UNIVERSITÉ DU QUÉBEC EN ABITIBI-TÉMISCAMINGUE

ÉTUDE DES FACTEURS MICROCLIMATIQUES ET ÉDAPHIQUES LIÉS À LA PRÉSENCE D'HERBACÉES AGRONOMIQUES QUI AFFECTENT LA COLONISATION NATURELLE ET LE DÉVELOPPEMENT JUVÉNILE DES ARBRES SUR LES PARCS À RÉSIDUS MINIERS ÉPAISSIS

THÈSE

PRÉSENTÉE

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AVANT-PROPOS

Cette thèse doctorale a été rédigée sous forme de trois articles pour publication dans les journaux en plus d'une introduction générale, une revue de littérature et une conclusion générale. Je suis la principale rédactrice de l'ensemble des articles et de la thèse, des relever de terrain et de l'analyse des données. Mon directeur et ma co-directrice ont contribué à la conception de l'étude, et m'ont guidé pour les analyses statistiques des données. En plus de leur précieuse collaboration pour la révision, leurs commentaires constructifs m'ont permis d'avancer mes connaissances scientifiques et académiques.

L'introduction générale (Chapitre 1) donne le contexte et l'intérêt de la recherche, suivi par une revue de littérature qui aborde les différentes thématiques du projet de recherche, puis l'objectif général et les objectifs et les hypothèses pour chacun des chapitres.

Le Chapitre 2, *The effects of agronomic herbaceous plants on the soil structure of gold mine tailings and the establishment of boreal forest tree seedlings*, est un article publié dans la revue *Water, Air, & Soil Pollution.* Il a été écrit en collaboration avec le professeur Philippe Marchand à l'UQAT, directeur de recherche, avec madame

Hermine Lore Nguena Nguefack, assistante en biostatistique, et avec la professeure Marie Guittonny co-directrice à l'UQAT. [1]

Le Chapitre 3, *The effects of agronomic herbaceous plants on the floristic composition at an early successional stage on gold mine tailings*, les résultats seront soumis à la revue *Ecoscience*. Il été écrit en collaboration avec Philippe Marchand et Marie Guittonny.

Le Chapitre 4, *The effects of agronomic herbaceous plants on microclimatic conditions and pioneer boreal tree seed germination and seedling survival on a gold mine tailings substrate*, les résultats seront soumis à la revue *Ecoscience*. Il a été co-écrit avec Philippe Marchand et Marie Guittonny.

La thèse se termine avec une conclusion générale (Chapitre 5), incluant des recommandations et perspectives.

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[1] Barrette, D., Marchand, P., Nguena Nguefack, H. L., & Guittonny, M. (2022). The effects of agronomic herbaceous plants on the soil structure of gold mine tailings and the establishment of boreal forest tree seedlings. *Water, Air, & Soil Pollution, 233*(1), 1-20.

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ACRONYMES, UNITÉS ET FORMULES

˚C	*Celsius degree* (Degré Celsius)
$D = 1-(\Sigma n_i (n_i - 1) / N(N-1))$	Formule pour l'Indice de diversité de Simpson
$H = -\Sigma p_i * \ln(p_i)$	Formule pour l'Indice de diversité de Shannon
ha	Hectare
kg	*Kilogram* (kilogramme)
G$	Milliard de dollars canadien
M$	Million de dollars canadien
n	Nombre
%	Pour cent (ou pourcentage)
WT %	Pourcentage par poids
VWC	*Soil volumetric water content* (teneur en eau du sol)
t	Tonne métrique

RÉSUMÉ

La végétalisation des résidus miniers représente un défi important en raison de leurs propriétés physico-chimiques uniques. Au Canada, la plupart des résidus miniers sont produits dans la région bioclimatique de la forêt boréale et leur végétalisation est donc primordiale pour réduire la fragmentation du territoire et l'empreinte écologique des activités minières. L'ensemencement des parcs à résidus miniers avec un mélange de graines de plantes herbacées agronomiques (légumineuses et graminées) est une pratique courante en première phase de la revégétalisation de ces résidus. Toutefois, peu d'études ont tenté de déterminer si cette pratique a un effet positif sur la succession végétale menant à un écosystème forestier, ou de quelle façon le choix des espèces semées influence l'établissement éventuel d'arbres boréaux.

En 2013, une aire expérimentale *in situ* de résidus miniers d'un hectare a été aménagée sur un site minier aurifère à Malartic (QC, Canada). Cette aire a été subdivisée en trois blocs de cinq placettes. Chaque placette a été ensemencée aléatoirement avec un des cinq traitements suivants : graminées agronomiques, légumineuses agronomiques, un mélange des deux, une mince couche de *topsoil*, ou un témoin (résidus seuls). En 2015, 30 semis de trois essences d'arbres boréaux pionniers; pin gris (*Pinus banksiana*), mélèze (*Larix laricina*), bouleau à papier (*Betula papyrifera*) et d'un cultivar de saule (*Salix miyabeana*) ont été plantés dans une aire représentant 1/3 de chaque placette. Des quadrats ont été délimités dans le reste de l'aire des placettes, une partie de ceux-ci pour évaluer la communauté de plantes vasculaires produite par colonisation spontanée, une autre partie pour déterminer le taux d'établissement de quatre essence (*P. banksiana, L. laricina, B., papyrifera et Salix discolor*) manuellement semées. Tous les résultats présentés sont basés sur des mesures prises l'année suivante (2016).

La première partie de la recherche portait sur les propriétés physico-chimiques du sol ainsi que la hauteur, la biomasse racinaire et la nutrition foliaire des semis plantés. Le traitement de *topsoil* a produit une teneur en matière organique et un point de flétrissement plus élevés, ainsi qu'une masse volumique apparente plus faible que les autres traitements, cependant les propriétés du sol des traitements herbacés n'étaient pas significativement différentes de celles du témoin. Toutes espèces confondues, la mortalité des semis plantés était plus faible pour les graminées (20 %) que pour les légumineuses (37 %) ou le mélange (28 %), à comparer avec 6 % pour le *topsoil* et 84 % pour le témoin. La biomasse racinaire, la croissance en hauteur et la teneur en N foliaire du saule étaient plus élevées dans les légumineuses que dans les autres traitements, incluant le *topsoil.*

La deuxième étape de la recherche visait à comparer la diversité des plantes spontanées entre les traitements. La richesse spécifique était plus élevée pour le *topsoil* que pour les traitements d'herbacées (moyenne de 17 espèces par placette contre 6), sans différence significative entre ces derniers. La diversité de Shannon était supérieure dans le *topsoil* comparé aux légumineuses. Une analyse multivariée a montré que 60 % de la variation entre communautés (distance de Bray-Curtis) était expliquée par les traitements; notamment, les arbres pionniers de la famille des salicacées étaient plus abondants dans le traitement de légumineuses, mais demeuraient rares en général.

La troisième étape de la recherche consistait à évaluer les effets du microclimat (température et humidité du sol, transmission de la lumière) produit par les traitements sur la germination de semences des quatre essences forestières. Au niveau du microclimat, la seule différence significative observée était une teneur volumétrique en eau du sol plus grande pour le *topsoil* (près de 40 % à 5 cm de profondeur, vs. 30 % ou moins pour les autres traitements). Néanmoins, la germination et l'établissement du pin gris et du mélèze étaient significativement plus élevés dans le traitement des graminées que dans les autres traitements. Les semis de bouleau à papier étaient quant à eux absents de tous les traitements.

Malgré le peu d'années écoulées depuis l'ensemencement des herbacées agronomiques, cette étude a démonté des apports complémentaires des graminées (survie des semis) et des légumineuses (azote foliaire, croissance et établissement des salicacées) qui dépassaient dans plusieurs cas les apports du traitement témoin positif (*topsoil*). Ces résultats justifient l'ensemencement d'un mélange de graminées et de légumineuses, avec une proportion au moins égale de légumineuses. De plus, l'association positive entre les légumineuses et les arbres pionniers de la famille des salicacées, démontrée par une plus grande croissance des semis de saule plantés ainsi qu'un nombre plus grand d'arbres volontaires des genres *Salix* et *Populus*, permet de recommander la plantation de ces essences à croissance rapide sur des résidus miniers préalablement ensemencées de légumineuses agronomiques.

Mots clés: biodiversité, plantes herbacées agronomiques, parcs à résidus miniers, revégétalisation minière, *topsoil.*

ABSTRACT

Revegetation of mine tailings represents a major challenge due to their unique physicochemical properties. In Canada, most mine tailings are produced in the boreal forest bioclimatic region, so revegetation is essential to reduce land fragmentation and the ecological footprint of mining activities. Seeding tailings sites with a mixture of agronomic herbaceous plant seeds (legumes and grasses) is a common practice in the first phase of tailings revegetation. However, few studies have attempted to determine whether this practice has a positive effect on plant succession leading to a forest ecosystem, or how the choice of seeded species influences the eventual establishment of boreal trees.

In 2013, a one-hectare experimental in situ tailings area was set up on a gold mine site in Malartic (QC, Canada). The area was subdivided into three blocks of five plots. Each plot was randomly seeded with one of five treatments: agronomic grasses, agronomic legumes, a mixture of the two, a thin layer of topsoil, or a control (tailings alone). In 2015, 30 seedlings of three pioneer boreal tree species; jack pine (*Pinus banksiana*), tamarack (*Larix laricina*), paper birch (*Betula papyrifera*) and a willow cultivar (*Salix miyabeana*) were planted in an area representing 1/3 of each plot. Quadrats were delineated in the remainder of the plot area, part of them to assess the volunteer vascular plant community, the rest to determine the rate of establishment of four manually seeded species (*P. banksiana*, *L. laricina*, *B., papyrifera* and *Salix discolor*). All results presented are based on measurements taken the following year (2016).

The first part of the research focused on the physicochemical properties of the soil, as well as the height, root biomass and foliar nutrition of the planted seedlings. The topsoil treatment produced a higher organic matter content and wilting point, as well as a lower bulk density than the other treatments, however the soil properties of the herbaceous treatments were not significantly different from those of the control. All species considered, mortality of planted seedlings was lower for grasses (20%) than for legumes (37%) or the mixture (28%), compared with 6% for topsoil and 84% for the control. Root biomass, height growth and foliar N content of willow were higher in legumes than in the other treatments, including topsoil.

The second stage of the research compared the diversity of volunteer plants between treatments. Species richness was higher in the topsoil than in the herbaceous treatments (mean of 17 species per plot vs. 6), with no significant differences between the latter. Shannon diversity was higher in topsoil than in legumes. A multivariate analysis showed that 60% of the variation between communities (Bray-Curtis distance) was explained by the treatments; in particular, pioneer trees of the Salicaceae family were more abundant in the legume treatment but remained rare in general.

The third stage of the research consisted in assessing the effects of the microclimate (soil temperature and moisture, light transmission) produced by the treatments on seed germination of the four forest tree species. In terms of microclimates, the only significant difference observed was a higher volumetric soil water content for topsoil (nearly 40% at 5 cm depth, vs. 30% or less for the other treatments). Nevertheless, germination and establishment of jack pine and tamarack were significantly higher in the grass treatment than in the other treatments. Paper birch seedlings were absent from all treatments.

Despite the few years that have elapsed since the agronomic grasses were sown, this study demonstrated complementary contributions from grasses (seedling survival) and legumes (foliar nitrogen, growth, and establishment of Salicaceae) that in several cases exceeded the contributions of the positive control treatment (topsoil). These results justify seeding a mixture of grasses and legumes, with at least an equal proportion of legumes. In addition, the positive association between legumes and pioneer trees of the Salicaceae family, demonstrated by greater growth of planted willow seedlings and a greater number of volunteer trees of the *Salix* and *Populus* genera, means that we can recommend planting these fast-growing species on mine tailings previously seeded with agronomic legumes.

Key words: agronomic herbaceous plants, biodiversity, mine tailings parks, mining revegetation, topsoil.

CHAPITRE 1

INTRODUCTION

1.1 Contexte

L’exploitation des gisements miniers fait partie intégrale du patrimoine canadien depuis plusieurs décennies déjà et il est prévu que la demande en minéraux continue d’augmenter, principalement dû à la transition vers les sources renouvelables d’énergie et les besoins de fabrication des batteries de véhicules électriques (VE) ou autres (RNCan, s.d.; Simard, 2018). En 2021, la contribution directe et indirecte de l’industrie minière des minéraux et des métaux à l’économie canadienne représentait 91 G$, soit 4 % du produit intérieur brut (PIB) (RNCan, 2021). Au Québec, la valeur totale des livraisons minérales est passée de 8 G$ en 2016 à près de 12 G$ en 2019, dont la plus grande partie est composée de minerai de fer (38 %) et d’or (30 %) (Madore, 2021).

Les gisements miniers exploités au Québec se trouvent en grande majorité dans les régions plus nordiques et bien souvent au cœur de la zone bioclimatique de la forêt boréale (NRCan, 2016(b) ; Simard, 2018). En effet, trois régions administratives principalement situées dans la zone boréale, soit l’Abitibi-Témiscamingue (23,3%), la Côte-Nord (25,4 %) et le Nord-du-Québec (26,5 %), contribuent 75,2 % des livraisons minérales du Québec (Madore, 2021).

Ceci ne va pas sans causer certains inconvénients, tels que le morcellement du territoire forestier, l’altération des habitats des espèces fauniques et floristiques, la pollution atmosphérique par les particules solides, les gaz à effet de serre (GES), et la quantité et superficie importante de déchets miniers (comprenant les résidus et les stériles miniers) (AMQ, s.d.; Donato *et al*., 2007; Simard, 2018). À l'échelle mondiale, un groupe de recherche a estimé que l'extraction de 10,2 milliards de tonnes de minerai pour la seule année 2016 a généré la production de 72 milliards de tonnes de stériles et 8,9 milliards de tonnes de résidus (Baker *et al.*, 2020).

Historiquement, plusieurs minières qui exploitaient les ressources du sous-sol québécois pliaient bagages sans décontaminer, ni restaurer leurs sites d'exploitation. Cet héritage a laissé plus de 350 sites miniers à restaurer dans la province du Québec (MRNF, 2023).

Ainsi, afin de réduire l'empreinte environnementale des entreprises minières et d'augmenter leur acceptabilité sociale, le gouvernement du Québec a codifié et encadré plusieurs lois, règlements et normes environnementales. Notamment, ces lois imposent aux sociétés d'exploitation minière la responsabilité complète de la restauration des sites miniers durant leur exploitation et à la fin de vie de la mine. Ces sociétés doivent déposer un plan d'aménagement et de restauration au ministère de l'Énergie et des Ressources naturelles (MERN) qui doit être approuvé par celui-ci avant même de commencer l'exploitation du gisement. Elles doivent aussi déposer une garantie financière au gouvernement afin de couvrir l'ensemble des coûts de restauration (AMQ, s.d.).

Afin d'assister les sociétés minières dans la préparation de leur plan d'aménagement et de restauration des sites miniers (durant et après la vie active d'exploitation du minerai), le MERN a aussi créé un outil de travail intitulé *Le Guide de préparation du plan de réaménagement et de restauration des sites miniers au Québec* (à la suite appelé Guide) (MERN, 2017). Le Guide comprend plusieurs items importants que doivent respecter les minières afin de se conformer à la Loi sur les mines du gouvernement du Québec.

Parmi les items du Guide, la section 4.2 « Mise en végétation » indique que tous les terrains et sites de bâtiments qui ont été affectés par les activités d'exploitation minière doivent faire l'objet d'une mise en végétation. Ceci inclut notamment les superficies occupées par les stériles miniers et les parcs à résidus miniers. Cette mise en végétation vise à « contrôler l'érosion et redonner au site un aspect naturel en harmonie avec le milieu environnant » (MERN, 2017). Tel que mentionné plus haut, dans le cas des mines exploitées au Québec, le milieu environnant se trouve le plus souvent dans la forêt boréale. Toujours selon le Guide, la végétation doit être « viable à long terme et ne nécessiter aucun amendement ou entretien pour en assurer le maintien ». Autrement dit, la végétation mise en place doit être autosuffisante au niveau des cycles nutritifs, se régénérer naturellement, etc.

Les résidus miniers, souvent appelés déchets ou rejets miniers, sont des sous-produits de l'extraction finale des roches concassées/broyées dont on a extrait le minerai de valeur commerciale (Lottermoser, 2007; Ripley *et al.,* 1996). Après le processus d'extraction du minerai de valeur, les rejets minéraux sont mélangés à l'eau pour être transportés et transférés dans un bassin de décantation (parc à résidus miniers). La particularité des résidus miniers, dont la taille varie de celle d'un grain de sable à celle d'une particule de limon, est qu'ils sont dépourvus de matière organique, ont une texture pauvre en aération, un mauvais drainage, parfois un taux de salinité élevé et peu ou pas de microorganismes que l'on retrouve naturellement dans les sols (Bradshaw et Chadwick, 1980; Guittonny-Larchevêque *et al.,* 2016a). Ces sols anthropogéniques représentent donc des conditions très difficiles de croissance pour la majorité des plantes vasculaires (Guittonny-Larchevêque et Pednault, 2016). Par exemple, la Figure 1.1 montre les résultats de la plantation de semis d'arbres pionniers directement plantés dans les résidus miniers (placette témoin) à la fin de la saison de croissance d'été.

Figure 1.1 Résultat de la plantation de semis d'arbres pionniers plantés directement dans les résidus miniers épaissis (placette témoin) durant une même saison de croissance d'été.

Toutefois, les plantes herbacées agronomiques (légumineuses, graminées), particulièrement certains cultivars, possèdent des traits fonctionnels tels que la tolérance à la salinité ou la tolérance à la sécheresse (Elias et Chadwick, 1979), ce qui leur permet de croître dans les résidus miniers (Burger et Zipper, 2018; Guittonny-Larchevêque *et al.,* 2016b). C'est pourquoi les minières utilisent les plantes herbacées agronomiques pour contrôler rapidement l'érosion éolienne sur les surfaces asséchées des parcs à résidus miniers. De plus, elles sont faciles à manipuler, à semer et sont peu dispendieuses (Tischew *et al.,* 2011).

1.2 Intérêt de la recherche

Tel que mentionné ci-dessus, il est coutume pour l'industrie minière au Québec de revégétaliser les parcs à résidus miniers avec des plantes herbacées agronomiques mixtes, c'est-à-dire avec des légumineuses et des graminées de mélange commercial à bas prix. Toutefois, peu d'informations existent présentement dans la littérature scientifique sur les effets abiotiques et biotiques à court et à long terme de ce procédé d'ensemencement dans un contexte de résidus miniers. Voici certaines des questions relatives à cette pratique qui pourraient être élucidées par des recherches scientifiques: Est-ce que le mélange commercial (légumineuses et graminées) produit les propriétés physiques et chimiques du sol les plus favorables à une succession forestière? Est-ce que le mélange commercial permet une bonne captation des graines aériennes pour augmenter la biodiversité floristique du site minier et sa résilience? Est-ce que le mélange commercial permet une bonne germination et survie des graines d'essences pionnières de la forêt boréale? Cette étude permettra d'acquérir de nouvelles connaissances pratiques en ce qui concerne les conditions optimales à l'établissement de la végétation et à la création d'écosystèmes boréaux diversifiés et fonctionnels sur les sites post-miniers et ce à la suite de l'ensemencement de plantes herbacées agronomiques.

1.3 Revue de littérature

Cette revue de littérature débute par la définition de quelques concepts liés aux résidus miniers et à leur revégétalisation, suivie par une présentation de la théorie écologique pertinente à l'étude de la succession primaire sur un site minier. Après une revue des études existantes contrastant la revégétalisation des résidus miniers avec ou sans intervention humaine, les sections suivantes présentent les connaissances spécifiques sur les traitements (*topsoil* et herbacées agronomiques) qui ont été choisis pour cette étude; les propriétés des arbres de la forêt boréale pertinentes dans un contexte de revégétalisation minière; et finalement les caractéristiques des quatre essences forestières choisies pour cette étude.

1.3.1 Définitions

Les résidus miniers sont des sous-produits (déchets) provenant de la dernière étape de broyage ou concassage du minerai terrestre, une fois que le minerai de valeur commerciale a été extrait. Règle générale, la taille des particules des résidus de broyage varie du sable au limon-argile (SME, s.d.). Les résidus miniers n'ont pas tous la même composition physico-chimique. Leur composition dépend de plusieurs facteurs tels que la composition du minerai parent, la taille des particules résultant du processus de broyage ou concassage, ainsi que le type de produit chimique utilisé lors de l'extraction du métal de valeur (Lottermoser, 2007).

Au Canada, en général, les résidus miniers provenant de l'usine sont transportés vers le parc à résidus dans des tuyaux convoyeurs sous forme de boue minérale contenant moins de ~30 % en poids solide (Blowes et al., 2005). Une nouvelle technologie de boues de résidus miniers épaissies (>60 % en poids solide) est maintenant utilisée par certaines minières. Les résidus miniers épaissis possèdent plusieurs avantages; ils requièrent moins d'eau, donnent des grains minéraux plus uniformes et permettent un séchage plus rapide des résidus miniers déposés dans le parc (Robinsky, 1999). La Figure 1.1 plus haut présente une photographie de résidus miniers épaissis à pH neutre.

Le retour d'un écosystème fonctionnel sur un ancien site minier peut prendre plusieurs formes. Bradshaw (1997) distingue les concepts de restauration, de réclamation et de réhabilitation de sites :

- la restauration vise à ramener si possible le site à son état original ou du moins à le restaurer pour qu'il atteigne les pleines fonctions biologiques du sol;
- la réclamation consiste à convertir un site soit à son état original ou du moins un état productif tels que terre cultivable, forêts, prairies, etc.;
- la réhabilitation suit un plan prédéterminé afin que le site atteigne au moins partiellement les fonctions d'un écosystème stable.

Le terme dégradation d'un site, implicite à l'exploitation minière à ciel ouvert, indique la destruction totale de l'écosystème original, incluant l'architecture initiale du paysage, le régime hydrique, la faune, la flore, le sol et son environnement biologique (Bradshaw, 1992). La dégradation du sol dû à l'exploitation minière réfère à la perte des fonctions biologiques du sol ('*ecosystem function'* i.e. décomposition de la matière organique, teneur et cycles des éléments nutritifs, etc.) et à la perte des fonctions structurelles du sol ('*ecosystem structure'* porosité, texture du sol, etc.) (Bradshaw, 1992). La restauration des sols miniers vise à redonner au site les fonctions biologiques et structurelles du sol, c'est-à-dire que la restauration a pour objectif d'atteindre l'état original du sol. Tandis que les pratiques de réclamation et réhabilitation sont des pratiques partielles de l’amélioration des structures biologiques et/ou structurelles du sol mais elles ne visent pas nécessairement à atteindre l’état original du sol avant l’exploitation minière (Bradshaw, 1997). La restauration d’un site comparativement au processus de succession naturelle est plus rapide, du moins, pour redonner les fonctions biologiques du sol (Bradshaw, 1997). Le processus de succession naturelle du site peut retrouver les services écosystémiques pré exploitation minière mais celui-ci peut s’échelonner sur une longue période particulièrement si le site à restaurer est très contaminé.

Pour ce qui est de la revégétalisation, elle « vise la reconstitution du couvert végétal d'un terrain dénudé par l'action de l'humain ou par l'effet de catastrophes naturelles » (Office québécoise de la langue française, 2022).

Quand on parle des caractéristiques d’espèces végétales qui les rendent plus ou moins bien adaptées à un milieu, on réfère souvent aux traits fonctionnels, qui sont définis comme les attributs phénotypiques d'un organisme, sa réponse aux changements environnementaux et son effet sur les processus écosystémiques (Hooper & Vitousek, 1998).

1.3.2 Succession primaire et modèles écologiques

Il existe dans la littérature scientifique plusieurs définitions du terme « succession primaire ». Dans le cadre de cette recherche, elle sera définie comme le changement progressif de la formation du sol et des espèces (fauniques et floristiques), à partir d’un tout nouveau substrat, échelonné dans le temps (Walker et del Moral, 2003; Walker et del Moral, 2009). Cependant, il y a une différence substantielle entre un nouveau substrat formé par des perturbations anthropiques (par exemple, le sol de résidus miniers) et un qui serait créé à la suite de perturbations naturelles (par exemple, le sol exposé à la suite d'un feu de forêt) (Shrestha et Lal, 2011; Zhang et Biswas, 2017). Ainsi dépendamment du type de nouveau substrat (anthropique ou naturel), la succession primaire et l'assemblage des communautés végétales seront différentes.

Des modèles écologiques ont été proposés afin de mieux comprendre et prédire la succession primaire et l'assemblage des communautés végétales associées aux nouveaux substrats anthropiques. Pour des grandes surfaces perturbées, tels que les parcs à résidus miniers, et à condition qu'il n'y ait plus de perturbations abiotiques ou changements majeurs qui surviennent à la suite du début du processus de revégétalisation, Connell et Slatyer (1977) proposent un modèle écologique de facilitation. Celui-ci suggère que seules les espèces qui ont les traits fonctionnels adaptés à des milieux de succession précoce sont capable de coloniser les nouvelles surfaces exposées. Ces premières espèces colonisatrices (ou espèces pionnières) modifieront à leur tour et cela de façon positive, les conditions microclimatiques et édaphiques de leur environnement, facilitant la venue et l'établissement d'espèces de succession ultérieure. Les espèces de succession ultérieure modifieront à leur tour leur environnement, le rendant moins adaptables pour les espèces pionnières, et avec le temps laisseront place aux espèces de fin de succession.

Un autre modèle est l'hypothèse du gradient de stress (*stress-gradient hypothesis*) proposée par Bertness et Callway (1994). Selon ce modèle, les sites présentant un grand niveau de stress abiotique, tels que les sites miniers, peuvent induire des interactions positives entre les espèces, par exemple arbres et herbacées, afin de compenser pour ce stress.

Un autre modèle écologique est celui des règles d'assemblage et de réponse, aussi appelé filtre écologique, de Keddy (1992). Celui-ci postule que l'environnement fonctionne comme un filtre, qui exclut les plantes n'ayant pas les traits fonctionnels ou combinaisons de traits nécessaires pour survivre dans cet environnement. Ceci permet d'avoir un modèle prédictif pour déterminer quel type de communauté végétale s'établira dans un environnement caractérisé par un filtre spécifique, par exemple des sols de résidus miniers.

1.3.3 Revégétalisation des parcs à résidus miniers avec ou sans intervention humaine

À la fin de vie de l'exploitation d'un parc à résidus miniers, on peut s'interroger sur la colonisation naturelle et le recouvrement végétatif de ces parcs laissés à eux-mêmes, c'est-à-dire sans intervention humaine (pas d'ensemencement, ni reboisement). Comment les parcs à résidus miniers se comportent-ils si on laisse la nature suivre son cours? Quel est le succès de la revégétalisation naturelle?

Chaque parc à résidus miniers a ses propres particularités tant au niveau du processus de succession végétale que de la communauté végétale qui en résulte. La rapidité à laquelle la végétation va coloniser un parc dépend de plusieurs paramètres tels que la composition du gisement exploité (roche mère), la technologie utilisée, les conditions édaphiques du sol minier prévalent, la proximité et le type de végétation adjacente au parc depuis sa fermeture (Lottermoser, 2007; Szarek-Lukaszewska, 2009). Le temps d'obtention d'un couvert végétal est particulièrement long pour les parcs à résidus miniers laissés à eux-mêmes (Bradshaw, 1997). Parmi les facteurs qui rendent ces milieux inhospitaliers pour la croissance végétale, particulièrement celle des essences arborescentes, notons la quantité faible ou inexistante de matière organique et d’éléments nutritifs, ainsi que le manque d’aération du sol (Young *et al*., 2013; Tordoff *et al*., 2000).

Young *et al*. (2013) ont examiné la colonisation spontanée de la végétation et ses effets édaphiques sur le parc à résidus miniers d’une mine d’or, abandonnée depuis 1942 et située en forêt boréale. Malgré les 70 ans qui se sont écoulés depuis la fin de vie de la mine, le couvert végétal ne représentait qu’environ 11 % de la superficie totale du parc à résidus. Les transects d’échantillons végétaux analysés ont démontré que le gradient de succession primaire allait d’une communauté de plantes herbacées vivaces dominée par la prêle des marais (*Equisetum palustre*) et les fougères, vers une communauté de peuplier baumiers (*Populus balsamifera*) et de saules (*Salix* spp.), suivie par une communauté de mélèzes (*Larix laricina*) pour se terminer avec une communauté de fin de succession arborescente, soit un peuplement forestier d’épinettes noires (*Picea mariana*). À chaque stade de cette succession, une augmentation de la matière organique a été observée. Un effet accélérateur de la décompaction du sol et de l’augmentation du N inorganique a aussi été observé pour la première communauté de prêles et fougères. Cet effet diffère de celui des premières successions végétales que l’on retrouve habituellement dans les parcs de résidus miniers, qui sont plus souvent composées d’une grande proportion de plantes herbacées annuelles et qui dominent plus longtemps le site minier (Bradshaw, 1997; Juge et al., 2021; Martínez-Ruiz et al., 2007).

Une autre étude par Skousen *et al.* (2006) a examiné la colonisation naturelle et spontanée des arbres sur une ancienne mine de charbon à ciel ouvert qui n’a pas été reboisée par l’humain. Leurs résultats démontrent que 20 ans après la fermeture de la mine, les endroits n’ayant pas reçu d’ensemencement en herbacées ont une forêt presque aussi dense et diversifiée que la forêt naturelle. Dans ce cas, les auteurs concluent que les espèces d'herbacées utilisées initialement pour le contrôle de l'érosion étaient trop compétitives envers la venue spontanée des arbres.

Comme exemple d'intervention humaine dans le processus de revégétalisation, Shrestha et Lal (2007, 2011) ont mené plusieurs études sur les propriétés physico-chimiques de sols restaurés à la fin de vie de mines de charbon. Une de leurs études comparait les propriétés chimiques et physiques des sols réclamés (*reclaimed mine sites,* RMS) récemment restaurés (moins d'un an) à celle des sols adjoints non perturbés (Shrestha et Lal, 2011). La restauration des sites consistait au remblayage du site avec les rejets miniers, suivi du dépôt d'une couche de 20 à 30 cm de *topsoil*, puis de l'ensemencement avec un mélange de graines de plantes agronomiques (graminées et légumineuses) et du paillage de résidus agronomiques pour conserver l'humidité du sol. Les RMS présentaient une masse volumique apparente plus grande de 54 % que le sol non perturbé, avec une perte importante de la teneur en C organique (83 % de moins) et en N (75 % de moins). Les auteurs attribuent ces changements à la manipulation du *topsoil* lors de son extraction, son stockage et son épandage, ce qui montre que des précautions sont nécessaires dans la manipulation de ses sols pour préserver leurs propriétés.

Dans une autre étude, Shrestha et Lal (2007) ont examiné l'effet sur les propriétés physico-chimiques du sol de trois types d'aménagement sur des sols récemment réclamés, soit un aménagement forestier (en forêt exploitée), un aménagement en terre à foin (récolté chaque année) et en un pâturage (brouté par les bovins). Les propriétés de ces sols 28 ans après restauration des mines de charbon ont été comparées à celles de sols d'une plantation forestière elle aussi vieille de 28 ans, mais n'ayant pas subi de coupe ou perturbation. Les concentrations de C organique et de N étaient plus grandes sous le pâturage et la terre à foin que sous les forêts, mais les données des deux forêts étaient semblables, montrant qu'après 28 ans, les sols restaurés se comparent avantageusement à des sols forestiers non perturbés.

La revue de littérature de Bradshaw (1997) indique que la restauration des sites miniers est possible sans nécessairement une grande intervention humaine. Toutefois, il est important d'identifier et d'atténuer les conditions physiques extrêmes des sols miniers tels que les métaux lourds, l'acidité extrême et le manque en nutriments essentiels à la croissance des plantes. Ainsi, à la suite de la remédiation de ses conditions extrêmes, le développement biologique du sol minier est possible et peut suivre son court à travers la succession primaire. Bradshaw (1997) spécifie que la vitesse de la formation du sol, par exemple la formation pédologique des horizons du sol et la dégradation de la roche parent, peuvent prendre plusieurs milliers d'années; par contre, le développement biologique du sol, c'est-à-dire les processus de cycles nutritifs, la formation de litière et d'humus, la formation de la matière organique (C organique), l'apport en éléments nutritifs essentiels aux plantes (N, P, K) et la microfaune du sol peuvent être atteignables à l'échelle de décennies.

Bradshaw (1997) indique que le développement du sol sur les sites miniers requiert souvent un apport externe initial (il recommande l'ensemencement de plantes herbacées indigènes ou agronomiques, et/ou de la plantation d'arbres et arbustes) afin d'atténuer les conditions extrêmes. Une de ces conditions extrêmes est le manque de N, puisque cet élément n'est pas produit par la dégradation minérale du sol. La principale contribution en N au sol provient de sa forme biologique grâce à la symbiose des racines des légumineuses et microorganismes (*Rhizobium* et actinomycètes). Ce déficit en N doit donc être pallié soit artificiellement ou par la plantation d'espèces végétales (herbacées ou ligneuses) fixatrices de N.

Finalement, pour évaluer le potentiel de revégétalisation spontanée, Bradshaw (1997) recommande de porter une attention particulière à la distance de la source en végétation semencière, par exemple une forêt ou une prairie, particulièrement les espèces à graines lourdes avec une distance de dispersion plus limitée.

Une étude de Guittonny-Larchevêque et Pednault (2016) a démontré que des arbres pionniers typiques de la forêt boréale, plantés directement dans des résidus miniers épaissis à faible teneur en soufre, ne survivent même une année à la suite de la plantation. Dans cet écosystème, d'autres étapes préalables sont donc nécessaires pour rendre le sol propice à l'établissement d'essences forestières. Deux options pour ce faire, l'ajout de *topsoil* ou d'herbacées agronomiques, sont discutées dans les sections qui suivent.

1.3.4 Utilisation du *topsoil* pour la revégétalisation de sites miniers

Si le minerai de valeur se trouve sous le terrain forestier de la forêt boréale, il faut donc pour l'atteindre abattre et enlever les arbres de la forêt puis excaver le sol forestier. La première couche de sol superficielle excavée (environ les 30 premiers cm de profondeur) est empilée et mise de côté pour une utilisation future post-minière en vue de la revégétalisation. C'est la terre la plus riche du sol forestier (horizons O et A) contenant de l'humus, de la matière organique, des semences enfouies et les microorganismes du sol forestier (Guittonny-Larchevêque et Pednault, 2016; Lynch et Brown, 2001). Cette terre, communément appelée *topsoil* dans les publications scientifiques, est souvent considérée comme un témoin positif (i.e., un traitement déjà reconnu comme ayant un effet positif sur la végétalisation) lors de comparaisons de traitements (Amoako *et al.*, 2017 ; Bendfeldt *et al.*, 2001; Brown *et al.*, 2014).

Dans cette étude-ci, l'application d'une mince couche de *topsoil* (2 cm d'épaisseur) sera utilisée comme témoin positif, reflétant le fait que la disponibilité de cette ressource est parfois limitée lorsqu'elle doit être utilisée pour restaurer un site de grande superficie.

1.3.5 Les plantes herbacées agronomiques commerciales

Dans le cadre de cette recherche, les plantes herbacées agronomiques commerciales réfèrent à des plantes vasculaires, qui font partie de la famille des fabacées (communément appelées légumineuses) et des poacées (communément appelées graminées) et sont utilisées en agronomie commerciale au Canada. Il s'agit de cultivars qui ont été sélectionnés, par exemple, pour leur capacité à mieux utiliser les nutriments du sol, leurs tolérances au sel et à la sécheresse, ainsi que de leur adaptation à divers stress climatiques (Diederichsen et Davidson, 2022). Les plantes herbacées agronomiques commerciales utilisées dans cette étude excluent les herbacées des familles des lycopodiacées, des juncacées, des équisétacées, des onocléacées et des cypéracées.

Il est coutume pour les sociétés minières de végétaliser les résidus miniers avec des plantes herbacées agronomiques mixtes, c'est-à-dire un mélange de graminées et légumineuses agronomiques. Le pourcentage de graminées et de légumineuses varie d'un fournisseur à l'autre. L'utilisation de ces plantes vise à pallier les carences des sols miniers : absence de matière organique, structure de sol mal aérée et manque de nutriments essentiels et de microorganismes pour les plantes (Burger et Zipper, 2002; Guittonny-Larchevêque et Pednault, 2016). Notamment, les herbacées affectent la structure du sol par leurs activités racinaires : capacité de pénétration des racines, capacité d'extraction de l'eau, micro et macro-agrégats et formation de macroporosités (Angers et Caron, 1998; Ibrahim et Goth, 2005).

1.3.5.1 Les graminées agronomiques

Les graminées agronomiques sont des plantes qui possèdent les traits fonctionnels nécessaires pour s'établir sur les résidus miniers. Plusieurs avantages sont associés à l'utilisation des semences de graminées agronomiques pour la végétalisation des résidus miniers; elles sont facilement disponibles sur le marché et relativement peu dispendieuses. Elles sont faciles de manipulation, d'entretien et rapides d'application. Les caractéristiques fonctionnelles des graminées agronomiques sont nombreuses; elles croissent rapidement, certains cultivars sont très résistants à la sécheresse, elles résistent bien aux maladies et demandent peu en nutriments du sol (Agriculture and Agri-Food Canada, 2020). La plupart des espèces de graminées produisent une bonne quantité de biomasse aérienne et souterraine qui enrichit les résidus miniers en matière organique (Cooke et Johnson, 2002; Maiti et Maiti, 2014). Doormar et Foster (1991) ont observé que des micro-agrégats (2-20 µm) étaient formés par le *Lolium perenne* vivace à travers les racines associées à des particules minérales, du gel racinaire, des fragments de racines et des polysaccharides extracellulaires microbiens (Dexter, 1991). En effet, les racines fibreuses (souvent de dimension égale) des graminées agrippent les particules libres du sol, permettant un meilleur contrôle de l'érosion de celui-ci (Maiti et Maiti, 2014). Guittonny-Larchevêque *et al.* (2016) ont déterminé que les graminées amélioraient la macroporosité des résidus miniers deux mois après l'ensemencement dans un contexte de chambre contrôlée et *in situ*, mais n'ont pas noté d'effets pour la masse volumique apparente. La graminée *Bromus inermis* Leyss, reconnue pour ses racines fibreuses, rhizomateuses et profondes, a été la plus efficace pour l'amélioration de la macroporosité des résidus miniers comparativement aux autres graminées : *Lolium perenne* L*., Agrostis gigantea* Roth, *Phalaris arundinacea* L. et *Festuca rubra* L. L'étude a démontré dans l'ensemble que *B. inermis* avait le meilleur taux de germination et de survie par rapport aux autres graminées de l'étude et constitue une bonne candidate pour l'amélioration des sols de résidus miniers.

Malgré ces effets positifs sur la structure du sol, plusieurs recherches considèrent les graminées comme une compétition limitant l'établissement des espèces ligneuses, dû en partie à leur réseau racinaire rhizomique développé et leurs stolons, leur permettant de compétitionner de façon efficace pour les nutriments et l'humidité du sol (Bouchard *et al.,* 2018; Burger *et al.,*2008).

1.3.5.2 Les légumineuses agronomiques

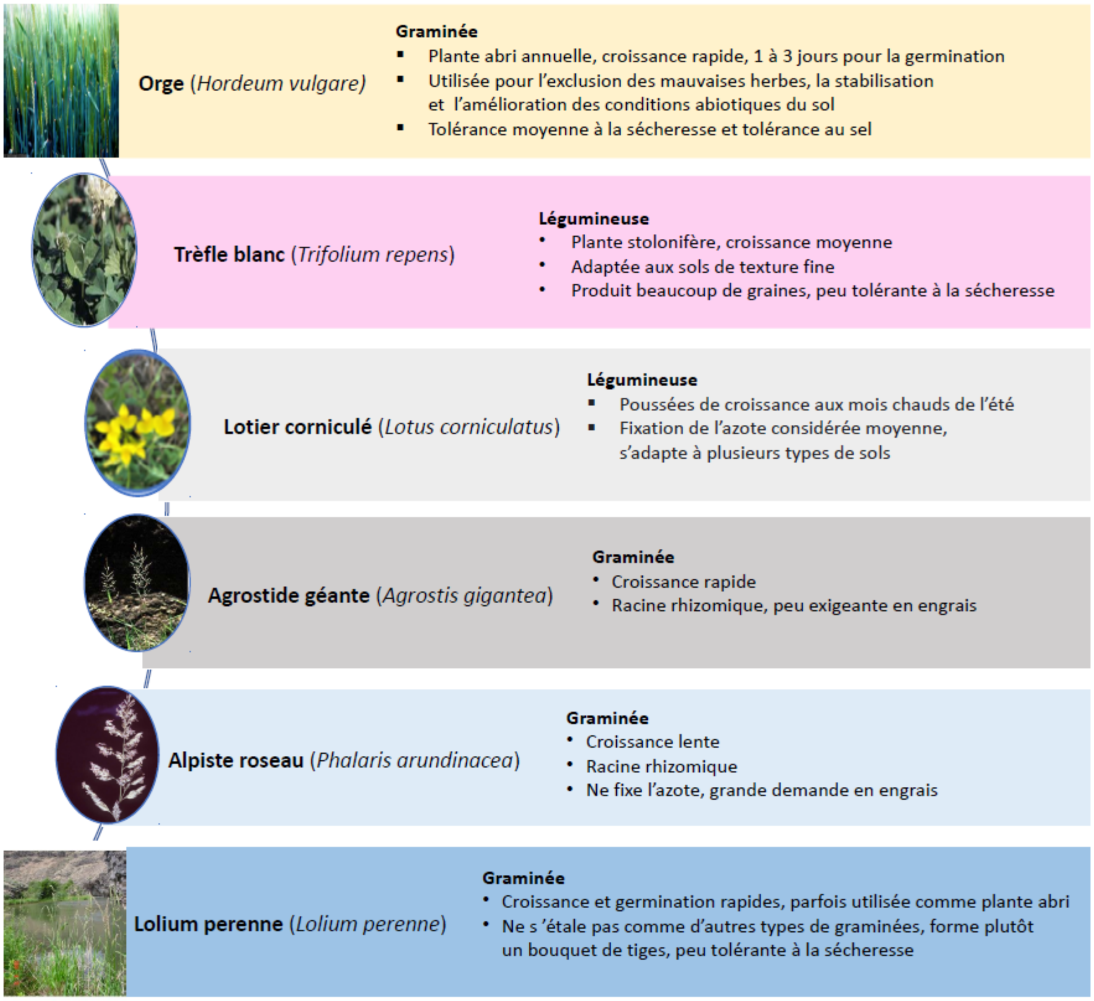
Les racines des légumineuses sont généralement profondes. Ces racines peuvent former des relations symbiotiques avec les bactéries rhizobia (*Rhizobiaceae, α-Proteabactéria*) (Sorensen and Sessitsch, 2007) fixatrices de N qui fournissent de le N à la plante. En retour, la décomposition des légumineuses enrichit le sol en N (Domingo et David, 2014; Ledgard et Steele, 1992; Maiti et Maiti, 2014).

1.3.5.3 Les herbacées agronomiques utilisées dans cette étude

La Figure 1.2 présente les principales caractéristiques des plantes herbacées agronomiques utilisées dans cette étude. Il s'agit d'un groupe d'espèces utilisées couramment en revégétalisation minière, sur la base des études de Burger *et al.* (2017b), qui recommandent des graminées et des légumineuses à croissance lente et moins compétitrices pour la survie et la venue des semis d'arbres. Ils suggèrent en particulier d'éviter les plantes herbacées agronomiques de saison froide (*cool-season grass*) à croissance rapide telles que les légumineuses *Sericea lespedeza* et *Securigera varia* ou les graminées *Schedonoris arundinaceus* et *Poa pratensis*.

Bradshaw (1997) recommande l'utilisation d'une plante abri (graminée de type céréale, annuelle et à croissance rapide) à semer avec les autres graminées agronomiques vivaces et de croissance plus lente. Cette plante abri sert à réduire la compétition des autres mauvaises herbes, stabiliser le sol et améliorer les conditions abiotiques de celui-ci, tout en n'étant pas compétitrice envers les autres graminées vivaces (Espeland et Perkins, 2013). Par exemple, l'orge, une graminée annuelle à croissance rapide, est souvent utilisée comme plante abri en revégétalisation minière; elle sera donc ajoutée à chacun des traitements d'herbacées agronomiques dans cette étude.

Les trois traitements appliqués dans cette étude (tous les % sont en poids des graines semées) incluent un traitement de graminées (10 % orge, 40 % lolium perenne, 40 % agrostide géante, 10 % alpiste roseau), un traitement de légumineuses (10 % orge, 45 % lotier corniculé, 45 % trèfle blanc,) et un mélange des deux (10 % orge, 20 % trèfle blanc, 20 % lotier corniculé, 25 % lolium perenne, 25 % alpiste roseau).



Orge (*Hordeum vulgare)*

Graminée
- Plante abri annuelle, croissance rapide, 1 à 3 jours pour la germination
- Utilisée pour l'exclusion des mauvaises herbes, la stabilisation et l'amélioration des conditions abiotiques du sol
- Tolérance moyenne à la sécheresse et tolérance au sel

Trèfle blanc (*Trifolium repens*)

Légumineuse
- Plante stolonifère, croissance moyenne
- Adaptée aux sols de texture fine
- Produit beaucoup de graines, peu tolérante à la sécheresse

Lotier corniculé (*Lotus corniculatus*)

Légumineuse
- Poussées de croissance aux mois chauds de l'été
- Fixation de l'azote considérée moyenne, s'adapte à plusieurs types de sols

Agrostide géante (*Agrostis gigantea*)

Graminée
- Croissance rapide
- Racine rhizomique, peu exigeante en engrais

Alpiste roseau (*Phalaris arundinacea*)

Graminée
- Croissance lente
- Racine rhizomique
- Ne fixe l'azote, grande demande en engrais

Lolium perenne (*Lolium perenne*)

Graminée
- Croissance et germination rapides, parfois utilisée comme plante abri
- Ne s 'étale pas comme d'autres types de graminées, forme plutôt un bouquet de tiges, peu tolérante à la sécheresse

Figure 1.2 Plantes herbacées agronomiques utilisées dans cette étude. Source : USDA-NCRS (2023) Images : L. Alain (trèfle, alpiste, lolium), D. Barrette (lotier), Smithsonian Institution (orge), R. Soreng (agrostide).

1.3.6 Importance des hautes plantes vasculaires (arbustes et arbres) pour la revégétalisation

La succession primaire est un long processus de changements graduels à la végétation et au sol à la suite d’une perturbation naturelle ou anthropique qui a mis le sol à nu (Walker et Moral, 2003). Il serait donc plausible théoriquement de laisser les sols minéraux se reconstituer avec le temps. Toutefois, il y a plusieurs particularités associées aux sols miniers qui ne permettent pas de laisser tout le temps que prendrait une succession primaire; les sols miniers, s’ils ne sont pas revégétalisés, peuvent contaminer les réseaux hydriques avec les lixiviats de métaux lourds ou acides. Les métaux lourds en particulier ne sont pas biodégradables, demeurent longtemps dans l’environnement et sont bioaccumulés (Iatan, 2021). Une étude récente de Yin *et al.*, (2023a) a démontré que l’empreinte écologique des mines en forêt boréale s’étend jusqu’à 0,2 km autour de celles-ci. Il est reconnu que les arbres et arbustes apportent plusieurs bénéfices pour les cycles nutritifs des sols, la protection de la qualité des eaux, le contrôle des eaux pluviales, le stockage du carbone, la diversité de la faune et des plantes et leur protection (Clements, 1971; Dallaire et Skousen, 2019; Zipper *et al.*, 2011). Aux États-Unis, dans la région de la forêt appalachienne, plusieurs chercheurs ont établi une méthodologie pour restaurer la forêt avec des arbres de haute qualité à la suite de la fermeture des sites miniers de charbon (Burger *et al.*, 2017b; Burger et Zipper, 2018). Une autre étude de Parrotta *et al.*, (1997) démontre que la plantation d’arbres aurait même un effet catalyseur (effet d’accélération) sur la restauration et la productivité des sites affectés par l’activité minière de la forêt tropicale.

1.3.7 Revue sur les semences d’essences forestières de la forêt boréale

En forêt boréale, les variations de production, de dispersion, de recrutement et de germination des graines provenant des différentes essences forestières indiquent des capacités inégales à coloniser les sites à la suite de perturbations naturelles ou de nouveaux substrats tels que les résidus miniers. Selon Greene et al. (1999), la production de graines dans un milieu donné est proportionnelle à la surface terrière de l’arbre et une taille minimale de tige est nécessaire à la production des graines. De plus, la masse des graines est inversement proportionnelle à la production annuelle de graines (les espèces à grosses graines produisent moins de graines que les espèces à petites graines) et la capacité de dispersion est inversement proportionnelle à la masse des graines (Greene *et al.*, 1999). La vitalité des graines est aussi reliée à leur taille; les grosses graines d’une même espèce ont plus de vitalité que les petites (Black, 1957; Susko et Cavers, 2008) et règle générale pour des espèces différentes, celles qui produisent une grande quantité de petites graines, leurs graines ont un plus faible taux de survie à ce stade que les espèces qui produisent de plus grosses graines, mais compensent en produisant un plus grand nombre de graines annuellement (Densmore et Zasada, 1983; Gage et Cooper, 2005). Par exemple, les graines de saule sont très petites (2-3 mm), mais sont produites en grande quantité (~7650 graines / m^2) (Gage et Cooper, 2005; Kuzovkina et Quigley, 2005), tandis que celle du pin gris sont plus grosses (4-5 mm), mais moins de graines sont annuellement produites (100 à 400 graines / m^2) (Green et Johnson, 1999; Rudolf, 1965; USDA-NCRS, 2023). La capacité de dormance des graines est aussi une autre stratégie (*bet-hedging*) qu’une espèce utilise pour sa survie (Fan *et al.*, 2018; Kildisheva *et al.*, 2020).

L'abondance des graines diffère également selon les espèces d'arbres; certaines espèces produiront plus ou moins de graines en fonction de nombreux facteurs tels que le climat, les perturbations et les stress hydriques. Seuls deux arbres de la forêt boréale, le pin gris (*Pinus banksiana* Lamb.) et l'épinette noire (*Picea mariana* Mill.), possèdent des cônes sérotineux à longue durée de vie, qui donnent des graines en abondance à la suite de la chaleur (feu ou une haute température estivale) (Greene *et al.,* 1999). La plupart des arbres de la forêt boréale, à l'exception du bouleau à papier (*Betula papyrifera* Marsh.), ont une dormance limitée ne dépassant généralement pas un an (Greene *et al*., 1999). Le piégeage des graines est un autre paramètre déterminant pour la régénération naturelle. Il est principalement influencé par le relief de la surface du sol et l'humidité de celui-ci (Gage et Cooper, 2005; Greene *et al*., 1999). Les graines dispersées par le vent auront plus de chances d'être piégées sur des surfaces rugueuses que sur des surfaces lisses et par la hauteur et densité de la végétation en place. Il est difficile de faire la distinction entre les facteurs abiotiques qui prévalent comme déterminants de la germination des graines. Pour les plantes vasculaires, les conditions du lit de semence qui sont le plus souvent déterminées par des facteurs abiotiques (exposition au sol minéral, humidité, température et lumière) seront les facteurs clé permettant la germination des graines (Chen et Popadiouk, 2002). À la suite de la germination des graines, les semis d’espèces pionnières à croissance rapide, tels que le bouleau à papier, le pin gris, le mélèze (*Larix laricina* Du Roi), les peupliers (*Populus* sp.) et les saules (*Salix* sp.), seront avantagés par rapport aux semis à croissance plus lente tels que ceux des espèces tolérantes à l'ombre (Chen et Popadiouk, 2002). Sur les résidus miniers de pH neutre ou acide, le développement des semis est difficile, notamment en raison des carences en éléments nutritifs et de la mauvaise structure du substrat qui limitent la croissance des racines (Larchevêque et al., 2013).

1.3.8 Essences forestières sélectionnées pour la recherche

Les quatre essences forestières choisies pour la recherche devaient répondre à certains critères de sélection : être de la zone bioclimatique de la forêt boréale, être intolérantes ou semi-intolérantes à l’ombre (espèces pionnières), inclure plusieurs essences résineuses et feuillues, et idéalement avoir fait l’objet d’autres études en revégétalisation minière.

- Mélèze (*Larix laricina* Du Roi)

Le mélèze est une essence pionnière de taille moyenne (10 m de largeur de la couronne x 20 m de haut) avec une croissance moyenne. Il est l’un des rares conifères qui s’adaptent à plusieurs types de sol, mais on le retrouve souvent dans les milieux humides (AFSQ, 2022; Johnston 1990).

- Pin gris (*Pinus banksiana* Lambert)

Le pin gris est une essence pionnière réputée pour s’établir sur sols minéraux mis à nu à la suite de feux forestiers. Ses cônes sérotineux s’ouvrent sous l’effet de la chaleur du feu, mais peuvent aussi s’ouvrir sous l’effet de la chaleur intense et les graines ailées peuvent être distribuées par la gravité, le vent et les petits mammifères, tels que les écureuils, lièvres et certains oiseaux, qui consomment les graines et les distribuent (USDA-NCRS, 2023). Un peuplement de pin gris peut contenir jusqu’à 5 millions de graines par hectare (USDA-NCRS, 2023). Les semences germent rapidement en environ 10 jours, sous une bonne chaleur, et les meilleures conditions de germination sont des sols minéraux avec moins de 1 cm de matière organique (USDA-NCRS, 2023). Selon les études de Chrosciewicz (1988), l’établissement des nouvelles semences germées se fait mieux au printemps et tôt en été qu’à l’automne. Petit arbre à croissance lente, le pin gris a la capacité de s'adapter aux sols minéraux et pauvres en nutriments (AFSQ, 2022; USDA-NCRS, 2023).

- Bouleau à papier (*Betula papyrifera* Marshall)

Le bouleau à papier ou le genre *Betula* est une essence forestière très prisée pour la revégétalisation minière (Bierza *et al.,*2020; Santala et Ryser, 2009; Theriault et Nkongolo, 2016).

Elle est une essence à croissance rapide, avec des racines superficielles, mais qui exigent un sol de type limon sablonneux et une humidité moyenne (AFSQ, 2022). Les expériences de Joseph (1929) sur la germination des semences de bouleau à papier ont démontré qu'une courte période de dormance (~2 mois) dans un substrat humide de tourbe granulaire et placé à une température de 10 °C, produirait le plus grand succès de germination.

- Saule discolore (*Salix discolor* Muhl) et le cultivar *Salix miyabeana* Seemen Sx64 clone

Le saule est une essence dioïque de plus en plus utilisée pour la recherche en végétalisation des sites miniers. Il peut être propagé par bouturage, il pousse rapidement et son réseau racinaire en fait l'une des rares essences forestières capables de former une association symbiotique autant avec les endomycorhizes que les ectomycorhizes (Dhillion, 1994).

Cette essence a aussi été spécifiquement étudiée pour sa capacité de décontamination dans les sites miniers pollués par les métaux lourds (Iori *et al.,* 2015; Tőzsér *et al.*, 2017; Wahsha *et al.*, 2012). Il s'agit aussi d'une essence pionnière qui fait souvent partie du processus de succession primaire.

Nous avons spécifiquement choisi pour cette recherche les semences du saule discolore, dû à sa proximité des sites miniers de l'Abitibi et son omniprésence comme espèce indigène en région boréale.

Les boutures de saule miyabeana utilisé dans l'étude, ont très bien réagi au sol des résidus miniers. Le saule miyabeana SX64 clone est reconnu pour son habilité à pousser rapidement dans plusieurs types de sol et de produire beaucoup de biomasse (Gomes *et al.*, 2015; Hénault-Ethier *et al.*, 2017a).

1.4 Objectifs de la recherche

L'objectif général de cette recherche est d'évaluer quelles sont les familles de plantes herbacées agronomiques (légumineuses ou graminées) qui, lorsque ensemencées dans un parc à résidus miniers aurifères, favoriseraient l'établissement optimal (c'est-à-dire un meilleur taux de germination, de survie et de croissance) des essences boréales pionnières.

Les objectifs spécifiques pour les trois chapitres de la recherche sont les suivants:

Chapitre 2

- Évaluer les effets des différents traitements des plantes herbacées agronomiques; 100 % légumineuses, 100 % graminées, un mélange des deux, le *topsoil* et le témoin sur certaines propriétés physico-chimiques du sol de résidus miniers.

- Évaluer si les différents traitements ont un effet sur la croissance, la survie, la hauteur, la biomasse racinaire et la nutrition foliaire des semis d'arbres pionniers plantés dans les différents traitements.

Hypothèses:

- H2.1 : Le traitement des graminées, avec son plus grand volume de racines et de fibrilles, aurait un effet plus important sur les propriétés physico-chimiques des sols à résidus miniers.
- H2.2 : Le traitement des légumineuses, grâce à son supplément en N inorganique via le processus de nitrification, va en retour favoriser la croissance et la concentration foliaire azotée des essences arboricoles plantées dans ce traitement.

Chapitre 3

- Mesurer l'effet des plantes herbacées agronomiques ensemencées sur l'abondance des espèces, la richesse spécifique et l'indice de diversité des nouvelles plantes vasculaires s'établissant spontanément sur le site.
- Évaluer quels traitements (légumineuses, graminées, le mixte ou le *topsoil*) favorisent le plus l'arrivée spontanée des arbustes et/ou arbres pionniers.

Hypothèses:

- H3.1 : Après le traitement *topsoil* qui devrait avoir la plus grande diversité (dû aux quantités plus élevées de nutriments et de banques de semences préexistantes), le traitement 100 % légumineuses aura des espèces plus diverses et plus abondantes en raison de l'apport en N inorganique des légumineuses.

- H3.2 : Dû à l'apport en N inorganique par les légumineuses, le traitement 100 % légumineuses permettra l'établissement et la survie des espèces arboricoles plus exigeantes en N.

Chapitre 4

- Déterminer si les différents traitements de plantes herbacées agronomiques créent des différences au niveau de certaines variables microclimatiques mesurées telles que la transmission de la lumière au sol, la température et l'humidité du sol.
- En fonction des conditions microclimatiques créées par les traitements herbacés agronomiques, déterminer quels traitements produisent un meilleur taux de germination des graines des quatre essences pionnières manuellement semées.

Hypothèses:

- H4.1 : Le traitement de graminées, en raison de leurs caractéristiques structurelles spécifiques telles que des longues tiges verticales, leur formation en talles et l'apport de paillis, augmentera l'humidité du sol.
- H4.2 : En retour, la meilleure humidité du sol apportée par le traitement de graminées va fournir un meilleur lit de germination et de survie des semis, en particulier pour les conifères qui sont plus exigeants au niveau de l'humidité pour la germination de leurs graines.

CHAPITRE 2

EFFECTS OF AGRONOMIC HERBACEOUS PLANTS ON THE SOIL STRUCTURE OF GOLD MINE TAILINGS AND THE ESTABLISHMENT OF BOREAL FOREST TREE SEEDLINGS

(EFFETS DES PLANTES HERBACÉES AGRONOMIQUES SUR LA STRUCTURE DU SOL DES RÉSIDUS MINIERS D'UNE MINE D'OR ET L'ÉTABLISSEMENT DE SEMIS D'ARBRES DE LA FORÊT BORÉALE)

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Abstract

In Canada, low-grade ore mines generate large amounts of mineral waste, such as mine tailings. To control erosion of the fine-grained tailings particles as quickly as possible, it is common practice for the mining industry to revegetate the mine tailings with agronomic herbaceous plants. However, it is unclear whether this practice is consequential to the natural establishment of boreal species. The first objective of this study was to evaluate which families of agronomic herbaceous plants (legumes or grasses) result in the most favorable physical and chemical soil properties for the establishment of boreal species. The second objective was to determine the effect of the agronomic herbaceous plants on the growth and foliar nutrient concentration on three indigenous boreal forest seedlings; jack pine (*Pinus banksiana* Lambert), tamarack (*Larix laricina* Du Roi), paper birch (*Betula papyrifera* Marshall) and a willow cultivar (*Salix miyabeana* Seemen).

In 2013, a 1-ha *in situ* experimental surface of mine tailings was set up on the gold mine site in Malartic, Abitibi-Témiscamingue, Quebec. The experimental site was subdivided into three blocks, each further divided in five plots. Each plot was randomly seeded as follows: 100% grass, 100% legumes, a mixture of both, topsoil, and a control (tailings only, no seeding). In the 2015 spring season, 30 seedlings of the three boreal tree species and cuttings of the willow cultivars were planted in each treatment plot. Seedling height and root biomass were measured at the end of the 2016 growing season.

Soil sample analyses indicated significant differences for bulk density, wilting point and organic matter content between the topsoil and the different agronomic herbaceous and control treatments; however, no significant differences were found between the different herbaceous treatments and the control for soil pH, bulk density, wilting point, macroporosity and organic matter content. The mortality rate of jack pine, tamarack, and paper birch seedlings was higher in the control plots compared to all other treatments. Root biomass and height of the willow cultivar were significantly higher in the legumes compared with topsoil treatment. Among the four pioneer tree seedlings studied, this research indicates that the combination of the willow cultivar with the legume treatment produces the best seedling growth and survival in the highly abiotic and stressful environments inherent to mine tailings.

Keywords: grasses, legumes, mine tailing reclamation, topsoil treatments, *Salix,* shade-intolerant seedlings.

Résumé

Au Canada, les mines de minerai à faible teneur génèrent de grandes quantités de déchets minéraux, tels que les résidus miniers. Pour contrôler l'érosion des particules fines des résidus le plus rapidement possible, il est courant pour l'industrie minière de revégétaliser les résidus miniers avec des plantes herbacées agronomiques. Cependant, il n'est pas clair si cette pratique favorise ou non l'établissement naturel d'espèces boréales. Le premier objectif de cette étude est d'évaluer quelles familles de plantes herbacées agronomiques (légumineuses ou graminées) présentent les propriétés physiques ou chimiques du sol les plus favorables à l'implantation d'espèces boréales. Le deuxième objectif était de déterminer l'effet des plantes herbacées agronomiques sur la croissance et la concentration foliaire des nutriments de trois semis indigènes de la forêt boréale ; le pin gris (*Pinus banksiana* Lambert), le mélèze laricin (*Larix laricina* Du Roi), le bouleau à papier (*Betula papyrifera* Marshall) et un cultivar de saule (*Salix miyabeana* Seemen).

En 2013, une surface expérimentale *in situ* de résidus miniers d'un hectare a été aménagée sur le site de la mine d'or de Malartic, en Abitibi-Témiscamingue, au Québec. Le site expérimental a été subdivisé en trois blocs, chacun divisé en cinq parcelles. Chaque parcelle a été ensemencée aléatoirement avec un des traitements suivants : 100 % de graminées, 100 % de légumineuses, un mélange des deux, du *topsoil* ou un témoin (résidus seulement, pas d'ensemencement). Au printemps 2015, 30 semis des trois espèces d'arbres boréaux et des boutures de cultivars de saule ont été plantés dans chaque parcelle de traitement. La hauteur des semis et la biomasse des racines ont été mesurées à la fin de la saison de croissance 2016.

Les analyses d'échantillons de sol ont indiqué des différences significatives au niveau de la masse volumique apparente, du point de flétrissement et de la teneur en matière organique entre le *topsoil* et les autres traitements (herbacées agronomiques et témoin). Cependant, aucune différence significative n'a été observée entre les différents traitements herbacés et le témoin pour le pH du sol, la masse volumique, le point de flétrissement, la macroporosité et de la teneur en matière organique.

Le taux de mortalité des semis de pin gris, de mélèze et de bouleau à papier était plus élevé dans les parcelles témoins que dans tous les autres traitements. La biomasse racinaire et la hauteur du cultivar de saule étaient significativement plus élevées dans le traitement de légumineuses que dans le traitement de *topsoil*. Parmi les quatre semis d’arbres pionniers étudiés, cette recherche indique que la combinaison du cultivar de saule avec le traitement de la légumineuse produit la meilleure croissance et la meilleure survie des semis dans les environnements hautement abiotiques et stressants inhérents aux résidus miniers.

Mots-clés : espèces intolérantes à l'ombre, graminées, légumineuses, revégétalisation de parcs à résidus miniers, *Salix*, *topsoil*.

2.1 Introduction

The mining industry generates large amounts of mineral wastes during the extraction and treatment of commercial ore (Dudka & Adriano, 1997; Hester & Harrison, 1994; Lottermoser, 2007; Ripley et al., 1996). Among the different types of mine waste, mine tailings are of particular concern for their reclamation challenges (Lottermoser, 2007).

Mine tailings are defined as fine crushed, milled, and grounded ore from which the valuable mineral has been chemically recovered. The mine tailings' mixture of sand grain to silt size particles is then pumped with water in a slurry paste with some chemical residues and deposited into a tailing storage facility (Edraki et al., 2014; Huang et al., 2012; NRCan, 2016a). The physical and chemical soil properties (such as bulk density, macroporosity and pH) of the mine tailings depend on the processing technology, the parent ore, the size of particles resulting from the milling process, and the type of chemical used for the extraction of the valuable mineral (Lottermoser, 2007).

In 2016, 63 active metal ore mines were in operation in Canada, producing 118 million cubic meters of tailings ponds (MAC 2017; NRCan, 2016a). Environmental impacts generated by the mine tailings include dust particle pollution, surface and groundwater contamination, acid leaching, wildlife habitat destruction (Donato et al., 2007) and human health impacts (Dudka & Adriano, 1997; Edraki et al., 2014; Sanchez-López et al., 2015). As such, mine tailings reclamation ("Mine reclamation entails restoring these disturbed areas to a previous natural resource setting, such as forest or agricultural land uses, while minimizing environmental impacts", Indiana Geological and Water Survey, n.d.) is a crucial intervention to mitigate their environmental impacts, with revegetation being the most common and least expensive treatment option (Sheoran et al., 2010; Wang et al., 2017). Revegetation used for mine tailings reclamation offers many benefits, including the creation of a soil profile and structure, an increase in soil fertility through the plants' decomposition, and a reduction of airborne fine dust particles from wind erosion (Bradshaw, 1997; Bradshaw & Chadwick, 1980; Bradshaw et al., 1978).

After a mine's closure, or when the tailings facilities area is filled up, revegetation is commonly used to control erosion and to improve mine soil substrate. Since mine tailings are anthropogenic soils, they are devoid of most benefits and services inherent to natural soils such as soil carbon sequestration and nutrient supply (Bronick & Lal, 2005; Capra et al., 2015; Dazzi & Lo Pappa, 2015; Macdonald et al., 2012). Moreover, mine tailings offer poor soil structure, poor drainage and aeration, high salinity, and lack organic matter as well as some essential nutrients for plant growth (Bradshaw & Chadwick, 1980; Wang et al., 2017). With some exceptions, most vascular plants do not have the physiological traits to survive such soil conditions (Khasa et al., 2002, 2005). Past research efforts thus focused on the addition of organic amendments to compensate for these deficiencies, with the objective of inducing some biotic soil functions and establishing the microbiome environment (Li & Fung, 1998). For examples, amendment with biosolids, composts, wood chips, municipal and pulp and paper sludge have all demonstrated some promising achievements in the pursuit of mine tailings reclamation (Asensio et al., 2013, 2014; Guittonny-Larchevêque & Pednault, 2016; Larchevêque et al., 2013; Renault et al., 2007; Sheoran et al., 2010; Young et al., 2015).

Another technique used in mine tailings reclamation is the application of topsoil material (up to 50 cm depth). At the mine's location, topsoil amendments consist of the stripped, saved, and stockpiled upper layers of the soil (i.e., a mixture of wood debris, humus, organic soil, and some trace of mineral soil) once the initial vegetation is removed. There is an ongoing understanding from recent mining reclamation studies that topsoil salvage is a valuable amendment to increase the speed of the soil ecosystem recovery and function (Shrestha & Lal, 2011). Cooke and Johnson (2002) suggest that if the topsoil amendment is available, the post-mining reclamation can be achieved much faster with its use compared to alternatives. Similarly, Skousen et al. (2011) suggest that coal mine soil reconstruction in the Appalachian Forest ecosystem can also be achieved faster with topsoil amendments, including the addition of overburden weathered rocks to reclaim mine land. These authors explain that topsoil material, including logging residues, is a soil "living resource" inoculum that contains seed banks, organic matter, and microorganisms. Many studies have used topsoil as a reference (positive control) to weigh against other categories of amended mine tailings or to compare the vegetation response of the topsoil to other amended mine tailings (Bendfeldt et al., 2001; Boyter et al., 2009; Guittonny-Larchevêque & Pednault, 2016; Guittonny-Larchevêque et al., 2016a, 2016b; Shrestha et al., 2009). Importantly, while topsoil amendments show promising results for forest restoration, their availability can be limited, and challenges arise when large areas must be reclaimed. Moreover, its stockpile residence time and its manipulation with heavy equipment (soil compaction) can adversely affect its potential for ecological restoration. Indeed, the stockpile residence time may affect topsoil properties necessary for soil restoration, such as its biological activity (e.g., decreased mycorrhizal potential and earthworm population) (Abdul-Kareem & McRae, 1984; Sheoran et al., 2010; Shrestha & Lal, 2011).

In Canada, open-pit and underground mining operations are mostly conducted within the boreal shield ecozone (Brandt et al., 2013; NRCan, 2016a). In the province of

Quebec, the mining industry is required to provide a reclamation plan and a financial guarantee to alleviate the environmental impacts of mining activities (Mining Act, chapter M-13.1). One of the reclamations' objectives is to restore mining sites to a natural setting in harmony with the surrounding environment; in Canada, the surrounding environment is most often a boreal forest (MERN, 2017). Consequently, mining reclamation often aims to mitigate the environmental impacts of biodiversity loss, land fragmentation, and other ecosystems benefits associated with the boreal forest biome. As the first step of mine tailings reclamation, it is common practice for the mining industry to start the process by vegetating their mineral waste with agronomic herbaceous seeds (grasses and legumes), often referred to as "conventional seed mix" (Fields-Johnson et al., 2012; Sheoran et al., 2010). Furthermore, the cost, availability, physiological traits, and the ease of using agronomic herbaceous plants allow for rapid vegetation cover establishment and stabilization of the mineral surface.

Vegetation affects soil structure and chemistry through its root activities: root penetration capability, water extraction ability, soil aggregates, macroporosity formation, organic matter deposition (root turnover) and root exudates (Bardgett et al., 2014; Grevers & Jong, 1990; Ibrahim & Goh, 2005; Lafleur et al., 2013). In general, grasses (graminoids) are water demanding species producing large amounts of roots and fine roots (Robinson, 1972). By growing within existing pores or through the soil matrix, roots exert compression and shear stress which in turn create macropores (Angers & Caron, 1998). The stability of soil aggregates is largely dependent on organic materials (organic binding agents) such as polysaccharides, roots, fungal hyphae, and polymers (Tildall & Oades, 1982). Most grass species produce large above and belowground biomass that enriches tailings in organic matter (Cooke & Johnson, 2002). Grasses have the capacity of tailoring new shoots with their fibrous, adventitious roots (Freams, 1905). The fibrous roots activity of grasses changes soil properties by creating soil aggregates. Doormar and Foster (1991) observed that microaggregates (2-

20 μm) were formed by perennial ryegrass (*Lolium perenne* L.) through roots associated to mineral particles, root gel, root fragments and microbial extra-cellular polysaccharides. In a controlled nursery condition, a study by Guittonny-Larchevêque et al., (2016a, 2016b) showed an increased benefit on mine tailing soil macroporosity related to perennial grass after two growing seasons. Despite the positive effects on soil structure, much of the literature has treated grasses as plant competition, limiting the woody species establishment (Carnevale & Montagnini, 2002; Sheoran et al., 2010).

The roots of agronomic herbaceous legumes can form symbiotic relationships with atmospheric nitrogen-fixing *Rhizobium* bacteria that supply the plant with nitrogen (N). In return, legume decomposition enriches the soil with N (Ledgard & Steele, 1992). In general, most agronomic legume plants have tap roots for soil deep water intake (OMAFRA, 2017). Studies have shown that in a soil environment where there is a high amount of organic matter and N, the efficiencies of legumes as N suppliers can be inhibited. Conversely, in soils with low organic matter content and N stock, such as in mine tailing environments, legumes can be an effective agent for soil improvement and vegetative succession (Bradshaw 1997; Domingo & David, 2014; Government of Saskatchewan, n.d.; Maiti & Maiti, 2014; Sheoran et al., 2010).

However, in the context of mine tailing reclamation within the boreal forest biome, it is still unclear whether these agronomic herbaceous plants, grasses and/or legumes, are conducive or competitive towards the afforestation of shade intolerant boreal forest trees species.

This paper has two main objectives; first, we aimed to measure the effects of different agronomic herbaceous treatments (grass, legumes, or a mix of the two) and the topsoil treatment on selected physical and chemical soil properties, including macroporosity, bulk density, wilting point, organic matter, and pH. Second, we aimed to identify the effects of the different agronomic herbaceous treatments on the survival, height, root biomass, and foliar nutrition of three native planted boreal forest tree species and one willow cultivar. We used willow cuttings as one of the selected species because of their phytoremediation potential shown by previous studies (Boyter et al., 2009; Kuzovkina & Quigley, 2005; Mosseler & Major, 2017). We hypothesized that: (1) the grass treatment, with its greater roots volume and fibrous roots physiology potential, would have a greater effect on the mine tailing soil physico-chemical properties; (2) a higher N foliar concentration would be found in the legume treatments regardless of the tree seedling species and subsequently would translate in improved growth of the planted seedlings for the legume treatment.

2.2 Materials and methods

2.2.1 Mine site

The *in situ* study site was located at an open pit gold mine site, south of Malartic, in the Abitibi-Témiscamingue region of northwestern Québec, Canada (48° 06'N, 78° 08'W). The study site is located within the balsam fir (*Abies balsamea* (L.) Miller) - paper birch (*Betula papyrifera* Marshall) bioclimatic domain (MFFP, 2016). The climate is characterized by long, cold winters, with short, moderately warm summers. For the 1981- 2010 period, mean annual rainfall is 985 mm with daily average temperature of -13.3, -17.2, -15.3 °C for December, January, and February and 14.4, 17.2, 15.8 °C for June, July, and August (Government of Canada, n.d.). The area is included within the last glacial advance of Wisconsin glaciation region (Vincent et Hardy, 1977) with a dominant grey luvisol soil type (Agriculture and Agri-Food Canada 2015).

The gold mine is still an active open-pit gold mine in 2021 with low grade ore and substantial deposition of mineral waste.

2.2.2 Experimental site description and design

The experimental site area (1 ha) is located on a flat surface of natural soil. Mine roads surround the north and west sides of the experimental site. Across the mine road at the west side (ca. 75 m from the experimental site) are patches of boreal forests consisting mainly of black spruce (*Picea mariana* (Miller) BSP (60-79%)) and balsam fir. Small slopes of waste rock berms surround the east side of the experimental site.

In May 2013, thickened tailings (50-70% solids by mass at deposition, (Bussière 2007) were excavated from the gold mine tailings facility to fill the 1 ha area of the experimental site. Thickened tailings are a technology used by mining industries to decrease water consumption during transportation and deposition of the mine tailings (Robinsky et al., 1991). Thickened tailings are mainly composed of 86% mineral particles (< 90 µm) and contain calcite and low sulfur content (~1% S); the ore at the mine site consisted of a mineralized greywacke.

The experimental site was set up in a randomized complete block design, consisting of three blocks (Fig. 2.1a). Each block was further subdivided into five experimental plots (20 m × 15 m), each 5 m apart. Each plot received one of the following treatments randomly applied within the block:

1) 100% perennial Poaceae (hereinafter referred to as grasses)

2) 100% perennial Fabaceae (hereinafter referred to as legumes)

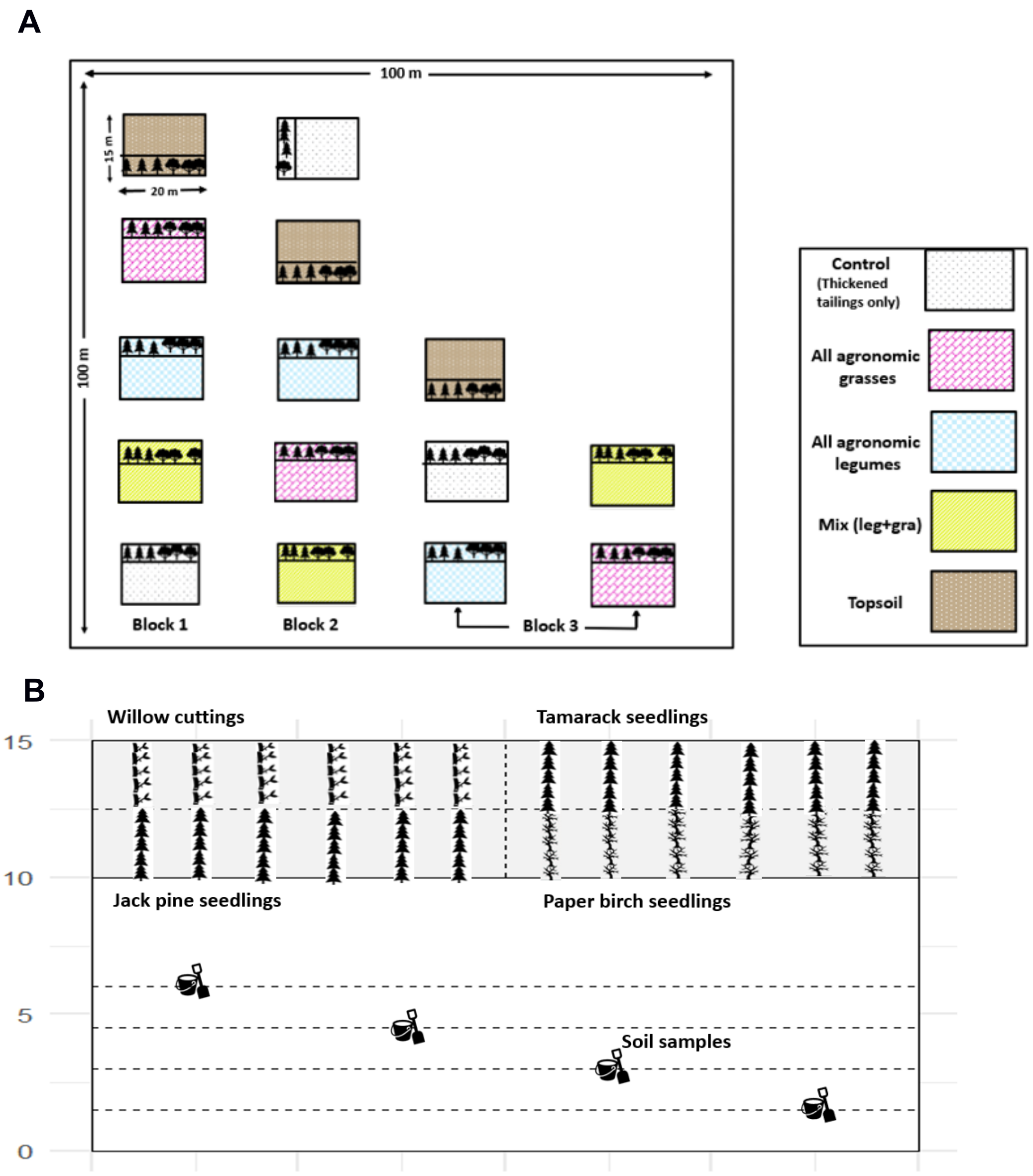

Figure 2.1 (a) Experimental site layout of the treated plots in each block (set up in 2013, not drawn to scale). In 2015, one third of each treated plot were afforested with 30 seedlings of jack pine, tamarack, paper birch and willow. (b) Treatment plot design (to scale) including 30 tree seedlings/species and soil samples' location.

3) An equal mix of grasses and legumes
4) Topsoil amendment (details are provided in section 2.2.3.1)
5) Control (thickened tailings only)

2.2.3 Soil materials

2.2.3.1 Topsoil and thickened tailings (control treatment)

Prior to the gold mine exploitation, the existing forest stand was harvested, and the upper layers of organic soil (topsoil) and its underneath mineral soils were salvaged and stockpiled (for 30 to 36 months) in two separates 7-m-high piles (2.5H:1V slope) for future use. The topsoil stockpile consisted of the first 30-cm dark (20% organic matter) soil layer (O- and A-horizons). The forest soil prior to mining was classified as a luvic gleysol (CSSC, 1998). In May 2013, the topsoil stockpile was excavated and spread over (2 cm thick layer) one plot (20 m × 15 m) of each block as showed in Fig. 2.1a and an example of one treatment plot design including the 30 tree seedlings/species and the location where the soil samples were picked up (Fig. 2.1b).

In June 2013, one soil sample (0-10 cm) of each topsoil treatment plot (1 plot per block, 3 blocks, n=3) and of each control treatment (thickened tailings only) (1 plot per block, 3 blocks, n=3) were collected for soil nutrient and metals analysis. Both topsoil and thickened tailings soil samples were oven-dried (at 50 °C for 48 h), grounded and sieved (2 mm mesh) for total N (Dumas combustion method, CNS 2000; LECO) and organic C (thermogravimetric method, TGA; LECO) analyses. Organic matter concentration was computed as 1.72 × total C (Allison, 1965). Total cations and metals were extracted by HNO_3^- digestion and analyzed by inductively coupled plasma-atomic emission spectrometry (ICP-AES). Total P was extracted using the Olsen method (Olsen, 1954) and analyzed by spectrophotometry. The initial topsoil and thickened tailings characteristics are summarized in Table 2.1.

Table 2.1 Characteristics of initial thickened tailings and topsoil.

Parameters	Thickened tailings	Topsoil	Regulatory threshold [a] (Industrial lands)
pH	7.9 (0.4)	6.2 (0.2)	–
EC [b] (cS m^{-1})	8.3 (1.1)	6.6 (1.2)	_
OM [b] (%)	0.74 (0.67)	14.9 (1.5)	_
Total N (%) [c]	0.04 (0.01)	0.34 (0.02)	_
Total S (%) [c]	1.0 (0.1)	0.32 (0.05)	_
Total K (g kg^{-1}) [c]	7.6 (1.7)	3.5 (0.31)	_
Total Mg (g kg^{-1}) [c]	14.8 (0.3)	13.2 (1.4)	_
Total P (g kg^{-1}) [c]	0.68 (0.03)	0.65 (0.01)	_
Total Ca (g kg^{-1}) [c]	14.4 (2.6)	8.6 (0.20)	_
Total Al (g kg^{-1})	13.4 (0.4)	12.4 (0.62)	_
Total Fe (g kg^{-1})	32.0 (2.7)	28.6 (1.07)	_
Total Na (g kg^{-1})	0.54 (0.18)	0.30 (0.09)	_
Total B (mg kg^{-1})	2.4 (1.1)	3.2 (0.51)	_
Total As (mg kg^{-1})	5.1 (1.2)	6.8 (1.73)	50.0
Total Cd (mg kg^{-1})	0.11 (0.1)	0.21 (0.06)	20.0
Total Cr (mg kg^{-1})	194.0 (29.4)	197.4 (32.5)	800.0
Total Cu (mg kg^{-1})	53.4 (2.4)	45.1 (0.94)	500.0
Total Mn (mg kg^{-1})	442.1 (8.6)	459.6 (35.7)	2200.0
Total Ni (mg kg^{-1})	78.5 (12.5)	83.8 (11.0)	500.0
Total Pb (mg kg^{-1})	42.9 (22.7)	76.0 (15.8)	1000.0
Total Zn (mg kg^{-1})	83.9 (6.8)	105.0 (5.8)	1500.0

Note: Mean (SE); n=3. All values are expressed on a dry mass basis

[a] MELCC, 2021

[b] *EC:* Electrical Conductivity, *OM*: Organic Matter

[c] Plant macronutrients

Initial data from 2013

2.2.4 Plant materials.

2.2.4.1 Agronomic herbaceous seeds

In June 2013, commercial agronomic herbaceous seeds (forage seeds) were obtained from Lanexco Inc. (Amos, QC). The company sowed the seeds using a manual sowing instrument according to the experimental design on the freshly hand-raked tailings. An annual nurse crop of barley seeds (*Hordeum vulgare*) was added to all seed mixes for soil stabilization, and to reduce weeds establishment (Espeland & Perkins, 2013). The legume treatment plots included barley 10% by mass, white clover (*Trifolium repens L.*) 45% by mass, and bird foot trefoil (Lotus corniculatus) 45% by mass. Legume seeds used for the experimental site were offhand coated with soil bacterium *Rhizobium* inoculant to enhance biological nitrogen fixation. The grass treatment plots included barley 10% by mass, ryegrass (*Lolium perenne*) 40% by mass, redtop (*Agrostis gigantea*) 40% by mass, reed canary grass (*Phalaris arundinacea* L.) 10% by mass. The mix legumes/grasses treatment plots included barley 10%, white clover 20%, bird foot trefoil 20%, reed canary grass 25% and ryegrass 25%. Seeding mixtures were applied at a rate of 100 kg ha-1. Mineral fertilizer 8-32-16 (Nitrogen, Phosphorus, 229 and Potassium) was applied once at 750 kg ha-1 rate to all treatment plots (except for the control and topsoil plots). Commercial MYKE® promycorrhizal inoculant (Premier Tech biotechnologies, Rivière-du-Loup) was also added to all treatment plots (except for the control and topsoil plots) in compliance with the manufacturer application chart.

2.2.4.2 Tree seedlings

In May 2015, two-year-old seedlings in containers (cells) of jack pine (*Pinus banksiana* Lambert) and tamarack (*Larix laricina* Du Roi) were obtained from the nearby Trécesson nursery of the Ministère des Forêts, de la Faune et des Parcs (MFFP). At the same time, two-year-old bareroot paper birch seedlings were obtained from the Berthier MFFP nursery. Willow cuttings (31 cm) were obtained from clones (*Salix miyabeana* Seemen, Sx64 clone) of a parent tree from a nearby nursery (La Morandière, QC, Canada). Tree seedlings were stored in a cold chamber until planting time. The initial average height (cm, measured above the soil) and the initial average diameter (mm, measured at 10 cm above soil) for the 450 seedlings/species are as followed: Paper birch (51 cm, 3.6 mm), Jack pine (34 cm, 4.6 mm), Tamarack (45 cm, 4.3 mm), willow's cutting (31 cm, 8.1 mm). In early June 2015, thirty seedlings (cuttings for Sx64) of each of the four tree species were planted by monospecific clusters (5 rows of 6 seedlings, planted 50 cm apart) at the end of each experimental plot (30 seedlings × 15 treatments × 4 species = 1800 seedlings) as per Fig. 2.1b.

2.2.5 Soil measurements

2.2.5.1 Soil core sampling

In September 2016, after the removal of the top surface layer of grass or legumes material, using a double-cylinder soil sampler, two undisturbed soil cores (100 cm^3) of the 0-10 cm soil depth and two soil cores of the 10-20 cm soil depth were systematically collected on four parallel transects located at 1.5, 3.0, 4.5, and 6.0 m from the bottom edge of the plot. Therefore, a total of 16 undisturbed soil cores (eight of 0-10 cm and eight of 10-20 cm) per plot were collected for bulk density and macroporosity analyses. One bag of loose samples at the 0-10 cm and at the 10-20 cm depth soil layers were also collected for organic matter, permanent wilting point, and pH measurements. All samples were kept in a refrigerator (4 °C) until processed.

2.2.5.2 Soil physical and chemical properties

Soil bulk samples at the 0-10 cm and the 10-20 cm soil depth were randomly collected in each treatment plots. Samples were then air-dried and sieved (2 mm), and organic matter (OM) content was determined using the loss-on-ignition method (Ball 1964) for each soil depth sampled. Excess soil samples were used for pH determination (10 g soil: 5 ml demineralized water, 2w:1w) (Sobek et al., 1978). For permanent wilting point, extra samples were collected, dried, and sieved (2 mm) before being saturated and brought to equilibrium with a pressure of -1500 kPa using a pressure membrane apparatus (Soil Moisture Equipment Corp., Santa Barbara, CA) and weighed (W_1). The samples were dried for 48 h at 105 °C and weighed again (W_2). The permanent wilting point (PWP) was estimated as:

$$PWP\left(\%\frac{w}{w}\right) = \frac{W_1 - W_2}{W_2} \times 100$$

For macroporosity and bulk density measurements, 16 undisturbed soil samples (5 cm diameter, 100 cm^3) collected in each of the fifteen plots were brought to saturation under vacuum and weighed (W_3, macroporosity). Samples were set on the porous surface of a sand-box apparatus (Eijkelkamp Agrisearch Equipment, Giesbeek, the

Netherlands), and brought to equilibrium at a tension of -10 kPa (field capacity). The samples were weighed again (W_4), then oven-dried (105 °C for 48 h) and weighed again for the last time (W_5). Macroporosity and bulk density (BD) were estimated as follows:

$$Macroporosity\ (\%) = \frac{W_3 - W_4}{100\ cm^3} \times 100$$

$$BD = \frac{W_5}{100\ cm^3}$$

2.2.6 Plant measurements.

2.2.6.1 Above-ground measurements of tree seedlings

The 30 seedlings of each species planted in 2015 (Fig. 2.1(a)) were measured in May 2016 and in October 2016 for height (cm), root collar diameter (mm) and seedling survival. The planted seedlings were marked as dead when they showed less than 5% greenery (twigs and leaves included). The height difference (cm) was measured for all surviving seedlings from the base of the seedling to the top leader.

2.2.6.2 Root biomass of tree seedlings

In each plot, we unearthed seedlings of rows 2 to 4 of each species (three seedlings per species) chosen randomly on the row for root biomass measurement. Seedling roots were water washed to remove any extra dirt. Tangled roots from other plants were removed. Seedling roots were cut from the main stem and placed in paper bags to dry for 48 h at 60 °C and weighed for root biomass within the same day.

2.2.6.3 Foliar analysis

Foliar samples were collected on the experimental site at the end of the seedlings' growing season (late August to early September) in 2016. Five to ten leaves were haphazardly sampled on each live stem of each plot and placed in brown paper bags for further foliar analysis. For the coniferous species, we took a random cluster of needles on different twigs and different twigs' height place on the seedling.

Leaves and needles from the same species and plot were combined, dried at 60 °C for 24 h, then ground for analysis. Foliar samples were analyzed for total N and C (CNS combustion by Elementar CHNS), total cations and metals (Nitric-Hydrochloric Acid Digestion and inductively coupled plasma atomic emission spectrometry (ICP-AES) analysis method) and moisture content (% weight loss method). Foliar analyses and the initial soil analyses (before treatment) were conducted at the Forest Resources & Soil Testing laboratory (FoReST Laboratory) of Lakehead University (Thunder Bay, ON, Canada).

2.2.7 Statistical analyses

The additive effects of agronomic herbaceous treatments and soil depth (0-10 cm and 10-20 cm) on soil properties were analyzed using a two-way analysis of variance (ANOVA), followed by post-hoc comparisons with Tukey's range test.

The effects of herbaceous treatments on the root biomass, height and diameter increments of seedlings were estimated with linear mixed models, with plot as a random effect since the growing environment might be more similar between seedlings located in the same plot. Height increments were log(x+1) transformed to improve normality and homoscedasticity of the regression residuals. On the other hand, the effect of herbaceous treatments on seedling survival was estimated with mixed effects logistic regression, with plot as a random effect. Separate models were fitted for each seedling species. All mixed effects models were fitted with the lme4 package (Bates et al., 2015) in R (R Core Team, 2019) version 3.6.2 and the emmeans package (Lenth, 2020) was used to perform post-hoc comparisons between treatments, with a Tukey adjustment applied for multiple comparisons. Foliar nutrient concentrations were compared across treatments with a one-way ANOVA, followed by post-hoc comparisons with Tukey's range test. We used a significance threshold of $\alpha = 0.05$ for all analyses. In addition, principal component analyses using the FactoMineR package (Lê et al., 2008) were applied to the soil properties and foliar analysis results to visualize the multivariate structure of the data.

2.3 Results

2.3.1 Soil physico-chemical properties

The topsoil treatment showed a significantly higher content of organic matter, lower bulk density and significantly higher wilting point compared to all other treatments, whereas macroporosity did not differ among all treatments (Table 2.2). No significant differences in soil physico-chemical properties were found between the control and the grasses, legumes, and mix treatments. For the pH, the only significant difference occurred between the topsoil and control treatments (Table 2.2). All soil properties except macroporosity also show significant differences by soil depth (0-10 cm and 10-20 cm), however, among all the treatments, the topsoil treatment showed the highest difference between the two soil depths in regards of the physico-chemical soil properties (except for the macroporosity) (Fig. 2.2).

Table 2.2 Effects of the herbaceous treatments and depth on the mine tailing soil properties.

	pH	Organic Matter %	Bulk Density g/cm^3	Macroporosity %	Wilting Point %
Treatment					
Control	7.37 (0.07) a	0.31 (0.12) b	1.36 (0.02) a	5.47 (0.60) a	0.65 (0.28) b
Grasses	7.36 (0.07) ab	0.60 (0.12) b	1.44 (0.02) a	4.63 (0.80) a	0.42 (0.05) b
Legumes	7.32 (0.05) ab	0.45 (0.07) b	1.36 (0.01) a	5.18 (0.58) a	0.43 (0.13) b
Mix	7.30 (0.08) ab	0.47 (0.11) b	1.34 (0.01) a	4.41 (0.32) a	0.59 (0.10) b
Topsoil	7.05 (0.10) b	9.14 (1.84) a	1.11 (0.07) b	6.48 (1.04) a	6.58 (1.50) a
Depth (cm)					
0-10	7.20 (0.05) B	2.92 (1.32) A	1.29 (0.02) B	5.82 (0.52) A	2.39 (0.99) A
10-20	7.35 (0.05) A	1.46 (0.63) B	1.35 (0.03) A	4.65 (0.35) A	1.09 (0.38) B

Mean (std. error) values shown for each property. Values followed by the same letter show no significant differences at *p* <0.05 (two-way ANOVA followed by Tukey's range test). The organic matter content and wilting point were square root transformed for the ANOVA (*n* =5 / treatment).

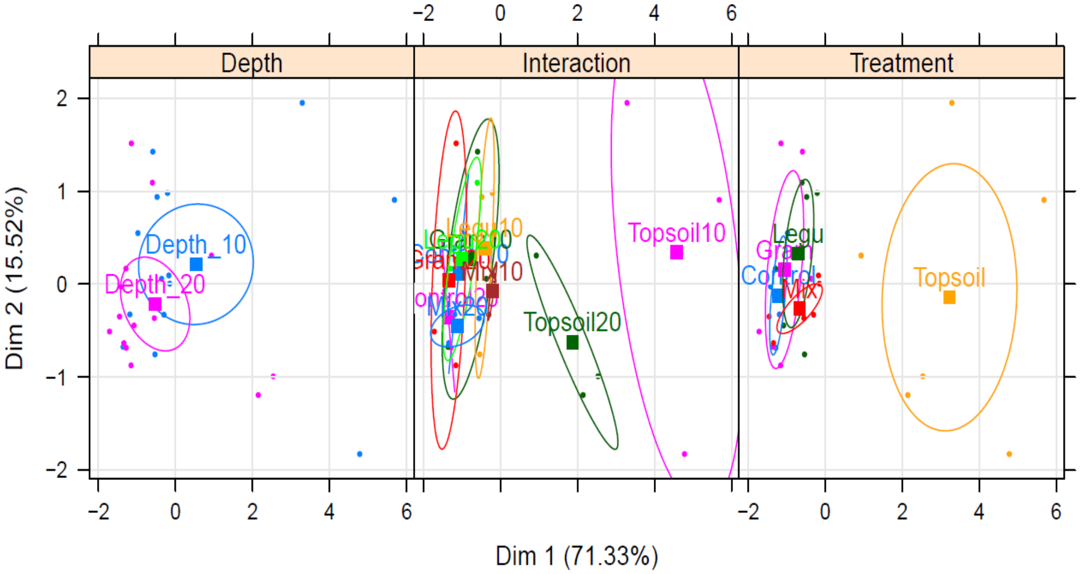

Figure 2.2 Principal component analysis of the physical and chemical soil properties measured for the Grasses (Gram), Legumes (Legu), Mix, Topsoil and Control treatments for each soil depth (0-10 cm and 10-20 cm). The Interaction panel shows the interaction of treatment and soil depth.

2.3.2 Seedling survival

Across all species, 626 of the 1800 planted seedlings were dead after two growing seasons in 2015-2016 (Table 2.3). The ranking of the treatments from the highest to lowest observed survival rate (irrespective of tree species) were as follows: topsoil > grasses > mixed > legumes > control (Table 2.3).

Figure 2.3 indicates the mean probability of survival estimated for tree seedlings within each treatment. For jack pine, a significant difference in the mean probability of survival was found between the grass, legumes, and control treatments (grasses > legumes > control). For tamarack seedlings, there was no significant difference among any of the treatments (except for the control treatment). For the willow and paper birch seedlings, results showed no significant difference in the survival probability between the control and legume treatments. Furthermore, the probability of survival of the tree seedlings, for all species except jack pine, did not vary significantly between the topsoil treatment and the herbaceous (legumes, grasses, and mix) treatments.

Table 2.3 Number of dead seedlings (out of 90 seedlings per species and treatment combination).

	Paper birch	Jack pine	Tamarack	Willow	Total
Treatments					
Topsoil	7	5	1	7	20
Grasses	12	25	16	18	71
Legumes	40	54	9	30	133
Mixed	22	29	12	37	100
Control	77	83	73	69	302
	158	196	111	161	626

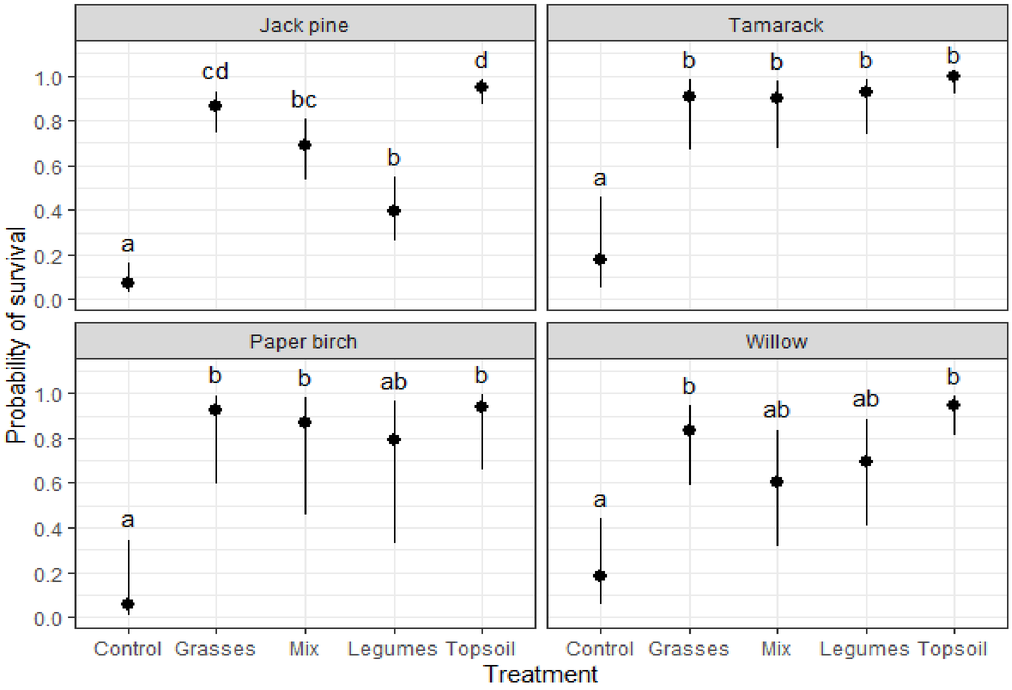

Figure 2.3 Mean probability of survival (with a 95% confidence interval) of the tree seedlings of each species within each treatment after two growing seasons (n = 90 seedlings per treatment and species combination). Shared letters indicate no significant difference (α = 0.05).

2.3.3 Seedling growth

2.3.3.1 Seedling roots biomass

After two growing seasons, the dry root biomass of jack pine seedlings showed no significant difference among treatments (Fig. 2.4). For tamarack seedlings, only the topsoil treatment significantly differs from the control treatment, with an 89% increase in root biomass. The willow seedlings (cuttings) had a significantly greater (over 100% increase) root biomass in the legume treatment compared with the topsoil treatment.

However, the control treatment did not significantly differ from the legume treatment. The roots of paper birch seedlings were very similar to the agronomic herbaceous plant roots and extremely tangled together. Therefore, were unable to accurately separate the paper birch roots from of the agronomic herbaceous roots and did not include paper birch in this analysis.

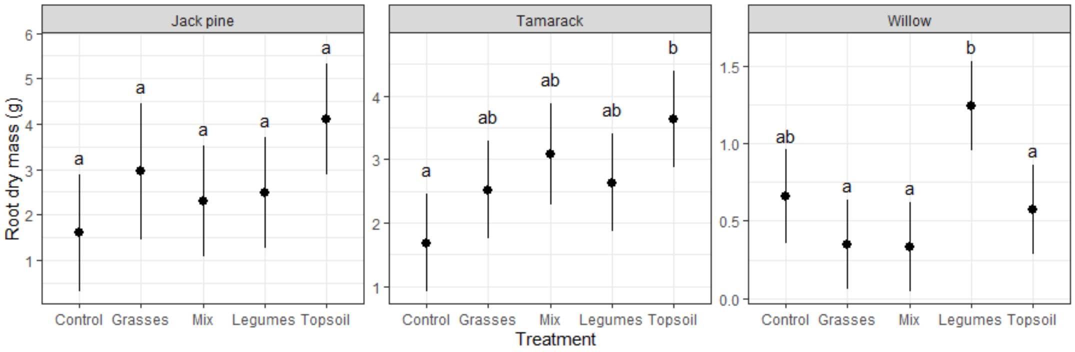

Figure 2.4 Mean root biomass (with a 95% confidence interval) of the jack pine, tamarack and willow tree seedlings after two growing seasons within each treatment (n = 90 seedlings per treatment and species combination). Shared letters indicate no significant difference (α = 0.05).

2.3.3.2 Seedling height growth

After two growing seasons, the seedling diameter increment did not significantly differ among treatments for any species planted (results not shown). However, the aboveground height increment of the willow seedlings was significantly greater in the legume treatment compared to the topsoil treatment (Fig. 2.5). No significant differences for the height increments were found in any other tree species between the different treatments.

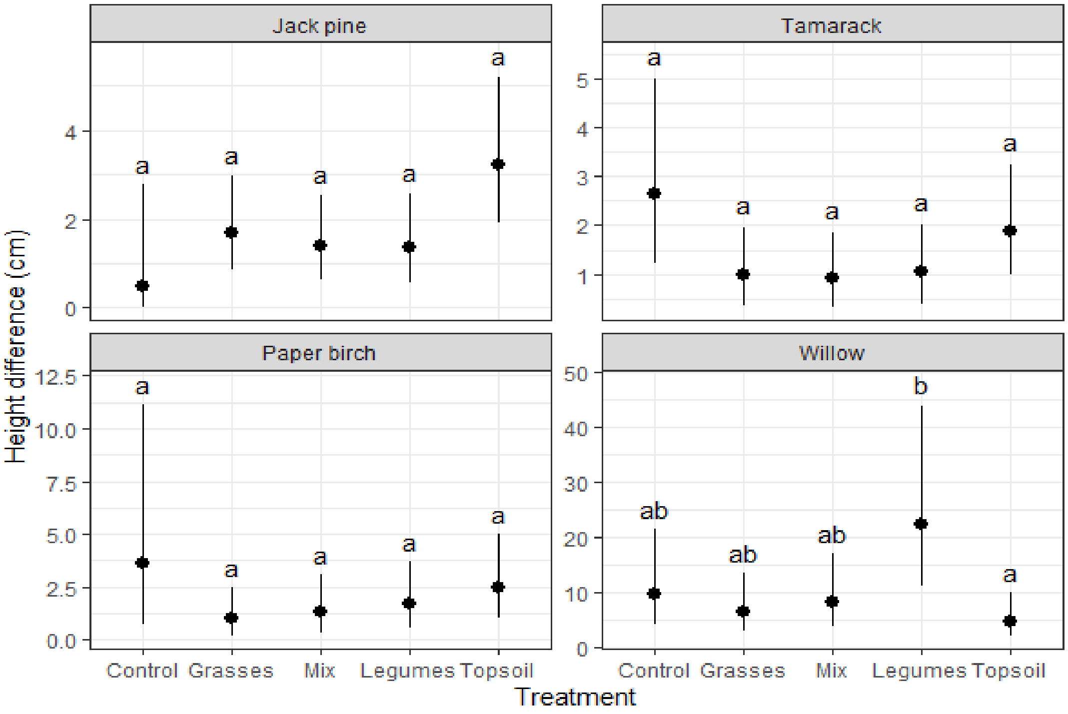

Figure 2.5 Mean height increment (with a 95% confidence interval) of the tree seedlings of each species within each treatment after two growing seasons (n = 90 seedlings per treatment and species combination). Shared letters indicate no significant difference (α = 0.05).

2.3.4 Foliar analyses

2.3.4.1 Heavy metals

After four years of weathering on the experimental site, no heavy metal accumulation of arsenic (As), cadmium (Cd), cobalt (Co), lead (Pb), nickel (Ni), and thallium (Tl) were found in the leaf tissues based on foliar analysis, regardless of the tree seedlings species and the applied treatments (Supplementary Information). Figure 2.6 indicates that the foliar concentration of calcium (Ca), copper (Cu), and zinc (Zn) showed a distinct pattern between the deciduous (willow and paper birch) compared to the coniferous needles (jack pine and tamarack). Furthermore, a higher portion of minerals are translocated to the leaves of the paper birch compared to the other species (Fig. 2.6).

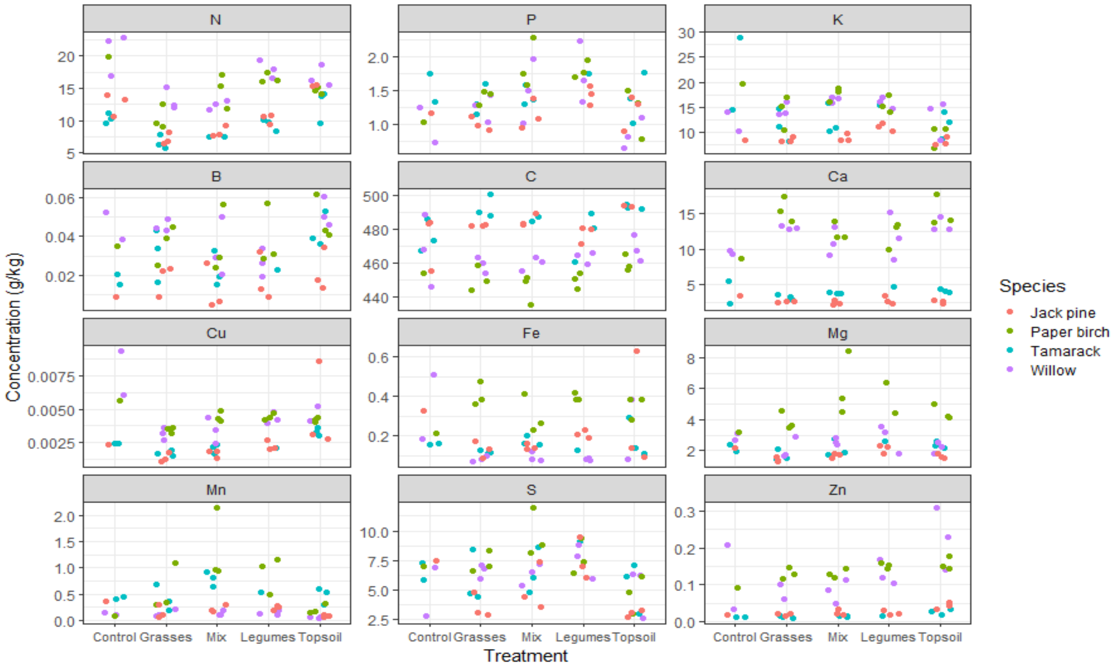

Figure 2.6 Foliar mineral concentration of the tree seedlings species. Elements N and C are in % and all other elements in the figure are in g/kg. Foliar analysis of elements; Al, Cr, Mo, Na, Ni, Sr, are excluded because data were below the detection limit (bdl). No available data for chloride. Each point corresponds to the pooled leaves samples from one plot (see text for details).

2.3.4.2 Foliar nitrogen concentration

For the jack pine seedlings, foliar N concentration results indicate a significant difference between the legume and the grass treatment (over 50%). However, the topsoil treatment had the most significant difference from the other treatments except with the control treatment; topsoil > legumes > mix > grasses (Fig. 2.7).

For the tamarack seedlings, foliar N concentration was not significantly different between the topsoil and the control, and there were no significant differences between the grass, legumes, and mix treatment (Fig. 2.7).

For both willow and paper birch, foliar N concentration results indicate no significant difference between the topsoil and the control. For the paper birch, but not the willow, there is a significant difference between the grass and legume treatment.

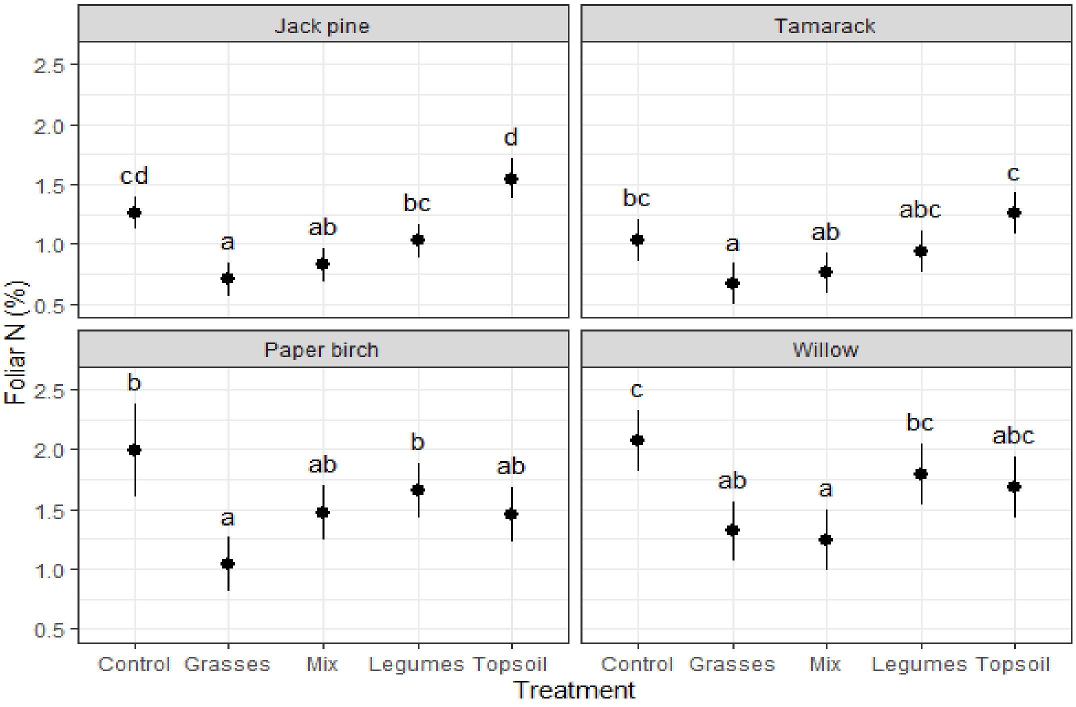

Figure 2.7 Mean foliar nitrogen concentration of the tree seedlings by species and treatment (with a 95% confidence interval). Shared letters indicate no significant difference (α = 0.05).

2.4 Discussion

2.4.1 Soil physico-chemical properties

The results of this study confirm a significant difference for most of the mine soil physico-chemical properties (except the macroporosity) in the upper soil depth 0-10 cm compared to the 10-20 cm depth for all treatments considered. This concurs with similar studies (Filcheva et al., 2000; Liu et al., 2017; Shrestha & Lal, 2007, 2011).

The topsoil treatment, which has a soil richer in organic matter and nutrients (Table 2.1) showed significant differences for most soil physico-chemical properties (except the macroporosity) compared to the grasses, legumes, mixed and control treatments (Table 2.2). Among the agronomic herbaceous plant treatments (grasses, legumes and mixed), we were unable to confirm our first hypothesis that the grass treatment with its greater roots volume and fibrous roots, would have a greater effect on the mine tailing soil physico-chemical properties. Guittonny-Larchevêque et al. (2016a, 2016b) conducted a study on the effect on soil macroporosity of metalliferous mine tailing using agronomic graminoids species in a controlled environment as well as in situ. The research has shown that within a controlled environment, the perennial grass has affected the mine tailing soil macroporosity with a significant difference even after two months growth. However, the study did not confirm any significant difference of the mine tailing soil macroporosity for the *in situ* experiment even after three growing seasons of the perennial grass.

Different factors may explain our results: there could have been unequal spreading and growth of the agronomic herbaceous grass, or the agronomic herbaceous grass species used for this research may not be optimal species for the mine tailings soil conditions. Further research is required to verify the efficiency of the agronomic herbaceous grass on the physico-chemical properties in an *in situ* mine tailing environment. For example, studies using indigenous grass species versus agronomic herbaceous grass, or different varieties of agronomic herbaceous grass with a longer pedogenic process time would be worth investigating.

2.4.2 Foliar nutrition

2.4.2.1 Foliar heavy metals

The pH of the tailings in our experimental site ranged from 6.6 to 7.7 for all included treatments. A low soil pH increases the solubility of most heavy metals, making metals available for plant uptake (Hodson, 2012; Masindi & Muedi, 2018). The mine tailings' neutral pH may have been a contributor in limiting heavy metal solubility and accumulation in plant tissue. We found no heavy metals or traces of heavy metals below the instrumentation detection limit in the foliage of any of the seedlings, regardless of applied treatments and despite 2 years of weathering (Supplementary Information). However, fast growing trees such as poplars, willows, and paper birch are known to have high rate of nutrient uptake (Lafleur et al., 2013) and the functional traits of paper birch may make them more sensitive to higher concentration of metal and metalloids as shown in Fig. 2.6.

2.4.2.2 Foliar nitrogen

Nitrogen is a macronutrient required in much higher quantities than micronutrient elements. Tree seedlings (except willow) that originated from nurseries may have been supplied with optimal N and soil conditions. Further, only the surviving seedlings were sampled, likely representing those strongest and best able to adapt in harsh environmental conditions. This may have created a bias in the results for the N foliar analyses, especially for the control treatment where mortality was high.

2.4.2.3 Foliar phosphorus

Phosphorus is an important nutrient for photosynthesis processes (Plaxton and Carswell, 2018). The P foliar analysis did not show any significant statistical difference for all treatments regardless of the seedling species. These results may be due to a sufficient concentration of P for plant growth even in the control treatment (mine tailings only) at the initiation of the experiment (Table 2.1). However, the mean P concentration is consistently slightly higher in the legume treatment, suggesting a possible different association with plant roots and living organisms in this treatment.

2.4.2.4 Foliar potassium

The initial soil analysis (Table 2.1) indicates that K was at a higher concentration in the control plot (7.6 g kg^{-1}) compared to the topsoil soil (3.5 g kg^{-1}). The K foliar analysis (Fig. 2.6) showed no significant difference in the leaf or needle content among the different treatments within the same seedlings' species, except for the paper birch and jack pine (Supplementary Information). However, it did not translate in height difference or root biomass increase across the treatment types, suggesting that this nutrient was not one of the key elements to account for the plant's growth (Fig. 2.5).

2.4.3 Seedling survival, seedlings root biomass and seedling height growth

Soil condition surfaces, lings are highly variable; depending on the season, they can have dry soil surfaces, wet stagnant surfaces, or saline crusted surfaces. All four tree seedling species – jack pine, tamarack, paper birch and willow – were selected based on their ability to grow under such variable conditions. Their pioneer qualities, tolerance to full sunlight and some drought, and their capability to populate oligotrophic and hypoxic sites are key to their resiliency (Kuzovkina & Quigley, 2005). Nevertheless, although these species (jack pine, tamarack, and paper birch) and the willow (Asian cultivar) may well be equipped with genotypes to sustain boreal forest climate and edaphic soil conditions, the study was able to identify which tree seedling species would be the most capable to survive and grow among agronomic herbaceous plants on a substrate such as mine tailing soil.

2.4.3.1 Jack pine

Among the four planted species, regardless of the applied treatment, results show that the jack pine seedlings had the lowest survival rate. The reasons for the poor jack pine survival on the mine soil could be related to the high mine tailing pH (ranging from 7.05 to 7.37) of this study. South (2016) conducted a review of studies on the optimum pH for growing pine seedlings. Based on the data, this author conclude that optimal pH would range from 4.5 to 5.0 for growing most common pine species (including jack pine) at sandy bareroot nurseries. Burger et al. (2007) conducted a study to evaluate seedlings responses of loblolly pine hybrid (*Pinus rigida* Mill. X *Pinus taeda* L.) on five different mine overburden mixes. Their results indicated that the loblolly pine hybrid grew much better on mine spoil that contained a high proportion of sandstone spoil rather than siltstone, and at a pH lower than 6.5. The authors indicated that pine growth decreased linearly as the pH increased within the 5.7 to 7.1 range.

Another reason that may also explain our results is that the mine tailing soil texture used for this study was not favorable to jack pine. Jack pines require a sandier soil with low organic matter (mineral soil) (South, 2017; USDA-NCRS, 2023). The combined effect of inadequate soil pH and soil texture may be to the reason for the jack pine's poor survival. The combined effect of inadequate pH and soil texture may have overshadowed any of the possible benefits of the applied treatments on the root biomass and seedling height (no significant difference within all applied treatment, Figs. 2.4 and 2.5).

Within the applied treatments, the probability of survival of jack pine showed a significant difference between the grass and legume treatments, but not between the grasses and the topsoil treatments. Since this was not translated by in any significant difference for the jack pine's growth or root biomass increases, it is difficult to explain these results without further research. Another avenue would be to study the interaction between grasses and jack pine seedlings at the rhizosphere level for possible mycorrhizal facilitation (Toju & Sato, 2018) between the two species.

2.4.3.2 Tamarack

Across all treatments, tamarack seedlings showed less mortality compared with paper birch, willow, and jack pine seedlings (Table 2.3). Tamarack seedlings showed a great resistance in surviving the mine tailing environment after two growing seasons.

However, it was within the topsoil treatment that the tamarack seedlings performed best (only one of 90 initial tamarack seedlings was reported dead). The height increment and root dry mass of tamarack seedlings were unaffected by the different applied treatments (except for topsoil > control). Similarly, the probability of survival also seems unaffected by the treatment type (except that it was significantly lower in the control treatment than all other treatments). Garbarino and al. (2010) used the deglaciation of the Ventina glacier as a surrogate to study spatial and temporal chronosequence establishment of the larch (*Larix decidua* Mill). Results showed that the age of trees was highly influenced by the terrain age or time since deglaciation and not by plant cover. Since the tamarack seedlings were planted in new mineral soil i.e., a soil that is “young soil” in the context of a chronosequence, their survival and growth may also not be affected by the plant cover (grass, mix or legumes), consistent with the results found in Garbarino et al. (2010) (Fig. 2.3).

2.4.3.3 Paper birch

Paper birch seedlings on the experimental site did not perform well. It is a species sensitive to harsh environmental conditions and pollution (USDA-NCRS, 2023). The control treatment (thickened tailings only) in which we planted 90 paper birch seedlings showed the highest mortality and a significant difference in survival probability compared to the topsoil treatment (Table 2.3, Fig.2.3). Those results may be attributed to a complexity of contributing factors such as low soil aeration and built-up salinity crust (field observations, results not shown), which may in return impede plant root development (Guittonny-Larchevêque et al., 2016a, 2016b). However, the main contributing factor that would explain our results is the low organic matter concentration found in the control treatment; 0.31% compared to 9.14% OM for the topsoil treatment (Table 2.2). Normal soil OM averages between 2 to 10% OM (Bot & Benites, 2005); the control treatment had very little OM, therefore little humic substances as well. Humic substances play an important role in binding metal(loid)s (Gagnon et al., 2020), as well as improving saline soil properties (Ouni et al., 2014). The combined effect of high pH with low humic substances may explain our results. The study of Gagnon et al. (2020) also demonstrated higher nutrient concentrations in paper birch plant tissue growing on gold mine tailing versus natural boreal forest soil (like topsoil) and corroborates our results. Our experiment was based on a very short study period (only two growing seasons); however, we note that on the scale of many generations, trees of the *Betula* genus can develop genetic adaptations to grow in a metal-contaminated region (Kirkey et al., 2012).

2.4.3.4 Willow cultivar

For the willow cultivar, the highest mortality was observed in the control treatment and the lowest in the topsoil treatment (Table 2.3). However, the probability of survival (Fig. 2.3) showed no significant difference between the control, mix and legume treatment, implying the ability of this species to survive within a soil that contains little organic matter, such as in the control and to a lower extent within the mix and legume treatment (Table 2.2). Willows are renowned to survived in harsh conditions and have been a preferred species in many phytoremediation projects for their abilities to survive in heavy metal spoils, organic contaminants, and polluted wetland areas (Kuzovkina et al., 2004a, 2004b; Yergeau et al., 2014). A study from Doty et al. (2009) have isolated beneficial nitrogen-fixing (diazotrophic) endophytes bacteria in the stem of black cottonwood (*Populus trichocarpa*) and willow (*Salix sitchensis*) that may explained the ability of these tree species to survive in limited N medium. Our study showed the willow species were able to survive and grow in all treatments including the control treatment and these endophytes microorganisms may have played a role that would explain our results. However, the willow cultivar in this study showed a significant higher root biomass in the 100% legume treatment compared to all the other treatments suggesting two possibilities. (1) The willow may have greatly benefited from the added N provided by the nitrification cycle processes of the legumes' decomposition. Jefferies et al. (1981) indicated that legumes are capable of N accumulation at a rate of approximately 100 kg ha^{-1} y^{-1} on derelict land, in a form which can be mineralized. The rhizospheres of the willow cultivars may be more capable to use this additional nitrogen whereas the other studied tree species grown in the legume treatment may not have the same genetic background to efficiently use the added nitrogen; or other parameters, such as soil pH, may have impeded the uptake of the additional nitrogen from the legumes' nitrification processes.

(2) Alternatively, the legumes' microorganism rhizosphere (including diazotrophic endophytes bacteria but not limited to *Rhizobium* sp.) provides a beneficial environment in which the willow unique genotype tree species is capable to harbor, exploit or interact to its full benefits (Afzal et al., 2019; Belimov et al., 2015; Doty et al., 2009; Mastretta et al., 2006; von Wuehlisch, 2011).The results of this study suggest this may be an important avenue for future research. Not all legume species act the same regarding nitrogen transfer, nor do all willow species have the same ability to interact efficiently with the legumes. However, within the realm of the study parameters, the willow cultivar and the legume treatment showed the most promising results for improving the soil conditions and may facilitate an easier natural establishment of other plant species.

2.5 Conclusion

This study demonstrates a positive interaction between the legume treatment and a willow cultivar despite only a few years of growth on anthropogenic gold mine tailings. Further investigation is required to determine if the increased willow biomass is associated with the nitrification benefits in the legume treatment or the diazotrophic microorganisms enabled by the legumes' rhizosphere environment.

The study did not show soil contamination with heavy metals after three years of weathering conditions. However, monitoring and further analysis would be needed to verify if the concentration of toxic chemicals may accumulate in the plant tissue. This study did not show a clear distinction between the grasses, legumes, and mixed treatments in terms of the soil physico-chemical properties. For the seedling growth, the willow was the species that benefitted most from the legume treatment. The foliar analysis shows that N may be appropriated by the agronomic grass, leaving little in the rhizosphere area for seedlings. Depending on the mine reclamation objective, the combination of the willow clones with the legume treatment may be a good first step to facilitate the natural succession of pioneer tree establishment.

Further studies at the site will evaluate the effects of grass and legume treatments on the mine tailings' plant biodiversity colonization on a mine tailing in situ conditions, as well as the microclimate and its potential for tree seed germination.

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CHAPITRE 3

THE EFFECTS OF AGRONOMIC HERBACEOUS PLANTS ON THE FLORISTIC COMPOSITION AT AN EARLY SUCCESSIONAL STAGE ON GOLD MINE TAILINGS

(EFFETS DES PLANTES HERBACÉES AGRONOMIQUES SUR LA COMPOSITION FLORISTIQUE EN DÉBUT DE SUCCESSION VÉGÉTALE SUR DES RÉSIDUS MINIERS D'UNE MINE D'OR)

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Abstract

Agronomic herbaceous plants are often used as a first step in the reclamation process of mine tailings during and/or after mine closure, including sites surrounded by forest ecosystems. Yet, there is a lack of research on how different compositions of agronomic plants can facilitate or impede (via competition for resources) the restoration of the site to a forested state. This study aims at evaluating which agronomic herbaceous plant treatment (grasses, legumes, or a mix of the two) will better promote plant diversity and volunteer establishment of early-successional boreal forest tree species at an early stage of the revegetation process. In 2013, an experimental area of one hectare of tailings was set up at a gold mine site in the Abitibi-Témiscamingue, Québec, Canada. The study site was subdivided into three blocks, each divided into five plots. Each plot was randomly seeded with one of the following treatments: 100% grass, 100% legumes, a mixture of both, topsoil, and a control (tailings only, no seeding). Treated plots were further divided into 10 quadrats to evaluate the effects of the treatments on volunteer plants colonization and its diversity.

Results on the volunteer plants evaluation in the treated plots indicated a significantly higher ($p < 0.05$) species richness in the topsoil treatment compared to the agronomic herbaceous treatment and the control. However, no significant difference was found for the species richness among the three agronomic herbaceous treatments. The Shannon and Simpson diversity indices showed no significant difference between the topsoil treatment and the three agronomic herbaceous treatments, except for the Shannon index being higher in the topsoil relative to the legume treatment. We found a significant difference in species composition assembly between treatments (PERMANOVA with $R^2 = 0.60$, $p < 0.001$). The topsoil plant community was dominated in abundance by introduced species (weeds). The community plant composition in the legume treatment was also different from those of the mix and grass treatments. One species, oakleaf goosefoot (*Oxybasis glauca* L.), was much more abundant in the legume treatment than in any other treatment, decreasing its diversity indices. The legume treatment also showed more volunteer pioneer trees of the Salicaceae family than the grass and mix treatment. These results suggest that when using an agronomic herbaceous seed mix for the first stage of mine tailings revegetation, a greater proportion of legumes rather than grasses may promote the establishment of deciduous pioneer trees.

Keywords: anthropogenic soils, biodiversity, grasses, legumes, mine tailings reclamation, topsoil.

Résumé

Les plantes herbacées agronomiques sont souvent utilisées comme première étape dans le processus de valorisation des résidus miniers pendant et/ou après la fermeture d’une mine, y compris sur les sites entourés d’écosystèmes forestiers. Cependant, il y a un manque de recherche sur la façon dont différentes compositions de plantes agronomiques peuvent faciliter ou entraver (via la compétition pour les ressources) la restauration de la forêt à un site. Cette étude vise à évaluer quel traitement agronomique des plantes herbacées (graminées, légumineuses ou un mélange des deux) favorisera le mieux la diversité végétale et l'établissement volontaire d'espèces d'arbres de forêt boréale en début de succession à un stade précoce du processus de revégétalisation. En 2013, une zone expérimentale d'un hectare de résidus a été aménagée sur le site d'une

mine d'or en Abitibi-Témiscamingue, Québec, Canada. Le site d’étude a été subdivisé en trois blocs, chacun divisé en cinq parcelles. Chaque parcelle a été ensemencée au hasard avec l'un des traitements suivants : 100 % de graminées, 100 % de légumineuses, un mélange des deux, une couche de *topsoil* et un témoin (résidus seulement, pas d'ensemencement). Les parcelles traitées ont ensuite été divisées en 10 quadrats pour évaluer les effets des traitements sur la colonisation des plantes spontanées et sur leur diversité.

Les résultats de l’étude des plantes spontanées dans les parcelles traitées ont indiqué une richesse spécifique significativement plus élevée ($p < 0{,}05$) dans le traitement de *topsoil* par rapport aux traitements d’herbacées agronomiques et au témoin. Cependant, aucune différence significative n’a été trouvée pour la richesse spécifique entre les trois traitements d’herbacées agronomiques. Les indices de diversité de Shannon et de Simpson n'ont pas montré de différence significative entre le traitement de *topsoil* et les trois traitements d’herbacées agronomiques, à l'exception de l’indice de Shannon qui était plus élevé dans le traitement de *topsoil* par rapport à celui des légumineuses. Nous avons trouvé une différence significative dans l'assemblage de la composition en espèces entre les traitements (PERMANOVA avec $R^2 = 0{,}60$, $p < 0{,}001$). La communauté végétale du *topsoil* était dominée en abondance par des espèces introduites (mauvaises herbes). La composition de la communauté végétale dans le traitement de légumineuses était également différente de celle du traitement de graminées et du mélange. Une espèce, le chénopode à feuilles de chêne (*Oxybasis glauca* L.), était beaucoup plus abondante dans le traitement de légumineuses que dans tout autre traitement, diminuant ainsi ses indices de diversité. Cependant, le traitement de légumineuses comprenait plus d’espèces d’arbres pionniers spontanés de la famille des Salicacées que le traitement de graminées et le mélange. Ces résultats suggèrent que lors de l’utilisation d’un mélange d’herbacées agronomiques pour la première étape

de la revégétalisation des résidus miniers, une plus grande proportion de légumineuses plutôt que de graminées peut favoriser l'établissement d'arbres feuillus pionniers.

Mots clés : biodiversité, graminées, légumineuses, sols anthropogéniques, *topsoil*, valorisation des résidus miniers.

3.1 Introduction

Mine closure is an important milestone in achieving sustainable development (Limpitlaw, 2004). It is now expected, socially, economically, and ecologically, that the closure of the mine will no longer be the vast abandoned land of the past but will instead be a safe and unpolluted rebuilt land. Ideally, post-mining sites aim for the use of viable lands, such as forest or agricultural land (Limpitlaw, 2004). Successful revegetation of mine sites is not only measured by their forested cover but also by the diversity of species, towards achieving self-sustaining ecosystem (Koch & Hobbs, 2007). However, starting from an anthropogenic reconstructed soil to a self-sustaining ecosystem is scientifically and technologically challenging.

Gold-mine tailings soil is a mineral waste consisting of fine-grained particles after the valued commercial ore has been removed (Lottermoser, 2007). This mineral soil is characterized by no organic and humic substances, poor structure and texture, and no nutrient cycles, providing a difficult substrate for most vascular plants to grow in (Bussière & Guittonny, 2020; Guittonny-Larchevêque & Pednault, 2016). In the absence of vegetation, mine tailings are subject to wind and waterborne erosion. To mitigate the erosion, one common practice is to sow agronomic herbaceous plants, such as graminoids (grasses) and/or leguminous (legumes), which have been selected for their ability to grow in poor nutrient substrates (Guittonny-Larchevêque et al., 2016b). Another method consists of using organic topsoil to cover the tailings (Huang et al., 2012) or amending the tailings' surface with different organic wastes such as pulp and paper wastes (Young et al., 2015), manure compost, green compost, and sewage sludge (Asensio et al., 2013; Chiu & Wong, 2006; Guittonny-Larchevêque & Pednault, 2016; Rakotonimaro et al., 2018). However, the topsoil and/or amended tailings may be expensive and less available; as a result, direct sowing of agronomic herbaceous plants is usually the preferred method (Nyenda et al., 2020).

Understanding the effects of sowing agronomic herbaceous plants, for example, using only grasses, legumes, or a mix of the two, on mine soil is important to predict what will be the resulting ecological community as well as the revegetation success of the mine soil (Keddy, 1992). Keddy (1992) proposed assembly and response rules with the goals of predictive community ecology. For example, if the species pool (presence or absence and abundance) is submitted to an environmental filter (mine tailings) and biotic filters (seeded plants), only certain species capable of tolerating the mine habitat conditions would be capable of forming a community. Many studies have shown that higher plant diversity provides better resilience against environmental change, such as climate change (Nelson et al., 2014) or alien invasive species (Eastburn et al., 2018), and improves nutrient cycling and ecosystem services (Errington & Pinno, 2015; Kirmer et al., 2012; Mace et al., 2012; Munford et al., 2020). Consequently, understanding and measuring early vegetation establishment and its evolution as well as competitive weed establishment are essential to determine the trajectory of the vegetation recovery and ecosystem assemblage community. Past research has mostly focused on the microbiology biodiversity of the mine soil (Londry & Sherriff, 2005) or the vegetation's biodiversity long after the mines have been closed (Anawar et al., 2013). However, little is known about plant species diversity during the early stage after seeding of agricultural herbaceous plants.

In a boreal forest context, the agronomic herbaceous treatments that would favor a higher quantity and diversity of pioneer tree regeneration and would keep invasive, competitive species at bay, should be the preferred treatment for future reclamation efforts. Many studies have shown that agronomic herbaceous plants are competitive towards naturally occurring tree seedlings. For example, Bouchard et al. (2018) have shown that agronomic herbaceous plants that were hydroseeded on mine waste rock were competitive towards spontaneous tree seedling establishment (*Salix* and *Populus*). Furthermore, Wade (1989) found that a reclamation mix (mostly comprised of grasses) was competitive towards a forest topsoil seed bank, producing a community with less total biomass, and fewer established native species than the one without the reclamation mix. Burger et al. (2017a) recommended a suitable ground cover such as natural topsoil or a plant cover compatible with trees rather than agricultural grasses and legumes that are often too competitive towards natural volunteer plants. We further note that invasive, competitive species undermine trees seedlings growth by competing for nitrogen (N) and water specially in low-N soil environments (Remaury et al., 2019; Woods & Smethurst, 1992).

Given the low-N conditions prevalent in most derelict land, the N fixating capacity of legumes has motivated their use in mine restoration studies. Jefferies and Bradshaw (1981) found that the use of legumes (*Trifolium repens*) grown with grasses can be effective in improving soil fertility. Domingo and David (2014) using herbaceous legume plants as ground cover to revegetate copper mine tailings, demonstrated good growth rate of the legumes despite the heavy copper metal concentration, the poor quality of the organic matter and the low plant nutrient content of the mine tailings substrate. Maiti and Maiti (2014) did a study using a mix of grass-legume cover on a waste from an integrated sponge iron plant. They used grasses as an annual mulch and legumes to restore soil moisture, and to enrich soil N and organic matter via decomposition of above and below ground plant components.

In this study, we measure and compare various indicators of plant community development on gold mine tailings revegetated with different treatments: a fine layer of topsoil, three agronomic herbaceous treatments (tailings seeded with 100% grasses, 100% legumes, or a mix of the two), as well as control plots with no seeding or amendment. Specifically, our objectives are (1) to compare the vascular plant species richness, biodiversity, and abundance four years after the application of the revegetation treatments; and (2) evaluate which treatments provide higher colonization of early successional shrub and tree species, as an important indicator of the possible evolution trajectory towards the native forest ecosystem. We hypothesize that apart from the topsoil treatment – which is expected to have the highest diversity due to higher nutrient amounts and pre-existing seed banks – the legume treatment will have more diverse and abundant species due to the N input from the legumes. We also hypothesize that this N input from the legumes will result in a greater abundance of early successional tree species, compared with the grasses that would compete with the trees for the limited N available.

3.2 Materials and methods

3.2.1 Mine site

The *in-situ* study site has been set up at an open-pit gold mine currently active and located at the south of the city of Malartic, in the Abitibi-Témiscamingue region of northwestern Québec, Canada (48° 06’N, 78° 08’ W) (Fig. 3.1). The mine site is within the boreal forest region with a climate characterized by long, cold winters, with short, moderately warm summers. The mean annual rainfall is 985 mm (1981-2010 data period), and a daily average temperature of -17 °C for January and 17 °C for July

(Government of Canada, n.d.). The area is included within the last glacial advance of Wisconsin glaciation region (Vincent & Hardy, 1977) with a dominant gray luvisol soil type (Soil classification working group, 1998). The study site is within the boreal and bioclimatic domains of the balsam fir (*Abies balsamea* (L.) Miller) - paper birch (*Betula papyrifera* Marshall) ecozone (MFFP, 2016).

Figure 3.1 Location of the experimental site (red star) within the Abitibi-Témiscamingue region of Quebec, Canada (Source: OpenStreetMap).

3.2.2 Experimental site description and design

The experimental site area was set up on a one-hectare area of moraine substrate that was set aside by the mine. Mine roads surround the north and west side of the experimental site, separating it (on the west) from patches of boreal forests consisting mainly of black spruce (*Picea mariana* (Miller) BSP) and balsam fir (MFFP, n.d.). Waste rock berms surround the east side of the experimental site.

In May 2013, thickened tailings (50–70% solids by mass at deposition) were withdrawn within the thickened tailings produce by the mine and applied to the 1-ha experimental site. Thickened tailings were mainly composed of 86% mineral particles with a diameter < 80 µm and contain low concentrations of neutralizing minerals and total sulfur (~ 1% S); the ore at the mine site consisted of a mineralized greywacke.

The experimental setting was organized in 2013 in a randomized complete block design, consisting of three blocks, each subdivided into five experimental plots (20 m x 15 m) placed 5 m apart. The following five treatments were randomly applied each to one plot per block:

1) Grasses (100% perennial Poaceae)

2) Legumes (100% perennial Fabaceae)

3) Mix of grasses (55% of seed mass) and legumes (45% of seed mass)

4) Topsoil amendment (details in Sect. 3.2.3)

5) Control (thickened tailings only)

From May 25 to June 9, 2015, 10 quadrats (1 m x 1 m) were delimited in each plot to measure volunteer plant colonization by seed rain, as we describe in detail in section 3.2.4.2 (Floristic survey).

3.2.3 Soil materials

Prior to the gold mine exploitation, the existing forest stand was harvested, and the upper layers of topsoil and its underneath mineral soils were salvaged and stockpiled (for 30 to 36 months) in two separate 7-m-high piles (2.5H:1 V slope) for future use. The topsoil stockpile consisted of the first 30-cm dark soil layer (O- and A-horizons). The forest soil prior to mining was classified as a luvic gleysol. In May 2013, the topsoil stockpile was excavated and some of the material spread over tailings in the topsoil plots (with a target thickness of 2 cm) according to the experimental site design. Detailed chemical analyses of the topsoil amendment as well as the thickened tailings (control) substrate are reported in our previous study at the same site (Barrette et al., 2022).

3.2.4 Plant materials

3.2.4.1 Agronomic herbaceous seeds

In June 2013, commercial agronomic herbaceous seeds (forage seeds) were sown by Lanexco Inc. (Dubuisson, QC, Canada) on the freshly hand raked tailings according to the experimental design. An annual nurse crop of barley seeds (*Hordeum vulgare* L.)

was added to all seed mixes to stabilize the soil and reduce the establishment of weeds (Espeland & Perkins, 2013). The legume treatment included white clover (*Trifolium repens* L., 45% by mass), bird's-foot trefoil (*Locus corniculatus* L., 45%) and barley (10%). A soil bacterium *Rhizobium* inoculant was applied to the legume seeds to facilitate N fixation. The grass treatment was composed of ryegrass (*Lolium perenne* L., 40% by mass), redtop (*Agrostis gigantea* Roth, 40%), reed canary grass (*Phalaris arundinacea* L., 10%) and barley (10%). The mix grass/legume treatment was composed of 25% reed canary grass, 25% ryegrass, 20% white clover, 20% bird's foot trefoil and 10% barley. Plots received seeds at a rate of 100 kg ha^{-1} as well as one application of mineral fertilizer 8–32–16 (N, P, K) at a 750 kg ha^{-1} rate to the plots receiving agronomic seed treatments. A commercial MYKE® promycorrhizal inoculant (Premier Tech biotechnologies, Rivière-du-Loup, Canada) was also added to the agronomic seed treatment plots in accordance with the manufacturer application chart.

3.2.4.2 Floristic composition survey

At the end of 2016 growing season, the fourth year after seeding, we determined the floristic composition of the 10 quadrats/plot used for volunteer colonization (10 quadrats/plots x 15 plots = 150 quadrats). A square wood frame of 1 m^2 was subdivided with elastic bands into 25 squares of 20 x 20 cm and was placed over each quadrat. Every vascular plant falling into each 20 x 20 cm subquadrat was identified, numbered, and recorded; the bryophyte percent cover (fraction of subquadrats with presence) was recorded for each plot, but bryophytes were not identified to a more precise level.

Once the data were gathered, we excluded any individuals that were too young to clearly identify to the species, for example a young two-leaf graminoid plant that could be confused with other grasses species. Vegetation that was mature enough was generally identified to the species level, except for some taxa that were identified to the genus level (see Table 3.1). Species that could not be identified on the experimental site were brought to the university lab for better identification using different Canadian identification guides such as VASCAN (Brouillet et al., 2022) and *"Fleurs sauvages du Québec"* (2014). All sown plants (grasses, legumes) listed in paragraph 3.2.4.1 were excluded from the counts (bird foot trefoil, rye grass, red top, reed canary grass, white clover).

3.2.5 Statistical analyses

The abundance of individuals in each species within the 10 1-m^2 quadrats in each plot were summed up for statistical analyses. For each plot, we calculated the species richness (number of individual species present), the Shannon diversity index and Simpson diversity index. An ANOVA was done to compare these three-diversity metrics among treatments, followed by post-hoc comparisons with Tukey's range test. We performed visual checks of the approximate normality and homoscedasticity of the ANOVA residuals. To visualize differences in species composition between treatments, we used a principal coordinate analysis (PCoA) based on the Bray-Curtis distance and tested the significance of compositional differences between treatments with a permutational multivariate ANOVA (PERMANOVA). All analyses were performed in R version 4.1.3 with RStudio version 1.0.136 (RStudio Team, 2016). We used the 'vegan' package in R (Oksanen et al., 2022) to calculate diversity indices and to perform the multivariate analyses, and the ‘emmeans’ package (Lenth, 2022) for post-hoc comparisons.

3.3 Results

3.3.1 Plants species composition and richness on the overall experimental setting

Four growing seasons from 2013 to 2016 were provided for the plants to colonize the experimental setting. This means little time was given for ecological succession and processes. Despite the limited time, a total of 27 volunteer vascular plant species were identified, belonging to twelve families (Table 3.1). Of the 27 species, 18 fell within the perennial forb (H) and annual forb (A/H) ecological guild categories, which are defined as species that require six hours or more of sunlight and are vascular plants with nonpersistent woody stems. The other species included six perennial grasses (G), two pioneer trees (PT) and one perennial shrub (S). Based on the USDA PLANTS Database (USDA-NCRS, 2023) and VASCAN (Brouillet et al., 2022) plant databases, 11 of the 27 species found are introduced (non-native to the study region) and 16 are native.

Table 3.1 Vascular plant species, families and ecological guilds surveyed within the overall experimental field.

Species code	**Scientific name**	**Common name**	**Family**	**Ecological guild**	**Native status**
A	*Aster* spp.	Hardy aster	Asteraceae	H	N
C	*Carex* spp.	Sedges	Cyperaceae	H	N
CF	*Cerastium fontanum* Baumg.	Mouse-ear chickweed	Caryophyllaceae	H	INT
EA	*Equisetum arvense* L.	Common sowthistle	Equisetaceae	H	N
EG	*Euthamia graminifolia* (L.)	Flat-top goldentop	Asteraceae	H	N
ER	*Elymus repens* (L.) Gould	Quackgrass	Poaceae/ Gramineae	G	INT
FA	*Festuca arundinacea* Schreb.	Tall fescue	Poaceae/ Gramineae	G	INT
FV	*Fragaria vesca* L.	Woodland strawberry	Rosaceae	H	N
J	*Juncus* spp.	Rushes	Joncaceae	H	N
OG	*Oxybasis glauca* (L.)	Oakleaf goosefoot	Chenopodiaceae	A/H	N
P	*Populus* spp.	Populus	Salicaceae	PT	N
PC	*Pilosella caespitosa* (Dumortier)	Meadow hawkweed	Asteraceae	H	INT
PM	*Plantago major* L.	Broadleaf plantain	Plantaginaceae	H	INT
PO	*Poa compressa* L.	Canada bluegrass	Poaceae/ Gramineae	G	INT
PP	*Phleum pratense* L.	Timothy (grass)	Poaceae/ Gramineae	G	INT
PV	*Panicum virgatum* L.	Switchgrass	Poaceae/ Gramineae	G	N
RI	*Rubus idaeus* L.	Red raspberry	Rosaceae	S	N
S	*Salix* spp.	Willow	Salicaceae	PT	N
SC	*Scirpus cyperinus* (L.) Kunth	Woolgrass	Cyperaceae	H	N
SM	*Sisyrinchium montanum* Greene	Strict blue-eyed grass	Iridaceae	H	N
SO	*Sonchus oleraceus* L.	Common sowthistle	Asteraceae	A/H	INT
SR	*Spergularia rubra* (L.)	Red sandspurry	Caryophyllaceae	H	INT
SU	*Solidago uliginosa* Nutt.	Bog goldenrod	Asteraceae	H	N
TF	*Tussilago farfara* L.	Coltsfoot	Asteraceae	H	INT
TO	*Taraxacum officinale* F.H.	Common dandelion	Asteraceae	H	INT
TS	*Trisetum spicatum* (L.)	Spike trisetum	Poaceae/ Gramineae	G	N
WP	*Lathyrus palustris* (L)	Marsh pea	Fabaceae/Leguminosae	H	N

Note. Ecological guild. A/H = annual forb; G = perennial grass; H = perennial forb; S = perennial shrub; PT = pioneer tree. Native Status. INT = introduced, N = native.

Table 3.2 Abundance of each vascular species in 30 1-m2 quadrats (10 quadrats x 3 plots) for each treatment. The last rows indicate the total vascular plant abundance, species richness, native species richness, as well as the percentage of 20 x 20 cm sub-quadrats where bryophytes were present.

	Scientific name	**Topsoil**	**Grasses**	**Mix**	**Legumes**	**Control**
A	*Aster spp.*	52	14	25	0	0
C	*Carex spp.*	74	0	0	0	0
CF	*Cerastium fontanum*	9	0	0	0	0
EA	*Equisetum arvense*	14	0	2	0	0
EG	*Euthamia graminifolia*	96	2	17	2	0
ER	*Elymus repens*	7	4	0	0	0
FA	*Festuca arundinacea*	98	18	0	1	0
FV	*Fragaria vesca*	104	0	2	0	0
J	*Joncus spp.*	4	0	0	11	0
OG	*Oxybasis glauca*	46	39	5	301	3
P	*Populus spp.*	1	0	0	3	0
PC	*Pilosella caespitosa*	85	32	2	2	0
PM	*Plantago major*	132	3	3	9	0
PO	*Poa compressa*	28	1	0	0	0
PP	*Phleum pratense*	4	0	2	1	0
PV	*Panicum virgatum*	19	0	0	0	0
RI	*Rubus idaeus*	1	0	0	0	0
S	*Salix spp.*	0	7	2	4	0
SC	*Scirpus cyperinus*	17	0	0	0	0
SM	*Sisyrinchium montanum*	2	0	0	2	0
SO	*Sonchus oleraceus*	22	13	3	2	0
SR	*Spergularia rubra*	2	0	0	61	0
SU	*Solidago uliginosa*	27	0	0	0	0
TF	*Tussilago farfara*	36	18	4	2	0
TO	*Taraxacum officinale*	7	1	0	0	0
TS	*Trisetum spicatum*	2	1	0	0	0
WP	*Lathyrus palustris*	21	0	0	0	0
	Total abundance	910	153	67	401	3
	Species richness	26	13	11	13	1
	Native species richness	15	5	6	6	1
	Bryophyte presence (%)	1	83	68	19	< 1

3.3.2 Effect of the experimental treatments on abundance, richness, and diversity of the volunteer vascular plants

The abundance of each species per applied treatment is recorded in Table 3.2. The topsoil treatment (positive control) had the highest total abundance (910 individuals), followed by the legume (401), the grass (153), the mix (67), and finally the control (3) treatment where few to no plants were observed in each plot. The topsoil treatment also presented the highest species richness (26 species across three plots, including 12 native species), whereas all three agronomic herbaceous plant treatments showed a similar level of species richness (11 to 13 species, 5 to 6 being native).

Among all pioneer tree species of the boreal forest surrounding the mine, only *Salix* sp. and *Populus* sp. were found as volunteer trees on the experimental field (Table 3.2). Volunteers of the genus *Salix* spp. (S), were found in the grass, mix and legume treatments, but not in the topsoil treatment. One individual of the genus *Populus* spp. (P) was found in the topsoil treatment and three were found in the legume treatment.

Table 3.3 compiles the Shannon and Simpson diversity indices of the volunteer vascular plants in each applied treatment. We note that one of the three control plots had no plant individuals recorded, so the species richness was 0 and the diversity indices were undefined; the Shannon and Simpson indices in the other two plots are 0 since a single species was present. Overall, the ordering of the treatments was similar for both Shannon and Simpson diversity, whether counting all species or native species only: the diversity was highest for the topsoil, followed by the grass and mix treatments, then the legume treatment and finally the control (Fig. 3.2). However, the only significant differences in the post-hoc test were found between the topsoil and some of the other treatments: the topsoil biodiversity was greater than that of the control under all metrics; then that of the legume treatment for all metrics except the Simpson index (all species); and finally, greater than that of the grass and mix treatments for the Shannon index (native species). The lower values of diversity for the legume treatment compared to the grass and mix treatments with similar species richness can be explained by the dominance of one plant species: oakleaf goosefoot (*Oxybasis glauca* L.), which accounted for approx. 75% of individuals in the legume plots. For the species richness, there was a significant difference between the topsoil (with a mean of 17 species and 9 native species per plot) and the four other treatments (each having a mean equal or less than 6 species and 3 native species per plot), with no significant difference among the latter (Fig. 3.3).

Table 3.3 Mean and standard deviation (between parentheses) of the species richness, Simpson index and Shannon index calculated at the plot level between treatments (n=3).

Treatment	Species richness	Simpson	Shannon
Topsoil	17.00 (1.00)	0.87 (0.04)	2.29 (0.22)
Grasses	6.00 (5.00)	0.48 (0.43)	1.07 (1.02)
Mix	5.00 (1.70)	0.57 (0.11)	1.14 (0.30)
Legumes	5.70 (2.10)	0.33 (0.24)	0.68 (0.46)
Control	0.67 (0.58)	0.00 (0.00)	0.00 (0.00)
All treatments included	6.87 (6.02)	0.48 (0.34)	1.11 (0.88)

Note. The Simpson and Shannon diversity results exclude one of the three control plots which had no individual recorded.

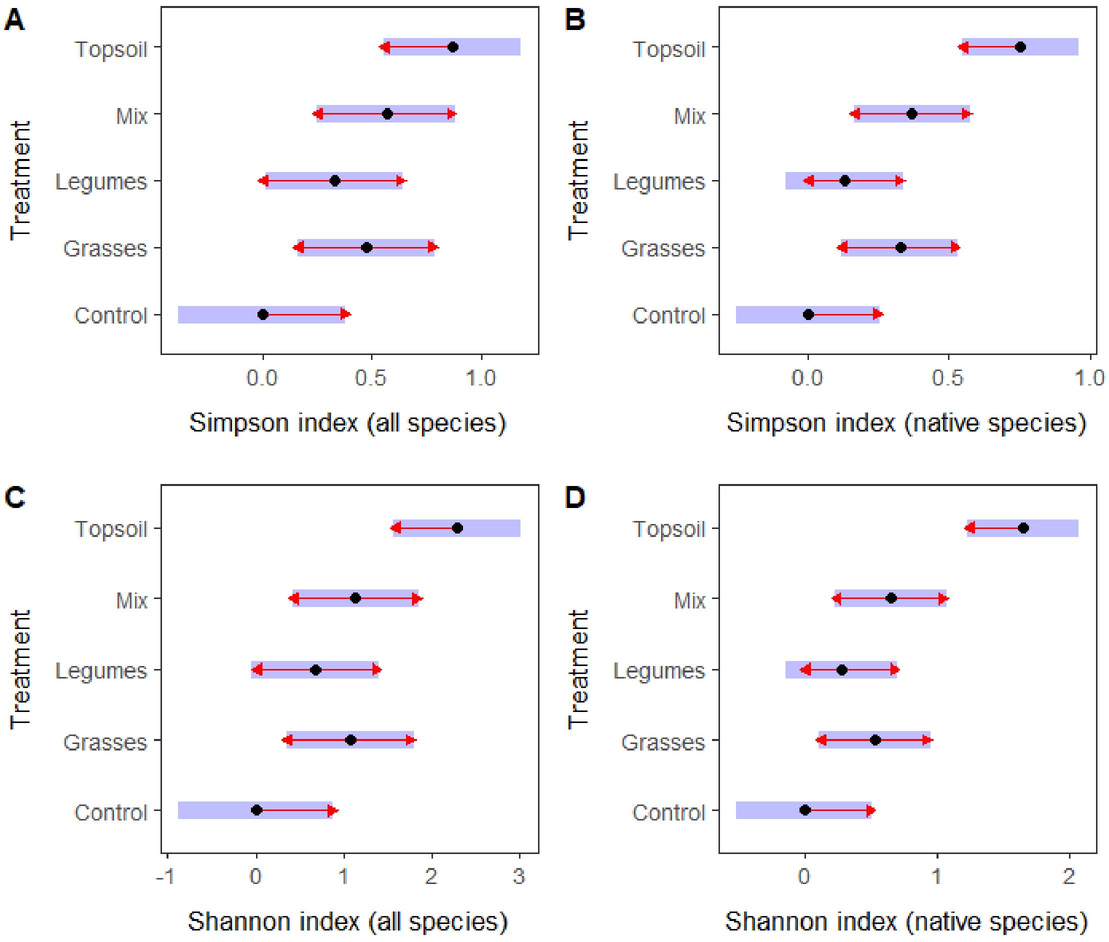

Figure 3.2 Estimated mean (black dot), 95% confidence interval (blue line) and post hoc test results (red arrows) of the Simpson and Shannon diversity indices for all species and native species per treatment. When the red arrows are overlapping along the x-axis, the mean values are not significantly different between those treatments.

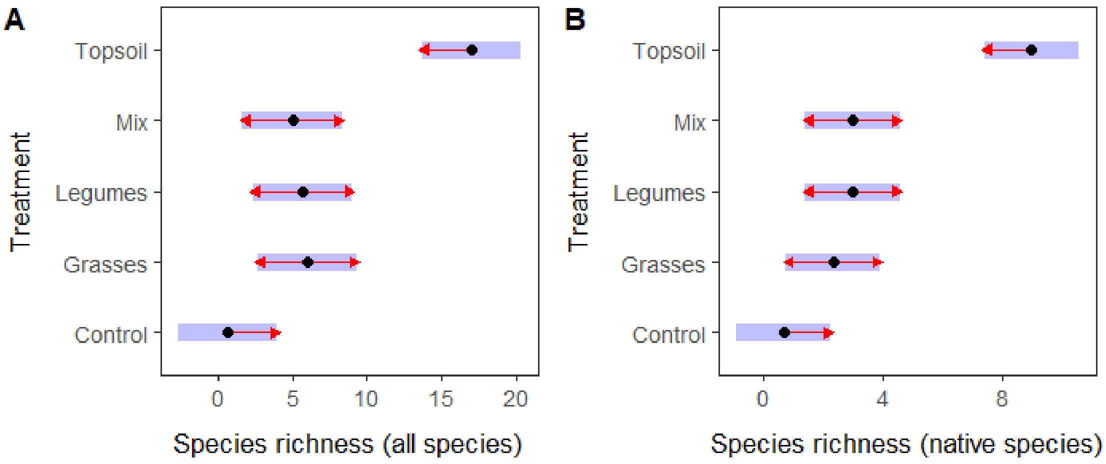

Figure 3.3 Estimated mean (black dot), 95% confidence interval (blue line) and post hoc test results (red arrows) for the species richness and native species richness per treatment. When the red arrows are overlapping along the x-axis, the mean values are not significantly different between those treatments.

3.3.3 Multivariate data analysis

The results of the principal coordinate analysis show a differentiation of the species composition between treatments (Fig. 3.4), as the plots for at least some of the treatments appear as close clusters in the PCoA plot. A significant difference in plant composition between treatments was also confirmed by the PERMANOVA ($R^2 = 0.60$, $p < 0.001$). The control plots (excluding the one with 0 abundance) are in the middle-left section, opposite to all species arrows, reflecting the near absence of vegetation. The grass and mix treatment plots (blue and green points) are more dispersed and appear in the line between the control and the topsoil, which may reflect sharing of some plant species with the topsoil plots, but an overall lower abundance and diversity.

The topsoil plots (red points, bottom-right corner) are in the direction pointed to by many species arrows, reflecting the higher richness and abundance in that treatment. The legume plots (top right corner) are differentiated from the other treatments and appear strongly associated with a few species, including two of the pioneer trees (S for *Salix* sp. and P for *Populus* sp.) and the previously mentioned oakleaf goosefoot (OG).

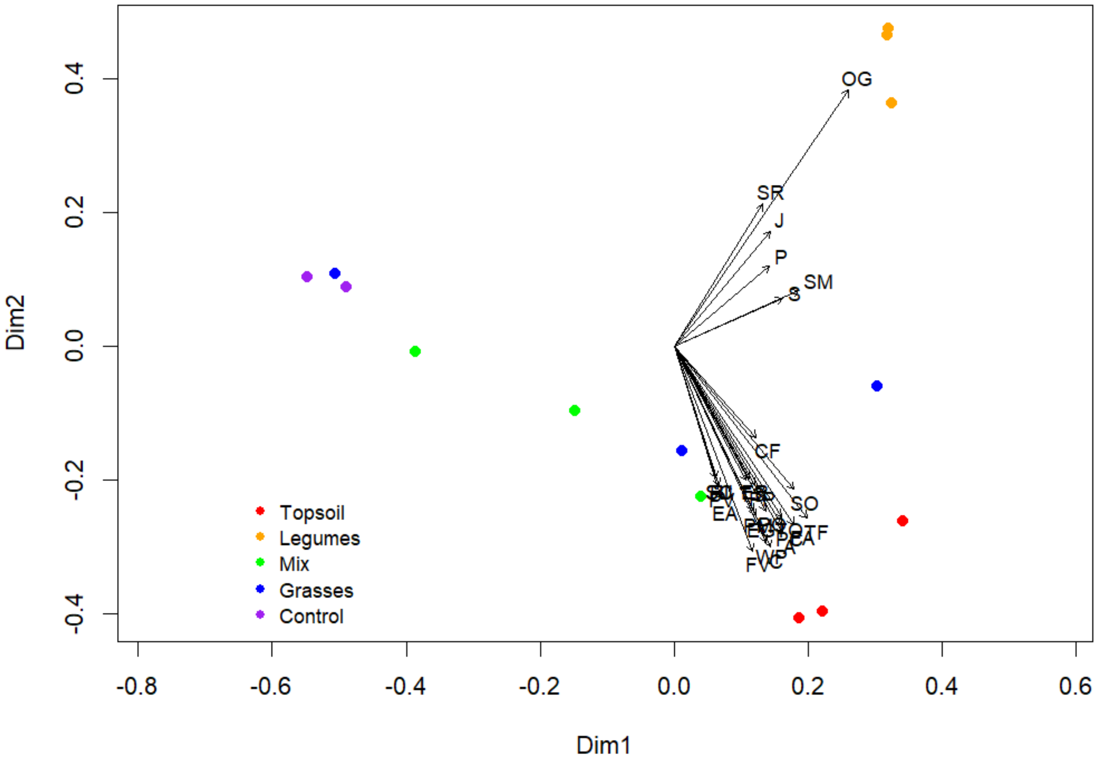

Figure 3.4 Principal Coordinate Analysis (PCoA) of the plant species composition based on the Bray-Curtis distance between plots, with point colors indicating the treatment applied to each plot and arrows representing the correlation of each species' abundance with the PCoA axes. Species codes at the end of arrows correspond to those in Table 3.1.

3.4 Discussion

3.4.1 Overall plant community and diversity in the experimental field

Most of the plant species surveyed were part of the perennial forb ecological guild (Table 3.1). The overall plant community assembly found at our experimental field was similar to that of another study by Gagnon et al., (2021), in the same province (QC, Canada), at the same latitude, as well as on non-acidic mine tailings (Niobec site). Their plant survey showed that forbs were the predominant ecological guild consisting of 12 families with Asteraceae being the most abundant, our study showed the forbs were the most important ecological guild with 18 families and Asteraceae also being the most abundant.

The different treatments including the mine tailings soil act as an ecological filter for plants that have functional traits capable of sustaining high levels of light exposure and low levels of soil nutrients, creating a different plant assembly than found in other common boreal forest disturbances such as wildfire, windthrow, or clearcutting (Dhar et al., 2018; Gagnon et al., 2021; Pinno & Hawkes, 2015; Roberts, 2004). The soil pH also affects the distribution and niches of boreal plant species establishment (Calvo-Polanco et al., 2017; Parraga-Aguado et al., 2013), but unlike more acidogenic gold mine tailings in the same region (e.g., Gagnon et al., 2020), the pH at our site was close neutral for all treatments (7 to 7.5, Barrette et al., 2022).

3.4.2 Effect of the experimental treatments on abundance, richness, and diversity

3.4.2.1 Topsoil treatment

The greater abundance and species richness of the topsoil treatment (Figs. 3.2 and 3.3, Table 3.3) was anticipated (see hypothesis section) as previous studies also support our findings (Cooke & Johnson, 2002; Lindemann et al., 1984; Skousen et al., 2011). The topsoil treatment had several advantages for the development of volunteer vegetation, given its higher content in organic matter and N (Barrette et al., 2022 for a previous study at this site), and may have contained buried seeds prior to our experiment. Nevertheless, the overall diversity measured by either the Shannon or Simpson index was not significantly different between the topsoil and the grass or mix treatment, and only the Shannon index was significantly higher in the topsoil than in the legume treatment. We note that among the five most abundant species in the topsoil plots: the broadleaf plantain (132 individuals), the woodland strawberry (104), the tall fescue (98), the flat-top golden (96) and the meadow hawkweed (85), only the woodland strawberry is a native species. Therefore, four out of five dominant volunteer species are introduced. Introduced species (in this research the term introduced refers to non-native plants that are often labelled as naturalized plants but are invasive (spread) and cause ecological disruption to natural ecosystems) are notoriously more competitive, their physical and/or chemical structure makes them better strategists to spread over a territory, precluding local diversity (Lloyd et al., 2002; Singh et al., 2021; Tischew et al., 2011). For example, the meadow hawkweed with its dense basal rosettes leaves makes patches that potentially displace other plants; research has also shown that it may be allelopathic, i.e. producing chemical agents to suppress competing species (Murphy, 2001).

3.4.2.2 Agronomic herbaceous plant treatments

Although the legume treatment did not result in a greater species richness or diversity than the other herbaceous treatments, it showed a greater abundance of vascular plants in general and pioneer trees, in line with our initial hypotheses (Table 3.2, Fig. 3.4). We associate these results to the N input provided by the decomposition of the N-rich legume plant organic components (aerial and rhizosphere parts), especially crucial in a N-deprived environment such as mine tailings soil. The N supplementation provided by legumes to other plants in their vicinity is confirmed by other studies (Domingo & David, 2014; Ledgard & Steele, 1992; Maiti & Maiti, 2014). Due to the small number of pioneer trees present and their absence from many of the plots and treatments, we could not perform a formal statistical analysis to test for differences in their abundance; therefore, these findings should be considered preliminary.

The lower diversity index of the legume plots is explained by the dominance of a single species, the oakleaf goosefoot. We note that even though we classified this plant as native to Canada in accordance with the USDA plant database, VASCAN (Canadian database) lists it as introduced. Had we chosen to list it as introduced, it would have probably changed our conclusions regarding the native species diversity. In their study, Newman and Redente (2001) showed that a native seeds mixture, compared to an introduced seeds mixture, tends to produce greater species richness and more aboveground biomass. Numerous studies have shown that native seedling provides many ecosystem services such as invasion resistance, native species richness and plant community diversity (Dyderski & Jagodzińsk, 2020; Rizza et al., 2007).

Due to their physical structure, the grass species create small islands of shade and soil moisture. Those patches in turn were occupied by bryophyte species (based on our observations in the field). We found that the number of subquadrats occupied by bryophytes was greater in the grass treatment than any of the other treatments (Table 3.2). From field observations, we expected that some coniferous species, at least the ubiquitous balsam fir (*Abies balsamea* (L.) Mill.) would have produced volunteers in our experimental setting (~hectare), at least near the moss-occupied patches. However, no coniferous seedlings were observed on the grass treatment, nor on the whole experimental field. This may be due to the fact no wooden debris were left on the mine tailings and very few on the topsoil. A study from Simard et al. (1998) showed that moss over wooden debris favors coniferous germination. Dhar et al. (2018) also confirm the importance of woody debris for mine reclamation site. A study of Kuuluvainen and Juntunen (1998) in the Finland boreal forest found that the pine seedlings that were clustered in the uprooting pits and mounds created by windthrow of fallen trees were also found on well decomposed woody debris.

Another possible reason why the coniferous seeds were not found on the natural colonization quadrats is their seeds' weight. Pioneer coniferous seeds are bigger and heavier than the pioneer Salicaceae seeds. The experimental field site was at least over 100 meters from the natural boreal forest stand (the stand, dominated by black spruce and balsam fir, surrounded only one side of the experimental field), decreasing chances that coniferous wind seeds would land on the experimental field. For example, balsam fir seeds may be disseminated between 100 to 160 meters, however most seeds fall close to the parent tree, from 25 to 60 meters (USDA-NCRS, 2023).

3.4.3 Effect of the applied treatments on vegetation community composition

Although we found no significant difference in richness or diversity between the three herbaceous agronomic treatments, the multivariate analysis (Fig. 3.4 and PERMANOVA) indicates a significant difference in the species composing the community of the plots with different applied treatments. In particular, Fig. 3.4 shows a cluster of six species with a preferential association with the legume treatment, including the only two pioneer tree genera found in the plots (*Populus* sp. and *Salix* sp., both in the Salicaceae family).

Trees are among the most efficient plants for ecosystem nutrient cycling; in this mine tailing reclamation context, they are especially important to restore the indigenous forested ecosystem. Treatments that benefit tree encroachment should be considered. Despite the highest species richness found in topsoil plots, we found almost no tree seedlings in those plots (1 *Populus* sp. seedling and no *Salix* sp.) (Table 3.2). This is surprising given prior studies (Bouchard et al., 2018; Burger et al., 2017a) that showed a topsoil treatment can be more favorable to tree establishment than agronomic plants, which compete with the trees for resources.

3.4.4 Study limitations and perspectives for future research

While our community analysis shows a preferential association of the two pioneer tree genera with the legume treatment, the abundance of pioneer trees was too low across all treatments to perform a standalone analysis of that important metric for restoration of the forest ecosystem. Therefore, even as our study provides preliminary evidence than some agronomic herbaceous seeding treatments (especially containing legumes) may not impede the establishment of pioneer trees, additional research would be required to identify under which conditions this result holds.

The study also did not investigate plant-microbe relationships at the early stage of the agronomic herbaceous plant seedings. Yet, there is evidence from previous research (e.g., Bauman et al., 2012) that existing vegetation can facilitate the establishment of new tree seedlings if the mycorrhizal fungi present are shared between the already present and establishing species.

3.5 Conclusion

Most revegetation processes use agricultural herbaceous plant seeding on tailings as the first step to rapidly control water and wind erosion. The early vegetation succession on the tailings environment is influenced by the composition of the seeding mixtures. Therefore, it is important to have the best agronomic herbaceous plant seeding strategy at this early stage to benefit the long-term plant biodiversity, ecosystem services, and resilience. Overall, the legume treatment showed a different plant community composition than the grass or the mix treatments, however, one species oakleaf goosefoot was in high numbers in the legume treatment, which reduced its species diversity. Agronomic seeding, especially grasses, favored bryophyte presence in the community. A thin topsoil layer (2 cm) resulted in a significantly higher abundance, species richness and diversity of vascular plants compared to the control and, depending on the metric, compared to the three agronomic herbaceous plant treatments. However, the topsoil treatment favored native and introduced plants alike, and contained very few pioneer tree species.

Volunteer pioneer tree species from the Salicaceae family were found mostly in the legume treatment, which may indicate that the agronomic legumes species (plant decomposition and plant-microorganisms interaction) alter the mine tailing soil to be more favorable to pioneer trees or be less competitive than grasses. Pioneer trees are especially important as an accelerator for ecosystem cycles and processes. When tailings topsoil or amendment is not available or affordable, we suggest that the agronomic seeds mix should include a higher percentage of legume seeds in the mix to favor volunteer pioneer trees.

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CHAPITRE 4

THE EFFECTS OF AGRONOMIC HERBACEOUS PLANTS ON MICROCLIMATIC CONDITIONS AND THE GERMINATION OF PIONEER TREE SEEDS MANUALLY SOWED ON A GOLDMINE TAILING SUBSTRATE

(EFFETS DES PLANTES HERBACÉES AGRONOMIQUES SUR LE MICROCLIMAT ET LA GERMINATION D'ESPÈCES D'ARBRES PIONNIERS SEMÉES SUR DES RÉSIDUS MINIERS D'UNE MINE D'OR)

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Abstract

Mining waste, like mine tailings, is a necessary byproduct of precious metal extraction. To rapidly control wind and water erosion, mining industries commonly sow the tailings with a mix of agronomic grasses and legumes. However, little is known on whether this practice creates favorable conditions for the establishment of boreal pioneer trees. This study aims to evaluate which agronomic herbaceous plant treatment (grasses, legumes, or a mix of the two) facilitates the germination and establishment of boreal pioneer trees seeds, at an early stage of the revegetation process. In 2013, a 1-ha experimental area was set up at a gold mine tailings site in the Abitibi-Témiscamingue region of Québec, Canada. Fifteen plots were delimited on the site and received one of five treatments in a randomized block design: grasses, legumes, a mixture of both, topsoil, and a control (tailings only, no seeding). Plots were hand-seeded in 2015 with 2000 seeds from four selected pioneer tree species; jack pine (*Pinus banksiana*), tamarack (*Larix laricina*), paper birch (*Betula papyrifera*), and pussy willow (*Salix discolor*). Viable seedlings were counted the next growing season to assess establishment success. Three microclimatic parameters: soil moisture, temperature, and light transmission were measured in the plots. Only the soil moisture varied significantly between the topsoil and the legume treatments at the 5 cm soil depth; no significant difference was found among treatments for temperature and light transmission. The seedling establishment of jack pine and tamarack were significantly higher in the grass treatment. Too few pussy willow seedlings were observed to draw clear conclusions, whereas no paper birch seedlings established in any of the treatments. Our results suggest that the structural characteristics of grasses provide a beneficial environment for the jack pine and tamarack germination and seedling establishment. A longer-term assessment is needed to evaluate the effect of competition from grasses on seedling survival in a resource-limited mine tailing environment.

Keywords: grasses, legumes, microclimatic factors, mine tailings reclamation.

Résumé

Les déchets miniers, tel que les résidus miniers, sont un sous-produit nécessaire de l’extraction des métaux précieux. Afin de contrôler rapidement l’érosion aérienne et hydrique, les industries minières ensemencent couramment les résidus avec un mélange de graminées et de légumineuses. Cependant, peu d’informations sont disponibles pour savoir si cette pratique crée des conditions microclimatiques propices à la germination et à l’établissement d’arbres pionniers boréaux. Cette étude vise à évaluer quel traitement agronomique de plantes herbacées (graminées, légumineuses ou un mélange des deux) facilite la germination et l'établissement des graines des arbres boréaux pionniers, à un stade précoce du processus de revégétalisation. En 2013, une zone expérimentale d'un hectare a été aménagée sur les résidus miniers d'une mine d'or de la région de l'Abitibi-Témiscamingue, Québec, Canada. Quinze parcelles ont été délimitées sur le site et ont reçu l'un des cinq traitements selon un plan en blocs randomisés : graminées, légumineuses, un mélange des deux, de la terre de couche arable (*topsoil*) et un témoin (résidus uniquement, pas d'ensemencement). Chaque parcelle a été ensemencée à la main en 2015 avec 2 000 graines provenant de quatre espèces d'arbres pionnières sélectionnées ; le pin gris (*Pinus banksiana*), le mélèze laricin (*Larix laricina*), le bouleau à papier (*Betula papyrifera*) et le saule discolore (*Salix discolor*) et les semis viables ont été comptés lors de la saison de croissance suivante afin d'évaluer le succès d'établissement. Trois paramètres microclimatiques : l'humidité du sol, la température et la transmission de la lumière ont été évalués parmi les traitements. Seule l'humidité du sol à une profondeur de 5 cm variait de manière significative entre le traitement de *topsoil* et celui des légumineuses; aucune différence significative n'a été observée entre les traitements pour la température et la transmission lumineuse. L'établissement des semis de pin gris et de mélèze étaient significativement plus élevés dans le traitement de graminées. Les semis de saule étaient trop peu nombreux pour tirer des conclusions claires, tandis qu'aucun semis de bouleau à papier ne s'est établi dans aucun des traitements. Nos résultats suggèrent que les caractéristiques structurelles des graminées offrent un environnement bénéfique pour la germination et l'établissement des semis du pin gris et du mélèze. Une évaluation à long terme est nécessaire pour évaluer l'effet compétitif des graminées sur la survie des semis dans le milieu pauvre en ressources des résidus miniers.

Mots-clés : facteurs microclimatiques, graminées, légumineuses, valorisation des résidus miniers.

4.1 Introduction

Mine tailing waste results from the crushing, grinding, and milling of hard metal rock from which the valuable ore has been extracted (Lottermoser, 2007). Once the extraction is completed, the mineral waste (tailing waste) is then transported with water and deposited into a tailing storage facility to decant. When the mineral surface dries up, the tailing fine-grained texture is prone to wind and water erosion. To rapidly alleviate the erosion, it is customary for the mining industries to sow agronomic herbaceous plants (grasses, legumes, or a mix of the two) as a first step of the revegetation process (Sheoran et al., 2010). Another reason for using agronomic herbaceous plants is that they have the physiological traits and the capacity to establish themselves on mineral soil (Elias & Chadwick, 1979).

Grass and legumes herbaceous plants have their own characteristics. Grasses have physical features unique to their family order such as bunch assembly, longer vertical stems, tillers, and mulch formation providing moisture to the soil (Maiti & Maiti, 2014). Legumes have organic decomposition (above and below ground) that enriches soil with nitrogen (Lindemann & Glover, 2003; Maiti & Maiti, 2014). Mine tailings soils are nutrient-deficient in nitrogen and any input could aid plant growth (Barrette et al., 2022).

Past studies have investigated different types of ground cover for mining revegetation: for example, Rizza et al. (2007) found that moderate amounts of ground cover with native warm season grass treatments provide the best results for the survival and growth of five planted native tree seedling species. Other studies, using agricultural herbaceous plants as a first step post-mining revegetation, have found agronomic herbaceous plants can be too competitive with the newly established tree seedlings (Bouchard et al.; 2018; Wade, 1989). However, in Canada, native herbaceous plant seeds have limited suppliers and are costly compared to agronomic seeds, thus they may not prevail as a cost-effective revegetation solution (Tischew et al., 2011).

In North America, most of the mining activity occurs within the boreal forest biome (Brandt et al., 2013), which includes pioneer trees species with seeds that are dispersed through wind or animal dispersion. A study by Kuuluvainen and Juntunen (1998) found that the seedlings of pioneer tree species such as *Betula* sp. and *Pinus sylvestris* have different establishment strategies within a microsite such as the uprooting pits and mounds formed by windthrow. Vodde et al. (2011) published a review of storm-induced microsites for the regeneration of seedlings of the boreal and hemiboreal forests. They found that the regeneration dynamics of tree seedlings are related to the types of storm-induced microsites. For the intermediate or severe events causing enough changes to modify edaphic conditions of the microsites, the dispersal traits of pioneer species will profit most of the new created microsites conditions such as mineral soil and elevated location needed for their establishment and growth.

Studies of pasture and heath ecosystems have established that legumes and grass herbaceous plants are affected by the soil biota (plant-microbe interaction) (Bender et al., 2015) and in return modify the soil environment (plant-soil interactions) (Dexter, 1991; Dexter, 2004; Maiti & Maiti, 2014). The plant community assembly can create a unique microclimate. For example, a study by D'odorico et al. (2013) on a woody-grass ecotone found that trees and shrubs are capable of modifying the microclimate conditions, particularly soil temperature, to encroach in the grassland and create suitable conditions (by lowering the soil temperature) for seedling germination and survival.

The types of agronomic herbaceous plants assemblages and topsoil used as ground cover for mine tailings create different microclimatic conditions that may affect seed germination and seedling emergence (Bouchard et al., 2018). Drought due to lack of soil moisture is a primary cause of mortality for most boreal seedlings (Greene et al., 1999). In mature boreal forests, woody debris such as decaying logs are preferred germination sites (Simard et al., 1998; Simard et al., 2003; USDA-NRCS, 2023), offering both sufficient moisture through their moss cover as well as a raised position from the competitive vegetation on the forest floor. This latter effect is more important for small-seeded species such as paper birch, which tend to be more sensitive to competition from herbaceous plants and shrubs compared to large-seeded species (Robert et al., 2012).

Seed germination is not only related to the above ground vegetation, which acts as a proxy for edaphic factors (light intensity reaching the soil surface, soil moisture and temperature), but also to the biology of the seed itself. Physiological traits and genetic adaptations of the seed species influence its germination ability; seed bet-hedging strategies (Fan et al., 2018; Kuuluvainen & Juntunen, 1998), seed size (Black, 1958; Susko & Cavers, 2008), and seed life persistence (Vodde et al., 2011) are fundamental to better predict the forest succession dynamics.

Previous studies on the revegetation of arsenical gold mine tailings have shown that the presence of pioneer trees and shrubs promotes higher nutrient concentrations and biodiversity (Munford et al., 2020), and natural reforestation by pioneer trees may be one of the best ways to accelerate the forest succession. Therefore, agronomic herbaceous plant treatments that facilitate and do not compete with the establishment of pioneer tree species should be favored in revegetation efforts. Yet, few studies have attempted to determine if agronomic herbaceous ground cover is compatible with pioneer tree seed germination and seedling survival at their early life stage. Moreover, the impact of herbaceous ground cover on tree seedling survival on a low-nutrient, anthropogenic soil such as mine tailings is likely different from what is observed in forest ecosystems.

In this study, we investigate the different microclimate sites (which are evaluated by three variables; soil moisture, temperature, and light transmission) created by agronomic herbaceous plant treatments (grasses, legumes, and a mix of the two) and their impact on the establishment success of four pioneer tree species native to the Canadian boreal forest: jack pine, tamarack, paper birch and pussy willow. The mass of jack pine (around 4 mg) and tamarack seeds (2 mg) is at least 10 times that of paper birch (0.2 mg) and willows (around 0.1 mg) (Charron, 1998; López-Fernández et al., 2018).

The objectives of this study are twofold:

1- Determine if there are differences in the soil edaphic conditions among the agronomic herbaceous plant treatments.

2- Determine which herbaceous plants treatments produce the highest rate of seed germination for the four pioneer species selected for this research.

We hypothesize that (1) the grass treatment, because of its specific structural features such as bunch assembly, longer vertical stems, tiller formations, and mulch formation will increase soil moisture; and thus (2) the grass treatment will increase tree seed germination and survival compared to the legume treatment and the control, more so for the larger-seeded species (jack pine, tamarack) that are less sensitive to competition from the grasses.

4.2 Materials and methods

4.2.1 Mine site

The *in-situ* study site has been set up at an open-pit gold mine currently active and located at the south of the Malartic city, in the Abitibi-Témiscamingue region of northwestern Québec, Canada (48° 06'N, 78° 08' W). The mine site is within the boreal forest region with a climate characterized by long, cold winters, with short, moderately warm summers. The mean annual rainfall is 985 mm (1981-2010 data period), with daily average temperatures of -17 °C for January and 17 °C for July (Government of Canada, n.d.). The area is included within the last glacial advance of Wisconsin glaciation region (Vincent & Hardy, 1977) with a dominant gray luvisol soil type (Soil classification working group, 1998). The study site is within the boreal and bioclimatic domains of the balsam fir (*Abies balsamea* (L.) Miller) paper birch (*Betula papyrifera* Marshall) ecozone (MFFP, 2016).

4.2.2 Experimental site description and design

The experimental site area was set up on a one-hectare area of moraine substrate that was set aside by the mine. Mine roads surround the north and west side of the experimental site, separating it (on the west) from patches of boreal forests consisting mainly of black spruce (*Picea mariana* (Miller) BSP) and balsam fir (MFFP, n.d.). Waste rock berms surround the east side of the experimental site.

In May 2013, thickened tailings (50–70% solids by mass at deposition, see Bussière, 2007) from the mine were applied to the 1-ha experimental site. Thickened tailings are mainly composed of 86% mineral particles with a diameter < 80 µm and contain low concentrations of neutralizing minerals and total sulfur (~ 1% S); the ore at the mine site consisted of a mineralized greywacke.

The experimental setting was organized in 2013 in a randomized complete block design, consisting of three blocks, each subdivided into five experimental plots (20 m x 15 m) placed 5 m apart. The following five treatments were randomly applied each to one plot per block:

1) Grasses (100% perennial Poaceae)

2) Legumes (100% perennial Fabaceae)

3) Mix of grasses (50% of seed mass) and legumes (40% of seed mass)

4) Topsoil amendment (details in section 4.2.3)

5) Control (thickened tailings only)

From May 25 to June 9, 2015, an area corresponding to two-thirds of each treatment plot was further subdivided into 26 quadrats (1 m x 1 m) spaced by one meter in a checkerboard pattern. The other one-third of the plot was reserved for tree planting for further experiments (Barrette et al., 2022). Of the 26 quadrats/plot, 16 quadrats received 500 seeds/quadrat of one of four boreal tree species (4 quadrats per species,): jack pine, tamarack, paper birch, and pussy willow. Given the small size of willow seeds, we weighed 100 hand-counted seeds and used this value to apply an approximate 500 seeds (by weight) in each quadrat. Therefore, 2000 seeds (4 quadrats x 500 seeds) per tree species were hand planted in each plot. The remaining 10 quadrats were set aside to measure natural colonization from windborne seeds (reported in Barrette et al., in prep.). The assignment of the 26 quadrats was randomized for the first plot and the same pattern was applied to all other plots.

4.2.3 Soil materials

Prior to the gold mine exploitation, the existing forest stand was harvested, and the upper layers of topsoil and its underlying mineral soil were salvaged and stockpiled (for 30 to 36 months) in two separate 7-m-high piles (2.5H:1 V slope) for future use. The topsoil stockpile consisted of the first 30-cm dark soil layer (O- and A-horizons). The forest soil prior to mining was classified as a luvic gleysol. In May 2013, the topsoil stockpile was excavated and some of the material spread over tailings in the topsoil plots (layer depth of 2 cm) according to the experimental site design. Detailed chemical analyses of the topsoil amendment as well as the thickened tailings (control) substrate are reported in our previous study at the same site (Barrette et al., 2022).

4.2.4 Plant materials

4.2.4.1 Agronomic herbaceous seeds

In June 2013, commercial agronomic herbaceous seeds (forage seeds) were sown by Lanexco Inc. (Dubuisson, QC, Canada) on the freshly hand raked tailings according to the experimental design. An annual nurse crop of barley seeds (*Hordeum vulgare* L.) was added to all seed mixes to stabilize the soil and reduce the establishment of weeds (Espeland & Perkins, 2013). The legume treatment included white clover (*Trifolium repens* L., 45% by mass), bird's-foot trefoil (*Locus corniculatus* L., 45% by mass) and barley (10% by mass). A soil bacterium *Rhizobium* inoculant was applied to the legume seeds to facilitate nitrogen fixation. The grass treatment was composed of ryegrass (*Lolium perenne* L., 40% by mass), redtop (*Agrostis gigantea* Roth, 40% by mass), reed canary grass (*Phalaris arundinacea* L., 10% by mass) and barley (10% by mass). The mix legumes/grasses treatment plots included barley 10%, white clover 20%, bird foot trefoil 20%, reed canary grass 25% and ryegrass 25%. Agronomic seeds were sown at a rate of 100 kg ha^{-1} along with one application of mineral fertilizer 8-32-16 (N, P, K) applied at a rate of 750 kg ha^{-1}, both seeds and fertilizer were applied to all treatment plots (except for the control and topsoil plots). A commercial MYKE® promycorrhizal inoculant (Premier Tech biotechnologies, Rivière-du-Loup, Canada) was also added to the agronomic seed treatment plots in accordance with the manufacturer application chart.

4.2.4.2 Pioneer tree seeds for field experiment and viability test

Pioneer tree seeds were provided by '*Ministère des Forêts, de la Faune et des Parcs*'(MFFP) except for the willow seeds which were collected in June 2015, directly from the parent pussy willow trees growing near the gold mine area. The seeds were hand seeded from May 25 until June 9, 2015, in the following manner: 10 shallow furrows received 50 seeds (10 x 50 seeds = 500 seeds/tree species) spaced by approximately 2 cm, each furrow separated by 10 cm. Seeds were not buried with soil to simulate natural wind-seed deposition, however, they were lightly sprinkled with minimal tailing soil, so as not to lose them from wind and runoff. Since willow seeds are very tiny, to sow them properly along the furrow, we use a salt and pepper shaker for practicality, at June end 2015.

We set aside some of the seeds donated by the MFFP to perform a vitability test, including some of the ones of the collected pussy willow seeds. Three batches of 100 seeds of each tree species (jack pine, paper birch, pussy willow, and tamarack) were placed in MiracleGro Seed Starting Soil (0.0-0.01-0.0) in a growth chamber, with temperature fixed at 20 ºC and 6 hours of daylight, at the Université du Québec en Abitibi-Témiscamingue (UQAT) Amos laboratory campus. Germinated seeds were counted after 20 days.

4.2.5 Microclimatic measurements

Three microclimate parameters: soil moisture, photosynthetically active radiation (PAR) at the soil level and soil temperature were measured during the 2015 and 2016 growing seasons (months of June to September). Soil moisture (volumetric water content (VWC), expressed as a percentage) was measured using two probes placed in the center of each of the 15 plots, one inserted at depth of 5 cm and the other at 10 cm (Em50 Data logger, ECH2O EC-5 cm moisture sensor probe, calibrated for the soil type used, Decagon Devices, Pullman, WA, USA). Since the topsoil layer was thin (approximately 3 to 5 cm), the first probe was in the topsoil layer but the second probe at the 10 cm was within the mine tailings soil underneath the topsoil. Measurements were taken hourly during both growing seasons and recorded to calculate weekly, monthly and season averages.

PAR measurements, measured by photosynthetic photon flux density (PPFD), were taken biweekly at ground level using a Sunfleck Ceptometer (Decagon Devices, Pullman, WA, USA), around noon time, under clear sky conditions.

Soil temperature was measured during the same period as PAR measurements, at 5 cm depth, using an acorn soil thermometer (Acorn^{TM} Temp 6 RTD meter, Oakton Instruments, Vernon Hills, Il, USA).

In each plot, PAR and soil temperature measurements were taken in the center of one of the tree-seeded quadrats for each pioneer species.

4.2.6 Establishment success of seeded tree species

From the initial 500 seeds sown in each quadrat, viable seedlings were counted, defined as those showing more than > 5% green, at the end of the following season (2016). Whereas the resulting counts depend on both germination rates and survival of seedlings up to that point, for simplicity we refer to the combination of both processes as establishment success.

4.2.7 Plant cover

Since the effect of the treatments, especially the agronomic herbaceous ones, on tree seedling establishment could depend on the realized plant cover in the plots, we assessed plant cover in each plot on a categorical scale. For each treatment plot in each block, we assigned one of the following cover classes: medium, light-medium, or very low plant cover.

4.2.8 Statistical analyses

Daily records of the soil moisture at 5 cm and 10 cm, as well as biweekly PAR and temperature measurements, were averaged in each plot for the full 2016 growing season. We note that partial or full soil VWC time series had to be excluded in some of the plots due to implausible values according to the sensor range (i.e., negative values or values >60% VWC). Due to sensor malfunctions in multiple plots, the majority of the VWC data had to be excluded for the 2015 growing season, thus we limited our analysis to 2016 data. A one-way ANOVA was performed for each edaphic factor to determine significant differences between treatments, followed by post hoc comparisons with Tukey's range test. We performed visual checks of the approximate normality and homoscedasticity of the ANOVA residuals. For each plot and each tree species, we combined the seedling counts from the four quadrats (out of 2000 initial seeds) and fitted separate models of seedling establishment success by treatment for each tree species using binomial logistic regression with bias reduction, as implemented in the brglm package (Kosmidis, 2021) in R. The bias reduction method is required due to observed establishment rates of 0 in some species-treatment combinations, which would cause unstable estimates in regular logistic regression. The emmeans package (Lenth, 2020) was used to perform post-hoc comparisons between treatments, with a Tukey adjustment applied for multiple comparisons. All analyses were performed in R version 4.1.3 with RStudio version 1.0.136 (RStudio Team, 2016).

4.3 Results

4.3.1 Effects of the agronomic herbaceous plant treatments on soil moisture, soil temperature and light transmission

Figure 4.1 displays the weekly average of soil volumetric water content (VWC) for each soil depth, averaging plots with the same treatment. Based on the results of the one-way ANOVA, the mean soil VWC during the 2016 growing season was significantly different among the treatments at the 5 cm soil depth; (*F* (4,8) = [5.35], p = 0.02) (see Table 4.1), however, no significant difference was found among the treatments for the 10 cm soil depth; (*F* (4,5) = [3.32], p = 0.11). The post hoc comparisons using Tukey's HSD indicate a significant difference in mean soil VWC at 5 cm between the topsoil treatment (highest soil VWC) versus the legume treatment (lowest value) only (p = 0.02, 95% C.I. = [2.48, 34.12]); no significant differences were found among the three agronomic herbaceous plant treatments (mix, legumes, and grasses) (Table 4.1, 4.2).

The mean soil temperature at 5 cm depth and light transmission (PAR) at soil surface for the 2016 growing season did not show any significant difference between the treatments (Annexe A).

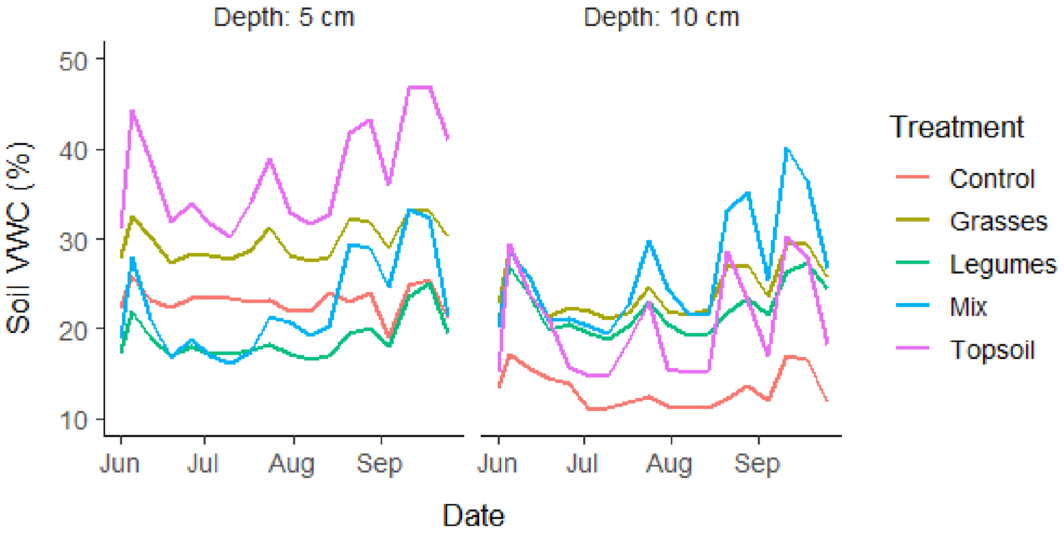


Figure 4.1 Weekly average of soil volumetric water content (VWC) per treatment at 5 cm and 10 cm soil depth, based on hourly measurements. Data gathered in the 2016 growing season.

Table 4.1 Results of the one-way ANOVA for the soil volumetric water content (VWC, in %) at 5 cm soil depth.

	df	Sum of squares	Mean square	*F* value	*p* Value
Treatment	4	448.30	112.08	5.35	0.02 *
Residual	8	167.70	20.96		

Note. * The mean difference is significant at the $p < 0.05$ level.

Table 4.2 Multiple comparison results (Tukey's range test) for the soil volumetric water content (VWC, in %) at 5 cm soil depth.

Treatments	Mean difference	95% confidence interval	*p* Adj.
Grasses-Control	6.5	(-6.4, 19.4)	0.46
Legumes-Control	-4.4	(-18.9, 10.0)	0.82
Mix-Control	-0.6	(-13.5, 12.4)	1.00
Topsoil-Control	13.9	(-0.6, 28.3)	0.06
Legumes-Grasses	-10.9	(-25.37, 3.5)	0.16
Mix-Grasses	-7.1	(-20.0, 5.8)	0.39
Topsoil-Grasses	7.4	(-7.1, 21.8)	0.45
Mix-Legumes	3.9	(-10.6, 18.3)	0.88
Topsoil-Legumes	18.3	(2.5, 34.1)	0.02 *
Topsoil-Mix	14.4	(-0.0, 28.9)	0.05

Note. * The mean difference is significant at the $p < 0.05$ level.

4.3.2 In vitro germination success of the tree species seeds.

A large majority of jack pine (94% across three batches of 100 seeds) and tamarack (79%) seeds successfully germinated under laboratory conditions (Table 4.3) In contrast, only 24% of paper birch seeds and 5% of pussy willow seeds germinated, indicating large differences between species under controlled conditions, which should be considered when interpreting the results of the field experiment.

Table 4.3 Germination rates of tree seeds under laboratory conditions.

Species	Batch 1 (%)	Batch 2 (%)	Batch 3 (%)	Average (n = 3)
Jack pine	95	92	95	94
Tamarack	86	86	65	79
Paper birch	8	34	30	24
Pussy willow	10	5	0	5

Note. Percentage of viable seeds (germinated) within three batches of 100 planted seeds of each tree species.

4.3.3 Effects of the agronomic herbaceous treatments and topsoil on seedling establishment

Whereas 24% of paper birch seeds germinated under laboratory conditions (potting mix soil), we observed no viable paper birch seedlings across the 15 plots (2000 seeds per plot) (tailing soil). Therefore, this species was excluded from the following analyses.

For jack pine, the highest establishment rate in the field (17%) was found in the grass treatment (Fig. 4.2). The post hoc comparisons showed significant differences between all pairs of treatments, ranked from highest to lowest establishment rate as follows: Grasses > Topsoil > Mix > Legumes > Control (see Table 4.4).

For the tamarack seedlings, after two growing seasons, the greatest establishment rates were found in the grass (6%) and mix (5%) treatments (Fig. 4.2). These two treatments were not significantly different from each other, and both had a significantly higher establishment rate than the topsoil, which in turn had a significantly higher establishment rate than the legume and control treatment.

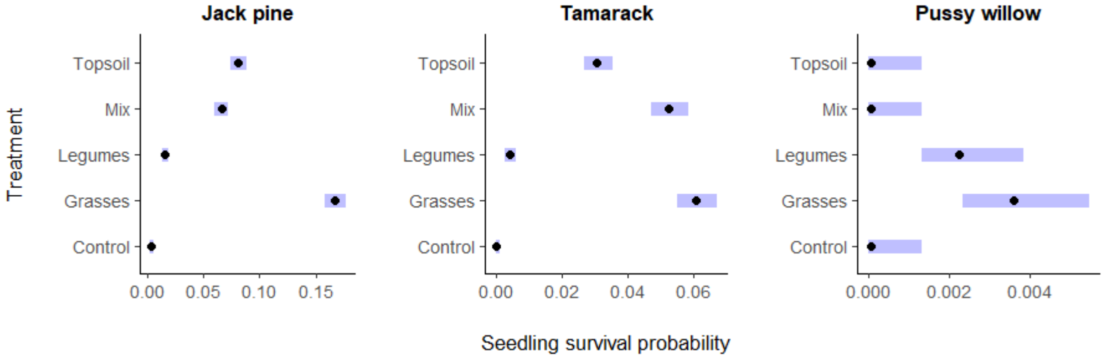
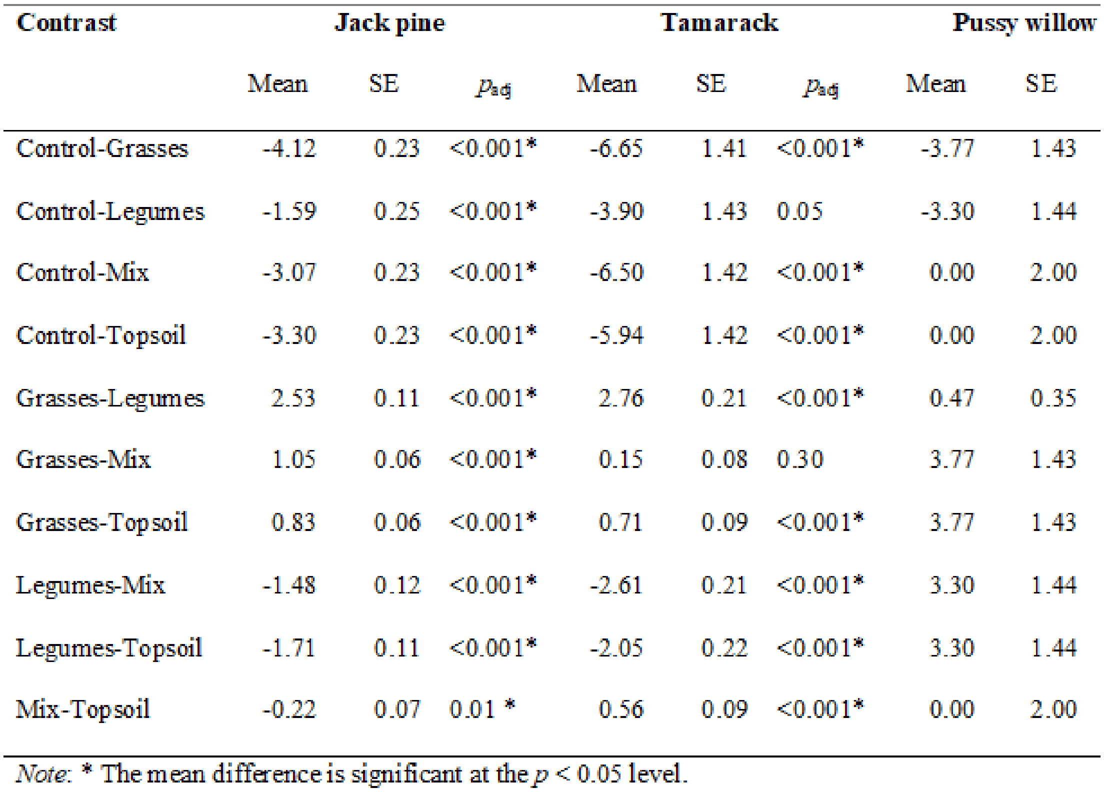


Figure 4.2 Estimated mean (black dot) and 95% confidence interval (blue line) of the seedling establishment rates for the different agronomic herbaceous plant treatments after two growing seasons.

Table 4.4 Multiple comparison results: mean, standard error (SE) and Tukey-adjusted *p*-values for differences in seedling establishment rates (on a logit scale) between treatments.



Contrast	**Jack pine**			**Tamarack**			**Pussy willow**	
	Mean	SE	p_{adj}	Mean	SE	p_{adj}	Mean	SE
Control-Grasses	-4.12	0.23	<0.001*	-6.65	1.41	<0.001*	-3.77	1.43
Control-Legumes	-1.59	0.25	<0.001*	-3.90	1.43	0.05	-3.30	1.44
Control-Mix	-3.07	0.23	<0.001*	-6.50	1.42	<0.001*	0.00	2.00
Control-Topsoil	-3.30	0.23	<0.001*	-5.94	1.42	<0.001*	0.00	2.00
Grasses-Legumes	2.53	0.11	<0.001*	2.76	0.21	<0.001*	0.47	0.35
Grasses-Mix	1.05	0.06	<0.001*	0.15	0.08	0.30	3.77	1.43
Grasses-Topsoil	0.83	0.06	<0.001*	0.71	0.09	<0.001*	3.77	1.43
Legumes-Mix	-1.48	0.12	<0.001*	-2.61	0.21	<0.001*	3.30	1.44
Legumes-Topsoil	-1.71	0.11	<0.001*	-2.05	0.22	<0.001*	3.30	1.44
Mix-Topsoil	-0.22	0.07	0.01 *	0.56	0.09	<0.001*	0.00	2.00

Note: * The mean difference is significant at the $p < 0.05$ level.

Just like its vitro germination rate, the seedling establishment rate of pussy willow in the field was very low (less than 0.5% for all treatments). Whereas more seedlings were established in the grass and legume treatments (Fig. 4.2), due to the small number of seedlings found among all treatments overall, the post hoc test showed no significant differences (Table 4.4).

4.3.4 Plant cover

The plant cover was similar in plots sharing the same treatment across the three blocks (Table 4.5). The legume treatment had slightly less plant cover due to the white clover species that did not growth well in the mine tailing soil; conversely, the bird's-foot trefoil did extremely well.

Table 4.5 Plant cover per treatment

Treatments	Block 1	Block 2	Block 3
Control	Very low	Very low	Very low
Grasses	Medium	Medium	Medium
Legumes	Light-medium	Light-medium	Light-medium
Mix	Medium	Medium	Medium
Topsoil	Medium	Medium	Medium

4.4 Discussion

4.4.1 Effects of the agronomic herbaceous plant treatments on the microclimate variables

Our results show that the topsoil treatment produced the highest values of soil VWC at a depth of 5 cm. It has been long recognized that organic matter and its associated humic substances hold soil moisture (Mosa et al., 2020; Dexter 2004; Dexter 1991) and this would account for these results.

Although the control plots appear to show a lower VWC at a depth of 10 cm (Fig. 4.1), multiple measurement errors (out of bound values for VWC) from the 10 cm sensors reduced the sample size to a point where we could not detect significant differences in VWC at the 10 cm depth.

Among the agronomic herbaceous treatments, there were no significant differences for any of the microclimatic variables. This lack of differentiation among the treatments, or between those treatments and the control plots, may be due in part to the short time scale of the study since measurements were taken only three years after sowing the herbaceous plants.

4.4.2 Effects of the treatments on seedling establishment

4.4.2.1 Jack pine

Both jack pine and tamarack achieved a high germination rate in the controlled chamber (laboratory environment) and high seedling establishment rates in the field. In the controlled chamber, the seed starting potting mix soil substrate, light, regular temperature, and well-kept moisture level, were all favorable to the coniferous seeds. A study from Haig (1959) on broadcast seeding with jack pine and white spruce seeds on a well-prepared mineral seedbed indicates a high mortality of jack pine seedlings following two years of drought conditions, and that the soil moisture regime was the most important predicament for the seedling survival. Sims (1970) tested three different seedbeds, one fresh, a moderately fresh, and a dry site for jack pine seedling establishment following broadcast seeding. Their results showed that establishment was significantly higher on the fresh seedbed than on the moderately fresh and dry ones, and that heat combined with drought was the main cause of the seedlings mortality. Another study from Dominy and Wood (1995) on the shelter spot seedling trials with jack pine, black spruce (*Picea mariana* [Mill] B.S.P.), and white spruce (*Picea glauca* [Moench] Voss), showed that "greenhouse effect" (keeping air humidity and soil moisture) led to greater germination and seedling survival of jack pine compared to the stocking of the bare seeds pots jack pine seedlings. Chrosciewicz (1990) did an extensive study on site conditions for jack pine seeding. His research indicates the best seedbed conditions for jack pine consist of a mineral soil exposed by scarification or burning with light humus accumulation such as mosses or dead plant litter (foliage, mulch from grass etc.) with some degree of shade that is essential. He explains that raw humus accumulation is not a suitable seedbed for jack pine because organic material has low thermal conductivities and exposure to direct sunlight temperature would create high surface temperature.

The grass treatment, due to its species' physiological characteristics such as bunch assembly, long stems, and mulch formation (Maiti & Maiti, 2014), provides shaded areas (increasing soil moisture) for seeds to germinate. Since the main criteria for jack pine seed to germinate include soil moisture, regular temperature (provided by mineral layer) and light humus, the microclimate created by grass treatment (with the highest seedling establishment rates) thus appears to provide the requirements for that species, and our results corroborate the previous findings.

4.4.2.2 Tamarack

Tamarack grows well on wet to moist organic soils such as woody peat, peatlands, swamps (Johnston, 1990). However, tamarack is a species capable of establishing a wide range of soil condition, pH and organic level to even extremely dry soil (Brown et al., 1988; Johnston, 1990). The germination and seedlings survival in both the mix and grass treatments are consistent with the ubiquitous nature of that species and its genotype adaptation to a wider range of microclimates.

4.4.2.3 Paper birch

We are not sure as to the reasons the paper birch seedling establishment failed on the thickened, pH neutral gold mine tailing soil and under the microclimate conditions created by the applied treatments. Birch sp. in other gold mine tailings studies has shown a good establishment (Borgegard & Rydin, 1989; Gagnon et al., 2020). Parameters or a combination of parameters specific to this study, may account for these results such as the mine tailings poor soil aeration, neutral pH, or soil moisture.

4.4.2.4 Pussy willow

Unlike the two coniferous species, willow presented low germination rates both in the lab and in the experimental field. It is known that small-seeded species have lower survival rates during establishment than large-seeded species (Moles & Westoby, 2004). Previous studies have shown that the seed vitality for many willows species is short-lived (Densmore & Zasada, 1983; Gage & Cooper, 2005). Therefore, perhaps the willow hand seeded in this study were insufficient in number to offset the small-seeded species and short-lived vitality characteristics to willow genotype.

4.5 Conclusion

Mine tailings soils are among the most challenging soils to reclaim. When using agronomic herbaceous plants at the early stage of the revegetation process, it is important to understand the microclimate conditions that these herbaceous plants may create for the natural forest succession. Our study showed that in regards of the selected and measured microclimatic parameters, no significant difference was found among the agronomic herbaceous plants. However, the study showed that the physiological characteristic of the grass with medium plant cover creates an environment that is beneficial to jack pine and tamarack hand-seeded establishment at their early life stage, achieving a greater establishment rate than even the topsoil-treated plots. Providing some grass cover could thus be beneficial when sowing these pioneer coniferous species for mine tailing restoration. Agronomic herbaceous treatments could also help the establishment of willow trees from the seed stage, but a greater seeding density would be needed due to the lower per-seed viability of willow. Our results would not suggest however sowing paper birch seeds, as they did not produce viable seedlings under any of the treatments.

Finally, some caution needs to be exercised when using agronomic grass treatments for mine revegetation, since previous studies have shown that agronomic herbaceous grasses are competitive towards established tree seedlings due to the limited resources of mine tailing environments. Further research could serve to determine which sowing density of grasses provides an acceptable tradeoff between improving germination success without creating excessive competition to trees. Parent tree species, density and distance surrounding the mine revegetation area should also be considered for seedbank availability.

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CHAPITRE 5

CONCLUSION

5.1 Principaux résultats et contributions de la recherche

Plusieurs points distinguent la recherche présentée ici des études existantes concernant la revégétalisation des parcs à résidus miniers. Tout d'abord, elle reflète la réalité de la pratique courante utilisé par l'industrie minière au Québec, du moins, à la première étape du processus de revégétalisation. Dans d'autres études portant sur l'utilisation des plantes herbacées agronomiques au tout début du processus de revégétalisation, les herbacées étaient ensemencées sur une couche de *topsoil* (Shrestha et Lal, 2007, 2011). Tel que discuté dans cette thèse, le *topsoil* n'est pas toujours disponible pour couvrir l'ensemble des parcs à résidus miniers et il représente une opération dispendieuse. Pour refléter la disponibilité limitée du *topsoil*, c'est une mince couche (2 cm) qui a été utilisée ici comme témoin positif, pour contraster l'effet de ce traitement aux traitements de plantes herbacées appliqués directement aux résidus miniers. L'étude permet donc de comparer expérimentalement différentes options dont l'application est réaliste dans le contexte industriel.

Du point de vue scientifique, cette étude contribue également aux connaissances sur le processus de succession primaire et l'assemblage de la communauté végétale sur un sol anthropogénique formé de résidus miniers. Il existe une grande variété de ces types de sols et un large éventail d'études est nécessaire pour comprendre les défis que chacun pose à l'établissement d'une nouvelle forêt; par exemple, les observations faites sur ce site de résidus miniers épaissis à pH neutre pourraient ne pas être reproduites sur d'autres types de résidus miniers.

Tableau 5.1 Sommaire des résultats des chapitres 2 à 4.

Propriété	**Résultats** (T = topsoil, G = graminées, M = mélange, L = légumineuses, C = témoin)			
Propriétés du sol (ch. 2)	T: + matière organique, + point flétrissement, - masse volumique apparente vs. autres traitements			
Microclimat (ch. 4)	T : + humidité du sol à 5 cm vs. L			
Végétation spontanée (ch.3)	T: + richesse spécifique totale et indigène vs. autres traitements, + diversité vs. C, L*, + diversité indigène vs. C, L, G*, M* (* indice de Shannon seulement) L: composition de la végétation différenciée des autres traitements, association avec *Salix*, *Populus* (mais très peu d'arbres dans tous les traitements) C: presque aucune végétation spontanée			
	Bouleau à papier	**Mélèze laricin**	**Pin gris**	**Saule**
Survie des semis plantés (ch. 2)	T, G, M > C	T, G, L, M > C	T > C, L, M G > C, L L, M > C	T, G > C
Biomasse racinaire (ch. 2)	(aucune donnée)	T > C		L > C, G, M
Croissance en hauteur (ch. 2)				L > T
N foliaire	L > G	T > G	T > G, L, M L > G	L > M
Établissement des semences (ch. 4)	(aucun semis vivant)	G, M > C, L, T T > C, L	G > T > M > L > C	

Le Tableau 5.1 résume les principaux résultats des trois études formant cette thèse. Le reste de cette section présente les points saillants de ces résultats en lien avec les hypothèses de recherche dans chacun des chapitres.

Si les propriétés physico-chimiques du sol (Chapitre 2) étaient significativement différentes entre le *topsoil* et les autres traitements, dû notamment à sa concentration élevée de matière organique, nous n'avons pas déterminé de différences significatives entre les traitements de plantes herbacées agronomiques ou entre ceux-ci et le témoin. Ces résultats pourraient être causés par le nombre limité de saisons de croissance entre l'ensemencement des herbacées agronomiques (2013) et les mesures effectuées (2016). L'hypothèse H2.1 n'est donc pas soutenue par les résultats.

- H2.1 : Le traitement des graminées avec son plus grand volume de racines et de fibrilles aurait un effet plus important sur les propriétés physico-chimiques des sols à résidus miniers.

Pour ce qui est des mesures prises sur les semis plantés dans les placettes au Chapitre 2, l'hypothèse H2.2 est soutenue en partie.

- H2.2 : Le traitement des légumineuses grâce à son supplément de N inorganique via le processus de nitrification, va en retour favoriser la croissance et la concentration foliaire azotée des essences arboricoles plantées dans ce traitement.

C'est pour le cultivar de saule (*Salix miyabeana*) que le traitement des légumineuses a eu le plus grand impact, augmentant significativement sa biomasse racinaire par rapport à trois autres traitements, sa croissance vs. le *topsoil* et sa concentration de N foliaire vs. le mélange. Il est possible que cette essence, présentant la croissance en hauteur la plus rapide parmi les quatre utilisées, puisse bénéficier le plus de l'apport en

N des légumineuses. Ceci donne une autre perspective à cette essence en tant que potentiel pour la revégétalisation minière de résidus miniers, tandis qu'elle était plutôt utilisée du moins au Québec en agroforesterie (Gomes *et al.*, 2015; Hénault-Ethier *et al.*, 2017a, 2017b).

Même si aucune hypothèse n'avait été émise sur la survie des semis plantés, notons qu'après le *topsoil* qui assurait une bonne survie pour toutes les essences (6 % de mortalité, voir Tableau 2.3), c'est le traitement de graminées qui assurait la plus grande survie pour trois des quatre essences, le mélèze étant l'exception. Le mélèze a bien survécu dans tous les traitements d'herbacées (14 % de moralité combinée), tandis que le pin gris a subi la plus grande moralité dans ces traitements (40 %), possiblement dû à sa préférence pour les sols plus acides que ces résidus à pH neutre. La mortalité était très élevée dans le témoin (84 %) et les mesures effectuées sur les arbres vivants (comme la concentration de N foliaire, relativement élevée dans le témoin) sont très affectées par le biais des survivants.

La comparaison de la biodiversité entre les traitements (Chapitre 3) n'appuie pas l'hypothèse H3.1, car après le *topsoil* qui contenait la richesse et diversité les plus élevées, les trois traitements d'herbacées agronomiques étaient équivalents.

- H3.1 : Après le traitement *topsoil* qui devrait avoir la plus grande diversité (dû aux quantités plus préexistantes), le traitement 100 % légumineuses aura des espèces plus diverses et plus abondantes en raison de l'apport de N inorganique des légumineuses.

La composition végétale des espèces était quant à elle différente dans le traitement des légumineuses comparée à celle des autres traitements. Une espèce dominante, le chénopode à feuilles de chêne ou chénopode glauque (*Oxybasis glauca* L.), a fortement influencé les résultats de diversité pour ce traitement. De plus, l'arrivée spontanée de saules et de peupliers semblent plus fréquente dans le traitement de légumineuses, mais le nombre limité d’individus observés ne nous permet pas d’avoir une conclusion définitive par rapport à l’hypothèse H3.2.

- H3.2 : Dû à l’apport en N inorganique par les légumineuses du traitement 100 % légumineuses, ce traitement permettra l’établissement et la survie des espèces arboricoles plus exigeantes en N.

Pour ce qui est du microclimat de germination (Chapitre 4), comme pour les propriétés du sol mesurées au Chapitre 2, seul le *topsoil* se démarque des autres traitements, avec une humidité plus élevée à une profondeur de 5 cm. Même si le traitement de graminées arrive deuxième pour cette mesure, l’absence de différence significative entre celui-ci et les autres traitements signifie que l’hypothèse H4.1 n’est pas soutenue.

- H4.1 : Le traitement de 100% graminées en raison de leurs caractéristiques structurelles spécifiques telles que des longues tiges verticales, leur formation en talles et l’apport de paillis augmentera l'humidité du sol.

En contrepartie, le traitement de graminées présentait une augmentation significative du taux d’établissement des graines semées pour deux espèces (pin gris et mélèze laricin), en accord avec l’hypothèse H4.2.

- H4.2 : En retour, une meilleure humidité du sol apportée par le traitement 100 % graminées va fournir un meilleur lit de germination et de survie des semis, en particulier pour les conifères qui dû à la plus grande taille de leurs graines, sont moins sensibles à la compétition produite par les herbacées.

Pour les deux autres espèces, le nombre de semis établis était nul (bouleau à papier) ou très faible (saule discolore). Dans le cas du saule, il est possible qu’une augmentation de la densité de graines semées aurait mené à des résultats plus concluants.

5.2 Limites de la recherche

Pour obtenir une meilleure compréhension des effets de l'utilisation des plantes herbacées agronomiques en début du processus de revégétalisation minière, il aurait été utile d'approfondir les connaissances suivantes:

- Nous n'avons pas effectué de mesure précise de la biomasse ou du couvert des plantes herbacées agronomiques. Les observations qualitatives indiquent un couvert similaire pour les placettes d'un même traitement et légèrement inférieur pour les légumineuses vis-à-vis des graminées et du mélange. Notre étude ne permet pas non plus de savoir si une variation du couvert herbacé détermine si ces herbacées sont plus facilitatrices ou compétitrices à l'établissement des arbres pionniers.

- Nous n'avons pas pu quantifier la densité des graines ailées se déposant sur les placettes, ce qui aurait permis de savoir si la faible colonisation spontanée des arbres était due à une limitation de cet apport de graines. Au début du protocole de recherche, nous avions mis des plats de captation de graines pour les compter et les identifier, mais la force des vents et les conditions climatiques difficiles ont causé la destruction de cette partie de l'expérience.

- L'utilisation d'un engrais minéral (8-32-16) à un taux de 750 kg ha^{-1} (8-32-16) pour les croissances des trois traitements de plantes herbacées agronomiques a donné un bon rendement ce celles-ci et demeure raisonnable dans un contexte de revégétalisation minière (Bradshaw, 1997). Cependant, celui-ci ainsi que l'inoculant mycorhizien auraient aussi pu bénéficier aux autres plantes situées sur ces mêmes placettes. Le fait que les semis et les graines des quatre essences étudiées aient été ajoutés aux placettes deux ans après l'ensemencement des herbacées, combiné au niveau de lessivage dans ces sols miniers, limite fortement la possibilité que ces essences aient bénéficié des intrants destinés aux herbacées, mais il aurait aussi été possible d'ajouter les mêmes apports aux placettes témoin pour tenir compte de ce possible effet confondant.

- Un autre type de parcelle témoin aurait pu être configurée en déposant des roches (stériles) concassées sur les résidus miniers. Des études comme celle Fields-Johnson *et al.* (2012) ont montré un apport minéral provenant du lessivage des stériles en plus du rôle de ces roches dans la captation de graines ailées pouvant faciliter une colonisation spontanée.

5.3 Recommandations et perspectives

Les résultats de l'étude ont montré les bénéfices complémentaires des légumineuses et graminées en première étape de la revégétalisation de résidus miniers épaissis à pH neutre (déficients en N). Le traitement de légumineuses a mené à une plus grande concentration foliaire de N pour les semis plantés, ainsi qu'une plus grande biomasse racinaire et croissance en hauteur pour le saule spécifiquement. Ces résultats corroborent des études existantes sur ce sujet (Bradshaw, 1997; Domingo et David, 2014; Jefferies *et al.*, 1981; Maiti et Maiti, 2014). Les graminées ont pour leur part mené à un meilleur établissement du pin gris et du mélèze semés, en plus d'un meilleur taux de survie pour les semis plantés. Ces résultats s'ajoutent à ceux de Guittonny-Larchevêque *et al.* (2016b) établissant l'importance des graminées pour l'amélioration de la macroporosité des résidus miniers.

En conséquence, nous recommandons l’utilisation d’un mélange de graminées et de légumineuses agronomiques, mais avec une plus grande proportion de légumineuses que le mélange utilisé dans cette étude (40 % de légumineuses), car les résultats pour celui-ci étaient peu différents de ceux du traitement de graminées. Inclure une majorité de légumineuses dans le mélange assurerait que leurs bénéfices se réalisent, tout en réduisant la compétition causée par les graminées, particulièrement pour les arbres produisant de petites graines, qui sont plus sensibles à cette compétition.

Au niveau du choix d’essences forestières et de la façon de les introduire sur un site (colonisation spontanée, ensemencement de graines ou plantation de semis), les résultats présentent plusieurs avantages en faveur du saule, notamment sa croissance rapide et le fait qu’il semble bénéficier le plus de la présence de légumineuses. Les salicacées (*Salix* et *Populus*) sont aussi les seuls arbres qui étaient présents spontanément sur l’aire d’étude, quoique leur abondance était très faible. Dans ce cas, dépendamment de l’abondance du saule au voisinage du site à restaurer, la plantation au stade de semis pourrait être nécessaire à son établissement. L’ensemencement serait aussi possible, bien que la densité de graines devrait être supérieure à celle utilisée dans cette étude, vu leur faible taux de germination. Le pin gris et le mélèze pouvait aussi bien s’établir dans les traitements herbacés à partir de graines ou de semis plantés, tandis que le bouleau à papier, pour l’ensemble de l’étude, est une essence qui ne semble pas bien pouvoir s’adapter à des résidus miniers épaissis de pH neutre.

Un aspect important affectant la biodiversité dans la revégétalisation minière des parcs à résidus miniers est la proximité d’espèces semencières et la physiologie de leurs graines. Dans cette étude, nous avions une forêt à moins 50 m de distance du site expérimental, mais les espèces dominantes (épinette noire, sapin baumier) ne se dispersent pas généralement à de grandes distances. Notons que si la proximité des écosystèmes forestiers affecte la revégétalisation, en contrepartie, le site minier affecte aussi la biodiversité de la forêt avoisinante (Yin et al., 2023a, 2023b).

L'application d'une mince couche de *topsoil*, considéré comme un témoin positif dans cette étude, a mené aux plus grands changements dans les propriétés du sol, à la plus grande richesse spécifique de plantes spontanées et à une plus grande survie des semis plantés. Dans les cas où cette ressource est disponible, ce traitement pourrait être considéré conjointement avec l'ensemencement d'herbacées. Toutefois, puisque le *topsoil* a aussi favorisé l'établissement de plusieurs plantes introduites (mauvaises herbes), il est important d'effectuer une surveillance des sites pour effectuer un contrôle de ses espèces au besoin.

Finalement, la revue de littérature et les résultats de l'étude soulèvent plusieurs questions pour la recherche future. Il serait notamment utile d'étudier :

- la trajectoire à plus long terme de l'établissement des graines ailées qui se déposent à la suite des traitements de plantes herbacées agronomiques, pour déterminer l'évolution de la diversité et l'effet de la compétition entre arbres et herbacées;

- le microbiome du sol pour chacun des traitements, en particulier leurs effets sur les communautés mycorhiziennes;

- les effets de la densité, de la distance et des types d'espèce végétale de la forêt boréale à proximité du site de revégétalisation minière sur l'influence de l'assemblage de la communauté végétale due aux traitements des plantes herbacées agronomiques.

Finalement, il serait utile de comparer l'ensemble des propriétés étudiées ici, ainsi que celles proposées comme sujets de recherche future, entre les traitements d'herbacées agronomiques et des traitements impliquant des herbacées indigènes à la région d'étude. Si les herbacées indigènes s'avéraient plus efficaces pour produire un écosystème forestier plus diversifié, résilient et plus proche de l'écosystème de la forêt boréale, alors un investissement dans l'ensemencement d'espèces herbacées indigènes locales serait tout à l'honneur des sociétés d'exploitation minière.

ANNEXE A

ANOVA ANALYSIS OF LIGHT TRANSMISSION AND SOIL TEMPERATURE

Results of the one-way ANOVA for the light transmission and soil temperature results

(a) Light transmission

	df	Sum of squares	Mean square	*F* value	*p* Value
Treatment	4	82485	20621	1.632	0.241
Residual	10	126345	12634		

(b) Soil temperature

	df	Sum of squares	Mean square	*F* value	*p* Value
Treatment	4	10.55	2.637	2.533	0.106
Residual	10	10.41	1.041		

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