Université de Montréal

Phytoremédiation d'un sol contaminé par des contaminants organiques et inorganiques

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Cette thèse intitulée

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Résumé

Le nombre important de sites contaminés au Québec (Canada) et partout dans le monde est une problématique de santé publique majeure en raison des risques toxicologiques qu'ils présentent pour la santé humaine et environnementale. Dans la municipalité de Varennes (Québec, Canada), située sur la rive sud de l'Île de Montréal, les activités d'une ancienne usine pétrochimique (Pétromont Inc.) ont conduit à l'accumulation de concentrations modérées à élevées d'éléments traces métalliques (ETMs), de biphényles polychlorés (BPCs), d'hydrocarbures pétroliers aliphatiques (C10-C50) et d'hydrocarbures aromatiques polycycliques (HAPs) sur les terrains de la compagnie. En 2010, une culture intensive de saule sur courtes rotations (CICR) a été établie sur le site, afin d'y conduire une expérience de phytoremédiation à grande échelle. Bien que cette plantation de *Salix miyabeana* ait été implantée dans une optique d'assainissement, aucun effet significatif n'a été signalé sur la concentration des contaminants du sol au cours des premières années de croissance. Les processus d'assainissement basés sur l'utilisation de végétaux peuvent être difficiles à prévoir en milieux naturels et nécessitent des améliorations afin d'en augmenter leur efficacité.

La fertilisation des sols avec des amendements organiques, ainsi que la manipulation du microbiome végétal, sont deux techniques agronomiques couramment utilisées pour la gestion des cultures traditionnelles, afin d'augmenter la production de biomasse et améliorer la santé générale des végétaux. Ces approches peuvent également influencer la mobilité et la biodisponibilité de certains composés du sol. Puisque de telles modifications sont connues pour avoir le potentiel d'améliorer considérablement l'efficacité des végétaux à éliminer ou à transformer certains contaminants du sol, ces deux techniques agronomiques présentent un intérêt grandissant dans le domaine de la phytoremédiation. Cette recherche doctorale vise donc à améliorer les connaissances scientifiques dans le domaine de la phytoremédiation appliquée à grande échelle en abordant certains aspects qui touchent à ces deux approches agronomiques.

En utilisant la plantation de saules déjà établie, une première étude a été réalisée afin d'évaluer l'impact d'un amendement de sol organique sur l'efficacité phytoremédiatrice des deux cultivars de saules ('SX61' et 'SX64'). À l'intérieur de cette plantation, le sol de certaines parcelles expérimentales a été recouvert de bois raméal fragmenté (BRF) de saules, combiné, ou non, avec du substrat de champignons épuisé (SCE) de *Pleurotus ostreatus*. Après trois saisons de croissance,

les résultats ont montré que l'ajout de SCE au BRF n'avait eu aucun effet sur la croissance des saules, ainsi que sur leur efficacité à extraire ou à réduire la concentration des contaminants présents sur le site. Les résultats suggèrent néanmoins que le BRF contribue à immobiliser certains HAPs dans le sol, en plus d'augmenter l'efficacité des saules à phytoextraire le Zn. La présence de saules semble avoir réduit de façon significative l'atténuation naturelle des C10-C50 sur le site. De plus, les concentrations de BPCs, de Cd, de Ni et de dix HAPs, ont montré des oscillations saisonnières, ce qui suggère que l'évapotranspiration qui a lieu à l'intérieur de la plantation de saules provoque un important flux d'eau et de contaminants solubles en direction des racines. Ainsi, la concentration de certains contaminants pourrait avoir tendance à augmenter à l'intérieur d'une dense plantation de saules au fil du temps.

Une deuxième étude a été réalisée à l'intérieur de cette même plantation, afin de vérifier si les augmentations de concentration observées précédemment pouvaient être liées à l'évapotranspiration qui a lieu à l'intérieur d'une plantation de saules. Dans l'optique d'éliminer l'effet de transpiration, des coupes de saules ont été effectuées dans certaines parcelles de la plantation, puis les concentrations des contaminants organiques et inorganiques ont été suivies au fil du temps (24 mois), et comparées avec celles observées dans les parcelles non coupées. Les résultats obtenus ont montré que l'élimination des saules avait bel et bien limité l'accumulation de certains contaminants à la surface du sol, tels qu'observé dans les parcelles non coupées. Ces résultats suggèrent donc encore une fois que la culture intensive de saules à courte rotation peut entrainer une migration de certains contaminants en direction des racines et ainsi augmenter leurs concentrations à la surface du sol près des zones racinaires. Très peu d'études ont rapporté des résultats qui semblent contredire les multiples avantages de purification qui sont habituellement mis de l'avant en phytoremédiation. Toutefois, de tels effets sur la mobilisation des contaminants pourraient être pertinents et souhaitables dans un contexte de gestion du risque.

La troisième et dernière étude présentée dans cette thèse explore la diversité des communautés microbiennes associées aux racines des deux cultivars de saules établis sur le site expérimental depuis plusieurs années (six années). Des études antérieures ont permis d'en apprendre davantage sur la composition du microbiome racinaire et rhizosphérique du saule poussant en milieux contaminés, mais la plupart de celles-ci ont été menées sur des individus relativement jeunes. Par conséquent, peu d'information existe concernant les associations

microbiennes des individus plus âgés qui ont été établis en milieux contaminés. La caractérisation des communautés fongiques, bactériennes et archéennes a permis de montrer des différences de composition entre les deux cultivars de saules, ainsi qu'entre leurs compartiments (i.e. racines et rhizosphère). Certains groupes taxonomiques, appartenant à chacun des trois domaines, se sont démarqués, de par leur abondance, ou par leurs fonctions écologiques déjà connues et potentiellement bénéfiques pour la survie des végétaux, ou pour augmenter la dégradation et l'extraction de divers contaminants. Cette étude fournit donc de précieuses informations qui pourront servir à l'amélioration de certaines approches d'ingénierie du microbiome favorisant l'établissement, la survie, la croissance et les performances d'assainissement de *Salix* spp. établis en milieux contaminés.

L'ensemble des résultats présentés dans cette thèse ont permis d'alimenter différentes réflexions sur l'intérêt d'utiliser certains amendements organiques et de caractériser le microbiome racinaire et rhizosphérique des saules afin d'améliorer les pratiques et la mise en œuvre de la phytoremédiation par des saules. Cette thèse met également en lumière un phénomène de migration des contaminants, influencé par la présence de plantes à croissance rapide, qui représente un obstacle pour l'évaluation des performances d'assainissement par des approches de phytoremédiation notamment par des saules.

Mots-clés: Salix, Pleurotus, Bois raméal fragmenté (BRF), Substrat de champignons épuisés (SCE), Microbiome, Culture intensive en courtes rotations (CICR), Phytoremédiation, Mycoremédiation, Contamination, Sol.

Abstract

The large number of contaminated sites in Quebec (Canada) and all around the world is a major public problem because of the toxicological risks they present for human and environmental health. In the municipality of Varennes (Quebec, Canada), located on the south shore of the Island of Montreal, the activities of a former petrochemical plant (Pétromont Inc.) have led to the accumulation of moderate to high concentrations of traces elements (TEs), polychlorinated biphenyls (PCBs), aliphatic petroleum hydrocarbons (C10-C50) and polycyclic aromatic hydrocarbons (PAHs) on the land. In 2010, a short rotation intensive culture (SRIC) of willow has been established on the site, in order to conduct a field-scale phytoremediation experiment. Although this plantation of *Salix miyabeana* was established with a remediation view, no significant effect was reported on the concentration of soil contaminants during the first years of growth. Plant-based remediation processes can be difficult to predict in the fiel and require improvement in order to increase their effectiveness.

Fertilization with organic amendments, as well as manipulating the plant microbiome, are two agronomic techniques commonly employed in traditional crop management, in order to increase biomass production and improve overall plant health. These approaches can also influence the mobility and bioavailability of some compounds in the soil. Since such modifications are known to have the potential to significantly improve the efficiency of plants in removing or transforming soil contaminants, these two agronomic techniques are of growing interest in the field of phytoremediation. This doctoral research aims to improve scientific knowledge in the field-scale phytoremediation application by addressing some aspects that affect these two agronomic approaches.

Inside the already established willow plantation, a first study was carried out to assess the impact of soil organic amendment on the phytoremediation efficacy of the two willow cultivars (‘SX61’ and ‘SX64’). The soil of some experimental plots was covered with ramial chipped wood (RCW) combined or not with spent mushroom substrate (SMS) of *Pleurotus ostreatus*. After three growing seasons, the results showed that the addition of SMS to the RCW had no effect on the growth of the willows, as well as on their effectiveness in removing or reducing the concentration of contaminants on the site. The results nevertheless suggest that RCW helps immobilize some PAHs in the soil, in addition to increasing the efficiency of willows to phytoextract Zn. The

presence of willows appears to have significantly reduced the natural attenuation of C10-C50 on the site. In addition, the concentrations of PCBs, Cd, Ni and ten PAHs, showed seasonal oscillations, which suggests that the evapotranspiration inside the willow plantation mobilized some contaminants towards the rooting zones. Thus, the concentration of certain contaminants may tend to increase within a dense willow plantation over time.

A second study was carried out inside the same plantation, in order to verify if the increases in concentration observed previously could be linked to the evapotranspiration that takes place inside a willow plantation. In order to eradicate the effect of plant transpiration, willows were harvested in certain plots of the plantation. The concentrations of organic and inorganic contaminants were followed over time (24 months) and compared with those observed in the unharvested plots. The results obtained showed that the removal of the willows limited the accumulation of certain contaminants on the soil surface, as observed in the uncut plots. These results suggested once again that the short rotation intensive culture of willows can lead to the migration of certain contaminants towards the roots and thus increase their concentrations on the soil surface near the root zones. Very few studies have reported results that seem to contradict the multiple purification benefits that are usually put forward in phytoremediation. However, such effects on contaminant mobilization could be relevant and suitable in a risk management context.

The third and final study presented in this thesis explores the microbial communities associated with the roots of the two willow cultivars established on the experimental site for several years (six years). Root and rhizosphere microbial communities of Salix spp. have been studied in contaminated environments, but most of studies have been carried out on relatively young hosts. Therefore, little information exists regarding the microbial communities associated with older willows established in contaminated environments. The characterization of fungal, bacterial and Archean communities has shown differences in composition between the two willow cultivars, as well as between their compartments (i.e., roots and rhizosphere). Some taxonomic groups, belonging to each of the three domains, caught our attention, either by their abundance, or by their ecological functions already known to be potentially beneficial for the plant survival, or for increasing the degradation and extraction of various contaminants. This study therefore provides valuable information that can be used to improve certain microbiome engineering approaches that

promote the establishment, survival, growth and phytoremediation performance of *Salix* spp. in contaminated environments.

All the results presented in this thesis have fueled various reflections on the interest of using soil organic amendments and characterizing the root and rhizosphere microbiome of willows in order to improve the practices and implementation of phytoremediation with willows. This thesis also highlights a phenomenon of contaminant migration, influenced by the presence of fast-growing woody plants, which represents an obstacle for the evaluation of phytoremediation performance approaches with willows.

Keywords: Salix, Pleurotus, Ramial chipped wood (RCW), Spent mushroom substrate (SMS), Microbiome, Short rotation intensive culture (SRIC), Phytoremediation, Mycoremediation, Contamination, Soil.

Table des matières

Liste des tableaux

Liste des figures

Liste des sigles et abréviations

(*En italique sont les termes en anglais.*)

ADN:	acide désoxyribonucléique
AMF:	*arbuscular mycorrhizal fungi*
ANOVA:	*analysis of variance*
ASV:	*amplicon sequence variant*
BCF:	*bioconcentration factor*
BG:	*bare ground*
bp:	*base pairs*
BPC:	biphényles polychlorés
BRF:	bois raméal fragmenté
BTEX:	benzène, toluène, éthylbenzène and xylènes
Cd :	cadmium
CEAEQ :	centre d'expertise en analyse environnementale du Québec
CICR:	culture intensive sur courtes rotations
CMA :	champignons mycorhiziens à arbuscule
Cr:	chrome/*chromium*
Ctrl:	contrôle/*control*
Cu:	cuivre/*copper*
DDT:	*dichlorodiphenyltrichloroethane*
DNA:	*desoxyribonucleic acid*
DNAPL:	*dense non-aqueous phase liquid*
DOC:	*dissolved organic carbon*
Dr:	*dormant*
EC:	*electrical conductivity*

EMF: *ectomycorrhizal fungi*

ET: *evapotranspiration*

ÉTM: Élément trace métallique

et al.: *et alia* (and company)

FA: *fulvic acids*

GC-MS: gas chromatography-mass spectrometry

GC-FID: gas chromatography-flame ionization detector

GERLED: Groupe d'études et de restauration des lieux d'élimination des déchets dangereux

Gr: *growing*

GV: *global variation*

HA: *humic acids*

HAP : hydrocarbures aromatiques polycycliques

HCP : hydrocarbures pétroliers

HSD: *honestly significant difference*

ICP-OES : *inductively coupled plasma-optical emission spectrometry*

i.e. : *« id est »*, expression latine signifiant « c'est-à-dire »

IV : *intermediate variation*

Kg: *kilogram*

K_{oe}: coefficient de partage octanol-eau

K_{ow}: *octanol-water partition coefficient*

LiPs: lignines peroxydases

LNAPL: *light non-aqueous phase liquid*

MDDELCC: ministère du développement durable, de l'environnement et de la lutte contre les changements climatiques

MELCC : ministère de l'environnement et de la lutte contre les changements climatiques

mg: *milligram*

mg ha^{-1} yr^{-1} : *milligram per hectare per year*

mg kg^{-1}: *milligram per kilogram*

Ni: nickel

odt ha^{-1} yr^{-1}: *oven dry tons per hectare per year*

MnPs: peroxydases dépendantes de manganèse

Pb: plomb/*lead*

PCB : polychlorinated biphenyls

PGPR: plant growth-promoting rhizobacteria

PHC: *petroleum hydrocarbon*

Ph. D.: *philosophiae doctor*

POP: polluants organiques persistants

RCW: *ramial chipped wood*

RDA: *redundancy analysis*

RLRQ: Recueil des lois et des règlements du Québec

SCE: substrat de champignons épuisé

SD: *standard deviation*

SRIC: *short rotation intensive culture*

SMC: *spent mushroom compost*

SMS: *spent mushroom substrate*

sp.: *specie*

spp.: *species*

SV: *seasonal variation*

TE: *trace element*

Tf: *final time*

Ti: *initial time*

TNT: trinitrotoluene

VF: *variation factor*

VR: *variation rate*

Vs.: *versus*

Zn: zinc

α: alpha

β: beta

μS cm^{-1}: microSiemens/cm

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Chapitre 1 | Introduction Générale

1.1 Mise en contexte

La révolution industrielle marque une période importante de l'histoire qui met en branle une cascade considérable d'inventions et d'innovations, ayant des conséquences grandement profitables pour la croissance économique et démographique de plusieurs grandes villes à travers le monde. À cette époque, l'impact des activités anthropiques sur les écosystèmes terrestres était ignoré des autorités, et très peu de lois régissaient la protection de l'environnement, laissant ainsi droit à des pratiques douteuses de gestions des déchets toxiques. La préoccupation du milieu juridique pour les questions environnementales a commencé à se faire sentir lorsque la communauté scientifique a débuté à établir des liens réels et potentiels entre la qualité de l'environnement et la santé humaine. Dans la juridiction québécoise, l'expression « pollution » apparaît pour la première fois lors de l'adoption de la loi de l'hygiène publique du Québec, en 1894 (refondue en 1901). Cette nouvelle législation comprenait des dispositions environnementales qui régissaient principalement la salubrité des immeubles, la suppression des nuisances, ainsi que le traitement, l'adduction et la pollution des eaux (Kenniff et Giroux, 1974). Malgré cette nouvelle loi de l'hygiène publique, la disposition des déchets toxiques demeurait tout de même problématique, puisqu'il n'existait encore aucun encadrement législatif à l'égard des sols. Les sols ont longtemps été considérés comme un réceptacle de déchets, dont on ne se préoccupait plus une fois déversées ou enterrées. Le déversement volontaire d'huile à moteur usée dans les sols était même considéré comme une pratique raisonnable et recommandée (Popular Science, 1963) (Figure 1.1). Suite à des catastrophes environnementales hautement médiatisées, comme celle de Love Canal (Niagara Falls, NY, USA), survenue dans les années 70, les normes environnementales nord-américaines ont commencé à devenir de plus en plus strictes. Malgré les nombreux progrès réalisés en matière de protection de l'environnement, il y a encore aujourd'hui de nombreux cas d'élimination sauvage et de déversement accidentel de matières dangereuses qui sont rapportés à l'attention du ministère de l'Environnement et de la lutte contre les changements climatiques du Québec (MELCC) (Beaulieu, 2016). Les milliers de litres d'huiles et de solvants qui ont été déversés un peu partout dans les écosystèmes terrestres font en sorte qu'aujourd'hui, la contamination et la pollution des sols représentent une problématique majeure pouvant avoir de lourdes conséquences sur la santé humaine et environnementale.

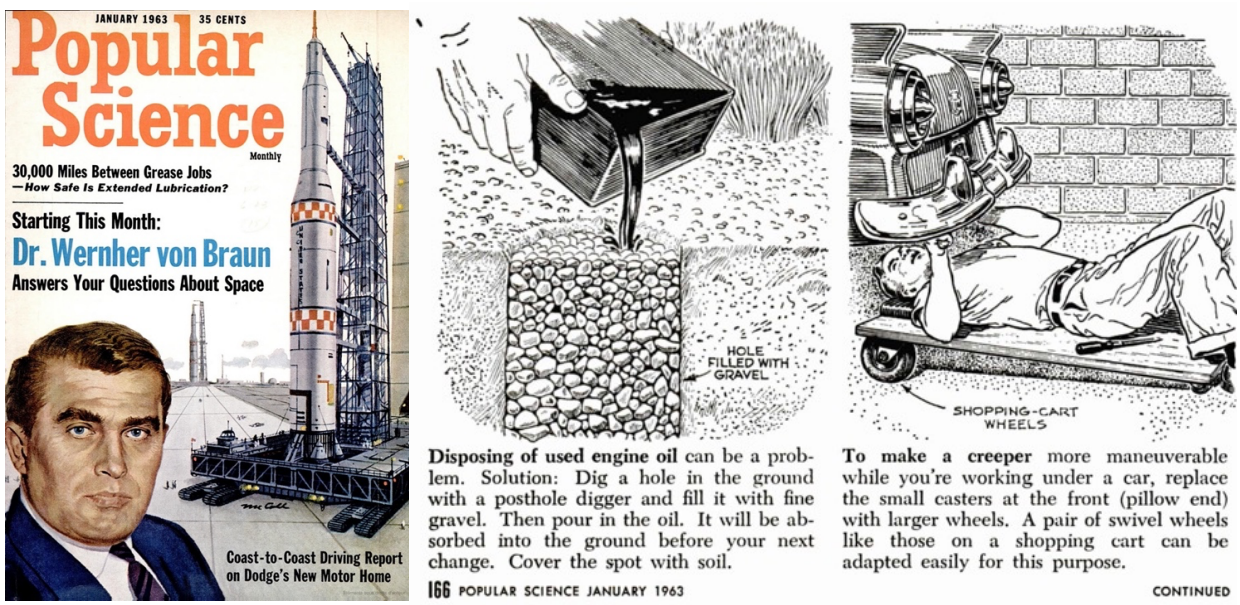

Figure 1.1 Gestion d'huile à moteur usée. Extrait tiré d'une revue américaine de science populaire publiée en janvier 1963 (Popular Science, 1963).

1.2 Contamination des sols

Le sol est une partie importante des écosystèmes terrestres qui joue un rôle fondamental dans la survie humaine. Il participe à l'équilibre et à la productivité des écosystèmes en accomplissant des fonctions essentielles de tampon, de filtrage, de stockage et de transformation. Malheureusement, ces fonctions essentielles ne sont pas illimitées et perdent de leur efficacité lorsque son équilibre naturel est perturbé, notamment par des contaminants et des polluants (Kabata-Pendias, 2010). La contamination et la pollution sont deux termes qui réfèrent à des concepts substantiellement différents, malgré qu'ils soient souvent utilisés de façon analogue dans la littérature. Un contaminant est défini comme une « substance » qui se trouve à des concentrations supérieures aux teneurs de fond, alors qu'un polluant est généralement défini comme un contaminant qui a le potentiel d'entrainer des effets biologiques néfastes pour les communautés résidentes (Chapman, 2007).

1.3 Types de contaminants

Au Québec, les terrains contaminés se caractérisent majoritairement par la présence de contaminants organiques potentiellement nocifs pour la santé humaine et environnementale. Une proportion inférieure, mais tout de même considérable, des terrains contaminés contiendraient des contaminants inorganiques ou une contamination mixte (Hébert et Bernard, 2013). Il n'est pas rare qu'un terrain contaminé contienne également des matières résiduelles, puisque dans le passé, des

débris de toutes natures ont été éliminés ou utilisés comme matériaux de remblais. De manière générale, les matériaux les plus fréquemment retrouvés sont issus de remblais de sables des fonderies, de scories métallurgiques, de résidus miniers, de scories de bouilloires, de mâchefers, de débris de construction et de démolition, ainsi que de déchets domestiques de toutes natures (Beaulieu, 2016; DRSP, 2016).

1.3.1 Hydrocarbures pétroliers (HCPs)

Les hydrocarbures pétroliers (HCPs) sont des molécules organiques, majoritairement composées d'atomes de carbone et d'hydrogène. Ces combustibles fossiles représentent une ressource énergétique et une matière première considérable pour beaucoup d'industries actuelles. Les rejets d'eaux usées industrielles et municipales, les activités pétrolières en mer et sur terre, ainsi que les déversements accidentels, contribuent à la contamination de l'environnement par les HCPs. Cette contamination affecte les écosystèmes et représente un risque pour la santé de la majorité des organismes vivants (Varjani, 2017). Les produits pétroliers sont des mélanges de molécules d'HCPs qui varient d'un à plus de cinquante atomes de carbone. En plus d'être très complexes, ces mélanges possèdent une constitution qui est unique d'un produit à un autre (Zakaria et al., 2001).

1.3.2 Les hydrocarbures aromatiques polycycliques (HAPs)

Les hydrocarbures aromatiques polycycliques (HAPs) sont des composés organiques d'origines pétrogéniques, pyrogéniques ou biologiques (Abdel-Shafy et Mansour, 2016). Les HAPs d'origines pyrogènes sont formés lorsque des composés organiques sont exposés à des températures très élevées dans des conditions d'oxygène faible ou absent. Ces composés se forment donc généralement lors d'une combustion incomplète de carburant (véhicules), de bois (feux de forêts et feux de foyers) et des mazouts (systèmes de chauffage). Les HAPs d'origines pétrogènes sont formés lors de la maturation du pétrole brut. Ils sont fréquents en raison de l'utilisation répandue des produits pétroliers. Leur propagation dans l'environnement est principalement due à des déversements accidentels, comme des fuites de réservoirs de stockage et le rejet d'essence et d'huile à moteur. Certains HAPs pourraient également être d'origine biologique en étant synthétisés par certaines plantes et bactéries ou formés lors de la dégradation biologique de la matière végétale (Abdel-Shafy et Mansour, 2016).

1.3.3 Biphényles polychlorés (BPCs)

Les biphényles polychlorés (BPCs) sont des molécules organiques synthétiques, composées de deux noyaux benzéniques liés, dont le nombre d'atomes d'hydrogène substitué par des atomes de chlore varie entre 1 et 10. Il existe donc 209 différents congénères de BPC, dont les propriétés chimiques varient en fonction du nombre d'atomes de chlore et de leur emplacement (Chatel et al., 2017). Leur utilisation répandue était essentiellement due à leurs propriétés diélectriques, leur ininflammabilité, leur point d'ébullition élevé, leur faible volatilité et leur miscibilité avec différents solvants organiques (Chatel et al., 2017; Vorkamp, 2016). En raison des risques toxicologiques associés aux BPCs, leur importation, leur fabrication et leur vente sont interdites au Canada depuis 1977. En raison de leur grande stabilité thermique, chimique et biologique, ces molécules sont très persistantes dans l'environnement, en plus d'être bioaccumulables et bioamplifiables (Li et al., 2006; Passatore et al., 2014; van Duursen et al., 2017). Les molécules de BPCs sont encore largement répandues dans l'environnement et environ 3% des inscriptions québécoises sont caractérisées par la présence de BPC (Hébert et Bernard, 2013).

1.3.4 Éléments traces métalliques (ÉTMs)

Les principaux contaminants inorganiques sont les métaux et métalloïdes. Dans la littérature, les termes « métaux lourds », « métaux toxiques » et « éléments traces » sont souvent utilisés sans distinction pour désigner des éléments métalliques qui présentent des risques écotoxicologiques (Duffus, 2002). En géochimie et en sciences biologiques, le terme « éléments traces » est généralement utilisé pour décrire les éléments chimiques aux propriétés différentes (i.e., métaux et métalloïdes) qui ont des teneurs de fond inférieures à 0,1% (1000 mg kg^{-1}) (Kabata-Pendias, 2010). Cependant, certains éléments, comme le fer, peuvent être considérés comme « traces » dans les tissus biologiques, alors qu'ils sont plutôt abondants en milieux terrestres. Le terme « métaux lourds » est aussi largement utilisé dans la littérature pour décrire certains éléments potentiellement toxiques. Il est généralement utilisé de façon large pour désigner des éléments regroupés sur la base de divers critères (i.e., poids atomique, numéro atomique, densité, propriétés chimiques) (Kabata-Pendias, 2010). Il existe plusieurs autres termes dans la littérature pour décrire les éléments de l'environnement, mais leur utilisation est souvent inadéquate, ce qui engendre beaucoup de confusion (Duffus, 2002). Dans le cadre de cette thèse, le terme « élément trace métallique » (ÉTM) sera utilisé pour faire référence aux métaux potentiellement toxiques.

Bien que les ÉTMs soient des éléments naturels de la croute terrestre, certaines activités anthropiques (i.e., industrielles, minières, agricoles) favorisent leur dispersion dans l'environnement. Puisqu'ils ne sont pas dégradables, les ÉTMs s'accumulent dans les écosystèmes, ce qui suscite des inquiétudes quant aux risques qu'ils présentent pour la santé humaine et environnementale (Tchounwou et al., 2012). Une fois dissipé à la surface du sol, leur devenir dépend principalement de leur spéciation, qui est fortement influencée par les propriétés physiques et chimiques du sol (Kabata-Pendias, 2010). En 2010, 26% des terrains inscrits au Répertoire des terrains contaminés du Québec présentaient une contamination en ÉTM.

1.4 **Méthodes de réhabilitation**

La réhabilitation des sols a pour objectif de redonner la qualité et les fonctions initiales à un site contaminé (Zabbey et al., 2017). En fonction de la nature des contaminants, des caractéristiques du site et des objectifs de travail, diverses stratégies peuvent être employées de façon *ex situ* ou *in situ* afin de s'attaquer à la problématique. Les traitements *ex situ* nécessitent l'extraction physique du milieu contaminé et un déplacement vers un autre endroit, alors que les traitements *in situ* se font directement sur place (Kuppusamy et al., 2016). Ces deux types de traitements ont bien évidemment des avantages et des coûts qui leur sont propres. Au Québec, l'excavation suivie de l'enfouissement (*Dig and dump*), est encore la technique de réhabilitation des sols la plus utilisée (Hébert et Bernard, 2013). Cette méthode consiste à cibler les points les plus contaminés d'un site, pour les excaver et les transporter vers des lieux d'enfouissement pour les éliminer ou les « valoriser » (Kuppusamy et al., 2016). Cette méthode offre une solution rapide et simple, mais les frais de manutention (i.e., excavation, transport et enfouissement) sont extrêmement élevés (Kuppusamy et al., 2016). Dans la province, la gestion hors site (*ex situ*) des sols contaminés excavés peut seulement être effectuée par enfouissement ou par traitement biologique, thermique et physico-chimique (Hébert et Bernard, 2013). Le recours à une approche *in situ* est généralement motivé par un accès restreint à la zone contaminée (i.e., sous un bâtiment ou un stationnement utilisé). Différentes méthodes physiques et chimiques peuvent être utilisées, mais la plupart ne conviennent pas vraiment à une contamination à grande échelle puisqu'elles nécessitent de la main-d'œuvre hautement qualifiée et sont très onéreuses (Zabbey et al., 2017). Certaines de ces techniques utilisent de l'eau, des réactifs et des solvants pour extraire ou dégrader les contaminants du sol, ce qui peut engendrer d'autres problèmes environnementaux (Lim et al., 2016).

1.5 Traitements biologiques

Le terme « bioremédiation » réfère à l'ensemble des stratégies biologiques utilisées pour éliminer ou transformer les déchets toxiques de l'environnement (Kumar et al., 2011). Des bactéries, des protistes, des plantes, des champignons et même des animaux peuvent être utilisés dans différentes approches de bioremédiation (Treu et Falandysz, 2017; Yao et al., 2012).

1.5.1 Biodisponibilité

Plusieurs organismes utilisés en bioremédiation doivent être en contact direct avec les contaminants de l'environnement pour les éliminer ou les transformer. Leur potentiel d'action dépendra donc fortement de la biodisponibilité des substances à traiter (Pilon-Smits, 2005). La fraction soluble des contaminants est généralement considérée comme la portion la plus disponible pour les organismes (Séguin et al., 2004). L'hydrophobicité et la volatilité des contaminants sont deux propriétés chimiques importantes qui influencent leur mobilité et leur biodisponibilité dans les sols. Les molécules de BPC, de HAP et d'hydrocarbure pétrolier C10-C50 sont en général très hydrophobes et ne sont pas très solubles dans l'eau. Celles-ci ont un coefficient de partage octanol:eau supérieure à 3 (log K_{ow}> 3) et sont généralement liées à la matière organique du sol. Ces liquides non aqueux peuvent atteindre les eaux souterraines et se retrouver au-dessous de l'aquifère (dense non-aqueous phase liquid (DNAPL)) ou au-dessus de l'aquifère (light non-aqueous phase liquid (LNAPL)), en fonction de leur densité. Les molécules qui ont un coefficient de partage octanol:eau inférieur à 3 (log K_{ow}< 3) sont hydrosolubles et sont capables de migrer dans l'eau interstitielle. Dans les sols, le partage des contaminants (organiques et ÉTMs) entre les phases solide et aqueuse est orchestré par une multitude de processus physicochimiques et biologiques (i.e., précipitation-dissolution, l'adsorption-désorption, la complexation et l'encapsulation) et de leur interaction. Ces processus sont très dynamiques et peuvent être modulés par différents facteurs, comme la nature des contaminants et leur spéciation, la structure du sol et sa pénétrabilité, l'humidité du sol, la température, le potentiel redox, le pH, la conductivité électrique (CE), la capacité d'échange cationique (CEC) et le pourcentage de matière organique et de carbone organique dissous (COD) (Carrillo-González et al., 2006; Ernst, 1996; Kabata-Pendias, 2010; Nguyen et al., 2017; Pilon-Smits, 2005; Rohrbacher et St-Arnaud, 2016).

1.5.2 Bactéries

Plusieurs bactéries ont la capacité naturelle de dégrader, de transformer et/ou d'immobiliser une panoplie de contaminants qui se trouvent dans l'environnement. Toutefois, pour y arriver, il

est nécessaire qu'elles soient en contact direct avec leur cible (Vidali, 2001). Certaines bactéries mobiles peuvent détecter les hydrocarbures à distance et présenter une réponse chimiotactique pour se déplacer dans leur direction (Thapa et al., 2012; Vidali, 2001). Elles peuvent également produire une grande variété de biosurfactants, dont certains restent attachés à la surface cellulaire pour favoriser l'attachement direct à un substrat hautement hydrophobe (Speight et El-Gendy, 2018), et d'autres sont libérés sous forme de molécules extracellulaires afin d'augmenter la solubilité, la mobilité et la biodisponibilité des contaminants (Varjani et Upasani, 2017). L'immobilisation des cellules bactériennes (i.e., sur de la perlite ou de la sciure de bois) favoriserait la production de biosurfactants (Emtiazi et al., 2005; Hazaimeh et al., 2014). Une fois dans la cellule, les contaminants peuvent être métabolisés pour être directement utilisés comme source de carbone et d'énergie, ou simplement cométabolisés (Dzionek et al., 2016; Speight et El-Gendy, 2018).

Bien que les milieux contaminés aux hydrocarbures pétroliers soient riches en carbone et en énergie, ils ne contiennent pas nécessairement les autres nutriments en quantités suffisantes pour supporter la croissance microbienne. La bioremédiation bactérienne peut donc se faire de façon naturelle (atténuation naturelle), mais elle peut aussi être stimulée (biostimulation) par l'ajustement des paramètres environnementaux, tels que la température, le pH, l'humidité, le niveau d'oxygène et le ratio (C:N:P:K) (Adams et al., 2015; Lim et al., 2016). La biodégradation des substances présentes dans le sol peut également être encouragée par un système (passif ou actif) qui favorise la circulation de l'air (bioventilation) (Hébert et Bernard, 2013; Speight et El-Gendy, 2018; Vidali, 2001). Lorsque le système comprend l'ajout d'un intrant (i.e., air chaud, vapeur, pointes chauffantes, etc.) qui permet d'augmenter la température du milieu jusqu'à une température maximale de 150 °C, on peut dire que la bioventilation est augmentée (bioventilation augmentée) (Hébert et Bernard, 2013). Des microorganismes (indigènes ou exogènes) peuvent être ajoutés (bioaugmentation) dans le milieu afin d'augmenter la biodégradation des substances présentes (Dzionek et al., 2016; Thapa et al., 2012). Les organismes individuels ont généralement la capacité de dégrader une variété plutôt limitée de composés organiques, alors qu'un consortium de populations mixtes présente des capacités enzymatiques globales beaucoup plus larges qui permettent de dégrader des mélanges d'organopolluants plus complexes (Cerqueira et al., 2011; Hazaimeh et al., 2014; Varjani, 2017; Varjani et Upasani, 2013).

Bien que certaines molécules très récalcitrantes puissent être dégradées dans des conditions anaérobies, la plupart des approches de bioremédiation bactériennes fonctionnent dans des conditions aérobies (Holliger et Zehender, 1996; Vidali, 2001). Ces deux voies métaboliques comportent une série d'étapes qui font appel à différentes enzymes impliquées dans des réactions de réduction, d'oxydation, d'hydroxylation et de déshydrogénation (Varjani, 2017). Les gènes qui codent pour ces enzymes peuvent être localisés sur un chromosome, mais la majorité provient de plasmides (Varjani, 2017).

Au Québec, le principe de biodégradabilité des hydrocarbures pétroliers a commencé à être appliqué à la fin des années 1970. Le premier projet de traitement biologique, porté à l'attention du ministère de l'Environnement, visait la décontamination de boues huileuses en utilisant une méthode d'épandage contrôlé des sols « *landfarming* » (Bégin et Prus, 1999). Cette méthode *ex situ* consistait à excaver des sols contaminés, puis à les épandre en minces couches d'épaisseur uniforme ou en andains sur un terrain receveur. L'activité des microorganismes aérobies pouvait être stimulée par un labour périodique, par l'ajustement de l'humidité, ou par l'ajout d'engrais, de minéraux et de nutriments (Bégin et Prus, 1999; Dzionek et al., 2016; Gouvernement of Canada, 2013). Cette approche entraînait une augmentation progressive de la contamination des terrains récepteurs et présentait des risques de transfert des contaminants dans l'air et l'eau. En raison des risques et de l'absence de contrôle rigoureux lié à cette pratique, le Ministère de l'Environnement du Québec a décidé de restreindre son utilisation à la fin des années 1980 (Bégin et Prus, 1999). Le « landfarming » demeure, malgré tout, accepté et utilisé dans d'autres provinces canadiennes (Gouvernement of Canada, 2013; Paudyn et al., 2008; Turgeon et al., 2017). Au Québec, la bioventilation est la principale technique de bioremédiation utilisée dans les différents centres régionaux de traitement de sols contaminés autorisés (Bégin et Prus, 1999; Hébert et Bernard, 2013; MDDELCC, 2020).

1.5.3 Champignons

La mycoremédiation, est une approche de bioremédiation qui profite essentiellement de la capacité qu'ont certains champignons saprotrophes à biodégrader une grande variété d'organopolluants. Les champignons de carie sont particulièrement intéressants pour cette approche puisqu'ils arrivent à dégrader efficacement les différentes composantes du bois, dont certaines ont une structure moléculaire qui s'apparente beaucoup à celle de certains contaminants. Le bois est principalement composé de cellulose, d'hémicellulose et de lignine. La lignine est très

résistante à la dégradation par la plupart des organismes. C'est un matériau amorphe de grande taille, qui est insoluble dans l'eau et non hydrolysable (Hammel, 1995). Ce polymère de phényle propane très complexe se combine chimiquement avec la cellulose et l'hémicellulose pour procurer une rigidité structurelle aux plantes ainsi qu'une protection contre de potentielles attaques microbiennes (Anastasi et al., 2013). En fonction de l'apparence du bois mort, il est possible de distinguer le type de champignon impliqué dans sa décomposition. Les champignons de carie blanche s'attaquent principalement à la lignine et y laissent la cellulose non dissoute, ce qui donne un aspect blanchi au bois. En revanche, les champignons de carie brune dégradent principalement la cellulose et y laissent la lignine, ce qui donne au bois une apparence brunâtre (Rhodes, 2014).

Les champignons de carie blanche ont la capacité de dégrader la lignine du bois, grâce à un système enzymatique de phénoloxydases, qui comprend des laccases, des lignines peroxydases (LiPs) et des peroxydases dépendantes de manganèse (MnPs) (Treu et Falandysz, 2017). Ces enzymes extracellulaires sont capables de minéraliser complètement la lignine et les glucides du bois en CO_2 et en H_2O. La lignine n'est pas utilisée comme source de carbone pour leur croissance, mais sa dégradation permet plutôt une ouverture de la structure du bois pour que les enzymes impliquées dans la dégradation des polysaccharides puissent le pénétrer (Anastasi et al., 2013; Hammel, 1995). Les champignons de carie blanche regroupent essentiellement des membres appartenant au phylum des basidiomycètes (Boulet, 2003), mais certains ascomycètes de la famille des *Xylariaceae* en feraient également partie (Anastasi et al., 2013; Pointing et al., 2003). Puisque leurs enzymes ligninolytiques sont extracellulaires et non spécifiques, les champignons de carie blanche peuvent attaquer efficacement un large spectre d'organopolluants environnementaux qui possèdent une structure moléculaire similaire à celle du bois (Anastasi et al., 2013; Hammel, 1995).

Le pleurote en huitre (*Pleurotus ostreatus*) est un champignon de carie blanche qui s'est montré efficace pour l'élimination d'hydrocarbures aliphatiques (Colombo et al., 1996), de plusieurs HAPs (Covino et al., 2010; Kadri et al., 2017) et d'une fraction considérable de différents BPCs (Cvančarová et al., 2012). Sa capacité à dégrader les contaminants environnementaux lui est essentiellement due aux différentes enzymes ligninolytiques oxydatives (LiP, MnP et laccase) qu'il possède et sécrète (da Luz et al., 2012). Un système enzymatique intracellulaire, qui inclut des cytochromes P-450 monooxygénases (CYP450), des aryl-alcool déshydrogénases, des aryl-

aldéhyde déshydrogénases et des époxydes hydrolases, pourrait également être impliqué dans la biotransformation des xénobiotiques (Bezalel et al., 1997; Cvančarová et al., 2012).

Les champignons de carie brune sont exclusivement des basidiomycètes, et ils représentent environ 6% de tous les champignons capables de dégrader le bois. Ils s'attaquent presque exclusivement à du bois de conifères et ne possèdent pas les phénols oxydases nécessaires pour dégrader la lignine (Anastasi et al., 2013). Ils ont malgré tout le potentiel de biodégrader divers contaminants organiques, tel que des HAPs et du DDT, grâce à un système catalytique non enzymatique de type Fenton qui leur sert normalement à dépolymériser partiellement la lignine avant de dégrader la cellulose et l'hémicellulose présentes dans le bois (Anastasi et al., 2013).

1.6 **Phytoremédiation**

La phytoremédiation est une technique de bioremédiation *in situ* qui utilise les plantes et les microorganismes qui leur sont associés pour éliminer, transformer ou immobiliser divers contaminants organiques et inorganiques qui se retrouvent dans l'air, dans l'eau ou dans le sol (Gerhardt et al., 2017). Cette approche montre un haut niveau d'acceptabilité sociale, puisqu'elle est efficace, abordable et sécuritaire (Weir et Doty, 2016).

Les plantes peuvent être utilisées pour empêcher la mobilisation ou le lessivage des ÉTMs du sol (phytostabilisation) en influençant leur sorption, leur précipitation et leur complexation. Dans la littérature, les termes "phytoséquestration", "phytoimmobilisation" et "phytoconfinement" sont parfois utilisés pour faire référence à la phytostabilisation (Roy et Pandey, 2020). Cette approche ne permet pas la décontamination des sols, mais elle représente une solution simple et économique pour la gestion du risque, en réduisant la biodisponibilité des contaminants dans l'environnement (Ali et al., 2013; Gerhardt et al., 2017). Les ÉTMs peuvent également être extraits du sol (phytoextraction) et accumulés dans les parties aériennes des végétaux (phytoaccumulation). Pour que ce processus ait lieu, les ÉTMs doivent être biodisponibles. La plante peut exsuder différents acides organiques, des protons, des biosurfactants et des biochélateurs qui vont augmenter la biodisponibilité des métaux au niveau de la rhizosphère. Les ÉTMs peuvent entrer dans les racines en suivant les mêmes voies d'absorption que les autres éléments nutritifs, soit par voies apoplastique, symplastique ou transmembranaire (Gerhardt et al., 2017). Une fois dans les cellules, les ÉTMs peuvent être séquestrés dans les parois cellulaires ou dans des vacuoles. Ils peuvent donc être stockés dans les racines ou dans les parties aériennes, s'il y a translocation (Ali

et al., 2013). Le mercure (Hg), le sélénium (Se) et l'arsenic (As) peuvent se volatiliser une fois transférés aux feuilles (phytovolatilisation) (Gerhardt et al., 2017).

Les mécanismes d'absorption des contaminants organiques sont différents de ceux qui s'appliquent aux ÉTMs, puisqu'il n'existe aucun transporteur membranaire pour ces molécules à la surface des cellules végétales. Leur entrée dans les cellules n'est possible que par diffusion. Les molécules organiques qui ont un log K_{ow} inférieur à 0,5 sont trop hydrophiles pour traverser la membrane des cellules végétales par diffusion. Les molécules qui ont un log K_{ow} entre 0,5 et 3, comme les BTEX, sont suffisamment hydrophobes pour traverser la bicouche lipidique des membranes cellulaires et sont suffisamment hydrophiles pour se déplacer dans les fluides cellulaires (Pilon-Smits, 2005). Ces molécules peuvent donc pénétrer les racines des plantes, pour ensuite être transférées aux feuilles. Une fois dans les cellules, ces xénobiotiques pourront être dégradées (phytodégradation), volatilisées (phytovolatilisation) ou simplement accumulées (phytoextraction) (Gerhardt et al., 2017). Les molécules organiques qui ont un log K_{ow} supérieur à 3 sont trop hydrophobes pour traverser complètement la membrane cellulaire. Par conséquent, elles peuvent être stabilisées (phytostabilisation) en restant emprisonnées dans les membranes et les parois cellulaires qui sont en périphérie des tissus racinaires (Pilon-Smits, 2005). Les plantes peuvent participer à la dégradation (rhizodégradation) de différents contaminants organiques du sol en exsudant des enzymes, comme des laccases, des phénols oxydases et des peroxydases qui vont directement catalyser des réactions d'oxydation (Rohrbacher et St-Arnaud, 2016). Toutefois, en phytoremédiation, c'est plutôt l'activité enzymatique d'origine microbienne, stimulée par l'exsudation racinaire (phytostimulation), qui serait la principale voie de dégradation des contaminants organiques (Martin et al., 2014).

1.6.1 Saules (Salix spp.)

La sélection judicieuse des végétaux est une étape critique qui aura une influence déterminante sur le succès d'un traitement par phytoremédiation. Plusieurs centaines de végétaux, incluant des plantes ligneuses et des herbacées, ont été étudiés et identifiés pour leur capacité phytoremédiatrice (Antoniadis et al., 2021; Fagnano et al., 2020; Khan et al., 2021; Pandey et Singh, 2020; Patra et al., 2021; Rosselli et al., 2003; Shang et al., 2020). Bien que toutes les plantes n'aient pas le même potentiel d'action sur les contaminants environnementaux, elles doivent, d'abord et avant tout, être bien adaptées aux conditions climatiques et édaphiques du site à traiter. Il est généralement souhaitable de sélectionner des plantes qui ont une croissance rapide; qui

produisent des quantités élevées de biomasse aérienne; qui ont un système racinaire largement répandu et ramifié; qui sont faciles à cultiver et à récolter; et qui sont tolérantes aux contaminants, aux ravageurs et aux autres facteurs de stress (Ali et al., 2013; Gerhardt et al., 2017).

La famille des *Salicaceae*, inclue des candidats particulièrement intéressants pour la phytoremédiation, notamment les saules (*Salix* spp.) et les peupliers (*Populus* spp.), qui en plus d'avoir prouvé leur efficacité pour traiter divers contaminants organiques et inorganiques (Courchesne et al., 2017a; Greger et Landberg, 1999; Hultgren et al., 2010; Janssen et al., 2015; Kersten, 2015; McIntosh et al., 2016; Mleczek et al., 2018; Padoan et al., 2020; Pavlíková et al., 2007), s'adaptent facilement à différentes conditions édaphiques; possèdent un système racinaire profond et dense; et donnent des rendements élevés en biomasse (Cristaldi et al., 2017; Greger et Landberg, 1999; Padoan et al., 2020). Le genre *Salix* comprend environ 450 espèces d'arbres et arbustes, qui sont principalement distribués dans l'hémisphère nord (Kuzovkina et Quigley, 2005). La plupart des espèces occupent des écosystèmes de basses terres qui se caractérisent par un apport hydrique relativement constant, comme des forêts riveraines, des plaines inondables et des milieux humides. Plusieurs espèces sont tolérantes aux conditions difficiles et jouent un rôle écologique important en tant qu'espèces pionnières de sites limités en éléments nutritifs, tels que des dunes de sable, des tourbières, et des bancs de graviers (Kuzovkina et Quigley, 2005). Les graines de *Salix* spp. ne sont généralement pas viables très longtemps (quelques semaines), et nécessitent des conditions hydriques particulières pour germer, ce qui complique leur établissement par semis. Les saules sont tout de même faciles à cultiver, puisque pratiquement toutes les espèces ont la capacité de croitre à partir de boutures (Pulford et Watson, 2003). En effet, à l'exception de *S. caprea* et *S. scouleriana*, tous les *Salix* spp. forment des primordia racinaires sur les nœuds de tige, ce qui facilite leur propagation végétative (Kuzovkina et Quigley, 2005). Des boutures en dormance non racinées (de 20-30 cm) sont fréquemment utilisées pour la culture intensive de saules à courtes rotations (CICR) (Labrecque et Teodorescu, 2006). Cette approche permet d'établir des plantations denses et productives pour des applications lucratives et des projets environnementaux (Kuzovkina et Quigley, 2005). Les tiges sont récoltées, tous les deux à quatre ans, le pied et son système racinaire demeure en place et la plantation peut être productive pendant des dizaines d'années (Labrecque et Teodorescu, 2006). Récemment, un intérêt a été porté pour le développement de méthodes de propagations alternatives, qui misent sur l'utilisation de microboutures de saules pour des applications environnementales (Guidi Nissim et Labrecque, 2016; Labrecque et al., 2020).

Les saules font incontestablement partie des plantes les plus polyvalentes pour des applications environnementales. Par exemple, ces phréatophytes à croissance rapide peuvent être utilisées pour filtrer des eaux usées municipales (Lachapelle-T. et al., 2019), pour traiter des lixiviats d'entrepôts et de décharges (Bialowiec et al., 2007; Lévesque et al., 2017), ou pour minimiser le lessivage de pesticides dans des champs agricoles (Hénault-Ethier et al., 2017; Lafleur et al., 2016). La biomasse de saule présente une valeur économique intéressante, en particulier pour les procédés bioénergétiques (Ul Hai et al., 2019), mais aussi pour la fabrication de matériaux ligneux, comme du BRF ou des clôtures en tiges de saules écorcées (Ramo, 2021). Ces secteurs d'activités donnent une valeur ajoutée aux approches de phytoremédiation, en offrant des revenus alternatifs attrayants (Ruttens et al., 2011). L'intérêt croissant pour le développement de cultures ligneuses sur des sites contaminés a incité plusieurs groupes de recherche à évaluer les performances de quelques centaines de taxons (i.e., espèces, cultivars et hybrides) de saules à travers le monde (Fischerová et al., 2006; Janssen et al., 2015; Landberg et Greger, 1996; Mleczek et al., 2017; Shang et al., 2020; Tlustoš et al., 2007). Au Québec, une grande variété de saules a également été testée sur des terrains agricoles, des terrains marginaux et des terrains contaminés (Grenier et al., 2015; Guidi Nissim et al., 2013; Guittonny-Larchevêque et Lortie, 2017; Labrecque et Teodorescu, 2005, 2006; Pray et al., 2018). De ce fait, les cultivars 'SX61' et 'SX64' (*Salix miyabeana*) ont été sélectionnés et reconnus comme étant parmi les plus productifs et performants dans l'est du Canada.

1.6.2 Microbiome végétal

Le terme « microbiome » ou « phytomicrobiome » fait généralement référence à l'ensemble des communautés microbiennes (i.e., champignons, levures, bactéries, algues, nématodes, archées, etc.) qui colonisent les différentes parties d'une plante (Bhatt et al., 2020; Quiza et al., 2015). Les microorganismes occupent la plupart des compartiments végétaux, notamment la surface des feuilles (phyllosphère), la surface des racines (rhizoplane), la zone de sol qui s'étend environ 1mm autour des racines (rhizosphère), ainsi que les parties internes des différents organes aériens et souterrains (endosphère) (Bhatt et al., 2020; Quiza et al., 2015; Yates et al., 2021). Le microbiome accomplit des fonctions complémentaires si importantes pour la croissance et la santé générale des végétaux, que la plante n'est plus considérée comme un simple individu à part entière, mais plutôt comme un « métaorganisme » ou un « holobionte » (Thijs et al., 2016; Vandenkoornhuyse et al., 2015).

La rhizosphère est un environnement fortement sélectif qui héberge une quantité impressionnante de microorganismes, dont les plus abondants sont les bactéries et les champignons (Hrynkiewicz et Baum, 2012; Quiza et al., 2015). En général, leur diversité y est plus faible que dans le sol adjacent « bulk soil », mais leur abondance peut y être de 2 à 20 fois supérieure (Hrynkiewicz et Baum, 2012), et l'activité métabolique d'origine microbienne peut y être stimulée de 10 à 100 fois (Ali et al., 2013). En plus d'agir comme support mécanique, les racines exsudent une multitude de métabolites primaires et secondaires (i.e., acides organiques, acides aminés, acides gras, glucides, vitamines, nucléotides, composés phénoliques, polysaccharides, protéines, etc.) qui attirent et nourrissent les microorganismes du sol (Rohrbacher et St-Arnaud, 2016). Il a été estimé qu'environ 11% du carbone fixé par photosynthèse était redistribué au sol sous forme d'exsudats racinaires (Jones et al., 2009). La quantité et la qualité des exsudats racinaires varient en fonction de plusieurs facteurs biotiques et abiotiques (i.e., espèce, cultivar, stade de développement, type de sol, pH) et jouent un rôle important dans la composition du microbiome rhizosphérique (Rohrbacher et St-Arnaud, 2016).

Les différents microorganismes qui composent le microbiome rhizosphérique forment un système complexe d'interactions biologiques, qui a des conséquences fondamentales sur les différents processus d'assainissement de leur hôte, notamment en affectant la croissance des plantes, mais aussi leur productivité et leur résilience face aux différents stress biotiques et abiotiques (Thijs et al., 2017). Ils peuvent également influencer les processus de phytoremédiation en agissant directement sur les différents contaminants organiques et inorganiques du sol, notamment en les dégradant, en les immobilisant, ou en modifiant leur mobilité et biodisponibilité (Gerhardt et al., 2017).

Certaines rhizobactéries sont connues pour favoriser la croissance des plantes (plant growth-promoting rhizobacteria (PGPR)), en agissant comme biofertilisant, notamment en fixant l'azote atmosphérique ou en solubilisant le phosphate inorganique. Elles peuvent également favoriser la croissance végétale, en agissant comme agents de lutte biologique, via une production d'antibiotiques et de composés antifongiques qui limitent la croissance d'organismes pathogènes (Quiza et al., 2015). Les rhizobactéries peuvent sécréter une variété de métabolites qui vont induire la résistance systémique chez la plante (Induced systematic resistance (ISR)). Ce mécanisme est

généralement activé pour augmenter la résistance face aux phytopathogènes, mais il améliorerait également la résistance des plantes face aux ÉTMs et aux polluants organiques (Prakash, 2021).

Des champignons du sol s'associent aux racines pour former des symbioses mycorhiziennes, qui reposent essentiellement sur des échanges mutuels d'éléments nutritifs indispensables à la croissance et à la survie des deux partenaires (Anastasi et al., 2013). Ces mycorhizes peuvent aussi favoriser la croissance des plantes en jouant un rôle de protection contre différents stress biotiques et abiotiques (Finlay, 2008). Les *Salicaceae* sont connues pour avoir la capacité de s'associer avec des champignons mycorhiziens arbusculaires (CMA), ainsi qu'avec des champignons ectomycorhiziens (ECM). Les CMA sont des biotrophes obligatoires appartenant au sous-phylum des Glomeromycotina (Berruti et al., 2014; Spatafora et al., 2016). Ce groupe monophylétique est composé d'un nombre relativement faible d'espèces (~150–200), qui forment des associations symbiotiques avec environ 72% de toutes les espèces de plantes vasculaires terrestres (Brundrett et Tedersoo, 2018). Sans pénétrer le plasmalemme, les hyphes des CMA forment des arbuscules à l'intérieur des cellules corticales des racines. Ces structures ramifiées augmentent la surface de contact et maximisent les échanges nutritionnels entre les deux partenaires (Finlay, 2008). Les ECM appartiennent principalement aux phylums des Basidiomycètes et des Ascomycètes. Ils sont très diversifiés (~10 000 espèces), mais ne s'associent qu'avec un nombre relativement limité de taxons végétaux, dont la plupart sont des arbres ou des arbustes (Brundrett, 2009; Finlay, 2008). Le mycélium des ECM forme une gaine ou un manteau autour des racines, et les hyphes pénètrent entre les cellules corticales pour former le réseau de Hartig, qui favorise les échanges de nutriments avec la plante (Finlay, 2008). Les hyphes extraradicaux des CMA et des ECM génèrent un vaste réseau mycélien qui explore le sol au-delà des zones d'épuisement nutritif qui entourent les racines. Le champignon y absorbe de l'eau et des nutriments (i.e., azote et phosphore) et les échange contre des composés carbonés d'origines photosynthétiques.

1.7 **Site expérimental**

Dans la municipalité de Varennes (Québec, Canada), située sur la rive sud de l'île de Montréal, une usine pétrochimique (Pétromont Inc.), a été présente pendant plus de 40 ans, avant de cesser ses activités en 2008 (Turcotte, 2009). Entre 1963 et 1975, trois bassins de décantation ont été aménagés à proximité des unités de production de l'usine, afin de contrôler les rejets liquides

issus des activités pétrochimiques (Figure 1.2). Entre 1972 et 1979, les boues de décantation ont été recueillies et épandues à quatre reprises, sur un terrain vacant situé à l'intérieur de la propriété de l'entreprise. Ces épisodes d'épandage ont été réalisés avec l'aval du service de protection de l'environnement de la direction générale de l'environnement industriel du gouvernement du Québec, et avaient pour objectif de décontaminer les boues en faisant appel à une approche de « landfarming » (1.5.2). Ces activités ont donc conduit à l'accumulation progressive de plusieurs contaminants (i.e., HAPs, BPCs, C10-C50 et certains ÉTMs) dans la couche superficielle de sol.

En juin 2010, une partie du secteur de l'ancienne aire d'épandage des boues a été utilisée comme site expérimental pour y débuter des études en phytoremédiation. Celui-ci fait référence au secteur GERLED (Groupe d'étude et de restauration des lieux d'élimination des déchets dangereux) dans l'étude de (Guidi et al., 2012). Le site utilisé était plutôt plat et avait une superficie totale de 5 840 m^2. Son centroïde se trouvait à moins de 350 mètres du fleuve Saint-Laurent et était environ 3,5 mètres au-dessus du niveau d'eau du fleuve (Figure 1.3). Deux cultivars de *Salix miyabeana* ('SX61' et 'SX64') ont été répartis en sept groupes de trois rangs, pour un total de 21 rangs, qui occupaient la presque totalité du site expérimental, soit 5 475 m^2. La plantation a été effectuée mécaniquement suivant une technique de culture intensive de saules en courtes rotations (CICR) (Labrecque et Teodorescu, 2006). Les boutures de saules ont été espacées de 1,8 m x 0,3 m, pour atteindre une densité de 18 500 plantes par hectare. Les saules ont été recepés pour une première fois en décembre 2010, puis trois ans plus tard, en décembre 2013, pour mettre fin au premier cycle de croissance végétale et à une première phase expérimentale. La Figure 2.2 du chapitre suivant montre les détails de cette plantation.

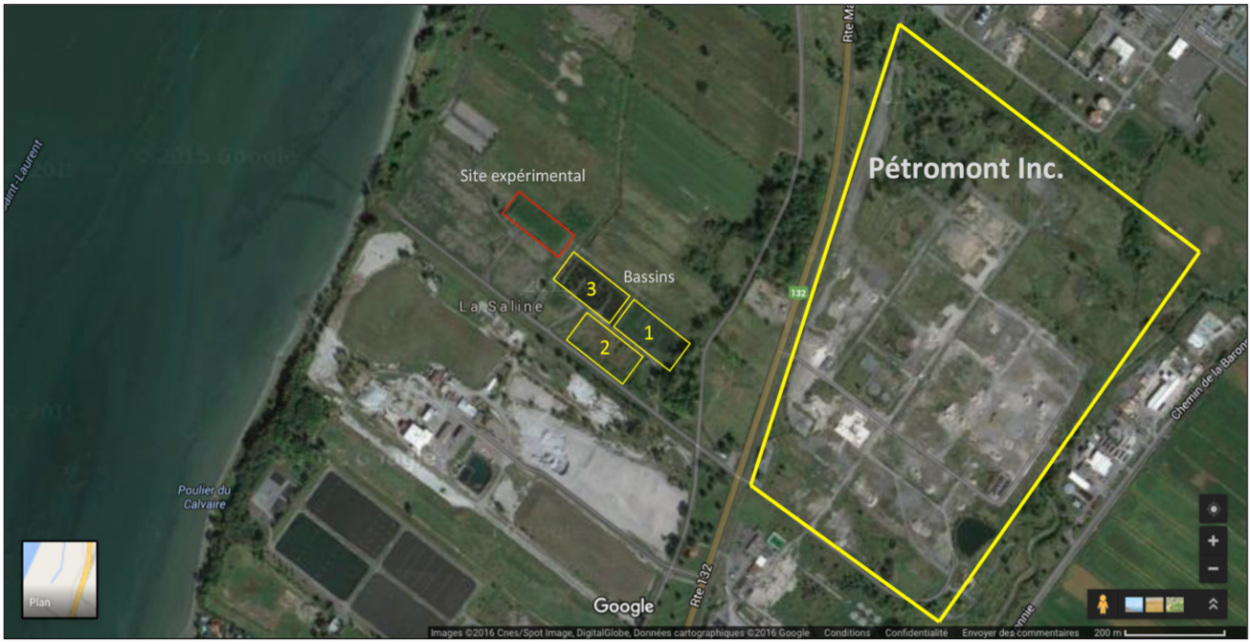

Figure 1.2 L'aire délimitée en rouge correspond à l'emplacement géographique du site expérimental (secteur de l'ancienne aire d'épandage des boues). Les aires délimitées en jaune réfèrent à l'emplacement des trois bassins de décantation, ainsi qu'aux unités de production de l'usine Pétromont Inc.

1.8 **Problématique du projet**

Au cours de la première phase expérimentale réalisée sur le secteur de l'ancienne aire d'épandage des boues, aucune diminution significative des contaminants n'a été observée dans le sol (données non publiées). Dans le rapport de recherche partagé à l'interne, il a été émis comme hypothèse que l'absence de résultat concluant pouvait être dû à la concomitance de plusieurs facteurs, qui incluent une méthode d'échantillonnage inadéquate; une hétérogénéité de la contamination dans chaque parcelle; un retour au sol des ÉTMs suite à la chute des feuilles; et une montée des contaminants vers la surface du sol, entrainée par la transpiration des plantes (IRBV, 2013).

Figure 1.3 Carte topographique du secteur à l'étude.

Malgré la compréhension croissante des processus de phytoremédiation et le succès de nombreuses études réalisées en laboratoire et en serre, il semble qu'il y ait encore beaucoup d'études de terrains qui soient également confrontées à des résultats peu concluants et difficiles à interpréter. Les variables environnementales et les méthodes d'évaluation sont souvent considérées comme les principaux facteurs d'embuche pour traduire la recherche de laboratoire à une échelle de terrain (Gerhardt et al., 2009).

La phytoremédiation est une approche alternative très attrayante pour la réhabilitation des sols, mais pour qu'elle devienne plus efficace et viable, il est nécessaire de développer des stratégies qui permettent d'atténuer le stress des plantes et maximiser leur croissance dans les sols contaminés. Il est également essentiel de bien connaitre les facteurs environnementaux qui ne peuvent pas être pris en compte dans les expériences de laboratoire, afin d'ajuster les méthodes actuelles de suivi aux conditions de terrain et d'établir des critères d'évaluation réalistes et fiables.

1.9 Objectifs de la thèse

Cette thèse doctorale comporte trois objectifs distincts qui s'insèrent dans une vision plus large visant à améliorer les stratégies de mise en œuvre, de suivi et d'évaluation d'une approche de phytoremédiation appliquée à grande échelle avec *Salix miyabeana*.

1.9.1 Chapitre 2 - Amendements de sol organiques

Objectif : Évaluer l'impact d'un amendement de sol organique, tel que du bois raméal fragmenté (BRF) de saules, et du substrat de champignons épuisé (SCE) de *Pleurotus ostreatus*, sur l'efficacité phytoremédiatrice des deux cultivars de saules ('SX61' et 'SX64'), établis depuis 4 ans sur le site contaminé de Varennes.

1.9.2 Chapitre 3 – Coupe de saules

Objectif : À l'intérieur de la plantation de saules établie depuis 7 ans, évaluer l'impact de l'élimination du couvert végétal évapotranspirant sur la variation des contaminants organiques et inorganiques.

1.9.3 Chapitre 4 - Communautés microbiennes

Objectif : Caractériser les communautés microbiennes (champignons, bactéries, archées) associées aux racines des deux cultivars de saules établis sur le site depuis 6 ans.

Chapitre 2 | Short Rotation Intensive Culture of Willow, Spent Mushroom Substrate and Ramial Chipped Wood for Bioremediation of a Contaminated Site Used for Land Farming Activities of a Former Petrochemical Plant

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2.1 Abstract

The aim of this study was to investigate the bioremediation impacts of willows grown in short rotation intensive culture (SRIC) and supplemented or not with spent mushroom substrate (SMS) and ramial chipped wood (RCW). Results did not show that SMS significantly improved either biomass production or phytoremediation efficiency. After the three growing seasons, RCW-amended S. miyabeana accumulated significantly more Zn in the shoots, and greater increases of some PAHs were found in the soil of RCW-amended plots than in the soil of the two other ground cover treatments' plots. Significantly higher Cd concentrations were found in the shoots of cultivar 'SX61'. The results suggest that 'SX61' have reduced the natural attenuation of C10-C50 that occurred in the unvegetated control plots. The presence of willows also tended to increase the total soil concentrations of PCBs. Furthermore, we found that many contaminant concentrations were subject to seasonal oscillations, showing average increases throughout the whole experimental site after a growing period, while showing significantly different variations, such as lesser increases or even decreases, after a dormant period. These observations suggest that contaminants may have leached or degraded faster in untreated conditions, and conversely to have mobilized towards trees through water flow driven by plant transpiration during growing seasons.

Keywords: willow; Salix; phytoremediation; short rotation intensive culture (SRIC), spent mushroom substrate (SMS), ramial chipped wood (RCW), soil contaminants

2.2 Introduction

Soil contamination with organic and inorganic pollutants is a widespread problematic around the world. Phytoremediation has been proposed as a cost-effective technique that uses plants and amendments to improve physical, biological and chemical soil properties, with the aim of reducing the risks for the environment and for human health (Fagnano et al., 2020; Gerhardt et al., 2017; Weir et Doty, 2016). Plants' ability to remediate pollutants effectively differs according to species and cultivars, but also depends on the microbiome composition in the rhizosphere (Bell et al., 2016; Fagnano et al., 2020; Wang et Greger, 2004). Soil properties as well as the nature of contaminants and their bioavailability are important factors that affect the mechanisms and efficiency of phytoremediation (Cristaldi et al., 2017; Mench et al., 1994). Polluted areas can be improved with plants and their associated microorganisms by implementing one or more phytoremediation techniques, which include phytoextraction, phytoaccumulation, phytotransformation, phytostabilization, rhizofiltration, phytovolatilization, phytodegradation and rhizodegradation (Cristaldi et al., 2017; Dagher et al., 2020b; Fagnano et al., 2020; Susarla et al., 2002). These plant-based remediation processes are lengthy compared to conventional approaches and the results are difficult to predict, requiring refinement to increase their efficiency. Nonetheless, phytoremediation is considered a safe remediation tool that has been attracting increasing interest and gaining social acceptability over the past 20 years (Gerhardt et al., 2017; Weir et Doty, 2016).

For successful phytoremediation, the plants selected need to be able to survive under the climate in the geographic region of a given site, and be well adapted to its edaphic conditions. Moreover, their tolerance to harsh conditions, such as contamination, pests and other stressors on the site must also be considered (Gerhardt et al., 2017). To date, several hundred plants have been studied around the world under different contaminated conditions, enabling identification of many herbaceous and woody plants able to improve the condition of contaminated sites (Antoniadis et al., 2021; Fagnano et al., 2020; Khan et al., 2021; Pandey et Singh, 2020; Patra et al., 2021; Rosselli et al., 2003; Shang et al., 2020).

The facility of willows (*Salix* spp.) and poplars (*Populus* spp.) in adapting to new environmental conditions, combined with their ability to rapidly produce high biomass yield as well as deep, dense roots, make them particularly interesting candidates for phytoremediation of

contaminated lands (Cristaldi et al., 2017; Greger et Landberg, 1999; Padoan et al., 2020). Many *Salix* spp. and *Populus* spp. have proven their efficacy in situ for the management of various organic and inorganic contaminants (Courchesne et al., 2017a; Greger et Landberg, 1999; Hultgren et al., 2010; Janssen et al., 2015; Kersten, 2015; McIntosh et al., 2016; Mleczek et al., 2018; Padoan et al., 2020; Pavlíková et al., 2007). Furthermore, the attractive economic value associated to the short rotation intensive culture (SRIC) of these woody plants, especially for bioenergy and biofuel processes, turns phytoremediation using these crops into a promising profitable activity (Janssen et al., 2015; Karp, 2014; Ruttens et al., 2011; Ul Hai et al., 2019). SRIC of willow and poplar is also increasingly considered as an effective solution to manage urban contaminated brownfields, because the resulting green infrastructures improve the landscape and provide ecosystem services, in addition to contributing to carbon sequestration (Cunniff et al., 2015; McHugh et al., 2015; Padoan et al., 2020).

Due to a growing interest in the development of woody crops under contaminated conditions, hundreds of *Salix* taxa were tested in many countries in order to compare their respective performance under different climates (Fischerová et al., 2006; Janssen et al., 2015; Landberg et Greger, 1996; Mleczek et al., 2017; Shang et al., 2020; Tlustoš et al., 2007). In Quebec (Canada), several studies conducted on agricultural land, abandoned farmland or contaminated land, have shown that 'SX61' and 'SX64' (*Salix miyabeana*) are two of the most biomass productive willow cultivars that thrive in eastern Canada (Grenier et al., 2015; Guidi Nissim et al., 2013; Guittonny-Larchevêque et Lortie, 2017; Labrecque et Teodorescu, 2005; Pray et al., 2018). Agronomic techniques that promote growth and tolerance of fast-growing species, and increase the mobility and bioavailability of contaminants, are of particular growing interest, because they could leads to overall better remediation (Fagnano et al., 2020; Gerhardt et al., 2017). Accordingly, several studies focused on inoculation with bacteria and both arbuscular mycorrhizal (AM) fungi and ectomycorrhizal (EM) fungi to enhance the phytoremediation efficacy of willows (Dagher et al., 2020b; Janssen et al., 2015; Pray et al., 2018; Yergeau et al., 2015). Organic soil amendments can also be employed to improve the ability of plants to remediate their environment. In recent years, the use of organic by-products as fertilizer or directly for the remediation of degraded areas has been the focus of several studies (Chirakkara et Reddy, 2015; Fagnano et al., 2020; Grobelak, 2016; Wiszniewska et al., 2016).

Spent mushroom substrates (SMS) are composted organic wastes, containing agronomic residues and active mycelia, which are a by-product of edible mushroom cultivation. SMS of *Pleurotus* spp. has been studied for potential use as a value-added product, especially as natural organic amendment in soils (Lou et al., 2017; Owaid et al., 2017) and as an available and cheap source of enzymes for bioremediation purposes (Buswell, 1994; Eggen et Sasek, 2002; Phan et Sabaratnam, 2012). *Pleurotus* spp. are among the white-rot fungi known for their abilities to degrade organic compounds through the production and secretion of ligninolytic enzymes such as lignin peroxidases, manganese peroxidases and laccases (Kadri et al., 2017). Ramial chipped wood (RCW) is a substrate made from branches, twigs and leaves, that can support the growth of white-rot fungi, which are the only known organisms capable of degrading all components of wood cell walls, including lignin (Caron et al., 1998; Goltapeh et al., 2013; Lemieux et Germain, 2000). Fungal wood decay processes play important functions in the humic system, and perform better when fungi are associated with other soil microorganisms (i.e., bacteria, algae and protozoa) (Caron, 1994). The organic soil components formed during RCW-decomposition processes are known to influence the physical, chemical and biological properties of soils, promoting the formation and maintenance of a fertile environment, by improving the water retention capacity and fixing nutrients or making them more available for plants (Goltapeh et al., 2013; Lemieux et Germain, 2000). Because of its decomposition-derived organic compounds, RCW has been investigated as a possible amendment for use in the phytoremediation of soils contaminated by trace elements (Hattab et al., 2014, 2015).

This study was conducted on a contaminated site which has historically (1972 to 1979) been used for land farming activities, specifically, for ex situ waste treatment of a site formerly occupied by a petrochemical refinery plant, located near Montreal (Quebec, Canada). The refinery's activities led to the accumulation of moderate to high concentrations of trace elements (TEs), polychlorinated biphenyls (PCBs), aliphatic compounds C10-C50 and polycyclic aromatic hydrocarbons (PAHs) in the soil. More recently (in 2010), two *Salix miyabeana* cultivars ('SX61' and 'SX64'), were successfully established on the site for remediation purposes, following a SRIC technique (Guidi et al., 2012). Over the initial years of growth, no significant decrease in soil contaminant concentration was reported for any of the compounds tested (unpublished data). The aim of this study was to investigate the effect of two different organic soil amendments (SMS of

Pleurotus ostreatus and RCW of *Salix* spp.), introduced in this willow plantation, on the bioremediation of TEs, PCBs, C10-C50 and PAHs present in the soil.

2.3 Results

2.3.1 Initial Soil Contaminant Concentrations (T0)

The initial soil samples (T0) revealed that PCBs, C10-C50, anthracene, benz[a]anthracene, Cr, Cu and Ni were the most problematic contaminants on the site, based on the provincial Land Protection and Rehabilitation Regulation, RLRQ, c. Q-2, r. 37, Sch. I (Table 2.1).

Table 2.1 Initial soil contaminant concentrations.

Parameters	Ctrl	SX61	SX64	*p*-value
PCBs	26.72±13.98 ^{B}	83.69±25.84 ^{A}	45.28±22.23 ^{B}	***0.006*** **
C10-C50	1757.60±1122.18 ^{B}	6189.33±2365.19 ^{A}	3097.87±1190.97 ^{B}	***<0.001*** ***
Cadmium	1.78±0.31 ^{B}	2.23±0.16 ^{A}	1.91±0.20 ^{B}	***0.019*** *
Chromium	411.00±200.10 ^{C}	912.73±143.32 ^{A}	623.13±157.63 ^{B}	***<0.001*** ***
Copper	791.80±382.98 ^{B}	2381.33±579.51 ^{A}	1279.47±559.65 ^{B}	***0.001*** **
Nickel	66.20±15.64 ^{C}	106.47±9.35 ^{A}	84.93±12.46 ^{B}	***<0.001*** ***
Zinc	234.20±75.27 ^{C}	479.27±54.00 ^{A}	337.87±66.96 ^{B}	***<0.001*** ***
Acenaphthene	0.31±0.21 ^{B}	2.25±3.75 ^{A}	0.67±0.25 ^{B}	***0.003*** **
Acenaphtylene	1.71±1.43 ^{B}	8.63±3.27 ^{A}	4.25±2.09 ^{B}	***<0.001*** ***
Anthracene	6.00±3.76 ^{B}	34.96±13.22 ^{A}	19.50±8.25 ^{B}	***0.001*** **
Benz[a]anthracene	0.23±0.16 ^{B}	1.14±0.54 ^{A}	0.52±0.25 ^{B}	***0.002*** **
Benzo[a]pyrene	0.11±0.08 ^{B}	0.48±0.25 ^{A}	0.22±0.11 ^{B}	***0.006*** **
Benzo[ghi]perylene	0.12±0.08 ^{B}	0.71±0.29 ^{A}	0.36±0.16 ^{B}	***0.002*** **
Chrysene	0.18±0.13 ^{B}	0.57±0.45 ^{A}	0.25±0.11 ^{B}	***0.004*** **
Fluoranthene	0.23±0.15 ^{B}	1.40±2.39 ^{A}	0.40±0.17 ^{B}	***0.004*** **
Fluorene	0.41±0.25 ^{B}	2.14±1.15 ^{A}	0.98±0.40 ^{B}	***0.004*** **
Indeno[1,2,3-cd]pyrene	0.09±0.07 ^{B}	0.49±0.19 ^{A}	0.24±0.12 ^{B}	***0.002*** **
Naphthalene	0.19±0.09 ^{B}	0.65±0.20 ^{A}	0.34±0.15 ^{B}	***<0.001*** ***
Phenanthrene	1.01±0.66 ^{B}	3.36±1.10 ^{A}	1.79±0.73 ^{B}	***<0.001*** ***
Pyrene	0.51±0.35 ^{B}	2.99±3.81 ^{A}	1.06±0.51 ^{B}	***0.006*** **
1-Methylnaphthalene	0.23±0.13 ^{B}	0.61±0.18 ^{A}	0.35±0.11 ^{B}	***<0.001*** ***
2-Methylnaphthalene	0.23±0.15 ^{B}	0.62±0.23 ^{A}	0.40±0.16 ^{B}	***0.002*** **
1,3-Dimethylnaphthalene	0.29±0.17 ^{B}	0.87±0.26 ^{A}	0.48±0.17 ^{B}	***<0.001*** ***
2,3,5-Trimethylnaphthalene	0.09±0.07 ^{B}	0.61±0.89 ^{A}	0.20±0.09 ^{B}	***0.001*** **

Values are the averages (mean±SD, n=5 for Ctrl; n = 15 for 'SX61'; n=15 for 'SX64') of contaminant concentrations (mg kg^{-1}), found in the initial soil samples (T0). Significance levels (*p*-value) are shown and asterisks ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$) indicate a significant difference in concentration between experimental conditions. Capital letters on the same row indicate differences according to Tukey's HSD test.

Data of Pb and some PAHs were removed from results due to concentrations below levels of concern (data not shown). All other targeted organic and inorganic contaminants have been found at much higher concentrations near plots planted with cultivar 'SX61' in the following order: 'SX61' > 'SX64' ≥ Ctrl. All contaminant concentrations found in 'SX61' plots were significantly

different ($p \leq 0.05$) from those found in 'SX64' and in Ctrl plots. Significant differences between 'SX64' and Ctrl plots were observed for the concentrations of Cr, Ni and Zn only.

The experimental design did not allow us to statistically compare values found at T0 with those observed in the 2010 soil characterization (Table 2.6 below). However, visual distributions are shown in the box plots of Figure S 2.1. Soil samples from 'SX61' plots generally showed higher mean values of contaminant concentration than those observed during soil characterization. Conversely, the samples collected in the unplanted plots (Ctrl) or in 'SX64' plots at T0 generally showed lower or similar values compared to those observed during soil characterization.

2.3.2 Intermediate Variation (IV) and Global Variation (GV) of Soil Contaminant Concentrations

Given the significant differences in soil contaminant concentrations observed between planted and unplanted plots in the initial soil samples (T0), we calculated the IV to compare the treatment effects over time. The mean contaminant concentrations per treatment, as well as their mean IV, are presented for each sampling time in Table S 2.1. Values for T5 can also be considered to be the GV observed over the course of this study. GV of eight compounds (i.e., PCBs, C10-C50 and six PAHs) are presented in Table 2.2. The IV of contaminant concentrations in soil was not significantly affected by treatments until the third growing season. Indeed, at T4, ground cover treatments showed an effect on the IV_{T4} of 1,3-dimethylnaphthalene. Its variations observed in the RCW plots were significantly different from those observed in the BG plots, with respective values of +52.66% vs. −33.49%. At the end of the third growing season (T5), GV of 1,3-dimethylnaphthalene remained significantly different between RCW plots and BG plots, with respective variations of +45.70% vs. +8.71% (Table 2.2). The GV of other PAHs, such as benzo[ghi]perylene, naphthalene and 1-methylnaphthalene, also showed significant differences between RCW plots and BG plots, with respective values of +68.66% vs. +16.89%, +31.06% vs. −25.83% and +36.60% vs. −18.58% (Table 2.2). The GV of 1-methylnaphthalene observed in the RCW (+36.60%) was also significantly different from its GV observed in the RCW+SMS plots (−6.81%). Over the course of this experiment, no significant effect of cultivar treatments was found concerning the IV of TEs, PCBs or PAHs in soil (Table S 2.1). However, after the third growing season, GV of C10-C50 were significantly different between the Ctrl plots and the 'SX61' plots, with respective values of −61.55% and −41.86% (Table 2.2).

Table 2.2 Global variation (GV) of soil PCBs, C10-C50 and six PAHs.

Treatments		PCBs	C10-C50	Benz[a]-anthracene	Benzo[ghi]-perylene	Naphthalene	1-Methyl-naphthalene	2-Methyl-naphthalene	1,3-Dimethyl-naphthalene
Ctrl	BG	23.87±22.33 (−16.06±53.42)	593.60±643.07 (−72.32±12.30)	0.14±0.15 (+85.00±343.97)	0.11±0.11 (+65.00±244.69)	0.08±0.07 (−1.67±168.68)	0.08±0.07 (−4.17±170.12)	0.08±0.07 (−0.83±168.85)	0.13±0.15 (+76.17±348.86)
	RCW	27.40±21.86 (+9.72±53.16)	674.80±402.98 (−55.82±26.21)	0.27±0.15 (+142.50±248.05)	0.14±0.11 (+55.00±144.05)	0.14±0.11 (+53.33±250.32)	0.15±0.07 (+23.33±157.83)	0.11±0.05 (−18.33±74.16)	0.25±0.21 (+61.33±176.24)
	RCW+SMS	33.19±22.54 (+11.33±38.73)	897.80±803.55 (−56.52±18.15)	0.20±0.15 (+100.00±335.88)	0.16±0.15 (+120.00±325.19)	0.11±0.11 (+41.67±256.24)	0.11±0.11 (+39.17±257.72)	0.11±0.11 (+42.50±256.18)	0.18±0.19 (+130.33±431.78)
SX61	BG	103.84±34.83 (+31.61±9.56)	3742.00±1327.96 (−35.62±14.07)	0.86±0.43 (−14.41±27.35)	0.70±0.42 (−6.33±27.24)	0.36±0.09 (-42.50±17.22)	0.42±0.13 (−26.57±30.23)	0.38±0.11 (-30.00±36.13)	0.62±0.08 (-14.38±35.33)
	RCW	89.78±24.35 (+29.81±26.09)	3528.00±821.87 (−29.38±18.78)	0.84±0.25 (−9.74±14.77)	0.70±0.25 (+12.30±21.31)	0.40±0.07 (-27.05±15.66)	0.44±0.09 (−17.52±12.04)	0.38±0.13 (−27.67±16.65)	0.60±0.12 (−24.13±17.29)
	RCW+SMS	106.90±46.26 (+4.45±35.25)	3416.00±1614.75 (−48.95±27.76)	0.86±0.40 (−21.08±49.18)	0.64±0.30 (−3.33±42.64)	0.32±0.08 (-51.90±14.72)	0.36±0.13 (−41.27±28.94)	0.36±0.09 (-42.93±26.73)	0.42±0.13 (−51.92±24.61)
SX64	BG	56.14±22.32 (+47.73±44.60)	1670.60±583.58 (−36.10±12.04)	0.40±0.12 (−14.17±42.25)	0.34±0.09 (−8.00±25.15)	0.22±0.04 (−33.33±20.41)	0.26±0.09 (−25.00±34.36)	0.20±0.07 (−50.33±29.59)	0.32±0.13 (−35.67±38.90)
	RCW	61.20±34.18 (+30.77±40.53)	1831.80±838.99 (-49.81±17.89)	0.54±0.19 (+281.56±681.36)	0.40±0.12 (+138.67±314.87)	0.26±0.05 (+66.90±242.45)	0.28±0.08 (+104.00±333.21)	0.22±0.04 (+15.71±159.19)	0.32±0.08 (+99.90±335.62)
	RCW+SMS	57.64±24.17 (+35.88±26.77)	1812.60±955.62 (-39.66±18.54)	0.44±0.21 (−11.11±43.21)	0.33±0.17 (−8.33±45.64)	0.19±0.09 (−36.67±21.73)	0.27±0.13 (−18.33±39.70)	0.23±0.11 (−35.00±28.50)	0.36±0.18 (−17.67±27.73)
p-value	Cover	0.782	0.835	0.057	***0.038****	***0.035****	***0.026****	0.059	***0.045****
	Cultivar	0.081	***0.048****	0.886	0.968	0.713	0.497	0.758	0.864
	Cover*Cultivar	0.305	0.216	0.796	0.530	0.830	0.741	0.820	0.475
Interpretation		-	**Ctrl^{B}**	-	**BG^{B}**	**BG^{B}**	**BG^{B}**	-	**BG^{B}**
		-	**SX61^{A}**	-	**RCW^{A}**	**RCW^{A}**	**RCW^{A}**	-	**RCW^{A}**
		-	**SX64^{AB}**	-	**RCW+SMS^{AB}**	**RCW+SMS^{AB}**	**RCW+SMS^{B}**	-	**RCW+SMS^{AB}**

The first value is the average (mean±SD, n=5) contaminant concentration (mg kg^{-1}) for each treatment at T5. The value in parentheses, below the preceding one, represents the average (mean±SD, n=5) GV (%). Negative values indicate a decrease in concentration. Significance levels (*p*-value) are shown and asterisks (∗$p \leq 0.05$, ∗∗$p \leq 0.01$, ∗∗∗$p \leq 0.001$) indicate a significant effect of Cover, Cultivar or Cover*Cultivar on GV values. Capital letters in the final row were used to identify significant differences between treatments according to Tukey's HSD test ($p \leq 0.05$).

Table 2.3 Seasonal variation (SV) of soil PCBs, nickel and eight PAHs.

Treatments			PCBs	Nickel	Benz[a]-anthracene	Benzo[ghi]-perylene	Chrysene	Naphthalene	Phenanthrene	1-Methyl-naphthalene	1,3-Dimethyl-naphthalene	2,3,5-Trimethyl-naphthalene
Growing period (Gr)	Ctrl	BG	17.56±75.04	−5.03±20.14	47.44±241.92	41.67±185.57	92.39±247.43	25.44±190.28	266.32±1007.36	28.33±191.40	86.74±344.78	25.00±137.26
		RCW	40.08±70.25	26.29±71.85	100.00±206.80	67.86±111.99	136.31±227.72	32.26±143.34	689.22±2424.26	41.92±149.79	196.89±669.04	80.00±310.00
		RCW+SMS	18.31±48.83	6.93±26.79	7.74±95.94	25.00±89.34	42.86±160.92	28.57±197.01	219.93±729.20	21.73±149.75	80.12±300.19	78.57±307.89
	SX61	BG	34.60±58.12	12.03±29.59	4.05±45.03	13.40±37.15	20.06±43.56	−5.87±49.87	42.61±94.61	−3.60±60.83	19.66±79.22	24.72±83.46
		RCW	26.55±29.69	12.92±31.02	−1.67±30.14	6.79±23.46	17.33±37.80	−14.97±26.78	26.73±106.34	−9.53±37.08	6.40±86.39	25.72±163.51
		RCW+SMS	35.20±56.89	10.38±27.87	−5.03±41.01	17.18±52.78	0.17±39.60	−16.63±40.32	13.01±93.42	−20.29±38.47	-9.35±66.81	4.18±153.05
	SX64	BG	31.03±96.53	13.97±42.99	32.93±186.42	36.22±133.09	41.56±140.34	-2.44±87.40	24.82±133.69	15.78±138.34	14.66±104.99	5.33±102.32
		RCW	29.46±27.87	9.28±21.25	34.30±134.01	29.44±80.96	59.78±136.71	1.25±86.64	184.57±697.84	9.03±87.98	25.25±138.93	−9.17±51.57
		RCW+SMS	25.50±31.96	8.04±15.96	−1.89±49.81	4.22±33.77	15.52±65.87	2.78±47.52	49.85±108.16	−6.48±35.52	34.82±113.74	15.83±97.08
Dormant period (Dr)	Ctrl	BG	1.79±57.36	0.77±34.50	5.00±47.96	−10.00±21.08	−5.67±75.63	1.67±49.35	-13.93±53.78	12.50±90.71	15.12±119.25	31.67±103.77
		RCW	−17.85±32.55	−23.63±20.91	−0.93±81.06	−17.59±52.45	−30.56±55.28	20.37±106.65	7.53±91.99	36.11±116.07	13.05±85.71	92.59±137.97
		RCW+SMS	−14.54±20.75	−16.68±12.32	28.70±111.89	−12.96±20.03	15.37±113.66	−3.70±42.92	1.26±102.21	18.33±109.66	15.19±77.05	57.41±146.75
	SX61	BG	−11.23±40.50	−13.06±12.52	21.83±95.71	−7.03±41.41	8.65±76.65	7.06±61.05	5.49±124.14	40.61±132.28	39.63±174.37	72.33±236.07
		RCW	−13.98±27.70	−14.78±6.01	13.29±52.95	5.71±43.37	−11.08±35.96	22.83±53.98	7.45±72.06	50.83±105.30	32.56±95.22	31.19±88.35
		RCW+SMS	−23.21±34.42	−17.33±7.16	14.63±63.10	−11.05±32.58	−2.24±28.35	14.67±50.67	13.85±76.20	62.67±145.55	38.70±112.60	73.25±148.85
	SX64	BG	18.76±69.77	−14.73±19.55	18.33±81.78	−2.50±52.42	3.21±64.43	30.00±69.30	12.57±55.10	26.00±73.41	14.83±56.73	60.00±90.68
		RCW	−16.39±34.73	−16.30±6.27	9.52±34.95	−1.50±20.07	−11.17±33.19	34.17±66.72	5.41±74.33	31.67±95.65	16.96±56.88	40.00±80.97
		RCW+SMS	−3.64±61.50	−14.07±6.92	61.00±203.73	−1.67±38.05	9.50±88.81	−6.67±43.18	0.94±87.90	36.67±120.20	5.11±93.11	92.62±239.59
p-value	Cover		0.571	0.681	***0.002*** **	***0.028*** *	***0.042*** *	***0.021*** *	***0.015*** *	***0.006*** **	***0.005*** **	0.405
	Cultivar		***0.024*** *	0.072	0.865	0.924	0.602	0.589	0.854	0.384	0.770	0.682
	Period		***<0.001*** ***	***0.024*** *	0.641	***0.007*** **	***0.009*** **	***0.028*** *	***0.012*** *	***0.025*** *	0.850	***0.015*** *
	Cover*Cultivar		0.165	0.767	0.846	0.890	0.698	0.919	0.842	0.755	0.825	0.402
	Cover*Period		0.255	0.207	0.412	0.635	0.491	0.811	0.998	0.853	0.912	0.941
	Cultivar*Period		0.725	0.994	0.838	0.726	0.253	0.890	0.655	0.803	0.825	0.817
	Cover*Cultivar* Period		0.601	***0.013*** *	0.589	0.214	0.855	0.549	0.562	0.970	0.560	0.549
Interpretation			**Ctrl^{B} SX61^{AB} SX64^{A}**	**Gr::Ctrl BG^{B} RCW^{A} RCW+SMS^{AB}**	**BG^{B} RCW^{A} RCW+SMS^{B}**	**BG^{B} RCW^{A} RCW+SMS^{AB}**	**BG^{AB} RCW^{A} RCW+SMS^{B}**	**BG^{B} RCW^{A} RCW+SMS^{B}**	**BG^{B} RCW^{A} RCW+SMS^{B}**	**BG^{B} RCW^{A} RCW+SMS^{B}**	**BG^{B} RCW^{A} RCW+SMS^{B}**	- - -
			Gr > Dr	**Gr > Dr**	-	**Gr > Dr**	**Gr > Dr**	**Gr < Dr**	**Gr > Dr**	**Gr < Dr**	-	**Gr < Dr**

Values are the mean SV_p (mean±SD) (%) for each treatment, observed either after a Growing period (Gr, n=15 for each treatment) or after a Dormant period (Dr, n=10 for each treatment). Negative values indicate a decrease in concentration. Significance levels (*p*-value) are shown and asterisks ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$) indicate a significant effect of Cover, Cultivar, Period, Cover*Cultivar, Cover*Period, Cultivar*Period and Cover*Cultivar*Period on SV. Capital letters and symbols of comparison (> or <) in the final row were used to identify significant differences between treatments according to Tukey's HSD test ($p \leq 0.05$).

2.3.3 Variation Rate (VR) of Soil Contaminant Concentrations

The VR was also calculated to compare the treatment effects over time in a different way. The mean contaminant concentrations and their mean VR per treatment are presented for each sampling time in Table S 2.2. Period type was added as a new two-level variable (growing and dormant) in the VR dataset. The mean VR per treatment was then calculated for each period type and referred to as the mean seasonal variation (mean SV_p). Table 2.3 presents the mean SV_p of ten contaminants (i.e., PCBs, nickel and eight PAHs), as well as the significant differences, according to the three-way ANOVA between VR values.

In general, PCBs concentrations significantly increased between two consecutive sampling times, moreso in 'SX64' than under Ctrl plots, with average VR of +17.03% vs +11.37%, respectively. The concentrations of benz[a]anthracene, benzo[ghi]perylene, chrysene, naphthalene, phenanthrene, 1-methylnaphthalene and 1,3-dimethylnaphthalene increased significantly more under RCW plots than under either one or both of the other ground cover treatments (BG and RCW+SMS) (Table 2.3). The period type had a significant effect on the variation rates of PCBs, Cd, Ni and ten (10) PAHs concentrations in soil. The positive VR of PCBs, Cd, Ni, anthracene, benzo[ghi]perylene and chrysene observed after a growing period, was significantly different from their negative VR observed after a dormant period. Fluoranthene, indeno[1,2,3-cd]pyrene, phenanthrene and pyrene, showed positive VR after both periods, but their mean VR after a growing one was significantly higher than observed after a dormant period. Naphthalene, 1-methylnaphthalene and 2,3,5-trimethylnaphthalene also showed positive VR after both periods, but a reverse pattern with significantly greater increases after a dormant period, than after a growing one. Values concerning SV_p of all contaminants, including Cd, anthracene, fluoranthene, indeno[1,2,3-cd]pyrene and pyrene, can be found in Table S 2.3.

Redundancy analysis (RDA) was conducted to evaluate the relationship between soil contaminant VR and ground cover treatments (BG, RCW or RCW+SMS), cultivar treatments (Ctrl, 'SX61' or 'SX64'), as well as periods (growing or dormant) (Figure 2.1). According to the permutation test, only period variables had significant impact on soil contaminant variations (r^2 = 0.06708, $p \leq 0.001$***), followed by cultivar treatments (r^2 = −0.00529, p = 0.972) and ground cover treatments (r^2 = -0.00683, p = 1.00). Only the first RDA axis was significant ($p \leq 0.001$***), explaining 7.18% of the cumulative percentage variance of all soil contaminant variations. The entire RDA model was significant ($p \leq 0.001$***).

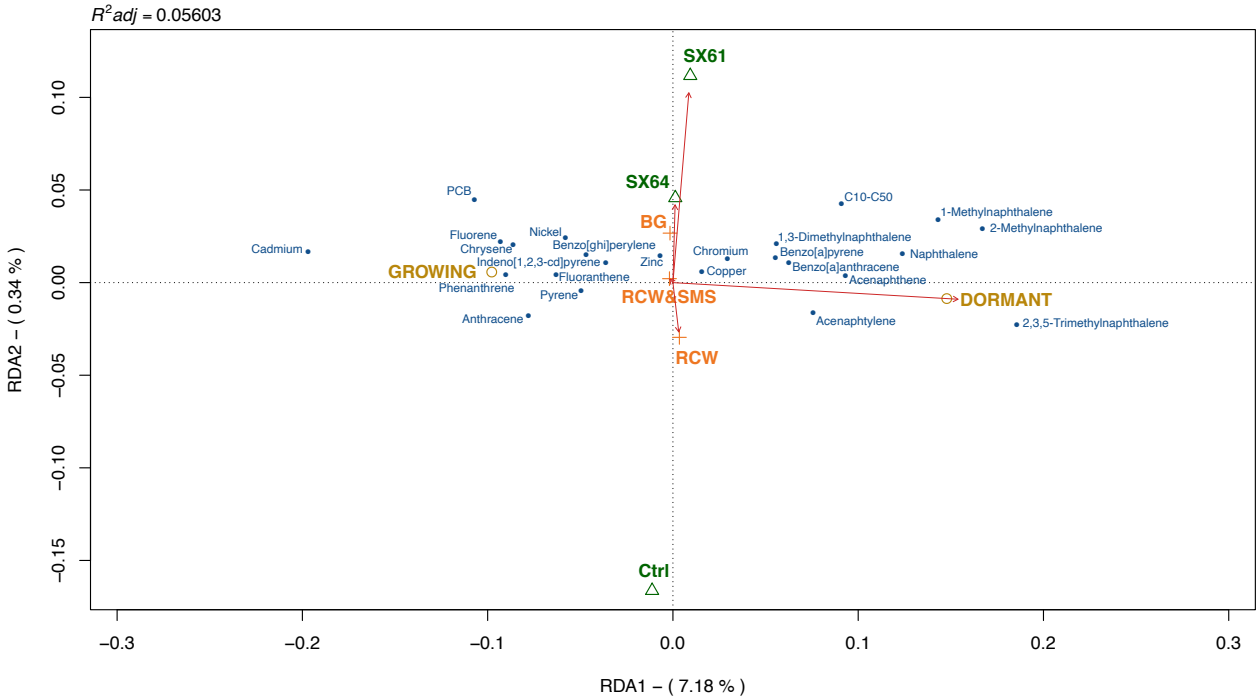

Figure 2.1 Redundancy analysis (RDA) showing the relationship between treatments, periods and the variation rates (VR) of contaminants in soil. Blue labels represent the VR of each "contaminant". Green open triangles, yellow open circles and orange cross symbol represent "factor centroids" of environmental variables. Red-line arrows represent the "biplot scores" of environmental variables. The length of each arrow indicates the contribution of the corresponding variable to the contaminant variation rates.

2.3.4 Water Extracted TEs, pH and EC

The water extracted fraction of TEs from soil samples, as well as pH and electrical conductivity (EC), were assessed in the RCW and BG subsections of 'SX61' and Ctrl treatments, at T0, T1 and T5 only (Table 2.4). The limited portion of the site was selected in order to evaluate the impact of willow trees and soil organic amendments on the bioavailability of TE. The ground cover and cultivar treatments (RCW and 'SX61') showed no significant effect on pH and EC at any sampling time. Among the six targeted TE, the water extracted concentration of Cu was the highest, followed by Cr, Zn, Ni and Pb (data not shown for Pb), while the water extracted concentrations of Cd were under the detection limit in all soil samples. All four water extracted TEs (i.e., Cr, Cu, Ni and Zn) were found in similar concentrations in 'SX61' and Ctrl plots in the initial soil samples (T0). After the first growing season (T1), the water extracted concentration of Cr was significantly higher in 'SX61' plots than in Ctrl plots, but only under the BG treatment. Its water extracted concentration was also significantly higher in RCW plots than in BG plots under the Ctrl plots only. After the third growing season (T5), the water extracted concentration of Cr rose to significantly higher levels in all RCW plots compared to BG plots and also rose to

significantly higher levels in all 'SX61' plots compared to all Ctrl plots. The Cu water extracted concentration was significantly higher in 'SX61' plots than in Ctrl plots, after the first and third growing seasons (T1 and T5). The water extracted concentration of Zn followed the same trend as Cr and Cu, but the differences between 'SX61' and Ctrl plots were significant only after the third growing season (T5). Conversely, the water extracted concentration of Ni showed significantly higher values in Ctrl than in 'SX61' plots, after the first and third growing seasons (T1 and T5).

2.3.5 Biomass production and TE phytoextraction

Willow biomass parameters measured after the third growing season (T5) are presented in (Table 2.5). The equivalent of 75.3 and 83.6 odt ha^{-1} was harvested for 'SX61' and 'SX64' respectively. On an annual basis, these values respectively represent yields of 25.1 and 27.9 odt ha^{-1} yr^{-1}. The differences between the two cultivars were not significant nor did the ground cover treatments impact biomass yield. Moisture levels inside the willow shoots varied significantly between the cultivars, with 1.94% more water in 'SX61' than in 'SX64'.

No trace of Cr, Ni or Pb was detected in the aerial tissues of the two willow cultivars. Zn was found in the highest concentrations, following by Cu and Cd. Significantly higher Cd concentrations were found in the shoots of 'SX61' than in 'SX64' shoots ($p = 0.024$). Therefore, no significant difference in the total extracted quantities of Cd was observed between the two willow cultivars. The BCF of Cd were also similar between both cultivars.

The average concentration of Zn in the willow shoots was significantly higher in RCW and RCW+SMS plots compared to BG plots ($p \leq 0.01$). Consequently, the total quantity of Zn extracted per hectare was significantly greater under RCW and RCW+SMS plots than in BG plots ($p = 0.027$). The Zn BCF was similar between ground cover treatments but was significantly higher in 'SX64' than in 'SX61' ($p = 0.028$). The Cu concentrations observed in the willow shoots did not vary significantly between cultivars or ground cover treatments, leading to similar potential extraction quantities per ha and year. The BCF of Cu was similar between all treatments.

Table 2.4 Mean water extracted concentration of TEs, pH and EC in soil over time.

Parameters	Times	Units	Ctrl		SX61		*p*-value			Interpretation
			BG	RCW	BG	RCW	Cover	Cultivar	Cover*Cultivar	
pH	T0	-	7.68±0.30	-	7.89±0.09	-	-	0.133	-	-
EC		µS cm^{-1}	127.50±37.96	-	120.45±9.09	-	-	0.675	-	-
Chromium		mg kg^{-1}	0.24±0.15	-	0.33±0.05	-	-	0.252	-	-
Copper		mg kg^{-1}	3.09±1.84	-	4.06±1.43	-	-	0.516	-	-
Nickel		mg kg^{-1}	0.04±0.02	-	0.03±0.00	-	-	0.102	-	-
Zinc		mg kg^{-1}	0.14±0.01	-	0.15±0.02	-	-	0.384	-	-
pH	T1	-	7.90±0.24	7.91±0.12	7.94±0.13	7.92±0.09	0.922	0.616	0.604	-
EC		µS cm^{-1}	47.02±10.91	91.09±33.67	68.57±11.74	74.14±18.91	0.070	0.637	0.061	-
Chromium		mg kg^{-1}	0.10±0.04	0.27±0.09	0.31±0.06	0.32±0.07	***0.039****	***0.009****	***0.040****	**Ctrl:BG < RCW & BG:Ctrl < SX61**
Copper		mg kg^{-1}	1.41±0.50	2.80±1.44	3.88±1.58	3.75±0.51	0.162	***0.047****	0.095	**Ctrl < SX61**
Nickel		mg kg^{-1}	0.03±0.01	0.05±0.01	0.03±0.01	0.03±0.01	0.147	***0.011****	0.289	**Ctrl > SX61**
Zinc		mg kg^{-1}	0.12±0.01	0.14±0.01	0.14±0.01	0.14±0.01	0.070	0.216	0.094	-
pH	T5	-	7.91±0.26	8.00±0.05	8.01±0.07	8.02±0.07	0.381	0.277	0.552	-
EC		µS cm^{-1}	47.56±17.81	72.58±39.63	67.72±7.20	76.34±16.76	0.071	0.271	0.269	-
Chromium		mg kg^{-1}	0.15±0.06	0.24±0.14	0.33±0.14	0.32±0.08	***0.012****	***0.008****	0.237	**BG < RCW & Ctrl < SX61**
Copper		mg kg^{-1}	1.78±0.70	1.92±0.77	4.08±1.08	3.77±0.54	0.684	***0.026****	0.305	**Ctrl < SX61**
Nickel		mg kg^{-1}	0.04±0.01	0.04±0.01	0.03±0.00	0.03±0.01	0.373	***0.008****	0.748	**Ctrl > SX61**
Zinc		mg kg^{-1}	0.12±0.01	0.12±0.01	0.13±0.01	0.13±0.02	0.936	***0.039****	0.987	**Ctrl < SX61**

Values are the average (mean ± SD, n = 5) concentrations (mg kg^{-1}) of water extracted TEs by treatment at T0, T1 and T5. Significance levels (*p*-value) are shown and asterisks (∗ $p \leq 0.05$, ∗∗ $p \leq 0.01$, ∗∗∗ $p \leq 0.001$) indicate a significant effect of Cover, Cultivar and Cover*Cultivar. Symbols of comparison (> or <) in the final column were used to identify significant differences between treatments according to Student's t-test ($p \leq 0.05$).

Table 2.5 Biomass parameters of willows after three seasons.

Parameters	Units	SX61			SX64			*p*-value			Interpretation
		BG	RCW	RCW + SMS	BG	RCW	RCW + SMS	Cover	Cultivar	Cover* Cultivar	
Biomass	odt ha^{-1} yr^{-1}	22.75±7.86	26.35±9.61	26.20±12.89	28.83±12.22	26.36±9.89	28.38±11.97	0.646	0.301	0.368	-
Humidity	%	55.85±1.00	56.26±1.00	55.44±1.00	53.94±1.00	53.84±1.00	53.98±1.00	0.387	***<0.001**** ***	0.076	**SX61^{A}, SX64^{B}**
Shoot [Cd]		2.24±0.00	2.42±0.00	2.50±1.00	2.02±1.00	2.30±1.00	2.36±1.00	0.526	***0.024**** *	0.844	**SX61^{A}, SX64^{B}**
Shoot [Cu]	mg kg^{-1}	5.60±1.00	5.60±1.00	5.60±1.00	5.00±0.00	5.20±1.00	5.20±1.00	0.922	0.311	0.873	-
Shoot [Zn]		64.80±4.00	78.20±8.00	75.60±11.00	68.60±4.00	81.40±13.00	77.60±8.00	***0.010**** **	0.312	0.921	**BG^{B}, RCW^{A}, RCW+SMS^{A}**
Cd extraction yield		51.42±16.71	63.22±15.44	67.16±33.96	61.25±31.79	59.51±18.58	69.07±30.56	0.368	0.685	0.288	-
Cu extraction yield	mg ha^{-1} yr^{-1}	129.46±41.98	149.38±47.13	148.88±61.75	144.15±33.14	133.82±34.27	145.75±29.23	0.613	0.953	0.430	-
Zn extraction yield		1470.03±247.55	2035.75±391.30	1956.97±654.48	1996.43±553.11	2114.64±598.14	2198.83±492.27	***0.027****	0.195	0.244	**BG^{B}, RCW^{A}, RCW+SMS^{A}**
BCF of Cd		1.04±0.19	1.09±0.15	1.07±0.20	1.11±0.36	1.13±0.20	1.27±0.33	0.699	0.089	0.299	-
BCF of Cu	Factor	0.0027±0.0037	0.0028±0.0021	0.0021±0.0028	0.0048±0.0013	0.0047±0.0005	0.0056±0.0005	0.893	0.079	0.207	-
BCF of Zn		0.14±0.03	0.17±0.02	0.15±0.02	0.22±0.05	0.23±0.04	0.26±0.12	0.711	***0.028****	0.407	**SX61^{B}, SX64^{A}**

Values are the averages (mean ± SD, n = 20 for biomass and humidity parameters; n=5 for all other parameters concerning TE) for each treatment. Significance levels (*p*-value) are shown and asterisks (∗ $p \leq 0.05$, ∗∗ $p \leq 0.01$, ∗∗∗ $p \leq 0.001$) indicate a significant effect of Cover, Cultivar and Cover*Cultivar. Capital letters in the final column were used to identify significant differences between treatments according to Tukey's HSD test ($p \leq 0.05$).

2.4 Discussion

2.4.1 Biomass Production

Biomass production of both cultivars, reached on a contaminated soil, compare favorably to other results obtained on farmland in southern Quebec (Guidi Nissim et al., 2013; Labrecque et Teodorescu, 2005). In their long-term trial, Labrecque and Teodorescu (2005) found no difference in growth between the same two cultivars ('SX61' and 'SX64') and reported respective annual yields of 20.4 and 23.7 odt ha^{-1} yr^{-1} for the second rotation cycle, and 21.3 and 24.3 odt ha^{-1} yr^{-1} for the third. Their recorded biomass yields were slightly lower than those observed in this study, but their estimates were based on a planting density of 18,000 cuttings per hectare, which is less than the 18,500 here. This high productivity, combined with the absence of visual symptoms of toxicity, together suggest that these two cultivars can be successfully cultivated in moderately contaminated soils under northern climatic conditions such as those found in Quebec. Also, it should be noted that the experimental site was polluted by activities that included spreading contaminated sediments of a decantation basins containing cocktails of organic and inorganic residues (land farming), but over a rich agricultural soil base. Many *Salix* spp. have demonstrated high biomass productivity and tolerance to petroleum hydrocarbons (PHCs) in southern Quebec (Grenier et al., 2015). Low concentrations of PAHs have even been reported to increase the growth and transpiration rate of some willow trees (Thygesen et Trapp, 2002).

Increasing soil organic matter has been reported to have positive effects on soil properties and crop performance (Bauer et Black, 1994). Stem biomass of *Salix viminalis* and *S. discolor* have been found to be significantly greater when receiving wastewater sludge treatment, resulting in productivity exceeding that of control plants by five to seven times (Labrecque et al., 1995). After a three-year growth cycle, *Salix dasyclados* also produced significantly greater biomass in organically amended plots than in control plots (Adegbidi et al., 2003). Because of this, it was expected that higher biomass yields would be observed after the application of soil organic amendments such as RCW and RCW+SMS. However, observation of plant growth over the three seasons of this study revealed no impact of these amendments on the biomass production of the two willow cultivars. These results may reflect the excellent agronomic conditions of the site as well as its adequate water supply, due to its proximity to the St. Lawrence River (less than 350 m) and low elevation (about 3.5 m above the river water level). Increased plant growth under SMS and RCW is usually attributed to improvement of the soil conditions and nutrients supplied, when

the amendments are the sole source of fertilizer, compared to the depleted soils used as controls (Frutos et al., 2017; Jonathan et al., 2011; Paula et al., 2017; Roy et al., 2015; Soumare et al., 2002).

2.4.2 TE Phytoextraction

Among the six-targeted TEs analyzed in the aboveground tissues, Zn was found in the highest concentration, followed by Cu and Cd, while Cr, Ni and Pb were not detected. These results compare well with previous phytoremediation field trials, which reported similar rankings of TE concentrations in the aerial parts of *Salix* spp., with little or no trace of Cr, Ni and Pb (Algreen et al., 2014; Courchesne et al., 2017a; Pulford et al., 2002).

The total concentration of TE in soil is an important factor that can influence the rate of phytoextraction (Courchesne et al., 2017a). For instance, at the beginning of the present study (T0), total concentrations of Cu and Zn in soil were relatively higher (from 792 to 2381 mg kg^{-1} and from 234 to 479 mg kg^{-1}), while Cd and Ni were relatively lower (from 1.8 to 2.2 mg kg^{-1} and from 66.2 to 106.5 mg kg^{-1}). This may partly explain why concentrations of Zn and Cu were higher than those of Cd and Ni in willow shoot samples.

Our results may also reflect the presence of H_2O-soluble TEs in soil. The metals present in the water extract can be considered as the fraction most readily bioavailable for plant uptake and are generally well correlated with phytoextraction (Séguin et al., 2004). The extent of elemental partitioning between the aqueous and solid phases in soils is very dynamic and depends on multiple physical, chemical and biological processes and their interaction. These processes include precipitation-dissolution, adsorption-desorption, complexation and encapsulation, which are mainly moderated by factors such as the nature of the element and their speciation, the structure and penetrability of soil, pH, electrical conductivity (EC), cation-exchange capacity (CEC) and percent of organic matter and dissolved organic carbon (DOC) (Carrillo-González et al., 2006; Ernst, 1996; Kabata-Pendias, 2010; Nguyen et al., 2017). It is also well known that total soil TE content largely determines the partitioning of TE between the solid and solution phases (Carrillo-González et al., 2006; Ernst, 1996). Consequently, the water extracted concentrations of all TEs followed the same order as their total concentrations in soil: Cu > Cr > Zn > Ni > Cd.

Although chromium was the second most abundant and bioavailable TE in soil, it did not seem to have been taken up by the plants to the same extent as other TEs, which highlights that phytoextraction is highly metal specific. Chromium is generally minimally translocated within

plants (Guidi et al., 2012; Kabata-Pendias, 2010; Laidlaw et al., 2012) and tends to be concentrated mainly in roots, apparently because of its propensity to bind to cell walls (Zayed et al., 1998).

In this experiment, Cd is the only element for which a BCF greater than 1 was calculated. BCFs for other TEs were rather low (from 0.14 to 0.26 for Zn and 0.0021 to 0.0056 for Cu). In a field experiment, Kacálková et al. (2015) found the BCF of Cd and Zn to be greater than 1 in *Salix smithiana* and *S. rubens*, and lower than 1 for Cu. Our results also compared well with those of Pitre et al. (2010), who reported a Zn BCF of 0.23 in *S. miyabeana* during a brownfield trial. A low Pb and Cu BCF, varying from 0.05 to 0.15, and a higher BCF for Zn and Cd, ranging between 2.0 and 7.0, were also reported in a study conduct on a military landfill (Courchesne et al., 2017a). During a pot experiment, Desjardins et al. (2016) found a slightly higher BCF for Zn (0.68), with higher concentrations in aerial tissues of *S. miyabeana* (119.96 mg kg^{-1}) compared to those reported here.

Willow species and cultivars may differ in their ability to translocate TEs from roots to shoots (Pulford et al., 2002; Pulford et Watson, 2003). In this study, significantly higher Cd concentrations were found in the shoots of 'SX61' than in 'SX64'. However, the quantities of Cd (ha^{-1} yr^{-1}) extracted were statistically similar. Extrapolating the amounts of TEs phytoextracted per unit of area represents a useful exercise to estimate the phytoextraction potential of various plants. Both cultivars showed similar concentrations of Zn and Cu in their aerial tissues, which may suggest similar extraction capacity for these TE, and greater Cd extraction capacity in 'SX61'. However, it is difficult to compare their phytoextraction efficacy, because the means of all TEs concentrations in soil were significantly higher in 'SX61' plots than in 'SX64' plots at the beginning of the study (T0). In view of this, it is reasonable to suggest that 'SX64' has a greater extraction capacity than 'SX61' for Zn, Cu and Cd, since the BCF of Zn was significantly higher in 'SX64' than in 'SX61' and the BCF of Cu and Cd showed a tendency to be higher under 'SX64' than in 'SX61' (p = 0.079 and p = 0.089). Although there is no significant differences in phytoextraction efficacy between the two cultivars, our results agree with the finding that *Salix* spp. are more suitable for the phytoextraction of Cd and Zn than Cu and Cr (Bissonnette et al., 2010; Labrecque et al., 2020; Laidlaw et al., 2012).

2.4.3 Soil Organic Amendment and TE Phytoextraction

Assessing the impact of soil amendments on willow phytoextraction efficiency revealed that the SMS did not affect TE phytoextraction in either willow cultivar. A reduction in the extraction rate was expected, since Gąsecka et al. (2019) found that content of Cu in roots, leaves and shoots of *Salix purpurea* × *viminalis* hybrid was reduced by the addition of *Pleurotus ostreatus* SMS. Although it differs in composition, spent mushroom compost (SMC) from the production of *Agaricus bisporus* was also effective in improving Cu phytostabilization by the same *Salix* hybrid in another hydroponic experiment (Magdziak et al., 2015). However, in small doses (addition of 10% SMC to 90% sand, instead of 30% SMC to 70% sand), it has been reported to have stimulated plant growth and efficiency of Cu accumulation of the same hybrid willow (Magdziak et al., 2015). In a greenhouse experiment carried out in plastic pots, Frutos et al. (2017) found that a mixture of SMC of *A. bisporus* and SMS of *P. ostreatus* reduced Cu, Cd and Pb translocation to the shoots of *Atriplex halimus*. They attributed their results to a progressive decrease of water-soluble fractions of TEs in the soil with increasing doses of SMC/SMS. They also found that roots of *A. halimus* accumulated significantly more Cu and Pb with increasing doses of SMC/SMS, despite the decrease of Cu and Pb in the leachate. It would have been interesting to measure TE concentrations in the roots of our willow cultivars to see if similar trends of phytostabilization would have been observed with the addition of SMS of *P. ostreatus*. However, the focus here was on monitoring the accumulation of TE in the shoots only.

In our study, RCW alone or mixed with SMS significantly increased the concentrations of Zn in willow shoots, by 17.27% after three seasons of treatment, suggesting its potential effect on Zn speciation and bioavailability. However, our results did not show any difference in water extracted concentrations of Zn between soil samples collected in BG and RCW plots. Nevertheless, a tendency towards higher dissolved concentrations of Zn (p = 0.070) was observed under the RCW plot at T1, once again suggesting a possible impact of RCW on Zn bioavailability. Such changes in Zn bioavailability in soil, combined with rapid uptake and translocation to plant shoots may explain the higher concentrations of Zn in the shoots of trees that grew under RCW alone or mixed with SMS. Simultaneous extractions of Zn may have masked a stronger effect of RCW on Zn bioavailability. Conversely, an exclusionary mechanism for Cr in *Salix* spp. may have led to a stronger effect of RCW on the bioavailability of this TEs in soil.

TEs bioavailability may have been increased by the RCW due to the formation of soluble organo-metallic complexes (Almås et al., 2000; Usman et al., 2004). Nguyen et al. (2017) also demonstrated a significant correlation between TEs bioavailability, notably Ni and Cu, and the concentrations of the dissolved organic carbon in soil. Organic amendments, such as composted sewage sludge or fresh RCW, are known to increase humic substances (HS) in soil, which can influence TEs speciation, thereby affecting their mobility and bioavailability (Hattab et al., 2014). Humic acids (HA) are known to be more insoluble and may contribute to TEs immobilization, while fulvic acids (FA) are more water-soluble and may be responsible for TEs solubilization (Kaschl et Chen, 2005). In soils, Zn seems to bond predominantly on FA (Borůvka et Drábek, 2004; Donisa et al., 2003; Hattab et al., 2014). The formation of Zn-FA complexes might then explain our phytoextraction results, by making Zn more bioavailable to willows. Hattab et al. (2015) reported that applying RCW favours TEs immobilization and reduces their phytoavailability, which differs from our results.

2.4.4 Soil Contaminants in the Initial Soil Samples (T0)

The initial soil samples (T0) showed greater concentrations of all organic and inorganic contaminants in planted plots ('SX61' and 'SX64') than in unplanted ones (Ctrl). This finding strongly suggests that the presence of willows on the site during the four years prior to the beginning of the current study influenced the spatial distribution of all contaminants in the soil. This observation seems to lie outside of the remediation objectives that motivated the establishment of this willow plantation on the site by Guidi et al. (2012). Unsuccessful and inconclusive field trials reporting similar patterns with organic contaminant, have usually attributed such results to lower degradative microorganism activity in the rhizosphere (Lalande et al., 2003; Vervaeke et al., 2003). However, because inorganics cannot be degraded, such explanations cannot account for the finding that TEs concentrations followed the same trend as organic compounds in the initial soil samples. Rather, this suggest that another phenomenon, such as soil contaminant migrations, may also have contributed to driving the differences in total contaminant concentrations in the soil.

Because most contaminants tended to be more concentrated under 'SX61' and less or similarly concentrated under Ctrl plots, than levels measured four years before (Figure S 2.1), such differences may either have been the result of greater decreases in the unplanted plots, greater increases in the planted plots or even a combination of both. Unfortunately, it was impossible to determine with certainty which of these phenomena would more likely explain the observed

pattern, since our experimental design did not allow us to statistically compare values between T0 and those from the 2010 soil characterization.

2.4.5 Willows and Global Variations (GV) of Contaminants in Soil

At the end of the present study, only GV of C10-C50 was significantly affected by the presence of willows. The observed differences in its GV suggest that cultivar 'SX61' reduced what could be described as the "natural attenuation" occurring in the unvegetated plots (Ctrl). The smaller decrease of C10-C50 in the 'SX61' plots align with the proposed explanations concerning its greater concentrations in planted plots than in unplanted ones previously observed in our initial soil samples (T0). However, it is difficult to determine if these smaller decreases were the result of less degradation or, alternatively, the result of less leaching.

The experimental site was an open system allowing vertical and/or horizontal water movement, which may have impacted contaminant migration in soil, as suggested in other studies (Matranga, 2012; Revitt et al., 2014). The willow plantation may have acted as a vegetated cap, preventing or minimizing leachate of contaminants, which is an important part of the natural attenuation process (Verginelli et Baciocchi, 2013). The foliage and canopy cover can physically slow down rainfall, thereby minimizing water infiltration into the soil. Additionally, willows are fast growing phreatophytic woody plants with a high transpiration rate, making them strong biological pumps for ground water in cool temperate regions (Ferro et al., 2003). When exploited as evapotranspiration cover in SRIC, they can strongly influence soil hydrological dynamics (Dimitriou et Busch, 2009) and reduce the deep percolation and leaching of toxic products into the environment (Bialowiec et al., 2007; Mirck et Volk, 2010; Pivetz, 2001; Rüth et al., 2007). Such impacts on soil hydrological dynamics may have contributed to the smaller reductions of C10-C50 observed under 'SX61'.

Our results revealed that the GV of all other contaminants was not statistically influenced by the willow plantation. However, we suspect that the GV of PCBs may also have been affected, to a lesser extent, by the presence of willows. The final concentrations of PCBs determined for Ctrl plots were, on average, +1.66% higher than those at T0, while they were found to be, on average, +21.96% and +38.13% higher in 'SX61' and 'SX64' plots, respectively. These GV were not significantly different between treatment (p = 0.081), possibly because of the high variability in our dataset. The standard deviations of the results well reflect the inherent heterogeneous

distribution of pollutants on the whole experimental site. To overcome the methodological problems associated with such heterogeneity, a value of 10% ($p \leq 0.1$), instead of 5% ($p \leq 0.05$), has been proposed as an acceptable level of significance in field studies (Gerhardt et al., 2009). Based on such a standard, the presence of willows can be considered to have increased the total soil concentrations of PCBs over the course of our study.

By removing a substantial amount of water from the groundwater, willows can increase the flux of dissolved contaminants from the water capture zone towards the rooting zone (Ferro et al., 2003). There is evidence that some plants can impact the movement of organic compounds such as PAHs, leading to their accumulation in the rhizosphere (Liste et Alexander, 2000). Similarly, elevated concentrations of Pb have been observed in the rooting zone of *Betula occidentalis* growing on contaminated soil collected from an abandoned mining site in Utah (Klassen et al., 2010). While, to our knowledge, this phenomenon has never been reported for PCBs, it may be a possible explanation for the trend in PCBs GV observed here. Such contaminant supply by migration could lead to apparent increases in concentrations over time, even if degradation and/or phytoextraction are actively occurring (Gerhardt et al., 2009; Klassen et al., 2010).

Our results show that considerable amounts of Zn, Cu and Cd were removed from the ground by phytoextraction. However, their GV in soil were not significantly different between the planted ('SX61' and 'SX64') and unplanted plots (Ctrl) after the duration of this study. The highly heterogeneous distribution of pollutants on the site may have masked significant results here, too. Simultaneous transfers, by water mass flow or diffusion toward the root zones, gradually reloading the soil in TEs in parallel to extraction, may also have contributed to mask significant results. The nature and intensity of changes in contaminant concentrations in the root zones may thus depend on the correspondence of contaminant supply by the adjacent bulk soil, and its elimination through plant uptake and/or by degradation. Consequently, it would be very difficult to monitor pollutant variations in the field by sampling surface soil that is extensively penetrated by roots (Liste et Alexander, 2000). Total plant uptake could be assessed as a measure of success in the field, rather than attempting to quantify decreases in soil contamination at the end of a growing season (Gerhardt et al., 2009).

2.4.6 Variation Rates (VR)

Monitoring soil contaminant variation according to the previous sampling time, revealed that many contaminant concentrations were subject to seasonal oscillations. Almost all contaminants (except C10-C50) showed average increases throughout the whole experimental site after a growing period, while many showed significantly different VR, such as lower average increases (i.e., fluoranthene, indeno[1,2,3-cd]pyrene, phenanthrene and pyrene), or even average decreases (i.e., PCBs, Cd, Ni, anthracene, benzo[ghi]perylene and chrysene), after a dormant period.

Because deciduous trees are dormant for part of the year, the impact of vegetation on water movement and contaminant mobilization (or immobilization) would be more likely to occur over a growing period, when roots are more active, evapotranspiration is maximal and precipitation is low (Ferro et al., 2003; Pivetz, 2001; Vervaeke et al., 2004). Although seasonal oscillations can be assumed to occur over the whole experimental site, including the Ctrl plots, they could still have been driven by the high transpiration rates occurring in the willow plantation throughout the growing seasons. It is important to mention that Ctrl plots were located only a few meters from the plantation (0-3 m), and that no physical barriers were installed to prevent willow roots from developing there over time and pumping a large amount of water into it.

Interestingly, naphthalene, 1-methylnaphthalene and 2,3,5-trimethylnaphthalene were the only contaminants to show the reverse pattern, with significantly higher increases after a dormant period than after a growing one. These two-ring naphthalenes may have increased as a result of migrating from the adjacent bulk soil, or due to degradation of other PAHs containing three or more rings in the molecule, as suspected by Gąsecka et al. (2012). Their study found rapid increases of naphthalene concentrations following the degradation of other PAHs (i.e., anthracene, pyrene, phenanthrene and fluoranthene) by *Agaricus bisporus* and *Lentinula edodes* in a 12-week experiment. These three naphthalenes have a low molecular weight and are among the more water-soluble PAHs (low Log K_{ow}), which makes them more susceptible than other PAHs to be taken up by plants or to be degraded. Based on our observations, we agree that sampling protocols for monitoring contaminant variations in field should account for potential seasonal fluctuations and specify that soil samples be compared between similar seasons (Ferro et al., 2003).

2.4.7 Willows and Water-Soluble Fractions in Soil

Willow growth seems to have affected TEs availability in our experimental site, because differences in the water extracted fraction of Cr, Cu, Ni and Zn were observed between 'SX61' and Ctrl plots at the end of two different growing seasons (T1 and/or T5). In the scientific literature, changes in labile soil TEs under trees are quite inconsistent. For example, under a cold climate field trial, Courchesne et al. (2017b) showed that the willow cultivars used ('Fish Creek' and 'SX67') significantly decreased the total, as well as the labile pool of As, Cd, Cu, Ni, Pb and Zn concentrations in soil, over a three-year period. Conversely, Pulford et al. (2002) found that the concentrations of EDTA extractable Cd, Cu, Ni and Zn were significantly higher in soil collected from under willows than from unplanted areas. Nguyen et al. (2017) reported that the labile pool of several trace elements was influenced by the presence of plants but varied according to the plant species.

Plant species have a distinct influence on the labile pools of TEs in soil, essentially by controlling pH and organic matter content (Séguin et al., 2004). Some pH changes can occur in the rhizosphere as a result of the differential uptake rates of cations and anions in plant roots (Nye, 1981). When the influx of cations is much higher than that of anions, protons are released to compensate for an excess of positive charges. Soil acidification invariably increased solubility of positively charged ions (such as Cd^{2+}) due to increased competition from H^+ ions at the negatively charged binding sites (Nguyen Thi Xuan, 2015). Conversely, hydroxyl or bicarbonate ions can be released by plants to compensate for an excess of negative charges in roots, increasing sorption and precipitation of TE cations, which decreases their availability in soil (Haynes, 1990). TEs solubility is then generally found to increase markedly under acidic conditions (Takáč et al., 2009). Plants can also alter the pH in soil with the release of carbon dioxide (CO_2) following the respiration of root cells and that of their associated microorganisms (Dunbabin et al., 1988; Marschner et al., 1987). Additionally, root exudation, which includes several forms of carbohydrates, proteins, organic acids, amino acids, and phenolic compounds, may affect TE solubility in soil, via direct complexation, or indirectly, by providing energy substrates for rhizosphere microflora, or by affecting pH (Marschner et al., 1987; Nguyen et al., 2017).

Results of our experiment indicated that pH values were close to 8 in all samples and remained stable over time. However, pH changes may have occurred very close to willow roots at a given time without being detected in the adjacent bulk soil. Supporting this point, Cd was

observed in willow shoots, with BCF values close to 1, even though its water extracted concentrations were under the detection threshold in all soil samples. Apparently, Cd is more soluble in soils within the range of pH 4.5-5.5, but more immobile above a pH of 7.5 (Kabata-Pendias, 2010). Cd may thus have been solubilized in the rhizosphere and/or in the hyphosphere (Taktek et al., 2016), taken up rapidly and translocated to plant shoots.

It has been proposed that if TEs were not taken up by plant roots, increases in soil TEs availability could lead them to leach into the environment (Laidlaw et al., 2015; Vervaeke et al., 2004; Zhu et al., 2010). We cannot exclude the possibility that such a phenomenon occurred on the study site in the past and may thus have helped drive the differences in their total concentrations in our initial soil samples (T0). However, this does not seem to have occurred during the current study, since the GV in soil concentrations of Cr, Cu and Zn showed no significant differences between treatments, although their water-soluble fractions were higher under planted plots ('SX61') than under unplanted ones (Ctrl).

Plants can also impact the dissolved chemical concentrations in the soil solution close to their roots as a result of water and nutrient uptake (Clothier et Green, 1997; Hinsinger, 1998). When elements are found in large concentrations in the soil solution, their enrichment can be expected in the root zones as a consequence of a transfer to the root-soil solution interface by mass flow, at a greater flux than required by the roots (Hinsinger, 1998). In fact, the water extracted fractions of Cr and Cu were the highest among the six TEs targeted in this study and they showed significantly higher concentrations under planted plots ('SX61') than under unplanted ones (Ctrl), after the first (T1) and third growing seasons (T5). The water extracted fractions of Zn were slightly lower than those of Cr and Cu, and only showed significant differences after the third growing season. Conversely, Ni had the lowest water extracted concentrations of the five targeted TE, and showed completely opposite results, suggesting that Ni may have been transferred at a lower flux than it was taken up by plant roots.

Forward diffusion or back-diffusion of the solutes through the concentration gradients can occur, following their depletion or enrichment in the root zones (Hinsinger, 1998). We believe that such a phenomenon may have occurred throughout our study, thereby tending to balance the water-soluble fractions of TEs throughout the site, more particularly during the dormant periods, as

observed in our initial soil samples (T0), collected after the winter months, when deciduous trees had been dormant for a long period.

2.4.8 Organic Amendment and Soil Contaminants Concentrations

Differences in the GV of contaminants were expected following application of SMS to the soil, but none were observed for any contaminant over the three seasons of the study. Soil contamination may have reduced the effectiveness of SMS for soil decontamination. García-Delgado et al. (2013) showed that TEs, such as Cd and Pb, inhibited laccases activity of *Pleurotus ostreatus* SMS, thus decreasing the biodegradation rate of PAHs. Here, it is also important to note that the RCW+SMS mixture was applied on the soil surface only, without being mixed deeply into the soil, which may have prevented contact between fungal enzymes and organic contaminants. The growth of *Salix planifolia* in plastic bins containing contaminated soil, previously mixed with woodchip colonized by *P. ostreatus*, led to higher rates of hydrocarbon removal than natural attenuation (Robichaud et al., 2019). Unfortunately, in the latter study, the effect of willow was not evaluated separately. It was therefore impossible to determine whether their amendment improved the performance of the willow in remediating their petroleum-contaminated soil.

Although RCW was mainly used to support the metabolic activities of SMS, its application alone affected the GV of PAHs over time, with significantly higher increases of benzo[g,h,i]perylene, naphthalene, 1-methylnaphthalene and 1,3-dimethylnaphthalene in RCW plots than in BG ones. Interestingly, among these four PAHs, three were two-ring naphthalenes. A high organic content appears to exert strong PAHs sorption capacities in soil with a more pronounced impact on the lighter PAHs, reducing their mobility (Revitt et al., 2014). Other PAHs (i.e., anthracene, benz[a]anthracene, fluorene, indeno[1,2,3-cd]pyrene, phenanthrene, pyrene and 2-methylnaphthalene) also showed a tendency to follow the same trend ($p \leq 0.1$), with either greater increases in RCW plots, or greater decreases in BG ones. Although the water extracted fraction of organic contaminants was not assessed in the present study, organic matter resulting from the humification of RCW may have induced the immobilization of some PAHs at the soil surface, resulting in their accumulation over time, after repeated groundwater movement. Incorporating SMS into RCW may have disrupted its degradation rate, thereby reducing its effect on the GV of PAHs.

2.5 Materials and Methods

2.5.1 Experimental Site

The experimental site is a flat area of 5,840 m^2, located in the municipality of Varennes, on the south shore of the St. Lawrence River, across from the Island of Montreal (Quebec, Canada, 45°42'02.8" N, 73°25'53.4" W). The region has a continental climate characterized by an annual average temperature of 6.6 ° C and annual average precipitation of 981 mm (MELCC, 2010). The petrochemical factory (PÉTROMONT INC.) that operated for many years on the site was shut down in 2008. During the period of industrial operation, settling ponds were built, to control liquid discharge from refining processes. Between 1972 and 1979, sludge collected from the bottom of these basins was spread on adjacent land (the current experimental site) according to land farming practices.

The soil on the site was characterized in 2010. Agronomic properties and contaminant concentrations are presented in Table 2.6. PCBs, Cu, Cr and anthracene were considered to be the most problematic contaminants on the site, based on the provincial Land Protection and Rehabilitation Regulation, RLRQ, c. Q-2, r. 37, Sch. I. These contaminants were found mainly in the surface soil (0-60 cm), as described in Guidi et al. (2012).

Table 2.6 Soil characteristics of the site.

Parameters	Units	Values	Parameters	Units	Values
Cation-exchange capacity	meq $100g^{-1}$	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
Ca[b]	mg kg^{-1}	7090.00	Fluoranthene[c]	mg kg^{-1}	0.54±0.20
P[b]	mg kg^{-1}	80.00	Fluorene[c]	mg kg^{-1}	0.94±0.21
K[b]	mg kg^{-1}	525.00	Indeno[1,2,3-cd]pyrene[c]	mg kg^{-1}	0.32±0.09
Mg[b]	mg kg^{-1}	800.00	Naphthalene[c]	mg kg^{-1}	0.42±0.13
Al[b]	mg kg^{-1}	48.00	Phenanthrene[c]	mg kg^{-1}	2.62±0.71
Zn[b]	mg kg^{-1}	85.60	Pyrene[c]	mg kg^{-1}	1.34±0.41
Cu[b]	mg kg^{-1}	417.00	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
B[b]	mg kg^{-1}	1.40	1,3-Dimethylnaphthalene[c]	mg kg^{-1}	0.55±0.18
Fe[b]	mg kg^{-1}	178.00	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 was performed by AGAT Laboratories Ltd (Montreal, QC) following the recommended provincial methods for environmental analyses (CEAEQ, 2004, 2008, 2014b, 2014a, 2016). Five (5) soil samples were collected at 0-30 cm below ground in each plot (P1, P2, P3 and P4, see Figure 2.2A). Values are averages (mean±SD, n=20). The table was adapted from Guidi et al. (2012).

2.5.2 Experimental Planting and Maintenance of the Plantation

A willow plantation of 5,475 m^2 was established on the site in mid-June 2010. The plantation included two willow cultivars, 'SX61' and 'SX64' (*Salix miyabeana*), randomly planted in seven groups of three rows for a total of 21 rows (Figure 2.2A). The planting was carried out mechanically, following a SRIC technique (Labrecque et Teodorescu, 2006). Plants were spaced 1.8 x 0.3 m, at a density of 18,500 plants per hectare (Guidi et al., 2012). The plantation was coppiced for the first time in December 2010 and again three years later, in December 2013.

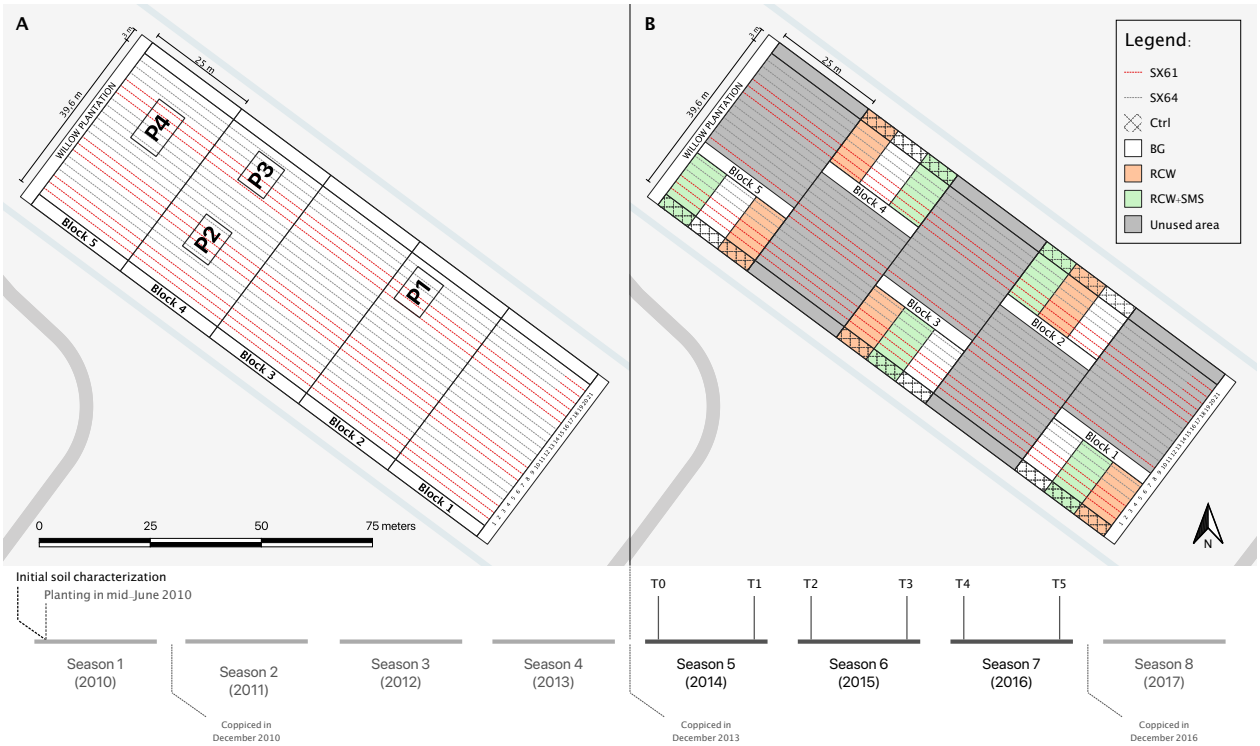

Figure 2.2 Evolution of the experimental design over time, including growth seasons, sampling times and coppicing times. (A) Experimental design of the first experimental phase, referred to as the GERLED site in Guidi et al. (2012). P1, P2, P3 and P4 were the sampling plots in their study; (B) Experimental design of the current experiment. T0 to T5 are the moments corresponding to the soil sampling. Adapted from Guidi et al. (2012).

The experimental setup was modified in July 2014 for the purposes of the current study. The new setup included five blocks of 13.8 x 25 m comprising three rows of 'SX61'; three rows of 'SX64'; and a control zone without willow, hereafter referred to as Ctrl (Figure 2.2B). The Ctrl

sections were located at the extremity of each block. Each section was split into three plots, to which three ground cover treatments were randomly applied: RCW (ramial chipped wood of *Salix* spp.); RCW+SMS (RCW mixed with spent mushroom substrate of *Pleurotus ostreatus*); and BG (bare ground). The RCW Agro Énergie, St-Roch-de-l'Achigan, Québec) was applied in a 10 cm thick layer. The SMS was obtained from a specialty mushroom farm (Advitam Inc., St-Ours, QC, Canada). A volume of 1.33 m^3 of SMS was manually incorporated to the RCW in every RCW+SMS subsection, each of which measured 115 m^2. Throughout the experiment, the plots without willows were mown and weeded to suppress vegetation using a string trimmer. The willow plantation was coppiced in December 2016 to end a third cycle of plant growth.

2.5.3 Soil Sampling

As shown in Figure 2.2A, soil samples were collected in June 2014 (T0) and at five other times over the course of the study: November 2014 (T1); June 2015 (T2); November 2015 (T3); June 2016 (T4); and November 2016 (T5). Each soil sample (one per treatment per block per time) was composed of three sub-samples collected at a depth of 0-30 cm. To limit the biases caused by the possible heterogeneous distribution of pollutants in the soil, each sample was collected within a 30 cm radius of its first corresponding sample (T0). Samples were placed in amber glass containers (System Plus Ltd., Baden, ON, Canada) and immediately sent to an external certified laboratory for chemical analysis (AGAT Laboratories Ltd., Montreal, QC, Canada) to assess the soil concentrations of polychlorinated biphenyls (PCBs) by GC-MS, petroleum hydrocarbons (C10-C50) by GC-FID, six TEs (i.e., Cd, Cr, Cu, Ni, Pb and Zn) by ICP-OES and polycyclic aromatic hydrocarbons (PAHs) by GC-MS, following the recommended provincial methods for environmental analyses (CEAEQ, 2004, 2008, 2014b, 2014a, 2016). Quality control and quality assurance were maintained for each method using duplicates, blanks and certified standard reference materials, following the provincial guidelines for analytical work in chemistry DR-12-SCA-01 (CEAEQ, 2018). Minimum frequency of insertion for blank and standard reference materials was one for each 10 samples. The recovery percentage for all standard reference materials, spiked blanks and fortified samples should be between 80 and 120% for all TEs and between 70 and 130% for C10-C50, PCBs and PAHs.

At T0, T1 and T5 only, extra soil samples were collected in RCW and BG subsections of 'SX61' and Ctrl sections, and put in 50 mL polypropylene tubes (Sarstedt Inc, Newton, NC, USA) to assess the soil trace element bioavailability according to a water extraction method described in

Courchesne et al. (2017b) and in Séguin et al. (2004). Briefly, in a ratio of 1:10 soil to ultra-pure water, each sample was shaken for 2 h and centrifuged (1400 g) for 15 min. Aliquot of the unfiltered solution was used for pH and electrical conductivity (EC) analyses. The remaining solution was filtered using a 0.45 µm nylon membrane (nylon plain, product code 1213776, GVS Magna™, Zola Predosa, BO, Italy). The water extracts were then acidified with 0.2% trace metal grade HNO_3 and stored at 4°C until TEs concentrations were measured by ICP-mass spectrometry (NexION® 300, Perkin Elmer, Guelph, ON, Canada).

2.5.4 Biomass Sampling

Sampling of willow biomass was carried out after autumn leaf drop, in November 2016 (T5). In each plot, four plants of each cultivar (for a total of 120 samples) were cut at the base of the trunk, using a forest brush cutter. Fresh weight was assessed in the field using a parcel scale (GRAM SAFIR X50, Barcelona, Spain) and subsamples from each plant were brought to the laboratory, then oven dried at 105°C to evaluate the percentage of moisture and estimate dry weight. These same subsamples were subsequently crushed and sent to an external laboratory (AGAT Laboratories Ltd., Montreal, QC, Canada) to analyze the TEs content (i.e., Cd, Cr, Cu, Ni, Pb and Zn) by ICP-OES following the recommended provincial methods for environmental analyses (CEAEQ, 2008, 2014a). Quality control and quality assurance were maintained following the provincial guidelines for analytical work in chemistry DR-12-SCA-01 (CEAEQ, 2018) as described for soil samples.

2.5.5 Data Analyses

Statistical analyses were performed using JMP® Pro V.15.0.0 (SAS Institute, Cary, NC, USA). Raw data (soil contaminant concentrations at T0, biomass yield, biomass TEs concentrations and soil TEs bioavailability) were submitted to a two-way analysis of variance (ANOVA) test, followed by multiple comparisons of means according to Tukey's Honestly Significant Difference (HSD) or simple comparisons of means according to Student's t-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. Bioconcentration factor (BCF) was used to describe shoot accumulation relative to soil concentration. BCF was calculated by the following equation:

$$\mathrm{BCF} = \frac{[TE]_{plant,i}}{[TE]_{soil,i}} \quad (1)$$

Given the significant differences in soil contaminant concentrations observed between raw values from planted and unplanted plots in the initial soil samples (T0) (see results), we decided to calculate the intermediate variation (IV) by plot at each subsequent sampling time (T1 to T5) to compare the treatment effects over time. The IV is the proportional variation of concentrations from a sampling time relative to the concentrations determined at T0. The IV was calculated by the equation:

$$\mathrm{IV_x} = \left(\frac{T_{x,i} - T_{0,i}}{T_{0,i}}\right) * 100 \quad (2)$$

where *x* is the subsequent sampling time (T1 to T5). The $\mathrm{IV_{T5}}$ can also be considered as the global variation (GV) over this study, since T5 refers to the final sampling time of the study. The IV was then submitted to a two-way ANOVA test, followed by multiple comparisons of means according to Tukey's HSD. The IV values were mostly presented as the mean IV per treatment at each sampling time (T1 to T5), calculated by:

$$\mathrm{Mean\ IV_x} = \frac{\sum_{i=1}^{n} \left(\frac{T_{x,i} - T_{0,i}}{T_{0,i}}\right) * 100}{n} \quad (3)$$

where *x* is the subsequent sampling time (T1 to T5) and *n* represents each replicate.

The variation rate (VR) was also calculated by plot at each subsequent sampling time (T1 to T5) to compare the treatment effects over time in a different way. The VR is the proportional variation between a given time (T1 to T5) and its corresponding previous one. The VR was calculated by the equation:

$$\mathrm{VR_x} = \left(\frac{T_{x,i} - T_{x-1,i}}{T_{x-1,i}}\right) * 100 \quad (4)$$

where *x* is the subsequent sampling time (T1 to T5). The VR values were also submitted to the same statistical analyses as IV. Values were mostly presented as the mean VR per treatment at each subsequent sampling time (T1 to T5), obtained by:

$$\text{Mean VR}_{\text{x}} = \frac{\sum_{i=1}^{n} \left(\frac{T_{x,i} - T_{x-1,i}}{T_{x-1,i}}\right) * 100}{n} \quad (5)$$

where *x* is the subsequent sampling time (T1 to T5) and *n* represent each replicate.

Period type has been added as a new two-level variable in the VR dataset in order to push the analysis further. The Growing level was attributed to VR values calculated over a growing period (*x* = T1, T3 and T5), while the Dormant level was attributed to VR values calculated over a dormant one (*x* = T2 and T4). The entire dataset of VR was then submitted to a three-way ANOVA test, followed by multiple comparisons of means according to Tukey's HSD or simple comparisons of means according to Student's t-test (when a period effect was observed) in order to compare values between ground cover treatments, cultivar treatments, and also by period type. The mean VR per treatment was calculated by period type and will referred as the mean seasonal variation (SV), which was calculated by the equation:

$$\text{Mean SV}_{\text{p}} = \frac{\sum_{i=1}^{n} VR_{p,i}}{n} \quad (6)$$

where *p* is the period (growing or dormant) and *n* represent each replicate.

Redundancy analysis (RDA) was performed on transformed variation factor (VF) values, using the vegan::rda function in R V.3.5.2 (R Core Development Team, 2020). VF was calculated by the equation:

$$\text{VF}_{\text{x}} = \frac{T_{x,i}}{T_{x-1,i}} \quad (7)$$

where *x* is the subsequent sampling time (T1 to T5). The VF data were normalized with a Hellinger transformation with the vegan::decostand function.

2.6 Conclusions

Two different cultivars of *Salix miyabeana* ('SX61' and 'SX64') supplemented with SMS of *Pleurotus ostreatus* and with RCW were tested in this study, with the goal of evaluating their impact on the in-soil variations of organic and inorganic contaminants over a period of three growing seasons. Results did not show that adding SMS as soil amendments improved the biomass

yield of either cultivar or would have potential for remediating a mixed-contaminated soil in a cool temperate region. Although RCW did not show any effect on willow biomass yield, its application increased the soil concentrations of some PAHs over three growing seasons and improved the phytoextraction of Zn on average by 17.27%. This suggests that RCW may cause changes in mobility and bioavailability of some contaminants in soil. Therefore, RCW may be a promising soil amendment to reduce the leaching of PAHs in soil and to increase the efficiency of *Salix* spp. for rehabilitating zinc-contaminated sites.

After three seasons of growth, similar concentrations of Zn and Cu accumulated in the shoots of both cultivars, but 'SX61' showed slightly yet significantly higher concentrations of Cd than 'SX64'. Both cultivars produced high biomass, up to 25 odt ha^{-1} yr^{-1}, highlighting their great growth potential in moderately contaminated soils. This finding may also reflect the excellent agronomic conditions of the study site. In addition to meeting direct remediation needs, similarly contaminated land with good agronomic properties could be used for high biomass production of renewable feedstock for bioenergy and bioproducts.

Determining soil contaminant concentrations at the beginning of the study and then monitoring their relative variations over time revealed that 'SX61' significantly reduced the "natural attenuation" of C10-C50. For all other soil contaminants, GV were not significantly impacted by the presence of willows, despite evidence that some TEs were substantially eliminated from the ground by plant uptake. To a lesser extent, the presence of willows is suspected to have accentuated the increases of PCBs in soil after the three seasons of growth. At first glance, the GV reported in this study could appear to fall short of the remediation objectives that generally motivate the establishment of willow plantation on contaminated sites. However, considering the inconsistency between the extracted quantities of some TEs and their GV over time, these results could rather indicate the complexity of monitoring soil contamination in the field.

Throughout this study, willows were suspected of having acted as an evapotranspiration cover, thus reducing the leaching of contaminants into the environment, or even mobilizing some contaminants towards the rooting zones, especially when the transpiration rate was maximal. Findings supporting this are that many contaminants were subject to seasonal oscillations, showing average increases after a Growing period, yet lower increases or even decreases after a Dormant

period. Such contaminant migration may have led to our inconclusive remediation results, even if degradation and/or phytoextraction processes would have been occurring simultaneously.

Our results highlight a phenomenon that certainly contributes to the difficulties of monitoring pollutant variations in the field over a long period of time, and that is rarely considered in phytoremediation studies. Based on the seasonal fluctuations reported here, we recommend comparing data from similar seasons, when monitoring soil contamination in the field, especially when soil is extensively penetrated by roots. Further investigation is underway in our laboratory to better understand the impact of SRIC of willow on the migration dynamics of soil contaminants.

Supplementary Materials: The following are available online at www.mdpi.com/22237747/10/3/520/s1, Figure S 2.1: Visual distribution of contaminant concentrations found in the initial soil samples and in those from the 2010 soil characterization,Table S 2.1: Mean intermediate variations of soil contaminant concentrations by treatment at each subsequent sampling time, Table S 2.2: Mean variation rates of soil contaminant concentrations by treatment at each subsequent sampling time. Table S 2.3: Seasonal variation (SV) of soil PCBs, C10-C50, PAHs and TEs.

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2.7 **Supplementary Materials**

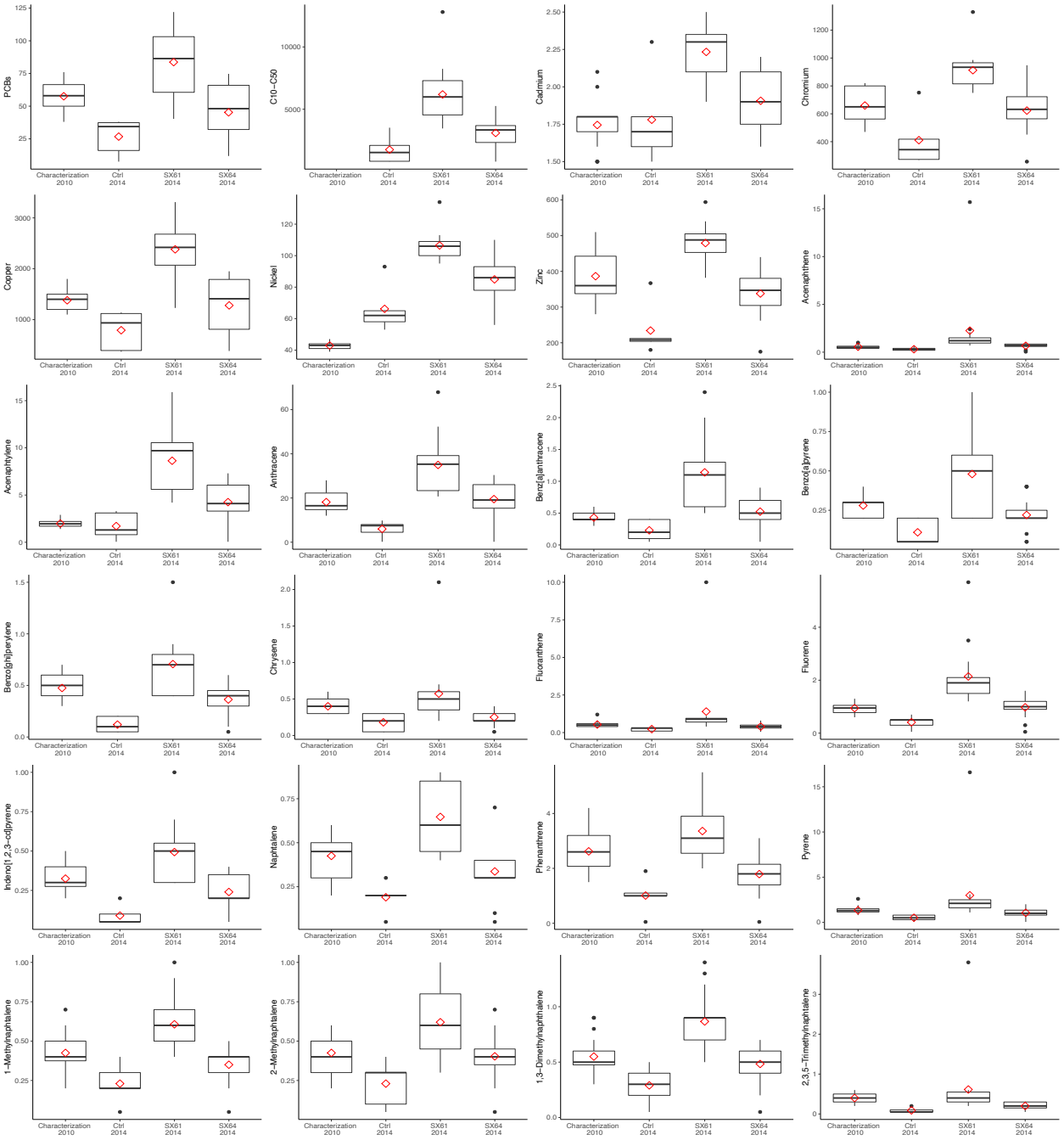

Figure S 2.1 Visual distribution of contaminant concentrations found in the initial soil samples and in those from the 2010 soil characterization. The box plots display the distribution of contaminant concentrations (mg kg^{-1}) by sample group (n=5 for Ctrl; n=15 for 'SX61'; n=15 for 'SX64' and n=20 for 2010 characterization). In each plot, the box boundaries represent the 25th and 75th percentiles, the horizontal thin black line represents the median and the red diamond symbol refers to the mean. The whiskers represent 1.5 times the interquartile range of the distribution. The outliers are denoted as larger points outside the whiskers.

Table S 2.1 Mean intermediate variations (IV) of soil contaminant concentrations by treatment at each subsequent sampling time.

		Ctrl			SX61			SX64			*p*-value			
	Parameters	**BG**	**RCW**	**RCW+SMS**	**BG**	**RCW**	**RCW+SMS**	**BG**	**RCW**	**RCW+SMS**	**Cover**	**Cultivar**	**Cover* Cultivar**	**Interpretation**
IV$_{T1}$	PCBs	51.42±44.30 (+57.75±108.08)	35.18±22.99 (+22.01±30.32)	40.97±30.24 (+32.96±57.50)	121.72±42.96 (+56.89±32.07)	105.96±15.06 (+56.17±29.30)	120.86±29.34 (+21.09±22.02)	48.76±27.53 (+38.78±73.12)	62.30±28.57 (+33.33±26.20)	61.66±28.10 (+43.04±23.60)	0.555	0.959	0.513	-
	C10-C50	1779.60±1777.43 (-15.72±37.03)	1414.00±848.38 (-18.88±10.91)	1438.20±903.76 (-17.65±38.91)	4128.00±1407.19 (-28.47±13.33)	3876.00±797.30 (-22.60±19.72)	3840.00±726.77 (-42.78±19.70)	2102.00±529.41 (-16.24±22.97)	2172.00±892.26 (-43.01±7.55)	1998.00±758.99 (-24.15±19.58)	0.437	0.451	0.417	-
	Cadmium	2.06±0.51 (+15.29±17.03)	2.16±0.32 (+22.16±12.86)	2.06±0.36 (+16.47±15.58)	2.58±0.33 (+19.40±9.65)	2.58±0.15 (+16.41±7.25)	2.64±0.26 (+13.86±9.49)	2.24±0.15 (+22.03±6.47)	2.18±0.16 (+8.15±7.03)	2.20±0.23 (+19.00±11.69)	0.769	0.916	0.263	-
	Chromium	406.60±309.21 (-4.04±39.38)	470.00±161.44 (+19.62±19.32)	416.40±196.62 (+11.04±52.60)	887.80±178.74 (+1.99±12.53)	901.00±118.96 (+2.04±14.73)	904.00±182.03 (-5.56±22.73)	617.00±77.92 (+9.36±24.98)	617.40±86.82 (-8.90±13.73)	544.80±183.32 (-7.00±15.55)	0.849	0.731	0.165	-
	Copper	1005.00±856.14 (+16.92±76.40)	837.20±471.88 (+7.54±27.53)	1014.60±798.84 (+13.27±50.89)	2434.00±1020.90 (+2.63±15.62)	2022.40±621.88 (-3.89±16.99)	2564.00±809.65 (-8.26±23.09)	1157.60±464.72 (-4.23±20.39)	1105.20±676.76 (-19.97±20.56)	1175.40±646.85 (-8.36±12.10)	0.762	0.294	0.990	-
	Nickel	63.80±23.69 (-4.60±20.00)	64.80±11.39 (-1.06±8.51)	63.80±15.01 (-1.95±22.05)	96.80±10.64 (-5.79±7.87)	95.80±4.97 (-7.41±7.19)	97.80±9.09 (-12.76±10.84)	80.00±3.39 (-1.51±8.17)	77.20±7.89 (-12.66±6.63)	76.60±12.90 (-8.09±9.47)	0.689	0.615	0.369	-
	Zinc	239.80±131.48 (-1.59±23.66)	253.40±67.37 (+9.58±12.04)	247.40±85.92 (+7.29±30.96)	469.20±107.60 (+1.39±12.69)	456.20±59.61 (-1.46±10.96)	476.80±54.35 (-6.33±16.35)	321.20±15.34 (+2.92±16.42)	322.40±43.06 (-10.70±10.97)	300.00±79.28 (-7.89±12.91)	0.549	0.186	0.314	-
	Acenaphthene	0.40±0.29 (+291.67±678.64)	0.52±0.44 (+496.67±1120.29)	0.46±0.38 (+248.33±421.82)	1.16±0.61 (-9.30±9.58)	1.18±0.36 (-1.44±29.17)	0.98±0.31 (-36.23±42.82)	0.70±0.25 (-5.98±21.27)	0.70±0.14 (+171.11±407.61)	0.68±0.19 (+29.52±44.08)	0.487	0.504	0.430	-
	Acenaphtylene	2.36±1.68 (+1682.74±3811.02)	1.86±1.14 (+651.29±1480.77)	1.96±1.45 (+691.79±1570.42)	6.52±3.72 (-27.64±8.01)	5.54±1.40 (-21.62±21.82)	5.90±1.70 (-29.93±40.79)	3.16±0.95 (-26.59±26.70)	3.22±1.48 (+716.80±1667.70)	2.92±1.29 (-20.33±27.83)	0.296	0.398	0.748	-
	Anthracene	8.62±5.63 (+1726.39±3898.44)	9.38±3.47 (+1220.33±2699.90)	7.70±3.50 (+1119.51±2505.16)	35.08±16.33 (-7.58±10.74)	33.74±8.01 (+8.45±19.02)	31.20±6.49 (-1.09±41.34)	18.54±3.96 (-6.64±25.76)	20.44±6.17 (+1572.76±3537.06)	17.14±5.90 (-3.37±18.74)	0.252	0.506	0.795	-
	Benz[a]-anthracene	0.29±0.20 (+160.00±414.43)	0.24±0.11 (+55.00±137.39)	0.23±0.13 (+60.00±148.53)	0.88±0.39 (-16.05±10.70)	0.74±0.22 (-19.51±23.23)	0.74±0.19 (-30.18±41.92)	0.44±0.11 (-13.33±12.64)	0.44±0.17 (+79.06±235.41)	0.38±0.13 (-20.22±21.26)	0.346	0.448	0.941	-
	Benzo[a]-pyrene	0.16±0.11 (+100.00±223.61)	0.10±0.06 (+10.00±54.77)	0.12±0.08 (+20.00±44.72)	0.34±0.17 (-21.57±20.83)	0.30±0.07 (-9.67±36.64)	0.34±0.09 (-19.17±68.44)	0.22±0.04 (+15.00±48.73)	0.16±0.09 (-11.67±63.36)	0.17±0.07 (-10.00±22.36)	0.295	0.177	0.536	-
	Benzo[ghi]-perylene	0.18±0.14 (+130.00±319.37)	0.17±0.10 (+70.00±130.38)	0.16±0.11 (+60.00±138.74)	0.66±0.30 (-10.33±14.16)	0.62±0.13 (+3.06±27.85)	0.60±0.12 (-5.60±46.84)	0.32±0.08 (-12.33±31.13)	0.32±0.13 (+46.00±142.49)	0.30±0.14 (-10.00±32.49)	0.093	0.417	0.985	-
	Chrysene	0.40±0.19 (+273.33±367.73)	0.30±0.07 (+176.67±216.54)	0.32±0.13 (+180.00±214.22)	0.66±0.19 (+62.38±35.25)	0.58±0.18 (+40.00±40.00)	0.60±0.17 (+4.52±57.93)	0.42±0.18 (+73.33±54.77)	0.38±0.08 (+135.00±207.36)	0.36±0.09 (+63.33±81.99)	0.304	0.228	0.557	-
	Fluoranthene	0.32±0.19 (+228.33±491.30)	0.32±0.22 (+261.67±583.20)	0.31±0.24 (+193.33±328.84)	0.90±0.20 (+30.71±29.26)	0.82±0.19 (+5.65±39.72)	0.70±0.19 (-22.93±52.27)	0.56±0.27 (+52.00±74.45)	0.44±0.11 (+96.00±226.70)	0.42±0.13 (+14.33±33.32)	0.196	0.637	0.727	-
	Fluorene	0.52±0.38 (+360.95±861.83)	0.52±0.26 (+326.48±767.92)	0.58±0.33 (+214.29±398.13)	1.80±1.25 (-12.85±23.21)	1.52±0.49 (-6.91±31.82)	1.48±0.46 (-31.62±34.72)	0.90±0.29 (-2.53±36.94)	0.92±0.26 (+208.83±498.68)	0.82±0.28 (-8.28±28.00)	0.454	0.557	0.827	-
	Indeno[1,2,3-cd]pyrene	0.16±0.11 (+120.00±216.79)	0.10±0.06 (+20.00±44.72)	0.12±0.10 (+50.00±100.00)	0.50±0.25 (-6.00±8.94)	0.44±0.11 (+6.67±20.55)	0.48±0.11 (-0.38±41.41)	0.24±0.05 (-1.67±32.49)	0.24±0.11 (+45.00±144.05)	0.21±0.10 (+5.00±27.39)	0.876	0.386	0.677	-
	Naphthalene	0.18±0.14 (+103.33±335.03)	0.19±0.11 (+75.00±239.79)	0.21±0.14 (+118.33±328.53)	0.42±0.13 (-35.28±12.56)	0.38±0.04 (-30.90±8.78)	0.40±0.12 (-31.90±48.08)	0.26±0.05 (-23.33±13.69)	0.26±0.09 (+29.76±151.36)	0.22±0.08 (-21.67±21.73)	0.544	0.740	0.900	-
	Phenanthrene	1.06±0.57 (+756.02±1757.72)	1.66±1.67 (+1802.93±4079.30)	1.94±2.40 (+619.81±1188.08)	3.78±1.59 (+21.76±27.44)	3.58±0.84 (+9.05±31.58)	3.18±1.30 (-6.41±26.69)	2.36±1.00 (+25.83±39.29)	2.28±0.80 (+539.22±1207.98)	2.44±1.03 (+60.76±47.17)	0.734	0.683	0.847	-
	Pyrene	0.84±0.54 (+582.00±1296.19)	0.68±0.31 (+417.67±940.73)	0.70±0.39 (+299.56±578.41)	2.32±0.59 (+23.65±30.56)	2.06±0.54 (+7.99±33.92)	1.90±0.46 (-11.07±58.78)	1.22±0.47 (+18.51±40.00)	1.14±0.33 (+339.66±760.67)	1.00±0.35 (+3.44±34.39)	0.275	0.577	0.808	-
	1-Methyl-naphthalene	0.19±0.13 (+103.33±335.03)	0.19±0.09 (+68.33±242.84)	0.25±0.17 (+105.83±237.31)	0.50±0.28 (-20.24±19.53)	0.48±0.13 (-11.86±11.47)	0.48±0.19 (-23.05±36.15)	0.30±0.00 (-15.00±13.69)	0.30±0.10 (+37.00±147.55)	0.30±0.10 (-1.67±36.51)	0.824	0.927	0.901	-
	2-Methyl-naphthalene	0.21±0.14 (+108.33±331.87)	0.23±0.11 (+78.33±236.41)	0.25±0.11 (+122.50±235.92)	0.60±0.29 (+0.33±22.99)	0.58±0.18 (+10.00±20.55)	0.50±0.17 (-21.40±37.63)	0.30±0.07 (-27.00±27.75)	0.32±0.08 (+69.76±240.70)	0.30±0.07 (-10.00±13.69)	0.632	0.835	0.699	-
	1,3-Dimethyl-naphthalene	0.31±0.24 (+228.00±599.99)	0.46±0.48 (+481.33±1128.75)	0.51±0.58 (+280.33±460.82)	0.86±0.36 (+10.10±33.65)	0.86±0.15 (+7.86±18.28)	0.78±0.40 (-17.74±35.96)	0.48±0.08 (-7.67±28.42)	0.50±0.19 (+92.48±228.76)	0.60±0.34 (+41.67±57.13)	0.543	0.844	0.771	-
	2,3,5-Trimethyl-naphthalene	0.10±0.11 (+75.00±239.79)	0.17±0.24 (+205.00±501.37)	0.20±0.23 (+265.00±488.49)	0.34±0.11 (+16.67±58.93)	0.34±0.09 (-1.67±43.46)	0.30±0.14 (-49.89±27.60)	0.22±0.04 (+23.33±52.17)	0.22±0.11 (+13.33±55.78)	0.21±0.12 (+55.00±103.68)	0.801	0.514	0.137	-

Table S 2.1 (continued)

	Parameters	Ctrl			SX61			SX64			*p*-value			Interpretation
		BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	Cover	Cultivar	Cover* Cultivar	
IV12	PCBs	28.38±21.11 (-4.31±38.24)	21.77±14.99 (-11.59±37.82)	32.19±22.12 (+9.08±39.86)	84.86±30.11 (+7.43±13.49)	73.04±19.84 (+5.60±20.43)	68.12±31.23 (-32.32±27.89)	44.56±21.20 (+13.76±29.59)	41.02±23.61 (-15.41±21.64)	44.08±21.42 (-1.33±17.93)	0.422	0.819	0.121	-
	C10-C50	1029.60±847.71 (-48.37±27.04)	870.00±654.30 (-50.60±16.95)	1338.40±1002.80 (-27.45±39.22)	3728.00±1068.70 (-34.94±7.52)	3320.00±835.31 (-34.90±12.00)	2968.00±1199.49 (-56.14±20.21)	1782.00±667.96 (-31.23±14.83)	1686.00±600.65 (-54.80±6.30)	1707.40±770.08 (-41.73±8.37)	0.590	0.994	0.092	-
	Cadmium	1.00±0.50 (-44.59±26.58)	1.19±0.50 (-33.78±23.53)	1.09±0.41 (-38.90±20.09)	1.68±0.19 (-22.19±6.20)	1.66±0.17 (-25.16±7.40)	1.60±0.16 (-30.82±7.67)	1.32±0.19 (-28.27±8.00)	1.28±0.19 (-36.39±10.54)	1.26±0.21 (-32.32±5.36)	0.792	0.282	0.150	-
	Chromium	336.20±221.56 (-19.90±45.57)	380.00±264.18 (-11.59±37.31)	323.20±224.41 (-23.07±31.76)	897.80±141.00 (+3.34±7.08)	875.80±50.95 (-0.90±7.67)	795.20±75.65 (-16.47±16.46)	541.40±154.18 (-7.38±16.87)	539.00±79.43 (-20.08±16.06)	486.00±161.91 (-15.24±18.05)	0.315	0.586	0.513	-
	Copper	787.40±812.22 (-11.41±63.80)	612.20±543.70 (-22.71±40.21)	623.80±470.35 (-23.24±33.05)	2312.00±723.58 (-0.72±3.15)	1876.00±553.61 (-10.16±13.27)	2392.00±769.66 (-14.41±22.67)	1173.00±760.65 (-11.76±23.63)	1018.40±577.72 (-25.21±17.79)	1045.20±556.02 (-15.69±12.56)	0.364	0.799	0.934	-
	Nickel	53.20±15.12 (-19.68±13.80)	46.80±21.68 (-30.47±26.01)	49.00±12.79 (-25.91±8.01)	83.60±7.37 (-18.61±4.77)	79.60±3.36 (-23.07±5.88)	79.80±3.27 (-28.73±7.61)	65.40±9.37 (-20.14±6.63)	64.00±6.56 (-27.47±7.17)	63.40±8.99 (-23.52±8.80)	0.198	0.912	0.574	-
	Zinc	193.40±93.25 (-17.97±32.18)	189.80±116.16 (-21.60±34.35)	186.00±87.55 (-22.01±18.91)	438.40±73.64 (-4.74±7.01)	428.00±47.56 (-7.58±7.01)	410.80±47.55 (-19.43±13.00)	281.00±84.36 (-13.22±12.94)	276.80±38.23 (-23.22±11.03)	259.20±65.85 (-19.43±14.51)	0.378	0.602	0.626	-
	Acenaphthene	0.22±0.13 (+105.00±334.25)	0.28±0.22 (+180.00±514.43)	0.33±0.22 (+215.00±499.18)	0.76±0.23 (-35.78±16.03)	0.76±0.30 (-34.00±38.04)	0.82±0.42 (-44.70±37.23)	0.62±0.28 (-13.26±42.29)	0.52±0.11 (+149.84±419.56)	0.56±0.34 (-11.43±36.63)	0.784	0.573	0.717	-
	Acenaphtylene	1.30±1.10 (+776.36±1858.13)	1.20±0.73 (+702.92±1675.59)	1.64±1.21 (+951.85±2207.31)	5.26±2.78 (-39.18±13.46)	4.04±0.79 (-40.93±19.82)	4.46±1.34 (-47.57±29.11)	2.62±0.84 (-39.26±20.30)	2.52±0.83 (+546.15±1315.87)	2.44±1.09 (-37.99±18.04)	0.530	0.560	0.568	-
	Anthracene	4.88±2.94 (+818.98±1946.00)	6.32±4.16 (+1180.11±2722.46)	7.00±5.15 (+1380.96±3141.49)	30.14±19.09 (-24.42±13.79)	27.20±13.67 (-17.97±11.48)	25.12±9.79 (-24.43±24.51)	15.84±4.68 (-21.80±19.02)	16.76±6.30 (+1001.39±2291.22)	13.74±6.00 (-26.93±4.52)	0.405	0.710	0.612	-
	Benz[a]-anthracene	0.23±0.18 (+40.00±148.53)	0.16±0.11 (+27.50±155.72)	0.21±0.17 (+110.00±331.95)	0.70±0.31 (-31.17±18.07)	0.62±0.30 (-35.35±19.31)	0.72±0.32 (-36.12±30.88)	0.36±0.11 (-29.17±18.63)	0.40±0.16 (+36.83±147.27)	0.39±0.21 (-29.00±28.32)	0.795	0.691	0.782	-
	Benzo[a]-pyrene	0.07±0.03 (-20.00±27.39)	0.07±0.03 (-5.00±67.08)	0.09±0.07 (+5.00±62.25)	0.36±0.25 (-20.43±23.47)	0.26±0.09 (-24.00±25.10)	0.26±0.11 (-45.83±31.46)	0.16±0.09 (-25.00±25.00)	0.17±0.10 (-21.67±21.73)	0.15±0.07 (-20.00±27.39)	0.612	0.663	0.399	-
	Benzo[ghi]-perylene	0.12±0.08 (+40.00±147.48)	0.09±0.07 (+25.00±156.12)	0.14±0.11 (+50.00±145.77)	0.56±0.38 (-29.00±11.94)	0.50±0.19 (-20.44±14.86)	0.52±0.22 (-23.73±29.18)	0.24±0.09 (-37.33±12.78)	0.30±0.10 (+42.67±144.50)	0.27±0.13 (-26.67±18.07)	0.751	0.729	0.199	-
	Chrysene	0.16±0.15 (+5.00±66.56)	0.12±0.08 (+26.67±155.72)	0.21±0.14 (+105.00±230.61)	0.46±0.15 (+16.67±47.14)	0.36±0.13 (-14.67±20.22)	0.46±0.11 (-23.81±35.60)	0.26±0.09 (+6.67±14.91)	0.26±0.11 (+25.00±50.00)	0.26±0.11 (+0.00±0.00)	0.870	0.793	0.281	-
	Fluoranthene	0.13±0.12 (+46.67±253.97)	0.16±0.11 (+56.67±248.50)	0.35±0.25 (+286.67±577.28)	0.52±0.11 (-19.66±40.22)	0.56±0.21 (-29.68±27.47)	0.56±0.15 (-38.88±34.55)	0.38±0.08 (+8.00±42.53)	0.34±0.05 (+76.00±237.31)	0.49±0.39 (+14.67±72.60)	0.956	0.637	0.208	-
	Fluorene	0.30±0.19 (+136.95±426.81)	0.36±0.21 (+186.10±511.55)	0.44±0.30 (+244.57±592.49)	1.24±0.61 (-36.47±8.69)	1.08±0.34 (-33.00±26.47)	1.12±0.31 (-46.38±30.02)	0.80±0.28 (-9.12±49.45)	0.72±0.16 (+153.33±417.66)	0.66±0.29 (-32.53±11.23)	0.601	0.601	0.698	-
	Indeno[1,2,3-cd]pyrene	0.07±0.03 (+0.00±61.24)	0.07±0.03 (+0.00±61.24)	0.11±0.08 (+65.00±145.34)	0.44±0.34 (-24.67±16.26)	0.32±0.13 (-25.33±15.92)	0.36±0.17 (-27.90±33.99)	0.18±0.08 (-31.67±20.75)	0.19±0.11 (-20.00±20.92)	0.17±0.07 (-10.00±22.36)	0.347	0.693	0.713	-
	Naphthalene	0.10±0.06 (+6.67±164.30)	0.15±0.10 (+58.33±247.35)	0.15±0.10 (+61.67±246.76)	0.34±0.11 (-47.22±14.96)	0.34±0.05 (-36.57±20.91)	0.34±0.11 (-43.10±39.50)	0.28±0.08 (-18.33±17.08)	0.26±0.05 (+31.90±150.45)	0.19±0.09 (-36.67±21.73)	0.420	0.693	0.453	-
	Phenanthrene	0.52±0.22 (+212.94±608.04)	1.02±1.01 (+431.33±1045.64)	0.98±0.68 (+603.56±1396.90)	1.72±0.37 (-40.47±20.51)	2.50±1.83 (-25.43±59.81)	2.32±2.03 (-37.66±38.79)	2.26±1.34 (+18.14±59.33)	1.58±0.60 (+511.22±1223.65)	1.60±1.28 (-5.76±47.15)	0.722	0.600	0.930	-
	Pyrene	0.36±0.25 (+174.00±517.95)	0.42±0.25 (+265.44±690.44)	0.78±0.65 (+656.22±1479.30)	1.56±0.54 (-16.90±31.07)	1.34±0.38 (-30.03±21.96)	1.46±0.33 (-36.34±34.67)	0.94±0.27 (-7.77±24.52)	0.78±0.16 (+233.84±596.14)	1.02±0.70 (-11.19±30.21)	0.861	0.690	0.471	-
	1-Methyl-naphthalene	0.10±0.06 (+4.17±165.94)	0.14±0.11 (+11.67±162.51)	0.16±0.11 (+65.83±246.44)	0.32±0.11 (-46.43±12.37)	0.30±0.12 (-41.76±28.35)	0.36±0.25 (-43.43±43.11)	0.30±0.12 (-18.33±24.58)	0.22±0.04 (+20.00±156.68)	0.21±0.10 (-36.67±28.01)	0.940	0.732	0.726	-
	2-Methyl-naphthalene	0.09±0.07 (+2.50±166.97)	0.12±0.08 (+10.00±162.70)	0.15±0.10 (+75.83±248.89)	0.34±0.05 (-32.83±39.85)	0.30±0.07 (-38.50±27.36)	0.34±0.21 (-47.45±36.84)	0.24±0.11 (-41.00±32.31)	0.22±0.04 (+15.24±159.33)	0.19±0.11 (-45.00±32.60)	0.670	0.997	0.322	-
	1,3-Dimethyl-naphthalene	0.19±0.11 (+62.33±247.26)	0.26±0.18 (+106.67±332.62)	0.31±0.19 (+182.00±411.55)	0.48±0.04 (-34.13±23.52)	0.58±0.37 (-25.20±55.19)	0.62±0.55 (-40.09±41.52)	0.52±0.34 (-5.33±57.28)	0.42±0.11 (+116.48±326.49)	0.41±0.34 (-16.00±50.92)	0.713	0.623	0.797	-
	2,3,5-Trimethyl-naphthalene	0.05±0.00 (-25.00±35.36)	0.11±0.08 (+50.00±141.42)	0.12±0.08 (+115.00±181.66)	0.20±0.00 (-30.00±29.81)	0.26±0.17 (-28.33±55.78)	0.25±0.22 (-49.40±38.31)	0.24±0.17 (+13.33±46.25)	0.18±0.04 (+33.33±150.92)	0.19±0.13 (+16.67±55.28)	0.844	0.176	0.250	-

Table S 2.1 (continued)

	Parameters	Ctrl			SX61			SX64			*p*-value			Interpretation
		BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	Cover	Cultivar	Cover* Cultivar	
IVT3	PCBs	29.79±32.07 (+2.39±79.92)	33.66±21.92 (+21.33±33.86)	33.73±32.33 (+10.77±70.18)	93.38±36.64 (+17.26±14.54)	75.50±17.47 (+10.11±25.09)	101.50±27.06 (+1.04±16.01)	40.82±20.52 (+9.81±55.81)	48.24±24.56 (+3.69±27.61)	50.18±27.92 (+10.64±29.06)	0.968	0.985	0.895	-
	C10-C50	862.20±1055.91 (-62.23±22.85)	846.80±600.89 (-54.76±12.92)	772.80±708.86 (-58.47±33.05)	3374.00±1424.60 (-42.01±17.42)	2600.00±706.12 (-48.11±14.81)	3016.00±494.30 (-55.45±13.80)	1596.00±745.84 (-40.60±13.60)	1401.00±559.83 (-62.65±6.14)	1516.20±795.60 (-50.56±16.53)	0.540	0.327	0.310	-
	Cadmium	1.76±0.46 (-1.55±14.44)	1.98±0.33 (+11.77±11.92)	1.80±0.44 (+0.95±17.03)	2.14±0.22 (-0.54±10.31)	2.20±0.20 (-0.79±8.59)	2.32±0.47 (+0.01±18.49)	1.78±0.16 (-3.14±5.80)	1.96±0.19 (-2.54±12.05)	1.88±0.23 (+1.32±6.75)	0.672	0.411	0.657	-
	Chromium	344.00±255.00 (-21.99±24.90)	478.40±229.45 (+18.28±22.24)	390.40±240.02 (+1.44±57.98)	852.80±156.55 (-1.76±12.34)	812.40±33.07 (-7.67±11.51)	849.60±161.20 (-10.86±22.07)	507.20±58.84 (-11.67±7.95)	599.40±64.47 (-10.98±16.19)	514.00±189.08 (-12.82±13.67)	0.432	0.594	0.342	-
	Copper	761.60±703.44 (-7.70±62.52)	864.40±513.41 (+6.56±22.02)	902.40±815.99 (+6.70±59.71)	2204.00±890.19 (-6.16±14.91)	1905.20±554.54 (-8.71±16.25)	2406.00±801.95 (-13.39±26.95)	1052.40±542.01 (-16.71±10.39)	1187.20±588.65 (-11.46±17.95)	1117.00±567.78 (-9.27±9.76)	0.908	0.461	0.964	-
	Nickel	53.40±17.26 (-20.16±8.15)	59.20±14.48 (-10.52±8.59)	54.80±17.88 (-16.48±23.75)	85.00±9.14 (-17.20±7.64)	82.40±2.61 (-20.28±6.85)	86.60±13.01 (-22.45±14.99)	65.60±4.77 (-19.47±4.53)	70.40±3.91 (-20.15±5.58)	68.00±10.58 (-18.23±7.91)	0.863	0.645	0.678	-
	Zinc	216.00±111.22 (-10.77±18.55)	262.80±93.57 (+11.54±11.62)	238.00±110.56 (+1.60±37.00)	474.20±131.97 (+2.36±19.13)	424.00±28.04 (-8.08±8.80)	459.40±75.07 (-9.69±19.26)	286.00±38.65 (-9.69±6.43)	339.20±44.46 (-5.51±16.57)	294.80±78.06 (-9.48±11.59)	0.649	0.492	0.472	-
	Acenaphthene	0.21±0.17 (+130.00±430.66)	0.30±0.20 (+196.67±506.13)	0.22±0.21 (+140.83±427.35)	1.00±0.72 (-21.06±42.41)	0.72±0.41 (-40.00±34.40)	0.72±0.16 (-57.56±23.12)	0.40±0.16 (-45.74±20.03)	0.60±0.14 (+273.33±686.00)	0.66±0.62 (+2.86±80.91)	0.286	0.703	0.435	-
	Acenaphtylene	1.44±1.33 (+1373.45±3201.26)	1.82±1.33 (+955.70±2205.07)	1.64±1.50 (+988.62±2298.65)	6.34±3.48 (-29.32±15.84)	3.88±1.23 (-43.36±20.93)	5.64±1.27 (-36.19±24.84)	2.64±0.80 (-38.32±24.58)	3.12±0.90 (+798.44±1845.78)	2.84±1.36 (-29.20±11.55)	0.345	0.579	0.457	-
	Anthracene	6.98±6.39 (+1754.65±4022.33)	9.92±6.65 (+1946.11±4362.59)	8.00±6.64 (+1733.60±3922.63)	39.90±26.29 (+2.08±47.45)	25.92±9.61 (-18.87±16.04)	32.26±11.08 (-3.44±25.54)	17.40±7.01 (-15.99±29.50)	21.52±8.73 (+1776.45±3982.30)	16.52±6.72 (-10.01±15.07)	0.315	0.631	0.478	-
	Benz[a]-anthracene	0.19±0.18 (+135.00±427.78)	0.23±0.16 (+110.00±330.53)	0.19±0.19 (+97.50±338.47)	0.86±0.50 (-23.64±18.28)	0.58±0.16 (-36.93±13.77)	0.78±0.19 (-29.71±34.22)	0.34±0.11 (-32.50±20.92)	0.48±0.13 (+125.56±321.78)	0.38±0.19 (-26.56±27.77)	0.327	0.873	0.594	-
	Benzo[a]-pyrene	0.09±0.07 (+35.00±151.66)	0.12±0.08 (+50.00±141.42)	0.11±0.08 (+45.00±146.20)	0.38±0.20 (-15.86±17.12)	0.22±0.08 (-36.33±25.34)	0.30±0.07 (-33.33±47.14)	0.11±0.05 (-45.00±27.39)	0.14±0.05 (-13.33±68.11)	0.15±0.10 (-20.00±44.72)	0.856	0.348	0.405	-
	Benzo[ghi]-perylene	0.15±0.15 (+115.00±328.63)	0.18±0.13 (+100.00±226.38)	0.15±0.14 (+85.00±236.91)	0.66±0.36 (-11.67±31.10)	0.46±0.13 (-25.24±15.91)	0.58±0.15 (-11.51±36.19)	0.30±0.12 (-21.67±21.73)	0.38±0.08 (+102.67±225.42)	0.29±0.14 (-21.67±21.73)	0.137	0.657	0.588	-
	Chrysene	0.14±0.11 (+66.67±244.10)	0.19±0.11 (+83.33±233.93)	0.14±0.11 (+31.67±154.16)	0.46±0.21 (+8.81±32.90)	0.38±0.08 (-8.00±10.95)	0.46±0.09 (-26.19±33.59)	0.22±0.11 (-10.00±32.49)	0.32±0.04 (+115.00±217.66)	0.28±0.16 (+6.67±36.51)	0.236	0.771	0.498	-
	Fluoranthene	0.21±0.17 (+140.00±425.02)	0.28±0.13 (+181.67±405.53)	0.23±0.20 (+155.00±419.39)	0.84±0.50 (+11.69±51.50)	0.60±0.23 (-26.57±23.89)	0.66±0.18 (-31.30±45.69)	0.30±0.07 (-18.67±18.50)	0.60±0.16 (+310.00±666.29)	0.58±0.48 (+35.67±82.27)	0.333	0.614	0.284	-
	Fluorene	0.29±0.32 (+244.10±702.29)	0.28±0.29 (+247.05±700.69)	0.29±0.29 (+249.05±699.61)	1.08±0.97 (-36.95±51.75)	0.88±0.41 (-43.62±31.79)	0.90±0.55 (-62.75±27.40)	0.50±0.35 (-49.06±27.21)	0.52±0.26 (+252.75±697.64)	0.67±0.54 (-41.61±38.99)	0.366	0.804	0.716	-
	Indeno[1,2,3-cd]pyrene	0.11±0.11 (+80.00±236.11)	0.12±0.08 (+60.00±134.16)	0.11±0.08 (+65.00±145.34)	0.42±0.26 (-24.00±23.02)	0.30±0.10 (-26.67±22.11)	0.38±0.08 (-24.38±15.45)	0.19±0.09 (-26.67±30.84)	0.22±0.04 (+45.00±144.05)	0.17±0.07 (-10.00±22.36)	0.257	0.311	0.795	-
	Naphthalene	0.11±0.11 (+41.67±256.24)	0.22±0.19 (+150.00±420.94)	0.15±0.15 (+93.33±340.82)	0.44±0.28 (-34.72±30.30)	0.32±0.11 (-41.10±21.03)	0.34±0.09 (-48.65±15.62)	0.24±0.11 (-31.67±23.86)	0.24±0.05 (+30.71±152.90)	0.25±0.13 (-18.33±35.55)	0.243	0.815	0.713	-
	Phenanthrene	0.66±0.40 (+412.38±1055.29)	0.98±0.46 (+635.37±1489.81)	0.72±0.66 (+652.61±1592.00)	3.58±2.54 (+17.72±77.34)	2.84±2.00 (-9.31±79.85)	2.26±0.83 (-32.18±26.72)	1.36±0.46 (-23.04±32.81)	2.16±1.23 (+573.12±1301.41)	2.34±2.07 (+49.39±141.52)	0.222	0.856	0.610	-
	Pyrene	0.52±0.40 (+427.94±1046.63)	0.66±0.36 (+410.72±944.52)	0.54±0.47 (+351.50±866.83)	2.12±1.21 (-0.57±29.41)	1.58±0.36 (-18.11±18.65)	1.72±0.34 (-26.39±42.76)	0.78±0.31 (-26.72±12.23)	1.30±0.34 (+585.97±1294.07)	1.20±0.81 (+6.82±35.02)	0.259	0.682	0.432	-
	1-Methyl-naphthalene	0.10±0.06 (+1.67±166.81)	0.15±0.10 (+55.83±249.09)	0.13±0.12 (+50.83±253.63)	0.44±0.29 (-28.57±44.03)	0.28±0.08 (-48.10±11.30)	0.30±0.07 (-54.54±4.44)	0.24±0.15 (-33.33±36.80)	0.26±0.09 (+30.00±152.64)	0.21±0.10 (-38.33±20.92)	0.379	0.846	0.699	-
	2-Methyl-naphthalene	0.10±0.06 (+5.00±165.37)	0.20±0.15 (+72.50±242.95)	0.17±0.17 (+62.50±249.34)	0.54±0.33 (-18.67±22.19)	0.34±0.11 (-36.83±3.35)	0.46±0.18 (-23.43±56.72)	0.24±0.11 (-43.67±28.73)	0.32±0.11 (+35.24±148.82)	0.26±0.11 (-26.67±18.07)	0.245	0.963	0.592	-
	1,3-Dimethyl-naphthalene	0.22±0.13 (+98.67±336.29)	0.30±0.12 (+166.33±412.16)	0.20±0.20 (+134.50±429.48)	0.82±0.61 (+4.54±70.26)	0.58±0.36 (-27.14±44.61)	0.58±0.22 (-38.42±25.28)	0.40±0.19 (-22.00±46.58)	0.52±0.18 (+207.43±499.83)	0.50±0.35 (+14.33±79.49)	0.119	0.738	0.541	-
	2,3,5-Trimethyl-naphthalene	0.06±0.02 (-5.00±67.08)	0.09±0.07 (+45.00±146.20)	0.09±0.07 (+55.00±152.48)	0.34±0.24 (+6.67±69.32)	0.30±0.23 (-13.33±82.83)	0.22±0.08 (-62.09±24.10)	0.13±0.07 (-18.33±72.26)	0.22±0.08 (+80.00±236.41)	0.25±0.27 (+40.00±119.37)	0.606	0.403	0.061	-

Table S 2.1 (continued)

	Parameters	Ctrl			SX61			SX64			*p*-value			Interpretation
		BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	Cover	Cultivar	Cover* Cultivar	
IV14	PCBs	36.20±35.92 (+13.57±90.66)	24.55±14.98 (+12.10±45.94)	35.47±41.03 (+13.11±91.31)	91.74±38.18 (+27.68±62.59)	78.54±33.93 (+10.56±24.04)	93.98±24.37 (-4.81±25.96)	54.36±40.73 (+34.21±63.41)	52.60±35.87 (+6.96±49.01)	55.82±29.00 (+30.46±79.51)	0.709	0.841	0.921	-
	C10-C50	1293.00±1521.48 (-44.26±43.52)	702.50±417.41 (-57.22±15.98)	835.00±589.10 (-48.36±27.32)	3738.00±988.62 (-29.12±32.31)	3748.00±1829.87 (-30.45±13.72)	3504.00±1723.09 (-53.90±9.57)	2453.60±2421.46 (-20.22±52.47)	1877.00±852.89 (-51.18±11.72)	2122.20±1821.66 (-30.08±49.35)	0.382	0.510	0.620	-
	Cadmium	1.76±0.48 (+0.29±29.16)	1.83±0.29 (+2.04±7.27)	1.82±0.36 (+0.44±12.38)	2.18±0.33 (+2.03±20.69)	2.20±0.19 (-0.87±7.01)	2.20±0.25 (-5.13±9.67)	1.55±0.65 (-13.37±38.34)	1.92±0.19 (-4.63±10.13)	1.84±0.19 (-0.53±9.29)	0.930	0.712	0.828	-
	Chromium	593.00±410.93 (+56.92±122.16)	448.25±148.93 (+4.93±14.08)	475.00±251.30 (+26.37±75.82)	961.40±190.56 (+13.24±29.37)	940.00±116.14 (+6.93±18.40)	867.40±132.49 (-9.70±17.75)	635.80±389.15 (+7.59±56.96)	667.20±143.91 (-2.71±14.00)	589.00±236.49 (-1.62±23.58)	0.455	0.442	0.870	-
	Copper	1343.00±926.36 (+85.16±151.45)	641.00±372.05 (-5.91±29.13)	1076.50±1127.83 (+29.36±78.77)	2116.00±646.40 (-2.09±34.61)	2166.00±728.58 (+4.56±21.57)	2324.00±619.38 (-16.06±17.53)	1279.80±1066.33 (+1.08±57.49)	1215.00±749.35 (-10.68±24.17)	1144.60±745.10 (-11.97±20.65)	0.451	0.192	0.557	-
	Nickel	58.80±20.07 (-9.21±34.10)	48.75±6.99 (-27.22±7.70)	53.25±13.74 (-18.92±21.65)	72.80±7.73 (-28.83±9.29)	71.80±5.31 (-30.53±7.73)	71.60±7.50 (-36.22±7.93)	58.80±19.51 (-28.18±21.67)	59.60±8.47 (-32.85±5.17)	60.20±9.98 (-27.47±9.82)	0.414	0.216	0.770	-
	Zinc	305.00±158.82 (+32.96±72.13)	240.00±68.63 (+1.15±8.95)	264.25±107.03 (+13.25±44.27)	468.80±88.91 (+3.67±23.11)	478.00±79.59 (+3.67±18.31)	446.40±51.07 (-12.72±13.17)	333.40±192.42 (+2.87±51.37)	338.00±53.62 (-6.70±11.02)	316.20±107.35 (-4.64±16.38)	0.513	0.426	0.858	-
	Acenaphthene	0.32±0.41 (+335.00±875.60)	0.48±0.43 (+497.92±1068.11)	0.31±0.19 (+222.92±455.79)	1.22±0.33 (+21.74±81.59)	1.18±0.52 (-1.56±41.47)	1.26±0.65 (-33.64±39.24)	0.70±0.58 (-10.74±62.67)	0.84±0.35 (+525.40±1215.71)	0.80±0.67 (+22.86±85.33)	0.274	0.889	0.514	-
	Acenaphtylene	2.24±2.29 (+2149.10±4892.04)	1.70±1.06 (+1459.24±2960.81)	1.92±1.65 (+1073.21±2151.84)	8.04±2.52 (+6.15±52.65)	6.64±1.95 (-7.32±18.68)	7.86±2.59 (-12.25±33.62)	5.02±4.00 (+4.15±61.68)	4.86±1.80 (+2095.84±4698.10)	4.98±4.08 (+22.42±100.06)	0.348	0.629	0.594	-
	Anthracene	7.32±7.95 (+2001.06±4583.41)	9.55±6.62 (+2216.73±4455.67)	7.60±4.67 (+1523.65±3051.39)	30.88±7.60 (-1.69±54.12)	33.92±12.69 (+10.89±42.72)	30.84±10.19 (-2.86±42.95)	26.08±22.01 (+14.45±80.58)	24.84±7.76 (+3086.23±6911.56)	24.66±23.45 (+14.01±75.09)	0.151	0.618	0.751	-
	Benz[a]-anthracene	0.28±0.33 (+260.00±693.86)	0.24±0.15 (+162.50±361.42)	0.24±0.15 (+131.25±256.07)	0.90±0.35 (+9.77±74.95)	0.78±0.23 (-11.43±33.45)	0.94±0.34 (-19.72±43.02)	0.57±0.42 (+8.33±79.77)	0.62±0.26 (+252.22±585.94)	0.76±0.84 (+65.56±215.93)	0.496	0.733	0.750	-
	Benzo[a]-pyrene	0.13±0.12 (+85.00±234.25)	0.08±0.03 (+12.50±62.92)	0.10±0.07 (+25.00±50.00)	0.40±0.10 (+18.57±85.21)	0.32±0.13 (-7.33±36.77)	0.38±0.08 (-19.17±42.04)	0.29±0.25 (+30.00±69.37)	0.24±0.11 (+50.00±141.42)	0.23±0.16 (+10.00±54.77)	0.795	0.731	0.875	-
	Benzo[ghi]-perylene	0.15±0.15 (+115.00±328.63)	0.14±0.08 (+75.00±150.00)	0.12±0.09 (+62.50±160.08)	0.54±0.17 (-15.67±41.62)	0.58±0.22 (-5.00±27.39)	0.50±0.10 (-22.54±30.97)	0.41±0.37 (-1.67±70.81)	0.38±0.11 (+132.67±318.16)	0.31±0.19 (-15.00±48.02)	0.080	0.530	0.866	-
	Chrysene	0.21±0.19 (+171.67±413.14)	0.16±0.08 (+66.67±156.35)	0.19±0.10 (+131.25±197.25)	0.52±0.13 (+47.14±81.54)	0.42±0.08 (+5.33±30.33)	0.52±0.08 (-19.29±36.25)	0.33±0.22 (+35.00±89.44)	0.36±0.11 (+155.00±305.37)	0.36±0.24 (+48.33±114.62)	0.812	0.779	0.433	-
	Fluoranthene	0.33±0.34 (+345.00±765.98)	0.28±0.17 (+212.50±458.94)	0.29±0.17 (+229.17±354.44)	0.78±0.33 (+29.26±82.77)	0.68±0.15 (-15.33±18.67)	0.78±0.33 (-26.17±53.44)	0.47±0.37 (+24.67±110.69)	0.54±0.21 (+292.50±675.09)	0.54±0.35 (+52.33±120.87)	0.748	0.661	0.523	-
	Fluorene	0.15±0.20 (+110.19±441.53)	0.28±0.29 (+271.90±685.48)	0.16±0.11 (+74.17±284.96)	0.90±0.35 (-42.51±37.70)	0.88±0.64 (-45.14±44.39)	0.82±0.45 (-68.45±13.82)	0.82±1.23 (-32.67±83.32)	0.44±0.26 (+282.75±792.30)	0.65±0.70 (-43.66±52.41)	0.246	0.968	0.834	-
	Indeno[1,2,3-cd]pyrene	0.15±0.15 (+130.00±319.37)	0.10±0.07 (+62.50±160.08)	0.08±0.03 (+25.00±50.00)	0.44±0.09 (+0.00±51.37)	0.36±0.13 (-14.67±20.22)	0.38±0.08 (-19.43±36.06)	0.35±0.33 (+23.33±86.08)	0.26±0.05 (+90.00±229.54)	0.25±0.19 (+20.00±75.83)	0.774	0.357	0.862	-
	Naphthalene	0.17±0.20 (+128.33±431.75)	0.24±0.19 (+197.92±469.06)	0.16±0.11 (+93.75±272.62)	0.44±0.09 (-21.94±44.10)	0.46±0.11 (-17.05±17.61)	0.46±0.13 (-30.63±23.50)	0.41±0.37 (+15.00±92.87)	0.36±0.13 (+157.62±415.02)	0.27±0.23 (-8.33±73.12)	0.130	0.868	0.886	-
	Phenanthrene	0.76±0.79 (+770.65±1861.33)	1.48±1.11 (+1503.52±3064.35)	0.82±0.75 (+836.32±1776.22)	3.26±1.73 (+22.54±89.77)	3.44±1.25 (+7.43±48.52)	3.20±1.71 (-0.79±54.03)	1.86±1.02 (+6.95±64.08)	2.38±1.15 (+1721.61±3901.06)	2.10±1.64 (+30.19±86.81)	0.136	0.909	0.855	-
	Pyrene	0.84±0.91 (+922.17±2112.27)	0.68±0.41 (+568.33±1154.96)	0.70±0.41 (+416.46±734.22)	1.98±0.59 (+23.53±77.43)	1.68±0.26 (-12.91±14.36)	1.96±0.60 (-23.11±47.86)	1.32±1.02 (+15.07±73.03)	1.34±0.40 (+700.29±1565.13)	1.42±0.98 (+45.93±107.23)	0.619	0.524	0.619	-
	1-Methyl-naphthalene	0.19±0.24 (+165.83±522.62)	0.30±0.27 (+291.67±672.27)	0.19±0.10 (+103.12±267.78)	0.62±0.18 (+17.48±69.84)	0.64±0.27 (+20.95±47.08)	0.68±0.29 (+13.17±66.63)	0.39±0.30 (+10.83±84.86)	0.42±0.18 (+242.00±591.61)	0.40±0.31 (+23.33±101.11)	0.079	0.846	0.898	-
	2-Methyl-naphthalene	0.32±0.45 (+379.17±962.52)	0.38±0.36 (+412.50±858.66)	0.29±0.20 (+194.79±346.57)	1.08±0.38 (+112.17±126.00)	0.92±0.26 (+92.67±100.01)	1.04±0.63 (+77.29±140.31)	0.56±0.42 (+49.33±144.13)	0.72±0.31 (+400.95±839.11)	0.60±0.64 (+83.33±218.26)	0.053	0.826	0.648	-
	1,3-Dimethyl-naphthalene	0.28±0.36 (+290.33±788.46)	0.50±0.41 (+513.75±1057.56)	0.29±0.23 (+256.67±565.15)	0.96±0.52 (+49.94±128.43)	1.08±0.63 (+34.17±73.32)	1.02±0.63 (+18.93±86.13)	0.54±0.38 (+2.33±73.69)	0.70±0.41 (+531.43±1212.47)	0.60±0.48 (+31.00±87.64)	***0.044****	0.944	0.808	**BG^{B}, RCW^{A}, RCW+SMS^{AB}**
	2,3,5-Trimethyl-naphthalene	0.13±0.15 (+125.00±323.07)	0.25±0.17 (+275.00±419.32)	0.18±0.17 (+231.25±352.00)	0.48±0.23 (+90.00±153.48)	0.50±0.32 (+36.67±96.03)	0.50±0.27 (-18.93±56.35)	0.29±0.17 (+78.33±150.88)	0.36±0.15 (+233.33±487.05)	0.33±0.28 (+150.00±308.22)	0.305	0.557	0.401	-

Table S 2.1 (continued)

	Parameters	Ctrl			SX61			SX64			*p*-value			Interpretation
		BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	Cover	Cultivar	Cover* Cultivar	
IVFS (GV)	PCBs	23.87±22.33 (-16.06±53.42)	27.40±21.86 (+9.72±53.16)	33.19±22.54 (+11.33±38.73)	103.84±34.83 (+31.61±9.56)	89.78±24.35 (+29.81±26.09)	106.90±46.26 (+4.45±35.25)	56.14±22.32 (+47.73±44.60)	61.20±34.18 (+30.77±40.53)	57.64±24.17 (+35.88±26.77)	0.782	0.081	0.305	-
	C10-C50	593.60±643.07 (-72.32±12.30)	674.80±402.98 (-55.82±26.21)	897.80±803.55 (-56.52±18.15)	3742.00±1327.96 (-35.62±14.07)	3528.00±821.87 (-29.38±18.78)	3416.00±1614.75 (-48.95±27.76)	1670.60±583.58 (-36.10±12.04)	1831.80±838.99 (-49.81±17.89)	1812.60±955.62 (-39.66±18.54)	0.835	***0.048****	0.216	**Ctrl^{B}, SX61^{A}, SX64^{AB}**
	Cadmium	1.80±0.37 (+1.19±12.27)	2.08±0.54 (+15.96±13.60)	2.06±0.58 (+14.70±18.26)	2.52±0.29 (+16.73±9.15)	2.58±0.24 (+16.25±8.76)	2.50±0.48 (+7.69±19.78)	2.10±0.32 (+13.91±10.48)	2.22±0.15 (+10.58±13.16)	2.24±0.35 (+20.60±12.89)	0.533	0.718	0.405	-
	Chromium	301.40±223.90 (-31.15±15.71)	405.00±171.29 (+0.66±12.27)	437.00±274.16 (+14.59±77.10)	953.40±126.96 (+10.00±5.83)	1000.20±254.59 (+11.84±20.45)	892.40±173.28 (-7.19±22.21)	607.80±117.29 (+5.17±13.54)	637.60±61.40 (-6.19±7.84)	645.00±261.99 (+5.99±19.72)	0.621	0.582	0.250	-
	Copper	607.20±476.43 (-23.94±41.81)	632.20±357.95 (-16.85±24.22)	1155.80±1169.01 (+23.25±88.31)	2504.00±855.27 (+7.21±10.00)	1870.00±570.75 (-10.86±11.80)	2428.00±943.57 (-13.86±24.15)	1195.40±487.39 (-2.79±17.19)	1159.80±545.57 (-13.60±9.29)	1240.20±697.54 (-3.22±15.45)	0.577	0.996	0.469	-
	Nickel	48.80±13.07 (-26.38±6.43)	55.20±9.26 (-15.29±12.03)	58.20±21.37 (-10.27±36.45)	99.60±15.53 (-3.22±12.09)	101.00±21.79 (-2.55±19.97)	96.00±17.39 (-13.70±20.51)	73.60±6.77 (-9.81±4.33)	76.40±6.07 (-13.12±10.41)	76.00±16.97 (-9.14±15.46)	0.842	0.180	0.502	-
	Zinc	190.00±91.67 (-20.88±13.76)	221.40±67.34 (-4.90±9.51)	253.60±125.41 (+8.24±47.58)	477.00±65.63 (+4.05±7.04)	509.80±110.96 (+9.37±17.26)	465.40±46.05 (-8.39±17.33)	322.80±70.32 (+0.88±11.36)	339.20±22.83 (-6.02±5.72)	348.00±123.60 (+3.69±17.45)	0.688	0.389	0.292	-
	Acenaphthene	0.13±0.15 (+75.83±349.06)	0.25±0.21 (+60.00±177.40)	0.14±0.15 (+80.00±346.94)	0.80±0.32 (-33.60±19.32)	0.72±0.36 (-40.56±28.28)	0.54±0.17 (-61.32±28.54)	0.32±0.08 (-54.24±17.78)	0.54±0.43 (+453.02±1144.31)	0.44±0.21 (-27.62±17.38)	0.136	0.820	0.493	-
	Acenaphtylene	0.86±0.76 (+760.23±1866.99)	0.90±0.64 (+259.51±693.78)	1.30±0.92 (+895.81±2126.65)	5.88±3.12 (-34.66±4.22)	4.96±1.29 (-31.16±9.70)	5.10±2.53 (-44.18±24.90)	2.40±0.49 (-42.59±20.47)	2.74±0.90 (+985.27±2300.22)	2.52±1.13 (-37.29±14.60)	0.170	0.868	0.550	-
	Anthracene	4.08±3.53 (+957.61±2287.72)	6.84±4.22 (+1188.10±2718.04)	6.50±6.14 (+1671.97±3844.95)	30.40±16.70 (-19.72±16.14)	36.38±15.92 (+10.74±5.62)	27.14±8.30 (-14.67±32.22)	16.88±4.09 (-16.00±20.11)	19.04±5.54 (+1814.27±4100.82)	17.48±9.32 (-11.20±16.45)	0.089	0.872	0.814	-
	Benz[a]-anthracene	0.14±0.15 (+85.00±343.97)	0.27±0.15 (+142.50±248.05)	0.20±0.15 (+100.00±335.88)	0.86±0.43 (-14.41±27.35)	0.84±0.25 (-9.74±14.77)	0.86±0.40 (-21.08±49.18)	0.40±0.12 (-14.17±42.25)	0.54±0.19 (+281.56±681.36)	0.44±0.21 (-11.11±43.21)	0.057	0.886	0.796	-
	Benzo[a]-pyrene	0.08±0.07 (+30.00±155.52)	0.09±0.07 (+5.00±62.25)	0.12±0.08 (+50.00±141.42)	0.44±0.23 (+0.29±30.48)	0.40±0.10 (+16.67±31.18)	0.38±0.16 (-18.33±48.02)	0.20±0.07 (+0.00±35.36)	0.22±0.08 (+78.33±236.41)	0.19±0.09 (+0.00±35.36)	0.488	0.938	0.352	-
	Benzo[ghi]-perylene	0.11±0.11 (+65.00±244.69)	0.14±0.11 (+55.00±144.05)	0.16±0.15 (+120.00±325.19)	0.70±0.42 (-6.33±27.24)	0.70±0.25 (+12.30±21.31)	0.64±0.30 (-3.33±42.64)	0.34±0.09 (-8.00±25.15)	0.40±0.12 (+138.67±314.87)	0.33±0.17 (-8.33±45.64)	***0.038****	0.968	0.530	**BG^{B}, RCW^{A}, RCW+SMS^{AB}**
	Chrysene	0.09±0.07 (+15.00±162.70)	0.18±0.14 (+161.67±340.22)	0.12±0.08 (+25.00±156.57)	0.50±0.17 (+27.86±47.88)	0.40±0.10 (-4.00±8.94)	0.50±0.26 (-19.52±53.83)	0.22±0.04 (-3.33±34.16)	0.34±0.22 (+250.00±587.37)	0.23±0.11 (-10.00±41.83)	0.179	0.743	0.544	-
	Fluoranthene	0.16±0.15 (+91.67±340.65)	0.55±0.66 (+471.67±752.72)	0.17±0.14 (+100.00±336.86)	0.86±0.27 (+38.00±92.30)	0.72±0.19 (-9.37±26.85)	0.74±0.29 (-19.87±61.96)	0.32±0.13 (-6.67±53.95)	0.74±0.82 (+839.50±1934.54)	0.40±0.20 (+7.00±60.17)	0.114	0.890	0.595	-
	Fluorene	0.20±0.18 (+119.05±436.72)	0.33±0.20 (+85.43±242.41)	0.28±0.25 (+207.24±610.94)	1.36±0.59 (-28.99±11.88)	1.28±0.32 (-22.69±16.49)	1.14±0.48 (-47.08±31.80)	0.62±0.16 (-30.77±27.84)	0.78±0.22 (+387.25±957.48)	0.70±0.30 (-28.51±13.65)	0.096	0.856	0.795	-
	Indeno[1,2,3-cd]pyrene	0.08±0.07 (+35.00±151.66)	0.11±0.08 (+50.00±141.42)	0.13±0.12 (+90.00±230.22)	0.50±0.31 (-6.00±26.08)	0.50±0.14 (+21.33±24.22)	0.50±0.24 (+3.05±48.16)	0.24±0.09 (+3.33±58.21)	0.28±0.13 (+95.00±228.04)	0.25±0.13 (+25.00±50.00)	0.096	0.891	0.688	-
	Naphthalene	0.08±0.07 (-1.67±168.68)	0.14±0.11 (+53.33±250.32)	0.11±0.11 (+41.67±256.24)	0.36±0.09 (-42.50±17.22)	0.40±0.07 (-27.05±15.66)	0.32±0.08 (-51.90±14.72)	0.22±0.04 (-33.33±20.41)	0.26±0.05 (+66.90±242.45)	0.19±0.09 (-36.67±21.73)	***0.035****	0.713	0.830	**BG^{B}, RCW^{A}, RCW+SMS^{AB}**
	Phenanthrene	0.60±0.62 (+602.79±1507.84)	1.26±1.13 (+548.02±1208.73)	0.66±0.65 (+609.56±1504.33)	2.74±1.02 (+0.16±59.55)	2.96±0.95 (-11.57±22.01)	2.04±1.02 (-36.49±34.29)	1.44±0.76 (-18.41±50.84)	1.60±0.71 (+629.58±1493.01)	1.78±0.51 (+18.91±17.95)	0.099	0.889	0.785	-
	Pyrene	0.38±0.37 (+327.33±879.21)	1.14±1.16 (+623.94±1027.16)	0.46±0.33 (+342.44±870.75)	2.08±0.54 (+16.76±52.11)	1.88±0.36 (-3.50±13.29)	2.02±0.74 (-10.16±66.74)	0.90±0.32 (-9.03±40.33)	1.74±1.73 (+1885.43±4256.72)	0.96±0.44 (-10.58±28.75)	0.091	0.849	0.619	-
	1-Methyl-naphthalene	0.08±0.07 (-4.17±170.12)	0.15±0.07 (+23.33±157.83)	0.11±0.11 (+39.17±257.72)	0.42±0.13 (-26.57±30.23)	0.44±0.09 (-17.52±12.04)	0.36±0.13 (-41.27±28.94)	0.26±0.09 (-25.00±34.36)	0.28±0.08 (+104.00±333.21)	0.27±0.13 (-18.33±39.70)	***0.026****	0.497	0.741	**BG^{B}, RCW^{A}, RCW+SMS^{B}**
	2-Methyl-naphthalene	0.08±0.07 (-0.83±168.85)	0.11±0.05 (-18.33±74.16)	0.11±0.11 (+42.50±256.18)	0.38±0.11 (-30.00±36.13)	0.38±0.13 (-27.67±16.65)	0.36±0.09 (-42.93±26.73)	0.20±0.07 (-50.33±29.59)	0.22±0.04 (+15.71±159.19)	0.23±0.11 (-35.00±28.50)	0.059	0.758	0.820	-
	1,3-Dimethyl-naphthalene	0.13±0.15 (+76.17±348.86)	0.25±0.21 (+61.33±176.24)	0.18±0.19 (+130.33±431.78)	0.62±0.08 (-14.38±35.33)	0.60±0.12 (-24.13±17.29)	0.42±0.13 (-51.92±24.61)	0.32±0.13 (-35.67±38.90)	0.32±0.08 (+99.90±335.62)	0.36±0.18 (-17.67±27.73)	***0.045****	0.864	0.475	**BG^{B}, RCW^{A}, RCW+SMS^{AB}**
	2,3,5-Trimethyl-naphthalene	0.08±0.07 (+35.00±151.66)	0.13±0.15 (+135.00±322.88)	0.08±0.07 (+35.00±151.66)	0.36±0.15 (+31.67±100.42)	0.28±0.04 (-21.67±21.73)	0.22±0.08 (-56.61±29.94)	0.12±0.08 (-23.33±75.78)	0.13±0.07 (+11.67±161.98)	0.15±0.10 (+13.33±106.33)	0.438	0.616	0.081	-

The first value is the average (mean±SD, n=5) contaminant concentrations (mg kg^{-1}). Value in parentheses in the same cell represent the mean IV_x (mean±SD, n=5) (%). Significance levels (*p*-value) are shown and asterisks ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$) indicate a significant effect of Cover, Cultivar or Cover*Cultivar on IV_x values. Capital letters in the final column were used to identify significant differences between treatments according to Tukey's HSD test ($p \leq 0.05$).

Table S 2.2 Mean variation rates (VR) of soil contaminant concentrations by treatment at each subsequent sampling time.

		Ctrl			SX61			SX64			*p*-value			
	Parameters	**BG**	**RCW**	**RCW+SMS**	**BG**	**RCW**	**RCW+SMS**	**BG**	**RCW**	**RCW+SMS**	**Cover**	**Cultivar**	**Cover* Cultivar**	**Interpretation**
VR_{T1}	PCBs	51.42±44.30 (+57.75±108.08)	35.18±22.99 (+22.01±30.32)	40.97±30.24 (+32.96±57.50)	121.72±42.96 (+56.89±32.07)	105.96±15.06 (+56.17±29.30)	120.86±29.34 (+21.09±22.02)	48.76±27.53 (+38.78±73.12)	62.30±28.57 (+33.33±26.20)	61.66±28.10 (+43.04±23.60)	0.555	0.959	0.513	-
	C10-C50	1779.60±1777.43 (-15.72±37.03)	1414.00±848.38 (-18.88±10.91)	1438.20±903.76 (-17.65±38.91)	4128.00±1407.19 (-28.47±13.33)	3876.00±797.30 (-22.60±19.72)	3840.00±726.77 (-42.78±19.70)	2102.00±529.41 (-16.24±22.97)	2172.00±892.26 (-43.01±7.55)	1998.00±758.99 (-24.15±19.58)	0.437	0.451	0.417	-
	Cadmium	2.06±0.51 (+15.29±17.03)	2.16±0.32 (+22.16±12.86)	2.06±0.36 (+16.47±15.58)	2.58±0.33 (+19.40±9.65)	2.58±0.15 (+16.41±7.25)	2.64±0.26 (+13.86±9.49)	2.24±0.15 (+22.03±6.47)	2.18±0.16 (+8.15±7.03)	2.20±0.23 (+19.00±11.69)	0.769	0.916	0.263	-
	Chromium	406.60±309.21 (-4.04±39.38)	470.00±161.44 (+19.62±19.32)	416.40±196.62 (+11.04±52.60)	887.80±178.74 (+1.99±12.53)	901.00±118.96 (+2.04±14.73)	904.00±182.03 (-5.56±22.73)	617.00±77.92 (+9.36±24.98)	617.40±86.82 (-8.90±13.73)	544.80±183.32 (-7.00±15.55)	0.849	0.731	0.165	-
	Copper	1005.00±856.14 (+16.92±76.40)	837.20±471.88 (+7.54±27.53)	1014.60±798.84 (+13.27±50.89)	2434.00±1020.90 (+2.63±15.62)	2022.40±621.88 (-3.89±16.99)	2564.00±809.65 (-8.26±23.09)	1157.60±464.72 (-4.23±20.39)	1105.20±676.76 (-19.97±20.56)	1175.40±646.85 (-8.36±12.10)	0.762	0.294	0.990	-
	Nickel	63.80±23.69 (-4.60±20.00)	64.80±11.39 (-1.06±8.51)	63.80±15.01 (-1.95±22.05)	96.80±10.64 (-5.79±7.87)	95.80±4.97 (-7.41±7.19)	97.80±9.09 (-12.76±10.84)	80.00±3.39 (-1.51±8.17)	77.20±7.89 (-12.66±6.63)	76.60±12.90 (-8.09±9.47)	0.689	0.615	0.369	-
	Zinc	239.80±131.48 (-1.59±23.66)	253.40±67.37 (+9.58±12.04)	247.40±85.92 (+7.29±30.96)	469.20±107.60 (+1.39±12.69)	456.20±59.61 (-1.46±10.96)	476.80±54.35 (-6.33±16.35)	321.20±15.34 (+2.92±16.42)	322.40±43.06 (-10.70±10.97)	300.00±79.28 (-7.89±12.91)	0.549	0.186	0.314	-
	Acenaphthene	0.40±0.29 (+291.67±678.64)	0.52±0.44 (+496.67±1120.29)	0.46±0.38 (+248.33±421.82)	1.16±0.61 (-9.30±9.58)	1.18±0.36 (-1.44±29.17)	0.98±0.31 (-36.23±42.82)	0.70±0.25 (-5.98±21.27)	0.70±0.14 (+171.11±407.61)	0.68±0.19 (+29.52±44.08)	0.487	0.504	0.430	-
	Acenaphtylene	2.36±1.68 (+1682.74±3811.02)	1.86±1.14 (+651.29±1480.77)	1.96±1.45 (+691.79±1570.42)	6.52±3.72 (-27.64±8.01)	5.54±1.40 (-21.62±21.82)	5.90±1.70 (-29.93±40.79)	3.16±0.95 (-26.59±26.70)	3.22±1.48 (+716.80±1667.70)	2.92±1.29 (-20.33±27.83)	0.296	0.398	0.748	-
	Anthracene	8.62±5.63 (+1726.39±3898.44)	9.38±3.47 (+1220.33±2699.90)	7.70±3.50 (+1119.51±2505.16)	35.08±16.33 (-7.58±10.74)	33.74±8.01 (+8.45±19.02)	31.20±6.49 (-1.09±41.34)	18.54±3.96 (-6.64±25.76)	20.44±6.17 (+1572.76±3537.06)	17.14±5.90 (-3.37±18.74)	0.252	0.506	0.795	-
	Benz[a]-anthracene	0.29±0.20 (+160.00±414.43)	0.24±0.11 (+55.00±137.39)	0.23±0.13 (+60.00±148.53)	0.88±0.39 (-16.05±10.70)	0.74±0.22 (-19.51±23.23)	0.74±0.19 (-30.18±41.92)	0.44±0.11 (-13.33±12.64)	0.44±0.17 (+79.06±235.41)	0.38±0.13 (-20.22±21.26)	0.346	0.448	0.941	-
	Benzo[a]-pyrene	0.16±0.11 (+100.00±223.61)	0.10±0.06 (+10.00±54.77)	0.12±0.08 (+20.00±44.72)	0.34±0.17 (-21.57±20.83)	0.30±0.07 (-9.67±36.64)	0.34±0.09 (-19.17±68.44)	0.22±0.04 (+15.00±48.73)	0.16±0.09 (-11.67±63.36)	0.17±0.07 (-10.00±22.36)	0.295	0.177	0.536	-
	Benzo[ghi]-perylene	0.18±0.14 (+130.00±319.37)	0.17±0.10 (+70.00±130.38)	0.16±0.11 (+60.00±138.74)	0.66±0.30 (-10.33±14.16)	0.62±0.13 (+3.06±27.85)	0.60±0.12 (-5.60±46.84)	0.32±0.08 (-12.33±31.13)	0.32±0.13 (+46.00±142.49)	0.30±0.14 (-10.00±32.49)	0.093	0.417	0.984	-
	Chrysene	0.40±0.19 (+273.33±367.73)	0.30±0.07 (+176.67±216.54)	0.32±0.13 (+180.00±214.22)	0.66±0.19 (+62.38±35.25)	0.58±0.18 (+40.00±40.00)	0.60±0.17 (+4.52±57.93)	0.42±0.18 (+73.33±54.77)	0.38±0.08 (+135.00±207.36)	0.36±0.09 (+63.33±81.99)	0.304	0.228	0.557	-
	Fluoranthene	0.32±0.19 (+228.33±491.30)	0.32±0.22 (+261.67±583.20)	0.31±0.24 (+193.33±328.84)	0.90±0.20 (+30.71±29.26)	0.82±0.19 (+5.65±39.72)	0.70±0.19 (-22.93±52.27)	0.56±0.27 (+52.00±74.45)	0.44±0.11 (+96.00±226.70)	0.42±0.13 (+14.33±33.32)	0.196	0.637	0.727	-
	Fluorene	0.52±0.38 (+360.95±861.83)	0.52±0.26 (+326.48±767.92)	0.58±0.33 (+214.29±398.13)	1.80±1.25 (-12.85±23.21)	1.52±0.49 (-6.91±31.82)	1.48±0.46 (-31.62±34.72)	0.90±0.29 (-2.53±36.94)	0.92±0.26 (+208.83±498.68)	0.82±0.28 (-8.28±28.00)	0.454	0.557	0.827	-
	Indeno[1,2,3]-cd]pyrene	0.16±0.11 (+120.00±216.79)	0.10±0.06 (+20.00±44.72)	0.12±0.10 (+50.00±100.00)	0.50±0.25 (-6.00±8.94)	0.44±0.11 (+6.67±20.55)	0.48±0.11 (-0.38±41.41)	0.24±0.05 (-1.67±32.49)	0.24±0.11 (+45.00±144.05)	0.21±0.10 (+5.00±27.39)	0.876	0.386	0.677	-
	Naphthalene	0.18±0.14 (+103.33±335.03)	0.19±0.11 (+75.00±239.79)	0.21±0.14 (+118.33±328.53)	0.42±0.13 (-35.28±12.56)	0.38±0.04 (-30.90±8.78)	0.40±0.12 (-31.90±48.08)	0.26±0.05 (-23.33±13.69)	0.26±0.09 (+29.76±151.36)	0.22±0.08 (-21.67±21.73)	0.544	0.740	0.900	-
	Phenanthrene	1.06±0.57 (+756.02±1757.72)	1.66±1.67 (+1802.93±4079.30)	1.94±2.40 (+619.81±1188.08)	3.78±1.59 (+21.76±27.44)	3.58±0.84 (+9.05±31.58)	3.18±1.30 (-6.41±26.69)	2.36±1.00 (+25.83±39.29)	2.28±0.80 (+539.22±1207.98)	2.44±1.03 (+60.76±47.17)	0.734	0.683	0.847	-
	Pyrene	0.84±0.54 (+582.00±1296.19)	0.68±0.31 (+417.67±940.73)	0.70±0.39 (+299.56±578.41)	2.32±0.59 (+23.65±30.56)	2.06±0.54 (+7.99±33.92)	1.90±0.46 (-11.07±58.78)	1.22±0.47 (+18.51±40.00)	1.14±0.33 (+339.66±760.67)	1.00±0.35 (+3.44±34.39)	0.275	0.577	0.808	-
	1-Methyl-naphthalene	0.19±0.13 (+103.33±335.03)	0.19±0.09 (+68.33±242.84)	0.25±0.17 (+105.83±237.31)	0.50±0.28 (-20.24±19.53)	0.48±0.13 (-11.86±11.47)	0.48±0.19 (-23.05±36.15)	0.30±0.00 (-15.00±13.69)	0.30±0.10 (+37.00±147.55)	0.30±0.10 (-1.67±36.51)	0.824	0.927	0.901	-
	2-Methyl-naphthalene	0.21±0.14 (+108.33±331.87)	0.23±0.11 (+78.33±236.41)	0.25±0.11 (+122.50±235.92)	0.60±0.29 (+0.33±22.99)	0.58±0.18 (+10.00±20.55)	0.50±0.17 (-21.40±37.63)	0.30±0.07 (-27.00±27.75)	0.32±0.08 (+69.76±240.70)	0.30±0.07 (-10.00±13.69)	0.632	0.835	0.699	-
	1,3-Dimethyl-naphthalene	0.31±0.24 (+228.00±599.99)	0.46±0.48 (+481.33±1128.75)	0.51±0.58 (+280.33±460.82)	0.86±0.36 (+10.10±33.65)	0.86±0.15 (+7.86±18.28)	0.78±0.40 (-17.74±35.96)	0.48±0.08 (-7.67±28.42)	0.50±0.19 (+92.48±228.76)	0.60±0.34 (+41.67±57.13)	0.543	0.844	0.771	-
	2,3,5-Trimethyl-naphthalene	0.10±0.11 (+75.00±239.79)	0.17±0.24 (+205.00±501.37)	0.20±0.23 (+265.00±488.49)	0.34±0.11 (+16.67±58.93)	0.34±0.09 (-1.67±43.46)	0.30±0.14 (-49.89±27.60)	0.22±0.04 (+23.33±52.17)	0.22±0.11 (+13.33±55.78)	0.21±0.12 (+55.00±103.68)	0.801	0.514	0.137	-

Table S 2.2 (continued)

	Parameters	Ctrl			SX61			SX64			*p*-value			Interpretation
		BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	Cover	Cultivar	Cover* Cultivar	
VRt2	PCBs	28.38±21.11 (-21.75±42.17)	21.77±14.99 (-24.97±35.78)	32.19±22.12 (-13.32±20.57)	84.86±30.11 (-29.72±14.98)	73.04±19.84 (-31.17±14.05)	68.12±31.23 (-43.52±20.32)	44.56±21.20 (+9.11±81.53)	41.02±23.61 (-36.55±10.03)	44.08±21.42 (-30.57±10.56)	0,325	0,617	0,307	-
	C10-C50	1029.60±847.71 (-36.44±28.76)	870.00±654.30 (-39.11±19.02)	1338.40±1002.80 (-14.60±16.21)	3728.00±1068.70 (-6.73±18.71)	3320.00±835.31 (-13.64±14.94)	2968.00±1199.49 (-24.58±17.74)	1782.00±667.96 (-16.61±11.76)	1686.00±600.65 (-20.52±7.10)	1707.40±770.08 (-17.59±27.73)	0,850	0,130	***0,046****	**RCW:(Ctrl^{B}, SX61^{A}, SX64^{A})**
	Cadmium	1.00±0.50 (-48.17±32.69)	1.19±0.50 (-46.31±17.51)	1.09±0.41 (-45.67±20.02)	1.68±0.19 (-34.74±4.04)	1.66±0.17 (-35.69±5.00)	1.60±0.16 (-39.31±3.54)	1.32±0.19 (-40.93±8.85)	1.28±0.19 (-41.43±6.34)	1.26±0.21 (-42.88±4.94)	0,893	0,456	0,965	-
	Chromium	336.20±221.56 (+2.50±79.94)	380.00±264.18 (-24.57±31.29)	323.20±224.41 (-18.67±34.30)	897.80±141.00 (+2.67±15.69)	875.80±50.95 (-0.96±18.65)	795.20±75.65 (-10.42±11.22)	541.40±154.18 (-9.04±36.19)	539.00±79.43 (-12.54±7.46)	486.00±161.91 (-9.01±11.96)	0,479	0,818	0,856	-
	Copper	787.40±812.22 (-2.85±91.41)	612.20±543.70 (-29.66±30.86)	623.80±470.35 (-20.45±36.15)	2312.00±723.58 (-1.73±12.95)	1876.00±553.61 (-5.31±12.47)	2392.00±769.66 (-6.71±11.49)	1173.00±760.65 (-2.96±40.34)	1018.40±577.72 (-5.71±10.31)	1045.20±556.02 (-7.01±17.63)	0,576	0,754	0,936	-
	Nickel	53.20±15.12 (-10.98±32.54)	46.80±21.68 (-29.40±25.90)	49.00±12.79 (-21.97±14.65)	83.60±7.37 (-13.11±9.00)	79.60±3.36 (-16.73±5.63)	79.80±3.27 (-18.08±4.45)	65.40±9.37 (-18.32±10.98)	64.00±6.56 (-17.03±3.31)	63.40±8.99 (-16.83±3.25)	0,413	0,825	0,326	-
	Zinc	193.40±93.25 (-8.76±53.84)	189.80±116.16 (-29.14±29.38)	186.00±87.55 (-22.34±24.35)	438.40±73.64 (-5.00±12.74)	428.00±47.56 (-5.12±14.20)	410.80±47.55 (-13.64±6.89)	281.00±84.36 (-12.58±26.13)	276.80±38.23 (-14.06±4.88)	259.20±65.85 (-12.71±7.97)	0,568	0,633	0,757	-
	Acenaphthene	0.22±0.13 (-16.67±71.69)	0.28±0.22 (-39.10±41.80)	0.33±0.22 (-13.88±56.53)	0.76±0.23 (-29.52±13.29)	0.76±0.30 (-31.51±33.01)	0.82±0.42 (-14.64±38.37)	0.62±0.28 (-10.94±30.11)	0.52±0.11 (-24.44±15.62)	0.56±0.34 (-20.67±46.40)	0,733	0,654	0,951	-
	Acenaphtylene	1.30±1.10 (-41.57±29.85)	1.20±0.73 (-31.07±32.51)	1.64±1.21 (-11.11±32.29)	5.26±2.78 (-16.31±13.28)	4.04±0.79 (-23.46±20.83)	4.46±1.34 (-24.76±4.23)	2.62±0.84 (-16.08±14.57)	2.52±0.83 (-17.87±16.03)	2.44±1.09 (-13.02±44.10)	0,427	0,604	0,359	-
	Anthracene	4.88±2.94 (-41.04±19.54)	6.32±4.16 (-36.38±26.68)	7.00±5.15 (-12.41±32.60)	30.14±19.09 (-18.09±13.55)	27.20±13.67 (-22.30±19.75)	25.12±9.79 (-21.40±19.18)	15.84±4.68 (-14.93±15.26)	16.76±6.30 (-19.37±9.40)	13.74±6.00 (-22.07±15.60)	0,481	0,428	0,247	-
	Benz[a]-anthracene	0.23±0.18 (-12.00±32.13)	0.16±0.11 (-31.67±35.55)	0.21±0.17 (-3.33±64.98)	0.70±0.31 (-18.57±13.18)	0.62±0.30 (-16.71±30.65)	0.72±0.32 (-5.56±25.13)	0.36±0.11 (-19.00±12.34)	0.40±0.16 (-9.52±14.68)	0.39±0.21 (-4.33±41.96)	0,446	0,931	0,915	-
	Benzo[a]-pyrene	0.07±0.03 (-36.67±36.13)	0.07±0.03 (-20.00±27.39)	0.09±0.07 (-15.00±33.54)	0.36±0.25 (+1.67±20.75)	0.26±0.09 (-11.67±28.63)	0.26±0.11 (-25.00±25.00)	0.16±0.09 (-30.00±27.39)	0.17±0.10 (+10.00±54.77)	0.15±0.07 (-10.00±22.36)	0,439	0,478	0,184	-
	Benzo[ghi]-perylene	0.12±0.08 (-20.00±27.39)	0.09±0.07 (-38.33±36.13)	0.14±0.11 (-10.00±22.36)	0.56±0.38 (-19.85±16.58)	0.50±0.19 (-20.24±19.53)	0.52±0.22 (-14.76±31.65)	0.24±0.09 (-23.33±22.36)	0.30±0.10 (-4.00±8.94)	0.27±0.13 (-10.00±37.91)	0,437	0,695	0,540	-
	Chrysene	0.16±0.15 (-64.67±19.05)	0.12±0.08 (-61.67±20.07)	0.21±0.14 (-32.33±50.60)	0.46±0.15 (-28.61±18.83)	0.36±0.13 (-37.50±13.82)	0.46±0.11 (-20.48±16.11)	0.26±0.09 (-33.57±21.37)	0.26±0.11 (-34.00±18.88)	0.26±0.11 (-28.00±28.44)	0,248	***0,009****	0,675	**Ctrl^{B}, SX61^{A}, SX64^{A}**
	Fluoranthene	0.13±0.12 (-59.17±21.73)	0.16±0.11 (-48.10±31.86)	0.35±0.25 (+28.57±73.15)	0.52±0.11 (-40.72±15.56)	0.56±0.21 (-26.46±36.59)	0.56±0.15 (-15.56±32.01)	0.38±0.08 (-21.11±33.94)	0.34±0.05 (-19.67±18.72)	0.49±0.39 (+12.00±97.38)	0,149	0,395	0,383	-
	Fluorene	0.30±0.19 (-25.00±35.36)	0.36±0.21 (-30.33±32.96)	0.44±0.30 (-15.56±40.49)	1.24±0.61 (-23.21±19.47)	1.08±0.34 (-24.35±28.34)	1.12±0.31 (-23.17±14.64)	0.80±0.28 (-11.16±17.03)	0.72±0.16 (-20.65±5.47)	0.66±0.29 (-20.59±29.74)	0,758	0,829	0,915	-
	Indeno[1,2,3]-cd]pyrene	0.07±0.03 (-38.33±36.13)	0.07±0.03 (-20.00±27.39)	0.11±0.08 (+3.33±58.21)	0.44±0.34 (-19.44±18.43)	0.32±0.13 (-28.00±19.80)	0.36±0.17 (-28.67±25.56)	0.18±0.08 (-26.67±25.28)	0.19±0.11 (-20.00±32.60)	0.17±0.07 (-13.33±18.26)	0,277	0,890	0,466	-
	Naphthalene	0.10±0.06 (-30.00±27.39)	0.15±0.10 (-16.67±23.57)	0.15±0.10 (-21.67±21.73)	0.34±0.11 (-19.00±12.34)	0.34±0.05 (-8.33±25.69)	0.34±0.11 (-13.33±26.74)	0.28±0.08 (+10.00±32.49)	0.26±0.05 (+5.00±27.39)	0.19±0.09 (-16.67±23.57)	0,567	0,196	0,242	-
	Phenanthrene	0.52±0.22 (-38.97±41.73)	1.02±1.01 (-8.71±117.00)	0.98±0.68 (-19.95±70.04)	1.72±0.37 (-50.87±13.90)	2.50±1.83 (-34.20±38.39)	2.32±2.03 (-25.63±51.01)	2.26±1.34 (-7.79±40.30)	1.58±0.60 (-27.90±21.41)	1.60±1.28 (-36.12±37.73)	0,934	0,547	0,804	-
	Pyrene	0.36±0.25 (-58.67±14.45)	0.42±0.25 (-37.68±25.33)	0.78±0.65 (+18.33±81.34)	1.56±0.54 (-33.00±14.46)	1.34±0.38 (-31.04±28.78)	1.46±0.33 (-20.66±19.46)	0.94±0.27 (-18.83±18.58)	0.78±0.16 (-29.61±14.49)	1.02±0.70 (-0.67±65.88)	0,115	0,597	0,286	-
	1-Methyl-naphthalene	0.10±0.06 (-25.00±70.71)	0.14±0.11 (-21.67±48.45)	0.16±0.11 (-27.00±37.35)	0.32±0.11 (-31.67±10.87)	0.30±0.12 (-33.33±31.18)	0.36±0.25 (-28.00±25.88)	0.30±0.12 (+0.00±40.82)	0.22±0.04 (-21.67±21.73)	0.21±0.10 (-30.00±32.60)	0,824	0,491	0,829	-
	2-Methyl-naphthalene	0.09±0.07 (-48.33±29.11)	0.12±0.08 (-41.67±30.05)	0.15±0.10 (-33.33±33.33)	0.34±0.05 (-35.73±24.46)	0.30±0.07 (-45.60±15.91)	0.34±0.21 (-35.50±16.43)	0.24±0.11 (-21.67±32.06)	0.22±0.04 (-28.33±18.26)	0.19±0.11 (-36.67±37.55)	0,834	0,375	0,388	-
	1,3-Dimethyl-naphthalene	0.19±0.11 (+21.90±158.36)	0.26±0.18 (-13.85±91.97)	0.31±0.19 (-10.00±43.46)	0.48±0.04 (-38.45±18.63)	0.58±0.37 (-32.89±39.34)	0.62±0.55 (-19.60±42.54)	0.52±0.34 (+6.33±64.81)	0.42±0.11 (-5.24±40.07)	0.41±0.34 (-33.60±39.76)	0,762	0,258	0,914	-
	2,3,5-Trimethyl-naphthalene	0.05±0.00 (-16.67±37.27)	0.11±0.08 (+36.67±150.19)	0.12±0.08 (+33.33±153.21)	0.20±0.00 (-35.33±22.80)	0.26±0.17 (-26.67±36.51)	0.25±0.22 (-13.50±55.10)	0.24±0.17 (+10.00±82.16)	0.18±0.04 (+0.00±61.24)	0.19±0.13 (+36.67±152.02)	0,788	0,233	0,922	-

Table S 2.2 (continued)

	Parameters	Ctrl			SX61			SX64			*p*-value			Interpretation
		BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	Cover	Cultivar	Cover* Cultivar	
VRr3	PCBs	29.79±32.07 (+2.46±54.54)	33.66±21.92 (+68.95±110.13)	33.73±32.33 (-1.33±38.66)	93.38±36.64 (+9.75±12.04)	75.50±17.47 (+4.28±11.46)	101.50±27.06 (+71.50±81.77)	40.82±20.52 (-6.77±22.59)	48.24±24.56 (+23.05±15.16)	50.18±27.92 (+11.24±14.06)	0,254	0,420	0,231	-
	C10-C50	862.20±1055.91 (-19.94±41.08)	846.80±600.89 (+7.88±65.36)	772.80±708.86 (-43.46±19.92)	3374.00±1424.60 (-9.61±31.40)	2600.00±706.12 (-18.86±24.27)	3016.00±494.30 (+11.44±34.66)	1596.00±745.84 (-11.66±20.90)	1401.00±559.83 (-17.47±4.95)	1516.20±795.60 (-16.00±24.45)	0,803	0,124	0,164	-
	Cadmium	1.76±0.46 (+118.17±112.61)	1.98±0.33 (+96.56±101.96)	1.80±0.44 (+100.71±138.19)	2.14±0.22 (+28.14±13.38)	2.20±0.20 (+32.92±9.28)	2.32±0.47 (+44.17±16.45)	1.78±0.16 (+36.61±19.78)	1.96±0.19 (+54.31±11.62)	1.88±0.23 (+50.34±13.90)	0,774	0,198	0,666	-
	Chromium	344.00±255.00 (+8.62±36.44)	478.40±229.45 (+63.04±108.61)	390.40±240.02 (+58.80±140.44)	852.80±156.55 (-4.61±12.81)	812.40±33.07 (-6.94±7.32)	849.60±161.20 (+6.35±11.70)	507.20±58.84 (-1.13±26.18)	599.40±64.47 (+12.20±13.43)	514.00±189.08 (+4.45±12.16)	0,228	0,444	0,713	-
	Copper	761.60±703.44 (+17.15±47.93)	864.40±513.41 (+97.23±171.07)	902.40±815.99 (+106.02±239.62)	2204.00±890.19 (-5.43±15.14)	1905.20±554.54 (+1.43±8.48)	2406.00±801.95 (+0.81±9.63)	1052.40±542.01 (-1.14±24.35)	1187.20±588.65 (+20.14±18.24)	1117.00±567.78 (+8.89±15.54)	0,191	0,414	0,901	-
	Nickel	53.40±17.26 (+1.26±16.08)	59.20±14.48 (+62.17±118.13)	54.80±17.88 (+13.96±36.02)	85.00±9.14 (+2.04±11.32)	82.40±2.61 (+3.60±3.42)	86.60±13.01 (+8.24±12.37)	65.60±4.77 (+1.53±11.93)	70.40±3.91 (+10.45±6.42)	68.00±10.58 (+7.11±2.89)	0,128	0,618	0,263	-
	Zinc	216.00±111.22 (+15.60±27.03)	262.80±93.57 (+92.44±157.62)	238.00±110.56 (+42.42±83.63)	474.20±131.97 (+7.48±17.66)	424.00±28.04 (-0.38±8.14)	459.40±75.07 (+11.76±11.88)	286.00±38.65 (+6.65±23.01)	339.20±44.46 (+23.09±11.94)	294.80±78.06 (+13.47±9.76)	0,177	0,391	0,374	-
	Acenaphthene	0.21±0.17 (-1.67±64.66)	0.30±0.20 (+45.00±79.84)	0.22±0.21 (-35.67±33.12)	1.00±0.72 (+28.59±75.73)	0.72±0.41 (+4.17±73.48)	0.72±0.16 (+10.97±69.35)	0.40±0.16 (-24.14±40.67)	0.60±0.14 (+19.29±34.57)	0.66±0.62 (+16.63±72.33)	0,503	0,319	0,428	-
	Acenaphtylene	1.44±1.33 (+19.64±42.14)	1.82±1.33 (+54.53±83.23)	1.64±1.50 (-2.50±31.08)	6.34±3.48 (+22.21±48.21)	3.88±1.23 (-4.18±24.70)	5.64±1.27 (+32.06±28.08)	2.64±0.80 (+4.36±27.56)	3.12±0.90 (+27.12±25.00)	2.84±1.36 (+20.88±31.36)	0,606	0,984	0,296	-
	Anthracene	6.98±6.39 (+40.51±55.19)	9.92±6.65 (+73.68±77.64)	8.00±6.64 (+11.61±26.94)	39.90±26.29 (+34.29±49.52)	25.92±9.61 (-0.16±21.47)	32.26±11.08 (+35.42±36.30)	17.40±7.01 (+9.01±29.62)	21.52±8.73 (+32.02±30.60)	16.52±6.72 (+23.49±20.80)	0,812	0,380	0,292	-
	Benz[a]-anthracene	0.19±0.18 (+7.33±86.23)	0.23±0.16 (+76.67±136.22)	0.19±0.19 (-20.00±27.39)	0.86±0.50 (+17.19±44.69)	0.58±0.16 (+3.50±32.44)	0.78±0.19 (+19.79±33.35)	0.34±0.11 (-1.67±32.49)	0.48±0.13 (+31.00±43.61)	0.38±0.19 (+17.67±55.32)	0,339	0,545	0,610	-
	Benzo[a]-pyrene	0.09±0.07 (+50.00±141.42)	0.12±0.08 (+60.00±54.77)	0.11±0.08 (+20.00±44.72)	0.38±0.20 (+15.00±48.73)	0.22±0.08 (-11.67±38.91)	0.30±0.07 (+31.67±48.02)	0.11±0.05 (-26.67±25.28)	0.14±0.05 (+3.33±58.21)	0.15±0.10 (+20.00±103.68)	0,539	0,245	0,506	-
	Benzo[ghi]-perylene	0.15±0.15 (+10.00±54.77)	0.18±0.13 (+110.00±134.16)	0.15±0.14 (+0.00±35.36)	0.66±0.36 (+25.00±38.19)	0.46±0.13 (-5.00±18.03)	0.58±0.15 (+27.14±49.59)	0.30±0.12 (+25.00±25.00)	0.38±0.08 (+33.33±31.18)	0.29±0.14 (+6.67±14.91)	0,319	0,708	0,147	Ctrl:(BG^{AB}, RCW^{A}, RCW+SMS^{B})
	Chrysene	0.14±0.11 (+42.50±110.26)	0.19±0.11 (+80.00±125.50)	0.14±0.11 (-31.67±20.75)	0.46±0.21 (-1.52±32.22)	0.38±0.08 (+15.33±40.52)	0.46±0.09 (+2.67±19.21)	0.22±0.11 (-16.67±23.57)	0.32±0.04 (+50.00±86.60)	0.28±0.16 (+6.67±36.51)	0,118	0,943	0,629	SX64:(BG^{B}, RCW^{A}, RCW+SMS^{AB})
	Fluoranthene	0.21±0.17 (+93.33±123.38)	0.28±0.13 (+143.33±145.11)	0.23±0.20 (-33.05±25.98)	0.84±0.50 (+66.67±110.71)	0.60±0.23 (+21.78±75.47)	0.66±0.18 (+24.50±48.94)	0.30±0.07 (-18.00±24.90)	0.60±0.16 (+81.67±64.12)	0.58±0.48 (+30.30±41.38)	0,190	0,788	***0,009****	RCW+SMS:(Ctrl^{B}, SX61^{A}, SX64^{A})
	Fluorene	0.29±0.32 (-17.00±51.67)	0.28±0.29 (-9.33±74.29)	0.29±0.29 (-35.48±32.63)	1.08±0.97 (+1.72±90.21)	0.88±0.41 (-7.60±58.82)	0.90±0.55 (-9.62±68.07)	0.50±0.35 (-33.66±51.75)	0.52±0.26 (-17.44±59.35)	0.67±0.54 (-11.19±66.67)	0,710	0,853	0,894	-
	Indeno[1,2,3]-cd]pyrene	0.11±0.11 (+40.00±89.44)	0.12±0.08 (+60.00±54.77)	0.11±0.08 (+0.00±0.00)	0.42±0.26 (+2.00±24.90)	0.30±0.10 (+2.67±40.24)	0.38±0.08 (+37.00±92.98)	0.19±0.09 (+10.00±54.77)	0.22±0.04 (+73.33±136.22)	0.17±0.07 (+0.00±0.00)	***0,026****	0,461	0,483	BG^{AB}, RCW^{A}, RCW+SMS^{B}
	Naphthalene	0.11±0.11 (+0.00±35.36)	0.22±0.19 (+33.33±58.93)	0.15±0.15 (-13.33±36.13)	0.44±0.28 (+28.33±61.69)	0.32±0.11 (-3.33±41.08)	0.34±0.09 (+7.00±33.47)	0.24±0.11 (-13.33±29.81)	0.24±0.05 (-3.33±34.16)	0.25±0.13 (+30.00±44.72)	0,932	0,841	0,291	-
	Phenanthrene	0.66±0.40 (+29.76±66.36)	0.98±0.46 (+63.52±98.03)	0.72±0.66 (-10.88±48.94)	3.58±2.54 (+113.49±140.95)	2.84±2.00 (+74.09±178.97)	2.26±0.83 (+71.28±147.65)	1.36±0.46 (-14.36±57.96)	2.16±1.23 (+39.66±70.66)	2.34±2.07 (+74.63±182.85)	0,821	0,346	0,472	-
	Pyrene	0.52±0.40 (+91.67±131.23)	0.66±0.36 (+67.50±58.36)	0.54±0.47 (-26.90±24.35)	2.12±1.21 (+34.75±71.47)	1.58±0.36 (+24.85±38.02)	1.72±0.34 (+19.66±21.00)	0.78±0.31 (-17.82±16.62)	1.30±0.34 (+71.97±49.01)	1.20±0.81 (+23.25±29.79)	***0,039****	0,965	0,080	BG^{AB}, RCW^{A}, RCW+SMS^{B}
	1-Methyl-naphthalene	0.10±0.06 (+10.00±54.77)	0.15±0.10 (+53.33±79.41)	0.13±0.12 (-15.00±33.54)	0.44±0.29 (+40.00±92.50)	0.28±0.08 (+5.33±56.06)	0.30±0.07 (+4.17±43.10)	0.24±0.15 (-9.33±53.67)	0.26±0.09 (+20.00±44.72)	0.21±0.10 (+3.33±29.81)	0,736	0,741	0,658	-
	2-Methyl-naphthalene	0.10±0.06 (+20.00±44.72)	0.20±0.15 (+115.00±174.64)	0.17±0.17 (+35.00±151.66)	0.54±0.33 (+61.67±97.47)	0.34±0.11 (+18.33±45.03)	0.46±0.18 (+75.24±113.00)	0.24±0.11 (+21.67±64.98)	0.32±0.11 (+50.00±61.24)	0.26±0.11 (+73.33±92.50)	0,850	0,769	0,590	-
	1,3-Dimethyl-naphthalene	0.22±0.13 (+50.00±102.74)	0.30±0.12 (+63.00±96.02)	0.20±0.20 (-31.00±42.49)	0.82±0.61 (+68.00±117.98)	0.58±0.36 (+41.00±146.35)	0.58±0.22 (+39.58±93.10)	0.40±0.19 (+25.71±111.35)	0.52±0.18 (+30.00±50.55)	0.50±0.35 (+88.89±181.73)	0,732	0,579	0,314	-
	2,3,5-Trimethyl-naphthalene	0.06±0.02 (+20.00±44.72)	0.09±0.07 (+5.00±62.25)	0.09±0.07 (-20.00±27.39)	0.34±0.24 (+70.00±120.42)	0.30±0.23 (+101.33±279.90)	0.22±0.08 (+110.00±243.70)	0.13±0.07 (-12.67±75.07)	0.22±0.08 (+20.00±27.39)	0.25±0.27 (+35.00±121.96)	0,933	0,398	0,611	-

Table S 2.2 (continued)

	Parameters	Ctrl			SX61			SX64			*p*-value			Interpretation
		BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	Cover	Cultivar	Cover* Cultivar	
VRt4	PCBs	36.20±35.92 (+25.33±65.11)	24.55±14.98 (-8.95±30.46)	35.47±41.03 (-16.06±24.04)	91.74±38.18 (+7.27±51.10)	78.54±33.93 (+3.21±28.11)	93.98±24.37 (-2.91±34.95)	54.36±40.73 (+28.42±63.82)	52.60±35.87 (+3.77±39.97)	55.82±29.00 (+23.28±81.15)	0,316	0,789	0,808	-
	C10-C50	1293.00±1521.48 (+43.65±80.82)	702.50±417.41 (+10.60±72.42)	835.00±589.10 (+33.11±81.75)	3738.00±988.62 (+33.16±66.34)	3748.00±1829.87 (+46.34±57.00)	3504.00±1723.09 (+19.04±66.66)	2453.60±2421.46 (+28.33±76.47)	1877.00±852.89 (+33.72±42.13)	2122.20±1821.66 (+45.20±97.92)	0,925	0,985	0,831	-
	Cadmium	1.76±0.48 (+1.93±25.39)	1.83±0.29 (-10.83±3.88)	1.82±0.36 (-6.70±3.45)	2.18±0.33 (+3.80±23.71)	2.20±0.19 (+0.24±7.33)	2.20±0.25 (-3.78±11.11)	1.55±0.65 (-11.45±38.50)	1.92±0.19 (-1.59±10.35)	1.84±0.19 (-1.49±11.62)	0,945	0,783	0,705	-
	Chromium	593.00±410.93 (+99.15±145.66)	448.25±148.93 (-3.58±26.01)	475.00±251.30 (+5.67±15.27)	961.40±190.56 (+17.90±37.26)	940.00±116.14 (+15.85±15.15)	867.40±132.49 (+2.96±11.50)	635.80±389.15 (+25.29±76.44)	667.20±143.91 (+12.31±25.46)	589.00±236.49 (+15.05±34.94)	0,125	0,731	0,350	-
	Copper	1343.00±926.36 (+132.82±201.97)	641.00±372.05 (-13.79±20.49)	1076.50±1127.83 (+2.32±15.91)	2116.00±646.40 (+9.40±45.60)	2166.00±728.58 (+17.27±29.56)	2324.00±619.38 (+0.23±16.85)	1279.80±1066.33 (+24.47±77.92)	1215.00±749.35 (+2.60±26.43)	1144.60±745.10 (+0.06±36.48)	0,115	0,638	0,231	-
	Nickel	58.80±20.07 (+12.52±35.68)	48.75±6.99 (-16.43±12.12)	53.25±13.74 (-10.07±3.64)	72.80±7.73 (-13.01±16.48)	71.80±5.31 (-12.84±6.32)	71.60±7.50 (-16.58±9.69)	58.80±19.51 (-11.14±26.59)	59.60±8.47 (-15.57±8.72)	60.20±9.98 (-11.32±8.85)	0,163	0,517	0,373	-
	Zinc	305.00±158.82 (+47.83±73.65)	240.00±68.63 (-7.75±14.98)	264.25±107.03 (-0.34±10.06)	468.80±88.91 (+4.53±31.25)	478.00±79.59 (+12.54±14.99)	446.40±51.07 (-1.80±11.97)	333.40±192.42 (+16.09±64.91)	338.00±53.62 (+1.31±21.54)	316.20±107.35 (+6.71±24.03)	0,204	0,900	0,418	-
	Acenaphthene	0.32±0.41 (+25.00±75.00)	0.48±0.43 (+77.08±159.48)	0.31±0.19 (+50.00±100.00)	1.22±0.33 (+92.94±152.72)	1.18±0.52 (+81.90±71.62)	1.26±0.65 (+75.83±69.25)	0.70±0.58 (+52.67±82.54)	0.84±0.35 (+41.29±45.60)	0.80±0.67 (+46.62±121.07)	0,823	0,380	0,981	-
	Acenaphtylene	2.24±2.29 (+45.00±66.39)	1.70±1.06 (+22.06±75.94)	1.92±1.65 (+21.88±66.86)	8.04±2.52 (+58.88±80.42)	6.64±1.95 (+94.22±109.53)	7.86±2.59 (+51.92±86.93)	5.02±4.00 (+73.65±104.88)	4.86±1.80 (+60.93±61.05)	4.98±4.08 (+81.53±160.16)	0,885	0,600	0,900	-
	Anthracene	7.32±7.95 (-1.87±42.52)	9.55±6.62 (+3.72±62.36)	7.60±4.67 (-6.93±53.13)	30.88±7.60 (+15.22±73.36)	33.92±12.69 (+48.35±87.54)	30.84±10.19 (+5.14±52.84)	26.08±22.01 (+34.75±91.48)	24.84±7.76 (+22.07±44.08)	24.66±23.45 (+33.43±104.40)	0,644	0,738	0,941	-
	Benz[a]-anthracene	0.28±0.33 (+22.00±58.48)	0.24±0.15 (+37.50±110.87)	0.24±0.15 (+68.75±154.62)	0.90±0.35 (+62.23±127.89)	0.78±0.23 (+43.29±55.84)	0.94±0.34 (+34.83±85.48)	0.57±0.42 (+55.67±106.82)	0.62±0.26 (+28.57±40.33)	0.76±0.84 (+126.33±284.52)	0,766	0,967	0,977	-
	Benzo[a]-pyrene	0.13±0.12 (+30.00±44.72)	0.08±0.03 (-25.00±28.87)	0.10±0.07 (-12.50±25.00)	0.40±0.10 (+33.33±76.38)	0.32±0.13 (+70.00±100.28)	0.38±0.08 (+35.00±50.14)	0.29±0.25 (+130.00±97.47)	0.24±0.11 (+80.00±83.67)	0.23±0.16 (+73.33±136.22)	***0,023****	0,167	0,284	**BG^{A}, RCW^{B}, RCW+SMS^{B}**
	Benzo[ghi]-perylene	0.15±0.15 (+0.00±0.00)	0.14±0.08 (+8.33±63.10)	0.12±0.09 (-16.67±19.25)	0.54±0.17 (+5.79±56.33)	0.58±0.22 (+31.67±46.55)	0.50±0.10 (-7.33±36.77)	0.41±0.37 (+18.33±67.80)	0.38±0.11 (+1.00±28.47)	0.31±0.19 (+6.67±40.57)	0,355	0,984	0,775	-
	Chrysene	0.21±0.19 (+53.33±61.69)	0.16±0.08 (+8.33±63.10)	0.19±0.10 (+75.00±150.00)	0.52±0.13 (+45.90±96.92)	0.42±0.08 (+15.33±31.21)	0.52±0.08 (+16.00±26.79)	0.33±0.22 (+40.00±74.16)	0.36±0.11 (+11.67±28.63)	0.36±0.24 (+47.00±115.84)	0,493	0,967	0,955	-
	Fluoranthene	0.33±0.34 (+111.00±225.23)	0.28±0.17 (-8.33±44.10)	0.29±0.17 (+63.75±157.87)	0.78±0.33 (+53.13±148.85)	0.68±0.15 (+23.00±41.17)	0.78±0.33 (+21.83±49.37)	0.47±0.37 (+45.00±109.23)	0.54±0.21 (-10.24±23.53)	0.54±0.35 (+36.73±97.70)	0,662	0,955	0,894	-
	Fluorene	0.15±0.20 (-42.50±27.39)	0.28±0.29 (-3.13±70.99)	0.16±0.11 (-28.12±32.87)	0.90±0.35 (+8.88±52.56)	0.88±0.64 (+1.81±43.01)	0.82±0.45 (+10.23±54.27)	0.82±1.23 (+39.29±110.37)	0.44±0.26 (-10.00±29.84)	0.65±0.70 (-8.00±47.47)	0,818	0,299	0,779	-
	Indeno[1,2,3]-cd]pyrene	0.15±0.15 (+26.67±43.46)	0.10±0.07 (-12.50±25.00)	0.08±0.03 (-25.00±28.87)	0.44±0.09 (+46.19±87.12)	0.36±0.13 (+33.33±73.83)	0.38±0.08 (+7.00±43.40)	0.35±0.33 (+60.00±89.44)	0.26±0.05 (+20.00±27.39)	0.25±0.19 (+40.00±82.16)	0,168	0,445	0,801	-
	Naphthalene	0.17±0.20 (+33.33±47.14)	0.24±0.19 (+66.67±156.35)	0.16±0.11 (+18.75±55.43)	0.44±0.09 (+33.11±80.85)	0.46±0.11 (+54.00±58.90)	0.46±0.13 (+42.67±55.70)	0.41±0.37 (+50.00±93.54)	0.36±0.13 (+63.33±84.49)	0.27±0.23 (+3.33±58.21)	0,464	0,931	0,823	-
	Phenanthrene	0.76±0.79 (+11.11±56.55)	1.48±1.11 (+27.84±57.67)	0.82±0.75 (+27.78±140.11)	3.26±1.73 (+61.85±162.91)	3.44±1.25 (+49.10±76.63)	3.20±1.71 (+53.34±81.03)	1.86±1.02 (+32.92±64.58)	2.38±1.15 (+38.71±95.91)	2.10±1.64 (+38.00±111.93)	0,837	0,705	0,956	-
	Pyrene	0.84±0.91 (+46.00±67.68)	0.68±0.41 (+2.69±52.88)	0.70±0.41 (+90.45±206.56)	1.98±0.59 (+46.52±128.70)	1.68±0.26 (+10.82±29.80)	1.96±0.60 (+18.74±43.89)	1.32±1.02 (+54.18±95.41)	1.34±0.40 (+5.17±28.10)	1.42±0.98 (+51.49±128.11)	0,478	0,963	0,943	-
	1-Methyl-naphthalene	0.19±0.24 (+50.00±100.00)	0.30±0.27 (+108.33±142.40)	0.19±0.10 (+75.00±150.00)	0.62±0.18 (+112.89±161.84)	0.64±0.27 (+135.00±79.14)	0.68±0.29 (+153.33±162.62)	0.39±0.30 (+52.00±93.65)	0.42±0.18 (+85.00±114.02)	0.40±0.31 (+103.33±142.59)	0,338	0,329	0,997	-
	2-Methyl-naphthalene	0.32±0.45 (+140.00±198.12)	0.38±0.36 (+143.75±139.01)	0.29±0.20 (+89.58±140.99)	1.08±0.38 (+200.28±220.35)	0.92±0.26 (+203.67±152.43)	1.04±0.63 (+156.19±216.01)	0.56±0.42 (+135.00±185.07)	0.72±0.31 (+144.00±146.86)	0.60±0.64 (+163.33±331.75)	0,546	0,389	0,999	-
	1,3-Dimethyl-naphthalene	0.28±0.36 (+8.33±82.50)	0.50±0.41 (+46.67±74.83)	0.29±0.23 (+46.67±104.56)	0.96±0.52 (+117.71±229.84)	1.08±0.63 (+98.00±90.26)	1.02±0.63 (+97.00±134.98)	0.54±0.38 (+23.33±53.49)	0.70±0.41 (+39.17±66.64)	0.60±0.48 (+43.81±119.08)	0,390	0,241	0,968	-
	2,3,5-Trimethyl-naphthalene	0.13±0.15 (+80.00±130.38)	0.25±0.17 (+162.50±94.65)	0.18±0.17 (+87.50±154.78)	0.48±0.23 (+180.00±309.66)	0.50±0.32 (+89.05±88.67)	0.50±0.27 (+160.00±167.33)	0.29±0.17 (+110.00±74.16)	0.36±0.15 (+80.00±83.67)	0.33±0.28 (+148.57±313.41)	0,902	0,938	0,823	-

Table S 2.2 (continued)

	Parameters	Ctrl			SX61			SX64			*p*-value			Interpretation
		BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	BG	RCW	RCW+SMS	Cover	Cultivar	Cover* Cultivar	
VR_{IS}	PCBs	23.87±22.33 (-7.54±44.31)	27.40±21.86 (+26.59±42.56)	33.19±22.54 (+24.56±53.65)	103.84±34.83 (+37.17±96.16)	89.78±24.35 (+19.20±17.61)	106.90±46.26 (+13.02±40.58)	56.14±22.32 (+61.09±154.17)	61.20±34.18 (+31.99±41.53)	57.64±24.17 (+22.21±46.58)	0,610	0,634	0,932	-
	C10-C50	593.60±643.07 (-26.86±50.20)	674.80±402.98 (+35.22±77.78)	897.80±803.55 (-12.63±40.32)	3742.00±1327.96 (+11.65±66.90)	3528.00±821.87 (+7.44±45.01)	3416.00±1614.75 (+19.44±83.22)	1670.60±583.58 (+39.76±134.35)	1831.80±838.99 (+1.65±28.73)	1812.60±955.62 (+34.98±129.64)	0,632	0,780	0,724	-
	Cadmium	1.80±0.37 (+7.48±30.71)	2.08±0.54 (+16.31±14.27)	2.06±0.58 (+16.54±15.47)	2.52±0.29 (+20.15±38.06)	2.58±0.24 (+17.90±14.04)	2.50±0.48 (+13.44±15.89)	2.10±0.32 (+102.67±209.82)	2.22±0.15 (+16.24±10.06)	2.24±0.35 (+21.22±6.50)	0,874	0,586	0,621	-
	Chromium	301.40±223.90 (-26.05±60.48)	405.00±171.29 (-2.49±15.23)	437.00±274.16 (-3.46±26.83)	953.40±126.96 (+5.43±41.67)	1000.20±254.59 (+9.42±39.96)	892.40±173.28 (+3.76±22.20)	607.80±117.29 (+58.77±168.62)	637.60±61.40 (-2.33±13.13)	645.00±261.99 (+9.89±19.14)	0,580	0,329	0,576	-
	Copper	607.20±476.43 (-25.82±70.65)	632.20±357.95 (-0.61±12.65)	1155.80±1169.01 (-4.03±27.09)	2504.00±855.27 (+29.21±72.97)	1870.00±570.75 (-12.54±18.27)	2428.00±943.57 (+3.82±24.74)	1195.40±487.39 (+59.31±170.39)	1159.80±545.57 (+0.63±18.94)	1240.20±697.54 (+11.66±11.65)	0,769	0,414	0,223	-
	Nickel	48.80±13.07 (-11.75±25.60)	55.20±9.26 (+15.63±11.12)	58.20±21.37 (+9.23±22.62)	99.60±15.53 (+39.85±37.22)	101.00±21.79 (+42.58±39.75)	96.00±17.39 (+35.66±31.22)	73.60±6.77 (+41.90±69.21)	76.40±6.07 (+30.05±18.77)	76.00±16.97 (+25.10±10.17)	0,367	0,061	0,571	-
	Zinc	190.00±91.67 (-26.56±36.54)	221.40±67.34 (-2.43±8.19)	253.60±125.41 (-0.68±19.86)	477.00±65.63 (+6.48±36.17)	509.80±110.96 (+9.88±36.54)	465.40±46.05 (+5.68±18.73)	322.80±70.32 (+39.52±123.10)	339.20±22.83 (+1.59±10.49)	348.00±123.60 (+9.47±13.83)	0,552	0,274	0,626	-
	Acenaphthene	0.13±0.15 (-19.50±73.92)	0.25±0.21 (+83.71±278.25)	0.14±0.15 (-43.54±40.49)	0.80±0.32 (-26.29±47.90)	0.72±0.36 (-37.04±23.04)	0.54±0.17 (-48.56±26.37)	0.32±0.08 (+0.64±115.42)	0.54±0.43 (-39.71±21.63)	0.44±0.21 (-18.00±53.31)	0,772	0,850	0,907	-
	Acenaphtylene	0.86±0.76 (-44.96±33.10)	0.90±0.64 (-19.72±52.15)	1.30±0.92 (-29.49±39.48)	5.88±3.12 (-22.97±42.19)	4.96±1.29 (-21.31±29.07)	5.10±2.53 (-32.10±30.99)	2.40±0.49 (-18.92±69.14)	2.74±0.90 (-41.37±13.24)	2.52±1.13 (-28.28±45.67)	0,861	0,725	0,915	-
	Anthracene	4.08±3.53 (-20.53±44.22)	6.84±4.22 (+3.07±42.26)	6.50±6.14 (-3.51±61.42)	30.40±16.70 (+3.40±58.28)	36.38±15.92 (+14.94±51.59)	27.14±8.30 (-2.41±46.72)	16.88±4.09 (+36.16±140.57)	19.04±5.54 (-19.90±19.53)	17.48±9.32 (-0.17±55.47)	0,882	0,755	0,897	-
	Benz[a]-anthracene	0.14±0.15 (-25.00±35.36)	0.27±0.15 (+185.42±348.43)	0.20±0.15 (-22.92±48.77)	0.86±0.43 (+11.00±64.85)	0.84±0.25 (+11.00±30.90)	0.86±0.40 (-4.70±38.17)	0.40±0.12 (+113.79±328.74)	0.54±0.19 (-7.14±29.46)	0.44±0.21 (-3.12±65.34)	0,305	0,932	0,600	-
	Benzo[a]-pyrene	0.08±0.07 (-21.67±33.12)	0.09±0.07 (+25.00±50.00)	0.12±0.08 (+12.50±62.92)	0.44±0.23 (+10.67±50.46)	0.40±0.10 (+35.00±41.83)	0.38±0.16 (-1.67±32.49)	0.20±0.07 (+35.71±151.02)	0.22±0.08 (-1.67±32.49)	0.19±0.09 (+5.00±57.01)	0,318	0,766	0,855	-
	Benzo[ghi]-perylene	0.11±0.11 (-15.00±22.36)	0.14±0.11 (+12.50±25.00)	0.16±0.15 (+12.50±62.92)	0.70±0.42 (+25.52±45.98)	0.70±0.25 (+22.30±18.28)	0.64±0.30 (+30.00±64.17)	0.34±0.09 (+96.00±229.85)	0.40±0.12 (+9.00±27.93)	0.33±0.17 (+16.00±47.75)	0,959	0,587	0,889	-
	Chrysene	0.09±0.07 (-38.67±37.31)	0.18±0.14 (+156.25±364.22)	0.12±0.08 (-35.42±41.04)	0.50±0.17 (-0.67±31.66)	0.40±0.10 (-3.33±24.01)	0.50±0.26 (-6.67±40.89)	0.22±0.04 (+68.00±242.92)	0.34±0.22 (-5.67±48.67)	0.23±0.11 (-23.43±47.75)	0,484	0,622	0,705	-
	Fluoranthene	0.16±0.15 (-21.11±74.58)	0.55±0.66 (+404.17±797.96)	0.17±0.14 (-35.42±41.04)	0.86±0.27 (+34.01±73.31)	0.72±0.19 (+13.97±52.92)	0.74±0.29 (+3.67±40.83)	0.32±0.13 (+148.89±420.86)	0.74±0.82 (+21.29±87.72)	0.40±0.20 (-15.76±50.67)	0,099	0,779	0,512	-
	Fluorene	0.20±0.18 (+80.00±130.38)	0.33±0.20 (+198.21±250.74)	0.28±0.25 (+70.83±82.07)	1.36±0.59 (+87.94±139.34)	1.28±0.32 (+84.17±80.39)	1.14±0.48 (+100.33±157.32)	0.62±0.16 (+149.33±214.68)	0.78±0.22 (+99.44±64.56)	0.70±0.30 (+204.41±308.02)	0,812	0,890	0,998	-
	Indeno[1,2,3]-cd]pyrene	0.08±0.07 (-25.00±35.36)	0.11±0.08 (+25.00±50.00)	0.13±0.12 (+37.50±110.87)	0.50±0.31 (+11.00±54.82)	0.50±0.14 (+45.33±31.41)	0.50±0.24 (+36.00±75.51)	0.24±0.09 (+44.44±146.78)	0.28±0.13 (+6.67±36.51)	0.25±0.13 (+31.00±95.16)	0,327	0,547	0,853	-
	Naphthalene	0.08±0.07 (-27.00±37.35)	0.14±0.11 (-22.50±26.30)	0.11±0.11 (-31.25±37.50)	0.36±0.09 (-10.67±46.57)	0.40±0.07 (-10.67±15.35)	0.32±0.08 (-25.00±34.36)	0.22±0.04 (+29.33±153.96)	0.26±0.05 (-22.67±20.87)	0.19±0.09 (+0.00±61.24)	0,886	0,491	0,955	-
	Phenanthrene	0.60±0.62 (+13.19±89.05)	1.26±1.13 (+79.21±225.50)	0.66±0.65 (+8.61±64.12)	2.74±1.02 (-7.41±27.51)	2.96±0.95 (-2.94±47.49)	2.04±1.02 (-25.84±37.82)	1.44±0.76 (+62.99±232.19)	1.60±0.71 (-25.16±42.02)	1.78±0.51 (+14.17±52.67)	0,994	0,707	0,784	-
	Pyrene	0.38±0.37 (-43.33±43.86)	1.14±1.16 (+356.55±729.93)	0.46±0.33 (-2.64±81.82)	2.08±0.54 (+16.11±50.42)	1.88±0.36 (+13.41±24.12)	2.02±0.74 (+7.09±40.20)	0.90±0.32 (+61.22±218.57)	1.74±1.73 (+17.89±83.95)	0.96±0.44 (-22.85±41.27)	0,154	0,581	0,573	-
	1-Methyl-naphthalene	0.08±0.07 (-28.33±38.91)	0.15±0.07 (-5.36±76.35)	0.11±0.11 (-37.50±43.30)	0.42±0.13 (-30.56±19.64)	0.44±0.09 (-22.06±32.55)	0.36±0.13 (-42.00±25.88)	0.26±0.09 (+71.67±240.92)	0.28±0.08 (-29.90±17.23)	0.27±0.13 (-21.11±42.15)	0,499	0,360	0,964	-
	2-Methyl-naphthalene	0.08±0.07 (-43.03±41.47)	0.11±0.05 (-43.06±36.96)	0.11±0.11 (-47.50±42.13)	0.38±0.11 (-60.03±22.96)	0.38±0.13 (-55.50±20.18)	0.36±0.09 (-54.01±24.57)	0.20±0.07 (-28.99±75.17)	0.22±0.04 (-65.88±11.55)	0.23±0.11 (-34.80±51.95)	0,940	0,938	0,892	-
	1,3-Dimethyl-naphthalene	0.13±0.15 (-17.78±72.37)	0.25±0.21 (+8.71±129.10)	0.18±0.19 (-31.25±32.19)	0.62±0.08 (-19.11±44.68)	0.60±0.12 (-29.66±35.29)	0.42±0.13 (-49.89±22.97)	0.32±0.13 (+25.92±156.33)	0.32±0.08 (-46.71±22.81)	0.36±0.18 (-26.10±25.36)	0,756	0,943	0,839	-
	2,3,5-Trimethyl-naphthalene	0.08±0.07 (-20.00±27.39)	0.13±0.15 (+17.50±188.35)	0.08±0.07 (-31.25±37.50)	0.36±0.15 (-12.50±45.07)	0.28±0.04 (-22.50±48.56)	0.22±0.08 (-47.56±28.90)	0.12±0.08 (+5.33±165.76)	0.13±0.07 (-60.83±20.33)	0.15±0.10 (-42.50±27.07)	0,553	0,510	0,751	-

The first value is the average (mean±SD, n=5) contaminant concentrations (mg kg^{-1}). Value in parentheses in the same cell represent the mean VR_x (mean±SD, n=5) (%). Significance levels (*p*-value) are shown and asterisks ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$) indicate a significant effect of Cover, Cultivar or Cover*Cultivar on VR_x values. Capital letters in the final column were used to identify significant differences between treatments according to Tukey's HSD test ($p \leq 0.05$).

Table S 2.3 Seasonal variation (SV) of soil PCBs, C10-C50, PAHs and TEs.

Treatments			PCBs	C10-C50	Cadmium	Chromium	Copper	Nickel	Zinc	Acenaphthene	Acenaphtylene	Anthracene	Benz[a]-anthracene	Benzo[a]-pyrene
Growing period (Gr)	Ctrl	BG	17.56±75.04	-20.84±40.20	46.98±81.86	-7.16±45.69	2.75±64.71	-5.03±20.14	-4.18±32.73	90.17±395.16	552.47±2199.01	582.12±2246.28	47.44±241.92	42.78±151.61
		RCW	40.08±70.25	6.13±57.00	47.06±69.05	28.81±67.70	37.24±106.95	26.29±71.85	35.74±98.25	217.37±673.00	246.44±881.15	463.02±1609.09	100.00±206.80	32.14±54.09
		RCW+SMS	18.31±48.83	-25.43±34.09	46.58±88.08	23.96±88.59	41.45±145.53	6.93±26.79	17.56±54.03	63.51±275.52	237.75±939.66	402.97±1496.44	7.74±95.94	17.86±46.44
	SX61	BG	34.60±58.12	-8.81±43.58	22.56±22.55	0.94±24.63	8.80±43.49	12.03±29.59	5.12±22.73	-2.33±53.70	-9.47±41.62	10.04±45.17	4.05±45.03	1.37±42.61
		RCW	26.55±29.69	-11.34±32.40	22.41±12.47	1.51±24.11	-5.00±15.29	12.92±31.02	2.68±21.51	-11.44±47.90	-15.70±24.96	7.75±32.20	-1.67±30.14	4.56±42.58
		RCW+SMS	35.20±56.89	-3.97±57.02	23.82±19.92	1.52±18.85	-1.21±19.55	10.38±27.87	3.70±16.66	-24.61±52.93	-9.99±43.85	10.64±42.64	-5.03±41.01	3.61±52.68
	SX64	BG	31.03±96.53	3.96±78.25	53.77±118.41	22.33±96.06	17.98±97.47	13.97±42.99	16.36±69.63	-9.83±67.27	-13.72±44.41	12.84±80.13	32.93±186.42	8.02±89.99
		RCW	29.46±27.87	-19.61±24.85	26.23±22.71	0.33±15.43	0.27±24.61	9.28±21.25	4.66±17.76	50.23±237.48	234.18±959.42	528.29±2039.56	34.30±134.01	-3.33±49.58
		RCW+SMS	25.50±31.96	-1.72±76.26	30.19±18.02	2.45±16.40	4.06±15.29	8.04±15.96	5.02±14.89	9.38±57.39	-9.25±39.94	6.65±35.45	-1.89±49.81	5.00±65.60
Dormant period (Dr)	Ctrl	BG	1.79±57.36	3.61±71.08	-23.12±38.19	50.82±121.92	64.98±164.19	0.77±34.50	19.53±67.74	4.17±72.57	1.71±66.61	-21.46±37.41	5.00±47.96	-3.33±52.00
		RCW	-17.85±32.55	-17.02±53.23	-30.54±22.55	-15.24±29.42	-22.60±26.52	-23.63±20.91	-19.63±25.35	12.54±119.00	-7.46±58.95	-18.55±47.55	-0.93±81.06	-22.22±26.35
		RCW+SMS	-14.54±20.75	6.60±57.18	-28.35±25.03	-7.85±28.99	-10.33±29.87	-16.68±12.32	-12.56±21.65	14.51±80.51	3.55±50.00	-9.97±39.98	28.70±111.89	-13.89±28.26
	SX61	BG	-11.23±40.50	13.22±50.53	-15.47±25.88	10.28±28.12	3.84±32.14	-13.06±12.52	-0.23±23.05	31.71±120.88	21.29±67.26	-1.43±52.74	21.83±95.71	17.50±55.34
		RCW	-13.98±27.70	16.35±50.43	-17.72±19.84	7.44±18.31	5.98±24.47	-14.78±6.01	3.71±16.62	25.20±79.61	35.38±96.80	13.02±70.47	13.29±52.95	29.17±81.77
		RCW+SMS	-23.21±34.42	-2.77±51.41	-21.54±20.28	-3.73±12.83	-3.24±14.08	-17.33±7.16	-7.72±11.13	30.60±71.13	13.58±70.71	-8.13±40.00	14.63±63.10	5.00±48.94
	SX64	BG	18.76±69.77	5.86±56.76	-26.19±30.58	8.12±59.22	10.76±60.26	-14.73±19.55	1.76±49.03	20.86±67.49	28.78±84.97	9.91±67.15	18.33±81.78	50.00±108.01
		RCW	-16.39±34.73	6.60±40.35	-21.51±22.51	-0.11±22.01	-1.55±19.41	-16.30±6.27	-6.38±16.81	8.42±47.25	21.53±59.13	1.35±37.15	9.52±34.95	45.00±76.19
		RCW+SMS	-3.64±61.50	13.80±75.49	-22.19±23.38	3.02±27.70	-3.47±27.27	-14.07±6.92	-3.00±19.74	12.97±93.43	34.26±121.44	5.68±76.21	61.00±203.73	31.67±101.97
p-value	Cover		0.571	0.819	0.421	0.831	0.814	0.681	0.951	0.057	0.137	0.068	***0.002***	0.287
	Cultivar		***0.024****	0.077	0.104	0.089	0.160	0.072	0.154	0.840	0.936	0.920	0.865	0.321
	Period		***<0.0001****	0.144	***<0.0001****	0.575	0.224	***0.024****	0.095	0.136	0.151	***0.008****	0.641	0.920
	Cover*Cultivar		0.165	0.689	0.586	0.721	0.938	0.767	0.517	0.778	0.884	0.966	0.846	0.675
	Cover*Period		0.255	0.934	0.966	0.346	0.515	0.207	0.358	0.724	0.806	0.808	0.412	0.920
	Cultivar*Period		0.725	0.976	0.168	0.852	0.677	0.994	0.687	0.460	0.416	0.407	0.838	0.176
	Cover*Cultivar*Period		0.601	0.315	0.871	***0.044****	0.122	***0.013****	***0.025****	0.607	0.951	0.884	0.589	0.909
Interpretation			**Ctrl^{B}**	-	-	-	-	Gr::Ctrl **BG^{B}**	-	-	-	-	**BG^{B}**	-
			SX61^{AB}	-	-	-	-	**RCW^{A}**	-	-	-	-	**RCW^{A}**	-
			SX64^{A}	-	-	-	-	**RCW+SMS^{AB}**	-	-	-	-	**RCW+SMS^{B}**	-
			Gr > Dr	-	**Gr > Dr**	-	-	**Gr > Dr**	-	-	-	**Gr > Dr**	-	-

Table S 2.3 (continued)

Treatments			Benzo[ghi]-perylene	Chrysene	Fluoranthene	Fluorene	Indeno[1,2,3-cd]-pyrene	Naphthalene	Phenanthrene	Pyrene	1-Methyl-naphthalene	2-Methyl-naphthalene	1,3-Dimethyl-naphthalene	2,3,5-Trimethyl-naphthalene
Growing period (Gr)	Ctrl	BG	41.67±185.57	92.39±247.43	100.19±293.32	141.32±495.34	45.00±140.85	25.44±190.28	266.32±1007.36	210.11±750.23	28.33±191.40	28.43±191.47	86.74±344.78	25.00±137.26
		RCW	67.86±111.99	136.31±227.72	260.12±519.33	169.90±468.70	35.71±49.72	32.26±143.34	689.22±2424.26	275.14±650.18	41.92±149.79	56.75±177.33	196.89±669.04	80.00±310.00
		RCW+SMS	25.00±89.34	42.86±160.92	47.13±216.01	84.10±250.45	28.57±80.18	28.57±197.01	219.93±729.20	96.62±359.73	21.73±149.75	42.68±172.01	80.12±300.19	78.57±307.89
	SX61	BG	13.40±37.15	20.06±43.56	43.80±74.59	25.60±100.73	2.33±33.32	-5.87±49.87	42.61±94.61	24.84±50.15	-3.60±60.83	0.66±75.24	19.66±79.22	24.72±83.46
		RCW	6.79±23.46	17.33±37.80	13.80±54.08	23.22±71.52	18.22±35.52	-14.97±26.78	26.73±106.34	15.41±31.00	-9.53±37.08	-9.05±44.54	6.40±86.39	25.72±163.51
		RCW+SMS	17.18±52.78	0.17±39.60	1.75±48.42	19.70±110.95	24.21±70.09	-16.63±40.32	13.01±93.42	5.23±41.78	-20.29±38.47	-0.06±86.33	-9.35±66.81	4.18±153.05
	SX64	BG	36.22±133.09	41.56±140.34	60.96±239.55	37.71±145.50	17.59±87.89	-2.44±87.40	24.82±133.69	20.64±123.70	15.78±138.34	-11.44±60.24	14.66±104.99	5.33±102.32
		RCW	29.44±80.96	59.78±136.71	66.32±138.49	96.94±287.05	41.67±111.40	1.25±86.64	184.57±697.84	143.17±435.00	9.03±87.98	17.96±146.62	25.25±138.93	-9.17±51.57
		RCW+SMS	4.22±33.77	15.52±65.87	9.63±43.94	61.65±198.80	12.00±54.77	2.78±47.52	49.85±108.16	1.28±38.21	-6.48±35.52	9.51±74.57	34.82±113.74	15.83±97.08
Dormant period (Dr)	Ctrl	BG	-10.00±21.08	-5.67±75.63	25.92±175.50	-33.75±31.21	-5.83±50.93	1.67±49.35	-13.93±53.78	-6.33±71.91	12.50±90.71	45.83±166.35	15.12±119.25	31.67±103.77
		RCW	-17.59±52.45	-30.56±55.28	-30.42±40.94	-18.24±51.37	-16.67±25.00	20.37±106.65	7.53±91.99	-19.74±42.69	36.11±116.07	40.74±131.33	13.05±85.71	92.59±137.97
		RCW+SMS	-12.96±20.03	15.37±113.66	44.21±111.20	-21.14±35.62	-9.26±47.22	-3.70±42.92	1.26±102.21	50.39±144.06	18.33±109.66	21.30±110.48	15.19±77.05	57.41±146.75
	SX61	BG	-7.03±41.41	8.65±76.65	6.21±111.37	-7.16±41.02	13.37±68.71	7.06±61.05	5.49±124.14	6.76±95.97	40.61±132.28	82.28±193.18	39.63±174.37	72.33±236.07
		RCW	5.71±43.37	-11.08±35.96	-1.73±45.03	-11.27±37.00	2.67±60.35	22.83±53.98	7.45±72.06	-10.11±35.35	50.83±105.30	79.04±166.43	32.56±95.22	31.19±88.35
		RCW+SMS	-11.05±32.58	-2.24±28.35	3.13±43.90	-6.47±41.40	-10.83±38.48	14.67±50.67	13.85±76.20	-0.96±38.15	62.67±145.55	60.35±176.25	38.70±112.60	73.25±148.85
	SX64	BG	-2.50±52.42	3.21±64.43	11.94±83.84	14.06±79.05	16.67±76.98	30.00±69.30	12.57±55.10	17.67±75.37	26.00±73.41	56.67±149.99	14.83±56.73	60.00±90.68
		RCW	-1.50±20.07	-11.17±33.19	-14.95±20.66	-15.32±20.99	0.00±35.36	34.17±66.72	5.41±74.33	-12.22±27.93	31.67±95.65	57.83±134.10	16.96±56.88	40.00±80.97
		RCW+SMS	-1.67±38.05	9.50±88.81	24.37±92.88	-14.30±37.93	13.33±62.76	-6.67±43.18	0.94±87.90	25.41±99.90	36.67±120.20	63.33±246.27	5.11±93.11	92.62±239.59
p-value		Cover	***0.028****	***0.042****	0.114	0.108	0.223	***0.021****	***0.015****	0.190	***0.006*****	0.154	***0.005*****	0.405
		Cultivar	0.924	0.602	0.891	0.806	0.732	0.589	0.854	0.847	0.384	0.567	0.770	0.682
		Period	***0.007*****	***0.009*****	***0.024****	0.107	***0.016****	***0.028****	***0.012****	***0.006*****	***0.025****	0.147	0.850	***0.015****
		Cover*Cultivar	0.890	0.698	0.736	0.812	0.835	0.919	0.842	0.737	0.755	0.981	0.825	0.402
		Cover*Period	0.635	0.491	0.090	0.731	0.606	0.811	0.998	0.141	0.853	0.900	0.912	0.941
		Cultivar*Period	0.726	0.253	0.828	0.559	0.569	0.890	0.655	0.775	0.803	0.619	0.825	0.817
		Cover*Cultivar*Period	0.214	0.855	0.244	0.889	0.936	0.549	0.562	0.643	0.970	0.930	0.560	0.549
Interpretation			**BG^{B}**	**BG^{AB}**	-	-	-	**BG^{B}**	**BG^{B}**	-	**BG^{B}**	-	**BG^{B}**	-
			RCW^{A}	**RCW^{A}**	-	-	-	**RCW^{A}**	**RCW^{A}**	-	**RCW^{A}**	-	**RCW^{A}**	-
			$RCW+SMS^{AB}$	**$RCW+SMS^{B}$**	-	-	-	**$RCW+SMS^{B}$**	**$RCW+SMS^{B}$**	-	**$RCW+SMS^{B}$**	-	**$RCW+SMS^{B}$**	-
			Gr > Dr	**Gr > Dr**	**Gr > Dr**	-	**Gr > Dr**	**Gr < Dr**	**Gr > Dr**	**Gr > Dr**	**Gr < Dr**	-	-	**Gr < Dr**

Values are the mean SV_p (mean±SD) (%) for each treatment, observed either after a Growing period (n=15 for each treatment) or after a Dormant period (n=10 for each treatment). Significance levels (*p*-value) are shown and asterisks ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$) indicate a significant effect of Cover, Cultivar, Period, Cover*Cultivar, Cover*Period, Cultivar*Period and Cover*Cultivar*Period on SV. Capital letters and symbols of comparison (> or <) in the final row were used to identify significant differences between treatments according to Tukey's HSD test ($p \leq 0.05$).

Chapitre 3 | Willows Used for Phytoremediation Increased Organic Contaminant Concentrations in Soil Surface

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3.1 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

3.2 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 (Guidi Nissim et al., 2013), often intended for bioenergy and biofuel processes (Ul Hai et al., 2019). Willows also show strong tolerance to several contaminants, such as nitrogen rich wastewater (Guidi Nissim et al., 2014), trace elements (TEs) (Desjardins et al., 2018), various petroleum hydrocarbons compounds (Grenier et al., 2015; Guidi et al., 2012), as well as pesticides (Pascal-Lorber et Laurent, 2011), making them effective riparian buffer strips in agricultural systems (Hénault-Ethier et al., 2017). More recently, their utilization has been extended to treatment wetlands (Lévesque et al., 2017) as well as vegetation filters designed to treat landfill leachate (Lachapelle-T. et al., 2019). These plants can additionally be used in phytoremediation to extract or degrade contaminants (Desjardins et al., 2018; Košnář et al., 2020), or, as evapotranspiration (ET) covers, to contain them in the soil (Mirck et Volk, 2010; Zalesny et al., 2019). 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 (Weir et Doty, 2016). However, successful soil decontamination by phytoremediation is often challenging to demonstrate clearly, especially in field studies (Gerhardt et al., 2009), characterized by many sources of variation that can influence the concentration and distribution of contaminants in soil (Kardanpour et al., 2015).

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 (Fortin Faubert et al., 2021b; Guidi et al., 2012), contrary to initial hypotheses and objectives. The main findings concerning the establishment (first year) of this plantation can be found in Guidi et al. (2012). 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. (2021b)), 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 (Guidi et al., 2012).

3.3 Materials and Methods

3.3.1 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) (MELCC, 2010). 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.

3.3.2 Previous Studies on the Site and Present Experimental Layout

3.3.2.1 Soil Characterization (2010)

Soil characterizations were carried out on the experimental part of the site in early 2010. Table 3.1 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. (2012), 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).

Table 3.1 Soil characteristics of the site in 2010.

Parameters	Units	Values	Parameters	Units	Values
Cation-exchange capacity	meq $100g^{-1}$	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 (CEAEQ, 2004, 2008, 2014b, 2014a, 2016). Five soil samples were collected at 0–30 cm below ground in each plot (P1, P2, P3 and P4, see Figure 3.1A). Values are the averages (mean ± SD, n = 20). Table was adapted from Guidi et al. (2012).

3.3.2.2 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. (2012). 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 3.1A). 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. (2012), is not shown in Figure 3.1A.

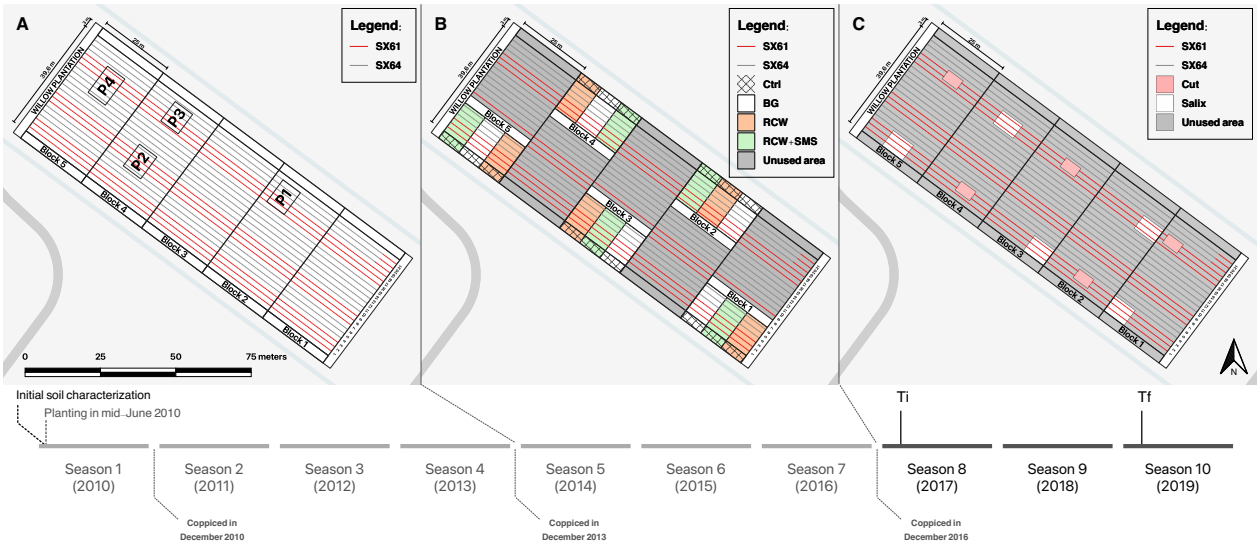

Figure 3.1 Evolution of the experimental design over time.(A) The first experimental phase (phase 1) on what is referred to as the GERLED site in Guidi et al. (2012). The 21 dotted lines inside the willow plantation refer to the rows planted with the cultivar 'SX61' (red lines) and with the cultivar 'SX64' (grey lines). P1 to P4 refer to the sampling plots in their study (Guidi et al., 2012); (B) the second experimental phase (phase 2) studied by Fortin Faubert et al. (2021b). 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. (2012).

3.3.2.3 Phase 2 (2014–2016)

The second experimental phase (Figure 3.1B) 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. (Fortin Faubert et al., 2021b). 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.

3.3.2.4 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 3.1C). 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. (2021b)).

3.3.3 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. (2021b).

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) and immediately sent to an external laboratory for chemical analysis (AGAT Laboratories Ltd., Montreal, Quebec) 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 (CEAEQ, 2004, 2008, 2014b, 2014a, 2016). 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 (CEAEQ, 2018).

3.3.4 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.

3.4 Results

3.4.1 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 3.2). 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 3.2). 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).

Table 3.2 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	0.666	0.258	0.936	0.177
Cr	991.00±134.28	962.60±110.21	1018.87±190.37	1074.6±139.24	0.709	0.640	0.816	0.235
Cu	2714.00±916.01	2552.00±668.93	2484.13±704.18	2688.60±834.20	0.587	0.676	0.531	0.745
Ni	89.80±6.65	85.00±9.57	95.93±23.16	75.20±25.80	0.439	0.392	0.653	0.574
Zn	503.00±75.41	481.20±37.57	526.27±61.21	579.60±86.20	0.650	0.341	0.676	0.166
PCBs	103.50±31.96	97.36±25.93	89.84±21.22	94.93±19.20	0.793	0.704	0.469	0.877
C10-C50	4000.00±1364.35	3640.00±1670.75 ^{B}	6231.33±2422.89	8191.33±1818.87 ^{A}	0.773	0.295	0.229	**0.026***
Acenaphthene	0.66±0.15	0.68±0.33 ^{B}	0.52±0.19 <	0.84±0.29 ^{A}	0.898	**0.025***	0.263	**0.053**
Acenaphthylene	4.00±1.74	3.72±2.18 ^{B}	3.58±1.32 <	6.75±2.89 ^{A}	0.813	**0.041***	0.134	**0.002****
Anthracene	26.34±8.88 ^{b}	20.36±13.17 ^{B}	33.45±9.38 ^{a}	35.55±14.23 ^{A}	0.310	0.872	**0.059**	**0.091**
Benzo[a]anthracene	0.56±0.19	0.46±0.29 ^{B}	0.49±0.17	0.66±0.24 ^{A}	0.519	0.179	0.236	**0.039***
Benzo[a]pyrene	0.28±0.13 ^{a}	0.31±0.24	0.19±0.10 ^{b} <	0.39±0.21	0.818	**<0.100**	**0.052**	0.116
Benzo[b]fluoranthene	0.30±0.16	0.32±0.23 ^{B}	0.25±0.13	0.43±0.24 ^{A}	0.887	0.236	0.130	**0.010****
Benzo[ghi]perylene	0.46±0.19 ^{a}	0.38±0.26 ^{B}	0.35±0.08 ^{b} <	0.61±0.25 ^{A}	0.597	**0.052**	**0.061**	**0.012***
Chrysene	0.36±0.11 ^{a}	0.38±0.26 ^{B}	0.28±0.10 ^{b} <	0.47±0.21 ^{A}	0.861	**0.050***	**0.073**	**0.020***
Fluoranthene	0.58±0.15	0.68±0.29	0.64±0.20	0.77±0.25	0.326	0.335	0.701	0.506
Fluorene	1.06±0.32	0.98±0.52 ^{B}	1.05±0.30 <	1.74±0.59 ^{A}	0.749	**0.056**	0.910	**<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	0.760	**0.058**	**0.051**	0.103
Naphthalene	0.34±0.09	0.32±0.16	0.32±0.07	0.41±0.10	0.778	0.155	0.665	0.118
Phenanthrene	2.22±0.75 >	1.88±0.65	2.45±1.02	2.91±1.19	**0.067**	0.288	0.665	0.116
Pyrene	1.68±0.54	1.86±0.92	1.93±0.56	2.31±0.90	0.691	0.582	0.386	0.358
1-methylnaphthalene	0.38±0.08	0.36±0.11 ^{B}	0.43±0.12 <	0.56±0.20 ^{A}	0.749	**0.044***	0.573	**0.032***
2-methylnaphthalene	0.30±0.07	0.30±0.12 ^{B}	0.43±0.10 <	0.59±0.15 ^{A}	1.000	**0.024***	0.115	**<0.001*****
1,3-dimetylnaphthalene	0.50±0.10	0.46±0.15 ^{B}	0.67±0.29 <	0.87±0.41 ^{A}	0.542	**0.094**	0.386	**0.041***
2,3,5-trimetylnaphthalene	0.22±0.04	0.20±0.07 ^{B}	0.31±0.15	0.39±0.15 ^{A}	0.374	0.129	0.310	**0.009****

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.

3.4.2 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 3.2), 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.

3.5 Discussion

3.5.1 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 3.2). 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 (Gerhardt et al., 2009). 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 (Gerhardt et al., 2009). 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 (Gerhardt et al., 2009). Following this recommendation, the *p*-values between 5% and 10% are presented in Table 3.2. 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 (Fortin Faubert et al., 2021b).

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. (2013) 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.

3.5.2 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 (Bengough, 2012; Chapman et al., 2012). Rhizospheric accumulations of these chemicals can then be expected, especially if the quantity transferred by water mass flow surpasses plant requirements (Hinsinger, 1998). Soil scientists generally describe this phenomenon in a macronutrient acquisition context, but it could also concern micronutrients (i.e., Cu, Mn, Zn and Co) (Linehan et al., 1989) and some TEs, as observed for Pb in Klassen et al. (2010). 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 (Hinsinger, 1998; Klassen et al., 2010). 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 (Desjardins et al., 2018) 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 (Liste et Alexander, 2000), which generally sorb to soil particles, decreasing their transport rate and increasing the time required for their remediation (Abdel-Shafy et Mansour, 2016). The release of organic acids with root exudates can increase their solubility and therefore their mobility (Rohrbacher et St-Arnaud, 2016). Colloids, as mobile bacteria, may also enhance the transport of PAHs in the subsurface of soil (Jenkins et Lion, 1993). Once in the rhizosphere, relatively hydrophobic compounds, such as high molecular weight (HMW) PAHs, can then adsorb and bind strongly to the roots (Parrish et al., 2005). Such adsorption to the roots can apparently increase with lipid content (Schwab et al., 1998), as well as with plant age, due to a greater total root mass (Schwab et al., 1998). Although it is generally accepted that low molecular weight (LMW) PAHs are potentially more soluble and therefore more mobile than heavier compounds (Iqbal et al., 2008), 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 (Liste et Alexander, 2000)), 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 (Frédette et al., 2019b). 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 (Ferro et al., 2003, 2013). 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 (Ferro et al., 2003). 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 (Frédette et al., 2019a, 2019b). 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.

3.5.3 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 (Mirck et Volk, 2010), 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. (2014) 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 (Tarroux et al., 2010). When tree root systems spread laterally and intermingle, connections between mature individuals may occur by grafting (Püttsepp, 2004). Natural root grafting is a fairly common phenomenon occurring among many trees including willows (Dallimore, 1917) and which has been observed under SRIC conditions in a past study conducted at a location near the study site (Fontana et al., 2020). 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 (Courchesne et al., 2017a; Desjardins et al., 2016, 2018; Košnář et al., 2020; Tőzsér et al., 2017; Vandecasteele et al., 2005). 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 (2002), 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 (Vervaeke et al., 2004). 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).

3.5.4 Results Interpretation and Implications for Field Trials

The challenges associated with monitoring changes in soil contamination over time are not new (Gerhardt et al., 2009). 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 (Gerhardt et al., 2009). 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 (Phytoremediation Action Team, 1999), to collect samples as close as possible to the same sampling point over time, and to be flexible when establishing statistical significance thresholds (Gerhardt et al., 2009).

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 (Mlinarić et al., 2017). Nevertheless, rather than remain unpublished, studies with such negative results should be available to the scientific community to help interpret other types of findings (Sharma et Verma, 2019).

Apparent increases of concentrations over time do not exclude the possibility that active degradation (Gerhardt et al., 2009) and extraction are occurring in the rhizosphere (Klassen et al., 2010). 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 (2000) 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 (Fortin Faubert et al., 2021b).

Finally, this experiment could be considered a case of pollutant containment, which has been proven effective elsewhere (Barac et al., 2009). 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.

3.6 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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Chapitre 4 | Roots and Rhizosphere Microbial Community of Willows growing under SRIC for six years in a mixed-contaminated soil from Quebec, Canada

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4.1 Abstract

There is a growing interest for the plant microbiomes engineering to optimize functions of interest in the plants used for soil phytoremediation. This study aimed at examining the microbial communities inhabiting the roots and rhizosphere of two *Salix miyabeana* cultivars that grown in short rotation intensive culture (SRIC) system for six years in a mixed-contaminated soil. DNA was extracted from roots and rhizospheric soil, and fungal internal transcribed spacer (ITS), as well as bacterial and archaeal 16S regions were amplified and sequenced with Illumina MiSeq. Cultivars 'SX61' and 'SX64' were found to harboured similar diversity of fungal, bacterial and archaeal amplicon sequence variants (ASVs). In general, greater microbial diversity was found in the rhizosphere than in the roots of both cultivars, but cultivar 'SX64' showed similar fungal diversity in both compartments. Microbial community structures were all found to be cultivars- and compartments-specific. The assemblages of fungi in root compartment of both cultivars were highly dominated by Basidiomycota (Agaricomycetes) and by Ascomycota (Pezizomycetes, Dothideomycetes, Sordariomycetes and Leotiomycetes). Only two fungal ASVs were found to be differentially abundant between both cultivars in this compartment. No sequences of arbuscular mycorrhizal fungi (AMF) were recorded in all our samples. Among the potentially ectomycorrhizal fungi (EMF), *Tuber borchii* appeared to be one of the most important partners of both cultivars. *Tomentella ellisii* and *Hymenogaster griseus* appeared to be other very important partner of 'SX61' and 'SX64', respectively. *Geopora* spp., *Hebeloma* spp. and *Serendipita* spp. were other potentially EMF that could be important, even if less abundant in both cultivars. Some ASVs among Dothideomycetes (*Leptosphaeria* Spp.), Leotiomycetes (*Cadophora luteo-olivacea*, *Cadophora orchidicola*) and Sordariomycetes (*Dactylonectria* Spp., *Ilyonectria* Spp., *Myrothecium* Spp.), were more abundant in the roots than in the rhizospheric soil samples, suggesting that both cultivars harbour nonmycorrhizal endophytic fungi. No archaeal sequence was obtained from all root samples of both cultivars. However, a high dominance of Thaumarchaeota and the negligible presence of Euryarchaeota, suggest that archaeal communities in the rhizosphere of both cultivars are involved in nutrient cycling, mostly as ammonia oxidizers and to a lesser extent as methanogens. Although the implication of some identified taxa for plant adaptability and biomass production capacity remains to be explored, this study provides valuable and useful information about microbes that could potentially favour the implantation and phytoremediation efficiency of *Salix miyabeana* in mixed contamination sites, in similar climatic environments.

Keywords: Willow, Phytoremediation, Short rotation intensive culture (SRIC), Soil contaminants, Rhizosphere, Root, Fungi, Bacteria, Archaea, Microbiome, Amplicon sequencing.

4.2 Introduction

The microbiome or phytomicrobiome referred to the consortium of microorganisms (i.e., fungi, bacteria and archaea) that collectively colonized most part of the plant, including the phyllosphere, rhizosphere, and endosphere (Quiza et al., 2015). The associated microbes are so important for plant health and growth, that plant and all of its microscopic partners are no longer seen as 'individual', but rather regarded as a metaorganism or holobiont (Thijs et al., 2016; Vandenkoornhuyse et al., 2015).

Interactions between plants and microorganisms are known to be very ancient, since association with arbuscular mycorrhizal fungi (AMF) is believed to have played a key role in the plant terrestrialization process, over about 460 million years ago (Redecker et al., 2000). Other associations between plant and fungi appeared later in the evolution and there would now be several other categories of mycorrhizal symbioses (i.e., arbuscular, arbutoid, ectendo, ecto, ericoid, monotropoid, orchid, and sebacinoid) (Finlay, 2008; Fortin et al., 2016). AMF and ectomycorrhizal fungi (EMF) have been extensively studied, because of their respective ubiquity and great diversity. Nowadays, it is estimated that AMF are still present in more than 72% of vascular land plant species and encompass specific group of primitive and little diversified fungi belonging to the subphylum Glomeromycotina (Brundrett et Tedersoo, 2018; Finlay, 2008; Fortin et al., 2016; Spatafora et al., 2016). While EMF would associate only with a small fraction of the total number of terrestrial plants, their disproportionate occupancy of the terrestrial land greatly increased the global importance of this type of mycorrhizas (Finlay, 2008; Smith et Read, 2008). AMF form highly branched hyphal structures (arbuscules) within cortical root cells (without penetrating the plasmalemma), while EMF envelop the root tip with sheath or mantle, and form a network of intercellular hyphae (Hartig net) between the cortical and epidermal cells (Finlay, 2008). The arbuscule and Hartig net are effective interfaces to give fungi a direct access to the plant's carbohydrates. Both AMF and EMF have extraradical mycelium with a great capacity to extend beyond the root zone, which greatly benefits the growth and health of plant, allowing it to absorb a greater amount of water and nutrients, which would not be accessible otherwise (Smith et Read, 2008). Association with mycorrhizal fungi is also known to provide plant protection against desiccation and pathogens that would enter through the roots. In many plant species, the inner parts of the roots are also frequently colonized by nonmycorrhizal ascomycetes, which collectively referred to dark septate endophytes (DSE) (Smith et Read, 2008). Even if their presence in plant

roots has been known for over a century, their function and taxonomic affinities, are still elusive (Knapp et al., 2018). Studies in experimental systems have suggested that DSE could be commensalistic or mutualistic symbionts, or even latent saprotrophs or pathogens (Knapp et al., 2018). Their prevalence in harsh conditions, as drought, nutrient-limited environments, or contaminated lands, suggests that DSE might be critical for plants health in unfavorable ecosystems (Barberis et al., 2021). Plant growth-promoting rhizobacteria (PGPR) are other beneficial microorganisms, which can be found in the rhizosphere, the rhizoplane as well as in the interior part of the roots. PGPR can directly enhance plant growth and health, in addition to offering protection to plants against phytopathogens by saturating the niche or by the induction of systemic resistance (Prakash, 2021). Induced systemic resistance (ISR) would also enhances plant resistance against trace elements (TEs) and organic pollutants (Prakash, 2021). Archaea are now considered as other important components of the plant microbiome (Akinola et Babalola, 2021; Buée et al., 2009; Taffner et al., 2018). Although less is known about their relations with plants, it is assume that they would interact positively due to their ubiquitous occurrence within the microbiome of healthy plants (Taffner et al., 2018).

In the last few years, there has been increasing interest for plant–microbiome manipulations in order to push beneficial interactions between plants and microbes toward enhanced specific outcomes, leading to a more sustainable agriculture (Pozo et al., 2021), or even to increased phytoremediation efficiency (Dessaux et al., 2016; Thijs et al., 2016). Very inconsistent results can be found in the scientific literature, revealing that manipulating the plant microbiome toward a certain type of community turned out to be very challenging (Quiza et al., 2015). Interactions between plants and microorganisms are very complex and not well understood. Microbial community often vary between plant species/cultivars, and also depend on many environmental factors, such as climatic conditions, edaphic properties and biological interactions (Quiza et al., 2015). Facing this complexity, it is crucial to improve our knowledge concerning the natural relationships between all partners belonging to an holobiont established in a particular environmental condition, before embarking on such microbiome engineering approaches.

Microbiome characterization is then a critical preliminary step to get there, and new sequencing technologies recently allow researchers to gain a new perspective on the microbial diversity associated with plants. Fast growing willow shrubs (*Salix* spp.) present attractive

economic values as woody crops for biomass production (Guidi Nissim et al., 2013; Padoan et al., 2020), and shown interesting versatility to be used in various environmental projects, to minimize leaching of pesticides from agricultural field (Hénault-Ethier et al., 2017; Lafleur et al., 2016), to treat contaminated leachate (Lachapelle-T. et al., 2019; Lévesque et al., 2017) or to remediate contaminated soil (Fortin Faubert et al., 2021b, 2021a; Kuzovkina et Quigley, 2005). Consequently, the microbial community of *Salix* spp. have been extensively studied in many different environmental conditions, as floodplain (Hashimoto et Higuchi, 2003), arable sites (Hrynkiewicz et al., 2012) and contaminated land (Dagher et al., 2019, 2020a). However, most studies concerning microbiome characterization of *Salix* spp. conducted in contaminated conditions, were mostly performed on relatively young hosts (rarely more than two years old), and less is known about the natural microbiome of willows established for several years on contaminated soil. The aim of this study was to determine the microbial communities found in the roots and the rhizosphere of mature willows growing under contaminated conditions. This study was then conducted inside the boundaries of a six-year-old willow plantation, containing two cultivars of *Salix miyabeana* ('SX61' and 'SX64'), that have been established and cultivated under SRIC system on a former industrial site in southern Quebec, Canada. Fungal, bacterial and archaeal communities inhabiting the roots and the rhizosphere were described using an Illumina MiSeq sequencing system. The relevant information could provide valuable and useful clues to improve some microbiome engineering approaches favoring the establishment, survival, growth, fitness, as well as remediation performances of *Salix* spp. on contaminated sites.

4.3 Materials and methods

4.3.1 Experimental site

This study was carried out in the municipality of Varennes (QC, Canada, 45°42'02.8" N, 73°25'53.4" W), located on the south shore of the St. Lawrence River, across from the Island of Montreal. The region has a temperate climate characterized by annual average temperature of 6.6 ^{o}C and annual average precipitation of 981 mm (MELCC, 2010). Less than 300 m separate the centroid of the experimental site from the St. Lawrence River. This site is a flat area of 5840 m^2, that rises approximately 3.5 m above river water level. Between 1972 and 1979, this site was used for the purpose of land farming practices to treat settling sludge, derived from liquid discharge of former petrochemical factory (PÉTROMONT INC.) that operated for many years before being shut down in 2008 (Turcotte, 2009).

In 2010, agronomic properties and contaminant concentrations were characterized in the soil (Table 4.1). Based on the provincial Land Protection and Rehabilitation Regulation, RLRQ, c. Q-2, r. 37, Sch. I, PCBs, Cu, Cr and anthracene were considered to be the most problematic contaminants on the site. As described in Guidi et al., (2012), the sector was mainly contaminated within the first 60 cm of soil.

Table 4.1 Soil characteristics of the site in 2010.

Parameters	Units	Values	Parameters	Units	Values
Cation-exchange capacity	meq $100g^{-1}$	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

[a]Water extraction. [b]Melich III method. [c]Chemical analysis was performed by AGAT Laboratories Ltd. (Montreal, QC, Canada) following the recommended provincial methods for environmental analyses (CEAEQ, 2004, 2008, 2014b, 2014a, 2016). Five (5) soil samples were collected at 0–30 cm below ground in each plot (P1, P2, P3 and P4, see Figure 4.1A). Values are averages (mean±SD, n = 20). The table was adapted from Guidi et al., (2012).

In mid-June 2010, two *Salix miyabeana* cultivars ('SX61' and 'SX64'), were successfully established on part of the site (5475 m^2) following a SRIC technique for remediation purposes (Guidi et al., 2012). The planting was carried out mechanically and plants were spaced 1.8 x 0.3 m, at a density of 18,500 plants per hectare (Guidi et al., 2012; Labrecque et Teodorescu, 2006). The plantation included seven randomly distributed groups of three rows, for a total of 21 rows (Figure 4.1A). A first cut was performed at the end of the first growing season (December 2010) and a second one at the end of the fourth growing season (December 2013).

4.3.2 Sample collection

In late August 2016, three individual plants of both cultivar ('SX61' and 'SX64') were collected in each of the five blocks (n=15 for each cultivar) (Figure 4.1B). In the field, root systems were dug up and vigorously stirred to remove excess soil, before being placed in 50 ml polypropylene tubes (Sarstedt Inc, Newton, NC, USA). In the laboratory, soil still attached to the roots was collected in 1.5 ml Eppendorf tubes (Eppendorf Canada Ltd., Mississauga, ON, CAN). This soil was considered as part of the rhizosphere and refer to the rhizospheric soil samples. Roots samples (n=15 for each cultivar) and rhizospheric soil samples (n=15 for each cultivar) were frozen and stored at -80°C before DNA extraction processing. Root samples from each cultivar will referred to Roots.SX61 and Roots.SX64, while the rhizospheric soil samples will referred to Rhizo.SX61 and Rhizo.SX64.

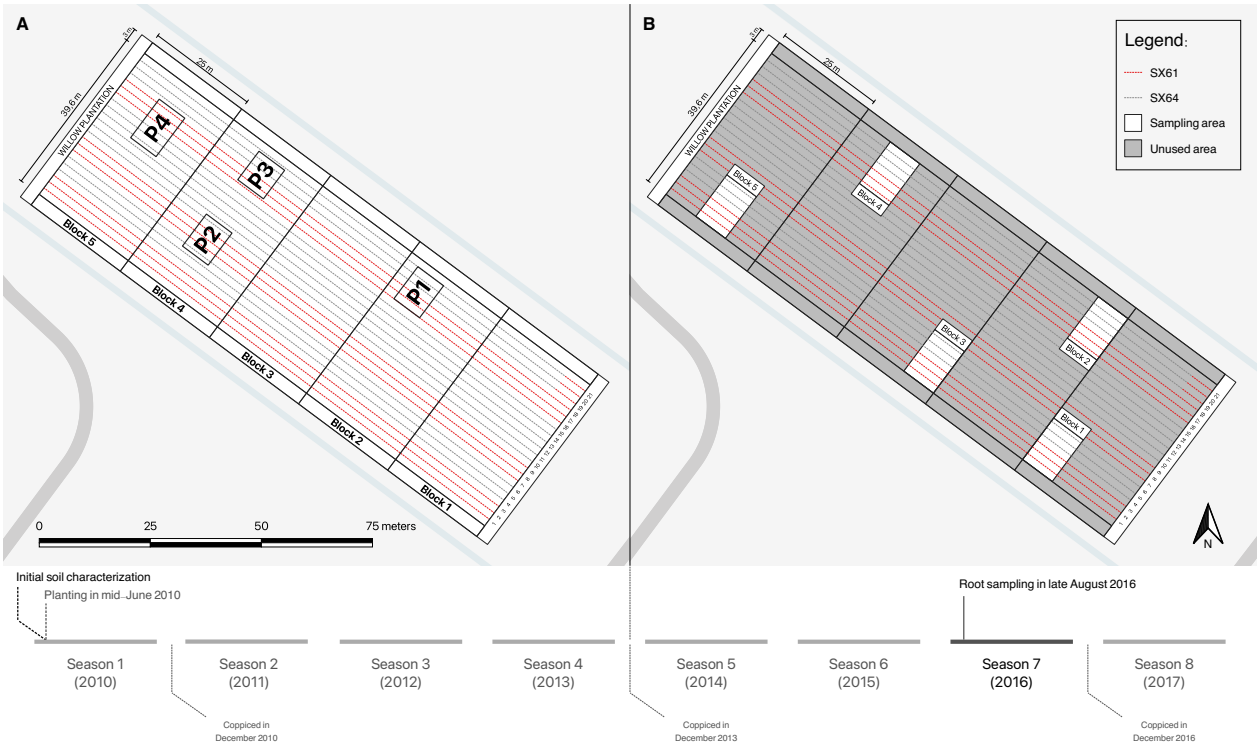


Figure 4.1 Evolution of the experimental design over time, including growth seasons and coppicing times. The 21 dotted lines inside the willow plantation refer to the rows planted with the cultivar 'SX61' (red lines) and with the cultivar 'SX64' (grey lines). (A) Experimental design of the first experimental phase, referred to as the GERLED site in Guidi et al., (2012). P1, P2, P3 and P4 were the sampling plots in their study; (B) Experimental design of the current experiment. White plots refer to the sampling areas. Although preserved as part of the plantation, the sections in dark grey were not used in the present study (Unused area). Adapted from Guidi et al., (2012).

4.3.3 DNA Extractions

Total rhizosphere genomic DNA was extracted from 0.3 g of soil (wet weight) using the MO BIO's PowerSoil DNA Isolation Kit (QIAGEN, Toronto, ON, CAN), following the manufacturer's instructions. The roots have been cleaned with tap water to remove soil particles and then crushed in liquid nitrogen using pestle and mortar. Roots genomic DNA was extracted from 0.1 g of wet material using the DNeasy Plant Mini Kit (QIAGEN, Toronto, ON, CAN), following the manufacturer's instructions. To ensure good quality of the extraction, DNA concentrations were measured by using a NanoDrop 2000 UV-visible spectrophotometer (Thermo Scientific, Wilmington, DE, USA). All extracts were stored at -21°C before PCR processing.

4.3.4 PCR amplifications and sequencing

The first step of PCR amplifications was carried out with the overhang adapter sequences CS1 (5'-ACACTGACGACATGGTTCTACA-3') and CS2 (5'-TACGGTAGCAGAGACTTGGTCT-3') attached to the fungal, bacterial and archaeal gene specific set of primers. The forward primers were designed as (5'-CS1-Gene specific primer-3') and the reverse primers as (5'-CS2-Gene specific primer-3'). The fungi-specific primers ITS1F (5'-CS1-CTTGGTCATTTAGAGGAAGTAA-3') and 58A2R (5'-CS2-CTGCGTTCTTCATCGAT-3') were used to amplify the ITS region of the fungal ribosomal DNA (Gardes et Bruns, 1993; Martin et Rygiewicz, 2005). The bacterial V3-V4 hypervariable region of the 16S rRNA gene was amplified using the specific primers 515F (5'-CS1-GTGCCAGCMGCCGCGGTAA 3') and 806R (5'-CS2-GGACTACHVGGGTATCTAAT-3') (Callahan et al., 2016; Klindworth et al., 2013). The archaea-specific primers Arch516F (5'-CS1-TGYCAGCCGCCGCGGTAAHACCVGC-3') and A806R (5'-CS2-GGACTACVSGGGTATCTAAT-3') were used to amplify the V3-V4 region of the archaeal 16S rRNA gene (Klindworth et al., 2013; Yergeau et al., 2015). PCR reactions were performed in 25µl volumes containing 0.14µl (0.7 unit/reaction) Taq DNA Polymerase (QIAGEN Toronto, ON, Canada) for 30 cycles (bacterial 16S) or 35 cycles (fungal ITS and archaea 16S) with annealing temperatures of 55°C (bacterial and archaea 16S) or of 45°C (fungal ITS). The same reaction conditions using nuclease-free water instead of extracted DNA has been used as negative control and the success of amplification products were checked on 2% agarose gel. Using this archaeal gene specific set of primers (Arch516F/A806R), PCR amplification was successful for all

rhizospheric soil samples, but it failed for all root samples. Dilutions were made on all extracts, to reduce the possible PCR inhibitor concentrations, but no amplicons were found again.

PCR products were shipped to Genome Quebec Innovation Center (McGill University, Montreal, QC, CAN) for the next steps. Unique barcode (index) and the sequence of Illumina adapters required for DNA binds to flow cell (i5 and i7) were added to each sample in the second PCR amplification. The reaction was performed with: (0.025 unit/µl) TAQ DNA Polymerase Roche FastStart High Fidelity PCR System (Sigma-Aldrich, Oakville, ON, CAN); 10 min at 95°C and 15 cycles of: 15 sec at 95°C, 30 sec at 60°C and 60 sec at 72°C, followed by a 3 min at 72°C at the end. The success of barcoding for each sample was verified on 2% agarose gel. DNA has been quantified with Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies, Burlington, ON, CAN). Libraries were then generated by pooling the same amount of each sample. The pool (or library) was cleaned up with a ratio of 0.85 of AMPure beads (Beckman Coulter Canada Inc, Montréal, QC, CAN). Libraries were then quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies, Burlington, ON, CAN) and the Kapa Illumina GA with Revised Primers-SYBR Fast Universal kit (Sigma-Aldrich, Oakville, ON, CAN). Average size fragment was determined using a LabChip GX (PerkinElmer®, Waltham, MA, USA) instrument. Before sequencing, 12% of Phix control library was spiked into the amplicon pool (loaded at a final concentration of 4.5pM) to improve the unbalanced base composition. Sequencing were done using the Illumina MiSeq PE250 system (Illumina Inc, San Diego, CA, USA) with the MiSeq Reagent Kit v2 500 cycles from Illumina. Sequencing was done with LNA™ modified custom primers (Exiqon, Woburn, MA, USA). Fungal amplicons were sequence in a separated run than bacterial and archaeal amplicons.

4.3.5 Sequence processing

Total of 2,993,007, 3,028,836 and 469,648 raw reads of fungi, bacteria and archaea were obtained from the whole dataset after sequencing runs. Theses sequencing datasets were individually processed using "DADA2" package (Callahan et al., 2016) in R V.3.5.1 (R Core Development Team, 2020). All primers and low-quality sequences were first trimmed and filtered from the raw reads with "filterAndTrim" function using a min Q score of 6 and max expected error of 2. Error rates were learned using "learnErrors" function, with default parameters, for the forward and reverse reads separately. Amplicon sequences were previously dereplicated from fastq files, using "derepFastq" function and the exact sequences were infer using the learned error rates to

remove the sequencing errors from dereplicated reads, using “dada” function. Forward and reverse reads were then merged using “mergePairs” function, without allowing mismatch in the overlap region. The smallest overlap regions were 12 bps in fungal dataset, 24 bps in bacterial dataset and 21 bps in archaeal dataset. Chimeras were removed with “removeBimeraDenovo” function. Singletons, doubletons and reads with prevalence < 2, were removed from the datasets because considered as artifacts. Using “assignTaxonomy” function, taxonomic assignment of fungi were done using the General Fasta release files version 7.2 from the UNITE ITS as reference database (Kõljalg et al., 2005). The Silva training set version 128 was used for bacterial and archaeal Taxonomic assignment (Pruesse et al., 2007). Archaea were also submitted to another taxonomic assignment, using the RDP database (Wang et al., 2007). Species-level annotation were then add to the bacterial and archaeal taxonomic table, using “addSpecies” function. After filtering, total of 1,492,944, 1,472,659 and 214,421 high-quality sequences of fungi, bacteria and archaea were recovered from the whole dataset (Figure S 4.1). The taxonomic assignations were visualized with the Krona tool (Ondov et al., 2011) through the “plot_krona” function in “psadd” package (Pauvert, 2020).

4.3.6 Statistical analysis

The rarefaction curves were calculated and visualized using the “ggrare” function in “ranacapa” package (Kandlikar et al., 2018) and Good's Coverage were calculated using “goods” function in “QsRutils” package (Quensen, 2020). All rarefaction curves tended towards their horizontal asymptotes (Figure S 4.2), and Good’s coverage estimations revealed that between 98.6–99.9%, 92.6–98.2% and 99.5–100.0% of fungal, bacterial and archaeal ASVs were obtained from their respective dataset, suggesting that the sequencing was adequate. Alpha diversity was assessed with Shannon index using “estimate_richness” function in “Phyloseq” package (McMurdie et Holmes, 2013). Group means of Shannon index were then submitted to a two-way analysis of variance (ANOVA) test, using JMP® Pro V.15.0.0 (SAS Institute, Cary, NC, USA). As recommended in McMurdie and Holmes (2014), a variance stabilizing transformation (VST) has been used to normalized the ASVs count data using “varianceStabilizingTransformation” function in “DESeq2” package (Love et al., 2014). VST count data was then submitted to a Permutational Analysis of Variance (PERMANOVA) using “adonis” function in “vegan” package (Oksanen et al., 2020), as well as to Permutational multivariate Analyses of Dispersion (PERMDISP) using the “betadisper” function in the “vegan” package. PERMANOVA and PERMDISP were both based on

the Euclidean distance matrix calculated using the "vegdist" function in "vegan" package. Euclidean distances among samples were visualized with principal component analysis (PCA). As suggested in McMurdie and Holmes (2014), differential abundance analysis based on the Negative Binomial Wald test has been used with "DESeq" function in "DESeq2" package (Love et al., 2014) to identify ASVs that were differentially abundant across two sample groups and visualized them on MA-plots (minus over average-plots) using "ggmaplot" function in "ggpubr" package (Kassambara, 2020). The common core microbiome was investigated at the ASV level and was defined here as the set of ASVs with > 0.1% of non-transformed reads abundance in at least 14 of the 15 samples in every groups. Venn diagrams were created using "venn" function in "venn" package (Dusa, 2020) to visualized the shared ASVs between the four groups.

4.4 Results

4.4.1 Fungal community structure

Over the 1,492,944 high-quality sequences, recovered from the fungal dataset, 1,292 ASVs were observed and divided into 9 phyla and 27 classes (Figure 4.2). 190 ASVs (containing ~1.09% of the total high-quality sequences) of the 1,292 ASVs, that could be identified as belonging to the fungal domain, remained unclassified at the phylum level. For the root samples 748 of those ASVs were observed among the two willow cultivars. Among the 7 observed phyla in roots samples, Basidiomycota and Ascomycota were the most dominant ones, representing 43% and 56% of the sequences in 'SX61' and 35% and 64% in 'SX64'. The abundances of other phyla such as Chytridiomycota, Kickxellomycota, Mortierellomycota, Olpidiomycota and Rozellomycota were <1%. In both cultivars, Pezizomycetes, Dothideomycetes, Sordariomycetes and Leotiomycetes were the most predominant classes of Ascomycota. Agaricomycetes was the only dominant class of Basidiomycota phylum in both cultivars, accounting for 43% of the fungal community in 'SX61' and 35% in 'SX64'. The abundances of other classes were <1%. In the rhizosphere samples, total of 1,288 ASVs, divided into 9 phyla and 27 classes, were observed among the two willow cultivars. In both of them, Basidiomycota, Ascomycota and Rozellomycota were the three most dominant phyla. Basidiomycota represented 43% of the sequences in 'SX61' and 47% in 'SX64'. Ascomycota also represented 43% of the sequences in 'SX61' and 47% in 'SX64'. Rozellomycota represented 11% of the sequences in 'SX61' and 4% in 'SX64'. The abundances of other phyla such as Chytridiomycota, Entomophthoromycota, Kickxellomycota, Mortierellomycota, Mucoromycota and Olpidiomycota were <1%. At the class level, Agaricomycetes,

Tremellomycetes and Microbotryomycetes were the three most dominant classes of the Basidiomycota phylum, with abundances of 40%, 3% and 0.5% in ‘SX61’ and 35%, 9% and 3% in ‘SX64’ respectively. Within Ascomycota, Sordariomycetes, Pezizomycetes, Leotiomycetes and Dothideomycetes were the four most dominant classes, accounting for 19%, 13%, 4% and 2% of the ‘SX61’ rhizospheric community, and for 15%, 24%, 3% and 3% of the ‘SX64’ rhizospheric community, respectively

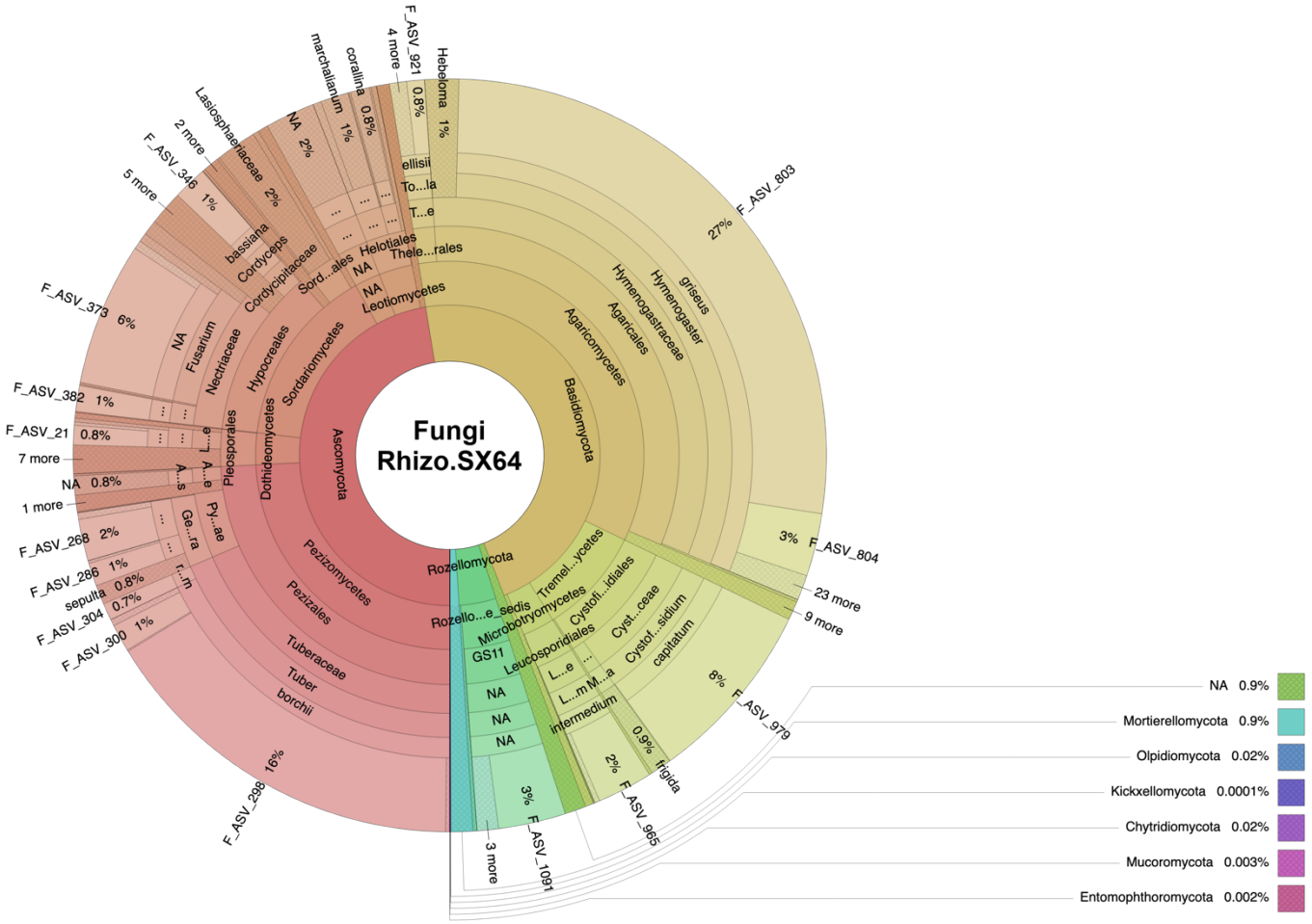


Figure 4.2 Krona charts of raw reads counts of all fungal ASVs in each compartments of both *Salix* cultivars. Arc length are proportional to the relative number of reads by group (Rhizo.SX64 = 684,598 reads; Rhizo.SX61 = 596,370 reads; Roots.SX64 = 95,896 reads and Roots.SX61 = 116,080 reads). The interactive Krona charts are available at https://github.com/MaximeFortinFaubert/Figure2/blob/main/README.md.

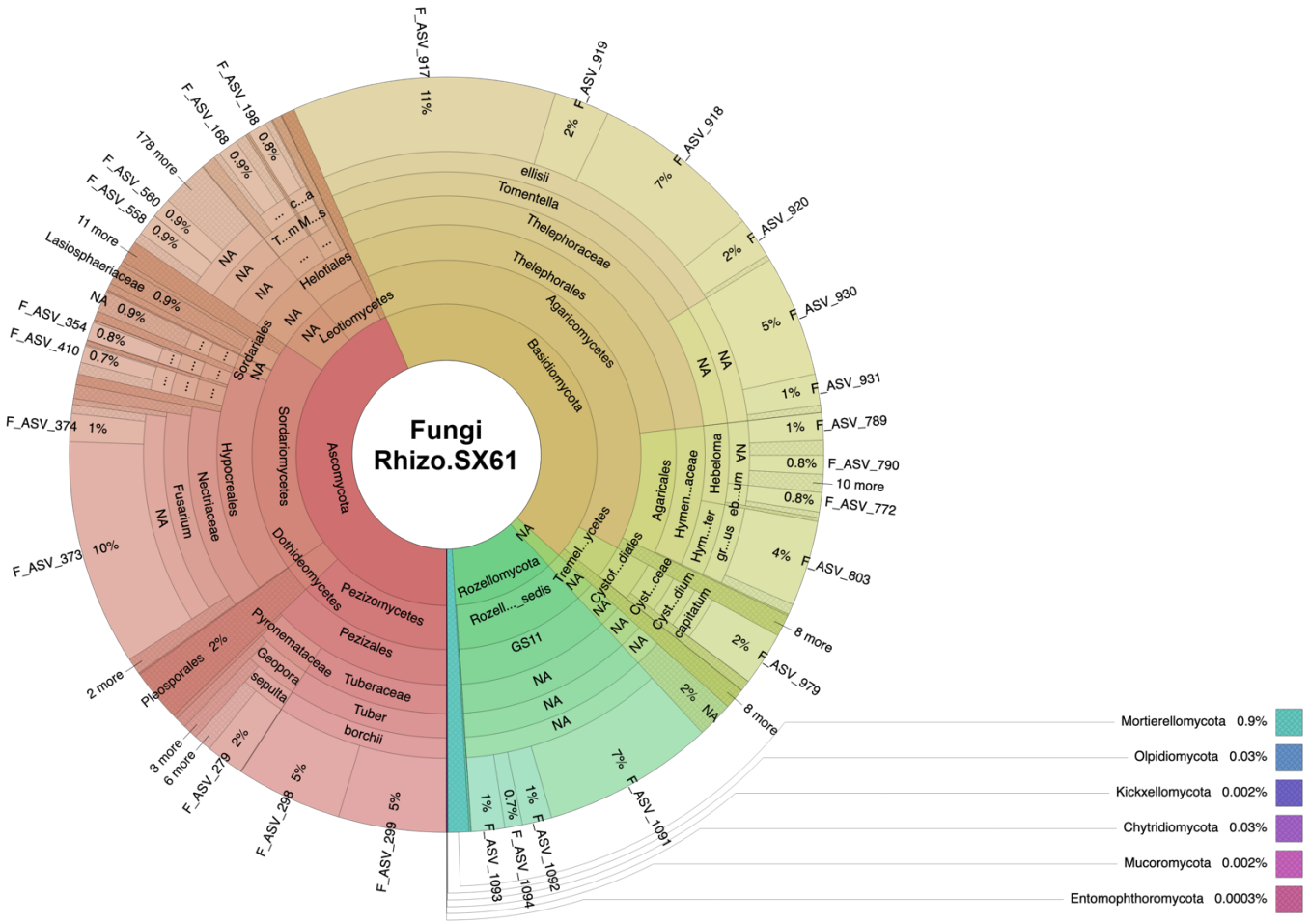


Figure 4.2 (continued)

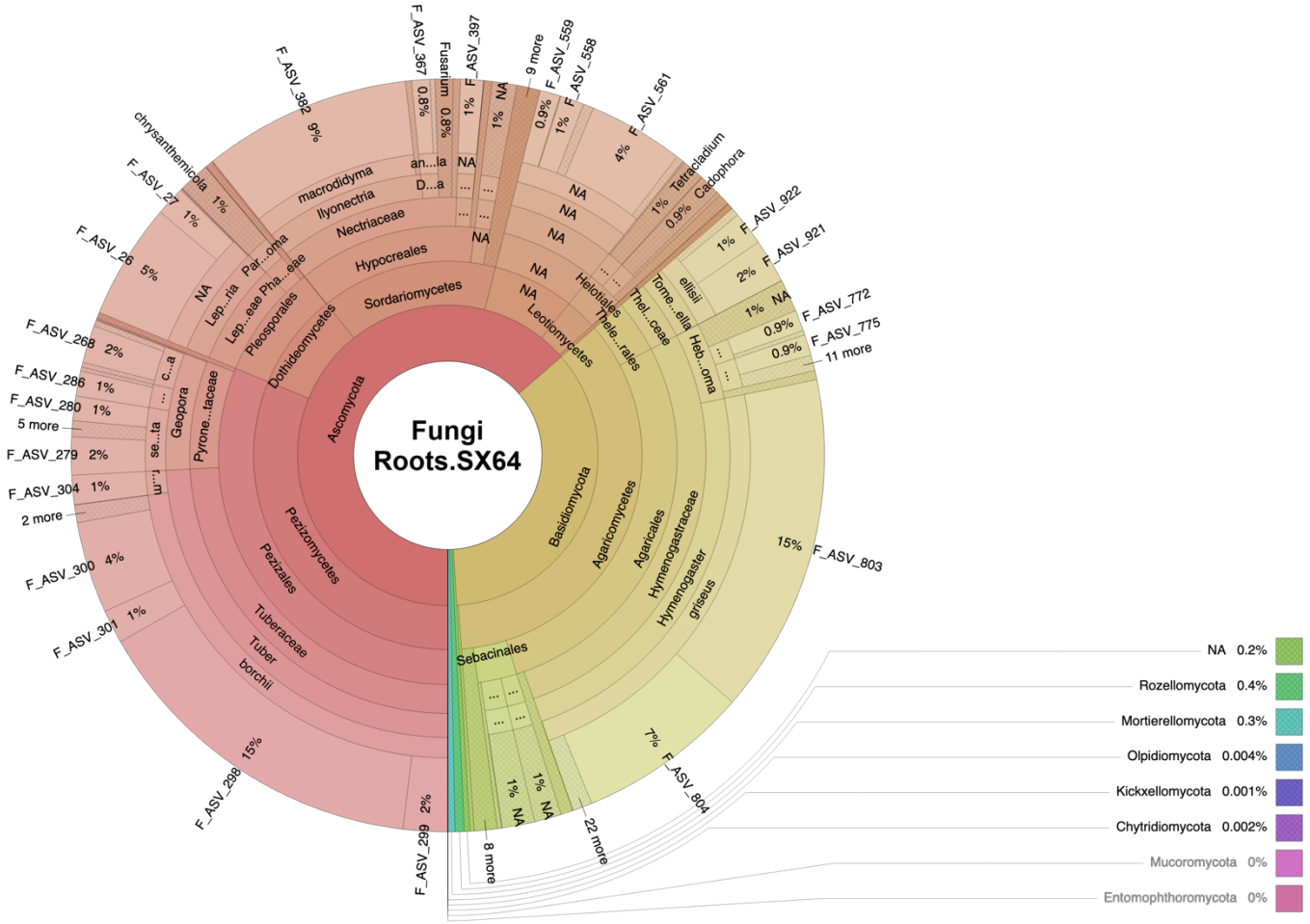


Figure 4.2 (continued)

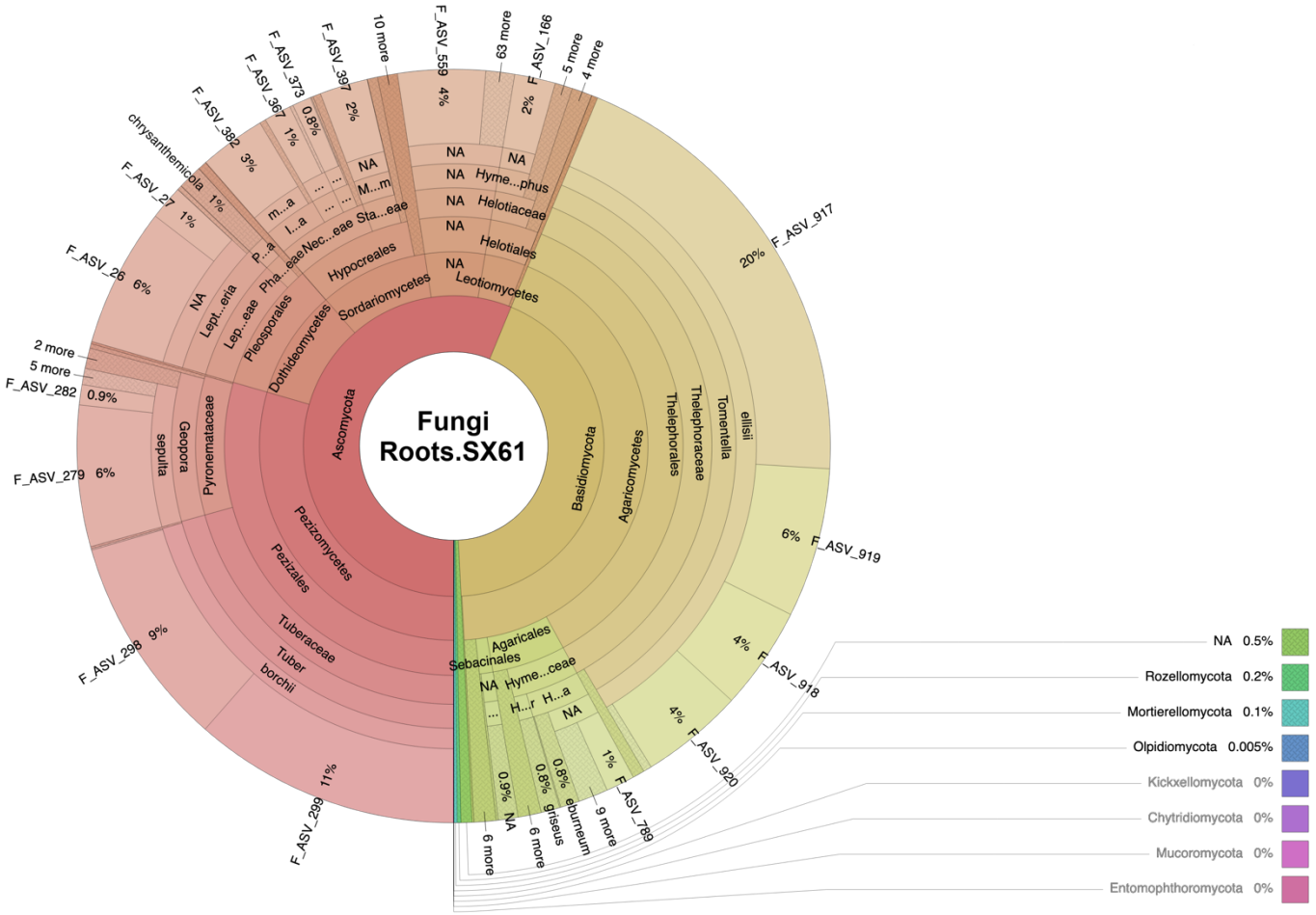


Figure 4.2 (continued)

4.4.2 Bacterial community structure

The 1,472,659 bacterial sequences, remaining from the whole dataset after sequences processing, were clustered into 6,079 ASVs (Figure 4.3). Only 83 ASVs (containing ~0.10% of the total high-quality sequences) of the 6,079 ASVs, that could be identified as belonging to the bacteria domain, remained unclassified at the phylum level. Total of 5,535 of these ASVS were present among the roots samples of both cultivars. The bacterial root community compositions of 'SX61' and 'SX64' were primarily dominated by the Proteobacteria (64% and 63%), followed by Actinobacteria (15% and 14%), Bacteroidetes (11% and 11%), Cyanobacteria (2% and 2%) and Acidobacteria (3% and 4%). Most of the Proteobacteria ASVs belonged to the Alphaproteobacteria, Gammaproteobacteria, Betaproteobacteria and Deltaproteobacteria. In the rhizosphere samples, the bacterial community compositions of both cultivars ('SX61' and 'SX64') included 6020 ASVs and were also dominated by the Proteobacteria (48% and 48%), followed by Actinobacteria (21% and 17%), Bacteroidetes (10% and 12%) and Acidobacteria (8% and 9%). Other phyla such as Verrucomicrobia (2% and 2%), Chloroflexi (3% and 3%), Gemmatimonadetes (3% and 3%), Planctomycetes (3% and 3%) were present in 'SX61' and 'SX64', respectively. Like in the roots samples, most of the ASVs associated to the Proteobacteria also belonged to the Alphaproteobacteria, Gammaproteobacteria, Betaproteobacteria and Deltaproteobacteria. Cyanobacteria were also present in rhizosphere of both Salix, but only in <1% abundance.

4.4.3 Archaeal community structure

In the rhizospheric dataset, 34 archaeal ASVs were recovered from the 214,421 high-quality sequences and grouped into 2 phyla and 3 classes (Figure 4.4). Only, one ASV (representing ~0.005% of the total high-quality sequences) remained unclassified at the phylum level. Thaumarchaeota was the dominant phylum in both cultivars, with almost 100% of the total read abundance. In both cultivars, all Thaumarchaeota ASVs were identified as member of the (soil Crenarchaeotic Group (SCG) according to the Silva training set. Another taxonomic assignment, using the RDP database, suggested that these ASVs were all *Candidatus* Nitrososphaera. Euryarchaeota was the only other archaeal phylum observed in the dataset with four (4) ASVs representing a total abundance < 0.1% in both cultivars. These four (4) ASVs were assigned to the genus level, *Methanosarcina*, *Methanocella* and *Methanobacterium* according to both databases (Silva and RDP). Seven (7) reads of *Methanocella* sp. were found in Rhizo.SX64 samples only, representing 8% of the Euryarchaeota.

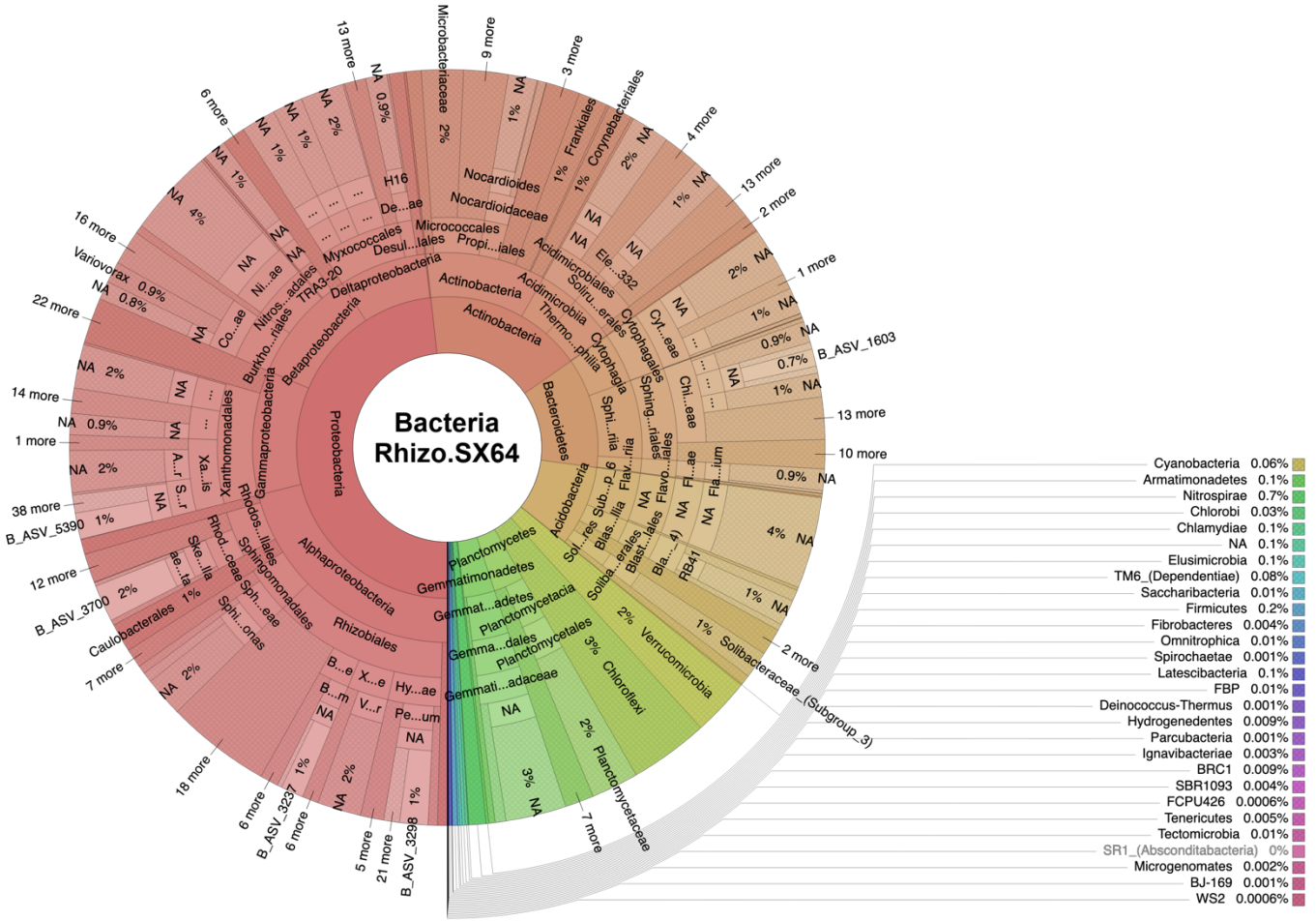


Figure 4.3 Krona charts of raw reads counts of all bacterial ASVs in each compartments of both *Salix* cultivars. Arc length are proportional to the relative number of reads by group (Rhizo.SX64 = 481,361 reads; Rhizo.SX61 = 448,274 reads; Roots.SX64 = 271,091 reads and Roots.SX61 = 271,933 reads). The interactive Krona charts are available at https://github.com/MaximeFortinFaubert/Figure3/blob/main/README.md.

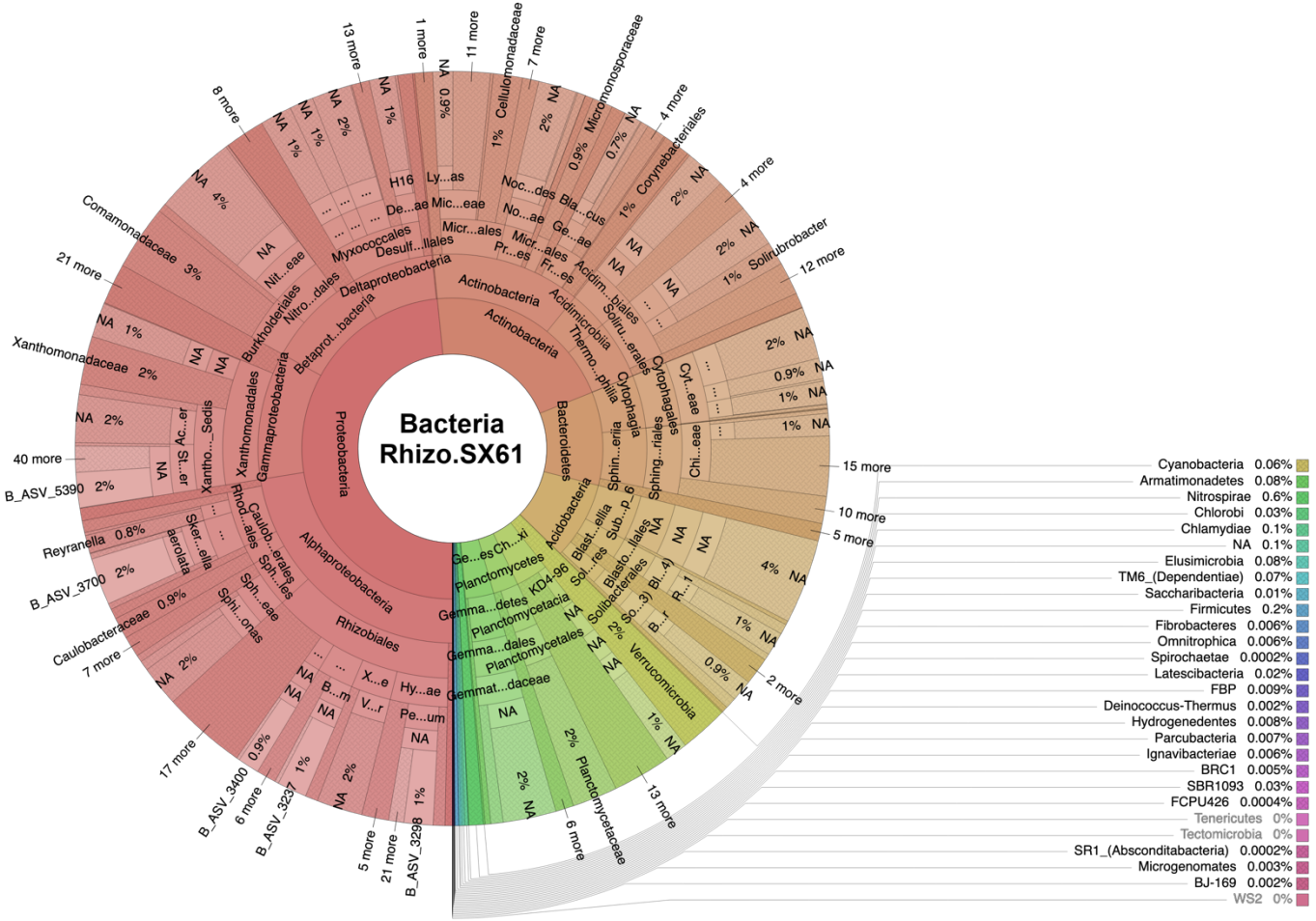
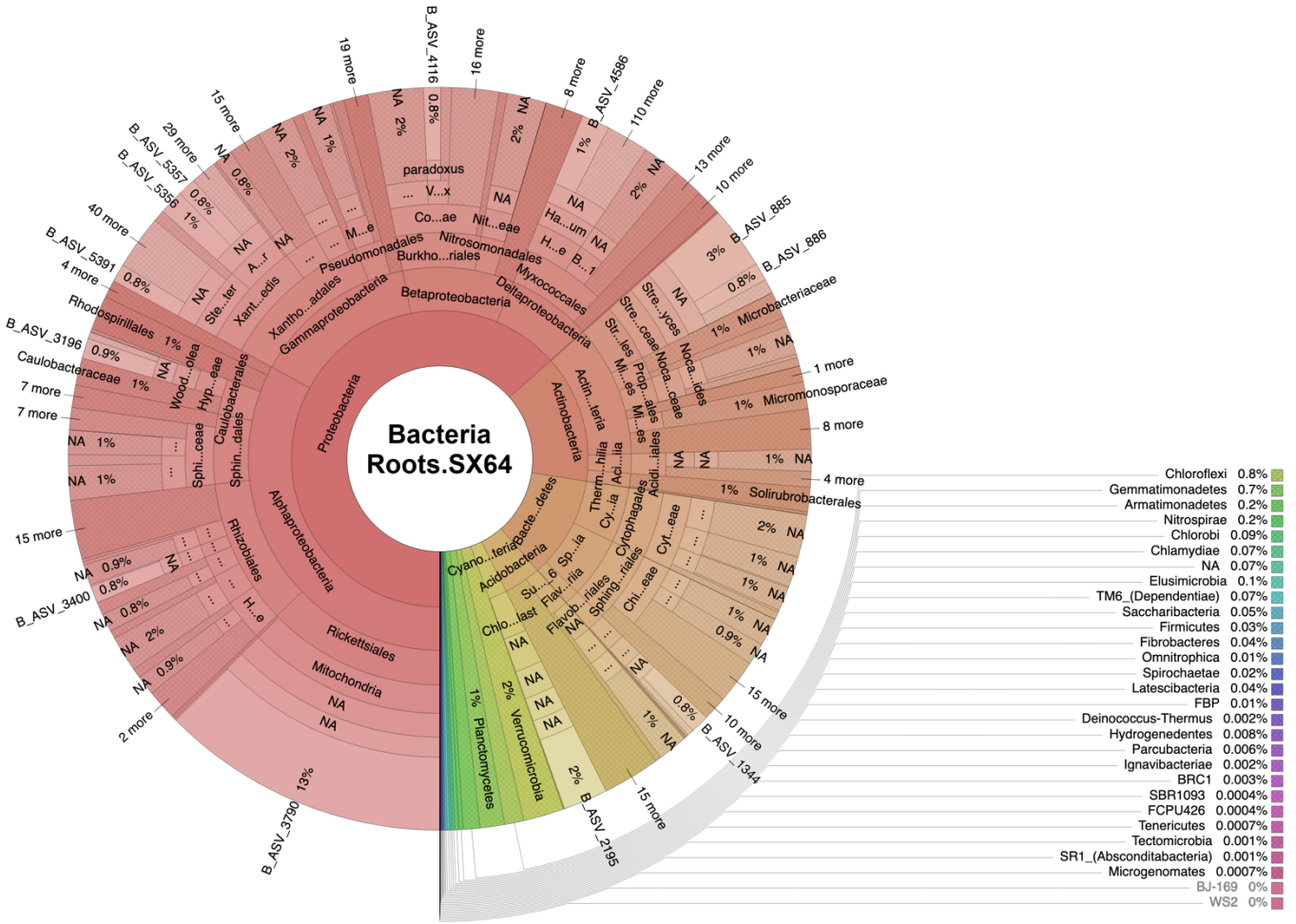


Figure 4.3 (continued)



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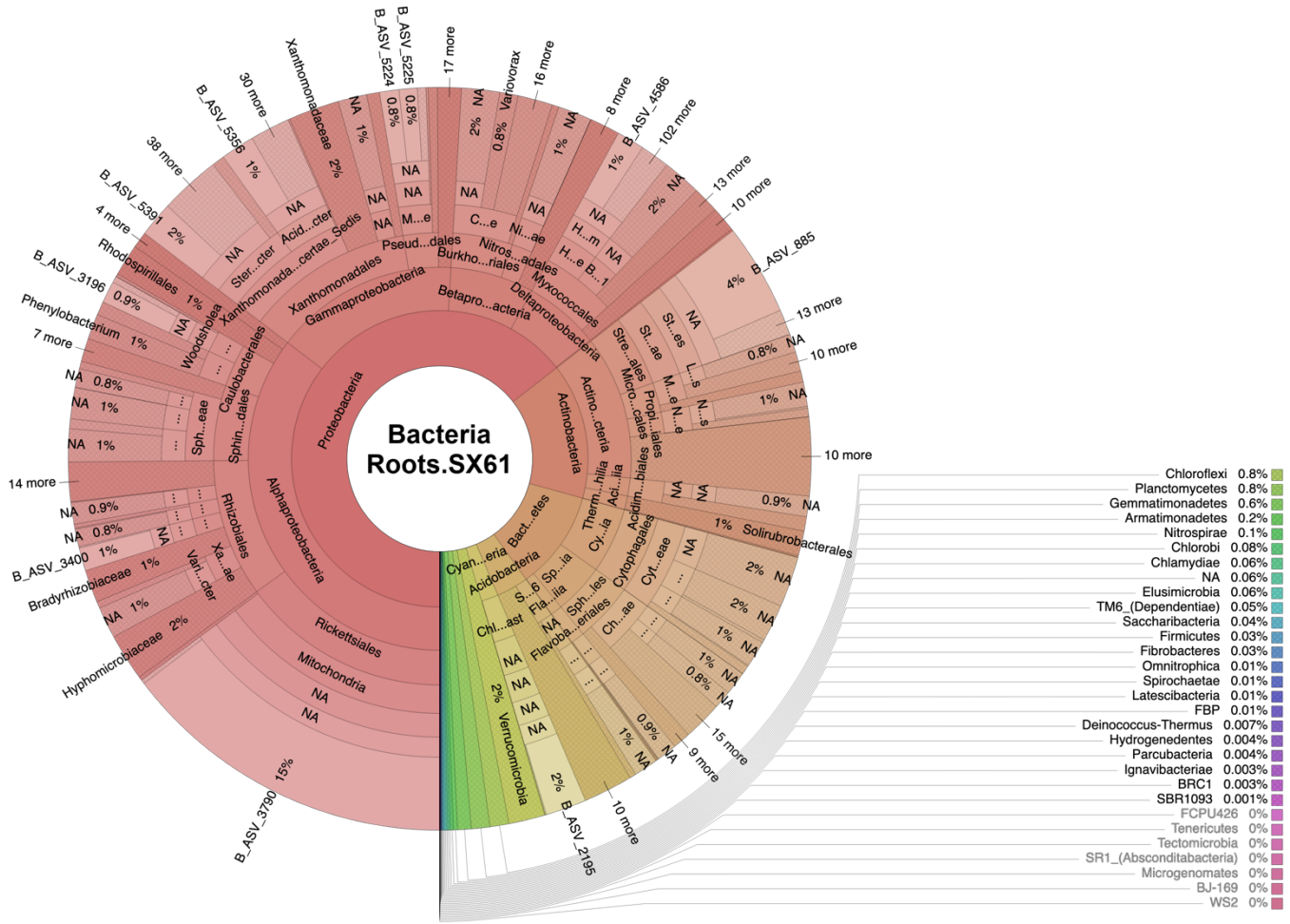

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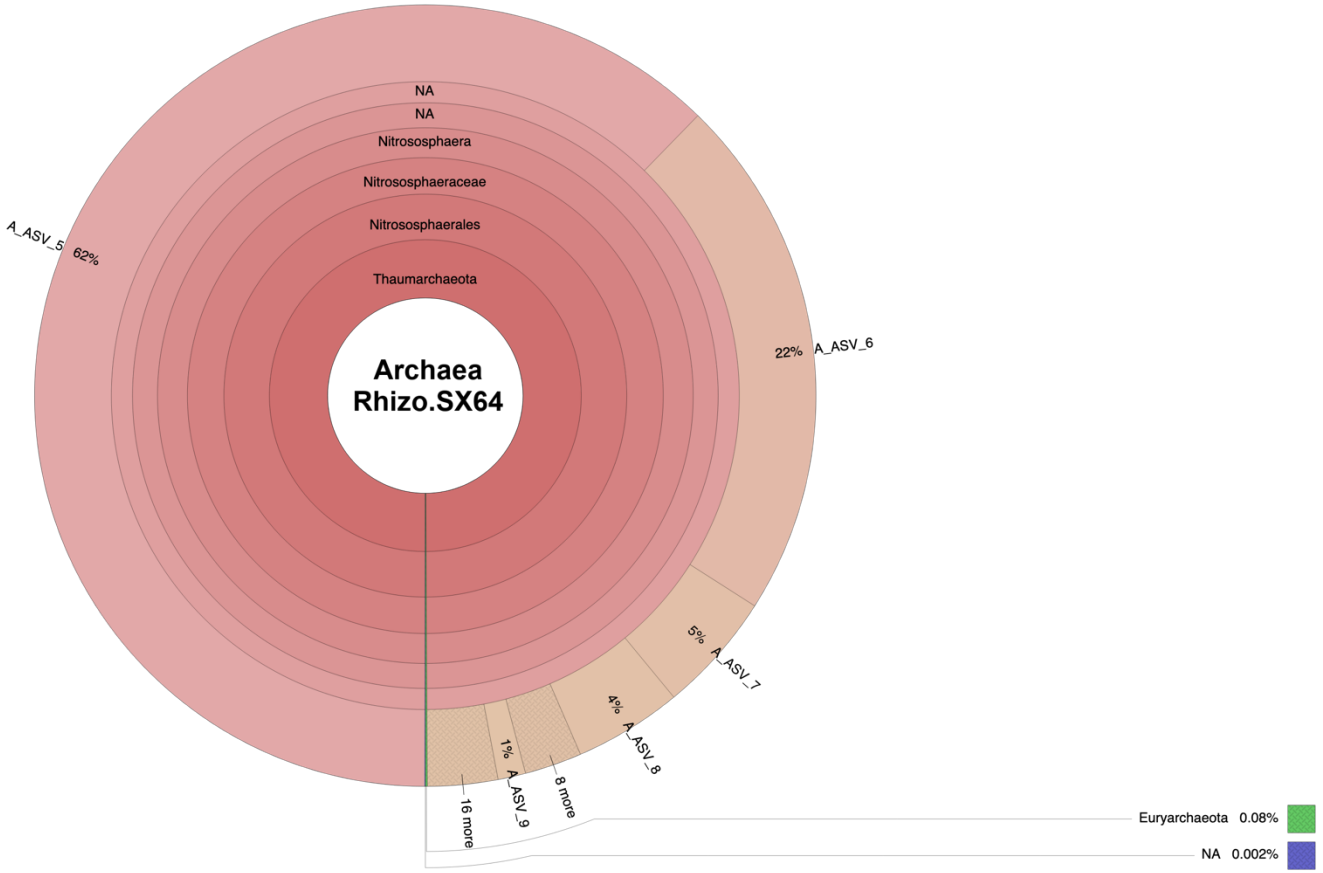

Figure 4.4 Krona charts of raw reads counts of all archaeal ASVs in each compartments of both *Salix* cultivars. Arc length are proportional to the relative number of reads by group (Rhizo.SX64 = 113,938 reads and Rhizo.SX61 = 100,483 reads). The interactive Krona charts are available at https://github.com/MaximeFortinFaubert/Figure4/blob/main/README.md.

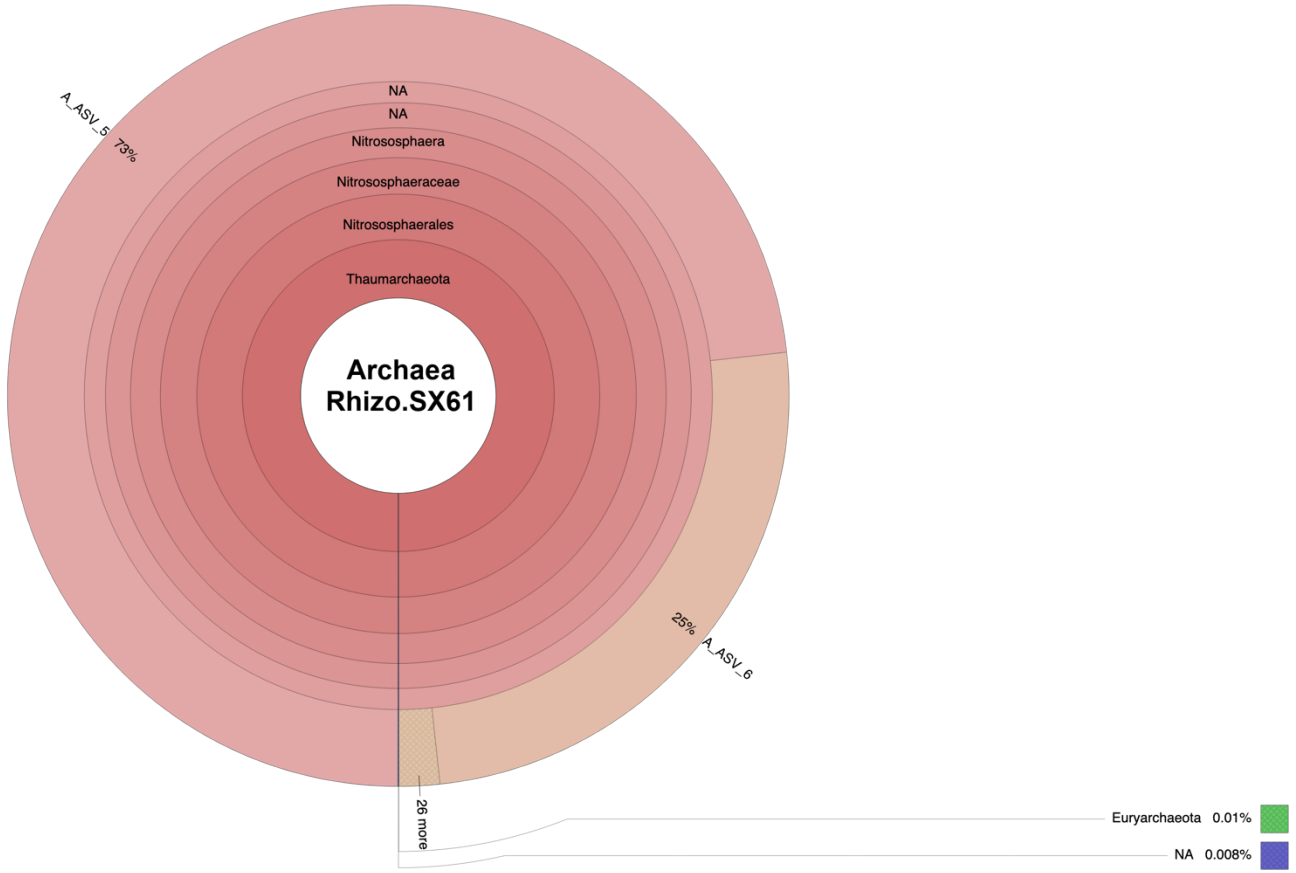

Figure 4.4 (continued)

4.4.4 Alpha diversity

In every group sample, bacterial community was significantly more diverse than the fungal one, which were both significantly more diverse than the archaeal one ($p < 0.001$ *** in every group sample). Table 4.2 shows that respective fungal, bacterial and archaeal communities were similarly diverse between both cultivars. Greater bacterial diversity was found in the rhizosphere than in the roots of both cultivars. The fungal communities of 'SX61' were more diverse in the rhizosphere than in the roots, while the ones of 'SX64' did not showed significant differences between its two compartments.

Table 4.2 Shannon diversity index calculated on ASVs.

	SX61		SX64		*p*-value			Interpretation
	Roots	**Rhizosphere**	**Roots**	**Rhizosphere**	**Cultivar**	**Compartment**	**Cultivar* Compartment**	
Fungi	1.95±0.84	2.87±0.63	2.52±0.75	2.67±0.89	0.385	**0.016***	**0.035***	**Roots.SX61 < Rhizo.SX61**
Bacteria	5.75±0.31	6.92±0.17	6.07±0.37	7.07±0.11	0.117	**<0.001*****	**0.040***	**Roots.SX61 < Rhizo.SX61** **Roots.SX64 < Rhizo.SX64**
Archaea	-	0.68±0.07	-	1.15±0.59	0.158	-	-	-

Values are the averages (mean±SD, n=15) of Shannon diversity index calculated on ASVs. Significance levels (*p*-value) are shown and asterisks ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$) indicate a significant difference between group samples.

4.4.5 Beta diversity

PERMANOVA analysis based on the Euclidean distance matrix showed that fungal and bacterial community composition were significantly influenced by plant compartment and by cultivar (Table 4.3). Archaeal community composition from the rhizosphere was also strongly influenced by cultivar. PERMDIPSD analysis showed that the dispersion of the fungal communities was significantly different between both compartments in each cultivar and that the dispersion of the archaeal communities was significantly different between both cultivars in the rhizospheric soil samples (Table S 4.1 and Figure S 4.3). Theses results suggest that the differences detected by the PERMANOVA can be artifact of heterogeneous dispersion.

Table 4.3 PERMANOVA analysis of the effects of the cultivar, plant compartment and their interaction on fungal community structure, based on Euclidean distance.

Factor	**Fungi**				**Bacteria**				**Archaea**			
	Df	***F*.Model**	**R^2**	**Pr(>F)**	**Df**	***F*.Model**	**R^2**	**Pr(>F)**	**Df**	***F*.Model**	**R^2**	**Pr(>F)**
Cultivar	1	2.7657	0.0363	**0.006****	1	4.1311	0.0489	**0.002****	1	6.0091	0.1767	**0.001*****
Compartment	1	16.3440	0.2145	**0.001*****	1	23.0238	0.2725	**0.001*****	-	-	-	-
Cultivar* Compartment	1	1.0759	0.0141	0.209	1	1.3507	0.0160	0.125	-	-	-	-
Residuals	56	-	0.7351	-	56	-	0.6627	-	28	-	0.8233	-
Total	59	-	1	-	59	-	1	-	29	-	1	-

Df, degree of freedom; *F*.Model, F-test value for model; R^2, R-squared; Pr(>F), *p*-value.

The PCA reflects those differences as fungal and bacterial roots samples showed separated cluster from the rhizospheric soil samples along the first canonical axis, which represent 23.4% and 28.3% of their total variability (Figure 4.5 A-B). Samples also showed separation between both cultivar along the second axis, which represent an additional 7.7% and 9.1% of their total variance, respectively. PCA of archaeal communities showed that rhizospheric soil samples from 'SX64' (Rhizo.SX64) were less clustered than those from 'SX61' (Rhizo.SX61), which typically clustered together along the first canonical axis, representing 72.6% of the total variability (Figure 4.5 C).

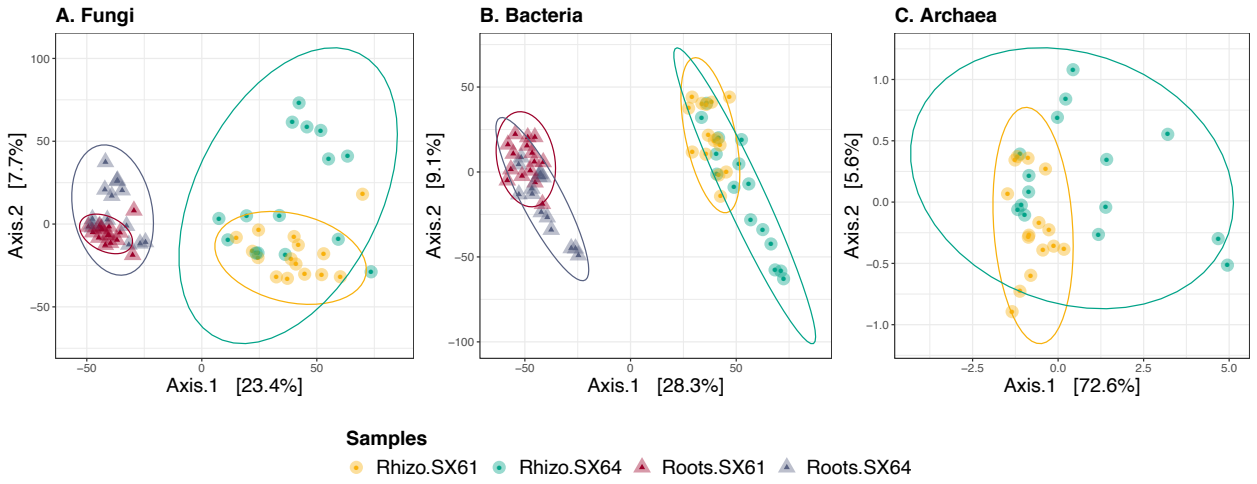

Figure 4.5 Principal component analysis (PCA) ordinations of microbial communities. Euclidean distances were calculated on the variance stabilizing transformed (VST) ASV counts in each: (A) fugal; (B) bacterial, and (C) archaeal datasets. Shapes (triangle and circle) represents the compartments and colors (red, blue, yellow and turquoise) represent samples groups. Samples closer together contain more homogeneous communities than samples farther apart. Ellipses were drawn around communities based on a 95% confidence interval.

4.4.6 Differential abundance of ASVs

Because our PCA plots revealed that some groups had much higher within-group variability than the others, it was more appropriate to create smaller datasets before running "DESeq2" function on our samples (Love et al., 2014). The root samples where then separately analysed from the rhizospheric soil samples, and all samples from 'SX61' where separately analysed from those of 'SX64'. Creating smaller datasets with only two groups without the others is supposed to be more sensitive than a model including all samples together (Love et al., 2014). In this case, "baseMean counts" is the average of the normalized count values, calculated over all samples of a subset datasets (n=30 for each cultivar and n=30 for each compartment), rather than over the entire dataset (n=60). Normalization was performed in the "DESeq2" package by dividing raw values with size factors (Love et al., 2014). Many ASVs were found to have significant differential

abundances (adjusted *p*-value < 0.05) between respective compartments of both cultivars (Rhizo.SX61 vs. Rhizo.SX64 and Roots.SX61 vs. Roots.SX64) and between compartments of the same cultivar (Rhizo.SX61 vs. Roots.SX61 and Rhizo.SX64 vs. Roots.SX64). All differential abundances can be visualized in MA-plots of Figure S 4.4. A visual summary of the most abundant ASVs (with a base mean > 10) with significant differential abundances between cultivars are presented in Figure 4.6.

Figure 4.6 Most abundant (A) fugal; (B) bacterial, and (C) archaeal ASVs showing significant differential abundances between two sample groups. Dots indicate ASV, where their size are scaled by "baseMean" abundance and their color represent the phylum to which ASVs belongs. The background color of each ASV indicates in which sample group these ones are more abundant. Only ASVs with adjusted *p*-values < 0.05 and estimated base mean > 10 were considered significantly differentially abundant and included in these plots.

4.4.7 Common core microbiome

The common core microbiome can be defined as the set of microbial taxa that occur within a host population above a particular occupancy frequency threshold (Risely, 2020). It can refer to various taxonomic ranks and have various dimensions (i.e., time and space), as well as different levels of complexity, as plant compartment, plant population and their phylogeny (Müller et al., 2016; Vandenkoornhuyse et al., 2015). Here, the microbial communities were further investigated to identify the most widespread microbial taxa within the compartments of both cultivars. As shown in Figure 4.7, only one (1) fungal and 12 bacterial ASVs were considered as common to the four libraries. The unique fungal ASV was identified as *Fusarium* sp., while the bacterial ones were identified as members of Actinobacteria (*Lysinimonas* sp.), Bacteroidetes (*Terrimonas* sp.) and Proteobacteria, including five Alphaproteobacteria, one Betaproteobacteria and four Gammaproteobacteria. The roots compartment of both cultivars shared three (3) other fungal ASVs, identified as *Leptosphaeria* sp., *Dactylonectria anthuriicola* and *Ilyonectria macrodidyma*, as well as 28 others bacterial ASVs, identified as members of Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria and Proteobacteria. Two (2) other fungal ASVs, identified as *Tetracladium marchalianum* and *Cystofilobasidium capitatum*, as well as 21 others bacterial ASVs, identified as members of Acidobacteria, Actinobacteria, Bacteroidetes, Gemmatimonadetes and Proteobacteria, and two (2) archaeal ASVs, identified as part of the Nitrososphaera family, were also common in the rhizosphere of both cultivars.

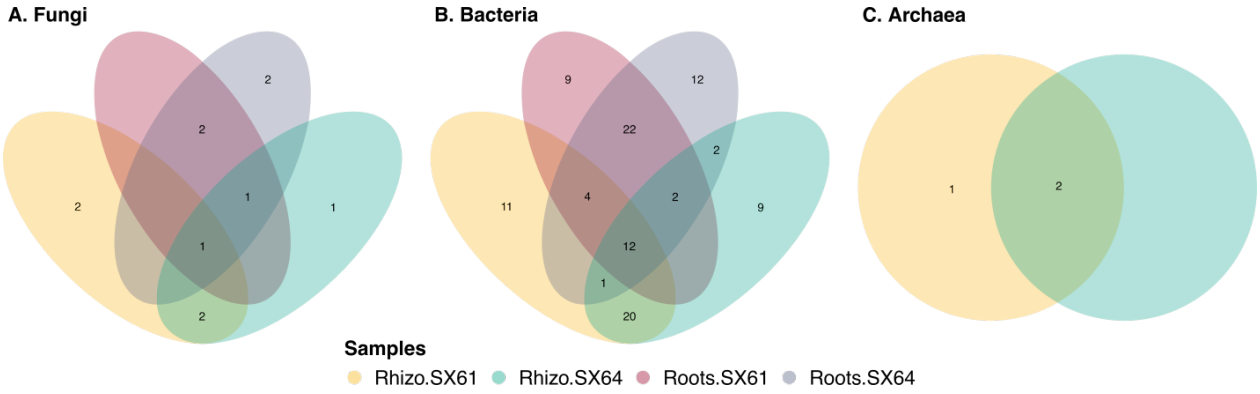

Figure 4.7 Venn diagram of shared (A) fungal; (B) bacterial and (C) archaeal ASVs between all group samples.

4.5 Discussion

4.5.1 Beta and Alpha Diversity

In this study, we examined the root and the rhizosphere microbiomes of two cultivars of *Salix miyabeana* ('SX61' and 'SX64') which grown under SRIC for six years in a mixed-contaminated soil. Multivariate analyzes revealed that fungal, bacterial and archaeal taxonomic composition significantly varied between cultivars, as well as between plant compartments. It is well known that plant identity is a strong driver of the abundance and structure of soil microbial communities (Dagher et al., 2019). Plants species and cultivars can differently influence their microbiome in the roots, in the rhizosphere, and in the adjacent bulk soil, through different exudation patterns of carbon compounds, in a way to attract or feed preferred partners, or even to deter unwanted pathogens and competitors (Bell et al., 2014; Quiza et al., 2015). The PCA also showed that microbial communities in the root samples were more clustered than in the rhizospheric soil samples. This could reflect the well known selective gradient that exists from the outside of the roots to the endosphere compartment (Müller et al., 2016).

The microbial diversity is also known to decline sequentially from the rhizosphere to the interior parts of the roots, due to an increase in competition between microorganisms as the habitat become more tightly defined (Müller et al., 2016; Tardif et al., 2016). It was then not surprising to find significantly lower diversity of bacterial ASVs in the roots of both cultivars than in their rhizosphere. The Archaeal diversity would also have been lower in the roots than in the rhizosphere of both cultivars, because 34 ASVs were identified from the rhizospheric soil samples, while none were obtained from the root's samples. Our results surprisingly showed that only cultivar 'SX61' harboured significantly lower diversity of fungal ASVs in the roots than in the rhizosphere. Iffis et al., (2017) also reported similar fungal diversity between the roots and the rhizosphere of other plant species (*Solidago canadensis*, *Populus balsamifera*, and *Lycopus europaeus*) that grown in petroleum hydrocarbon polluted sedimentation basins.

Although community compositions of each domain were found to be significantly different between both cultivars in each compartment, their alpha diversity remained statistically similar. This could reflect the differential abundances of some taxa. Increasing microbial diversity in roots and rhizosphere often leads to greater functional trait diversity, redundancy and complementarity, which would improve ecological services, as well as plant resistance to environmental changes and pathogen invasion (Bell et al., 2021; Blažková et al., 2021; Quiza et al., 2015). In contaminated

environment, higher diversification of the soil microbiome have been found to be more effective for the biodegradation of some crude oil fractions (Bell et al., 2016). Based on the similar alpha diversity between both willow cultivars, our results suggest a similar interest for their used in phytoremediation.

4.5.2 Arbuscular mycorrhizal fungi (AMF)

In addition to diversity, the presence and abundance of some taxa could be relevant for survival, growth and phytoremediation efficiency of some plant species. It is well known that *Salix* spp. can form symbiosis with both; AMF and EMF (Bell et al., 2015; Berruti et al., 2014; Jansa et al., 2003). Interestingly, in the present study, no ASVs were identified as AMF belonging to the subphylum Glomeromycotina (=Glomeromycota). Because the ITS region does not differentiate AMF very well, it would maybe be possible to observed some, if AMF-specific primers (such as AML1/AML2) were used, instead of universal fungal primers (Lee et al., 2008; Pray et al., 2018). The methodology used here could then partially explain the absence of AMF in our samples. As here, none ITS amplicons generated from the rhizosphere samples of 11 willow cultivars of 2 months-old, were assigned to Glomeromycota (Bell et al., 2014). However, some researchers still have successfully identified a great diversity of AMF in their samples by targeting ITS region (Bourdel et al., 2016; Iffis et al., 2017). Iffis et al., (2016) even reported a comparable taxonomic profile of AMF in term of abundance, between their ITS dataset and their specific AMF one, generated from the same samples. The use of the same primer set as us (ITS1F/58A2R), did not allow Yergeau et al., (2015) to identify AMF in the rhizosphere of *S. purpurea*, after 100 days of growth in contaminated soil (Varennes, QC, Canada). However, always with the same primer set, Bell et al. (2015) managed to identify some Glomeromycota in the rhizosphere of three willow cultivars, (i.e. *S. purpurea* 'Fish Creek', *S. miyabeana* 'SX67' and *S. dasyclados* 'SV1'), after 4 and 16 months of growth in a contaminated site (Valcartier, QC, Canada). All of these willows were however predominantly associated with EMF. By targeting the ITS region (ITS1F/ITS2), Tardif et al., (2016) also reported that Basidiomycota and Ascomycota occupied a greater relative abundance than Glomeromycota in the roots and in the rhizosphere of *S. purpurea* 'Fish Creek' and *S. miyabeana* 'SX67', after two seasons of growth on a contaminated site (Varennes, QC, Canada). The age of willow shrubs in our plantation could also partially explain the absence of AMF in samples. In *Salix* spp. AMF seem to be more important during early stages of growth and later largely replaced by EMF (Parádi et Baar, 2006; Van der Heijden, 2001). Such succession

could explain why Pray (2017) did not find any sequence of Glomeromycota in the roots of *S. miyabeana* ('SX61' and 'SX64'), after two growing seasons, even though they were inoculated with *Rhizoglomus irregulare* at the establishment stage of the plantation.

4.5.3 Ectomycorrhizal fungi (EMF)

In the present study, Ascomycota and Basidiomycota were the two most dominant fungal phyla in the roots, as well as in the rhizosphere of both willow cultivars belonging the 6-years-old plantation. Among these two phyla, several potentially EMF taxa were assigned to ASVs observed in both cultivars, suggesting that every single plant of the plantation, could have been colonized by multiple EMF, which in turn could have interacted with several other plants of both cultivars, to form a common mycorrhizal network (CMN) on the site (Bücking et al., 2016). EMF partners of both cultivars were essentially taxa belonging to Pezizales (*Tuber* spp. and *Geopora* spp.), Agaricales (*Hymenogaster griseus, Hebeloma* spp.) and Thelephorales (*Tomentella ellisii*).

Some studies have shown a strong link between soil contamination and the association of *Salix* spp. with ascomycota belonging to Pezizomycota (Bell et al., 2014; Tardif et al., 2016). It has been suggested that Pezizalean EMF could offer a more beneficial cost:benefit ratio to their hosts than Basidiomycota, because they would be less robust (Egger, 2006). The stout hyphae and thick-walled chlamydospores and ascospores of pezizalean may further contribute to their ability to persist under disturbed environmental conditions (Tedersoo et al., 2006). In our samples, the two most dominant Pezizales genera were *Tuber* spp. and *Geopora* spp. These two Pezizales genera have been reported to be among the most dominant EMF associated with *Salix* spp. (Hrynkiewicz et al., 2010, 2012).

Tuber borchii caught our attention, because it appeared to be one of the most important symbiotic partners of both willow cultivars, representing 21% and 23% of the sequences in the roots of 'SX61' and 'SX64', respectively. This hypogeous ascomycetous specie has been suggested to be potentially involved in phytoremediation, due to the presence of metallothioneins in its ascocarps, which may play a critical role in metal tolerance of both partners (Pierleoni et al., 2004). *T. borchii* also owns numerous genes that encode for proteins, which are already known to be involved in metal tolerance pathways in the yeast *Saccharomyces cerevisieae* (Smith et Read, 2008). White truffles sporocarps were harvested from the experimental site, and molecular identification, performed with another sequencing technology (Sanger method) and using another

primer set (ITS1F/ITS4), led us to assign the amplicons to *T. maculatum* (data not shown). In our root and rhizospheric soil samples, two other ASVs were assigned to another truffle specie (*T. ruffum*), which could also belong to the same specie as the other ASVs. To our knowledge, the presence of *T. ruffum*, *T. borchii* and *T. maculatum* have never been reported in Quebec, Canada. Additionally, we do not know any studies which revealed the presence of any *Tuber* spp. associated with willows growing in a contaminated soil from Quebec, Canada. *Tuber* spp. are however well known to form ectomycorrhizas with many *Salix* spp. (Hrynkiewicz et al., 2010, 2012; Marjanović et al., 2010; Parádi et Baar, 2006). Apparently, the soil water regime would be a very important factor determining the microenvironment of *Tuber* spp. and most would be very common to lowland ecosystems with shallow water table (Erlandson et al., 2016; Marjanović et al., 2010). Thereby, in the Netherlands, unidentified *Tuber* spp. have been found as the most abundant EMF associated with 10, 20 and 60 years old *S. alba* that grown in riparian edge forests (Parádi et Baar, 2006). The edaphic conditions of the experimental site, as its proximity and its low elevation from the south shores of the St-Lawrence River, could have favoured the symbiotic association of *Tuber* spp. with our *Salix miyabeana* cultivars. It has been suggested that more frequent harvests (shorter than 6-year rotation) of above-ground parts of *S. viminalis* in SRIC would promote the mycorrhiza formation with *Tuber* spp. (Hrynkiewicz et al., 2010). Therefore, the 3-year rotation length in our SRIC of willow plantation could have played a key role in the selective promotion of *Tuber* spp.

Geopora spp. (identified here as *G. sepulta* and *G. cervina*) appeared to be other important Pezizalean EMF partners of both *S. miyabeana* cultivars. *Geopora* spp. seem to include generalists EMF species, since many have been found to form associations with both angiosperm and gymnosperm hosts (Gehring et al., 2014). *Geopora* spp. were also reported to have saprotrophic abilities, which could allow them to occupy a variety of ecological niches and persist when they are less competitive than other EMF taxa for the root colonization (Tedersoo et al., 2010). This Pezizales genera seems to be commonly dominant in willows that grown in harsh conditions, as fly ash landfills (Hrynkiewicz et al., 2009), former landfill (Bell et al., 2015), or field containing high concentrations of petroleum hydrocarbons (Tardif et al., 2016). Interestingly, in our study, no sequence was assigned to any *Sphaerosporella* spp., another Pezizales genera that appeared to be commonly associated with willows growing under contaminated conditions (Bell et al., 2014, 2015; Iffis et al., 2017; Tardif et al., 2016), and that seems to have beneficial effects on the biomass

of *Salix miyabeana*, as well as on its efficiency to decrease the soil concentrations of Pb, Sn, and Zn (Dagher et al., 2020b).

Agaricales (*Hymenogaster griseus* and *Hebeloma* spp.) and Thelephorales (*Tomentella ellisii*) were the three most dominant basidiomycetous EMF found in our samples. Even if both cultivars hosted these taxa in their two compartments, our results showed that *T. ellisii* was mostly associated to 'SX61', while *H. griseus* was more dominant in 'SX64'. These two species were thereby assigned to the only two fungal ASVs showing significant differential abundances between the root samples of both cultivars. Such differences could suggest that both species would not cohabite very well and would compete each other to inhabit the same ecological niche. In a previous study conducted in marginal farm land in Québec, *Hymenogaster griseus* has been assigned to one of the 16 OTUs observed in the roots of the same two cultivars ('SX61' and 'SX64'), after one season of growth under SRIC conditions (Pray et al., 2018). In central Sweden, *H. griseus* was also identified as one of the most dominant taxa associated with other *Salix* spp. that also grown under SRIC conditions for 4 years (Hrynkiewicz et al., 2012). Interestingly, in the same study, *Salix* spp. from an older plantation (11-year-old) also harboured *H. griseus*, but in a marginal proportion compared to *Tomentella* spp., which were not observed in the youngest plantations (4-year-old). Although not addressed in their study, this may support the hypothesis that members of *Hymenogaster* spp. would not coexist very well with members of *Tomentella* spp. Besides this possibility, *Hymenogaster* spp. could be considered as pioneer or early-stage fungi, while *Tomentella* spp. could be more associated with older and mature host. *H. griseus* may then plays important roles in the establishment stage of *Salix* spp. by improving their tolerance to different contaminant. In northern Slovenia, the later secondary successional stages of a heavy metal polluted site, was characterized by the prominence of naturally established *Salix caprea* associated with *H. griseus* (Likar et Regvar, 2009).

Thelephorales (*Tomentella* spp.) have been found to be predominant in many mature (20-30 years old) *Salix* spp. from southern Switzerland (Arraiano-Castilho et al., 2020) and Argentina (Becerra et al., 2009). Low frequencies of *Tomentella* spp. have been found on the root tips of six-years-old *S. viminalis* and *S. dasyclados* (Püttsepp et al., 2004). *Tomentella ellisii* have been found to be an important symbiotic partner of many *Salix* spp. naturally distributed across different hydrologic gradients in temperate North America (Erlandson et al., 2016). Many results suggest

that *T. ellisii* is a generalist able to colonize several hosts, including 26-years-old *Pinus sylvestris* (Guo et al., 2020), *Carpinus betulus*, *Tilia cordata* (Rudawska et al., 2019), *Pinus pinaster* and *Quercus suber* (Buscardo et al., 2010). In eastern Serbia, *T. ellisii* have been found to be one of the four EMF associated with 40-year-old poplar trees (*Populus alba*, *P. nigra*, *P. tremula* and their hybrids) which naturally grown on pyrite tailings contaminated site, containing high levels of copper and zinc (Katanić et al., 2014). *T. ellisii* and other *Tomentella* spp. have been reported to be dominants in *Pinus massoniana* (~30-year-old) and *Quercus fabri* (5 and 8-year-old), growing in Xiangtan manganese mining area in China (Huang et al., 2014). Even if *Tomentella* spp. would not coexist very well with *Hymenogaster* spp., it seems to be commonly found together with Pezizales (*Tuber* spp. and *Geopora* spp.) in *Salix* spp., *Populus* spp. and *Quercus* spp. (Erlandson et al., 2016; Hrynkiewicz et al., 2010, 2012; Pruett et al., 2008), suggesting that these fungi do not have strong antagonistic effects against each other.

Hebeloma spp. appeared to be an other important potentially EMF partners of both cultivars, representing 1 to 5% of the total ASVs in each of the four-group sample. *Hebeloma* spp. are also considered to have broad host ranges, even if several preferentially grows with members of Salicaceae (Aanen et al., 2000). Fungi belonging this genus are generally described as early-stage mycorrhizal symbiont (Mason et al., 1983; Smith et Read, 2008). Because *Hebeloma* spp. have been found in the root tips of 6 years old *Salix dasyclados* and *S. viminalis*, in a short-rotation forestry plantation, Püttsepp et al., (2004) concluded that EMF communities of their willows were still at a relatively early successional stage, which could also be the case for the fungi communities of our 6 years old *S. miyabeana*. However, *Hebeloma* spp. happen to be reported in older stands of trees, especially when growing in harsh environments, such as in 20-30-years-old hybrid populations of *S. helvetica x purpurea* in alpine glacier forefield (Arraiano-Castilho et al., 2020), in 20 years old *Salix alba* in riparian edge forests (Parádi et Baar, 2006), or in 33 years old plantation of *S. caprea* in three former silver-mining sites (Hrynkiewicz et al., 2008). *Hebeloma* spp. could have important roles in contaminated soils since some has been found to owns genes that encode for proteins already known to be involved in metal tolerance pathways in the yeast *Saccharomyces cerevisieae* (Smith et Read, 2008). *Hebeloma* spp. are among the few EMF species to be commercially available as inoculum for agricultural applications (Pray et al., 2018; Wijesinghe, 2013). On marginal farm land, the inoculation of *H. longicaudum* does not impacted the biomass production of *Salix miyabeana* ('SX61' and 'SX64') cultivated in SRIC system (Pray

et al., 2018). In pot experiment conducted using Cd polluted soil, Sell et al., (2005) found that inoculation of *H. crustuliniforme* enhanced the biomass of *Populus canadensis*, while no such effect was observed concerning *Salix viminalis*. However, inoculated *S. viminalis* shown lower Cd concentrations in its leaves and higher Cd concentrations in its stems, than the non-inoculated controls plants. In another pot experiment, inoculation of *H. sinapizans* reduce Cd translocation from roots to shoots of *Pinus sylvestris*, and increased lengths of its roots and shoots, as well as its biomass (Kozdrój et al., 2007).

4.5.4 Nonmycorrhizal endophytic fungi

Total of eight (8) fungal ASVs were significantly more abundant in the roots than in the rhizospheric soil samples of, either one or both, willow cultivars. All these ASVs belonged to Dothideomycetes (*Leptosphaeria* spp.), Leotiomycetes (*Cadophora luteo-olivacea*, *Cadophora orchidicola*) and Sordariomycetes (*Dactylonectria anthuriicola*, *Ilyonectria macrodidyma*, *Myrothecium* spp.). All of these eight ASVs were similarly abundant between respective compartments of both cultivars, except for one, identified as *Ilyonectria macrodidyma*, which was significantly more abundant in Rhizo.SX64 than in Rhizo.SX61. Even if these eight ascomycetous ASVs were observed in the rhizospheric soil samples, their significantly higher abundance in the root samples suggest that these taxa could be endophytic fungi, inhabiting the roots of *Salix miyabeana*. Indeed, Dothideomycetes, Leotiomycetes and Sordariomycetes appear to contain taxa that are commonly found in the roots of *Populus* spp. and *Salix* spp. growing in a contaminated environment in Quebec (Canada) (Bell et al., 2014; Bourdel et al., 2016; Iffis et al., 2017; Tardif et al., 2016).

Leptosphaeria spp. represented 8% and 6% of the sequences in Roots.SX61 and Roots.SX64, respectively. This genus is well known to contains plant pathogenic species responsible of lesions on the leaves of many cruciferous species and invades their stem and causes severe cankers at their root necks and stem bases (Kaczmarek et Jędryczka, 2011). This kind of plant disease is a major threat to canola production in Canada (Dilantha Fernando et al., 2016). *Leptosphaeria* spp. have been found on the leaves of *Salix viminalis* cultivated in SRIC in southern Quebec, Canada, but it did not seem to be problematic to them (Vujanovic et Labrecque, 2002). As in the present study, *Leptosphaeria* have been reported to be the most dominant genus of Dothideomycetes in the roots of different plant species when the hydrocarbon concentrations were very high (Iffis et al., 2017). Although *Leptosphaeria* spp. seem to be potentially pathogenic fungi,

a strain have been described as dark septate endophytic (DSE) fungi with beneficial effects on the growth of *Ammopiptanthus mongolicus* under drought conditions (Li et al., 2018a).

Cadophora spp. represented 0.6% and 0.9% of the sequences in Roots.SX61 and Roots.SX64, respectively. Although some *Cadophora* spp. (as *C. finlandica*) are sometimes considered as EMF (Dos Santos Utmazian et al., 2007), members of this genus are generally described as DSE species (Knapp et al., 2018). *Cadophora* spp. have been found to be a very common DSE in the roots of *Salix* spp. growing in uncontaminated arable soils (Baum et al., 2018). Under axenic conditions, it has shown beneficial effects on the growth of cuttings of *Salix caprea* in soil enriched with Cd and Zn (Likar et Regvar, 2013).

Several nonmycorrhizal taxa were quite redundant in a relatively high abundance in our root samples (> 0.1% of non-transformed reads abundance in at least 14 of the 15 samples). Indeed, at the exception of only one ASV (assigned to the EMF *Hymenogaster griseus*), all other ASVs considered as part of the core microbiome of the root of either one or both cultivars, were assigned to *Fusarium* sp., *Ilyonectria macrodidyma*, *Leptosphaeria* spp. and *Dactylonectria anthuriicola*. The ASVs, associated to *Fusarium* sp. and *Ilyonectria macrodidyma* were also considered as part of the core microbiome of the rhizosphere of either one or both cultivars. Interestingly the only one fungal ASVs identified as part of the core microbiome in all group samples was assigned to *Fusarium* sp. and was significantly more abundant in the rhizosphere of both cultivars, than in their roots. *Fusarium* spp. are commonly considered as pathogens for some plants (Michielse et Rep, 2009; Poletto et al., 2020). However, nonpathogenic *Fusarium* spp. are gaining interest in agriculture as biocontrol agent to manage plant diseases (Wei et al., 2019). *Fusarium* is found to be among the most common and abundant genus in the roots of various plant species (Khalmuratova et al., 2015; Li et al., 2018b; Maciá-Vicente et al., 2008), including *Salix* spp. grown under SRIC (Corredor et al., 2012). Several endophytic fungi, are opportunistic and have the ability to occupy a variety of ecological niches (Jumpponen et Trappe, 1998). *Cadophora* also includes species that are frequently encountered as postharvest fruit pathogens of kiwi, apple and pear (Auger et al., 2018; Spadaro et al., 2011). Ilyonectria includes pathogenic species often reported as causative agent of root-rot disease and rusty symptoms in ginseng and olive trees (Farh et al., 2018; Úrbez-Torres et al., 2012). To our knowledge, these taxa have never been described as specific pathogens to *Salix* spp. However, as mentioned by Corredor et al., (2012), some of them may act

opportunistically and use the newly planted cuttings as temporal hosts, thus limiting the establishment of willow. *Cadophora luteo-olivacea*, *Ilyonectria* spp., and *Leptosphaeria sp.*, can also take advantage of the death tissues of willows to colonize it (Hirose et al., 2013). In addition, *Cadophora* spp. can be considered as potential threat to the health of *Salix* spp. during long cold-storage of cuttings (Hosseini-Nasabnia et al., 2016).

Our results indicate that healthy willows hosted a considerable abundance of nonmycorrhizal endophytic fungi, which are possible opportunistic or obligate endophytes or biotrophic pathogens taxa. These fungi and their interactions with plants show great potential for increasing plant growth and improving phytoremediation efficiency (Deng et Cao, 2017). All of them could then have beneficial function for *Salix miyabeana* growing under contaminated conditions. However, the use of Dothideomycetes, Leotiomycetes and Sordariomycetes as inoculum to increase the phytoremediation potential of willows should therefore be approached with caution and more studies are needed to fully understand the interactions of DSE with their hosts.

4.5.5 Archaeal communities

In our study, 34 archaeal ASVs, identified as members of Euryarchaeota and Thaumarchaeota, were found in the rhizosphere of *Salix miyabeana*. The hight dominance of Thaumarchaeota (almost 100% of sequences) and the negligible presence of Euryarchaeota, suggest that rhizospheric archaeal communities are involved in nutrient cycling, mostly as ammonia oxidizers (Thaumarchaeota) and to a lesser extent as methanogens (Euryarchaeota). Indeed, Thaumarchaeota (formerly known as Group I Crenarchaeota) has been described as a phylum containing aerobic ammonia oxidizing archaeon (AOA), inhabiting marine (Group I.1a/Nitrosopumilales, *Nitrosopumilus*, *Nitrosoarchaeum*, *Cenarchaeum*), and soil environments (Group I.1b/Nitrososphaerales, *Nitrososphaera*) (Pester et al., 2011). It is now known that this phylum also includes newly added lineages of archaea that have not the ability to oxidize ammonia (i.e., Group I.1c/Fn1 and Group I.1d/Beowulf and Dragon) (Adam et al., 2017). However, all identified Thaumarchaeota in our samples were affiliated to Soil Crenarchaeotic Group (SCG), also known as “Group I.1b”, which englobes key players for the global nitrogen cycle in soil ecosystems (Stahl et De La Torre, 2012). For their part, Euryarchaeota are well known to be strictly anaerobes involved in methanogenesis - a metabolic process specific to archaea (Thauer et al., 2008). In our study, the four (4) ASVs belonging to Euryarchaeota were identified at the genus

level, as members of Class I methanogens (*Methanobacterium* sp.) and Class II methanogens (*Methanocella* sp. and *Methanosarcina* spp.) (Adam et al., 2017).

To our knowledge, very few studies focused on the assessment of archaeal communities inhabiting the roots of *Salix* spp. Among them, Yergeau et al. (2015) used the same archaeal gene specific set of primers than us to assessed the archaeal communities in the rhizosphere of 100-days-old *Salix purpurea* that grown in pots containing petroleum-contaminated soil. In comparison to our study, they reported higher archaeal diversities, with high dominance of both Euryarchaeota and Thaumarchaeota phyla. Based on microbial expression profiles, another study revealed that Euryarchaeota were more active in contaminated bulk soil, whereas Crenarchaeota and Thaumarchaeota were more active in the rhizosphere of 6-months-old *S. purpurea* planted in non-contaminated soil (Yergeau et al., 2014).

The high dominance of Thaumarchaeota (SCG/I.1b Crenarchaeota) in the rhizosphere of both willow cultivars reported in our study, was in agreement with previous findings concerning many other plant species, including olive cultivars (*Olea europaea*) (Caliz et al., 2015), arugula (*Eruca sativa*) (Taffner et al., 2019), tomato plants (*Solanum* spp.) (Lee et al., 2019; Taffner et al., 2020), native alpine trees (*Picea crassifolia* and *Populus szechuanica*) (Zhang et al., 2020), maize (*Zea mays*) and soybean (*Glycine max*) (Nelson et al., 2010). Although reported in much lesser abundance than Thaumarchaeota, Euryarchaeota were found, in all these studies, to be the second or the third most dominant phylum in the rhizosphere of these plants. Nelson et al. (2010) found greater relative abundance of Euryarchaeota in the rhizosphere of soybean than in the maize and suggested that the release of H_2 by N_2-fixing bacteria inhabiting the root nodules of legumes, could promote the growth of Euryarchaeota, because H_2 is used as a reducing agent to convert CO_2 to methane during anaerobic respiration by methanogens (Nelson et al., 2010; Peoples et al., 2008; Thauer et al., 2008). However, due to their preferences for anoxic conditions, Euryarchaeota appear to be mostly dominant in the rhizosphere of crops that are typically cultivated in flooded soil, as rice (*Oryza sativa*) (Breidenbach et al., 2016).

Compared to fungi and bacteria, little is known about the ecological roles of archaea inhabiting plant microbiomes, because most of them remain undetectable using conventional culture-based methods. In the last few years, next generation sequencing methods, as well as omics (i.e., metabolomics, metatranscriptomics, and metagenomics) has greatly increased the number of

studies assessing archaeal communities, as well as their roles in plant proximity (Akinola et Babalola, 2021). Consequently, archaeal community is now considered as an important component of the plant rhizosphere microbiome (Akinola et Babalola, 2021; Buée et al., 2009; Taffner et al., 2018). Although the relations of archaea with plants remained largely unclear, their ubiquitous occurrence on healthy plants have been suggested to be the reflect of positive interactions between both partners (Taffner et al., 2018). Some archaeal taxa (i.e., *Methanosarcina*, SAGMA group, MCGCL group and *Methanomassiliicoccus*) have been reported to be positively correlated with shoot and root biomass of *Salix purpurea* that grown under highly petroleum-contaminated soil (Yergeau et al., 2015). However, such positive interactions would not be generalized to all archaea, since other taxa (i.e., Unidentified Methanomicrobiales, *Methanobacterium*, pGrfC26) have been reported in the same study to be negatively correlated with willow growth. It is expected that AOA and methanogens archaeon play important roles in nutrient cycling within the rhizosphere (Akinola et Babalola, 2021). Thereby, AOA could fulfil key functions in the context of soil bioremediation, since nutrients, as nitrogen, are often limiting in petroleum contaminated soil due to an unbalanced C:N ratio (Yergeau et al., 2014). Based on metagenomic mining, it has been deduced that archaeal community inhabiting the rhizosphere could potentially fulfill other important functions involved in plant growth promotion, as through auxin biosynthesis, nutrient supply, and protection against abiotic stress (Taffner et al., 2018).

In our study, no archaeal sequence was obtained from all root samples of both cultivars. Following the roots DNA extractions, serial dilutions were performed to reduce the possible PCR inhibitor concentrations that can be found in plant material, such as polysaccharides and humic acids (Bessetti, 2007), but no amplicons were found again. Too much PCR inhibitor concentration combined with a very low archaeal DNA concentration, may have caused the failure of generating archaeal amplicons from all our root samples. However, our results could also basically reveal that no archaea lived inside the roots of our two willows. To our knowledge, endophytic archaea have never been reported in *Salix* spp. However, few studies conducted on other plants species reported internal tissue colonization by archaea, as in coffee cherries of *Coffea arabica* (Oliveira et al., 2013), in the roots of maize (*Zea mays*) (Chelius et Triplett, 2001), in leaves, stems and roots of common reed (*Phragmites australis*) (Ma et al., 2013), rice (*Oryza sativa*) (Sun et al., 2008), leaves of olive trees (*Olea europaea*) (Müller et al., 2015), and in the roots of tomato plants (*Solanum* sp.) (Lee et al., 2019). As mentioned by Chelius and Triplett (2001), such results concerning root

samples can be questionable, considering that some taxa may have been incorrectly classified as endophytes, due to a possible detection of DNA from dead cells that reside on the rhizoplane after root surface sterilisation.

4.6 Conclusions

According to the presented results in this study, it could be concluded that the diversity of the fungal, bacterial and archaeal community was quite similar between both cultivars ('SX61' and 'SX64') of six-years-old *Salix miyabeana* that grown in a mixed contaminated soil from Southeast region of Canada. Microbial diversity generally decreased from the rhizosphere to the roots, at the exception of fungi that shown similar diversity between both compartments of 'SX64'. The general taxonomic structures of each microbial community were found to be cultivar- and compartment-specific. Although our universal fungal primers had the potential to amplify Glomeromycotina DNA, no sequences were assigned to this subphylum in all our samples, reinforcing the scientific idea that *Salix* spp. are mainly associated with EMF fungi. Among the fungi identified in our study, *Tuber borchii, Tomentella ellisii*, *Hymenogaster griseus, Geopora* spp. and *Hebeloma* spp., were found to be among the most dominant EMF partners of both cultivars. Some Dothideomycetes, Leotiomycetes and Sordariomycetes were found to be abundant in the roots of both cultivars, suggesting that they are important endophytes for health and growth of willows that grown under contaminated conditions. Our study is one of the few to provide information about archaea inhabiting the root zone of willows. It can be concluded that our two cultivars do not harbor endophytic archaea, since no sequence was obtained from PCR performed with all root samples. However, the high dominance of Thaumarchaeota in the rhizosphere suggested that most archaea associated with *S. miyabeana* are involved in nutrient cycling as ammonia oxidizers. This study gave a unique view into the root and rhizosphere microbiomes of *S. miyabeana* well established in a mixed contaminated soil for six years. Our findings and observations provide valuable and useful clues to a better understanding of plant- microbiome interaction, which could be used to improve agronomic techniques which rely on the use of microorganisms (inoculum and engineer) to increase performances of willows under contaminated soil.

Supplementary Materials: The following are available in the section 4.7, Figure S 4.1: Track visualisation, Figure S 4.2: Rarefaction curves, Figure S 4.3: Boxplot of distance to centroid, Figure S 4.4: MA-plots showing fold difference in the normalized count abundance ASVs, Table S 4.1 Tukey multiple comparisons of mean beta-dispersions for each group sample.

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4.7 Supplementary Materials

Figure S 4.1 Track visualisation of (A) fungal; (B) bacterial; and (C) archaeal reads recovered from the whole dataset at different bioinformatic steps.

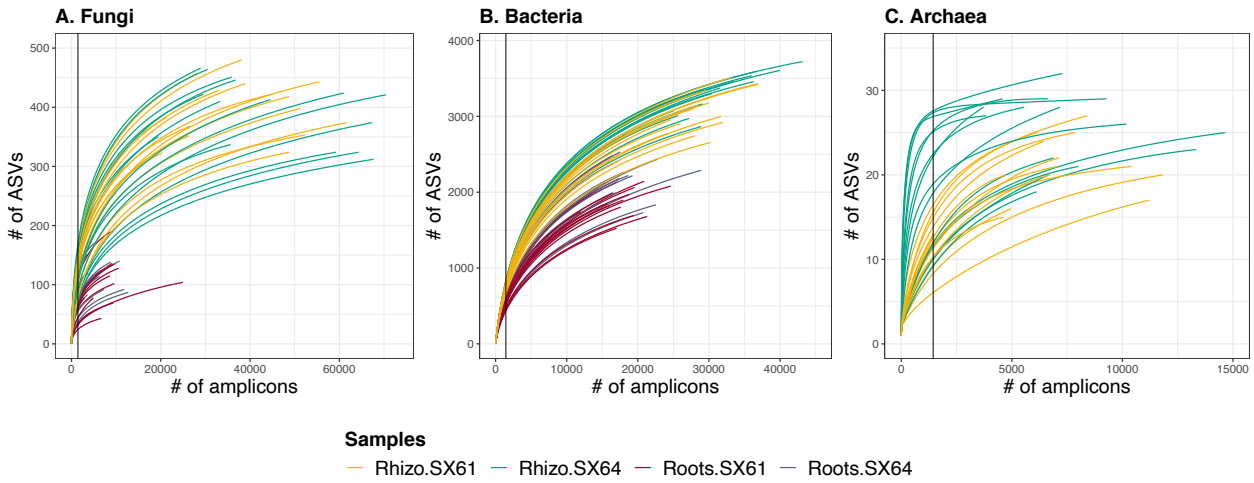

Figure S 4.2 Rarefaction curves of (A) fungal; (B) bacterial; and (C) archaeal ASVs by sequence sample size.

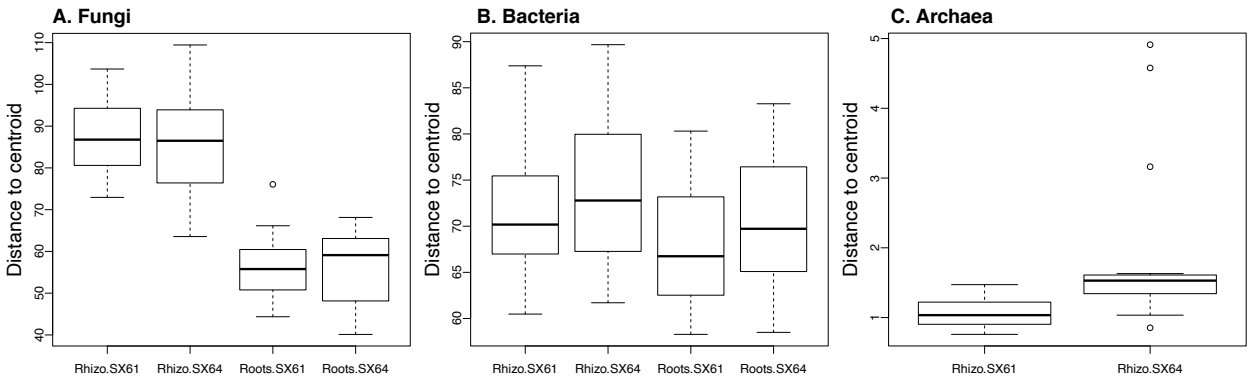

Figure S 4.3 Boxplot of distance to centroid based on beta-dispersion analysis of (A) fungal; (B) bacterial; and (C) archaeal community in each group sample.

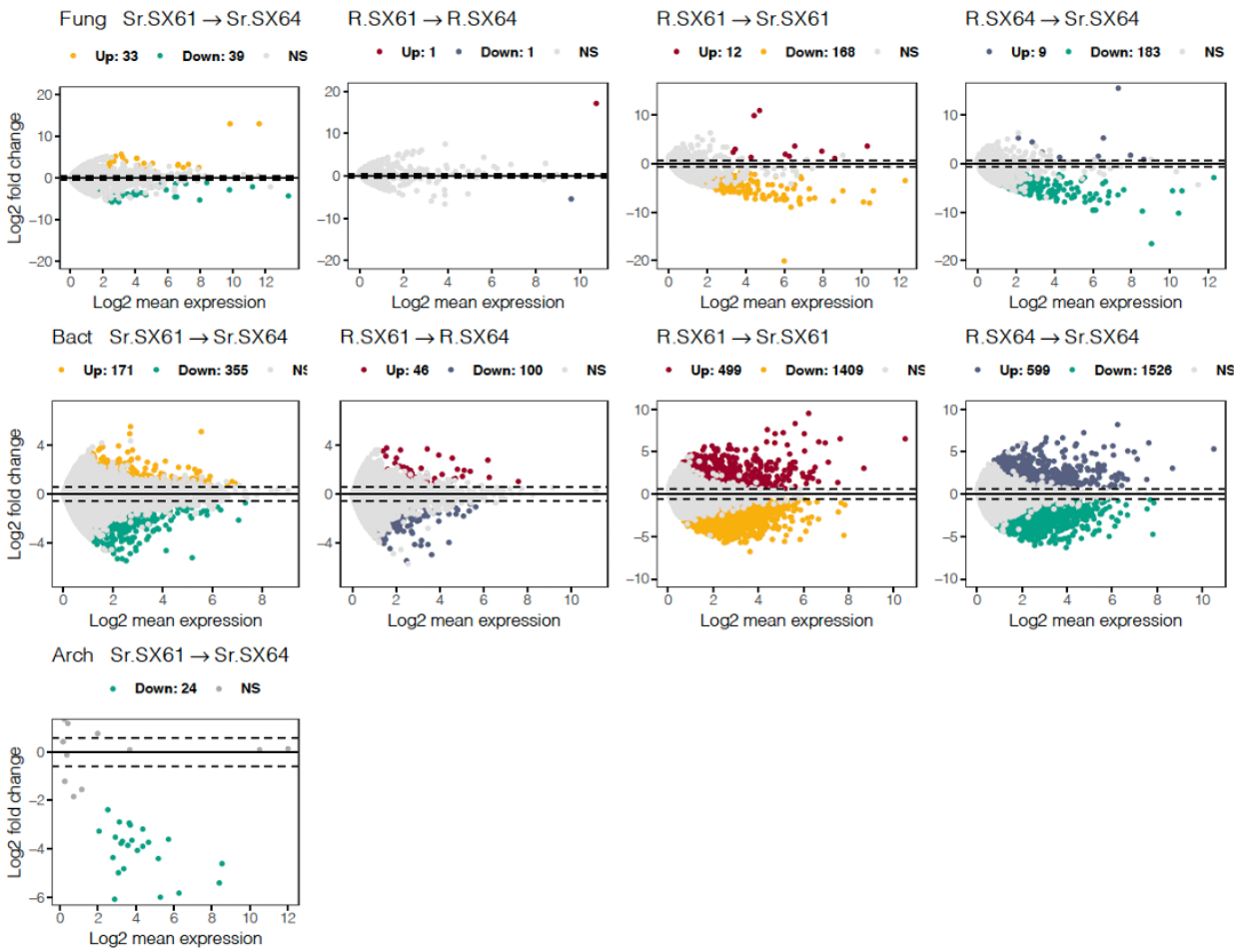

Figure S 4.4 MA-plots showing fold difference in the normalized count abundance of ASVs between both cultivars and between their respective compartments. All fungal (first line), bacterial (second line) and archaeal (third line) ASVs that showed significant differences between two groups (p_value_adj < 0.05) are highlighted in color according to the group hosting it higher normalized count abundance: Rhizo.SX61: Yellow; Rhizo.SX64: Green; Roots.SX61: Red and Roots.SX64: Blue. The gray dots are the other ASVs.

Table S 4.1 Tukey multiple comparisons of mean beta-dispersions for each group sample.

Group sample	Interaction	Difference	Lower limit	Upper limit	p adj
Fungi	Rhizo.SX64 - Rhizo.SX61	-2,20859	-11,8735	7,456335	0.930
	Roots.SX61 - Rhizo.SX61	-31,1987	-40,8637	-21,5338	**<0.001*****
	Roots.SX64 - Rhizo.SX64	-29,2571	-38,922	-19,5921	**<0.001*****
	Roots.SX64 - Roots.SX61	-0,26691	-9,93183	9,398017	0.100
Bacteria	Rhizo.SX64 - Rhizo.SX61	2,063289	-5,12985	9,256429	0.872
	Roots.SX61 - Rhizo.SX61	-4,00992	-11,2031	3,183216	0.459
	Roots.SX64 - Rhizo.SX64	-2,86515	-10,0583	4,32799	0.718
	Roots.SX64 - Roots.SX61	3,208063	-3,98508	10,4012	0.641
Archaea	Rhizo.SX64 - Rhizo.SX61	0,871322	0,198514	1,54413	**0.013***

Significance levels (p-value) are shown and asterisks ($*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$) indicate a significant difference between two group samples.

Chapitre 5 | Synthèse générale

5.1 Rappel des objectifs et synthèse des principaux résultats

5.1.1 Chapitre 2

La première étude réalisée dans le cadre de cette thèse visait à évaluer l'impact d'un apport en BRF et en SCE sur l'efficacité phytoremédiatrice des deux cultivars de saules ('SX61' et 'SX64'), établis depuis quelques années (4 ans) sur un site contaminé. Les résultats ont montré que le recouvrement de sol avec du BRF n'avait eu aucun effet sur la croissance des deux cultivars de saules. Cet amendement organique semble néanmoins avoir contribué à immobiliser certains HAPs dans le sol, en plus d'avoir augmenté l'efficacité des deux cultivars à phytoextraire le Zn. L'ajout de SCE au BRF n'a montré aucun effet significatif sur la croissance des saules, ainsi que sur leur efficacité à extraire ou à modifier (réduire ou augmenter) la concentration des contaminants présents sur le site. La présence du cultivar 'SX61' semble avoir réduit l'atténuation naturelle des C10-C50. La concentration de tous les autres contaminants dans le sol n'aurait pas été affectée par la présence des saules, malgré les évidences que certains ÉTMs ont été substantiellement éliminés du milieu par phytoextraction. Les concentrations de BPCs, de Cd, de Ni et de dix HAPs, ont montré des oscillations saisonnières sur le site expérimental, ce qui suggère que l'évapotranspiration qui a lieu à l'intérieur de la plantation de saules peut affecter la mobilité de certains contaminants.

5.1.2 Chapitre 3

La deuxième étude présentée dans cette thèse a été conduite afin de vérifier si les augmentations de concentrations observées précédemment pouvaient être liées à la transpiration des saules. L'objectif de recherche était donc d'évaluer l'impact de l'élimination du couvert végétal évapotranspirant sur la variation des contaminants organiques et inorganiques à la surface du sol. Tel qu'attendu, les résultats obtenus ont montré que l'élimination du couvert végétal avait bel et bien minimisé l'augmentation de concentration de certains contaminants à la surface du sol, comme observé dans les parcelles intactes (non coupées) de la plantation. Ces résultats viennent appuyer l'hypothèse proposée dans le deuxième chapitre de cette thèse, selon laquelle les saules pourraient faciliter la migration des contaminants vers leurs racines, possiblement grâce à leur taux d'évapotranspiration élevé. L'augmentation des concentrations de contaminants qui en découle peut paraître surprenant et incompatible avec les objectifs de phytoremédiation. Toutefois, ce type de phénomène est fort probablement observable parce que certaines diminutions se produisent

ailleurs, ce qui est pertinent et souhaitable dans un contexte de gestion du risque à faible investissement.

5.1.3 Chapitre 4

Une troisième et dernière étude a été réalisée dans cette thèse, afin d'améliorer les connaissances sur le microbiome de saules bien établis en milieu contaminé depuis plusieurs années. L'objectif de cette recherche était de caractériser les communautés microbiennes (champignons, bactéries, archées) associées aux racines des deux cultivars de saules établies sur le site contaminé depuis six ans. Les résultats de cette étude descriptive ont révélé des différences de composition microbienne, entre les deux cultivars de saules, ainsi qu'entre leurs compartiments. L'abondance et la fonction écologique connue de certains microorganismes, a permis d'identifier quelques taxons fongiques, bactériens et archéens qui pourraient présenter un potentiel intéressant pour améliorer les performances phytoremédiatrices de *Salix miyabeana* en milieu naturel.

5.2 Questionnements soulevés

5.2.1 Phytoremédiation à grande échelle

La phytoremédiation se révèle être une approche alternative très prometteuse pour la décontamination des sols. Elle est peu onéreuse, par rapport aux méthodes conventionnelles et elle bénéficie d'une forte acceptabilité sociale (Weir et Doty, 2016). Au cours des dernières décennies, notre compréhension des mécanismes impliqués s'est beaucoup enrichie, et de nombreuses études réalisées en laboratoires et en serres ont montré des résultats très encourageants. Toutefois, il est important d'admettre qu'il y a encore beaucoup de travail à faire, puisque très peu d'études de terrains aboutissent à des conclusions aussi prometteuses et fructueuses (Gerhardt et al., 2017). Il est donc impératif d'évaluer de manière critique, les raisons de cette dichotomie existante entre les études de terrains et celles qui sont réalisées en milieux contrôlés (i.e., laboratoires et serres).

Dans la littérature, les défis de terrain sont généralement attribués, d'une part, aux facteurs environnementaux, qui sont moins présents en laboratoires et en serres, et d'autre part, aux méthodes d'évaluation qui peuvent ne pas être adéquates pour montrer l'efficacité réelle des plantes pour réhabiliter de grandes surfaces de sol en milieu naturel (Gerhardt et al., 2009). Un des problèmes les plus fréquemment rencontrés dans les études de terrains est la répartition inégale des contaminants dans le sol, qui complexifie la démonstration statistique d'effets significatifs d'un traitement par rapport à un autre (Courchesne et al., 2017b; French et al., 2006). Afin de limiter les

biais causés par une telle distribution hétérogène des contaminants, il devrait être impératif que tous les échantillons de sol soient collectés à proximité de leur premier échantillon respectif (moins de 30 cm), tel que décrit et recommandé dans les deux premiers chapitres de cette thèse (Fortin Faubert et al., 2021b, 2021a). Augmenter le nombre d'échantillons analysés par parcelle est une autre façon d'atténuer le problème de la variabilité spatiale, mais cette option est généralement peu réaliste dû à des contraintes de temps et de ressources (Gerhardt et al., 2009). Il peut néanmoins être envisageable et judicieux de considérer, à la fois les valeurs de 5% ($p < 0,05$) et de 10% ($p < 0,10$) comme seuil statistique pour identifier les différences « significatives » et « marginalement significatives » (Gerhardt et al., 2009; Yates et al., 2021).

Dans le deuxième et troisième chapitre de cette thèse, une telle flexibilité dans les analyses statistiques a certainement aidé à identifier des tendances et un schéma général qui suggèrent fortement qu'une culture intensive de saules à courtes rotations consomme et transpire suffisamment d'eau pour entrainer une migration de certains contaminants en direction des racines, et ainsi augmenter leurs concentrations au fil du temps (Fortin Faubert et al., 2021b, 2021a). Puisque ce phénomène de pompage se déroule en période de croissance, lorsque le taux de transpiration des végétaux est à son maximum, il pourrait également être responsable d'oscillations saisonnières, tel qu'observé chez de nombreux contaminants dans le premier chapitre de cette thèse (Fortin Faubert et al., 2021b). À la lumière de ces résultats, il semblerait sage d'utiliser des jeux de données qui proviennent d'échantillons récoltés au même moment de l'année, afin de suivre l'évolution des contaminants sur une échelle d'années, plutôt que de saisons. Cette fréquence d'échantillonnage semble peu commune dans la littérature scientifique, mais elle permettrait certainement de minimiser les sources d'erreurs liées aux facteurs saisonniers.

Les résultats présentés dans le deuxième chapitre de cette thèse montrent que la présence de saules n'a pas eu d'effet significatif sur les variations globales (GV) des concentrations en ÉTM dans le sol, malgré les évidences que des quantités considérables de Cd, de Cu et de Zn ont été éliminées du sol par phytoextraction (Fortin Faubert et al., 2021b). Cette incohérence traduit bien la complexité liée à l'évaluation de l'efficacité réelle des plantes pour éliminer les contaminants en milieu naturel. La migration de certains contaminants pourrait donc masquer les effets de phytoextraction et/ou de dégradation et conduire à des résultats d'assainissement peu concluants. L'impact des amendements de sol organiques sur la dynamique des contaminants dans le sol

pourrait également avoir été masqué par ce phénomène. Par conséquent, une méthode d'évaluation basée uniquement sur des échantillons de sol, collectés à proximité des arbustes, pourrait ne pas donner une estimation précise de l'efficacité des processus de phytoremédiation.

Les conséquences d'une plantation de saules sur l'hydrologie du sol et sur la migration des contaminants seraient marginales dans les expériences réalisées en laboratoires et en serres (système fermé). Cependant, elles contribueraient, non seulement aux difficultés d'évaluer l'efficacité réelle des plantes pour décontaminer les sols en milieux naturels (système ouvert), mais elles pourraient également se traduire par des augmentations de concentrations de certains contaminants, tel que rapporté dans cette thèse. Ce phénomène a rarement été abordé dans les études de phytoremédiation, ce qui pourrait être dû aux conditions édaphiques particulières et nécessaires à son observation, ou encore parce qu'il a tendance à générer des résultats d'assainissement peu concluants. Il est possible que des résultats ne suggérant aucun effet, voire même des effets opposés à la décontamination des sols (augmentation), aient été observés à maintes reprises, mais oubliés au fond d'un tiroir (effet de tiroir), car considérés comme incomplets et impropres à leur publication (Ashkarran et al., 2021; Lishner, 2021). Par conséquent, la littérature scientifique est généralement dominée par des études qui parviennent à confirmer une hypothèse, produisant ainsi un biais de publication (Mlinarić et al., 2017; Sharma et Verma, 2019). Un tel biais dans le domaine de la phytoremédiation, pourrait en partie contribuer aux succès limités des études de terrains, pour évaluer l'efficacité réelle des plantes à décontaminer les sols.

5.2.2 Analyse des communautés microbiennes

La caractérisation des communautés microbiennes par « metabarcoding » présente un potentiel intéressant par rapport aux approches traditionnelles basées sur la culture en laboratoire, ou l'observation microscopique et macroscopique. Il est cependant important de reconnaître que cette technique comporte une série d'étapes (i.e., échantillonnage, extraction d'ADN, amplification d'ADN, séquençage, analyses bio-informatiques et analyses statistiques), dont les composantes varient grandement d'une étude à l'autre. Certains biais peuvent donc être introduits à chacune de ces étapes, ce qui soulève de nombreux questionnements quant à la reproductibilité et la validité des résultats obtenus.

Il est bien connu que la structure des communautés microbiennes est fortement influencée par une panoplie de facteurs biotiques et abiotiques (Dagher et al., 2019; Rohrbacher et St-Arnaud,

2016). Puisque plusieurs de ces facteurs varient en fonction des saisons, il est raisonnable d'assumer que les résultats obtenus dans cette thèse auraient fort probablement pu être différents si les échantillons avaient été récoltés à un autre moment de l'année, tel que rapporté dans différentes études (Hrynkiewicz et al., 2010; Marjanović et al., 2015; Snyder, 2005). Comme pour la plupart des études qui examinent le microbiome racinaire, ce sont les racines fines, à proximité du tronc, qui ont été échantillonnées, broyées et homogénéisées, avant d'être caractérisées. Une différente approche d'échantillonnage aurait pu modifier considérablement nos observations, puisque le diamètre et l'ordre hiérarchique des racines se trouvent à être des facteurs qui influencent fortement la composition des communautés microbiennes (King et al., 2021; Yates et al., 2021).

Au moment de l'extraction d'ADN, différents produits, tels que des métaux, des polysaccharides et des acides humiques, peuvent s'introduire dans les échantillons et influencer l'étape d'amplification d'ADN par PCR. Ces inhibiteurs de PCR peuvent interagir directement avec l'ADN ou interférer avec les polymérases (Bessetti, 2007). Dans cette thèse, différents kits d'isolement d'ADN (i.e., MO BIO's PowerSoil (QIAGEN, Toronto, ON, CAN) et DNeasy Plant Mini Kit (QIAGEN, Toronto, ON, CAN)) ont été utilisés, afin de minimiser l'introduction de tels inhibiteurs spécifiques aux sols et aux tissus végétaux. Les résultats de cette thèse suggèrent qu'aucune archée ne vivait dans les parties internes des racines de saules. Toutefois, une concentration élevée en inhibiteur de PCR, combinée à une faible concentration d'ADN d'archée, pourraient avoir provoqué l'échec de la génération d'amplicons d'archée à partir de tous les échantillons de racines. À l'aide d'un spectrophotomètre UV-visible NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA), un contrôle de qualité a été effectué sur tous les échantillons d'ADN (données non présentées). La moyenne des ratios 260/280 obtenues sur les extraits de sol rhizosphérique (n = 30) était de ~1,8, ce qui est généralement accepté comme « pur » pour l'ADN (*Interpretation of Nucleic Acid 260/280 Ratios*, 2012). En contrepartie, la moyenne des ratios mesurée sur les extraits de racines (n = 30) était sensiblement inférieure (~1,4), ce qui peut indiquer la présence d'inhibiteurs de PCR, tels que des protéines, des phénols ou d'autres contaminants qui absorbent fortement à une longueur d'onde près de 280 nm (Thermo Scientific, 2015).

Les amorces utilisées pour l'amplification d'ADN par PCR peuvent grandement influencer la composition des communautés microbiennes observées. La sélection de la meilleure paire d'amorces est un défi de taille, puisqu' il existe une panoplie de combinaisons possibles, capables

de couvrir des diversités globales très variées, en ciblant diverses régions d'ADN pour générer des amplicons de différentes tailles (Klindworth et al., 2013). Les amorces qui visent la région ITS sont largement utilisées pour l'identification taxonomique des champignons (Martin et Rygiewicz, 2005; Toju et al., 2012). Toutefois, cette région d'ADN n'est pas très efficace pour capter la diversité des gloméromycètes et les amorces qui visent la région de l'ARNr 18S seraient beaucoup mieux adaptées pour l'étude de ces derniers (Lee et al., 2008). Tel que mentionné dans le quatrième chapitre de cette thèse, la sélection d'amorces visant la région ITS, pourrait en partie expliquer qu'aucun gloméromycète n'ait été détecté dans les échantillons de racines et de rhizosphère de *Salix miyabeana*. Chez les bactéries et les archées, ce sont les régions hypervariables du gène codant pour l'ARNr 16S qui sont ciblées pour l'identification taxonomique (Klindworth et al., 2013; Takahashi et al., 2014). L'idéal aurait été d'utiliser une combinaison de plusieurs amorces, mais les contraintes de temps et de ressources nous ont forcé à en utiliser une seule par domaine (i.e., champignons, bactéries et archées). Les trois paires d'amorces utilisées dans cette thèse avaient le potentiel de couvrir un pourcentage élevé de taxons fongiques, bactériens et archéens (Klindworth et al., 2013; Toju et al., 2012). De plus, ils ont fait leurs preuves dans d'autres travaux (Bell et al., 2015, 2021; Dagher et al., 2019; Danovaro et al., 2016; Davis et al., 2016; Hays et al., 2016; Javurek et al., 2016; Jiang et al., 2016; Nunoura et al., 2010; Pray, 2017; Robichaud et al., 2019; Sands et al., 2014; Santelli et al., 2008; Ye et al., 2009; Yergeau et al., 2015), et la taille des amplicons générés (~319 bp pour les champignons, ~291 bp pour les bactériens et ~290 bp pour les archées) semblait idéale pour permettre aux deux lectures de 250 bp (Illumina MiSeq PE250), d'être fusionnées avec la plus grande superposition possible, et ainsi assurer la fiabilité des résultats. Cette dernière stratégie semble avoir fonctionné, puisque les plus petites régions de chevauchement observées après les traitements bio-informatiques étaient de 12 bp chez les champignons, de 24 bp chez les bactéries et de 21 bp chez les archées.

Plusieurs types de mutation (i.e., substitutions, insertions, délétions, inversions) peuvent survenir pendant les processus de synthèse d'ADN. Lors d'une synthèse biologique, des systèmes de vérification et de réparation des séquences permettent de minimiser ces erreurs (Lynch, 2010). Toutefois, lors d'une synthèse chimique, il n'y a aucun système qui vérifie et répare les séquences d'ADN, ce qui augmente la fréquence de mutation, qui serait de l'ordre de 1 sur 5000 lors d'une réaction PCR avec une *Taq* polymérase (Potapov et Ong, 2017), de 1 sur 1000 lors d'un séquençage par synthèse avec la technologie illumina MiSeq PE250 (Illumina, 2021), et de 1 sur 100 lors de la

synthèse d'une amorce (Alpha DNA, 2019). Bien qu'il existe des méthodes de purification supplémentaires, aucune d'elles ne permet d'éliminer toutes les molécules mutantes, suite à une synthèse chimique (Alpha DNA, 2019).

Une sortie (output) de séquençage par synthèse du système Illumina, contient donc des centaines de milliers de séquences erronées, qui doivent être filtrées ou corrigées, afin d'améliorer la fiabilité des analyses subséquentes de caractérisation des communautés microbiennes. Plusieurs approches tentent de corriger ces erreurs par un filtrage de qualité, suivi par la construction d'unités taxonomiques opérationnelles (OTU), qui regroupent les séquences en fonction de leur similitude (généralement 97%) (Dagher et al., 2020a; Lee et al., 2019; Shao et al., 2019). Le regroupement des séquences en OTU a ses avantages, mais il exclut la possibilité d'identifier des variations à échelle fine. Ce problème peut être résolu en utilisant le progiciel DADA2, qui permet de modéliser et corriger les erreurs à l'intérieur des séquences, afin de différencier celles qui varient d'un seul nucléotide (Callahan et al., 2016).

Une fois les tables de séquences variantes d'amplicons (ASV) en main, il est possible de calculer divers indices de diversité alpha, qui possèdent tous des forces et des faiblesses. Les analyses de diversité beta peuvent également être effectuées de différentes façons. Il existe en effet plusieurs possibilités pour transformer les données, pour calculer leurs distances, ainsi que pour en visualiser le produit. Dans cette thèse, ce sont les distances euclidiennes qui ont été calculées sur les données de comptage d'ASV normalisées en VST, avant d'être soumises à une PERMANOVA et visualisées à l'aide d'analyses des composantes principales (PCA). Afin de valider l'interprétation des résultats, d'autres combinaisons de transformation et de calculs de distances ont été testées (données non présentées). Malgré les légères différences observées sur les sorties de PERMANOVA (i.e., R^2, F.Model et Pr(>F)) et les appréciations visuelles des ordinations, les interprétations statistiques sont demeurées inchangées.

La présente section ne présentait que certains exemples qui contribuent aux limites méthodologiques liées à la caractérisation des communautés microbiennes par « metabarcoding ». Bien que la méthodologie utilisée dans cette thèse adopte des pratiques rigoureuses qui permettent de réduire au maximum les sources d'erreur, il aurait été simple et judicieux d'appliquer la démarche d'identification taxonomique sur une « mock community », afin d'effectuer un contrôle de qualité. La caractérisation des communautés microbiennes, par « metabarcoding », est

certainement une approche imparfaite, mais elle demeure très intéressante puisqu'elle permet de mieux décrire la composition des communautés microbiennes en identifiant des taxons, qui étaient auparavant négligés ou simplement inconnus.

5.3 Perspectives et opportunités d'avenir

Bien que la phytoremédiation soit une approche alternative très prometteuse pour la décontamination des sols, nous sommes forcés de constater qu'elle est encore sous-utilisée et peine à être commercialisée partout dans le monde (Gerhardt et al., 2017), incluant au Québec (Hébert et Bernard, 2013). Il semblerait que cette approche ne soit pas priorisée, en partie attribuable au fait que les parties prenantes ont des connaissances limitées quant à son potentiel d'action (Cundy et al., 2013; Onwubuya et al., 2009). Il est donc important d'utiliser les médias conventionnels et réseaux sociaux afin d'éduquer et impliquer un public plus large. Il va de soi qu'un meilleur transfert des connaissances se fasse également au sein de la communauté scientifique, notamment en publiant les résultats qui semblent moins concluants, afin de minimiser les biais de publication dû à l'effet de tiroir.

L'industrie semble frileuse face à l'idée de dépenser des ressources financières, sans être en mesure de garantir le succès et les délais d'un système biologique, dont les performances dépendent fortement de variables incontrôlables (i.e., météo, maladie, ravageurs). Si les résultats présentés dans les deux premiers chapitres de cette thèse avaient été observés par une firme d'experts en réhabilitation des sols, celle-ci aurait probablement été très embarrassée d'annoncer à leur client que le sol traité par phytoremédiation présente maintenant des concentrations de contaminants plus élevées qu'avant l'intervention. Des résultats semblables pourraient décourager l'industrie à poursuivre ses activités en phytoremédiation, puisqu'ils cadrent difficilement dans une réglementation basée sur des délais et des critères génériques très stricts. Toutefois, un cadre règlementaire plus flexible basé sur des analyses de risques pourrait considérer les bénéfices de confinement d'un couvert végétal évapotranspirant, et ainsi favoriser l'utilisation des plantes comme approche alternative pour la « phytogestion » des sols contaminés (Gerhardt et al., 2017).

Les résultats présentés dans le deuxième et troisième chapitre de cette thèse, suggèrent fortement que les taux d'évapotranspiration de la plantation de saules étaient suffisamment élevés pour confiner, voir même attirer certains contaminants du sol près des racines. Ce phénomène mérite une attention particulière puisqu'il pourrait non seulement contribuer à minimiser le

lessivage et le ruissellement de certains contaminants vers les terrains avoisinants et les écosystèmes aquatiques, mais aussi parce qu'il pourrait permettre de réduire la charge de contamination des sols adjacents ou des masses d'eau souterraine. Ces impacts sont sans aucun doute souhaitables pour le développement d'une stratégie de gestion du risque nécessitant peu d'investissement. De plus, considérant les effets potentiels du saule sur l'attraction des contaminants du sol, il serait intéressant d'explorer la possibilité d'utiliser la culture intensive de saule comme stratégie pour contrôler la migration de certains contaminants, et favoriser leur accumulation un endroit précis dans l'espace. Cette approche pourrait servir de prétraitement pendant quelques années et ainsi réduire les quantités de sols à excaver.

Toutes les grandes municipalités du Canada sont confrontées à la problématique de contamination des sols (FCM, 2015). Bien évidemment, parmi l'ensemble des sites contaminés, plusieurs ne peuvent pas être entièrement décontaminés par une approche de phytoremédiation. Cependant, puisque plusieurs d'entre eux sont vacants et abandonnés en raison des frais exorbitants associés aux méthodes conventionnelles nécessaires à leur décontamination (FCM, 2015, 2016; MDDELCC, 2017; Tremblay, 2016), ces municipalités pourraient incontestablement valoriser ces espaces perdus en y implantant une végétation à court, moyen ou long terme.

L'utilisation de saules et de peupliers en culture intensive à courtes rotations est de plus en plus considérée comme une solution économique simple et avantageuse pour la phytogestion de friches contaminées en contexte urbain (McHugh et al., 2015; Padoan et al., 2020). En plus d'être en mesure de répondre à des besoins directs d'assainissement, les plantes qui composent ces infrastructures vertes améliorent la gestion des eaux de ruissellement, tout en limitant le lessivage et la propagation des contaminants vers les eaux souterraines et les terrains avoisinants, contribuant ainsi à une meilleure stratégie de gestion du risque (Ferro et al., 2003; Fortin Faubert et al., 2021a; Mirck et Volk, 2010; Rüth et al., 2007). De plus, une telle transformation du paysage en milieu urbain apporte des changements durables, notamment en augmentant la séquestration des gaz à effet de serre (Cunniff et al., 2015; McHugh et al., 2015), en réduisant l'effet d'îlots de chaleur (Al-Saadi et al., 2020; Pearsall, 2017), en améliorant la biodiversité (Pandey et Maiti, 2020; Villaseñor et al., 2020) et en atténuant la pollution de l'air (Nowak et al., 2006; Prigioniero et al., 2021; Weyens et al., 2015). Tous les services écosystémiques associés au verdissement urbain deviennent particulièrement importants dans un contexte de développement durable et de résilience des villes

face aux changements climatiques (Maure et al., 2018; Pandey et Maiti, 2020). Puisque ce contexte climatique fait pression grandissante sur les décideurs et parties prenantes pour qu'ils adoptent des pratiques durables et respectueuses de l'environnement, il est concevable et réaliste de croire que la demande pour la phytoremédiation et la phytogestion des sites contaminés soit à la hausse dans les prochaines années. L'ensemble de cette thèse fournit de précieuses informations qui contribueront certainement à améliorer les stratégies de mise en œuvre, de suivi et d'évaluation de ces approches appliquées à grande échelle avec *Salix* spp.

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