Université de Montréal

Bioremédiation de sols en milieu nordique

Des ressources locales pour traiter une variété d'hydrocarbures pétroliers et autres contaminants avec la phytoremédiation, la mycoremédiation et l'aide de matières résiduelles fertilisantes

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Résumé

Les hydrocarbures pétroliers sont expédiés et utilisés dans pratiquement toutes les parties du monde et sont devenus l'un des contaminants parmi les plus communs et les plus répandus dans les sols. Les éléments traces sont parfois associés aux hydrocarbures, ce qui peut augmenter la toxicité d'un site, compliquer les voies possibles de remédiation, et augmenter les coûts de traitements traditionnels. Dans les régions nordiques, où des déversements sont recensés dans de nombreux sites, le temps de nettoyage des contaminants et les coûts des méthodes d'assainissement traditionnelles peuvent s'avérer considérablement plus élevés que dans les régions tempérées. Des recherches alternatives visant une remédiation plus rapide et moins coûteuse, adaptée à des climats subarctiques, sont nécessaires et l'utilisation de plantes et champignons indigènes à l'écosystème local sont des approches prometteuses. En parallèle, les gouvernements de multiples pays visent à réduire les émissions de gaz à effet de serre, notamment par la réduction des déchets putrescibles voués à l'enfouissement. En utilisant ces matières résiduelles fertilisantes obtenues localement dans le processus de décontamination, une opportunité se présente pour valoriser ces matières tout en décontaminant des sites.

Cette recherche doctorale vise à développer des méthodes de bioremédiation adaptées aux conditions locales, avec des intrants indigènes aux régions d'étude, pour des sols contaminés aux HCP au sein de trois projets de recherche à grande échelle sur le terrain, dont un également contaminé aux éléments traces.

Deux projets ont été effectués dans un climat subarctique au 60° parallèle (Whitehorse, Yukon), en utilisant une approche novatrice de phytoremédiation assistée de champignons et compost municipal; nous qualifions cette technique intégrée d'approche par « microsystème écologique ». Comme le volume de sol affecte beaucoup l'efficacité des méthodes de bioremédiation, des volumes considérables de 0.15 m^3 et 1 m^3 ont été utilisés (au premier et deuxième site, respectivement), pour maximiser la pertinence des résultats lors de transfert d'échelles futures. Au premier site, l'efficacité des différentes composantes du microsystème a été évaluée en bacs dans quatre différentes combinaisons et comparée au

traitement de base habituel (fertilisant) dans un sol contaminé par un déversement accidentel de diésel. La plante choisie était le saule *Salix planifolia* et le champignon *Pleurotus ostreatus*. Les résultats indiquent qu'après une saison de traitement, le microsystème était le traitement avec le taux d'élimination du diésel le plus rapide. Après trois saisons, les traitements contenant un ou plusieurs éléments du microsystème avaient des taux de contamination sous les normes pour des sols agricoles et étaient plus efficaces que le traitement au fertilisant ou que l'atténuation naturelle. Le deuxième site était une fosse à huiles usées située sur une pile de stériles miniers au cœur d'un ancien dépotoir. Le microsystème a été implanté dans des cellules au sol avec une doublure à l'épreuve du lessivât de contaminants. Les saules *Salix alaxensis* et *Salix planifolia* furent utilisés en combinaison avec le champignon *Trametes versicolor*. Cette approche, dans un sol hautement contaminé, a réussi à diminuer de façon considérable les hydrocarbures pétroliers (plus de 65 à 75 %). Le potentiel d'accumulation des métaux dans les tissus aériens des plantes a également été mesuré. Les deux espèces de saules ont démontré des taux d'accumulation d'éléments traces distincts et des stratégies racinaires différentes. Un champignon de la famille des *Psathyrellaceae* fut observé pour la première fois sur un site si hautement contaminé, puis est apparu de façon récurrente sur les cellules du microsystème pendant quatre ans. Les deux premiers sites répondent à un besoin de développer des méthodes de bioremédiation efficaces, passives et applicables en climat subarctique.

Le troisième site de recherche porte sur l'utilisation de deux matières résiduelles fertilisantes (bois raméal fragmenté et drêche) et de fumier pour la dégradation de l'huile à moteur dans un climat continental humide à une latitude moyenne (Neuville, Québec). Des mésocosmes de 0.76 m^3 avec aération contrôlée furent utilisés. L'utilisation de bois raméal fragmenté et de drêche de brassage à cette échelle, de même que l'acquisition des matières résiduelles dans un rayon très rapproché du centre de traitement afin d'explorer une approche d'économie circulaire dans un tel contexte, constituent les volets novateurs de cette étude. Les résultats démontrent que le fumier est plus efficace que le traitement habituel au fertilisant. La drêche de brassage et le bois raméal fragmenté sont utiles pour conserver l'humidité dans les sols (un paramètre de bioremédiation important). Cette étude, menée en collaboration avec un partenaire industriel, s'intègre dans la politique canadienne et québécoise de réductions des

gaz à effets de serre et la philosophie de l'économie circulaire en valorisant des matières organiques résiduelles locales.

Cette thèse a permis de démontrer qu'une combinaison de stratégies de bioremédiation avec des composantes locales est une méthode efficace dans un climat subarctique. Deux nouvelles espèces de saules (*Salix planifolia* et *Salix alaxensis*) ont été utilisées avec succès pour la bioremédiation d'hydrocarbures pétroliers et pour l'accumulation de certains éléments traces. Lors d'une collaboration avec un partenaire industriel, il a été possible de démontrer l'applicabilité de certains concepts d'économie circulaire et d'approche écosystémique en bioremédiation.

Mots-clés : Bioremédiation, Climat nordique, Phytoremédiation, Mycoremédiation, Hydrocarbures pétroliers, Éléments traces, Matières résiduelles fertilisantes.

Abstract

Petroleum hydrocarbons are shipped and used in virtually all parts of the world and have become one of the most common and widespread contaminants in soils. Trace elements are sometimes associated with them, and they can increase the toxicity of a site, complicate remediation, and increase the costs of traditional treatments. In northern areas where spills occur at multiple sites, the clean-up time and cost of traditional remediation methods can be significantly higher than in temperate regions. Alternative research aimed at faster and cheaper remediation adapted to subarctic climates is needed and the use of native plants and fungi integrated into the local ecosystem are promising approaches. Concurrently, governments in multiple countries aim to reduce greenhouse gas emissions, namely through the reduction of putrescible waste destined for landfills. By using locally-sourced residual fertilizing materials for decontamination processes, an opportunity arises to valorize these materials while restoring soils.

This doctoral research aims to develop locally-adapted bioremediation methods, with indigenous plant and fungal inputs, to treat petroleum hydrocarbon contaminated soils in three large-scale field research projects, including one also contaminated with trace elements.

Two projects were carried out in a subarctic climate at the 60 ° parallel (Whitehorse, Yukon), using an innovative approach of phytoremediation assisted by mushrooms and municipal compost; we call this integrated technique the "ecological microsystem" approach. Because soil volume has significant impacts on the efficiency of bioremediation methods, considerable volumes of 0.15 m^3 and 1 m^3 were used (at the first and second sites, respectively) to maximize the relevance of results in the event of a scale-up operation. At the first site, the effectiveness of the various components of the microsystem were evaluated in four different combinations, as well as compared to the usual basic treatment (fertilizer) in soil contaminated by an accidental diesel spill. The plant species chosen was *Salix planifolia* and *Pleurotus ostreatus* was selected for the fungus. Results indicate that after one treatment season, the microsystem was the treatment with the fastest diesel removal rates. After three seasons, treatments containing one or more elements of the microsystem were below standards for agricultural soils and were more effective than fertilizer treatment or natural attenuation.

The second northern site was a waste oil pit located on top of a mine waste rock pile, at the heart of an old landfill. The microsystem was implanted into ground-level cells with a contaminant-proof leachate liner. The plant species *Salix alaxensis* and *Salix planifolia* were used in combination with the fungus *Trametes versicolor*. This approach in a highly contaminated soil was able to significantly reduce petroleum hydrocarbons (65 to 75%). The potential for metal accumulation in aerial plant tissues was also measured. Both willow species demonstrated distinct trace element accumulation patterns and different rooting strategies. A fungus of the *Psathyrellaceae* family was observed for the first time at a site so highly contaminated and was recurrent on the cells of the microsystem for 4 years. The first two sites contribute to the development of efficient and passive bioremediation methods applicable in subarctic climates.

The third research site focused on the use of two residual fertilizing materials (fragmented rameal wood and brewer's spent grain) and manure for the degradation of motor oil in a humid continental climate at a medium latitude (Neuville, Quebec). Mesocosms of 0.76 m^3 with controlled aeration were used. Innovative aspects of this project include the use of rameal wood and spent grain at this scale, as well as the acquisition of residual materials in a very close radius of the treatment center to explore how a circular economy approach could apply in such a context. Results indicate that the addition of manure is more effective than the usual fertilizer treatment alone. Brewer's spent grain and fragmented rameal wood were useful for maintaining soil moisture (an important bioremediation parameter) but did not increase degradation. This study, conducted in close collaboration with an industrial partner, falls under the objectives set by Canadian and Quebec policy on greenhouse gas reductions and circular economy approaches by working on the valorization of local residual organic matter.

This thesis has demonstrated that combining strategies with local biological components is an effective bioremediation method in a subarctic climate. Two new willow species (*Salix planifolia* and *Salix alaxensis*) have been successfully used for the bioremediation of petroleum hydrocarbons and for the accumulation of certain trace elements. In collaboration with an industrial partner, it has been possible to demonstrate the applicability of certain circular economy concepts and the ecosystemic approach to bioremediation.

Keywords: Bioremediation, Cold climate, Phytoremediation, Mycroremediation, Petroleum hydrocarbons, Trace elements, Residual fertilizing materials.

Table des matières

Liste des tableaux

CHAPITRE 1

CHAPITRE 2

CHAPITRE 3

CHAPITRE 4

Liste des figures

CHAPITRE 1

CHAPITRE 2

CHAPITRE 3

CHAPITRE 4

Liste des abréviations

(En italique sont les termes en anglais.)

ABH :	Site de recherche Arctic Backhoe
BRF :	Bois Raméal Fragmenté
CA :	Certificat d'Authorisation
CCME :	Conseil Canadien des Ministres de l'Environnement
CDB :	Secrétariat De La Convention Sur La Diversité Biologique
ET :	Élément Trace
Etc. :	Et cætera
HCP :	Hydrocarbures Pétroliers
HAM :	Hydrocarbures Aromatiques Monocycliques
HAP :	Hydrocarbures Aromatiques Polycycliques
i.e. :	« id est », expression latine signifiant « c'est-à-dire »
LTU :	*Land Treatment Unit* (Unité de traitement des sols contaminés)
MELCC :	Ministère de l'Environnement et de la Lutte contre les changements climatiques
MRF :	Matières Résiduelles Fertilisantes
PEx :	Expérience en pots située au YRC
PHC :	*Petroleum Hydrocarbons*
SD :	*Standard Deviation*
SOL :	Expérience en gros cylindres de béton au LTU de Solneuf
TE :	*Trace Elements*
TOC :	*Total Organic Carbon*
Y2C2 :	*Yukon Youth Conservation Corps*
YRC :	*Yukon Research Center*
WOP :	Site de recherche Son of War Eagle Landfill's Waste Oil Pit

Je dédie cette thèse à

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CHAPITRE 1. | Introduction Générale

Les hydrocarbures pétroliers et les éléments traces sont des contaminants problématiques et très répandus. Cette thèse explore des méthodes de bioremédiation avec des champignons, des plantes indigènes au Yukon et des matières résiduelles fertilisantes locales, pour s'insérer dans une philosophie de transition vers des technologies vertes et durables.

La problématique des sols contaminés

Un site pollué[1] est un endroit qui contient des substances à des concentrations supérieures à ce que l'on retrouve dans des sites non atteints, et qui sont susceptibles d'avoir des effets néfastes pour la santé humaine ou l'environnement (Newman, 2014). Ces sites peuvent être en milieux urbains ou industrialisés, mais aussi en zones rurales et dans des régions éloignées comme le Grand Nord. La contamination de ces sites peut provenir d'une multitude de sources, notamment des dépotoirs, des usines et opérations industrielles, des bases militaires, des activités minières, ainsi que l'exploitation du pétrole et les déversements liés à son transport et son utilisation. Il existe beaucoup de sites contaminés issus d'une époque où les normes environnementales n'étaient pas aussi sévères qu'aujourd'hui (BVGC, 2012). Malgré tout, de nombreux déversements accidentels ont toujours lieu. La pollution anthropique est un problème croissant qui exerce des facteurs de stress considérables sur les écosystèmes dans le monde entier (Tortella et al., 2005).

L'Agence de Protection de l'Environnement des États-Unis (US EPA) estime qu'il y a entre 10 et 25 millions gallons d'hydrocarbures pétroliers (HCP) déversés chaque année aux États-Unis seulement (2015). En 2011, 22,000 sites contaminés (confirmés ou présumés) étaient inscrits dans l'inventaire fédéral canadien. Entre 2005 et 2012, le gouvernement canadien a dépensé plus de 1,5 milliard de dollars pour gérer ce problème, et il est estimé qu'il y a un passif environnemental d'au moins 7,7 milliards au Canada seulement (BVGC, 2012). Ce passif est lié au fait que beaucoup de sites contaminés restent abandonnés, en grande part à cause des coûts très élevés associés à leur remédiation. Les frais de gestion et les impacts environnementaux peuvent perdurer des décennies après la contamination initiale, et ce, particulièrement en milieu nordique. En 2013, le programme des sites contaminés du Nord a signalé la présence de 166 sites contaminés dans le nord-ouest du Canada (Yukon, TN-O. et Nunavut), d'une valeur équivalente à 2,3 milliards de dollars (AANDC, 2013). La gestion de

[1] Les termes « contaminant » et « polluant » sont souvent utilisés comme synonymes, mais il existe quelques distinctions entre les deux. Un contaminant est une substance qui est relâchée par l'humain, tandis qu'un polluant est une substance qui a un impact négatif mesurable (Newman, 2014). Néanmoins, les deux termes sont utilisés de façon plutôt interchangeable au sein du domaine des sols contaminés et il a été fait de même dans cette thèse.

sites contaminés peut s'avérer plus dispendieuse dans le Nord si l'accès aux sites est restreint, car ils se trouvent en région éloignée. De plus, le temps de remédiation peut être plus long à cause des climats froids qui ralentissent l'activité biologique liée à l'assainissement des sites (i.e. les processus microbiens et la croissance des plantes) (Filler et al., 2006).

Cette thèse s'est penchée sur le développement et l'application de méthodes passives et potentiellement moins coûteuses, applicables à des régions éloignées et urbaines, en se basant sur l'utilisation de ressources locales (organismes biologiques indigènes et matières organiques), en accord avec les principes décrits dans la section suivante.

Approche écosystémique & Économie circulaire

Certains processus d'assainissement des sols consomment beaucoup d'énergie et, dans certains cas, le transport des matériaux peut être la principale cause des émissions de carbone (Khan et al., 2004; Sanscartier et al., 2010). US EPA reconnait le besoin d'adopter des approches plus vertes et durables dans l'industrie du nettoyage des sols contaminés. Ils ont identifié les éléments clés afin améliorer l'impact environnemental des projets de remédiation de sites contaminés sur leur page « Greener Clean-up » (US-EPA, 2016). Les principales actions à entreprendre sont résumées dans la Figure 1.1.

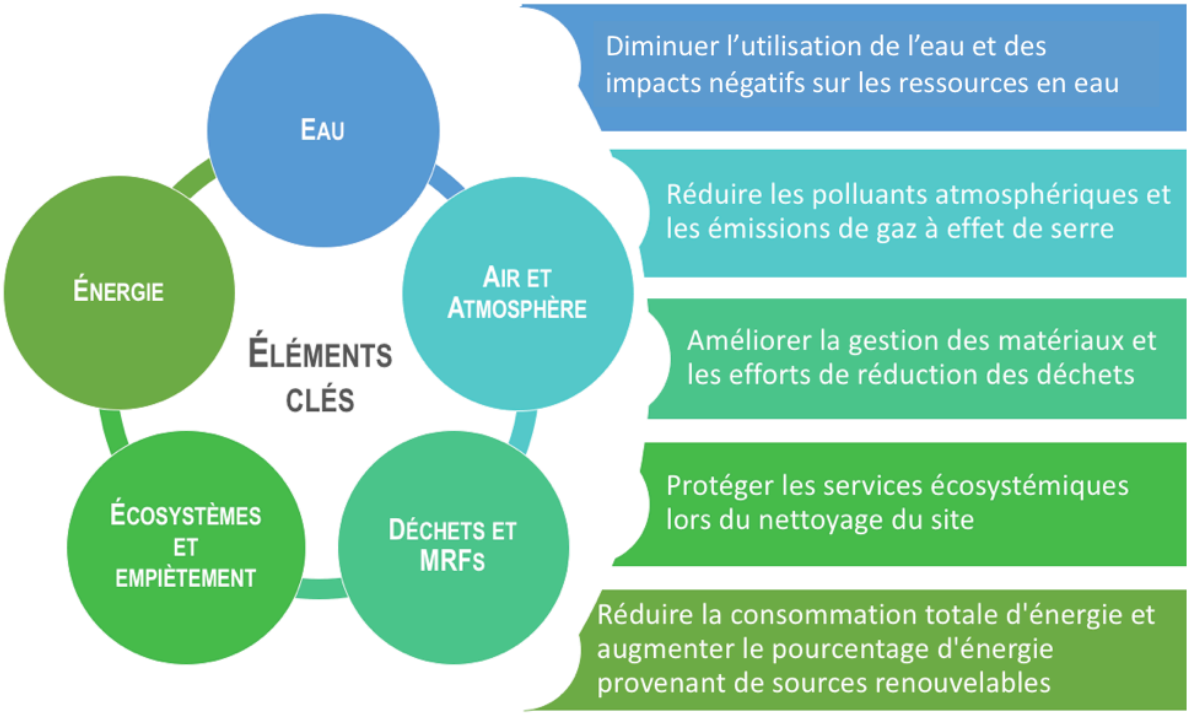

Figure 1.1 Les éléments clés pour améliorer l'impact environnemental des projets de remédiation de sites contaminés (adapté de US-EPA, 2011).

Réduire les intrants et améliorer les pratiques tout en respectant l'intégrité écologique sont les grandes lignes de ces recommandations et elles sont plus aisées à réaliser avec une équipe pluridisciplinaire. La communauté scientifique reconnaît de plus en plus l'importance de la recherche multidisciplinaire et Robertson et al. (2007) souligne que c'est également souhaitable pour les régions boréales. Il existe deux grandes écoles de pensées dans ce domaine, soit l'économie circulaire (Sauvé et al., 2016) et l'approche écosystémique (Christensen et al., 2013) qui ont guidé le cadre décisionnel et moral de cette thèse en sciences biologiques (Figure 1.2). L'économie circulaire est un concept émergent qui examine comment consommer des biens et des services sans dépendre de l'extraction de ressources naturelles brutes, fermant ainsi les boucles et empêchant l'élimination des matériaux dans les décharges à ordures. Contrairement au modèle « d'économie linéaire » habituel, les impacts de la consommation de ressources sont pris en compte (Sauvé et al., 2016). L'approche écosystémique quant à elle, souligne le fait que la planète est un milieu clos où tout est lié. Lors de la gestion des ressources, cette dernière approche accorde une importance équivalente

à une bonne gestion de l'environnement, aux facteurs économiques et aux aspirations de la communauté. L'approche classique tend plutôt à hiérarchiser ces besoins en priorisant d'abord les questions économiques, puis les projets de la communauté, souvent au détriment de l'environnement (Christensen et al., 2013; Lebel, 2003).

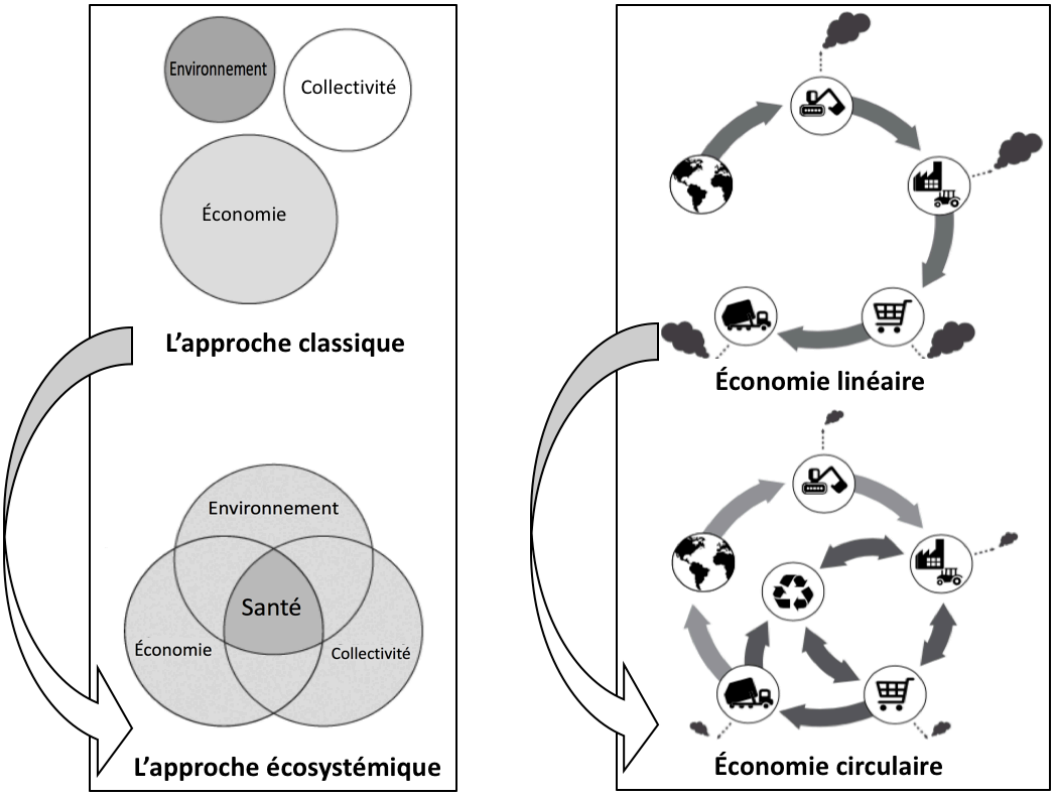

Figure 1.2 Modèles comparatifs entre l'approche classique et l'approche écosystémique (gauche) et entre l'économie linéaire et l'économie circulaire (droite) (adapté de : Lebel, 2003, et Sauvé et al., 2015).

Le but de cette thèse n'est pas de prouver ou de réfuter ces concepts, mais plutôt de les appliquer dans la mesure du possible, et d'intégrer dans la prise de décision, non seulement les impacts environnementaux des méthodes de bioremédiation choisies, mais aussi les engagements envers la communauté et les entreprises locales. Ce sont des concepts émergents en développement et il n'est pas évident de les appliquer à l'intérieur d'un cadre rigide. Néanmoins, la prise de décision tout au long de cette thèse a été influencée par ces deux approches. Ces concepts ont également orienté l'approche de bioremédiation en « microsystème écologique » utilisée au Yukon. Plus de détails sur cette approche se trouvent à

la fin de la section suivante (Figure 1.4). Finalement, une explication détaillée des retombées se trouve dans la conclusion.

Méthodes de remédiation de sols contaminés

La remédiation[2] est l'action de nettoyer et revitaliser des sols contaminés par les hydrocarbures pétroliers (HCP) et les éléments traces (ET), en les retirant du sol, en les immobilisant ou en les transformant en produits moins nocifs. Il existe une grande variété d'approches et de procédés pour décontaminer les sols qui sont groupés en trois grandes classes de méthodes : chimiques, physiques et la bioremédiation. L'organigramme de la Figure 1.3 présente un aperçu de celles-ci en spécifiant le type de contaminants visé par chacune des méthodes. Le choix de la technologie de décontamination est spécifique à chaque site et basé sur une multitude de facteurs. Parmi ces facteurs, mentionnons la nature du sol, les propriétés du site (par ex. l'hydrogéologie), les types de contaminants et leurs concentrations, la profondeur de la contamination, le budget disponible, les contraintes de temps, l'accès au site (par ex. s'il est en région éloignée), l'utilisation future du site, la réglementation locale et l'évaluation des risques pour la santé humaine et les écosystèmes environnants (Mulligan et al., 2001).

[2] Le terme « assainissement » est en quelque sorte un synonyme du terme « remédiation », la nuance étant que l'assainissement est souvent utilisé lors du processus, tandis qu'une remédiation est l'étape finale visée. Toutefois, les deux termes sont utilisés de façon plutôt interchangeable au sein du domaine des sols contaminés et il a été fait de même dans cette thèse.

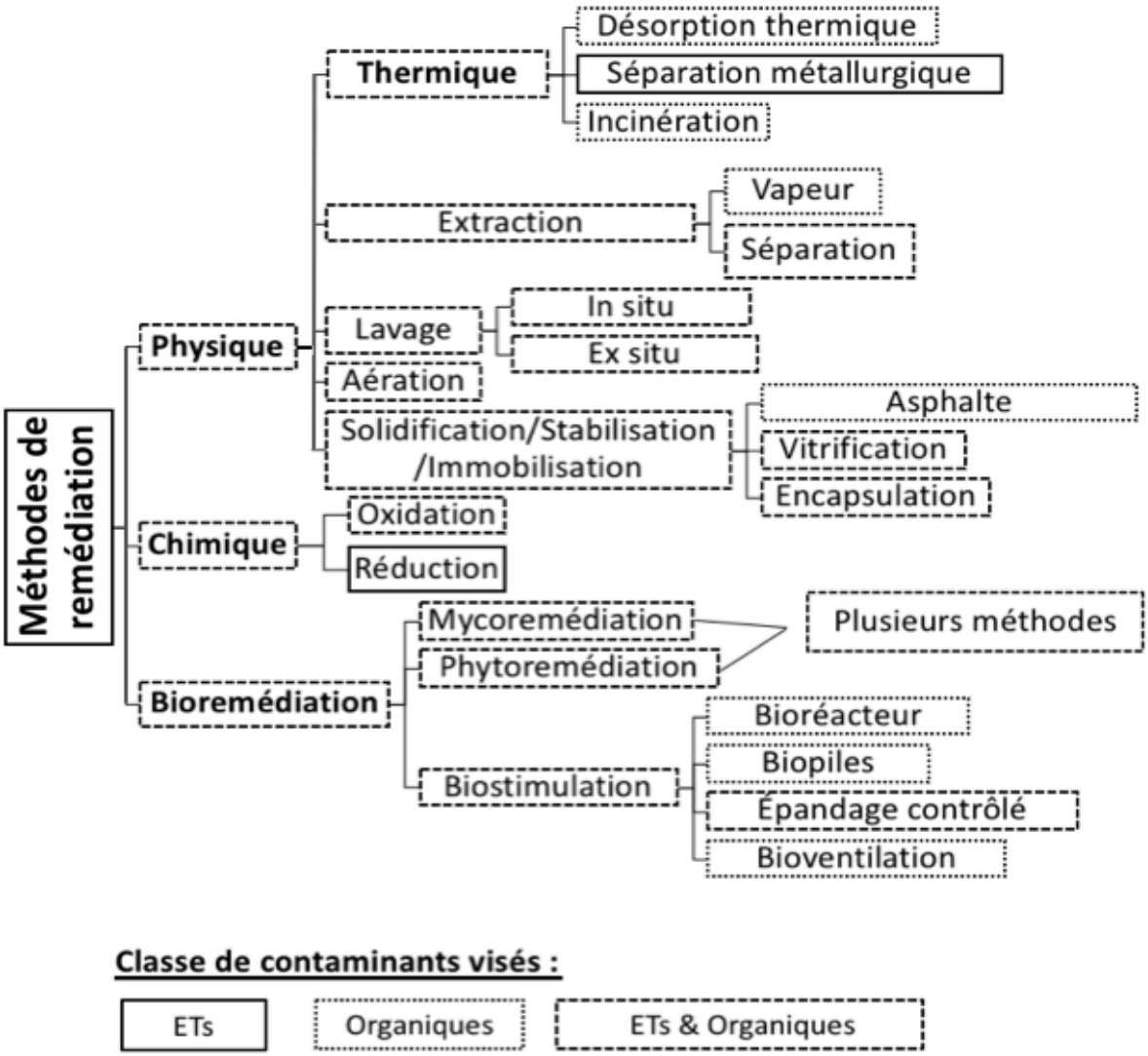

Figure 1.3 Méthodes de remédiation de sols contaminés aux éléments traces (ETs), aux composés organiques ou aux deux (données de: Ivshina et al., 2015; Khan et al., 2004; Mani and Kumar, 2014; C. Mulligan et al., 2001).

La majorité des approches physiques et chimiques requièrent des intrants extérieurs importants (combustible, chaleur, fertilisants, équipement spécialisé, etc.) et peuvent donc s'avérer hautement énergivores (Ivshina et al., 2015; Khan et al., 2004). Dans le cadre de cette thèse, une importance particulière a été portée sur le développement de technologies ayant le moins d'impact écologique possible. Par conséquent, nous avons choisi la méthode de la bioremédiation, car elle est généralement moins énergivore que les autres approches, tout en respectant davantage les principes de l'approche écosystémique. La bioremédiation est une

approche définie par l'utilisation de plantes, de champignons et de consortiums microbiens pour dégrader ou détoxifier des contaminants environnementaux (Juwarkar et al., 2010; Peter, 2011). Les communautés microbiennes du sol sont les acteurs-clés des systèmes de bioremédiation ; elles peuvent agir directement sur les contaminants et moduler leur disponibilité aux plantes et à certains champignons (Srivastava et al., 2014). Dans la section suivante, les éléments de bioremédiation utilisés, soit la phytoremédiation, la mycoremédiation et la biostimulation (du type biopiles) seront discutés plus en détail.

Phytoremédiation

La bioremédiation faisant appel aux plantes est appelée phytoremédiation. Il existe de nombreux mécanismes par lesquels les plantes peuvent effectuer de la décontamination. Ces mécanismes entrent dans les catégories suivantes (Juwarkar et al., 2010; Peter, 2011) :

- **Phytotransformation** – décomposition de contaminants organiques via des processus métaboliques au sein de la plante ou à l'extérieur (i.e. enzymes) ;
- **Rhizodégradation et stimulation** – l'assainissement des contaminants dans la zone des racines ;
- **Phytostabilisation** - immobilisation de contaminants organiques et inorganiques ;
- **Phytoextraction** - extraction et accumulation de contaminants inorganiques dans les tissus de la plante ;
- **Phytovolatilisation** - dispersion de substances volatiles à travers les feuilles ;
- **Évapotranspiration** - contrôle du débit hydraulique sur le site contaminé.

Plusieurs plantes sont de bonnes candidates pour la phytoremédiation, et des travaux récents démontrent que l'utilisation concomitante de plusieurs espèces pourrait être bénéfique pour l'assainissement de sols contaminés aux HCP et ETs (Desjardins et al., 2018). Dans le cadre de cette thèse, deux espèces appartenant au genre *Salix* ont été choisies et implantées en boutures, mais aucun désherbage n'a été effectué pour permettre l'établissement spontané d'autres espèces qui pourraient contribuer à la remédiation. Les *Salix* ont été sélectionnés pour les raisons suivantes : ce sont des plantes résistantes à croissance rapide; elles sont originaires

de la zone de recherche; et elles sont couramment utilisées dans un large éventail de projets de bioremédiation. Dans les régions nordiques, les *Salix* ont démontré un bon potentiel pour la dégradation des BPC (biphényles polychlorés) (Slater et al., 2011). Des recherches menées au Yukon indiquent que les espèces de saules en général sont de bons indicateurs de concentrations environnementales élevées en zinc (Zn) et cadmium (Cd) (Pugh et al., 2002). Généralement, plus les concentrations de métaux dans le sol sont élevées, plus les saules en accumuleront. Toutefois, cela n'est pas applicable de façon unilatérale à toutes les espèces ni aux individus qui poussent sur des sites fortement contaminés, car ceux-ci développent parfois une résistance, qui peut résulter en une accumulation réduite. La présence de certains métaux tels que le zinc (Zn) peut augmenter l'absorption d'autres métaux tels que l'arsenic (As) et le cadmium (Cd) dans les feuilles (Meers et al., 2007; Roy et al., 2005; Vyslouzilova et al., 2003). La définition d'une plante hyperaccumulatrice est qu'elle doit accumuler plus de 1000 mg kg^{-1} (masse sèche) d'un métal (Cook et al., 2010), mais plusieurs espèces de plantes qui en accumulent en moindre quantité, peuvent néanmoins être très utiles. Certaines espèces du genre *Salix* ont également démontré un certain potentiel pour l'assainissement des hydrocarbures. Cook (2010) a démontré avec succès l'implantation de saules sur un site contaminé aux HCP : 97% des saules plantés ont survécu en comparaison à 63% de survie chez le peuplier (Cook et al., 2010). Une autre étude a mis en évidence une dégradation du phénanthrène et du pyrène de 100% et 80% respectivement, avec la présence de *Salix viminalis*, contre seulement 68% et 63% en son absence (Hultgren et al., 2009). Par contre, les recherches ne sont pas toutes concluantes à ce sujet. Une étude de Roy et al. (2005) n'a démontré aucune dégradation significative des hydrocarbures aromatiques polycycliques (HAP) dans un sol alcalin lors de l'utilisation *Salix viminalis.* Les saules sont également connus pour leur capacité à fortement stimuler les populations microbiennes dans les sols, ce qui peut conduire à une amélioration de la bioremédiation, principalement dans la zone racinaire (Hultgren et al., 2009).

Dans le cadre de cette thèse, nous avons identifié les principaux rôles et avantages de la phytoremédiation : 1) Encourager la dégradation des contaminants organiques dans leur

rhizosphère en formant des relations mycorhiziennes[3] et en favorisant la formation d'un habitat pour les bactéries. 2) Réduire l'accumulation d'eau par processus d'évapotranspiration. 3) Favoriser la phytoextraction et l'accumulation des ET du sol. Pour remplir l'une ou plusieurs de ces fonctions, deux espèces indigènes au Yukon furent choisies :

Salix alaxensis longistylis: Ce saule, communément connu sous le nom de saule d'Alaska, varie de la taille d'un arbuste jusqu'à 10 m de haut. Cette espèce est fortement recommandée pour les projets de végétalisation, en particulier dans les sols humides, car elle se propage facilement à partir de boutures (Collet, 2004). Cette espèce n'est pas très connue pour la bioremédiation, mais elle s'implante facilement et des botanistes expérimentés suggèrent qu'il n'est pas nécessaire de récolter des boutures d'hiver en dormance pour utiliser cette espèce (Benett, B. Communication personnelle, mai-juin 2013). Slater (2011) a constaté que *S. alaxensis* serait une plante efficace pour la rhizoremediation de certains des biphényles polychlorés (BPC) via la libération de composés phytochimiques lors de la mort des radicelles. *S. alaxensis* a donc été choisi parce qu'il s'implante facilement, semble prometteur pour modifier positivement la structure de la communauté microbienne du sol et représente une des seules espèces nordiques à stimuler la dégradation de composés chlorés.

Salix planifolia : Le nom commun pour ce saule est « saule à feuille plane ». C'est un arbuste dont la hauteur varie de 0.15 à 9 m. Cette espèce est recommandée pour les projets de végétalisation à l'aide de boutures récoltées lors de leur dormance en hiver. Dans une étude portant sur l'accumulation des métaux, *S. planifolia* occupe le dernier rang sur huit espèces comparées pour l'accumulation dans les pousses et les racines (Meiman et al., 2011). Très peu de littérature est disponible sur l'utilisation de cette espèce pour la bioremédiation. (Pour plus de détails sur le choix des espèces de *Salix*, voir l'Annexe I).

[3] Association entre les hyphes du mycélium d'un champignon et les racines d'une plante.

Mycoremédiation

La bioremédiation à l'aide de champignons est appelée mycoremédiation. Comme les champignons tiennent des rôles clés dans la biosphère et représentent jusqu'à 75% de la biomasse microbienne du sol, il est stratégique de les utiliser comme alliés dans la décontamination des sols. Tortella et al. (2005), ont rapporté qu'environ 72,000 espèces de champignons ont été nommées et que près de 700 à 1500 nouvelles espèces s'ajoutent à ce nombre chaque année. À l'aide de leurs hyphes à croissance rapide, ils influencent la structure du sol avec des mécanismes électrostatiques, adhésifs et structuraux. De plus, ils jouent un rôle majeur dans la décomposition de la matière organique (Harms et al., 2011). Leurs hyphes effectuent une importante translocation de composés variés grâce à des mécanismes de diffusion active et passive. Cela permet aux champignons de transporter des nutriments entre des régions à fortes concentrations dans le sol (source) vers des régions à faibles concentrations (puits). Cette capacité peut être mise au profit de la bioremédiation en effectuant le transport de contaminants; en fait, il a été démontré que des hyphes mycéliens peuvent activement transporter des contaminants tels que les HAP à partir de sites à toxicité létale pour les bactéries, jusqu'à des sites plus éloignés où se trouvaient des bactéries capables de dégrader lesdits contaminants (Furuno et al., 2010). Furuno et al. (2012) ont démontré que des hyphes pouvaient transporter les HAP comme le phénanthrène à une vitesse moyenne de 13 ± 9 μm min^{-1}, ce qui est équivalent à un transport de 4.9 nmol h^{-1}. Il est également reconnu que les champignons jouent un rôle important dans le nettoyage de sols contaminés aux HCP (Aranda, 2016, Zytner et al., 2006). Lors de travaux portant sur les biopiles, Grace Liu et al. (2011) ont démontré que les communautés bactériennes dominent le système de dégradation des HCP dans un premier temps, mais qu'après une période de 70 jours, ce sont alors les champignons qui dominent la dégradation de composés plus récalcitrants.

Les types de champignons sélectionnés pour ce projet sont les carries blanches *Pleurotus ostreatus* et *Trametes versicolor* (WRF: White Rot Fungi) qui ont maintes fois démontré leur potentiel de bioremédiation (Haritash and Kaushik, 2009; Okparanma et al., 2011; Pointing, 2001; Young et al., 2015). Les WRF forment un groupe fonctionnel plus que taxonomique; ils se spécialisent dans la dégradation de la lignine. Bien que la lignine soit l'un des principaux constituants de toutes les plantes vasculaires et représente le deuxième

polymère le plus abondant dans la nature, elle est une structure complexe et hétérogène qui est difficile à dégrader pour la plupart des organismes. Les WRF sont donc des recycleurs de carbone essentiels dans la plupart des écosystèmes terrestres (Gargulak et al., 2015; Wong, 2009). Comme la structure de la lignine est semblable à certains contaminants organiques tels que les HAP, les WRF ont également la capacité de les dégrader. Les WRF dégradent généralement la lignine en exsudant les enzymes ligninolytiques oxydantes suivantes: la lignine peroxydase, le manganèse peroxydase, des peroxydases polyvalentes et finalement des laccases (Cvančarová et al., 2012). Ces enzymes sont intrinsèquement agressives et non spécifiques. Les peroxydases produisent du peroxyde d'hydrogène (H_20_2) qui catalyse la dépolymérisation par oxydation de la lignine (Yateem et al., 1998). C'est habituellement une certaine carence nutritionnelle (notamment en azote) qui stimule la production de ces peroxydases. La dégradation de la lignine et des HCP s'effectue donc dans un processus métabolique secondaire (à l'exception de l'espèce *Trametes versicolor* qui semble être en mesure de dégrader les HCP même s'il n'y a pas de carence en azote) (Yateem et al., 1998). Les WRF sont des organismes robustes, et une fois qu'ils sont établis, ils peuvent généralement tolérer des concentrations de contaminants plus élevées que les bactéries (Tortella et al., 2005). *P. ostreatus* dégrade préférentiellement la lignine tandis que *T. versicolor* est moins spécifique et dégrade également d'autres composantes de la paroi cellulaire telles que l'hémicellulose et la cellulose (Wong, 2009). Une des observations importantes lors de l'incorporation de WRF dans l'approche en microsystème de ce projet, est que la dégradation du diésel atteint 96% en 6 semaines, lorsque *P. ostreatus* est couplé avec un consortium bactérien (Facundo et al., 2001).

Dans le cadre de cette thèse, des copeaux de bois ont été ajoutés pour les traitements contenant du mycélium de champignons. Le rôle des copeaux est de servir de source de nourriture et de plateforme de lancement pour aider à propager le mycélium dans le sol. Une expérience en serre, réalisée au printemps 2013, a démontré que le champignon *Pleurotus* sp. colonisait le sol de façon nettement plus efficace en présence de copeaux de bois que seul. (Se reporter à l'Annexe II pour le rapport complet).

Évaluation des risques

Il est important d'évaluer les répercussions écosystémiques indésirables que peut avoir un projet de bioremédiation sur la faune locale. La phytoremédiation peut notamment bioaccumuler des contaminants au sein d'espèces de plantes consommées par les animaux. En utilisant cette méthode, l'accumulation et l'immobilisation des ETs dans la biomasse souterraine des plantes posent peu de risque de mouvement des contaminants à partir de la matrice du sol. Au contraire, l'accumulation des ETs dans la biomasse aérienne, en particulier lorsque des techniques physiques de dépollution *in situ* sont appliquées, peut créer une voie d'exposition pour la faune locale. Les saules sont des espèces fourragères importantes pour de nombreuses espèces animales indigènes du Yukon, telles que l'orignal, le caribou des bois, le lièvre d'Amérique, le castor, le lagopède et le tétras (Argus, 2004; Collet, 2004; Pugh et al., 2002). Un suivi est nécessaire afin de s'assurer que la faune locale ne court pas de risque de transfert d'ETs en consommant des espèces végétales utilisées dans un projet de phytoremédiation. Une évaluation des risques peut être effectuée à partir des concentrations d'ETs dans les parties aériennes des plantes, de la dose journalière moyenne de fourrage par espèce animale et des valeurs de référence de toxicité (Jimmo et al., 2018). En suivant le principe des « Hazard Quotients », cette évaluation permet de fixer un seuil : si la valeur finale du calcul est >1, alors les plantes peuvent poser des risques inacceptables à la faune locale (la méthodologie est détaillée dans le chapitre 3). Un autre risque à évaluer est l'introduction d'organismes non indigènes à la région et potentiellement envahissants dans un écosystème qui ne leur avait pas encore fait face, ce qui est un problème répandu avec la globalisation et l'introduction de cultivars exotiques (Lowe et al., 2004). Un exemple est la restauration de stériles miniers en milieu boréal avec des espèces de graminées non indigènes habituellement retrouvées dans des prairies. Il a été démontré que cette végétation herbacée formait de denses couvertures de végétation pouvant empêcher une colonisation par les arbres indigènes à la région pendant des décennies, ainsi qu'un retour à l'habitat forestier original (Macdonald et al., 2015). Peu d'exemples de champignons sont cités comme étant envahissants, à l'exception de pathogènes sévères de plantes et d'animaux (Lowe et al., 2004), mais une diligence raisonnable devrait être appliquée dans le choix d'espèces fongiques pour la bioremédiation.

Apports organiques et MRF

Les ajouts organiques tels que le compost, le fumier et les matières résiduelles fertilisantes (MRF) à un sol contaminé peuvent s'avérer très avantageux dans le processus d'assainissement. Ils peuvent favoriser une aération accrue et plus uniforme; fournir des apports nutritifs et encourager les populations microbiennes en stimulant leur croissance et en apportant de nouvelles espèces (Battelle and NFESC, 1996; Brady and Weil, 1996a). L'utilisation de tels amendements organiques peut également s'avérer avantageuse sur le plan économique, car ils font partie des solutions les moins dispendieuses (Cole, 1998). Il existe deux grandes façons d'utiliser des amendements organiques lors de la bioremédiation : 1) utiliser du compost mature ou de la matière qui servira d'agent gonflant (bulking agent) ajouté au sol (10% à 30%) (Hupe et al., 1996); 2) utiliser des matières organiques très fraiches (restes de table, fumier frais, etc.) en plus grande proportion pour stimuler une action de co-compostage (50% à 95%) (Cole, 1998, Namkoong et al., 2002, Kirchmann and Ewnetu, 1998). Le compost, le fumier, le bois raméal fragmenté (BRF) et la drêche de brassage ont été utilisés comme amendements organiques dans le cadre de cette thèse.

Compost : Un gramme de compost mature peut contenir de 9 à 69 fois plus de bactéries qu'un terreau fertile et de 3 à 17 fois plus de champignons (Cole, 1998). Il a été démontré qu'il augmente l'aération du sol et stimule significativement une panoplie de microorganismes actifs dans la bioremédiation d'un large spectre de contaminants organiques (Antizar-Ladislao et al., 2004; Hupe et al., 1996; Margesin and Schinner, 1997). Il favorise également la phase très importante de l'établissement des plantes (Guidi et al., 2012) et peut diminuer la mobilisation de certains métaux (Zubillaga et al., 2012).

Fumier de cheval : Kirchmann and Ewnetu (1998) ont trouvé qu'un co-compostage à l'aide de fumier de cheval pouvait réduire une contamination de grosses molécules de paraffine de 80% en 110 jours, alors que les résultats pour la dégradation de résidus pétroliers atteignaient des taux de dégradation de 93% en 50 jours. De son côté, Cole (1998) rapporte que le fumier mature (6 mois) est préférable dans la majorité des cas qu'il a étudiés.

Bois raméal fragmenté : Le BRF a fait ses preuves dans l'accroissement de la fertilité du sol et se distingue des copeaux de bois réguliers parce qu'il est issu de branches de moins

de 7 cm de diamètre et non de troncs entiers (bois caulinaire), ce qui lui confère un ratio d'azote beaucoup plus riche (C :N 50-175 :1 versus 400-600 :1) (Lemieux, 1986). L'utilisation du BRF est très peu citée en bioremédiation, contrairement aux copeaux de bois caulinaire qui sont largement utilisés comme agent gonflant. Hattab et al. (2015) ont tout de même observé qu'il réduisait la toxicité de ETs envers les plantes, cette dernière étant déterminée par la mobilité et la phytodisponibilité.

La drêche de brassage : La drêche est la masse résiduelle de grains humides issue du procédé de fabrication de bière. La drêche est riche en nutriments et contient de 77 à 81% d'eau (g g^{-1}) (Santos et al., 2003). Abioye (2011) mentionne la drêche comme un agent prometteur pour la bioremédiation de sols contaminés aux HCP et aux métaux. Dans une expérience de phytoremédiation à l'aide de la plante *Jatropha curcas* (noix des Barbades), Agamuthu (2010) a démontré qu'en ajoutant de la drêche de brassage à un sol contaminé à l'huile de graissage à des taux de 1% et 2.5%, les taux de dégradation des HCP atteignaient respectivement 96.6% et 89.6%.

L'approche du « Microsystème écologique »

Un écosystème est un ensemble où une communauté biologique est en relation dynamique avec les composantes biotiques et abiotiques de son milieu. Les différentes composantes de l'écosystème (autotrophes, hétérotrophes et l'environnement abiotique) transforment et échangent de l'énergie et de la matière entre elles. (Smith and Smith, 2009a) Donc, dans la nature, les organismes ne fonctionnent pas seuls et il existe des interactions complexes entre eux et leur milieu physique. Habituellement, un système plus complexe est davantage résilient. (El Amrani et al., 2015; Harms et al., 2011) Finalement, un écosystème est défini par des limites spatiales qui sont déterminées par des changements de l'environnement physique sous-jacent et/ou pas la composition des espèces. Bien sûr, à cause des entrées et sorties naturelles de composantes, les frontières en milieu naturel sont souvent floues. (Smith and Smith, 2009b).

Dans le cadre de cette thèse, le principe d'écosystème a été appliqués à la bioremédiation. L'objectif était de créer des petits systèmes se rapprochant d'un modèle naturel, avec une diversité écologique provenant d'au moins trois règnes, qui pourraient

assainir des sols contaminés. Le terme « micro » a été choisi pour illustrer que cette approche est appliquée à une échelle plus petite qu'un écosystème complet. Le terme « écologique » a été choisi car c'est l'écologie est la science des relations entre les organismes et leur environnement. Le microsystème écologique est donc une intégration de multiples composantes biotiques (telles que des plantes, des mycètes, la pédofaune et la pédoflore) et abiotiques (incluant les composantes du sol, la température, l'oxygène, les nutriments, et etc.) visant à stimuler toutes les composantes biologiques vivants en contact avec le sol dans un objectif de bioremédiation de contaminants (Figure 1.4). Les fertilisants inorganiques sont à éviter dans le cadre de cette approche tandis que les composantes biologiques locales et natives à la région de recherche sont privilégiées (voir la section « Méthodes de remédiation de sols contaminés »). Ces microsystèmes sont situés dans un espace défini, préférablement avec des mesures en place pour empêcher le ruissèlement des contaminants (ou autre forme de déplacement), mais ils restent ouverts à des intrants extérieurs comme par exemple les précipitations naturelles et des graines de plantes locales. Lors d'un projet de remédiation, il est important de considérer les propriétés du site, du sol et des contaminants présents. Ces éléments influenceront le choix des composantes biologiques à incorporer. L'approche du microsystème écologique s'imbrique bien dans l'approche écosystémique qui intègre les grandes sphères de la santé et de l'environnement, ainsi que dans l'approche de l'économie circulaire qui valorise les déchets (Figure 1.2).

Les contaminants

Maintenant que les méthodes de remédiation et leurs composantes associées ont été introduites, les contaminants impliqués seront décrits.

Il existe de façon générale deux grandes classes de contaminants : 1) les contaminants organiques qui sont constitués de molécules à base de carbone; et 2) les contaminants inorganiques, qui sont essentiellement des métaux et métalloïdes du tableau périodique des éléments (éléments traces (ETs)) (Newman, 2014). Les molécules de la première classe peuvent être dégradées en composés plus petits lors des processus de remédiation. Par contre, les ETs ne peuvent être dégradés en éléments plus petits. Les modes de gestion sont donc très différents pour ces deux grandes classes. La décontamination est particulièrement difficile

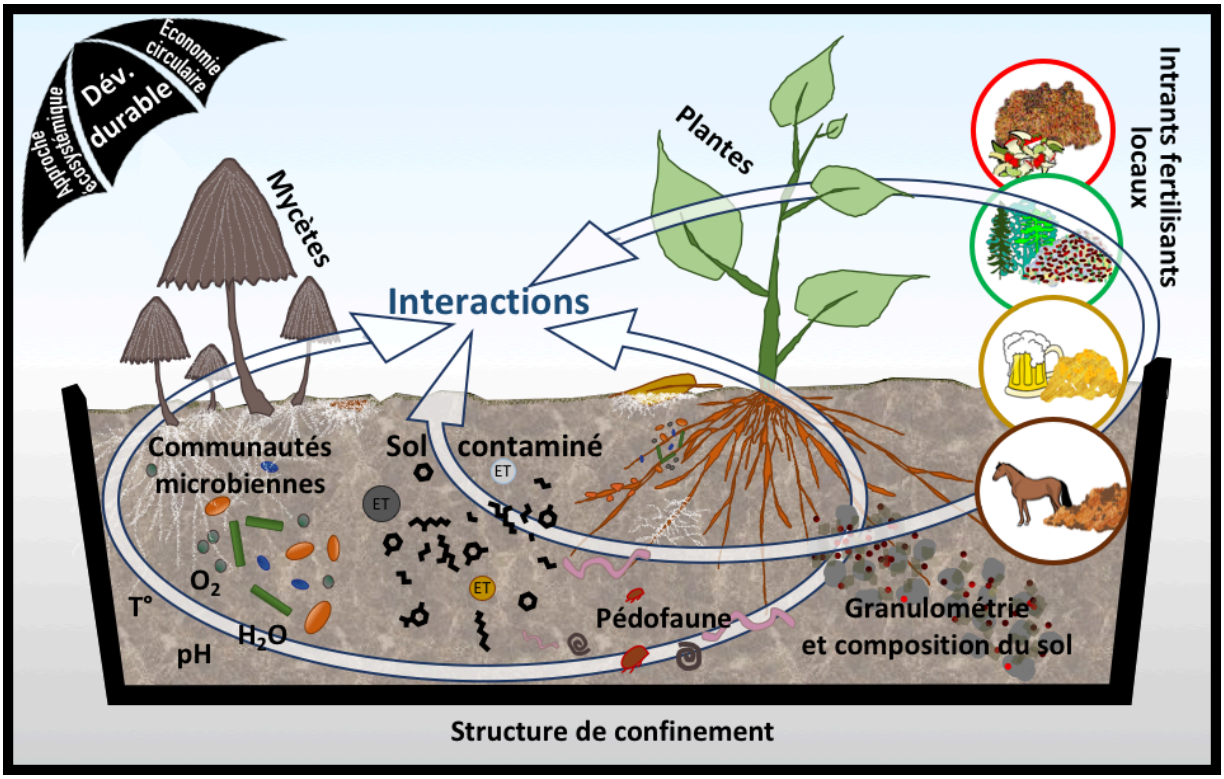

Figure 1.4 Les grandes composantes du microsystème écologique dans un cadre de bioremédiation de sol contaminé.

lorsqu'une contamination dite « contamination mixte », regroupe à la fois plusieurs contaminants organiques et inorganiques. Ce type de contamination est fréquent sur des sites industriels et des dépotoirs. Il est important de mentionner que lorsque plusieurs composés (considérés comme contaminants ou non) sont présents ensemble, leurs interactions peuvent entraîner des seuils de toxicité supérieurs aux effets cumulés de chaque composé respectif (Wuana and Okieimen, 2011). De plus, le nettoyage des sols contenant à la fois des ETs et des contaminants organiques pose des défis supplémentaires tels que l'inhibition des bactéries dégradant les HCP par les ETs, ou encore l'utilisation de méthodes de traitement séquentielles (Dong et al., 2013; Morkin et al., 2000). Des stratégies novatrices et prometteuses, telles que l'utilisation de silicate, sont développées en laboratoire pour la remédiation de sols à contamination mixte. Par contre, elles doivent encore être optimisées pour la réhabilitation des HCP et faire leurs preuves sur le terrain (Camenzuli et al., 2017). Parfois, même après excavation et transport hors site, ces sols à contaminants mixtes sont enfouis plutôt que traités en raison des difficultés techniques et des coûts associés à leur assainissement.

Organiques : Hydrocarbures pétroliers

La famille des hydrocarbures pétroliers (HCP) constitue l'un des nombreux contaminants problématiques actuellement. Les HCP sont des combustibles fossiles tels que le bitume et le gaz naturel. Comme le laisse entendre leur nom, les HCP sont des molécules organiques composées majoritairement de carbone et d'hydrogène. Cependant, dépendamment de leurs origines et du degré de raffinage, les HCP peuvent également contenir de l'azote, du soufre, de l'oxygène et des centaines de composés chimiques, dont certains ajoutés lors du processus de raffinement (ATSDR, 1999). Ils sont expédiés et utilisés dans pratiquement toutes les parties du monde pour alimenter notre mode de vie moderne, notamment comme ressources énergétiques et comme matières premières pour la fabrication des plastiques. En raison de leur grande utilisation et de leur large distribution, y compris les déversements associés, les HCP sont devenus l'un des contaminants les plus communs et les plus répandus sur la planète (Cozzarelli and Baehr, 2005). Par conséquent, la contamination des sols par HCP devient de plus en plus courante dans le monde entier (Peter, 2011). Les HCP raffinés comme l'essence, le diésel et le kérosène sont des types communs d'HCP déversés accidentellement près des communautés nordiques. Mentionnons qu'il n'y a pas nécessairement d'exploitation dans ces communautés, mais bien une dépendance énergétique à ces composés.

Il existe deux grandes classes de molécules chez les HCP : les aliphatiques et les aromatiques (Figure 1.5). Les hydrocarbures aromatiques (HA) sont généralement classés comme plus toxiques (Battelle and NFESC, 1996). Les HA comportent une configuration cyclique souvent représentée par un ou plusieurs noyaux de benzène (HAM: hydrocarbures aromatiques monocycliques; HAP: hydrocarbures aromatiques polycycliques), ou par des hétéroarènes (molécules à arrangement cyclique, où un atome de carbone est remplacé par un atome d'oxygène, d'azote ou de soufre). Les molécules aromatiques contiennent souvent des groupes fonctionnels tels qu'un groupe d'alkyle, un halogène, ou un groupe azoté par exemple. Elles sont généralement moins solubles. Les molécules aliphatiques peuvent être linéaires, ramifiées ou cycliques, mais elles ne contiennent pas de noyaux aromatiques. Ces molécules sont classées en trois groupes structurellement différents: les alcanes (≡C-C≡), les

alcènes (=C=C=) et les alcynes (-C≡C-) (Snape et al., 2008, Aislabie and Foght, 2008, CCME, 2008, 2001).

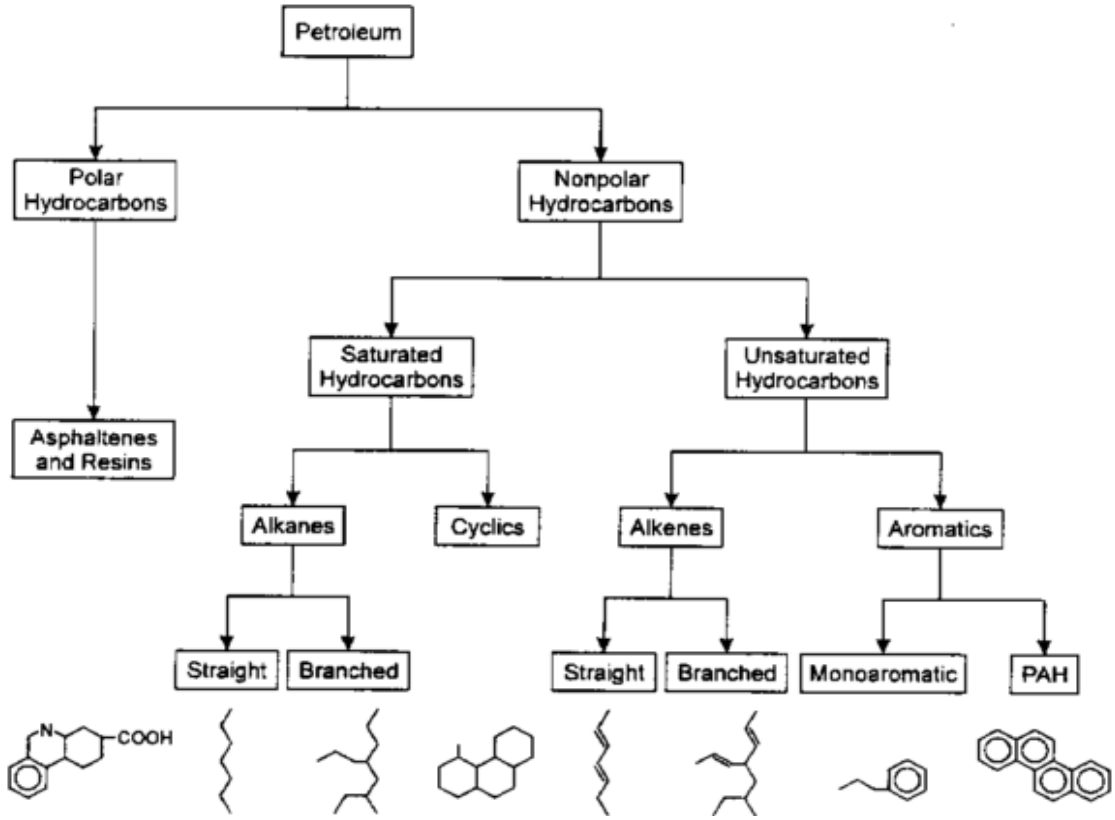

Figure 1.5 Illustration des types de composés organiques dans le pétrole (Battelle and NFESC, 1996).

Selon le Conseil canadien des ministres de l'Environnement (CCME) (2008), le relâchement d'HCP dans l'environnement peut causer plusieurs préoccupations environnementales: 1) La plupart des HCP sont toxiques dans une certaine mesure (par exemple, certains déversements sont responsables de la perte de sources d'eau potable et les molécules aromatiques, qui sont particulièrement toxiques et parfois mutagènes, ont tendance à se bioaccumuler). 2) Ils peuvent dégrader la qualité du sol en interférant avec ses propriétés de rétention d'eau et les mouvements hydrauliques naturels, ainsi qu'avec l'équilibre et l'approvisionnement en éléments nutritifs. 3) Les fractions d'HCP avec une masse moléculaire plus importante et une structure ramifiée sont persistantes dans l'environnement. 4) Les fractions les plus légères sont très mobiles dans le sol, l'eau et l'air, ce qui signifie qu'elles

peuvent parcourir des distances considérables à partir du point d'émission. Les stratégies de bioremédiation des contaminants organiques comportent une multitude d'approches (phytoremédiation, mycoremédiation et biostimulation) et de techniques (biopiles, bioréacteurs, bioventilation et épandage contrôlé) (Figure 1.5).

Organiques : Composés chlorés

Le trichloréthylène (TCE) et le tétrachloréthylène (PCE) (Figure 1.6) ont été largement utilisés comme dégraissants métalliques, mais tous deux sont soupçonnés d'être carcinogènes. Ils sont difficiles à gérer, car ces composés chlorés sont à la fois volatils et plus denses que l'eau. Comme ils possèdent une densité supérieure à 1 (Chemwatch, 2009), cela leur permet de pénétrer profondément dans les aquifères, jusqu'aux sédiments, et de parcourir ainsi de longues distances. Ces molécules se volatilisent également très facilement, mais se dégradent difficilement dans l'atmosphère. Elles sont donc persistantes et problématiques dans l'environnement et c'est pour ces raisons, en plus de leur toxicité, que des taux réglementaires très bas leur sont appliqués, même dans les sols à usage industriel (respectivement 0.01 et 0.6 mg kg^{-1} pour le TCE et le PCE) (CCME, 2007). L'inhalation de vapeurs peut provoquer des somnolences, des vertiges, une irritation des voies respiratoires et des lésions pulmonaires. Le TCE peut aussi produire des effets mutagènes chez l'homme (Chemwatch, 2009; Ryoo et al., 2001; Watts, 2006). Certaines souches bactériennes du genre *Pseudomonas* ont démontré un potentiel de dégradation des TCE et PCE. Cependant, en conditions anaérobiques, le PCE peut être transformé par dés-halogénation réductrice en TCE et autres composés hautement toxiques: le dichloroéthylène et le chlorure de vinyle. Pour éviter la formation de ces sous-produits toxiques, il est préférable de favoriser une dégradation des TCE et des PCE en aérobie (Ryoo et al., 2001; Vogel and McCarty, 1985).

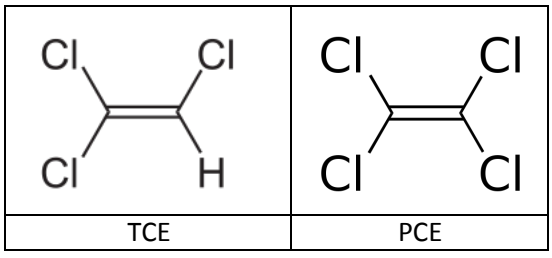

Figure 1.6 Structure moléculaire des composés chlorés rencontrés lors de cette thèse. ("Perchloroéthylène," 2016, "Trichloroethylene," 2016, Wikimedia Commons)

Inorganiques : Éléments traces

Contrairement aux contaminants organiques, les métaux et métalloïdes sont élémentaires et ne peuvent être dégradés en molécules inoffensives. La terminologie varie en fonction des éléments concernés, mais dans le cadre de cette thèse le terme « élément trace » (ET) sera utilisé. Certains ET examinés de plus près dans cette thèse sont considérés comme des contaminants prioritaires par l'agence de protection de l'environnement aux É-U (US EPA), notamment l'arsenic (As), le cadmium (Cd), le cuivre (Cu), le plomb (Pb) et le zinc (Zn) (Cameron, 1992). L'exposition aux ETs par l'eau, le sol et l'air peut avoir de nombreuses conséquences néfastes sur la santé animale et végétale, notamment par des processus de bioaccumulation. La présence simultanée de plusieurs contaminants peut également affecter leurs propriétés. Vysloužilová (2003) a montré que les cinq ETs énumérés peuvent interagir entre eux de façon à modifier leurs taux respectifs de bioaccumulation ainsi que l'emplacement de l'accumulation dans les tissus végétaux. Les champignons mycorhiziens joueraient également un rôle important dans la médiation des ETs absorbés par les racines des plantes (Rajkumar et al., 2012).

Propriétés du sol et biodisponibilité

La concentration d'un contaminant dans un sol ne dicte pas à elle seule s'il sera délétère à la vie végétale ou animale. Un facteur qui influence largement la bioaccumulation et les effets toxiques des contaminants est la biodisponibilité, qui elle-même est fortement affectée par les propriétés du sol. La biodisponibilité est la quantité d'une substance accessible à un organisme en vue de son absorption ou adsorption. L'argile, le limon, le sable, le gravier et la matière organique sont les grandes composantes de base de tous les sols, et elles déterminent l'infiltration et la rétention de l'eau dans la matrice du sol. (Brady and Weil, 1996b) (Figure 1.7).

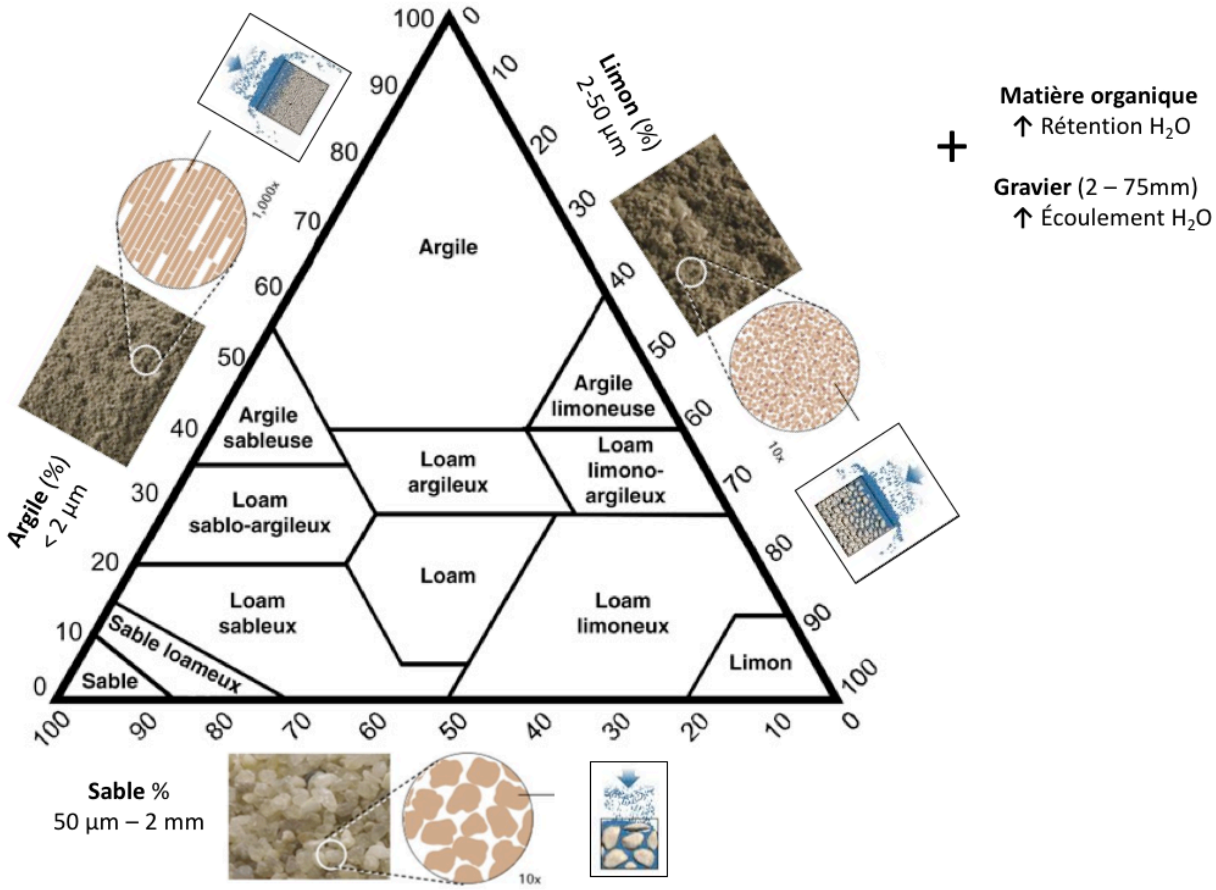

Figure 1.7 Grandes composantes du sol et comment elles influencent l'infiltration et la rétention de l'eau (adapté de COMET, 2015 et MAAARO, 2012).

Ces différentes composantes auront également une incidence directe sur la rétention et la biodisponibilité des contaminants grâce à leurs charges et affinités électrochimiques pour certaines molécules organiques ou ETs (Brady and Weil, 1996b). Le pH a un effet direct sur ces affinités, car il joue sur l'équilibre H^+ et OH^- du sol. Par exemple, un pH acide mènera à une plus grande solubilité et biodisponibilité de la majorité des ETs (Brady and Weil, 1996a). La composition de base de sols accidentellement contaminés est hors du contrôle des intervenants, mais certains paramètres comme le pH, l'humidité et l'aération peuvent être modifiés avec certains amendements (Hawkins et al., 2013).

Réglementations

Plusieurs réglementations environnementales sont en vigueur en Amérique du Nord. Au Canada, en plus de lignes directrices au niveau fédéral, chaque province régit ce secteur avec ses propres lois. Les normes pancanadiennes sont établies par le Conseil Canadien des Ministres de l'Environnement (CCME). Le Yukon utilise la réglementation de la Colombie-Britannique « Contaminated Sites Regulation » (CSR). Au Québec c'est la « Politique de protection des sols et de réhabilitation des terrains contaminés » du MELCC qui est en vigueur (MDDELCC, 1998). Les analyses requises sont similaires dans ces trois réglementations, mais les seuils admissibles de même que le fractionnement des tailles de HCP ne sont pas exactement les mêmes (Tableau 1.1). Les fractions F1 à F4 du CCME sont déterminées par leurs propriétés physico-chimiques pertinentes (solubilité, point d'ébullition, etc.) et leurs caractéristiques toxicologiques (CCME, 2008a; Yukon-Regulations, 2002). Lors de la présentation des résultats obtenus dans les deux projets au Yukon (chapitres 2 et 3), la norme du CCME a été choisie, car elle donne plus de détails sur les hydrocarbures et est applicable partout au Canada, alors que la CSR ne présente actuellement pas d'équivalent pour les fractions F4 et F4G (composantes importantes de la contamination sur le site WOP). Les ETs évalués dans cette thèse seront également évalués en fonction des normes CCME (Table 2.2). Lors de la présentation du projet effectué au Québec (chapitre 4), les normes du MELCC seront utilisées, car elles régissent la région où le projet a eu lieu.

Table 1.1 Sommaire de trois réglementations canadiennes sur les hydrocarbures pétroliers, en vigueur pour des terrains à vocation commerciale, non situés dans un secteur résidentiel et pour des terrains à usage industriel.

	CCME Regulation			CSR Regulation			MELCC Regulation		
Typically found in :	**Name$^{\Delta}$**	**Carbon** (n)	***Norm†***	**Name$^{\Delta}$**	**Carbon** (n)	***Norm†***	**Name$^{\Delta}$**	**Carbon** (n)	***Norm†***
Gasoline	**F1 :** volatile	C_6-C_{10}	*320*	**VPH :** volatile PHC	C_6-C_{10}	*200*			
Diésel, kerosene, jet fuel, No. 2 fuel oil	**F2 :** semi-volatile	C_{10}-C_{16}	*260*	**LEPH :** light extractable	C_{10}-C_{19}	*2000*	Paramètre intégrateur	C_{10}-C_{50}	*3500*
Lubricating and motor oils, No. 4 fuel oil,	**F3**	C_{16}-C_{34}	*1700*	**HEPH :** heavy extractable	C_{19}-C_{32}	*5000*			
Heavy lubricants, greases, waxes	**F4 :** low mobility	C_{34}-C_{50}	*3300*						
Heavy: lubricants, greases, waxes, tar.	**F4G* :** extremely heavy	>C_{50}							

* Analyzed gravimetrically rather than by gas chromatography.
Δ Fractions include aliphatic and aromatic fractions.
† Norms are in mg kg -1

Objectifs de la thèse et contributions

Cette thèse regroupe les éléments préalablement discutés dans trois projets distincts, réalisés sur des sites différents. Chaque projet fait l'objet d'un chapitre où sont décrits les objectifs, hypothèses et éléments novateurs particuliers.

Chapitre 2. Site de recherche : Arctic Backhoe LTU (Yukon, CAN)

Objectif : Évaluer l'efficacité d'une approche de bioremédiation simultanée avec *Pleurotus ostreatus*, *Salix alaxensis* et du compost municipal pour le traitement d'un sol contaminé au diésel en milieu subarctique.

Hypothèses :

1. L'utilisation de trois composantes biologiques conjointement augmentera significativement la rapidité et le pourcentage total de la dégradation du diésel comparativement à l'utilisation d'une seule composante.
2. Des traitements à base de composantes biologiques seront plus efficaces que des méthodes traditionnelles telles que l'atténuation naturelle ou l'utilisation de fertilisant inorganique.
3. L'espèce de saule choisie sera apte à végétaliser un site subarctique contaminé au diésel à partir de boutures dormantes implantées directement dans le sol.

À notre connaissance, aucune recherche n'a exploré l'utilisation de champignons du type « carries blanches » pour la bioremédiation d'HCP dans un climat subarctique. Les conditions climatiques froides énumérées dans la plupart des études étaient généralement dans une plage comprise entre 0°C et 25°C. Les capacités bioremédiatrices des saules, des carries blanches et du compost sont fréquemment citées dans la littérature, mais à notre connaissance ces trois éléments n'ont jamais été utilisés simultanément avant le début de notre projet en 2013. Cette étude ouvre la voie à une nouvelle méthode d'assainissement des sols en milieu subarctique.

Chapitre 3. Site de recherche : Son of War Eagle waste oil pit (Yukon, CAN)

Objectif : Évaluer l'efficacité de l'approche du microsystème pour la bioremédiation de contaminants organiques d'un sol à contamination mixte en milieu subarctique et déterminer le facteur de risque pour les brouteurs lié à l'accumulation des éléments trace dans les saules.

Hypothèses :

1. Les composantes biologiques dans le traitement actif favoriseront une plus grande dégradation des HCP que l'atténuation naturelle.
2. Les deux espèces de saules implantées accumuleront des métaux dans leurs tissus aériens.
3. La toxicité du sol sera nettement plus basse dans le sol traité que dans le contrôle.

Comme pour le projet décrit dans le chapitre 2, nous utilisons des éléments de bioremédiation connus (*Trametes versicolor* et *Salix* spp.), mais, à notre connaissance, ils n'ont pas été utilisés conjointement avant le début de ce projet. Ce projet ouvre également la voie à une nouvelle méthode d'assainissement des sols à contaminants mixtes (hydrocarbures, composés chlorés, métaux et métalloïdes) en milieu subarctique. De plus, cette recherche se distingue par les très hauts taux de contamination du site, des taux surpassant les niveaux habituellement recommandés pour la bioremédiation.

Chapitre 4. Site de recherche : Plateforme de traitement Solneuf (Québec, CAN)

Ce projet s'inscrit dans un stage effectué chez l'entreprise Akifer dans le cadre du programme Mine de Savoir. Trois projets ont eu lieu au cours de deux années. Un de ces projets était voué à la publication scientifique (présenté dans cette thèse), tandis que les deux autres étaient de nature plus confidentielle. Le rapport résumant ces deux projets non destinés à la publication est présenté dans l'Annexe IV pour la phase de révision par le jury, mais devra être retiré avant la publication de la thèse pour respecter l'entente conclue avec Akifer.

Objectifs académiques : 1) Quantifier la performance de différentes matières résiduelles fertilisantes locales pour la dégradation de l'huile à moteur ; 2) Appliquer des approches plus vertes et durables à l'industrie du nettoyage des sols contaminés.

Objectifs industriels :

1) Remplir les conditions pour l'obtention d'un nouveau certificat d'autorisation (CA) : Démontrer que les installations et méthodes du centre de traitement de Solneuf ont la capacité de dégrader les hydrocarbures pétroliers (HP) lourds (huile à moteur neuve ou usée) pour l'obtention d'un nouveau CA.

2) Atteindre rapidement les critères « C »: Démontrer qu'il est possible d'abaisser le niveau de contamination pour les HCP des fractions C_{10} à C_{50} au-dessous du critère environnemental « C » (3500 mg kg^{-1}) du ministère de l'Environnement et de la Lutte contre les changements climatiques (MDDELCC) du Québec à l'intérieur d'une saison de traitement.

3) Maximiser l'efficacité des traitements : Déterminer à quel point les amendements de matières résiduelles fertilisantes (MRF) et le taux de fertilisation influencent la vitesse et le taux de dégradation des HP lourds.

Hypothèses :

1.1 L'utilisation de matières résiduelles fertilisantes locales permettra de dégrader l'huile à moteur plus rapidement que la pratique courante à base de fertilisants inorganiques.

1.2 Les différentes matières résiduelles fertilisantes locales auront des efficacités respectives.

3. Lors de l'analyse des C_{10}-C_{50} séparés en six fractions, une dégradation inégale des fractions d'HCP sera observée à cause de fractions récalcitrantes.

L'utilisation de bois raméal fragmenté et de drêche de brassage pour un projet de bioremédiation d'huile à moteur à cette échelle, et dans un climat continental humide, est peu commune. De plus, dans ce projet nous avons tenté d'appliquer certains principes de l'économie circulaire lors du traitement en favorisant des matières résiduelles abondantes et disponibles à proximité du site de traitement. Ce chapitre s'intègre dans la politique canadienne et québécoise de réduction des gaz à effets de serre et adhère à la philosophie de l'économie circulaire en valorisant des matières organiques résiduelles locales, dont certaines semblent novatrices, à moyenne échelle.

CHAPITRE 2. | Bioremédiation d'un sol contaminé au diésel dans le Nord canadien à l'aide de compost municipal, champignons et saules locaux

Local fungi, willow and municipal compost effectively remediate petroleum-contaminated soil in the Canadian North

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Abstract

Low energy-input alternatives based on locally available products are needed for treating petroleum-hydrocarbon spills in northern regions. We tested the efficacy of three local biological components (municipal compost, white-rot fungus: *Pleurotus ostreatus* and willow: *Salix planifolia*) to remediate diesel-contaminated soils in a subarctic climate (Whitehorse, YT, Canada), and compared their efficacy to natural attenuation and chemical fertilizers (industry standard). After the first growing season, biologically amended treatments (BAT) that contained >2 biological components, had decreased 69-73 % of the diesel's F2 fraction (C_{10}-C_{16}), which is more than natural attenuation or fertilizer (48 and 51 %). By the third growing season, the BAT dropped below the Canadian Council of Ministers of the Environment's (CCME) Agricultural & Residential/Parkland guideline (<150 mg kg-1) and 86% of willows had survived and developed extensive roots. MiSeq amplicon sequencing of fungal (ITS) and bacterial (16S) rRNA genes showed the BAT's microbial communities were significantly more abundant and diverse. We found 132 bacterial and 35 fungal genera unique to the BAT. Readily-available local biological components such as municipal compost, fungi and willows may provide an effective alternative to applications of imported chemical fertilizers for the bioremediation and revegetation of diesel-contaminated soil in northern environments.

TOC/ABSTRACT ART

KEY WORDS

Northern bioremediation; soil microbiome; mycoremediation; phytoremediation; petroleum hydrocarbons

HIGHLIGHTS

- Local compost, willows and fungi were tested for diesel treatment in northern soils
- Municipal compost removed a higher percentage of diesel than inorganic fertilizers
- Re-vegetation took place concomitantly with decontamination
- Microbial communities in biologically-amended soils were most abundant and diverse
- This locally-sourced approach works well in the North

Introduction

Petroleum hydrocarbons (PHC) have become one of the most common and widely distributed contaminants worldwide due to their extensive use, distribution, and associated spills (Aislabie et al., 2008; Peter, 2011). In northern regions, human activities rely heavily on petroleum fuels, and spills have occurred at many sites (Mohn et al. 2001; Aislabie et al. 2006). Attenuation of PHC occurs naturally through volatilization and degradation by endemic microorganisms, particularly bacteria and fungi. Nonetheless, this is a slow process; especially in the North (above 50° latitude), where cold temperatures are present for the majority of the year (a frost-free period of 50 to 90 days $year^{-1}$) (Britannica, 2018). These low temperatures increase PHC viscosity, which limits their bioavailability to degrading microbes and slows down enzymatic reactions. Other factors limiting microbial activity in these environments include poor moisture and nutrient availability (Aislabie et al., 2008; Walworth et al., 2007; Walworth and Ferguson, 2008). Conventional northern soil remediation methods in North America include the addition of nutrients in the form of synthetic fertilizers (often with repetitive tilling), which stimulate PHC degradation by native microorganisms. In northern soils, fertilizer amendments have shown varying results from very effective to detrimental (Juwarkar et al., 2010; Lladó et al., 2012; Paudyn et al., 2008; Walworth et al., 2007); results are often site-specific and linked to initial PHC concentration (Walworth and Ferguson, 2008). This could be due to varying requirements between microbial taxa since many cold-climate microbes are adapted to oligotrophic soil conditions (Aislabie and Foght, 2008). The most important drivers for PHC-degradation seem to be microbial competition, initial community structure, and soil properties (Bento et al., 2005), which can all be manipulated through site-specific soil amendments. In 2013, the Northern Contaminated Sites Program reported that there were 166 contaminated sites in northern Canada (Yukon, NWT, and Nunavut), worth $2.3Billion in liability (AANDC). While there is a clear need for remediation and revegetation of contaminated soils in northern regions, spill sites are often remote and traditional remediation can be prohibitively costly (Mohn et al. 2001). Additionally, traditional strategies may be questionable in terms of broader environmental impacts such as: (1) tilling-induced volatilization which can simply displace the problem from soil to air; (2) greenhouse gas emissions related to soil displacement and the transport of chemical fertilizers; and (3) impact

of fertilizer mining of finite resources or energy-intensive synthesis (Rafiqul et al., 2005; Sanscartier et al., 2010). The need for combining complementary approaches in order to effectively decontaminate soils in the North has been recognized for some time (Robertson et al., 2007). Low energy-input, *in situ* materials adapted to northern climates and based on locally available products may provide affordable and effective alternative remediation strategies.

Soil treatment approaches using multiple biological components have been shown to be effective for contaminant-remediation in other studies (Asemoloye et al., 2017; Germaine et al., 2015; Palmroth et al., 2006). Based on the literature, we identified (1) local municipal compost, (2) fungi and (3) willows as potential biological agents of interest that might complement each other when used for northern remediation. (1) Compost provides microbial communities and slow-release nutrients that can accelerate contaminant degradation, and it acts as a physical support for other bioremediation elements such as willows and fungi (Aislabie et al., 2008; BATTELLE Environmental Restoration Department, 1996; Guidi et al., 2012; Zubillaga et al., 2012). Additionally, municipal compost represents a key opportunity for cities to increase waste diversion from burdened landfills (Giroux, 2014). (2) Mycoremediation is the use of fungi for decontamination. White-rot fungi, like *Pleurotus ostreatus* are saprotrophs that specialize in the degradation of the complex plant polymer lignin, through the exudation of aggressive and non-specific lignolytic enzymes such as laccases and peroxidases (Stamets, 2005). These enzymes have been shown to target organic contaminants like aromatic and aliphatic PHC (Colombo et al., 1997; Pointing, 2001). Although *P. ostreatus* is a wood saprotroph, its mycelium is adapted for some soil colonization (Baldrian, 2008; Kendrick, 2000). (3) Phytoremediation is the use of plants to assist in contaminant-remediation. Willows are fast-growing hardy plants that are extensively used for revegetation and in a wide range of bioremediation projects (Cunha et al., 2012; Pugh et al., 2002; Slater et al., 2011). *Salix planifolia* was chosen because it was identified as a PHC-resistant species (Kershaw and Kershaw, 1986; McKendrick, 2002, 1987) and it was the most vigorous of four local species we examined in growth trials (data not shown). It has been used in revegetation projects (Collet, 2004), but to our knowledge represents a new species for the direct use of cuttings in a PHC bioremediation project.

The goal of this project was to test the efficacy of these three biological components (municipal compost, fungi and willow) individually and together, in a subarctic climate, and to compare their efficacy to a fertilizer treatment as well as natural attenuation. Since the components were chosen to each fill a different role in the plant-soil system, it was hypothesized that their respective actions and contributions would strengthen the system as a whole, making it more resilient over time, and more effective for decreasing PHC contamination. To obtain a profile of the soil microbial community, we used MiSeq amplicon sequencing of fungal and bacterial markers (ITS and 16S respectively). Our aim was to identify the influence the different biological components had on the microbial communities, and if certain changes in community composition and diversity could be linked to greater PHC reduction. Finally, we compared degradation between treatments and related the contamination levels to regulatory guidelines from Canada (Canadian Council of Ministers of the Environment (CCME, 2008a)). The approach examined here could be advantageous because it is less energy-intensive than the traditional tilling and fertilizing methods and could be implemented at remote sites, eventually as a low-maintenance *in-situ* technology.

Materials and methods

Site description

The experimental trial occurred at a Land Treatment Unit (LTU) owned by Arctic Backhoe Services Ltd. (60° 40' 15.29" N 135° 6' 8.93" W) located near the City of Whitehorse, Yukon, Canada, from June 2013 to July 2015. Starting total PHC concentration in the soil was 2200 mg kg^{-1}. The initial soil used for the trial was received in August 2012 from the Klondike area (YT), where an accidental diesel spill had just occurred. The CCME (2008) defines their environmental guideline fractions as: F2 (C_{10}-C_{16}), F3 (C_{16}-C_{34}), and F4 (C_{34}-C_{34}). Since F3 and F4 fractions were present in low concentrations and were well below the guidelines for Commercial and Industrial sites (C&I), our target for remediation was the F2 fraction, which was above CCME C&I guidelines at the beginning of the trial (Table 2.1).

Initial soil pH was 7.72 ± 0.08 (tabletop Oakton PCD650, equipped with an Oakton epoxy pH probe. H_2O:soil ratio of 1:2 (Kalra and Maynard, 1991)). The soil contained 1.5 %

organic matter (loss on incineration) and its textural description was sandy muddy gravel, unsorted with a median size of 0.1075 mm and a density of 1.39 g cm^{-3} (ASTM Standard test method (*Standard Test Method for Particle-Size Analysis of Soils. Designation: D 422 – 63*, 2008), analysed with GRADISTAT (Blott and Pye, 2001)). Initial moisture was 7.5 ± 0.6 % by mass (determined gravimetrically).

Experimental design

Treatment descriptions and amendments

Treatments included: C-Compost (15 % mature municipal compost (v/v)); CW-Compost and Willow (15 % compost (v/v) and six dormant *S. planifolia* cuttings); CF-Compost and Fungi (15 % compost and 15 % fungal treatment (composed of 5 % pure sawdust spawn of *P. ostreatus*, 7.1 % sundried *Populus* spp. woodchips, and 2.9 % naturalized *P. ostreatus* mycelium (v/v)); CWF-Compost, Willow and Fungi (15 % compost, 15 % fungal treatment and six *S. planifolia* cuttings); FERT-Fertilizer (C:N:P ratio 100:9:1)) and CTRL-Control (no amendment added). When the four biologically amended treatments (C, CW, CF, CWF) are mentioned together, they are hereinafter referred to as BAT, and the FERT and CTRL as NoC (no compost). Due to logistical constraints, it was not possible to conduct all 9 possible combinations of willow, compost and fungi. The choice was made to add compost to the fungal and willow treatments to act as a colonization support for the willow and fungi in this nutrient-poor soil. Furthermore, we hypothesized that the effects of these biological components would be additive in PHC-reducing efficiencies.

Since heterogeneous distribution of PHC in contaminated soils if often observed (Aislabie et al. 2008; Rike et al. 2008), our six treatments were replicated seven times each. They were then placed in seven randomly distributed blocks (n=42) (see Figure. 2.S1A for ground layout). A total of 42 Rubbermaid polypropylene bins (189 L each) were perforated with 0.64 cm holes at regular intervals to promote drainage and aeration (Fig. 2.S1B). Each bin received 150 L of soil.

Municipal compost produced by Boreal Compost Enterprises Ltd. was used. It was analyzed by A&L Canada Laboratories Inc. and met the CCME (CCME 2008) and the Bureau

de Normalisation du Québec (BNQ) (2005) standards for maximum allowable microbiological levels and trace metal content in Category A compost. It was fine textured (99.9 % < 0.64 cm (1/4 in.)), with a pH of 8.8, initial moisture of 7.3 %, C:N ratio of 12:1 and qualified as mature compost (CO2 respiration <0.01 mg CO2-carbon g-1 organic matter day-1) (see SI for A&L report).

Salix planifolia cuttings were harvested near Whitehorse (YT) in their dormant state in March 2013 and stored in thick black plastic bags for 3 months at -18 °C. Willow branches (1.3 to 2.5 cm wide) were thawed and cut into 30 cm-long segments. They were then soaked in 20 cm of water for 24 h prior to planting 15 cm deep in the experimental bins (soaking time based on lab trials and practicality). Essentially no other plants colonized the soil surface. No additional management (such as weeding) was required.

For the fungal amendment, woodchips were soaked for 24 h in tap water and then inoculated with pure sawdust spawn of *P. ostreatus* (spawn origin: Champignons Advitam inc. Qc, Canada). This was incubated for 28 days (18-22 °C) to allow the mycelium to colonize the woodchips (hereinafter referred to as "naturalized mycelium"). The objective of naturalization is to acclimatize the mycelium to a non-sterile environment and hence stimulate its immune system (Stamets 2005). A second woodchip mix was incorporated directly in the treatment piles to serve as a primary carbon source for the fungi. (See SI for full details on fungi source and woodchip origin and handling.)

Nitrogen and phosphorus are the two most commonly added nutrients in fertilizers. Reported ratios vary from C:N 200:1 to 9:1 and C:P 500:1 to 12:1 (Walworth & Ferguson 2008). In this experiment, urea (46-0-0) and Teeble Super phosphate (0-45-0) were added to a ratio of 100:9:1.

Soil preparation

The diesel-contaminated soil was thoroughly mixed in an attempt to reduce the heterogeneous distribution of PHC and then split in six piles. Each pile received the amendments for one treatment and was then turned over nine times. The amended soils were randomly loaded in wheelbarrows by hand shovels and placed in their respective treatment bins.

Four temperature sensors (Thermacron ibuttons) were placed at a depth of 25 cm in the center of CTRL and CWF treatment bins to monitor their core temperature over 12 months, from June 2013 to June 2014 (Figure 2.SI.B).

During the willow and fungi establishment phase, irrigation was conducted twice with watering cans, to simulate a remote-site condition where irrigation frequency may be limited. The water was from the City of Whitehorse's municipal supply. In 2013, 2.4 L were applied to each of the 42 bins on June 28, while 8 L were applied on July 15. Moisture readings were taken gravimetrically (oven-dry, 105 °C) on PHC sampling days.

Sampling

Soil samples for PHC concentrations were collected eight times over three years, when the ground was not frozen. Two soil-column samples were taken from a depth of 5 to 25 cm and homogenized into one composite sample for each treatment bin. The homogenized samples were placed in a 125 mL glass jar with a Teflon-lined lid and kept at -20 °C until analysis (n = 7 per treatment, per sampling time). Sub-samples of that frozen soil were taken from the beginning and end of the growing seasons of the first two years to conduct the DNA extraction (n = 4 per treatment, per sampling time). Willow cuttings were monitored for leaf emergence at the beginning of the growing seasons and for survival at the end of each season. The dead cuttings were replaced once in the spring after the first growing season. In July of the last year of treatment, the willow cuttings were pulled from the ground and the shoots growing from the initial cuttings were cut and weighed separately from the cuttings to measure the biomass produced without the initial introduced biomass (dry weight).

Hydrocarbon quantification

The CCME fractions F2, F3, and F4 (C_{10}-C_{16}, C_{16}-C_{34} and C_{34}-C_{50}, respectively) (2008) were extracted by rotary evaporator methods and then analyzed by GC-FID (Gas Chromatography – Flame Ionization Detector, Agilent 6890 GC systems). (See Supplementary Methods in SI). Only alkanes are reported since analysis revealed that aromatic hydrocarbons were absent from the site (data not shown). Samples were preserved at -20 °C until time of analysis.

Amplicon sequencing of fungal ITS and bacterial 16S DNA

DNA extraction and sequencing

Fungal and bacterial DNA was extracted from soil samples using the NucleoSpin® Soil Kit from Macherey-Nagel. Primers ITS1F CS1 (CTTGGTCATTTAGAGGAAGTAA) and 58A2R CS2 (CTGCGTTCTTCATCGAT) were used for fungi, while primers 341F CS1 (CCTACGGGNGGCWGCAG) and 805R CS2 (GACTACHVGGGTATCTAATCC) were used for the V3-V4 region of the 16S rRNA gene in bacteria. Extracted DNA was sent for paired-end next generation sequencing by Illumina MiSeq PE250 (Genome Quebec, See Supplementary Methods in SI.)

Sequence processing and analyses

Primers were trimmed and only sequences with a Q score greater than Q33 (16S) and Q30 (ITS) were retained. Chimeric sequences were identified and removed (vsearch{} (Rognes et al., 2016)). Open-reference OTU (Operational Taxonomic Unit) picking was performed and OTU tables were rarefied to 20,000 and 7,700 reads/sample for 16S and ITS, respectively (QIIME (Caporaso et al., 2010)) (see Supplementary Methods in SI). Raw 16S and ITS sequences have been deposited in GenBank-NCBI_under study ID 384633 (http://www.ncbi.nlm.nih.gov/bioproject/384633).

Statistical analysis

A linear mixed-effects repeated measures model fitted by Restricted Maximum Likelihood (REML) with blocking was performed with nlme{} (Pinheiro et al., 2017) in R (R Core Team et al., 2016) to compare : decreases in PHC concentrations (LOG of [F2]) ; temperature; and moisture levels between treatments. Treatment was considered a fixed factor and blocking was a random one. Measures were repeated eight times over three years. Post-hoc multiple comparison tests were performed with the HSD Tukey method (lsmeans{} (Lenth, 2016) and mltcompview{} (Hothorn et al., 2008)).

Fungal and bacterial alpha diversity as well as differences between degradation rates (k) were also tested with a linear mixed effects model followed by the HSD Tukey method. Principal coordinates analyses (PCoA) followed by permutational multivariate analysis of variance (PERMANOVA in vegan{}) (Oksanen et al., 2016) were conducted to compare fungal and bacterial beta diversity metrics (the compare_categories.py script in QIIME was also used for 16S). Assumptions of normality and homoscedasticity were met for all tests. Figures were built with ggplot2{} (Wickham, 2009) and phyloseq{} (McMurdie and Holmes, 2013) in R (R Core Team et al., 2016).

Results and discussion

Diesel removal

Temporal trends

This study shows that local biological components (compost, plants and fungi) led to higher rates of hydrocarbon removal than inorganic fertilizer or natural attenuation for the field remediation of diesel-contaminated soil in a subarctic climate. The Biologically amended treatments (BAT) brought the contamination level below 260 mg kg^{-1} (the CCME's Commercial and Industrial (C&I) guideline,) in 14 months and below 150 mg kg^{-1} (CCME's Agricultural & Residential/Parkland limit (A&R/P)) within three growing seasons. We chose to compare our results to the national guideline values for our study sites. Note that comparisons with guidelines from other jurisdictions is difficult, because of the different purposes underlying these guidelines (see Cavanagh & Halloran (2002) for a discussion on guidelines). For instance, the purpose of a guideline may be to reach a remediation goal or assess the urgency of remediation. We present F2 results on a yearly basis, to highlight PHC removal in the remediation system (Fig. 2.1).

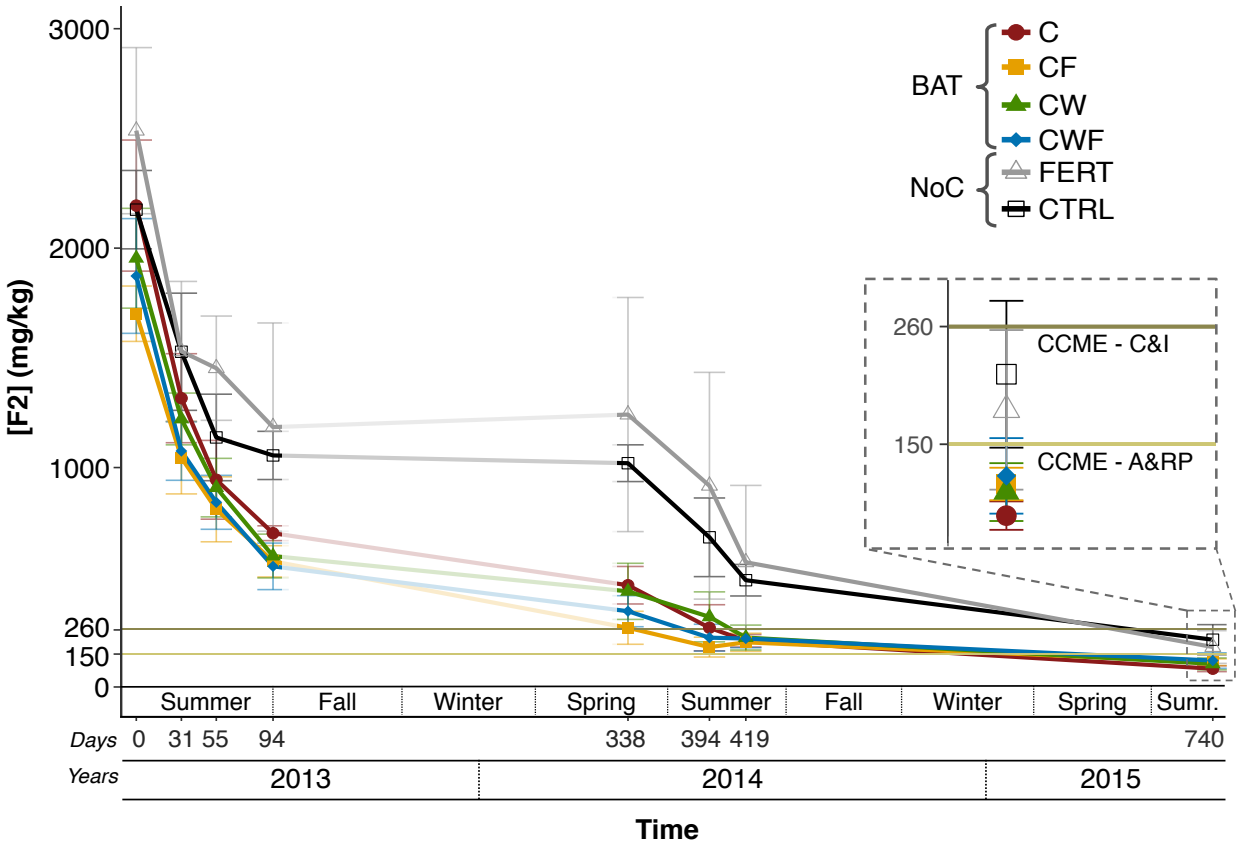

Figure 2.1 Diesel fraction F2 (C_{10}-C_{16}) removed over 740 days for the six treatments at the ABH site. Error bars represent standard deviation (SD, n=7). The dashed lines during the first winter indicate a change in PHC extraction method (Text S2). For graphical representation only, year one values were increased 30 % to palliate lower recovery rates of that year. The lines at 260 and 150 mg kg^{-1} indicate the CCME's Commercial & Industrial (C&I), and Agricultural & Residential/Parkland (A&RP) limits, respectively.

Table 2.1 Initial PHC concentrations measured (mg kg^{-1} dry soil) across hydrocarbon fractions in different treatments (n=7). The contamination is a 12-month old diesel fuel in soil. In the listed CCME fractions for Commercial & Industrial limits, the C indicates the number of carbons in the molecules. Letters denote the ranks of the statistical differences between treatments, determined by a Tukey HSD test ($p < 0.05$), following a linear mixed-effects model. SD stands for standard error.

Fraction	**F2** (C_{10}-C_{16})	**F3** (C_{16}-C_{34})	**F4** (C_{34}-C_{50})
CCME limit	*260 mg kg^{-1}*	*1700 mg kg^{-1}*	*3300 mg kg^{-1}*
Detection limit	*30 mg kg^{-1}*	*50 mg kg^{-1}*	*50 mg kg^{-1}*
Treatment	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
CTRL	1697 ± 178	482 ± 36	43 ± 17
C	1715 ± 298	496 ± 65	66 ± 9
CW	1475 ± 227	476 ± 71	58 ± 23
CF	1222 ± 126	447 ± 34	86 ± 27
CWF	1394 ± 261	480 ± 72	73 ± 12
FERT	2057 ± 378	547 ± 68	42 ± 16

C= Compost; F=Fungi; W=Willow; CTRL=Control; FERT=Fertilizer.

After the first growing season, treatments containing two or more biological components had lost significantly more F2 (71.4 ± 2.6 % (CWF), 72.9 ± 2.8 % (CF) and 67.8 ± 2.0 % (CW)) ($p < 0.05$), than the NoC treatments (51.1 ± 9.1 % (FERT), and 48.2 ± 2.1 % (CTRL)) (Table 2.2). These results pointed to the additive efficacy of multiple biological components used concomitantly. Biodegradation followed pseudo first-order kinetics (linear slopes were obtained with: $Ln[F2]t / Ln[F2]t_0 = -kt$). The BAT treatments generally had steeper slopes (faster removal rates) than the NoC. The fastest removal rate in year one was observed for the CWF treatment (0.78 ± 0.08 day^{-1}), which was significantly faster than the NoC treatments ($p < 0.05$) (Table 2.2). Our results indicate that within one growing season in a subarctic setting, our approach, incorporating multiple biological components, performed better than a chemical fertilizer or natural attenuation. One year and two months following treatment, the remediation target was reached for all the BAT treatments; the CCME's C&I guideline (260 mg kg^{-1}) (Fig. 2.1, Table 2.1). The BAT led to significantly more F2 reduction (C: 87.5 ± 2.2 %, CW: 84.4 ± 4.6 %, CF: 83.2 ± 3.6 % and CWF: 84.0 ± 2.5 %) than both the CTRL (71.0 ± 5.6 %) and the FERT (72.4 ± 18.4 %) treatments. A final sampling was performed a year later to see the long-term effectiveness of the remediation methods. At this

point, the CTRL, representing natural attenuation, showed the lowest overall F2 removal (87.5 ± 1.1 %). The BAT's F2 removal was statistically equivalent, and the C treatment led to significantly more than the two NoC ($p < 0.05$) for a total of 95.0 ± 0.4 % removal, the highest overall (Table 2.2). By the end of the experiment, the BAT had fallen below the CCME's A&RP limits (150 mg kg^{-1}), while the NoC were below C&I limits (Fig. 2.1).

Table 2.2 Cumulative percent decreases (% dcr.) in the concentrations of the F2 fraction in relation to the first sampling event (June 2013) are presented for the beginning and end of each year. Removal rates (over the first 94 days are presented with the constant k, which represents mean percent removal in mg kg^{-1} per day. Letters denote the ranks (r) of the statistical differences between treatments' F2 concentrations, determined by a Tukey HSD test ($p < 0.05$), following a linear mixed-effects model with repeated measures. Soil moisture (%) means represent the averages measured per treatment between June 27, 2013 and July 4, 2015 (n=56 per treatment, measure on the same days as PCH-sampling occurred), as well as the minimum and maximum moisture concentrations measured.

Date	2013				2014		2015				
	June	Sept.		Removal rates		May		Aug.		Sept.		Soil Moist. (%)		
Treatment	% dcr.	% dcr.	r	k (day^{-1})	r	% dcr.	r	% dcr.	r	% dcr.	r	mean	min	max
CTRL	0	48 ± 5	a	0.51 ± 0.06	c	40 ± 4	c	71 ± 6	b	88 ± 3	c	9	3	16
C	0	69 ± 6	b	0.73 ± 0.06	ab	72 ± 7	b	88 ± 2	a	95 ± 1	a	12	6	20
CW	0	71 ± 7	b	0.76 ± 0.07	a	70 ± 10	b	84 ± 5	a	93 ± 2	a	11	4	27
CF	0	68 ± 5	b	0.72 ± 0.06	ab	78 ± 5	a	83 ± 4	a	91 ± 1	ab	15	7	21
CWF	0	73 ± 7	b	0.78 ± 0.08	a	75 ± 5	ab	84 ± 3	a	91 ± 3	ab	13	5	35
FERT	0	51± 24	a	0.54 ± 0.26	bc	40 ± 25	c	71 ± 6	b	91 ± 4	bc	9	5	17

C= Compost; F=Fungi; W=Willow; CTRL=Control; FERT=Fertilizer.

Early in the experiment, the highest removal rates and percentages where observed for the CWF treatment. Bell et al. (2013) determined that the natural evolution of a contaminated soil's microbial community structure does not always naturally foster an environment conducive to organisms which possess the strongest biodegradation efficacies. This type of selection may have occurred in the CWF soil, as it did not keep the lead for the following years. Additionally, it is possible that during the last 12 months of the study a nutrient deficiency occurred in the CWF, leading to increased competition between the organisms and a decrease in their ability to degrade PHCs.

Multiple factors can impact the rate and total PHC-removal achieved in a given system, and field studies regularly yield different results than laboratory studies (Aislabie and Foght, 2008; Facundo et al., 2001; Paudyn et al., 2008; Rike et al., 2008). While most studies have focussed on approaches using one or two techniques, we tested the efficacy of phytoremediation, mycoremediation and compost-aided bioremediation, simultaneously. In a field experiment conducted over 94 days in Ontario, Canada, Gomez and Sartaj (2013) found that the addition of compost and a microbial consortium stimulated a removal of 82 % (of the initial 940 mg kg^{-1} of heating fuel) and their compost-only treatment yielded 52 % (Gomez and Sartaj, 2013). In the same time span our compost-only treatment had removed 69 % and our CWF treatment had removed 73 % (Table 2.2). In Finland, a local species of pine was used to treat soil contaminated with 5000 mg kg^{-1} diesel. In 330 days, this phytoremediation resulted in the removal of at least 88% of original fuel (Palmroth et al., 2002). Over a longer period of time, the course of two years, research conducted in the Canadian Arctic (Resolution Island, Nunavut) by a landfarming approach (with tilling every 4 days), attained removal rates >90 % (Paudyn et al., 2008). Our passive compost-only treatment led to a 95 % decrease of the initial 1715 mg kg^{-1} of F2 from diesel, in two years and 10 days. The age of contaminants can also have an impact on the remediation as weathered contaminants tend to be more recalcitrant to bioremediation. As contaminants become less available to degradation they can also be less available to local flora and fauna, hence possibly reducing risks (Brassington et al., 2007). A large advantage of our method resides in the fact that no amendment addition, tilling or handling is required after initial set-up and additionally re-vegetation takes place concomitantly with decontamination.

Effect of compost vs. fertilizer

FERT led to a smaller F2 percent decrease than the four BAT ($p < 0.05$). The 234 mg kg^{-1} of nitrogen added to our soil is well below the reported inhibitory levels of 1200 mg N kg^{-1} in sub-polar soils (Walworth et al., 2007) and within the commonly used C:N range of 100:9 (Snape et al. 2008a; Karppinen et al. 2017b). The addition of fertilizer has been reported to yield mixed degradation results, from very good to inhibitory, especially in cold climates where soil microorganisms are usually adapted to low nutrient levels (Lladó et al., 2012; Paudyn et al., 2008; Walworth et al., 2007). Compost on the other hand, can not only provide

a moisture-retaining matrix in the soil, but also nutrients and microbial communities that can accelerate contaminant degradation as well as serve as a physical support for other bioremediation elements such as willows and fungi (Aislabie et al., 2008; BATTELLE Environmental Restoration Department, 1996; Guidi et al., 2012; Zubillaga et al., 2012). Vouillamoz et al., (2001) also found that the addition of compost reduced the toxicity of diesel to plants and increased its degradation. Similarly, Chirakkara and Reddy (2015) determined that biomass amendments like compost improved plant growth in PHC-contaminated soil. Although we did not have willows growing in a control soil, we hypothesize their good survival rates could be linked in part to compost addition, in accordance with the previous author's findings. Overall, compost appears to play a key role in our approach. Although not all northern towns and cities have composting facilities, in Canada there is a growing trend to increase diversion of waste from landfill through composting (a 125% increase between 2000 and 2010) (Giroux, 2014). Diversion of 66% of residential waste can usually be achieved, while reducing the production of greenhouse gases, such as methane (produced by the anaerobic degradation of compostable materials within a landfill), which is very relevant within the current global climate change context (Giroux, 2014; Lou and Nair, 2009).

In remote northern locations the long-term efficacy of in-situ treatments, as well as impact on the ecological integrity of the surrounding environment, absolutely need to be considered. Leewis et al. (2013) observed that initial plant colonization of a contaminated site had long-term influences on the site's future plant-community composition, and most importantly that fertilizer applications favoured non-native plant establishment, even 15 years after treatment (Leewis et al., 2013). The use of locally available organic materials, such as compost rather than inorganic fertilizers, may be used to effectively improve PHC degradation and promote recovery of native vegetation. Further, fertilizer transport has been shown to be one of the main sources of pollution (Sanscartier et al., 2010). By using local materials, which reduced transport, we also aimed to minimize this project's carbon footprint.

Effect of environmental drivers in a cold climate

Moisture and temperatures above freezing are important environmental drivers of microbial activity in the soil. Microorganisms require water for their metabolic functions; a

system with adequate moisture will encourage their growth. In our 42 bins, mean soil moisture varied between 8.5 and 14.6 % in the sampling months (Table 2.2). The BAT treatments generally had higher moisture levels than the NoC ($p < 0.05$). This is most likely explained by the presence of additional organic matter in the soil and possible water retention by fungal mycelium.

No temperature differences were denoted between the two treatments monitored (CTRL, CWF) ($p > 0.05$). The bins were aboveground, and comparisons of our recorded temperatures with those of a nearby weather station indicate that bin core temperatures followed air temperatures (Fig. 2.S2). The center of the bins was buffered against the most extreme temperatures but were still subjected to a wide range of temperatures (-27 to 28 °C). The key temperature-related consideration in the North is the limited season when the ground, and its water content, are not frozen. Studies in northern locations, including near our research site in Whitehorse, have shown that the addition of certain amendments such as biochar could extend PHC-remediation into the winter months by maintaining pockets of liquid water in the soil where microbes remain active, even when the temperature is below zero (Karppinen, et al. 2017a; Karppinen et al. 2017b). It should be noted that it is challenging to assess the role of soil temperature alone in the study of PHC-degradation since temperature will influence the properties of both soil and petroleum hydrocarbons (Aislabie and Foght, 2008; Rike et al., 2008).

Progression of biological components

Salix planifolia

Of the 84 *S. planifoli*a cuttings planted, 100 % showed bud and leaf emergence, and 79 % survived the first winter. Dead cuttings were replaced in the spring of the second year (May 2014) since the priority was to measure their influence on remediation, not their survival rates. A total of 96 % were alive before going into the second winter and 86 % survived into the third growing season. The total aboveground dry biomass per cutting (all new branches and leaves but excluding the initial cutting) was an average of 4.60 ± 0.56 g and 4.18 ± 0.44 g, for CW and CWF respectively. No differences between treatments were observed ($p > 0.05$).

Although underground biomass was not quantified, the root systems that developed were extensive and traveled throughout the bins.

The presence of petroleum in soil can slow or hinder plant growth or germination (Gillian and Harry, 2002; van Gestel et al., 2001). Despite the presence of diesel and large temperature fluctuations observed in our bins, good post-winter survival rates were observed for *S. planifolia* (79 (CWF) and 86% (CW)). These survival rates in diesel-contaminated soil are within the range that Houle and Babeux (1994) observed in non-contaminated soil (77 % and 96 %) at two different sites in subarctic Canada. Different authors have observed this species' ability to survive or colonize petroleum-contaminated soils. Kershaw and Kershaw (1986) found this species to be one of the dominant colonizers on a 35-year-old crude oil spill in the Northwest Territories of Canada and McKendrick (1987) found it was one of the few shrub species that survived a diesel spill in the Alaskan Arctic. Although it has not yet been investigated, we hypothesize that *S. planifolia's* success for diesel-remediation may result from its ability to structure the microbial community in its rhizosphere, as it was recently described by Leewis et al. (2016) for *Salix alaxensis* in diesel-contaminated soil. We found 9 fungal and 1 bacterial genera unique to the treatments that contained willow cuttings, but we did not specifically analyze the rhizosphere soil. Our results confirm this species' resilience to petroleum-contaminated soil; its resistance to relatively dry soil conditions; and most importantly, the possibility for the direct use of its cuttings in diesel-contaminated soil in the North.

Pleurotus ostreatus

Large fruiting bodies appeared in multiple locations on the surface, as well as from aeration holes on all CF and CWF bins at the end of the first summer (see abstract image), indicating mycelial growth was distributed throughout the bins. Sporophores also appeared in 2014, but they were much smaller and scattered. This second fruiting confirmed the survival of the fungi, but their trifled state could indicate a reduced access to nutrients. Our results show that *P. ostreatus* can grow in a northern bioremediation system. To our knowledge, white-rot fungi has rarely been used for the bioremediation of diesel in subarctic climates (Camenzuli and Freidman, 2015; Winquist et al., 2014), and our work confirms its potential.

Low-nutrient soils that support smaller densities of microbes may favour the initial establishment of white rot fungi, which can be more successful at colonizing sterile soils because of reduced competition (Baldrian, 2008). Other field-scale studies at lower latitudes have found this species to be an effective degrader of PHC both in *in vitro* and *in situ* settings. Thomas et al. (1998) found that the addition of *P. ostreatus* to large outdoor biopiles noticeably altered the appearance of the soil in comparison to the control and to bacterial bioaugmentation treatments over four months. The scent of oil disappeared, multiple large fungi fruiting bodies regularly flushed, and by the 10th week volunteer vegetation had begun colonizing the pile, while the other two treatments remained black with a smell of PHC (Stamets, 2005; Thomas et al., 1998). *Pleurotus ostreatus* is specialized in the primary degradation of dead wood (Kendrick, 2000) and a willow cutting end can offer an entry way for the fungi. The replacement rate of willows in the treatment with fungi (28.5 %) was double than without fungi (14.2 %). Planting seedlings or using rooted cuttings could possibly diminish this additional loss in the presence of a white rot fungus. *Pleurotus ostreatus* proved to be an adequate species in this climate, but because of the naturalization process used, we found it required more handling than municipal compost. Although we ultimately deemed the addition unnecessary in our particular case, *P. ostreatus* may still be an appropriate and profitable option for other diesel spills, especially where toxic and recalcitrant organic polycyclic aromatic hydrocarbons (PAH) molecules are present (we initially expected them in our soil).

Soil fungal and bacterial communities

The different biological components added to the soil had clear and lasting impacts on the soil fungal and bacterial community composition and diversity. It comes to no surprise that the initial addition of amendments would have some effect, since the BAT supply their own new microbial communities. Nonetheless, since most bio-augmentation experiments yield little success in community manipulation over time (Thompson et al., 2005), we were surprised to see the fungal and bacterial communities remain very different in the BAT versus the NoC, even by the end of the second growing seasons (Fig. 2.2).

In total, 390 fungal genera were identified of which 35 are exclusive to the BAT treatments. The most abundant phyla across all treatments were *Ascomycota* and *Basidiomycota* followed by *Zygomycota*, *Chytridiomycota* and *Rozellomycota*, which make up a small portion of the remaining diversity (Fig. 2.S3A). Compost does not appear to be a driver of fungal richness within bins (alpha diversity) in our experimental trial ($p > 0.05$), but the presence of *P. ostreatus* reduced fungal richness (Fig. 2.S4A, "Observed"). The FERT has the largest range of observed OTUs (≈175 to ≈450); this may be linked to the high heterogeneity of PHC removal performance discussed above. Compost shaped the fungal community's structure and composition with clear clusters separating NoC (cluster CA.1; Fig. 2.2A) and BAT treatments (Cluster CA.2) (beta diversity calculated with Jensen-Shanon Divergence metric, Fig. 2.2A. PERMANOVA, $p < 0.05$). Interestingly this strong difference was maintained over two years and the community did not appear to move back to its original composition. On the other hand, there is a clear change over time in the treatments amended with *P. ostreatus* (cluster CA.3); they display a steady decrease in total fungal genera present (269, 244, 219, and 204 for the four sampling times; a 25% decrease). The ITS sequence of the genus *Pleurotus* is clearly detectable at the start of the experiment in the CF and WF treatments (6.8 ± 3.2 % of the total abundance), but it drops to nearly zero (0.03 ± 0.04 %) by the end of the second growing season. This indicates that the addition of *P. ostreatus* can have a lasting effect on a soil's fungal community structure and composition (Fig. 2.2A, cluster CA.3), by decreasing diversity, even after it is no longer present in the system.

We found there were 132 bacterial genera unique to the BAT treatments (absent from NoC). These may not be bacteria directly responsible for petroleum degradation, but may promote degradation pathways, and further work should be conducted to examine the presence and role of these bacteria. The presence of compost had a clear impact on the number of observed bacterial OTUs (Kruskal-Wallis test, Bonferroni-corrected, $p < 0.05$) (alpha diversity, Fig. 2.S4.B). The FERT reduced the minimum richness below the CTRL; again this is possibly linked to soil pockets with higher fertilizer composition, which could have reduced richness. The BAT had markedly more observed OTUs than the NoC (≈1660 to 2200 compared to ≈800 to 1500, respectively) ($p < 0.05$). We used the Bray-Curtis dissimilarity index to measure similarity across groups. The PCoA on bacterial beta diversity (based on

Bray-curtis) indicates that compost is a strong driver of community structure and composition as clear differences are visible between the BAT and the NoC (PERMANOVA, $p < 0.05$) (Fig. 2.2B). At the start, the CTRL and FERT clustered together, and then gradually moved in opposite direction through time, indicating that the fertilizer is likely driving bacterial community structure and composition (Fig 2.2B, clusters CB.2 and CB.3). Although there is a slight divergence of the treatments with *P. ostreatus*, the BAT treatments mainly cluster together (Fig 2.2B, cluster CB.1). Six commonly recognized cold climate PHC-degrading bacterial genera were identified in all treatments: *Acinetobacter*, *Arthrobacter*, *Pseudomonas*, *Rhodococcus*, *Sphingomonas* and *Variovorax*.

Our results indicate that the addition of compost had an immediate and lasting influence (over two years) on fungal and bacterial community composition and structure. *Pleurotus ostreatus* also had a dramatic impact on the fungal community's structure. Overall, the biologically amended treatments (BAT) led to higher F2 removal than fertilizer or natural attenuation. Higher soil microbial diversity does not always lead to greater PHC degradation (Bell et al., 2013). But in this case the BAT, which led to the greatest F2 percent-decrease, also had significantly more diverse and abundant fungal and bacterial communities. In our results, *S. planifolia* was not as important as compost for stimulating PHC-removal, but it proved to be an adequate species for the re-vegetation of diesel-contaminated soils whilst bioremediation occurred.

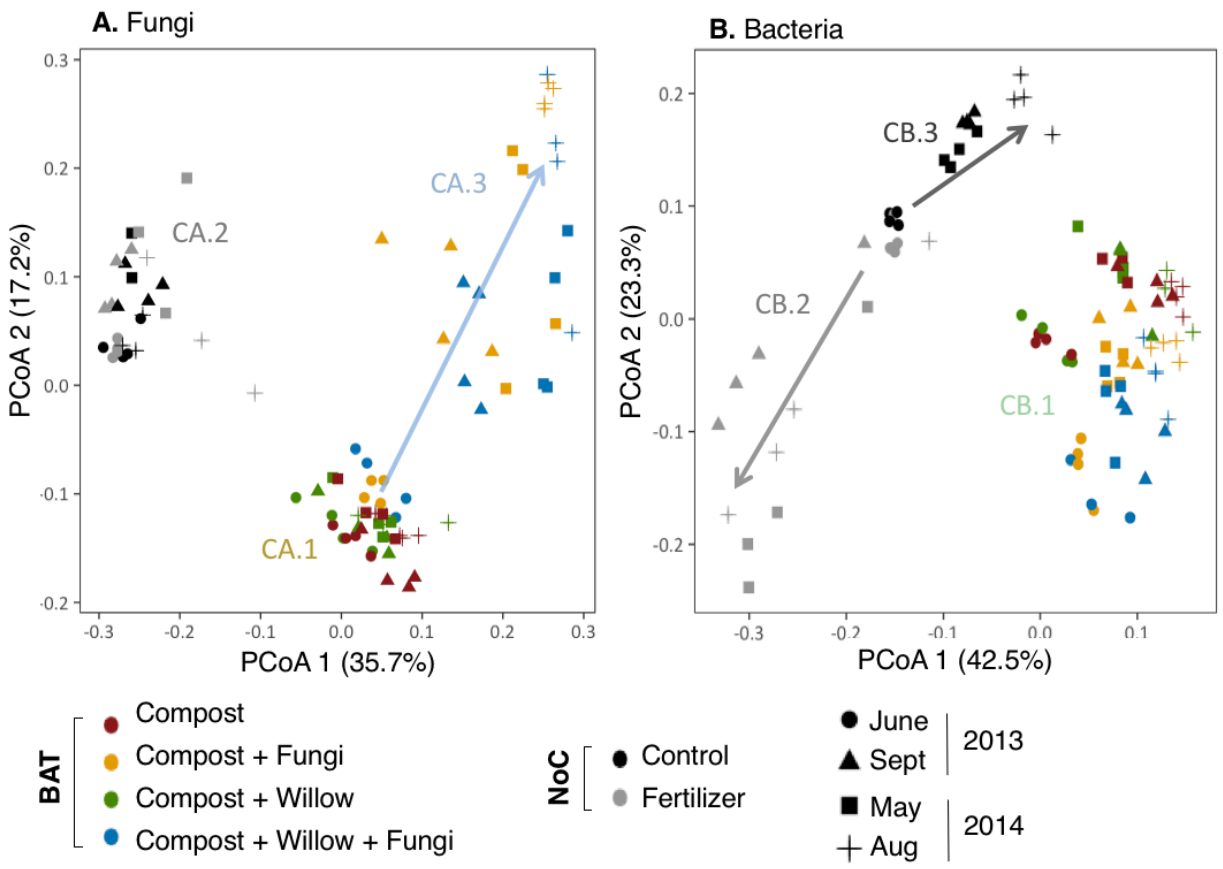

Figure 2.2 A. A principal coordinates analysis (PCoA) was performed on fungal OTU datasets using Jensen-Shannon divergence. **B.** Another PCoA was performed on bacterial OTU datasets using Bray-Curtis dissimilarity. Arrows indicate temporal shifts.

This study shows that a diesel-contaminated subarctic soil can be successfully remediated utilizing compost alone or with plants and fungi simultaneously. Despite promising research results with local organic amendments in northern regions, the fertilization and tilling method is still the most widely used for soil remediation in North America. This research paper proposes another angle on existing bioremediation tools by using multiple local biological amendments concomitantly, to best serve a specific site's requirements. As global resources continue to decrease and carbon emissions linked to global warming are taken more into account, research and remediation projects working to reduce their environmental footprints will become increasingly necessary (Sanscartier et al., 2010; Sauvé et al., 2016, 2015). Circular economy practices (Sauvé et al., 2016) and ecosystemic principle (Secretariat

De La Convention Sur La Diversite Biologique, 2004) can serve as guiding tools to increase sustainability in research practices; they helped guide our approach using local materials, rather than synthetic fertilizers. Finally, a variety of different local resources surrounds each northern contaminated site, and the use of site-specific amendments is recommended.

Declaration of interests

This project was funded through NSERC's College and Community Innovation (CCI) grant to S. Mooney (Cold Climate Innovation, YRC); an NSERC CREATE Mine of knowledge scholarship to KR; NSERC Discovery grants to MA and MH; NSERC northern supplement and Canada Research Chair grants to MA; the Northern Scientific Training Program to KR, and in-kind contributions from Boreal Compost Enterprises Ltd. and Arctic Backhoe Services Ltd, who also provided land space.

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Supplementary figures

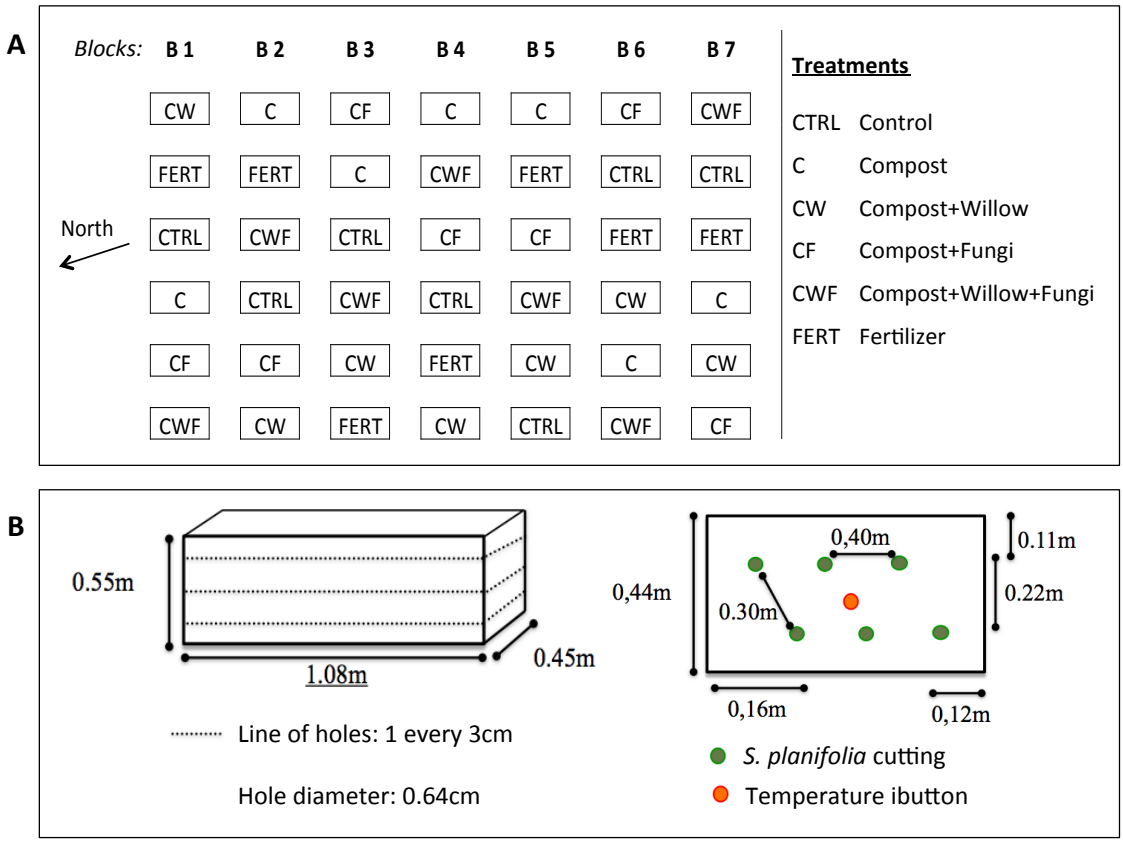

Figure 2.S1 A. Field layout of the 42 treatment bins, grouped by block. **B.** Experimental bin dimensions, placement of willow cuttings and temperature monitoring ibuttons. The bin on the left is a lateral view, while the bin on the right is as viewed from the top.

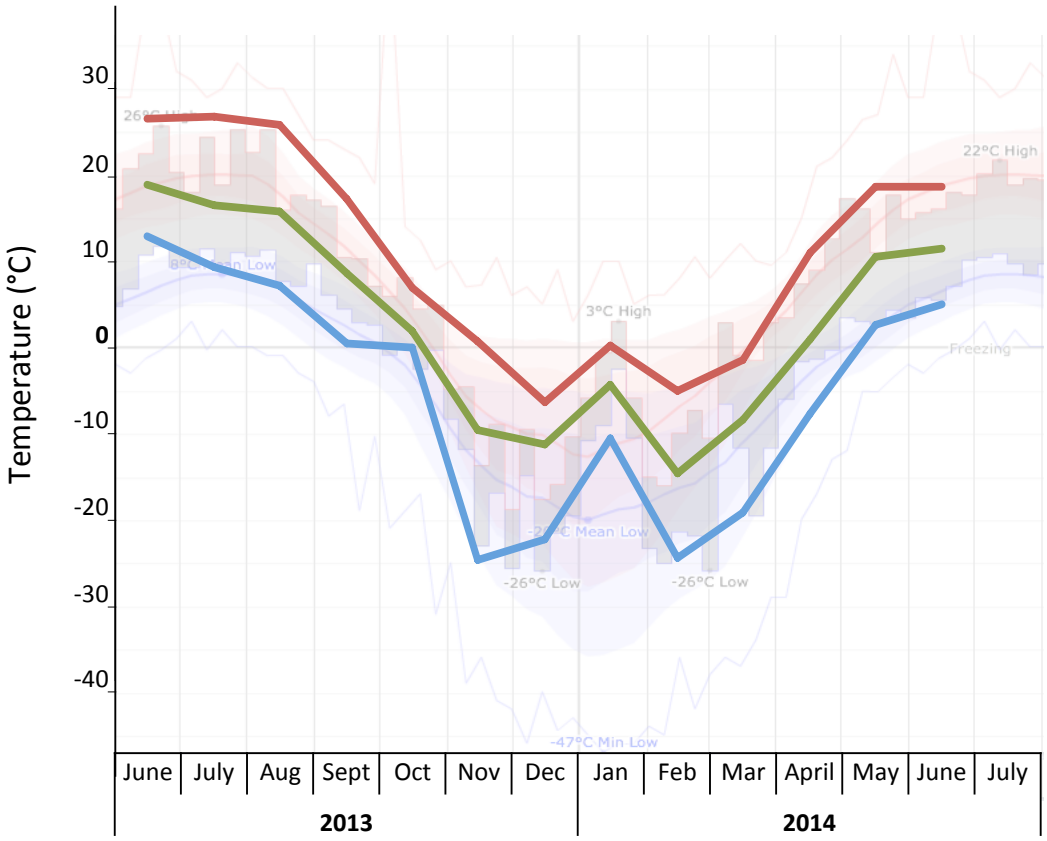

Figure 2.S2 Air and soil temperatures from June 2013 to June 2014 inclusively. The thick lines indicate the monthly highs (red), averages (green) and lows (blue), in the center of four experimental bins, while the back shaded area correspond to air temperatures recorded at the weather station located at the nearby Erik Nielsen Whitehorse International airport (Shaded backdrop of the graph extracted from: https://weatherspark.com/averages/28430/Whitehorse-Yukon-Territory-Canada). Soil temperatures narrowly follow air temperatures, indicating that there was essentially no temperature buffering effect from the soil, except from extremes.

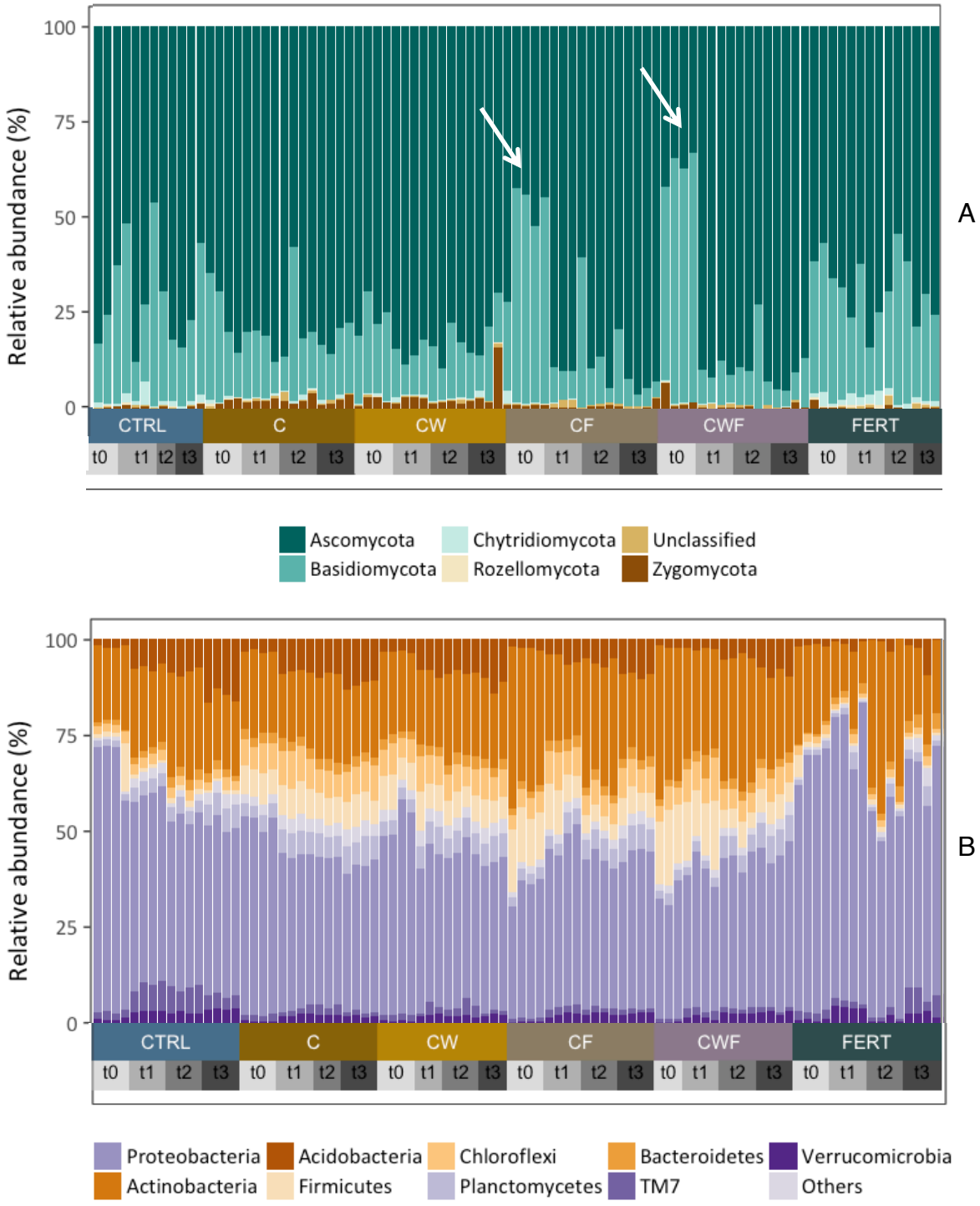

Figure 2.S3 Relative abundance of major phyla identified in the experimental soil. Data is presented here as a time evolution within each treatment. **A.** The five most prevalent fungal phyla. A higher relative abundance of *Basidiomycota* is visible at time zero for the two treatments that received an amendment of the Basidiomycete *Pleurotus ostreatus* (white arrows). **B.** The nine most prevalent bacterial phyla.

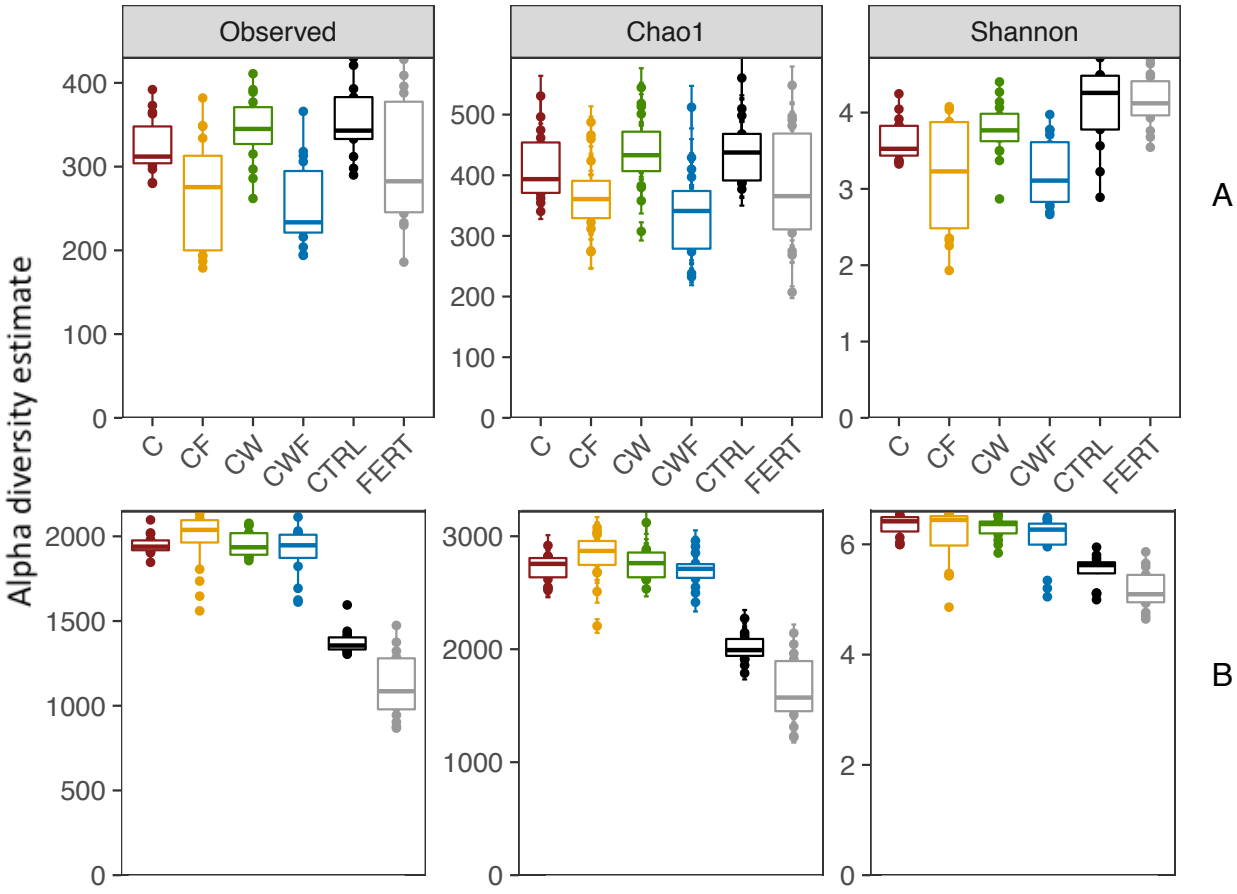

Figure 2.S4 Alpha diversity metrics used were Observed (OTU), Chao1 and Shannon. **A. Fungal alpha diversity**. Compost is not the driver of species abundance, but the addition of *P. ostreatus* does reduce abundance ($p < 0.05$). **B**. **Bacterial alpha diversity**. Compost significantly increases the observed community diversity (1600 to 2200 Operational Taxonomic Units (OTUs)) compared to the treatments without compost (500 to 1500) ($p < 0.05$. Results are the same on all three diversity metrics).

Supplementary methods

SM.1. AMMENDEMENTS

White rot fungi

The strain of white *P. ostreatus* (a naturally occurring species in the Whitehorse area) was purchased from Champignons Advitam (St-Ours, QC, Canada). *Pleurotus ostreatus* was grown on hardwood sawdust from liquid culture by Advitam.

A woodchip mix, from the City of Whitehorse's waste management services, composed of predominantly *Populus tremuloides* and *Populus balsamifera* (80 %) with a lesser portion of *Picea mariana* and *Pinus contorta* (20 %) was soaked for 24 h in tap water and then inoculated with pure sawdust spawn of *P. ostreatus*. This was left to incubate for 28 days at 18-22° C in 189 L polypropylene Rubbermaid containers with aeration holes. The mycelium colonized the woodchips (hereafter referred to as "naturalized mycelium") and was used in treatments CF and CWF. The objective of naturalization is to habituate the mycelium to be in a non-sterile environment and hence stimulate its immune system (Stamets, 2005). A second wood chip mix composed of *P. tremuloides* and *P. balsamifera* was directly incorporated in the treatment piles to serve as a primary carbon source for the fungi. These woodchips were freshly cut and chipped trees, which originated from roadside clearing activities on the Hotsprings Road, near Whitehorse, YT. They were air-dried in a well-ventilated area for 2 weeks for conservation and soaked for 24 h in tap water and drained before experiments began.

SM.2. QUALITY ASSURANCE

Table 2.SM.2.1 Compost quality report from A&L Canada on organic chemistry parameters.

PARAMETER	RESULT	UNIT	DETECTION LIMIT	METHOD REFERENCE
PCB	BDL*	ug/g	0.1	Gas Chromatography
Aldrin	BDL*	ug/g	0.01	GC/MS
b -BHC	BDL*	ug/g	0.01	GC/MS
g - BHC	BDL*	ug/g	0.01	GC/MS
a - BHC	BDL*	ug/ml	0.01	GC/MS
y - BHC	BDL*	ug/g	0.01	GC/MS
Dieldrin	BDL*	ug/g	0.01	GC/MS
Endrin Aldehyde	BDL*	ug/g	0.01	GC/MS
Endosulfan II	BDL*	ug/g	0.01	GC/MS
Endosulfan I	BDL*	ug/g	0.01	GC/MS
Endrin	BDL*	ug/g	0.01	GC/MS
Endosulfan Sulphate	BDL*	ug/g	0.01	GC/MS
Heptachlor	BDL*	ug/g	0.01	GC/MS
Heptachlor epoxide	BDL*	ug/g	0.01	GC/MS
Methoxychlor	BDL*	ug/g	0.01	GC/MS
Acenaphthene	BDL*	ug/g	1	GC/MS
Acenaphthylene	BDL*	ug/g	1	GC/MS
Antharacene	BDL*	ug/g	1	GC/MS
Benzo(a)anthracene	BDL*	ug/g	1	GC/MS
Benzo(a)fluoranthene	BDL*	ug/g	1	GC/MS
Benzo(ghi)perylene	BDL*	ug/g	1	GC/MS
Benzo(k)fluoranthrene	BDL*	ug/g	1	GC/MS
Benzo(a)pyrene	BDL*	ug/g	1	GC/MS
Chrysene	BDL*	ug/g	1	GC/MS
Dibenzo(ah)anthracene	BDL*	ug/g	1	GC/MS
Fluorene	BDL*	ug/g	1	GC/MS
Fluoranthene	BDL*	ug/g	1	GC/MS
Indeno(123-cd)pyrene	BDL*	ug/g	1	GC/MS
Naphthalene	BDL*	ug/g	1	GC/MS

Phenanthrene	BDL*	ug/g	1	GC/MS
Pyrene	BDL*	ug/g	1	GC/MS
Benzene	BDL*	ug/g	0.01	Gas Chromatography
4, 4 - DDT	BDL*	ug/g	0.01	GC/MS
Toluene	BDL*	ug/g	0.01	Gas Chromatography
Ethylbenzene	BDL*	ug/g	0.01	Gas Chromatography
4,4 - DDE	BDL*	ug/g	0.01	GC/MS
Xylene (Total)	BDL*	ug/g	0.01	Gas Chromatography
4,4 - DDD	BDL*	ug/g	0.01	GC/MS

* BDL - Below detectable levels

Table 2.SM.2.2 Compost quality report from A&L Canada on soil parameters.

Sample Number	Lab Number	pH	Lime Index	Available Organic Matter %	Phosphorus P ppm	Potassium K ppm	Magnesium Mg ppm	Calcium Ca ppm
101210	**9863**	**8.8**		**28.8**	**340**	**2754**	**774**	**6830**

Sulfur S ppm	Zinc Zn ppm	Manganese Mn ppm	Iron Fe ppm	Copper Cu ppm	Boron B ppm	Sodium Na ppm	Nitrate-N NO3-N ppm	Soluble Salt ms/cm	Nitrogen (Total) (%)	Moisture %
31	**43.9**	**44**	**144**	**3.2**	**4.2**	**762**	**45**	**2.8**	**1.58**	

INTERPRETATION

CEC	Percent Base Saturation					Proportional Equivalents (meq)				Cation Ratio		C/N Ratio
meq/100g	% BS	% K	% Mg	% Ca	% Na	K	Mg	Ca	Na	Mg/K	Ca/Mg	
77.3	**65.8**	**9.14**	**8.24**	**44.18**	**4.29**	**7.06**	**6.37**	**34.15**	**3.31**	**1:1**	**5:1**	
Optimum Range:		3 - 5	8 - 20	60 - 80		0.5 - 1.3				7:1	5:1	

Table 2.SM.2.3 Compost quality report from A&L Canada on metal concentrations.

PARAMETER	RESULT	UNIT	DETECTION LIMIT	METHOD REFERENCE
Arsenic	7.80	ug/g	1.00	TMECC.04.13
Cadmium	BDL*	ug/g	1.00	TMECC.04.06
Chromium	25.23	ug/g	1.00	TMECC.04.06
Cobalt	5.75	ug/g	1.00	TMECC.04.06
Copper	43.13	ug/g	1.00	TMECC.04.06
Lead	20.20	ug/g	1.00	TMECC.04.06
Mercury	BDL*	ug/g	0.10	TMECC.04.13A
Molybdenum	BDL*	ug/g	2.00	TMECC.04.06
Nickel	12.34	ug/g	1.00	TMECC.04.06
Selenium	BDL*	ug/g	1.00	TMECC.04.13
Zinc	147.10	ug/g	1.00	TMECC.04.06

Table 2.SM.2.4 Compost quality report from A&L Canada.

PARAMETER	RESULT	UNIT	DETECTION LIMIT	METHOD REFERENCE
Fecal Coliform	<3	MPN/g		TMECC.07.01
Salmonella	<3	MPN/4g		TMECC.07.02
Total Sharp Inert Materials (> 3.0mm)	BDL*	%	0.01	TMECC.03.08
Total Inerts (NonBio. >8 mesh)	BDL*	%	0.01	TMECC.03.08
Total Plastic Inerts (>8 mesh)	BDL*	%	0.01	TMECC.03.08
OM @ 550 deg C	35.80	%	0.10	LOI@550C
Moisture	7.30	%	0.10	TMECC.03.09
C:N Ratio	12:1			TMECC.05.02
Sieve 2 Inch (% Passing)	100.00	%	0.10	ASTMD422
Sieve 1 Inch (% Passing)	100.00	%	0.10	ASTMD422
Sieve 1/2 Inch (% Passing)	100.00	%	0.10	ASTMD422
Sieve 3/8 Inch (% Passing)	100.00	%	0.01	ASTMD422
Sieve 1/4 Inch (% Passing)	99.90	%	0.01	ASTMD422
Compost Stability Index	8	---		TMECC.05.08-B
Respiration-CO2-C/g OM/day	BDL*	mgCO2	0.01	TMECC.05.08-B
Respiration - CO2-C/g TS/day	BDL*	mgCO2	0.01	TMECC.05.08-B

SM.2.3 QUANTIFICATION OF HYDROCARBONS

The CCME fractions F2, F3 and F4 (C_{10}-C_{16}, C_{16}-C_{34} and C_{34}-C_{50}, respectively) were extracted by rotary evaporator methods and then analyzed by GC-FID (Gas Chromatography – Flame Ionization Detector).

In 2013, PHC extractions were conducted with a mechanical shaking method as described by Schwab *et al.* (1999). Approximately 2 g of soil per sample was placed in a glass scintillation vial. Acetone (10 mL) was added to each vial, which was then placed on the reciprocating shaker (E-6010) for 1 hr at 120 cycles min^{-1}. The contents were then transferred to a Falcon tube and centrifuged for 10 min at 1500 rpm. The liquid was then transferred to a second glass scintillation vial. Another 10 mL of acetone was added, and the shaking process was repeated. A hydrocarbon clean up procedure outlined by CCME was followed to remove water and polar organic compounds from each sample (CCME, 2001a). All glassware was cleaned by rinsing 3 times with hexane and once with acetone. A sodium sulphate clean-up was used to purify the sample. Soil was added to the column and rinsed with 10 mL of hexane, which was then collected in a round bottom flask. Toluene (1.8 mL) was added to the flask and the solution was evaporated to ~2 mL using a rotary evaporator (R-210, Buchi Labs, Switzerland). A silica gel cleanup was then performed to further purify the sample then done. Glass wool was inserted into the tip of the column and 5 g of silica gel (previously baked at 100° C for 24 h and stored in a desiccator) was added and then topped with 2.5 g of sodium sulfate. The column was rinsed with 1:1 hexane:DCM (dichloromethane). The sample was then transferred to the column and 10 mL of hexane was added and collected in a round bottom flask. Then, the contents of the flask were evaporated to 1-1.8 mL using the rotavapor. The volume was recorded, and the sample was transferred to a 2 mL GC vial. There was an unfortunate delay between extraction and analysis on GC-FID, and the recovery rates varied between 54.4 and 97.3 %. There were no significant recovery differences between treatments ($p < 0.05$) and the mean recovery rate across all treatments was 70.8 ± 10.5 %.

The PHC extraction for the 2015 and 2016 seasons were carried out in accordance to the "Reference Method for the Canada-Wide Standard for Petroleum Hydrocarbon in Soil – Tier 1 Method, Canadian Council of Ministers of the environment" (CCME, 2001a). In summary, the fractions F2, F3 and F4 were extracted with 1:1 hexane:acetone using a rotary

extractor. The extracts underwent a silica-gel clean-up to remove polar compounds in preparation for the on-column GC/FID. The recovery rates varied between 87.2 and 109.7 %.

SM.2.4 GENOMIC ANALYSES

A. DNA extraction, library prep and sequencing

Fungal and bacterial DNA was extracted from soil samples using the NucleoSpin Soil Kit (Macherey-Nagel).

For fungal DNA, library preparation was done using a two-step PCR method to amplify the ITS region of the ITS rRNA gene. In step-1, PCR primers ITS1F_CS1 (5' CTTGGTCATTTAGAGGAAGTAA 3') and 58A2R_CS2 (5' CTGCGTTCTTCATCGAT 3') were used to target ITS1 and to add step-2 priming sites. Step-1 PCR reactions were performed following this protocol: 0.1 µL of 5 U μL^{-1} Taq (Hot Start, Quiagen) and 1.25 µL DMSO (Sigma), with 2.5 µL 10X + 18mM MgCl2 buffer, 0.5 µL 10mM dNTPs, 5 µL of 0.5 µM ITS1F-CS1 and 5 µL of 0.5 µM 58A2R-CS2, 1 µL of DNA diluted 1/10 were combined in a 25 µL reaction. PCR reactions were performed as follows: initialization step at 96 °C for 900 seconds, denaturation at 96 °C for 30 seconds, annealing at 52 °C for 30 seconds, extension at 72 °C for 60 seconds and final elongation at 72 °C for 600 seconds, over 35 cycles.

During step 2 (ITS), 10- bp barcodes for sample identification and Illumina adaptor sequences were added to step-1 products. Step-2 primers were PE1_CS1_Fwr (5' AATGATACGGCGACCACCGAGATCTACACTGACGACATGGTTCTACA 3') and CS2 FLD0001 to FLD0384 (5' Illumina i7 sequence (CAAGCAGAAGACGGCATACGAGAT) + barcode (variable 10 bases in reverse complement) + CS2 (TACGGTAGCAGAGACTTGGTCT) 3'). Step-2 PCR reactions were performed following this protocol: 0.2 µL of Taq and 1.00 µL DMSO (5 %), with 4 µL 10X + MgCl2 buffer, 0.4 µL dNTPs, 1 uL of 2 uM PE1_CS1_Fwr and 1 uL of 2 uM CS2 FLD0001 to FLD0384 1µL of step 1 DNA diluted to 1/200 were combined in a 20 µL reaction. PCR reactions were performed as follows: initialization step at 95 °C for 900 seconds, denaturation at 95 °C for 15 seconds, annealing at 58 °C for 30 seconds, extension at 72 °C for 60 seconds and final

elongation at 72 °C for 180 seconds, over 15 cycles. Samples were purified using AMPure XP beads from Beckman Coulter following the manufacturer's protocol.

For bacterial DNA, library preparation was done using a two-step PCR method to amplify the V3-V4 region of the 16S rRNA gene. In step-1, PCR primers 341F_CS1 (5' CCTACGGGNGGCWGCAG 3') and 805R_CS2 (5' GACTACHVGGGTATCTAATCC 3') were used to target the V3-V4 region and to add step-2 priming sites. Step-1 PCR reactions were performed following this protocol: 0.1 µL of Taq Roche 5U/ul (FastStart High Fidelity PCR System, Sigma) and 1.5 µL of DMSO (Sigma) with 2.5 µL 10X+18mM MgCl2, 0.5 µL 10 mM dNTPs, 5 µL of 3 µM 341F and 805R, 9.65 µL H2O, 1 µL of DNA diluted 1/10 were combined in a 25 µL reaction. PCR reactions were performed as follows: initialization step at 94°C for 120 seconds, denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds and final elongation at 72°C for 420 seconds, over 30 cycles.

During step 2 (16S), 10-bp barcodes for sample identification and Illumina adaptor sequences were added to step-1 products. Step-2 primers were PE1_CS1_Fwr (5' AATGATACGGCGACCACCGAGATCTACACTGACGACATGGTTCTACA 3') and CS2 FLD0001 to FLD0384 (5' Illumina i7 sequence (CAAGCAGAAGACGGCATACGAGAT) + barcode (variable 10 bases in reverse complement) + CS2 (TACGGTAGCAGAGACTTGGTCT) 3'). Step-2 PCR reactions were performed following this protocol: 0.1 µL of Taq Roche 5U/ul and 2 µL of DMSO with 2 µL 10X+18mM MgCl2 buffer, 0.4 µL 10mMdNTPs, 1 uL of 2 uM forward and 1 uL of 2 uM reverse primers, 1 uL (14 ng) of DNA were combined in a 20 uL reaction. PCR reactions were performed as follows: initialization step at 95°C for 600 seconds, denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 60 seconds and final elongation at 72°C for 180 seconds, over 15 cycles. Samples were purified using AMPure XP beads from Beckman Coulter following the manufacturer's protocol.

ITS Library size was confirmed at 394-bp with BioAnalyzer (including 103 bp of primers and sequencing adapters). Libraries were quantified with a Qubit (Thermo Fisher Scientific High sensitivity kit), then pooled and denatured with NaOH, following the Illumina protocol. **Paired-end sequencing** (2 x 250bp) was performed with a MiSeq reagent Kit V2

(500 cycles) (Illumina). Sequencing was done in one run, with an average Q score greater than Q30 for 78.0 % of reads, and an average cluster density of 547 ± 19 K mm^{-2}.

16S Library size was confirmed at 577-bp with BioAnalyzer (including 103 bp of primers and sequencing adapters). Libraries were quantified with a Qubit, then pooled and denatured with 10µL 0.2N NaOH, following the Illumina protocol. **Paired-end sequencing** (2 x 250bp) was performed with a MiSeq reagent Kit V2 (500 cycles) (Illumina). Sequencing was done in one run, with an average Q score greater than Q30 for 80.6 % of reads, and an average cluster density of 618 ± 40 K mm^{-2}.

B. Sequence processing

For ITS sequences: Pre-processing was performed with FastQC (v0.11.5) (Andrews, 2010) to evaluate the quality of raw paired-end reads with mkdir fastqc_out and fastqc -t 4 raw_data/* -o fastqc_out/. PEAR (v0.9.10) (Zhang et al., 2014) was used for stitching of unambiguous read pairs together with run_pear.pl -p 4 -o stitched_reads raw_data/*. To confirm that read stitching was been correctly performed, FastQC was run again on the stitched reads. Based on these cutoffs, FASTX- Toolkit (v0.0.14) (Gordon, 2009) was used to keep only sequences with a Q score greater than Q30 (over at least 90 % of the read). Reads shorter than 200pb were filtered out as well as reads that did not exactly match the known primer sequences at the 5" and 3" ends using BBMap (v35.85) (Bushnell, 2014) with read_filter.pl. Chimeric sequences were identified and removed using VSEARCH (v1.11.1) (Rognes et al., 2016) with chimera_filter.pl, which implements the UCHIME algorithm (Edgar et al., 2011). This left us with a total of 2,782,582 reads for 97 samples, with an average of 28,686 ± 8,718 reads per sample. QIIME wrapper scripts (v1.91)(Caporaso et al., 2010) were used to perform open-reference OTU picking with pick_open_reference_otus.py at 97 % identity level (UNITE database) (Caporaso et al., 2012). The open-source methods SortMeRNA (v2.0-dev time stamped 29/11/2014) (Kopylova et al., 2012) and SUMACLUST (v1.0.00) (Mercier et al., 2013) were used for the reference-based and de novo clustering steps, respectively. Singletons and low-confidence OTUs (likely due to MiSeq bleed-through between runs) were removed with remove_low_confidence_otus.py (<0.1 % of reads). The

OTU table was rarefied to 7,700 reads with single_rarefaction.py, leaving us with a final data set of 92 samples and 1,527 OTUs.

For 16S sequences, primers were trimmed and only sequences with a Q score greater than Q33 were retained. The rest of data processing was performed with QIIME (version 1.9.0) (Caporaso et al., 2010). Paired-end reads were concatenated with the join_paired_ends.py script, and libraries were de-multiplexed with split_libraries_fastq.py. Chimeric sequences were identified and removed using identify_chimeric_seqs.py and filter_fasta.py using usearch 61. This left us with a total of 2,803,607 reads for 96 samples, with an average of 29,204 ± 5,882 reads per sample. Open-reference OTU picking was performed with pick_open_reference_otus.py at 97 % identity level using Greengenes taxonomy (version 13.8). Unassigned reads and OTUs with fewer than 10 observations across all samples were removed with filter_otus_from_otu_table.py. The OTU table was rarefied to 20,000 reads with single_rarefaction.py, leaving us with a final data set of 92 samples and 11,105 OTUs.

S3 C. Data analyses

For ITS and 16S, diversity analyses were performed using phyloseq{} (McMurdie and Holmes, 2013) in R (R Development Core Team, 2016). For alpha diversity, we used observed species as a count for OTUs, chao1 as a richness estimator, and Shannon's diversity index to measure richness and community evenness. Alpha diversity was compared across groups using a mixed model (nlme{} in R, Pinheiro *et al.*, 2017). Beta diversity analyses were visualized with PCoA using ggplot2{} in R(Wickham, 2009). We used Bray-Curtis dissimilarity to measure similarity across groups with vegan{} in R (Oksanen et al., 2016). Groups were compared with adonis() (analysis of variance with permutation test with pseudo-F ratios), permanova() in vegan{} in R (Oksanen et al., 2016) (the compare_categories.py script in QIIME was also used for 16S).

CHAPITRE 3. | Un microsystème écologique pour gérer une fosse à huiles usées : L'utilisation de plantes indigènes, de champignons et de compost pour assainir un sol à contamination mixte dans un climat froid

An ecological microsystem to treat waste oil contaminated soil: Using phytoremediation assisted by fungi and local compost on a mixed-contaminant site, in a cold climate

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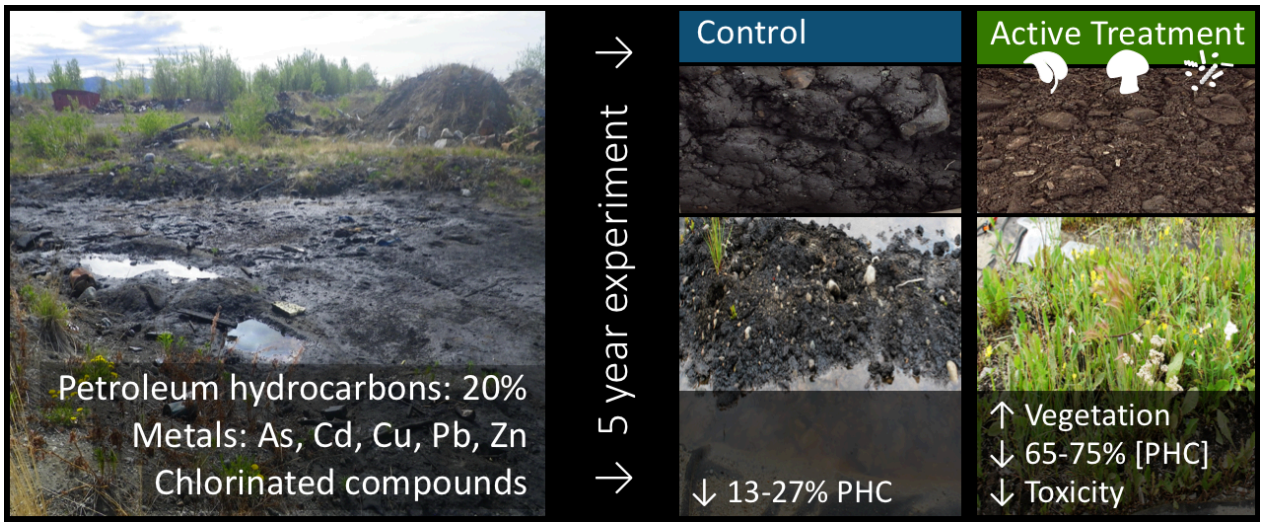
Abstract

As a result of anthropization and industrialization, northern remote communities face issues of soil contamination by mixtures of organic and inorganic contaminants. Soil bioremediation in cold environments is particularly challenging because of slower degradation rates, slower production of biomass for phytoextraction of trace elements (TEs), and remoteness, which can complicate logistics and inflate costs. This study evaluated a decontamination approach integrating indigenous willows, fungi and compost in a northern community. The site was a waste oil pit and its soil was initially contaminated with petroleum hydrocarbons (PHC) exceeding 200 g kg^{-1} and TEs including As, Cd, Co, Cr, Cu, Pb and Zn. In under five years, 65 and 75% of PHC (C_6-C_{50} and > C_{50}) were degraded, compared to 27 and 13% for the untreated control soil. We found contrasting TE translocation patterns to the aboveground biomass for the willow species used (*Salix planifolia* and *Salix alaxensis*), as well as distinctive rooting strategies. Hazard quotients were calculated to assess the risk plant material could pose to local wildlife. The highest TE concentration measured was Zn in *S. planifolia*, which exceeded Canadian soil guidelines. Results indicate toxicity risks to animals linked to TEs in *Salix* leaves is generally unlikely. The fungus *Trametes versicolor* inoculated into the soil did not fruit, however fruiting bodies of *Psathyrella* sp. were observed consistently (four out of five years). Biological tests indicated that in five growing seasons soil toxicity significantly decreased compared to the untreated soil used as control. This was demonstrated by vegetation cover (137 vs 11% cover), toxicity assays on earthworms (*Eisenia andrei*) (0 vs 33% mortality) and barley seed germination (*Hordeum vulgare*) (86 vs 62% germination). The proposed decontamination approach, without the use of synthetic fertilizers, is promising for the PHC remediation of mixed-contaminants on cold climate sites.

Graphical abstract



KEYWORDS:

Co-contamination, north, bioremediation, phytoremediation, mycoremediation.

HIGHLIGHTS:

- Local plants, fungi and compost can remediate highly contaminated subarctic soils.
- Petroleum over 150 g kg^{-1} can be decreased by 65–75%, without fertilizers.
- The microsystem decreased soil toxicity significantly compared to untreated control.
- The willows used have distinctive rooting strategies and TE accumulation patterns.
- Hazard Quotients to local animals were variable for different TE in willow leaves.

Introduction

The North has a history of contamination events linked to periodic exploration for natural resources and their exploitation, temporary installations, dump sites, and industrial activities (Poland et al., 2003). Many of these sites have historically remained untreated either because of their isolated locations or costs associated with treatment (Poland et al., 2003). Excavation and transportation are often prohibitive in terms of cost and questionable in terms of broader environmental impacts (Camenzuli and Freidman, 2015; Sanscartier et al., 2010). Natural contaminant degradation rates in the North can also be significantly slower than in temperate regions due to cold temperatures and limited moisture which can further slow microbial processes (Sanscartier et al., 2009; Snape et al., 2008; Walworth et al., 2007). Additionally, for historically polluted sites, bioavailability of weathered petroleum hydrocarbon (PHC) can be an important limiting factor in degradation processes (Mohn and Stewart, 2000; Ramadass et al., 2018).

Bio-stimulation of indigenous soil microbes, through landfarming and biopiles with aeration and nutrient amendments such as fertilizers, has been largely used for cleaning up PHC-contaminated sites in cold regions (Aislabie et al., 2008; Paudyn et al., 2008). Other methods have also been utilized for TE contaminated soil using permeable reactive barriers, chemical fixation, electrokinetic separation, land capping and lining, pump and treat systems as well as bioremediation and phytoremediation (Camenzuli et al., 2013; Yadav et al., 2018). The remediation of mixed-contaminants soils, with both TE and organic contaminants, comprises supplementary challenges such as the inhibition of PHC-degrading bacteria by TE (Dong et al., 2013) or the need for sequential treatment (Lu et al., 2010). Innovative lab-based strategies, such as the use of silicate to treat mixed contaminants under cold temperatures are promising, but still need to be scaled up and optimized for the remediation of PHC (Camenzuli et al., 2017). Sometimes, even after excavation and transport, these soils are buried rather than treated due to the technical challenges and costs associated with their remediation. From both economical and ecological standpoints, there is a clear need for the development of on-site cold-climate adapted alternatives to treating mixed contaminants sites. Technology coupling can be advantageous in such sites (Camenzuli and Freidman, 2015). For instance, bioremediation methods using plants (phytoremediation) and fungi (mycoremediation) are interesting because they are low-tech

remediation methods that can be applied *in situ* to isolated regions for both organic and inorganic contaminants (Harms et al., 2011; Juwarkar et al., 2010).

Plants can not only enhance the degradation of petroleum hydrocarbons (PHC) by releasing root exudates into the soil that stimulate microbial activity, but also they can absorb and accumulate trace elements (TE) in their tissues (Juwarkar et al., 2010). Willows (*Salix spp*.) are hardy plants that are used extensively in a wide range of bioremediation projects as well as for revegetation. Their rapid growth, large biomass and high accumulation capacity for TEs make them interesting for phytoremediation (Vondráčková et al., 2017). *Salix spp*. are found in abundance in many geographical locations requiring remediation, notably in northern regions, and locally adapted shrubs that will grow rapidly under the local pedoclimatic conditions are essential for phytoremediation success (Rockwood et al., 2004). Furthermore, implementation of native trees and shrubs that are well adapted to local conditions can increase long-term petroleum removal (Leewis et al., 2013). Accumulation and immobilization of TE in belowground biomass pose little to no risk for movement of contaminants from the soil matrix. On the contrary, accumulation in aboveground biomass may create an exposure pathway to local wildlife, especially where *in situ* remediation techniques are deployed. Using plant species that are forage for local wildlife requires monitoring to ensure there are no risks of TE transfer to wildlife. *Salix spp*. are an important forage species to many northern animal species such as moose, woodland caribou, snowshoe hare, ptarmigan and grouse (Pugh et al., 2002). To measure that these technologies do not pose significant risks to the surrounding environment, hazard quotients (HQ) can be calculated based on the leaf concentrations, the average daily intake (ADI) of forage by local animal species, and toxicity reference values (TRVs) for TEs of concern.

Fungi are ubiquitous in soil environments, even in polar regions (Koltz et al., 2018). Although they are usually under-investigated in northern contaminated soils, they may play an important role in the degradation of PHC (Aislabie and Foght, 2008). Their use has been limited because they are difficult to establish in large-scale field sites, but their potential in bioremediation has been clearly demonstrated (Harms et al., 2011). Wood-digesting fungi have an arsenal of enzymes able to digest a wide range of organic contaminants including polycyclic aromatic hydrocarbons (PAHs), chlorinated compounds and pesticides (Harms et al., 2011; Marco-Urrea et al., 2008; Pozdnyakova et al., 2018). Other fungi have shown the ability to concentrate incredibly high concentrations of TEs in their fruiting bodies (over 650 mg kg -1 of

each Cu, Cd, Pb, and Zn from a soil initially contaminated at 50 mg kg^{-1}) (Damodaran et al., 2013).

The addition of organic matter in contaminated soils, especially compost, can accelerate the degradation of organic contaminants and reduce TE mobility (Zubillaga et al., 2012). It improves soil structure to allow better aeration and acts as a reservoir of microbes (Brady and Weil, 1996a) while fostering the important phases of plant establishment (Guidi et al., 2012) and revegetation (Palmroth et al., 2006). Additionally compost acts as a nutrient reserve and structural support for the organisms involved in soil bioremediation (Wyszkowski and Ziólkowska, 2009). To tie it all together, synergistic bioremediation mechanisms have even been established between plants and fungi to accelerate PHC degradation, while reducing soil TE concentrations (Asemoloye et al., 2017). In the end though, many of these methods have yet to be used concomitantly on large-scale mixed-contaminants projects in northern regions. Here, we propose a novel bioremediation approach to northern mixed contaminants sites, using a combination of exclusively local biological components.

A highly contaminated site at the Son of War Eagle landfill (Yukon, Canada) was chosen to test the combination of northern plants, fungi and municipal compost. From 1968 to 1989, large quantities of waste oil, lubricants, sewage trucks and other waste such as oil filters and drums were disposed of in an unregulated manner at this location, which is City of Whitehorse's former landfill site. The waste oil pit itself was placed on top of a large waste rock pile produced by copper mining at the beginning of the 20th century (Fig. 3.A.1).

The objective of this study was to assess the phytoremediation efficiency by combining plants, fungi and compost to clean up a historically polluted site in the Canadian Subarctic. This approach is an integrated strategy that is cost-effective and that we coined as the "Ecological Microsystem" approach. We established and conducted the experiment over five years with the aim to clean up a mixed contaminated soil that showed extreme contamination level of PHC (> 200 000 mg kg^{-1}). A control treatment was used for comparison. We sought to quantify the TE accumulation patterns of two indigenous *Salix* species (*S. planifolia* and *S. alaxensis*) to assess their risk of being vectors of contamination to the surrounding wildlife. We also assessed the viability of the aggressive ligninolytic fungi *Trametes versicolor* in a large-scale waste oil-pit setting. The soils' toxicity to plants and invertebrates was tested.

Materials and methods

Site description

This experiment took place over five growing seasons from 2013 to 2017, at the Son of War Eagle landfill (60°44'31.3"N, 135°10'19.9"W), a site that had been contaminated for more than 40 years. The initial soil contained multiple contaminants in concentrations above the Canadian Environmental Guidelines for Industrial sites (Canadian Council of Ministers of the Environment (CCME-I)). The contaminants included up to 200 000 mg kg^{-1} of total petroleum hydrocarbons (TPHC) (aliphatic and aromatic), various TEs (including As, Cd, Cu, Pb and Zn) and chlorinated compounds (tricholoethylene and tetrachloroehtylene).

The soil's appearance was a dense hybrid of clay and tar (see graphical abstract) with an initial pH of 7.16 ± 0.16 (1:2 soil:water) and no visible organic matter. Despite stagnant water on the surface, the initial soil moisture was only 11.7 ± 1.7 % by mass, determined gravimetrically (105°C for 6 hours minimum). No water, other than natural precipitation, was added throughout the five-year duration of the project to mimic real field application where water availability may be limited. Mean annual precipitations for this area are 262 mm and the mean temperature of the hottest month (July) is 14.3 °C (data from the Whitehorse airport weather station for years 1981-2010 (Whitehorse A)).

Experimental design

Salix cuttings were harvested close to the experimental site from native *Salix* growing naturally. The two species selected originated from 2-3 small clusters each. They were in their dormant state in March prior to the start of the project and stored for 3 months at -18 °C (in thick black plastic bags). The branches were then thawed and cut into 30 cm-long segments (1.3 to 2.5 cm wide) and soaked in 20 cm of water for 24 h prior to planting 15 cm deep in the experimental cells (based on lab trials, data not shown). Twelve individual cells (1m (L) x 2m (W) x 0.5m (H)) were built up with gravel on a flat area just outside of the waste oil pit. The gravel was covered with sand, geotextile and a Hazguard® 1 000 liner (Layfields, Table 3.A1) to prevent leaching of the contaminants in the soil below. A final layer of geotextile was then applied to protect the liner from solar radiation. Soil ($12m^3$) was excavated from the pit with a backhoe and mixed thoroughly. For the untreated soil treatment (UNTRD), six batches of 1 m^3 of soil were mixed

individually (to further homogenize the dense and sticky soil). Each cubic meter of soil was then placed in one of the six cells reserved for the UNTRD, which served as a control for natural attenuation. For the "Ecological Microsystem" treatment (ECOS) soil was mixed in the same matter, with the addition of the local biological amendments. These were added to the soil to a ratio of: 30 % compost; 13 % *Trametes versicolor* fungal spawn (incubated on oak sawdust, moisture 60%); and 17 % wood chips from road-side clearing activities to support the fungi (a sun-dried mix of green *Populus* spp. (60%) and *Picea mariana* (40%)). Eight cuttings *of Salix alaxensis* var. *longistylis* (referred to as *S. alaxensis* further in the text) were planted in each ECOS cell. The amendments were added by volume (60:40, amendments:contaminated soil), but since their density was much lower than the soil, the additions totaled approximately 20% by mass. Spontaneous growth of other fungi on the treatment plots was visually assessed and the frequency was noted.

In the second growing season we introduced what we named "Life Pockets" to replace the first year's all-dead *Salix* cuttings. They consisted of small jute bags filled with compost and clean topsoil (50:50 ratio, ≈3 L each) and were introduced to promote plant establishment and survival. Eight Life Pockets were placed in each cell and two un-rooted *Salix* cuttings were planted in each (one *S. planifolia* and one *S. alaxensis*) (Fig. 3.A.2).

Sampling

Soil samples were taken from the experimental cells nine times over five years, when the ground was not frozen. Each time, soil cores (n = 4) were taken in each treatment cell, from a depth of 5 to 35 cm, and homogenized into one composite sample. The homogenized samples were placed in a 250 mL glass jars with a Teflon-lined lid and kept at -20 °C until analysis for petroleum hydrocarbons, TE and chlorinated compounds. At the end of the experiment, 1 L of soil was taken from each of the twelve plots (following the above-mentioned method) for biological tests (a test for measuring emergence and growth of plant seeds and test for toxicity of contaminated soil to earthworms). This soil was not frozen but kept refrigerated until time of analysis. Above and below ground plant tissue of living *S. planifolia* and *S. alaxensis* were extracted by shovel in the last year of the study to assess their structure and TE accumulation. They were kept frozen at -20°C until analysis of biomass and TE content was performed on leaves.

Contaminants quantification

For organic contaminants, the CCME petroleum hydrocarbon fractions F2, F3, F4 and F4G (C_{10-16}, C_{16-34}, C_{34-50}, and $>C_{50}$ respectively) as well as PAHs were extracted by rotary extractor methods. F1 (C_{6-10}) and VOCs (including chlorinated compounds) were extracted with methanol and the headspace was isolated. The F1 to F4 fractions were analyzed by GC-FID (Gas Chromatography – Flame Ionization Detector), while F4G was determined gravimetrically. PAHs were analyzed by capillary column gas chromatography followed by mass spectrometric detection (GC/MS), which was also used for VOCs (for detailed methods see 3.B3.). All PHC value met the recovery % (± 30%) or were re-analyzed (Table 3.B1). TE present in the soil at the very beginning of the project were analyzed with procedures adapted from the CSR Analytical Method "Strong Acid Leachable Metals (SALM) in Soil" (CSR, 2017a) and procedures adapted from EPA Method 200.2 (Martin et al., 1994) (Appendix 2.B1. Soil metals quantification). *Salix* leaves TE concentrations were obtained with a digestion conducted with the Microwave Accelerated Reaction System followed by ICP-MS (inductively coupled plasma-mass spectrometry) quantification (samples were from the last sampling date of the study). The elements that did not meet the % recovery and measured value were not further analyzed (within ± 20% of the certified reference material (spinach leaves SRM 1570a, NIST), n = 4). Not all TEs were certified in the reference material (B2. for detailed methods).

Risk assessment

The transfer of TEs from *Salix* spp. leaves to the surrounding wildlife whose diet includes *Salix*, was assessed with a hazard quotient (HQ) approach, where HQ = Exposure Concentration/Reference Concentration. The calculations used in Table 3.3 were adapted from Jimmo et al. (2018). The herbivorous receptor's chosen were moose (*Alces alces*) and snowshoe hare (*Lepus americanus*), which consume *Salix* spp. in their diet and are commonly found around the field site. A HQ < 1 means likelihood of risk is acceptable while an HQ ≥ 1 means the likelihood of risk is not acceptable (US-EPA, 2018). Safety factors are not included in calculations.

Biological tests

Percent vegetation cover was visually assessed by species, and then cumulated for total vegetation cover per plot. In the fifth and last summer of this project, two common model

organisms were used to assess the toxicity of the UNTRD and ECOS soils. The earthworm *Eisenia Andrei*'s survival over 14 days (10 worms per sample) was conducted in accordance to the reference method MA. 500 – VTL 1.0 (CEAEQ, 2012). Barley's (*Hordeum vulgare*) germination rates were tested (25 seeds per sample) in accordance to the reference method MA. 500 – GCR 1.0 (CEAEQ, 2003). An uncontaminated soil was used as a clean control in the all of the trials (n = 12 per biological test).

Statistical analysis

A linear mixed-effects repeated measures model fitted by Restricted Maximum Likelihood (REML) was performed with the nlme{} (Pinheiro et al., 2017) in R (R Core Team et al., 2016) to compare: decreases in PHC concentrations, moisture levels, toxicological data, and pH between treatments. Post-hoc multiple comparison analysis were performed with the HSD Tukey method (lsmeans{}(Lenth, 2016) and mltcompview{} (Hothorn et al., 2008)). Welch's two sample t-test was used to measure PHC concentrations differences between two times (within a single treatment) as well as for TE concentrations in *Salix* leaves between species (R Core Team et al., 2016). Assumptions of normality and homoscedasticity were met for all tests.

Results

Decline of organic contaminants

Petroleum hydrocarbons

The soil initially contained nearly 200 000 mg kg^{-1} TPHC. This represents a substantial 20% of the soil mass (w/w). After five growing seasons, the ECOS soil contained significantly less PHC than the UNTRD soil for all fractions measured (mixed model, $p < 0.05$) (Fig. 3.1A). PHC chains from 6 to 50 carbons (CCME's F1 – F4) had decreased by 74.9 ± 4.9 % in the ECOS, compared to 26.7 ± 19.5 % in the UNTRD, which represented natural attenuation. In the ECOS, the F4 fraction was now below the CCME's guidelines for industrial sites (6 600 mg kg^{-1}). The heaviest fraction (F4G) composed of carbon chains greater than 50 carbons long were also degraded in greater proportions in the ECOS (65.0 ± 7.9 %) than in the UNTRD (13.3 ± 15.8 %)

(mixed model, $p < 0.05$) (Fig. 3.1B). The 13% degradation measured in the UNTRD over five years was not statistically significant (t-test, $p > 0.05$) (Table 3.1).

Table 3.1 Concentration of petroleum hydrocarbon fractions in the soil at the beginning (July 2013) and end (August 2017) of the study. Values are in mg kg^{-1} (± standard deviation). BDL stands for below detection limit.

		UNTRD		ECOS	
Parameter		**beginning**	**end**	**beginning**	**end**
pH		7.16 ± 0.16	6.93 ± 0.04	7.51 ± 0.122	7.2 ± 0.06
Moisture (%)		11.7 ± 1.5	19.1 ± 3.3	25.1 ± 2.4	37.7 ± 2.2
PHC	**CCME**				
F1 (C6-C10)	320	323 ± 52	BDL ± BDL	244 ± 117	BDL ± BDL
F2 (C10-C16)	260	5 000 ± 797	1 183 ± 678	3 872 ± 748	750 ± 0
F3 (C16-C34)	2 500	66 283 ± 7 833	51 117 ± 13 329	54 100 ± 7 063	13 467 ± 2 104
F4 (C34-C50)	6 600	19 717 ± 1 738	14 217 ± 4 528	15 717 ± 2 139	4 033 ± 1 115
F4G (>C50)	-	92 417 ± 9 318	79 533 ± 13 369	76 600 ± 10 783	26 433 ± 4 495

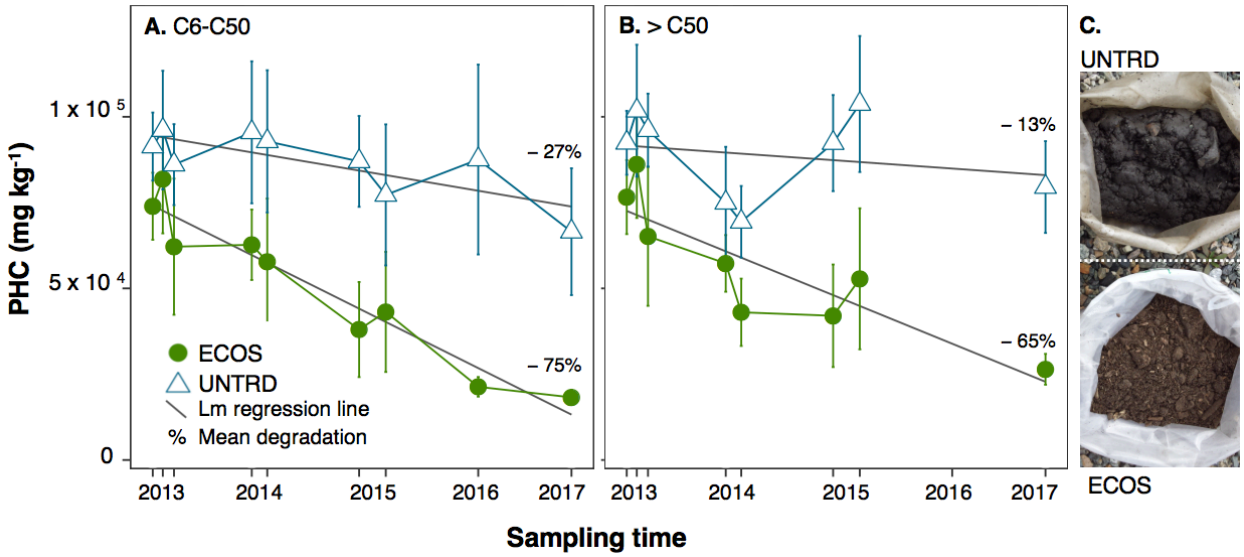


Figure 3.1 Petroleum Hydrocarbon concentrations over time (reported in mg kg^{-1}). **A.** F1 to F4 fractions ($C_6 - C_{50}$). **B.** The heaviest PHC fraction measured (F4G, > C_{50}). Error bars represent standard deviation. The slopes are linear fitted regression lines. The percentages presented are the mean decreases based on the respective initial concentrations of PHC. The active treatment (ECOS) led to significantly more reduction in PHC than the control (UNTRD) for all fractions measured (mixed model, $p < 0.05$). **C.** Visual appearance of soils in September 2016.

Chlorinated compounds and aromatic hydrocarbons

Two years and four months after initial set-up, the monocyclic aromatic hydrocarbons (MAHs), which initially exceeded the CCME-I limits in at least two samples (benzene, toluene and xylene) were all below detection limits (0.005, 0.05 and 0.075 mg kg^{-1} respectively), with the exception of toluene in the UNTRD. More precisely, toluene was present in all the UNTRD cells (0.19 ± 0.16 mg kg^{-1}) and below detection limit (BDL) for all ECOS cells. The polycyclic aromatic hydrocarbons (PAHs) initially measured were all below the CCME-I guidelines and were not tested again (Table 3.C.2). The chlorinated compound trichloroethylene was now BDL (< 0.01 mg kg^{-1}) for both treatments. Tetrachloroethylene was also BDL (0.05 mg kg^{-1}) for the ECOS, but not for three cells of the UNTRD (0.17 ± 0.07 mg kg^{-1}).

Trace elements in soil and leaves

Soil TE concentrations were measured at the beginning of the experiment to get baseline concentrations. Of the 31 elements tested, four TE ions of concern (As, Cu, Pb and Zn) (Nordberg et al., 2007) were above the CCME-I guidelines in at least two soil samples (Table 3.2). While the rest did not exceed that guideline, their concentrations were generally high. When handling the leaves there was no visual signs of detrimental effects from the TEs, but our results clearly indicate translocation from the soil to aboveground biomass. 19 essential and non-essential TEs were measured in the *Salix* leaves. None of the leaf material exceeded the ECOS total initial soil concentrations for either *Salix* species. Concentrations of TEs that are usually reported as poorly transported to leaf material such as Pb were low in our samples, while TEs reported as more mobile such as Cr and Zn were present in higher concentrations (Verbruggen and Hermans, 2013). There is a clear separation in the preferential accumulation of different TEs between the two species. *S. alaxensis* accumulated significantly more As, Co, Cr, Fe, Pb, Sb, and U, while *S. planifolia* accumulated more Cd, Cu, Mn, Zn (t-test, $p < 0.05$). The TEs Ag, Ba, Mo, Ni and Sr were statistically equivalent between species (Fig. 3.2A) (t-test, $p > 0.05$). TEs in the *Salix spp*. leaves were below the CCME-I soil guidelines, except for *S. planifolia*'s Zn concentrations, which exceeded them (361.2 ± 74.3 mg kg^{-1} of Zn (dry leaf tissue) from soil containing 589.8 ± 72.1 mg kg^{-1}) (Table 3.2).

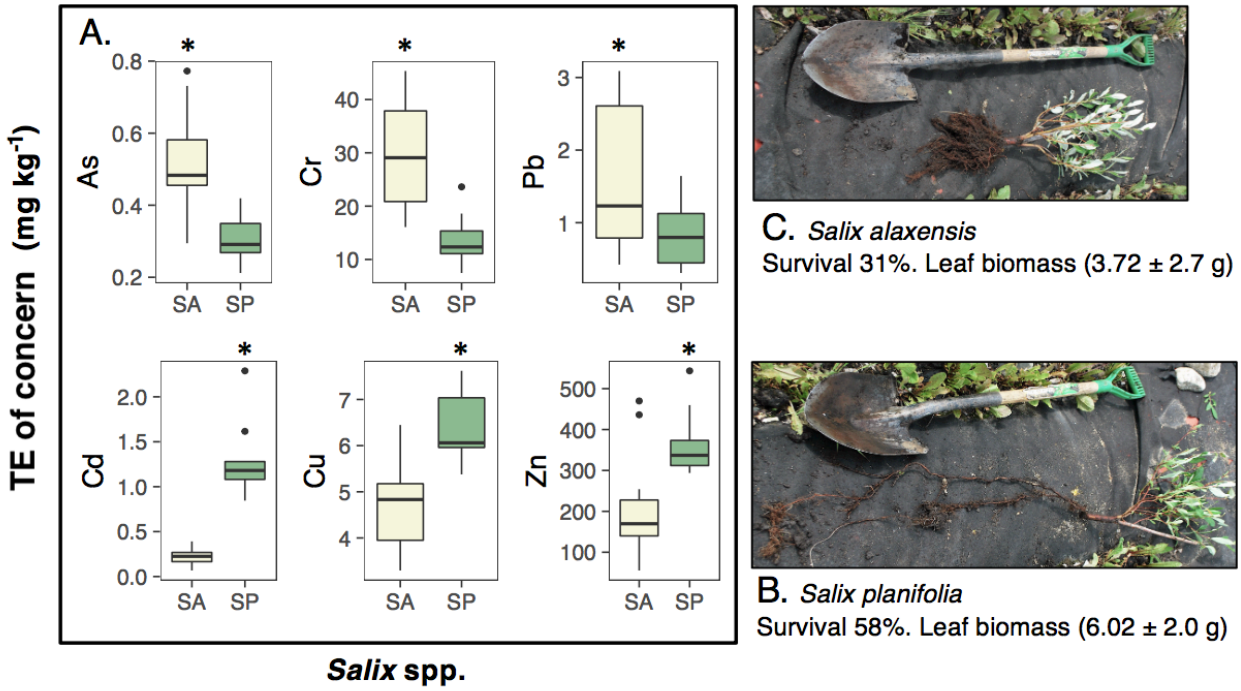


Figure 3.2 A. *Salix* leaves concentrations of the following TE: As, Cd, Cr, Cu, Pb and Zn. All values are in mg kg^{-1}. SA stands for *Salix alaxensis* and SP for *S. planifolia*. The * symbols denote statistically higher concentrations in one species (Welch's t-test, $p < 0.05$) **B.** Guerilla growth style of *S. planifolia* with its long runners. **C.** Clustered roots of *S. alaxensis* in phalanx growth form. The reported percent survival of the *Salix* is four years after the cuttings were planted and leaf biomass means are reported per plant (± SD) (n = 48 per species).

Risk assessment

The hazard quotients (HQs) indicated that there is some likelihood of risk to one ecological receptor from consumption of TE accumulated in *Salix spp*. through their diet. The chronic exposition HQ values for *L. americanus* are >1 for Zn in both *S. alaxensis* and *S. planifolia* (Table 3.3). This value above one indicates that the likelihood of risk is not acceptable for this animal and further investigation is required. For *A. alces* the acute and chronic HQ for both Cr and Zn are <1 for both *Salix* spp., which indicates the likelihood of risk is acceptable (Table 3.3).

Table 3.2 Trace element concentrations in soil at the beginning of the experiment (July 2013) and in the two species of *Salix* leaves at the end (August 2017). The presented CCME environmental guideline is for industrial sites and experimental soil values exceeding them are in bold case. All values are in mg kg $^{-1}$ (± SD).

	D.L.	CCME	UNTRD	ECOS	*Salix planifolia*	*Salix alaxensis*
Ag	0.1	40	1.5 ± 0.4	1.2 ± 0.2	0.004 ± 0.002	0.008 ± 0.007
Al	50	-	9978.3 ± 281	8943.3 ± 501		
As	**0.1**	**15**	**15.53 ± 4.39**	**12.57 ± 0.76**	0.31 ± 0.06	0.51 ± 0.14
Ba	0.5	400	202.5 ± 32.3	186.5 ± 16.2	3.01 ± 0.75	3.45 ± 1.38
Be	0.2	8	0.3 ± 0	0.3 ± 0		
Bi	0.2	-	1.6 ± 0.2	1.3 ± 0.4		
Ca	50	-	18433 ± 11216	24283 ± 5578		
Cd	0.1	1.5	3.57 ± 2.15	2.07 ± 0.25	1.26 ± 0.38	0.23 ± 0.1
Co	0.1	300	7.5 ± 0.2	7 ± 0.5	1.24 ± 0.25	1.95 ± 0.6
Cr	0.5	60	36.7 ± 1.8	32.6 ± 1.7	13.7 ± 4.3	29.8 ± 11
Cu	**0.5**	**90**	**235 ± 30.8**	**186.3 ± 56**	6.4 ± 0.7	4.68 ± 0.9
Fe	50	-	18000 ± 369	15967 ± 480	234.4 ± 63.2	357.5 ± 89.7
Hg	0	40	0.391 ± 0.159	0.31 ± 0.09		
K	100	-	1152 ± 90	2165 ± 99		
Li	5	-	9.6 ± 0.7	8.9 ± 0.5		
Mg	20	-	6125 ± 175.2	6186.7 ± 432		
Mn	1	-	269.2 ± 12.2	294.8 ± 6	65.5 ± 22.5	30.8 ± 13.1
Mo	0.5	40	8.3 ± 5.3	4.7 ± 0.4	3.14 ± 0.46	3.39 ± 1.13
Na	100	1000	266.7 ± 28	521.7 ± 40.2		
Ni	0.5	500	25.8 ± 1.5	23.9 ± 3	0.135 ± 0.023	0.121 ± 0.033
P	50	-	1325 ± 86	1803 ± 77		
Pb	**0.5**	**100**	**709.8 ± 107.4**	**536.5 ± 164**	0.86 ± 0.48	1.62 ± 1
Sb	0.1	40	3.2 ± 0.8	3 ± 1.9	0.023 ± 0.006	0.045 ± 0.014
Se	0.2	10	0.5 ± 0.1	0.4 ± 0		
Sn	2	300	112.8 ± 18.4	66.5 ± 13.3	0.07 ± 0.05	0.169 ± 0.141
Sr	0.5	-	54.8 ± 5.1	72.6 ± 11.3	14.7 ± 3.3	13.7 ± 4.6
Ti	1	-	599.2 ± 43	490.7 ± 54.3	4.69 ± 0.86	8.02 ± 1.99
Tl	0.1	-	0.13 ± 0.02	0.11 ± 0.01	0.002 ± 0.001	0.002 ± 0.001
U	0.1	-	1.22 ± 0.09	1.18 ± 0.34	0.005 ± 0.002	0.009 ± 0.005
V	0.2	-	41.1 ± 1.2	36 ± 2		
Zn	**1**	**150**	**779.7 ± 165.3**	**589.8 ± 72.1**	361.2 ± 74.3	206.1 ± 129.3

Table 3.3 Risk assessment calculations for two local ecological receptors based on their potential intake of two TEs though *Salix* spp. HQ values > 1 indicate the likelihood of risk is unacceptable.

		Ingestion rate[†]	Max [TEl] in Salix leaves	ADI[Δ]	ACUTE[§] TRV[¥]	ACUTE[§] HQ[∂]	CHRONIC[Ø] TRV[¥]	CHRONIC[Ø] HQ[∂]
		g *Salix* dw day-1	mg ai g^{-1} Salix	mg ai kg^{-1} bw day^{-1}	mg ai kg^{-1} bw day^{-1}		mg ai kg^{-1} bw day^{-1}	
Salix alaxensis	***Alces alces***							
	Cr	38.4	45.4	0.9	183*	0	768	0
	Zn		469.9	8.8	89.8	0.1	44.9	0.2
	Lepus americanus							
	Cr	3.6	45.4	12.8	183*	0.1	2011	0
	Zn		469.9	132.8	235.2	0.6	117.6	***1.1***
Salix planifolia	***Alces alces***							
	Cr	38.4	23.6	0.4	183*	0	768	0
	Zn		543.4	10.2	89.8	0.1	44.9	0.2
	Lepus americanus							
	Cr	3.6	23.6	6.7	183*	0	2011	0
	Zn		543.4	153.6	235.2	0.1	117.6	***1.3***

dw = dry weight. ai = active ingredient. bw = body weight.

[†] Food ingestion rate for *A. alces* obtained from McArt et al. (2009) and *L. americanus* from Ellsworth and Reynolds (2006).

[Δ]ADI (Average Daily Intake) is calculated on a per Kg basis: (Food ingestion rate[†] x Maximum observed TE concentrations in *Salix* leaves) / (Animal body weight[‡] x % average Salix consumption in animal diet [††]). Calculations adapted from Jimmo et al. (2018).

[‡] Body weight for *A. alces* obtained from Welch et al. (2015); Body weight for *L. americanus* from Ellsworth and Reynolds (2006).

[††] Average % Salix composition in moose diet (21%) from Welch et al. (2015) and in hare diet from Seccombe-Hett and Turkington (2008) (11%).

[¥] Estimated TE TRV (toxicological reference values) for mammalian receptors from Sample et al. (1996). *A. alces* TRV values based on white tail deer estimates. *L. americanus* TRV values based on cottontail rabbit estimates.

[*] Acute TRV values processed in a comparable manner to the chronic values were not available.

[§] Acute values are calculated from LOAEL (Lowest observed adverse effect level) (Sample et al., 1996)

[Ø] Chronic values are calculated from NOAEL (No observed adverse effect level) (Sample et al., 1996)

[∂] HQ = ADI/TRV

Toxicological effects of soil

Changes in soil properties

Over time, drastic visual changes took place in the ECOS soil as it changed from a very dense, dark and sticky soil, to brown in color, filled with obvious fine organic matter and a lighter crumbling texture, somewhat like potting soil (Fig. 3.1C) The ECOS' pH always remained significantly higher (7.42 ± 0.15) than the UNTRD (6.98 ± 0.14) (mixed model, $p < 0.05$) (Table 3.1). On every visit throughout the experiment, stagnant water was observed in the UNTRD experimental cells, at least upon sampling, but not in the ECOS the last two years (except during spring thaw). Contrastingly, the moisture inside the soil matrix of the ECOS (31 ± 8 %) always remained significantly higher than the UNTRD (15 ± 3 %) (mixed model, $p < 0.05$) (Table 3.1).

Plant survival in the field

Salix

The first year of the experiment, every *Salix* cutting planted (*S. alaxensis*) produced leaves, but 100 % mortality was observed by the end of the growing season. The leaves showed clear signs of stress and none of the cuttings were able to produce roots. The following year, "Life Pockets" were introduced to offer a buffer zone between the *Salix* cuttings and the highly contaminated soil. This led to better survival and growth of the *Salix*; after four growing seasons, 58 % of the *S. planifolia* (n = 8 per plot) and 31 % of the *S. alaxensis (*n=8 per plot) cuttings had survived (n = 48 per species). The two species exhibited markedly different rooting strategies from compact formation to long runners (Fig. 3.2). The production of aboveground biomass per plant (dry leaves) was significantly higher for *S. planifolia* (6.02 ± 2.0 g) than for *S. alaxensis* (3.72 ± 2.7 g) (t-test, $p < 0.05$).

Spontaneous revegetation

No plant removal (weed control) was performed in this experiment. During the five growing seasons, multiple plant species spontaneously grew on the experimental cells (12 species on the ECOS and 7 on the UNTRD) (Table 3.C3). The total vegetation cover on the ECOS plots (137 ± 9 %) was significantly higher than the UNTRD (11 ± 8 %) (mixed model, $p < 0.05$). The

ECOS vegetation cover surpasses 100% as there were multiple canopy layers to the vegetation (see graphical abstract).

Fungi occurrence

Not a single fruiting event took place for the fungi *Trametes versicolor,* but another species thrived. The first summer following the field experiment set-up, and every year afterwards, multiple fruiting events of a small mushroom from the *Psathyrellaceae* family (*coprinellus* or *coprinopsis* genus) occurred exclusively on the ECOS cells (Fig. 3.3). This polyphyletic family possesses few easily discernable characters but are generally saprotrophic and work as primary or secondary decomposers in terrestrial ecosystems (Padamsee et al., 2008). Since our specimen's mycelium was intertwined in woodchips it is most likely a wood-decomposer.

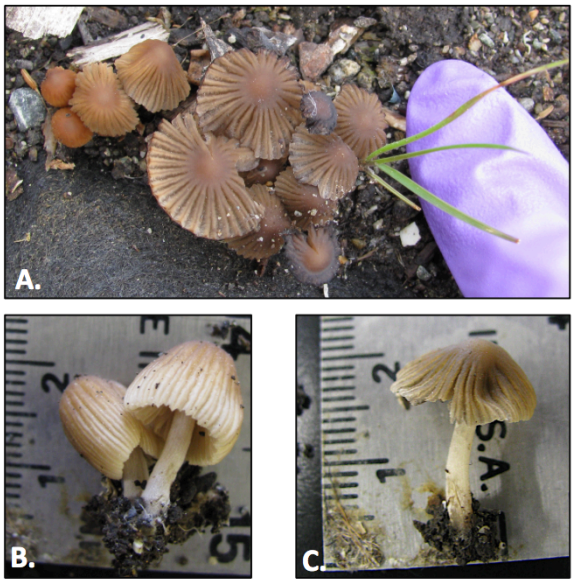


Figure 3.3 Spontaneous *Psathyrellaceae* fungi growth. A. Young and old sporophores with finger and grass as size indication. **B. and C.** Fruiting bodies with centimeter scale.

Biological assays

In order to measure the soil's toxicity status at the end of the experiment, soils were taken from the ECOS and UNTRD at the last sampling event and their effects on common model organisms (earthworms and barley seeds) were compared to a clean soil. After a 14-day exposure, 60 out of 180 *Eisenia andrei* placed in the UNTRD were dead (equivalent to 33 ± 44%

of the 10 worms placed in each of the 18 experimental pots). All those placed in the ECOS and experimental clean control (CLEAN-C) survived (Fig. 4.4A). Although lethality tests are the least sensitive of toxicology tests, there is a clear difference in the acute toxicity between the UNTRD and the ECOS soils. Tests measuring sublethal effects were performed with *Hordeum vulgare*. Germination rates were highest in the CLEAN-C (4.9 ± 0.3 seeds /5, n=300), closely followed by the ECOS (4.3 ± 0.8 seeds /5, n=150), and lastly the UNTRD which had significantly lower germination rates (3.1 ± 1.7 seeds /5, n=150) (mixed model, $p < 0.05$) (Fig. 4.4B). Dry leaf biomass was equivalent for the CLEAN-C and the ECOS (16.6 ± 1.8 mg and 15.8 ± 1.9 mg respectively), but significantly lower in the UNTRD (8.8 ± 4.5 mg) ($p < 0.05$) (Fig. 4.4C). Finally, the length of the longest root and longest shoot revealed that the ECOS yielded significantly longer plants (roots: 15.3 ± 2.0 cm, shoots: 16.7 ± 2.9 cm) than both the CLEAN-C (roots: 12.0 ± 1.7 cm, shoots: 15.7 ± 2.8 cm) and the UNTRD (roots: 5.4 ± 3.3 cm, shoots: 9.7 ± 3.7 cm) (mixed model, $p < 0.05$) (Fig. 4.4D and E).

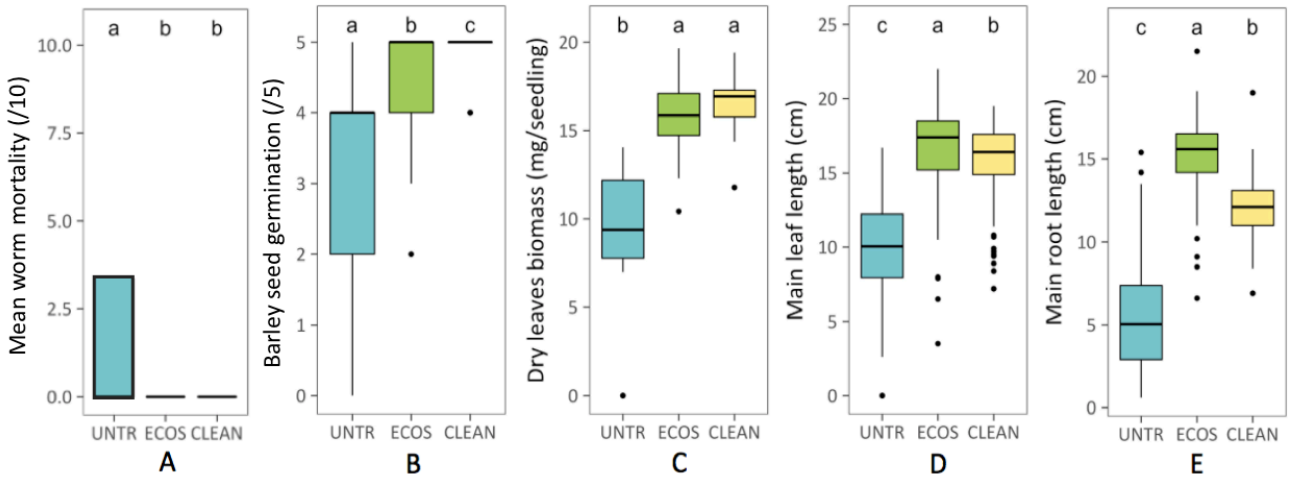


Figure 3.4 Toxicity measures. A. Mean *E. andrei* mortality out of ten worms (n = 180 treatment^{-1}). **B.** Mean number of *H. vulgare* seeds, which germinated out of five (n = 150 for ECOS and UNTRD, n = 300 for CLEAN-C). **C.** Mean dry leaf biomass (mg seedling^{-1}). **D.** Overall mean of the longest leaf on each seedlings (cm). **E.** Overall mean of the longest root on each seedlings (cm). For all tests: UNTRD n = 6; ECOS n = 6, CLEAN-C = 12. Different minuscule letters within boxplots indicate statistical differences ($p < 0.05$).

Discussion

Soil properties

Among the most important parameters that influence the degradation of organic pollutant by microbes is pH because many chemical and biological reactions are dependent upon it. Namely, pH changes the charge and mobility of TEs and the adsorption of some organic contaminants in the soil matrix, making them more or less available (Brady and Weil, 1996b). The optimal pH for bacterial degradation of petroleum hydrocarbon pollutants, in temperate soils, is believed to range between 7.0 and 7.8 (Aislabie and Foght, 2008). While the optimal pH range for this cold climate soil's particular microbial community is unknown, the ECOS' pH always remained in the reported middle range, and significantly higher (7.42 ± 0.15) than the UNTRD (6.98 ± 0.14) ($p < 0.05$) (Table 4.1). Another important parameter for bioremediation processes is moisture. Organic matter increases water retention, which can in turn facilitate soil microbial processes (Battelle and NFESC, 1996; Brady and Weil, 1996a). With the addition of clean material (such as organic matter) it is primordial to consider the impact of dilution and the possibility of contaminating a larger volume of material if decontamination is not successful. At first glance our amendments can appear substantial, but this is because the measured values are by volume (60%), not by mass. Our method choice was informed by field-scale logistics in remote locations, which favor volume measurements (e.g. loader buckets). Initial PHC concentrations in the ECOS soil were 18 ± 5 % lower than in the UNTRD. This indicates the dilution by mass was not 60%, but in fact just under 20%.

Nutrients and fertilizers

Nutrients are also key and the availability of nutrients (such as N and P) are generally a limiting factor for PHC-degrading microbes. Synthetic fertilizers are undoubtedly the current dominant method used for PHC-remediation, but highly contaminated sites require higher fertilizer dosages that can have toxic effects if added at once (Mohn and Stewart, 2000; Walworth and Ferguson, 2008). They have shown good performance in some cold-climate settings, but dosing is very site-specific and varying concentrations have shown detrimental effects on the bioremediation of some sites (Juwarkar et al., 2010; Mohn and Stewart, 2000). Soils near polar regions are usually nutrient-poor and it would be reasonable to assume indigenous microbes are

adapted to oligotrophic soil conditions (Aislabie and Foght, 2008). At least one psychrotolerant microbe from a polar region has been found to degrade a wide range of petroleum products without the addition of nutrients (Baraniecki et al., 2002). Considering 1) the possibly untapped potential of indigenous microbes, 2) the energy-intensive mining and synthesis of fertilizers (Rafiqul et al., 2005), as well as 3) inorganic fertilizers' potential for opening the door to invasive plant species through feedback loops in historically nutrient-poor soils (Kueffer, 2009), the authors prioritized the elaboration of a system that excluded them. Other northern projects also achieved good results without chemical fertilizers (Palmroth et al., 2006), but working solely with local materials remains marginal at the moment.

The decline of organic contaminants

The amount of PHC degraded over 5 years is remarkably high in the ECOS cells (65 to 75% of the initial ≈150 500 mg kg^{-1} in the ECOS soil). This is noteworthy not only because of the cold climate setting, but also because soils contaminated with more than 50 000 mg kg^{-1} PHC are usually viewed as highly polluted and difficult to clean up in a biopile remediation setting (Battelle and NFESC, 1996). Contaminant removal through natural attenuation was expected because the UNTRD soil was handled analogously to the ECOS (the initial mixing aerates the soil and homogenizes contaminants, which can prompt volatilization and stimulate biodegradation by endemic microbes) (Tomei and Daugulis, 2013). Nonetheless, PHC removal was significantly less for the UNTRD than the ECOS. Further, the UNTRD had no significant decrease in the F4G fraction over the course of the study (t-test, $p > 0.05$). Some cold-climate phytoremediation studies have demonstrated that the use of compost and plants were successful for PHC-degradation (Ferrera-Rodríguez et al., 2012; Palmroth et al., 2002; Robichaud et al., 2019), however, none of these studies investigated the efficiency of soil so highly contaminated with PHC. A field study at a mixed-contaminants site in Finland found compost with plants (clover and grasses, with or without trees) led to the degradation of 57 to 60 % of the initial 11 400 ± 4 300 mg kg^{-1} aged PHC (C_{21}-C_{40}) (Palmroth et al., 2006). In Sweden another field study found that an alfalfa monoculture was more efficient than co-planting with sunflower for the remediation of C_{10}-C_{40} PHC at a mixed-contaminant site (40% of the initial 10 233 ± 465 mg kg^{-1}), explicitly indicating that the choice of plant combinations are important (Marchand et al., 2018).

Heavier petroleum fractions are recalcitrant to degradation (Brown et al., 2017), therefore, we expected a markedly slower degradation of the PHC > C_{50} compared to the C_6-C_{50} range. Our results show that the degradation of heavy fraction of PHC was slightly slower (65% versus 75%) (mixed model, $p < 0.05$). Bearing in mind the waste oil pit had been in place for over 40 years, it is likely that the indigenous microbial community was already shaped to tolerate the toxicity of these contaminants whose biodegradation had been limited because of the lack of optimal parameters such as soil structure and aeration (Roy et al., 2014). The ECOS amendments appear to have favored the microbial communities' bioremediation abilities for the degradation of light to very heavy PHC fractions inclusively.

In our study, we also measured chlorinated compounds and aromatic hydrocarbons because they are known to be highly toxic in the environment and their degradation by microbes is very difficult (CCME, 2008). Of the compounds monitored, benzene, trichloroethylene, and xylene dropped below detection limit (BDL) in both treatments, indicating that the initial mixing of the soil provided suitable conditions for dissipation and/or degradation by the indigenous microbial population. Toluene and tetrachloroethylene were removed to BDL in the ECOS, but not from the UNTRD soil. This may be linked to different starting concentrations, but plant rhizosphere microbes have been found to be good tetrachloroethylene and BTEX (benzene, toluene, ethylbenzene and xylenes) degraders (CCME, 2008; Wongbunmak et al., 2017). Hence, it is possible that the presence of the plants in the ECOS cells favored some of the toluene and tetrachloroethylene removal.

Plant establishment

The presence of PHC can have negative impacts on plant survival and we hypothesize the 100% mortality of our *Salix* cuttings' in the first year was due to hydrophobic conditions caused by high PHC concentrations, as observed by Zalesny et. al (2005). Differences between the visual observations of stagnant water in the UNTRD versus the analytical data showing ECOS had a higher percentage of moisture is most likely due to the saturation of soil pores by petroleum in the initial soil, which can lead to the exclusion of water. Organic matter amendments change the soil matrix's properties, which can then facilitate the establishment of plants (Brady and Weil, 1996a; Guidi et al., 2012). Some authors successfully tested either pre-rooted plant cuttings, or clean soil around the cuttings or seeds to promote plant establishment in contaminated field sites

(Guidi et al., 2012; Palmroth et al., 2006; Zalesny et al., 2005). In Finland, compost has also been shown to encourage greater vegetative coverage (than inorganic fertilizers) at a mixed-contaminant site (Palmroth et al., 2006). Employing small amounts of clean soil for plant establishment in highly contaminated soils has proven advantageous at multiple sites, including ours. The "Life Pockets" we introduced to offer a buffer zone between the *Salix* cuttings and the highly contaminated soil were successful as they led to better survival and growth of the *Salix* after four growing seasons. We hypothesize that the higher survival rates were linked to the ability of plants to form roots and establish themselves prior to direct contact to the waste oil pit soil. In our setting, we found the pre-filled jute bags convenient and easy to handle in the field.

Both *Salix* species used in our research contributed to the vegetation of the ECOS cells and exhibited complimentary rooting strategies, from compact formation to long runners. *S. planifolia* spread long adventious roots throughout the cells, often following the bottom of the cells (Fig. 3.2C), which was intermittently flooded. This type of growth enables plants to spread quickly and find suitable microhabitats (Ye et al., 2006). The other species, *S. alaxensis* grew shorter, more compact roots with a fibrous ball formation (Fig. 3.2B). This type of monopolization strategy can increase the plants' tolerance to stressful conditions while making better use of locally abundant resources (Ye et al., 2006). These different strategies can confer different advantages to each species in heterogeneous environments for contaminant tolerance and resource supply (Ye et al., 2006). Plants native to any given research area may not always be as performant as selected cultivars and clones, but they present the advantages of being integrated in the local ecosystem and adapted to the climate, and they do not pose the risk of being an invasive species (Kueffer, 2009; Leewis et al., 2013; Rockwood et al., 2004).

In addition to *Salix* plants, we allowed the natural colonization by other species to occur on all plots (Table C3). The added benefit of a spontaneous plant cover (namely: evapotranspiration, root microbiomes, soil structure) was judged to outweigh the advantage of weeding (reduced competition to the *Salix*). ECOS soils were colonized at greater rates and by more species than the UNTRD. This large difference in plant colonization and diversity suggests a reduced toxicity of the ECOS. Most plant species foster beneficial microbes in their rhizosphere, which can help further contaminant degradation, leading to more plant colonization through a positive feedback loop (Asemoloye et al., 2017). This mixed plant species approach is

supported by Desjardins et. al (2018) who demonstrated that plant complementarity was the most robust approach for the remediation of multiple TEs concomitantly.

Fungi presence

The fungus *Trametes versicolor* did not produce fruiting bodies on our experimental site. Although its mycelium may remain in the soil in our project, this is not the first experiment where translations of lab results to the field are not an obvious success because of difficulties maintaining proper parameters for growth in a field setting (aeration, moisture, growth substrate, temperature, etc.). *T. versicolor* is an aggressive white-rot fungus (WRF). It is a known organic-contaminant degrader and has successfully mineralized TCE (Marco-Urrea et al., 2008) as well as PAHs in a soil environment (Borràs et al., 2010) even at field scale (Winquist et al., 2014). The use of live WRF in large-scale soil bioremediation projects is notoriously challenging since this environment is not their optimal niche (Gao et al., 2010). Nonetheless it is possible in sub-arctic climates, as demonstrated by Robichaud et al. (2019) who obtained large fungi fruiting bodies with another white-rot species, but on a site where aeration was not as restricted (above-ground bins and coarser soil granulometry). Although *Trametes versicolor* does not appear to be the good candidate for this study, we cannot assert whether its presence may have favored or deterred the establishment of other fungi, since there is sequential colonization of woody habitats in natural systems (Rajala et al., 2012). Perhaps *Pleurotus ostreatus* or other fungi better suited for dense soil conditions would have been a better alternative.

The spontaneous presence of the *Psathyrellaceae*' fruiting bodies on the ECOS treatment cells is noteworthy, and perhaps linked to the addition of the unsterilized woodchips. As far as we know, *Psathyrella* spp. have never been used nor considered in mycoremediation studies. Our experiment brings new evidence that *Psathyrella* spp. can grow in highly polluted soils with petroleum hydrocarbons, although its efficiency for petroleum hydrocarbon degradation remains to be demonstrated. Although its fruiting bodies are small (≈ 2 cm tall), other small fungi have shown the ability to accumulate metals of concern (Cu, Cd, Pb, Zn concomitantly) up to 10 times the soil concentration (Damodaran et al., 2013). Little is known about such Agaricomycetes growing on northern contaminated sites and further experiments considering indigenous fungi in bioremediation are needed. This new information contributes a new cold climate family, tolerant to high levels of metals and PCH, to the blooming field of mycoremediation.

Traces elements

Trace element (TE) concentrations were measured in the soil at the beginning of the experiment to get baseline concentrations. TEs cannot be degraded and the impermeable liner at the bottom of our treatment cells meant they could not be leached. At the last sampling period of the study, TE concentrations in *Salix* leaves were measured to determine if there had been translocation from the soil, and if they posed a threat to local wildlife. The 19 TEs we examined in plant tissues are seldom volatile.

Though numerous TE ions are required for basic metabolic needs of plants and soil microorganisms, high concentrations of essential and non-essential ions can be detrimental to biotic processes (Nordberg et al., 2007). The root system is the main point for their entry and accumulation in shoots is largely controlled by root activity and strategies, as well as bacterial presence and translocation though mycelia of symbiotic fungi such as mycorrhizal fungi and endophytes (Hrynkiewicz et al., 2018). Information on the distribution of TE throughout the rooting systems is lacking (Vondráčková et al., 2017), but the different root systems uncovered between the two Salix species in this project may be linked to the plants' distinct TE accumulation patterns. *Salix*, are known to accumulate TE ions (Roy et al., 2005) and it is interesting to note the Cu, Cd and Zn concentrations found in our leaves were within the ranges found by Pugh et al. (2002) for native *Salix* (*spp*. unspecified) at large Yukon mine site (Faro), a notably different environment. TE mobility can influence their uptake by plants. Lower-mobility elements such as As, Cd and Pb are usually stored in roots and are not effectively translocated to the aboveground biomass, (Verbruggen and Hermans, 2013; Vondráčková et al., 2017), as was observed in our samples. From a management point of view, if plants accumulate enough TEs, they can then be harvested and handled suitably (by controlled incineration for example) to gradually remove these ions from the site and lower toxicity over time (Vanek et al., 2010).

Risk to local herbivores

Because *Salix* spp. can act as accumulators of certain TEs in their leaves and shoots, they may act as vectors of contaminants to other biological systems, which may cause adverse effects in local animal populations (Pugh et al., 2002). In this study, because they were present at high concentrations in the leaves, the metals of concern Cr and Zn were the most likely to cause unacceptable risks to local herbivores who use *Salix* spp. as forage (moose and snowshoe hare).

To err on the side of caution, worst-case scenarios are often considered when conducting a risk assessment. First, we used total concentrations of TEs rather than the bioavailable fractions (which were not measured), which can inflate the risks. Secondly, the TRVs used in our calculations imply the herbivorous receptors must consume the contaminated *Salix* spp. leaves every day for the rest of their lives, and that the leaves must consistently make up 11 and 21% of their daily diet (hare and moose respectively) (Seccombe-Hett and Turkington, 2008; Welch et al., 2015). Relatively few studies have measured the toxicological effects of Cr on wildlife or livestock in controlled experiments (CCME, 1999). Neither acute nor chronic values for Cr showed a HQ > 1, hence it was deduced that the likelihood of Cr posing a risk is low. Our risk assessment indicated that the chronic HQ values for Zn in both *Salix* spp. for snowshoe hare exceed 1, which implies the likelihood of risk could be unacceptable and further assessment may be required for a larger-scale project. It is unlikely that wildlife would be exposed to contaminated foliage from *in situ* remediation on a daily basis for the rest of their lives, but improving our TRV values for wildlife, for both Zn and Cr would be helpful for estimating risk. Since herbivores also browse shoots, which can compose 62% of plant mass (Courchesne et al., 2017), future research should also examine their TE content.

Decrease in soil toxicity with the ecological microsystem

Assessment of soil toxicity is important because even once contaminants are below environmental guidelines, they can still be problematic to plant and animal life. This is especially true when multiple contaminants are present concomitantly, as they can have additive toxicological actions (Millward et al., 2004) and predictions of toxicity based solely on compiled previous laboratory studies are rarely realistic (Chapman, 2007). Furthermore, contaminant concentrations alone do not provide bioavailability data, which is pivotal in determining if a contaminant will be immobilized to the soil matrix, or easily available for microbial and plant uptake. Lower pH values will increase the solubility of some TEs (namely Cu, Cd, Pb, Zn) while a pH near neutral will lead to their highest adsorption and reduced availability (Brady and Weil, 1996b). Organic matter in the soil increases TE retention by increasing cation exchange capacity (CEC) and through the formation of organometallic ligands (Antoniadis et al., 2017). Organic matter was added to the ECOS soil and its pH was significantly higher than the UNTRD ($p <$ 0.05), potentially reducing contaminant bioavailability. This is why, after measuring the decline

in organic contaminants and translocation of some TEs in plants, we assessed the soil's toxic potential. The UNTRD soil still had evident detrimental impacts on both plant and worm health and survival, despite the fact that *Eisenia sp*. can live and reproduce in PHC-contaminated soil (Martinkosky et al., 2017). The plant length results are noteworthy, because while CLEAN-C contains no known growth inhibitors it also does not have added nutrients. The fact that ECOS surpassed its performance in plant lengths and was similar to it in terms of biomass, are good indicators that overall toxicity of the ECOS soil has been greatly reduced in comparison to UNTRD. Furthermore, ECOS even contains enough nutrients to support active growth, if initial establishment is successful, just as was observed in the field with the "Life Pockets". Overall, our "Ecological Microsystem" approach led to a reduction of soil toxicity to plants and soil animals, as well as promoting spontaneous revegetation by neighboring species, which can lead the way to further TE phytoextraction and organic contaminant degradation.

Summary and conclusions

This study evaluated the concerted performance of local willows, fungi and municipal compost (ECOS) for the remediation of a waste oil pit soil contaminated with both TEs and extremely high levels of PHC, in a subarctic climate. Although most fractions remain above the CCME-I guidelines, the ECOS approach yielded excellent results. Within five years the soil structure was clearly modified, and considerable organic contaminant removal was measured, despite potential interference by the presence of TEs. The two *Salix* species used showed satisfactory survival rates (especially *S. planifolia*) and exhibited different TE accumulation patterns as well as differing rooting strategies. Although the hare HQ calculated for chronic Zn toxicity was > 1, the likelihood of these animals supplementing all of their dietary needs on *Salix spp*. solely on this small site, is unsubstantiated. Nevertheless, in the event of a scale-up operation, considerations should include precautionary measures to minimize wildlife accessing the site. We established the "Life Pockets" we developed are a good strategy for early plant implementation leading to long-term colonization of the highly contaminated waste oil pit soil. Increased natural plant colonization compared to the control (UNTRD), as well as assays on barley seedlings and earthworms, confirmed the soil's toxicity had been significantly reduced. This site has remained highly contaminated for over 40 years, and in less than five years we reached excellent PHC degradation results whilst fostering revegetation. The use of the

"Ecological Microsystem" approach to treat the substantial volume of soil remaining at the waste oil pit would be a good first step for organic contaminants removal. Further work at this site should focus on increasing plant biomass and on the phytoextraction of TE. The presence of a *Psathyrella* spp. fungus indicates that there may be more northern fungi to consider in future bioremediation ventures. Few cold climate projects have tackled such heavily and mixed contaminated sites. The high PHC-remediation efficiency of this approach opens the door to more locally-based and fertilizer-free bioremediation methods in subarctic climates.

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Author contributions

Designed the experiments and provided expertise on the design: KR, MA, ML, MH, KS. Performed the experiments: KR, JC (leaf TE). Data was analyzed by KR. Wrote the paper: KR, MA. All authors have edited and given approval to the final version of the manuscript.

Appendices for chapter 3.

APPENDIX A. Site features

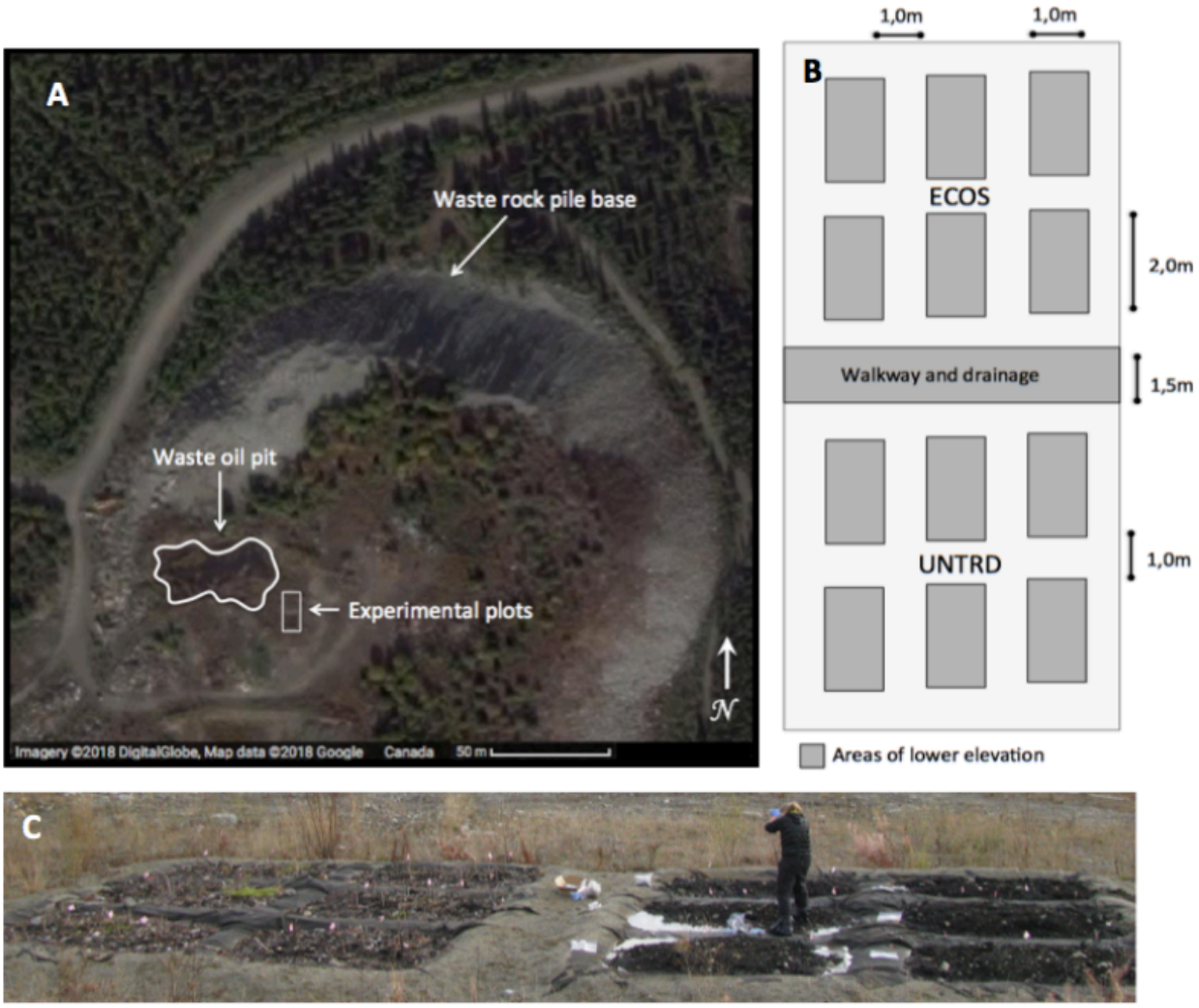

Figure 3.A1 Site layout. A. Satellite image of the Copper mine's waste rock pile with the location of the waste oil pit and experimental plots located on top. **B.** Dimensions and layout of the experimental plots. The ECOS and UNTRD cells were separated to avoid cross-contamination from the annual cells overflowing which occurs every spring during thaw-out. **C.** Picture of the cells, while soil sampling in October 2015.

Table 3.A1 HAZGARD® liner properties.

	HAZGARD® Minimum Material Properties	
Style	ASTM	HAZGARD 1000
Thickness (Nominal)	D1593	27 mil 0.68 mm
Thickness Minimum	D1593	26 mil 0.65 mm
Tensile Strength	D882 D751	350 lbs^2 1555 N
Elongation	D882, D751	25 %2
Tear Strength	D1004, D751B	150 lbs^2 667 N
Low Temperature	D1790, D2136	-45°F2 -43°C

Note: Product manufactured by Layfield.

Figure 3.A2 Life pockets in the ECOS treatment.

APPENDIX B. Supplementary methods

B1. Soil metals quantification

The analysis was carried out with procedures from CSR Analytical Method: "Strong Acid Leachable Metals (SALM) in Soil", BC Ministry of Environment, 26 June 2009 (CSR, 2017b), and procedures adapted from EPA Method 200.2 (Martin et al., 1994). The samples were manually homogenized and dried at 60° C. They were then sieved through a 2 mm sieve and a subsample of the dry material was weighed. The sample were digested at 95° C for 2 hours with concentrated nitric and hydrochloric acids, by block digester. The instrumental analysis of the digested extract is performed by collision cell inductively coupled plasma - mass spectrometry (ICP-MS) (modified from EPA Method 6020A). The reference used material was STSD-3 (CCRMP, 1990). Although this method is not a total digestion technique, it is a very strong acid digestion that is intended to dissolve metals, which could be environmentally available. Elements bound in silicate structures are usually not dissolved by this procedure, as they are usually not usually mobile in the environment. Samples who did not meet quality control were re-run until they fell within set parameters (± 20% recovery).

B2. Metal quantification in willow leaves.

Leaves of the two randomly chosen plants of each species from each active treatment plot (n=24) (n=12, *Salix planifolia*) (n=12, *Salix alaxensis*), were collected from the shoots, and washed with Milli-Q water and patted dry with paper towel. Leaves were freeze dried at -30^{o} C for 43 hours prior to determining the dry weight of the leaves for each sample. Leaves were homogenized by hand with a spatula in a plastic capsule then ground into a powder using a ball grinder (Mixer Mill 400 from Retsch, Germany) for 5 minutes at 22.5 Hz in order to be digested and analyzed for heavy metals.

Powdered leaf samples were digested using the Microwave Accelerated Reaction System (MARS-5) with maximum power of 1600 W. Approximately 1 g of powdered leaf tissue was transferred to a large Teflon Xpress Vessels that were nitric acid washed and Barnstead water rinsed. Concentrated 69% Nitric acid (1 mL) and 30% hydrogen peroxide (2 mL) were added to the Teflon tubes. Samples, including blanks and standard reference material (1570a, spinach leaves, obtained from the National Research Council Canada), were digested using the

microwave for 2 hours, which included 1.5 hours of cooling and 3 stages. After digestion, samples where filtered with a 0.45µm filter membrane (25 mm syringe filter with polyethersulfone membrane) and diluted with Barnstead water to 2% nitric acid.

Table 3.B1 Method quality assurance/quality control of certified reference material. DL is the instrumental detection limit. Metals in red did not meet the % recovery (± 20%) or were not present in either reference material and were not further analyzed (values in mg kg^{-1}).

		Instrumental QA/QC - Certified Reference Material 1640 (spinach leaves) (*n=5*)			**Method QA/QC - Certified Reference Material 1570a (natural water) (*n=4*)**		
Element	**DL (ug kg-1)**	**Recovery (%)**	**±**	**SD**	**Recovery (%)**	**±**	**SD**
B	0.585	98.45	±	4.99	104.60	±	3.38
Al*	0.013	101.78	±	3.03	42.11	±	0.50
Ti*	0.080	n/c	±	n/c	n/c	±	n/c
V	0.003	99.84	±	0.95	70.19	±	0.52
Cr	0.069	97.84	±	1.59	n/c	±	n/c
Mn	0.035	96.34	±	1.50	96.47	±	3.75
Fe	0.044	100.32	±	2.78	n/c	±	n/c
Co	0.069	97.05	±	2.16	115.63	±	0.35
Ni	0.007	95.92	±	4.04	86.47	±	3.84
Cu	0.001	96.93	±	4.90	101.98	±	5.62
Zn	0.028	100.03	±	3.52	98.17	±	2.16
As	0.011	97.04	±	4.65	94.97	±	6.80
Se*	0.023	100.23	±	1.46	123.84	±	4.31
Sr	0.001	92.19	±	4.63	92.86	±	3.05
Mo	0.003	97.55	±	1.84	n/c	±	n/c
Ag	0.000	90.40	±	0.68	n/c	±	n/c
Cd	0.001	92.56	±	1.57	90.45	±	3.67
Sn*	0.039	n/c	±	n/c	n/c	±	n/c
Sb	0.003	91.02	±	1.18	n/c	±	n/c
Ba	0.019	97.65	±	1.17	n/c	±	n/c
Hg*	0.008	n/c	±	n/c	65.17	±	1.72
TI	0.002	94.12	±	1.03	n/c	±	n/c
Pb	0.007	95.62	±	0.76	92.99	±	12.63
U	0.001	94.22	±	0.94	98.86	±	4.55

* Metals not further analyzed.
n/c = element not certified in certified reference material.
The instrumental certified reference material is 1640a, trace elements in natural water, obtained from the National Institute of Standards and Technology.
The method certified reference material is 1570a, spinach leaves, obtained from the National Research Council Canada.

Metal concentrations were determined in mg/kg using multi-element Agilent Technologies 8800 ICP-MS (inductively coupled plasma-mass spectrometry) Triple Quad. A large metal suite including Al, Sb, As, Ba, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Hg, Mo, Ni, Se, Ag, Sr, Tl, Sn, Ti, U, V and Zn were analyzed. Hg, Al, Se, and V did not meet the % recovery for the method QA/QC (recovery and measured value must be within ± 20% of certified value) using the certified reference material (1570a) and 4 replicates, therefore those metals were not further analyzed (Table 3.B1).

B3. Quantification of petroleum hydrocarbons in soil

The VOCs analysis was carried out in accordance to the BC MOE (2017) "Volatile Organic Compounds in Soil" method. In summary, the methanol extract is added to reagents and water. The sealed vial is heated and brought to equilibrium. Once completed, the headspace is transferred to a gas chromatograph where target compound concentrations are measures using GC/MS. PAHs were analyzed using procedures adapted from "Test Methods for Evaluating Solid Waste" SW-846, Methods 3545 & 8270 (US-EPA, 2007). A subsample of a sediment/soil mixture (1:1 with hexane and acetone) is obtained through mechanical shaking. The extract is solvent-exchanged to toluene and the final extract is analyzed by capillary column with Gas Chromatography Mass Spectrometry Detection (GC/MS). Samples who did not meet quality control were re-run until they fell within target parameters (± 30% recovery).

The PHC extraction for the fractions F1, F2, F3, F4 and F4G were carried out in accordance to the "Reference Method for the Canada-Wide Standard for Petroleum Hydrocarbon in Soil – Tier 1 Method, Canadian Council of Ministers of the environment [CCME]" (CCME, 2001b). For the F1 fraction, briefly the method involves a methanol extraction of the soil, which is then added to water and reagents. This solution is then heated in a sealed vial to equilibrium and the headspace from the vial is transferred to a gas chromatograph. The fraction is then measured with flame ionization detection (GC/FID). For the fractions F2-F4, in summary, the fractions were extracted with 1:1 hexane:acetone using a rotary extractor. The extracts underwent a silica gel clean-up to remove polar compounds in preparation for the on-column GC/FID. Finally, for the F4G fraction, a sample of the sediment/soil extract (as for PAHs) is extracted with a rotary extractor. The extract undergoes a silica-gel clean-up to remove polar compounds and is

then analyzed gravimetrically. Any samples that did not meet quality control were re-run until they fell within target parameters (± 30% recovery).

APPENDIX C. Results

Table 3.C2 Volatile Organic Compounds (VOC) and Polycyclic Aromatic Hydrocarbons (PAH) in the CTRL and ECOS soils. The initial testing was done at implementation time (July 2013). Some VOCs were above CCME guidelines for Commercial and Industrial sites, they were tested again in October 2015 (**bold underlined**). Since no PAHs were above guidelines at the start they were not tested again.

				July 2013						October 2015							
Class	**Analyte**	**D.L.**	**CCME***	**UNTRD**		**n***	**ECOS**		**n***	**UNTRD**		**n***	**ECOS**			**n***	
VOC	Benzene [1,2]	0.005	0.04	0.125	± 0.118	6	0.079	± 0.121	4	BDL				BDL			
	Ethylbenzene [1,2]	0.015	7	0.835	± 0.560	6	0.428	± 0.708	6	0.031	± 0.000	1		BDL			
	Toluene [1,2]	0.05	2.5	2.830	± 2.465	6	1.414	± 2.125	6	0.188	± 0.156	6		BDL			
	ortho-Xylene [1,2]	0.05	-	5.198	± 1.477	6	3.855	± 3.514	6	0.312	± 0.327	2		BDL			
	meta- & para-Xylene [1,2]	0.05	-	5.588	± 4.225	6	2.835	± 5.233	6	0.110	± 0.087	3		BDL			
	Xylenes [1,2]	0.075	20	10.788	± 5.671	6	6.690	± 8.745	6	0.451	± 0.429	2		BDL			
	1,2-Dichlorobenzene [2,3]	0.05	10	0.378	± 0.131	6	0.449	± 0.088	6	BDL				BDL			
	1,3-Dichlorobenzene [2,3]	0.05	10	0.074	± 0.010	5	0.090	± 0.010	6	0.057	± 0.010	2		BDL			
	1,4-Dichlorobenzene [2,3]	0.05	10	0.783	± 0.304	6	1.125	± 0.297	6	0.421	± 0.315	6	0.063	±	0.016	2	
	Dichloromethane [3]	0.3	50	BDL			0.350	± 0.057	2	BDL				BDL			
	Tetrachloroethylene [3]	0.05	5	0.430	± 0.106	6	0.299	± 0.206	6	0.170	± 0.069	4		BDL			
	Trichloroethylene [3]	0.01	0.015	0.022	± 0.005	6	0.016	± 0.009	3	BDL				BDL			
PAH	Benz(a)anthracene	0.01	10	BDL			1.290	± 0.396	2								
	Benzo(a)pyrene	0.01	1.4	1.280	± 0.118	3	1.202	± 0.141	6								
	Benzo(g,h,i)perylene	0.01	-	1.001	± 0.054	3	0.853	± 0.121	6								
	Fluoranthene	0.01	180	3.237	± 0.579	6	2.515	± 0.413	6								
	Fluorene	0.01	180	2.417	± 0.488	6	1.865	± 0.446	6								

P	Indeno(1,2,3-c,d)pyrene	0.01	10	0.827	± 0.146	6	0.611	± 0.101	6	
	2-Methylnaphthalene	0.01	-	17.083	± 5.218	6	8.805	± 5.231	6	
	Naphthalene	0.01	22	5.147	± 1.745	6	2.562	± 1.621	6	
	Phenanthrene	0.01	50	7.978	± 1.539	6	5.368	± 1.591	6	
	Pyrene	0.01	100	4.097	± 0.609	6	3.412	± 0.548	6	

VOC - Volatile Organic Compounds
D.L. Detection Limit
CCME** - Guidelines for Industrial sites (CCME, 2008b).
* n > D.L. used to calculate means
BDL: Below Detection Limit

PAH - Polycyclic Aromatic Hydrocarbons
[1] BTEX (Benzene, Toluene, Ehtylbenzene, Xylenes)
[2] MAH (Monocyclic aromatic hydrocarbons)
[3] Chlorinated hydrocarbons
Compounds above guidelines are **bold and underlined**.

Table 3.C3 Vegetation cover. Percent vegetation cover split by species. SD represents standard deviation (n = 6 cells / treatment). ECOS exceeds 100% since there are canopy layers formed by different plant species. NP indicates that the plant species was Not Present.

	ECOS		UNTRD	
Plant species	%	SD	%	SD
Taraxacum spp.	55.2	± 10.3	8.0	± 5.7
Melilotus albus	24.4	± 18.9	0.0	± 0.0
Crepis tectorum	17.8	± 9.0	1.1	± 1.2
Salix planifolia	12.8	± 3.5	NP	
Elymus trachycaulus	12.0	± 6.6	0.9	± 0.9
Achillea millefolium	6.0	± 3.3	0.1	± 0.2
Elymus repens	5.0	± 2.0	NP	
Salix alexensis	2.5	± 1.4	NP	
Hordeum Jubatum	2.3	± 2.7	0.6	± 1.2
Leymus innovatus	1.7	± 2.6	NP	
Chamerion angustifolium	1.2	± 1.0	0.5	± 1.2
Potentilla gracilis	0.2	± 0.4	NP	
Bromus inermus	0.2	± 0.4	NP	
TOTAL	**137.2**	**± 8.6**	**11.2**	**± 7.8**

CHAPITRE 4. | Bioremédiation de l'huile à moteur avec des matières résiduelles fertilisantes de proximité

Bioremediation of engine-oil contaminated soil using local residual organic matter

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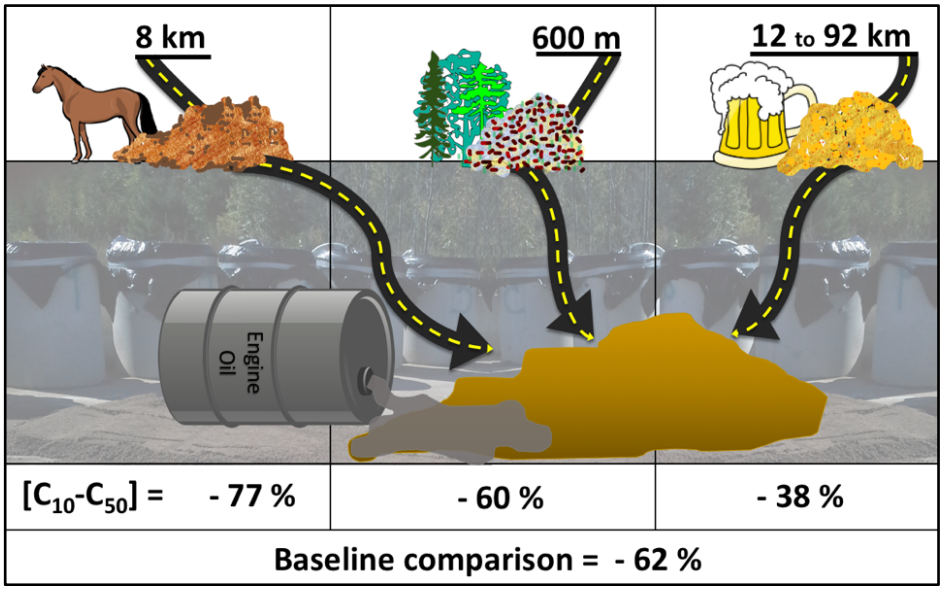
Graphical abstract



SUBJECTS: Ecotoxicology, Environmental Contamination and Remediation.

KEYWORDS: Petroleum, Bioremediation, Residual organic matter, Circular economy, Valorisation.

Abstract

Soil remediation industries continue to seek technologies to speed-up treatment and reduce operating costs. Some processes are energy intensive and, in some cases, transport can be the main source of carbon emissions. Residual fertilizing materials (RFM), such as organic residues, have the potential to be beneficial bioremediation agents. Following a circular economy framework, we investigated the feasibility of sourcing RFMs locally to reduce transport and assess possible bioremediation efficiency gains. RFMs were recruited within 100 km of the treatment site: ramial chipped wood (RCW), horse manure (MANR) and brewer spent grain (BSG). They were added to the land treatment unit's baseline fertilizer treatment (FERT, "F") to measure if they improved the remediation efficiency of an engine oil-contaminated soil (7,500 ± 100 mg kg-1). Results indicate that MANR-F was the only amendment more effective than FERT for petroleum hydrocarbons (PHC) reduction, while emitting the least CO2 overall. RCW-F was equivalent to FERT but retained more moisture. Although BSG contributed the most nitrogen to the soil, BSG-F retained excessive moisture, emitted more volatile organic compounds, contained less soil O2, and was less effective than the baseline treatment. Significantly more of the C16–C22 fraction was removed (63% ± 22%) than all other fractions (C22–C28, C28–C34, C34–C40), which were equally removed. Microbial community-level physiological profiling was conducted with Biolog EcoplatesTM, and catabolic diversity differed between treatments (utilization rates of31 carbon sources). MANR-F has the potential to increase PHC-remediation speed and efficiency compared to inorganic fertilizer alone. Other RFM promote moisture retention and diverse microbial catabolic activity. A variety of RFM are present across the globe and some can offer low-cost amendments to boost remediation efficiency, while reducing treatment time compared to traditional fertilizer-only methods.

Introduction

Soil treatment centers are constantly seeking new soil remediation technologies to speed up treatment and reduce their production costs. Conversely, organic waste diversion from landfills and processing strategies for sustainable waste management practices are actively being put in place across Europe and North America (CEC, 2017; European Commission, 2010). Organic waste makes up a significant portion of the waste stream going to landfills where its anaerobic decomposition produces polluting gases, like methane, which not only affect human health and air quality, but also contribute to climate change (Lou and Nair, 2009). In Canada, all levels of government aim to reduce greenhouse gas emissions, namely through the reduction of putrescible landfill waste (Environment Canada, 2014). For example, the Quebec Ministry of Environment and Fight Against Climate Change (MELCC) encourages the valorization of residual fertilizing materials (RFM) to divert them from landfills where they become putrescible organic residues. These include, but are not limited to, residential food scraps, agricultural and industrial wastes and wood residues. This diversion, which reached 1.6 million tons in 2012, can be used to revegetate degraded sites and serve as fertilizer in agriculture, thereby helping to reduce greenhouse gas emissions (Larose et al., 2014). Several studies demonstrate the potential of RFM as efficient bioremediation agents when compared to controls (Abioye, 2011; Cole, 1998; Guidi et al., 2012; Hupe et al., 1996; Margesin and Schinner, 1997; Onwosi et al., 2017; Zubillaga et al., 2012). However, the MELCC (2014) reports that only 8% of salvaged RFM were used on degraded sites and usage on soils contaminated with petroleum hydrocarbons (PHC) was not mentioned. We reported the current practices in the province of Quebec, where our project takes place, but the main principles of organic waste reduction apply across Canada, the US, Mexico and Europe (CEC, 2017; European Commission, 2010).

Further, these main principles are embedded into a circular economy framework. Circular economy is an emerging concept which considers how we can consume goods and services without depending on the extraction of raw natural resources, thereby closing loops that prevent the disposal of materials in landfills (Sauvé et al., 2016). In contrast to the usual ‘linear economy’ model, the impacts of resource consumption are taken into account. Governments around the world are adopting the concept of circular economy (e.g. China and

Europe; see Sauvé et al., 2015). This concept applies to soil remediation practices because some remediation approaches are energy-intensive or require large areas of land (landfarming, soil vapor extraction, thermal desorption, etc.) and many depend on the introduction of inorganic fertilizers which rely on energy-intensive synthesis and mining of non-renewable resources, such as phosphorus (Daneshgar et al., 2018; Khan et al., 2004; Rafiqul et al., 2005).

A popular soil remediation approach to large-scale PHC-contamination is the use of low-tech and relatively low-cost biopiles (Ivshina et al., 2015). This remediation method is normally based on the stimulation of endemic microbes for contaminant degradation (Khan et al., 2004). It is optimized through different manipulations such as the addition of nutrients, the modification of soil structure and exerting some control over moisture content and air supply (Juwarkar et al., 2010). In Quebec, regulations require that biopiles must be covered and equipped with a pulled-air system and an air treatment system for volatile organic carbon contaminants, like BTEX (benzene, toluene, ethylbenzene and xylenes), methane, hexane and other light PHC molecules having less than 12 carbon atoms (C_{12}), prior to release in the atmosphere.

Appropriate nutrient input and timing of application are key for effective bioremediation of PHC-contaminated soils (Nwankwegu et al., 2016). The main sources for nutrients are inorganic fertilizer and organic matter amendments to the contaminated soil. Nitrogen is the most common limiting nutrient, but depending on its form, it may or may not be available for the microorganisms in the soil that biodegrade contaminants (Schulten and Schnitzer, 1997). However, accurately monitoring different forms of N is sometimes difficult because the microbe-moderated nitrogen cycle in the soil is rapid and complex. Microbes are at the heart of bioremediation techniques like biopiles and it is advantageous to understand how soil amendments may influence them. Community-level physiological profiling (CLPP) is an approach commonly used in ecology for assessing microbial community profiles in soils (Jones et al., 2018). Biolog Ecoplates™ give a sensitive fingerprinting tool for communities' catabolic diversity. The microplates contain 31 different carbon sources, along with a redox dye which turns purple when the substrate is consumed (Biolog, 2018). Based on a specific soil's heterotrophic bacterial community, different carbon sources are consumed at varying rates, offering a unique look at the functional carbon use in a given soil, which can

lead to the statistical differentiation of soils based on Ecoplates™ data alone (Jones et al., 2018). The method's limits include the issue of poor laboratory cultivability of certain bacterial strains and the challenges associated with the huge amount of data generated. Nonetheless, the data obtained is information-rich and reproducible (Bradley et al., 2006).

For this study, we aimed to find three close-proximity RFM which could be further valorized as biological agents to stimulate the biodegradation of PHCs. 1) Wood residues have been used as bulking agents in bioremediation projects (Battelle and NFESC, 1996; Kauppi et al., 2011). We opted for ramial chipped wood (RCW), a material that has been proven to increase soil fertility (Lemieux, 1986). Its chemical composition has rich ratio of polysaccharides to proteins (C:N) varying between 50:1 and 175:1 (varying across species and seasons) compared to woodchips from stem wood which have a C:N ratio of 400:1 to 600:1 under the same conditions (Lemieux, 1986). RCW is rarely cited in bioremediation, but Hattab et al. (2015) found that it reduced the toxicity (determined by mobility and phytoavailability) of trace metals in plants. 2) Animal manure can contain high levels of nutrients such as N, P and K (Moreno-Caselles et al., 2002). Kirchmann and Ewnetu (1998) found that co-composting with horse manure could reduce the concentration of large paraffin molecules by 80% in 110 days, as well as 93% of petroleum residues in 50 days. Cole (1998) reported that mature manure (six months) was efficient and reduced treatment time from the typical six months or more to two months or less for oily sludges (PHC in the engine oils molecular range). 3) Brewers' Spent Grain (BSG), which is the mass of wet grains remaining after the beer manufacturing process, is a fast-growing RFM in our region. The number of micro-breweries has more than doubled (105 new) in the last seven years (AMBQ, 2018). The production of BSG for micro-breweries in Quebec is approximately 970000 kg $year^{-1}$. BSG is rich in nutrients and contains 77 to 81% water (w w^{-1}) (Santos et al., 2003). Abioye (2011) suggests that BSG is a promising agent for the bioremediation of soil contaminated with PHC and metals. Research by Santos et al. (2003) shows that the BSG resulting from different types of beer within the same brewery was fairly homogenous, thus ensuring greater replicability.

In bioremediation, laboratory results do not always correspond with field observations since the dynamics of large-scale bioremediation systems are different. Key soil parameters (such as moisture, aeration, temperature, homogeneity, etc.) become more difficult to control

with increasing size, and bioremediation dynamics do not seem to follow a linear transfer in efficiency from small to medium to large scale (Khan et al., 2015; Ko et al., 2007). Furthermore, the legal regulations for industrial applications do not need to be respected in laboratory experiments. The scaling up of experiments must respect the legal framework applicable to the region or go through a process of application for approval, which can slow down technology transfer. In this study, we worked in partnership with an industrial soil remediation operator (Akifer for the SolNeuf treatment site) who projected to treat heavy PHC fractions (PHC molecules with more than 20 carbon atoms (C_{20})) on their platform. The remediation target for the contaminant chosen (engine oil) was set to the province of Quebec's 'C' criteria (commercial use, less than 3500 mg kg^{-1} for the PHC molecules in the C_{10}-C_{50} range), and was to be achieved within one treatment season in pilot scale (0.76 m^3) experimental units, using different local residual organic matter found close to the treatment platform. We aimed to quantify if there was preferential degradation of some PHC fractions among treatments. We sought to determine how the amendments changed the metabolic activity of the soil's bacterial communities and if distinctive 'fingerprints' could be identified.

Materials and methods

Soil preparation

This experiment was conducted on a certified treatment platform for contaminated soils owned by SolNeuf Inc., and operated by AKIFER Inc., located in Neuville, Quebec, Canada (46° 44' 5.0352" N, 71° 41' 2.634" W). The soil used was sandy (98.4 ± 0.3% sand, 2.5 ± 0.2% gravel, 0.9 ± 0.1% silt and loam), with a pH of 6.4 (1:1, soil:H_2O). It contained less than 0.3% organic matter (combustion) and 34.4 mg kg^{-1} phosphorus (P). The soil was free of PHC as measured by GC-FID (Gas Chromatography with Flame Ionization Detector) according to the method MA. 400 - HYD. 1.1 (CEAEQ, 2016). In order to control the concentration and homogeneity of the contamination, the initial soil was artificially contaminated with unused engine oil to 7500 mg kg^{-1} of PHC (C_{10}-C_{50}) and thoroughly mixed for 1h by excavator. The oil was RUBIA LD 10W30, manufactured by Total for diesel motors. The PHC concentrations were followed from June to November 2015. The target for

remediation was the Government of Quebec's 'C' Criteria (less than 3500 mg kg^{-1} in the C_{10}-C_{50} range) (Beaulieu, 2016).

RFM soil amendments

For the RFM materials, we defined as 'local' the ones that originated from a distance of 100 km or less of the soil treatment platform. This action was guided by a circular economy framework and the ITRC's Regulatory Guidance for Green and Sustainable Remediation to minimize the project's carbon footprint by reducing fuel consumption associated with transport (ITRC, 2011). A recycling and triage center located just 600 meters from the treatment platform was the first location where RFM were sought out. Ramial chipped wood (RCW) of mixed origin (unspecified mix of deciduous trees and conifers, excluding *Thuja* sp.) was chosen. RCW differs from regular wood chips because it originates from branches less than 7 cm in diameter and not whole trunks (stem wood) (Lemieux, 1986). The second closest available RFM was excess horse manure from stables 8 km away (homogeneous mix of fresh to eight months old manure). The last RFM was brewer's spent grain (BSG). Initially intended to be sourced 12 km away, the BSG used for the project had to be sourced 92 km away because of time constraints. The spent grain used in this project was four days old and had been stored outdoors in barrels.

Experimental design

The four treatments used were: 1 – Ramial Chipped Wood (**RCW-F**), 2 – Brewer's Spent Grain (**BSG-F**), 3 – Horse manure (**MANR-F**), and 4 – Fertilizer alone (**FERT**) (88.6% calcium mononitrate (27 N : 2.7 Mg : 4.6 Ca) and 11.4% diammonium phosphate (18 N : 46 P : 0 K) to achieve a C : N : P ratio of 100 : 2.5 : 0.5). This fertilizer dosage is the usual treatment at this treatment platform, and it was also added as a nutrient baseline to all other treatments ('F'). Due to space and logistical constraints, no bins were tested without fertilizer. The organic amendments were added at a ratio of 30% by volume for each local organic amendment and were mixed for 10 minutes by excavator, followed by 10 minutes of hand-shovel mixing (see Fig 4.S1 for design). For each treatment, three concrete cylinders (0.76 m^3) were placed outdoor and filled with 760 L of the resulting mixtures of soil and amendments.

The twelve cylinders were randomly distributed in a straight-line oriented southwest-northeast. Following the province of Quebec's regulations, the tops of the bins were covered with a thick plastic and a pulled-air system was connected to a perforated 2" PVC pipe placed 48 cm from the bottom of each bin (10% below the center). The air was drawn at a rate of 1.5 ± 0.26 m^3 h^{-1} and passed through a biofilter prior to release to the atmosphere. The rate was set to be proportional to the airflow in large biopiles on this site. Finally, all water runoff was collected and sent for treatment at a registered facility. The targeted soil moisture concentration was set at 70% of field capacity. When the moisture levels dropped significantly below that target, watering events took place (n=3).

Sampling

The concentrations of C_{10}-C_{50} PHC in the soil were monitored three times over 5 months, on June 10, August 31 and November 2, 2015. On the first and last sampling dates, the C_{10}-C_{50} PHC fractions were split into 6 smaller fractions (C_{10}-C_{16}, C_{16}-C_{22}, C_{22}-C_{28}, C_{28}-C_{34}, C_{34}-C_{40}, and C_{40}-C_{50}) to quantify if there was preferential fraction degradation. At each sampling event two new holes were drilled with a manual auger (5 cm diameter). Samples were taken from 30 cm and 60 cm depths in the first hole, and from 60 cm and 90 cm depths in the second hole. These four samples were homogenized and sub-sampled for all analyses. Nutrient measurements were conducted from the first and last sampling events described above. For moisture readings, the soil was sampled eight times and was measured gravimetrically (oven dry). Gas measurements were taken from a tube linked to a perforated PVC pipe capped on both ends and placed in the center of each bin. Carbon dioxide (CO_2), oxygen (O_2), and volatile organic compounds (VOCs) were measured with a portable gas detector (Eagle model, RKI Instruments). The tube's end was equipped with a clamp, which was released to measure the gases present in the bins. Temperature loggers (Levelogger Gold model 3001, by Solinst) were placed inside the PVC pipes used for gas monitoring in each experimental unit. Temperature was recorded every hour throughout the experiment. The loggers were calibrated at the beginning of the experiment and the deviation between loggers did not exceed 0.3°C (0.14 ± 0.03°C (air) and 0.08 ± 0.03°C (water)).

Analytical methods

The PHC fractions were measured by GCFID according to the MA. 400 - HYD. 1.1 method (CEAEQ, 2016). Soil samples were first dried with acetone (CAS no 67-64-1) and then extracted with hexane (CAS no 110-54-3) using a 'paint mixer' type extraction system. Subsequently, silica gel (60-200 mesh grade 62 (CAS no 112926-00-8), SiO_2) was added to the extract to adsorb the polar substances. Finally, the supernatant hexane was analyzed by GC-FID. The concentration of hydrocarbons present in the sample was determined by comparing the total area of all peaks from n-C_{10} to n-C_{50} with the surfaces of the standards used to establish the calibration curve under the same assay conditions (diesel standard solution altered to 50% at 5000 µg ml^{-1} ('Diesel fuel No. 2'), by Restek). The methodological limit of quantification was 100 mg kg^{-1} and PHC recovery rates were all between 82 and 94%.

The determination of ammoniacal nitrogen was a two-step process conducted according to method MA 300-N 2.0 (CEAEQ, 2014a). The first step was an extraction in the presence of potassium chloride (CAS no 7747-40-7). Secondly, the ammonium ion reacted with sodium salicylate (CAS #54-21-7), nitroferricyanide (CAS no 13755-38-9) and dichloroisocyanuric acid (CAS no 2893-78-9) to form a blue alkaline complex with an absorbance at 660 nm, which is proportional to the concentration of ammoniacal nitrogen. Ammoniacal nitrogen recovery rates were between 95 and 96%. Phosphorus concentrations were determined according to method MA. 300 – NTPT 2.0 (CEAEQ, 2014b). Nitrate and nitrite ions were analyzed in accordance with method MA. 300 – Ions 1.3 (CEAEQ, 2014c). The soil was mixed with water in order to dissolve the extractable anions. For leached nitrates and nitrites, the extraction was done with the leaching buffer as specified in the Hazardous Materials Regulations and described in method MA. 100 – Lix.com. 1.1 (CEAEQ, 2012). Subsequently, anions are were separated by an ion exchange column using the following eluent solution: 0.0027 M sodium carbonate (CAS no 497-19-8) and 0.0003 M sodium bicarbonate (CAS no 144-55-8)). The retention time differs for each anion, which makes it possible to identify and dose them. Nitrates and nitrites were measured using a conductivity sensor and the measured conductivity was proportional to the concentration of the anion in the sample. Recovery rates were between 97-104%.

Total phosphorus was determined in two steps. First, an acid digestion with sulfuric acid (CAS no 7664-93-9), which transforms phosphorus into orthophosphate. Secondly, orthophosphate ions were assayed by an automated system. The orthophosphate ion reacted with molybdate (CAS no 12054-85-2) and antimony (CAS no 28300-74-5) ions to form a phosphomolybdate complex. The latter was reduced with ascorbic acid (CAS no 50-81-7) to trigger the appearance of molybdenum blue, whose absorbance at 660 nm is proportional to the concentration of the orthophosphate ion. The detection limit was 200 mg kg^{-1} and recovery rates were within ± 15%.

Total organic carbon (TOC) was measured by combustion according to method MA. 310-CS 1.0 (CEAEQ, 2013) at 1360°C for a maximum of 600 seconds (oxygen at 30 lb in^2). Recovery rates were between 112-113%. The water used for the preparation of reagents and standard solutions was distilled or demineralized water. All samples were kept at 4° C until analysis (less than 14 days).

Community-level physiological profiling

The 96-well microplates contained 31 individual carbon sources from 6 classes (amine, amino acids, carbohydrates, carboxylic acids, phenolic compounds and polymers) (replicated 3 times in each plate) along with a clear tetrazolium dye, which gets irreversibly reduced to purple formazan dye when the bacteria consume the carbon (Bochner, 1989). One plate was used for each treatment cylinder (n = 9 carbon sources per treatment). A soil-aqueous solution was made according to methods previously described (Choi and Dobbs, 1999). All optical densities (OD) were brought below 0.350 nm (0.264 ± 0.009 nm) through dilutions to normalize the optical density at time zero. The well color development was monitored every day for 6 days by spectrophotometer (590 nm) and raw data was normalized by subtracting the control wells (water) present within each plate's three replicates. The result were interpreted by the rate of color change in the wells, the area under the curve and the functional richness as described in Choi & Dobbs (1999) and Garland (1997). The average well color development (AWCD) was calculated with the following formula: AWCD = $\sum \frac{(C-R)}{n}$, where C is color production, R is the absorbance of the control (water) and n is the number of substrates (n=31). To independently estimate color development, a curve-integration (CI) approach was

used. The area under the curve (the trapezoid area) was calculated as: $\sum_{i=1}^{n} \frac{v_i+v_{i-1}}{2} \times (t_i - t_{i-1})$ where v is optical density at time t. Functional richness is defined by the number of positive wells (OD_{final}-$OD_{initial}$ > 0.25 nm).

Statistical analysis

Decreases in PHC concentrations, moisture levels, EcoPlates™ data, and temperature between treatments were assessed with a linear mixed-effects repeated measures model fitted by Restricted Maximum Likelihood (REML), performed with the nlme{} (Pinheiro et al., 2017) in R (R Core Team et al., 2016). Post-hoc multiple comparison analysis were performed with the Tukey HSD method (lsmeans{} (Lenth, 2016) and mltcompview{} (Hothorn et al., 2008)). Assumptions of normality and homoscedasticity were met for all mixed model tests. Gas readings (O_2, CO_2, VOC) were analyzed with the Kruskal-Wallis test by ranks for non-parametric data agricolae{} (Mendiburu, 2017). Multiple comparisons were corrected with Bonferroni for all parametric and non-parametric tests. Figures were built with ggplot2{} (R Core Team et al., 2016; Wickham, 2009).

Results

Soil properties

In the first month, BSG-F temperatures in two bins spiked at least 14°C above the other bins. Overall, the maximum temperatures reached were 45°C, 31°C, 29°C and 28°C for BSG-F, MANR-F, RCW-F and FERT respectively. There was variability between bins of the same treatment and between treatments, but for the first 90 days, the mean temperature across bins was 22.9 ± 2.7°C (n = 25920). Subsequently, the bins' internal temperatures gradually dropped with the advent of the fall season (Fig. 4.1A). At this point, BSG-F remained significantly higher than the other treatments (with the exception of the 10-day increment reported at 140 days) (mixed model, $p < 0.05$). The addition of engine oil (Total Base Number: 10) raised the initial soil pH by around one unit, to approximately 7.4 across all treatments. Subsequently, all treatments remained stable with a pH between 7.2 and 7.6, except for the BSG, (6.8 ± 0.4, after oil addition, and increased to 7.6 ± 0.2 by the end of the

experiment). Water requirements varied between treatments. To maintain soil moisture near 70% of field capacity, three watering events took place during the course of the experiment. FERT had little water-holding capacity and needed watering each time. RCW-F required watering during the first two events, but not the third. MANR-F remained within a desirable moisture range (70% of field capacity) for the duration of the experiment and did not require any additional watering. Finally, BSG-F did not require any watering either and it contained the highest moisture levels of all treatments. There was a strong correlation between the amount of moisture (%) and the organic carbon content in the soil (excluding PHC-based carbon). (Fig. 4.1B).

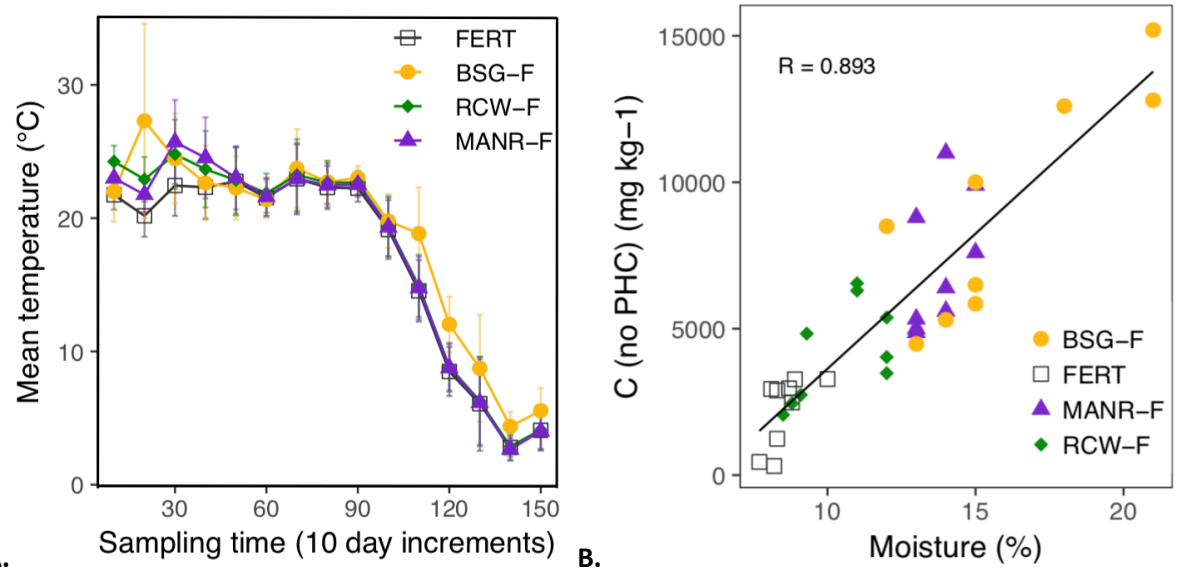


Figure 4.1 Soil temperature and moisture readings. A. Mean temperature in the center of the treatment bins in 10-day increments (n=720). Error bars are SD. **B.** Correlation (R = 0.893) between soil carbon and moisture (%) in the experimental bins from June, September and November data. The carbon contents attributable to PHC concentrations were removed, as they do not contribute to water retention in the soil.

Nutrient balance

Raw brewer's spent grain (BSG) was the amendment richest in nitrogen whereas ramial chipped wood (RCW) contained the least nutrients (Table 4.1). All treatments experienced a sharp decline in available nitrogen (NO_2^-, NO_3^- and NH_4^+), which was likely assimilated by soil microorganisms, with the exception of BSG-F, where a sharp increase in NH_3 and NH_4^+ was observed between June and November. Treatments all show relatively low

total nitrogen loss (<15%), except BSG-F that lost nearly 25%. For FERT, an increase in total nitrogen was measured. Since there were no nitrogen inputs over the course of the project, this is probably linked to a heterogeneous distribution of the fertilizer in the soil.

Table 4.1 Nutrient and organic matter content in raw amendments prior to incorporation in soil as well as measurements in the soil matrix at the beginning (June) and end of the experiment (November). TOC stands for total organic carbon. (Values ±SD). There is low variability within the treatments' initial PHC concentrations and they are statistically different from each other. Reduction presents the percent PHC removed and the lowercase letters denote statistical rank (mixed model, $p < 0.05$).

RAW LOCAL ORGANIC AMMENDMENTS

Source	Analyte material	NH_{3-4}	Total N	Total P	Total K	Org. matter	C:N
units		*mg kg^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*	*%*	*ratio*
MANR		0.14 ± 0.02	2.8 ± 0.0	1.00 ± 0.01	4.75 ± 0.22	66 ± 2.5	36 ± 2.6
RCW		0.20 ± 0.05	2.2 ± 0.75	0.21 ± 0.03	0.61 ± 0.10	85 ± 0.5	74 ± 20
BSG		0.12 ± 0.02	5.8 ± 0.20	1.08 ± 0.03	0.39 ± 0.02	96 ± 0.0	18 ± 0.9

SOIL MEASUREMENTS

Treatment	Analyte *units*	NH_{3-4} *mg kg^{-1}*	NO_{3-4} *mg kg^{-1}*	Total N *mg kg^{-1}*	TOC : N *ratio*	C_{10}-C_{50} *mg kg^{-1}*	Reduction *%, rank*
FERT	*June*	61 ± 29	78 ± 8	196 ± 50	44:02.5	7 500 ± 100	62 ± 4 b
	Nov.	10 ± 0	0.5 ± 0	243 ± 12	01:02.5	2833 ± 252	
MANR-F	*June*	76 ± 13	60 ± 5	420 ± 60	18:02.5	6500 ± 173	77 ± 3 a
	Nov.	10 ± 0	1.3 ± 0.8	363 ± 12	45:02.5	1 500 ± 200	
RCW-F	*June*	66 ± 16	68 ± 13	330 ± 12	10:02.5	6767 ± 58	60 ± 6 b
	Nov.	10 ± 0	0.5 ± 0	283 ± 22	00:02.5	2700 ± 400	
BSG-F	*June*	10 ± 0	40 ± 16	990 ± 87	52:02.5	7133 ± 58	38 ± 11 c
	Nov.	80 ± 35	4 ± 3.5	733 ± 55	33:02.5	2700 ± 400	

Engine oil decrease

A sharp decrease in the first 82 days, followed by a plateau was observed for all treatments, except the BSG, which maintained a slower but constant reduction over the five-month experiment (Fig. 4.2A). There is little variability within each treatment for the initial PHC data before RFMs addition (± 57 to ± 173 mg kg^{-1}, which represents a variability range of 0.8 to 2.7%), indicating a homogeneous initial distribution of the engine oil in the soil. However, PHC concentrations were different for all treatments at the start ($p < 0.05$), which is likely due to uneven dilution from the different RFMs since they were added on a volume basis and PHC concentrations are expressed on a mass basis (Table 4.1). MANR-F was significantly more effective at driving a PHC reduction (77 ± 3% reduction) in the soil than all other treatments (BSG-F: 38 ± 11%, RCW-F: 60 ± 6%, and FERT: 62 ± 4%) (mixed model, $p < 0.05$). On August 31, while the platform's usual fertilizer treatment (FERT) was fluctuating around the Commercial & Industrial sites' regulatory guideline (3500 mg kg^{-1}), MANR-F was well below it and approaching the Residential & Recreational guideline (700 mg kg^{-1}) (Fig. 4.2A). The analysis of engine oil by fraction revealed the lightest (C_{10}-C_{16}) and heaviest (C_{40}-C_{50}) fractions were either close to or below the detection limit (100 mg kg^{-1}) and were excluded from further analysis. The next lightest fraction (C_{16}-C_{22}) decreased significantly more (63 ± 22%) than the rest of the fractions (C_{22}-C_{28}, C_{28}-C_{34}, C_{34}-C_{40}), which were removed at equivalent rates (42 ± 17, 39 ± 16 and 44 ± 15%, respectively), despite differences in molecular size (Fig. 4.2B).

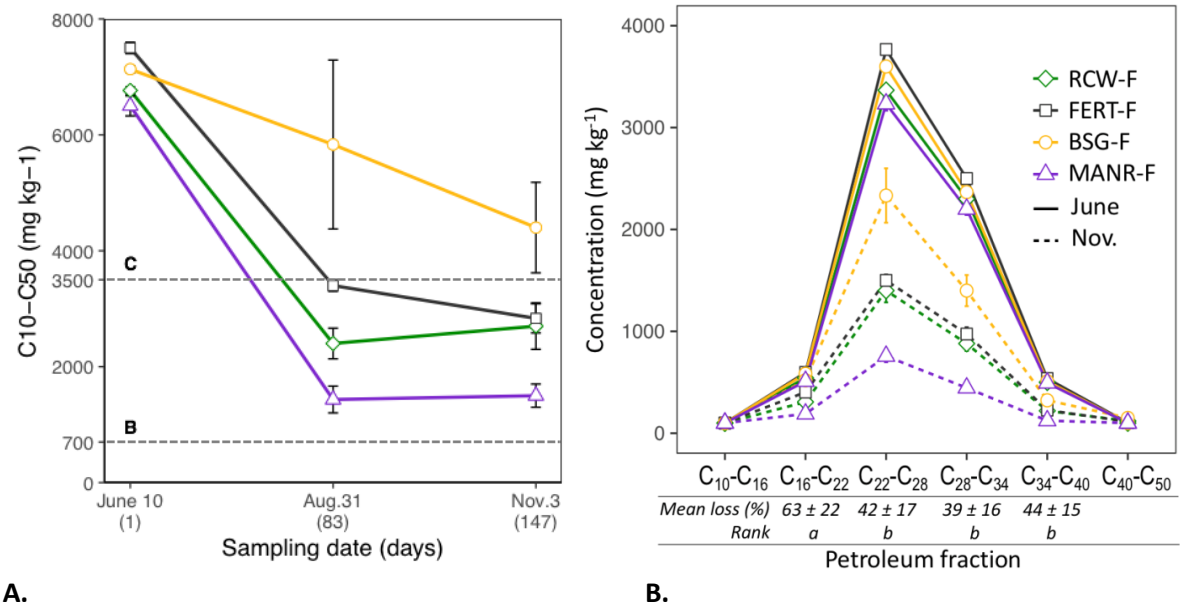


Figure 4.2 Petroleum hydrocarbon concentrations. A. Concentrations of engine oil fraction C_{10} to C_{50} over 147 days. The lines at 3500 and 700 mg kg^{-1} indicate the Quebec environmental guidelines for Commercial & Industrial sites ('C') and Residential & Recreational sites ('B') respectively (Beaulieu, 2016). **B**. Concentrations of the six petroleum hydrocarbon fractions monitored in the soil. The dotted lines represent the last sampling event (November). Mean loss compares the percent decreases in the different fractions. The rank is Tukey HSD (Bonferroni correction applied for multiple comparisons, after a mixed model analysis ($p < 0.05$)). For both figures, reported errors are standard deviation (SD, n=3).

Outgassing

There were notable differences in the gas emissions monitored among the different treatments, despite high variability within the data (Fig. 4.3). With a mean of 7.9 ± 3.6%, BSG-F contained significantly less O_2 than the other treatments, which all showed averages between 18 and 19% ($p < 0.05$). CO_2 emissions by FERT and BSG-F were always near the upper detection limit of the equipment (6000 ppm), while MANR-F was the treatment with the lowest mean emission (2695 ± 2871 ppm) ($p < 0.05$). VOCs were very low in all treatments (means below 250 ppm), except for BSG-F (3636 ± 3318 ppm), which released significantly more than all other treatments ($p < 0.05$). The VOCs emitted in this case could likely be methane from the breakdown of organics in waterlogged pockets of the cylinder which had become anoxic.

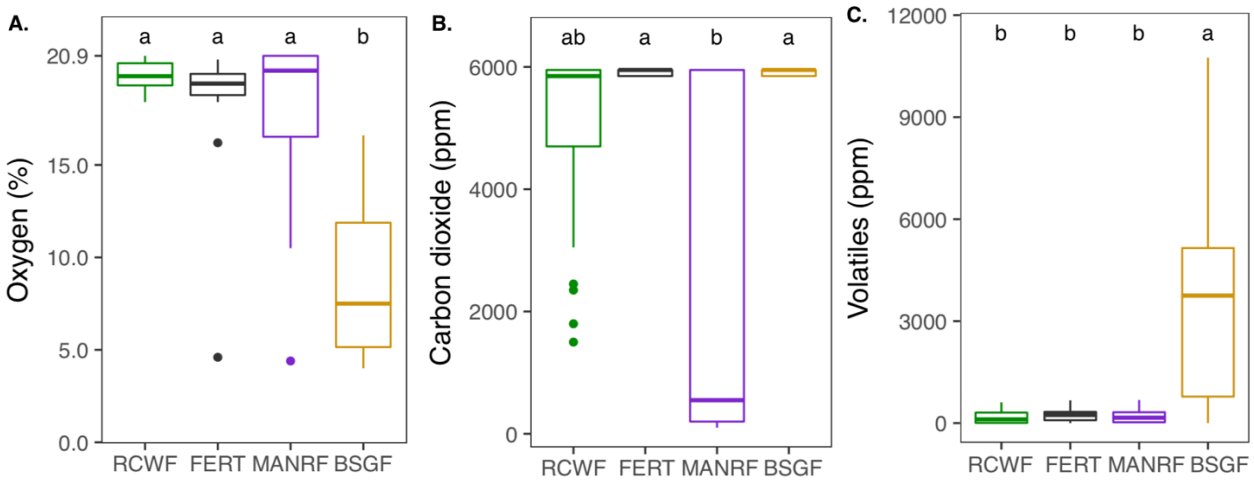


Figure 4.3 Gas concentrations in the center of the treatment bins measured seven times over 45 days. A. Oxygen (%). **B.** Carbon dioxide (ppm). **C.** Volatiles organic compounds (ppm). Error bars represent SD. Lower case letters denote statistical ranking (Kruskal-Wallis, $p < 0.05$, Bonferroni correction applied for multiple comparisons. n=21).

Community-level physiological profiling

The global metabolic activity of the plates (AWCD) showed that the soils with amendments were more active than the FERT by the third day and by the fourth day, BSG-F had more AWCD than all others (Fig. 4.4A) (mixed model, $p < 0.05$). All treatments followed a sigmoidal shape, presenting a standard bacterial growth curve. The curve-integration approach also revealed that FERT-F was less active than the soils amended with local RFM. There were differences in carbon usage between treatments. For example, BSG-F was able to use polymer carbon sources more effectively than the other treatments and FERT-F used carbohydrates less effectively than all other treatments (mixed model, $p < 0.05$). Multiple significant differences were also noted within the usage of the carbohydrates, where bacteria in RCW-F were able to actively use more C sources, more rapidly than all other treatments. There was less functional richness in FERT (240 active wells out of 288) than in MANR-F (270), RCW-F (270) and BSG-F (273). Finally, the PCA indicates a clear separation between BSG-F and the other three treatments, which form over-lapping but clear clusters (Fig. 4.4B). This confirms the different soils' bacterial community's catabolic activity fingerprint.

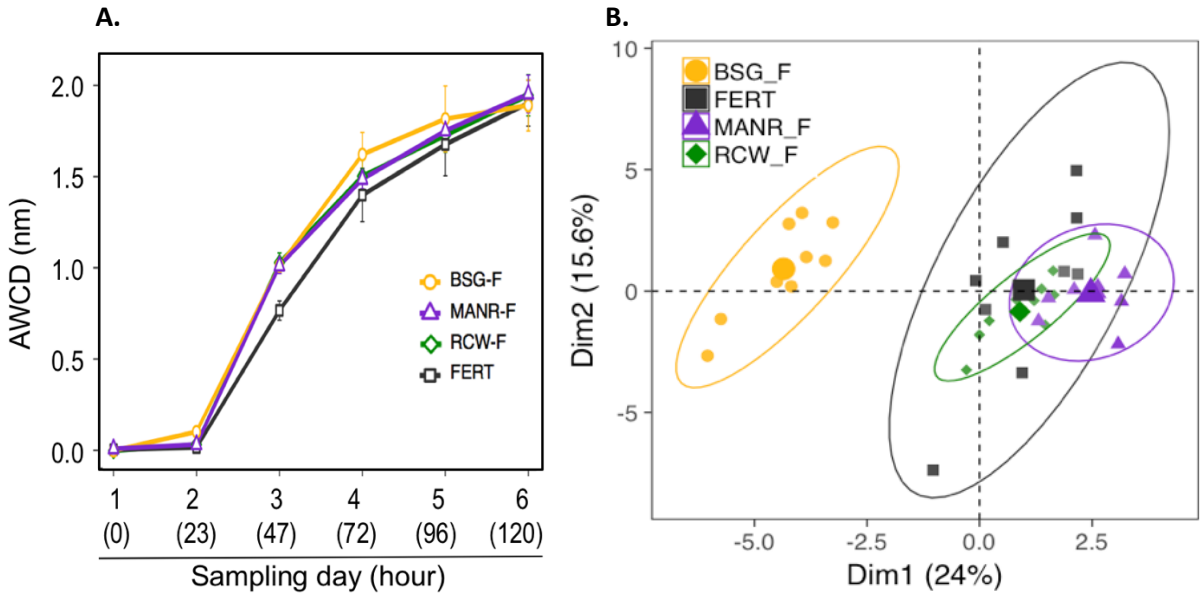


Figure 4.4 Ecoplates results. A. Average well color development (AWCD) (nm) over six days for soil from the four treatments. Error bars represent standard deviation.
B. PCA: Principal component analysis of the consumption of the 31 carbon sources in relation to the four treatments.

Discussion

CLPP and soil properties

Community-level physiological profiling (CLPP) conducted with the Ecoplates™ indicated that the biological amendments increased the metabolic activity and functional richness of the soils compared to the fertilizer control (FERT). No well-defined links between PHC-degradation efficiency and Ecoplates-derived metabolic activity were observed. Nonetheless, there were clear clusters in the PCA defining the catabolic capabilities of the soils based on amendment. Microbial activity is optimal between 20 and 40°C for petroleum remediation and slows when soil temperatures drop close to 0 °C (Battelle and NFESC, 1996). In this project, overall higher temperatures were not linked to higher rates of contaminant removal. Other authors have found that temperature had a great influence on the reduction of petroleum concentrations (Van Gestel et al., 2003), or that higher temperatures could lead to increased volatilization (but unlikely with heavy PHC) (Namkoong et al., 2002). In our case, BSG-F exhibited the highest temperatures, but was the least effective at PHC-removal. This

was most likely due to the heat generated during fermentation (anaerobic breakdown) of the BSG. Soil pH is influenced by microbes, and can also be influenced by their activity (Nwankwegu and Onwosi, 2017). Over the course of this experiment, pH remained stable in all treatments, except in the BSG-F. Other studies have reported pH variations in most treatments; this difference in our work could be linked to the soil's pH buffering capacity or the large-scale set-up, which has different dynamics than microcosms (Nwankwegu and Onwosi, 2017; Prince, 2015). Moisture is an important factor for bioremediation: concentrations too low inhibit microbial activity, and excessive moisture levels can promote hypoxic conditions that are less conducive to rapid biodegradation of PHC (Khan et al., 2004). A moisture content of 50 to 70% of field capacity is often cited as optimal (ATSDR, 1999; Khan et al., 2015). In the sandy soil used in the trials, 70% of the field capacity corresponds to about 15% moisture on a mass basis (g g^{-1}). Field capacity varies with soil type and organic matter in soil can improve water retention (Brady and Weil, 1996a). The various local organic amendments used in this project clearly demonstrated this point, with the strong correlation between non-PHC carbon in the soil and moisture levels, and with the different watering requirements of the treatments. FERT had virtually no organic matter and required moisture addition throughout the project. RCW-F required watering the first two events, but not the third, indicating that the wood had started to retain water in the soil. BSG-F retained moisture slightly above levels for optimal degradation (20 ± 1.7% (g g^{-1}) at the start), but the aspect of most concern was that this high moisture was coupled with a fine particle and compact amendment structure which limited air circulation in the bins, fostering hypoxic conditions which are not ideal for rapid PHC degradation. Further, the BSG supplied soil microbes with an abundance of easily accessible food which may have been easier to target than the engine oil. Finally, MANR was the optimal RFM input for moisture since MANR-F did not require any watering throughout the project, while maintaining values close to 70% field capacity. Reduction in soil management operations can be advantageous in large-scale settings.

Nitrogen balance and engine oil removal

In this study, nitrogen came from two main sources: an inorganic fertilizer and the three RFMs (soil nitrogen content was negligible). No nitrogen was added during the course of the study. We had aimed to keep the soils under aerobic conditions where volatile forms of N

are not normally emitted, hence total nitrogen should have remained stable throughout the monitoring. It did, with the exception of BSG-F where a nearly 25% drop in nitrogen occurred. Other than heterogeneous distribution in soil which can skew the results, total nitrogen loss in soils may be related to leaching of soluble forms (although bins were largely protected from rainfall) or a denitrification process resulting in the formation of nitrogen gas and its loss to the atmosphere. This last process may indicate conditions in soils that are too low in oxygen. The reduced forms of nitrogen (NH_4^+ and NH_3) present in the BSG-F soil in November support the presence of sub-optimal oxygen levels since these reduced forms of nitrogen are usually observed when the soil is deficient in oxygen. Whereas, when the soil is well aerated, nitrogen is found in oxidized forms such as nitrate and nitrite (Brady and Weil, 1996b). There was an initial input of ammonium as diammonium phosphate in the inorganic fertilizer, but this was not detected in the BSG bins at the beginning of the experiment. The concentrations detected in November were likely mineralized from the organic matter in the amendment and remained in this form due to lack of oxygen for conversion to oxidized forms (NO_3^- and NO_2^-) by the nitrification process. Reduced forms of nitrogen are most prevalent under hypoxic soil conditions (Britto and Kronzucker, 2002) and effective aliphatic PHC-degradation usually occurs under aerobic conditions (Khan et al., 2004).

Larger PHC molecules were expected to be biodegraded at slower rates than the lighter ones, but this was not generally the case in our study (Battelle and NFESC, 1996, Khan et al., 2004). The C_{16}-C_{22} fraction was indeed removed faster than the other fractions monitored, but the decrease of the other three fractions (between C_{22} and C_{40}) was statistically equivalent ($p > 0.05$). PHC can move out of soil through volatilization, but other authors found mainly fractions below 12 to 16 carbons to be volatile while heavier compounds were mainly biodegraded (Namkoong et al., 2002, Gallego et al., 2010). Bacteria usually reduce PHC molecules down to CO_2 (Brady and Weil, 1996c). After 82 days of the different treatments, MANR-R had significantly decreased PHC concentrations and was approaching the Residential and Recreational guideline (700 mg kg^{-1}), while the LTU's usual FERT treatment was fluctuating around the Commercial and Industrial guideline (3500 mg kg^{-1}) of the Quebec government regulations. From this point on to the end of the experiments all treatments plateaued, and little degradation took place in the last 65 days. Since varying levels of carbon

from the PHC remained in the soil, it would be possible to optimize the treatments to stimulate degradation beyond the plateaus. This could be done through better control over parameters such as aeration, moisture and nutrients, and using known correlations for estimating petroleum biodegradation rates in soils, which are especially useful for scaling-up experiments (Khan et al., 2015).

BSG-F did not exhibit the same plateau but was far less effective than the other treatments for engine oil removal (38 ± 11%). Other authors have found BSG to be an effective soil amendment. In a phytoremediation experiment using the plant *Jatropha curcas*, Agamuthu et al. (2010) found that when BSG was added to a soil contaminated with 2.5% and 1% of lubricating oil, the degradation rates of the PHCs were 89.6% to 96.6% respectively. Oruru (2014) demonstrated not only the efficiency but also the sustainability of BSG as an amendment to treat diesel-contaminated soil. The reduced efficiency of the BSG-F in this study appears to be linked to the soil structure and excess moisture which restricted aeration. Despite its lower PHC-removal performance in this project, the application of BSG could be further considered for the remediation of soils with low nutrients and low water retention capacities, as long as aerobic conditions are maintained to minimize nitrogen loss and VOC output. Currently, farmers supplement their animals' regular feed with BSG, but rapid disposable is primordial for breweries because it ferments rapidly causing serious odor problems. This leads many city-based breweries to send their BSG to the landfill (Santos et al., 2003).

RCW-F was equivalent to FERT for PHC-removal and its measured contributions within this project are limited to improvements of soil parameters (moisture and microbial activity). Horse manure (MANR-F) was unequivocally the most successful soil amendment in this project. It led to the most PHC-reduction in the shortest time period. From a soil clean-up facility's perspective, this represents a faster turnaround of the soil and potential increases in profitability. MANR-F maintained adequate moisture and oxygen levels, while releasing the least amount of CO_2, which is interesting in a global climate change context. The bacterial degradation of PHC emits CO_2 and since the most PHC-removal took place in this treatment, it should have released more than the rest. Since it was not the case, we hypothesized a form of carbon sequestration in the microbial biomass, but this was not tested further.

Cost of RFM and opportunities for circular economy?

Within the scope of this project, horse manure (MANR) was available nearby and free of cost, but in other regions such manure may be sold at a higher price or used directly on adjacent farmlands, potentially reducing the application of our results to some regions. Ramial chipped wood (RCW) did not significantly increase remediation speed compared to fertilizer alone, but it promoted long term moisture retention, which can alleviate the management of biopiles through reduced watering needs. Brewer's spent grain (BSG) led to less PHC removal than the LTU's usual fertilizer treatment by limiting aeration due to excess moisture. In retrospect, the addition of 30% BSG for this soil was excessive. We hypothesize that a smaller proportion of the dense and nutritious BSG, coupled with the addition of a bulking agent such as RCW could create favorable PHC remediation conditions at an advantageous cost. We conclude that there is no one-size-fits-all approach for the use of RFMs, and each remediation site will need to evaluate the RFMs available in their respective region.

In their Vision 2050 project, the World Business Council for Sustainable Development envisions making the concept of waste obsolete as a normal business practice (WBCSD, 2010). This research highlights a particularly interesting opportunity for soil treatment industries to actively participate in this vision, by up-cycling degraded 'waste soil' through remediation and introducing it into a circular economy loop. Further, RFM are present across the globe and can offer low-cost amendments to boost remediation efficiency while reducing treatment time.

Conclusion

Soil remediation industries can benefit from the addition of RFM in their operations, while diverting RFMs from landfill, thereby contributing to waste diversion objectives set by European and North American governing agencies. Each locality has regulations to limit the quantity of material that can be incorporated into contaminated soil, to avoid excessive dilution. Overall, RFM's main functions are improving moisture retention and soil structure, contributing nutrients and a greater diversity of microorganisms, and to enhance the growth matrix for microbial life in the soil, which can, in some cases, enhance PHC remediation as compared to fertilizers alone. These conclusions are unsurprising in the sense that adding

organic matter to contaminated soil has been shown to foster bioremediation in the past, but we view the process undertaken in this project as another step towards more partnerships between academia and industry, in a manner that incorporates considerations of the broader environmental impacts of bioremediation work. The circular economy objective of reducing pollution and waste as much as possible served as a guiding principle to sourcing the three residual fertilizing matter (RFM) amendments within 100 km of the study site. Due to performance pressures, inorganic fertilizer use was maintained in the scope of this project, but future work should include the exploration of RFMs and processes which may allow for a move away from this traditional approach, which requires resources that are synthesized by high-energy processes and sourced from non-renewable resources (such as P). Overall, choice of the RFM treatment should be made considering multiple factors such as treatment effects, cost, time, accessibility by users, and sustainable supplying, to fall into the concept of a circular economy.

Author contributions

Designed the experiments and provided expertise on the design: KR, ML, SM, MA. Performed the experiments: KR, ML. Data was analyzed by KR. Wrote the paper: KR, MA. All authors have edited and given approval to the final version of the manuscript.

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CHAPITRE 5. | Synthèse générale

La nature évolue depuis 3.4 milliards d'années; c'est amplement de temps pour développer des solutions aux problèmes comme ceux que nous engendrons aujourd'hui avec la pollution des écosystèmes à l'échelle planétaire.

Les solutions sont là.

Il est primordial de les découvrir avant de, et surtout sans, les détruire…

L’état des comptes

La contamination des sols est une problématique d’envergure internationale où les hydrocarbures pétroliers (HCP) dominent. Les éléments traces (ET) sont également répandus et présentent des défis de taille. La présence concomitante des HCP et des ETs rend l’assainissement plus difficile et plus coûteux. Il est reconnu que les opérations d’assainissement des sols peuvent elles-mêmes créer des impacts environnementaux négatifs tels que l’utilisation de grandes quantités de combustibles fossiles et de ressources non renouvelables, ainsi que l’introduction d’espèces de plantes envahissantes, pour ne nommer que ceux-là. De plus, on ne peut ignorer l’importance d’ancrer des projets scientifiques académiques dans le tissu social de la communauté où ils prennent place. En climats nordiques et en régions éloignées, des défis additionnels s’appliquent aux sites contaminés et font augmenter les coûts associés au traitement des sols. Par exemple, les taux de dégradation des contaminants organiques sont ralentis au froid et la saison de croissance des plantes est beaucoup plus courte qu’au sud. Il est donc nécessaire d’avoir des méthodes d’assainissement abordables, adaptées au climat et en respect de l’environnement écologique et humain qui entoure les sites.

Les technologies d’assainissement passives telles que la phytoremédiation, la mycoremédiation et l’incorporation de matières organiques fertilisantes sont des méthodes éprouvées pour la bioremédiation des sols, mais leurs pratiques peuvent encore être optimisées et améliorées. Ces technologies sont généralement sous-utilisées dans des projets à grande échelle qui pourraient en bénéficier. Les trois approches sont rarement utilisées de façon concomitante. Cela peut être dû au fait que la complexification des systèmes peut engendrer une hausse des coûts et une augmentation de la difficulté d’interprétation des résultats. En général, ces technologies sont jugées non applicables à des sites très fortement contaminés, car la croissance et la survie des plantes et des microorganismes peuvent être compromises par des seuils de toxicité élevés. Des cultivars de plantes résistantes à de forts taux de contamination et avec un bon potentiel d’extraction de métaux sont en développement continu. Ces plantes ont des applications positives indéniables et nécessaires, mais une certaine attention doit être portée afin d’éviter de les implanter dans des environnements où elles ne sont pas indigènes et où elles pourraient éventuellement devenir envahissantes dans l’écosystème environnant. Avec

l'utilisation de la phytoremédiation, il est également important de tenir compte de l'accès au site par les espèces fauniques locales qui pourraient consommer les plantes servant à la décontamination, engendrant ainsi des conséquences délétères si les plantes contiennent des taux d'ETs élevés. L'utilisation de champignons est encore très limitée à grande échelle et à l'extérieur et, à notre connaissance, aucun cas d'espèce fongique envahissante n'a été rapporté suite à un projet de bioremédiation. Néanmoins, avant d'implanter toute espèce, il serait sage de respecter le vieil adage disant « dans le doute, abstiens-toi ». Le choix des espèces de champignons pourrait aussi être limité par leur possibilité d'être cultivées (les symbiotes obligatoires peuvent par exemple poser certains défis). L'utilisation d'espèces de plantes et de champignons indigènes dans les écosystèmes environnants les sites de remédiation permet d'éviter le problème d'espèces envahissantes.

Les propriétés du sol ont une grande incidence sur la biodisponibilité et la bioremédiation des contaminants. De multiples sources de matières organiques ont la capacité d'améliorer les propriétés du sol, incluant notamment l'aération, le pH, la rétention d'humidité et l'apport de nutriments. Ces matières organiques augmentent ainsi l'efficacité des processus de bioremédiation médiés par les communautés bactériennes et fongiques du sol. Chaque année, d'énormes quantités de matières organiques putrescibles sont enfouies dans des dépotoirs. Certains gouvernements d'Europe et d'Amérique du Nord mettent en œuvre des stratégies pour détourner ces matières et favoriser leur valorisation, notamment pour réduire l'émission de gaz à effets de serre dans un contexte de changements climatiques. Un pont peut être fait entre des centres de traitement de sols contaminés et des sources de matières résiduelles fertilisantes (MRF) pour que tous en tirent des bénéfices, tout en réduisant les impacts environnementaux.

Retour sur les hypothèses et faits saillants

Lors de cette thèse, la performance combinée de saules, de champignons et de compost municipal a été évaluée pour l'assainissement de deux sols dans un climat subarctique. Le premier était contaminé au diésel et le deuxième était issu d'une fosse à huiles usées fortement contaminée à la fois par des ETs et des niveaux extrêmement élevés d'HCP. La performance

de MRF locales a également été évaluée pour la remédiation d'un sol contaminé à l'huile à moteur.

Tout en considérant l'environnement écologique et humain dans lequel les projets avaient lieu, des recherches ont été faites, lorsque cela était possible, pour appliquer une approche écosystémique et d'économie circulaire. Nous avons voulu, pour ces projets situés en région subarctique, recréer une certaine structure écologique et une diversité biologique dans les sols contaminés. Nous avons donc utilisé simultanément plusieurs composantes biologiques en incorporant des organismes issus de trois règnes différents (plantes, champignons, bactéries). Cette incorporation simultanée est qualifiée de « microsystème écologique » dans les chapitres 2 et 3. Au cours d'un projet de recherche effectuée dans un climat tempéré humide au sud du Québec, des mesures ont été prises pour obtenir des amendements organiques à proximité du site de traitement (chapitre 4). Dans le cadre de cette thèse, des efforts ont été mis de l'avant pour réduire l'utilisation de fertilisants inorganiques. C'est la méthode de traitement la plus courante, mais la synthèse de ces engrais est très énergivore et requiert l'exploitation de ressources non renouvelables. De plus, ils peuvent avoir des impacts délétères sur les écosystèmes en région nordique, et leur efficacité pour la bioremédiation est parfois remise en question.

Les recherches élaborées au cours de cette thèse se retrouvent sous l'ombrelle de l'état des comptes présenté plus haut. Toutefois, comme elles présentent toutes des conditions particulières, chacune sera décrite séparément, suivie d'une synthèse plus générale sur leurs retombées communes.

Chapitre 2 | Développement d'un microsystème écologique pour la remédiation du diésel dans un climat subarctique

Le premier objectif était d'évaluer l'efficacité d'une approche de bioremédiation avec l'utilisation simultanée de *Pleurotus ostreatus*, *Salix alaxensis* et de compost municipal pour le traitement d'un sol contaminé au diésel en milieu subarctique. Ce microsystème a également été comparé à l'utilisation de fertilisant inorganique, la méthode la plus répandue dans le domaine de bioremédiation des sols. L'hypothèse que l'utilisation conjointe de trois composantes biologiques augmenterait significativement la rapidité et le pourcentage total de

la dégradation de diésel comparativement aux composantes isolées a été validée pour la première saison de traitement. Pour les années subséquentes, les traitements à base de composantes biologiques ont été plus efficaces que l'atténuation naturelle ou l'utilisation de fertilisants inorganiques. Cela a confirmé la deuxième hypothèse qui comparait les traitements avec ou sans composantes du microsystème. Cette étude a démontré qu'un sol subarctique contaminé par du diésel peut être assaini avec succès conformément aux normes commerciales et industrielles du CCME en 14 mois, en utilisant simultanément du compost ou plusieurs composantes biologiques. Si les zones résidentielles et agricoles sont visées, trois saisons de croissance suffisent. Finalement, il a été démontré que l'espèce *S. planifolia* peut être utilisée en boutures directes pour effectuer la végétalisation d'un site subarctique contaminé au diésel.

Limites et perspectives

Chaque projet de recherche scientifique a ses limites et peut être continuellement amélioré. Le microsystème instauré dans un sol oligotrophe en milieu subarctique a semblé être limité par la quantité de nutriments disponibles après la première saison, ce qui a pu stimuler une certaine compétition entre les différentes composantes du microsystème. Une source additionnelle de nutriments ou bien l'implantation de plantes fixatrices d'azote pourraient être des moyens de soutenir plus longuement tous les éléments biologiques. Des nutriments sous forme de MRF ou composts pourraient, par exemple, être étalés en surface au début de la deuxième année. Si la végétalisation du site n'est pas une priorité, nos résultats indiquent que l'application seule de compost peut favoriser une dégradation plus rapide que l'usage de fertilisants inorganiques. Un projet complémentaire au chapitre 2 a été conduit pour mesurer l'efficacité du microsystème dans un cadre de diversification des sols, les résultats sont présentés dans l'Annexe III.

Chapitre 3 | Application du microsystème dans un sol à forte contamination mixte dans un milieu subarctique

Le deuxième objectif fut d'évaluer l'efficacité de l'approche du « microsystème écologique » pour la bioremédiation de contaminants organiques et la phytoextraction de métaux et métalloïdes (éléments traces (ETs)) d'un sol à contamination mixte en milieu

subarctique. Bien que la plupart des fractions restent au-dessus des normes industrielles du CCME, l'approche en microsystème a donné d'excellents résultats. Au bout de cinq ans, la structure du sol s'était clairement modifiée et une élimination considérable des contaminants organiques était mesurée, malgré les interférences potentielles dues à la présence d'ETs. La première hypothèse supposant que les composantes biologiques dans le traitement actif favoriseraient une plus grande dégradation des HCP comparativement à l'atténuation naturelle a été démontrée sans équivoque. Il a également été démontré que les deux espèces de saules implantées ont accumulé des ETs dans leurs tissus aériens. Les « life pockets » ont favorisé l'implantation des plantes et les deux espèces de *Salix* utilisées ont démontré des taux de survie satisfaisants (en particulier *S. planifolia*), et ont présenté des profils d'accumulation de TE distincts ainsi que des stratégies d'enracinement différentes. Une question s'est ajoutée au cours du processus de recherche pour déterminer si les ET accumulés dans les feuilles des saules pouvaient présenter un danger potentiel pour la faune locale. Les résultats démontrent que, sur ce site, une exposition avec un facteur de risque élevé (HQ >1) était improbable, mais qu'il s'agirait d'une mesure importante à considérer s'il y avait une mise à l'échelle de ce projet (i.e. considérer l'installation de clôtures temporaires pour limiter l'accès à la faune). La colonisation naturelle élevée sur les cellules du traitement avec microsystème par rapport au témoin, ainsi que les tests effectués sur des plants de seigle et des vers de terre ont confirmé que la toxicité du sol avait été considérablement réduite. En fait, la toxicité du sol était significativement plus basse dans le sol traité avec le microsystème que dans le contrôle.

Limites et perspectives

Une limite claire de l'étude est la méthodologie liée aux mesures des métaux. Une meilleure évaluation de la distribution des métaux devrait être effectuée en échantillonnant les tiges et les racines en plus des feuilles des plantes. Les analyses de métaux sont coûteuses et le budget peut être un facteur limitant, mais si la phytoextraction est envisagée pour l'assainissement des sols, il serait pertinent d'étudier plus attentivement la distribution des métaux au sein des plantes. Une autre limite de l'étude est au niveau de la somme des métaux extraits par les plantes (du moins dans les parties mesurées). Si un assainissement des ET à l'aide de la phytoremédiation est envisagé, la quantité de métaux extraits devrait augmenter. Ceci peut être accompli par une plus grande production de biomasse ou par une plus forte

concentration d'ET dans les tissus des plantes. Un accroissement de la biomasse végétale produite pourrait y contribuer. L'utilisation de boutures de saules ayant déjà un système racinaire développé dans des « life pockets » pourrait permettre un meilleur établissement, et donc une croissance plus rapide qui pourrait augmenter la biomasse. D'autres espèces de plantes locales pourraient être évaluées pour leur potentiel d'hyperaccumulation, surtout celles déjà présentes en bordure de la fosse à huiles usées. Finalement, l'utilisation de plusieurs espèces de plantes ayant des modes d'extraction d'ETs complémentaires pourrait être considérée en milieu subarctique, comme cela a été fait récemment en région tempérée.

La présence du champignon volontaire de la famille de *Psathyrellaceae* est intrigante et l'étude de son potentiel en tant qu'espèce biorémédiatrice serait très pertinente. Les questions à explorer pourraient inclure : 1) la capacité du champignon à dégrader des composés organiques; 2) ses mécanismes de tolérance à de hauts taux de contaminants; et 3) son potentiel d'immobilisation ou d'accumulation d'ET. Les taux d'ETs dans les fructifications de cette plante n'ont pas été évalués au cours de cette étude.

Cette étude effectuée dans une fosse à huiles usées d'un ancien dépotoir se distingue des autres expériences de bioremédiation en climat subarctique par l'ampleur de la contamination du site. Ce site est resté fortement contaminé pendant plus de 40 ans et, en moins de cinq ans, nous avons obtenu d'excellents résultats en termes de dégradation des HCP, tout en favorisant la végétalisation. Cette étude ouvre donc la porte à la bioremédiation de fosses à huiles usées et autres sites industriels abandonnés en milieu subarctique. Le recours à une telle approche pour traiter le volume substantiel de sol restant dans la fosse à huiles usées, serait une bonne première étape pour l'élimination des contaminants organiques.

Chapitre 4 | Collaboration industrielle : Optimiser le traitement d'huile à moteur en intégrant des MRF locales

Le troisième grand objectif était d'évaluer la performance de différentes matières résiduelles fertilisantes (MRF) locales pour la dégradation de l'huile à moteur, en collaboration avec un partenaire industriel, tout en intégrant des considérations sur les impacts environnementaux plus larges, notamment en s'approvisionnant de MRF locales. La première hypothèse a été confirmée en partie seulement. En effet, les différentes matières résiduelles

fertilisantes utilisées ont eu des efficacités variables, mais une seule, soit l'usage de fumier, a été significativement plus efficace que la pratique courante d'utiliser uniquement du fertilisant inorganique. Pour le deuxième objectif, une dégradation légèrement inégale des fractions d'hydrocarbures pétroliers a été observée dans le sol lors de l'analyse des C_{10}-C_{50} fractionnés. Cette étude n'a pas permis d'identifier là où se trouvent les fractions les plus récalcitrantes, outre le fait que la fraction la plus légère (mesurée en quantité considérable) a été retirée à des taux plus élevés que les quatre fractions suivantes, qui elles ont subi une réduction équivalente. Quant aux objectifs industriels, une bonne partie de la démonstration que les installations et méthodes du centre de traitement ont la capacité de dégrader les hydrocarbures pétroliers (HP) lourds (huile à moteur neuve ou usée) pour l'obtention d'un nouveau certificat d'autorisation (CA) a été effectuée, mais à ce jour, le processus est toujours en cours. Il a été démontré avec succès qu'il est possible en une saison de traitement d'abaisser le niveau de contamination pour les C_{10}-C_{50} sous le critère environnemental « C » (3,500 mg kg^{-1}) du Ministère de l'Environnement et de la Lutte contre les changements climatiques (MELCC) du Québec, en tenant compte de la contamination initiale de 7,500 mk kg^{-1}. Finalement, l'influence de MRF locales sur la vitesse et le taux de dégradation des HP lourds a été quantifiée.

Limites et perspectives

Le projet de recherche visant à trouver une MRF locale pour la bioremédiation de sols contaminés à l'huile à moteur a été effectué en collaboration avec un partenaire industriel pour répondre à certains objectifs d'expansion de leur plateforme de traitement. Le but premier n'était pas de repousser la limite du savoir scientifique en bioremédiation, mais bien la limite de la plateforme. De prime abord, ceci peut sembler étrange comme composante d'une thèse en sciences biologiques, mais il est important de considérer les répercussions plus larges, dont l'intégration des approches écosystémiques et d'économie circulaire à grande échelle. Ces retombées sont pertinentes, car elles peuvent servir d'outils de base pour accroître la durabilité et l'applicabilité des pratiques de recherche.

Il est essentiel pour l'auteur de faire le pont entre la science fondamentale et le monde industriel. Ce partenariat a offert une belle opportunité d'effectuer un projet de bioremédiation

concret en mettant de l'avant l'usage de matières résiduelles fertilisantes locales. L'objectif ultime était de valoriser les matériaux locaux pour éventuellement diminuer l'utilisation d'engrais synthétiques. Les résultats obtenus à ce jour ne le permettent pas encore pour l'huile à moteur en milieu tempéré, car la plateforme doit maintenir un roulement de base minimum. Alors que les ressources mondiales continuent de diminuer et que les émissions de carbone liées au réchauffement planétaire sont mieux prises en compte, les projets de recherche et de réhabilitation visant à réduire leur empreinte environnementale sont de plus en plus nécessaires. Cela répond également aux objectifs gouvernementaux de réacheminement et de valorisation de déchets putrescibles voués à l'enfouissement pour aider à réduire les émissions de gaz à effet de serre. En utilisant ces matières résiduelles fertilisantes (MRF) dans des centres de traitement des sols pour améliorer les processus de décontamination, une opportunité de répondre à ces deux objectifs se présente.

Retombées des approches utilisées

L'objectif premier de cette thèse était de mettre en pratique des méthodes de bioremédiation novatrices en prenant en considération l'état des comptes présenté plus haut lors leur application, notamment la combinaison d'éléments du milieu nordique, l'utilisation de MRF et des partenariats avec l'industrie de l'assainissement des sols. Plus tôt dans la thèse, il a été question de l'importance de développer des méthodes applicables à des régions éloignées. La méthode la moins dispendieuse et la plus pratique en région éloignée est habituellement l'approche de traitement *in situ*. Cela peut sembler utopique à première vue de faire des expériences en bacs et en cellules pour ensuite implanter ces techniques *in situ.* Toutefois, ces recherches constituent une première étape réaliste et très importante pour effectuer des tests contrôlés et choisir avec soin les traitements pour une mise à l'échelle. Il est primordial de considérer les critères suivants : 1) le travail a été effectué à l'extérieur, et non en laboratoire; 2) les volumes de sols utilisés allaient jusqu'à 1 m^3, ce qui est plus réaliste que des expériences en petits pots; et 3) les sols utilisés étaient issus de vrais sites contaminés. Ces critères augmentent tous la faisabilité de transfert sur le terrain.

Pour maximiser l'applicabilité concrète des résultats scientifiques, les trois projets de recherche ont été menés en collaboration avec trois entreprises locales, soit deux au Yukon

(Arctic Backhoe Services et Boreal Compost Enterprise), ainsi qu'une au Québec (Akifer inc.). De plus, au Yukon, la ville de Whitehorse et le Yukon Research Center ont été impliqués directement dans les projets de recherche. Plus de 24 étudiants locaux ont été recrutés et ont participé activement au projet, particulièrement sur le terrain. Par souci de partage et de transparence, des présentations sur la science de la bioremédiation et l'avancement du projet ont été données à cinq reprises aux étudiants, aux décideurs et au public général.

En conclusion, cette thèse ouvre la voie à de nouvelles stratégies intégrées dans le domaine de la bioremédiation de sols contaminés aux HCP et aux ET en milieu subarctique, à l'aide de plantes, champignons et compost municipal, formant ainsi le « microsystème écologique ». De même, elle identifie certaines opportunités pour l'intégration de l'approche écosystémique et de l'économie circulaire dans un cadre de valorisation de matières résiduelles fertilisantes pour l'assainissement des sols. Chaque région possède des ressources locales en flore, champignons et MRF uniques qui pourraient être mises au profit de l'assainissement des sols contaminés. Dans la nature, tout est intégré, et il semble logique d'appliquer ce principe aux approches de bioremédiation.

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ANNEXE I | Justification du choix des espèces de *Salix*

The choice of which species to use was guided by three main criteria:

1. Occurrence: The species needed to be already growing in the general area of the study (in the extended area of the city of Whitehorse).
2. Ability to grow from cuttings.
3. If possible, found in the literature reviews of promising species: Some species such as *Salix viminalis* and other well-developed cultivars are commonly used in phytoremediation. None of those were either native to the area or found in the willow collection field survey that was conducted in the late winter of 2013.

Bruce Benett, a Yukon botanist provided this list of Salix species found in the Whitehorse area: *alaxensis* (both ssp.) X, *arbusculoides* X, *arctica**, *athabascensis*?, *barclayi* x, *barrattiana**, *bebbiana* X, *candida*?, *commutata**, *glauca* X, *myrtillifolia* X, *niphoclada*, *planifolia* X, *polaris**, *pseudomonticola* x, *pseudomyrsinites* x, *pulchra* X(dominates in alpine), *reticulata**, *richardsonii**, *scouleriana* X.

X = common lowland species	* = alpine only
x = uncommon lowland species	? = reported but rare

Glauca, planifolia, scoulerriana, and *barclayi* were found in the spring survey. In May 2013 a test trial was performed using winter cuttings. The species showing best overall growth after X days was *Salix planifolia* (previously soaked for X days in deionized water).

Salix alaxensis was identified as an interesting species in literature reviews. It was found once summer had begun and hence summer cuttings were used. Cuttings also performed very well in test trials conducted in potting soil prior to the implementation on the project site.

ANNEXE II | Expérience sur la croissance du mycélium

21 avril 2013

Résultat d'expérience – Observation du succès de croissance du mycélium du champignon *Pleurotus pulmonarius* avec différents volumes de vermicompost et copeaux de bois dans un sol semi-argileux.

But

Le but de cette expérience était de déterminer les pourcentages de vermicompost (compost) et copeaux de bois à utiliser pour favoriser la croissance du mycélium d'un champignon, afin de prendre des décisions éclairées lors de la mise en place sur le terrain d'un projet de recherche similaire.

Les objectifs étaient spécifiquement de déterminer le pourcentage minimum de vermicompost et copeaux de bois nécessaire pour:

1) permettre la survie du mycélium
2) obtenir une croissance optimale du mycélium.

Méthode

- Jeudi le 21 mars, les copeaux de bois et la terre pour l'expérience (un mélange sableux et argileux avec peu de matière végétale) furent déterrés de sous la neige de deux piles près du jardin des jeunes, au Jardin Botanique. Les espèces d'arbres d'où proviennent les copeaux de bois sont inconnues. Le compost a été acheté à Santropol Roulant pour $5.

- Lundi le 25 mars 2013, la terre fut mélangée avec du sable et du turface (argile de montmorillonite) jusqu'à l'obtention d'un sol avec une texture semi-granuleuse (moins compacte que simplement le sol argileux). 8 combinaisons de sol, copeaux et compost furent mélangées et placées dans des pots de plantes à ouverture de 8 pouces. Un volume total de 2L fut placé dans chaque pot, puis un bloc de mycélium de 125mL de *Pleurotus pulmonarius* fut ajouté au centre de 7 des 8 combinaisons (A à G). Le mycélium avait été cultivé sur de la sciure de bois franc par la compagnie Advitam. Les combinaisons de A à E furent répliquées trois fois tandis que les combinaisons de F et G furent répliquées deux fois. H n'a eu qu'un réplica (car ce pot non planifié était composé des restes de matériel). Le tableau 1 définit les combinaisons utilisées.

- Par inadvertance les pots furent tous trop arrosés le jour le l'empotage. Par la suite, les pots furent arrosés de nouveau seulement lorsqu'il y avait besoin, pour maintenir un niveau d'humidité moyen dans les pots. Le mycélium de six pots fut déterré et enlevé par des souris au début de l'expérience, ce qui a rendu ces pots inutilisables pour l'expérience. Voir le tableau 1 pour l'identification de ces pots.

- Vendredi le 19 avril, 25 jours après le début de l'expérience, tous les pots furent renversés et l'expansion du mycélium jugée de façon visuelle.

Résultats

Tableau 1. Combinaisons utilisés et résultats		
Pot	**Combinaison**	**Résultats et observations**
A1	Compost 5% Copeaux 5% Mycélium 6%	A1 Le mycélium a été retiré par les souris.
A2		A2 et A3 Le bloc au centre est vivant, a commencé à coloniser les copeaux qui le touchaient mais n'a pas colonisé le sol.
A3		
B1	Compost 10% Copeaux 5% Mycélium 6%	B1 et B3 Le bloc au centre est vivant, a commencé à coloniser les copeaux qui le touchaient mais n'a pas colonisé le sol.
B2		
B3		B2 Le mycélium a été retiré par les souris.
C1	Compost 5% Copeaux 10% Mycélium 6%	C1 et C2 Plus de mycélium observé que dans les pots précédents. Il suit les copeaux et est visible jusqu'aux côtés et fond du pot. Un peu de sol est également colonisé.
C2		
C3		C3 Le mycélium a été retiré par les souris.
D1	Compost 10% Copeaux 10% Mycélium 6%	D1 Pas très bien colonisé mais aucuns copeaux de bois sont visibles.
D2		D2 et D3 Le mycélium est en très bonne santé. Colonisation visible jusqu'aux côtés du pot. Les copeaux mais aussi des morceaux de compost sont colonisés.
D3		
E1	Compost 8% Copeaux 8% Mycélium 6%	E1 Le mycélium a été retiré par les souris.
E2		E2 et E3 Colonisation des copeaux de bois a débuté et un « nuage » de colonisation est visible autour du bloc de mycélium mais pas une bonne croissance comme C ou D.
E3		
F1	Compost 8% Copeaux 12% Mycélium 6%	F1 et F2 le sol mouillé à « capacité de champ » (plus que tout les autres pots) mais le mycélium va très bien, il colonise les copeaux et la terre commence à faire tenir la terre en blocs. (Voir image 2).
F2		
G1	Compost 100% Mycélium 6%	G1 et G2 Le mycélium a été retiré par les souris.
G2		
H	Compost 33% Copeaux 33% Mycélium 33%	Une excellente colonisation du pot. Création d'un beau « pain » solide. Tout commence à être colonisé sauf 1 pouce à la surface. (Voir image 3).

Limites de l'expérience

Les champignons qui seront utilisés sur le terrain (*Pleurotus ostreatus et Trametes versicolor*) seront différents de l'espèce utilisée dans cette expérience (*Pleurotus pulmonarius*). Par contre, il est fort probable que *Pleurotus ostreatus* est assez similaire à *Pleurotus pulmonarius* pour que les résultats soient comparables et *Trametes versicolor* est considérée comme une espèce plus agressive. Donc, l'hypothèse qu'elle sera tout aussi performante, sinon plus, pourrait être émise.

L'observation visuelle de la colonisation des pots est une façon subjective de quantifier les résultats. Pour une expérience où l'on désirerait avoir des chiffres quantitatifs précis une autre méthode devrait être envisagée, mais dans le cadre de ce projet, ce type d'observation a rempli son rôle de façon satisfaisante.

Conclusion et discussion

Les résultats démontrent que plus il y a de copeaux de bois, plus la colonisation du milieu par le champignon est rapide. Le traitement H a été le pot largement mieux colonisé que tous les autres. Dans ce traitement deux facteurs diffèrent beaucoup des autres traitements : 1. le mycélium a été mélangé au sol au lieu d'être mis en boule au centre et 2. 33% de mycélium a été utilisé comparativement à 5% dans les autres traitements. Également, beaucoup plus de copeaux de bois ont été utilisés : 33% comparativement au maximum de 12% dans les autres traitements. Donc, il semblerait que de mélanger le mycélium soit une meilleure option que de l'appliquer en blocs. Il semblerait également que d'utiliser un plus grand volume de copeaux de bois favoriserait la colonisation du substrat de façon plus efficace. Il est intéressant de noter que le pot H n'avait pas été planifié, mais comme il restait du matériel le tout à été mélangé « pour voir » et ce pot a en fait fournit de bonnes informations.

Le fait que les souris puissent être assez attirées par le mycélium pour le déterrer et tout l'enlever est un élément qui était inconnu de l'auteur et il sera très important à considérer pour le projet à venir sur le terrain. Si des souris ont été attirées, peut-être que d'autres petits animaux comme les écureuils pourraient également l'être. Certaines stratégies comme des agents répulsifs pour souris ou de mélanger le mycélium directement au sol pourraient minimiser la quantité de mycélium retiré par de petits rongeurs comme des souris.

Il est intéressant de noter que plusieurs graines originaires du compost ont germé dans les pots. Ceci n'a probablement pas eu d'impact sur l'expérience mais supporte l'idée que des plantes puissent pousser avec du mycélium de *Pleurotus pulmonarius* présent (voir image 1). Ceci est bon signe pour le travail sur le terrain qui inclura des plantes.

Il pourrait être intéressant de mener cette expérience pour une période plus longue que 25 jours pour savoir si les champignons dans les pots qui n'ont pas bien été colonisés arrivent éventuellement à coloniser tout le substrat.

Images

Image 1. Graine de plante qui a germé proche du bloc de mycélium du pot E2.

Image 2. Le mycélium à colonisé le substrat jusqu'aux abords du pot F2.

Image 3. Le mycélium issu du pot H a colonisé la majorité du substrat et le maintient en place.

Un grand merci à Dimitri Dagher pour tout ton aide durant le projet !

ANNEXE III | Résumé d'un projet complémentaire au chapitre 2 |

PEx : Mesure de l'efficacité du microsystème avec une diversification des sols

Ce projet (PEx) est une extension du projet ABH (chapitre 2). Après la première année de recherche (2013) qui semblait indiquer une plus grande efficacité du modèle à trois composantes biologiques, nous avons décidé d'évaluer l'efficacité de cette combinaison novatrice sur trois sols typiques du Yukon (Table AI.1, Figure AI.1), dont un avec d'autres types d'amendements (le biochar et le carbonate de calcium). Un bref résumé de ce projet est présenté ici.

Objectif

Implanter le microsystème composé de *Salix planifolia*, *Pleurotus ostreatus* et de compost municipal, dans cinq différents types de sols contaminés au diésel pour mesurer à quel point la composition sol aura un impact sur son efficacité de bioremédiation.

Hypothèses

1. Les traitements à base des trois composantes biologiques seront encore une fois plus efficaces que les contrôles pour la bioremédiation du diésel, et ce peu importe le type de sol.
2. Les types de sols auront des impacts significatifs sur les taux d'élimination du diésel.

Design expérimental et méthodologie

Le site de recherche est situé dans une enceinte extérieure au Yukon Research Center (YRC). Cette expérience hors terre est contenue sur une bâche protégeant le sol environnant des lessivats. Trois types de sols typiques du Yukon avec différents amendements furent choisis : un sable acide typique des forêts de conifères; une glaise limoneuse; et un sol très riche en matière organique (typique seulement le long de certains cours d'eau). La

contamination artificielle au diésel visait 10,000 mg kg^{-1}. L'expérience a eu lieu de juin 2014 à juillet 2015.

Table AI.1 Propriétés des sols utilisés pour PEx.

Type de sol	pH	Humidité (%)	Densité sèche (g cm^{-3})	Couleur humide d'après la charte Munsell
Organique	7.2	53.8	0.34	Black
Argile limoneuse	7.8	3.1	1.08	Dark Brown
Sableux	6.2	5.2	1.20	Light Olive Brown

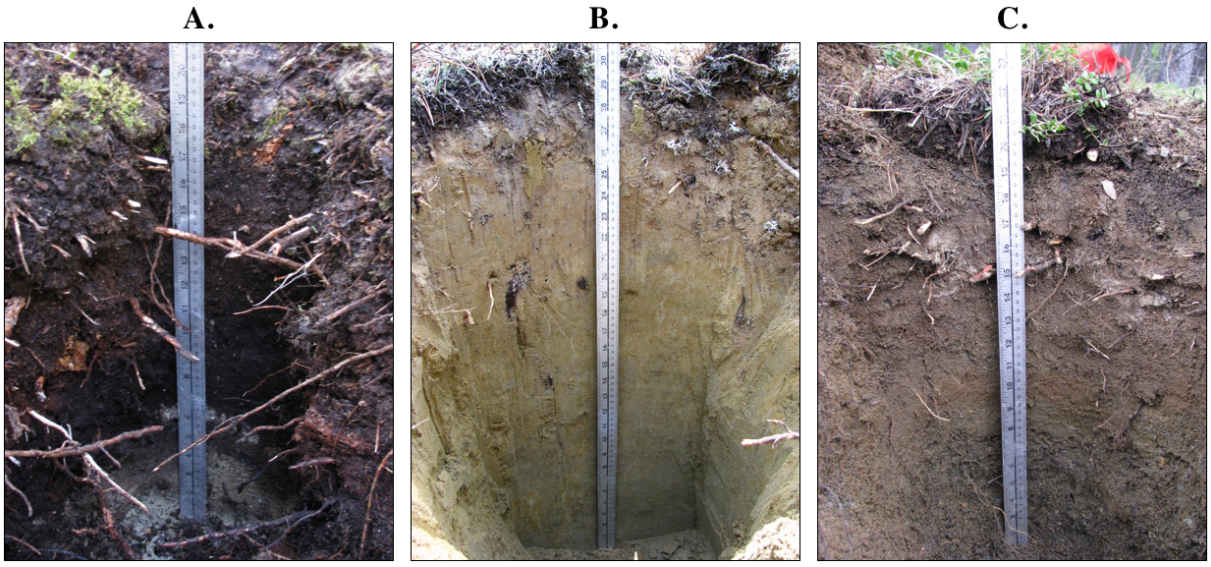

Figure AI.1 Photo de l'origine des sols utilisés dans l'étude. **A.** Organique. **B.** Argile limoneuse. **C.** Sableux.

Les unités expérimentales choisies étaient beaucoup plus petites que celles utilisées dans les chapitres 2, 3, et 4. Des bacs de 4L de polypropylène contenant 3.5L de sols artificiellement contaminés furent utilisés (Figure AI.2). Pour chacun des cinq sols, nous avons effectué un contrôle et un traitement actif, avec 5 réplicats, pour un total de 50 unités expérimentales (Table AI.2 pour la liste des traitements). Ces unités furent placées sur une épaisse bâche « Enviro Liner 6030^{HD} » à l'épreuve des HCP pour protéger les sols environnants. Le Enviro Liner fut à son tour recouvert d'une simple bâche gris pâle pour augmenter la réflexion de la lumière et minimiser la surchauffe du système à cause de la radiation solaire. Les 50 bacs en polypropylène furent placés dans 5 blocs aléatoires et l'échantillonnage eut lieu trois fois au fil des 13 mois de l'expérience.

Figure AI.2 Unités expérimentales placées de façon aléatoire juste après la mise en place de projet.

Les saules furent traités de la même façon que dans ABH et trois boutures furent placées dans chaque bac. Le mycélium de *Pleurotus ostreatus* utilisé pour cette expérience est la souche « K1 » de la compagnie Sylvan Inc. Les amendements utilisés sur des réplicats de sol sableux furent du biochar (cité comme agent favorisant la dégradation des HCP (Qin et al., 2013, Bushnaf et al., 2011) et du carbonate de calcium ($CaCO_3$) pour augmenter le pH acide du sable et combler certains besoins en calcium du champignon (Phan and Sabaratnam, 2012) (Table AI.3.).

Table AI.2 Énumération et codes utilisés pour les dix traitements.

Sols pour les traitements	Codes d'identification	
	Contrôle	Traitement actif
Organique	OrC	OrAT
Sableux alcalin ($CaCO_3$)	SaC	SaAT
Sableux acide	SbC	SbAT
Argile limoneuse	LmC	LmAT
Sableux + Biochar	BcC	BcAT

Table AI.3 Description des amendements utilisés dans les sols de l'expérience PEx.

Alcalin	$CaCO_3$ issu de coquilles d'œufs lavées, séchées et broyées. 1% (volumique)
Biochar	1% (volumique) Préparé par le Collège Langara à base SPF (épinette, pin et sapin) 4h à 600°C. Taille <1cm.
Traitement actif	70% sol, 15% compost (volumique), 10% mycélium stérile de P. ostreatus + 5% copeaux de bois inoculés de *P. ostreatus*.

Analyse statistique

Un modèle linéaire à effets mixtes, ajusté en fonction du maximum de vraisemblance restreint (REML) a été réalisé avec le nlme{} (Pinheiro et al., 2017) dans R (R Development Core Team, 2016) pour comparer: la diminution des concentrations de HCP entre les types de sol et entre les traitements. Des analyses de comparaisons multiples post-hoc ont été effectuées avec la méthode Tukey HSD (lsmeans{}(Lenth, 2016) and mltcompview{} (Hothorn et al., 2008)). Les hypothèses de normalité et d'homoscédasticité ont été satisfaites pour les tests susmentionnés. Pour les données non paramétriques, le test de Kruskal-Wallis a été utilisé pour mesurer les différences de pourcentage de dégradation de l'AT et du C, au sein des types de sols types avec agricolae{} (De Mendiburu, 2009).

Résultats

Les HCP furent mesurés dix jours après la mise en place du projet et les résultats de ces premières mesures sont présentés dans la Figure AI.3.

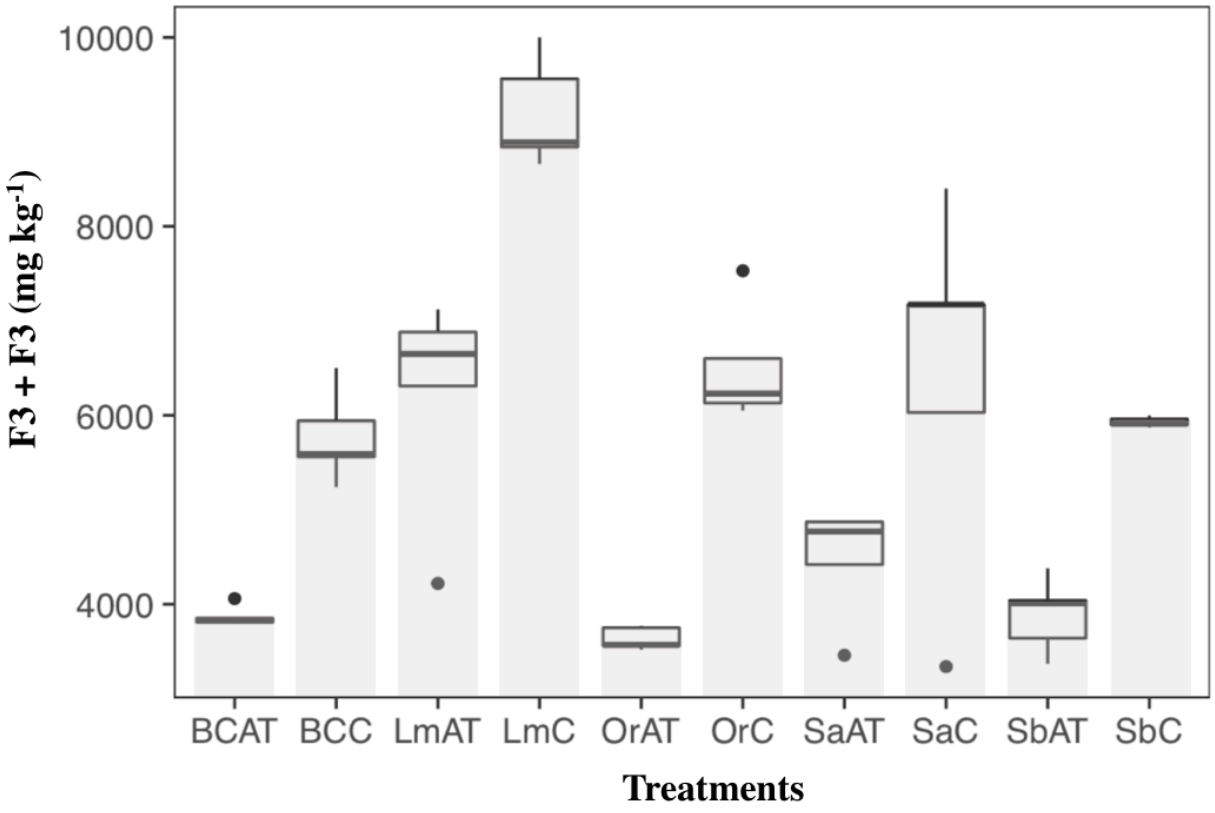

Figure AI.3 Concentrations d'HCP (fractions F2 et F3) mesurées dans les sols dix jours après la mise en place du projet.

Le pourcentage d'HCP retirés du sol a été calculé entre la première mesure (juin 2014) et la dernière (juillet 2015). Les résultats sont présentés dans la Table AI.4 et dans la Figure AI.4.

Table AI.4 Pourcentage de diésel retiré. Les lettres indiquent les différences statistiques entre le traitement actif (ACTV) et le contrôle (CTRL) dans un même sol.

	ACTV		**CTRL**	
Argile limoneuse	**60.3% ± 2.4%**	**a**	47.2% ± 4.6%	b
Organique	70.3% ± 3.8%	b	**89.5% ± 1.0%**	**a**
Sable acide	**70.4% ± 6.3%**	**a**	53.1% ± 20.9%	b
Sable + CaCO3	72.4% ± 7.1%	a	66.9% ± 3.7%	a
Sable + Biochar	54.9% ± 9.2%	a	52.0% ± 6.6%	a

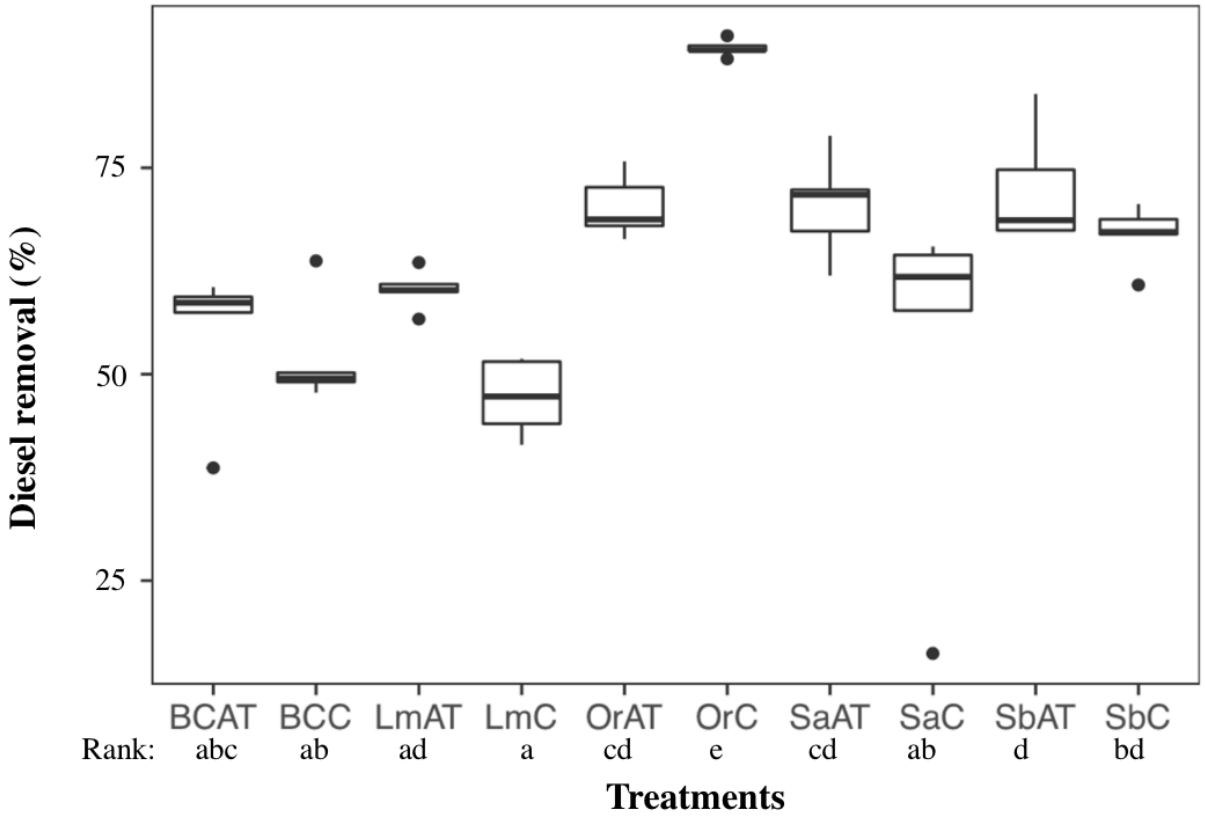

Figure AI.4 Pourcentage de diésel retiré du sol lors de l'expérience par traitement. Les lettres minuscules représentent les différences statistiques (modèle mixte, $p < 0.05$).

Les différences entre les types de sols ont été examinées pour voir l'impact des sols sans tenir compte des traitements AT et C. Voici les tendances générales pour les sols : BC (a), Lm (a), Sa (ab), Sb (bc) and Or (c) ($p < 0.05$).

Discussion et conclusion

En dépit d'une contamination manuelle contrôlée au millilitre près et un brassage intégral, les taux d'HCP sont très hétérogènes dans cette expérience. Cela est très probablement lié à l'attente de 10 jours post-contamination avant d'échantillonner. Cette décision avait été prise pour réduire le taux de toxicité aiguë causée par le relâchement initial des volatiles qui aurait pu être nocif pour les plantes et champignons. C'est également une pratique que nous jugions plus représentative d'un cas réel de déversement là où il y aurait un certain délai entre le moment du déversement et celui du traitement. Les résultats obtenus indiquent que le type de sol organique aurait une plus grande influence sur le taux de

dégradation du diésel que le traitement actif lui-même. Cependant, les concentrations d'HCP sont tellement variables lors de la première prise de mesures que nous hésitons à tirer des conclusions de ces données. À cause du délai, il est impossible de savoir si les baisses par rapport au 10 000 mg kg^{-1} visé étaient dues à de la variabilité dans le dosage, à des taux de volatilisation plus élevés dans certains sols lors du brassage, ou encore à une rapide dégradation microbienne des HCP. En conclusion, les données obtenues ne nous permettent pas de répondre avec confiance aux hypothèses émises.