UNIVERSITÉ DU QUÉBEC EN ABITIBI-TÉMISCAMINGUE

DÉTERMINER LES EFFETS HORS SITE DES MINES ET LEUR EMPREINTE EN UTILISANT LA VÉGÉTATION ET LA PHYLLOSPHÈRE : INTÉGRATION DU TYPE D'ÉCOSYSTÈME ET DU CYCLE DE VIE DES MINES

THÈSE

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Boisvert, R., Yin, X., & Fenton, J. N. 2021. Offsite effects of mining on the frequency and abundance of five herbaceous species in western Québec (Canada). *Botany*, 99(7): 449-455. doi: 10.1139/cjb-2020-0158. (ANNEXE C)

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Yin, X., Martineau, C., Boisvert, R. & Fenton, J. N., 2021. The offsite impacts of mines on plant diversity in boreal areas." 22e colloque annuel de la Chaire AFD. December 2020, Rouyn-Noranda, Canada.

Effets hors site des mines

comme la propagation du COVID-19 par les éternuements et la toux.

à l'abri des regards

mais le virus dans l'air ambiant

comme les poussières qui recouvrent les lunettes

petites, voire minuscules

mais qui deviennent grandes

quand on les néglige ou qu'on les laisse reposer

comme une pomme pourrie

intacte et belle à l'extérieur

mais qui commence à pourrir de l'intérieur

comme un diamant qui sort de terre

en enlevant seulement une particule

mais qui pourrait avoir plus de valeur qu'une tonne de roche.

“It's the little details that are vital. Little things make big things happen.” — John Wooden

TABLE DES MATIERES

LISTE DES FIGURES

LISTE DES TABLEAUX

RÉSUMÉ

Les plantes et les microorganismes de leur phyllosphère contribuent à la biodiversité et aux services écologiques dans différents biomes forestiers, dont la forêt boréale. Ils jouent un rôle important dans les réservoirs de carbone mondiaux et la fixation de l'azote. L'augmentation de l’activité minière dans le but de satisfaire les besoins en minéraux constitue une menace potentielle pour les services écosystémiques fournis par les plantes de sous-bois et le microbiote de la phyllosphère. Ces menaces ne concernent pas seulement les zones directement perturbées, mais pourraient également s'étendre à des paysages hors site relativement intacts. Les changements prévus dans le type d'écosystème dans le cadre des scénarios de changements globaux constituent une autre perturbation potentielle pour les communautés du sous-bois boréal. Les effets qui se produisent dans les paysages hors site (effets hors site) sont facilement et souvent négligés dans les évaluations d'impact car ils se situent en dehors de l'emplacement immédiat des activités minières. Par conséquent, on en sait peu sur la façon dont les effets hors site des mines sur les communautés du sous-étage et sur le rôle du type d'écosystème sur ces effets.

Afin d'améliorer notre compréhension des effets hors site des mines sur les plantes boréales et le microbiote de la phyllosphère, nous avons étudié la diversité des espèces du sous-étage et l'ADN microbien de la phyllosphère d'une espèce de mousse à plumes (*Pleurozium schreberi* (Brid.) Mitt. dans des paysages en mosaïque de 1 km contenant quatre types d'écosystèmes (conifères, feuillus, forêts mixtes et milieux ouverts) autour de six mines à différents stades d'exploitation (trois mines en exploitation et trois mines non exploitées) dans la zone boréale du Canada. Le métabarcodage du gène de l'ARNr 16S bactérien et la région ITS2 fongique ont été utilisés pour la caractérisation taxonomique du microbiome. Nos résultats ont confirmé la présence d'effets hors site des mines sur la diversité et la composition de la phyllopshère des plantes vasculaires de sous-bois, des bryophytes et des mousses à plumes. L'ampleur des effets hors site était associée au stade d'exploitation et au type d'écosystème. Les effets combinés du type d'écosystème et de l'exploitation minière dans les paysages hors site étaient généralement synergiques pour les plantes vasculaires de sous-bois et les bryophytes, mais pas pour la phyllosphère de la mousse à plumes. Nous avons également constaté que les microhabitats pouvaient atténuer les effets hors site des mines sur la diversité du sous-étage (c'est-à-dire la richesse en bryophytes). Les effets hors site des mines les plus forts se sont produits à une distance de 0,2 km des mines, en particulier près des mines en exploitation.

Compte tenu des changements prévus dans les écosystèmes de la forêt boréale, avec l'empiètement des espèces à feuilles caduques sur les forêts de conifères, et la sensibilité accrue des forêts mixtes et à feuilles caduques, la zone touchée par les effets hors site des mines pourrait augmenter dans le futur. La résistance des forêts pourrait être réduite dans les paysages hors site par la réduction de la diversité des plantes vasculaires et des bryophytes, l'augmentation des différences de composition et la diminution de l'abondance de groupes fonctionnels importants (c'est-à-dire les mousses à plumes et les sphaignes).

Les effets hors site sur la diversité et la composition du sous-étage observés étaient généralement faibles et semblaient négligeables dans la biodiversité globale de la forêt boréale. Cependant, la perte d'espèces clés, l'invasion d'espèces introduites et les impacts cumulatifs à grande échelle peuvent augmenter l'impact des effets hors site des mines sur les services écosystémiques. Par conséquent, les effets hors site des mines devraient être inclus dans les évaluations écologiques futures afin de maintenir la biodiversité et les services écologiques dans la forêt boréale. La création d'une zone tampon autour des mines pourrait permettre de compenser les effets hors site potentiels. D'autres études seront nécessaires pour évaluer comment ces effets hors site des mines sur la structure du sous-étage et de la phyllosphère affectent les services des écosystèmes boréaux et pour développer des stratégies d'atténuation appropriées.

Mots-clés : impacts indirects, végétation de sous-bois, surface foliaire, bactéries, champignons, écologie du paysage, indicateurs, taïga forestière.

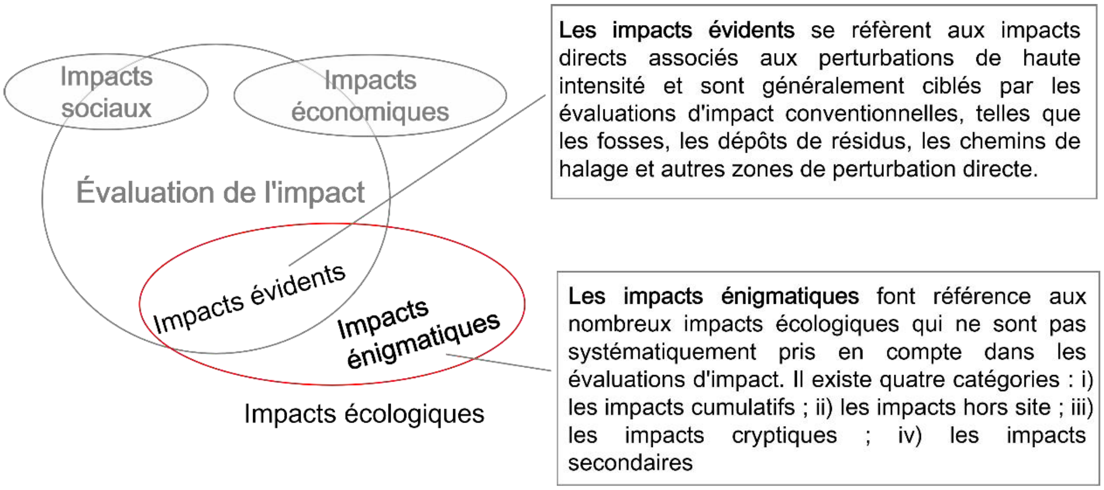
CHAPITRE I

INTRODUCTION GÉNÉRALE

1.1 Mise en contexte

Les activités humaines sont devenues un moteur essentiel de la dynamique de la biodiversité mondiale dans l'Anthropocène (Ellis et al., 2012 ; Seddon et al., 2016 ; Storch et al., 2021). Les activités minières constituent un héritage à long terme de l'industrialisation propre à l'époque (McMahon et Moreira, 2014 ; Zhang et al., 2019) et ont un impact considérable sur la biodiversité par le biais de divers mécanismes (par exemple, la dégradation de l'habitat, les espèces envahissantes, le rejet de déchets et la pollution) et à travers les échelles spatiales (Sonter et al., 2018). En général, les mines et les gisements ne sont pas répartis de manière aléatoire, mais concentrés dans des zones présentant une biodiversité intermédiaire à élevée (Murguia et al., 2016). Les besoins croissants en matière de production d'énergie renouvelable vont également exacerber les menaces minières sur la biodiversité (Sonter et al., 2020). Par exemple, les éléments de terres rares sont de plus en plus nécessaires pour les technologies propres telles que les véhicules électriques et les éoliennes (Zhou et al., 2017), ce qui entraînera une augmentation des zones influencées directement et indirectement par l'exploitation minière (Goodenough et al., 2018).

Les impacts de l'exploitation minière sur la biodiversité sont susceptibles d'être modulés par des changements globaux tels que les changements prévus dans la composition des forêts dans les zones boréales. La prédominance relative des arbres à feuilles caduques devrait augmenter au cours des prochaines décennies, la composition des peuplements passant des conifères aux feuillus sous l'effet des changements climatiques et des incendies de forêt (Gray et He, 2009 ; Mack et al., 2021 ; Mekonnen et al., 2019). En outre, le passage des tourbières aux peuplements forestiers est également un scénario pour les changements de végétation prévus dans les forêts boréales (Ratcliffe et al., 2017 ; van der Velde et al., 2021), en particulier dans le sud des forêts boréales où le boisement des tourbières est causé par des perturbations naturelles et anthropiques, telles que les changements climatiques, l'agriculture et l'exploitation minière (Howson et al., 2021 ; Lachance et al., 2005 ; Lavoie et al., 2005). Les effets de l'exploitation minière et des changements prévus dans le type d'écosystème sur la biodiversité ont été largement étudiés séparément, mais peu d'études sont disponibles sur les effets de leur interaction sur la biodiversité, en particulier pour les impacts miniers hors site potentiels. Par conséquent, on ne sait toujours pas si les changements prévus dans la composition des forêts exacerberont les effets de l'exploitation minière sur la biodiversité des paysages environnants. Les effets hors site des mines sur la biodiversité appartiennent à la catégorie des impacts énigmatiques (figure 1.1) car ils sont souvent négligés dans les évaluations d'impact (Raiter et al., 2014). Les effets hors site sont les impacts qui se produisent loin de l'emplacement immédiat de la perturbation ou de la zone d'étude et qui peuvent s'infiltrer loin dans des paysages apparemment intacts (Raiter et al., 2014). Des études sur les effets hors site ont été réalisées à proximité des sites de diverses activités humaines telles que les routes (Jakobsson et al., 2018), l'exploitation forestière (Brudvig et al., 2009) et l'exploitation minière (Weir et al., 2007), cependant, nos connaissances sur les effets hors site des mines sur la diversité végétale et microbienne sont encore limitées, en particulier dans les paysages boréaux. La recherche d'indicateurs appropriés pour surveiller l'étendue de la zone affectée par les mines (empreinte hors site) constitue également un défi dans l'évaluation des effets hors site. Nous prévoyons que les types d'écosystèmes et le cycle de vie des mines sont des facteurs déterminants pour la présence et l'ampleur des effets hors site des mines sur la biodiversité. Trois groupes de communautés d’organismes de sous-bois, les plantes vasculaires, les bryophytes et le microbiote de la phyllosphère (micro-organismes vivant à la surface des feuilles), ont été utilisés dans le projet pour confirmer cette prédiction et pour sélectionner des groupes d'indicateurs appropriés.



Les impacts évidents se réfèrent aux impacts directs associés aux perturbations de haute intensité et sont généralement ciblés par les évaluations d'impact conventionnelles, telles que les fosses, les dépôts de résidus, les chemins de halage et autres zones de perturbation directe.

Les impacts énigmatiques font référence aux nombreux impacts écologiques qui ne sont pas systématiquement pris en compte dans les évaluations d'impact. Il existe quatre catégories : i) les impacts cumulatifs ; ii) les impacts hors site ; iii) les impacts cryptiques ; iv) les impacts secondaires

Figure 1.1 Le concept d'impact énigmatique

1.1.1 Effets des types d'écosystèmes sur la biodiversité du sous-bois

Les forêts de conifères, les forêts de feuillus, les forêts mixtes et les milieux ouverts (sans arbres de canopée, constitués principalement de tourbières) sont les quatre principaux types d'écosystèmes de la forêt boréale en fonction de la composition de leur canopée (Figures 1.2). Les types d'écosystèmes peuvent déterminer la structure du sous-étage en créant des conditions variées pour la croissance des espèces qui y vivent. En général, les arbres à feuilles caduques permettent une transmission plus élevée des nutriments foliaires et de la lumière que les conifères (Kembel et Dale, 2006 ; Qian et al., 2003), tandis que les peuplements de conifères créent un pH plus faible, des cations basiques plus bas et une humidité de l'air plus élevée (Augusto et al., 2002). Les forêts mixtes créent généralement des environnements plus hétérogènes que les forêts monospécifiques (Pollastrini et al., 2017). Les milieux ouverts, sans arbres de la canopée, reçoivent plus de lumière, de précipitations, de rayonnement solaire et de dépôts atmosphériques que les forêts à canopée fermée. Ces différences abiotiques influencent la composition du sous-étage dans chaque type d'écosystème. Les peuplements de conifères sont généralement considérés comme moins favorables à la diversité et à la couverture des plantes vasculaires que les peuplements de feuillus (Kembel et Dale, 2006), et tendent à supporter une couverture plus élevée de bryophytes (Bartels et al., 2018b ; Légaré et al., 2005). Les forêts mixtes ne favorisent ni les strates vasculaires ni les bryophytes (Cavard et al., 2011) et présentent une diversité de sous-étage similaire à celle des peuplements de conifères et de feuillus (Brassard et al., 2008). Les zones ouvertes ont tendance à abriter des communautés de lichens ou de bryophytes en fonction du taux d'humidité (Horstkotte et Moen, 2019). En outre, certaines études ont révélé que les forêts de conifères et de feuillus soutiennent différents groupes microbiens spécifiques dans leur couche de sous-bois. Dans la phyllopshère de la mousse à plumes, les cyanobactéries étaient davantage associées aux forêts de feuillus (*Populus tremuloides* Michx.) qu'aux forêts de conifères (*Picea mariana* (Mill.) Britton, Sterns & Poggenb., Rodríguez-Rodríguez et al., 2022), tandis que la forêt de conifères supportait une plus grande abondance relative du phylum bactérien WPS-2 (Jean et al., 2020b).

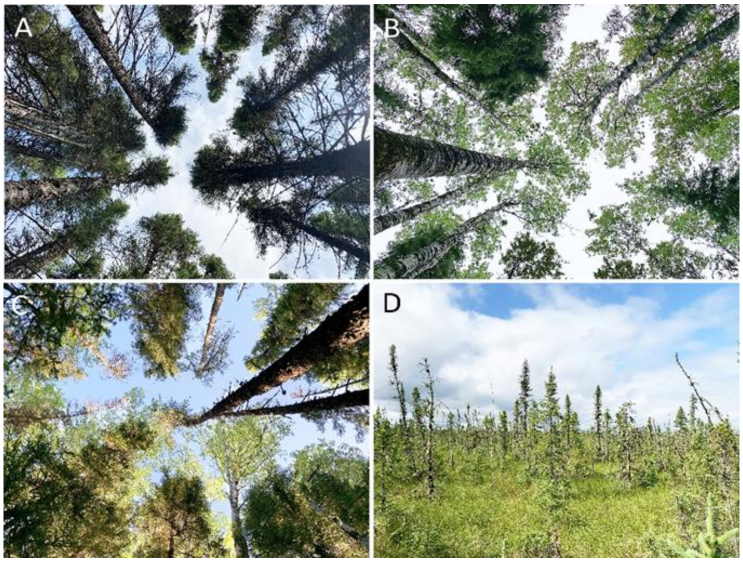


Figure 1.2 Principaux types d'écosystèmes dans les paysages boréaux. A: forêt de conifères. B: forêt de feuillus. C: forêt mixte. D: milieux ouverts (tourbières).

Les différences dans la structure de la canopée et la composition des espèces du sous-étage contribuent à la résistance variable de chaque type d'écosystème aux perturbations humaines (par exemple, le dépôt de la pollution atmosphérique provenant de l'exploitation minière) et aux changements climatiques. Les espèces végétales soutenues par les canopées de feuillus sont généralement intolérantes à l'ombre et exigeantes en nutriments, tandis que les conifères favorisent l'établissement d'espèces végétales tolérantes à l'ombre et à faible teneur en nutriments du sol, comme les bryophytes (Su et al., 2019), ce qui indique que l'écosystème de conifères pourrait être plus résistant aux changements climatiques naturels et aux perturbations anthropiques que l'écosystème de feuillus. En outre, les canopées de conifères peuvent tamponner les variations environnementales et éliminer la pollution atmosphérique grâce aux caractéristiques de la longueur de la période de défoliation et de la forte densité du couvert arboré (Barbé et al., 2020 ; De Jalón et al., 2019). Dans les milieux ouverts, malgré une exposition directe aux changements environnementaux avec l'absence d'arbres de la canopée, les données paléoécologiques montrent une résistance relativement élevée des tourbières aux changements climatiques du passé (Łuców et al., 2020) car elles sont protégées par un microclimat spécifique avec des conditions humides générées par la tourbe accumulée (Augusto et al., 2002). Cependant, la résistance des tourbières aux activités humaines pourrait être faible, et la dégradation des tourbières par l'exploitation minière, les routes, la foresterie et l'agriculture par le drainage et l'extraction de la tourbe (Glina et al., 2019 ; Page et Baird, 2016 ; Williams-Mounsey et al., 2021) a été signalée. Par conséquent, nous pensons que la structure du sous-étage dans chaque type d'écosystème pourrait présenter une résistance différente aux effets hors site des mines.

1.1.2 Dynamique des perturbations au cours du cycle de vie de l'exploitation minière

L'intensité et l'échelle des perturbations minières évoluent de manière dynamique avec le cycle de vie de l'exploitation. Le processus d'exploitation minière se compose de quatre grandes étapes de développement: 1) l'exploration; 2) la construction; 3) l'exploitation; 4) la fermeture et la réhabilitation (Gorman et Dzombak, 2018). La force et les échelles de perturbations ne sont pas les mêmes à chaque étape. La tâche principale de l'étape d'exploration est de sonder pour trouver des minéraux, y compris des relevés sur le terrain et par satellite, ainsi que la collecte d'échantillons de minéraux pour une évaluation économique. Les perturbations (par exemple, les camps et l'équipement sur le terrain) sur les paysages sont limitées à ce stade. L'intensité des perturbations augmente au cours de la phase de construction, lorsque les bâtiments et les infrastructures sont développés et que la végétation et le sol de surface sont défrichés, ce qui détruit directement la structure et la fonction des écosystèmes de surface. L'intensité des perturbations (c'est-à-dire les effets sur les milieux environnants) et les échelles (c'est-à-dire les zones influencées) continuent de s'étendre au stade de l'exploitation, car les opérations d'extraction et de traitement des minéraux sont les principales activités sur les sites miniers en exploitation, comme le forage, le dynamitage, la ventilation, l'assèchement, le chargement et le transport, le concassage et le broyage, et les séparations (Norgate et Haque, 2010). De nombreux polluants (par exemple, les métaux lourds, la poussière et le drainage minier acide) sont créés et libérés dans le milieu environnant au cours de ces activités (Punia, 2021), ce qui entraîne une pollution atmosphérique, une pollution de l'eau et une dégradation des sols dans les paysages environnants (Li et al., 2014 ; Tolvanen et al., 2019). Au stade de la fermeture et de la réhabilitation, les perturbations commencent à être réduites en raison de la fermeture de toutes les opérations minières, du retrait des équipements et de la revégétalisation (Limpitlaw, 2004).

En général, étant donné que l'étendue et l'intensité des perturbations minières changent avec le temps, le cycle de vie minier peut être classé en deux groupes principaux en fonction de l'intensité des perturbations : exploitation et non-exploitation (y compris l'exploration, la construction, ainsi que la fermeture et la réhabilitation). Les différences de perturbations minières à différentes étapes peuvent conduire à des effets distincts des mines sur la biodiversité environnante entre les étapes.

1.1.3 Rôles écologiques des communautés de sous-bois dans l'écosystème boréal

La forêt boréale est l'un des plus grands biomes sur Terre, représentant 11% de la surface terrestre globale, qui assure de multiples fonctions écologiques à l'échelle locale et globale (Gauthier et al., 2015). Les forêts boréales canadiennes, qui occupent environ 30 % des forêts boréales du monde entier, jouent des rôles essentiels dans le cycle global du carbone, la régulation du climat et la conservation de la biodiversité (Peng et al., 2011 ; Venier et al., 2014). Les communautés végétales du sous-bois (y compris les espèces vasculaires et bryophytes, Figure 1.3 présentant la biodiversité du sous-bois dans la forêt boréale) apportent une contribution indispensable à ces services écologiques, notamment la succession forestière (Royo et Carson, 2006) et le cycle des nutriments (Nilsson et Wardle, 2005), la nourriture et les habitats pour les espèces sauvages et même les médecines traditionnelles pour les communautés autochtones (Uprety et al., 2012). En outre, le microbiote résidant dans ou sur les tissus végétaux d'espèces de sous-bois (comme les bryophytes) soutient également la biodiversité et les services écologiques dans les paysages boréaux (Compant et al., 2019 ; Trivedi et al., 2020).

Figure 1.3 Biodiversité du sous-étage dans les paysages boréaux. A-D: la couche de sous-bois des forêts de conifères, de feuillus, mixtes et de milieux ouverts (de A à D); E: *Chamerion angustifolium* (L.) Holub; F: *Gaultheria hispidula* (L.) Muhl. ex Bigelow; G: *Pteridium aquilinum* (L.) Kuhn; H: *Vaccinium myrtilloides* Michx.; I: *Rubus idaeus* L.; J: *Ribes glandulosum* Grauer; K: *Sarracenia purpurea* L.; L: *Rhododendron groenlandicum* (Oeder) Kron & Judd; M: *Drosera rotundifolia* L.; N: *Cladonia cristatella* Tuck.; O: *Sphagnum fuscum* (Schimp.) Klinggr.; P: *Sphagnum capillifolium* (Ehrh.) Hedw.; Q: *Riccardia chamedryfolia* (With.) Grolle.; R: *Pleurozium schreberi* (Willd. ex Brid.) Mitt.; S: *Marchantia polymorpha* L. subsp. *ruderalis* Bischl. & Boissel.-Dub.; T: *Aneura pinguis* (L.) Dumort.; U-V: champignons.

La végétation de sous-bois est la composante la plus diversifiée des communautés végétales boréales (Hart et Chen, 2006). Les plantes de sous-bois représentent une faible proportion de la biomasse totale par rapport aux arbres, mais elles contribuent de manière significative aux cycles du carbone et des nutriments et à la succession des écosystèmes boréaux, avec des taux de renouvellement plus élevés que les arbres de la canopée (Kumar et al., 2018 ; Nilsson et Wardle, 2005). Par exemple, les mousses à plumes peuvent représenter jusqu'à 30 % de la productivité primaire nette des forêts boréales (Wardle et al., 2012) et leur phyllosphère peut contribuer jusqu'à > 50 % des sources totales d'azote dans les forêts boréales de conifères (Jean et al., 2021). Les espèces du sous-étage sont aussi généralement utilisées comme indicateurs biologiques pour évaluer les perturbations humaines, car leur diversité et la composition des communautés sont sensibles aux activités anthropiques, notamment l'exploitation forestière, l'exploitation minière et le pâturage (Ekka et Behera, 2011; Kariuki et al., 2006 ; Metzger et al., 2005). Les espèces végétales envahissantes sont des indicateurs typiques qui sont généralement utilisés pour évaluer la gravité et la fréquence des perturbations (Dyderski et Jagodziński, 2018; Lavoie, 2010). En outre, les bryophytes ont également été largement utilisés comme indicateurs fiables de la pollution atmosphérique, des changements climatiques (température et humidité), des dépôts de métaux lourds et d'autres perturbations (Balabanova et al., 2017; Ceschin et al., 2012; Gignac, 2001; Mahapatra et al., 2019; Szczepaniak et Biziuk, 2003). En effet, elles sont dépourvues de véritables racines, de systèmes vasculaires et de cuticules épaisses, et sont donc très sensibles aux changements du milieu environnant, notamment aux microhabitats, aux types d'écosystèmes et aux propriétés physicochimiques.

La microflore (ou microbiote) de la phyllosphère (la surface des feuilles) est une autre composante importante de la couche de sous-bois qui maintient les services écologiques des écosystèmes boréaux. Des études ont révélé que la microflore de la phyllosphère joue un rôle critique dans la résistance aux agents pathogènes et aux herbivores (Ab Rahman et al., 2018 ; Saleem et al., 2017), la tolérance au stress (Arun et al., 2020), la promotion de la croissance et de la reproduction (Canto et Herrera, 2012 ; Stone et al., 2018), la dégradation du méthanol (Galbally et Kirstine, 2002 ; Iguchi et al., 2012) et la fixation de l'azote (Abril et al., 2005 ; Fürnkranz et al., 2008). Dans les écosystèmes boréaux, les micro-organismes de la phyllopshère des mousses jouent un rôle important dans les services écologiques. En particulier, les associations mousse à plumes-cyanobactéries (Rousk et al., 2013) et *Sphagnum*-

méthanotrophes (Putkinen et al., 2012) sont des moteurs importants du cycle du carbone et de l'azote dans les forêts de conifères et les tourbières. Une étude a montré que *Pleurozium schreberi* (une espèce commune de mousse à plumes) pouvait à elle seule, grâce à l'activité des bactéries fixatrices d'azote de la phyllosphère (par exemple, les genres *Nostoc* et *Scytonema*), fixer 1,5 à 2,0 kg d'azote ha^{-1} an^{-1} dans les forêts boréales (DeLuca et al., 2002). En outre, les communautés microbiennes (la plupart des études portent sur le microbiome du sol) sont sensibles aux changements environnementaux causés par les activités humaines, notamment la teneur en métaux lourds et les dépôts atmosphériques (Karimi et al., 2017 ; Li et al., 2017 ; Sharma et al., 2010). Les micro-organismes sensibles aux perturbations (par exemple, la toxicité des métaux lourds) vont diminuer en abondance, tandis que les espèces tolérantes deviennent prédominantes (Guo et al., 2017 ; Zhang et al., 2022). Les changements dans la structure de la communauté microbienne peuvent sérieusement affecter les services écologiques fournis par les micro-organismes (Giller et al., 1998). Les changements dans le microbiome de la phyllosphère pourraient également être un indicateur sensible du stress de l'hôte par les perturbations humaines, y compris l'exploitation minière (Xu et al., 2020). Cependant, peu d'études ont jusqu'à présent permis de comprendre comment l'exploitation minière affecte les communautés de la phyllosphère.

1.2 Objectifs et hypothèses de recherche

L'objectif général de cette thèse de doctorat est de comprendre et de caractériser les effets hors site des mines sur les communautés du sous-étage et de la phyllosphère dans les paysages boréaux environnants et d'identifier si la composition de la forêt et le cycle de vie de l'exploitation minière influencent ces effets. Pour cet objectif global de recherche, nous avons sélectionné trois groupes : les communautés végétales du sous-étage au sol, les bryophytes dans les microhabitats et les micro-organismes de la phyllosphère des mousses à plumes. Les objectifs spécifiques et les hypothèses pour chaque groupe de recherche sont les suivants :

1.2.1 Communautés générales du sous-bois

Objectifs :

1) Évaluer comment le type d'écosystème et le cycle de vie des mines affectent les effets hors site des mines ;

2) Evaluer la taille de l'empreinte hors site des mines sur le sous-étage.

Hypothèses:

1) Étant donné que plus d'effets environnementaux (par exemple, les émissions) se produisent pendant la phase d'exploitation, on s'attend à ce que la diversité globale du sous-étage soit plus faible à proximité des sites d'exploitation que des sites non exploités ;

2) On s'attend à ce que les communautés du sous-étage diffèrent dans leur sensibilité aux effets hors site de l'exploitation minière en fonction du type d'écosystème parce que les différents écosystèmes favorisent des groupes distincts qui pourraient avoir des niveaux variables de tolérance aux perturbations ; en outre, les différences dans la structure de la canopée entre les types d'écosystèmes pourraient fournir différents

niveaux de protection aux communautés du sous-étage contre les perturbations des mines;

3) Si l'on considère que les effets hors site se produisent dans des paysages relativement intacts qui ne sont pas visuellement distincts, les changements et la distance influencés par les mines devraient être relativement faibles, mais détectables si l'on considère la richesse et la composition du sous-étage au niveau de la communauté, et leur empreinte cumulative pourrait être significative.

1.2.2 Bryophytes sur les microhabitats

Objectifs:

1) Évaluer le rôle des microhabitats dans la médiation des effets d'interaction des types d'écosystèmes et de l'exploitation minière sur la structure des communautés de bryophytes.

2) Évaluer si les types d'écosystèmes et l'exploitation minière affectent les services écologiques des bryophytes en modifiant l'abondance de groupes fonctionnels importants (i.e. les mousses à plumes et les sphaignes).

Hypothèses:

1) On s'attend à ce que les types d'écosystèmes affectent la présence et l'ampleur des effets des mines sur la structure des bryophytes environnantes, étant donné les effets variables des différents types d'écosystèmes sur la diversité des bryophytes ;

2) Les interactions entre les effets hors site des mines et les types d'écosystèmes sur les bryophytes devraient également être influencées par les microhabitats, étant donné que les microhabitats présentent des capacités différentes pour amortir les changements environnementaux ;

3) Les changements de type de mines et d'écosystèmes pourraient affecter les services écologiques des paysages hors site en réduisant l'abondance de groupes fonctionnels importants (c'est-à-dire les mousses à plumes et les sphaignes), car les mousses à plumes et les sphaignes sont principalement soutenues par les forêts de conifères et les tourbières par rapport aux forêts de feuillus et mixtes.

1.2.3 Microbiote de la phyllosphère de la mousse de plumes

Objectifs :

1) Déterminer la présence d'effets hors site des mines sur le microbiote de la phyllosphère des mousses à plumes dans les paysages environnants des mines.

2) Évaluer les effets du type d'écosystème, du stade d'exploitation minière et de leur interaction sur les effets hors site de l'exploitation minière sur la structure de la communauté microbienne de la phyllosphère des mousses.

3) Déterminer la distance d'influence des effets hors site des mines sur le microbiote de la phyllosphère des mousses.

Hypothèses:

1) En se basant sur les résultats prédits selon lesquels la structure des communautés de bryophytes serait affectée par le stade de l'exploitation minière (Hypothèse en 1.2.2), on s'attend à ce que les effets sur la diversité microbienne, la structure des communautés et la composition de la phyllosphère soient plus importants près des mines en exploitation que près des sites non exploités.

2) Étant donné que le type d'écosystème est censé être un déterminant clé de la structure des communautés de bryophytes (hypothèse du point 1.2.2), les effets des mines sur le microbiote de la phyllosphère de la mousse à plumes devraient également être influencés par les types d'écosystèmes.

1.3. Sites étudiés

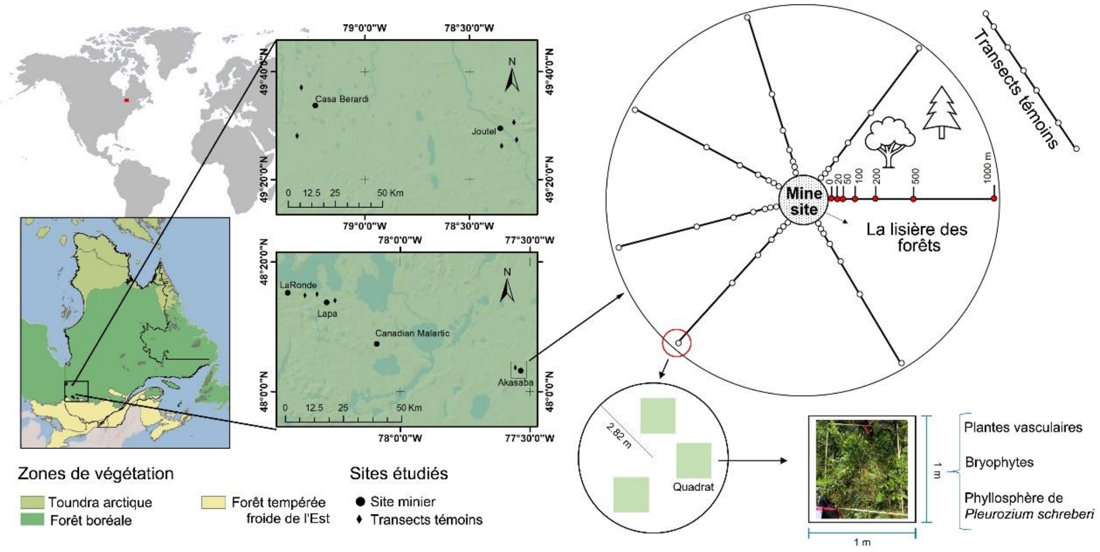


Figure 1.4 Sites étudiés. À gauche, carte du Canada et du Québec montrant les principales zones de végétation. À droite, carte de la région d'étude indiquant la position des parcelles d'échantillonnage sur six sites de mines d'or et 9 transects de contrôle

Six sites miniers ont été sélectionnés en Abitibi-Témiscamingue et dans le Nord-du-Québec, dans la province de Québec au Canada (Figure 1.4): Akasaba (48°3′12″N; 77°32′8″W, Agnico Eagle Mines Ltd), Canadian Malartic (48°7′21″N; 78°5′23″W, Canadian Malartic Corp.), Casa Berardi (49°33′43″N; 79°14′8″W, Hecla Québec Inc.), Joutel (49°29′28″N; 78°21′8″W, Agnico Eagle Mines Ltd), Lapa (48°13′45″N; 78°17′1″W, Agnico Eagle Mines Ltd), LaRonde (48°15′14″N; 78°25′59″W, Agnico Eagle Mines Ltd). Trois d'entre eux (Casa Berardi, en exploitation depuis 1988 ; LaRonde, depuis 1988 et Canadian Malartic, depuis 2005) sont en exploitation et les trois autres (Akasaba, en cours d’établissement ; Joutel, fermé depuis 1988 et Lapa, fermé depuis 2018) sont hors exploitation. Casa Berardi et Joutel sont situés dans le domaine bioclimatique *Picea mariana* - mousses, tandis que les quatre autres sites miniers sont tous situés dans le domaine bioclimatique *Abies balsamea* - *Betula* *papyrifera*. Neuf transects non perturbés ont été sélectionnés comme sites témoins : cinq dans le domaine bioclimatique *Picea mariana* - mousses et quatre dans le domaine bioclimatique *Abies balsamea* - *Betula papyrifera*.

Les sites d'étude se trouvent dans une région qui est caractérisée par un climat continental froid et humide avec une température moyenne comprise entre -14 et -10°C en hiver et entre 16 et 18°C en été (Environnement Canada, 2021). Les précipitations annuelles moyennes vont de 790 à plus de 1 300 mm (Wang et al., 2013).

1.4. Plan de la thèse

Cette thèse est structurée en six chapitres, comme le montre la figure (Figure 1.5). Le contenu principal de ces six chapitres est le suivant :

Le chapitre I (ce chapitre) présente le contexte de la recherche, les objectifs, la méthodologie et le plan de la thèse.

Le chapitre II présente les effets hors site des mines sur la richesse, la couverture et la composition des espèces du sous-étage entourant les paysages boréaux (zones de 1 km près des sites miniers) au niveau des communautés (richesse, couverture et composition des espèces de plantes vasculaires herbacées et ligneuses ainsi que la couverture des bryophytes et des lichens) et évalue les rôles potentiels du cycle de vie minier (mines en phase d'exploitation et de non-exploitation) et des types d'écosystèmes comme facteur déterminant de ces paramètres. La distance influencée par les effets hors site est déterminée en utilisant différents groupes de sous-étage.

Le chapitre III présente les effets d'interaction de l'exploitation minière et de la composition de la forêt sur la structure des communautés de bryophytes dans les paysages boréaux entourant les mines en utilisant la richesse et la composition en espèces de toutes les bryophytes, mousses et hépatiques. Ensuite, les effets tampons des microhabitats (c'est-à-dire le sol, les arbres, les billes, les souches, les chicots et les rochers) sur ces effets d'interaction ont été évalués. Enfin, l'abondance des mousses à plumes et des sphaignes a été utilisée pour estimer les impacts potentiels des effets d'interaction sur les services écologiques des forêts boréales. Dans ce chapitre, nous avons constaté que la couverture végétale de la mousse à plumes a des effets significatifs en fonction du stade d'exploitation et du type d'écosystème. Nous avons donc choisi d’étudier le microbiote de la mousse à plumes dans le chapitre IV pour évaluer comment les effets hors site des mines affectent la structure de la communauté microbienne de la phyllosphère.

Le chapitre IV présente les effets des mines sur la diversité et la composition du microbiote de la phyllopshère des mousses à plumes (indices de richesse et de diversité bactérienne et fongique ainsi que l'abondance relative des principaux phyla) dans les paysages boréaux et évalue l'influence potentielle du du cycle de vie minier et des types d'écosystèmes. Les taxons indicateurs de chaque type d'écosystème et de chaque stade d'exploitation minière (exploitation, non-exploitation et contrôles) ont été évalués, respectivement. Ensuite, la distance influencée par les effets hors site des mines a été déterminée en utilisant la diversité et la structure de la communauté du microbiote de la phyllopshère ainsi que l'abondance relative des taxons individuels (principaux phyla bactériens et fongiques, genres et ASVs, amplicon sequence variant).

Le chapitre V résume brièvement les résultats obtenus dans la thèse, discute de leur signification, identifie les limites de la recherche et propose des orientations futures pour la recherche.

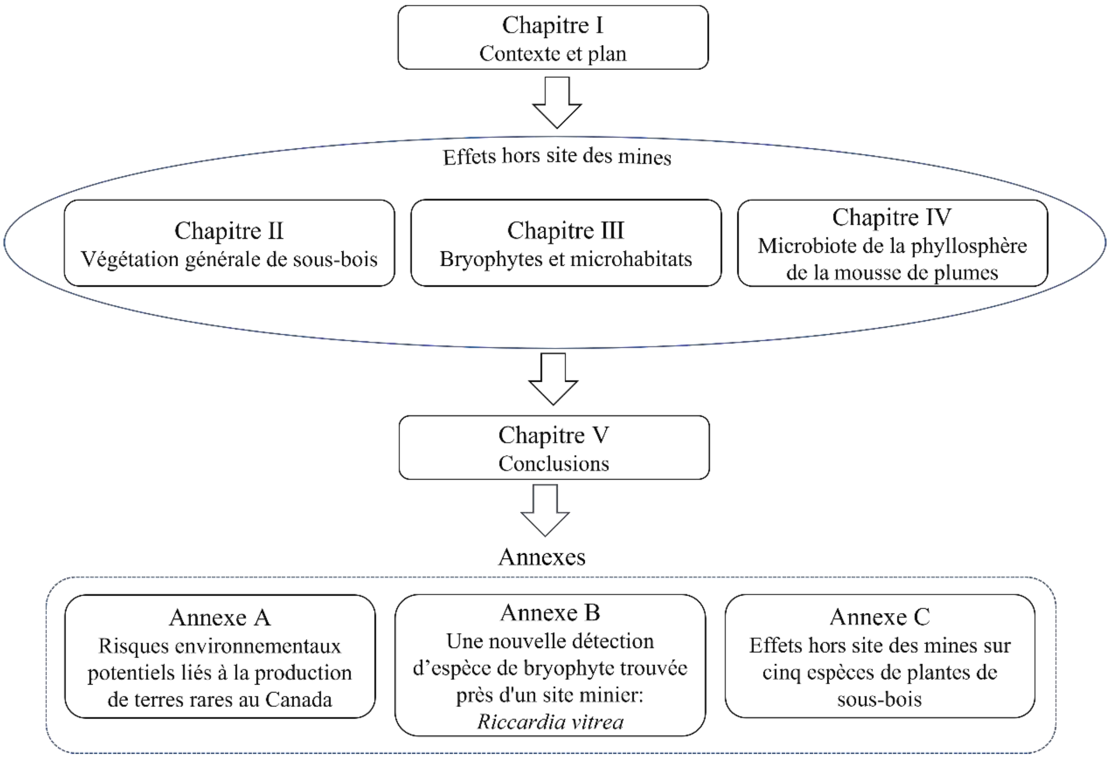


Figure 1.5 Le plan de la thèse

En outre, trois chapitres supplémentaires sont inclus dans la section ‘Annexe’ de cette thèse. Le premier chapitre annexé (annexe A) concerne les risques environnementaux potentiels associés à ‘activités minière en utilisant la production de terres rares comme cas. Il traite des impacts potentiels de l'exploitation des terres rares sur les services écologiques et la santé humaine au Canada, ce qui fournit une revue de la littérature en lien avec le contexte de la thèse. Le deuxième chapitre annexé (annexe B) décrit une nouvelle détection d’espèce (*Riccardia vitrea*) en Amérique du Nord. Cette espèce a été trouvée près d'un site minier, ce qui suggère que l'exploitation minière pourrait fournir un habitat assez spécifique pour certaines espèces. L'annexe B présente la contribution de cette thèse au nombre d'espèces de bryophytes présentes au Canada. Le troisième chapitre annexé (annexe C) porte sur les effets hors site des mines sur cinq espèces de sous-bois, ce qui constitue nos résultats préliminaires. Les résultats de l'annexe C ont confirmé la présence d'effets hors site des mines au niveau des espèces, ce qui fournit des bases pour des études ultérieures dans les chapitres principaux.

CHAPITRE II

QUELLE EST LA TAILLE DE L'EMPREINTE ? QUANTIFIER LES EFFETS HORS SITE DES MINES SUR LES COMMUNAUTÉS VÉGÉTALES BORÉALES

HOW BIG IS THE FOOTPRINT? QUANTIFYING OFFSITE EFFECTS OF MINES ON BOREAL PLANT COMMUNITIES

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2.1 Résumé

Les menaces que l'exploitation minière fait peser sur la biodiversité et les services écologiques des forêts boréales augmentent à mesure que la demande de minéraux s'accroît à l'échelle mondiale. Cependant, on en sait beaucoup moins sur la façon dont les effets hors site des mines affectent les communautés du sous-étage, car ils se produisent en dehors de l'emplacement immédiat de la mine et sont souvent négligés lors des évaluations écologiques. Nous avons mené une étude approfondie sur le terrain le long de transects d'un kilomètre autour de six sites miniers à différents stades d'exploitation (en exploitation ou non), qui traversent quatre types d'écosystèmes (feuillus, conifères, forêts mixtes et canopée ouverte) dans la forêt boréale canadienne. Les effets hors site des mines sur le sous-étage ont été quantifiés en utilisant des plantes vasculaires (ligneuses et herbacées), des bryophytes et des lichens. Les effets hors site des mines ont eu un impact sur la diversité et la composition du sous-étage. La richesse et la couverture du sous-étage étaient plus réduites à proximité des mines en exploitation que des mines non exploitées. Le stade d'exploitation minière a principalement altéré de manière significative la diversité du sous-étage et la structure de la communauté dans les forêts à feuilles caduques et mixtes, tandis que les communautés du sous-étage étaient plus résistantes aux effets hors site dans les forêts de conifères. L'empreinte a été quantifiée en utilisant la distance influencée et les effets les plus forts se situaient généralement à moins de 0,2 km des mines. Étant donné les changements prévus dans les écosystèmes de la forêt boréale avec l'empiètement des espèces à feuilles caduques sur les forêts de conifères et la sensibilité accrue des forêts mixtes et à feuilles caduques, la zone affectée par les effets hors site des mines pourrait augmenter à l'avenir. Nous suggérons que les effets hors site soient inclus dans les évaluations écologiques.

Mots-clés: impacts énigmatiques, empreinte minière, végétation de sous-bois, activités humaines.

2.2 Abstract

Threats from mining to the biodiversity and ecological services of boreal forests are increasing as demand for minerals increases globally. However, much less is known about how offsite effects of mines affect understory communities as they occur outside the immediate location of mining and are often overlooked during ecological evaluations. We conducted an extensive field survey along 1-km transects surrounding six mine sites of different mining stages (operating vs non-operating), that crossed four ecosystem types (deciduous, coniferous, mixed forests and open canopy) in Canada's boreal forest. The offsite effects of mines on the understory were quantified using vascular plants (woody and herbaceous), bryophytes and lichens. Mine offsite effects impacted understory diversity and composition. Understory richness and cover was more reduced near operating than non-operating mines. Mining stage mainly significantly altered understory diversity and community structure in deciduous and mixed forests while understory communities were more resistant to the offsite effects in coniferous forest ($P > 0.05$). The footprint was quantified using the influenced distance and the strongest effects were generally within 0.2 km from mines. Given the predicted changes in boreal forest ecosystems with encroachment of deciduous species into coniferous forests and the increased sensitivity of mixed and deciduous forests, the area affected by offsite effects of mines could grow in the future. We suggest that offsite effects should be included in ecological evaluations.

Keywords: enigmatic impacts, mine footprint, understory vegetation, human activities

2.3 Introduction

Ecological degradation, particularly biodiversity loss, is a global process with mining activities as one of the main drivers. This is due to habitat degradation, invasive species propagation, and pollution (Sonter et al., 2018; Tolvanen et al., 2019; Žibret et al., 2018). Currently, studies focus on impacts within industrial mine sites, however the effect of mine sites on surrounding landscape has not been studied. Offsite effects are impacts that extend beyond the edge of the immediate mine location reaching into undisturbed areas (Raiter et al. 2014). They are often overlooked in ecological evaluations and decision-making processes as they are outside of the designated project area and their impacts are often less visually distinct. The offsite landscapes surrounding mines are exposed to various disturbances including deposition of heavy metals, salt, and excess nutrients that are generated by mining operations, transportation, forest fragmentation and edge effects (Raiter et al., 2014; Sonter et al., 2018). A few studies report offsite effects on plants in the areas surrounding mines (Bech et al., 2012; Boojar and Tavakkoli, 2011; Christou et al., 2017). However, the potential for characteristics of mine sites (e.g., mining stages) and surrounding landscapes (e.g., ecosystem types over hundreds of meters) to modify the strength of offsite effects remains unstudied to our knowledge. This is particularly true in the boreal forest where increasing mining activity is becoming a highly visible threat to biodiversity (Venier et al. 2014; Musetta-Lambert et al. 2019).

Mine sites at different stages of the mining lifecycle generate different environmental disturbances. Generally, the mining lifecycle includes establishment (including surface vegetation cleared, building roads and processing facilities), operation (including extracting, processing, grinding and smelting), closure and rehabilitation (including removing waste and hazardous material and establishing new landforms and vegetation), which could be grouped into two stages of operating (operation) and non-operating (i.e., establishment, closure and rehabilitation) basing on the strength of mining activities. At operating mine sites, wind, water and moving vehicles transport large amounts of particulate matter that are generated by excavation, roads, and residues (Csavina et al., 2012; Ochieng et al., 2010; Patra et al., 2016). Non-operating mine sites (i.e., mine sites at the stage of establishment, closure, and rehabilitation) have no intensive mining activities; however, clearing of surface vegetation and soil and residual pollution are their main sources of pollutants. Therefore, mine sites at different stages could lead to different effects on surrounding understory plant communities.

Deciduous, mixed (deciduous and coniferous trees), coniferous forests, and open canopy areas (i.e., peatlands without canopy trees) are four ecosystem types frequently found in the Canadian boreal forest, and their different environmental characteristics result in a varying diversity and composition of understory plant communities (Barbier et al., 2008; Kembel and Dale, 2006; Qian et al., 2003). Broadleaf overstories usually support higher understory cover and richness than conifer overstories (Chen et al., 2018a). Thus, factors that drive changes in ecosystem types will likely cause concomitant changes in understory communities and canopy structure. Coniferous forest ecosystems currently dominate many boreal forest landscapes, but global change is predicted to result in an increased abundance of deciduous and mixed forests (Boisvert-Marsh and de Blois, 2021; Mack et al., 2021). A shift towards deciduous and mixed forests is predicted to inhibit nonvascular species growth, increase vascular species establishment and lower canopy closure (Hart and Chen, 2008; Turetsky et al., 2010). Nonvascular groups (bryophytes and lichens) are typically more resistant to mining disturbances (e.g., heavy metals) than vascular species (Salemaa et al., 2004), whereas lower canopy closure can result in more dust falling on the ground (Amezaga et al., 1997). However, whether ecosystem type has the potential to modulate offsite effects of mines on understory diversity and composition remains unknown.

Our objective is to determine the offsite effects of mines on understory plant communities. To do this, we quantified the diversity and composition of understory vegetation (the presence and abundance of wood and herbaceous species as well as bryophyte and lichen abundance) along 1-km transects near six gold mines in Abitibi-Témiscamingue and Nord-du-Québec (Quebec, Canada) to examine whether community diversity and composition is associated with mining stage and ecosystem type. We also quantified the distance up to which impacts from the mine sites were detected. We tested three hypotheses: 1) more elements of the understory in terms of diversity and composition are expected to be affected surrounding operating than non-operating mine sites (H1); 2) understory communities differ in their sensitivity to offsite effects of mines among different ecosystem types (H2); 3) the influenced distance and understory community changes are expected to be small (H3) as previous studies found that the mean distance of edge influence on forest variables was less than 50 m (Franklin et al., 2021). By examining these three hypotheses, we will characterize the offsite effects of mines in the boreal forest and highlight their importance in future ecological evaluations and forest management planning.

2.4.1 Study area

Six mine sites were selected in the regions of Abitibi-Témiscamingue and Nord-du-Québec in the province of Québec, Canada (Fig. 1): Akasaba (48°3′12″N; 77°32′8″W, Agnico Eagle Mines Ltd), Canadian Malartic (48°7′21″N; 78°5′23″W, Canadian Malartic Corp.), Casa Berardi (49°33′43″N; 79°14′8″W, Hecla Québec Inc.), Joutel (49°29′28″N; 78°21′8″W, Agnico Eagle Mines Ltd), Lapa (48°13′45″N; 78°17′1″W, Agnico Eagle Mines Ltd), LaRonde (48°15′14″N; 78°25′59″W, Agnico Eagle Mines Ltd). The selected sites are different in size, mining method, life stage and bioclimatic domain, but together represent the main characteristics of mines in Canada’s boreal forest. Lapa is a small underground mine, while Canadian Malartic is one of the largest open pit mines in North America. Three sites (Casa Berardi, LaRonde and

Canadian Malartic) are operating and the rest (Akasaba, Joutel and Lapa) are non-operating. Casa Berardi and Joutel are located in the *Picea mariana* –moss bioclimatic domain, while the other four are in the *Abies balsamea – Betula papyrifera* bioclimatic domain. Study sites are in the region that is characterized by a cold and humid continental climate with the mean temperature between −14 to −10°C in winter and between 16 to 18°C in summer (Environment Canada, 2021). Mean annual precipitation ranges from 790 to over 1,300 mm (Wang et al., 2013). More detailed information about individual mines is available in Table S1.1.

2.4.2 Fieldwork design and sampling

Six to eight transects (the number selected based on the size of each mine area, Fig. S1.1) were established perpendicular to each mine periphery through different ecosystem types in 2017-2019. Considering the environmental effects (e.g., dust) of mining are usually detected more than 1 km from mines (Gillings et al., 2022; Mattielli et al., 2009), the transects were established to 1 km from the edge of mine sites to make sure sampling plots were in the potential area influenced by the mine sites. Transects were positioned in different cardinal directions, but to avoid other non-mining disturbances (e.g., highways and towns), not all four cardinal directions are represented in some mine sites. This is particularly true at the Canadian Malartic and Lapa mine sites; to the north of the Canadian Malartic mine is the town of Malartic and to the south of Lapa mine is a highway, a natural gas pipeline and a hydroelectric corridor (Fig. S1.1). In addition, nine control transects with seven plots per transect were established in undisturbed natural sites (Fig. S1.1), five in the *Picea mariana* – moss bioclimatic domain and four in the *Abies balsamea – Betula papyrifera* bioclimatic domain.

Seven vegetation plots (3 m in radius) were established along each transect at distances from the edge of adjacent forest at 0, 20, 0.02, 0.1, 0.2, 0.5 and 1 km from the mine. However, the plot in control transects did not follow the distance pattern of the transects around the mine and they were selected depending on ecosystem types to have balance the types in the controls. The forest stand of each plot was categorized into one of four ecosystem types based on tree species composition and size (diameter at breast height (DBH) ≥ 10cm) within a prism plot (2M basal area factor) centered on the vegetation plot: coniferous (> 70% of coniferous tree stems, N=173), deciduous (>70% deciduous tree stems, N=48), mixed forests (coniferous tree stems between 69% and 31%, n=38), and open canopy ecosystem (DBH of all trees below 10 cm, primarily peatlands in our study area, N=110, Table S1.2). In total, 371 plots were established, but 369 plots were used because two control plots were destroyed by logging.

Understory composition in each vegetation plot was determined based on the Canadensys Database (www.canadensys.net) during the summer of 2019. In total, 172 vascular species (60 woody species and 112 herbaceous species) were identified (species list in Table S1.3). Cover (%) of each vascular species, total bryophyte and total lichen was determined and value in three random 1 m^2 quadrats were averaged by plot. The cover of individual species often overlapped and, therefore, total cover sometimes exceeded 100%.

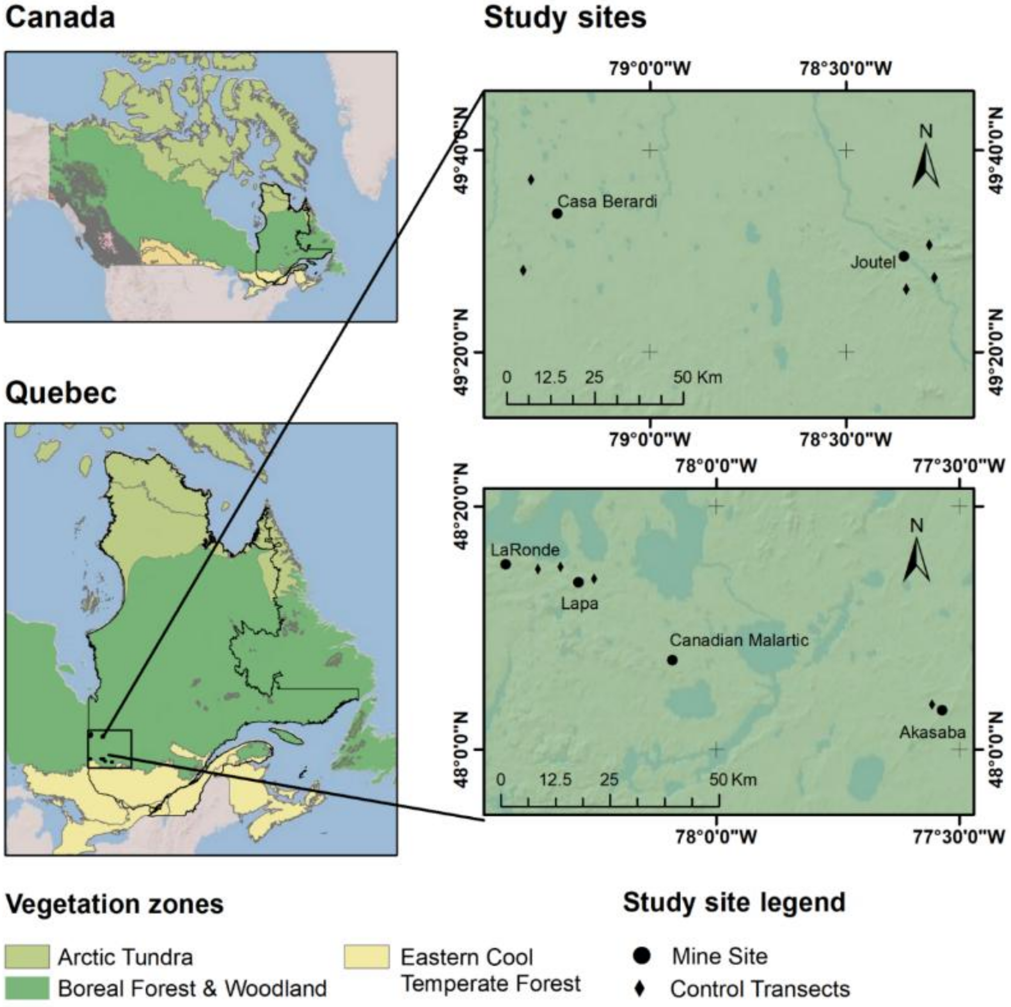


Figure 2.1 Study area. Left, map of Canada and Quebec showing the main vegetation zones. Right, map of study region indicating the position of sampling sites at six gold mine sites and 9 control transects (graph by Enrique Hernández-Rodríguez using GIS, August 9th, 2021)

Furthermore, 13 structural variables (i.e., ground cover of leaf litter, woody debris, organic matter, water and rock, soil humidity and organic soil depth, canopy openness, conifer proportion, live crown ratio and basal areas) were measured at each plot (listed in Table S1.4). A principal component analysis (PCA) for structural variables illustrates that the 13 variables differed significantly among ecosystem types

(ANOVA on the first two axis scores, $p < 0.001$, Fig. 2.2), therefore, structural variables were grouped into ecosystem types in the analysis. Furthermore, no differences in almost all structural variables were found between distances along the transects (Table S1.5), which supports our sampling design and confirms that the plots were established in relatively intact landscapes.

2.4.3 Statistical analysis

2.4.3.1 The impact of mining stage and ecosystem type on understory structure in the mine offsite landscapes

To describe patterns of understory diversity affected by mining stage and ecosystem type in mine offsite landscapes, we fitted a generalized linear mixed model (GLMM, “glmer” function from the *lme4* R package, Bates et al., 2018, and “glmmTMB” function from *glmmTMB* package, Brooks et al., 2017). Richness, cover and alpha diversity indices (“diversity” function from *vegan* package, Oksanen et al., 2013) of all vascular species, woody and herbaceous richness and cover and bryophyte and lichen cover were used as the response variables (Table S1.6). The predictor variables included mining stage, ecosystem type and their interaction as fixed effects. To avoid spatial autocorrelation, the nested terms representing the sampling design (site, transect and plot) were included to take into account the spatial location of the data. Different distribution functions were used, Conway-Maxwell-Poisson (“glmmTMB” function and family= “compois”) for underdispersion of woody richness counts, Negative Binomial (“glmer” function and family=MASS::negative.binomial) for overdispersed of all vascular and herbaceous richness counts, Gaussian (“glmmTMB” function and family=gaussian) for continuous number with normally distributed residuals of Shannon Index, bryophyte cover, all vascular, woody and herbaceous covers, Gamma (“glmer” function and family=Gamma) for continuous number with not normally distributed residuals of Simpson Index, and Zero-inflated beta distributions (“glmmTMB” function and family=beta_family) for Zero-Inflated data of lichen cover. Marginal significance of the predictors was tested by type II analysis of variance with the “Anova” function in *car* package (Fox et al., 2012). Pairwise comparisons for all response variables among mine stages and ecosystem type were conducted with Tukey’s post hoc test (“emmeans” function in the *emmeans* package, Lenth et al., 2018). When an interaction term was significant, pairwise comparisons were performed between mine stages within each ecosystem type, whereas pairwise comparisons between mine stages and between ecosystem types were performed independently when the interaction term was not significant. All analyses were performed with R 3.4. Results were visualized with the ggplot2 package (Wickham et al., 2015).

To assess variations in understory composition in mine offsite landscapes, the differences in species composition patterns of woody and herbaceous plants among mining stages and their interactions with ecosystem types were tested by permutational multivariate analysis of variance (PERMANOVA, “adonis” function from the *vegan* package, Anderson, 2005) on Bray–Curtis dissimilarity matrices with 999 permutations. Dissimilarity between plots was further visualized by distance-based Principal co-ordinates analysis (PCoA, “PCOA” function in the *vegan* package, Oksanen et al., 2013). We also ran pairwise PERMANOVAs (999 permutat, “pairwise.adonis” function in the package *RVAidememoire*, Hervé, 2017) to separately compare the mining stages with a multiple comparison correction based on Benjamini-Hochberg method (Benjamini and Hochberg, 1995). In addition, to further define communities specifically associated with each mining stage, indicator species analysis was performed with the “multipatt” function in the *indicspecies* package (De Caceres et al., 2016).

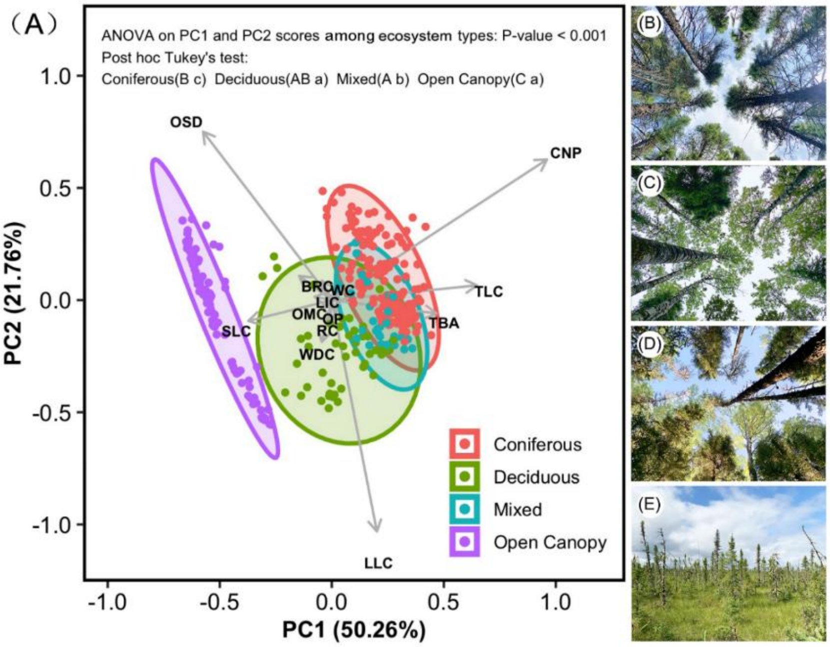


Figure 2.2 Biplot of principal component analysis (PCA) of structural variables across plots and forest types. Each ecosystem type (coniferous (B), deciduous (C), mixed forest (D) and open canopy ecosystem(E)) is represented with a specific color and ellipse. All plots are displayed (small circles). SLC: live crown ratio for sapling trees; TLC: live crown ratio for canopy trees; SBA: basal area for sapling trees; TBA: basal area for canopy trees; OP: Openness; LLC: leave litter ground cover; WDC: woody debris ground cover; WC: water ground cover; RC: rock ground cover; OMC: organic matter ground cover; OSD: depth of organic soil; CNP: conifer proportion; HM: humility; BRC: bryophyte ground cover; LIC: lichen ground cover.

2.4.3.2 Understory diversity patterns along distance from mine sites and footprint

To assess the patterns of understory diversity with distance from mine sites, generalized linear mixed models were performed. As the distance between plots in control transects and mine sites varied and to ensure the independence of predictors, control transects were not included in the models. Response variables (understory richness, cover and alpha diversity indexes) were the same as those used in 2.3.1 while nested terms (mine site, transect and plot) and ecosystem types were included as random factors. The predictor variables included the distance from mine sites and its interaction with mining stage. When the graphs ("ggplot" function in *ggplot2* package) of responses present a non-linear relationship with distance, a second-order polynomial of distance ("poly" function in *stats* package) was included in the predictors to account for unimodal patterns (all vascular and herbaceous plant cover). As we expected the disturbance from mines to decrease with increased distance, only linear and second-order polynomial patterns were considered. The insignificant interactions and quadratic terms were removed from the final models. As the presence of ecosystem types with distance was random, generated by the structure of the landscape (Fig. S1.1, Table S1.2) and unbalanced, ecosystem type and cardinal direction were included as random factors.

To determine influenced distances, we calculated means and plotted all responses against the distance using the ggplot2 package. Based on the general average influenced distance, the offsite footprint of the selected six mine sites were calculated with the Google Earth Engine Platform. As the selected mine sites in our project varied in size, shape, mining method, stage of mining lifecycle and bioclimatic domain, their average offsite footprint was considered representative and was further used to estimate total offsite footprint of operating mine sites in the boreal biome of Canada. To avoid overestimation of influence area, we used the distance that had the strongest effects in estimation of average offsite footprint.

2.5 Results

2.5.1 Effects of mine stage and ecosystem type

The effect of mining stage on understory diversity was not consistent among the ecosystem types in the offsite landscapes. The richness and cover of all vascular and herbaceous plants as well as the cover of bryophytes and lichens were affected by the interaction between mining stage and ecosystem type (the results of GLMMs, ANOVA type II sums, $P < 0.05$, Table S1.7 and S1.8). All vascular and herbaceous plants had lower richness and cover in deciduous or mixed forests near operating mines than in controls (the results of Tukey HSD pairwise comparisons in Figure 2.3). Notably, herbaceous richness and cover were also lower near non-operating sites than near controls (Figure 2.3). Unlike vascular plants, bryophyte cover differed between mining stages in coniferous forests and lichen cover was affected in open canopy ecosystems. These trends were observed near both operating and non-operating sites (Figure 2.3).

The composition of offsite understory vascular communities was also significantly associated with mining stage, ecosystem type and their interaction for both woody and herbaceous plants (see results of PERMANOVAs in Figure 2.4), although the effects were small (the sum contribution of mining stage, ecosystem type and their interaction in PERMANOVA results, $R^2 < 0.11$, Table S1.9). This pattern was visible in the principal component analysis (PCoA), where the communities clustered by mining stage in each ecosystem type (Figure 2.4). Ecosystem type was the largest contributing variable to community composition of woody ($F=9.37$, $R^2 = 0.071$, $p < 0.001$) and herbaceous species patterns ($F=5.71$, $R^2 = 0.046$, $p < 0.001$), while mining stage (woody and herbaceous, $F=2.898$, $R^2 = 0.015$, $p < 0.001$ and $F=4.209$, $R^2 = 0.023$, $p < 0.001$, respectively) and their interaction (woody and herbaceous, $F=1.445$, $R^2 = 0.022$, $p = 0.007$ and $F=1.555$, $R^2 = 0.025$, $p = 0.002$, respectively) were also significant but contributed less (PERMANOVA results in Table S1.9). Pairwise PERMANOVA revealed that woody community composition differed among mining stages in coniferous and mixed forests, while herbaceous composition differed more among mining stages in deciduous forests and open canopy ecosystems than in coniferous and mixed forests (pairwise PERMANOVA results in Table S1.10).

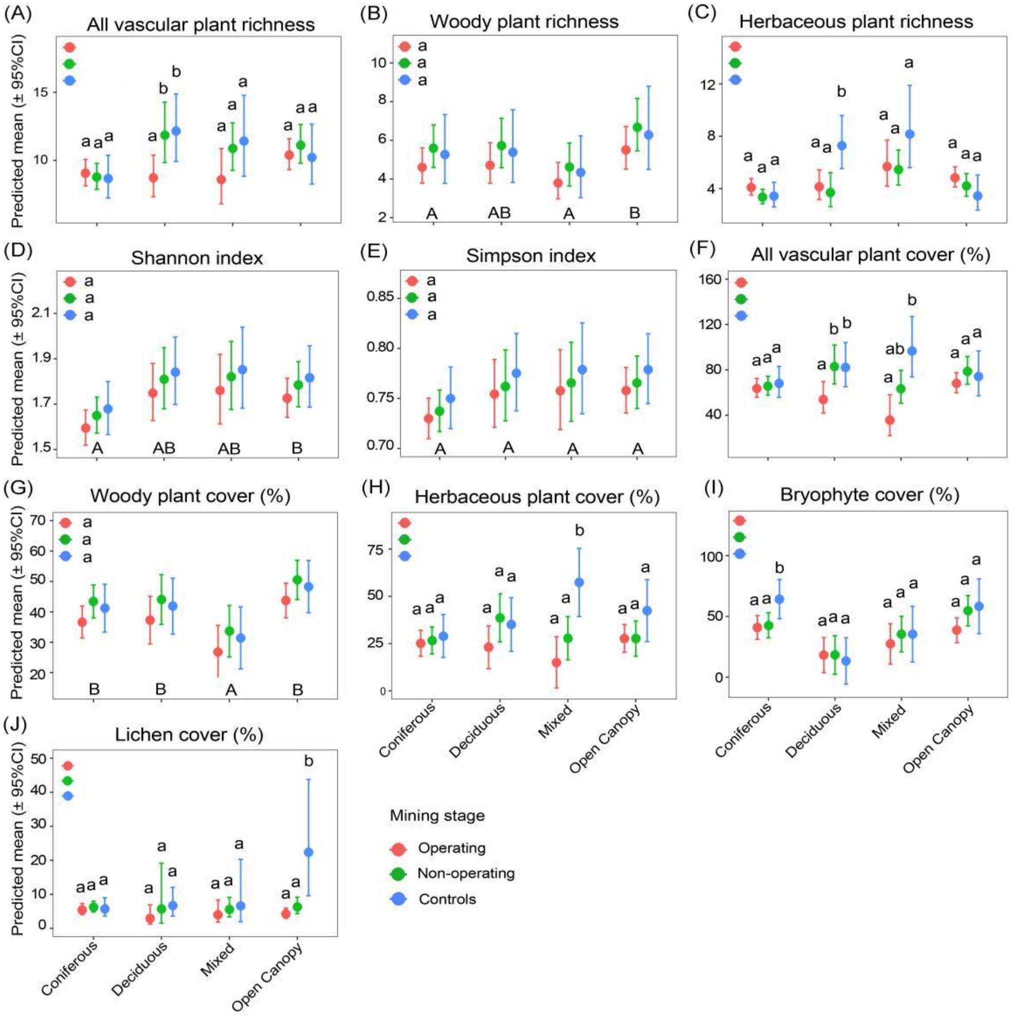


Figure 2.3 Comparison of understory diversity between mining stages for each ecosystem type. Points show means for all samples; bars show a 95% confidence interval around the mean. Different lowercase letters indicate significantly different means between groups based on Tukey HSD pairwise comparisons (α = 0.05) from the results of generalized linear mixed model (Table S1.4 and Table S1.5). When interactions between ecosystem type and mining stage were significant, pairwise comparisons were used between mine stage in each ecosystem type, while when the interactions were not significant, the interaction terms were removed from the models and pairwise comparison were used between forest types and mining stages, respectively.

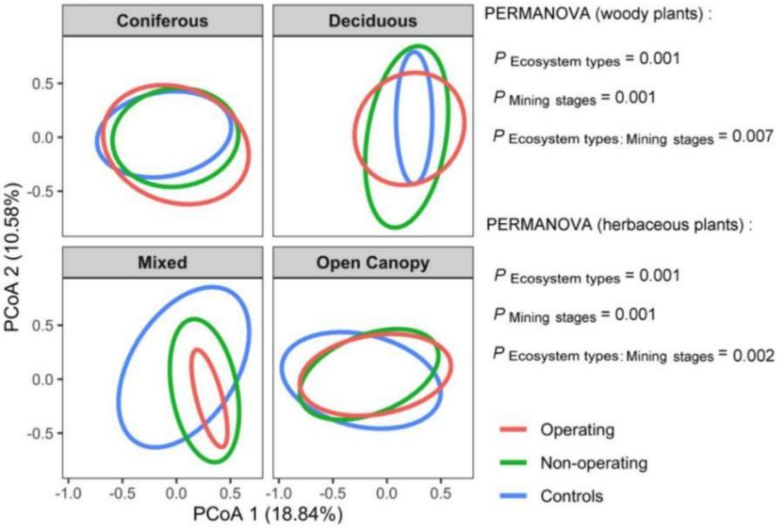
Indicator species analysis indicated that more species were associated with controls than non-operating and operating mine sites (Table 2.1). Two non-native species (*Pilosella aurantiaca* (L.) FW Schultz & Sch. Bip. and *P. caespitosa* (Dumort.) P. D. Sell & C. West.) and one non-native species (*Galium sylvaticum* L.) were closely associated with operating and non-operating sites, respectively. However, all indicator species for controls were native species (Table 2.1).

Table 2.1 List of indicator species for the landscape near sites at different mining stage (only significant species are shown, $P<0.05$)

Species	Types	Operating (5)		Non-operating		Controls (15)	
		Stat	*P* value	stat	*P* value	stat	*P* value
Amelanchier arborea	Woody			0.172	0.003		
Aralia nudicaulis	Herbaceous					0.182	0.003
Athyrium filix-femina	Herbaceous					0.136	0.013
Coptis trifolia	Herbaceous	0.129	0.045				
Cornus sericea	Woody			0.134	0.02		
Corylus cornuta	Woody					0.137	0.028
Dendrolycopodium	Herbaceous					0.14	0.015
Dryopteris carthusiana	Herbaceous					0.217	0.001
Epilobium angustifolium	Herbaceous			0.147	0.01		
Eriophorum vaginatum	Herbaceous			0.125	0.047		
*Galium sylvaticum**	Herbaceous			0.118	0.029		
Gymnocarpium dryopteris	Herbaceous					0.13	0.018
Lonicera hirsute	Woody					0.146	0.023
Maianthemum canadense	Herbaceous					0.137	0.029
Moneses uniflora	Herbaceous			0.141	0.018		
Pilosella aurantiaca *	Herbaceous	0.129	0.046				
Pilosella caespitosa *	Herbaceous	0.12	0.047				
Rhododendron canadense	Woody			0.142	0.013		
Ribes hudsonianum	Woody					0.166	0.003
Rubus chamaemorus	Herbaceous					0.133	0.041
Salix bebbiana	Woody	0.15	0.009				
Sorbus Americana	Woody	0.14	0.026				
Sparganium americanum	Herbaceous					0.169	0.005
Spinulum annotinum	Herbaceous					0.169	0.005
Vaccinium myrtilloides	Woody			0.151	0.017		
Viburnum lentago	Woody					0.188	0.001
Viburnum nudum	Woody					0.132	0.037

Notes: Stat is the association statistic and *P* value is the *p*-value of the permutational test. “*” indicates the non-native plant species.

(A) Woody plants



(B) Herbaceous plants

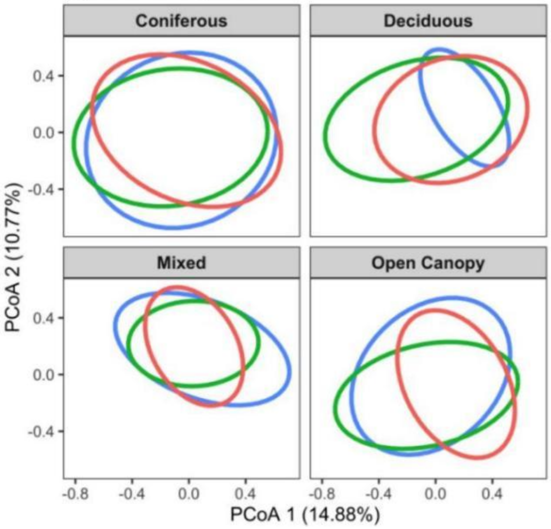


Figure 2.4 Ordination diagrams and PERMANOVA results of understory vascular plant composition at the community level. Principal co-ordinates analysis (PCoA) for woody plants (A) and herbaceous plants (B) based on Bray–Curtis dissimilarity. Ellipses represent 95% confidence intervals (individual data points not shown for clarity). Texts on the right side present the results of PERMANOVA.

2.5.2 Distance influenced by offsite effects of mines and offsite footprint

The impacts of mining activities on understory diversity generally decreased with increasing distance from operations, although the pattern was mediated by mining stage (the results of GLMM in Table S2.11 and Fig. 2.5). All six response variables (richness of all vascular, woody, and herbaceous plant, Shannon and Simpson indices and bryophyte cover) were associated with distance except for two response variables (all vascular, herbaceous richness) that varied not only with distance but also with by mining stage (operating vs. non-operating, Table S2.11).

The variations with distance occurred mostly in 0.2-0.5 km for Shannon and Simpson indices, 0.2 km for woody plant richness and 0.1 m for bryophyte cover near all mine sites, according to the pattern of mean values with the distance (Fig. 2.5). Moreover, the richness of all vascular and herbaceous plants had no significant patterns with distance near operating mines but there was a pattern near non-operating sites where richness of all vascular plants showed a slight threshold (turning point) at 0.5 km (Fig. 2.5A), and herbaceous richness gradually increased without a threshold (Fig. 2.5B).

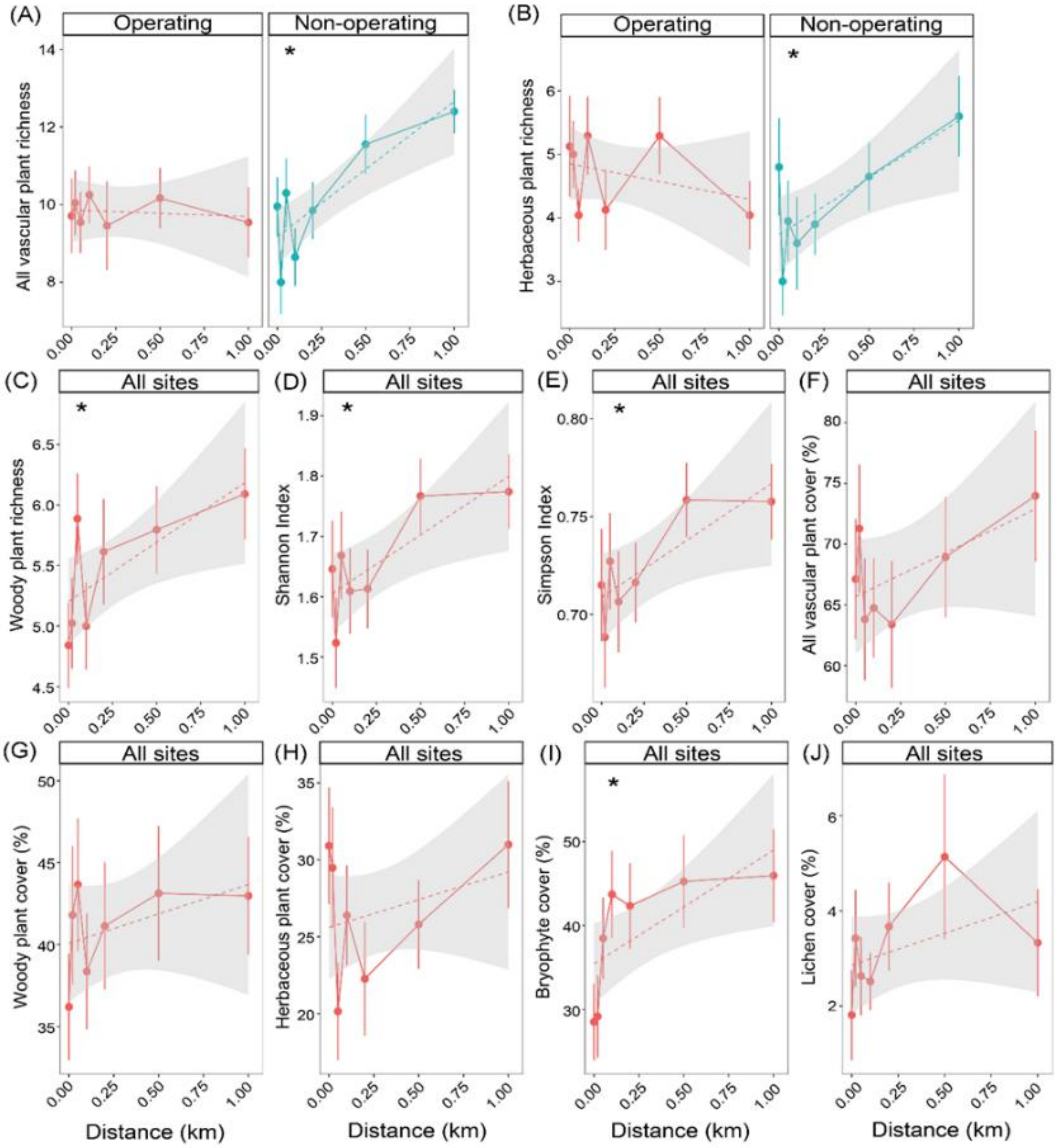
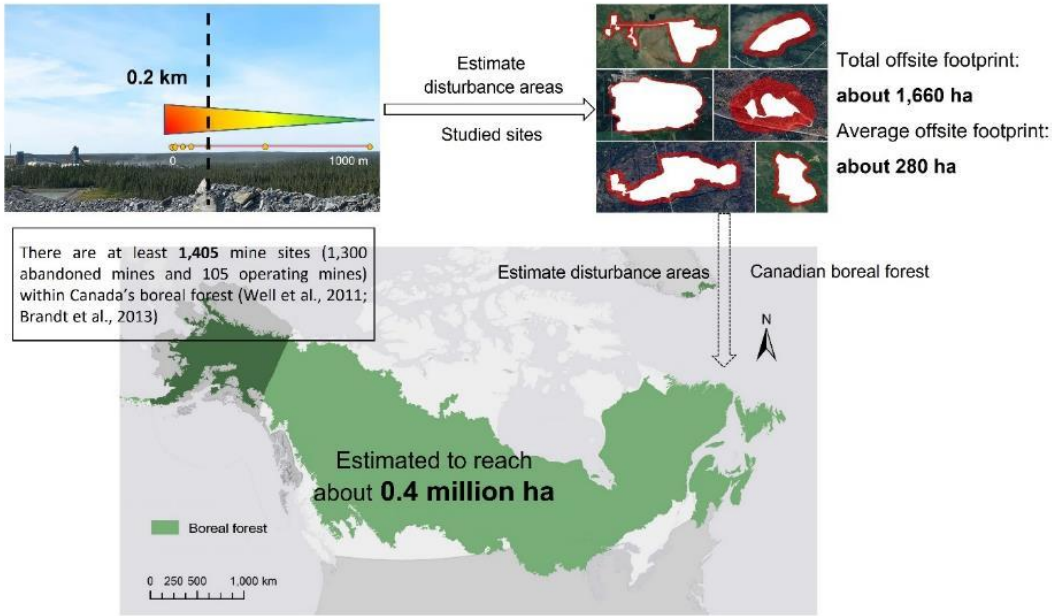


Figure 2.5 Effects of distance from mine site on understory diversity. Points show means for all samples; bars show standard error (SE). The significant associations were examined based on the results of (generalized) linear mixed model (Table S1.8). Dashed lines show the fitted trends and asterisks show significant correlations ($P < 0.05$).



There are at least **1,405** mine sites (1,300 abandoned mines and 105 operating mines) within Canada's boreal forest (Well et al., 2011; Brandt et al., 2013)

Figure 2.6 Total disturbance offsite footprint of mines in Canada's boreal forest. The offsite footprint was calculated within the Google Earth Engine Platform. The data of operating and abandoned mines is from previous reports (Brandt et al., 2013; Wells et al., 2010)

2.6. Discussion

2.6.1 Determinants of understory diversity and composition in surrounding landscapes of mine sites

The presence of offsite effects of mines on understory communities was confirmed. The offsite effects decreased understory diversity (richness and cover) in the surrounding landscapes, but the severity of the effects was modulated by mining stage and ecosystem type. As expected in H1, reduced richness and cover were mainly found near operating rather than near non-operating mines, which suggests potential recovery in plant diversity after mine closure. Alternatively, mining activities including digging, blasting and grinding mainly occurred during the operating and not the non-operating stage, which could lead to overall stronger effects on understory communities near operating mines because of higher heavy metal contents and dust deposition (David et al. 2021). However, some offsite effects were still detected near non-operating sites (e.g., lower herbaceous, bryophyte and lichen cover, Fig. 2.3). This may be the result of residual pollution from the operating period that may persist after closure (Festin et al., 2019; Frouz et al., 2001). Some studies reported that the residuals effects on onsite surface vegetation diversity could persist over 35 years after mine closure (Gould, 2012; Holl, 2002; Norman et al., 2006) and our results confirmed that persistent residual effects of mining might be also remaining surrounding intact landscapes.

Notably, herbaceous plants, bryophytes and lichens were more sensitive to the offsite effects than woody plants. With high water absorption efficiency and a greater ability to store nutrients (Craine and Dybzinski, 2013), woody plants usually have a higher resilience to environmental changes than other understory groups (Kirk et al., 2019; Salemaa et al., 2001; Watkinson et al., 2021). Bryophytes and lichens do not have roots and conductive tissues and absorb mineral salts as well as heavy metal ions from precipitation and dry deposition on their surface (Kłos et al., 2018; Korzeniowska et al., 2021). Bryophytes and lichens have been widely reported as indicators of air and metal pollution (Gerdol et al., 2014; Onianwa, 2001; Szczepaniak and Biziuk, 2003). Therefore, they have the potential to be applied in future offsite effect evaluation as specific ecological indicators of mining, but more work is needed to explore which species are the most reliable.

Ecosystem type was also an important factor associated with the offsite effects as we expected in H2. Understory communities showed variable resistance to the offsite effects among ecosystem types. Vascular plant diversity and composition changed in deciduous and mixed but not in coniferous forests, which may be due to the possibility that coniferous trees intercept more dust, heavy metals and other pollutants than deciduous trees (Augusto et al., 2002; Barbier et al., 2008; Nguyen et al., 2015) because of higher canopy cover, leaf area index and a more persistent foliage (Augusto et al., 2002). Mixed and deciduous forests had similar responses to the offsite effects, which could be due to their similar canopy structure with high seasonal variation in canopy gaps because deciduous trees drop their leaves in autumn (Brassard et al., 2008; Chávez and Macdonald, 2010).Furthermore , deciduous trees can translocate high amounts of heavy metals into the foliage which can then result in more heavy metal accumulation in topsoil (Van Nevel et al., 2011). Consequently, acidic deposition (e.g., sulfides and carbonates) from mining could lead to an increase in available heavy metals in the soil, resulting in greater effects on the understory species in deciduous and mixed forests.

Both woody and herbaceous plants were more resistant in open canopy ecosystems, potentially because the vegetation in these ecosystems (acidic and nutrient poor) develops unique adaptations to survive (Williams-Mounsey et al., 2021) and therefore is less vulnerable to the offsite effects. An example are the insectivorous species *Sarracenia purpurea* L. and *Drosera rotundifolia* L. (Table S1.3) are able to tolerate low oxygen, acidity and poor nutrient availability (Ellison and Gotelli, 2001; Hoyo and Tsuyuzaki, 2014).

In contrast to the findings on vascular plants, bryophyte cover was affected in coniferous forests while lichen cover was affected in open canopy ecosystems. Reduced bryophyte cover in coniferous forests may be due to canopy cover loss near mine sites given that forest-dwelling bryophytes are considered to be shade-tolerant (Tinya et al., 2009). Similarly, previous studies have shown that bryophyte abundance can be used to determine the depth of edge effects, which could be attributed to the removal of large trees and canopy disruption (Benítez et al., 2015, Stewart and Mallik, 2006). The negative offsite effects of mines on lichen cover in open canopy ecosystem is consistent with previous studies that showed a decrease in lichen cover near a diamond mine in the boreal forest (Watkinson et al., 2021). This is probably a consequence of the high sensitivity of lichens to dust deposition (Farmer, 1993; Salemaa et al., 2004).

Three non-native species (*Pilosella aurantiaca*, *P. caespitosa* and *Galium sylvaticum*) were identified as indicators of offsite effects, supporting the finding of earlier studies that mining activities promote the establishment and expansion of non-native species (Boyce, 2002; Calinger et al., 2015; Lemke et al., 2013). Removal of native vegetation, soil, soil microbes, and seed banks from the surface of mine sites (Dhar et al., 2018) may have provided opportunities for non-native species. With small plant height and greater capacity to utilise soil resources than native species, non-native species usually can establish rapidly and grow under harsh conditions such as nutrient-poor soils (Jo et al., 2015; Mathakutha et al., 2019; Schumacher et al., 2009). Our results indicate that these non-native species growing on the surface of mines could reach into surrounding intact forests. Therefore, mine offsite effects would also be an important driver of the spread of non-native species.

Our results supported our first and second hypothesis. Mining stage and ecosystem type were important factors affecting the magnitude of mine offsite effects. The combination of canopy cover loss, soil contamination as well as species differences in the tolerance to environmental changes might uncover the mechanisms how offsite effects of mines affect understory diversity and composition.

2.6.2 Determinants the footprint of offsite effects and their total impacts

Understory diversity indexes increased with the distance from the edge of mine sites following as expected for a point-source of pollution where disturbance and potential pollutants (such as acidic depositions, heavy metals, and other toxins) extend from the mine sites to offsite landscapes. Higher heavy metal content, for example, has been reported in soil and dust near mines, and their bioavailability decreased when moving away from the disturbance sources (Elmayel et al., 2020; Tang et al., 2020).

Unfortunately, in our study, information on wind direction or dust emissions by mine sites, which would have allowed us to assess correlations between the spread of contaminants and the observed offsite effects on plant communities, was not available, but should be considered in future studies.

As hypothesized in H3, the changes in understory diversity caused by offsite effects, although statistically significant, impacted a relatively small area (strongest effects occurred within 0.2 km) and weak (e.g., only 1-3 species varied along the distance). Offsite effects are considered enigmatic impacts as they are often overlooked in ecological evaluations of mining projects (Raiter et al., 2014). However, for some sensitive groups or species such as bryophytes, which had their cover reduced by about 15 % in forests near mines compared to distant plots (Fig. 2.5I), the offsite effects could be large.

Furthermore, when individual offsite effects are considered together, the cumulative impacts could be significant. The total disturbance footprint was estimated to up about 1,660 ha (Fig. 2.6) for the six selected mine sites (calculated with the Google Earth Engine Platform based on 0.2 km areas surrounding each site). The total offsite footprint could reach up to about 0.4 million ha (using the average offsite footprint value 280 ha of the six mine sites) across Canada's boreal forest, based on previous reports indicating that there were approximately 1,405 mine sites (105 operating and 1,300 abandoned mines within Canada's boreal forest, Brandt et al., 2013; Wells et al., 2020). Since mine sites were only selected in Canada's boreal forest, future work in other regions will be needed to predict the total footprint of offsite effects at a larger scale. Offsite effects of mines are a disturbance (like roads and gas pipelines) that have small effects at the scale of individual sites but that occur in many sites across the globe. Another concern is that the cumulative effects with other disturbances (e.g., logging, climate changes and forest fire) could be greater.

2.6.3 Management implications

Based on the results obtained in this study, we propose that mine offsite landscapes should be included in future ecological evaluations, and ecosystem types and mining lifecycle should be considered in the process. Furthermore, the predicted shifts in forest composition from coniferous to deciduous dominance with global change in boreal areas could exacerbate the offsite effects of mines on understory communities. Given that the strongest offsite effects mainly occurred within 0.2 km from mine sites, a buffer zone around mines is recommended to offset these effects. Keeping and planting conifer trees surrounding mine sites might be a reasonable option considering coniferous forest is the dominant stand type in boreal forest and showed a relatively high resistance to the offsite effects. Future work should determine the width of buffer zone and forest management strategies within the zone.

2.7 Conclusion

Offsite effects of mine sites were confirmed in boreal understory communities and their severity was associated with ecosystem type and mine lifecycle stage. Mine offsite effects on understory diversity and composition were generally small and seemed negligible in the overall biodiversity of boreal forest, especially in coniferous forest. However, key species loss, non-native species invasion, and total impacts at a large scale may increase the impact of mine offsite effects on ecosystem services. With global climate change and the predicted changes in boreal forest ecosystems, including the encroachment of deciduous species into coniferous forests, the areas that are affected by offsite effects of mines could grow in the future given the increased sensitivity of mixed and deciduous forests compared to coniferous forests. Furthermore, the increasing number of mining projects will lead to more landscapes being exposed to offsite effects. Our findings encourage future studies to evaluate how these changes by offsite effects affect ecological services of the boreal forest and their cumulative impacts with other anthropogenic and natural disturbances at a large scale.

2.8 Acknowledgements

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CHAPTER III

EFFETS SYNERGIQUES DANS PAYSAGE HORS SITE DU SITES MINIER : LES CHANGEMENTS PRÉVUS DANS LES ÉCOSYSTÈMES POURRAIENT EXACERBER LES EFFETS DE L'EXPLOITATION MINIÈRE SUR LA STRUCTURE DES COMMUNAUTÉS DE BRYOPHYTES.

SYNERGISTIC EFFECTS IN MINE OFFSITE LANDSCAPES: PREDICTED ECOSYSTEM SHIFTS COULD EXACERBATE MINING EFFECTS ON BRYOPHYTE COMMUNITY STRUCTURE

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3.1 Résumé

Le changement global modifie l'abondance relative des types d'écosystèmes dans les forêts boréales, tandis que la transition énergétique verte entraîne une augmentation des activités minières dans le monde entier. L'interaction et les effets conséquents de ces deux tendances sur la biodiversité n'ont pas été examinés en profondeur. Les espèces de bryophytes peuvent être utilisées comme indicateurs pour mesurer ces effets car elles jouent des rôles écologiques clés dans les forêts boréales. Nous avons identifié et évalué l'interaction entre le type d'écosystème (c.-à-d. conifère, feuillu, forêt mixte et canopée ouverte) et l'exploitation minière sur la diversité et la composition des bryophytes à l'échelle du microhabitat dans des paysages d'un kilomètre autour de six sites miniers à différentes étapes du cycle de vie minier dans la forêt boréale canadienne. Indépendamment du type de microhabitat, les effets combinés du type d'écosystème et du stade d'exploitation minière étaient interactifs sur les bryophytes. La richesse en bryophytes et la composition de la communauté ont été affectées négativement par les effets hors site des mines uniquement dans les forêts de feuillus et mixtes. Les effets interactifs sur la richesse en bryophytes se sont produits principalement dans les microhabitats du sol. Nous avons également constaté que les forêts de feuillus, les forêts mixtes (forêt de conifères comme référence) et les mines avaient un impact négatif sur l'abondance des mousses à plumes et des sphaignes. En outre, des espèces indicatrices ont été identifiées pour les zones affectées par les mines (*Pohlia nutans* et *Dicranum polysetum*) et pour les zones témoins (*Sphagnum angustifolium* et *Plagiomnium cuspidatum*). Nos résultats suggèrent que les changements d'écosystème prévus avec les changements globaux, des forêts de conifères aux forêts de feuillus, pourraient potentiellement augmenter les effets de l'exploitation minière sur la résistance de l'écosystème forestier à travers les changements de la structure de la communauté de bryophytes. L'ajout de microhabitats (c'est-à-dire l'ajout de débris ligneux grossiers) près des sites miniers est une stratégie potentielle pour maintenir la richesse des espèces. Collectivement, ces résultats font progresser notre compréhension de la façon dont l'exploitation minière affecte la biodiversité et soulignent l'importance de prendre en compte les paysages hors des sites miniers dans les futures évaluations environnementales des projets de développement dans le contexte des changements mondiaux.

Mots_clés : Activités minières, changements climatiques, impacts indirects, épiphytes, microhabitats, taïga.

3.2 Abstract

Global change is shifting ecosystem type relative abundance in boreal forests, while the green energy transition results in increased mining activities around the globe. The interaction and consequent effects of these two trends on biodiversity have not been examined in depth. Bryophytes species can be used as indicators to measure these effects because they play key ecological roles in boreal forests. We identified and evaluated the interaction between ecosystem type (i.e., coniferous, deciduous, mixed forest and open canopy) and mining on microhabitat scale bryophyte diversity and composition in 1-km landscapes surrounding six mine sites at different stages of the mining lifecycle in the Canadian boreal forest. Irrespective of microhabitat type, the combined effects of ecosystem type and mining stage were interactive on bryophytes. Bryophyte richness and community composition were negatively affected by offsite effects of mines in only deciduous and mixed forests. The interacted effects on bryophyte richness mainly occurred on the ground r microhabitats. We also found that deciduous, mixed forests (coniferous forest as a reference) and mines had a negative impact on the abundance of feather mosses and sphagna. Furthermore, indicator species were identified for areas affected by mines (*Pohlia nutans* and *Dicranum polysetum*) and for control areas (*Sphagnum angustifolium* and *Plagiomnium cuspidatum*). Our results suggest the predicted ecosystem shifts with global changes, from coniferous to deciduous forests, could potentially increase the effects of mining on forest ecosystem resistance through the changes in bryophyte community structure. Adding microhabitats (i.e., adding coarse woody debris) near mine sites is a potential strategy in maintaining species richness. Collectively, these findings advance our understanding of how mining affects biodiversity and highlight the importance of considering mine offsite landscapes in future environmental evaluations of development projects in the context of global changes.

Key words: Mining activities, climate changes, indirect impacts, epiphytes, microhabitats, Taiga

3.3 Introduction

Global changes are modifying the distribution of ecosystem types across the globe. Changes include shifts from boreal coniferous to deciduous forest and from northern peatlands to forests (Hirota et al., 2011, van der Velde et al., 2021). How the effects of these global changes will interact with accelerated ongoing human activities is an important area of study. A synergistic effect appears when the combined effects of two disturbances is greater than their sum (Coors and De Meester, 2008, Raiter et al., 2014). An example is the interactions between global changes and forest fires (Hessburg et al., 2021), logging (Anderson et al., 2017, Cusack et al., 2016), and agricultural activity (Anderson et al., 2017, Cusack et al., 2016, Danneyrolles et al., 2019), which all present synergistic results in effects on biodiversity and the provision of ecosystem services. However, the effects of mining and their potential interactions with global changes have received little attention despite the fact that mines are a significant source of disturbances in forest ecosystems globally (Martins and Lima, 2020, Maynard et al., 2014). Numerous mines and mineral deposits are located in the boreal forest (Fig. 3.1) and more mining projects will be established in this area in coming decades as the energetic transition increases the world’s demand for minerals such as rare earths for clean technologies and uranium for power generation (Sonter et al., 2020, Watari et al., 2021, Yin et al., 2021). This will lead to more intact boreal landscapes being exposed to mining disturbances while they are also facing the consequences of global changes.

Coniferous, deciduous and mixed forests as well as open canopy (primarily peatlands) are four main ecosystem types in boreal landscapes. Predicted shifts from coniferous needle-leaved to deciduous broad-leaved tree dominance and from peatlands to forests are two scenarios under global change (Boisvert-Marsh and de Blois, 2021, Mack et al., 2021). The shifts could affect the response of forest biodiversity and function to mining disturbances through changes in plant community structure, given the plant communities of different ecosystems generally differ in tolerance and resilience capacities under stressors (Barbier et al., 2008, Jean et al., 2017). For example, low light intensity below coniferous trees appear to support high cover of bryophyte (Király and Ódor, 2010), while bryophyte cover in deciduous and mixed forest is constrained by the fall of broadleaf litter (Bartels et al., 2018). Furthermore, coniferous canopies buffer environmental variations (e.g., temperature, moisture, irradiance and wind in the understory) in the understory to a greater degree than deciduous forests, as persistent foliage and dense branching patterns; (Barbé et al., 2020, De Jalón et al., 2019). Boreal forests, including coniferous forest and peatlands, store large amounts of carbon (Bradshaw and Warkentin, 2015), while predicted ecosystem shifts and increasing mining activities (Fig. 3.1) could interact and affect ecological services via plant diversity and composition, including the bryophyte layer.

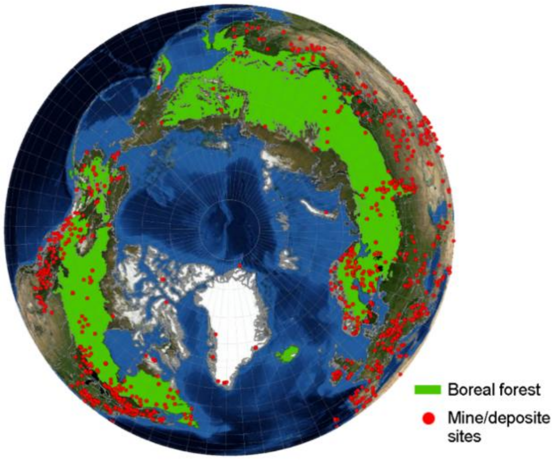


Fig. 3.1. The distribution of mines and deposits of major mineral commodities in global boreal forests (March 9th, 2022). Datasets for mine sites was retrieved from Mineral resources online spatial data, United States Geological Survey (Labay et al., 2017).

Bryophytes are widely distributed globally and particularly contribute ecological services in boreal ecosystems such as carbon and nitrogen cycling, water balance and forest succession (Arróniz-Crespo et al., 2014, Nilsson and Wardle, 2005, Rousk et al., 2013, Xia et al., 2020). Particularly, two most abundant groups, feather mosses and Sphagna, shape ecological function and resilience. Feather mosses can account for as much as 30 % of net primary productivity and contribute over 50 % of total nitrogen sources in boreal coniferous forests (Wardle et al., 2012, Jean et al., 2021). *Sphagnum* is the ecosystem engineer of peatlands (Rydin and Jeglum, 2013), which store around 25 % of global soil carbon and represent important resources to mitigate climate changes (Turetsky et al., 2015). Despite the critical roles bryophytes play in the ecosphere, they lack true roots, vascular systems and thick cuticles and are therefore highly sensitive to the surrounding environment. These characteristics result in bryophytes being widely used as indicators of natural and human disturbances including climate changes and heavy metal deposition (Balabanova et al., 2017, Mahapatra et al., 2019, Printarakul and Meeinkuirt, 2022).

The type and abundance of microhabitats have been shown to be more important than mesohabitats for bryophyte diversity (Cole et al., 2008, Király and Ódor, 2010). Microhabitats for bryophytes are generally defined as the smallest subunit of forest habitat including pieces of deadwood and rocks (Barbé et al., 2020). Microhabitats can protect bryophytes from the effects of global changes and anthropogenic disturbances (Paquette et al., 2016, Scheffers et al., 2014). Usually, microhabitats provide a relatively stable substrate in terms of temperature and moisture to effectively buffer extreme climate events and environmental variations, for example bryophyte composition was more dependent on the characteristics of coarse woody debris than environmental features in boreal coniferous stands (Barbé et al., 2020).

The negative effects of mining on biodiversity and ecological services have been well documented in directly disturbed areas (Maus et al., 2020, Odell et al., 2018, Sonter et al., 2018), but offsite effects that occur in relatively intact landscapes surrounding mine sites (mine offsite landscapes) have received little attention (Raiter et al 2014). The main mechanisms of offsite effects associated with mines are dust, salt, excess nutrients, or other contaminants that move from mines to surrounding ecosystems via air, water or human activities (e.g., vehicle movement, Raiter et al., 2014). An increase in the number of mining projects will lead to the exposure of more landscapes to offsite effects. Furthermore, the disturbance of biodiversity and ecological services associated with mining is also dependent on the mining lifecyle as operating mine sites with more activities (e.g., digging, blasting, transportation) usually have a larger influence on surrounding biodiversity than non-operating sites (Adesipo et al., 2020; Bartels et al., 2019).

The aim of this study is to evaluate whether the effects of mining and predicted ecosystem shifts could be synergistic in mine offsite landscapes, resulting in changes in the bryophyte community at the microhabitat scale. Bryophytes were sampled from various microhabitats (i.e. ground, trees, logs, snags, stumps and rocks) in the boreal forest of Québec (Canada) inside 1-km intact landscapes surrounding six mine sites (from operating and non-operating stages). The offsite landscapes were grouped into coniferous, deciduous, mixed forest and open canopy ecosystems. We hypothesized that mine sites at the operating stage have more offsite effects on bryophyte community structure (diversity and composition) than those at the non-operating stage (H1) and that ecosystem type affects the presence and magnitude of the offsite effects of mines (H2). The interactions between offsite effects of mines and ecosystem type on bryophytes are affected by microhabitats (H3). We expect a reduced abundance of important functional groups (i.e., feather mosses and sphagna) as a consequence of offsite effects of mines and the predicted shifts in ecosystem type (H4). How bryophyte structure is altered in boreal offsite mine landscapes and potential factors under global changes have not been addressed in previous studies; therefore, our study provides a first look at these industrial impacts.

3.4. Material and methods

3.4.1 Study area

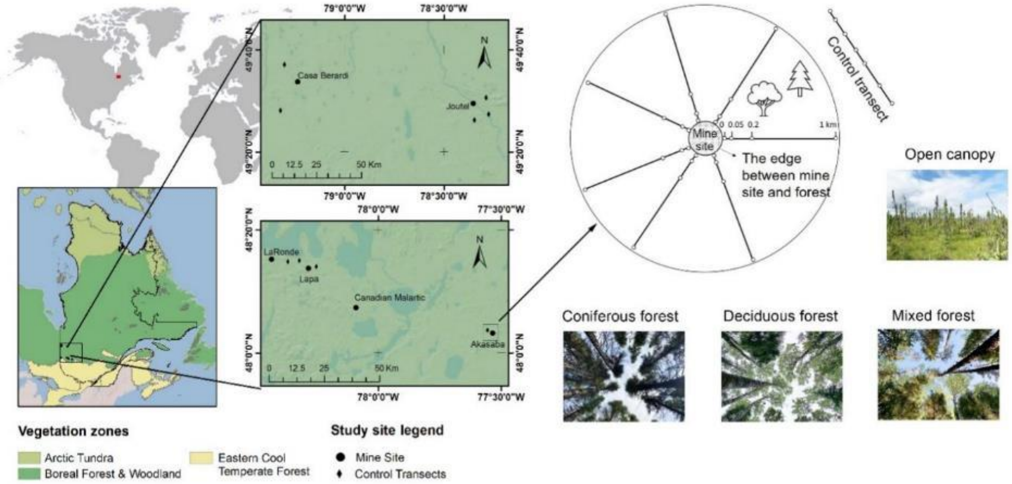


Figure 3.2 Study area. Left, map of Canada and Quebec showing the main vegetation zones. Center, map of study region indicating the position of sampling sites at six gold mine sites and 9 control transects (August 9th, 2021).

The study was conducted in six mine sites in the regions of Abitibi-Témiscamingue and Nord-du-Québec in the province of Québec, Canada (Figure 3.2): Akasaba (48°3′12″N; 77°32′8″W, Agnico Eagle Mines Ltd), Canadian Malartic (48°7′21″N; 78°5′23″W, Canadian Malartic Corp.), Casa Berardi (49°33′43″N; 79°14′8″W, Hecla Québec Inc.), Joutel (49°29′28″N; 78°21′8″W, Agnico Eagle Mines Ltd), Lapa (48°13′45″N; 78°17′1″W, Agnico Eagle Mines Ltd), LaRonde (48°15′14″N; 78°25′59″W, Agnico Eagle Mines Ltd). They differ in size, mining method, life stage and bioclimate domain. Lapa is a small underground mine, while Canadian Malartic is one of the largest open pit mines in North America. Three of them (Casa Berardi, LaRonde and Canadian Malartic) are operating and the other three (Akasaba, Joutel and Lapa) are non-operating. Furthermore, Casa Berardi and Joutel are located in the *Picea mariana*- moss bioclimatic domain, while the other four mine sites are all located in the *Abies balsamea* - *Betula papyrifera* bioclimatic domain. This region is characterized by a cold and humid continental climate with the mean temperature between −14 to −10°C in winter and between 16 to 18°C in summer (Environment Canada, 2021). Mean annual precipitation ranges from 790 to over 1,300 mm (Wang et al., 2013). More detailed information about the six mines is available in Table S2.1.

3.4.2 Fieldwork design and sampling

Fieldwork design

In each mine site, 6-8 transects (the number of transects was selected based on the size of each mine area, see Fig. S1.1) were established perpendicular to each mine periphery through different ecosystem types (See Table S2.2) in 2017-2019. Nine control transects (over three 3 km from the mine sites) were established in undisturbed natural sites, avoiding down wind from the mines, five in the *Picea mariana* – moss bioclimatic domain and four in the *Abies balsamea* – *Betula papyrifera* bioclimatic domain. As the environmental effects of mines (deposition of dust or heavy metals) are usually felt farther than 1 km from mines (Gillings et al., 2022; Mattielli et al., 2009), the 1 km long transects were deigned to be within the potentially influenced areas. In order to avoid other non-mining disturbances (like highways and towns), transects were not established in all cardinal directions at each mine. This is particularly true for two sites, Canadian Malartic and Lapa, as north of Canadian Malartic mine is the town of Malartic and south of the Lapa mine is a main highway, natural gas pipeline and hydro-corridor (See Fig. S1.1).

Seven vegetation plots (3 m in radius) were established along each transect at distances from the edge of the area directly disturbed by mining activities at 0, 0.02, 0.05, 0.1,0.2, 0.5, 1 km. Three 1-m^2 quadrats per plot were randomly established to determine the cover of each macroscopic terricolous bryophyte species and different substrates (leaf litter, woody debris, organic matter, water, rock, lichen). The forest stand of each plot was categorized into one of four types based on tree species (diameter at breast height (DBH)≥10cm) composition in a prism plot with a 2M basal area factor centered on each vegetation plots: coniferous forest (> 70% of coniferous trees, N=173), deciduous forest (>70% deciduous trees, N=47), mixed (coniferous tree stems between 69% and 31%, n=37), and open ecosystems (DBH of all trees below 10 cm, primarily peatlands in our study area, N=107). In total, 371 plots were established, but 364 plots were analyzed because two control plots were destroyed by logging and some missing data.

Bryophyte sampling

The bryophyte community was sampled using a modified floristic habitat sampling technique (Newmaster et al., 2005) in the summer of 2019 and 2020. All bryophytes on each microhabitat (ground, trees, logs, snags, stumps and rocks, classification criteria in Table S2.2) within each vegetation plot were sampled and stored in individual paper bags with plot and microhabitat information. Samples were identified to the species level based on the "Flore des bryophytes du Québec-

Labrador" (Faubert, 2012) at the bryophyte lab of Université du Québec en Abitibi-Témiscamingue. Species Latin names in our database were checked using The Plant List database in the R package Taxon stand version 2.4 (Cayuela et al., 2012). In total, 186 bryophyte species (132 moss species and 54 liverwort species) belonging to 89 genera were identified (species list see Table S2.3) and the feather moss *Pleurozium schreberi* (Willd. ex Brid.) Mitt. and *Sphagnum angustifolium* (Warnst.) C.E.O. Jensen were the dominant species with 8.96 % and 7.09% ground cover, respectively.

Environmental variable sampling

Canopy openness and organic soil depth were measured three times in each plot, and shrub diameter at breast height (DBH, measured 1.3 m from ground level) was measured in the same plot as the tree basal area. Conifer proportion (%) was calculated by the percentage number of individuals of coniferous trees in the prism plot. The live crown ratio of each shrub (vegetation plot) and trees (prism plot) were measured and recorded (Table S2.4). Furthermore, environmental variables were compared among mine sites in Table S2.5.

3.4.3 Statistical analysis

3.4.3.1 Effect of ecosystem type and mining stage on bryophyte community structure

The sum of species occurring in all microhabitats per plot was used as the species richness of the bryophyte community at each plot. This study took the species richness for diversity indices as its high sensitivity to environmental changes (Wilsey and Stirling, 2007; Andersen et al., 2020). Bryophyte species were classified by taxonomy (moss and liverwort), so in each plot (N=364), we obtained the species richness and composition for three groups: total bryophytes, mosses and liverworts.

Species richness. Generalized linear mixed models (GLMMs, "glmmTMB" function from *glmmTMB* package, Brooks et al., 2017) were used to determine if mining stage, ecosystem type and their interaction influenced total bryophyte, moss and liverwort richness. The richness, referring to the species richness per plot, is the total number of bryophytes/moss/liverwort species identified in each plot. Considering the nested structure of the sampling design, “plot” was nested in “site” as random effects, as microhabitat was the smallest sampling unit. All models were first performed with Poisson error distribution and a log-link function, but when overdispersion was detected, a negative binomial distribution was used in final models. One model (moss richness) showed convergence errors and was corrected by optimization of the model via the Broyden–Fletcher–Goldfarb–Shanno (BFGS) algorithm (Dai, 2002) in the function of the “glmmTMBControl” from the *glmmTMB* package. Significance of the predictors (mining stage, ecosystem type and their interaction) was tested by type II Wald chi-square (χ^2) tests with the “Anova” function in *car* package (Fox et al., 2012). A Tukey post hoc test (“emmeans” function in the *emmeans* package, Lenth et al., 2018) was performed to assess interaction terms (the differences between mining stage in each ecosystem type).

Community composition. Permutational multivariate analysis of variance with 999 permutations (PERMANOVA, “adonis” function from the *vegan* package, Oksanen et al., 2010) were run to assess the interacting effects of ecosystem type and mining stage on bryophyte community composition. in the PERMANOVA analysis, non-significant interaction terms were removed in final analysis. Results were visualized using principal coordinates analysis (PCoA, “PCOA” function in the *vegan* package) with Bray–Curtis dissimilarity matrices. The “envfit” function in *vegan* was then used to fit vectors of environmental parameters (microhabitat number, stand structure and soil properties) onto the ordinations with p-values (>0.05) derived from 999 permutations. When significant interactions between mining stage and ecosystem type were detected, the interaction effects on bryophyte composition shifts were qualified by calculating the Euclidean distances between centroids of PCoA cluster for each mining stage (distances between centroids of operating mines and controls, non-operating mines and controls as well as operating and non-operating mines, respectively) within each ecosystem type following a changed method by Martineau et al. (2020). The calculated distances were used to determine whether the combined effects of predicted ecosystem shifts and mining stage were synergistic or not (coniferous forest as references, details in Fig. S2.1). In addition, to identify species that characterized the communities of each mining stage, point biserial-correlation coefficient (phi) was calculated for indicator species analysis ("multipatt" function in the *indicspecies* package, De Caceres et al., 2016). The value of "stat" (the point-biserial-serial-correlation-coefficient) was used to measure the strength of the co-occurrence pattern observed between a bryophyte species and each ecological state of mining stage. Moreover, generalized linear mixed models were also used to test the differences in 17 environmental variables between mining stages in each ecosystem type (Table S2.6).

3.4.3.2 Role of microhabitats in mediating the effects of ecosystem type and mining stage on bryophyte structure

Bryophyte richness for each microhabitat in each plot was calculated as the sum of all species detected on each microhabitat of a given type in the plot. Composition on each microhabitat type (trees, logs, snags, stumps and rocks) per plot was determined using relative species frequency, where $F_m = n_m / N_t * 100$, F_m is the relative frequency of species *m* on each microhabitat type; n_m is the number of occurrences of species *m* in each microhabitat per plot; N_t is the total number of all microhabitat types per plot (ground counted as one individual microhabitat. Considering it is impossible to count the number of ground microhabitat elements, the bryophyte composition on the ground was determined using species raw cover measured in the $1m^2$ quadrats.

The analysis process for richness and composition per microhabitat was similar to the description in 3.4.3.1. However, there are some differences in models for richness associated with the characteristics of each dataset. Considering the lack of certain microhabitats in some plots, zero-inflated (ZI) models (“zi=~.” used in “glmmTMB” function) were used in all models for bryophyte richness on each microhabitat. The model for bryophyte richness on rocks was analyzed with Poisson error distribution instead of negative binomial distribution because overdispersion was not detected. Furthermore, non-significant interaction terms (ecosystem type * mining stage) were removed from the final models (richness on trees, logs, stumps, snags and rocks).

3.4.3.3 Does a shift in forest composition and mining disturbance affect the ecological roles of bryophytes through abundance of important functional groups?

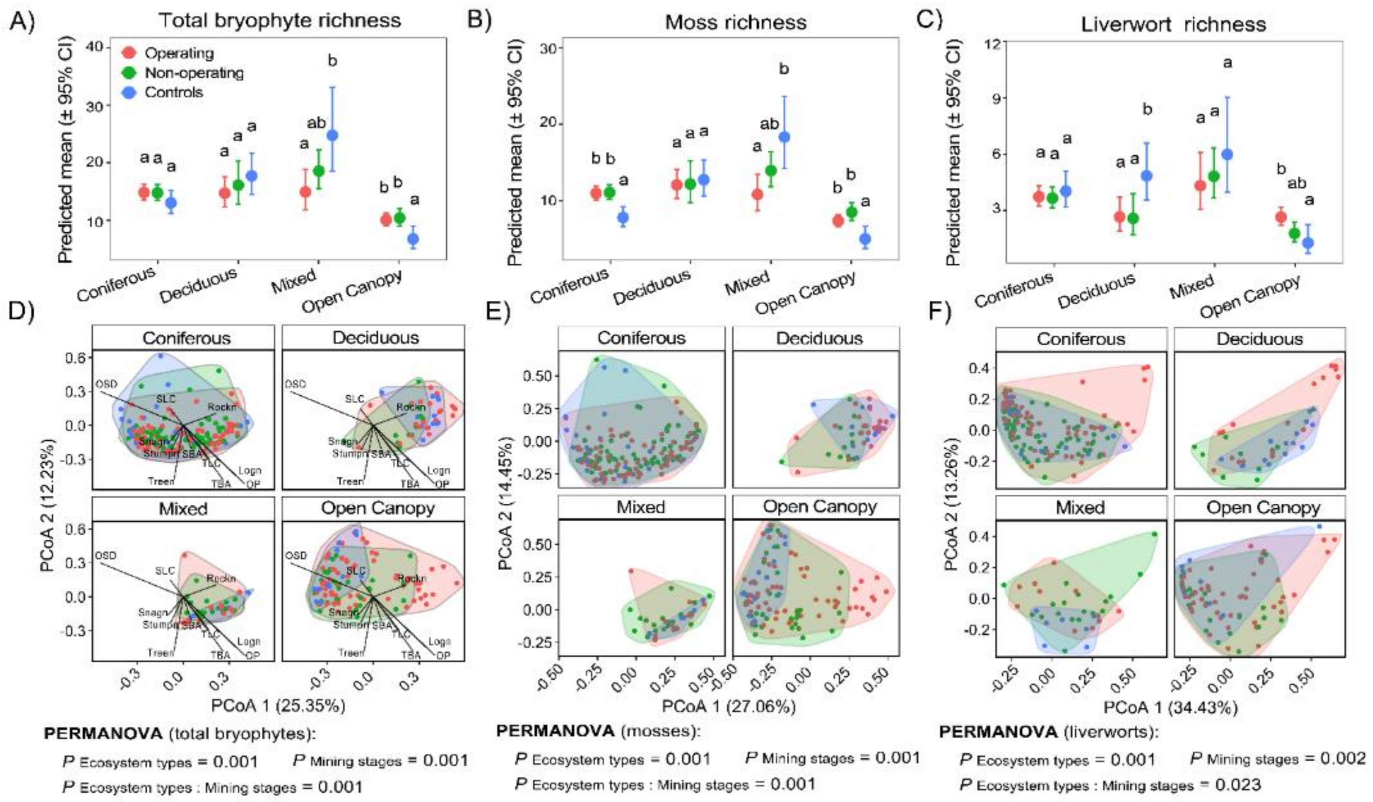
Structural equation modeling (SEM, “sem” function in *lavaan* package, Rosseel, 2012) was used to obtain a mechanistic understanding of how differences in ecosystem type, mining stage and microhabitats mediated changes the abundance of feather mosses and *Sphagnum*. A good model fit was evaluated using: 1) a Root Mean Square Error of Approximation (RMSEA) equal to or higher than 0.07 (Steiger, 2007) and 95% confidence interval (CI) = 0.054–0.086; 2) Comparative Fit Index (CFI) equal to or higher than 0.96; and 3) Tucker-Lewis Index (TLI) equal to or lower than 0.96 (Hu and Bentler, 1999). All analyses were performed with R 4.0.5 (2021-03-31). Results were visualized with the *ggplot2* package (Wickham, 2011).

3.5. Results

3.5.1 Synergistic effects of ecosystem type and mining stage on bryophyte richness and composition

Bryophyte richness in mine offsite landscapes was affected by the interaction between ecosystem type and mining stage (the results of GLMMs for total bryophyte, moss and liverwort richness, ANOVA type II sums, $P < 0.05$, Table S2.7). Generally, mines present a positive effect on bryophyte richness in coniferous forests and open canopies, but a negative influence in deciduous and mixed forests (the results of

Tukey HSD pairwise comparisons, Fig. 3 A-C). Specifically, moss richness was higher near both operating and non-operating sites than in controls in coniferous forest and open canopy, while it was significantly lower near operating sites than controls in mixed forest (Figure 3.3 B). Furthermore, more liverwort species were also found near operating sites than controls in open canopy, but less liverwort species were observed near both operating and non-operating sites in deciduous forests (Figure 3.3 A).



PERMANOVA (total bryophytes):
P Ecosystem types = 0.001 P Mining stages = 0.001
P Ecosystem types : Mining stages = 0.001

PERMANOVA (mosses):
P Ecosystem types = 0.001 P Mining stages = 0.001
P Ecosystem types : Mining stages = 0.001

PERMANOVA (liverworts):
P Ecosystem types = 0.001 P Mining stages = 0.002
P Ecosystem types : Mining stages = 0.023

Figure 3.3 Comparison of bryophyte community structure between mining stages for each ecosystem type. A) Total bryophyte richness; B) Moss richness; C) Liverwort richness; D) Total bryophyte composition; E) Moss composition; F) Liverwort composition. Significant effects based on the results of generalized linear mixed model. Different letters denote significant differences ($\alpha \leq 0.05$) between mining stages (plots near operating mines, N=167; plots near non-operating sites, N=136; plots in controls, N=61) within each ecosystem type (plots in coniferous forest, N=173); plots in deciduous forest, N=47; plots in mixed forest, n=37; plots in open canopy, N=107) from generalized linear mixed models with Tukey HSD pairwise comparisons (A, B, C). Principal co-ordinate analysis is used to visualise composition structure and present the results of PERMANOVA based on Bray–Curtis dissimilarity in D, E, F.

Table 3.1. List of indicator species for each mining stage with their point biserial correlation coefficient and p value (only significant species shown, $P < 0.05$).

Species	**Abbreviation**	**Type**	**stat**	***P* value**
Operating (6)				
Calliergonella lindbergii (Mitten) Hedenas	Cal.lind	Moss	0.144	0.03*
Hygroamblystegium varium (Hedwig) Mönkemeyer	Hyg.vari	Moss	0.144	0.018*
Hypnum cupressiforme Hedw.	Hyp.cupr	Moss	0.17	0.01**
Mylia anomala (Hook.) J.J. Engel & Braggins	Myl.anom	Liverwort	0.185	0.002**
Plagiothecium laetum Schimp.	Pla.laet	Moss	0.154	0.01**
Pohlia nutans (Hedw.) Lindb.	Poh.nuta	Moss	0.207	0.002**
Non-operating (2)				
Brachythecium erythrorrhizon Schimp.	Bra.eryt	Moss	0.165	0.006**
Dicranum polysetum Sw.	Dic.poly	Moss	0.175	0.009*
Controls (12)				
Barbilophozia attenuata (Nees) Loeske	Bar.atte	Liverwort	0.17	0.006**
Barbilophozia barbata (Schreb.) Loeske	Bar.barb	Liverwort	0.14	0.017*
Brachythecium reflexum (Starke) Schimp.	Bra.refl	Moss	0.138	0.036*
Breidleria pratensis (Koch ex Spruce) Loeske	Bre.prat	Moss	0.125	0.03*
Mnium spinulosum Bruch & Schimp.	Min.spin	Moss	0.163	0.01**
Mylia taylorii (Hook.) Gray	Myl.tayl	Liverwort	0.147	0.01**
Plagiomnium cuspidatum (Hedw.) T.J. Kop.	Pla.cusp	Moss	0.231	0.002**
Scapania mucronata H. Buch	Sca.mucr	Liverwort	0.141	0.024*
Sphagnum angustifolium (Warnst.) C.E.O. Jensen	Sph.angu	Moss	0.235	0.001***
Sphagnum capillifolium (Ehrh.) Hedw.	Sph.capi	Moss	0.151	0.024*
Sphagnum magellanicum Brid.	Sph.mage	Moss	0.185	0.005**
Sphagnum quinquefarium (Lindb.) Warnst.	Sph.squa	Moss	0.142	0.014*

Notes: *ssignificance at 0.05 level, ** significance at 0.01 level and *** significance at 0.001 level.

Similarly, bryophyte community composition near mine sites was also affected by the interaction between ecosystem type and mining stage (PERMANOVA results in Figure 3.3 D-F and Table S2.8). Overall, ecosystem type (PERMANOVA, $8.97\% < R^2 < 13.42\%$, Table S2.8) explained more differences in richness of total bryophytes, moss and liverwort species than mining stage (PERMANOVA, $1.42\% < R^2 < 1.58\%$, Table S2.8) and the interaction ecosystem type - mining stage interaction (PERMANOVA, $2.43\% < R^2 < 3.1\%$, Table S2.9). Specifically, there were greater differences in bryophyte composition among mining stages in deciduous and mixed forests, and open canopy (pairwise PERMANOVA results for total bryophytes, mosses and liverworts, $3\% < R^2 < 11\%$, Table S2.9) than in coniferous forest (pairwise PERMANOVA, $R^2 < 3$, Table S2.9). Furthermore, liverwort composition shifts present more synergistic effects (Table S2.9). The Euclidean distances between centroids of mining stages of liverworts in deciduous and mixed forests almost were greater than those in coniferous forest, while the synergistic effects were only detected in deciduous forests for mosses (Table S2.10). Also, the results were supported in PCoA of liverworts where some plots near operating mines are in the top right corner far from the plots near non-operating sites in mixed and deciduous forests (Figure 3.3F), while in moss composition, differences between operating and non-operating sites were visible only in open canopy (Figure 3.3E).

Forest structure, the number of individual microhabitat types, and organic soil depth were all correlated with total bryophyte species composition (Figure 3.3D). Canopy openness (OP, $R^2=0.45$, $p=0.001$, Table S2.11), organic soil depth (OSD, $R^2=0.40$, $p=0.001$, Table S2.11) and number of logs (logn, $R^2=0.33$, $p=0.001$, Table S2.11) appeared three significant environmental gradients structuring the community. OSD was more related with bryophyte composition in open canopy, while OP, tree basal area (TBA) and logn was associated with the composition in coniferous and mixed forests (Figure 3.3D).

Six indicator species were identified near mine operating mine sites (Table 3.1, five moss species and one liverwort species), two indicator species (both moss species) were identified for non-operating sites and twelve indicator species (eight moss species and four liverwort species) for controls. Almost all indicators for mining sites (operating and non-operating) were moss species and *Pohlia nutans* (Hedw.) Lindb. (stat=0.207, p = 0.002) and *Dicranum polysetum* Sw. (stat=0.175, p = 0.009) were the best indicators for operating and non-operating sites, respectively, while *Sphagnum angustifolium* (Warnst.) C.E.O. (stat=0.235, p = 0.001, Table 3.1) was the best indicator for control sites.

3.5.2 Role of microhabitats in mediating bryophyte response to ecosystem type and mining stage

No statistically significant interactions were found for bryophyte richness on all microhabitats except ground (results of GLMMs, ANOVA type II sums, Table S2.12). Ecosystem type influenced bryophyte richness in most microhabitats (except stumps) with generally, more species in deciduous and mixed forest than that in coniferous and open canopy (the results of Tukey HSD pairwise comparisons, Fig. 3.4 A-F). In contrast to richness, bryophyte composition was affected by the interaction between mining stage and ecosystem type on all microhabitats except rocks (PERMANOVA results in Fig. 3.3 G-L and Table S2.13). Generally, the Euclidean distances between centroids of mining stage were greater in deciduous, mixed forest and open canopy ecosystems than those in coniferous forest for all microhabitat although some opposite patterns were detected (e.g., Euclidean distances between centroids of operating and non-operating stages in mixed forest were lower than that in coniferous forest for total bryophytes on ground, trees and logs, Table S3.13). The interaction explained a greater proportion of differences in bryophytes species composition on snags (PERMANOVA, R2 = 7.8%, Table S2.13) than on other microhabitats (PERMANOVA, R2 < 4.0%, Table S2.13). Bryophyte composition in coniferous forest was only different between mining stages on the ground, while in deciduous forest and open canopy, differences between mining stages were found on the ground, trees, logs and stumps (pairwise PERMANOVA, Table S2.14). Bryophyte composition in mixed forest was only affected by mining stages on stumps (pairwise PERMANOVA, Table S2.14).

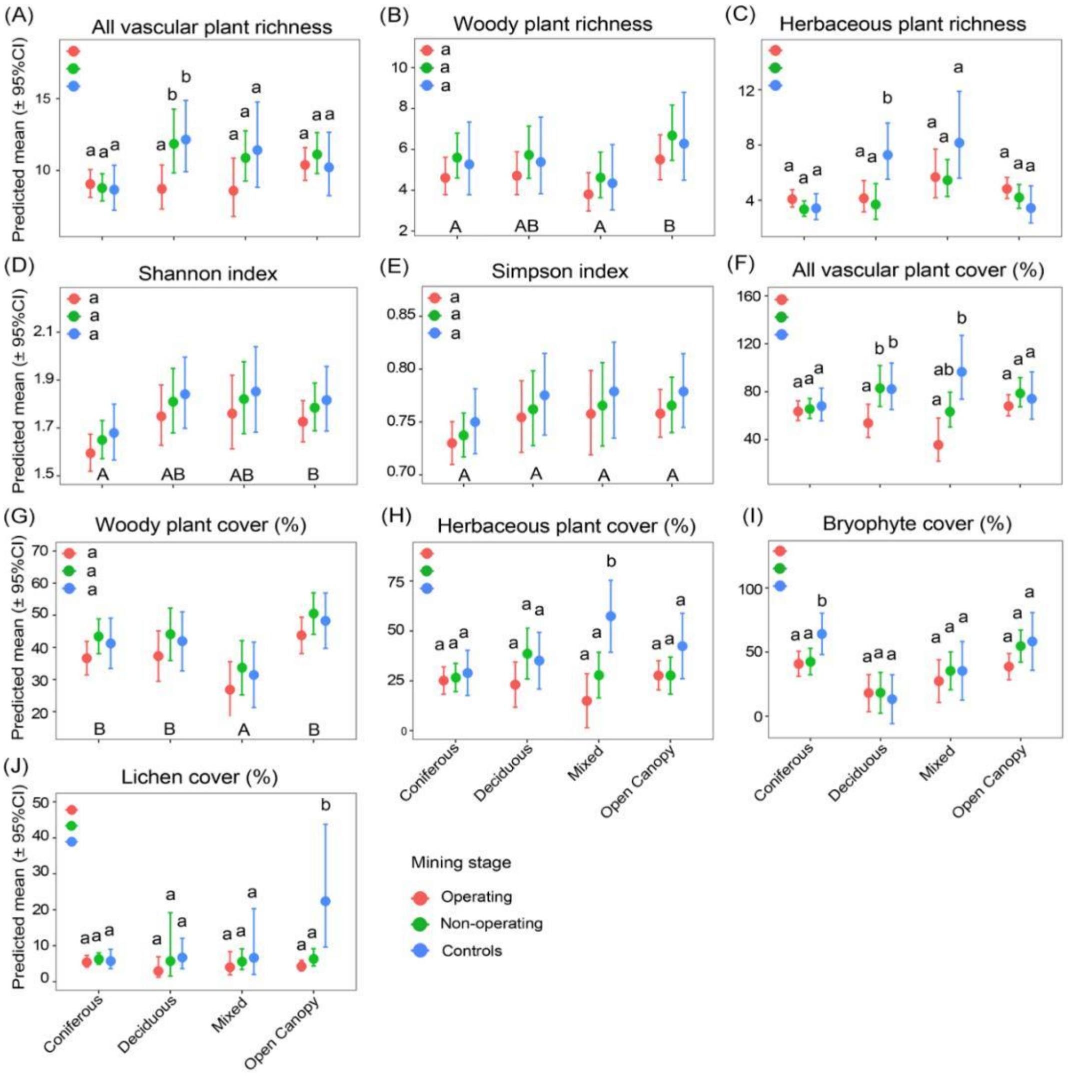


Figure 3.4 Comparison of the richness and composition of bryophytes on each microhabitat between mining stages for each ecosystem type. A) Total bryophyte richness on ground; B) Total bryophyte richness on trees; C) Total bryophyte richness

on logs; D) Total bryophyte richness on snags; E) Total bryophyte richness on sumps; F) Total bryophyte richness on rocks; G) Total bryophyte composition on ground; H) Total bryophyte composition on trees; I) Total bryophyte composition on logs; J) Total bryophyte composition on snags; K) Total bryophyte composition on sumps; L) Total bryophyte composition on rocks. Principal co-ordinates analysis and present the results of PERMANOVA using the Bray–Curtis distance in G, H, I, J, K, L.

3.5.3 Effects of ecosystem type and mining stage on the abundance of major bryophyte functional groups

The SEM explained 21.3% and 22% of the variation in the ground cover of feather mosses and sphagna, respectively (Figure 3.5). Deciduous (β (standardized coefficient) = -0.212) and mixed (β = -0.113) forests were negatively correlated with feathermoss ground cover, with coniferous forest as reference (Figure 3.5A). Operating mine sites (β =-0.183) also had a negative effect on surrounding feathermoss cover, while the numbers of trees (β = 0.234), snags (β = 0.185) and stumps (β = 0.126) were positively correlated with feathermoss cover but the number of logs (β = -0.148) was negatively correlated with feather moss cover (Figure 3.5A).

Similarly, compared to coniferous forest, *Sphagnum* cover was reduced in deciduous (β=-0.125) and mixed (β=-0.068, Figure 3.5B) forests. However, it was increased in open canopy (β=0.288, Figure 3.5B). Both operating and non-operating sites had negative effects on *Sphagnum* cover in the surrounding landscape. Only log number (β=-0.190, Figure 3.5B) was associated with the ground cover of *Sphagnum* among microhabitat types. Open canopy and operating sites were the most parsimonious explanatory variables for *Sphagnum* cover near mine sites. Therefore, ecosystem type and microhabitats were more important than mining stages in determining feather moss cover near mine sites, while mining stages were the most important variable influencing S*phagnum* cover in the study.

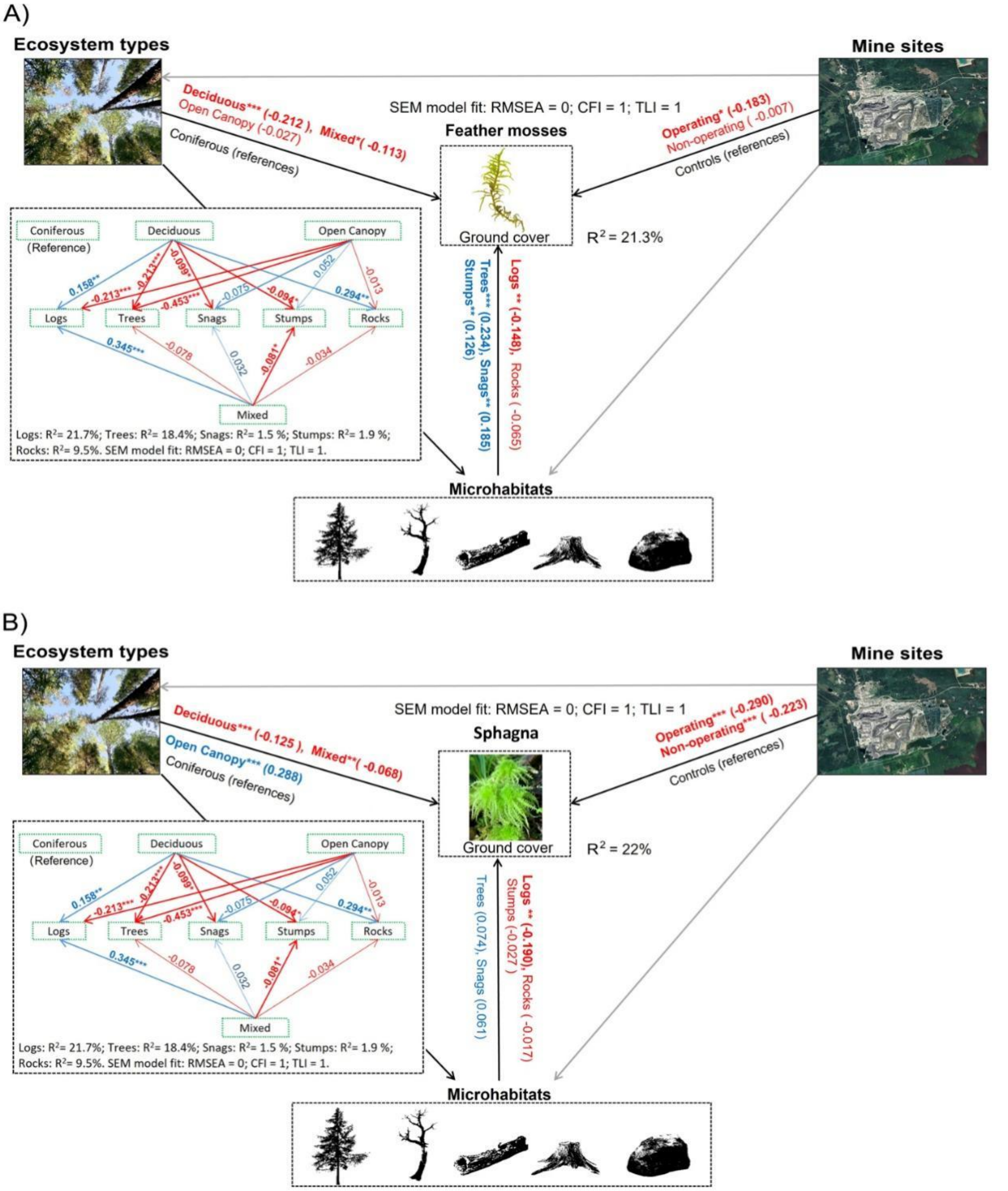


Figure 3.5 Structural equation models (SEM) of ecosystem type, mining stage and microhabitats as predictors of the ground cover of important functional bryophyte groups (i.e., feather mosses and sphagna). A) Feather moss ground cover; B) *Sphagnum* ground cover. Red text represents negative effects, blue text represents positive effects and significant parameters (≤ 0.05) are indicated in bold. The associations between ecosystem type and the number of individual microhabitats were evaluated in a separate structural equation model because of poor fit when all

elements were included in one model. Overall fit of piecewise SEM was evaluated using RMSEA (root mean square error of approximation), CFI (comparative fit index) and TLI (Tucker-Lewis index).

3.6 Discussion

3.6.1 Mine offsite effects and their synergistic interaction with ecosystem type on bryophytes

Offsite effects of mine sites on bryophytes were confirmed based on the differences in richness and composition of bryophyte communities in 1km radius landscapes near mine sites (operating or non-operating) compared to controls. The results extend offsite effects of mines from vascular plants (Boisvert et al., 2021; Chen et al., 2017; Dyer et al., 2001) to cryptogamic community structure. At the same time, operating mines had more effects on bryophyte structure (richness and composition) than non-operating ones (e.g., total bryophyte richness was affected by operating mines in mixed forest but not by non-operating sites in Fig. 3.3A). Compared with non-operating sites, more mining associated activities (e.g., blasting, digging, and transporting) occur in operating sites, which lead to more disturbances (e.g., atmospheric particulate matters and heavy metals) in offsite landscapes (Betancourt et al., 2005; Corriveau et al., 2011; Wang and Mulligan, 2006) and consequently bryophytes were more affected, confirming H1.

The presence and magnitude of the offsite effects is largely dependent on ecosystem type based on the significant interaction effects, which confirms H2. Offsite effects of mines reduced bryophyte richness in deciduous and mixed forest sites, however, the opposite pattern was observed in coniferous forest and open canopy sites. Combined with the microhabitat results (Fig. 3.4), we found that the increase in species richness mainly occurred on the ground in coniferous forest and in open canopy ecosystems. On the one hand, changes in species interactions may contribute to the positive impact of mine offsite effects. Reduced ground cover of “large” dominating moss species (i.e., feather mosses and sphagna Fig. 3.5) caused by the offsite effects in coniferous and open canopy ecosystems might lead to less competition in disturbed habitats and provide opportunities for smaller bryophytes, such as *Pohlia nutans*, *Plagiothecium laetum* Schimp., *Hypnum cupressiforme* Hedw. and *Hygroamblystegium varium* (Hedw.) Mönk. to establish (Table 3.1). This finding is consistent with an earlier result where disturbed areas with less competitive species supported more bryophyte species (Zielińska et al., 2016). However, this mechanism did not apply to mixed and deciduous forests, possibly because larger bryophytes are not a limiting factor for other bryophyte species in these environments (Jean et al., 2020; Oechel and Van Cleve, 1986). The high ground cover of broadleaf litter (up to over 70 % near operating and non-operating sites, see Table S2.5) could mainly contribute to reduced bryophyte richness (Márialigeti et al., 2009; Saetre et al., 1997) near mined sites in deciduous and mixed forests. Low decomposition rates due to low activity of microorganisms in polluted soil near mine sites could lead to an increase in the ground cover and thickness of deciduous litter (Freedman and Hutchinson, 1980; Horodecki and Jagodziński, 2017; Strojan, 1978). On the other hand, the differences in resistance of each ecosystem type can be also attributed to the canopy structure and environmental characteristics. Conifers have higher canopy cover, leaf area index, and a more persistent foliage than deciduous trees, which may intercept more dust, heavy metals, and other pollutant emissions generated by mining (Augusto et al., 2002; Barbier et al., 2008; Nguyen et al., 2015). Furthermore, deciduous trees can translocate high amounts of heavy metals into the foliage which can then result in more heavy metal accumulation in topsoil than under coniferous trees (Van Nevel et al., 2011).

Bryophyte indicator species were identified for offsite landscapes at different mining stages. *Calliergonella lindbergii* (Mitt.) Hedenäs, *Hygroamblystegium varium*, *Hypnum cupressiforme*, *Mylia anomala* (Hook.) Gray, *Plagiothecium laetum* and *Pohlia nutans* were indicators of operating mines, and they are tolerant or pioneer species (Barrett and Watmough, 2015; Petschinger et al., 2021; Rydgren et al., 2004;

Salemaa et al., 2001). In particular, *P. nutans* had the highest indicator value in operating sites and has been reported as one of most tolerant moss species growing in the immediate vicinity of mines and associated disturbed areas (Gignac and Beckett, 1986; Helmisaari et al., 1995; Salemaa et al., 2001). More species (13 species) occurred more abundantly in undisturbed landscapes (controls), suggesting that they have low tolerance to mining disturbances and high indicator values for intact landscapes. Four *Sphagnum* species including *Sphagnum angustifolium*, which had the highest indicator value, suggests that the *Sphagnum* group could be particularly sensitive to offsite effects, which is consistent with previous results that *Sphagnum* was a key indicator of mining effects on vegetation (Mullan-Boudreau et al., 2017; Spratt and Wieder, 1988) . Interestingly, only two indicator species were detected for non-operating sites, which suggests that bryophyte species composition near non-operating sites was generally similar with that either near operating mines or in controls and few specific species were associated with this condition where neither tolerant nor sensitive groups were particularly supported.

Therefore, ecosystem types and mining stages are two drivers for offsite effects of mines on bryophyte richness and composition. The combined effects of predicted ecosystem shift from coniferous to deciduous forest dominance and mining on bryophytes could be synergistic in the offsite boreal landscapes. Meanwhile, indicators for the offsite effects have potential value for future ecological assessment in mining projects

3.6.2 Microhabitats mitigated the synergistic effects of ecosystem type and mining on bryophyte structures

Whether microhabitats can mitigate effects from predicted global change on bryophytes was still unclear, but our results did confirm their potential buffer roles in the scenario of predicted ecosystem shifts and increases of mining projects (H3). Some types of microhabitats mitigated the offsite effects of mines and their synergistic effects with predicted ecosystem shifts, which indicates that they could play the role of mining-refugia in offsite landscapes. The possible explanation is that microhabitats, and especially coarse woody debris (logs, snags and stumps), are specialised substrates which retain moisture, offering a more stable microclimate environment than soil (Haughian and Frego, 2017; Jönsson and Jonsson, 2007). Furthermore, offsite effects of mines on bryophyte richness were only found on the ground and rocks (Fig. 3.4A, F). These results indicate that bryophytes growing in microhabitats located near or on the forest ground are more vulnerable to offsite effects of mines than those colonizing vertical microhabitats (i.e., trees, stumps, snags). Microhabitats in the forest ground layer could accumulate higher amounts of dust deposition than standing microhabitats considering that dust deposited on tree and shrub leaf surfaces will finally deposit on the forest ground through the action of wind, rainwater and defoliation (Sase et al., 2012). Meanwhile, loss of canopy cover by the edge affects mainly communities on the ground, where the level of incident light, soil moisture and nutrient availability can be changed by canopy openness, while non-ground microhabitats having vertical dimensions might lead to less exposure probabilities to the changes in environmental variables (e.g., lightness, treefalls and soil moisture).

In contrast to richness, microhabitats generally did not mitigate the interaction and synergistic effects on bryophyte composition, indicating that community composition is generally much more vulnerable to environmental changes than species richness, a response that has been found after other disturbances (Oldén et al., 2014). Species richness is an emergent property of ecosystems and it is relatively constant after disturbances if local compensatory colonisations occurred (Legendre et al., 2005; Parody et al., 2001). Nevertheless, our results firstly confirmed the buffer roles of microhabitats for bryophytes in mine offsite landscapes, although the interaction and synergistic effects could still be detectable in community composition.

3.6.3 The offsite effects of mine sites on important functional bryophyte groups

Predicted shifts in ecosystem composition and mining could both limit the abundance of feather mosses and sphagna in mine offsite landscapes, which supports H4. Lower feather moss and *Sphagnum* abundances in deciduous and mixed forests than coniferous forest and open canopy ecosystems can be attributed to their strong environmental preferences. Feather mosses occur in closed-canopy, well-drained coniferous forests and sphagna occur in the more open-canopy, poorly-drained coniferous forests and peatlands (Bisbee et al., 2001; Peckham et al., 2009). Furthermore, compared with feather moss abundance, sphagna was more effected by offsite effects of mines. It indicates that *Sphagnum* abundance should be highly sensitive to mining activities, which might be attributed to the fact that *Sphagnum* growth is more susceptible to water and peat depth than feather mosses (Bergeron et al., 2009; Bisbee et al., 2001) since the landscapes near mine sites are characterized by low water table and depletion of organic soil content (Glina et al., 2019). Feather mosses occurred in a wider range of microhabitats than sphagna. The dominating habitat of sphagna is the ground while feather mosses can colonize various microhabitats (e.g. logs and rocks) which can serve as shelter from environmental changes (e.g. water content and temperature) by providing microclimatic buffering for species growing on them (Dražina et al., 2016; Haughian, 2018). The results reveal predicted ecosystem shifts and increasing mining activities might affect ecological services of boreal forests through reducing abundances of important functional groups.

Although the offsite effects of mines on bryophyte diversity, community structure and composition and drivers (ecosystem types, mining stages and microhabitats) were confirmed, further research is required to identify the source of the stress and that other studies (e.g., measuring heavy metal concentrations in the soil, installing dust samplers to see the amounts of dust and level of contamination of the dust coming from the mines) will be needed in order to do that.

3.7 Conclusion

Our study provides the evidence that mine offsite landscapes could be a growing threat to boreal biodiversity of sensitive groups such as bryophytes as offsite effects extended mining effects beyond the areas originally degraded. Overall, our results revealed that ecosystem type and mining stage are the predictor of bryophyte community structure in mine offsite landscapes and their combined effects are generally interacted, although microhabitats have the potential to mitigate the interaction effects in maintaining bryophyte richness. Bryophyte communities in coniferous forest seemed more resistant to the offsite effects than other ecosystem types. Reduced abundance of important functional groups in offsite landscapes could be a challenge for ecological services in boreal forests, especially in coniferous forest and open canopy ecosystems. With global changes, the predicted shifts in ecosystem type might exacerbate the effects of mining on biodiversity and ecological services in mine offsite landscapes. Cumulative areas exposed to mine offsite effects could be large at global scales, and therefore mine offsite landscapes should be considered in future impact evaluations and landscape management. A certain level of coarse woody debris storage near mine sites could be an effective conservation strategy. Our findings further encourage future studies to evaluate how these changes in bryophyte structure affect ecological services (e.g., carbon and nitrogen cycle) of the boreal forest and the microhabitat refugia under the global change.

3.8 Acknowledgements

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CHAPTER IV

OUT OF SITE, OUT OF MIND: CHANGES IN FEATHER MOSS PHYLLOSPHERE MICROBIOTA IN MINE OFFSITE BOREAL LANDSCAPES

HORS DU SITE, HORS DE L'ESPRIT : CHANGEMENTS DANS LE MICROBIOTE DE LA PHYLLOSPHÈRE DES MOUSSES À PLUMES DANS LES PAYSAGES BORÉAUX HORS SITE DES MINES

Ce chapitre est en préparation pour *Environmental Microbiology*

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Highlights:

- Impact of mines on microbial communities of the feather moss phyllosphere were studied in mine offsite boreal landscapes.
- Mining lifecycle and ecosystem type both affected the diversity, community structure and composition of feather moss phyllosphere microbiota.
- Ecosystem type did not interact with the offsite effects of the mine lifecycle on the feather moss phyllosphere microbiota.
- The majority of effects occurred within 0.2 km surrounding mine sites, especially near operating mines.

4.1 Résumé

Les interactions plantes-microorganismes, incluant les associations entre les mousses à plumes des forêts boréales et le microbiote de leur phyllosphère, contribuent à la biodiversité et aux services écologiques dans les biomes forestiers. L'augmentation de l'exploitation minière pour répondre aux besoins en minéraux constitue une menace potentielle pour la biodiversité. Cependant, l’impact de l'exploitation minière sur la biodiversité dans les paysages hors sites des mines est encore peu connu, en particulier pour les microbiomes. Nous avons échantillonné *Pleurozium schreberi* (Brid.) Mitt. dans des sites témoins et le long de transects d'un kilomètre autour de six sites miniers à différents stades d'exploitation (en exploitation ou non) qui traversent quatre types d'écosystèmes (conifères, feuillus, forêts mixtes et canopée ouverte) dans la forêt boréale canadienne. Les effets hors site des mines sur le microbiote de la phyllosphère ont été détectés à l'aide de l'ADN microbien récupéré à partir de la phyllosphère de *P. schreberi*. Le stade d'exploitation et le type d'écosystème ont tous deux affecté la diversité alpha et bêta ainsi que l'abondance relative de certains taxons, généralement sans interaction entre les deux facteurs. Les taxons microbiens individuels ont été plus affectés par les effets hors site que la diversité au niveau de la communauté. Les effets les plus forts se sont produits à moins de 0,2 km des mines en exploitation. Ces résultats indiquent que les sites miniers structurent les communautés microbiennes de la phyllosphère de la mousse à plumes dans les paysages boréaux et que ces effets sont modulés par le stade d'exploitation et le type d'écosystème. Les résultats impliquent également que les changements prédits de la dominance des conifères vers les feuillus dans la forêt boréale dans le contexte des changements globaux ne modifieraient pas l'impact des mines sur le microbiote de la phyllosphère dans les paysages hors site. D'autres études seront nécessaires pour évaluer comment ces effets hors site sur la diversité de la phyllosphère et la structure des communautés affectent le fonctionnement des écosystèmes boréaux et pour développer des stratégies d'atténuation appropriées.

Mots-clés : phyllosphère, écologie du paysage, indicateurs microbiens, bryophytes, taïga forestière

4.2 Abstract

Plant-microbe interactions, including associations between feather mosses from boreal forests and their phyllosphere microbiota, contribute to biodiversity and ecological services in forest biomes. Increases in mining for mineral requirements are potential threats to biodiversity. However, knowledge of impacts of mining on biodiversity in mine offsite landscapes is still poor, especially for microbiomes. We sampled *Pleurozium schreberi* (Brid.) Mitt. in control sites and within 1-km radial landscapes (away from the edge of each mine site) containing four ecosystem types of the Canadian boreal forest (i.e., coniferous, deciduous, mixed forests and open canopy peatlands) in six mines at different stages of the mine lifecycle. Offsite effects of mines on the phyllosphere microbiota were detected using microbial DNA recovered from the phyllosphere of *P. schreberi*. Mining stage and ecosystem type both affected alpha and beta diversity as well as the relative abundance of some taxa, generally without interactions between the two factors. Individual microbial taxa were more affected by offsite effects than community-level diversity. The strongest effects occurred within 0.2 km from operating mines. These results indicate that mine sites are structuring microbial communities of the feather moss phyllosphere in boreal landscapes, and that these effects are driven by mining stage and ecosystem type. The results also imply that predicted shifts from coniferous to deciduous dominance in boreal forest under global changes would not modify the impact of mines on the phyllosphere microbiota in offsite landscapes. Further studies are needed to assess how these offsite effects on the phyllosphere diversity and community structure affect the functioning of boreal ecosystems and to develop appropriate mitigation strategies.

Keywords: phyllosphere, landscape ecology, microbial indicators, bryophytes, taiga forest

4.3 Introduction

The phyllosphere — the aerial surfaces of plants — represents a widespread and diverse habitat for various groups of microorganisms, such as bacteria and fungi (Bashir et al., 2021; Perreault and Laforest-Lapointe, 2021; Vorholt, 2012). Organisms in the phyllosphere not only promote host plant fitness and nutrient acquisition, but also play important roles in global biodiversity and biogeochemical cycles (Peñuelas and Terradas, 2014; Perreault and Laforest-Lapointe, 2021; Stone et al., 2018). In boreal forests, where nutrient availability is limited, the moss phyllosphere microbiota plays a major role in carbon and nitrogen cycling, as exemplified by the feather mosses-cyanobacteria (Rousk et al., 2013) and *Sphagnum*–methanotrophs (Putkinen et al., 2012) associations. *Pleurozium schreberi* (one of the most widespread boreal feather mosses) alone, through the activity of its phyllosphere nitrogen-fixing bacteria (e.g. Cyanobacteria), was shown to fix 1.5 to 2.0 kg nitrogen ha^{-1} yr^{-1} in boreal forests (DeLuca et al., 2002).

Increasing anthropogenic activities such as mining are main threats to biodiversity and ecosystem services in the Anthropocene (Chester et al., 2019; Corlett, 2015). Currently, the area occupied by mines extends to about 57,300 km^2 across the globe (Maus et al., 2020). Areas directly disturbed by mining (e.g., open pits, tailings and waste rock) have been extensively studied (Blackmore et al., 2018; Chung et al., 2019; Feng et al., 2019; Gagnon et al., 2020; Thavamani et al., 2017). Meanwhile, ecological degradation in mine offsite landscapes (i.e., relatively intact ecosystems surrounding mines) remains poorly characterized. Effects occurring in offsite landscapes are identified as offsite effects (Raiter et al., 2014) and are thought to be caused by the spread of contaminants (e.g., dust, salt, heavy metals and excess nutrients) through the air, water or human activities (e.g., vehicle movement). There is increasing evidence that these "out of site" landscapes impacts should not stay out of mind as both plant and animal species are affected by distance to mines (Boisvert et al., 2021; Duarte et al., 2015; Watkinson et al., 2021; Wu et al., 2021). However, to the best of our knowledge, changes in microbial communities, in particular those inhabiting plants in mine offsite landscapes, have not been studied. Understanding the effects of mines on microbial communities of the phyllosphere is necessary to fully understand the impacts of mining on ecosystem services.

The phyllosphere composition of feather moss (i.e., *P. schreberi*) has been described in previous studies. Proteobacteria, Acidobacteriota, Bacteroidota and Actinobacteriota were the most abundant bacterial phyla (Cutler et al., 2017; Holland-Moritz et al., 2021; Holland-Moritz et al., 2018; Jean et al., 2020a; Rodríguez-Rodríguez et al., 2022), while Ascomycota and Basidiomycota were dominant fungal phyla (Cutler et al., 2017). These studies found that forest type was the main factor affecting the community composition of feather mosses through the different environmental conditions shaped by canopy structure such as temperature, leaf litter input, pH, and availability of nitrogen, moisture, and light (Holland-Moritz et al., 2021; Jean et al., 2020a; Rodríguez-Rodríguez et al., 2022). Proteobacteria, Acidobacteriota and WPS-2 (*Candidatus* phylum Eremiobacterota; Ji et al., 2017) were found at higher relative abundances in feather moss phyllosphere in coniferous than in deciduous forests, while deciduous forests supported higher relative abundances of Bacterioidota and Cyanobacteria (Jean et al., 2020a; Rodríguez-Rodríguez et al., 2022).

Deciduous, mixed, and coniferous forests, and open canopy areas (consisting mainly of peatlands) are four dominant ecosystem types in boreal landscapes. The resistance (i.e. the degree to which community composition remains unchanged in the face of a disturbance, Allison and Martiny, 2008) of the ecosystem types to environmental changes is conditioned by their structure and physicochemical characteristics. Generally, coniferous trees have higher canopy cover, leaf area index, less light transmission, and a more persistent foliage than deciduous trees, which leads to a relatively high resistance to environmental changes in coniferous forest and a relatively stable understory environment (Augusto et al., 2002; Eugster et al., 2000; Sánchez-Pinillos et al., 2019). Mixed forests usually have a more heterogeneous understory environment than monospecific forests (Chávez and Macdonald, 2012) and its resistance depends on the tree species composition of the mixture (Jactel et al., 2017). Although open-canopy peatlands receive more light, precipitation, solar radiation and atmospheric depositions than closed-canopy forests, they have a relatively high resistance to environmental changes (Łuców et al., 2020) because of humid microclimatic conditions generated by the accumulated peat (Augusto et al., 2002). From coniferous needle-leaved to deciduous broad-leaved tree dominance is the main ecosystem shift scenario in boreal forests in response to global changes (Boisvert-Marsh and de Blois, 2021; Mack et al., 2021; van der Velde et al., 2021). In a previous study, we have shown that this predicted shift could exacerbate mining effects on bryophyte community structure (Yin et al., 2022). Therefore, we predict that the effects of mines on the feather moss phyllosphere could also be mediated by the predicted shifts in ecosystem types.

Generally, the severity of mining disturbance varies with mine lifecycle from the establishment, operation to closure, and rehabilitation.The mine lifecycle might therefore also be a factor affecting the offsite effects. Emissions occur at each stage of the lifecycle but are especially abundant during operational activities where more pollutants (e.g., heavy metals, dust and acid mine drainage) are created and released into the surrounding environment (Punia, 2021). In contrast, the main risks in non-operating sites (i.e., establishment, closure and rehabilitation) are surface vegetation and soil clearing and residual pollution. Results from previous studies have indicated that offsite effects of mines were more obvious near operating mines than near non-operating sites in the community structure of understory vascular plants and bryophytes (Boisvert et al., 2021, Yin et al., 2022). Therefore, we expect to find the same pattern in the feather moss phyllosphere microbiota.

The objective of this study was to identify and characterize the impacts of mining on the feather moss phyllosphere microbiota in mine offsite boreal landscapes. To address the objective, we asked the following specific questions: Q1) Is there an offsite effect of mining on feather moss phyllosphere microbiota? Q2) What is the main driver influencing the phyllosphere microbiota in mine offsite landscapes: ecosystem type, mining stage or their interaction? Q3) At what distance can we detect the offsite effects of mines on the phyllosphere microbiota? To answer these questions, six mine sites at different stages of the mining lifecycle (grouped into operating mines and non-operating sites) were selected in the boreal forest of Quebec, Canada (Fig.4.1) and 1-km offsite landscapes around each mine site were used as study sites where *P. schreberi* shoots were collected. Plots in mine offsite landscapes were classified in four ecosystem types (i.e., coniferous, deciduous, mixed forests and open canopy areas). We expect that our results will help understand the impacts of mine sites on the spatial patterns of microbial communities in the moss phyllosphere and contribute to the identification of potential indicators of offsite effects of mines.

4.4 Materials and methods

4.4.1 Study sites and design

To conduct the study, six mine sites were selected in the Abitibi-Témiscamingue and Nord-du-Québec regions of the province of Québec, Canada (Fig. 4.1), including three operating mines (Casa Berardi, LaRonde and Canadian Malartic mine) and three non-operating sites (Akasaba, Joutel and Lapa mine sites). More detailed information about the six selected mines is available in Table S1.1. Using a stratified selection process, 6-8 transects (the number of transects was selected based on the size of each mine area, see Fig. S3.1) were established perpendicular to each mine periphery. Four plots were established along each transect with distance from the edge of intact forest at 0, 0.05, 0.2, and 1 km. Furthermore, six control transects with 7-8 plots per transect (44 plots in total) near the six mine sites were established in natural forests. The ecosystem of each plot was categorized into one of four types based on tree species (diameter at breast height (DBH) ≥10cm) composition: coniferous forest (> 70% of coniferous tree stems), deciduous forest (>70% deciduous tree stems), mixed (coniferous tree stems between 69% and 31%) and open canopy forests (DBH of all trees below 10 cm, peatlands in our studied areas). More detailed information about the design is shown in our previous studies (Boisvert et al., 2021; Yin et al., 2022).

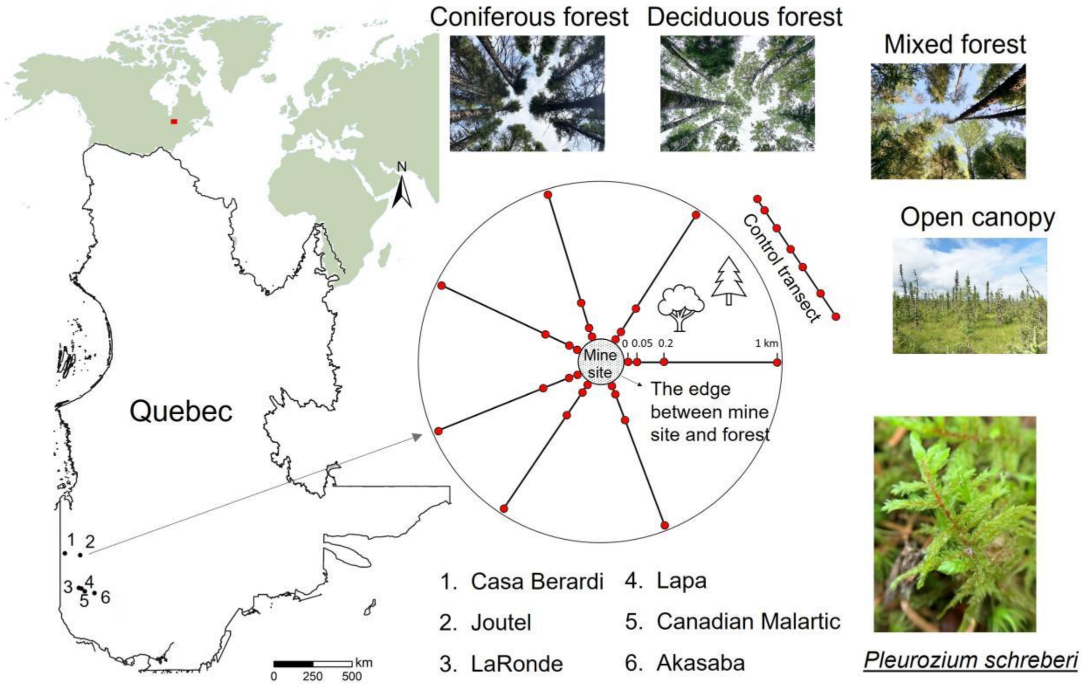

Figure 4.1 Study sites. Left, map of Quebec showing the location of the six gold mine sites. Right, sampling design. The four sampling plots at 0, 0.05, 0.2 and 1 km from the border of mine sites along transects at each study site are indicated by red circles. Six control transects were established near mine sites (see Figure S3.1).

4.4.2 Sampling

Between end of June and beginning of September of 2019, three colonies (a group of shoots of one species that live and interact closely with each other) of *P. schreberi* were randomly selected around the center point of each plot. The distance between colonies was approximately 10 m. At least 20 shoots per colony were picked, and debris and other plants were removed in the field. Only the top green parts (the last two years' growth, 0-3 cm from the tip of each shoot, Street et al., 2013) of each individual shoot were collected to avoid contamination from soil particles. All samples from one plot were pooled in sterile plastic roll bags and stored on ice in a cooler until reaching the laboratory, where samples were stored at -20°C until further processing. In total, 211 samples were collected from the 220 plots (no *P. schreberi* was found in 9 plots). In addition, forest environmental variables were also measured in the field as previously described (Yin et al., 2022). Principal component analysis (PCA) analysis showed that the environmental variables differed significantly among ecosystem types (ANOVA, $p < 0.001$, Fig. S3.2), therefore, environmental variables were grouped into the four ecosystem types in further analyses.

4.4.3 DNA library preparation and sequencing

The DNA extraction process followed the protocol of Kembel et al. (2014), with some modifications. Briefly, 10-15 shoots were selected from the collected samples, placed into sterile 50 mL Falcon-tubes, and covered with phosphate buffered saline supplemented with Tween 20 (PBST) (30-35 ml). Shoots were removed from the tube after shaking on vortex for 30 seconds and on a stirrer plate for 5 minutes. Microbial cells were recovered from the PBST by centrifuging at 4000 × g for 20 minutes at 4 °C. The supernatant was discarded and the pellet was transferred to a PowerBead tube of the DNeasy Power Soil DNA Isolation Kit (Qiagen,Valencia, CA) for DNA extraction, following the manufacturer's instructions. DNA extracts were quantified with the Qubit dsDNA HS assay kit using a Qubit Fluorometer 2.0 (Thermo Fisher Scientific, Watham, MA, USA). Bacterial and fungal communities were characterized by amplifying and sequencing the V4-V5 regions of the 16S ribosomal RNA gene with the primer pair 515F-Y (5'-GTGYCAGCMGCCGCGGTAA-3') / 926R (5'-CCGYCAATTYMTTTRAGTTT-3') (Parada et al., 2016) and the ITS2 region of nuclear ribosomal DNA with the primer pair ITS9 (5'-GAACGCAGCRAAIIGYGA-3') (Menkis et al., 2012) / ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Bartels et al., 2018a), respectively. Library preparation for Illumina sequencing was performed according to the manufacturer's instructions for user-defined primers (Bokulich et al., 2013). The first amplification was performed in a 25 μl reaction mix composed of 9 μl of UltraPure™

DNase/RNase-Free distilled water (Gibco, Thermo Fisher Scientific), 200 μM of each dNTP, 1.5mM of Mg2+, 200 nM of each primer, 1 U of HotStarTaq Plus DNAPolymerase (Qiagen, Valencia, CA, USA), and 2.5 μl of DNA extract. Thermocycling conditions were as follows: initial denaturation step at 95°C for 5 min, 34 (bacteria) or 40 (fungi) cycles at 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min, and a final elongation step at 72°C for 10 min. PCR products were purified with magnetic beads (Agencourt AMPure XP, Beckman Coulter, Mississauga, ON, CA). Unique codes were added to each PCR product using the Nextera XT Index Kit following the manufacturer's instructions (Illumina). Indexed amplicons were purified with magnetic beads, quantified with the Qubit dsDNA BR Assay Kit (Thermo Fisher Scientific) and pooled at equimolar concentration. Sequencing was performed on an Illumina MiSeq platform with a MiSeq Reagent Kit v3 (600 cycles) at the Next Generation Sequencing Platform of the centre hospitaliers universitaires de Québec-Université Laval Research Centre. Sequence data from this study are available at the NCBI Sequence Read Archive (SRA) under the BioProject PRJNA800026.

4.4.4 Bioinformatic processing

The DADA2 Pipeline (1.16 version for 16S and ITS Pipeline Workflow 1.8 version for ITS, Callahan et al., 2016) on R platform was used to exclude primer sequences, filter and de-noise sequences, de-replicate unique amplicon sequence variants (ASVs, similar to 100%-identity operational taxonomic units), and remove chimeric sequences. Those ASVs were classified (minBoot=80) with the SILVA 138 (Quast et al., 2013) and UNITE 8.2 (Koljalg et al., 2005) taxonomic databases for 16S and ITS identification, respectively. Then, we identified and removed non-bacterial and non-fungal ASVs from bacterial and fungal ASV tables including ASVs classified as Archaea, Mitochondria, Chloroplast, Eukaryote and Rickettsioses. After the processing, 38,901 and 20,086 reads remained for bacteria and fungi, respectively.

The tables generated by the DADA2 Pipeline were imported into R (3.4) as a phyloseq object (*phyloseq* package version 1.12.3, McMurdie and Holmes, 2013) for further quality filtering steps. The removal of sequences identified as contaminants was used by the *decontam* R package (Davis et al., 2018), and then singletons, doubletons and ASV with less than 10 reads were removed to control for the potential influence of rare sequences. One sample from the LaRonde site showed a usual and much higher fungal richness than other samples based on visual inspection of rarefaction curves and was removed from further analyses. To analyze the alpha diversity of the phyllosphere microbiota, the datasets were rarefied to the minimum sample size (2,383 reads per sample for bacteria and 6,153 reads per sample for fungi, Weiss et al., 2017), whereas, for community similarity analysis, library size normalization was carried out using the geometric mean of pairwise ratios (Chen et al., 2018b).

4.5 Statistical analyses

To answer the three specific questions, statistical analyses were conducted on the bacterial and fungal dataset separately, and in two sections: 1) Examine offsite effects of mines on the phyllosphere microbiota as well as their potential drivers (ecosystem type and mine stage, Q1 and Q2); 2) Determine the distance influenced by the offsite effects (Q3). All analyses were performed with the statistical platform R 4.0.5 (2021-03-31) with R Studio software. Results were visualized with the *ggplot2* package (Wickham, 2011).

4.5.1 Effects of ecosystem type and mine stage on the phyllosphere microbiota

To test the presence of offsite effects of mines on the phyllosphere microbiota (Q1) and identify potential drivers of microbial diversity, community structure and composition (Q2), alpha, beta diversity and relative abundance of individual taxa were analyzed. For alpha diversity analysis, generalized linear mixed models (GLMMs, “glmer” function from the *lme4* R package version 1.1-23, Bates et al., 2018) were applied on mining stage, ecosystem types and their interaction as predictors. Considering the nested structure of the sampling design, the nested terms site, transect and plot were included as random effects. Observed richness (number of ASVs), Shannon and InvSimpson indexes (Shannon and InvSimpson indexes were calculated with “estimate_richness” function from *phyloseq* package) were selected as the response variables for bacterial and fungal alpha diversity, separately. Based on the inherent statistical characteristics of response variables, Negative Binomial (overdispersed count data, observed bacterial and fungal richness, with “glmer.nb” function in *lme4* R package) and Gaussian (continuous number with normally distributed residuals, Shannon and InvSimpson Indexes with “glmer” function with family=gaussian and a log link) distribution were used in the models. Marginal R squared of GLMMs were calculated with the function “r.squaredGLMM” of the *MuMIn* R package R package 1.15.6 (Barton and Barton, 2015). Significant p-values for the effects of mining stage, ecosystem type and their interactions were calculated with ANOVA using Type II sum of squares (Type II sum of squares is more powerful than Type III for ANOVAs with unbalanced data, Langsrud, 2003, “Anova” function in the package “car” version 3.0-2 (Fox and Weisberg, 2019). When interaction terms were not significant, they were removed from the final models. Multiple pairwise comparisons for all response variables among mine stages and ecosystem types and when the interaction term was significant, pairwise comparisons were performed between mine stages in each ecosystem type. When the interactions were not significant, pairwise comparisons between each mine stage and between each ecosystem type, respectively were conducted with TukeyHSDtest (“emmeans” function in the *emmeans* package version 1.3. 5.1, Lenth et al., 2019).

Similarly, the effects of ecosystem type and mining stage on phyllosphere beta diversity were tested by permutational multivariate analysis of variance

(PERMANOVA, “adonis” function from the *vegan* package version 2, Oksanen et al., 2013) on Bray-Curtis distance matrices (“vegdist” function in the *vegan* package) with 999 permutations. PERMANOVA tests were performed for the bacteria and fungi ASV matrices as the response and ecosystem type, mining stage and their interactions as the explanatory variables and site factor as a ‘strata’ term to reduce randomizations across all sites. Bray-Curtis distance matrices were further visualized using non-metric Multi-dimensional Scaling (NMDS, “metaMDS” function in the *vegan* package) with three dimensions. We also used post-hoc pairwise PERMANOVAs (999 permutations, “pairwise.adonis” function in the package *RVAidememoire* version 0.9-45-2, Hervé, 2014) with a multiple comparison correction based on Benjamini-Hochberg method (Benjamini and Hochberg, 1995) to compare differences between mining stages.

Then, relative abundance of main bacterial and fungal phyla (average relative abundance across all samples > 0.5 %) was used as response variables to determine the effects of mining stage and ecosystem type (similar analysis process for alpha diversity). Microbiome relative abundances are compositional data that range between 0 and 1 and are also generally zero-inflated (Ho et al., 2019; Peng et al., 2016), therefore zero-inflated beta mixed models (ZIBMMs, and “glmmTMB” function with ziformula=~. and family=beta_family in *glmmTMB* package version 0.1.3, Brooks et al., 2017) were used instead of GLMMs here. The models that showed convergence errors were corrected by optimization of the model algorithm via Broyden–Fletcher–Goldfarb–Shanno (BFGS) algorithm (Dai, 2002) method in the function of the “glmmTMBControl” from *glmmTMB* package. Marginal R squared of ZIBMMs were calculated with the function “r2” of the *sjmisc* package version 2.4.0 (Lüdecke and Lüdecke, 2019). Furthermore, to identify the taxa (ASVs) driving differences in community composition between offsite landscapes of operating, non-operating mine sites and controls, indicator species analysis (“multipatt” function in the *indicspecies* package version 1.7. 6, De Caceres et al.,

2016) with point biserial-correlation coefficient (phi) was applied.

4.5.2 Effects of the distance from mines on the phyllosphere microbiota

The effects of distance from the mines on alpha and beta diversity of bacterial and fungal communities (Q3) and on the relative abundance of individual taxa (i.e., main phyla, top 10 main genera through all samples, and common ASVs found in at least 90 % of samples for bacteria and 70 % of samples for fungi) were analysed. Control transects were removed from these analyses given that the control transects were designed for landscape-level analysis (Q1 and Q2). Firstly, to assess variations in diversity and community structure (alpha and beta diversity) along the transects, alpha diversity indices (Observed ASV richness, Shannon and InvSimpson) and Bray–Curtis dissimilarity matrices were used as response variables in GLMMs and Pairwise PERMANOVAs, respectively. The effect of distance and its interaction with mining stage (only operating and non-operating here) were used as predictors. The analysis process (error distribution, ANOVA test and post hoc test) was similar to the models we used in Q1 and Q2, but here transects were nested into sites, and ecosystem type were used as random factors. Distance influenced by offsite effects of mines were determined based on the results of TukeyHSD tests and graphs (showing the position of threshold point, “ggplot” function in *ggplot2* package). Considering the very large ranges of variation (orders of magnitude) between taxa and zeros in relative abundance datasets, row data were replaced by log (x + l) transformation to present the spatial pattern with the distance in graphs. Finally, the offsite footprint of mines was evaluated and estimated using the influenced distance for alpha and beta diversity of bacterial and fungal communities as well as the relative abundance of individual taxa.

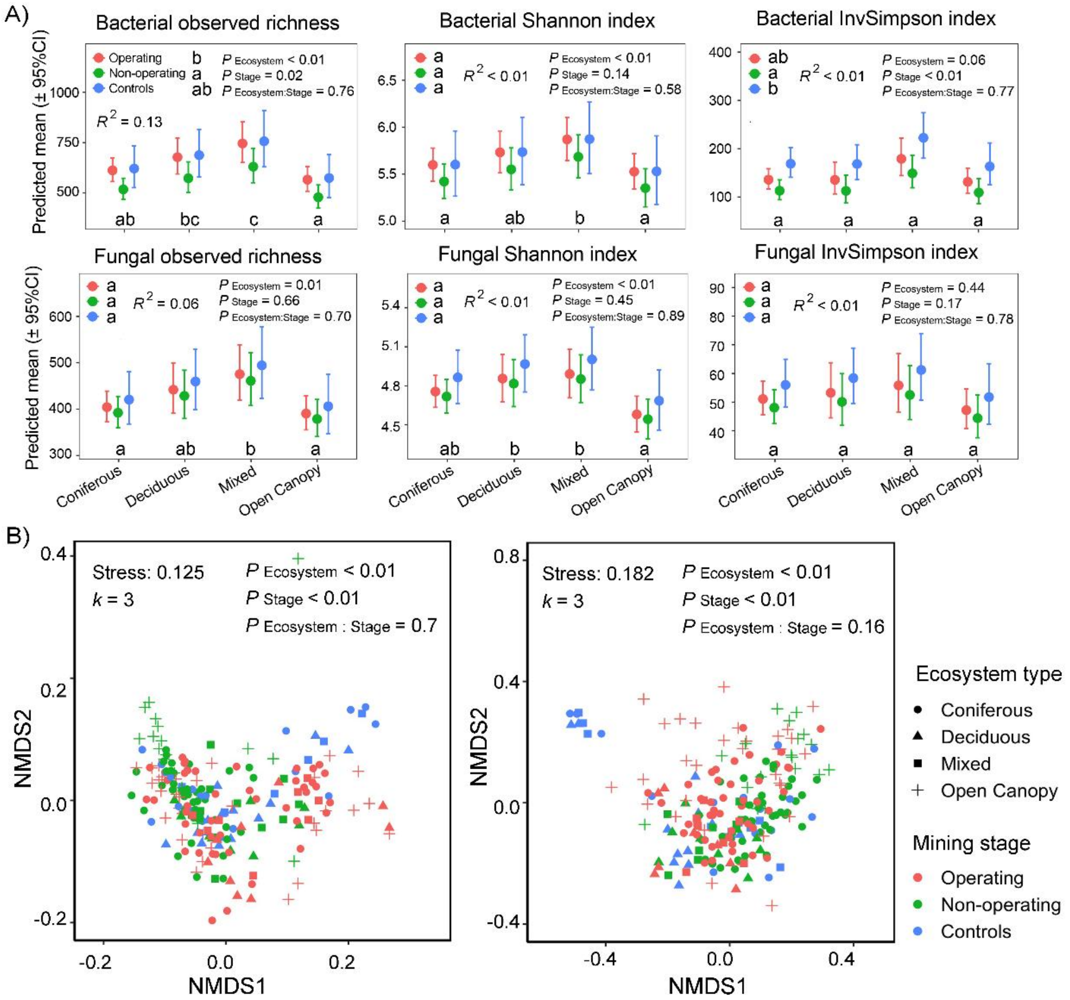

Figure 4.2 Changes in the phyllosphere alpha and beta diversity in mine offsite landscapes. A) Alpha diversity for both bacterial and fungal communities at different mining stages (plots surrounding operating sites, N= 92; plots surrounding non-operating sites, N=78; plots in controls, N=40) in four ecosystem types (coniferous forest, N=101; deciduous forest, N=33; mixed forest, N=26; open canopy, N=50). Points show means for all samples; bars show a 95% confidence interval around the mean. Different lowercase letters indicate significantly different means across mining stage and ecosystem type, respectively. P value based on generalized linear mixed models (type II sum of squares) and Tukey HSD pairwise comparisons ($\alpha = 0.05$) were used to test the differences across ecosystem types and mining stages, respectively. B) Beta diversity of bacterial and fungal community structure. Non-metric Multi-dimensional Scaling (NMDS) are based on the Bray–Curtis dissimilarity. Tests in the top side of the panels present the results of PERMANOVA.

4.6 Results

4.6.1 Effects of mining stage and ecosystem type on the phyllosphere microbiota

Mining stage and ecosystem type both affected *P. schreberi* phyllosphere alpha and beta diversity in mine offsite landscapes without interaction between the two factors. Bacterial alpha diversity differed among mining stages (observed richness and InvSimpson index, ANOVA type II sums, $P_{\text{Stage}} < 0.05$, Fig. 4.2A). No significant differences in alpha diversity were found between operating sites and controls (post hoc test results in Fig. 4.2A), but both had higher alpha diversity values than non-operating sites (Fig. 4.2A). The effects of mining stage on bacterial alpha diversity did not depend on ecosystem type (ANOVA type II sums, $P_{\text{Ecosystem:Stage}} > 0.05$, Fig. 4.2A), but ecosystem type also affected the alpha diversity (bacterial observed richness and Shannon index, ANOVA type II sums, $P_{\text{Ecosystem}} < 0.05$, Fig. 4.2A). Generally, deciduous and mixed forests had a higher alpha diversity than coniferous forests and open canopy in the offsite landscapes (bacterial observed richness and Shannon index, Fig. 4.2A). Similar patterns were found in fungal alpha diversity with higher alpha diversity found in deciduous and mixed forests (fungal observed richness and Shannon index, Fig. 4.2A) and the lack of interaction between mining stage and ecosystem type. But in contrast to bacteria, none of the fungal diversity indices measured was significantly affected by the mining stage (ANOVA type II sums, $P_{\text{Stage}} > 0.05$, Fig. 4.2A). Furthermore, only 0-13% of shifts in bacterial and fungal alpha diversity were explained by fixed factors (mining stage and ecosystem types) in the models (see R^2 in Fig. 4.2A).

Similarly, the analysis of the beta diversity of phyllosphere bacterial and fungal communities showed a significant effect of both the mining stage (bacteria, R^2= 4.43%, fungi, R^2= 2.62%, PERMANOVAs in Fig. 4.2B and Table S3.1) and ecosystem type (bacteria, R^2= 5.83%, fungi, R^2= 4.63%, PERMANOVAs in Table S3.1) without interaction between the two factors (PERMANOVAs, $P_{\text{Ecosystem:Stage}} >$

0.05, Fig. 4.2B) Furthermore, all pair-wise comparisons for mining stage and ecosystem type showed significant differences in bacterial and fungal community structure (Pairwise PERMANOVA tests, $P < 0.01$, Table S3.2 and Table S3.3).

For most phyla detected in the moss phyllosphere, mining stage and ecosystem type both had a significant effect on the relative abundance. The effect of mining stage was variable depending on the phylum. The relative abundance of the bacterial phylum Acidobacteriota and the fungal phylum Ascomycota were lower in offsite landscapes of operating sites than in non-operating sites across all samples (see Tukey HSD pairwise comparisons in Fig. 4.3), while the relative abundances of the bacterial phyla Bacteroidota and Myxococcota were higher near operating sites than non-operating sites (Fig. 4.3). The relative abundance of the Armatimonadota was higher in both operating and non-operating sites than in the controls (Fig. 4.3). The relative abundance of dominant phyla also differed between ecosystem types. Higher relative abundance of Acidobacteriota was detected in coniferous forest and open canopy ecosystems than in deciduous and mixed forests, while deciduous and mixed forests had a higher relative abundance of Bacteroidota. A significant interaction effect between the mining stage and ecosystem type on the relative abundance of four bacterial phyla (i.e., Proteobacteria, Cyanobacteria, WPS-2 and Olpidiomycota, Fig. 4.3) was observed. Interestingly, the relative abundance of two groups of photosynthetic microorganisms, the Cyanobacteria phylum and the WPS-2 candidate phylum, were negatively affected by operating mines in either coniferous forests or open canopy, but not in deciduous and mixed forests (Fig. 4.3). Overall, 5-25% of the shifts in the relative abundance of bacterial and fungal phyla were explained by fixed factors (mining stage and ecosystem type) in the models (see R^2 in Fig. 4.3).

Indicator species analysis was performed to identify fungal and bacterial taxa driving the differences in microbial communities based on mining stage. Phyllosphere of *P. schreberi* near operating sites, non-operating sites and control sites were characterized respectively by 42 (8 phyla), 44 (6 phyla) and 22 (4 phyla, Fig. 4 and

Table S3.5) bacterial indicator species, and 54 (4 phyla), 23 (4 phyla) and 78 (5 phyla) fungal indicator species (Fig 4.5 and Table S3.6) (in this case, the term 'species' refers to ASVs). Bacterial ASV_84 (stat=0.318, Table S3.5) and ASV_101 (stat=0.318, Table S3.5) assigned to the genera *Acidiphilium* and *Candidatus Solibacter*, respectively, were the best indicator species for operating sites (Table S3.6), whereas bacterial ASV_8 (stat=0.279, family Acetobacteraceae, Table S3.5) and ASV_361 (stat=0.514, genus *Cupriavidus*, Table S3.5) were the best indicators of non-operating sites and controls, respectively (Table S3.5). Fungal ASV_10 (stat=0.326, genus *Phenoliferia*, Table S3.6) was the best indicator species for operating mines, while fungal ASV_136 (stat=0.314, order Orbiliales, Table S3.6) and ASV_429 (stat=0.397, genus *Thelephora*, Table S3.6) were the best indicator species of non-operating sites and controls, respectively.

4.6.2 Effects of distance from the mine on the phyllosphere and offsite footprint

Phyllosphere alpha and beta diversity were associated with the distance from mines without interaction with the mining stage (ANOVA type II sums for GLMMs in bacterial and fungal observed richness and Shannon indices, $P_{Distance} < 0.05$, $P_{Stage} < 0.05$ and $P_{Distance:Stage} > 0.05$; PERMANOVAs in bacteria and fungi, $P_{Distance} < 0.001$, $P_{Stage} < 0.001$ and $P_{Distance:Stage} > 0.05$, Table S3.7 and S3.8). Bacterial alpha diversity (observed richness and Shannon index) showed a decreasing trend with the distance from mine sites. Observed richness and Shannon index were respectively about 13% and 4% higher at 0 km than at 1 km, although only the 0 km value was significantly higher compared to the 0.2 and 1 km values (TukeyHSDtest results in Table S3.7 and Fig. 4.5). In contrast, fungal alpha diversity (observed richness and Shannon index) followed a non-linear pattern with the distance, with no significant difference between 0 and 1 km but with significant differences between 0 and 0.05 or 0.2 km (TukeyHSD tests in Table S3.7 and Fig. 4.5). For beta diversity, bacterial community structure at 0 and 0.05 km differed from that at 1 km (Fig. 4.5B), while fungal community structure differed between 0 and 1 km, but no significant difference was found between 0.05 and 1 km (Fig. 4.5B). Therefore, in most cases, the variation of phyllosphere alpha and beta diversity was only detectable until 0.05 km into the forest interior.

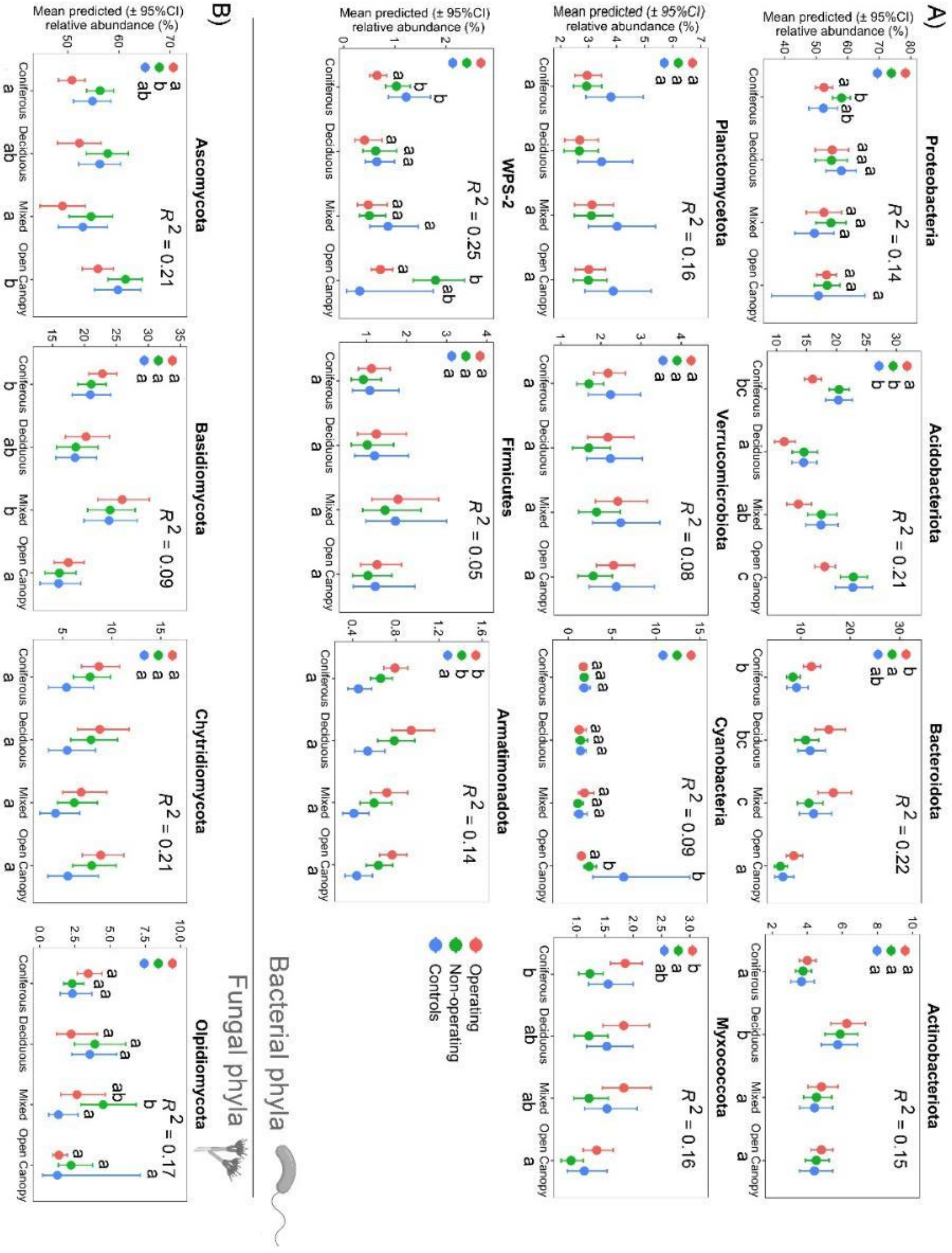
A)
B)
Mean predicted (± 95%CI) relative abundance (%)
Coniferous
Deciduous
Mixed
Open Canopy
Proteobacteria
$R^2 = 0.14$
Acidobacteriota
$R^2 = 0.21$
Bacteroidota
$R^2 = 0.22$
Actinobacteriota
$R^2 = 0.15$
Planctomycetota
$R^2 = 0.16$
Verrucomicrobiota
$R^2 = 0.08$
Cyanobacteria
$R^2 = 0.09$
Myxococcota
$R^2 = 0.16$
WPS-2
$R^2 = 0.25$
Firmicutes
$R^2 = 0.05$
Armatimonadota
$R^2 = 0.14$
Ascomycota
$R^2 = 0.21$
Basidiomycota
$R^2 = 0.09$
Chytridiomycota
$R^2 = 0.21$
Olpidiomycota
$R^2 = 0.17$
Operating
Non-operating
Controls
Bacterial phyla
Fungal phyla

Figure 4.3 Differences in relative abundance of main phyllosphere phyla (relative abundance > 0.5 %) at each mining stage in four ecosystem types in mine offsite landscapes. Zero-inflated beta regression models were used to determine responses of relative abundance of main bacterial and fungal phyla to ecosystem types, mining stages and their interaction. Points show means for all samples; bars show a 95% confidence interval around the mean. Different lowercase letters indicate significantly different means across groups based on Tukey HSD pairwise comparisons (α = 0.05). When interactions between ecosystem type and mining stage were significant, pairwise comparisons were used between mine stage in each ecosystem type, while when the interactions were not significant, the interaction terms were removed from the models and pairwise comparison were used between ecosystem type and mining stage, separately.

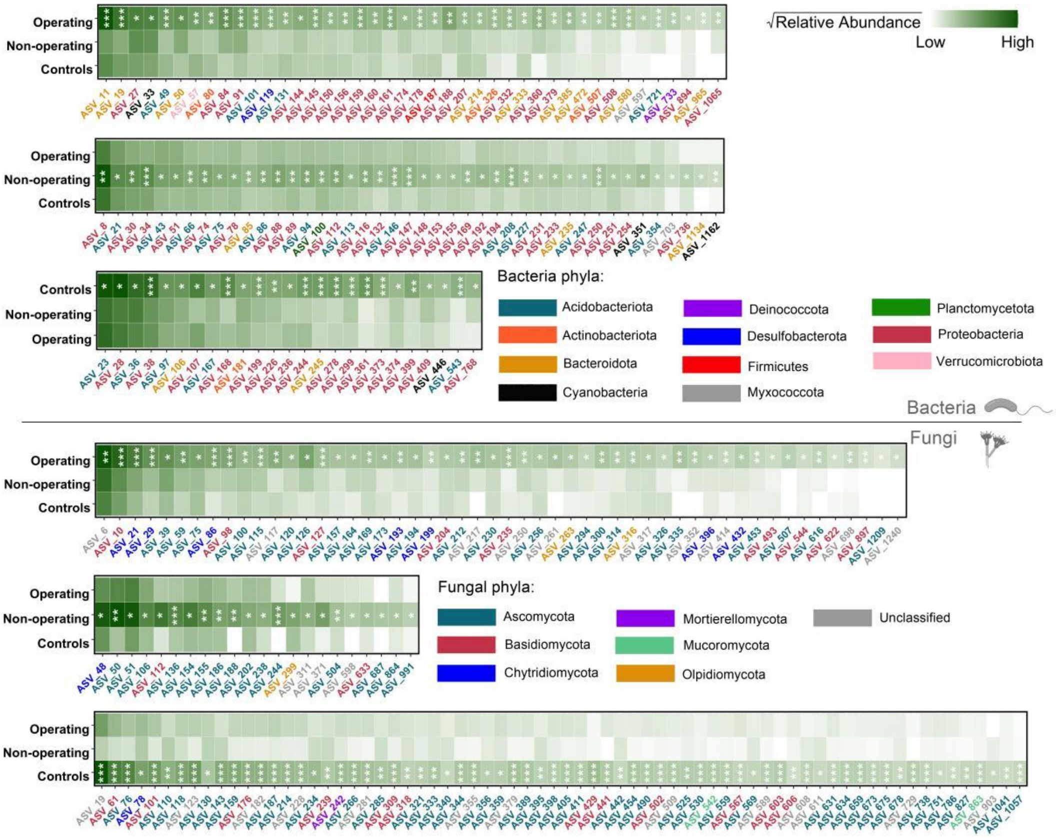

Figure 4.4 Heatmaps of the average relative abundance of indicator ASVs for mining stages. Only ASVs with total relative abundance > 0.05 % were included in the indicator analysis. * $P < 0.05$; **$P < 0.01$; *** $P < 0.001$. Note relative abundance of each bacterial and fungal ASV is shown for each mine stage when not an indicator ASV in that stage.

In contrast to alpha and beta diversity results, analysis of the relative abundance of individual taxa detected significant interactions between the distance and the mining stage as well as significant effects of mining up to over 0.2 km. In total, for five phyla, four genera and four ASVs among bacterial taxa, as well as one fungal phylum and two fungal ASVs, the effect of distance on the relative abundance based on GLMMs depended on whether the mine site was operating (Fig. 4.5C, Table S3.9, S3.10 and S3.11). Relative abundance of three bacterial phyla (i.e., Bacteroidota, Actinobacteriota and Armatimonadota), one bacterial genus (i.e., *Sphingomonas*) and one fungal ASV (ASV 10, *Phenoliferia* see Table S3.7) were positively influenced by mines, with highest values (between 1.4 and 3.14-fold higher that those at 1 km) detected at 0 km and decreasing with the distance near operating mines. No linear associations with distance were found near non-operating sites for these taxa (Fig. 4.5C). For another four ASVs (bacterial ASV 15, *Sphingomonas*, and fungal ASV 21, Spizellomycetales order, ASV 22, *Cladosporium*, ASV 66, *Aureobasidium*, Fig. 4.5C and Table S3.11), the relative abundance was positively affected by mines near both operating and non-operating sites (1.63-7 fold changes). Furthermore, two bacterial phyla (i.e., Acidobacteriota and WPS-2), three bacterial genera (i.e., *Granulicella*, *Leptothrix* and *Terriglobus*) and six bacterial ASVs, as well as two fungal genera (i.e., *Epithamnolia* and *Thoreauomyces*) and four fungal ASVs were negatively affected by either operating mines or all mine sites (both operating and non-operating, Fig. 4.5C).

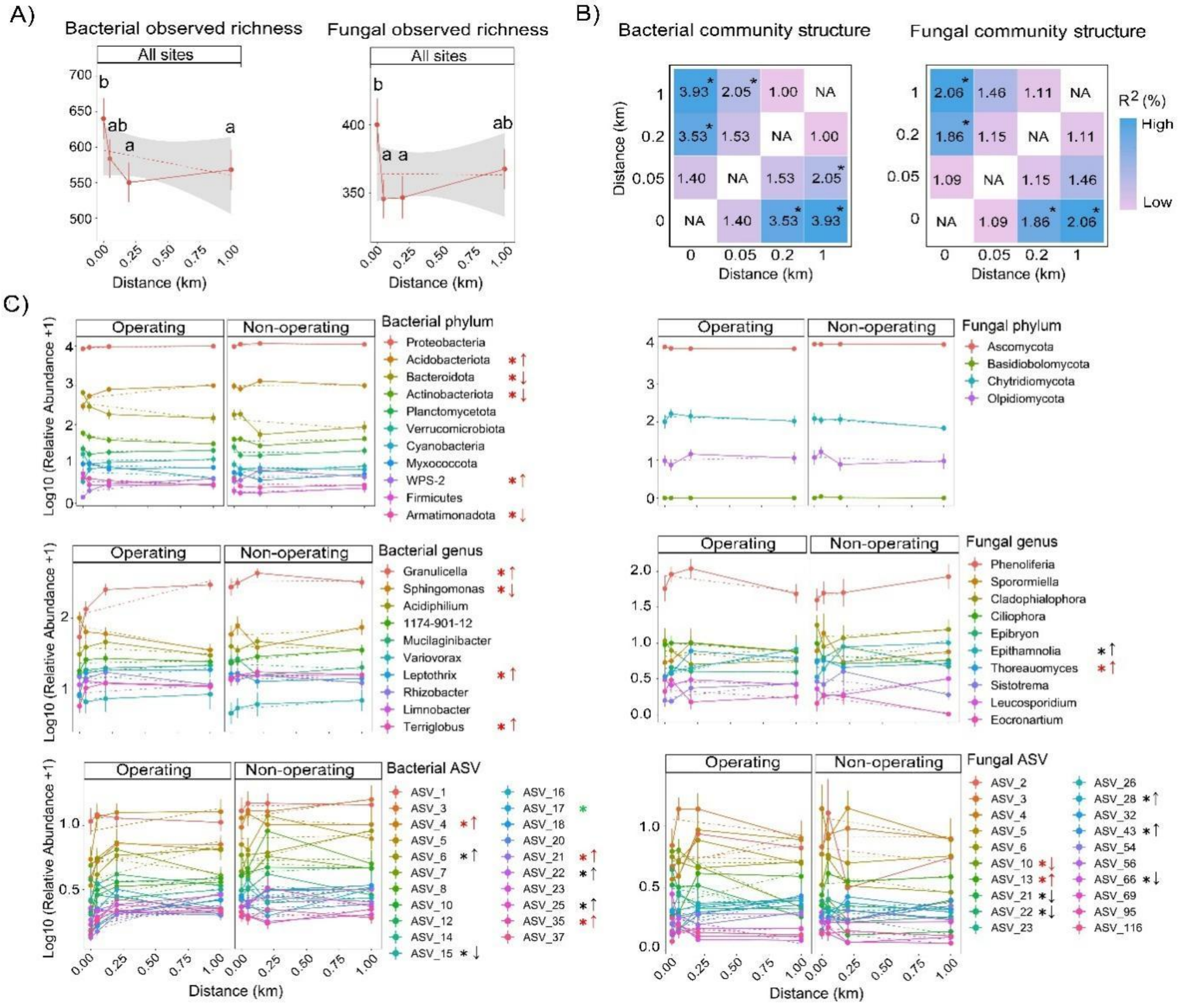

Figure 4.5 Changes in phyllosphere microbial communities with distance from mine sites. A) Bacterial and fungal observed richness. Different lowercase letters indicate significantly different means between different distances at 0 (N=39), 0.05 (N=44), 0.2 (N=43) and 1 km (N=44) from mine sites (results based on generalized linear mixed models and Tukey HSD pairwise comparisons, α = 0.05). B) Bacterial and fungal community structure differences between distances based on the results of pairwise PERMANOVA on Bray-Curtis dissimilarity matrices, * $P < 0.05$. C) Relative abundance of bacterial and fungal taxa along the distance at phylum, genus and ASV levels. Points show means for relative abundance after log (x + l) transformation; bars show standard error (SE). Black asterisks indicate significant association with distance at both mining stages based on the zero-inflated beta mixed regression models and post-hoc tests with Tukey HSD pairwise comparisons. Red

asterisks indicate the interaction between the distance and mining stage was significant and the effects occurred near operating mines, while green asterisks indicate the effects occurred near non-operating sites. Arrows used for linear patterns: up arrows, relative abundance is increasing with the distance; down arrows relative abundance is decreasing with the distance.

4.7 Discussion

4.7.1 The presence of offsite effects of mines on the feather moss phyllosphere microbiota

Our results confirmed the presence of offsite effects of mines on the feather moss phyllosphere microbiota (Q1) based on the differences in alpha and beta diversity, relative abundance of individual taxa, and indicator species of mined sites (operating or non-operating) and controls. These findings extend the offsite effects of mines on surrounding ecosystems to microbiomes, as only vegetation and animals were used to determine the effects in previous studies (Boisvert et al., 2021; Dyer et al., 2001; Watkinson et al., 2021; Wu et al., 2021, Yin et al., 2022). Surprisingly, similar levels of bacterial alpha diversity were detected in operating mines and controls, while the diversity in non-operating sites was lower (Fig. 4.2), which is contrary to our expectations. Integrating these results with those obtained for individual taxa, we suggest that this result is explained by taxon-specific responses to offsite effects. Some taxa (e.g., Armatimonadota, Fig. 4.3) were favored near mine sites, although some other (e.g., Acidobacteriota, Fig. 4.3) were suppressed, leading to similar levels of diversity as in the control sites. These results seem to support the novel 'niche flip' mechanism in microbial ecology, where alpha diversity is shaped by both disturbance frequency and intensity (Mancuso et al., 2021). In this mechanism, the microbial communities follow a U-shaped diversity dependence on the disturbance intensity, with the lowest level of diversity occurring at an intermediate level of disturbance (Mancuso et al., 2021). Here, controls, non-operating and operating sites represent zero, low and intermediate mining disturbance intensity, respectively, and followed this high-low-high (U-shaped) pattern in alpha diversity. Further work will be needed to confirm the mechanisms underlying these observations, especially regarding the fluctuation and intensity of the offsite effects, which were not characterized in this study.

Differences in phyllosphere fungal and bacterial beta diversity between mining stages confirmed that the mining stage is a driver of microbial community structure in mine offsite landscapes. Previous studies have identified pH, total organic carbon, total phosphorus and heavy metal content as the main influencing factors in the variation of soil microbial community structure in mining polluted areas (Dimitriu and Grayston, 2010; Kane et al., 2020; Wu et al., 2022). These factors could also partly explain the variation observed in the moss phyllosphere in this study considering that feather moss grows in close contact with the soil. The concentration of metals (aluminium, copper) was also significantly correlated with variations in microbial functions in a study comparing the phyllosphere microbiota of various tree species (Lajoie et al., 2020). Considering that metal concentrations could be higher in the phyllosphere near operating than non-operating mines and that mosses are known to accumulate metals (Blagnytė and Paliulis, 2010; Stanković et al., 2018), we can expect that metal concentration in the moss phyllosphere could be responsible for the differences in community structure observed here between mining stages. For example, the drastic decline of Acidobacteriota near operating mines compared with non-operating and control sites indicated the sensitivity of Acidobacteria to heavy metals (Guo et al., 2019; Macdonald et al., 2011; Zheng et al., 2019). In contrast, the higher relative abundances of Bacteroidota near operating mines suggests that these landscapes could provide favorable conditions for some microorganisms. Bacteroidota usually harbour the majority of heavy metal resistance genes (Yan et al., 2020), and is able to quickly adapt and endure high heavy metal stresses in mining polluted areas (Zhao et al., 2020a; Zhao et al., 2020b; Zou et al., 2021). Characterizing soil properties, assessing dust emissions from mines, and measuring heavy metal concentrations in mosses would be important next steps to further identify the environmental drivers responsible for changes in phyllosphere community structure observed in mine offsite landscapes.

Furthermore, operating mines supported some specific species, as highlighted by the indicator species analysis. Bacterial ASVs 101 and 84 as well as the fungal ASV 10 were the best indicators of operating mines (Table S3.5 and S3.6) and belonged to Candidatus *Solibacter, Acidiphilium* and *Phenoliferia*, respectively. The ability of members from the first two bacterial taxa to overcome stressful conditions (including high metal concentrations) have been reported in mining environments (Böhmer et al., 2020; Li et al., 2020). Furthermore, members of the fungal genus *Phenoliferia* can degrade phenolic compounds (Pakarinen et al., 2021; Perini et al., 2019) and its higher relative abundance near operating mines might be driven by a higher phenolic compound content of feather moss, as these compounds are usually produced by plants as a defense mechanism under heavy metal stress (Maleki et al., 2017). More studies are needed to explore which environmental variable or plant traits (such as heavy metals and phenolic compounds content) lead to the increased relative abundance of Fungal ASV 10 (*Phenoliferia*) near mines. Nevertheless, fungal ASV 10, with its relatively high abundance (about 1.5 % at 0 m near operating mines, Table S3.11) and its sensitivity to mining stage and distance, could be a reliable indicator to assess the presence and magnitude of offsite effects of mines. Surprisingly, one of the best indicator ASV for control sites (bacterial ASV361) was assigned to the *Cupriavidus* genus, which contains several species or strains known for their heavy metal resistance and adaptation to metal-contaminated environments (Mijnendonckx et al., 2013; Reith et al., 2009; Van Houdt et al., 2018). The species or strain identified here in the moss phyllosphere may lack the specific genes required for heavy metal resistance, which could be confirmed by further characterizing the moss phyllosphere DNA using shotgun metagenomics approaches.

Our results confirmed that offsite effects are usually cryptic because inherent limitations of impact evaluation (e.g., experimental design, detection methods, spatial scales, statistical power) led to those effects being overlooked. If our experimental design had included operating mines only, and if the focus of our statistical analyses had been limited to alpha diversity, substantial offsite effects could have been undetected because taxa-specific responses were masked in the alpha diversity response (especially for fungi, Fig. 4.2). This study also highlights that the use of environmental DNA (eDNA) tools can reveal impacts of disturbances on groups of living organisms (here bacteria and fungi, but many others could be targeted) that are typically overlooked in impact evaluations because of the absence of efficient methods to detect them. The performance of these tools to assess biodiversity and measure the environmental impacts of disturbances is becoming widely accepted by the scientific community (Edge et al., 2020; Pawlowski et al., 2021) and hopefully, this will lead to a wider application of these tools in impact assessment in the near future.

4.7.2 Ecosystem type and mining stage affected phyllosphere microbiota in mine offsite landscapes

Our results provide further evidences that feather moss phyllosphere diversity and composition differed between ecosystem types (Jean et al., 2020b; Rodríguez-Rodríguez et al., 2022). However, no differences in alpha diversity (bacterial and fungal observed richness and bacterial Shannon index, Fig. 4.2B) were found between deciduous and coniferous forests in contrast with Rodríguez-Rodríguez et al. (2022) who found higher bacterial alpha diversity of the feather moss phyllosphere in coniferous stands (black spruce) than in deciduous stands (trembling aspen). To avoid a loss of statistical power by creating too many categories, coniferous forests and deciduous forests in this study were not monospecific stands, which may have led to these differences. Our observations of a higher relative abundance of the bacterial phylum Acidobacteria in coniferous forests and open canopy and a higher relative abundance of Bacteroidota in deciduous and mixed forests were, however, consistent with findings from Rodríguez-Rodríguez et al. (2022). Despite the limitation that most forest stands in this study were located near mine sites and not in natural conditions, our results still provide insights into the relationships between ecosystem types and moss phyllosphere communities in boreal forests.

Surprisingly, the effects of mining stage on the alpha, beta diversity and relative abundance of most phyla were not ecosystem type-dependent. It suggests that ecosystem types did not influence the ability of the moss phyllosphere to resist to the offsite effects, which is in contrast with a previous study indicating that bryophyte diversity was more affected by mining stage in deciduous forest than in coniferous forest (Yin et al., 2022). Compared with deciduous forest, coniferous trees with high canopy cover, leaf area index and persistent foliage intercept more pollutants in atmospheric deposition (Barbier et al., 2008; Nguyen et al., 2015). Here, this mechanism did not did not play an obvious role in reducing the impact of mines on the moss phyllosphere microbiota as the effects were similar in coniferous and deciduous forests. To our knowledge, no study to date has directly compared the sensitivity of bryophyte communities to that of phyllosphere microbial communities to dust, but microorganisms colonizing the leaf surface are know to be strongly exposed to adverse conditions, including air pollutants (Bringel and Couée, 2015), and have been found to be more sensitive to environmental conditions than leaf endophytes (Sivakumar et al., 2020). A study also found that the overall reduction of dustfall by coniferous trees was between 38-42%, while deciduous trees reduced dustfall by 27-30% (Dochinger, 1980). So over 50 % of dustfall still could pass through the canopy structure, leading to loads of heavy metals and other toxins that may have impacted the phyllosphere microbial communities without reaching, in coniferous forests, the level at which bryophyte diversity would also be significantly impacted. On the other hand, it is also possible that an interaction between ecosystem types and mining would have emerged if the phyllosphere was sampled later in the season after leaf fall, when the differences in canopy structure were more striking than in the summer. Further studies should be done to examine these two potential explanations. Overall, the results indicate that predicted shifts in ecosystem types would not generally modify the offsite effects of mines on the phyllosphere microbiota in boreal landscapes.

Notably, the offsite effects on the relative abundance of two photosynthetic bacterial phyla, WPS-2 and Cyanobacteria, were affected by mining stage-ecosystem type interactions. Indeed, no effect of the mining stage on the relative abundance of WPS-2 and Cyanobacteria was detected in both deciduous and mixed forests, which can be expected as these ecosystem types are known to be unfavorable for the two groups (Jean et al., 2020a; Qu et al., 2020; Rousk et al., 2013) because of relatively low air moisture, high soil pH and broadleaf litter inputs (Augusto et al., 2002; Qian et al., 2003). However, the relative abundance of these phyla was lower near operating mines in coniferous forest and open canopy peatland, which is in contrast with previous studies highlighting the resistance of Cyanobacteria and WPS-2 to metal contamination (Al-Amin et al., 2021; Cui et al., 2021; Grasby et al., 2013; Kavehei et al., 2021). This may be attributable to the differences between the soil and phyllosphere habitats (Leveau, 2019). However, there is almost no literature to explain these findings. The current knowledge on how environmental stress affects phyllosphere communities is very limited, especially for mosses, and more studies on this topic are needed. Overall, although mechanisms are still unclear, our results suggest that the ecological services supported by these taxonomic groups, such as the fixation of carbon and nitrogen, could be impacted in offsite landscapes of operating mines, especially in coniferous forests and open canopy peatlands. We also found fungi were more resistant to the offsite effects than bacteria. According to previous studies, fungi appear to be more tolerant to heavy metals than bacteria (Kelly et al., 2003; Zeng et al. 2020), and extreme conditions under mining disturbances imposing selection pressure may trigger additional evolutionary adaptation in the fungal communities (Adriaensen et al. 2005).

4.7.3 The footprint of offsite effects of mines on feather moss phyllosphere

Determining the offsite footprint of mines is challenging but necessary for policy maker and governments to assess ecological impacts of mining projects and, subsequently, develop mitigation strategies. Our findings indicated that phyllosphere alpha and beta diversity were affected by the distance from the mine site, but the effect was only detectable up to a distance between 0 - 0.05 km from mine sites, which is much lower than what was detected using the relative abundance of individual taxa (up to 1 km, such as fungal ASV10 near operating mines, Table S3.11). Therefore, if the offsite footprint is determined at the community-level, sizes of the offsite footprint and their impacts on ecological services could be underestimated.

Based on all results of alpha, beta diversity and relative abundance of individual taxa, we found that significant effects are detectable within 0.2 km of mine sites for most taxa, especially for operating mines. Therefore, if the average width of the offsite footprint surrounding mines is set to 0.2 km, the total area for footprint calculation would be about 4.5 km^2 (average 1.5 km^2/mine, measured through Google Earth Pro 7.1) for the three operating mines in our study. With more than 6,000 active mines across the globe (Maus et al., 2020), the estimated global offsite footprint of active mines on surrounding phyllosphere microbiota could be up to 9,000 km^2 (about the land area of Cyprus). Considering non-operating sites still have effects on surrounding phyllosphere structure, the actual offsite effects of mines could be even larger. Although the mining method, size, shape and local environmental conditions could affect the range of offsite effects, our results still provide justification to include offsite footprints of mines when evaluating the total footprint of mining on landscapes.

4.8 Conclusion

Our findings highlight the presence of offsite effects of mines on microbiomes, as exemplified here in the feather moss phyllosphere. Although both mining lifecycle and forest composition were identified as factors affecting the offsite effects of mines, our study indicates that these effects are cryptic and can easily be ignored at the community level. Specific taxa such as Bacteroidota and fungal ASV10 (*Phenoliferia*) have higher indicator roles than community-level diversity to determine the presence and footprint of offsite effects of mines. Though the average distance influenced by the offsite effects is small (about 0.2 km) near individual mines, the sum might be large and impactful across the globe. Moreover, as more mining projects will be established in the coming decades with the energy transition, more landscapes will be exposed to the offsite effects of mines. The global offsite footprint of mines is therefore expected to expand and become a problem over larger areas. Further studies are needed to assess how these changes in microbial communities of the phyllosphere in mine offsite landscapes affect ecosystem functioning, including carbon and nitrogen cycling in boreal ecosystems, and to develop appropriate mitigation strategies.

4.9 Acknowledgements

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CHAPITRE V

CONCLUSION GÉNÉRALE

5.1 Résumé des principaux chapitres

Cette thèse a été conçue pour identifier et caractériser les effets hors site des mines sur la biodiversité des paysages boréaux environnants et pour déterminer si la composition de la forêt et le cycle de vie des mines sont des moteurs de l'effet hors site. Dans le chapitre II, différents groupes de végétation de sous-bois de plantes ligneuses, herbacées, bryophytes et lichens ont d'abord été utilisés pour déterminer les effets hors site. Les résultats ont confirmé la présence d'effets hors site de la mine sur les communautés de sous-bois, et le stade d'exploitation et le type d'écosystème ont été des facteurs déterminants pour l'ampleur des effets hors site. Cependant, nous avons également constaté que les différents groupes de végétation ont réagi différemment aux effets hors site et, par rapport aux autres catégories, les bryophytes semblent être plus touchées en raison de la réduction de la couverture végétale près des sites miniers. Compte tenu des rôles des bryophytes dans les services écologiques de la forêt boréale et de leur grande sensibilité aux changements dans les microhabitats et la pollution, les bryophytes ont été sélectionnées pour déterminer les effets des microhabitats sur les effets hors site et les impacts potentiels sur les groupes fonctionnels (c'est-à-dire les mousses à plumes et les sphaignes) dans le chapitre III. Dans les conclusions du chapitre III, il a été observé que les bryophytes au sol étaient plus affectées par les effets hors site que les groupes sur les autres types de microhabitats. En outre, la couverture au sol de la mousse à plumes a présenté une réponse significative aux types d'écosystèmes et au stade d'exploitation. Sur la base des résultats du chapitre III, nous pensons que les mousses à plumes poussant sur le sol pourraient recevoir plus de contaminants, nous nous sommes donc demandés si le microbiote de la phyllosphère des mousses est également touché ? Pour répondre à cette question, dans le chapitre IV, nous avons sélectionné une espèce de mousse à plumes, *Pleurozium schreberi* pour identifier et déterminer les effets hors site des mines sur le microbiote de la phyllosphère. La thèse a permis d'élargir la compréhension de différents groupes vivants sur les effets des mines sur la biodiversité et a fourni des implications pour la gestion forestière autour des sites miniers. Les trois chapitres présentent les principales informations relatives aux effets hors site des mines sur les plantes et les microbiomes associés de la phyllosphère dans le sous-étage de la forêt boréale : 1) l'effet hors site individuel est relativement faible et limité dans l'espace, mais son accumulation dans l'espace pourrait être substantielle ; 2) les effets sont plus nombreux à proximité des mines en exploitation que des sites non exploités ; 3) les effets hors site dépendent également du type de forêt et des communautés. Les plantes vasculaires (couverture totale et richesse) étaient plus touchées dans les forêts de feuillus que de conifères, tandis que les bryophytes étaient plus touchées dans les forêts de conifères. Les communautés bactériennes et fongiques de la phyllosphère ont été affectées de manière similaire par les mines, quel que soit le type de forêt ; 3) Les microhabitats peuvent atténuer les effets hors site. Les effets hors site des mines affectent principalement les microhabitats proches du sol ; 4) les effets hors site offrent des opportunités pour certaines espèces tolérantes (y compris la tolérance aux métaux lourds et à la luminosité) de se développer dans les paysages hors site des mines ; 5) les capacités indicatrices (sensibilité) des différents groupes de sous-bois aux effets hors site ont été trouvées : pour les plantes de sous-bois, bryophytes > plantes herbacées > plantes ligneuses ; pour le microbiote, bactéries > champignons. Par conséquent, nos résultats ont atteint les objectifs généraux et spécifiques que nous avions proposés et ont caractérisé les effets hors site des mines dans les paysages boréaux.

5.2 Synthèse des résultats

Les objectifs généraux et spécifiques du projet ont été atteints dans les trois chapitres centraux (chapitre II, III et IV), mais en examinant les résultats des chapitres ensemble pourrait nous aider à mieux comprendre les caractéristiques de ces effets dans les paysages hors site minier. Nous avons comparé les différentes réponses des trois groupes aux effets hors site et leurs facteurs influencés, le type d'écosystème, le stade d'exploitation minière et les microhabitats. De plus, l'empreinte hors site des mines sur les communautés de sous-bois a finalement été déterminée sur la base de tous les résultats des trois groupes. Selon l'objectif global de notre recherche, la synthèse des résultats comprend deux parties : 1) les effets du stade d'exploitation et du type d'écosystème sur les communautés de sous-bois hors site et le rôle des microhabitats sur ces effets ; 2) la détermination de l'empreinte hors site des mines ; 3) la sensibilité de chaque groupe aux effets hors site des mines.

5.1.1 Les effets du stade d'exploitation et du type d'écosystème

L'interaction des effets combinés du stade d'exploitation et du type d'écosystème dépend des différentes communautés et microhabitats. Des effets significatifs des deux paramètres et de leur interaction ont généralement été trouvés dans les communautés de plantes vasculaires et de bryophytes du sous-bois en termes de composition de la communauté, mais les effets d'interaction sur la richesse des espèces ont été médiés par les microhabitats (Figure 5.1). En effet, seule la richesse des communautés de sous-étage (c'est-à-dire les plantes vasculaires et les bryophytes) sur le sol a démontré des effets d'interaction du stade d'exploitation et du type d'écosystème, tandis que les effets sur la richesse des bryophytes des microhabitats comme des rochers, des débris ligneux, des souches, des chicots et des arbres étaient tamponnés. Nous avons également constaté que seules les communautés des microhabitats inférieurs (c.-à-d. le sol, les rochers et la phyllosphère de la mousse de plumes) ont démontré les effets hors site des mines sur la richesse et la composition des communautés (Figure 5.1). Ces résultats suggèrent que les macrohabitats (type d'écosystème) pourraient être un moteur des effets hors site pour les communautés de sous-bois au sol, tandis que les microhabitats pourraient être le principal moteur pour ceux qui colonisent les microhabitats hors sol, ce qui est cohérent avec des études précédentes montrant que les communautés de sous-bois au sol et les microhabitats hors sol avaient des réponses différentes aux changements environnementaux (Barbé et al., 2020 ; Caners et al., 2013). Ce phénomène pourrait être attribué à une combinaison de dépôts de poussière accumulée et de changements dans les structures de la canopée. Les communautés du sous-étage sur les microhabitats inférieurs étaient plus exposées aux effets hors site en raison de la poussière atmosphérique (y compris les métaux lourds, les métaux traces et autres polluants) déposée sur les microhabitats supérieurs et les surfaces des feuilles qui sont finalement déposées sur le sol de la forêt par le vent, l'eau de pluie et la défoliation (Sase et al., 2012). Tandis que les microhabitats hors sol ayant des dimensions verticales, pourraient être moins exposés aux changements des variables environnementales (par exemple, la disponibilité de la lumière, la chute des arbres et l'humidité du sol). Par conséquent, nous concluons que l'interaction des effets du stade d'exploitation et du type d'écosystème sur les effets hors site dépend largement des types de microhabitats.

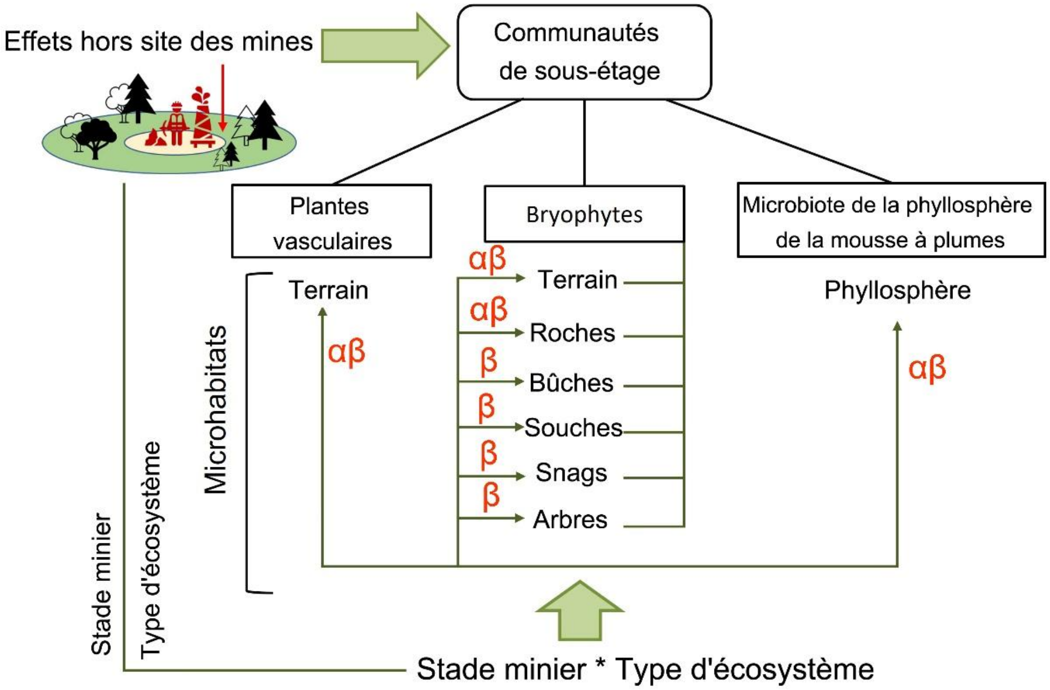


Figure 5.1 Effets de l'interaction stade d'exploitation - type d'écosystème sur les effets

hors site sur les communautés du sous-étage. α signifie que la richesse a été affectée et β signifie que la composition de la communauté a été affectée.

5.1.2 Détermination de l'empreinte hors site des mines

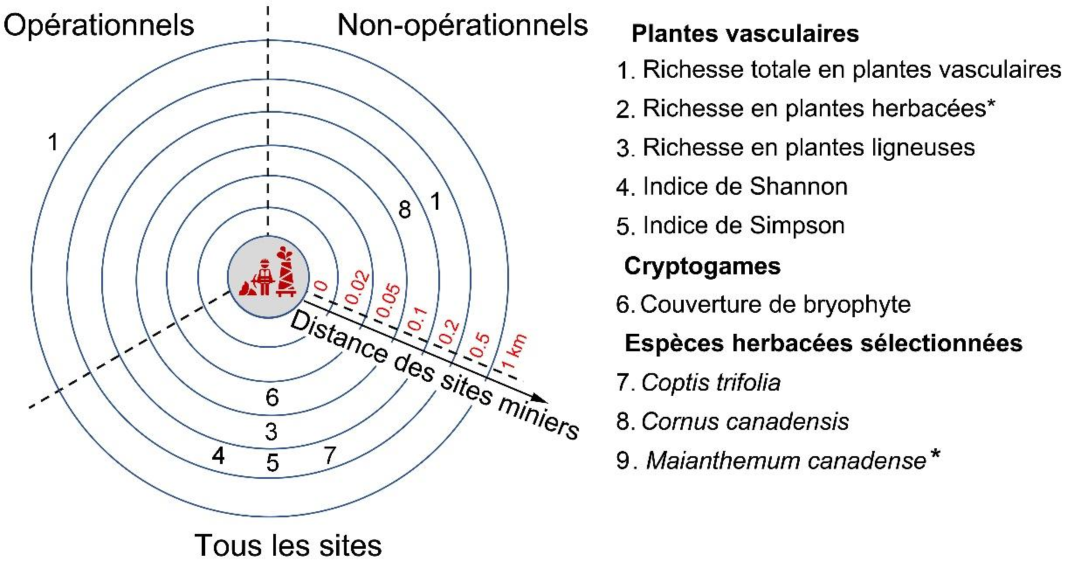


Figure 5.2 La distance d'influence des mines sur les communautés du sous-étage. Seuls les indices qui ont été associés à la distance sont présents dans les graphiques. * Indique qu'aucune distance influencée n'a été détectée

L'évaluation de l'empreinte hors site des mines est un défi dans les évaluations de l'impact écologique des mines, car les effets hors site sont subtils et hors de vue (Raiter et al., 2014). Dans cette étude, l'empreinte hors site a été déterminée en utilisant la distance influencée sur la diversité et la composition des plantes vasculaires, des bryophytes et du microbiote de la phyllosphère des mousses à plumes dans les couches de sous-bois de la forêt boréale. La distance d'influence des effets hors site pouvait atteindre plus d'un kilomètre, en particulier à proximité des mines en exploitation, mais les effets les plus forts se situaient à moins de 0,2 km des mines (Figures 5.2 et 5.3). Par conséquent, les résultats combinés confirment la taille de l'empreinte hors site des mines dans les forêts boréales, ce qui est cohérent avec les résultats des chapitres 2 et 3. Les résultats indiquent que les mesures d'atténuation des effets hors site devraient se concentrer sur la plage de 0 à 0,2 km de la lisière de la forêt.

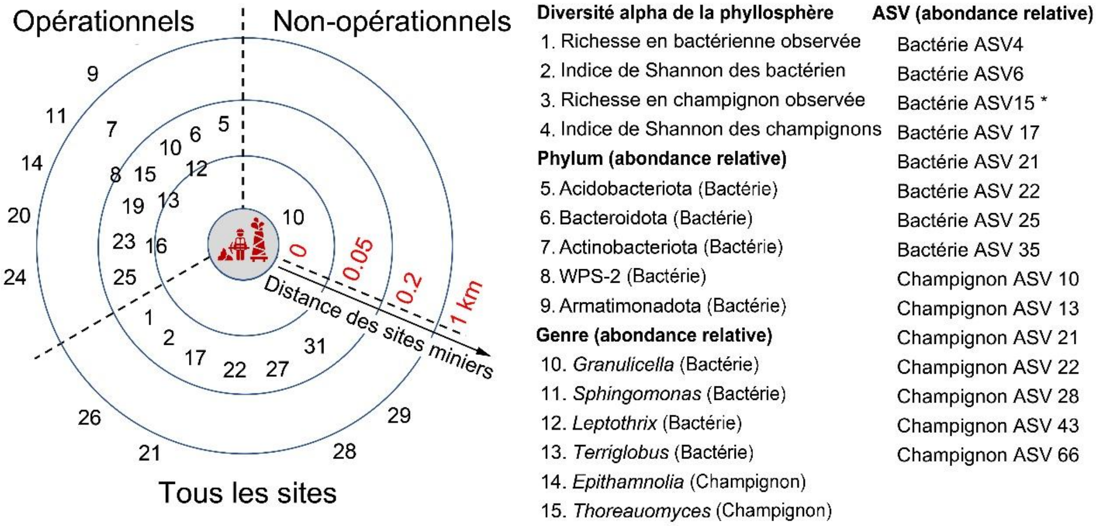


Figure 5.3 Influence de la distance des mines sur les communautés microbiennes de la phyllosphère de Pleurozium schreberi. Seuls les groupes/taxa associés à la distance sont présents. * Indique qu'aucune distance influencée n'a été détectée.

5.1.3 Structure des communautés bryophytes et microbiote de la phyllosphère des mousses à plumes

La structure de la communauté bryophyte (couverture et richesse) pourrait être associée au microbiote de la phyllosphère d'après les résultats des chapitres II, III et IV. Premièrement, le couvert total des mousses (chapitre II) et la couverture végétale de la mousse de plumes (chapitre III) ont été réduites dans la forêt de conifères et l'écosystème de canopée ouverte près des mines en exploitation, tandis que certains groupes bactériens fonctionnels de WPS-2 et de Cyanobactéries habitant dans la phyllosphère de la mousse de plumes ont également été réduits dans ces deux écosystèmes près des mines en exploitation. Deuxièmement, la richesse en bryophytes au sol a présenté une forme de "Ո" dans la forêt de conifères et l'écosystème de canopée ouverte avec une valeur plus élevée près des sites non exploités que des sites d'exploitation et de contrôle (chapitre III), tandis que la richesse bactérienne de la phyllosphère a présenté une forme de "U" avec une valeur plus élevée près des sites non exploités. Ces deux liens suggèrent que la structure de la communauté voisine (par exemple, la couverture végétale et la richesse) pourrait être un moteur pour la structure de la communauté microbienne de la phyllosphère. Une étude a révélé que le taxon bactérien sur l'érable à sucre était positivement corrélé avec l'abondance relative de cet hôte dans le paysage (Lajoie et Steven, 2021). Une autre étude a révélé que l'abondance bactérienne de la phyllosphère était négativement associée à la richesse de la communauté voisine (Meyer et al., 2022). Dans cette étude, pour la diversité alpha de la phyllosphère (par exemple, la richesse), la couverture végétale de l'espèce hôte (*Pleurozium schreberi*) pourrait être un facteur positif, tandis que la richesse totale en bryophytes (richesse de la communauté voisine) pourrait être un facteur négatif. Les liens potentiels indiquent une voie indirecte des effets hors site des mines sur le microbiote de la phyllosphère par le biais de la structure des communautés voisines. D'autres analyses seront effectuées pour confirmer les liens entre la structure de la communauté de la phyllosphère de la mousse à plumes et son voisinage de bryophytes.

5.2 Contributions

Cette thèse propose une approche analytique et méthodologique pour identifier les effets hors site des mines et déterminer leur empreinte dans les paysages boréaux. La contribution de l'étude comprend :

1) Confirmation de la présence d'effets hors site des mines sur les communautés du sous-étage des forêts boréales. Les paysages hors site sont relativement intacts et sont facilement et souvent négligés dans les évaluations des impacts (Raiter et al., 2014), mais nos résultats ont quantifié les effets se produisant dans les paysages hors site des

mines. Bien que ces effets soient subtils, ils sont détectables à l'aide des plantes de sous-bois et des microbiomes associés, en particulier pour certains groupes spécifiques, tels que les espèces végétales introduites et les bactéries tolérantes aux métaux. Par conséquent, nos résultats présentent non seulement les effets hors site qui ne doivent pas être oubliés, mais fournissent également des indicateurs potentiellement utiles pour surveiller les effets hors site.

2) Une compréhension préliminaire des caractéristiques clés des effets hors site des mines dans les forêts boréales. Les effets hors site sont une sorte d'impact "énigmatique", hors site et de faible ampleur, mais qui peuvent être importants à l'échelle mondiale. Le cycle de vie de l'exploitation minière, la composition de la forêt et les microhabitats peuvent tous affecter leur force, ce qui permet de déterminer les éléments à inclure dans le futur cadre d'évaluation des effets hors site des mines.

3) Quantification de l'empreinte hors site des mines. L'identification des zones d'influence est essentielle pour évaluer et atténuer les effets hors site des mines. Nos résultats montrent que les effets les plus importants se produisent à moins de 0,2 km des sites miniers. Nous avons estimé l'empreinte hors site des mines à l'aide de ces données. Bien que les zones influencées puissent varier d'une mine à l'autre, notre estimation de l'empreinte constitue une référence pour les décideurs politiques et les parties prenantes dans les processus décisionnels.

4) Stratégies potentielles proposées pour réduire les effets hors site des mines. Étant donné que la forêt de conifères est le peuplement dominant de la forêt boréale et qu'elle présente, selon nos résultats, une résistance relativement élevée aux effets hors site, nous proposons d'utiliser des plantations de conifères indigènes comme zones tampons autour des sites miniers. De plus, un certain niveau de stockage de débris ligneux grossiers près des sites miniers est proposé pour compenser les effets hors site potentiels des mines sur les bryophytes, étant donné leur rôle dans le maintien de la richesse en bryophytes.

5.3 Limites et recherches futures

5.3.1 Limites de la recherche

1) Le plan d'échantillonnage nous a limité dans l'exploration des effets de la direction du vent, de la taille de la mine et des installations adjacentes (p. ex. bassin de résidus, amas de stériles, bâtiments et fosses) sur la distance d'influence.

2) Il est impossible d'explorer les effets du type d'écosystème sur la distance influencée dans les paysages réels car les paysages entourant les mines sélectionnées sont caractérisés par une mosaïque de parcelles de forêts de feuillus et de conifères.

3) La source de pollution des effets hors site des mines n'a pas été détectée dans l'étude. La concentration de poussière à la surface des plantes (en particulier pour les mousses à plumes), les métaux lourds et les nutriments du sol doivent être déterminés pour identifier la source de pollution.

4) Les associations entre les variables environnementales (par exemple, les métaux lourds) et la diversité du sous-étage près des sites miniers n'ont pas été évaluées.

5.3.2 Recherches futures

Sur la base des résultats de cette thèse, des recherches supplémentaires sont nécessaires pour mieux comprendre les effets hors site des mines sur les communautés du sous-étage dans les paysages boréaux :

1) Évaluer comment ces effets hors site des mines sur le sous-étage et la structure de la phyllosphère affectent les services écologiques des écosystèmes boréaux. En particulier, il est essentiel d'évaluer les effets hors site des mines sur la dynamique du carbone et de l'azote dans la forêt boréale, étant donné que la forêt boréale est manifestement limitée par l'azote et que la végétation et le sol de la forêt boréale

représentent l'un des plus grands réservoirs mondiaux de carbone (Näsholm et al., 1998 ; Strömgren et Mjöfors, 2012).

2) D'évaluer les effets combinés des effets hors site et d'autres perturbations dans les paysages hors site de la mine. L'interaction entre les effets hors site et les autres effets pourrait se faciliter mutuellement. Par exemple, nous avons déjà évalué les effets synergiques entre les effets hors site et les changements prévus dans la composition des forêts. Cependant, d'autres perturbations, notamment les incendies de forêt, la déforestation et les changements globaux de température et de précipitations, devraient être prises en compte dans les recherches futures sur les effets hors site des mines.

3) D'inclure davantage de sites miniers dans les recherches futures. Les sites miniers de différentes régions boréales, y compris l'Europe du Nord et l'Asie du Nord, sont essentiels pour l'acquisition de données complètes, qui peuvent fournir une estimation plus précise de l'empreinte hors site globale des mines dans la forêt boréale.

5.4 Sommaire

Les effets hors site des mines ont affecté les communautés boréales de plantes de sous-bois et le microbiote de la phyllosphère. Les effets ont été déterminés à la fois par le type d'écosystème et le cycle de vie des mines, et les effets les plus forts ont été discutables dans un rayon de 0,2 km des paysages hors site des mines. L'effet énigmatique (c'est-à-dire les effets hors site) a été confirmé et caractérisé pour la première fois dans les paysages boréaux. Ce projet a atteint l'objectif fixé, mais il ne représente que la "pointe de l'iceberg" des effets hors site des mines. Il reste encore beaucoup à découvrir sur les paysages hors site des mines et nos résultats devraient justifier d'autres études associées pour la biodiversité et les services écologiques.

“... This journey has a goal, but it does not stop at any goal, for it has come to realise that today's goal is only the starting point of tomorrow's journey.” - Sri Chinmoy

APPENDICE A

MATÉRIEL SUPPLÉMENTAIRE DU CHAPITRE II

Table S1.1 The information about the selected respective mines

	Akasaba	Canadian Malartic	Casa Berardi	LaRonde	Lapa	Joutel
Stage	Establishing	Operation	Operation	Operation	Closing	Restored
Main disturbances	Roads, deforestation and some human activities	Activities associated with mining (e.g., blasting, digging and transportation)			Residual contaminants and some human activities	Residual contaminants (e.g., tailings)
Mine type	Open Pit	Open Pit	Underground & Open Pit	Underground	Underground	Open pit
Commodities	Gold, Copper	Gold, Silver	Gold, Silver	Gold, Silver, Copper, Zinc	Gold	Gold, Copper
Owned/operated by	Agnico Eagle Mines Ltd.	Canadian Malartic Corp.	Hecla Quebec Inc.	Agnico Eagle Mines Ltd	Agnico Eagle Mines Ltd	Agnico Eagle Mines Ltd
Num. Employees	-	699 (2017)	580 (2018)	833 (2017)	165 (2018)	-
Mine life	Preparation since 2014	Operation since 2005	Operation since 1988	Operation since 1988	Closed since2018	Closed since 1988
Mining Method	-	▪ Truck & Shovel/ Loader	▪ Truck & Shovel/ Loader ▪ Longhole stoping ▪Timbered stoping ▪ Longitudinal stoping	▪ Transverse open stoping ▪ Longitudinal retreat ▪ Cemented backfill ▪ Paste backfill	▪ Transverse open stoping ▪ Longitudinal retreat ▪ Cemented backfill	-

Notes: information from the platform of Mining Data Online (https://miningdataonline.com/).

Table S1.2 Description of the sampling design. The number of plots of each ecosystem type encountered at different distance along transect was included.

Distance (m)	Coniferous	Deciduous	Mixed	Open Canopy	Sum
0	20	9	2	13	44
20	19	7	2	16	44
50	20	4	8	12	44
100	27	2	4	11	44
200	24	1	5	14	44
500	15	5	6	18	44
1000	21	6	4	13	44
Controls	27	14	7	13	61
Sum	173	48	38	110	Total:

Table S1.3 The list of all vascular species identified in all samples (only plants identified at the species level shown)

ID	Species	Genus	Family	Group	Duration	Introduced	Habit
1	*Abies balsamea* (L.) Mill.	*Abies*	Pinaceae	Gymnosperm	Perennial	Native	Woody
2	*Acer rubrum* L.	*Acer*	Aceraceae	Dicot	Perennial	Native	Woody
3	*Acer spicatum* Lam.	*Acer*	Aceraceae	Dicot	Perennial	Native	Woody
4	*Achillea millefolium* L.	*Achillea*	Asteraceae	Dicot	Perennial	Introduced	Herbaceous
5	*Actaea pachypoda* Elliott.	*Actaea*	Ranunculaceae	Dicot	Perennial	Native	Herbaceous
6	*Actaea rubra* (Aiton) Willd.	*Actaea*	Ranunculaceae	Dicot	Perennial	Native	Herbaceous
7	*Agrostis gigantea* Roth	*Agrostis*	Poaceae	Monocot	Perennial	Introduced	Herbaceous
8	*Alnus alnobetula* (Ehrh.) K.Koch	*Alnus*	Betulaceae	Dicot	Perennial	Native	Woody
9	*Alnus incana* (L.) Moench	*Alnus*	Betulaceae	Dicot	Perennial	Native	Woody
10	*Alnus viridis* A.Gray	*Alnus*	Betulaceae	Dicot	Perennial	Native	Woody
11	*Amelanchier arborea* (F.Michx.) Fernald	*Amelanchier*	Rosaceae	Dicot	Perennial	Native	Woody
12	*Amelanchier bartramiana* (Tausch) M.Roem.	*Amelanchier*	Rosaceae	Dicot	Perennial	Native	Woody
13	*Amelanchier canadensis* Darl.	*Amelanchier*	Rosaceae	Dicot	Perennial	Native	Woody
14	*Amelanchier laevis* Wieg.	*Amelanchier*	Rosaceae	Dicot	Perennial	Native	Woody

Table S1.3 continued

15	*Anaphalis margaritacea* (L.) Benth. & Hook.f.	*Anaphalis*	Asteraceae	Dicot	Perennial	Native	Herbaceous
16	*Andromeda glaucophylla* Link	*Andromeda*	Ericaceae	Dicot	Perennial	Native	Woody
17	*Anemonastrum canadense* (Juz.) Holub	*Anemonastrum*	Ranunculaceae	Dicot	Perennial	Native	Herbaceous
18	*Anemone quinquefolia* L.	*Anemone*	Ranunculaceae	Dicot	Perennial	Native	Herbaceous
19	*Apocynum androsaemifolium* L.	*Apocynum*	Apocynaceae	Dicot	Perennial	Native	Herbaceous
20	*Aralia hispida* Vent.	*Aralia*	Araliaceae	Dicot	Perennial	Native	Herbaceous
21	*Aralia nudicaulis* Blume	*Aralia*	Araliaceae	Dicot	Perennial	Native	Herbaceous
22	*Arctostaphylos uva-ursi* (L.) Spreng.	*Arctostaphylos*	Ericaceae	Dicot	Perennial	Native	Woody
23	*Athyrium filix-femina* (L.) Roth	*Athyrium*	Dryopteridaceae	Fern	Perennial	Native	Herbaceous
24	*Betula papyrifera* Marshall	*Betula*	Betulaceae	Dicot	Perennial	Native	Woody
25	*Betula pumila* L.	*Betula*	Betulaceae	Dicot	Perennial	Native	Woody
26	*Calamagrostis canadensis* (Michx.) P.Beauv.	*Calamagrostis*	Poaceae	Monocot	Perennial	Native	Herbaceous
27	*Caltha palustris* L.	*Caltha*	Ranunculaceae	Dicot	Perennial	Native	Herbaceous
28	*Capnoides sempervirens* (L.) Borkh.	*Capnoides*	Fumariaceae	Dicot	Perennial	Native	Herbaceous
29	*Carex disperma* Dewey	*Carex*	Cyperaceae	Monocot	Perennial	Native	Herbaceous
30	*Carex magellanica* Lam.	*Carex*	Cyperaceae	Monocot	Perennial	Native	Herbaceous
31	*Carex oligosperma* Michx.	*Carex*	Cyperaceae	Monocot	Perennial	Native	Herbaceous

Table S1.3 continued

32	*Carex platyphylla* J.Carey	*Carex*	Cyperaceae	Monocot	Perennial	Native	Herbaceous
33	*Carex retrorsa* Schwein.	*Carex*	Cyperaceae	Monocot	Perennial	Native	Herbaceous
34	*Carex stipata* Muhl. ex Willd.	*Carex*	Cyperaceae	Monocot	Perennial	Native	Herbaceous
35	*Carex tenera* Dewey	*Carex*	Cyperaceae	Monocot	Perennial	Native	Herbaceous
36	*Chamaedaphne calyculata* (L.) Moench	*Chamaedaphne*	Ericaceae	Dicot	Perennial	Native	Woody
37	*Cicuta bulbifera* L.	*Cicuta*	Apiaceae	Dicot	Perennial	Native	Herbaceous
38	*Cinna latifolia* (Trevir.) Griseb.	*Cinna*	Poaceae	Monocot	Perennial	Native	Herbaceous
39	*Circaea alpina* L.	*Circaea*	Onagraceae	Dicot	Perennial	Native	Herbaceous
40	*Clintonia borealis* (Aiton) Raf.	*Clintonia*	Liliaceae	Monocot	Perennial	Native	Herbaceous
41	*Comarum palustre* L.	*Comarum*	Rosaceae	Dicot	Perennial	Native	Herbaceous
42	*Coptis trifolia* (L.) Salisb.	*Coptis*	Ranunculaceae	Dicot	Perennial	Native	Herbaceous
43	*Cornus canadensis* L.	*Cornus*	Cornaceae	Dicot	Perennial	Native	Herbaceous
44	*Cornus sericea* L.	*Cornus*	Cornaceae	Dicot	Perennial	Native	Woody
45	*Corylus americana* Walter	*Corylus*	Betulaceae	Dicot	Perennial	Native	Woody
46	*Corylus cornuta* Marshall	*Corylus*	Betulaceae	Dicot	Perennial	Native	Woody
47	*Cylactis pubescens* (Raf.) W.A.Weber	*Cylactis*	Rosaceae	Dicot	Perennial	Native	Herbaceous

Table S1.3 continued

48	*Cyperus esculentus* L.	*Cyperus*	Cyperaceae	Monocot	Perennial	Native	Herbaceous
49	*Cypripedium acaule* Aiton	*Cypripedium*	Orchidaceae	Monocot	Perennial	Native	Herbaceous
50	*Danthonia compressa* Austin	*Danthonia*	Poaceae	Monocot	Perennial	Native	Herbaceous
51	*Delphinium carolinianum* Walter	*Delphinium*	Ranunculaceae	Dicot	Perennial	Native	Herbaceous
52	*Dendrolycopodium dendroideum* (Michx.) A. Haines	*Dendrolycopodium*	Lycopodiaceae	Lycopod	Perennial	Native	Herbaceous
53	*Dendrolycopodium obscurum* (L.) A. Haines	*Dendrolycopodium*	Lycopodiaceae	Lycopod	Perennial	Native	Herbaceous
54	*Diervilla Lonicera* Mill.	*Diervilla*	Caprifoliaceae	Dicot	Perennial	Native	Woody
55	*Drosera rotundifolia* L.	*Drosera*	Droseraceae	Dicot	Perennial	Native	Herbaceous
56	*Dryopteris carthusiana* (Vill.) H.P. Fuchs	*Dryopteris*	Dryopteridaceae	Fern	Perennial	Native	Herbaceous
57	*Elymus canadensis* L.	*Elymus*	Poaceae	Monocot	Perennial	Native	Herbaceous
58	*Elymus repens* (L.) Gould	*Elymus*	Poaceae	Monocot	Perennial	Introduced	Herbaceous
59	*Epigaea repens* L.	*Epigaea*	Ericaceae	Dicot	Perennial	Native	Woody
60	*Epilobium angustifolium* L.	*Epilobium*	Onagraceae	Dicot	Perennial	Native	Herbaceous
61	*Epilobium leptophyllum* Raf.	*Epilobium*	Onagraceae	Dicot	Perennial	Native	Herbaceous
62	*Epilobium palustre* L.	*Epilobium*	Onagraceae	Dicot	Perennial	Native	Herbaceous
63	*Epipactis helleborine* (L.) Crantz	*Epipactis*	Orchidaceae	Monocot	Perennial	Introduced	Herbaceous

Table S1.3 continued

64	*Equisetum arvense* L.	*Equisetum*	Equisetaceae	Horsetail	Perennial	Native	Herbaceous
65	*Equisetum fluviatile* L.	*Equisetum*	Equisetaceae	Horsetail	Perennial	Native	Herbaceous
66	*Equisetum pratense* Ehrh.	*Equisetum*	Equisetaceae	Horsetail	Perennial	Native	Herbaceous
67	*Equisetum sylvaticum* L.	*Equisetum*	Equisetaceae	Horsetail	Perennial	Native	Herbaceous
68	*Eriophorum vaginatum* L.	*Eriophorum*	Cyperaceae	Monocot	Perennial	Native	Herbaceous
69	*Eurybia macrophylla* (L.) Cass.	*Eurybia*	Asteraceae	Dicot	Perennial	Native	Herbaceous
70	*Fallopia cilinodis* (Michx.) Holub	*Fallopia*	Polygonaceae	Dicot	Perennial	Native	Herbaceous
71	*Fragaria vesca* L.	*Fragaria*	Rosaceae	Dicot	Perennial	Native	Herbaceous
72	*Fragaria virginiana* Mill.	*Fragaria*	Rosaceae	Dicot	Perennial	Native	Herbaceous
73	*Galium aparine* L.	*Galium*	Rubiaceae	Dicot	Annual	Introduced	Herbaceous
74	*Galium asprellum* Michx.	*Galium*	Rubiaceae	Dicot	Perennial	Native	Herbaceous
75	*Galium sylvaticum* L.	*Galium*	Rubiaceae	Dicot	Perennial	Introduced	Herbaceous
76	*Gaultheria hispidula* (L.) Muhl. ex Bigelow	*Gaultheria*	Ericaceae	Dicot	Perennial	Native	Woody
77	*Gaultheria procumbens* L.	*Gaultheria*	Ericaceae	Dicot	Perennial	Native	Woody
78	*Gentiana linearis* Froel.	*Gentiana*	Gentianaceae	Dicot	Perennial	Native	Herbaceous
79	*Geocaulon lividum* (Richardson) Fernald	*Geocaulon*	Santalaceae	Dicot	Perennial	Native	Herbaceous

Table S1.3 continued

80	*Glyceria striata* (Lam.) Hitchc.	*Glyceria*	Poaceae	Monocot	Perennial	Native	Herbaceous
81	*Goodyera repens* (L.) R.Br.	*Goodyera*	Orchidaceae	Monocot	Perennial	Native	Herbaceous
82	*Gymnocarpium dryopteris* (L.) Newman	*Gymnocarpium*	Dryopteridaceae	Fern	Perennial	Native	Herbaceous
83	*Huperzia lucidula* (Michx.) Trevis.	*Huperzia*	Lycopodiaceae	Lycopod	Perennial	Native	Herbaceous
84	*Hypericum perforatum* L.	*Hypericum*	Clusiaceae	Dicot	Perennial	Introduced	Herbaceous
85	*Ilex mucronata* (L.) M. Powell, Savol. & S. Andrews	*Ilex*	Aquifoliaceae	Dicot	Perennial	Native	Woody
86	*Juncus effusus* L.	*Juncus*	Juncaceae	Monocot	Perennial	Native	Herbaceous
87	*Kalmia angustifolia* L.	*Kalmia*	Ericaceae	Dicot	Perennial	Native	Woody
88	*Kalmia microphylla* (Hook.) A. Heller	*Kalmiamicrophylla*	Ericaceae	Dicot	Perennial	Native	Woody
89	*Lactuca canadensis* L.	*Lactuca*	Asteraceae	Dicot	Perennial	Native	Woody
90	*Larix laricina* (Du Roi) K.Koch	*Larix*	Pinaceae	Gymnospern	Perennial	Native	Woody
91	*Leucanthemum vulgare* (Vaill.) Lam.	*Leucanthemum*	Asteraceae	Dicot	Perennial	Introduced	Herbaceous
92	*Linaria vulgaris* Mill.	*Linaria*	Scrophulariaceae	Dicot	Perennial	Introduced	Herbaceous
93	*Linnaea borealis* L.	*Linnaea*	Caprifoliaceae	Dicot	Perennial	Native	Herbaceous
94	*Lonicera caerulea* L.	*Lonicera*	Caprifoliaceae	Dicot	Perennial	Native	Woody
95	*Lonicera canadensis* Bartram ex Marshall	*Lonicera*	Caprifoliaceae	Dicot	Perennial	Native	Woody

Table S1.3 continued

96	*Lonicera hirsuta* Eaton	*Lonicera*	Caprifoliaceae	Dicot	Perennial	Native	Herbaceous
97	*Lotus corniculatus* L.	*Lotus*	Fabaceae	Dicot	Perennial	Introduced	Herbaceous
98	*Lysimachia borealis* (Raf.) U.Manns & Anderb.	*Lysimachia*	bees	Herb	Perennial	Native	Herbaceous
99	*Lysimachia terrestris* (L.) Britton, Sterns & Poggenb.	*Lysimachia*	Primulaceae	Dicot	Perennial	Native	Herbaceous
100	*Maianthemum canadense* Desf.	*Maianthemum*	Liliaceae	Monocot	Perennial	Native	Herbaceous
101	*Maianthemum trifolium* (L.) Sloboda	*Maianthemum*	Liliaceae	Monocot	Perennial	Native	Herbaceous
102	*Matteuccia struthiopteris* (L.) Tod.	*Matteucciastruthiopteris*	Dryopteridaceae	Fern	Perennial	Native	Herbaceous
103	*Melilotus albus* Medik.	*Melilotus*	Fabaceae	Dicot	Perennial	Introduced	Herbaceous
104	*Mitella nuda* L.	*Mitella*	Saxifragaceae	Dicot	Perennial	Native	Herbaceous
105	*Moneses uniflora* A.Gray	*Moneses*	Pyrolaceae	Dicot	Perennial	Native	Herbaceous
106	*Oclemena nemoralis* (Aiton) Greene	*Oclemena*	Asteraceae	Dicot	Perennial	Native	Herbaceous
107	*Oenothera biennis* L.	*Oenothera*	Onagraceae	Dicot	Biennial	Native	Herbaceous
108	*Onoclea sensibilis* L.	*Onoclea*	Dryopteridaceae	Fern	Perennial	Native	Herbaceous
109	*Oxalis acetosella* L.	*Oxalis*	Oxalidaceae	Dicot	Perennial	Native	Herbaceous
110	*Petasites frigidus* (L.) Fr.	*Petasites*	Asteraceae	Dicot	Perennial	Native	Herbaceous
111	*Phegopteris connectilis* (Michx.) Watt	*Phegopteris*	Thelypteridaceae	Fern	Perennial	Native	Herbaceous

Table S1.3 continued

112	*Picea glauca* (Moench) Voss	*Picea*	Pinaceae	Gymnosperm	Perennial	Native	Woody
113	*Picea mariana* (Mill.) Britton, Sterns & Poggenb.	*Picea*	Pinaceae	Gymnosperm	Perennial	Native	Woody
114	*Pilosella aurantiaca* (L.) F.W.Schultz & Sch.Bip.	*Pilosella*	Asteraceae	Dicot	Perennial	Introduced	Herbaceous
115	*Pilosella caespitosa* (Dumort.) P.D.Sell & C.West	*Pilosella*	Asteraceae	Dicot	Perennial	Introduced	Herbaceous
116	*Pinus banksiana* Lamb.	*Pinus*	Pinaceae	Gymnosperm	Perennial	Native	Woody
117	*Poa compressa* L.	*Poa*	Poaceae	Monocot	Perennial	Introduced	Herbaceous
118	*Populus balsamifera* L.	*Populus*	Salicaceae	Dicot	Perennial	Native	Woody
119	*Populus deltoides* Marshall	*Populus*	Salicaceae	Dicot	Perennial	Native	Woody
120	*Populus tremuloides* Michx.	*Populus*	Salicaceae	Dicot	Perennial	Native	Woody
121	*Potentilla norvegica* L.	*Potentilla*	Rosaceae	Dicot	Perennial	Native	Herbaceous
122	*Prunus pensylvanica* L.f.	*Prunus*	Rosaceae	Dicot	Perennial	Native	Woody
123	*Prunus virginiana* L.	*Prunus*	Rosaceae	Dicot	Perennial	Native	Woody
124	*Pteridium aquilinum* (L.) Kuhn	*Pteridium*	Dennstaedtiaceae	Fern	Perennial	Native	Herbaceous
125	*Pyrola asarifolia* Michx.	*Pyrola*	Pyrolaceae	Dicot	Perennial	Native	Woody
126	*Rhamnus alnifolia* L'Hér.	*Rhamnus*	Rhamnaceae	Dicot	Perennial	Native	Woody
127	*Rhododendron canadense* (L.) Torr.	*Rhododendron*	Ericaceae	Dicot	Perennial	Native	Woody
128	*Rhododendron groenlandicum* (Oeder) Kron & Judd	*Rhododendron*	Ericaceae	Dicot	Perennial	Native	Woody

Table S1.3 continued

129	*Ribes hirtellum* Michx.	*Ribes*	Grossulariaceae	Dicot	Perennial	Native	Woody
130	*Ribes hudsonianum* Richardson	*Ribes*	Grossulariaceae	Dicot	Perennial	Native	Woody
131	*Ribes lacustre* (Pers.) Poir.	*Ribes*	Grossulariaceae	Dicot	Perennial	Native	Woody
132	*Ribes ruizii* Rehder	*Ribes*	Grossulariaceae	Dicot	Perennial	Native	Woody
133	*Ribes triste* Pall.	*Ribes*	Grossulariaceae	Dicot	Perennial	Native	Woody
134	*Rosa acicularis* Lindl.	*Rosa*	Rosaceae	Dicot	Perennial	Native	Woody
135	*Rosa blanda* Aiton	*Rosa*	Rosaceae	Dicot	Perennial	Native	Woody
136	*Rubus chamaemorus* L.	*Rubus*	Rosaceae	Dicot	Perennial	Native	Herbaceous
137	*Rubus idaeus* L.	*Rubus*	Rosaceae	Dicot	Perennial	Native	Woody
138	*Salix balsamifera* Barratt ex Andersson	*Salix*	Salicaceae	Dicot	Perennial	Native	Woody
139	*Salix bebbiana* Sarg.	*Salix*	Salicaceae	Dicot	Perennial	Native	Woody
140	*Sambucus racemosa* L.	*Sambucus*	Caprifoliaceae	Dicot	Perennial	Native	Woody
141	*Sarracenia purpurea* L.	*Sarracenia*	Sarraceniaceae	Dicot	Perennial	Native	Herbaceous
142	*Scheuchzeria palustris* L.	*Scheuchzeria*	Scheuchzeriaceae	Dicot	Perennial	Native	Herbaceous
143	*Scirpus microcarpus* J.Presl & C.Presl	*Scirpus*	Cyperaceae	Monocot	Perennial	Native	Herbaceous
144	*Scirpus pendulus* Muhl.	*Scirpus*	Cyperaceae	Monocot	Perennial	Native	Herbaceous
145	*Scutellaria galericulata* L.	*Scutellaria*	Lamiaceae	Dicot	Perennial	Native	Herbaceous

Table S1.3 continued

146	*Solidago brendae* Semple.	*Solidago*	Asteraceae	Dicot	Perennial	Native	Herbaceous
147	*Solidago canadensis* L.	*Solidago*	Asteraceae	Dicot	Perennial	Native	Herbaceous
148	*Solidago rugosa* Mill.	*Solidago*	Asteraceae	Dicot	Perennial	Native	Herbaceous
149	*Solidago uliginosa* Nutt.	*Solidago*	Asteraceae	Dicot	Perennial	Native	Herbaceous
150	*Sonchus oleraceus* (L.) L.	*Sonchus*	Asteraceae	Dicot	Annual	Introduced	Herbaceous
151	*Sorbus americana* Marshall	*Sorbus*	Rosaceae	Dicot	Perennial	Native	Woody
152	*Sparganium americanum* Nutt.	*Sparganium*	Typhaceae	Monocot	Perennial	Native	Herbaceous
153	*Spinulum annotinum* (L.) A. Haines	*Spinulum*	Lycopodiaceae	Lycopod	Perennial	Native	Herbaceous
154	*Spiranthes romanzoffiana* Cham.	*Spiranthes*	Orchidaceae	Dicot	Perennial	Native	Herbaceous
155	*Symphyotrichum puniceum* (L.) Á.Löve & D.Löve	*Symphyotrichum*	Asteraceae	Dicot	Perennial	Native	Herbaceous
156	*Symphyotrichum ciliolatum* (Lindl.) Á.Löve & D.Löve	*Symphyotrichum*	Asteraceae	Dicot	Perennial	Native	Herbaceous
157	*Taraxacum officinale* (L.) Weber ex F.H.Wigg.	*Taraxacum*	Asteraceae	Dicot	Perennial	Native	Herbaceous
158	*Taxus canadensis* Marshall	*Taxus*	Taxaceae	Gymnosperm	Perennial	Native	Woody
159	*Thalictrum pubescens* Pursh	*Thalictrum*	Ranunculaceae	Dicot	Perennial	Native	Herbaceous
160	*Thuja occidentalis* L.	*Thuja*	Cupressaceae	Gymnosperm	Perennial	Native	Woody
161	*Trifolium pratense* L.	*Trifolium*	Fabaceae	Dicot	Perennial	Introduced	Herbaceous

Table S1.3 continued

162	*Trillium grandiflorum* (Michx.) Salisb.	*Trillium*	Liliaceae	Monocot	Perennial	Native	Herbaceous
163	*Tussilago farfara* L.	*Tussilago*	Asteraceae	Dicot	Perennial	Introduced	Herbaceous
164	*Typha latifolia* L.	*Typha*	Typhaceae	Monocot	Perennial	Native	Herbaceous
165	*Vaccinium angustifolium* Aiton	*Vaccinium*	Ericaceae	Dicot	Perennial	Native	Woody
166	*Vaccinium myrtilloides* Michx.	*Vaccinium*	Ericaceae	Dicot	Perennial	Native	Woody
167	*Vaccinium oxycoccos* L.	*Vaccinium*	Ericaceae	Dicot	Perennial	Native	Woody
168	*Viburnum lentago* L.	*Viburnum*	Caprifoliaceae	Dicot	Perennial	Native	Woody
169	*Viburnum nudum* L.	*Viburnum*	Caprifoliaceae	Dicot	Perennial	Native	Woody
170	*Vicia cracca* L.	*Vicia*	Fabaceae	Dicot	Perennial	Introduced	Herbaceous
171	*Viola macloskeyi* F.E. Lloyd	*Viola*	Violaceae	Dicot	Perennial	Native	Herbaceous
172	*Viola rostrata* Pursh	*Viola*	Violaceae	Dicot	Perennial	Native	Herbaceous

Table S1.4 The list of environmental and stand structure variables (continuous variables with minimum, maximum, and average values; categorical variables with count number).

Variables/Treatment	Variable Type	Unite	Range
Distance from mine edge	Continuous	m	0, 20, 50, 100, 200, 500, 1000 m (N=44 for each distance)
Live crown ratio for shrubs [1]	Continuous	%	Min=0; Max=100; Average=21.54
Live crown ratio for trees [1]	Continuous	%	Min=0; Max=100; Average=24.14
Base areas for shrubs	Continuous	m^2/ha	Min=0; Max=7.084746; Average=1.27
Base areas for trees	Continuous	m^2/ha	Min=0; Max=58; Average=14.34
Canopy openness [2]	Categorical	-	Min=1; Max=4; Average=1.13; 4 levels *
Leave litter ground cover	Continuous	%	Min=0; Max=100; Average=34.60
Woody debris ground cover	Continuous	%	Min=0; Max=92.67; Average=14.43
Water ground cover	Continuous	%	Min=0; Max=24.33; Average=2.60
Rock ground cover	Continuous	%	Min=0; Max=88; Average=7.17
Organic matter ground cover	Continuous	%	Min=0; Max=70.33; Average=5.44
Depth of organic soil	Continuous	cm	Min=0; Max=128.333; Average=30.92
Conifer proportion [3]	Continuous	%	Min=0; Max=100; Average=44.30
Humidity	Continuous	-	Min=1; Max=10; Average=2.58
Bryophyte cover	Continuous	%	Min=0; Max=100; Average=34.83
Lichen cover	Continuous	%	Min=0; Max=78.33; Average=8.08
Ecosystem types	Categorical	-	Coniferous (N=173), Mixed (N=38), Deciduous (N=48), Open Canopy (N=110)
Mining stages	Categorical	-	Operating (N=168), Non-operating (N=140), Controls (N=61)

Notes: 1. Live crown ratio (%) was calculated as (H-HCB)/H, where H is the total height of the tree/shrub, and HCB is the height from crown top to base (Kuprevicius, Auty et al. 2013). 2. Canopy openness was measured with a spherical densiometer at 1 m height above ground and recorded as 1-4 levels (L1, 0 % (0 grid); L2, 1% - 40 % (1 - 9.6 grids); L3, 40 % - 70 % (9.7 - 16.8 grids); L4: > 70% (>16.8 grids). 3. Conifer proportion (%) was calculated by the number of individuals of coniferous trees in each plot.

Table S1.5 Comparison and differences in measured forest environmental and stand structure variables at different distance along transects (mean (SD)).

	0 m N=44	20 m N=44	50 m N=44	100 m N=44	200 m N=44	500 m N=44	1000 m N=44	Controls N=61	chi-squared	p-value
Soil humidity	5.227(3.124)	4.752(3.152)	4.917(2.801)	4.5(2.429)	4.345(2.292)	4.235(2.025)	4.322(2.403)	4.454(2.323)	2.518	0.926
Live crown ratio for shrubs	38.824 (17.744)	42.385 (19.591)	45.211 (21.197)	43.787 (20.909)	44.189 (24.894)	49.132 (23.896)	43.558 (20.764)	44.031 (22.458)	4.102	0.768
Base areas for shrubs	1.868 (1.45)	2.016 (1.509)	1.815 (1.026)	1.687 (1.236)	1.746 (1.289)	1.619 (1.423)	1.497 (1.177)	1.317 (1.002)	10.666	0.154
Live crown ratio for trees	29.648 (26.468)	31.876 (28.039)	27.893 (22.837)	30.45 (23.771)	27.869 (25.679)	23.498 (22.214)	29.247 (22.444)	26.961 (22.563)	3.006	0.885
Base areas for trees	11.636 (12.592)	11.773 (12.819)	15.473 (15.14)	16.209 (16.335)	15.302 (16.914)	12.338 (14.899)	14.697 (15.387)	12.689 (11.248)	3.799	0.803
Depth of organic soil	27.455 (25.116)	37.944 (33.116)	37.032 (28.279)	39.109 (32.347)	39.051 (31.301)	36.465 (31.706)	36.92 (32.281)	46.97 (31.251)	12.840	0.076
Leave litter ground cover	53.83 (34.017)	62.242 (33.076)	51.318 (30.767)	46.432 (31.485)	48.389 (33.662)	45.909 (36.674)	47.788 (35.97)	41.216 (37.617)	13.305	0.065
Rock ground cover	3.22 (13.553)	0.587 (3.669)	2.617 (8.866)	1.182 (5.812)	0.023 (0.151)	2.182 (9.742)	0.773 (4.775)	0.17 (1.062)	19.610	**0.006**
Water ground cover	0.833 (2.936)	0.417 (1.802)	0.561 (3.668)	0.811 (3.235)	0.235 (1.558)	0.106 (0.537)	0.235 (1.07)	0.992 (3.536)	7.485	0.380
Woody debris ground cover	17.799 (18.719)	16.836 (12.862)	18.654 (15.126)	17.773 (16.277)	14.183 (11.22)	15.623 (16.448)	14.872 (14.429)	15.179 (10.321)	4.935	0.668
Organic matter ground cover	1.595 (3.952)	0.288 (0.852)	1.547 (4.041)	1.102 (3.531)	0.492 (1.729)	2.386 (8.372)	2.318 (11.238)	0.563 (1.727)	5.291	0.625
Conifer proportion	43.612 (42.937)	48.438 (45.266)	54.454 (43.808)	62.561 (44.798)	57.841 (44.687)	41.635 (43.498)	49.795 (44.67)	51.78 (44.181)	6.969	0.432

Notes: ANOVA analysis with significant at $P < 0.05$.

Table S1.6 The list of understory diversity indexes (continuous variables with minimum, maximum and average values; Categorical variables with count number).

Responses	Variable Type	Unite	Range
All vascular plant richness	Count	-	Min=2; Max=26; Average=3.89
Woody plant richness	Count	-	Min=0; Max=13; Average=2.43
Herb plant richness	Count	-	Min=0; Max=14; Average=2.93
Shannon	Continuous	-	Min=0.03; Max=2.62; Average=0.46
Simpson	Continuous	-	Min=0.03; Max=2.61; Average=1.67
All vascular plant cover	Continuous	%	Min=3.33; Max=171.33; Average=33.30
Woody plant cover	Continuous	%	Min=0; Max=121; Average=24.38
Herb plant cover	Continuous	%	Min=0; Max=127; Average=24.82
Bryophyte cover*	Continuous	%	Min=0; Max=100; Average=34.83
Lichen cover*	Continuous	%	Min=0; Max=78.33; Average=8.08

* The cover of bryophytes and lichens are both response and explanatory variables (for vascular plants)

Table S1.7 Results of mixed models for the plant richness and diversity indices testing for the effect of mine stage, ecosystem type and their interaction. Non-significant interaction terms were determined by Anova (Type=II) and deleted from the final models. Significant parameters (≤ 0.05) are indicated in bold.

	All vascular richness		Simpson		Shannon		Woody richness		Herbaceous richness	
	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*
(Intercept)	2.159	**<0.001**	-0.288	**<0.001**	0.518	**<0.001**	1.661	**<0.001**	1.230	**<0.001**
Mining stages (Reference: Controls)										
Operating	0.043	0.684	-0.027	0.215	-0.051	0.177	-0.133	0.493	0.181	0.254
Non-operating	0.013	0.904	-0.017	0.452	-0.018	0.648	0.060	0.756	-0.019	0.906
Ecosystem types (Reference: Coniferous)										
Deciduous	0.338	**<0.001**	0.033	0.165	0.092	**0.014**	0.023	0.725	0.755	**<0.001**
Mixed	0.277	**0.037**	0.038	0.168	0.098	**0.025**	-0.191	0.021	0.870	**<0.001**
Open Canopy	0.166	0.136	0.038	**0.033**	0.079	**0.006**	0.177	<0.001	0.009	0.966
Mining stages: Ecosystem types										
Deciduous: Operating	-0.375	**0.007**	-	-	-	-	-	-	-0.744	**0.001**
Deciduous: Non-operating	-0.038	0.791	-	-	-	-	-	-	-0.658	**0.008**
Mixed: Operating	-0.328	0.068	-	-	-	-	-	-	-0.543	**0.043**
Mixed: Non-operating	-0.062	0.692	-	-	-	-	-	-	-0.384	0.129
Open Canopy: Operating	-0.027	0.829	-	-	-	-	-	-	0.156	0.508
Open Canopy: Non-operating	0.072	0.584	-	-	-	-	-	-	0.215	0.383
Anova (Type=II)										
Factors	Chisq.	*P*	Chisq.	*P*	Chisq.	*P*	Chisq.	*P*	Chisq.	*P*
Mining stages	1.03	0.596	1.59	0.451	2.28	0.319	1.98	0.371	4.53	0.104
Ecosystem types	25.71	**<0.001**	5.79	0.122	12.72	**0.005**	22.70	**<0.001**	32.74	**<0.001**
Mining stages: Ecosystem types	12.67	**0.049**	5.05	0.538	5.09	0.533	5.93	0.431	18.80	**0.005**

Table S1.8 Results of mixed models on the plant cover testing for the effect of mining stages, ecosystem types and their interactions. Non-significant interaction terms were determined by Anova (Type=II) and deleted from the final models. Significant parameters (≤ 0.05) are indicated in bold.

	All vascular cover		Woody cover		Herbaceous cover		Bryophyte cover		Lichen cover	
	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*	Estimate	*p*
(Intercept)	-0.385	**<0.001**	0.408	**<0.001**	0.272	**<0.001**	0.642	**<0.001**	-2.802	**<0.001**
Mining stages (Reference: Controls)										
Operating	-0.067	0.582	-0.036	0.591	-0.034	0.611	-0.233	**0.016**	-0.052	0.851
Non-operating	-0.038	0.750	0.014	0.835	-0.021	0.754	-0.215	**0.027**	0.087	0.750
Ecosystem types (Reference: Coniferous)										
Deciduous	0.191	0.161	0.021	0.562	0.056	0.410	-0.508	**<0.001**	0.163	0.678
Mixed	0.353	**0.021**	-0.114	**0.005**	0.278	**0.021**	-0.288	**0.008**	0.164	0.809
Open Canopy	0.088	0.570	0.052	0.059	-0.002	0.978	-0.059	0.608	1.556	**0.006**
Mining stages: Ecosystem types										
Deciduous:	-0.356	**0.068**			-0.100	0.243	0.280	**0.013**	-0.806	0.184
Deciduous: Non-operating	0.047	0.791			0.102	0.259	0.264	**0.022**	-0.252	0.751
Mixed: Operating	-0.929	**0.002**			-0.394	**0.004**	0.152	0.257	-0.491	0.539
Mixed: Non-operating	-0.385	**0.050**			-0.287	**0.030**	0.215	0.091	-0.278	0.705
Open Canopy: Operating	-0.020	0.909			0.048	0.559	0.036	0.772	-1.827	**0.002**
Open Canopy: Non-operating	0.095	0.593			0.002	0.982	0.179	0.160	-1.534	**0.011**
Anova (Type=II)										
Factors	Chisq.	*P*	Chisq.	*P*	Chisq.	*P*	Chisq.	*P*	Chisq.	*P*
Mining stages	5.86	0.053	0.75	0.687	1.08	0.583	7.29	**0.026**	6.94	**0.031**
Ecosystem types	6.25	0.100	14.92	**0.002**	3.40	0.335	44.40	**<0.001**	2.70	0.439
Mining stages: Ecosystem types	16.13	**0.013**	1.812	0.936	17.44	**0.008**	18.21	**0.006**	12.90	**0.045**

Notes: in each case the interaction term was not significant and was dropped from the model.

Table S1.9 Influence of mining stage, ecosystem type, and their interaction on understory vascular community structure. Permutational multivariate analysis of variance (PERMANOVA) are used based on the Bray-Curtis dissimilarity index for all vascular, woody, and herbaceous plants.

Assemblage	Variable	Df	Mean square	*F* statictics	R^2	*P*
All vascular plants	Ecosystem types	3	3.142	8.588	0.065	**0.001**
	Mining Stages	2	1.219	3.331	0.017	**0.001**
	Ecosystem: Stages	6	0.545	1.490	0.022	**0.002**
	Residuals	357	0.366		0.896	
Woody plants	Ecosystem types	3	3.388	9.370	0.071	**0.001**
	Mining Stages	2	1.048	2.898	0.015	**0.001**
	Ecosystem: Stages	6	0.522	1.445	0.022	**0.007**
	Residuals	348	0.362		0.892	
Herbaceous plants	Ecosystem types	3	2.228	5.714	0.046	**0.001**
	Mining Stages	2	1.642	4.209	0.023	**0.001**
	Ecosystem: Stages	6	0.606	1.555	0.025	**0.002**
	Residuals	331	0.390		0.906	

Table S1.10. PERMANOVA post hoc pair-wise comparisons of mine stages on understory structure. Community structure of woody (A), herbaceous (B) plants was sued in each ecosystem type based on 999 permutations with Benjamini-Hochberg adjustemt (BH).

A) Woody plants

Pairs	Df	Sums of squares	F.Model	R^2	*p*	*p.adj*
Coniferous forest						
Operating vs Nonoperating	1	0.74	2.01	0.01	0.018	**0.026**
Operating vs Controls	1	0.82	2.32	0.02	0.015	**0.023**
Nonoperating vs Controls	1	0.64	1.73	0.02	0.045	0.057
Mixed forest						
Operating vs Nonoperating	1	0.40	1.09	0.04	0.328	0.328
Operating vs Controls	1	0.98	2.57	0.13	0.004	**0.007**
Nonoperating vs Controls	1	0.70	1.81	0.07	0.031	**0.044**
Deciduous forest						
Operating vs Nonoperating	1	0.71	1.71	0.05	0.041	0.054
Operating vs Controls	1	0.68	1.65	0.05	0.053	0.065
Nonoperating vs Controls	1	0.74	1.90	0.07	0.042	0.054
Open canopy ecosystem						
Operating vs Nonoperating	1	0.49	1.42	0.02	0.145	0.154
Operating vs Controls	1	0.59	1.70	0.02	0.069	0.083
Nonoperating vs Controls	1	0.47	1.57	0.03	0.106	0.121

(B) Herbaceous plants

Pairs	Df	Sums of squares	F.Model	R^2	*p*	*p.adj*
Coniferous forest						
Operating vs Nonoperating	1	0.99	2.61	0.02	0.004	**0.006**
Operating vs Controls	1	0.39	0.98	0.01	0.443	0.443
Nonoperating vs Controls	1	0.41	1.11	0.01	0.306	0.311
Mixed forest						
Operating vs Nonoperating	1	0.63	1.82	0.07	0.044	0.055
Operating vs Controls	1	0.52	1.44	0.09	0.132	0.145
Nonoperating vs Controls	1	0.48	1.33	0.06	0.179	0.194
Deciduous forest						
Operating vs Nonoperating	1	1.01	2.56	0.08	0.008	**0.012**
Operating vs Controls	1	0.46	1.18	0.04	0.270	0.278
Nonoperating vs Controls	1	1.10	3.15	0.11	0.003	**0.005**
Open canopy ecosystem						
Operating vs Nonoperating	1	1.65	3.91	0.04	0.001	**0.002**
Operating vs Controls	1	1.29	3.00	0.04	0.001	**0.002**
Nonoperating vs Controls	1	1.18	3.09	0.06	0.001	**0.002**

Table S1.11 Results of mixed models on the understory diversity testing for the effect of distance from mine sites as well as its interaction with mining stages. Non-significant interaction terms were deleted from the final model. Significant parameters (≤ 0.05) are indicated in bold.

	(Intercept)		Distance		Distance: Mining stage (Operating)	
	Estimate	*P*	Estimate	*p*	Estimate	*p*
All vascular richness	2.222	<0.001	0.322	<0.001	-0.349	0.001
Simpson	-0.346	<0.001	0.082	0.025	-	-
Shannon	0.487	<0.001	0.105	0.012	-	-
Woody richness	1.708	<0.001	0.168	0.006	-	-
Herbaceous richness	1.367	<0.001	0.381	0.001	-0.506	0.001
Bryophyte cover	0.347	<0.001	0.137	0.001	-	-
Lichen cover	-2.878	<0.001	0.167	0.346	-	-
All vascular cover	-0.421	<0.001	0.101	0.168	-	-
Woody cover	0.421	<0.001	0.036	0.356	-	-
Herbaceous cover	0.273	<0.001	0.036	0.319	-	-

Notes: Non-operating sites as references for mining stages.

Table S1.12 Disturbed areas by mines including directed mined areas and estimated offsite disturbance areas (ha).

	Akasaba	Canadian Malartic	Casa Berardi	Lapa	LaRonde	Joutel	Total	Average (precentage)
Mining Stage	Non-operating	Operating	Operating	Non-operating	Operating	Non-operating		
Directed disturbance areas	103.90	2046.37	449.39	13.06	552.59	229.31	3394.61	565.77 (67.18%)
Offsite disturbance areas (0.2 km	86.10	473.70	536.79	52.59	345.28	163.87	1658.32	276.39 (32.82%)
Total disturbance areas	189.99	2520.07	986.18	65.64	897.87	393.18	5052.94	842.16

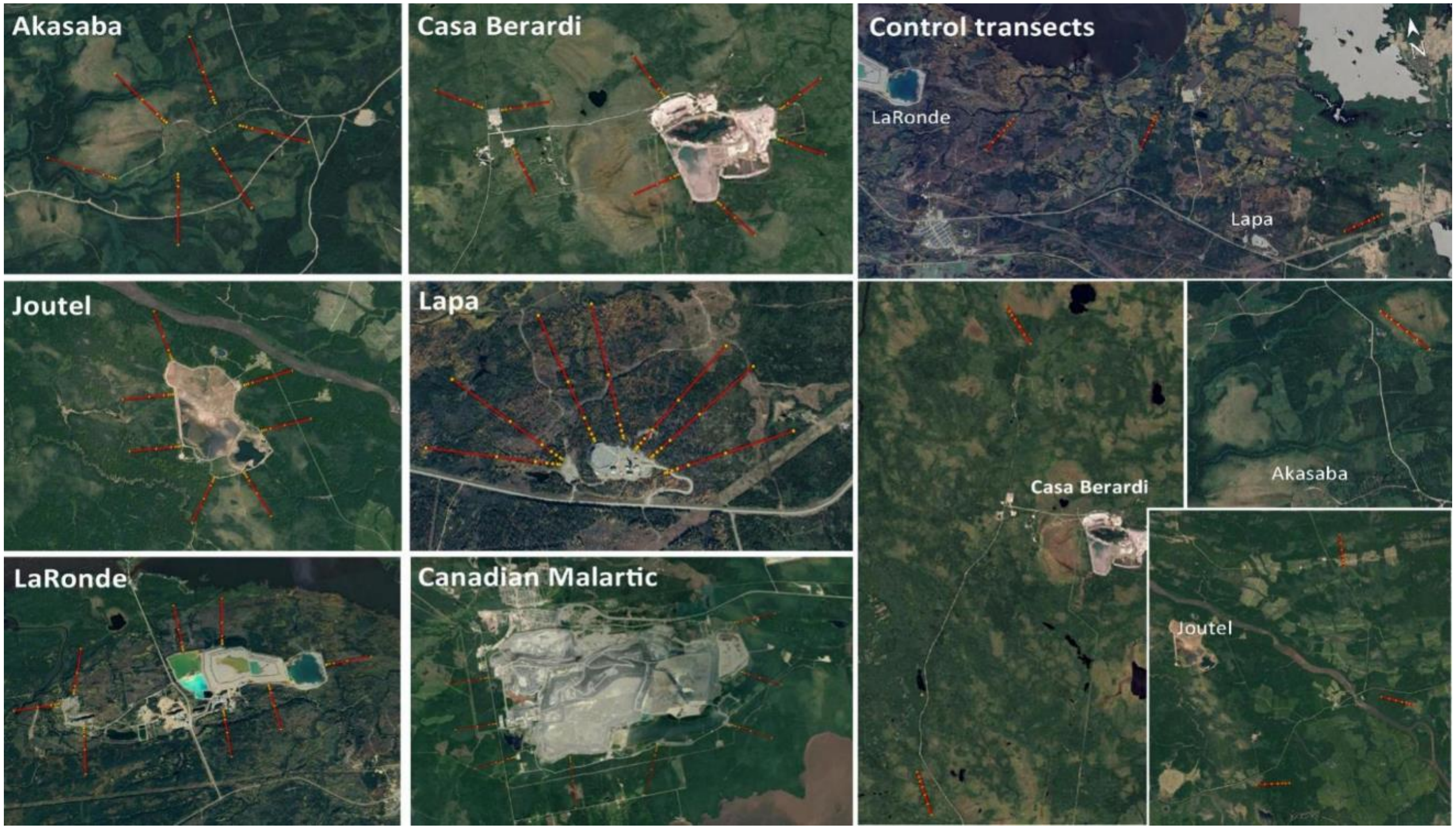


Figure S1.1 The maps of six selected mine sites with the position of transects. Scale was not present because the length of transect is 1 km which could be as references to the maps.

APPENDICE B

MATÉRIEL SUPPLÉMENTAIRE DU CHAPITRE III

Table S2.1 Description of the sampling design. Only the number of plots of each ecosystem type encountered at different distance along transect was included.

Distance (m)	Coniferous	Deciduous	Mixed	Open Canopy	Sum
0	20	9	2	13	44
20	19	7	2	14	42
50	20	4	7	12	43
100	27	2	4	11	44
200	24	1	5	13	43
500	15	4	6	18	43
1000	21	6	4	13	44
Controls	27	14	7	13	61
Sum	173	47	37	107	Total: 364

Table S2.2 Criteria used to classify the six microhabitats investigated for bryophytes

Microhabitat	Criteria
Ground	Forest floor not occupied by other microhabitats
Tree (trunk)	DBH ≥ 8 cm
Logs	DBH ≥ 8 cm and length ≥ 1 m
Stump	Diameter ≥ 8 m (measured at top) and height < 1.5 m
Snags	DBH ≥ 8 cm and height ≥ 1.5 m
Rocks	Shortest diameter ≥ 20 cm (measured at top)

Table S2.3 Species list in all sampled plots in all studied sites

ID	Species	Abbreviation	Group	Genus
1	*Amblystegium serpens* (Hedw.) Schimp.	Amb.serp	Moss	*Amblystegium*
2	*Anastrophyllum hellerianum* (Nees ex Lindenb.) R.M. Schust.	Ana.hell	Moss	*Anastrophyllum*
3	*Anastrophyllum michauxii* (F. Weber) H. Buch ex A. Evans	Ana.mich	Moss	*Anastrophyllum*
4	*Aneura pinguis* (L.) Dumort.	Ane.ping	Liverwort	*Aneura*
5	*Atrichum angustatum* (Brid.) Bruch & Schimp.	Atr.angu	Moss	*Atrichum*
6	*Aulacomnium palustre* (Hedw.) Schwägr.	Aul.palu	Moss	*Aulacomnium*
7	*Barbilophozia attenuate* (Nees) Loeske	Bar.atte	Liverwort	*Barbilophozia*
8	*Barbilophozia barbata* (Schreb.) Loeske	Bar.barb	Liverwort	*Barbilophozia*
9	*Barbilophozia kunzeana* (Huebener) K. Müller	Bar.kunz	Liverwort	*Barbilophozia*
10	*Bazzania denudata* (Torr. ex Gottsche, Lindenb. & Nees) Trevis.	Baz.denu	Liverwort	*Bazzania*
11	*Bazzania trilobata* (L.) Gray	Baz.tril	Liverwort	*Bazzania*
12	*Blepharostoma trichophyllum* (L.) Dumort.	Ble.tric	Liverwort	*Blepharostoma*
13	*Brachythecium acutum* (Mitt.) Sull.	Bra.acut	Moss	*Brachythecium*
14	*Brachythecium albicans* (Hedw.) Schimp.	Bra.albi	Moss	*Brachythecium*
15	*Brachythecium campestre* (Müll. Hal.) Schimp.	Bra.camp	Moss	*Brachythecium*
16	*Brachythecium curtum* (Lindb.) Limpr.	Bra.oedi	Moss	*Brachythecium*
17	*Brachythecium erythrorrhizon* Schimp.	Bra.eryt	Moss	*Brachythecium*
18	*Brachythecium falcatum* (Grout) H.A. Crum	Bra.falc	Moss	*Brachythecium*
19	*Brachythecium populeum* (Hedw.) Schimp.	Bra.popu	Moss	*Brachythecium*
20	*Brachythecium reflexum* (Starke) Schimp.	Bra.refl	Moss	*Brachythecium*
21	*Brachythecium rivulare* Schimp.	Bra.rivu	Moss	*Brachythecium*
22	*Brachythecium rotaeanum* De Not.	Bra.rota	Moss	*Brachythecium*

Table S2.3 continued

23	*Brachythecium rutabulum* (Hedw.) Schimp.	Bra.ruta	Moss	*Brachythecium*
24	*Brachythecium starkei* (Brid.) Schimp.	Bra.star	Moss	*Brachythecium*
25	*Brachythecium velutinum* (Hedw.) Schimp.	Bra.velu	Moss	*Brachythecium*
26	*Breidleria pratensis* (Koch ex Spruce) Loeske	Hyp.prat	Moss	*Breidleria*
27	*Bryum creberrimum* Taylor	Bry.creb	Moss	*Bryum*
28	*Bryhnia novae-angliae* (Sull. & Lesq.) Grout	Bry.nova	Moss	*Bryhnia*
29	*Bryum pseudotriquetrum* (Hedw.) P. Gaertn., B. Mey. & Scherb.	Bry.pseu	Moss	*Bryum*
30	*Bryoeruthrophyllum recurvirostrum* (Hedwig) P. C. Chen	Bry.recu	Moss	*Bryoeruthrophyllum*
31	*Calliergon cordifolium* (Hedw.) Kindb.	Cal.cord	Moss	*Calliergon*
32	*Calypogeia fissa* (L.) Raddi	Cal.fiss	Liverwort	*Calypogeia*
33	*Callicladium haldanianum* (Grev.) H.A. Crum	Cal.hald	Moss	*Callicladium*
34	*Calypogeia integristipula* Stephani	Cal.inte	Liverwort	*Calypogeia*
35	*Calypogeia muelleriana* (Schiffner) K. Müller	Cal.muel	Liverwort	*Calypogeia*
36	*Calypogeia neesiana* (C. Massal. & Carestia) K. Müller	Cal.nees	Liverwort	*Calypogeia*
37	*Calliergon richardsonii* (Mitt.) Kindb.	Cal.rich	Liverwort	*Calliergon*
38	*Calypogeia sphagnicola* (Arnell & J. Perss.) Warnst. & Loeske	Cal.spha	Liverwort	*Calypogeia*
39	*Campyliadelphus chrysophyllus* (Brid.) R.S. Chopra	Cam.chry	Moss	*Campyliadelphus*
40	*Campylophyllum halleri* (Sw. ex Hedw.) M. Fleisch	Cam.hall	Moss	*Campylophyllum*
41	*Campylophyllum hispidulum* (Brid.) Hedenäs	Cam.hisp	Moss	*Campylophyllum*
42	*Campylium stellatum* (Hedw.) C.E.O. Jensen	Cam.stel	Moss	*Campylium*
43	*Cephalozia bicuspidata* (L.) Dumort.	Cep.bicu	Liverwort	*Cephalozia*
44	*Cephalozia catenulata* (Huebener) Lindb.	Cep.cate	Liverwort	*Cephalozia*
45	*Cephalozia connivens* (Dicks.) Lindb.	Cep.conn	Liverwort	*Cephalozia*

Table S2.3 continued

46	*Cephalozia elachistsa* (J.B. Jack ex Gottsche & Rabenh.) Schiffn.	Cep.elac	Liverwort	*Cephalozia*
47	*Cephalozia loitlesbergeri* Schiffner	Cep.loit	Liverwort	*Cephalozia*
48	*Cephalozia lunulifolia* (Dumort.) Dumort.	Cep.lunu	Liverwort	*Cephalozia*
49	*Cephalozia planiceps* (Aust.) Lindb.	Cep.plen	Liverwort	*Cephalozia*
50	*Cephaloziella rubella* (Nees) Warnst.	Cep.rube	Liverwort	*Cephaloziella*
51	*Ceratodon purpureus* (Hedw.) Brid.	Cer.purp	Moss	*Ceratodon*
52	*Chiloscyphus coadunatus* (Sw.) R.M. Schust. & J.J. Engel	Chi.coad	Liverwort	*Chiloscyphus*
53	*Chiloscyphus cuspidatus* (Nees) J.J. Engel & R.M. Schust.	Chi.cusp	Liverwort	*Chiloscyphus*
54	*Chiloscyphus profundus* (Nees) J.J. Engel & R.M. Schust.	Chi.prof	Liverwort	*Chiloscyphus*
55	*Cladopodiella fluitans* (Nees) Jörg.	Cla.flui	Liverwort	*Cladopodiella*
56	*Climacium dendroides* (Hedw.) F. Weber & D. Mohr	Cli.dend	Moss	*Climacium*
57	*Dicranum flagellare* Hedw.	Dic.flag	Moss	*Dicranum*
58	*Dicranum fulvum* Hook.	Dic.fulv	Moss	*Dicranum*
59	*Dicranum fuscescens* Turner	Dic.fusc	Moss	*Dicranum*
60	*Dicranella heteromalla* (Hedw.) Schimp.	Dic.hete	Moss	*Dicranella*
61	*Dicranum leioneuron* Kindb.	Dic.leio	Moss	*Dicranum*
62	*Dicranum majus* Turner	Dic.maju	Moss	*Dicranum*
63	*Dicranum montanum* Hedw.	Dic.mont	Moss	*Dicranum*
64	*Dicranum ontariense* W.L. Peterson	Dic.onta	Moss	*Dicranum*
65	*Dichodontium pellucidum* (Hedw.) Schimp.	Dic.pell	Moss	*Dichodontium*
66	*Dicranum polysetum* Sw.	Dic.poly	Moss	*Dicranum*
67	*Dicranum scoparium* Hedw.	Dic.scop	Moss	*Dicranum*
68	*Dicranum spurium* Hedw.	Dic.spur	Moss	*Dicranum*
69	*Dicranum undulatum* Schrad. ex Brid.	Dic.undu	Moss	*Dicranum*

Table S2.3 continued

70	*Distichium capillaceum* (Hedwig) Bruch & Schimper	Dis.capi	Moss	*Distichium*
71	*Drepanocladus aduncus* (Hedw.) Warnst.	Dre.adun	Moss	*Drepanocladus*
72	*Eurhynchium pulchellum* (Hedw.) Jenn.	Eur.pulc	Moss	*Eurhynchium*
73	*Fissidens osmundoides* Hedw.	Fis.osmu	Moss	*Fissidens*
74	*Frullania oakesiana* Austin	Fru.oake	Liverwort	*Frullania*
75	*Geocalyx graveolens* (Schrad.) Nees	Geo.grav	Liverwort	*Geocalyx*
76	*Gymnocolea inflata* (Huds.) Dumort.	Gym.infl	Liverwort	*Gymnocolea*
77	*Herzogiella striatella* (Brid.) Z. Iwats.	Her.stri	Moss	*Herzogiella*
78	*Herzogiella turfacea* (Lindb.) Z. Iwats.	Her.turf	Moss	*Herzogiella*
79	*Homalia trichomanoides* (Hedw.) Schimp.	Hom.tric	Moss	*Homalia*
80	*Hygroamblystegium varium* (Hedwig) Mönkemeyer	Hyg.vari	Moss	*Hygroamblystegium*
81	*Hylocomium brevirostre* (Brid.) Schimp.	Hyl.brev	Moss	*Hylocomium*
82	*Hylocomium splendens* (Hedw.) Schimp.	Hyl.sple	Moss	*Hylocomium*
83	*Hypnum cupressiforme* Hedw.	Hyp.cupr	Moss	*Hypnum*
84	*Hypnum curvifolium* Hedw.	Hyp.curv	Moss	*Hypnum*
85	*Hypnum fauriei* Cardot	Hyp.faur	Moss	*Hypnum*
86	*Hypnum imponens* Hedw.	Hyp.impo	Moss	*Hypnum*
87	*Hypnum lindbergii* Mitt.	Cal.lind	Moss	*Hypnum*
88	*Hypnum pallescens* (Hedw.) P. Beauv.	Hyp.pall	Moss	*Hypnum*
89	*Jamesoniella autumnalis* (DC.) Stephani	Jam.autu	Liverwort	*Jamesoniella*
90	*Jungermania crenuliformis* Austin	Jun.cren	Liverwort	*Jungermania*
91	*Jungermannia leiantha* Grolle	Jun.leia	Liverwort	*Jungermannia*
92	*Kurzia pauciflora* (Dicks.) Grolle	Kur.pauc	Liverwort	*Kurzia*
93	*Leiomylia anomala* (Hook.) J.J. Engel & Braggins	Myl.anom	Liverwort	*Leiomylia*

Table S2.3 continued

94	*Lepidozia reptans* (L.) Dumort.	Lep.rept	Liverwort	*Lepidozia*
95	*Leptodictyum riparium* (Hedw.) Warnst.	Lep.ripa	Moss	*Leptodictyum*
96	*Lophozia longidens* (Lindb.) Macoun	Lop.long	Liverwort	*Lophozia*
97	*Lophozia sudetica* (Nees ex Huebener) Grolle	Lop.sude	Liverwort	*Lophozia*
98	*Lophozia ventricosa* (Dicks.) Dumort.	Lop.vent	Liverwort	*Lophozia*
99	*Marchantia polymorpha* L.	Mar.poly	Liverwort	*Marchantia*
100	*Mnium spinulosum* Bruch & Schimp.	Mni.spin	Moss	*Mnium*
101	*Mnium marginatum* (Dicks. ex With.) P. Beauv.	Mni.marg	Moss	*Mnium*
102	*Mylia taylorii* (Hook.) Gray	Myl.tayl	Liverwort	*Mylia*
103	*Nowellia Curvifolia* (Dicks.) Mitt.	Now.curv	Liverwort	*Nowellia*
104	*Orthotrichum anomalum* Hedw.	Ort.anom	Moss	*Orthotrichum*
105	*Orthotrichum elegans* Schwägr. ex Hook. & Grev.	Ort.eleg	Moss	*Orthotrichum*
106	*Orthotrichum obtusifolium* Brid.	Ort.obtu	Moss	*Orthotrichum*
107	*Orthotrichum ohioense* Sull. & Lesq.	Ort.ohio	Moss	*Orthotrichum*
108	*Orthotrichum speciosum* Nees	Ort.spec	Moss	*Orthotrichum*
109	*Orthotrichum stellatum* Brid.	Ort.stel	Moss	*Orthotrichum*
110	*Palustriella falcata* (Brid.) Hedenäs	Pal.falc	Moss	*Palustriella*
111	*Pallavicinia lyellii* (Hook.) Gray	Pal.lyel	Liverwort	*Pallavicinia*
112	*Pellia epiphylla* (Dicks.) Dumort.	Pel.epip	Liverwort	*Pellia*
113	*Plagiothecium cavifolium* (Brid.) Z. Iwats.	Pla.cavi	Moss	*Plagiothecium*
114	*Platydictya confervoides* (Brid.) H.A. Crum	Pla.conf	Moss	*Platydictya*
115	*Plagiomnium cuspidatum* (Hedw.) T.J. Kop.	Pla.cusp	Moss	*Plagiomnium*
116	*Plagiothecium denticulatum* (Hedw.) Schimp.	Pla.dent	Moss	*Plagiothecium*

Table S2.3 continued

117	*Plagiomnium drummondii* (Bruch & Schimp.) T.J. Kop.	Pla.drum	Moss	*Plagiomnium*
118	*Plagiomnium ellipticum* (Brid.) T.J. Kop.	Pla.elli	Moss	*Plagiomnium*
119	*Plagiothecium laetum* Schimp.	Pla.laet	Moss	*Plagiothecium*
120	*Plagiothecium latebricola* Schimp.	Pla.late	Moss	*Plagiothecium*
121	*Plagiomnium medium* (Bruch & Schimp.) T.J. Kop.	Pla.medi	Moss	*Plagiomnium*
122	*Platygyrium repens* (Brid.) Schimp.	Pla.repe	Moss	*Platygyrium*
123	*Platydictya subtilis* (Hedw.) H.A. Crum	Pla.subt	Moss	*Platydictya*
124	*Pleurozium schreberi* (Willd. ex Brid.) Mitt.	Ple.schr	Moss	*Pleurozium*
125	*Pohlia nutans* (Hedw.) Lindb.	Poh.nuta	Moss	*Pohlia*
126	*Pohlia wahlenbergii* (F. Weber & D. Mohr) A.L. Andrews	Poh.wahl	Moss	*Pohlia*
127	*Polytrichastrum alpinum* (Hedw.) G.L. Sm.	Pol.alpi	Moss	*Polytrichastrum*
128	*Polytrichum commune* Hedw.	Pol.comm	Moss	*Polytrichum*
129	*Polytrichum juniperinum* Hedw.	Pol.juni	Moss	*Polytrichum*
130	*Polytrichastrum longisetum* (Sw. ex Brid.) G.L. Sm.	Pol.long	Moss	*Polytrichastrum*
131	*Polytrichum ohioense* (Renauld & Cardot) G.L. Sm.	Pol.ohio	Moss	*Polytrichum*
132	*Polytrichum piliferum* Hedw.	Pol.pili	Moss	*Polytrichum*
133	*Polytrichum strictum* Menzies ex Brid.	Pol.stri	Moss	*Polytrichum*
134	*Pseudotaxiphyllum elegans* (Brid.) Z. Iwats.	Pse.eleg	Moss	*Pseudotaxiphyllum*
135	*Pseudoleskeella nervosa* (Brid.) Nyholm	Pse.nerv	Moss	*Pseudoleskeella*
136	*Pseudocampylium radicale* (P. Beauv.) Vanderp. & Hedenäs	Pse.radi	Moss	*Pseudocampylium*
137	*Ptilidium ciliare* (L.) Hampe	Pti.cili	Liverwort	*Ptilidium*
138	*Ptilium crista-castrensis* (Hedw.) De Not.	Pti.cris	Moss	*Ptilium*

Table S2.3 continued

139	*Ptilidium pulcherrimum* (Weber) Hampe	Pti.pulc	Liverwort	*Ptilidium*
140	*Pylaisia intricata* (Hedw.) Schimp.	Pyl.intr	Moss	*Pylaisia*
141	*Pylaisia polyantha* (Hedw.) Schimp.	Pyl.poly	Moss	*Pylaisia*
142	*Pylaisia selwynii* Kindb.	Pyl.selw	Moss	*Pylaisia*
143	*Racomitrium microcarpum* (Hedw.) Bednarek-Ochyra & Ochyra	Buc.micr	Moss	*Racomitrium*
144	*Radula complanata* (L.) Dumort.	Rad.comp	Liverwort	*Radula*
145	*Rhizomnium gracile* T.J. Kop.	Rhi.grac	Moss	*Rhizomnium*
146	*Rhizomnium magnifolium* (Horik.) T.J. Kop.	Rhi.magn	Moss	*Rhizomnium*
147	*Rhizomnium pseudopunctatum* (Bruch & Schimp.) T.J. Kop.	Rhi.pseu	Moss	*Rhizomnium*
148	*Rhizomnium punctatum* (Hedw.) T.J. Kop.	Rhi.punc	Moss	*Rhizomnium*
149	*Rhynchostegium serrulatum* (Hedw.) A. Jaeger	Rhy.serr	Moss	*Rhynchostegium*
150	*Rhytidiadelphus subpinnatus* (Lindb.) T.J. Kop.	Rhy.subp	Moss	*Rhytidiadelphus*
151	*Rhytidiadelphus triquetrus* (Hedw.) Warnst.	Rhy.triq	Moss	*Rhytidiadelphus*
152	*Riccardia latifrons* (Lindb.) Lindb.	Ric.lati	Liverwort	*Riccardia*
153	*Riccardia multifida* (L.) Gray	Ric.mult	Liverwort	*Riccardia*
154	*Riccardia vitrea* Furuki	Ric.vitr	Liverwort	*Riccardia*
155	*Sanionia uncinata* (Hedw.) Loeske	San.unci	Moss	*Sanionia*
156	*Scapania apiculata* Spruce	Sca.apic	Liverwort	*Scapania*
157	*Scapania irrigua* (Nees) Nees	Sca.irri	Liverwort	*Scapania*
158	*Scapania mucronata* H. Buch	Sca.mucr	Liverwort	*Scapania*
159	*Schistidium agassizii* Sull. & Lesq.	Sch.agas	Moss	*Schistidium*
160	*Schistochilopsis incisa* (Schrad.) Konstantinova	Sch.inci	Liverwort	*Schistochilopsis*

Table S2.3 continued

161	*Schistochilopsis laxa* (Lindb.) Konstantinova	Sch.laxa	Liverwort	*Schistochilopsis*
162	*Sphagnum angustifolium* (Warnst.) C.E.O. Jensen	Sph.angu	Moss	*Sphagnum*
163	*Sphagnum capillifolium* (Ehrh.) Hedw.	Sph.capi	Moss	*Sphagnum*
164	*Sphagnum centrale* C.E.O. Jensen	Sph.cent	Moss	*Sphagnum*
165	*Sphagnum cuspidatum* Ehrh. ex Hoffm.	Sph.cusp	Moss	*Sphagnum*
166	Sphagnum fallax H. Klinggr.	Sph.fall	Moss	Sphagnum
167	*Sphagnum fuscum* (Schimp.) H. Klinggr.	Sph.fusc	Moss	*Sphagnum*
168	*Sphagnum girgensohnii* Russow	Sph.girg	Moss	*Sphagnum*
169	*Sphagnum magellanicum* Brid.	Sph.mage	Moss	*Sphagnum*
170	*Sphagnum quinquefarium* (Lindb.) Warnst.	Sph.quin	Moss	*Sphagnum*
171	*Sphagnum rubellum* Wilson	Sph.rube	Moss	*Sphagnum*
172	*Sphagnum russowii Warnst.*	Sph.russ	Moss	Sphagnum
173	*Sphagnum squarrosum* Crome	Sph.squa	Moss	*Sphagnum*
174	*Sphagnum subfulvum* Sjors	Sph.subf	Moss	*Sphagnum*
175	*Sphagnum wulfianum* Girg.	Sph.wulf	Moss	*Sphagnum*
176	*Splachnum ampullaceum* Hedw.	Spl.ampu	Moss	*Splachnum*
177	*Tetraphis pellucida* Hedw.	Tet.pell	Moss	*Tetraphis*
178	*Thuidium delicatulum* (Hedw.) Schimp.	Thu.deli	Moss	*Thuidium*
179	*Thuidium recognitum* (Hedw.) Lindb.	Thu.reco	Moss	*Thuidium*
180	*Tomenthypnum falcifolium (Renauld ex Nichols) Tuom.*	Tom.falc	Moss	*Tomenthypnum*
181	*Tomenthypnum nitens* (Hedw.) Loeske	Tom.nite	Moss	*Tomenthypnum*
182	*Tortella humilis* (Hedw.) Jenn.	Tor.humi	Moss	*Tortella*

Table S2.3 continued

183	*Trematodon ambiguus* (Hedw.) Hornsch.	Tre.ambi	Moss	*Trematodon*
184	*Ulota coarctata* (P. Beauv.) Hammar	Ulo.coar	Moss	*Ulota*
185	*Ulota crispa* (Hedw.) Brid.	Ulo.cris	Moss	*Ulota*
186	*Warnstorfia fluitans* (Hedw.) Loeske	War.flui	Moss	*Warnstorfia*

Table S2.4 The list of environmental and stand structure variables as well as treatment (continuous variables with minimum, maximum, and average values ± standard deviation; categorical variables with count number).

NO.	Variables/Treatment	Variable Type	Unite	Range
Stand structure				
1	Live crown ratio for shrubs [1]	Continuous	%	Min=0; Max=100; Average=21.6 ± 43.8
2	Live crown ratio for trees [1]	Continuous	%	Min=0; Max=100; Average=24.2 ± 28.3
3	Base areas for shrubs	Continuous	m^2/ha	Min=0; Max=7.084746; Average=1.27 ± 1.68
4	Base areas for trees	Continuous	m^2/ha	Min=0; Max=58; Average=14.4 ± 13.7
5	Canopy openness [2]	Continuous	-	Min=1; Max=4; Average=1.12 ± 2.85
6	Conifer proportion [3]	Continuous	%	Min=0; Max=100; Average=44.3 ± 52.4
7	Depth of organic soil	Continuous	cm	Min=0; Max=128.33; Average=31 ± 38.4
The cover of ground substrates				
8	Leave litter ground cover	Continuous	%	Min=0; Max=100; Average=34.6 ± 49.2
9	Woody debris ground cover	Continuous	%	Min=0; Max=92.67; Average=13.4 ± 15.8
10	Water ground cover	Continuous	%	Min=0; Max=24.33; Average=2.60 ± 0.553
11	Rock ground cover	Continuous	%	Min=0; Max=88; Average=6.36 ± 1.07
12	Organic matter ground cover	Continuous	%	Min=0; Max=70.33; Average=5.48 ± 1.27
The number of microhabitats				
13	Number of trees per plot	Continuous	-	Min=0; Max=20; Average=3.29 ± 4.11
14	Number of logs per plot	Continuous	-	Min=0; Max=15; Average=8.13 ± 3.39
15	Number of stumps per plot	Continuous	-	Min=0; Max=78.33; Average=2.41 ± 2.02
16	Number of snags per plot	Continuous	-	Min=0; Max=5; Average=0.82 ± 0.44
17	Number of rocks per plot	Continuous	-	Min=0; Max=8; Average=0.854 ± 0.24

Notes: 1. Live crown ratio (%) was calculated as (H-HCB)/H, where H is the total height of the tree/shrub, and HCB is the height from crown top to base. 2. Canopy openness was measured with a spherical densiometer at 1 m height above ground and recorded as 1-4 levels (L1, 0 % (0 grid); L2, 1%-40 % (1-9.6 grids); L3, 40 %-70 % (9.7 - 16.8 grids); L4: > 70% (>16.8 grids). 3. Conifer proportion (%) was calculated by the number of individuals of coniferous trees in each plot.

Table S2.5 the variables grouped by mine sites (mean with standard deviation) with the results of Kruskal-Wallis Test.

Variables	Akasaba N=42	Casa Berardi N=56	Joutel N=49	Lapa N=49	LaRonde N=56	Canadian Malartic N=56	Controls N=61	chi-squared	p-value
Stand structure									
Live crown ratio for shrubs	52.125 (26.571)	49.911 (23.057)	49.876 (18.601)	38.482 (14.138)	45.632 (22.015)	30.428 (14.772)	44.031 (22.458)	45.631	<0.001
Live crown ratio for trees	23.682 (27.286)	20.447 (29.206)	31.502 (24.358)	37.322 (16.691)	29.399 (28.061)	29.673 (15.494)	26.961 (22.563)	21.281	0.001
Base areas for shrubs	1.545 (1.21)	1.448 (1.282)	2.303 (1.409)	1.639 (1.156)	1.611 (1.114)	1.896 (1.464)	1.317 (1.002)	18.174	0.006
Base areas for trees	5.984 (7.146)	3.13 (4.608)	19.959 (18.45)	22.98 (14.359)	12.264 (15.125)	18.75 (13.165)	12.689 (11.248)	84.59	<0.001
Canopy openness	2.29 (1.2)	2.16 (1.01)	2.96 (1.1)	3.36 (0.77)	3.09 (1.09)	3.34 (0.86)	2.77 (1.2)	52.648	<0.001
Conifer proportion	53.571 (49.869)	35.268 (47.14)	54.917 (42.572)	69.494 (33.382)	38.728 (43.466)	58.516 (41.006)	51.78 (44.181)	19.525	0.002
Depth of organic soil	52.472 (18.524)	73.406 (23.289)	36.194 (30.283)	12.088 (10.18)	14.944 (9.351)	30.024 (27.111)	47.336 (31.383)	165.01	<0.001
The cover of ground substrates									
Leave litter ground cover	25.278 (20.365)	21.598 (24.432)	51.204 (33.28)	68.687 (25.027)	77.723 (20.028)	56.458 (33.775)	41.216 (37.617)	111.92	<0.001
Woody debris ground cover	11.81 (10.362)	13.727 (14.112)	15.756 (11.233)	19.005 (18.964)	22.083 (20.09)	15.854 (10.286)	15.179 (10.321)	15.369	0.012
Water ground cover	0.079 (0.514)	0.476 (1.812)	0.918 (4.305)	0.211 (1.476)	0.238 (1.401)	0.75 (2.718)	0.992 (3.536)	8.8924	0.18
Rock ground cover	0 (0)	0.089 (0.668)	0.122 (0.604)	4.493 (12.313)	3.899 (13.613)	0.289 (1.232)	0.17 (1.062)	39.411	<0.001

Table S2.5 continued

Organic matter ground cover	1.063 (4.209)	1.193 (2.219)	3.063 (8.506)	0.765 (2.689)	0.274 (1.39)	2.03 (10.011)	0.563 (1.727)	19.345	0.004
The number of microhabitats									
Number of trees per plot	3.76(2.38)	3.95 (2.81)	3.84 (3.09)	3.09 (2.17)	3.38 (3.02)	6.82 (4.53)	3.64 (2.76)	29.981	<0.001
Number of logs per plot	1.29 (1.47)	0.71 (1.24)	1.9 (2.56)	2.31 (2.6)	2.45 (2.35)	2.77 (2.25)	2.54 (3.08)	37.718	<0.001
Number of stumps per plot	1.86 (1.42)	1.51 (1.77)	1.24 (1.36)	0.62 (0.83)	0.87 (1.11)	1.27 (1.45)	0.92 (1.14)	24.84	<0.001
Number of snags per plot	0.38 (0.62)	0.62 (1.01)	0.37 (0.7)	0.31 (0.7)	0.35 (0.87)	0.54 (0.83)	0.49 (0.89)	6.5313	0.366
Number of rocks per plot	0.05 (0.22)	0.07 (0.54)	0.02 (0.14)	0.31 (0.6)	0.67 (1.62)	0.34 (0.94)	0.16 (0.61)	26.949	<0.001

Table S2.6 Mean values of environmental variables near mine sites for each mining stages in four identified ecosystem types.

Variables	Coniferous			Deciduous			Mixed forest			Open Canopy		
	Operating	Non-operating	Controls	Operating	Non-operating	Controls	Operating	Non-operating	Controls	Operating	Non-operating	Controls
Stand structure												
Live crown ratio for shrubs	37.8 ± 2.5 a	43.5 ± 2.5 ab	40.2 ± 4.0 ab	36.3 ± 4.8 a	43.0 ± 5.8 ab	52.8 ± 5.6 ab	32.6 ± 6.1 a	41.2 ± 4.9 ab	41.7 ± 7.9 ab	50.4 ± 2.9 ab	57.2 ± 3.7 b	43.7 ± 5.8 ab
Live crown ratio for trees	41.4 ± 1.8 bc	44.2 ± 1.8 bc	37.4 ± 3.0 bc	48.9 ± 3.5 c	40.3 ± 4.3 bc	30.6 ± 4.1 b	31.8 ± 4.4 bc	32.4 ± 3.6 bc	29.4 ± 5.8 bc	0.0 ± 2.0 a	0.0 ± 2.7 a	0.0 ± 4.3 a
Base areas for shrubs	1.9 ± 0.1 b	2.1 ± 0.1 b	1.4 ± 0.2 ab	2.3 ± 0.3 b	1.8 ± 0.3 ab	1.6 ± 0.3 ab	1.8 ± 0.4 ab	1.8 ± 0.3 ab	1.1 ± 0.5 ab	1.1 ± 0.2 a	1.5 ± 0.2 ab	1.0 ± 0.3 ab
Base areas for trees	16.7 ± 1.3 b	18.9 ± 1.2 b	15.0 ± 2.0 b	18.3 ± 2.4 b	21.1 ± 2.9 b	15.9 ± 2.8 b	27.0 ± 3.0 bc	36.3 ± 2.5 c	20.9 ± 4.0 b	0.0 ± 1.4 a	0.0 ± 1.8 a	0.0 ± 2.9 a
Canopy openness	3.3 ± 0.1 bc	3.1 ± 0.1 b	2.9 ± 0.2 b	3.9 ± 0.2 c	3.4 ± 0.2 bc	3.5 ± 0.2 bc	3.5 ± 0.2 bc	3.7 ± 0.2 bc	3.6 ± 0.3 bc	1.9 ± 0.1 a	1.7 ± 0.1 a	1.2 ± 0.2 a
Conifer proportion	94.6 ± 1.0 d	95.5 ± 1.0 d	96.2 ± 1.6 d	10.4 ± 1.9 b	17.7 ± 2.4 b	10.9 ± 2.3 b	54.5 ± 2.5 c	54.2 ± 2.0 c	53.6 ± 3.2 c	0.0 ± 1.1 a	0.0 ± 1.5 a	0.0 ± 2.4 a
Depth of organic soil	36.9 ± 3.1 bcd	33.4 ± 3.2 abcd	46.6 ± 5.2 cde	12.9 ± 6.2 a	10.4 ± 7.5 ab	26.8 ± 7.2 abcd	19.2 ± 8.1 abc	15.6 ± 6.3 ab	19.9 ± 10.2 abcde	54.8 ± 3.5 e	52.0 ± 4.7 de	84.0 ± 7.5 f
The cover of ground substrates												
Leave litter ground cover	51.7 ± 3.6 cd	50.6 ± 3.6 cd	52.4 ± 5.9 cd	86.5 ± 6.9 e	71.9 ± 8.5 de	50.8 ± 8.2 bcd	75.3 ± 8.9 de	72.2 ± 7.3 de	38.0 ± 11.6 abcd	36.7 ± 3.9 abc	25.5 ± 5.4 ab	9.3 ± 8.5 a
Woody debris ground cover	14.9 ± 1.56 a	14.4 ± 1.6 a	15.8 ± 2.6 a	18.4 ± 3.0 a	16.9 ± 3.7 a	15.0 ± 3.6 a	21.8 ± 3.9 a	19.7 ± 3.2 a	13.6 ± 5.1 a	17.6 ± 1.7 a	12.7 ± 2.3 a	14.9 ± 3.7 a

Table S2.6 continued

Water ground cover	0.7 ± 0.3 a	0.2 ± 0.3 a	0.0 ± 0.5 a	0.6 ± 0.6 a	0.0 ± 0.7 a	0.0 ± 0.7 a	0.1 ± 0.7 a	0.0 ± 0.6 a	0.0 ± 1.0 a	0.3 ± 0.3 a	1.3 ± 0.4 a	4.7 ± 0.7 b
Rock ground cover	0.2 ± 0.7 a	0.4 ± 0.7 a	0.3 ± 1.2 a	3.7 ± 1.4 a	2.2 ± 1.8 a	0.17 ± 1.7 a	0.0 ± 1.8 a	4.3 ± 1.5 a	0.0 ± 2.4 a	2.5 ± 0.8 a	0.1 ± 1.1 a	0.0 ± 1.8 a
Organic matter ground cover	1.0 ± 0.6 a	0.7 ± 0.6 a	0.8 ± 1.0 ab	0.3 ± 1.2 ab	0.8 ± 1.5 ab	1.0 ± 1.4 ab	0.2 ± 1.6 ab	0.0 ± 1.3 a	0.0 ± 2.0 ab	1.8 ± 0.7 ab	5.2 ± 0.9 b	0.0 ± 1.5 ab
The number of microhabitats												
Number of trees per plot	6.8 ± 0.3 e	4.4 ± 0.3 cd	4.6 ± 0.5 bcd	3.7 ± 0.6 abc	2.8 ± 0.8 abc	3.4 ± 0.8 abc	7.2 ± 0.8 de	3.4 ± 0.7 abc	3.4 ± 1.1 abcde	2.1 ± 0.4 a	2.3 ± 0.5 ab	2.0 ± 0.8 abc
Number of logs per plot	2.4 ± 0.2 bcd	1.6 ± 0.2 abc	1.9 ± 0.4 abcd	2.9 ± 0.5 cde	2.2 ± 0.6 abcd	4.0 ± 0.6 def	3.7 ± 0.6 cdef	4.9 ± 0.5 ef	6.1 ± 0.8 f	0.9 ± 0.3 a	0.6 ± 0.4 a	0.4 ± 0.6 ab
Number of stumps per plot	1.4 ± 0.2 a	1.2 ± 0.2 a	1.0 ± 0.3 a	0.9 ± 0.3 a	0.9 ± 0.4 a	0.8 ± 0.4 a	0.8 ± 0.4 a	0.9 ± 0.3 a	1.0 ± 0.5 a	1.3 ± 0.2 a	1.6 ± 0.2 a	0.7 ± 0.4 a
Number of snags per plot	0.7 ± 0.1 a	0.4 ± 0.1 a	0.4 ± 0.2 a	0.3 ± 0.2 a	0.2 ± 0.2 a	0.3 ± 0.2 a	0.3 ± 0.2 a	0.4 ± 0.2 a	1.3 ± 0.3 a	0.4 ± 0.1 a	0.2 ± 0.1 a	0.5 ± 0.2 a
Number of rocks per plot	0.2 ± 0.1 a	0.2 ± 0.1 a	0.0 ± 0.2 a	1.6 ± 0.2 b	0.3 ± 0.2 a	0.4 ± 0.2 a	0.3 ± 0.2 a	0.1 ± 0.2 a	0.0 ± 0.3 a	0.2 ± 0.1 a	0.0 ±0.1 a	0.3 ± 0.2 a

Notes: Significant differences ($p = 0.05$) between mining stages crossing ecosystem types are indicated by different letters (generalized linear mixed model followed by Tukey HSD test).

Table S2.7 Results of generalized linear mixed models (GLMM) on the bryophyte richness testing for the effect of mine stages, ecosystem types and their interactions. Non-significant interaction terms were deleted from the final model. Significant parameters (≤ 0.05) are indicated in bold.

	All bryophyte richness (N=364)		Moss richness (N=364)		Liverwort richness (N=364)	
	Estimate	*p*	Estimate	*p*	Estimate	*p*
(Intercept)	2.54	**<0.01**	2.00	**<0.01**	1.40	**<0.01**
Mining Stage (Reference: Controls)						
Operating	0.13	0.15	0.35	**<0.01**	-0.08	0.59
Non-operating	0.13	0.17	0.36	**<0.01**	-0.10	0.50
Ecosystem type (Reference: Coniferous)						
Deciduous	0.31	**0.02**	0.50	**<0.01**	0.18	0.35
Mixed	0.65	**<0.01**	0.87	**<0.01**	0.40	0.10
Open Canopy	-0.68	**<0.01**	-0.46	**0.01**	-1.15	**<0.01**
Mining Stage: Ecosystem type						
Deciduous: Operating	-0.32	0.06	-0.41	**0.01**	-0.52	**0.05**
Deciduous: Non-operating	-0.22	0.22	-0.40	**0.03**	-0.54	0.07
Mixed: Operating	-0.65	**<0.01**	-0.89	**<0.01**	-0.25	0.42
Mixed: Non-operating	-0.42	**0.04**	-0.64	**<0.01**	-0.12	0.68
Open Canopy: Operating	0.28	0.13	0.05	0.80	0.81	**0.02**
Open Canopy: Non-operating	0.32	0.10	0.19	0.34	0.43	0.22
Anova (Type=II)						
Factors	Chisq.	P	Chisq.	P	Chisq.	P
Mining stage	0.91	0.63	7.36	0.03	3.98	0.14
Ecosystem type	88.12	<0.01	95.34	<0.01	44.89	<0.01
Mining stage: Ecosystem type	17.70	0.01	27.89	<0.01	16.40	0.01

Table S2.8 Influence of mining stage, ecosystem type and their interactions on bryophyte community composition using permutational multivariate analysis of variance (PERMANOVA) based on the Bray-Curtis dissimilarity index for total bryophyte, moss and liverwort species.

Assemblage	Variable	Df	Mean square	F statictics	R^2 (%)	*P*
Total bryophyte species	Mining Stages	2	0.64	3.32	1.41	**0.001**
	Ecosystem types	3	3.81	19.84	12.60	**0.001**
	Sites	4	2.13	11.09	9.39	**0.001**
	Mining Stages: Ecosystem types	6	0.44	2.31	2.93	**0.001**
	Residuals	348	0.19		73.67	
Moss species	Mining Stages	2	0.73	3.57	1.49	**0.001**
	Ecosystem types	3	4.36	21.45	13.45	**0.001**
	Sites	4	2.25	11.06	9.25	**0.001**
	Mining Stages: Ecosystem types	6	0.50	2.46	3.09	**0.001**
	Residuals	348	0.20		72.73	
Liverwort species	Mining Stages	2	0.51	3.23	1.58	**0.002**
	Ecosystem types	3	1.92	12.27	8.97	**0.001**
	Sites	4	1.45	9.30	9.06	**0.001**
	Mining Stages: Ecosystem types	6	0.26	1.66	2.43	**0.023**
	Residuals	320	0.16		77.97	

Table S2.9 PERMANOVA (Bray-Curtis) post hoc pair-wise comparisons of mine stages in each ecosystem type on composition of total bryophytes, mosses and liverworts respectively based on 999 permutation and Benjamini-Hochberg adjustment (BH).

Pairs	Total bryophytes		Mosses		Liverworts	
	R^2 (%)	p.adjusted	R^2 (%)	p.adjusted	R^2 (%)	p.adjusted
Coniferous						
Operating vs Nonoperating	0.91	0.20	0.96	0.17	1.57	0.05
Operating vs Controls	2.08	**0.04**	2.55	**0.02**	1.14	0.32
Nonoperating vs Controls	2.26	**0.02**	2.80	**0.01**	1.17	0.33
Deciduous						
Operating vs Nonoperating	6.25	**0.03**	6.53	0.03	9.49	0.04
Operating vs Controls	6.40	**0.01**	6.72	0.01	5.48	0.13
Nonoperating vs Controls	9.60	**0.01**	10.86	0.01	6.42	0.14
Mixed						
Operating vs Nonoperating	6.07	**0.03**	7.08	**0.01**	4.40	0.28
Operating vs Controls	8.40	**0.06**	8.92	**0.06**	7.62	0.21
Nonoperating vs Controls	5.48	0.20	4.89	0.28	7.01	0.13
Open Canopy						
Operating vs Nonoperating	3.39	**<0.01**	2.95	**0.01**	4.23	**0.01**
Operating vs Controls	4.25	**<0.01**	4.78	**<0.01**	1.96	0.30
Nonoperating vs Controls	6.07	**0.01**	6.50	**0.01**	3.31	0.23

Table S2.10 The results of the Euclidean distances (D) between mining stage centroids [operating(op), non-operating (nop) and controls (con)] for an ecosystem type. Composition dataset of liverwort species used in the diagram to describe the methodology.

	Bryophyte groups			Total bryophytes on each microhabitat				
	Total	Moss	liverworts	Ground	Trees	Logs	Snags	Stumps
$D_{\text{op-con}}$								
Coniferous	0.099	0.115	0.082	0.095	0.146	0.125	0.113	0.100
Deciduous	0.029↓	0.035↓	0.160↑	0.234↑	0.256↑	0.235↑	0.604↑	0.216↑
Mixed	0.067↓	0.053↓	0.160↑	0.270↑	0.220↑	0.221↑	0.071↓	0.067↓
Open Canopy	0.208↑	0.225↑	0.136↑	0.370↑	0.376↑	0.464↑	0.379↑	0.391↑
$D_{\text{non-con}}$								
Coniferous	0.123	0.150	0.072	0.047	0.113	0.073	0.113	0.114
Deciduous	0.172↑	0.182↑	0.196↑	0.425↑	0.520↑	0.355↑	0.498↑	0.414↑
Mixed	0.073↓	0.089↓	0.179↑	0.168↑	0.118↑	0.249↑	0.283↑	0.097↓
Open Canopy	0.183↑	0.203↑	0.139↑	0.636↑	0.477↑	0.388↑	0.572↑	0.487↑
$D_{\text{op-non}}$								
Coniferous	0.040	0.067	0.089	0.134	0.127	0.057	0.003	0.049
Deciduous	0.145↑	0.147↑	0.277↑	0.193↑	0.272↑	0.153↑	0.147↑	0.234↑
Mixed	0.007↓	0.036↓	0.070↓	0.104↓	0.109↓	0.033↓	0.273↑	0.118↑
Open Canopy	0.132↑	0.107↑	0.168↑	0.371↑	0.101↓	0.125↑	0.251↑	0.104↑

Notes: only groups affected significantly by the interactions between mining stage and ecosystem type were present in the table see PERMANOVA results in Table S2.7 and S2.12. "↑" means synergistic effects and "↓"means antagonistic effects.

Table S2.11. Nonmetric multi-dimensional scaling (NMDS) showing the associations between the composition of all bryophytes and selected variables. The significant relationships are in bold, and R^2 is the fit index of the model.

Variables	Abbreviation	PCOA1	PCOA2	R^2	*P*
Canopy openness	OP	0.61	-0.79	0.45	**0.001**
Live crown ratio for shrubs	SLC	-0.48	0.88	0.03	**0.005**
Base areas for shrubs	SBA	0.24	-0.97	0.05	**0.003**
Live crown ratio for trees	TLC	0.50	-0.87	0.12	**0.001**
Base areas for trees	TBA	0.47	-0.88	0.32	**0.001**
Depth of organic soil	OSD	-0.87	0.48	0.40	**0.001**
Number of trees per plot	Treen	-0.12	-0.99	0.25	**0.001**
Number of logs per plot	Logn	0.60	-0.80	0.33	**0.001**
Number of stumps per plot	Stumpn	-0.34	-0.94	0.06	**0.001**
Number of snags per plot	Snagn	-0.52	-0.85	0.04	**0.002**
Number of rocks per plot	Rockn	0.90	0.45	0.06	**0.001**

Table S2.12 Results of generalized linear mixed models (GLMM) on total bryophyte richness on each microhabitat testing for the effect of mine stages, ecosystem types and their interactions. Non-significant interaction terms were deleted from the final models. Significant parameters (≤ 0.05) are indicated in bold.

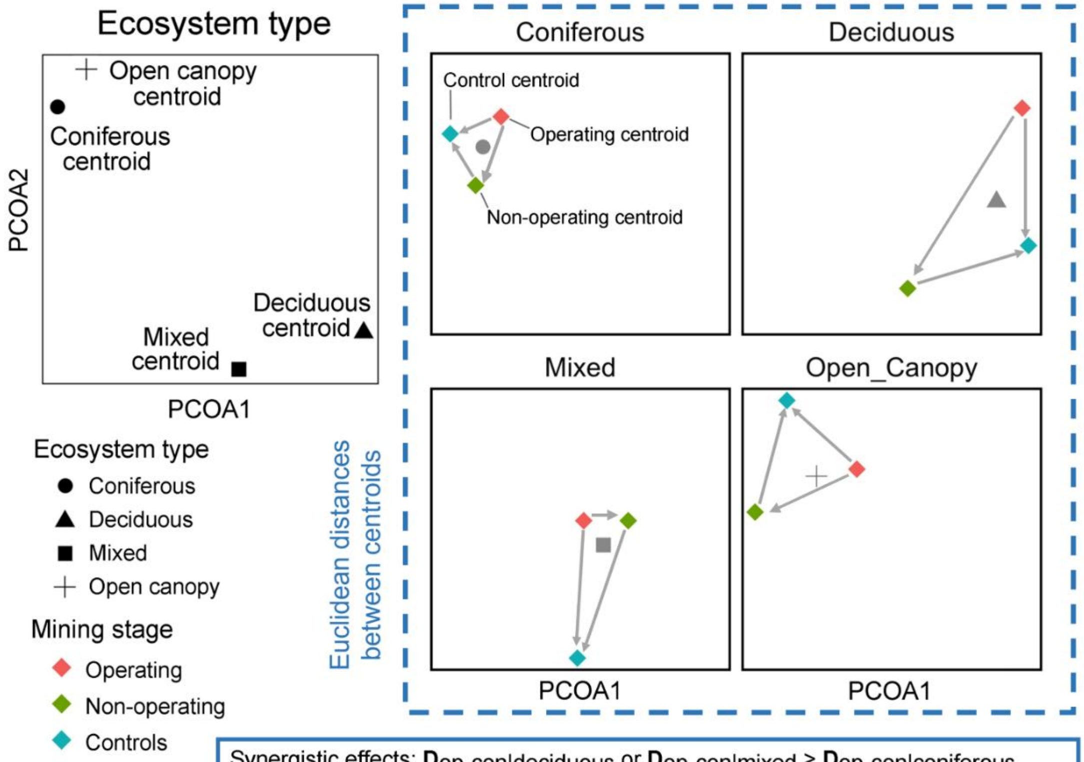
	Ground		Trees		Logs		Stumps		Snags		Rocks	
	Estimat	*P*	Estimat	*P*	Estimat	*P*	Estimat	*P*	Estimat	*P*	Estimat	*P*
(Intercept)	1.80	**<0.0**	1.99	**<0.0**	2.11	**<0.**	1.72	**<0.**	1.48	**<0.0**	1.00	**<0.0**
Mining Stage												
Operating	0.20	**0.01**	0.12	0.20	-0.55	0.24	<0.01	1.00	-0.22	0.37	0.62	**0.02**
Non-operating	0.27	**0.03**	0.04	0.70	-0.19	0.69	0.04	0.73	-0.31	0.21	0.36	0.23
Ecosystem type												
Deciduous	0.18	0.27	-0.02	0.85	0.31	**0.02**	0.08	0.55	0.03	0.90	0.54	**<0.0**
Mixed	0.26	0.23	0.20	**0.04**	0.54	**<0.**	0.09	0.54	0.60	**<0.0**	0.57	**0.03**
Open Canopy	-0.40	**0.04**	-0.77	**<0.0**	-0.98	**<0.**	-0.20	0.05	-0.48	**0.02**	-0.19	0.58
Mining Stage: Ecosystem type												
Deciduous:	-0.44	**0.04**										
Deciduous: Non-	-0.72	**<0.0**										
Mixed: Operating	-0.25	0.35										
Mixed: Non-	-0.24	0.34										
Open Canopy:	0.18	0.41										
Open Canopy:	0.25	0.26										
Anova (Type=II)												
Factors	Chisq.	*P*	Chisq.	*P*	Chisq.	*P*	Chisq.	*P*	Chisq.	*P*	Chisq.	*P*
Mining stage	4.01	0.13	2.33	0.31	4.73	0.19	0.25	0.88	1.71	0.43	0.19	0.91
Ecosystem type	16.08	**<0.0**	88.67	**<0.0**	52.73	**<0.**	5.92	0.12	18.90	**<0.0**	16.07	**<0.0**
Mining stage:	14.29	**0.03**	6.28	0.39	4.55	0.60	6.31	0.39	11.13	0.08	9.64	0.14

Table S2.13 Influence of mining stages, ecosystem types and their interactions on bryophyte community dissimilarity on different microhabitat using permutational multivariate analysis of variance (PERMANOVA) based on the Bray-Curtis dissimilarity index for trees, logs, stumps, snags and rocks.

Microhabitats	Variable	Df	Mean square	F statictics	R^2(%)	*P*
Ground (N=345)	Mining Stages	2	1.26	3.66	1.91	**0.001**
	Ecosystem types	3	3.55	10.33	8.06	**0.001**
	Mining Stages: Ecosystem types	6	0.71	2.07	3.22	**0.001**
	Residuals	334	0.34	0.87		
Trees (N=312)	Mining Stages	2	0.57	2.45	1.4	**0.003**
	Ecosystem types	3	3.09	13.32	11.1	**0.001**
	Mining Stages: Ecosystem types	6	0.50	2.16	3.6	**0.001**
	Residuals	301	0.23	0.84		
Logs (N=226)	Mining Stages	2	0.59	2.37	1.9	**0.004**
	Ecosystem types	3	1.74	6.96	8.4	**0.001**
	Mining Stages: Ecosystem types	6	0.34	1.37	3.3	**0.035**
	Residuals	215	0.25	0.86		
Stumps (N=212)	Mining Stages	2	0.52	1.79	1.6	**0.01**
	Ecosystem types	3	1.58	5.45	7.1	**0.001**
	Mining Stages: Ecosystem types	6	0.42	1.46	3.8	**0.012**
	Residuals	201	0.29	0.88		
Snags (N=103)	Mining Stages	2	0.50	1.90	3.3	**0.017**
	Ecosystem types	3	1.00	3.77	9.7	**0.001**
	Mining Stages: Ecosystem types	6	0.40	1.51	7.8	**0.017**
	Residuals	92	0.26	0.79		
Rocks (N=39)	Mining Stages	2	0.58	1.78	8.3	**0.015**
	Ecosystem types	3	0.59	1.81	12.6	**0.003**
	Residuals	34	0.33	0.79		

Table S2.14. PERMANOVA (Bray-Curtis) post hoc pair-wise comparisons of mine stages in each ecosystem type on composition of all bryophyte species on each microhabitat respectively based on 999 permutation and Benjamini-Hochberg adjustment (BH).

Pairwise PERMANOVA	Ground			Trees			Logs			Stumps			Snags		
	R^2 (%)	p.value	p.adjusted	R^2 (%)	p.value	p.adjusted	R^2 (%)	p.value	p.adjusted	R^2 (%)	p.value	p.adjusted	R^2 (%)	p.value	p.adjusted
Coniferous															
Operating vs Nonoperating	0.62	0.59	0.59	0.90	0.22	0.24	0.68	0.68	0.71	0.70	0.85	0.87	4.10	0.02	0.08
Operating vs Controls	2.90	<0.01	**<0.01**	1.38	0.22	0.24	1.22	0.48	0.55	1.04	0.84	0.87	5.54	0.02	0.07
Nonoperating vs Controls	2.46	0.01	**0.01**	2.06	0.06	0.07	1.33	0.54	0.59	0.93	0.89	0.90	6.09	0.09	0.17
Deciduous															
Operating vs Nonoperating	7.91	<0.01	**0.01**	3.87	0.37	0.38	5.27	0.24	0.30	5.25	0.69	0.74	17.73	0.26	0.33
Operating vs Controls	5.33	0.06	0.07	5.69	0.06	0.07	8.89	<0.01	**0.01**	12.11	0.01	**0.03**	20.45	0.70	0.72
Nonoperating vs Controls	9.97	<0.01	**<0.01**	13.12	0.01	**0.01**	11.62	0.01	**0.01**	11.94	0.08	0.11	16.01	0.27	0.34
Mixed															
Operating vs Nonoperating	4.88	0.13	0.14	7.05	0.05	0.06	6.37	0.06	0.08	8.56	0.15	0.18	23.49	0.10	0.17
Operating vs Controls	5.16	0.55	0.56	9.27	0.11	0.12	4.63	0.66	0.70	19.32	0.04	0.06	18.53	0.60	0.63
Nonoperating vs Controls	5.44	0.19	0.21	7.76	0.05	0.07	4.38	0.55	0.59	13.04	0.03	**0.05**	18.07	0.12	0.18
Open Canopy															
Operating vs Nonoperating	2.88	0.01	**0.01**	5.37	<0.01	**<0.01**	9.24	0.01	**0.01**	3.82	0.02	**0.04**	8.57	0.18	0.25
Operating vs Controls	8.50	<0.01	**<0.01**	5.00	0.02	**0.02**	5.56	0.48	0.55	3.89	0.08	0.11	5.99	0.71	0.72
Nonoperating vs Controls	11.37	<0.01	**<0.01**	3.91	0.23	0.25	5.84	0.42	0.50	6.12	0.13	0.16	10.31	0.50	0.53



Synergistic effects: $\mathbf{D}_{op\text{-}con|deciduous}$ or $\mathbf{D}_{op\text{-}con|mixed}$ > $\mathbf{D}_{op\text{-}con|coniferous}$
OR $\mathbf{D}_{nop\text{-}con|deciduous}$ or $\mathbf{D}_{nop\text{-}con|mixed}$ > $\mathbf{D}_{nop\text{-}con|coniferous}$
OR $\mathbf{D}_{op\text{-}nop|deciduous}$ or $\mathbf{D}_{op\text{-}nop|mixed}$ > $\mathbf{D}_{op\text{-}nop|coniferous}$
Antagonistic effects: $\mathbf{D}_{op\text{-}con|deciduous}$ or $\mathbf{D}_{op\text{-}con|mixed}$ > $\mathbf{D}_{op\text{-}con|coniferous}$
OR $\mathbf{D}_{nop\text{-}con|deciduous}$ or $\mathbf{D}_{nop\text{-}con|mixed}$ > $\mathbf{D}_{nop\text{-}con|coniferous}$
OR $\mathbf{D}_{op\text{-}nop|deciduous}$ or $\mathbf{D}_{op\text{-}nop|mixed}$ > $\mathbf{D}_{op\text{-}nop|coniferous}$

Figure S2.1 Testing for Interaction effects for community composition shifts in the study. op, operating centroid; nop, non-operating centroid; con, control centroid. $D_{op\text{-}con|deciduous}$ means Euclidean distances between operating centroid and control centroids in deciduous forest.

APPENDICE C

MATÉRIEL SUPPLÉMENTAIRE DU CHAPITRE IV

Table S3.1. Results of PERMANOVA showing the relative importance of forest type, mining stage and their interaction in structuring the phyllosphere communities based on Bray-Curtis dissimilarity.

	Df	Sums Of Sqs	Mean Sqs	F.Model	R^2 (%)	*P*
Bacterial community						
Mining stage (Stage)	2	2.32	1.16	4.57	4.43	< **0.001**
Forest type (Forest)	3	3.04	1.01	3.98	5.83	< **0.001**
Forest: Stage	6	1.56	0.26	1.02	2.72	0.70
Residuals	198	50.31	0.25	0.88		
Total	209	57.22	1.00			
Fungal community						
Mining stage (Stage)	2	2.39	1.20	3.38	2.62	< **0.001**
Forest type (Forest)	3	4.10	1.37	3.86	4.63	< **0.001**
Forest: Stage	6	2.41	0.40	1.13	3.06	0.16
Residuals	197	69.77	0.35	0.89		
Total	208 [1]	78.67	1.00			

Notes: 1, one outlier was removed from analysis (LR2A plot)

Table S3.2 Results of pairwise PERMANOVA for the community composition between mining stages with Benjamini-Hochberg adjustment.

Pairwise PERMANOVA	Bacterial community			Fungal community		
	F	R^2(%)	*P*.adj	*F*	R^2(%)	*P*.adj
Operating vs. Non-operating	5.18	2.99	**0.001**	2.34	1.38	**0.001**
Operating vs. Controls	4.50	3.35	**0.001**	3.27	2.47	**0.001**
Non-operating vs. Controls	4.38	3.64	**0.001**	3.24	2.72	**0.001**

Table S3.3 Results of pairwise PERMANOVA for the community composition between forest types with Benjamini-Hochberg adjustment.

Pairwise PERMANOVA	Bacterial community			Fungal community		
	F	R^2(%)	*P*.adj	*F*	R^2(%)	*P*.adj
Coniferous vs. Open Canopy	4.48	2.92	**0.002**	4.30	2.80	**0.002**
Coniferous vs. Mixed	2.51	1.97	**0.005**	1.84	1.45	**0.007**
Coniferous vs. Deciduous	5.10	3.72	**0.002**	3.60	2.67	**0.002**
Deciduous vs. Open Canopy	6.10	7.00	**0.002**	3.72	4.44	**0.002**
Deciduous vs. Mixed	1.78	3.03	**0.019**	1.95	3.37	**0.004**
Mixed vs. Open Canopy	4.74	6.03	**0.002**	4.14	5.30	**0.002**

Table S3.5 List of bacterial indicators for mining stages with their taxonomic information (only significant species are shown, $P < 0.05$ and stat ≥ 0.2). “Stat” is the association statistic (the square root of the indicator value) and “P value”is the p-value of the permutational test.

ASV	stat	P value	Kingdom	Phylum	Class	Order	Family	Genus
Operating								
ASV_11	0.29	0.002	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	NA
ASV_19	0.276	0.002	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Microscillaceae	*OLB12*
ASV_84	0.318	0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acidiphilium*
ASV_101	0.318	0.001	Bacteria	Acidobacteriota	Acidobacteriae	Solibacterales	Solibacteraceae	*Candidatus Solibacter*
ASV_159	0.317	0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*Methylobacterium-Methylorubrum*
ASV_119	0.297	0.002	Bacteria	Desulfobacterota	Desulfuromonadia	Geobacterales	Geobacteraceae	*Geobacter*
ASV_91	0.29	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*1174-901-12*
ASV_145	0.287	0.002	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*
ASV_326	0.284	0.001	Bacteria	Actinobacteriota	Actinobacteria	Frankiales	Nakamurellaceae	*Nakamurella*
ASV_49	0.276	0.004	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae	*Occallatibacter*
ASV_178	0.268	0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Hyphomonadaceae	*SWB02*
ASV_156	0.267	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*Pseudorhodoplanes*
ASV_360	0.266	0.004	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acidiphilium*
ASV_80	0.26	0.003	Bacteria	Actinobacteriota	Thermoleophilia	Solirubrobacterales	67-14	NA
ASV_721	0.258	0.001	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae	*Bryocella*
ASV_160	0.251	0.004	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*
ASV_385	0.249	0.001	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	*Aurantisolimonas*

Table S3.5 continued

ASV_150	0.246	0.005	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Nitrosomonadaceae	*IS-44*
ASV_507	0.246	0.001	Bacteria	Actinobacteriota	Actinobacteria	Micromonosporales	Micromonosporaceae	*Actinoplanes*
ASV_161	0.245	0.001	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Oxalobacteraceae	*Massilia*
ASV_187	0.244	0.006	Bacteria	Firmicutes	Desulfitobacteriia	Desulfitobacteriales	Desulfitobacteriaceae	*Desulfosporosinus*
ASV_214	0.244	0.005	Bacteria	Bacteroidota	SJA-28	NA	NA	NA
ASV_580	0.244	0.001	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Spirosomaceae	*Huanghella*
ASV_472	0.239	0.001	Bacteria	Bacteroidota	Bacteroidia	Cytophagales	Spirosomaceae	*Spirosoma*
ASV_57	0.238	0.006	Bacteria	Verrucomicrobiota	Verrucomicrobiae	Opitutales	Opitutaceae	*Lacunisphaera*
ASV_207	0.238	0.005	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	NA
ASV_332	0.23	0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*1174-901-12*
ASV_131	0.226	0.011	Bacteria	Acidobacteriota	Acidobacteriae	Bryobacterales	Bryobacteraceae	*Bryobacter*
ASV_333	0.226	0.006	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	*Ferruginibacter*
ASV_379	0.221	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*
ASV_733	0.218	0.002	Bacteria	Deinococcota	Deinococci	Deinococcales	Deinococcaceae	*Deinococcus*
ASV_508	0.217	0.004	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*1174-901-12*
ASV_1065	0.215	0.007	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acidiphilium*
ASV_188	0.209	0.002	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acidiphilium*
ASV_27	0.204	0.017	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*
Non-operating								
ASV_8	0.279	0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	NA
ASV_34	0.275	0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	PMMR1
ASV_147	0.266	0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	NA

Table S3.5 continued

ASV_208	0.266	0.001	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae	*Granulicella*
ASV_132	0.262	0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Novosphingobium*
ASV_146	0.26	0.003	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae	*Bryocella*
ASV_116	0.25	0.003	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	NA
ASV_194	0.245	0.004	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	NA
ASV_30	0.243	0.004	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	NA
ASV_227	0.241	0.001	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae	*Granulicella*
ASV_88	0.233	0.006	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	NA
ASV_169	0.231	0.004	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	*Novosphingobium*
ASV_85	0.23	0.004	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	*Aurantisolimonas*
ASV_66	0.228	0.01	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae	*Granulicella*
ASV_250	0.222	0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA
ASV_86	0.221	0.011	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae	*Granulicella*
ASV_112	0.221	0.011	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acidisoma*
ASV_89	0.217	0.007	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA
ASV_94	0.214	0.007	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae	*Bryocella*
ASV_51	0.213	0.005	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acidiphilium*
ASV_100	0.213	0.01	Bacteria	Planctomycetota	Planctomycetes	Isosphaerales	Isosphaeraceae	*Tundrisphaera*
ASV_74	0.209	0.009	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acidisoma*
ASV_354	0.204	0.013	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae	*Granulicella*
ASV_148	0.203	0.022	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acidisphaera*
ASV_192	0.203	0.015	Bacteria	Proteobacteria	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	NA

Table S3.5 continued

Controls								
ASV_361	0.514	0.001	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	*Cupriavidus*
ASV_543	0.386	0.001	Bacteria	Acidobacteriota	Acidobacteriae	Subgroup 2	NA	NA
ASV_373	0.364	0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	*Roseiarcus*
ASV_244	0.358	0.001	Bacteria	Proteobacteria	Gammaproteobacteria	Salinisphaerales	Solimonadaceae	*Nevskia*
ASV_278	0.358	0.001	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	*Stenotrophomonas*
ASV_245	0.347	0.001	Bacteria	Bacteroidota	Bacteroidia	Chitinophagales	Chitinophagaceae	*Puia*
ASV_299	0.307	0.001	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Rhodanobacteraceae	*Rhodanobacter*
ASV_38	0.299	0.001	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*Bradyrhizobium*
ASV_199	0.299	0.001	Bacteria	Proteobacteria	Gammaproteobacteria	WD260	NA	NA
ASV_226	0.258	0.001	Bacteria	Proteobacteria	Gammaproteobacteria	Burkholderiales	Comamonadaceae	*Variovorax*
ASV_168	0.241	0.001	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*
ASV_167	0.212	0.006	Bacteria	Acidobacteriota	Acidobacteriae	Acidobacteriales	Acidobacteriaceae	*Granulicella*
ASV_106	0.208	0.01	Bacteria	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	*Mucilaginibacter*

Table S3.6 List of fungal indicators for mining stages with their taxonomic information (only significant species are shown $P < 0.05$ and stat ≥ 0.2). “Stat” is the association statistic (the square root of the indicator value) and “P value”is the p-value of the permutational test.

ASV	stat	*P*	Kingdom	Phylum	Class	Order	Family	Genus
Operating								
ASV_10	0.326	0.001	Fungi	Basidiomycota	Microbotryomycetes	Kriegeriales	Kriegeriaceae	*Phenoliferia*
ASV_21	0.32	0.001	Fungi	Chytridiomycota	Spizellomycetes	Spizellomycetales	NA	NA
ASV_86	0.32	0.001	Fungi	Chytridiomycota	Spizellomycetes	Spizellomycetales	Powellomycetaceae	*Thoreauomyces*
ASV_115	0.314	0.001	Fungi	Ascomycota	Dothideomycetes	Mytilinidiales	Mytilinidiaceae	*Lophium*
ASV_212	0.259	0.001	Fungi	Ascomycota	Dothideomycetes	Mytilinidiales	Mytilinidiaceae	*Lophium*
ASV_98	0.256	0.001	Fungi	Basidiomycota	Tremellomycetes	Tremellales	NA	NA
ASV_235	0.254	0.001	Fungi	Basidiomycota	Microbotryomycetes	Sporidiobolales	Sporidiobolaceae	*Rhodosporidiobolus*
ASV_453	0.253	0.002	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	*Devriesia*
ASV_29	0.247	0.003	Fungi	Chytridiomycota	Rhizophydiomycetes	Rhizophydiales	Rhizophydiaceae	*Rhizophydium*
ASV_193	0.245	0.004	Fungi	Chytridiomycota	Rhizophydiomycetes	Rhizophydiales	Rhizophydiaceae	*Rhizophydium*
ASV_314	0.245	0.001	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Setomelanomma*
ASV_250	0.241	0.004	Fungi	NA	NA	NA	NA	NA
ASV_616	0.24	0.004	Fungi	Ascomycota	Dothideomycetes	Dothideales	Dothideaceae	*Rhizosphaera*
ASV_414	0.237	0.003	Fungi	NA	NA	NA	NA	NA
ASV_127	0.233	0.004	Fungi	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	*Mrakia*
ASV_59	0.228	0.002	Fungi	Ascomycota	NA	NA	NA	NA

Table S3.6 continued

ASV_100	0.226	0.01	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Neodevriesiaceae	NA
ASV_217	0.223	0.005	Fungi	NA	NA	NA	NA	NA
ASV_39	0.221	0.011	Fungi	Ascomycota	Leotiomycetes	Leotiales	Mniaeciaceae	*Epithamnolia*
ASV_199	0.219	0.004	Fungi	Chytridiomycota	Spizellomycetes	Spizellomycetales	NA	NA
ASV_698	0.219	0.006	Fungi	NA	NA	NA	NA	NA
ASV_169	0.218	0.009	Fungi	Ascomycota	Dothideomycetes	Capnodiales	Teratosphaeriaceae	*Capnobotryella*
ASV_897	0.218	0.012	Fungi	Basidiomycota	Tremellomycetes	Tremellales	NA	NA
ASV_75	0.217	0.006	Fungi	Ascomycota	Pezizomycotina_cls_Incertae_sedis	Pezizomycotina_ord_Incertae_sedis	Pezizomycotina_fam_Incertae_sedis	*Ciliophora*
ASV_6	0.217	0.013	Fungi	NA	NA	NA	NA	NA
ASV_117	0.214	0.003	Fungi	NA	NA	NA	NA	NA
ASV_164	0.209	0.005	Fungi	Ascomycota	Dothideomycetes	Venturiales	Venturiaceae	*Venturia*
ASV_622	0.208	0.003	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	NA
ASV_1240	0.207	0.007	Fungi	NA	NA	NA	NA	NA
ASV_352	0.204	0.007	Fungi	NA	NA	NA	NA	NA
ASV_544	0.204	0.011	Fungi	Basidiomycota	Agaricomycetes	Cantharellales	Ceratobasidiaceae	*Ceratobasidium*
ASV_316	0.2	0.012	Fungi	Olpidiomycota	GS18	NA	NA	NA
Non-operating								
ASV_136	0.314	0.001	Fungi	Ascomycota	Orbiliomycetes	Orbiliales	NA	NA
ASV_186	0.245	0.004	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	NA	NA
ASV_50	0.222	0.005	Fungi	Ascomycota	Dothideomycetes	Dothideales	NA	NA
ASV_244	0.222	0.001	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*
ASV_155	0.22	0.005	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	NA

Table S3.6 continued

ASV_504	0.212	0.009	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Cladophialophora*
ASV_188	0.208	0.01	Fungi	Ascomycota	Leotiomycetes	Helotiales	NA	NA
Controls								
ASV_429	0.397	0.001	Fungi	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Thelephora*
ASV_634	0.395	0.001	Fungi	Ascomycota	Pezizomycotina_cls_Incertae_sedis	Pezizomycotina_ord_Incertae_sedis	Pezizomycotina_fam_Incertae_sedis	*Ciliophora*
ASV_729	0.395	0.001	Fungi	NA	NA	NA	NA	NA
ASV_659	0.391	0.001	Fungi	Ascomycota	Pezizomycotina_cls_Incertae_sedis	Pezizomycotina_ord_Incertae_sedis	Pezizomycotina_fam_Incertae_sedis	*Ciliophora*
ASV_502	0.389	0.001	Fungi	Basidiomycota	Tremellomycetes	Filobasidiales	Piskurozymaceae	*Solicoccozyma*
ASV_673	0.388	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	NA	NA
ASV_675	0.386	0.001	Fungi	Ascomycota	Eurotiomycetes	Eurotiales	Trichocomaceae	*Talaromyces*
ASV_441	0.384	0.001	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Inocybaceae	*Inocybe*
ASV_490	0.384	0.001	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Phaeosphaeriaceae	*Paraphoma*
ASV_542	0.384	0.001	Fungi	Mucoromycota	Umbelopsidomycetes	Umbelopsidales	Umbelopsidaceae	*Umbelopsis*
ASV_509	0.383	0.001	Fungi	NA	NA	NA	NA	NA
ASV_603	0.383	0.001	Fungi	Basidiomycota	Agaricomycetes	Russulales	Russulaceae	*Lactifluus*
ASV_559	0.382	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	NA
ASV_405	0.376	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Ascocoryne*
ASV_76	0.373	0.001	Fungi	Ascomycota	Pezizomycetes	Pezizales	Pyronemataceae	*Sphaerosporella*
ASV_389	0.373	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	NA	*NA*
ASV_266	0.372	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiales_fam_Incertae_sedis	*Leptodontidium*
ASV_123	0.371	0.001	Fungi	NA	NA	NA	NA	NA

Table S3.6 continued

ASV_355	0.371	0.001	Fungi	NA	NA	NA	NA	NA
ASV_379	0.371	0.001	Fungi	NA	NA	NA	NA	NA
ASV_19	0.37	0.001	Fungi	NA	NA	NA	NA	NA
ASV_454	0.369	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Hyaloscypha*
ASV_159	0.368	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Meliniomyces*
ASV_61	0.365	0.001	Fungi	Basidiomycota	Microbotryomycetes	Leucosporidiales	Leucosporidiaceae	*Leucosporidium*
ASV_738	0.364	0.001	Fungi	Ascomycota	Leotiomycetes	Thelebolales	Pseudeurotiaceae	NA
ASV_101	0.363	0.001	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Strophariaceae	*Hypholoma*
ASV_176	0.363	0.001	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Tricholomataceae	*Mycena*
ASV_411	0.362	0.001	Fungi	Ascomycota	Leotiomycetes	Thelebolales	NA	NA
ASV_182	0.359	0.001	Fungi	NA	NA	NA	NA	NA
ASV_530	0.359	0.001	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	NA
ASV_589	0.358	0.001	Fungi	NA	NA	NA	NA	NA
ASV_228	0.354	0.001	Fungi	NA	NA	NA	NA	NA
ASV_281	0.353	0.001	Fungi	NA	NA	NA	NA	NA
ASV_118	0.352	0.001	Fungi	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Hypoxylon*
ASV_214	0.351	0.001	Fungi	Ascomycota	Sordariomycetes	Xylariales	Xylariaceae	*Hypoxylon*
ASV_309	0.349	0.001	Fungi	Basidiomycota	Microbotryomycetes	Kriegeriales	Kriegeriaceae	*Phenoliferia*
ASV_242	0.347	0.001	Fungi	Mortierellomycota	Mortierellomycetes	Mortierellales	Mortierellaceae	*Mortierella*
ASV_631	0.345	0.001	Fungi	Ascomycota	Saccharomycetes	Saccharomycetales	Saccharomycetales_fam_Incertae_sedis	*Nadsonia*
ASV_567	0.333	0.001	Fungi	Basidiomycota	Agaricomycetes	Agaricales	Hymenogastraceae	*Hebeloma*
ASV_187	0.296	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	Hyaloscyphaceae	*Hyaloscypha*

Table S3.6 continued

ASV_398	0.287	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiales_fam_ Incertae_sedis	*Leptodontidium*
ASV_525	0.273	0.001	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	NA	NA
ASV_143	0.259	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiaceae	*Collophora*
ASV_344	0.256	0.001	Fungi	Ascomycota	Leotiomycetes	Helotiales	Helotiales_fam_ Incertae_sedis	*Cadophora*
ASV_395	0.252	0.003	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	Herpotrichiellaceae	*Rhinocladiella*
ASV_333	0.246	0.001	Fungi	Ascomycota	NA	NA	NA	NA
ASV_442	0.244	0.005	Fungi	Ascomycota	Dothideomycetes	Capnodiales	NA	NA
ASV_110	0.241	0.005	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	NA	NA
ASV_318	0.239	0.003	Fungi	Basidiomycota	Agaricomycetes	Thelephorales	Thelephoraceae	*Thelephora*
ASV_678	0.232	0.002	Fungi	Ascomycota	Dothideomycetes	Venturiales	Venturiaceae	*Venturia*
ASV_321	0.231	0.002	Fungi	Ascomycota	NA	NA	NA	NA
ASV_608	0.229	0.001	Fungi	NA	NA	NA	NA	NA
ASV_606	0.22	0.007	Fungi	Basidiomycota	Tremellomycetes	Cystofilobasidiales	Mrakiaceae	*Mrakia*
ASV_239	0.218	0.006	Fungi	Basidiomycota	Agaricomycetes	Polyporales	Ganodermataceae	*Ganoderma*
ASV_356	0.207	0.013	Fungi	Ascomycota	Leotiomycetes	Helotiales	Leotiaceae	*Pezoloma*
ASV_285	0.202	0.01	Fungi	Ascomycota	Eurotiomycetes	Chaetothyriales	NA	NA
ASV_78	0.2	0.012	Fungi	Chytridiomycota	Spizellomycetes	Spizellomycetales	NA	NA

Table S3.7 Phyllosphere alpha diversity at each distance level from mine sites. Mean ± SD are shown. Significant p-values for the effects of the distance, mining stage and their interactions were calculated with ANOVA using Type II sum of squares based on the results of GLMMs. Different lowercase letters represent significant multiple compositions with Tukey's post hoc tests at the 0.05 level. Significant relationships are highlighted in bold.

Alpha diversity	ANOVA (type II sums)		Distance from mine sites (km)			
	Distance	Distance* Stage	0 (N=39)	0.05 (N=44)	0.2 (N=43)	1 (N=44)
Bacteria						
Observed	**p<0.01**	p=0.63	640.85 ± 180.97 b	584.09 ± 180.41 ab	550.28 ± 183.82 a	568.25 ± 190.34 a
Shannon	**p<0.01**	p=0.86	5.72 ± 0.43 b	5.55 ± 0.47 ab	5.45 ± 0.50 a	5.51 ± 0.43 a
InvSimpson	**p=0.03**	p=0.81	153.42 ± 78.92 a	127.28 ± 68.16 a	115.77 ± 59.95 a	119.45 ± 63.43 a
Fungi						
Observed	**p<0.01**	p=0.47	462.50 ± 142.32 b	382.16 ± 104.14 a	396.40 ± 129.17 a	404.80 ± 113.62 ab
Shannon	**p=0.01**	p=0.64	4.88 ± 0.38 b	4.62 ± 0.47 a	4.69 ± 0.41 ab	4.69 ± 0.48 ab
InvSimpson	p=0.33	p=0.42	55.31 ± 21.65 a	45.84 ± 25.13 a	47.96 ± 22.99 a	48.34 ± 25.71 a

Table S3.8 PERMANOVA results showing the relative importance of distance from mines, mining stage and their interactions in structing the phyllosphere communities based on Bray-Curtis dissimilarity.

	Df	SumsOfSqs	MeanSqs	F.Model	R^2 (%)	Pr(>F)
Bacterial community						
Mining stage (Stage)	1	1.34	1.34	5.09	3%	**< 0.001**
Distance	1	0.66	0.66	2.50	1%	**< 0.001**
Distance: Stage	1	0.33	0.33	1.26	1%	0.07
Residuals	166	43.58	0.26	0.95		
Total	169	45.91	1.00			
Fungal community						
Mining stage (Stage)	1	0.97	0.97	2.67	2%	**< 0.001**
Distance	1	0.64	0.64	1.74	1%	**< 0.001**
Distance: Stage	1	0.42	0.42	1.14	1%	0.06
Residuals	165	60.17	0.36	0.97		
Total	168	62.20	1.00			

Table S3.9 Relative abundance of main phyllosphere phyla (i.e. relative abundance > 0.5 through all samples) at each distance from mine sites. Mean ± SD are shown. Zero-inflated beta mixed regression models were used to compare mean relative abundance of phyllosphere phylum. Lowercase letters represent significant differences based on Tukey's test at the 0.05 level. Significant relationships (significant differences in Tukey's test at 0.05 level) are highlighted in bold.

Phylum	Mining Stage	Distance (km)			
		0	0.05	0.2	1
Bacteria					
Proteobacteria		52.28 ± 9.7 a	54.49 ± 8.56 a	55.43 ± 7.89 a	55.2 ± 8.01 a
Acidobacteriota	Operating	11.96 ± 5.69 a	15.28 ± 5.63 ab	18.07 ± 6.02 b	19.52 ± 4.8 b
	Non-Operating	19.82 ± 7.07 a	18.48 ± 6.53 a	22.36 ± 6.78 a	20.13 ± 7.3 a
Bacteroidota	Operating	16.74 ± 5.98 b	12.68 ± 6.77 a	10.34 ± 6.06 a	9.57 ± 6.06 a
	Non-Operating	10.09 ± 5.52 a	10.13 ± 5.9 a	5.68 ± 3.83 a	7.43 ± 5.16 a
Actinobacteriota	Operating	5.33 ± 2.22 b	5.22 ± 3.58 ab	4.29 ± 2.02 ab	3.82 ± 1.75 a
	Non-Operating	4.38 ± 1.88 a	4.39 ± 1.62 a	3.53 ± 1.45 a	4.45 ± 2.04 a
Planctomycetota		3.45 ± 1.86 a	2.75 ± 1.65 a	2.77 ± 1.4 a	3.14 ± 1.69 a
Verrucomicrobiota		2.46 ± 1.71 a	1.86 ± 1.51 a	1.84 ± 1.5 a	2.14 ± 1.61 a
Cyanobacteria		0.98 ± 1.06 a	1.8 ± 1.95 a	2.33 ± 4.04 a	1.33 ± 1.46 a
Myxococcota		1.59 ± 0.95 a	1.74 ± 1.82 a	1.22 ± 0.75 a	1.4 ± 0.71 a
WPS-2	Operating	0.17± 0.15 a	0.40 ± 0.31 ab	0.77 ± 0.80 b	1.01 ± 0.88 b
	Non-Operating	0.89 ± 0.79 a	0.971 ± 0.92 a	1.54 ± 1.20 a	1.13 ± 0.86 a
Firmicutes		1.04 ± 1.27 a	0.72 ± 1.19 a	0.71 ± 1.16 a	0.86 ± 1.35 a
Armatimonadota	Operating	0.96 ± 0.6 b	0.94 ± 0.52 b	0.81 ± 0.41 ab	0.61 ± 0.36 a
	Non-Operating	0.92 ± 0.38 b	0.56 ± 0.22 a	0.53 ± 0.35 a	0.61 ± 0.28 ab
Fungi					
Ascomycota		56.42 ± 10.23 a	55.21 ± 12.86 a	54.72 ± 10.61 a	54.32 ± 12.08 a
Basidiomycota		18.53 ± 9.75 a	19.84 ± 10.94 a	21.31 ± 9.72 a	22.41 ± 10.26 a
Chytridiomycota		8.98 ± 7.1 a	9.02 ± 5.84 a	9.95 ± 8.6 a	7.59 ± 6.4 a
Olpidiomycota		2.76 ± 2.94 a	2.61 ± 2.68 a	2.63 ± 2.87 a	3.06 ± 4.21 a

Table S3.10 Relative abundance of top 10 phyllosphere genera at each distance from mine sites. Mean ± SD are shown. Zero-inflated beta mixed regression models were used to compare mean relative abundance of phyllosphere genera. Lowercase letters represent significant differences based on Tukey's test at the 0.05 level. Significant relationships (i.e. significant differences in Tukey test at 0.05 level) are highlighted in bold.

Genus	Mining Stage	Distance (km)			
		0	0.05	0.2	1
Bacterial genus					
Granulicella	Operating	6.33 ± 5.3 a	8.57 ± 4.71 b	11.07 ± 4.56 bc	11.77 ± 4.32 c
	Non-Operating	11.91 ± 5.12 a	11.94 ± 4.06 a	13.75 ± 4.08 a	12.15 ± 4.28 a
Sphingomonas	Operating	7.03 ± 3.61 b	5.85 ± 3.39 ab	5.55 ± 3.32 ab	4.19 ± 2.63 a
	Non-Operating	5.61 ± 2.87 a	6.68 ± 3.74 a	4.43 ± 2.5 a	6.3 ± 3.53 a
Acidiphilium		4.36 ± 2.68 a	4.17 ± 2.03 a	4.85 ± 2.92 a	3.8 ± 1.74 a
g117490112		3.06 ± 1.71 a	3.3 ± 1.54 a	3.44 ± 1.53 a	3.5 ± 1.39 a
Mucilaginibacter		2.88 ± 1.48 a	3.01 ± 1.42 a	2.64 ± 1.2 a	2.99 ± 1.6 a
Variovorax		1.72 ± 2.2 a	1.84 ± 2.49 a	2.38 ± 3.71 a	2.7 ± 4.06 a
Leptothrix	Operating	1.72 ± 1.22 a	2.74 ± 1.75 b	2.68 ± 1.22 b	3.1 ± 2.47 b
	Non-Operating	2.63 ± 1.43 a	2.56 ± 1.32 a	2.26 ± 1.4 a	2.41 ± 1.48 a
Rhizobacter		2.51 ± 1.69 a	2.37 ± 1.44 a	2.63 ± 1.46 a	2.09 ± 1.26 a
Limnobacter		2.27 ± 1.52 a	2.37 ± 1.36 a	2.25 ± 1.14 a	2.19 ± 1.22 a
Terriglobus	Operating	1.3 ± 1.03 a	2.11 ± 2.12 b	2.06 ± 1.04 b	1.96 ± 1.1 b
	Non-Operating	2.23 ± 1.18 a	2.39 ± 0.96 a	2.62 ± 1.39 a	2.47 ± 1.2 a
Fungal genus					
Phenoliferia		6.08 ± 5.89 a	6.67 ± 4.9 a	7.65 ± 5.89 a	6.73 ± 5.14 a
Sporormiella		2.41 ± 3.79 a	4.19 ± 8.67 a	3.46 ± 6.64 a	3.93 ± 8.62 a
Cladophialophora		2.96 ± 3.03 a	1.98 ± 1.97 a	2.16 ± 3.34 a	2.19 ± 2.47 a
Ciliophora		2.22 ± 2.26 a	1.72 ± 1.45 a	1.63 ± 1.3 a	1.54 ± 1.43 a
Epibryon		1.28 ± 1.89 a	1.41 ± 1.52 a	1.47 ± 1.43 a	1 ± 0.89 a
Epithamnolia		0.93 ± 1.15 a	1.26 ± 1.99 ab	1.58 ± 1.55 ab	2.14 ± 1.99 b
Thoreauomyces	Operating	0.93 ± 1.2 a	0.98 ± 0.96 a	1.96 ± 2.14 b	1.61 ± 1.69 ab
	Non-Operating	1.25 ± 0.69 a	1.35 ± 1 a	1.03 ± 0.86 a	1.4 ± 1.62 a
Sistotrema		0.79 ± 1.95 a	0.62 ± 1.66 a	1.78 ± 5.23 a	1.92 ± 7.65 a
Leucosporidium		0.89 ± 1.74 a	0.6 ± 1 a	0.63 ± 0.79 a	0.96 ± 1.84 a
Eocronartium		1.17 ± 4.19 a	1.46 ± 5.17 a	0.52 ± 1.56 a	0.4 ± 1.56 a

Table S3.11 Relative abundance of phyllosphere common ASVs at each distance from mine sites. Mean ± SD are shown. Zero-inflated beta mixed regression models were used to compare mean relative abundance of phyllosphere ASVs. Lowercase letters represent significant differences based on Tukey's test at the 0.05 level. Significant relationships (significant differences in Tukey test at 0.05 level) are highlighted in bold. Common ASVs were identified based on the presence in at least in 90 % of the samples for bacteria and 70 % of the samples for fungi.

ASV	Taxonomic information			Mining Stage	Distance (km)			
	Order	Family	Genus		0	0.05	0.2	1
Bacterial ASV								
ASV1	Burkholderiales	Burkholderiaceae	*Limnobacter*		0.02 ± 0.01 a	0.02 ± 0.01 a	0.02 ± 0.01 a	0.02 ± 0.01 a
ASV3	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*		1.45 ± 1.17 a	1.84 ± 1.64 a	1.88 ± 1.23 a	2.07 ± 1.53 a
ASV4	Burkholderiales	Comamonadaceae	*Leptothrix*	Operating	1.29 ± 1.02 a	2.23 ± 1.54 b	2.2 ± 1.12 b	2.38 ± 1.64 b
				Non-Operating	2.13 ± 1.21 a	2.15 ± 1.03 a	1.98 ± 1.2 a	1.96 ± 1.19 a
ASV5	Acetobacterales	Acetobacteraceae	*Acidiphilium*		1.35 ± 1.47 a	1.36 ± 0.98 a	1.94 ± 1.72 a	1.52 ± 1.04 a
ASV6	Burkholderiales	Burkholderiaceae	*Caballeronia*		0.91 ± 1.31 a	1.13 ± 0.89 ab	1.4 ± 1.18 b	1.65 ± 1.17 b
ASV7	Burkholderiales	Comamonadaceae	*Rhizobacter*		1.21 ± 0.81 a	1.07 ± 0.68 a	1.32 ± 0.74 a	0.97 ± 0.66 a
ASV8	Acetobacterales	Acetobacteraceae	*NA*		0.61 ± 0.8 a	0.88 ± 0.77 a	1.37 ± 1.44 a	1.04 ± 0.91 a
ASV10	Acidobacteriales	Acidobacteriaceae	*Terriglobus*		0.67 ± 0.57 a	0.97 ± 1.59 a	0.93 ± 0.63 a	0.97 ± 0.71 a
ASV12	Burkholderiales	Comamonadaceae	*Rhizobacter*		0.85 ± 0.9 a	0.77 ± 0.58 a	0.97 ± 0.7 a	0.72 ± 0.56 a
ASV14	Acidobacteriales	Acidobacteriaceae	*Granulicella*		0.53 ± 0.38 a	0.76 ± 0.64 a	0.56 ± 0.33 a	0.7 ± 0.35 a
ASV15	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*		0.59 ± 0.44 ab	0.78 ± 0.55 b	0.6 ± 0.5 ab	0.48 ± 0.37 a
ASV16	WD260	NA	*NA*		0.39 ± 0.37 a	0.39 ± 0.37 a	0.53 ± 0.46 a	0.66 ± 0.6 a
ASV17	Sphingomonadales	Sphingomonadaceae	*Sphingomonas*	Operating	0.51 ± 0.63 a	0.44 ± 0.33 a	0.78 ± 0.81 a	0.46 ± 0.35 a
				Non-Operating	0.57 ± 0.33 ab	0.53 ± 0.44 ab	0.38 ± 0.34 a	0.71 ± 0.45 b
ASV18	WD260	NA	*NA*		0.39 ± 0.39 a	0.37 ± 0.26 a	0.54 ± 0.42 a	0.7 ± 0.77 a

Table S3.11 continued

ASV20	Acidobacteriales	Acidobacteriaceae	*Granulicella*		0.39 ± 0.37 a	0.48 ± 0.42 a	0.41 ± 0.27 a	0.44 ± 0.32 a
ASV21	Acidobacteriales	Acidobacteriaceae	*Granulicella*	Operating	0.14 ± 0.16 a	0.21 ± 0.24 a	0.4 ± 0.44 ab	0.7 ± 0.69 b
				Non-Operating	0.49 ± 0.42 a	0.55 ± 0.43 a	0.6 ± 0.4 a	0.54 ± 0.42 a
ASV22	Rhizobiales	Beijerinckiaceae	*1174-901-12*		0.41 ± 0.36 a	0.49 ± 0.39 ab	0.6 ± 0.47 ab	0.66 ± 0.44 b
ASV23	Acidobacteriales	Acidobacteriaceae	*Granulicella*		0.46 ± 0.42 a	0.39 ± 0.27 a	0.38 ± 0.37 a	0.4 ± 0.35 a
ASV25	Acidobacteriales	Acidobacteriaceae	*Terriglobus*		0.41 ± 0.47 a	0.44 ± 0.44 a	0.66 ± 0.71 b	0.5 ± 0.51 ab
ASV35	Acidobacteriales	Acidobacteriaceae	*Granulicella*	Operating	0.16 ± 0.14 a	0.3 ± 0.26 ab	0.57 ± 0.4 c	0.44 ± 0.29 bc
				Non-Operating	0.36 ± 0.26 a	0.36 ± 0.22 a	0.29 ± 0.28 a	0.46 ± 0.39 a
ASV37	Acetobacterales	Acetobacteraceae	*NA*		0.31 ± 0.28 a	0.35 ± 0.42 a	0.44 ± 0.34 a	0.39 ± 0.38 a
Fungal ASV								
ASV2	Pleosporales	Sporormiaceae	*Sporormiella*		2.15 ± 3.66 a	3.76 ± 8.04 a	2.84 ± 6.2 a	3.53 ± 8.13 a
ASV3	Kriegeriales	Kriegeriaceae	*Phenoliferia*		1.64 ± 1.64 a	2.27 ± 1.67 a	2.71 ± 3.03 a	2.06 ± 2.36 a
ASV4	Chaetothyriales	NA	*NA*		2.38 ± 3.14 a	1.73 ± 2.5 a	2.51 ± 2.38 a	1.95 ± 1.99 a
ASV5	Kriegeriales	Kriegeriaceae	*Phenoliferia*		1.52 ± 2.08 a	1.39 ± 1.39 a	1.52 ± 1.28 a	1.44 ± 1.49 a
ASV6	NA	NA	*NA*		1.24 ± 1.43 a	1.56 ± 1.94 a	1.05 ± 1.09 a	1.13 ± 1.5 a
ASV10	Kriegeriales	Kriegeriaceae	*Phenoliferia*	Operating	1.57 ± 1.69 b	1.43 ± 1.12 b	1.15 ± 0.99 b	0.5 ± 0.53 a
				Non-Operating	0.73 ± 1.42 a	0.66 ± 0.91 a	0.33 ± 0.35 a	0.53 ± 0.52 a
ASV13	Spizellomycetales	Powellomycetaceae	*Thoreauomyces*	Operating	0.37 ± 0.47 ab	0.42 ± 0.52 a	1.05 ± 1.02 bc	1.05 ± 1.07 c
				Non-Operating	0.67 ± 0.53 a	1.01 ± 0.8 a	0.85 ± 0.87 a	1.04 ± 1.32 a
ASV21	Spizellomycetales	NA	*NA*		0.71 ± 0.76 a	0.65 ± 0.9 a	0.63 ± 1.13 ab	0.32 ± 0.74 b
ASV22	Capnodiales	Cladosporiaceae	*Cladosporium*		0.93 ± 1.42 b	0.61 ± 0.7 ab	0.37 ± 0.34 ab	0.4 ± 0.4 a
ASV23	Chaetothyriales	Epibryaceae	*Epibryon*		0.4 ± 0.38 a	0.63 ± 0.73 a	0.53 ± 0.51 a	0.51 ± 0.48 a

Table S3.11 continued

ASV26	Chaetothyriales	NA	*NA*	0.39 ± 0.68 a	0.4 ± 1.06 a	0.59 ± 1.04 a	0.58 ± 1.09 a
ASV28	Capnodiales	NA	*NA*	0.42 ± 0.53 a	0.43 ± 0.49 ab	0.48 ± 0.43 ab	0.54 ± 0.52 b
ASV32	Dothideales	Dothioraceae	*Hormonema*	0.39 ± 0.54 a	0.34 ± 0.34 a	0.51 ± 0.63 a	0.51 ± 0.73 a
ASV43	Helotiales	Hyaloscyphaceae	*Polydesmia*	0.24 ± 0.7 a	0.29 ± 0.36 ab	0.47 ± 1.12 ab	0.72 ± 1.06 b
ASV54	Chaetothyriales	Herpotrichiellaceae	*Capronia*	0.29 ± 0.32 a	0.33 ± 0.46 a	0.26 ± 0.24 a	0.44 ± 0.44 a
ASV56	NA	NA	*NA*	0.22 ± 0.28 a	0.35 ± 0.45 a	0.43 ± 0.45 a	0.41 ± 0.59 a
ASV66	Dothideales	Aureobasidiaceae	*Aureobasidium*	0.77 ± 1.47 b	0.27 ± 0.73 ab	0.09 ± 0.13 a	0.11 ± 0.16 a
ASV69	NA	NA	*NA*	0.25 ± 0.43 a	0.24 ± 0.38 a	0.26 ± 0.36 a	0.26 ± 0.39 a
ASV95	Pleosporales	Pleosporaceae	*Alternaria*	0.27 ± 0.5 b	0.26 ± 0.64 ab	0.09 ± 0.1 ab	0.11 ± 0.17 a
ASv116	Orbiliales	Orbiliaceae	*NA*	0.13 ± 0.22 a	0.19 ± 0.24 a	0.18 ± 0.2 a	0.13 ± 0.18 a

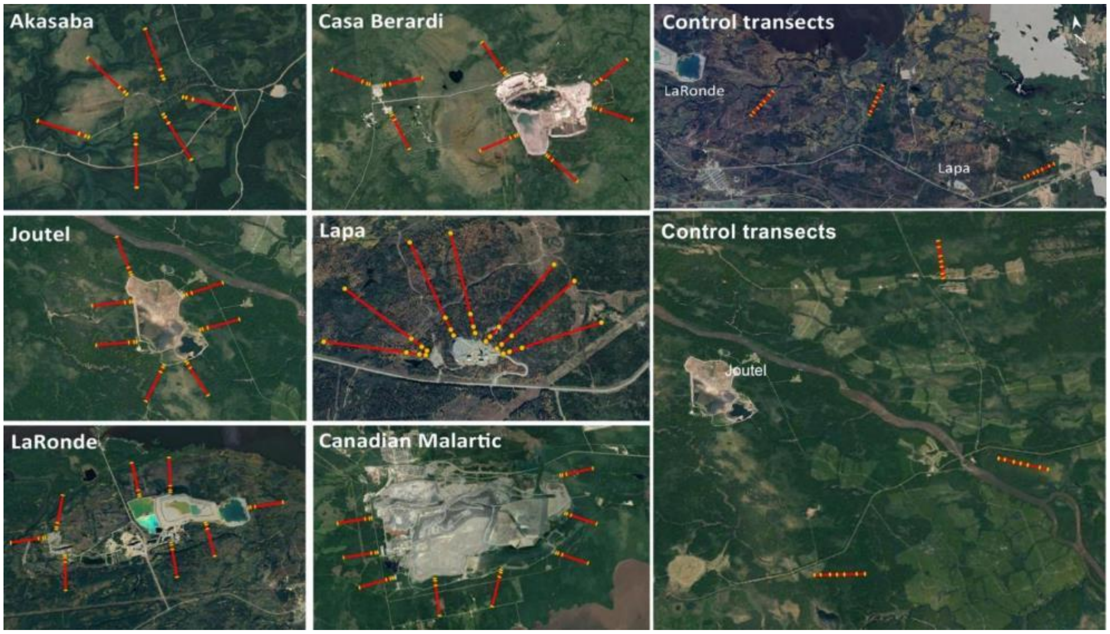


Figure S3.1 Maps of the study sites and six selected mine sites and controls showing the position of transects. Six selected mine sites and controls: Akasaba site, 6 transects, 24 plots; Casa Berardi site, 8 transects, 32 plots; Joutel site, 7 transects, 28 plots; Lapa site, 7 transects, 28 plots; LaRonde site, 8 transects, 32 plots; Canadian Malartic site, 8 transects, 32 plots; Control transects, 6 transects, 44 plots. Scales are not shown because the 1 km transects can be used as a reference in each map.

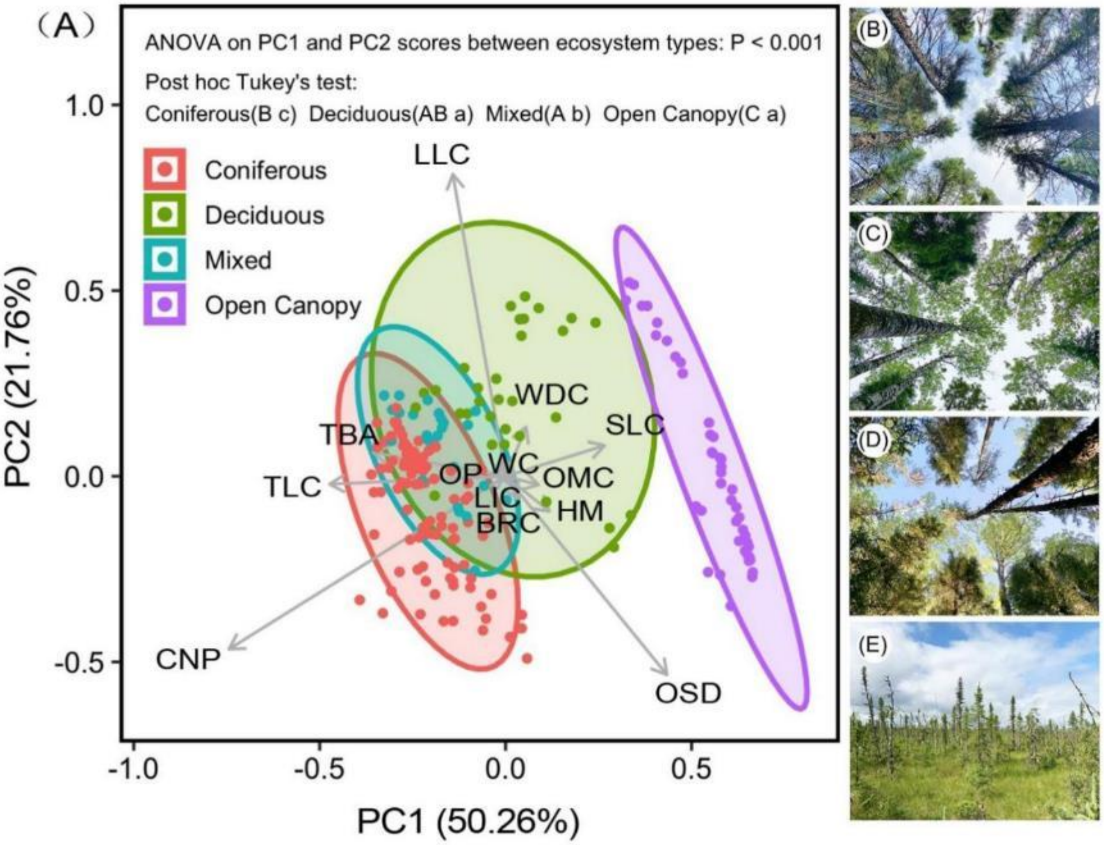


Figure S3.2 Biplot of principal component analysis (PCA) of forest structures and environmental variables across plots and forest types. Each ecosystem type (coniferous (B), deciduous (C), mixed forest (D) and open canopy ecosystem(E)) is represented by a different color and ellipse, and all plots are displayed (small circles). SLC: live crown ratio for sapling trees; TLC: live crown ratio for canopy trees; SBA: base areas for sapling trees; TBA: base areas for canopy trees; OP: Openness; LLC: leave litter ground coverage; WDC: woody debris ground coverage; WC: water ground coverage; RC: rock ground coverage; OMC: organic matter ground coverage; OSD: depth of organic soil; CNP: conifer proportion; HM: humility; BRC: bryophyte ground coverage; LIC: lichen ground coverage.

ANNEXE A

LES RISQUES ENVIRONNEMENTAUX POTENTIELS ASSOCIÉS AU DÉVELOPPEMENT DE LA PRODUCTION D'ÉLÉMENTS DE TERRES RARES AU CANADA

THE POTENTIAL ENVIRONMENTAL RISKS ASSOCIATED WITH THE DEVELOPMENT OF RARE EARTH ELEMENT PRODUCTION IN CANADA

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A1.1 Résumé

Le développement de la production de métaux du groupe des terres rares (MTR) au Canada pourrait générer des avantages économiques importants, mais il présente aussi des risques sérieux pour l’environnement. Les métaux du groupe des terres rares ont été largement utilisés dans la vie moderne et les industries, et ils sont même indispensables dans certaines technologies de pointe essentielles (par exemple, les aimants permanents). La demande croissante et les tensions commerciales actuelles entre les États-Unis et la Chine offrent des occasions de développement économique commercial au Canada, qui dispose de riches ressources en MTR, pour développer son propre secteur. Cependant, les problèmes d’environnement et de santé causés par la production de MTR constituent des défis auxquels le Canada doit faire face, puisque des impacts environnementaux importants ont été rapportés ailleurs (par exemple en Chine). Il existe peu de documentation sur les risques environnementaux potentiels associés au développement de la production de MTR au Canada. Il est important de savoir quels problèmes environnementaux, en particulier ceux générés par les MTR eux-mêmes, pourraient survenir au Canada dans le futur. Par conséquent, trois aspects majeurs sont évalués et résumés à partir de perspectives multidisciplinaires dans cet article : (1) un modèle conceptuel général du transport des MTR en tant que groupe dans l’environnement est établi ; (2) les niveaux de toxicité, les mécanismes biochimiques et les effets physiologiques des MTR sur différents organismes sont examinés, et des études de cas provenant de zones d’exploitation minière des MTR existantes sont brièvement mises en évidence ; et (3) en tenant compte des conditions environnementales spécifiques et des facteurs de risque, les risques environnementaux auxquels le Canada pourrait être confronté dans les développements futurs des MTR sont identifiés et discutés. Cette synthèse se termine par une macro-identification des risques environnementaux potentiels associés au développement de la production de MTR au Canada, en tenant compte de la santé humaine et écologique. Les auteurs notent que l'ingestion, l'inhalation et l'exposition cutanée chez les travailleurs et les résidents avoisinants (y compris les communautés autochtones potentielles), ainsi que les conditions climatiques subarctiques et arctiques pourraient accroître les risques pour la santé humaine et écologique dans le développement futur de la production de MTR au Canada. Enfin, des orientations de recherche futures sont proposées qui pourraient être appliquées à la fois au contexte canadien et à d'autres contextes géographiques.

Mots-clés : lanthanides, industrie minière, contaminants émergents, risques écologiques, régions boréales.

A1.2 Abstract

The development of rare earth element (REE) production in Canada could generate significant economic benefits, but also poses serious potential risks to the environment. Rare earth elements have been widely used in modern life and industries, and even are indispensable in some crucial advanced technologies (e.g., permanent magnets). Increasing demand and the context of current US-China trade tensions provide a commercial economic development opportunity for Canada, which has rich resources of REEs, to develop its own sector. However, environmental and health issues caused by REE production are challenges Canada has to face, given that significant environmental impacts have been reported elsewhere (e.g., China). Little literature is available on the potential environmental risks associated with the development of REE production in Canada. It is important to know what environmental issues, particularly those generated by REEs themselves, may happen in Canada in the future. Therefore, three major aspects are evaluated and summarized from multidisciplinary perspectives in this paper: 1) a general conceptual model of the transport of REEs as a group in the environment is established; 2) toxicity levels, biochemical mechanisms, and physiological effects of REEs on different organisms are reviewed, and case-studies from existing REE mining areas are briefly highlighted; and 3) Considering specific environmental condition and risk factors, environmental risks Canada may face in future REE developments are identified and discussed. This review concludes with a macro-identification of potential environmental risks associated with the development of REE production in Canada considering both human and ecological health. We note that ingestion, inhalation and dermal exposure for workers and surrounding residents (including potentially indigenous communities), and sub-arctic/arctic climate conditions could increase the risks to human and ecological health in future REE production development in Canada. Finally, future research directions are proposed that could be applied to both Canadian and other geographical contexts.

Key words: lanthanides, mining industry, emerging contaminants, ecological risks, boreal areas

A1.3 Introduction

Increasing demand for rare earth elements (REE) is stimulating an increasing number of production projects outside of China, which is currently the largest global REE producer. Several countries are planning to develop their own REE industry including Canada, which holds rich REE reserves representing about 7% of raw global resources (Zhou et al. 2017). There are large potential economic and strategic advantages for Canada in the future development of the REE industry, especially in the context of current US-China trade tensions and the economic crisis caused by COVID-19. However, REE industries can produce serious health and environmental issues, such as REE bioaccumulation (Li et al. 2014), radiation exposure (Ault et al. 2015), species invasion (Boojar and Tavakkoli 2011) and biodiversity loss (Degtjarenko et al. 2016) (see Figure A1.1). Many of these environmental issues are also present in other types of mining activities, but radioactive pollution and REE toxicity are specific environmental risks associated with REE production that are attracting more attention (Gwenzi et al. 2018; Ma et al. 2019).

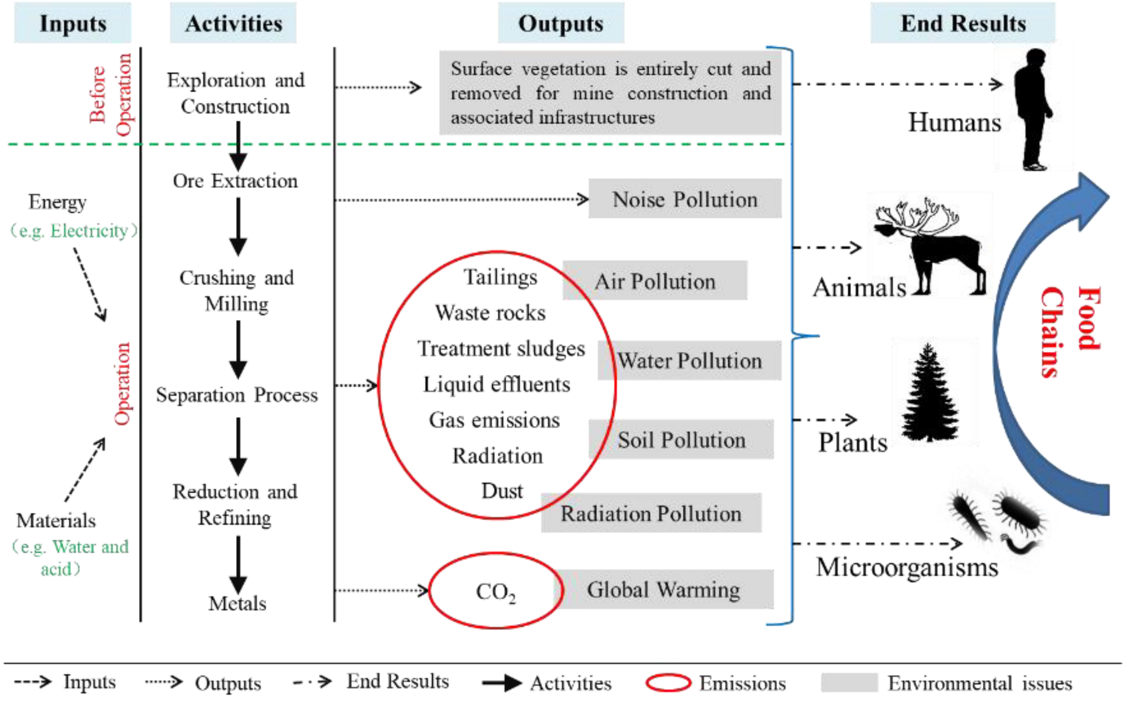

Figure A1.1 The conceptual model of the environmental issues associated with REEs production (Peiró and Méndez, 2013; Edahbi et al., 2019; Pell et al., 2019).

REE mining and processing affect the geochemical behaviour and fate of REEs in the environment, which can lead to them becoming toxicants (Figure A1.1). Firstly, products and tailings containing residual REEs generated production directly increase the content of REEs in surface environments. Secondly, mining activities could change natural soil and water physicochemical conditions (e.g., pH and redox states). Acidity can solubilize otherwise stable REE fractions, increasing their bioavailability and migration in the environment. As a result, high concentrations of REEs have been detected in soils (Liu et al. 2019), vegetables (Li et al. 2013) and other crops (Zhuang et al. 2017) and even human bodies (Li et al. 2014) near mining and processing operations. The number of REE-contaminated areas is expected to steadily increase over the next decades with increasing REE production. Increasing REE accumulation in organisms has the potential to exceed organisms' toxicity thresholds, therefore causing health damage (e.g., growth inhibition or illness). Due to increasing numbers of exposure sources, such as tailings, wastewater and dust from mining, e-waste, REE-containing products and medical applications (Pagano et al. 2015b; Gwenzi et al. 2018; Pagano et al. 2019), the toxicity of REEs is receiving increased attention (Sauvé and Desroirers 2014; Gwenzi et al. 2018).

The development of REE production in Canada will increase REE concentrations in the environment in the coming decades. This review focuses on the associated potential risks to ecological and human health in Canada. Increased REE development adversely affecting ecosystem services has been reported in those countries with REE mining and associated processing and downstream industries (Balaram 2019, Ali 2014, Yang et al. 2013), thus the development of REE sectors will pose new environmental risks in Canada. However, few primary studies have been carried out in Canada related to the environmental concerns associated with REE production. Given that over 50 REE projects are in development in Canada (Orris et al. 2018), it is urgent to review and summarize knowledge on REE transfer and toxicity, with an integration of the characteristics of Canadian landscapes. Here, a broad range of studies on the environmental risks of REE production are reviewed, Canada-specific contexts are evaluated, potential health and ecological risks in Canada are presented, and future research directions are proposed.

A1.4 Introduction to REEs and their uses

REEs include 15 lanthanide elements in the periodic table as well as yttrium (Y) and scandium (Sc) as defined by the International Union of Pure and Applied Chemistry (IUPAC). REEs were considered as non-essential elements for organisms (Pang et al. 2002) until the discovery of a lanthanide-dependant enzyme for methane metabolism of methanotrophic bacteria found in extreme acidic environments (Pol et al. 2014, Picone & den Camp, 2019). According to their atomic numbers, these 17 chemical elements can be divided into heavy rare earths (HREEs) of terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), lutetium (Lu), and yttrium (Y), as well as light rare earth elements (LREEs) of lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), promethium (Pm), samarium (Sm), europium (Eu), gadolinium (Gd) and scandium (Sc) (Haque et al. 2014). The REEs share chemical and physical characteristics and therefore, they are considered as a geochemically coherent group. The configuration of the valence electrons of the outermost shell is the same for lanthanides, and most of them are electropositive (+3), which results in their unique phosphorescent, magnetic, and catalytic properties (Balaram 2019). They are widely used in magnets, catalysts, alloys, phosphors, and glass production and in polishing (reviewed by Balaram, 2019; Haque et al. 2014; Weng et al. 2013). Furthermore, REEs are used as antibiotic feed additives in animal feed and fertilizers in agricultural production (Pang et al. 2002; Abdelnour et al. 2019).

A1.5 Specific environmental issues associated with REE production

REE mineral deposit types can strongly influence environmental impacts, as processing methods vary based on the physico-chemical properties of deposit (Weng et al. 2013). Significant use of chemicals (e.g., acids and alkalis), REE content in tailings and radioactive element (Th and U) concentration in ores are arguably the most common concerns (Weng et al. 2013). REE in mineral deposits are usually hosted by a wide and diverse range (more than 200) of REE-bearing minerals (the main REE-bearing minerals are reviewed by Weng et al. 2013 and Batapola et al. 2020). The REE deposits exist primarily in four geologic environments (Weng et al. 2013; Dostal, J. 2017): carbonatites, alkaline igneous systems, ion-absorption clay deposits (mainly in China), and monazite-bearing placer deposits. Carbonatite and alkaline rocks constitute the majority of the world REE resources, but LREEs are relatively more abundant in carbonatites than HREEs, and alkaline igneous rocks have relatively low total REE grades and contain small amounts of U and Th. In Canada, the major REE-containing mineral types are carbonatite and alkaline igneous (the mineral types and geological province are listed in Table A1). Therefore, both the generation of mine tailings and the potential release of radionuclides are environmental risks for REE development in Canada.

The principal flow sheets of REE production includes five steps: 1) Mining of REE-bearing mineral ore including silicates, fluor carbonates, oxides/hydroxides, and phosphates; 2) Beneficiation of rare earth ore mixtures; 3) Separation of REE ores into individual REE minerals; 4) Reduction of REE minerals to individual REE metals; 5) Refining of individual REE metals to purify REEs (reviewed by Chi et al, 2004, Khan et al. 2017 and Wu et al. 2018). REE production and processing (mainly milling and smelting operations) are characterized by high energy and resource consumption, and high levels of pollution, as well as complicated sequential processes compared with other metals (Balomenos et al. 2017; Haque et al. 2014). For example, the extraction of 1 tonne of REE requires 4.41 tonnes of sulphuric acid, 12.32 tonnes sodium chloride, 1.64 tonnes of sodium hydroxide, 1.17 tonnes of hydrochloric acid, 1.90 tonnes of water, and milling of almost 50 tonnes of mineral ore at Bayan Obo in Mongolia, and generally, the energy used to obtain 1 tonne of an individual REE varies from 38 GJ to 48 GJ, except for scandium (148 GJ/tonne) and yttrium (75 GJ/tonne) (Peiró, & Méndez, 2013). Many potential environmental and health issues are generated by this processing with significant radionuclides, heavy metals and acids in water discharge, tailings and air emissions, as well as a high energy cost.

The radioactive pollution and REE toxicity caused by mining and processing are unique environmental risks that are different from most other mining contexts. The radioactive pollution generated during REE production is mainly caused by the co-occurrence of REE deposits with radioactive elements such as U (uranium), Th (thorium), and their decay products (e.g., Radium) (Golev et al. 2014). The natural radionuclide content of the main REE minerals was summarized by García et al. (2020). Usually, there is a higher content of radioactive elements in the environment near a REE mining site. For example, the mean content of airborne Th in total suspended particulate ranged from 820 μBq m^{-3} in mining areas to 39,720 μBq m^{-3} in smelting areas in the Bayan Obo mining region, far higher than global background level of 0.5 μBq m^{-3} (Wang et al. 2016). As a result, biota near REE mines has higher health risks from exposure to high levels of radioactive elements. While radioactive pollution has been an important issue receiving attention in public health for some time, the direct toxicity of REEs is often overlooked because of the insignificant amounts found in the environment naturally, and the benefits of REEs in agricultural production. The growth in REE extraction and manufacturing has led to a strong increase of REE concentration in localized surface environments (Tepe et al. 2014; Hatje et al. 2016). For example, the concentrations of REEs in the Sidaosha River (passing through Baotou City where the Bayan Obo REE deposit is located) exceed 30,000 μg/L of suspended particles and surface sediments, respectively, which was 200 times higher than the average value of REEs of other rivers in Northern China (Liang et al. 2014). As emerging contaminants, their toxicity is attracting more attention (Gwenzi et al. 2018; Ma et al. 2019), especially in countries like Canada planning major REE exploitation projects in the near future.

A1.6 The transport of REEs in the environment

The mobility of REEs and their movement in the environment are similar to other potentially toxic metals and metalloids (e.g., Arsenic and Mercury) driven by three main mechanisms: 1) sorption-desorption, 2) precipitation-dissolution and 3) reduction-oxidation processes (Violante et al. 2010; Cao et al. 2001). Soil and solution components (e.g., ligands, anions, clay minerals, colloids and suspended matter), pH, and redox condition are considered as critical environmental parameters controlling these three mechanisms as they influence the transformations among REE fractions (see Table A1S1). Generally, REEs in the environment can be partitioned into six fractions based on their geochemical behaviours (see Table A1S2, Tessler et al. 1979; Cao et al. 2000): water soluble (R(ws)), ion-exchangeable (R(ie)), bound to carbonates (R(cb)), bound to iron and manganese oxides (R(fm)), bound to organic matter (R(om)), and residual (R(rd)). REE bioavailability and potential migratory capacity depends upon their fraction, ranked from strong to week as follows: R(ws) and R(ie)> R(cb)> R(fm)> R(om) > R(rd) (Wen et al. 2013). R(ws) and R(ie) are quite mobile and bioavailable to biota (Lu et al. 2013). In natural environments, R(rd) is a major form, which is stable and cannot be absorbed directly by organisms (Pang et al. 2002), but REEs exist in more active states in mining areas, such as R(ie) in soils and dissolved R(ws) in water bodies (Liang et al. 2014). Mining activities disrupt the spatial distribution and natural state of minerals, including notably turning deposits into smaller unconsolidated particles with a relatively higher specific surface area. More REE minerals are transported from subsurface to surfaces and these surficial mining areas become enriched in total REEs. Those REE minerals significantly increase the source supply of REEs in the earth's surface by exposing them to much higher rates of weathering and erosion (e.g., rain, wind, microbial decomposition) than seen in more consolidated subsurface locations. A conceptual model based on recently published literature (mainly adapted from Gwenzi et al. 2018) is presented in Figure A1.2. This model shows that hydrological and wind-driven (e.g., weathering, dust and atmospheric fallout) processes, as well as human activities (e.g., REE mining and international trade) play a key role in environmental mobilization and transport of REEs. At last, additional REEs by mining mostly settle into soil and water, leading to increased exposure for terrestrial and aquatic food webs.

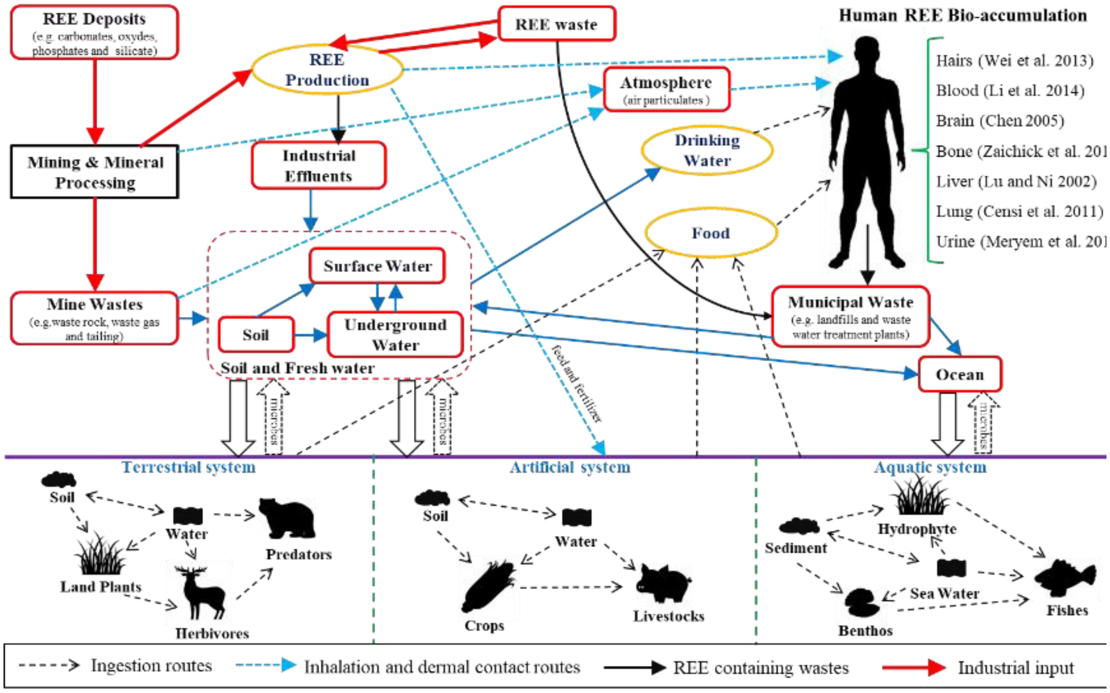

Figure A1.2 The conceptual model of the sources of REE inputs and their fate in the environment. Terrestrial system (MacMillan et al. 2017), Artificial System (Jiang et al. 2012), Marine system (Li et al., 2016).

REEs in soil can enter plant cells through soil-plant pathways. For most plants, REE concentrations generally follow the order of root > leaf > stem > flower > fruit (He et al. 2019a; Hu et al. 2004; Xu et al. 2002), but close to REE mining or other polluted areas, the REE content is higher in leaf surfaces because of dust fall (Zhuang et al. 2017). Water is the main exposure intermediary for aquatic organisms. Increased REEs by mining can enter adjacent aquatic systems through industrial and domestic wastewater as well as surface run-off, and then deposit in sediments or remain distributed in the water column depending on particle size and makeup and mixing regimes (Chua, 1998; Liang et al. 2014). For example, increased usage in Gd-based contrast agents (Gd-CA) for magnetic resonance imaging contributed to a more than one order of magnitude increase in anthropogenic Gd concentrations in San Francisco

Bay (from 8.27 to 112 pmol kg^{-1}) over the two decades from 1993 to 2013 (Hatje et al. 2016).

Ingestion, inhalation and dermal contact are the three main pathways for the entry of REEs in humans (Figure A1.2). Ingestion is mainly associated with REE-contaminated food and drinking water and medicine containing REEs. Gd-CA are a prominent cause of REE ingestion, and some gadolinium accumulation in brain and liver tissues have been reported (Kanda et al. 2015; Frenzel et al. 2017). On the other hand, most of Gd-CA are excreted and released into waste water systems and then are discharged into surface and groundwater, potentially subsequently reaching back to drinking water supplies (Aime and Peter, 2009; Bichler et al. 2016; Schmidt et al. 2019). Inhalation of airborne dust containing REEs is another pathway by which they can enter the human body. High concentrations of REEs (56.9 to 165.4 μg L^{-1}) were found in lung fluids of people working in REE associated industries (Censi et al. 2011). Further, REEs can enter the human body through dermal absorption (Sun et al. 2017), for example, through bathing using water containing high REEs and exposure to REE dust. Current research shows that REEs can accumulate in various human tissues: hair (Wei et al. 2013), blood (Li et al. 2013), brain (Chen et al. 2005), bone (Zaichick et al. 2011), liver (Chen & Zhu, 2009) and lungs (Censi et al. 2011). Risks are not explicitly well understood though, and it is likely that there could be subtle positive and negative health effects due to these emerging contaminants, as well as the largely negative oxidative stress effects that have been reported (Pagano et al. 2015).

A1.7 A review of REE toxicity on organisms

A1.7.1 Method

A literature review of published, peer-reviewed studies on the REEs affecting the environment available in Scopus (https://www.elsevier.com/solutions/scopus) was done in two steps, first in November 2019 and in January 2021 to update the review. Searches were conducted using the combined following keywords: “terbium” OR “dysprosium” OR “holmium” OR “erbium” OR “thulium” OR “ytterbium” OR “lutetium” OR “yttrium” OR “lanthanum” OR “cerium” OR “praseodymium” OR “neodymium” OR “promethium” OR “samarium” OR “europium” OR “gadolinium” OR “scandium” OR “lanthanide” OR “rare earth element*” AND “health risk∗” OR “health impact∗” OR “ toxicity*” OR “animals*” OR “plant*” OR “human*” OR “microbiome*” OR “environment*” OR “bacteria*” OR “fungus*” Also, Google Scholar (http://scholar.google.com) was searched to ensure as many publications as possible were collected, and 27,580 references were acquired from these searches. In a second screening stage eligibility and exclusion criterion were determined. First with regards to literature type, only published peer-reviewed journal articles of primary research and reviews were selected. Second, in order to avoid any confusion and difficulties of translation, only on articles published in English were kept. Thirdly, with regards to timeframe, articles published up to 2020 were selected to see the potentian growth/evolution of research and related publications. As the review process focused on REE toxicity to organisms, the subject areas were restricted in “biochemisty, genetics and molecular biology”, “environmental science”, “agricultural and biological sciences” and “immunology and microbiology”. Titles, keywords and abstracts were reviewed and screened following the above criteria. Finally, 1,228 articles remained and were used for the analysis of publication volume and subsequently classified into five topics of humans, plants, animals, microbiome and “others” (review articles and those about abiotic effects/environments).

A1.7.2 State-of-the-art in REE-associated environmental risks/effects

The number of published studies on REE environmental risks and impacts shows an increasing trend with time (Figure A1.3), with a faster pace since 2011, likely because of the higher number of REE projects recently undertaken outside of China.

The attention received by different target species or biotic groups in these studies is clearly uneven. The number of annual published articles generally followed the order of plants > animals > humans > microorganisms. Initially most articles were about plants and animals, then the effect of REEs on humans and microorganisms became the center of focus in an increasing number of publications. However, research on humans and microbes is still less abundant than for animals and plants up to 2020.

Review articles about environmental issues associated with REE production were focused on ecological risks (mainly animal and plant health), human health, and toxicity mechanisms of REEs. However, little information has been synthesized on environmental and individual factors that influence REE toxicity (Figure A1S1).

A1.7.3 The toxic effects of REEs on different target biota

Multifaceted problems caused by REEs have been reported by a body of literature (shown in Figure A1.3) and publications about REE-associated health effects mainly include acute toxicity and bio-accumulation studies. Globally, these studies have shown that when the content of REEs in organisms exceeds the background concentration or toxic threshold, there is a potential risk to the health of ecosystems and purportedly to humans.

REEs hormetic dose-response is the core of the toxicity research on various life forms (reviewed by Agathokleous et al. 2018; Agathokleous et al. 2019; Pagano et al. 2015a) and is characterized by biphasic concentration-related effects: low doses of REEs are beneficial for organisms, but inhibitory or toxic effects are present at high levels (Agathokleous et al. 2018; Pagano et al. 2015). For example, La^{3+} at doses of 0 - 25 μM increased shoot biomass of maize plants associated with increased photosynthetic rates and chlorophyll index values, but at a higher level (above 25 μM), photosynthesis was inhibited (Carolina et al. 2018). The mechanism of hormetic effects of REEs is still not clear, but the chronic accumulation of these elements in the environment could exceed the toxicity threshold of REEs.

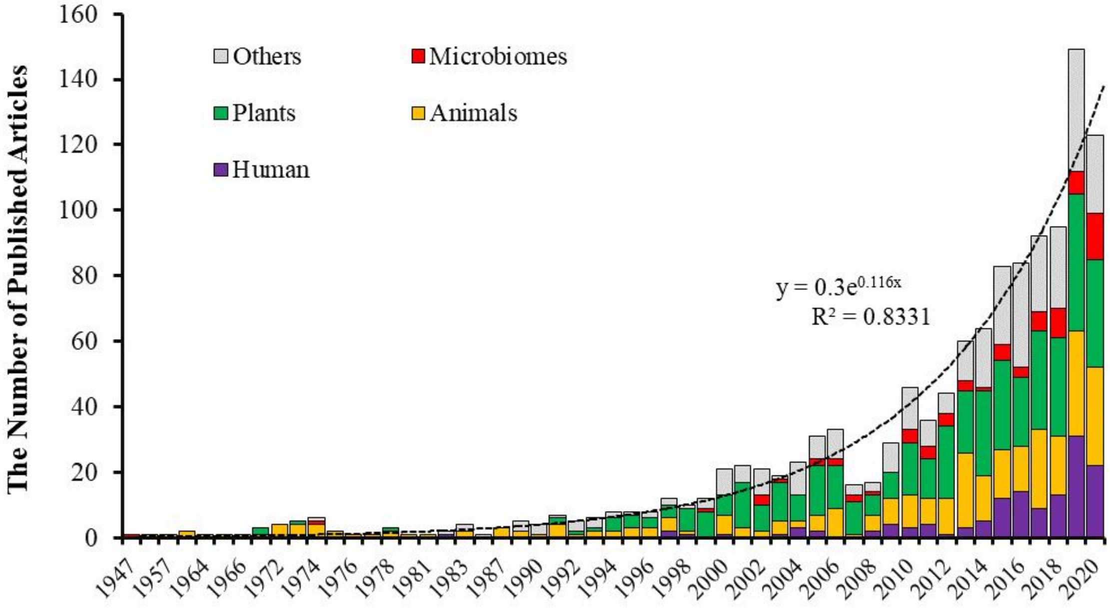

Figure A1.3 Annual rate of publication of journal articles on REE-associated environmental risks/effects for different groups of biotas.

LC50 (lethal concentration 50%) and EC50 (median effective concentration) are statistically derived doses used to evaluate toxicity threshold in the biotic ligand model (BLM) (Smith et al. 2015). Lower LD50 (Lethal Dose 50%)/LC_{50} value indicates higher acute toxicity. The toxicity threshold (LC50 and EC50) of REEs on aquatic organisms has been reviewed by Malhotra et al. (2020) and toxicity-modifying factors such as pH, dissolved organic carbon (DOC), temperature and potential competitors (e.g., Ca^{2+}) have a main impact on the bioavailability and toxicity of REEs to aquatic organisms. However, there is a significant gap in knowledge about LC50 and EC50 of REEs in some special exposure conditions (e.g., Canadian boreal and arctic environment) and in non-aquatic organisms. On the other hand, the lowest observable effect concentrations (LOECs) are used in chronic or long-term ecotoxicological studies, which is more helpful to better understand the actual ecotoxicological risk induced by REEs on the environment (Cardon et al. 2019). The traditional toxicity model using dissolved concentrations as the dose descriptor without considering its bioavailability, therefore failed to accurately predict the toxicity of REEs (Zhao et al. 2015; Gong et al. 2019b). REEs in the 3+ oxidation state are considered to be the dominant toxic chemical species and its concentration is a good indicator of toxicity (Aharchaou et al. 2020), therefore the LC50 and EC50 based on free ion activities should be widely used instead of dissolved concentrations. For example, Gong et al. (2019b) determined the EC50 of Ce and Y on the root elongation of wheat (*Triticum aestivum*) and found the values of EC50 of dissolved concentration were far higher than those of free-ion concentration: EC50[Y_{diss}] =6.08 μM and EC50[Ce_{diss}] =7.11 μM; EC50[Y_{ion}] =2.05 μM and EC50[Ce_{ion}] =2.28 μM. Furthermore, the analytical techniques for chemical characterization of REEs are also a key factor in determining data quality (Filella and Rodushkin, 2018) and these techniques have been reviewed by Gorbatenko and Revina (2015). Inductively coupled plasma mass spectrometry (ICP-MS) and instrumental neutron activation analysis (INAA) are commonly used for REE determination in environmental samples because of their multi-element capability, ease of operation, high sensitivity and accuracy.

The significant toxicity of high concentrations of REEs to plants, animals and microorganisms has been reviewed by Abdelnour et al. (2019) and Adeel et al. (2019), and effects have been identified on cell division (Kotelnikova et al. 2019), gene expression (Dubé et al. 2019, Qi et al. 2019), blood variables (Adua et al. 2013), photosynthesis (Xu et al. 2019, Zicari et al. 2018), metabolic capacity (Henriques et al. 2019, Pinto et al. 2019), reproductive capacity (Zhang et al. 2014), oxidative stress (Oral et al. 2017; Henriques et al. 2019) and various growth indices (Xu et al. 2019, recent studies listed in Table A1S3).

Current literature has found some human health problems caused by REEs (reviewed by Gwenzi et al. 2018, Adeel et al. 2019). Excess REEs have adverse impacts on organ function, as illustrated in Figure A1.4. In addition, interactive effects between REEs and other heavy metals have been identified. For example, Y was shown to increase the toxicity of chromium on oxidative damage to DNA in the blood of workers in a chromate plant (Bai et al. 2019). REEs and other heavy metals are usually concomitantly present in the environment, therefore, their interactive effects should be considered in environmental risk assessments.

The existing data to date on REE toxicity are mainly based on controlled experiments or laboratory tests, and although useful, typically only show acute effects in isolation (i.e., without multiple stressors). REE toxicity cases from operating REEs mining projects are summarized based on Scopus and the China National Knowledge Infrastructure (CNKI) database (see Table A1A2), which may facilitate better identification of potential environmental and health risks for Canada. We found that nearly all cases are from China and concentrated in Inner Mongolia where the largest LREE mines are located and Jiangxi with the largest HREE mines on the globe. Most of these studies focused on soil, water, human health and food, but data about wildlife is limited.

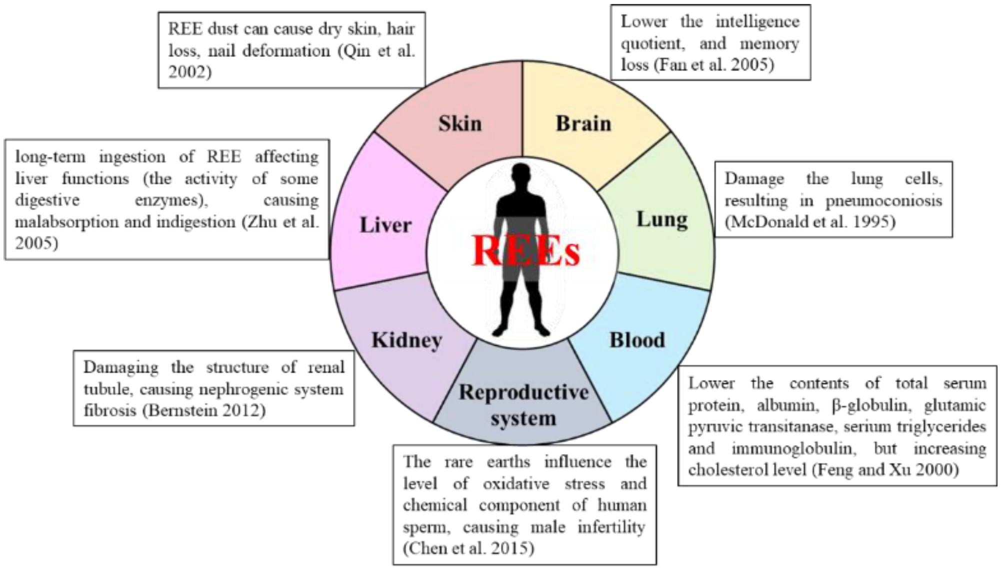

Figure A1.4 Effects of REEs on human organs/systems.

A1.7.4 The mechanisms and influential factors of REEs toxicity

Based on the collected published literature, the mechanisms of REEs toxicity and main environmental and individual factors influencing toxicity are summarized in Table A1.1 and A1.2, respectively. Except general metal-induced oxidative stress, the specific molecular mechanisms of REEs toxicity include: (1) acting as a substitute or antagonist for Ca^{2+}, (2) replacement of Mg^{2+}, (3) reaction with essential inorganic salt substances in the body, (4) altering endogenous hormonal balance, (5) effecting on expression level of genes. These mechanisms are described and summarized in Table A1.1. Compared with other heavy metals, the molecular mechanisms of REE toxicity to various life forms are still not well-defined and further work is required in this field. As discussed above, trivalent cationic REEs are the dominant toxic chemical species, and therefore drivers affecting the transfer of REE fractions to this more soluble/free form (e.g., pH) are also factors for REE toxicity by increasing bioavailability. The factors influencing REE toxicity can be divided into two categories based on collected references: (1) external environmental factors including interaction between REEs, exposure time, dose, potential competitors (e.g., Ca^{2+}) and pH, as well as (2) individual characteristics of the receptors including species, growth stage, and sex (see Table A1.2).

A1.8 Potential environmental risks associated with REE development in Canada

A1.8.1 The state of REE development in Canada

Currently, there are no REE mines in operation in Canada (Natural Resources Canada, 2018) although in the past some REEs were generated as a by-product during uranium and niobium production. The exploration activity in Canada is, however, quite high, with over 50 projects in development (Orris et al. 2018), accounting for up to 7 percent of REE projects worldwide (Figure A1.5). The REE deposits in Canada are dominated by REE-bearing carbonates, silicate phases and phosphates (Kalvig and Machacek 2018). Some advanced REE exploration projects are ongoing in Canada, and some of them have high concentrations of HREEs that are globally less abundant than LREEs, and particularly needed for many renewable energy technologies (Karen 2015). For example, the deposits of Strange Lake and the Nechalacho project have a high HREE proportion (HREEs/REEs) at 38%. It is anticipated that Canada would be a global leader in the production of REEs (especially for HREEs) by 2030 (Kalvig and Machacek 2018).

However, the pollution prevention and investment cost of REE production may be greater in Canada compared to China because of characteristics of the ore. This is because REE mineral phases for HREE production are different, with solid state minerals in Canada and ion-adsorption clay minerals in China. Ion-adsorption type REE minerals, which supplies most of HREEs in the world are found only in China. REEs in a trivalent cationic state are adsorbed onto clay minerals, and they are extracted by leaching with an aqueous electrolyte solution (sodium chloride or ammonium sulfate) via an ion-exchange process (Yang et al. 2013). The REE minerals in Canada are in solid state mineral phases, such as carbonates, and usually are associated with the radioactive elements uranium and thorium. The technological processes of extracting solid state minerals of REEs will be more complicated than ion-adsorption clay minerals. Therefore, limiting environmental risks and cost constitutes important challenges for future development of Canadian REE industry.

Table A1.1 Summary of the main molecular mechanisms of REE toxicity.

Mechanisms	Description	Reference
Substitute or antagonist for Ca^{2+}	REEs and Ca share similar chemical and physiological functions because of similar ionic radius. REEs can interfere with the normal physiological functions of Ca by replacing Ca in enzymatic reactions. For example, as a Ca^{2+} Channel antagonist, REEs can interact with membrane associated enzymes, and prevent the influx of other mineral elements, thereby disrupting elemental homeostasis.	Jakubek et al. (2009) Wang et al. (2008) Zeng et al., (2003)
Replacement of Mg^{2+}	Lanthanides could replace the magnesium atom of chlorophyll a and form a new REE-chlorophyll a, therefore influencing the photosynthesis of plants.	Chen et al. (2000) Wang et al. (2014b)
Reaction with essential inorganic salts in the body	Exogenous REEs can react with substances in the body, which can lead to physiological toxicity and even diseases. For example, inhalation of airborne particles including REEs can lead to pulmonary fibrosis because the reaction between the phosphate in lung fluid and REEs produces phosphate precipitation in lungs. Besides, REEs can lead to deposition by combination with negative ions (e.g. with phosphatic ion) in serum.	Censi et al. (2011) Wu et al. (2006)
Alter endogenous hormonal balance	REEs can alter endogenous hormone levels, which subsequently promote or inhibit the growth and physiological function. Generally, REEs at low concentration increase the level of Gibberellin A3 (GA3), trans-Zeatin-riboside (ZR) and indole-3-acetic acid (IAA) but decrease their level at high REE concentration because of reduced auxin carrier abundances.	Wang et al. (2014c) Liu et al. (2017) Cui et al. (2019) Luo et al. (2008)
Effecting expression level of genes	REEs can regulate the physiological function of organisms by affecting functional gene expression. For example, 1) La^{3+} can enhance endocytosis in root cells by regulating the expression level of genes encoding DNA methylases/demethylases; 2) Rare earth elements can activate the expression of silent or poorly expressed genes; 3) La^{3+} can increase the expression of auxin biosynthesis-related genes.	He et al. (2019b) Tanaka et al. (2010) Liu et al. (2016) Dubé et al. (2019)

Table A1.2 Key factors influencing REE toxicity to organisms.

Factors		Test models	Finding	References
Individual characteristics	Growth stage	Rice	Changes in the photosynthesis indexes of rice under the the effects of acid rain and La^{3+} were the booting stage > seedling stage > grain filling stage	Zhang et al. (2018)
		Mussel (*Mytilus galloprovincialis*)	La and Y are more toxic (mortality) to developing embryos and larvae than to juveniles	Mestre et al. (2019)
	Sex	Human	Total serum protein tended to decrease for male compared with female in a REE mining area in China	Zhang et al. (2000)
		Rat	REEs at high dose lead to renal and liver damage in rats. Female rats present a higher REE tolerance than males after 90-day exposure based on various metabolic profiles in the serum and urine.	Wu et al. (2006)
	Species	Rat and human	Kidney cell lines of rats were more sensitive than human in the strength of cell viability reduction, exposed to four lanthanides (La, Ce, Eu, and Yb).	Heller, et al. (2019)
Environmental factors	REEs interaction	Wheat (*Triticum aestivum*)	Additive toxicity was found for Y-La and La-Ce mixtures, whereas antagonistic interaction was observed for Y-Ce mixtures.	Gong et al. (2019)
	Potential competitors	Wheat (*Triticum aestivum*)	Ca^{2+} and Mg^{2+} increased the values of EC50 (Y^{3+}) by 19 and 2.24 folds respectively, but no significant increase with Na^{+}, K^{+} and H^{+}.	Gong et al. (2019)
	Exposure time	Rare minnow (*Gobiocypris rarus*)	The mortality increased with exposure time from 24 to 96h for the same concentration of $LaCl_3 \cdot 7H_2O$.	Hua et al. (2017)
	Dose	*Lemna minor*	The growth (e.g., levels of chlorophyll and carotenoid) of plants increased at lower Ce concentrations and decreased at higher Ce concentrations	Zicari et al. (2018)
			Low-dose La^{3+} (0.2, 2.0, and 4.0 μM) exposure increased the total content of microcystins produced by *Microcystis aeruginosa* (algae), while the production of microcystins was inhibited at high-dose La^{3+} (8.0, 20, 40, and 60 μM) exposure.	Shen et al. (2018)
	pH	Rice	At 81.6 μM $LaCl_3$, the photosynthesis was increased at pH 4.5, but toxic effects were observed at pH 3.5.	Wang et al. (2014b)

A1.8.2 The REE background levels in Canada

The natural REE background levels before the mass production of REEs are key reference points for environmental and health risk assessment of both current and future REE projects in Canada. In this section, many studies that have made contributions to understanding Canadian baseline data of REEs in natural resources and organisms are discussed (see Table A1.3). Data from two important reviews (Adeel et al. 2019; Bayon et al. 2015) were integrated with publications about Canadian REE background concentrations in soil/sediments found during the literature search (Casse et al. 2019; Cheung et al. 2009; Chiarenzelli et al. 2001; Johannesson and Hendry, 2000; MacMillan et al. 2018; Rasmussen et al. 2017; Romero-Freire et al.). The Canadian background concentration of each REE in water and soils/sediments was not above the levels found in most of the regions in the world (Figure A1A1 and A1A2). For example, in Canada, the average concentrations of total REE (total REEs =88.8 mg/kg) in house dust sampled from 1025 urban homes (Rasmussen et al. 2017) were below the average level of REEs (137.1 mg/kg) found in global soil (Kabata-Pendias, 2011). Therefore, the health risk of current REEs in the environment is low.

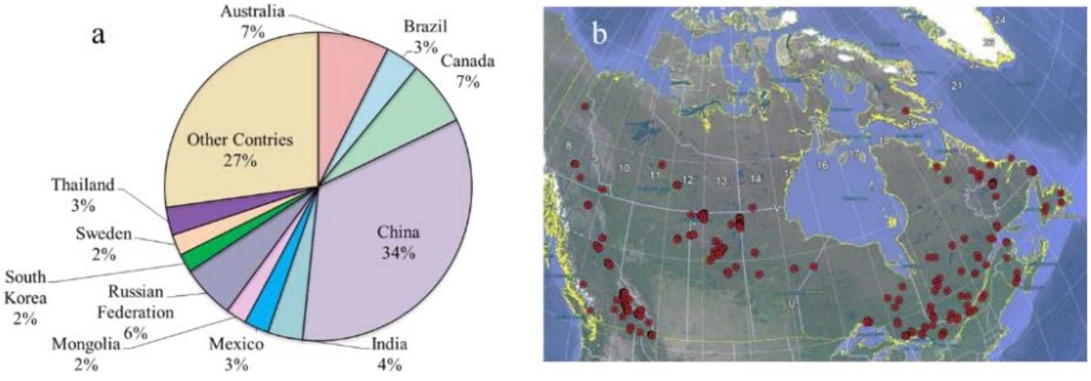

Figure A1.5 The global REE projects (a) and the REE deposits in Canada (b) (data from USGS 2019 edited with Google Earth).

The data of REE levels in biota in Canada was reviewed, and the selected recent studies are shown in Table A1.3. REE concentrations were significantly different among biotic groups, and higher REE levels were reported in mosses and lichens than other groups ($p<0.05$, Figure A1A3). Also, it appears that aquatic organisms can absorb and accumulate more REEs than terrestrial organisms (Table A1.3 and Figure A1A3). Currently, a generally acknowledged allowable daily intake of total and individual REEs in humans was not available, but Zhu et al. (1997) have proposed a daily allowable intake of 70 μg/kg•bw (4.9 mg/day, considering a 70 kg adult) for total REEs, which was determined from a human health study in REE mining areas and animal experiments. This value has been used in many subsequent studies (Zhuang et al. 2017a; Zhuang et al. 2017b. Yang et al. 2016), so it will be also used in this study to assess associated health risk Some aquatic animals, plants and most lichens and mosses have a high health risk for human food intake. In Table A1.3, for example, the REE concentration of blue mussel is 4.86 mg/kg and if 1 kg blue mussel is eaten, the REE intake will be more than the allowable daily intake. Furthermore, some traditional food of Indigenous people should be noted, for example a study shows that the REE content of Labrador tea (*Ledumgroen landicum*) varies from 42.3 to 171.6 mg/kg in a REE rich area in northern Saskatchewan (Dunn and Hoffman, 1986), which is a level representing a high risk for human health if consumed excessively. More studies about the ability of REE accumulation in similar important species (e.g., indigenous medicines and food) should be included, and then their health risks can be analyzed and compared.

A1.8.3 The potential environmental risks of REE development in Canada

REE development is a new sector in Canada, and radioactive contamination and REE toxicity are unique potential risks compared with other types of mines. Those potential risks are cryptic and of high risk for public health because there are few proven mitigation strategies appropriate for Canada to reduce or minimize their adverse impacts. For the identification of associated environmental risks in Canada in this review, risk is deemed to be “the probability of occurrence of a harmful event” (Scheer et al. 2014) and depends on both the level of exposure to the hazard and the vulnerability of the receptors (York et al. 2012). In this synthesis, “harmful events” are environmental issues caused by REE mining and processing such as elevated concentrations of REEs or other hazardous substances in the environment, and the receptors are biota living in Canada, therefore, potential environmental risks associated with REE development can be identified in different communities and ecosystems by analyzing the exposure routes, their influence factors, and vulnerability of various life forms in Canada. Then several typical REE projects are discussed as different scenarios.

As there are no Canadian federal water quality standards or guidelines for Rare Earth Elements, although a draft federal water quality guideline for Ce, La, Nd and Y will be presented soon (Dixit, 2020), the data from other countries is used to compare with available Canadian information to evaluate the risks of exposure to REEs via water. Illegal production in China represents more than 50 % of global REE production (Langkau and Erdmann 2020), which is much more likely to damage the local environment and people’s health (see Table A1.4) as there are no strict safety rules, regulated extraction procedures and accepted environmental strategies at these sites. However, illegal production is unlikely in Canada. Therefore, we follow the basic assumption that associated laws, regulations, rules and strategies will be strictly enforced at each REE project in Canada.

Table A1.3 REE background content (mg/kg) of living organisms in Canada.

	location	**Species**	**Group**	**Y**	**La**	**Ce**	**Pr**	**Nd**	**Sm**	**Eu**	**Gd**	**Tb**	**Dy**	**Ho**	**Er**	**Tm**	**Yb**	**Lu**	**References**
1	Kuujjuarapik-Whapmagoostui, Quebec	Benthos	Aquatic animals /plants		1.3821	1.664	0.22	0.71	0.0908	0.0239	0.0995	0.0094	0.0357	0.0066	0.0209	0.0022	0.0147	0.0021	MacMillan *et al.* 2017
2	Kuujjuarapik-Whapmagoostui, Quebec	Blue Mussel	Aquatic animals		1.394	1.9618	0.24	0.85	0.1262	0.0199	0.1302	0.0122	0.0533	0.0097	0.0299	0.0034	0.0221	0.0035	MacMillan *et al.* 2017
3	Kuujjuarapik-Whapmagoostui, Quebec	Brook Trout_1	Aquatic animals		0.0001	0.0001	0	0	0.0002	0	0	0	0	0	0	0	0	0	MacMillan *et al.* 2017
4	Kuujjuarapik-Whapmagoostui, Quebec	Brook Trout_2	Aquatic animals		0.0008	0.0014	0	0	0.0002	0	0.0002	0	0.0002	0	0	0	0	0	MacMillan *et al.* 2017
5	Near Montreal, Quebec	Brook Trout_3	Aquatic animals	0.0454	0.2074	0.1872	0.03	0.11	0.0146	0.0029	0.0207	0.0021	0.0101	0.0021	0.0057	0.0006	0.0028	0.0004	MacMillan *et al.* 2018
6	Near Montreal, Quebec	Mussel	Aquatic animals	0.0522	0.0666	0.0697	0.01	0.05	0.0102	0.0024	0.013	0.0016	0.0075	0.0015	0.004	0.0004	0.0024	0.0004	MacMillan *et al.* 2018
7	Kuujjuarapik-Whapmagoostui, Quebec	Ringed Seal	Aquatic animals		0.0003	0.0004	0	0	0.0002	0	0.0002	0	0	0	0	0	0	0	MacMillan *et al.* 2017
8	Kuujjuarapik-Whapmagoostui, Quebec	Sea Urchin	Aquatic animals		0.6974	0.6052	0.1	0.34	0.0497	0.0081	0.0554	0.0056	0.0267	0.0053	0.0164	0.002	0.0128	0.0019	MacMillan *et al.* 2017
9	Kuujjuarapik-Whapmagoostui, Quebec	Whitefish	Aquatic animals		0.0001	0.0001	0	0	0.0002	0	0.0002	0	0	0	0	0	0	0	MacMillan *et al.* 2017
10	Nomans River, Quebec	Typha latifolia	Aquatic plants		0.2294	0.477	0.05	0.2	0.0299	0.016	0.0256	0.0033	0.0141	0.0025	0.006	0.0008	0.0047	0.0006	Romero-Freire *et al.* 2019
11	Kuujjuarapik-Whapmagoostui, Quebec	Zooplankton_1	Aquatic plants		3.9405	4.87	0.75	2.59	0.3285	0.0643	0.3306	0.0334	0.146	0.0271	0.0867	0.0101	0.065	0.0095	MacMillan *et al.* 2017
12	Near Montreal, Quebec	Zooplankton_2	Aquatic plants	0.3651	0.3775	0.7903	0.09	0.4	0.0854	0.0268	0.0981	0.0122	0.0667	0.0124	0.0366	0.0046	0.0304	0.0044	MacMillan *et al.* 2018
13	Deslauriers Island, Quebec	Chroicocephalus ridibundus_female	Bird		0.0276	0.15	0.16	0.02	0.0728	0.0095	0	0.0087	0	0.0041	0.0009	0.0024	0.0016	0	Brown *et al.* 2019

Table A1.3 continued

14	Deslauriers Island, Quebec	Chroicocephalus ridibundus_ male	Bird		0.0053	0.19	0.21	0.02	0.0843	0.0094	0	0.007	0	0.001	0	0	0	0	Brown *et al.* 2019
15	Kuujjuarapik-Whapmagoostui, Quebec	Ptarmigan	Bird		0.0003	0.0003	0	0	0.0002	0	0.0002	0	0	0	0	0	0	0	MacMillan *et al.* 2017
16	Otter Lake area, Nunavut	Alectoria pubescens	Lichen	0.73	1.81	5.89	0.42	1.7	0.314	0.068	0.231	0.031	0.176		0.082		0.075	0.015	Chiarenzelli *et al.* 2001
17	Otter Lake area, Nunavut	Calcoplaca saxicola	Lichen	0.38	0.88	1.71	0.21	0.77	0.155	0.027	0.114	0.017	0.099		0.041		0.038	0.004	Chiarenzelli *et al.* 2001
18	Otter Lake area, Nunavut	Cetaria cucculatta	Lichen	0.24	0.56	1.15	0.13	0.5	0.09	0.018	0.076	0.01	0.052		0.027		0.024	0.004	Chiarenzelli *et al.* 2001
19	Otter Lake area, Nunavut	Cetaria nivalis	Lichen	0.05	0.1	0.2	0.02	0.09	0.02		0.011		0.011		0.004		0.005	0.002	Chiarenzelli *et al.* 2001
20	Otter Lake area, Nunavut	Cladonia rangiferina	Lichen	2	3.55	11.84	0.8	3.45	0.644	0.131	0.532	0.076	0.431		0.199		0.195	0.023	Chiarenzelli *et al.* 2001
21	Otter Lake area, Nunavut	Cladonia stellaris	Lichen	0.16	0.33	0.66	0.07	0.29	0.054	0.012	0.044	0.005	0.032		0.018		0.019	0.004	Chiarenzelli *et al.* 2001
22	Kuujjuarapik-Whapmagoostui, Quebec	Lichen spp.	Lichen		1.2467	2.4703	0.26	0.95	0.1406	0.029	0.1427	0.0142	0.0593	0.0109	0.0327	0.0039	0.0247	0.0033	Chiarenzelli *et al.* 2001
23	Otter Lake area, Nunavut	Masonhalea richardsonii	Lichen	0.6	1.46	3.24	0.35	1.3	0.212	0.052	0.17	0.022	0.117		0.068		0.056	0.009	Chiarenzelli *et al.* 2001
24	Otter Lake area, Nunavut	Nephroma arcticum	Lichen	0.74	2.11	4.27	0.49	1.82	0.334	0.074	0.23	0.033	0.173		0.088		0.092	0.011	Chiarenzelli *et al.* 2001
25	Otter Lake area, Nunavut	Parmelia fulinginosa	Lichen	2.21	4.92	16.73	1.06	4.44	0.89	0.24	0.58	0.115	0.506		0.275		0.25	0.037	Chiarenzelli *et al.* 2001
26	Otter Lake area, Nunavut	Parmelia mougoetti	Lichen	1.55	4.38	10.17	0.79	3.23	0.582	0.14	0.464	0.067	0.366		0.194		0.19	0.026	Chiarenzelli *et al.* 2001
27	Otter Lake area, Nunavut	Stereocaulon paschale	Lichen	0.16	0.53	1.17	0.13	0.49	0.069	0.015	0.052	0.007	0.034		0.017		0.017	0.004	Chiarenzelli *et al.* 2001
28	Otter Lake area, Nunavut	Umbilicaria polyphylla	Lichen	0.95	2.15	6.82	0.51	2.12	0.382	0.096	0.319	0.043	0.22		0.103		0.088	0.015	Chiarenzelli *et al.* 2001
29	Otter Lake area, Nunavut	Brachythecium velutinum	Mosses	1.56	3.54	8.1	0.67	2.72	0.527	0.141	0.44	0.067	0.355		0.201		0.205	0.043	Chiarenzelli *et al.* 2001
30	Otter Lake area, Nunavut	Dicranum scoparium	Mosses	0.72	2.13	7.38	0.46	1.85	0.337	0.073	0.225	0.031	0.168		0.09		0.083	0.016	Chiarenzelli *et al.* 2001
31	Otter Lake area, Nunavut	Hyalocomium splendens	Mosses	0.13	0.23	0.43	0.05	0.18	0.035	0.006	0.034	0.006	0.023		0.014		0.016	0.003	Chiarenzelli *et al.* 2001

Table A1.3 continued

32	Otter Lake area, Nunavut	Polytrichium juniperina	Mosses	0.7	2.02	5.71	0.4	1.6	0.253	0.088	0.189	0.04	0.141		0.076		0.075	0.008	Chiarenzelli *et al.* 2001
33	Otter Lake area, Nunavut	Grimmia apocarpa	Mosses	3.38	4.44	15.55	1.01	4.24	0.872	0.23	0.817	0.129	0.778		0.443		0.413	0.061	Chiarenzelli *et al.* 2001
34	Kuujjuarapik-Whapmagoostui, Quebec	Caribou	Terrestrial animals		0.0006	0.0004	0	0	0.0002	0	0.0002	0	0	0	0	0	0	0	MacMillan *et al.* 2017
35	Kuujjuarapik-Whapmagoostui, Quebec	Eider	Terrestrial animals		0.001	0.0018	0	0	0.0005	0.0002	0.0005	0	0.0002	0	0	0	0.0002	0	MacMillan *et al.* 2017
36	Kuujjuarapik-Whapmagoostui, Quebec	Hare	Terrestrial animals		0.0006	0.0003	0	0	0.0002	0	0.0002	0	0	0	0	0	0	0	MacMillan *et al.* 2017
37	Otter Lake area, Nunavut	Betula glandulosa	Terrestrial plants	0.03	0.05	0.07	0.01	0.03	0.007	0	0.006	0	0.005		0		0.005	0	Chiarenzelli *et al.* 2001
38	Otter Lake area, Nunavut	Empetrum nigrum	Terrestrial plants	0.05	0.08	0.16	0.02	0.07	0.014	0.004	0.009	0	0.01		0.007		0.007	0.001	Chiarenzelli *et al.* 2001
39	Nomans River, Quebec	Equisetum arvense	Terrestrial plants		1.1177	1.6159	0.19	0.68	0.0925	0.0464	0.0816	0.0105	0.0435	0.0077	0.0195	0.0025	0.0153	0.002	Romero-Freire *et al.* 2019
40	Otter Lake area, Nunavut	Ledum palustre	Terrestrial plants	0.04	0.06	0.12	0.01	0.05	0.011	0	0.009	0	0.007		0.006		0.006	0	Chiarenzelli *et al.* 2001
41	Kuujjuarapik-Whapmagoostui, Quebec	Plants spp.	Terrestrial plants		0.0317	0.0594	0.01	0.02	0.0035	0.0091	0.0033	0.0003	0.0015	0.0003	0.001	0	0.0007	0.0002	MacMillan *et al.* 2017
42	Otter Lake area, Nunavut	Salix arcticum	Terrestrial plants	0.04	0.12	0.19	0.02	0.08	0.021	0	0.009	0	0.012		0.005		0	0	Chiarenzelli *et al.* 2001
43	Otter Lake area, Nunavut	Stellaria ssp	Terrestrial plants	0.19	0.62	0.91	0.11	0.47	0.074	0.013	0.069	0.008	0.039		0.022		0.015	0.001	Chiarenzelli *et al.* 2001

Note: ADW, acceptable daily sample weight; HHR, human health risks (Low: ADW > 1.2 kg; Mid: 0.6 kg ≤ ADW ≥ 1.2 kg; High: ADW < 0.6 kg). For animals, the REE content was from muscle samples.

Table A1.4 List of main health important aspects in REE industry with concerned groups.

Exposure	Risks	Groups Involved	Planning & Control
Ingestion	Food sources	Indigenous people near REE mines or processing plants	Avoid collecting food/medicine sources from contaminated areas;
	Traditional medicines	Indigenous people near REE mines or processing plants	Guidelines for recommended daily limits.
	Drinking water	Surrounding residents and indigenous people near REE mines or processing plants	Water quality monitoring
Inhalation and dermal contact	Dust	Surrounding residents and workers in mines and processing plants	Strict preventive measures (e.g., anti-dust respirators and);
			Air quality monitoring;
	Radiation	Surrounding residents and workers in mines and processing plants	Determining the distance influenced by REE mines based on content of REEs and radioactive elements and moving residents from influenced areas.

A1.8.3.1 Potential risks to human health

1) Workers

Occupational exposure is generally the most significant risk to human health associated with REE production. Those working in the REE mining and processing industry can be easily and chronically exposed to multiple hazardous substances (e.g., REE-containing dust, heavy metals, radioactive materials and process chemicals) by ingestion, inhalation and dermal contact (Figure A1.1 and A1.2), and this exposure then has the potential to damage human organs or tissues after long-term exposure and potential bioaccumulation. Radiation risk is usually considered as the greatest threat for workers' health in REE mining areas. Cases from the Bayan Obo mine show that the risk of death from leukemia for workers increased significantly over ten years (Li et al. 1986). Although Canada, as one of the biggest producer and supplier of uranium in the world (Winde et al. 2017), has experience in the management of radioactive exposure during production and waste processing, new and increased exposure sources still have the potential to threaten workers' health if not managed properly. On the other hand, both inhalation and dermal contact are the main exposure route of REEs for people working in mining areas, especially for the inhalation of atmospheric particulates containing REEs, and long-term exposure of REE dust may cause rare earth pneumoconiosis and negative neurological effects (Wang et al. 2014a). Furthermore, snowfall in mining areas should be studied and monitored in Canada, because compared with raindrops, snowflakes with larger surface area and slower fall velocity may accumulate more REE dust (Cereceda-Balic et al. 2012), which increases the risk of REEs' dermal contact for workers and nearby residents. Environmental quality monitoring and strict occupational radiation protection should be able to effectively reduce the risk for workers and avoid acute damages.

2) Surrounding residents

REE mining and processing also pose significant potential risks for surrounding residents, primarily through the inhalation of REE dust and ingestion of food and water with higher REEs content, and some cases have been reported in Chinese REE mine areas (see Table A1A2). For example, Baotou City with the Bayan Obo REE mine, has suffered from considerable pollution issues from REE production and processing (Julio and Fu 2014). As most REE mine projects are far away from population centers and there strict mining waste management laws in Canada, health risks for the general public may be limited. However, there are still some potential risks: Food sources near REE mines or processing plants. Hazardous substances (e.g., REEs, heavy metals and radioactive elements) can disperse and leach into surrounding soil and water (for cases see Table A1A2). Vegetables growing in contaminated soil have the potential to increase the rate of REE bioaccumulation inside the human body, especially for root vegetables (such as potato and sugar beet) because they absorb more REEs from polluted soil than other vegetables, based on data from China (Zhuang et al. 2017). Aquatic products are also a potential health risk as data collected in Canada (Table A1.3) indicate that benthicorganisms (e.g., blue mussels) have higher background content of REEs and a large REE bioaccumulation capacity (Zhang et al. 2009; Squadrone et al. 2019). These and other food sources with a higher REE bioaccumulation capacity should be given special consideration during REE production development in Canada.

3) Indigenous People

Indigenous peoples near REE mining areas in Canada may be more affected by environmental issues associated with REE development than other groups, because they may rely more heavily on local biota for their daily lives (e.g., food, medicine and traditional activities on the land) (Sharma 2010; Wesche and Chan 2010). Potential health risks involve lands used for hunting, fishing, trapping, and food gathering being impacted by REE mines. For example, the Kipawa project (a proposed REE mine site) is located on the traditional lands of two Anicinabek First Nations in western Quebec. There are some potential risks for Indigenous peoples: 1) Traditional food from REE rich areas. An increase in REE projects will lead to more REE rich areas in Canada, which is a serious threat to their traditional territories and potentially health. The content of REEs and radioactive elements in traditional foods is expected to increase inside the influenced areas of REE mines 2) Traditional medicines. More than 500 medicinal plant taxa used by Indigenous peoples in Canada and the REE content of them near mines can be increased especially for those hyperaccumulator for REEs. For example, lichens (11 taxa from 4 families) and bryophytes (6 taxa from 2 families) with higher REE background levels (Table A1.3) and capacity of bioaccumulation (Agnan et al. 2014; Urošević et al. 2020) are used as traditional medicines (Uprety et al. 2012), which may increase the health risks for Indigenous people in the context of REE production. Under the basic assumption that associated laws, regulations, rules and strategies will be strictly enforced, the health risk from drinking water and inhalation may be low for Indigenous peoples. Avoiding collecting food/medicine sources from contaminated areas and guidelines for recommended daily limits of REE content could lower the risks significantly. Very importantly too, regulators should pre-emptively consult with Indigenous stakeholders to avoid siting potentially damaging extraction and processing operations/facilities where they may do harm.

REE projects as they may impact on indigenous communities, have recently received increasing attention. For example, a community-based environmental monitoring program (IMALIRIJIIT) was initiated prior to the start of a rare earth elements (REEs) mining project in Nunavik, Quebec (Gérin-Lajoie, et al., 2018). This will provide baseline data and reference points to better understand the diverse impacts of REE projects on socio-ecological systems, as well as to protect the health, well-being, and quality of life indigenous people in other as future REE projects arise near other northern communities

A1.8.3.2 Potential ecological risks in Canada

The geochemical behaviour of REEs in Canada is influenced by the geographical and geological location of the deposits (geological provinces of main REE projects in Table A1A1) The studies from Canada identified pH, dissolved organic carbon (DOC), free REE^{3+} concentration, active ligand concentration and temperature as the main drivers for REE transfer and bioaccumulation in Canadian aquatic ecosystems (MacMillan et al. 2018; Serrano et al. 2000). For example, a laboratory study on material from the Eldor REE project showed that low temperatures and freeze-thaw events may increase the concentration of elements leached from the tailings produced by the beneficiation of REE minerals in sub-arctic areas (Costis et al. 2020). The main potential ecological risks of Canadian REE projects for ecosystems are discussed below.

1) Wetland and aquatic ecosystems

Wetlands and lakes cover about 13% and 9 %, respectively, of the land area of Canada (Wulder et al. 2018) and support many ecosystem services. In wetlands and open-water aquatic ecosystems, reducing conditions and chemical reduction are a main factor controlling REE behaviour (Davranche et al. 2015; Mihajlovic et al. 2017). Organic matter is a major sink for REEs due to its high binding capacity for cationic metallic contaminants through ligation or electrostatic interactions (Davranche et al. 2015). In sulfide containing ore and waste environments, acid mine drainage solubilizes REEs, however they can be subsequently adsorbed by soil organic matter in wetlands. Furthermore, the suspended particles including REEs from mining sites can settle in surrounding wetlands and forests. Those REEs will be released to the water under reducing conditions such as seasonal floods caused by spring snowmelt (Davranche et al. 2015; Shiller, 2010). Soil and solution components (e.g., colloids and suspended matter), pH, and redox condition are considered as critical environmental parameters controlling the geochemical behaviors of REEs in the environment because they can increase or decrease cation exchange capacity (CEC). However, more research is needed on geochemical behaviours of REEs because little is known about REEs in different environments (e.g., vast boreal and sub-arctic peatlands in Canada).

Low pH values of certain environments can increase the toxicity of REE for plants and animals by increasing the solubility and bioavailability of REEs. For example, at the Bayan Obo REE mine, a study found water pollution from mine tailings reduced local toad (*Strauchbufo raddei*) population density by affecting the mating rate and causing oxidative damage to tissues and DNA damage in tadpole blood cells (Huang et al. 2019). In Canadian boreal areas, the low pH of bogs and acidic lakes has the potential to make them sinks for REEs following the release of REEs from future mining sites.

2) Boreal forest ecosystems

The Canadian boreal forests comprise about 30% of global boreal forests and play critical roles in the global carbon cycle and in biodiversity conservation (Peng et al. 2011; Venier et al. 2014). Many studies on fossil fuels and mineral mines (e.g., gold, coal and diamond) affecting boreal forest ecosystems have been published (Naeth and Wilkinson, 1997; Amalesh et al. 2018), however, little is known about REE mines. Dust and heavy traffic are probably the main REE risks for boreal forest ecosystems in Canada. In particular the significant number and quantity of radioactive elements (Wang et al. 2016), REEs (Wang and Liang 2014) and other toxic heavy metal elements (e.g., As, Cd and Pb) (Kexin et al. 2015) in atmospheric deposition from REE mining, and their impact may extend over 3-7 km from sources (Chang and Chang 2012; Degtjarenko et al. 2016). These dusts may enter the stomatal openings and impact physiological functions of plants notably because REEs can interfere with the normal physiological functions of Ca^{2+} and Mg^{2+}. Therefore, there is a potential risk to ecological services of forests near REE mines as photosynthesis and nutrient absorption may be disrupted (mechanisms see Table A1.1 and A1.2). The accumulation of these elements in plants also has potential health risks for other biota and humans via trophic transfers in local and regional food webs.

In addition, the large amount of chemicals and materials required for REE mining and processing increases traffic intensity (e.g., 7-10 tons of ammonium salts and over 10 tons of acid are used for the production of 1 ton rare-earth oxides based on the data from China, Huang et al. 2015), which will disturb roadside biota and animal movement by noise, dust, road accidents and fluid leakage, especially for some species at risks particularly sensitive to new linear disturbances (e.g., Caribou) in the boreal forest. For example, mountain woodland caribou avoided high-use roads and mines with a buffer of 2 km and low-use roads by 1 km based on the data in British Columbia, Canada (Polfus et al. 2011). Different species have different habitats, physiological features and ecological services, and potential risks caused by REE mining for species at risk in Canada require more specialists involved in assessments prior, during, and after development.

3) Sub-arctic/arctic environment

Many REE projects are at an advanced stage of development in northern regions of Canada (e.g., Strange Lake, Montviel, Eldor and Foxtrot projects), especially in sub-arctic and arctic regions. Environmental concerns related to the development of REE projects in the sub-arctic/arctic climate (extreme temperatures, timing and duration of snow and rain, and freeze-thaw period) should have been raised. Recent studies present that climate condition of cold regions can promote the mobilization of REEs by affecting main driving factors (pH, DOC and redox condition) of REE mobility and bioavailability. Firstly, high-latitude sub-arctic and arctic surface/inland waters often contain sizeable reservoirs of DOC because of the activity of microbial communities at low temperatures (Pokrovsky et al. 2018; Ritson et al. 2014). Furthermore, poor fens and bogs common in watersheds in these regions are highly acidic (typically with pore water pH less than 4.5) (Zoltai and Vitt 1995). As a result, the mobility and bioavailability REEs could be promoted with high DOC content and low pH. Secondly, the redox environments in the lower active layer in permafrost affected soils leads to DOC and REEs that cannot be consumed by the biota or depleted by abiotic reactions (Ji et al. 2021). REEs persist in suprapermafrost water and can be transported to the adjacent rivers or lakes by high surface flows, such as spring thaws (Ji et al. 2021). Thirdly, repeated freeze-thaw cycles could release more REEs and other heavy metals from tailings through intensive erosion process (Pokrovsky et al. 2018). For example, the leaching potential of elements present in flotation tailings was assessed at the Eldor project (Costis et al. 2020), and the results showed that freezing event increased leachate concentrations. As climate changes in high latitudes, the depth of active layer and freeze-thaw cycles are expected to be increased in the sub-arctic and arctic regions of Canada. Therefore, sub-arctic/arctic climate conditions may importantly influence the environmental risks caused by REE mining in Canada.

A1.9 Conclusions and next steps for research in Canada

The concentration of REEs in the environment has increased significantly in recent years, and this trend is expected to continue over the next decades as more REE projects are developed globally. REEs as emerging contaminants and radioactive elements (U and Th) are main ecological and health risks. Canada ranks among the top countries with rich REE deposits and is expected to be a global leader in the production of REEs in the coming decades. Although no REE mines are currently operating in Canada, many projects are at an advanced stage of development. To understand and assess potential environmental risks that Canada could face in the future, this paper examined environmental behaviours and fates of REEs and their toxicity on ecological and human health. Based on this overview, Canadian REE background studies and data from case studies in long standing REE mining areas, this paper integrated environmental conditions and Canadian specific risk factors. The risk assessment presented here suggests that ingestion exposure via traditional foods and medicines for Indigenous people as well as inhalation and dermal exposure to dust and radiation for workers and surrounding residents could be the main health risks. Considering the many advanced REE projects in sub-arctic/arctic regions in Canada, the environmental and climate condition (acidic waters, low temperature and repeated freeze-thaw cycles) promoting REE transportation is expected to influence ecological risks, which should be a matter of concern in REE mines. However, the environmental issues caused by REE development vary depending on deposit ore minerals, processing technology, waste management strategies, policies and regulations, and vulnerabilities of the local environment. Ion-adsorption rare earth deposits, for example, provide 80% of HREEs in the world, but this type of REEs ores with high HREE content are currently mainly found in China (Yang et al. 2013; Ram et al. 2019), while the HREEs of Canada are mainly present in carbonates or peralkaline ores. Although some data are available for environmental issues caused by HREEs development in China and other countries, their experience may also be only of limited use for the identification of associated potential environmental risks in Canada. Furthermore, unique climate, ecological systems and species at risk could generate new risks in Canada. Therefore, the following points should be addressed in Canada to determine accurate risk assessment in actual REEs project development:

(1) More REE baseline concentrations in Canada are required for soil, water, vegetation animals, and humans especially for species at risks, which will permit more detailed and accurate risk assessments

(2) Determining the toxicity of REEs on target species for food sources in Canada and then providing guidelines for recommended daily limits of REE content. For example, REE uptake, bioaccumulation and toxicity threshold of those species that are traditional food and medicines for Indigenous people.

(3) Mixed REEs, and not necessarily individual elements alone, should get more attention, because REEs usually transfer and accumulate as a group of contaminants. Additionally, multiple stressors, including effects of associated radionuclides and acidity (for the proposed sites with sulfidic substrates) with REEs should be concomitantly examined.

(4) Due to the geochemical behaviour of REEs in different physical and chemical conditions, more studies should be performed to better understand the environmental behaviour and fate of REEs under Canadian environmental conditions and in our

most keystone ecosystems, such as wetlands, acid-sensitive Precambrian Shield lakes, boreal forests and tundra.

(5) Developing cleaner technologies for exploiting Canadian REE deposit minerals, reducing the materials and energy consumption and waste discharge during REE mining and processing, which is the most effective way to limit potential environmental risks.

REFERENCES

Abdelnour, S.A., Abd El-Hack, M.E., Khafaga, A.F., Noreldin, A.E., Arif, M., Chaudhry, M.T., Losacco, C., Abdeen, A., and Abdel-Daim, M.M. 2019. Impacts of rare earth elements on animal health and production: Highlights of cerium and lanthanum. Sci. Total Environ. 672: 1021–1032. doi:10.1016/j.scitotenv.2019.02.270.

Adeel, M., Lee, J.Y., Zain, M., Rizwan, M., Nawab, A., Ahmad, M.A., Shafiq, M., Yi, H., Jilani, G., Javed, R., Horton, R., Rui, Y., Tsang, D.C.W., and Xing, B. 2019. Cryptic footprints of rare earth elements on natural resources and living organisms. Environ. Int. 127: 785–800. doi:10.1016/j.envint.2019.03.022.

Adua, O.A., Akinmuyisitana, I.W., and Gbore, F.A. 2013. Growth performance and blood profile of female rabbits fed dietary cerium oxide. J. Bio-Sci. 21: 69–75.doi: 10.3329/jbs.v21i0.22521.

Agathokleous, E., Kitao, M., and Calabrese, E.J. 2018. The rare earth element (REE) lanthanum (La) induces hormesis in plants. Environ. Pollut. 238: 1044–1047. doi:10.1016/j.envpol.2018.02.068.

Agathokleous, E., Kitao, M., and Calabrese, E.J. 2019. Hormetic dose responses induced by lanthanum in plants. Environ. Pollut. 244: 332–341. doi:10.1016/j.envpol.2018.10.007.

Aharchaou, I., Beaubien, C., Campbell, P.G., and Fortin, C. 2020. Lanthanum and cerium toxicity to the freshwater green alga *Chlorella fusca*: applicability of the Biotic Ligand Model. Environ. Toxicol. Chem. 39(5): 996-1005. doi: 10.1002/etc.4707.

Aime, S., and Caravan, P. 2009. Biodistribution of gadolinium-based contrast agents, including gadolinium deposition. J. Magn. Reson. 30(6): 1259–1267. doi: 10.1002/jmri.21969.

Ali, S. 2014. Social and environmental impact of the rare earth industries. Resources, 3(1), 123-134. doi:10.3390/resources3010123.

Alpay, S., Veillette, J.J., Dixit, A.S., and Dixit, S.S. 2006. Regional and historical distributions of lake-water pH within a 100-km radius of the Horne smelter in Rouyn-Noranda, Québec, Canada. Geochemistry Explor. Environ. Anal. 6: 179–186. doi: 10.1144/1467-7873/05-097.

Amalesh, D., Philip, G.C., Justine, K., Bradley, D.P., Scott, X.C., Anne, M.N., Robert, V. and Caroline, B. 2018. Plant community development following reclamation of oil sands mine sites in the boreal forest: a review. Environ. Rev. 26(3): 286-298. doi: 10.1139/er-2017-0091.

Ault, T., Krahn, S., and Croff, A. 2015. Radiological impacts and regulation of rare earth elements in non-nuclear energy production. Energies 8(3): 2066–2081. doi:10.3390/en8032066.

Bai, Y., Long, C., Hu, G., Zhou, D., Gao, X., Chen, Z., Wang, T., Yu, S., Han, Y., and Yan, L. 2019. Association of blood chromium and rare earth elements with the risk of DNA damage in chromate exposed population. Environ. Toxicol. Pharmacol. 72: 103237. doi:10.1016/j.etap.2019.103237.

Balaram, V. 2019. Rare earth elements: A review of applications, occurrence, exploration, analysis, recycling, and environmental impact. Geosci. Front. 10(4): 1285–1303. doi:10.1016/j.gsf.2018.12.005.

Bayon, G., Toucanne, S., Skonieczny, C., André, L., Bermell, S., Cheron, S., Dennielou, B., Etoubleau, J., Freslon, N., Gauchery, T. and Germain, Y. 2015. Rare earth elements and neodymium isotopes in world river sediments revisited. Geochim. Cosmochim. Acta, 170: 17-38. doi: 10.1016/j.gca.2015.08.001.

Balomenos, E., Taxiarchou, M., Panias, D., and Paspaliaris, I. 2017. Current and alternative routes in the production of rare earth elements. BHM. 162(7): 245–251. doi:10.1007/s00501-017-0610-y.

Batapola, N.M., Dushyantha, N.P., Premasiri, H.M.R., Abeysinghe, A.M.K.B., Rohitha, L.P. S., Ratnayake, N.P., Dissanayake, D.M.D.O.K., Ilankoon, I.M.S.K., and Dharmaratne, P.G.R. 2020. A comparison of global rare earth element (REE) resources and their mineralogy with REE prospects in Sri Lanka. J. Asian Earth Sci. 200: 104475. doi: 10.1016/j.jseaes.2020.104475.

Bernstein, E. J., Schmidt-Lauber, C., and Kay, J. 2012. Nephrogenic systemic fibrosis: a systemic fibrosing disease resulting from gadolinium exposure. Best Pract. Res. Cl. Rh. 26(4): 489-503. 10.1016/j.berh.2012.07.008.

Bichler, A., Muellegger, C., Brünjes, R., and Hofmann, T. 2016. Quantification of river water infiltration in shallow aquifers using acesulfame and anthropogenic gadolinium. Hydrol. Process. 30(11): 1742–1756. doi:10.1002/hyp.10735.

Boojar, M.M.A., and Tavakkoli, Z. 2011. Antioxidative responses and metal accumulation in invasive plant species growing on mine tailings in Zanjan, Iran. Pedosphere 21(6): 802–812. doi:10.1016/S1002-0160(11)60184-7.

Brown, L., Rosabal, M., Sorais, M., Poirier, A., Widory, D., and Verreault, J. 2019. Habitat use strategy influences the tissue signature of trace elements including rare earth elements in an urban-adapted omnivorous bird. Environ. Res. 168: 261-269. doi: 10.1016/j.envres.2018.10.004.

Casse, M., Montero-Serrano, J.C., St-Onge, G., and Poirier, A. 2019. REE distribution and Nd isotope composition of estuarine waters and bulk sediment leachates tracing lithogenic inputs in eastern Canada. Mar. Chem., 211: 117-130. doi: 10.1016/j.marchem.2019.03.012.

Cao, X., Wang, X., and Zhao, G. 2000. Assessment of the bioavailability of rare earth elements in soils by chemical fractionation and multiple regression analysis. Chemosphere, 40(1): 23–28. doi: 10.1016/S0045-6535(99)00225-8.

Cao, X., Chen, Y., Wang, X., and Deng, X. 2001. Effects of redox potential and pH value on the release of rare earth elements from soil. Chemosphere. 44(4), 655-661. doi: 10.1016/S0045-6535(00)00492-6.

Carolina, A., Duarte, O., Oliveira, C. De, Ramos, S.J., and De, E.M. 2018. Acta Scientiarum Lanthanum content and effects on growth, gas exchanges, and chlorophyll index in maize plants. Acta Sci. Biol. Sci. 40: e38469. doi:10.4025/actascibiolsci.v40i1.38469.

Cardon, P.Y., Triffault-Bouchet, G., Caron, A., Rosabal, M., Fortin, C., and Amyot, M. 2019. Toxicity and subcellular fractionation of yttrium in three freshwater organisms: Daphnia magna, Chironomus riparius, and Oncorhynchus mykiss. ACS omega, 4(9): 13747-13755. doi: 10.1021/acsomega.9b01238.

Censi, P., Tamburo, E., Speziale, S., Zuddas, P., Randazzo, L.A., Punturo, R., Cuttitta, A., and Aricò, P. 2011. Yttrium and lanthanides in human lung fluids, probing the exposure to atmospheric fallout. J. Hazard. Mater. 186(2–3): 1103–1110. doi:10.1016/j.jhazmat.2010.11.113.

Cereceda-balic, F., Palomo-marín, M.R., Bernalte, E., Vidal, V., Christie, J., Fadic, X., Guevara, J.L., Miro, C., and Gil, E.P. 2012. Impact of Santiago de Chile urban atmospheric pollution on anthropogenic trace elements enrichment in snow precipitation at Cerro Colorado, Central Andes. Atmos. Environ. 47: 51–57. doi:10.1016/j.atmosenv.2011.11.045.

Chang, C., and Chang, C. 2012. Assessment of influential range and characteristics of fugitive dust in limestone extraction processes. J. Air Waste Manag. Assoc. 54: 141–148. doi:10.1080/10473289.2004.10470889.

Costis, S., Coudert, L., Mueller, K.K., Cecchi, E., Neculita, C.M., and Blais, J.F. 2020. Assessment of the leaching potential of flotation tailings from rare earth mineral extraction in cold climates. Sci. Total Environ. 732: 139225. doi: 10.1016/j.scitotenv.2020.139225.

Chen, J., Xiao, H., Qi, T., and Chen, D. 2015. Rare earths exposure and male infertility: the injury mechanism study of rare earths on male mice and human sperm. Environ. Sci. Pollut. Res. 22: 2076–2086. doi:10.1007/s11356-014-3499-y.

Chen, W., Gu, Y., Zhao, G., Tao, Y., Luo, J., and Hu, T. 2000. Effects of rare earth ions on activity of RuBPcase in tobacco. Plant Sci. 152: 145–151. doi: 10.1016/S0168-9452(99)00235-6.

Chen, Z. 2005. Accumulation and toxicity of rare earth elements in brain and their potential effects on health. Rural Eco-Environ, 21(4): 72-73. doi: 10.1007/s10971-005-6694-y.

Chen, Z. and Zhu X. 2009. Accumulation and toxicity of rare earth elements in liver. J. Dom. Anim. Ecol., 30 (4): 98-102. Available from http://en.cnki.com.cn/article_en/cjfdtotal-jcst200904028.htm [accessed April 2009].

Cheung, K., Sanei, H., Klassen, P., Mayer, B., and Goodarzi, F. 2009. Produced fluids and shallow groundwater in coalbed methane (CBM) producing regions of Alberta, Canada: Trace element and rare earth element geochemistry. Int. J. Coal Geol. 77(3-4): 338-349. doi: 10.1016/j.coal.2008.07.012.

Chi, R., Zhang, X., and Wang, C. 2004. Recovery of rare earth from bastnasite by ammonium chloride roasting with fluorine deactivation. Miner. Eng. 17: 1037–1043. doi:10.1016/j.mineng.2004.04.010.

Chiarenzelli, J., Aspler, L., Dunn, C., Cousens, B., Ozarko, D., and Powis, K. 2001. Multi-element and rare earth element composition of lichens, mosses, and vascular plants from the central Barrenlands, Nunavut, Canada. Appl. Geochemistry 16(2): 245–270. doi:10.1016/S0883-2927(00)00027-5.

Chua, H. 1998. Bio-accumulation of environmental residues of rare earth elements in aquatic flora *Eichhornia crassipes* (Mart.) Solms in Guangdong Province of

China. Sci. Total Environ. 214(1-3): 79-85. doi: 10.1016/S0048-9697(98)00055-2.

Costis, S., Coudert, L., Mueller, K.K., Cecchi, E., Neculita, C.M., and Blais, J.F. 2020. Assessment of the leaching potential of flotation tailings from rare earth mineral extraction in cold climates. Sci. Total Environ. 732: 139225. doi: 10.1016/j.scitotenv.2020.139225.

Cui, W., Kamran, M., Song, Q., Zuo, B., Jia, Z., and Han, Q. 2019. Lanthanum chloride improves maize grain yield by promoting photosynthetic characteristics, antioxidants enzymes and endogenous hormone at reproductive stages. J. Rare Earths. 37(7): 781–790. doi:10.1016/j.jre.2018.12.006.

Davranche, M., Gruau, G., Dia, A., Marsac, R., Pédrot, M., and Pourret, O. 2015. Biogeochemical factors affecting rare earth element distribution in shallow wetland groundwater. Aquat. Geochem. 21(2–4): 197–215. doi:10.1007/s10498-014-9247-6.

Degtjarenko, P., Marmor, L., and Randlane, T. 2016. Changes in bryophyte and lichen communities on scots pines along an alkaline dust pollution gradient. Environ. Sci. Pollut. Res. 23: 17413–17425. doi:10.1007/s11356-016-6933-5.

Dixit, S. 2020. Canadian Federal Water Quality Guidelines for Rare Earth Elements. SETAC North America 41st Annual Meeting.

Dostal, J. 2017. Rare earth element deposits of alkaline igneous rocks. Resources, 6(3): 34. doi: 10.3390/resources6030034.

Dubé, M., Auclair, J., Hanana, H., Turcotte, P., Gagnon, C., and Gagné, F. 2019. Gene expression changes and toxicity of selected rare earth elements in rainbow trout juveniles. Comp. Biochem. Physiol. Part-C Toxicol. Pharmacol. 223: 88–95. doi:10.1016/j.cbpc.2019.05.009.

Dunn, C.E., and Hoffman, E. 1986. Multi-element study of vegetation from a zone of rare-earth rich allanite and apatite in northern Saskatchewan, Canada. Appl. Geochem. 1(3): 375-381. doi: 10.1016/0883-2927(86)90022-3.

Edahbi, M., Plante, B., and Benzaazoua, M. 2019. Environmental challenges and identification of the knowledge gaps associated with REE mine wastes management. J. Clean. Prod. 212: 1232–1241. doi:10.1016/j.jclepro.2018.11.228.

Fan, G.Q., Zheng, H.L., and Yuan, Z.K. 2005. Effects of thulium exposure on IQ of children. J. Environ. Health 22(4): 256-257. doi: 10.16241/j.cnki.1001-5914.2005.04.008.

Feng, J., and Xu, S.Q. 2000. Bio-effect of rare earths in RE-High Background Region. J. Rare Earth 18(4):356. doi: 10.3321/j.issn:1000-4343.2000.04.018.

Filella, M., and Rodushkin, I. 2018. A concise guide for the determination of less-studied technology-critical elements (Nb, Ta, Ga, In, Ge, Te) by inductively coupled plasma mass spectrometry in environmental samples. Spectrochim. Acta B, 141: 80-84. doi: 10.1016/j.sab.2018.01.004.

Frenzel, T., Apte, C., Jost, G., Schöckel, L., Lohrke, J., and Pietsch, H. 2017. Quantification and assessment of the chemical form of residual gadolinium in the brain after repeated administration of gadolinium-based contrast agents comparative study in rats. Invest. Radiol. 52(7): 396–404. doi:10.1097/RLI.0000000000000352.

García, A.C., Latifi, M., Amini, A., and Chaouki, J. 2020. Separation of Radioactive Elements from Rare Earth Element-Bearing Minerals. Metals, 10(11): 1524. doi: doi.org/10.3390/met10111524.

Gérin-Lajoie, J., Herrmann, T.M., MacMillan, G.A., Hébert-Houle, É., Monfette, M., Rowell, J.A., Anaviapik Soucie, T., Snowball, H., Townley, E., Lévesque, E. and Amyot, M. 2018. IMALIRIJIIT: a community-based environmental monitoring program in the George River watershed, Nunavik, Canada. Écoscience, 25(4): 381-399. doi: 10.1080/11956860.2018.1498226.

Golev, A., Scott, M., Erskine, P.D., Ali, S.H., and Ballantyne, G.R. 2014. Rare earths supply chains: Current status, constraints and opportunities. Resour. Policy 41(1): 52–59. doi:10.1016/j.resourpol.2014.03.004.

Gong, B., He, E., Qiu, H., Li, J., Ji, J., Peijnenburg, W.J., Liu, Y., Zhao, L. and Cao, X. 2019. The cation competition and electrostatic theory are equally valid in quantifying the toxicity of trivalent rare earth ions (Y^{3+} and Ce^{3+}) to *Triticum aestivum*. Environ. Pollut., 250: 456-463. doi: 10.1016/j.envpol.2019.04.075.

Gorbatenko, A.A., and Revina, E.I. 2015. A review of instrumental methods for determination of rare earth elements. Inorg. Mater., 51(14): 1375-1388. doi: 10.1134/S0020168515140058.

Gwenzi, W., Mangori, L., Danha, C., Chaukura, N., Dunjana, N., and Sanganyado, E. 2018. Sources, behaviour, and environmental and human health risks of high-technology rare earth elements as emerging contaminants. Sci. Total Environ. 636: 299–313. doi:10.1016/j.scitotenv.2018.04.235.

Haque, N., Hughes, A., Lim, S., and Vernon, C. 2014. Rare earth elements: overview of mining, mineralogy, uses, sustainability and environmental impact. Resources, 3(4): 614–635. doi:10.3390/resources3040614.

Hatje, V., Bruland, K.W., and Flegal, A.R. 2016. Increases in anthropogenic gadolinium anomalies and rare earth element concentrations in San Francisco Bay over a 20 year record. Environ. Sci. Technol. 50(8): 4159–4168. doi:10.1021/acs.est.5b04322.

He, H., Fan, C., Peng, Q., Wu, M., Zheng, J., and Wu, G. 2019a. Bioaccumulation and translocation of rare earth elements in two forage legumes grown in soils treated with coal fly ash. Chem. Geol. 528:119284. doi:10.1016/j.chemgeo.2019.119284.

He, D., Xia, B., Zhou, Q., Wang, L., and Huang, X. 2019b. Rare earth elements regulate the endocytosis and DNA methylation in root cells of *Arabidopsis thaliana*. Chemosphere, 227: 522–532. doi:10.1016/j.chemosphere.2019.04.076.

Heller, A., Barkleit, A., Bok, F., and Wober, J. 2019. Effect of four lanthanides onto the viability of two mammalian kidney cell lines. Ecotoxicol. Environ. Saf. 173: 469–481. doi:10.1016/j.ecoenv.2019.02.013.

Henriques, B., Coppola, F., Monteiro, R., Pinto, J., Viana, T., Pretti, C., Soares, A., Freitas, R., and Pereira, E. 2019. Toxicological assessment of anthropogenic Gadolinium in seawater: Biochemical effects in mussels Mytilus galloprovincialis. Sci. Total Environ. 664: 626–634. doi:10.1016/j.scitotenv.2019.01.341.

Hu, Z., Richter, H., Sparovek, G., and Schnug, E. 2004. Physiological and biochemical effects of rare earth elements on plants and their agricultural significance: A Review. J. Plant Nutr. 27(1): 183–220. doi:10.1081/PLN-120027555.

Hua, D., Wang, J., Yu, D., and Liu, J. 2017. Lanthanum exerts acute toxicity and histopathological changes in gill and liver tissue of rare minnow (*Gobiocypris rarus*). Ecotoxicology, 26(9): 1207–1215. doi:10.1007/s10646-017-1846-8.

Huang, T., Qiu, R., Lan, Z., Ren, Y., Yang, C., Qian, Y., Wang, C., He, Xi., and Si, W. 2019. The toxic effects of leakage water from rare earth tailings reservoir on the postembryonic development of *Strauchbufo raddei*. J. South. Agric. 50(2): 412–417. doi: 10.3969/j.issn.2095-1191.2019.02.28.

Huang, X., Long, Z., and Wang, L. 2015. Technology development for rare earth cleaner hydrometallurgy in China. Rare Met. 34(4): 215–222. doi:10.1007/s12598-015-0473-x.

Jakubek, L.M., Marangoudakis, S., Raingo, J., Liu, X., Lipscombe, D., and Hurt, R.H. 2009. The inhibition of neuronal calcium ion channels by trace levels of yttrium released from carbon nanotubes. Biomaterials, 30(31): 6351–6357. doi:10.1016/j.biomaterials.2009.08.009.

Ji, X., Abakumov, E., Polyakov, V., and Xie, X. 2021. Mobilization of geochemical elements to surface water in the active layer of permafrost in the Russian Arctic. Water Resour. Res. 57(1): e2020WR028269. doi: 10.1029/2020WR028269

Jiang, D.G., Yang, J., Zhang, S., and Yang, D.J. 2012. A Survey of 16 Rare Earth Elements in the Major Foods in China. Biomed. Environ. Sci. 25(3): 267–271. doi:10.3967/0895-3988.2012.03.003.

Johannesson, K.H., and Hendry, M.J. 2000. Rare earth element geochemistry of groundwaters from a thick till and clay-rich aquitard sequence, Saskatchewan, Canada. Geochim. Cosmochim. Ac. 64(9): 1493-1509. doi: 10.1016/S0016-7037(99)00402-0.

Julio, N., and Fu, Z. 2014. Life-cycle assessment of the production of rare-earth elements for energy applications a review. Front. Energy Res. 2: 45. doi: 10.3389/fenrg.2014.00045.

Kabata-Pendias A, 2011. Trace Elements in Soils and Plants, 4th ed. CRC Press, Boca Raton, Florida, USA.

Kalvig, P., and Machacek, E. 2018. Examining the rare-earth elements (REE) supply–demand balance for future global wind power scenarios. Surv. Denmark Greenl. Bull. 41: 87–90. doi:10.34194/geusb.v41.4350.

Kanda, T., Fukusato, T., Matsuda, M., Toyoda, K., Oba, H., Kotoku, J., Haruyama, T., Kitajima, K., and Furui, S. 2015. Gadolinium-based contrast agent accumulates in the brain even in subjects without severe renal dysfunction: Evaluation of autopsy brain specimens with inductively coupled plasma mass spectroscopy. Radiology, 276(1): 228–232. doi:10.1148/radiol.2015142690.

Karen, S.S. 2015. Heavy rare earths, permanent magnets, and renewable energies: An imminent crisis. Energ. Policy, 79: 1–8. doi:10.1016/j.enpol.2014.12.015.

Kexin, L.I., Tao, L., Lingqing, W., and Zhiping, Y. 2015. Contamination and health risk assessment of heavy metals in road dust in Bayan Obo Mining Region in

Inner Mongolia, North China. J. Geogr. Sci. 25(12): 1439–1451. doi:10.1007/s11442-015-1244-1.

Khan, A.M., Kartini, N., Bakar, A., Farid, A., and Bakar, A. 2017. Chemical speciation and bioavailability of rare earth elements (REEs) in the ecosystem: a review. Environ. Sci. Pollut. Res. 24: 22764–22789. doi:10.1007/s11356-016-7427-1.

Kotelnikova, A., Fastovets, I., Rogova, O., Volkov, D. S., and Stolbova, V. 2019. Toxicity assay of lanthanum and cerium in solutions and soil. Ecotoxicol. Environ. Saf. 167: 20-28. doi: 10.1016/j.ecoenv.2018.09.117.

Langkau, S., Erdmann, M. 2020. Environmental impacts of the future supply of rare earths for magnet applications. J. Ind. Ecol. 1-17. doi: 10.1111/jiec.13090.

Li, L., Wang, L., and Wen, S. 1986. Ten-year death cause analysis of radon-containing dust workers in the Bayan Obo mine. Chinese J. Radiol. Med. Prot. 6(6): 399–401. Available from http://www.cjrmp.net/ch/reader/view_abstract.aspx?file_no=19860614 [accessed 19 December 1984].

Li, X., Chen, Z., Chen, Z., and Zhang, Y. 2013. A human health risk assessment of rare earth elements in soil and vegetables from a mining area in Fujian Province, Southeast China. Chemosphere, 93(6): 1240-1246. doi: doi.org/10.1016/j.chemosphere.2013.06.085.

Li, X., Chen, Z., and Chen, Z. 2014. Distribution and fractionation of rare earth elements in soil–water system and human blood and hair from a mining area in southwest Fujian Province, China. Environ. Earth Sci. 72(9): 3599–3608. doi:10.1007/s12665-014-3271-0.

Li, J., Zheng, L., Sun, C., Jiang, F., Yin, X., and Chen, J. 2016. Study on ecological and chemical properties of rare earth elements in tropical marine organisms. Chinese J. Anal. Chem. 44(10): 1539–1546. doi:10.1016/S1872-2040(16)60963-5.

Liang, T., Li, K., and Wang, L. 2014. State of rare earth elements in different environmental components in mining areas of China. Environ. Monit. Assess. 186(3): 1499-1513. doi: 10.1007/s10661-013-3469-8.

Liu, W.S., Guo, M.N., Liu, C., Yuan, M., Chen, X.T., Huot, H., Zhao, C.M., Tang, Y.T., Morel, J.L., and Qiu, R.L. 2019. Water, sediment and agricultural soil contamination from an ion-adsorption rare earth mining area. Chemosphere, 216: 75–83. doi:10.1016/j.chemosphere.2018.10.109.

Liu, Y., Sun, L., Zhang, P., Wan, J., and Wang, R. 2017. Lanthanum inhibits primary root growth by repressing auxin carrier abundances in *Arabidopsis*. Front. Plant Sci. 8: 1661. doi:10.3389/fpls.2017.01661.

Liu, Y., Wang, R., Zhang, P., Sun, L., and Xu, J. 2016. Involvement of reactive oxygen species in lanthanum-induced inhibition of primary root growth. J. Experiemntal Bot. Bot. 67(21): 6149–6159. doi:10.1093/jxb/erw379.

Lu, R., and Ni, J. 2002. Mechanism of rare earth effect on liver. J. Rare Earths 20,193-198. doi: 10.3321/j.issn:1000-4343.2002.03.001.

Lu, A., Zhang, S., Shan, X.Q., Wang, S., and Wang, Z. 2003. Application of microwave extraction for the evaluation of bioavailability of rare earth elements in soils. Chemosphere, 53(9): 1067-1075. doi: 10.1016/S0045-6535(03)00672-6.

Luo, J., Zhang, J., and Wang, Y. 2008. Changes in endogenous hormone levels and redox status during enhanced adventitious rooting by rare earth element neodymium of *Dendrobium densiflorum* shoot cuttings. J. Rare Earths, 26(6): 869–874. doi:10.1016/S1002-0721(09)60023-5.

Ma, L., Dang, D., Wang, W., Evans, R., and Wang, W. 2019. Rare earth elements in the Pearl River Delta of China: potential impacts of the REE industry on water, suspended particles and oysters. Environ. Pollut. 244: 190–201. doi:10.1016/j.envpol.2018.10.015.

Malhotra, N., Hsu, H.S., Liang, S.T., Roldan, M.J.M., Lee, J.S., Ger, T.R., and Hsiao, C.D. 2020. An Updated review of toxicity effect of the rare earth elements (REEs) on aquatic organisms. Animals, 10(9): 1663. doi: 10.3390/ani10091663.

MacMillan, G.A., Chételat, J., Heath, J.P., Mickpegak, R., and Amyot, M. 2017. Rare earth elements in freshwater, marine, and terrestrial ecosystems in the eastern Canadian Arctic. Environ. Sci. Process. Impacts, 19(10): 1336–1345. doi:10.1039/c7em00082k.

MacMillan, G.A., Clayden, M.G., Chételat, J., Richardson, M.C., Ponton, D.E., Perron, T., Amyot, M., 2018. Environmental drivers of rare earth element bioaccumulation in freshwater zooplankton. Environ. Sci. Technol. 53(3): 1650-1660. doi: 10.1021/acs.est.8b05547.

Mcdonald, J.W., Ghio, A.J., Sheehan, C.E.,Bernhardt, P.F., Roggli, V.L. 1995. Rare earth (cerium oxide) pneumoconiosis: analytical scanning electron microscopy and literature review. Mod. Pathol. 8: 859–865. PMID: 8552576.

Mestre, N.C., Sousa, V.S., Rocha, T.L., and Bebianno, M.J. 2019. Ecotoxicity of rare earths in the marine mussel Mytilus galloprovincialis and a preliminary approach to assess environmental risk. Ecotoxicology, 28(3): 294–301. doi:10.1007/s10646-019-02022-4.

Meryem, B., Hongbing, J., Yang, G., Huajian, D., and Cai, L. 2016. Distribution of rare earth elements in agricultural soil and human body (scalp hair and urine) near smelting and mining areas of Hezhang, China. J. Rare Earth. 34(11): 1156-1167. doi: 10.1016/S1002-0721(16)60148-5.

Mihajlovic, J., Stärk, H. J., and Rinklebe, J. 2017. Rare earth elements and their release dynamics under pre-definite redox conditions in a floodplain soil. Chemosphere, 181: 313-319. doi: j.chemosphere.2017.04.036.

Naeth, M.A., and Wilkinson, S.R. 1997. Lichens as biomonitors of air quality around a diamond mine, northwest territories, Canada. J. Environ. Qual. 37: 1675–1684. doi:10.2134/jeq2007.0090.

Oral, R., Pagano, G., Siciliano, A., Gravina, M., Palumbo, A., Castellano, I., Migliaccio, O., Thomas, P.J., Guida, M., Tommasi, F., and Trifuoggi, M. 2017. Heavy rare earth elements affect early life stages in Paracentrotus lividus and Arbacia lixula sea urchins. Environ. Res. 154: 240–246. doi:10.1016/j.envres.2017.01.011

Orris, G.J., Seo, Y., Briggs, D.A., Dunlap, P., and Cocker, M.D. 2018. Global rare earth element occurrence database: U.S. Geological Survey data release. doi:10.5066/F7DR2TN4.

Pang, X., Li, D., and Peng, A. 2002. Application of rare-earth elements in the agriculture of China and its environmental behavior in soil. Environ. Sci. Pollut. Res. 9(2): 143. doi: 10.1007/bf02987462.

Pagano, G., Guida, M., Tommasi, F., and Oral, R. 2015a. Health effects and toxicity mechanisms of rare earth elements-Knowledge gaps and research prospects. Ecotoxicol. Environ. Saf. 115: 40–48. doi:10.1016/j.ecoenv.2015.01.030.

Pagano, G., Aliberti, F., Guida, M., Oral, R., Siciliano, A., Trifuoggi, M., and Tommasi, F. 2015b. Rare earth elements in human and animal health: State of art and research priorities. Environ. Res. 142: 215–220. doi:10.1016/j.envres.2015.06.039.

Pagano, G., Thomas, P.J., Di Nunzio, A., and Trifuoggi, M. 2019. Human exposures to rare earth elements: Present knowledge and research prospects. Environ. Res. 171: 493–500. doi:10.1016/j.envres.2019.02.004.

Polfus, J.L., Hebblewhite, M., and Heinemeyer, K. 2011. Identifying indirect habitat loss and avoidance of human infrastructure by northern mountain woodland caribou. Biol. Conserv., 144(11): 2637-2646. doi: 10.1016/j.biocon.2011.07.023.

Peiró, L.T., and Méndez, G.V. 2013. Material and energy requirement for rare earth production. JOM. 65(10): 1327-1340. doi: 10.1007/s11837-013-0719-8.

Pell, R., Wall, F., Yan, X., Li, J., and Zeng, X. 2019. Mineral processing simulation based-environmental life cycle assessment for rare earth project development: A case study on the Songwe Hill project. J. Environ. Manage. 249: 109353. doi:10.1016/j.jenvman.2019.109353.

Peng, C., Ma, Z., Lei, X., Zhu, Q., Chen, H., Wang, W., Liu, S., Li, W., Fang, X., and Zhou, X. 2011. A drought-induced pervasive increase in tree mortality across Canada's boreal forests. Nat. Clim. Chang. 1(12): 1–5. doi:10.1038/nclimate1293.

Picone, N., and den Camp, H.J.O. 2019. Role of rare earth elements in methanol oxidation. Curr. Opin. Chem. Biol. 49: 39-44. doi: 10.1016/j.cbpa.2018.09.019.

Pinto, J., Costa, M., Leite, C., Borges, C., Coppola, F., Henriques, B., Monteiro, R., Russo, T., Cosmo, A., Soares, A., Polese, G., Pereira, E., and Polese, G. 2019. Ecotoxicological effects of lanthanum in Mytilus galloprovincialis: biochemical and histopathological impacts. Aquat. Toxicol. 211: 181-192. doi: 10.1016/j.aquatox.2019.03.017.

Pol, A., Barends, T.R.M., Dietl, A., Khadem, A.F., Eygensteyn, J., Jetten, M.S.M., and Camp, H.J.M.O. Den. 2014. Rare earth metals are essential for methanotrophic life in volcanic mudpots. Environ. Microbiol. 1(16): 255–264. doi:10.1111/1462-2920.12249.

Polfus, J. L., Hebblewhite, M., and Heinemeyer, K. 2011. Identifying indirect habitat loss and avoidance of human infrastructure by northern mountain woodland caribou. Biol. Conserv. 144(11): 2637-2646. doi: 10.1016/j.biocon.2011.07.023.

Pokrovsky, O.S., Karlsson, J., and Giesler, R. 2018. Freeze-thaw cycles of Arctic thaw ponds remove colloidal metals and generate low-molecular-weight organic matter. Biogeochemistry 137(3): 321-336. doi: 10.1007/s10533-018-0421-6.

Qi, L., Ge, Y., Xia, T., He, J.Z., Shen, C., Wang, J., and Liu, Y.J. 2019. Rare earth oxide nanoparticles promote soil microbial antibiotic resistance by selectively enriching antibiotic resistance genes. Environ. Sci. Nano, 6(2): 456-466. doi: 10.1039/c8en01129j.

Qin, J., Chen, X., and Li, Z. 2002. Rare earth element' influence on human health. Guangdong Trace Elem. Sci. 9(6): 1–17. doi: 10.3969/j.issn.1006-446X.2002.06.001.

Ram, R., Becker, M., Brugger, J., Etschmann, B., Burcher-jones, C., Howard, D., Kooyman, P.J., and Petersen, J. 2019. Characterisation of a rare earth element- and zirconium-bearing ion- adsorption clay deposit in Madagascar. Chem. Geol. 522: 93–107. doi:10.1016/j.chemgeo.2019.05.011.

Rasmussen, P.E., Levesque, C., Chénier, M., and Gardner, H.D. 2017. Rare earth elements and select actinoids in the Canadian house dust study. Indoor air, 27(5): 965-976. doi: 10.1111/ina.12379.

Romero-Freire, A., Turlin, F., André-Mayer, A. S., Pelletier, M., Cayer, A., and Giamberini, L. 2019. Biogeochemical cycle of lanthanides in a light rare earth element-enriched geological area (Quebec, Canada). Minerals, 9(10): 573. doi: 10.3390/min9100573.

Ritson, J.P., Graham, N.J.D., Templeton, M.R., Clark, J.M., Gough, R., and Freeman, C. 2014. The impact of climate change on the treatability of dissolved organic matter (DOM) in upland water supplies: a UK perspective. Sci. Total Environ. 473: 714-730. doi: 10.1016/j.scitotenv.2013.12.095.

Sauvé, S., and Desroirers, M. 2014. A review of what is an emerging contaminant. Chem. Cent. J. 8(1): 1–7. doi:10.1016/j.marpolbul.2013.01.035.

Scheer, D., Benighaus, C., Benighaus, L., Renn, O., Gold, S., Bettina, R., and Gaby-fleur, B. 2014. The distinction between risk and hazard: understanding and use in stakeholder communication. Risk Anal. 34(7): 1270–1285. doi:10.1111/risa.12169.

Schmidt, K., Bau, M., Merschel, G., and Tepe, N. 2019. Anthropogenic gadolinium in tap water and in tap water-based beverages from fast-food franchises in six major cities in Germany. Sci. Total Environ. 687: 1401–1408. Elsevier B.V. doi:10.1016/j.scitotenv.2019.07.075.

Sharma, S. 2010. Assessing diet and lifestyle in the Canadian Arctic Inuit and Inuvialuit to inform a nutrition and physical activity intervention programme. J. Hum. Nutr. Diet. 23: 5–17. doi:10.1111/j.1365-277X.2010.01093.x.

Shen, F., Wang, L., Zhou, Q., and Huang, X. 2018. Effects of lanthanum on *Microcystis aeruginosa*: Attention to the changes in composition and content of cellular microcystins. Aquat. Toxicol. 196: 9–16. doi:10.1016/j.aquatox.2018.01.007.

Shiller, A.M. 2010. Dissolved rare earth elements in a seasonally snow-covered, alpine/subalpine watershed, Loch Vale, Colorado. Geochim. Cosmochim. Acta, 74(7): 2040-2052. doi:10.1016/j.gca.2010.01.019.

Smith, K.S., Balistrieri, L.S., and Todd, A.S. 2015. Using biotic ligand models to predict metal toxicity in mineralized systems. Appl. Geochemi. 57: 55-72. doi: 10.1016/j.apgeochem.2014.07.005.

Squadrone, S., Brizio, P., Stella, C., Mantia, M., Battuello, M., Nurra, N., Sartor, R.M., Orusa, R., Robetto, S., Brusa, F., Mogliotti, P., Garrone, A., and Abete, M.C. 2019. Rare earth elements in marine and terrestrial matrices of Northwestern Italy: Implications for food safety and human health. Sci. Total Environ. 660: 1383–1391. doi:10.1016/j.scitotenv.2019.01.112.

Sun, G., Li, Z., Liu, T., Chen, J., Wu, T., and Feng, X. 2017. Rare earth elements in street dust and associated health risk in a municipal industrial base of central China. Environ. Geochem. Health, 39(6): 1469–1486. doi:10.1007/s10653-017-9982-x.

Tanaka, Y., Hosaka, T., and Ochi, K. 2010. Rare earth elements activate the secondary metabolite–biosynthetic gene clusters in *Streptomyces coelicolor* A3 (2). J. Antibiot. Res. 63(8): 477-481. doi:10.1038/ja.2010.53.

Tessler, A., Campbell, P.G.C., and Bisson, M., 1979. Sequential extraction techniques for the speciation of particulate trace metals. Anal. Chem. 51(7): 844–851. doi: 10.1021/ac50043a017.

Tepe, N., Romero, M., and Bau, M. 2014. High-technology metals as emerging contaminants: Strong increase of anthropogenic gadolinium levels in tap water of Berlin, Germany, from 2009 to 2012. Appl. Geochem. 45: 191–197. doi:10.1016/j.apgeochem.2014.04.006.

Uprety, Y., Asselin, H., Dhakal, A., Julien, N. 2012. Traditional use of medicinal plants in the boreal forest of Canada: review and perspectives. J. Ethnobiol. Ethnomed. 8(1): 1-14. doi: 10.1186/1746-4269-8-7.

Venier, L.A., Thompson, I.D., Fleming, R., Malcolm, J., Aubin, I., Trofymow, J.A., Langor, D., Sturrock, R., Patry, C., Outerbridge, R.O., Holmes, S.B., Haeussler, S., Grandpré, L. De, Chen, H.Y.H., Bayne, E., Arsenault, A., and Brandt, J.P. 2014. Effects of natural resource development on the terrestrial biodiversity of Canadian boreal forests 1. Environ. Rev. 490: 457–490. doi: 10.1139/er-2013-0075.

Violante, A., Cozzolino, V., Perelomov, L., Caporale, A. G., & Pigna, M. 2010. Mobility and bioavailability of heavy metals and metalloids in soil environments. J. Soil. Sci. Plant Nutr. 10(3), 268-292. doi: 10.4067/S0718-95162010000100005.

Wang, L., and Liang, T. 2014. Accumulation and fractionation of rare earth elements in atmospheric particulates around a mine tailing in Baotou, China. Atmos. Environ. 88: 23–29. doi:10.1016/j.atmosenv.2014.01.068.

Wang, L., Huang, X., and Zhou, Q. 2008. Chemosphere effects of rare earth elements on the distribution of mineral elements and heavy metals in horseradish. Chemosphere, 73: 314–319. doi:10.1016/j.chemosphere.2008.06.004.

Wang, L., Liang, T., Zhang, Q., and Li, K. 2014a. Rare earth element components in atmospheric particulates in the Bayan Obo mine region. Environ. Res. 131: 64–70. doi:10.1016/j.envres.2014.02.006.

Wang, X., Shan, X., Zhang, S., Wen, B. 2014b. Distribution of rare earth elements among chloroplast components of hyperaccumulator *Dicranopteris dichotoma*. Anal. Bioanal. Chem. 376(6): 913–917. doi:10.1007/s00216-003-2014-y.

Wang, L., Wang, W., Zhou, Q., and Huang, X. 2014c. Combined effects of lanthanum (III) chloride and acid rain on photosynthetic parameters in rice. Chemosphere, 112: 355–361. doi:10.1016/j.chemosphere.2014.04.069.

Wang, L., Zhong, B., Liang, T., Xing, B., and Zhu, Y. 2016. Atmospheric thorium pollution and inhalation exposure in the largest rare earth mining and smelting area in China. Sci. Total Environ. 572: 1–8. doi:10.1016/j.scitotenv.2016.07.192.

Wei, B., Li, Y., Li, H., Yu, J., Ye, B., and Liang, T. 2013. Rare earth elements in human hair from a mining area of China. Ecotoxicol. Environ. Saf. 96: 118-123. doi: 10.1016/j.ecoenv.2013.05.031.

Weng, Z.H., Jowitt, S.M., Mudd, G.M., and Haque, N. 2013. Assessing rare earth element mineral deposit types and links to environmental impacts. Appl. Ear. Sci. 122(2): 83-96. doi:10.1179/1743275813Y.0000000036.

Wesche, S.D., and Chan, H.M. 2010. Adapting to the impacts of climate change on food security among Inuit in the western Canadian Arctic. Ecohealth, 7: 361–373. doi:10.1007/s10393-010-0344-8.

Winde, F., Brugge, D., Nidecker, A., and Ruegg, U. 2017. Uranium from Africa - An overview on past and current mining activities: Re-appraising associated risks and chances in a global context. J. African Earth Sci. 129: 759–778. doi:10.1016/j.jafrearsci.2016.12.004.

Wu, H., Li, X., Feng, J.H., Li, W., Li, W., Li, Z., Liao, P., Wu, Y., Pei, F. 2006. Comparison of biochemical effects induced by Changle between male and female rats using NMR and ICP-MS techniques. J. Rare Earth. 24, 108-114

Wu, S., Wang, L., Zhao, L., Zhang, P., and El-shall, H. 2018. Recovery of rare earth elements from phosphate rock by hydrometallurgical processes – A critical review. Chem. Eng. J. 335: 774–800. doi:10.1016/j.cej.2017.10.143.

Wulder, M.A., Li, Z., Campbell, E.M., White, J.C., Hobart, G., Hermosilla, T., and Coops, N.C. 2018. A National Assessment of Wetland Status and Trends for Canada's Forested Ecosystems Using 33 Years of Earth Observation Satellite Data. Remote Sens. 10: 1623. doi:10.3390/rs10101623.

Xu, Q., Zhang, W., Sha, S., Yang, Y., Su, C., and Hu, D. 2019. Subcellular distribution and physiological responses of Potamogeton crispus to yttrium. Acta Physiol. Plant. 41(6). doi:10.1007/s11738-019-2857-2.

Xu, X., Zhu, W., Wang, Z., and Witkamp, G.J. 2002. Distributions of rare earths and heavy metals in field-grown maize after application of rare earth-containing fertilizer. Sci. Total Environ. 293(1–3): 97–105. doi:10.1016/S0048-9697(01)01150-0.

Yang, X.J., Lin, A., Li, X.L., Wu, Y., Zhou, W., and Chen, Z. 2013. China's ion-adsorption rare earth resources, mining consequences and preservation. Environ. Dev. 8(1): 131–136. doi:10.1016/j.envdev.2013.03.006.

Yang, L., Wang, X., Nie, H., Shao, L., Wang, G., and Liu, Y. 2016. Residual levels of rare earth elements in freshwater and marine fish and their health risk assessment from Shandong, China. Mar. Pollut. Bull. 107(1): 393-397. doi: 10.1016/j.marpolbul.2016.03.034.

York, N., Allen, T., Stone, J., Brooks, W., and Beck, M.W. 2012. Assessing future risk: quantifying the effects of sea level rise on storm surge risk for the southern shores of Long. Nat. hazards, 60(2): 727–745. doi:10.1007/s11069-011-0046-8.

Zaichick, S., Zaichick, V., Karandashev, V., and Nosenko, S. 2011. Accumulation of rare earth elements in human bone within the lifespan. Metallomics, 3(2): 186-194. doi: 10.1039/c0mt00069h.

Zeng, F., Tian, H.E., Wang, Z.P., An, Y., Gao, F.Y., Zhang, L.J., Li, F.M., and Shan, L. 2003. Effect of rare earth element europium on amaranthin synthesis in

Amarathus caudatus seedlings. Biol. Trace Elem. Res. 93: 271–282. doi:10.1385/BTER:93:1-3:271.

Zeng, X.Y., Li, S.W., Leng, Y. and Kang, X.H., 2020. Structural and functional responses of bacterial and fungal communities to multiple heavy metal exposure in arid loess. *Science of The Total Environment*, 723,138081. doi: 10.1016/j.scitotenv.2020.138081

Zhang, D.Y., Shen, X.Y., Ruan, Q., Xu, X.L., Yang, S.P., Lu, Y., Xu, H.Y., and Hao, F.L. 2014. Effects of subchronic samarium exposure on the histopathological structure and apoptosis regulation in mouse testis. Environ. Toxicol. Pharmacol. 37(2): 505–512. doi:10.1016/j.etap.2014.01.007.

Zhang, F., Hu, H., Wang, L., Zhou, Q., and Huang, X. 2018. Chemosphere Effects of rare earth and acid rain pollution on plant chloroplast ATP synthase and element contents at different growth stages. Chemosphere, 194: 441–449. doi:10.1016/j.chemosphere.2017.12.001.

Zhang, H., Deng, L., Yang, J., Jiang, J., Shen, Z., and Xie, J. 2009. Rare earth elements in marine organisms from Shenzhen coastal region. J. Hyg. Res. 38(5): 543–545. Available from http://www.cnki.com.cn/Article/CJFDTotal-WSYJ200905010.htm [accessed 10 February 2009]. PMID: 19877510.

Zhang, H., Feng, J., Zhu, W., Liu, C., Xu, S., Shao, P., Wu, D., Yang, W., and Gu, J. 2000. Chronic toxicity of rare-earth elements on human beings: Implications of blood biochemical indices in REE-high regions, South Jiangxi. Biol. Trace Elem. Res. 73(1): 1–17. doi:10.1385/bter:73:1:1.

Zhao, C.M., and Wilkinson, K.J. 2015. Biotic ligand model does not predict the bioavailability of rare earth elements in the presence of organic ligands. Environ. Sci. Technol. 49(4): 2207-2214. doi: 10.1021/es505443s.

Zhou, B., Li, Z., and Chen, C. 2017. Global potential of rare earth resources and rare earth demand from clean technologies. Minerals, 7(11): 203. doi:10.3390/min7110203.

Zhu, W., Xu, S., Shao, P., Zhang, H., Wu, D., Yang, W., Feng, J., Feng, L. 2005. Investigation on liver function among population in high background of rare earth area in South China. Biol. Trace Elem. Res. 104, 1–7. doi: 10.1385/BTER:104:1:001.

Zhu,W.,Xu, S., Shao, P., Zhang, H., Feng, J., Wu, D., and Yang, W. 1997. Investigation on intake allowance of rare earth - A study on bio-effect of rare earth in South Jiangxi. Chin. Environ. Sci. 17(1): 63-66.

Zhuang, M., Wang, L., Wu, G., Wang, K., Jiang, X., Liu, T., Xiao, P., Yu, L., Jiang, Y., Song, J., and Zhang, J. 2017a. Health risk assessment of rare earth elements in cereals from mining area in Shandong, China. Sci. Rep. 7: 1-6. doi: 10.1038/s41598-017-10256-7.

Zhuang, M., Zhao, J., Li, S., Liu, D., and Wang, K. 2017b. Chemosphere Concentrations and health risk assessment of rare earth elements in vegetables from mining area in Shandong, China. Chemosphere, 168: 578–582. doi:10.1016/j.chemosphere.2016.11.023.

Zicari, M.A., d'Aquino, L., Paradiso, A., Mastrolitti, S., and Tommasi, F. 2018. Effect of cerium on growth and antioxidant metabolism of Lemna minor L. Ecotoxicol. Environ. Saf. 163: 536–543. doi:10.1016/j.ecoenv.2018.07.113.

Zoltai, S.C., and Vitt, D.H. 1995. Canadian wetlands: environmental gradients and classification. Plant Ecol. 118(1): 131-137. doi: 10.1007/BF00045195.

A1.10 Appendix

Table A1A1 Advanced Rare Earth Elements Exploration Projects in Canada.

Project Name	Province	Geological Province	Deposit Type	Size (Million tonnes)	Total REE%	HREE % (HREE/ REEs)
Montviel Project	Quebec	Superior	CAR	250	1.45%	3%
Hoidas Lake Project	Saskatchewan	Churchill	CAR	2.9	2.57%	4%
Two Tom Project,	Labrador	Grenville	CAR	40	1.18%	6%
Clay Howells Project,	Ontario	Superior	ALK	8.5	0.73%	10%
Eldor Project	Quebec	Churchill	CAR	250	1.90%	10%
Echo-Ridge Project	Ontario	Superior	PLA	60	0.16%	15%
Grande Vallee Project	Quebec	Appalachian Orogen	SLE	1,040	0.01%	20%
Foxtrot Project	Labrador	Grenville	ALK	10	1.10%	20%
Strange Lake Project	Quebec	Churchill	ALK & PEG	300	0.93%	38%
Nechalacho Project	North West Territories	Slave	ALK	320	1.70%	38%
Zeus-Kipawa Project	Quebec	Grenville	ALK	16	0.51%	37%

Notes: CAR – Carbonatite; ALK – Alkaline Complex; PEG – Pegmatites; SLE – Shale; ALU/PLA – Alluvial or Placers and modified Placers; G&R - Granites and Rhyolites. (Natural Resources Canada, 2014; Weng et al., 2013; Zhou et al., 2017)

Table A1A2 The summary of the REEs toxicity cases in REEs mining areas.

NO.	Country	Mine/Province	Component affected	Finding	Reference
1	China	Bayan Obo mine / Inner Mongolia	Atmospheric aerosol particles	These values of REEs associated with PM2.5 are much higher than those in non-REEs mining areas. Long-term exposure to PM2.5 has been consistently linked to cardiovascular and cardiopulmonary diseases.	Li et al. (2018)
2	China	Bayan Obo mine / Inner Mongolia	Children' urine	The content of Y, La, Ce, Pr, Nd, and Sm in the urine of children in the RE mining area were significantly higher than that in the control group.	Liang et al. (2018)
3	China	Bayan Obo mine / Inner Mongolia	Groundwater	The contamination of RE tailings impoundment leakage on the surrounding groundwater. The pollution dispersion plume in groundwater on the south side of the RE tailings was over a distance of about 5.7 km.	Si et al. (2016)
4	China	Bayan Obo mine / Inner Mongolia	Human hair	The REE concentrations in the hair of people from mining are higher than those in control area.. The mean concentrations of REEs were higher in hair of males than that of females from the mining area.	Wei et al. (2013)
5	China	Weishan rare earth mine / Shandong	Vegetables	REE concentration in vegetables of mining area (94.08 μg/kg) was significant higher than that of control area (38.67 μg/kg). REE concentration declined in the order of leaf vegetable > taproot vegetable > alliaceous vegetable > gourd vegetable.	Zhuang et al. (2017a)
6	China	-/ Fujian	Soil, Water, Human blood and hair	The concentrations of REEs in soil, water and human blood and hair of mining area were higher than those of other regions.	Li et al. (2014)
7	China	Bayan Obo mine / Inner Mongolia	Human Urine	Urinary heavy metals and uranium in the population increased concomitantly with increasing REE level. Sex, age, educational level, alcohol and smoking habit were major factors contributing to inter-individual variation. Males were more exposed to metals than females.	Hao et al. (2015)

Table A1A2 Continued

8	China	Bayan Obo mine / Inner Mongolia	Soil	Tailing ponds are the main source of REE in the surrounding soil and the concentration of REEs, decreased with the distance from tailing ponds.	Zheng et al. (2016)
9	China	-/ Jiangxi	Human liver functions	Long-term intake of low-dosage REEs could affect liver function of people living in REE mining area, influencing the activity of a variety of enzymes necessary in the digestion, absorption, and metablization.	Zhu et al. (2005)
10	China	Bayan Obo mine / Inner Mongolia	Soil	1. The REE concentrations in soil from mining area were significantly higher than the reference. 2. The REE transportation led to the soil REE contamination along the railway, and its distribution was affected by the dominant wind direction.	Guo et al. (2013)
11	China	XinLong rare earth mine / Jiangxi	Water	The surface water and shallow groundwater of mining area were characterized by low pH, high total nitrogen, mangenese and REEs. Samples investigated showed 88 % water of pond and stream were unsuitable for agricultural use and aquaculture water supply, and 50 % of well and spring water were unsuitable for drinking water	Hao et al. (2016)
12	China	Weishan rare earth mine / Shandong	Crops	The REE concentrations of cereals, wheat and maize from mining area were higher than control areas. The health risk assessment demonstrated that the estimated daily intakes of REEs through cereal consumption were considerably lower than the acceptable daily intake (70 μg/kg body weight). The damage to adults can be neglected	Zhuang et al. (2017b)

Table A1A2 Continued

13	China	-/ Fujian	Soil, Vegetable, Human hair and blood	1. The farmlands in vicinity of mining site were contaminated with higher REEs level. 2. Accumulation levels of REEs differ significantly among vegetable species. 3. Elevated concentration of REEs in human hair and blood is associated with soil. 4. The distance from mining site determines the REEs exposure level of local residents.	Li et al. (2013a)
14	China	-/ Jiangxi	Human brain	REE was difficult to accumulate in the brain stem, but it was susceptible to cerebral cortex, thus causing subclinical damage (sensory conduction was interfered at the cerebral cortex).	Zhu et al. (1997)
15	China	Bayan Obo mine / Inner Mongolia	Dust	REEs are the most abundant elements, followed by Mo, Cd, Pb, and Ag in the road dust around the REE mine. Considerable proportions of polluted metals came directly from the mine, and pollution levels increased with decreasing distance and size of particles.	Tian et al. (2018)
16	China	-/ Jiangxi	Human blood	Blood biochemical indices (total serum protein, albumin, β-globulin, glutamic pyruvic transitanase, serium triglycerides, and immunoglobulin) of people from REE-high regions were lower than normal region.	Zhang et al. (2000)
17	China	-/ Jiangxi	Soil	High concentrations of REEs in tailings (392 mg/kg), stream waters (4460 μg/L), sediments (462 mg/kg) and farmland soils (928 mg/kg) were detected in comparison with control sites. REEs contamination in the farmland soils was mainly originated from the stream water contaminated by the leaching from the tailings.	Liu et al. (2019)
18	China	-/ Jiangxi	Children's IQ and blood	1. The score of IQ was significantly lower in the exposure group than in the control. 2. The quantity of blood REEs of the children was lower in exposed group compared to control group.	Fan et al. (2004)

Table A1A2 Continued

19	China	-/ Fujian	Soil, Vegetables	1. The concentration of REEs and heavy metals of the farmland soil near REEs mining area were high, especially for Cd, Cu and As, exceeding 10, 1, 0.3 times higher than Chinese National Soil Quality Standard. 2. The concentrations of Gd and As concentration in vegetables were higher than references.	Li et al. (2013b)
20	China	-/ Shandong	Fruits, Chicken eggs	1. The REEs concentration of fruits from the mining area (142.5 μg/kg) were significantly higher than control area (16.1 μg/kg) and pome fruits and berry fruits were easier to accumulate REEs. 2. For chicken eggs, there were no significant differences between mining areas and control area.	Zhuang et al. (2016)
21	China	-/ Shandong	Crops	There were no significant differences between mining areas and non-mining area in the REEs concentration of crops. Rare earth elements ingested via wheat, corn, and soybeans do not endanger human health.	Liu et al. (2017)
22	China	-/ Inner Mongolia	Toad (*Strauchbufo raddei*)	Water leakage pollution from tailings pond can reduce the tadpole population density by affecting the mating rate， and the main influencing mechanism of pollution on the *Strauchbufo raddei* postembryonic development is to cause oxidative damage of tissues and DNA damage of blood cells.	Huang et al. (2019)
23	China	-/ Fujian	Soil, Vegetable, Human blood, Water	1. The concentrations of REEs of cultivated soils, well water, vegetables from the mining area exceeded the references. 2. REE concentrations in local resident's blood and hair were nearly 156 and 10 times higher than those of the general population, respectively. 3. The average daily intake of REEs of local inhabitants was estimated to be 12.5 mg/kg.day, exceeding the maximum daily intake to maintain human health (6.0-6.7 mg/kg.day in China).	Li et al. (2013c)

Table A1A2 Continued

24	China	Bayan Obo mine / Inner Mongolia	Human leukemia	The workers at REEs mine who die from leukemia significantly increased. The chromosomal aberration rate of lymphocytes of miners was higher than that of the control. The chromosomal aberration rate of the miners was higher than that of the maintenance workers, and the distortion rate increased with working time.	Li et al. (1986)
25	China	Bayan Obo mine / Inner Mongolia	Human lung cancer	The dust-exposed miners after long-term (more than 30 years) exposure to high air concentrations of thorium containing rare -earth dusts and thoron short -lived progeny could induce excess lung cancer	Chen et al. (2002)

Figure A1A1 Spatial difference of REEs in soils/dusts and sediments in different countries. Data are compiled from two reviews (Adeel et al. 2019; Bayon et al. 2015) and collected Canadian data (Romero-Freire et al. 2019; Casse et al. 2019; MacMillan et al. 2018; Rasmussen et al. 2017; Chiarenzelli et al. 2001).

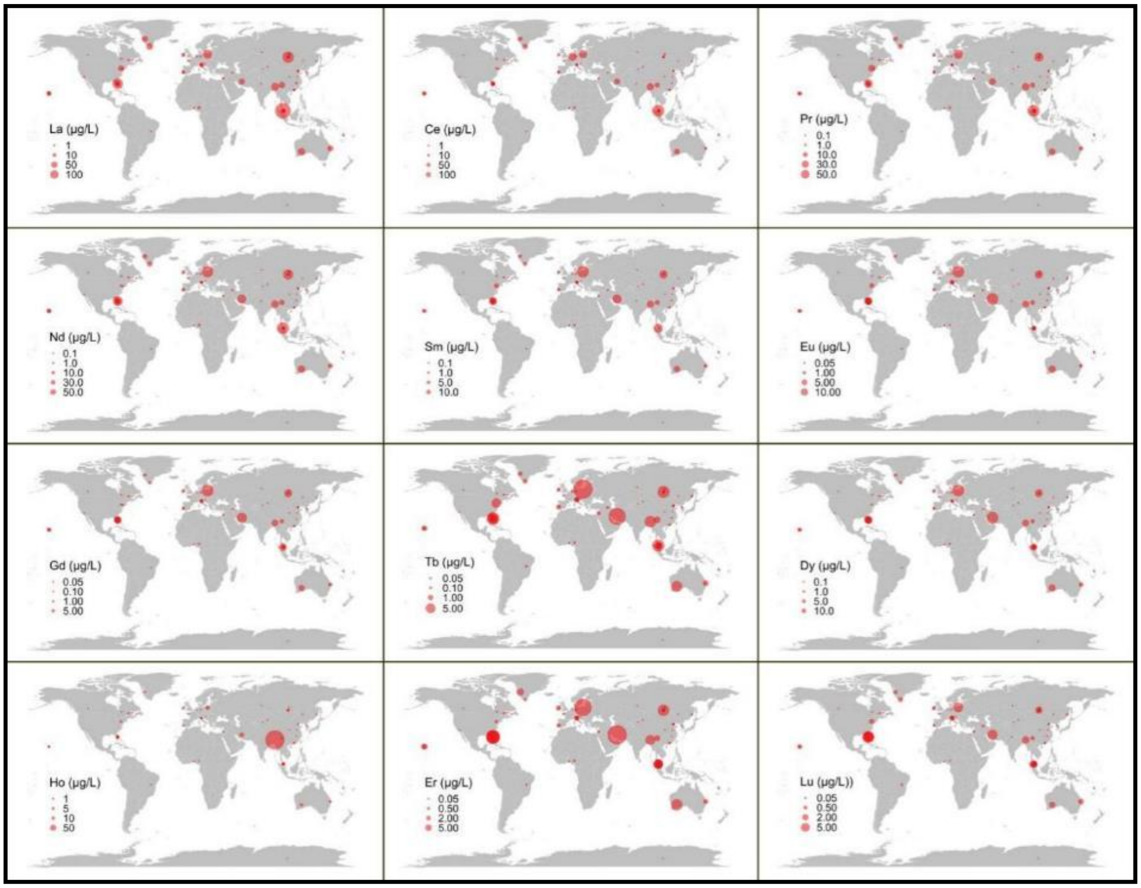

Figure A1A2 Spatial difference of REEs in various water matrices in different countries. Data are compiled from two reviews (Adeel et al. 2019; Bayon et al. 2015) and collected Canadian data (Romero-Freire et al. 2019; Casse et al. 2019; Cheung et al. 2009; Johannesson and Hendry 2000).

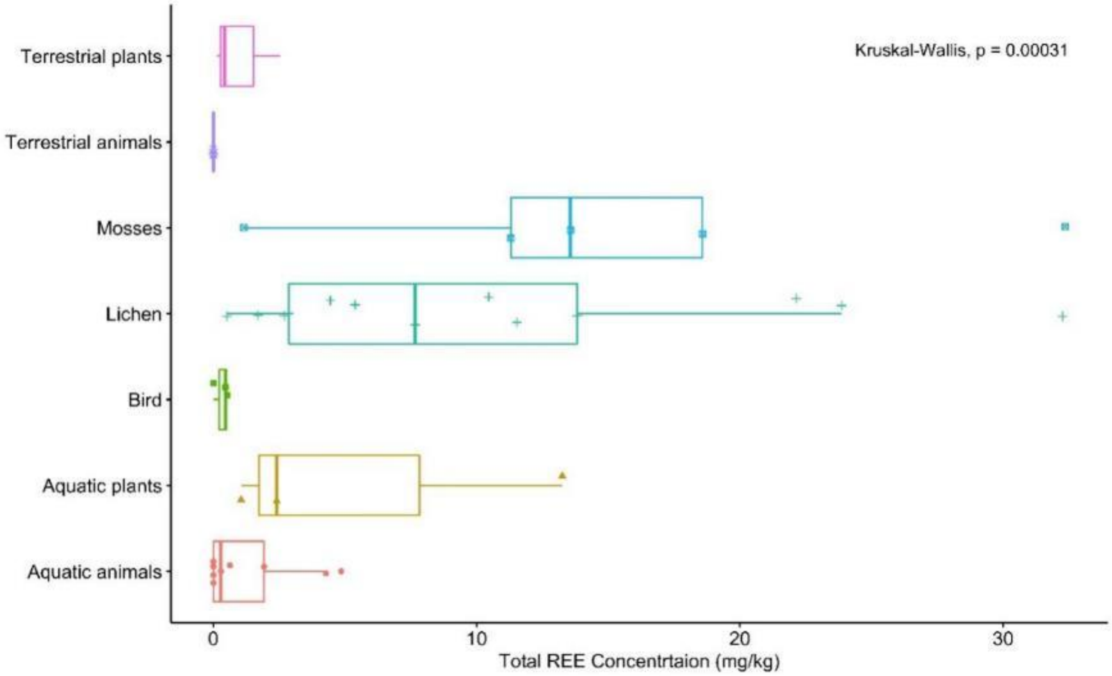

Figure A1A3 The REE concentration in different groups of living organisms in Canada based on Table A1.3.

REFERENCES

Adeel, M., Lee, J.Y., Zain, M., Rizwan, M., Nawab, A., Ahmad, M.A., Shafiq, M., Yi, H., Jilani, G., Javed, R., Horton, R., Rui, Y., Tsang, D.C.W., and Xing, B. 2019. Cryptic footprints of rare earth elements on natural resources and living organisms. Environ. Int. 127: 785–800. doi:10.1016/j.envint.2019.03.022.

Bayon, G., Toucanne, S., Skonieczny, C., André, L., Bermell, S., Cheron, S., Dennielou, B., Etoubleau, J., Freslon, N., Gauchery, T. and Germain, Y. 2015. Rare earth elements and neodymium isotopes in world river sediments revisited. Geochim. Cosmochim. Acta, 170: 17-38. doi: 10.1016/j.gca.2015.08.001.

Casse, M., Montero-Serrano, J.C., St-Onge, G., and Poirier, A. 2019. REE distribution and Nd isotope composition of estuarine waters and bulk sediment leachates tracing lithogenic inputs in eastern Canada. Mar. Chem., 211: 117-130. doi: 10.1016/j.marchem.2019.03.012.

Cheung, K., Sanei, H., Klassen, P., Mayer, B., and Goodarzi, F. 2009. Produced fluids and shallow groundwater in coalbed methane (CBM) producing regions of Alberta, Canada: Trace element and rare earth element geochemistry. Int. J. Coal Geol. 77(3-4): 338-349. doi: 10.1016/j.coal.2008.07.012.

Chen, X., Han, X., Cheng, Y., Feng, Z., Feng, G., Bao, X., Zhang, J., Dong, Y., Ma, L., Zhang, G., and Wu, C. 2002. Study of the health effects exposure to thorium dusts of the miners in China. Chinese J. Radiol. Heal. 11(1): 6–7. Available from http://en.cnki.com.cn/Article_en/CJFDTotal-REDI200201004.htm.

Chiarenzelli, J., Aspler, L., Dunn, C., Cousens, B., Ozarko, D., and Powis, K. 2001. Multi-element and rare earth element composition of lichens, mosses, and vascular plants from the central Barrenlands, Nunavut, Canada. Appl. Geochemistry 16(2): 245–270. doi:10.1016/S0883-2927(00)00027-5.

Fan, G., Yuan, Z., Zheng, H., and Liu, Z. 2004. Study on the effects of exposure to rare earth elements and health-responses in children aged 7-10 years. J. Hyg. Res. 33(1): 23–28.

Guo, W., Fu, R., Zhao, R., Zhao, W., Guo, J., and Zhang, J. 2013. Distribution Characteristic and Current Situation of Soil Rare Earth Contamination in the Bayan Obo Mining Area and Baotou Tailing Reservoir in Inner Mongolia. Environ. Sci. 34(5): 1895–1900. doi:10.13227/j.hjkx.2013.05.034.

Hao, X., Wang, D., Wang, P., Wang, Y., and Zhou, D. 2016. Evaluation of water quality in surface water and shallow ground water: a case study of a rare earth mining area in southern Jiangxi Province , China. Environ. Monit. Assess. 188: 24. doi:10.1007/s10661-015-5025-1.

Hao, Z., Li, Y., Li, H., Wei, B., Liao, X., Liang, T., and Yu, J. 2015. Levels of rare earth elements, heavy metals and uranium in a population living in Baiyun Obo, Inner Mongolia, China: A pilot study. Chemosphere 128: 161–170. doi:10.1016/j.chemosphere.2015.01.057.

Huang, T., Qiu, R., Lan, Z., Ren, Y., Yang, C., Qian, Y., Wang, C., He, Xi., and Si, W. 2019. The toxic effects of leakage water from rare earth tailings reservoir on the postembryonic development of Strauchbufo raddei. J. South. Agric. 50(2): 412–417. doi: 10.3969/j.issn.2095-1191.2019.02.28.

Johannesson, K.H., and Hendry, M.J. 2000. Rare earth element geochemistry of groundwaters from a thick till and clay-rich aquitard sequence, Saskatchewan, Canada. Geochim. Cosmochim. Ac. 64(9): 1493-1509. doi: 10.1016/S0016-7037(99)00402-0.

Li, K., Liang, T., Wang, L., and Tian, S. 2018. Inhalation exposure and potential health risk estimation of lanthanides elements in PM 2.5 associated with rare earth mining areas: a case of Baotou city, northern China. Environ. Geochem. Health, 40(6): 2795–2805. doi:10.1007/s10653-018-0146-4.

Li, L., Wang, L., and Wen, S. 1986. Ten-year death cause analysis of radon-containing dust workers in the Bayan Obo mine. Chinese J. Radiol. Med. Prot. 6(6): 399–401. Available from http://www.cjrmp.net/ch/reader/view_abstract.aspx?file_no=19860614 [accessed 19 December 1984].

Li, X., Chen, Z., Chen, Z., and Zhang, Y. 2013a. A human health risk assessment of rare earth elements in soil and vegetables from a mining area in Fujian Province, Southeast China. Chemosphere, 93(6): 1240–1246. doi:10.1016/j.chemosphere.2013.06.085.

Li, X., Chen, Z., Chen, Z., Zhang, Y., Wang, Q., and Huang, M. 2013b. Concentrations and health risk assessment of heavy metals in soil and vegetables from REEs mining area, Fujian Province. J. Soil Water Conserv. 27(1): 146–151. Available from http://www.cnki.com.cn/Article/CJFDTotal-TRQS201301031.htm. [accessed February 2013].

Li, X., Chen, Z., Zhang, Y., and Chen, Z. 2013c. Concentrations and health risk assessment of rare earth elements in soil and vegetables from a mining area in Fujian Province. Acta Sci. Circumstantiae 33(3): 835–843. Available from http://www.actasc.cn/hjkxxb/ch/reader/create_pdf.aspx?file_no=20120812003 [accessed March 2013]

Li, X., Chen, Z., and Chen, Z. 2014. Distribution and fractionation of rare earth elements in soil–water system and human blood and hair from a mining area in southwest Fujian Province, China. Environ. Earth Sci. 72(9): 3599–3608. doi:10.1007/s12665-014-3271-0.

Liang, Q., Yin, H., Li, J., Zhang, L., Hou, R., and Wang, S. 2018. Investigation of rare earth elements in urine and drinking water of children in mining area. Medicine, 97(40): e12717. doi: 10.1097/MD.0000000000012717.

Liu, D., Zhuang, M., Zong, J., Yang, L., Wang, K., Xiao, P., and Zhao, J. 2017. Rare earth elements in crops and their health risk in a light rare earth elements mine in Shandong. J. Environ. Heal. 34(7): 595–598. doi: 10.16241/j.cnki.1001-5914.2017.07.009.

Liu, W.S., Guo, M.N., Liu, C., Yuan, M., Chen, X.T., Huot, H., Zhao, C.M., Tang, Y.T., Morel, J.L., and Qiu, R.L. 2019. Water, sediment and agricultural soil contamination from an ion-adsorption rare earth mining area. Chemosphere 216: 75–83. doi:10.1016/j.chemosphere.2018.10.109.

MacMillan, G.A., Chételat, J., Heath, J.P., Mickpegak, R., and Amyot, M. 2017. Rare earth elements in freshwater, marine, and terrestrial ecosystems in the eastern Canadian Arctic. Environ. Sci. Process. Impacts 19(10): 1336–1345. doi:10.1039/c7em00082k.

Rasmussen, P.E., Levesque, C., Chénier, M., and Gardner, H.D. 2017. Rare earth elements and select actinoids in the Canadian house dust study. Indoor air, 27(5): 965-976. doi: 10.1111/ina.12379.

Romero-Freire, A., Turlin, F., André-Mayer, A. S., Pelletier, M., Cayer, A., and Giamberini, L. 2019. Biogeochemical cycle of lanthanides in a light rare earth element-enriched geological area (Quebec, Canada). Minerals, 9(10): 573. doi: 10.3390/min9100573.

Si, W., He, X., Li, A., Liu, L., Li, J., and Gong, D. 2016. Application of an integrated biomarker response index to assess ground water contamination in the vicinity of a rare earth mine tailings site. Environ. Sci. Pollut. Res. 23: 17345–17356. doi:10.1007/s11356-016-6728-8.

Tian, S., Liang, T., Li, K., and Wang, L. 2018. Source and path identification of metals pollution in a mining area by PMF and rare earth element patterns in road dust. Sci. Total Environ. 633: 958–966. doi:10.1016/j.scitotenv.2018.03.227.

Weng, Z.H., Jowitt, S.M., Mudd, G.M., and Haque, N. 2013. Assessing rare earth element mineral deposit types and links to environmental impacts. Appl. Ear. Sci. 122(2): 83-96. doi:10.1179/1743275813Y.0000000036.

Wei, B., Li, Y., Li, H., Yu, J., Ye, B., and Liang, T. 2013. Rare earth elements in human hair from a mining area of China. Ecotoxicol. Environ. Saf. 96: 118–123.

Zhang, H., Feng, J., Zhu, W., Liu, C., Xu, S., Shao, P., Wu, D., Yang, W., and Gu, J. 2000. Chronic toxicity of rare-earth elements on human beings: Implications of blood biochemical indices in REE-high regions, South Jiangxi. Biol. Trace Elem. Res. 73(1): 1–17. doi:10.1385/bter:73:1:1.

Zheng, C., Zhang, Z., Liu, Q., Chen, M., Cheng, H., and Zhang, X. 2016. Rare Earth Distribution in the Soil around Rare Earth Tailings. J. Rare Earth. 37(2): 73–80. doi: 10.16533/J.CNKI.15-1099/TF.201602012.

Zhu, W., Xu, S., Shao, P., Zhang, H., Wu, D., Yang, W., and Feng, J. 1997. Bioelectrical activity of the central nervous system among populations in a rare earth element area. Biol. Trace Elem. Res. 57(1):71-77.

Zhu, W., Xu, S., Shao, P., Zhang, H., Wu, D., Yang, W., Feng, J., and Feng, L. 2005. Investigation on liver function among population in high background of rare earth area in South China. Biol. Trace Elem. Res. 104(1): 1–7.

Zhuang, M., Zhao, J., Li, S., Liu, D., and Wang, K. 2017a. Concentrations and health risk assessment of rare earth elements in vegetables from mining area in Shandong, China. Chemosphere, 168: 578–582. doi:10.1016/j.chemosph Zhuang, M., Wang, L., Wu, G., Wang, K., Jiang, X., Liu, T., Xiao, P., Yu, L., Jiang, Y., Song, J., Zhang, J., Zhou, J., Zhao, J., and Chu, Z. 2017b. Health risk assessment of rare earth elements in cereals from mining area in Shandong, China. Sci. Rep. 7(1): 9772. doi:10.1038/s41598-017-10256-7.ere.2016.11.023.

Zhuang, M., Zhao, J., Li, S., Liu, D., Zhang, J., Zhang, Y., Yan, J., Zong, S., Yan, J., Zhou, J., and Chu, Z. 2016. Contents and health risk of rare earth elements in fruits and eggs from a REEs mining area, Shandong. Mod. Prev. Med. 43(23): 4255–4259.

Zhou, B., Li, Z., & Chen, C. 2017. Global potential of rare earth resources and rare earth demand from clean technologies. Minerals 7(11): 203. doi: doi.org/10.3390/min7110203.

A1.11 Supplementary Materials

Table A1S1. The main driving factors (pH, DOC and Redox condition) for REEs mobility and bioavailability.

Factors	Finding	References
pH	Low pH and redox potential favored the REEs released from exchangeable fraction and Fe–Mn oxide fraction.	Cao et al. (2001)
	REEs in carbonate bound and Fe–Mn oxide bound fractions were negatively correlated with soil pH.	Zhang and Shan (2001)
	More of the REEs became adsorbed onto the organic matter with increased soil pH in acid sulfate soils.	Sukitprapanon et al. (2019)
Dissolved organic carbon	Humic substances enhanced REE adsorption at low pH range (i.e., pH < 4) and decreased REE adsorption when pH was above 4.	Tang and Johannesson (2010)
	There was a stronger connection between REEs and DOC than REEs and pH in a moderately acidic water (pH of samples: 3.61-6.42) of the Great Dismal Swamp watershed. Dissolved REEs were positively correlated to DOC concentrations and inversely correlated with pH.	Johannesson et al. (2004)
Redox condition	The major REE carriers were variable during alternating redox conditions. The REEs were bound to colloidal and dissolved organic matter under reducing conditions, while they were bound to big size particulates, colloidal, organic and Fe-enriched fractions in oxidizing conditions.	Guénet et al. (2018)
	Significant amounts of REEs were released during anoxic incubation (at pH=5)) of the wetland soil, while oxic incubation at pH=7 can stimulate soil desorption.	Davranche et al. (2011)

Table A1S2. The description of each REE fraction (Mseddi et al. 2010; Tessier et al. 1979)

Fractions	Description
Water soluble	Free REE^{3+} dissolved in water or soil solution.
Ion-exchangeable	The REEs adsorbed on clay minerals by electrostatic interaction. They can be released easily into leaching liquor upon ion exchange with NH^{4+}, Mg^{2+}, Fe^{2+} and Fe^{3+}.
Bound to Carbonates	Carbonate dissolution into soil porewaters increases soil pH, potentially enhancing the fixation of trace metals to carbonates via incorporation of cations in the carbonate unit cell. This fraction would be susceptible to changes of pH.
Bound to Iron and Manganese Oxides	Iron and manganese oxides exist as nodules, concretions, cement between particles, or simply as a coating on particles; these oxides are excellent scavengers for trace metals and are thermodynamically unstable under anoxic conditions.
Bound to Organic Matter	Trace metal may be bound to various forms of organic matter: living organisms, detritus, coating on mineral particles, etc. The complexation and peptization properties of natural organic matter (notably humic and fulvic acids) are well recognized, as is the phenomenon of bioaccumulation in certain living organisms. Under oxidizing conditions in natural waters, organic matter can be degraded leading to a release of soluble trace metals
Residual	Once the first four fractions have been removed, the residual solid should contain mainly primary and secondary minerals, which may hold trace metals within their crystal structure. These metals are not expected to be released in solution over a reasonable time span under the conditions normally encountered in nature.

Table A1S3 Recent studies about the REE toxicity to different groups of biotas.

Life forms	Species	Elements	REE Compound and Treatment	Toxicity	References
Vertebrate	Rat	Y	YCl_3 (12mmol/L and 24mmol/L)	Neuronal cell death (+)	Ding et al. (2017)
	Rat	La	$LaCl_3$ (0, 0.125, 0.25, 0.5, and 1%)	Spatial learning and memory (-), lactate transport (-)	Jin et al. (2017)
	Rat	La	$La(NO_3)_3$ (0, 1.5, 6.0, 24.0, and 144.0 mg/kg body weight)	Growth index: body weight (-), organ weight (-) and food consumption (-)	Fang et al. (2018)
	Rainbow trout (*Oncorhynchus mykiss*)	Ce, Er, Gd, La, Nd, Sm, Y	$CeCl_3$, $ErCl_3$, $GdCl_3$, $LaCl_3$, $NdCl_3$, $SmCl_3$ and YCl_3 (0.064, 0.32, 1.6, 8 and 40 mg/L)	Gene expression changes (- or +)	Dubé et al. (2019)
Invertebrate	Mussels (*Mytilus galloprovincialis*)	Gd	$GdCl_3$ (0, 15, 30, 60, 120 μg/L)	Metabolic capacity (-), lipid peroxidation (+), antioxidant and biotransformation defences (+)	Henriques et al. (2019)
		La, Y	$LaCl_3$(0.01, 0.1, 1, 10, 100 mg/L) YCl_3 (0.001, 0.01, 0.1, 1, 10 mg /L)	Embryogenesis success (-)	Mestre et al. (2019)
		La	$LaCl_3$ (0, 0.1, 1, 10 mg/L)	Metabolic capacity (-), antioxidant and biotransformation defences (+), Neurotoxicity: activity of Acetylcholinesterase (-), Reproductive capacity: female spawning (-)	Pinto et al. (2019)
	Nematode (*Caenorhabditis elegans*)	Nd, Pr, Sc	$NdCl_3$, $PrCl_3$ and $ScCl_3$ (0,10, 50, 75,100, 200 mg/L)	Neurotoxicity: motor behaviors (-), dopaminergic and GABAergic neurons (-)	Xu et al. (2017a)
	Bivalve (*Corbicula fluminea*)	La	$LaCl_3$ (0, 50, 100 and 200 μM)	Metabolic capacity (-)	Zhao and Liu (2018)
	Sea Urchin (*Paracentrotus lividus* and *Arbacia lixula*)	Dy, Ho, Er, Yb, Lu, Ce	REE salts (10-4 - 10-7 M)	Mitotic activity of embryos (-), nitric oxide (+), antioxidant defences (+), Fertilization success (-), offspring development (-)	Oral et al. (2017)
	Sea Urchin (*Sphaerechinus granularis*)	Dy, Ho, Er, Yb, Lu, Ce	$DyCl_3$, $HoCl_3$, $ErCl_3$, $YbCl_3$, $LuCl_3$ and $CeCl_3$ (10-4 - 10-7 M)	Fertilization success (-), offspring development (-)	Gravina et al. (2018)

Table A1S3 continued

Vascular Plant	Soybean (*Glycine max*)	La	$LaCl_3$ (0.40 and 1.20 mM)	Population respiration (-) and the respiratory key enzymes (-)	Zhang et al. (2019)
		La	$LaCl_3$ (0.40 and 1.20 mM)	Root growth (-), photosynthesis parameters (-)	Zhang et al. (2018)
	Curlyleaf-pondweed (*Potamogeton crispus*)	Y	YCl_3(0, 10, 20, and 30 μM)	Photosynthetic pigments (-), growth rates (-), protein content (-), leaf area damage (+), elemental homeostasis (- or +)	Xu et al. (2019)
	Brassica chinensis and *Helianthus annuus*	La, Nd	La_2O_3 and Nd_2O_3 (20 mg/kg, 100 mg/kg, 200 mg/kg, and 300 mg/kg MS media)	plant biomass (-), total chlorophyll (-)	Rezaee et al. (2018)
	Greater duckweed (*Spirodela polyrhiza*)	Ce	$Ce(NO_3)_3{\cdot}6H_2O$ (0, 10, 20, 40 and 60 μM)	Growth (-), photosynthetic pigment (-), protein contents (-), cell death (+)	Xu et al. (2017b)
	Duckweed *(Lemna minor)*	Ce	Ce nitrate (0, 2.5, 5, 10, 20 μM and 0.1, 0.5, 1 mM) Ce chloride and Ce sulphate (0.5 and 1mM)	Photosynthetic pigments (-), hydrogen peroxide (+), antioxidant activity (+)	Zicari et al. (2018)
	Onion (*Allium cepa*)	La, Ce	$LaCl_3$ and $CeCl_3$ (0, 10, 20, 50, 100, and 200 mg/kg air-dry soil)	Cell division (-), aberrant cells (+)	Kotelnikova et al. (2019)
	Sweetpotato (*Ipomoea batatas*)	Ce	$CeCl_3$ (10, 20, 40 and 80 mg/L)	Photosynthesis (-), activities of antioxidant enzymes (+)	Jiang et al. (2017)
Non-vascular Plant	Algea (*Chlorella Vulgaris* and *Phaeodactylum Tricornutum*)	La	$La(NO_3)_3{\cdot}$ $6H_2O$ (0, 2, 4, 6, 8, and 10 mg/L)	Photosynthetic system (-), activities of antioxidant enzymes (+)	Sun et al. (2019)
Microbiome	Soil microbial communities	La_2O_3, Nd_2O_3, Gd_2O3	La_2O_3, Nd_2O_3, Gd_2O (10, 50, 100 mg/kg soil)	The proliferation of antibiotic resistance genes (+)	Qi et al. (2019)
	Fungus (*Shiraia bambusicola*)	La	$LaCl_3$ (0.0-1.4 g/L)	Hydrogen peroxide (+), hypocrellin A (+)	Lu et al. (2019)

Note: (-) means negative impacts; (+) means positive impact.

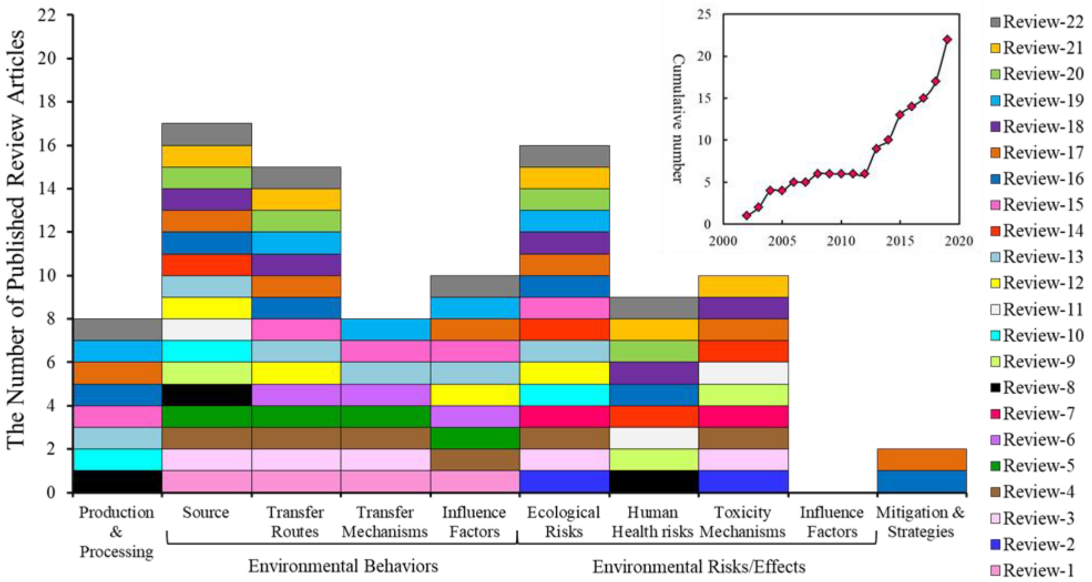

Figure A1S1 The topics reported in review literatures

Note: Review-1 (Pang etal. 2002), Review-2 (Tomonori 2003), Review-3 (Hu et al. 2004), Review-4 (Tyler 2004), Review-5 (Hu et al. 2006), Review-6 (Liang et al. 2008), Review-7 (Zhang et al. 2013), Review-8 (Weng et al. 2013), Review-9 (Rim et al. 2013), Review-10 (Haque et al. 2014), Review-11 (Pagano et al. 2015a), Review-12 (Pagano et al. 2015b), Review-13 (Migaszewski and Gałuszka 2015), Review-14 (Rim 2016), Review-15 (Khan et al. 2017), Review-16 (Gwenzi et al. 2018), Review-17 (Turra 2018), Review-18 (Adeel et al. 2019), Review-19 (Edahbi et al. 2019), Review-20 (Pagano et al. 2019), Review-21 (Abdelnour et al. 2019), Review-22 (Balaram 2019), Review-23 (Malhotra et al. 2020), Review-24 (Langkau et al. 2020).

REFERENCES

Abdelnour, S.A., Abd El-Hack, M.E., Khafaga, A.F., Noreldin, A.E., Arif, M., Chaudhry, M.T., Losacco, C., Abdeen, A., and Abdel-Daim, M.M. 2019. Impacts of rare earth elements on animal health and production: Highlights of cerium and lanthanum. Sci. Total Environ. 672: 1021–1032. doi:10.1016/j.scitotenv.2019.02.270.

Adeel, M., Lee, J.Y., Zain, M., Rizwan, M., Nawab, A., Ahmad, M.A., Shafiq, M., Yi, H., Jilani, G., Javed, R., Horton, R., Rui, Y., Tsang, D.C.W., and Xing, B. 2019. Cryptic footprints of rare earth elements on natural resources and living organisms. Environ. Int. 127: 785–800. doi:10.1016/j.envint.2019.03.022.

Balaram, V. 2019. Rare earth elements: A review of applications, occurrence, exploration, analysis, recycling, and environmental impact. Geosci. Front. 10(4): 1285–1303. doi:10.1016/j.gsf.2018.12.005.

Cao, X., Chen, Y., Wang, X., and Deng, X. 2001. Effects of redox potential and pH value on the release of rare earth elements from soil. Chemosphere 44: 655–661. doi: 10.1016/S0045-6535(00)00492-6.

Davranche, M., Grybos, M., Gruau, G., Pédrot, M., Dia, A., and Marsac, R. 2011. Rare earth element patterns: A tool for identifying trace metal sources during wetland soil reduction. Chem. Geol. 284: 127–137.doi: 10.1016/j.chemgeo.2011.02.014.

Ding, Y., Tian, Y., Zeng, Z., Shuai, P., Lan, H., Zhu, X., Zhong, Y., Wu, L., Fan, X. 2017. YCl_3 promotes neuronal cell death by inducing apoptotic pathways in rats. Biomed Res.Int. ID:2183658. doi: 10.1155/2017/2183658.

Dubé, M., Auclair, J., Hanana, H., Turcotte, P., Gagnon, C., and Gagné, F. 2019. Gene expression changes and toxicity of selected rare earth elements in rainbow trout juveniles. Comp. Biochem. Physiol. Part-C Toxicol. Pharmacol. 223: 88–95. doi:10.1016/j.cbpc.2019.05.009.

Edahbi, M., Plante, B., and Benzaazoua, M. 2019. Environmental challenges and identification of the knowledge gaps associated with REE mine wastes management. J. Clean. Prod. 212: 1232–1241. doi:10.1016/j.jclepro.2018.11.228.

Fang, H.Q., Yu, Z., Zhi, Y., Fang, J., Li, C.X., Wang, Y.M., Peng, S.Q., and Jia, X.D. 2018. Subchronic Oral Toxicity Evaluation of Lanthanum: a 90-day, Repeated

Dose Study in Rats. Biomed. Environ. Sci. 31, 363–375. doi: 10.3967/bes2018.047.

Guénet, H., Demangeat, E., Davranche, M., Vantelon, D., Pierson-Wickmann, A.C., Jardé, E., Bouhnik-Le Coz, M., Lotfi, E., Dia, A., and Jestin, J. 2018. Experimental evidence of REE size fraction redistribution during redox variation in wetland soil. Sci. Total Environ. 631–632, 580–588.doi: 10.1016/j.scitotenv.2018.03.005.

Gwenzi, W., Mangori, L., Danha, C., Chaukura, N., Dunjana, N., and Sanganyado, E. 2018. Sources, behaviour, and environmental and human health risks of high-technology rare earth elements as emerging contaminants. Sci. Total Environ. 636: 299–313. doi:10.1016/j.scitotenv.2018.04.235.

Gravina, M., Pagano, G., Oral, R., Guida, M., Toscanesi, M., Siciliano, A., Di, A., Petra, N., Daniel, B., Philippe, M.L., and Marco, J.T. 2018. Heavy rare earth elements affect Sphaerechinus granularis sea urchin early life stages by multiple toxicity endpoints. Bull. Environ. Contam. Toxicol. 100, 641–646. doi: 10.1007/s00128-018-2309-5.

Haque, N., Hughes, A., Lim, S., Vernon, and C. 2014. Rare earth elements: overview of mining, mineralogy, uses, sustainability and environmental Impact. Resources, 3: 614–635. doi: 10.3390/resources3040614.

Henriques, B., Coppola, F., Monteiro, R., Pinto, J., Viana, T., Pretti, C., Soares, A., Freitas, R., and Pereira, E. 2019. Toxicological assessment of anthropogenic Gadolinium in seawater: Biochemical effects in mussels Mytilus galloprovincialis. Sci. Total Environ. 664: 626–634. doi:10.1016/j.scitotenv.2019.01.341.

Hu, Z., Richter, H., Sparovek, G., and Schnug, E. 2004. Physiological and Biochemical Effects of Rare Earth Elements on Plants and Their Agricultural Significance: A Review. J. Plant Nutr. 27(1): 183–220. doi:10.1081/PLN-120027555.

Hu, Z., Silvia, H., Gerd, S., and Ewald, S. 2006. Rare Earth Elements in Soils. Commun. Soil Sci. Plant Anal. 37(9): 1381–1420. doi:10.1080/00103620600628680

Khan, A.M., Kartini, N., Bakar, A., Farid, A., and Bakar, A. 2017. Chemical speciation and bioavailability of rare earth elements (REEs) in the ecosystem: a review. Environ. Sci. Pollut. Res. 24: 22764–22789. doi:10.1007/s11356-016-7427-1.

Kotelnikova, A., Fastovets, I., Rogova, O., Volkov, D. S., and Stolbova, V. 2019. Toxicity assay of lanthanum and cerium in solutions and soil. Ecotoxicol. Environ. Saf. 167: 20-28. doi: 10.1016/j.ecoenv.2018.09.117.

Jin, C., Gao, L., Li, Y., Wu, S. and Lu, X. 2017. Lanthanum damages learning and memory and suppresses astrocyte – neuron lactate shuttle in rat hippocampus. Exp. Brain Res. 235, 3817–3832. doi: 10.1007/s00221-017-5102-5.

Jiang, J., Hu, J., Xie, Z., Cao, Q., Ma, D., Han, Y. 2017. The trivalent cerium-induced cell death and alteration of ion flux in sweetpotato [Ipomoea batatas (L.) Lam]. J. Rare Earths 35, 1273–1282. doi: 10.1016/j.jre.2017.06.006.

Johannesson, K.H., Tang, J., Daniels, J.M., Bounds, W.J., and Burdige, D.J. 2004. Rare earth element concentrations and speciation in organic-rich blackwaters of the Great Dismal Swamp, Virginia, USA. Chem. Geol. 209, 271–294. doi: 10.1016/j.chemgeo.2004.06.012.

Langkau, S., and Erdmann, M., 2020. Environmental impacts of the future supply of rare earths for magnet applications. Journal of Industrial Ecology.1-17. doi: 10.1111/jiec.13090.

Liang, T., Ding, S., Song, W., Chong, Z., Zhang, C., and Li, H. 2008. A review of fractionations of rare earth elements in plants. J. Rare Earths, 26: 7–15. doi: 10.1016/S1002-0721(08)60027-7

Lu, C., Ma, Y., and Wang, J. 2019. Lanthanum elicitation on hypocrellin A production in mycelium cultures of Shiraia bambusicola is mediated by ROS generation. J. Rare Earths 37, 895–902. doi: 10.1016/j.jre.2018.10.010.

Malhotra, N., Hsu, H.S., Liang, S.T., Roldan, M.J.M., Lee, J.S., Ger, T.R., and Hsiao, C.D. 2020. An Updated Review of Toxicity Effect of the Rare Earth Elements (REEs) on Aquatic Organisms. Animals, 10(9): 1663. doi: 10.3390/ani10091663.

Mestre, N.C., Sousa, V.S., Rocha, T.L., and Bebianno, M.J. 2019. Ecotoxicity of rare earths in the marine mussel Mytilus galloprovincialis and a preliminary approach to assess environmental risk. Ecotoxicology, 28(3): 294–301. doi:10.1007/s10646-019-02022-4.

Migaszewski, Z.M., and Gałuszka, A. 2015. The characteristics, occurrence, and geochemical behavior of rare earth elements in the environment: A review. Crit. Rev. Environ. Sci. Technol. 45(5): 429–471. doi:10.1080/10643389.2013.866622.

Mseddi, H., Ben Mammou, A., and Oueslati, W. 2010. Methodology for the extraction of carbonate-bound trace metals from carbonate-rich soils: application to Lakhouat soils, Tunis, Tunisia. Chem. Spec. Bioavailab., 22(3): 165-170. doi: 10.3184/095422910X12827488554310.

Oral, R., Pagano, G., Siciliano, A., Gravina, M., Palumbo, A., Castellano, I., Migliaccio, O., Thomas, P.J., Guida, M., Tommasi, F., and Trifuoggi, M. 2017. Heavy rare earth elements affect early life stages in Paracentrotus lividus and Arbacia lixula sea urchins. Environ. Res. 154: 240–246. doi:10.1016/j.envres.2017.01.011.

Pang, X., Li, D. and Peng, A. 2002. Application of Rare-earth Elements in the Agriculture of China and its Environmental Behavior in Soil. Environ. Sci. Pollut. Res. 9(2): 143–148. doi: 10.1007/BF02987462.

Pagano, G., Aliberti, F., Guida, M., Oral, R., Siciliano, A., Trifuoggi, M., and Tommasi, F. 2015a. Rare earth elements in human and animal health: State of art and research priorities. Environ. Res. 142: 215–220. doi:10.1016/j.envres.2015.06.039.

Pagano, G., Guida, M., Tommasi, F., and Oral, R. 2015b. Health effects and toxicity mechanisms of rare earth elements-Knowledge gaps and research prospects. Ecotoxicol. Environ. Saf. 115: 40–48. doi:10.1016/j.ecoenv.2015.01.030.

Pagano, G., Thomas, P.J., Di Nunzio, A., and Trifuoggi, M. 2019. Human exposures to rare earth elements: Present knowledge and research prospects. Environ. Res. 171: 493–500. doi:10.1016/j.envres.2019.02.004.

Pinto, J., Costa, M., Leite, C., Borges, C., Coppola, F., Henriques, B., Monteiro, R., Russo, T., Cosmo, A., Soares, A., Polese, G., Pereira, E., and Polese, G. 2019. Ecotoxicological effects of lanthanum in Mytilus galloprovincialis: biochemical and histopathological impacts. Aquat. Toxicol. 211: 181-192. doi: 10.1016/j.aquatox.2019.03.017.

Qi, L., Ge, Y., Xia, T., He, J.Z., Shen, C., Wang, J., and Liu, Y.J. 2019. Rare earth oxide nanoparticles promote soil microbial antibiotic resistance by selectively enriching antibiotic resistance genes. Environ. Sci. Nano, 6(2): 456-466. doi: 10.1039/c8en01129j.

Rezaee, A., Hale, B., Santos, R.M., Chiang, Y.W. 2018. Accumulation and toxicity of Lanthanum and Neodymium in horticultural plants (*Brassica chinensis* L. and *Helianthus annuus* L.). Can. J. Chem. Eng. 96, 2263–2272.

Rim, K. 2016. Effects of Rare Earth Elements on the Environment and Human Health: A Literature Review. Toxicol. Environ. Health Sci. 8(3): 189–200. doi:10.1007/s13530-016-0276-y.

Rim, K.T., Koo, K.H., and Park, J.S. 2013. Toxicological evaluations of rare earths and their health impacts to workers: A literature review. Saf. Health Work 4(1): 12–26. doi:10.5491/SHAW.2013.4.1.12.

Sun, D., He, N., Chen, Q., Duan, S. 2019. Effects of Lanthanum on the Photosystem II energy fluxes and antioxidant system of *Chlorella Vulgaris* and *Phaeodactylum Tricornutum*. Int. J. Environ. Res. Public Health 16, 2242. doi: 10.3390/ijerph16122242.

Sukitprapanon, T., Suddhiprakarn, A., Kheoruenromne, I., and Gilkes, R.J. 2019. Rare earth elements in acid sulfate soils under long-term paddy rice cultivation in Thailand. Geoderma Reg. 17, e00216. doi: 10.1016/j.geodrs.2019.e00216.

Tang, J., and Johannesson, K.H. 2010. Ligand extraction of rare earth elements from aquifer sediments: implications for rare earth element complexation with organic matter in natural waters. Geochim. Cosmochim. Acta, 74(23): 6690-6705. doi: 10.1016/j.gca.2010.08.028.

Tessler, A., Campbell, P.G.C., and Bisson, M., 1979. Sequential extraction techniques for the speciation of particulate trace metals. Anal. Chem. 51, 844–851.doi: 10.1021/ac50043a017.

Tomonori, K. 2003. Biological actions of rare earth element ions in plants: A review. ITE Lett. 4(1): 44–48.

Turra, C. 2018. Sustainability of rare earth elements chain: from production to food – a review. Int. J. Environ. Health Res. 28(1): 23–42. doi: 10.1080/09603123.2017.1415307.

Tyler, G. 2004. Rare earth elements in soil and plant systems - A review. Plant Soil, 267(1–2): 191–206. doi:10.1007/s11104-005-4888-2.

Weng, Z.H., Jowitt, S.M., Mudd, G.M., and Haque, N. 2013. Assessing rare earth element mineral deposit types and links to environmental impacts. Appl. Earth Sci. 2(122): 83–96. doi:10.1179/1743275813Y.0000000036.

Xu, T., Zhang, M., Hu, J., Li, Z., Wu, T., Bao, J., Wu, S., Lei, L., He, D. 2017a. Behavioral deficits and neural damage of *Caenorhabditis elegans* induced by three rare earth elements. Chemosphere 181, 55–62. doi: 10.1016/j.chemosphere.2017.04.068.

Xu, Q., Jiang, Y., Chu, W., Su, C., Hu, D., Lu, Q., Zhang, T., 2017b. Response of *Spirodela polyrhiza* to cerium: subcellular distribution, growth and biochemical changes. Ecotoxicol. Environ. Saf. 139, 56–64. doi: 10.1016/j.ecoenv.2017.01.012.

Xu, Q., Zhang, W., Sha, S., Yang, Y., Su, C., and Hu, D. 2019. Subcellular distribution and physiological responses of *Potamogeton crispus* to yttrium. Acta Physiol. Plant. 41(6). doi:10.1007/s11738-019-2857-2.

Zhao, L. and Liu, L., 2018. Assessing the impact of lanthanum on the bivalve *Corbicula fluminea* in the Rhine River. Sci. Total Environ. 640–641, 830–839. doi: 10.1016/j.scitotenv.2018.05.351.

Zhang, C., Li, Q., Zhang, M., Zhang, N., and Li, M. 2013. Effects of rare earth elements on growth and metabolism of medicinal plants. Acta Pharm. Sin. B, 3(1): 20–24. doi:10.1016/j.apsb.2012.12.005.

Zhang, S., and Shan, X.Q., 2001. Speciation of rare earth elements in soil and accumulation by wheat with rare earth fertilizer application. Environ. Pollut., 112(3): 395-405.doi: 10.1016/S0269-7491(00)00143-3.

Zhang, F., Li, Y., Shi, Y., Wang, L., Zhou, Q., Huang, X., 2018. A novel evaluation of the effect of lanthanum exposure on plant populations. Chemosphere 202, 377–386. doi: 10.1016/j.chemosphere.2018.03.086.

Zhang, R., Yin, X., Ding, H., Wang, L., Zhou, Q., Huang, X., 2019. Analysis for effects of lanthanum (III) on the aboveground modules and respiration of soybean populations. Ecotoxicol. Environ. Saf. 167, 196–203. doi: 10.1016/j.ecoenv.2018.10.004.

Zicari, M.A., d'Aquino, L., Paradiso, A., Mastrolitti, S., and Tommasi, F. 2018. Effect of cerium on growth and antioxidant metabolism of *Lemna minor* L. Ecotoxicol. Environ. Saf. 163: 536–543. doi:10.1016/j.ecoenv.2018.07.113.

ANNEXE B

RICCARDIA VITREA (ANEURACEAE), UNE ESPÈCE D'HÉPATIQUE NOUVELLE EN CHINE ET EN AMÉRIQUE DU NORD

RICCARDIA VITREA (ANEURACEAE), A LIVERWORT SPECIES NEW TO CHINA AND NORTH AMERICA

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A2.1 Résumé

L'espèce d'hépatique Riccardia vitrea Furuki (Aneuraceae) est signalée pour la première fois en Chine et en Amérique du Nord (Canada). Ce rapport est principalement axé sur sa présence au Canada. Riccardia vitrea est caractérisé par de nombreux petits corps huileux dans les cellules épidermiques et internes, et par des pinnules ultimes prostrées, larges et peu développées, et se distingue ainsi facilement des autres espèces canadiennes du genre. Ceci constitue une extension significative de son aire de répartition connue, de l'Asie orientale à l'Amérique du Nord. Cette espèce pourrait être négligée en raison du fait que les spécimens secs sans corps gras sont souvent utilisés pour l'identification, et elle est probablement plus répandue que ce qui est actuellement connu dans les forêts boréales du Canada. Des illustrations et des descriptions basées sur les spécimens canadiens sont fournies avec une clé pour les espèces canadiennes du genre.

Mots-clés : Bryophytes, hepaticae, hépatiques thalloïdes simples, forêt boréale, Québec.

A2.2 Abstract

The liverwort species *Riccardia vitrea* Furuki (Aneuraceae) is reported for the first time for China and North America (Canada). This report is primarily focused on its occurrence in Canada. *Riccardia vitrea* is characterized by numerous small oil bodies in both epidermal and inner cells, and by prostrate, wide and poorly developed ultimate pinnules, and is thus easily distinguished from other Canadian species of the genus. This constitutes a significant extension of its known range from East Asia to North America. This species could be overlooked owing to the fact that dry specimens without oil bodies are often used in identification, and it is probably more widespread than currently known in the boreal forests of Canada. Illustrations and

descriptions based on the Canadian specimens are provided with a key for Canadian species of the genus.

Key words: Bryophytes, hepaticae, simple thalloid liverworts, boreal forest, Quebec.

A2.3 Introduction

Riccardia vitrea Furuki is a simple thalloid liverwort species in the family Aneuraceae, which was first recorded in Japan (Honshu, Shikoku, Kyushu, Furuki 1991). Its known range was subsequently expanded to China (Yin 2017, not published) and Russia (Bakalin 2018). To date, *R. vitrea* has not been reported from outside of East Asia. However, a new population was observed during recent fieldwork on bryophyte diversity in boreal forests at a post-mine site in north-west Quebec, Canada. Combining morphological and phylogenetic analyses, this new record was confirmed as an addition to the bryophyte flora of Canada and North America. Although *Riccardia* Gray is the largest genus of simple thalloid liverworts, with more than 250 names currently accepted (Gradstein & Cuvertino 2015, Söderström et al. 2016), only four species had been recorded in Canada, *R. chamedryfolia* (With.) Grolle, *R. latifrons* (Lindb.) Lindb., *R. palmata* (Hedw.) Carruth., and *R. multifida* (L.) Gray (Stotler & Crandall- Stotler 2017, Haughian et al. 2016, Faubert 2012, Damsholt 2007, Hong 2007, Brassard & Williams 1975), and they are all common species in the global temperate and boreal zones. *Riccardia vitrea* can be easily confused with these other species when it is dry, because of the great polymorphism of this genus (Reeb & Bardat 2014) and the loss of oil bodies which provide key characteristics for taxonomy in genus *Riccardia*. The shape, position, size and number of oil bodies are important in this genus for identification at the species level. Many bryologists have suggested the use of fresh material with oil bodies in this genus for more reliable identification (Reeb & Bardat 2014, Bakalin

2018, Schuster 1989). Short morphological and ecological descriptions of the species based on the specimens collected in Canada are provided in this study.

A2.4 Materials and Methods

The materials were collected during a field survey in Joutel, James Bay, Quebec, Canada, during the summers of 2019 and 2020. The study area is located in the *Picea mariana* feather moss bioclimatic domain and is influenced by cold and dry arctic air during the winter. The mean temperature is -20 °C in January and 16 °C in July. Mean annual precipitation is approximately 850mm (Bergeron et al. 2004). Collected samples were examined under a microscope (Olympus BX 43) and a stereomicroscope (Olympus SZX 16). The photos were taken with an Olympus DP 22 digital camera.

Figure A2.1 Habitat of *Riccardia vitrea* at Joutel, Quebec, Canada (Sample 201909 Joutel10 (UQAT)). A – Deciduous forest in which *R. vitrea* was collected. B–D – The microhabitat of *R. vitrea* at this site.

A2.5 Taxonomic description and discussion

Riccardia vitrea Furuki, J. Hattori Bot. Lab. 70: 327. 1991. (Figures A2.1 and A2.2)

Thallus medium to large, in dense radial patches, closely adjacent to the substratum, prostrate along main axis, irregularly pinnately or palmately branched, fresh-green to brown-green when fresh, whitish brown when dry, smooth and flat at apices. The main axis up to 9mm long, 0.3 – 0.8 mm wide, biconvex to plano-convex, in cross-section 5 –7 cells (98 –150µm) thick, obtuse at margins. Ultimate branches prostrate, poorly developed, wide, stuck to the substratum, up to 1.5mm long, 0.3 – 0.6mm wide, biconvex to plano-convex, only 5 –7 cells (92–130 µm) thick in cross-section, acute to obtuse at margins. Epidermal cells of thallus thin walled, 28 –92 × 22– 45 µm, about 1/3 –1/2 size of inner cells (Figure A2.2F, G). Inner cells 53 –107 × 20 – 61 µm. Oil bodies brownish to grayish brown, globose to ovoid, indistinctly granular. Oil bodies in epidermal cells (0 –)1–9/cell, 3.64 –11.74 × 3.16 –7.22 µm. Oil bodies in inner cells (0 –)2–14/cell, 4.44 –9.97 × 5.58 –14.98 µm. Mucilage hairs in 2 rows on the ventral surface of thallus only at apices, 16.66 – 46.31µm long, 7.56 –18.58 µm thick. Rhizoids are often present on the ventral surface of the thallus. Dioicous. Female and male branches lateral on the main axis or base of primary. Few mature calyptrae and developed male branches were found in collected samples. Based on collected samples, paraphyses fringed, scale-like, 5– 8 cell high (100 –200 µm), 2– 4 cells wide at the base. Elaters 85 –230 µm. Spores 11.74 –13.30 µm in diam. These descriptions are based on collected Canadian samples of 202007-CT6E and 201909-Joutel10. The description of Chinese samples was presented in Yin (2017), but figures are attached in the supporting document (Figures A2S1 and A2S2 in Supplementary documents online).

Habitat: On wet sandy soil and rock at open sites (in Japan, China and Russia). On decaying logs in deciduous and mixed forests (in Russia and Canada). In Canada, this species is found on moist decaying logs without bark (Figure 1), growing with other bryophyte species including *Syzygiella autumnalis* (DC.) K.Feldberg, Váňa, Hentschel & Heinrichs, *Geocalyx graveolens* (Schrad.) Nees, *Lophocolea bidentata* (L.) Dumort., and *Sanionia uncinata* (Hedw.) Loeske.

Range: Japan (Furuki 1991, Furuki & Higuchi 2006), China (Yin 2017), Russia (Bakalin 2018) and Canada (present report)

Phylogenetic analysis: This new record of the species is strongly supported by a molecular phylogenetic analysis based on DNA sequences data of one plastid regions *rbc*L and available sequences for other species downloaded from GenBank (Bainard et al. 2013, Masuzaki et al. 2010, Forrest et al. 2006). The phylogenetic study includes *R. vitrea* samples from Japan (GenBank: AB476596, Masuzaki et al. 2010), China (GenBank: OK302921 and OK302922) and Canada (GenBank: OK302923) as well as for the similar species *R. latifrons* and *R. palmata* that are recorded in Canada. The Canadian sample was placed in the same clade with *R. vitrea* from China and Japan and has a clearly different topology from other morphologically similar species known from Canada (phylogenetic tree and primers used shown in Figure A2S3 and Table A2S1 in Supplementary documents online).

Specimens examined from Canada: CANADA. Quebec, James Bay, Joutel, 49°30'35.64"N/78°17'16.8"W, 300m, on decaying logs, in mixed forest, 8 July 2020, X. B. Yin, X. St-Amant & T. Kim 202007-CT6E (UQAT); Canada. Quebec, James Bay, Joutel, 49°29'2.04"N/78°23'13.92"W, 300 m, on decaying logs, in deciduous forest, 27 September 2019, X. B. Yin & R. Boisvert 201909-Joutel10 (UQAT).

Discussion: The main characters of *R. vitrea* include 1) thallus closely adjacent to the substratum (Figure A2.2A), 2) numerous small oil bodies in epidermal and inner cells (Figure A2.2F, G), 3) prostrate, wide and poorly developed ultimate pinnules (Figure A2.2D), 4) large epidermal cells, similar size with inner cells seen in the cross-section of the thallus (Figure A2.2I, J), 5) obtuse to acute margins in cross-section of thallus

(Figure A2.2J). This species was already recorded in Japan (Furuki 1991, Furuki & Higuchi 2006), China (Anhui, Fujian, Hainan, Yunnan provinces, Yin 2017) and Russia (Far East, Bakalin 2018). Although it was first believed to be endemic to Japan when found by Furuki (1991), more findings were recorded outside Japan. At the same time, its known habitat has been extended from open moist soil and rock to include decaying logs in forests, and its biogeographical range includes subtropical and tropical ecosystems to sub-arctic northern ecosystems (Bakalin 2018). This species could be widely overlooked due to the fact that dry specimens without oil bodies are often used in identification, and it is probably more widespread than currently known. Misidentification with *R. latifrons* may be the main reason for its current limited recorded distribution in Canada. In the field, we found *R. latifrons* and *R. vitrea* at the same place, but the presence of a brown-green thallus and prostrate, wide ultimate pinnules make *R. vitrea* significantly different from *R. latifrons*, with narrow and ascending to erect ultimate pinnules. Another unique character of *R. latifrons* is the apparent lack of oil bodies, which allows easy separation from other species in Canada. However, identification can be difficult when material is dry due to the loss of morphological traits (e.g., oil bodies, thallus shape). Examining fresh material is best for accurate identification in genus *Riccardia*. This species is also similar to *R. palmata* in having small and ovoid oil bodies. However, oil bodies in North American samples are 1–2 per inner cell, and mostly absent from the epidermal cells (Schuster 1987). It is worth mentioning that we found that oil bodies can be kept for more than five months in a 4 °C fridge. Based on collected samples and references (Furuki 1991, Faubert 2012, Schuster 1987), the key to *Riccardia* species present in Canada is as follows.

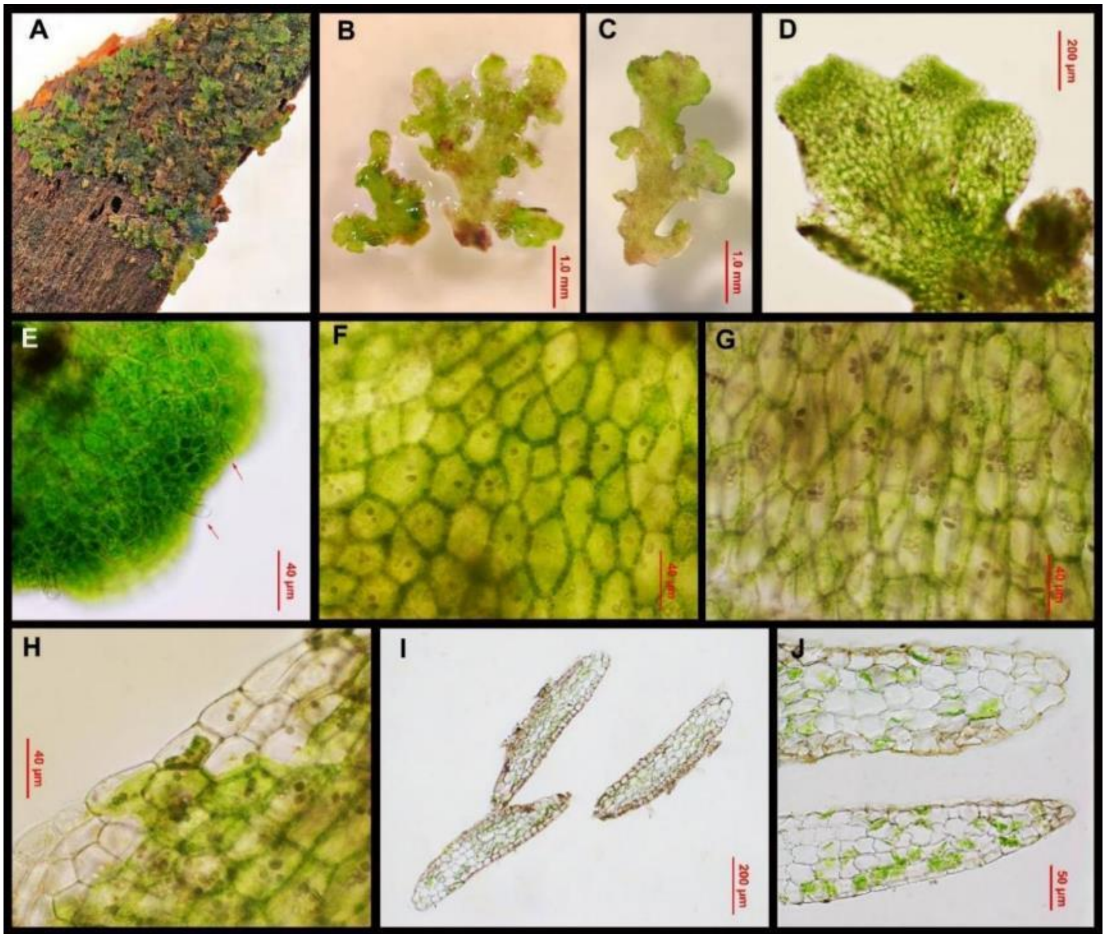

Figure A2.2 *Riccardia vitrea* Furuki. A – A colony of *R. vitrea* on decaying log. B–D – Thalli. E – Apex of ultimate pinnule with mucilage hairs. F– Epidermal cells with oil bodies. G – Inner cells with oil bodies. H – Marginal cells of ultimate pinnule. I–J – Cross sections of thallus (I – Cross sections of ultimate pinnules; J – Cross section of main axes). All from 201909-Joutel10 (UQAT).

1 Thallus regularly to irregularly pinnately to tripinnately branched; having moderately to well-developed pinnae; winged at the margin of the ultimate pinnule, unistratose alar portion more than 2 cells wide; ultimate pinnules cross-sections 3 –5 cells thick; usually on wet soil or rocks, rarely on wet decaying logs **2**

1* Thallus irregularly pinnately to tripinnately branched, having poorly to moderately developed pinnae; acute to winged at the margin of ultimate pinnule, unistratose alar portion less than 2 cells wide; ultimate pinnules cross-sections 4 –7 cells thick; usually on wet decaying logs or peatland **3**

2 Autoicous; flat cells along margin; oil bodies (1–3) in each epidermal, inner and wing cell .... ***R. chamedryfolia***

2* Heteroicous; crenulate cells along margin; oil bodies (1–2) in each inner cell, absent or rare in epidermal and wing cells .... ***R. multifida***

3 Monoicous; oil bodies absent or rare in all cells .... ***R. latifrons***

3* Dioicous; oil bodies present at least in inner cells **4**

4 Ultimate pinnules prostrate to ascending, moderately developed, less than 0.4mm wide; emarginate to bifid at apices; oil bodies usually 1–2 per inner cell, mostly absent from the epidermal cells, diameter over 15µm .... ***R. palmata***

4* Ultimate pinnules prostrate, poorly developed, more than 0.4mm wide; rounded at apices; oil bodies more than 2 per inner and epidermal cells, diameter less than 10µm .... ***R. vitrea***

A2.6 Acknowledgements

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REFERENCES

Bainard, J. D., Forrest, L. L., Goffinet, B. & Newmaster, S. G. 2013. Nuclear DNA content variation and evolution in liverworts. – Molecular Phylogenetics and Evolution 68: 619 – 627.

Bakalin, V. a. 2018. The review of Aneuraceae in the Russian Far East. – Botanica Pacifica: a Journal of Plant Science and Conservation 7: 3 –21.

Brassard, G. R. & Williams, H. 1975. The liverworts of Labrador, Canada. – Lindbergia 3: 83 – 88.

Bergeron, Y., Gauthier, S., Flannigan, M. & Kafka, V. 2004. Fire regimes at the transition between mixed wood and coniferous boreal forest in northwestern Quebec. – Ecology 85: 1916 –1932.

Damsholt, K. 2007. Liverworts of the Reindeer Preserve, Northwest Territories, Canada. – Lindbergia 31: 88 –100.

Faubert, J. 2012. Flore des Bryophytes du Québec-Labrador. Volume 1: Anthocérotes et Hépatiques. – Saint-Valérien: Société québécoise de bryologie.

Forrest, L. L., Davis, E. C., Long, D. G., Crandall-Stotler, B. J., Clark, A. & Hollingsworth, M. L. 2006.

Unraveling the evolutionary history of the liverworts (Marchantiophyta): multiple taxa, genomes and analyses. – The Bryologist 109: 303 –334.

Furuki, T. 1991. A taxonomical revision of the Aneuraceae (Hepaticae) of Japan. – Journal of the Hattori Botanical Laboratory 70: 293 –397.

Furuki, T. & Higuchi, M. 2006. Bryophytes of the Izu-Oshima Island, the Southern Sagami Sea, Japan. – Memoirs of the Natural Science Museum 42: 37–52.

Gradstein, S. R. & Cuvertino, J. 2015. Observations on the liverwort flora of the surroundings of Santiago, Central Chile. – Cryptogamie, Bryologie 35: 47–75.

Haughian, S. R., Bagnell, B. A., Daley, E., Frego, K. A., Smith, L. & Clayden, S. R. 2016. A checklist of the Marchantiophyta and Anthocerotophyta of New Brunswick, Canada. – Northeastern Naturalist 23: 1–35.

Hong, W. S. 2007. Checklist of liverworts for the Queen Charlotte Islands (Haida Gwaii), British Columbia, Canada. – Lindbergia 32: 69 –74.

Masuzaki, H., Shimamura, M., Furuki, T., Tsubota, H., Yamaguchi, T., Majid, H. M. A. & Deguchi, H. 2010.

Systematic position of the enigmatic liverwort *Mizutania* (Mizutaniaceae, Marchantiophyta) inferred from molecular phylogenetic analyses. – Taxon 59: 448 – 458.

Reeb, C. & Bardat, J. 2014. Studies on African *Riccardia* types and related material. – Cryptogamie, Bryologie 35: 47–75.

Schuster, R. M. 1987. Studies on Metzgeriales: I. North American Aneuraceae. – Journal of the Hattori Botanical Laboratory 62: 299 –329.

Schuster, R. M. 1989. Studies on the hepatic flora of the Prince Edward Islands. I. Aneuraceae. – Journal of the Hattori Botanical Laboratory 67: 59 –108.

Söderström, L., Hagborg, A., von Konrat, M., Bartholomew-Began, S., Bell, D., Briscoe, L., Brown, E., Cargill, D. C., Costa, D. P., Crandall-Stotler, B. J., Cooper, E. D., Dauphin, G., Engel, J. J., Feldberg, K., Glenny, D., Gradstein, S. R., He, X. H., Heinrichs, J., Hentschel, J., Ilkiu-Borges, A. L., Katagiri, T., Konstantinova, N. A., Larraín, J., Long, D. G., Nebel, M., Pócs, T., Puche, F., Reiner-Drehwald, M.E., Renner, M. A. M., Sass-Gyarmati, A., Schäfer-Verwimp, A., Segarra Moragues, J. G., Stotler, R. E., Sukkharak, P., Thiers, B. M., Uribe, J., Váňa, J., Villarreal, J. C., Wigginton, M., Zhang, L. & Zhu, R.-L. 2016. World checklist of hornworts and liverworts. – PhytoKeys 59: 1– 828.

Stotler, R. E. & Crandall-Stotler, B. 2017. A Synopsis of the Liverwort Flora of North America North of Mexico. – Annals of the Missouri Botanical Garden 102: 574 –709.

Yin, X. B. 2017. A taxonomical revision of the Aneuraceae of China [中国绿片苔科 Aneuracea 的分类学修订]. – Master dissertation, East China Normal University (in Chinese). https://cdmd.cnki.com.cn/Article/CDMD-10269–1017081621.htm.

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A2.7 Supplementary documents

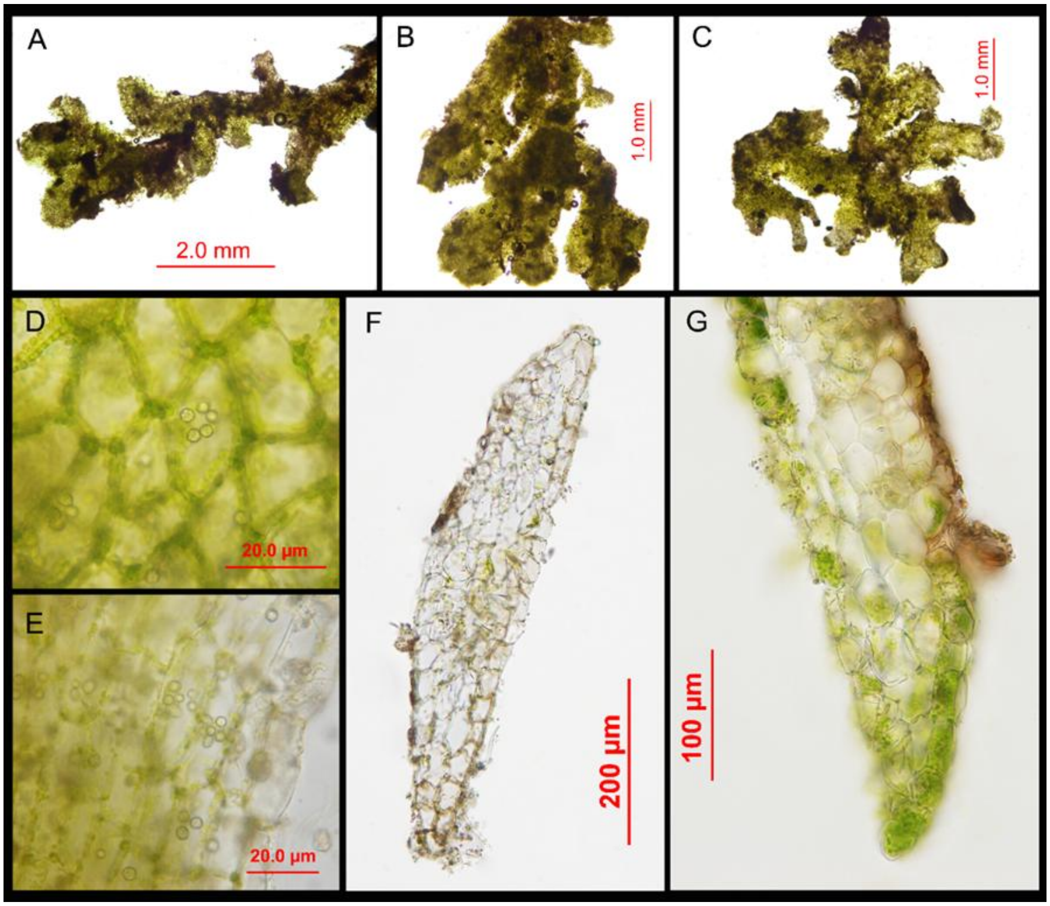

Figure A2S1 *Riccardia vitrea* Furuki. A–C–Thalli; D–E–Epidermal cells with oil bodies; F–G–Cross-sections of the thallus (F – Cross-sections of ultimate pinnules, G – Cross-section of main axes). D, E, F from Shi et al., 20150920-16 (HSNU); A, B, G from Shi et al., 20150922-7; C from Shi et al., 20150922-31. Voucher specimen for Chinese materials (Herbaria of East China Normal University, China. HSNU): 1 – China, Fujian Province, Dehua County, Jiuxian Mountain, Wang et al., 20090730-49; 2 – China, Anhui Province, Qingliangfeng National Nature Reserve, Shi et al., 20150920-16, 20150922-7, 20150922-31; 3 – China, Yunnan Province, Mengla County, Peng et al., 20150130-23, 2016042-10.

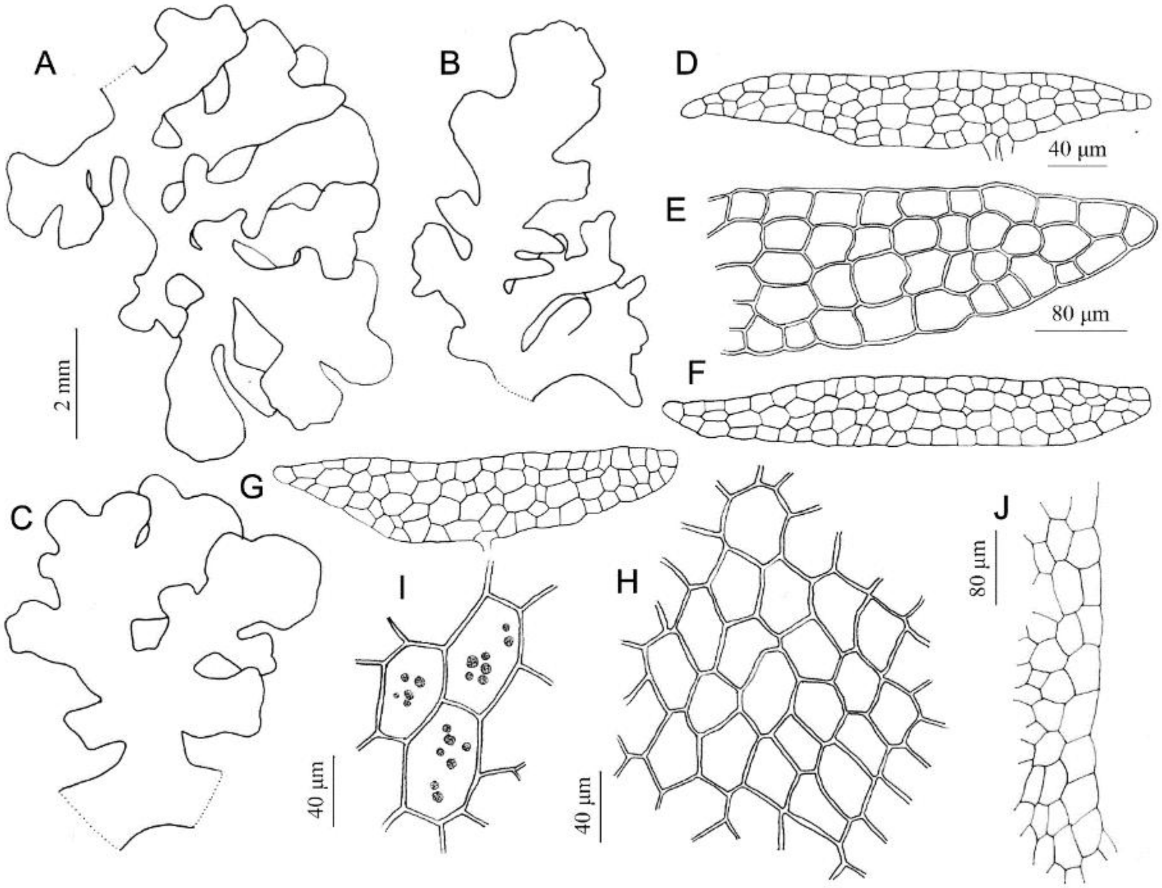

Figure A2S2. *Riccardia vitrea* Furuki. A–C – Thalli. D-G – Cross sections of thallus (D–E. ultimate pinnules; F–G. main axes). H – Epidermal cells of median part of ultimate pinnule. I – Epidermal cells of main axes, with oil bodies. J – Marginal cells of ultimate pinnule. All from Shi et al.20150920–16 (HSNU). The figure from Yin (2017).

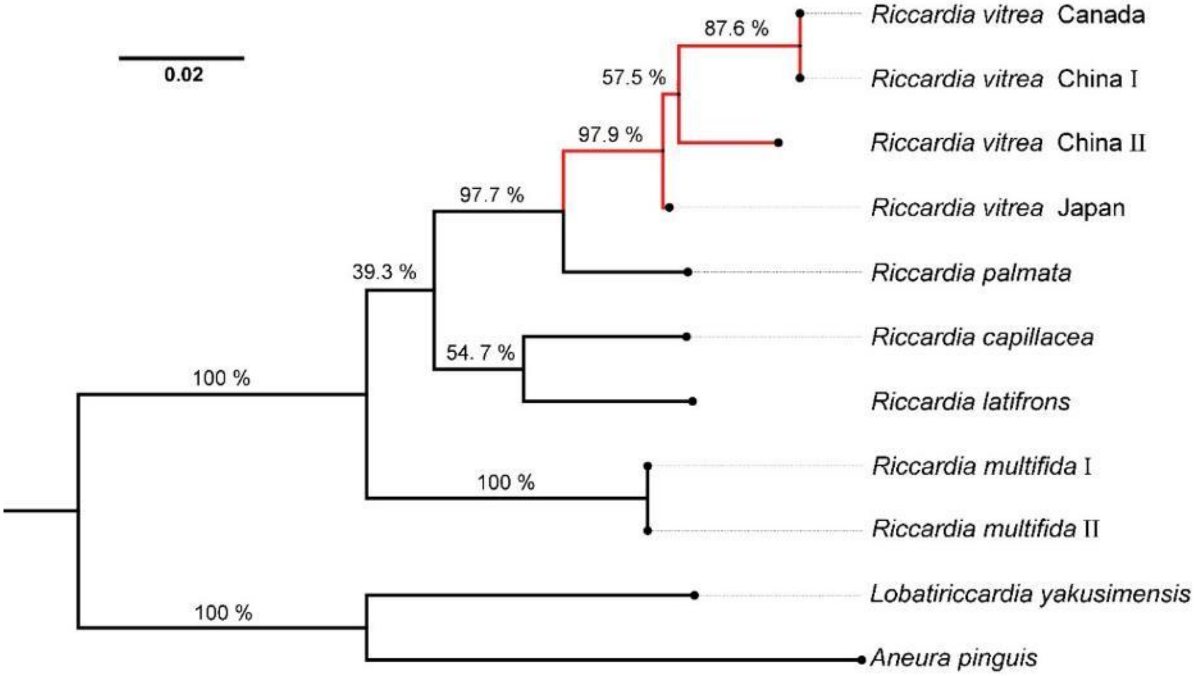

Figure A2S3 A simple maximum likelihood tree was constructed based on the *rbcL* gene of *R. vitrea* and similar species in Canada (*R. palmat* and *R. latifrons*). Primer sequences for *rbc*L are lised in Table A2S1. A phylogenetic tree with more species and sequences will be shown in a future article about the revision of Aneuraceae in China. Sequences ID from Genbank are listed in Table A2S2. The maximum likelihood (ML) method as implemented by PhyML version 3.0 (GUINDON et al. 2010). The best-fit model was GTR+G+I determined using ModelTest (POSADA & KEITH 1998). The proportion of invariant sites and the gamma distribution parameter alpha were estimated from the data. Likelihood bootstrap searches (MLBS) were performed with 1000 times.

Table A2S1 Primer sequences used for *rbc*L.

	Primer	Sequence（5'–3'）	Direction	Reference
*rbc*L	1plF	ATGTCACCACAAACGGAGACTAAARCAGGT	Forword	WILSON et al. 2004
	600R	CCAACGCATAAATGGTTGAG	Reverse	GRADSTEIN et al. 2006
	210F	TGGACTACGGTTTGGACTGA	Forword	GRADSTEIN et al. 2006
	1200R	TGYCCYAAAGTTCCACCACC	Reverse	WILSON et al. 2004
	640F	CTCAACCATTTATGCGTTGG	Forword	GRADSTEIN et al. 2006
	1390R	CTTTCCAWAYTTCRCAAGCAGCRG	Reverse	WILSON et al. 2004

Table A2S2 Sequences ID from Genbank present in the phylogenetic tree.

Species	Location	Genbank ID
Riccardia vitrea	Japan: Honshu, Tokyo, Ohshima	AB476596.1
R. palmata	USA: Pennsylvania	KC305704.1
R. capillacea	Venezuela: Merida	AY877392.1
R. latifrons	United Kingdom	KC305702.1
R. multifida I	USA: North Carolina	KC305703.1
R. multifida II	Canada: Vancouver Island	DQ268980.1
Lobatiriccardia yakusimensis	Japan: Kagoshima	AB673209.1
Aneura pinguis	Poland	KY705822.1
Unpublished sequences		
R. vitrea	Canada: Quebec	OK302923
R. vitrea I	China: Anhui	OK302922
R. vitrea II	China: Anhui	OK302921

REFERENCES

Posada, D. & Keith A. C. 1998. Modeltest: testing the model of DNA substitution. – Bioinformatics (Oxford, England) 14(9): 817–818. doi:10.1093/bioinformatics/14.9.817.

Guindon, S., Jean-François D., Vincent L., Maria A., Wim H. & Olivier G. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. – Systematic biology 59(3): 307–321. doi:10.1093/sysbio/syq010.

Gradstein, S.R., Wilson, R., Ilkiu-Borges, A.L., & Heinrichs, J. 2006. Phylogenetic relationships and neotenic evolution of Metzgeriopsis (Lejeuneaceae) based on chloroplast DNA sequences and morphology. Botanical journal of the Linnean society, 151(3): 293–308. doi:10.1111/j.1095-8339.2006.00531.x.

Wilson, R., Gradstein, S.R., Heinrichs, J., Groth, H., Ilkiu-Borges, A.L. & Hartmann, F.A. 2004. Phylogeny of Lejeuneaceae: A cladistic analysis of chloroplast gene rbcL sequences and morphology with preliminary comments on the mitochondrial nad4-2 spacer region. Monographs in systematic botany from the Missouri Botanical Garden 98: 189–202.

ANNEXE C

EFFETS HORS SITE DE L'EXPLOITATION MINIÈRE SUR LA FRÉQUENCE ET L'ABONDANCE DE CINQ ESPÈCES DE PLANTES DE SOUS-BOIS DANS L'OUEST DU QUÉBEC (CANADA)

OFFSITE EFFECTS OF MINING ON THE FREQUENCY AND ABUNDANCE OF FIVE UNDERSTOREY PLANT SPECIES IN WESTERN QUÉBEC (CANADA)

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Botany, DOI: 10.1139/cjb-2020-0158

A3.1 Résumé

Bien que l’industrie minière fournisse des minéraux et des métaux pour le marché mondial et représente d’importantes occasions économiques, elle constitue aussi une perturbation anthropique majeure dans les écosystèmes où elle prend place. Toutefois, on sait peu de choses sur ses impacts hors site sur les communautés végétales. Les auteurs ont évalué la fréquence et l’abondance de cinq espèces de plantes de sous-bois boréales communes (*Coptis trifolia* (L.) Salisb., *Cornus canadensis* L., *Linnaea borealis* L., *Lysimachia borealis* (Raf.) U.Manns & Anderb., and *Maianthemum canadense* Desf.) à différentes distances autour de six mines en Abitibi-Témiscamingue et dans le Nord-du-Québec (Québec). Leurs résultats ont montré que *Coptis trifolia* et *M. canadense* étaient affectés négativement par la présence des mines, ce qui suggère qu’ils pourraient être sensibles à la perte du couvert forestier, au dépôt de particules et à la contamination du sol induits par l’exploitation minière. À l’inverse, l’activité minière a favorisé, *Cornus canadensis*, ce qui indique sa résilience au stress causé par les mines et sa capacité à exploiter l’espace de niche laissé par les espèces plus sensibles. Aucun effet sur *Linnaea borealis* et *Lysimachia borealis* n’a été observé. Cette étude confirme l’existence d’impacts hors site des mines sur les communautés végétales. Des études supplémentaires portant sur les impacts non conventionnels devraient être réalisées afin de développer notre compréhension de l’empreinte environnementale plus large de l’activité minière. [Traduit par la Rédaction]

Mots-clés : forêt boréale, impacts non conventionnels, impacts hors site, communautés végétales, stress.

A3.2 Abstract

Although the mining industry provides minerals and metals to the global market and represents important economic opportunities, it also constitutes a major anthropogenic disturbance in the ecosystems where it takes place. However, little is known about its offsite impacts on plant communities. We assessed the frequency and the abundance of five common boreal understorey plant species (*Coptis trifolia* (L.) Salisb., *Cornus canadensis* L., *Linnaea borealis* L., *Lysimachia borealis* (Raf.) U.Manns & Anderb., and *Maianthemum canadense* Desf.) at different distances around six mines in Abitibi-Témiscamingue and Nord-du-Québec (Québec). Frequency and (or) abundance of *Coptis trifolia* and *M. canadense* decreased near mines, suggesting that they might be sensitive to the loss of forest cover, particle deposition, and soil contamination induced by mining. Conversely, the frequency and abundance of *Cornus canadensis* increased near active mines, which indicates its resilience to the stress caused by mines and its ability to exploit the niche space left by more sensitive species. No effects on *Linnaea borealis* and *Lysimachia borealis* were observed. Our study suggests the possible presence of offsite impacts of mines on plant communities. Further studies considering enigmatic impacts should be conducted in order to develop our understanding of the broader environmental footprint of mining activity.

Key words: boreal forest, enigmatic impacts, offsite impacts, plant communities, stress.

A3.3 Introduction

Although the mining industry provides minerals and metals to the global market and represents important economic opportunities, it also constitutes a major anthropogenic disturbance in the ecosystems where it takes place. In fact, mining development has significant ecological impacts, contributing to the loss, degradation, and conversion of wildlife habitats (Johnson and Skousen 1995; Simmons et al. 2008; Gould 2011). These impacts are partly the result of land-cover change at the mining site and are direct and easily assessable. However, ecological impacts are not restricted to the immediate location of the mines. Offsite impacts are substantial, although little is known about their extent. Raiter et al. (2014) included offsite impacts as one of their four categories of enigmatic impacts, which they define as the "impacts that are easily and often overlooked in impact evaluations".

Various offsite impacts are associated with mining activity. Loss of canopy cover can be observed around mines due to the edge effects they generate. In fact, anthropogenic development not only destroys vegetation at its immediate location, but it also induces tree mortality in the surrounding area (Nascimento and Laurance 2004). Anthropogenic openings have been shown to provoke more significant edge effects than natural openings in boreal forests (Vepakomma et al. 2018). Generation of dust is another issue related to mining (Ghose and Majee 2000). The transport of dust particles by wind (particles that can contain metals and metalloids) leads to their deposition in a large perimeter around the mine site and can have negative effects on vegetation. (Ghose and Majee 2000; Csavina et al. 2012; Li et al. 2018). Soil contamination is also a major risk generated by the mining industry, principally by acid mine drainage. The acidification of soil causes the solubilization of toxic heavy metals, which then become available for plants (Zhuang et al. 2009). Significant heavy metal concentrations can be observed several kilometers away from the mine sites (Escarré et al. 2011). The combination of these impacts might have an effect on surrounding plant communities by inducing the loss of native species thus creating a favourable environment for the introduction of exotic ruderal species, which then alters the functioning of the ecosystems (Hooper et al. 2005).

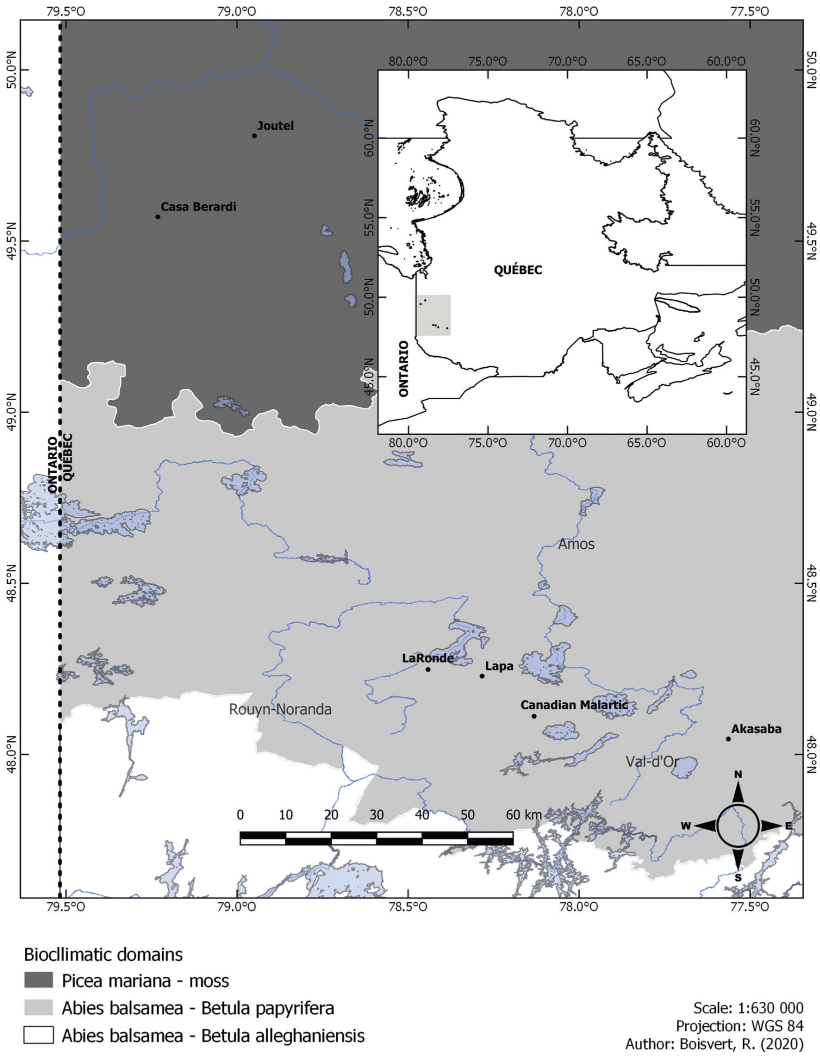

Figure A3.1 Geographic position of the six mines sampled: Casa Berardi (Hecla Québec Inc.), Joutel (Agnico Eagle Mines Ltd.), Akasaba (Agnico Eagle Mines Ltd.), Canadian Malartic (Canadian Malartic Corp.), Lapa (Agnico Eagle Mines Ltd.) and LaRonde (Agnico Eagle Mines Ltd.). Province boundary data from Statistics Canada (2011). Municipality data from Ministère de l'Energie et des Ressources Naturelles (2018). Water system data from Ministère de l'Energie et des Ressources Naturelles (2019). Bioclimatic domain data from Ministère des Forêts, de la Faune et des Parcs (2016). Themap was created in QGIS (QGIS Development Team 2020). [Colour online.]

Table A3.1: Description of the sampling design, including the number of transects sampled, the number of plots of each ecosystem type encountered around the mine and the number total of plots sampled for each mine.

Mines	Akasaba	Canadian Malartic	Lapa	LaRonde	Joutel	Casa Berardi	Total
Number of transects	6	8	7	8	7	8	44
Number of plots per ecosystem type	22 coniferous 1 deciduous 6 mixedwood 13 wetland	17 coniferous 9 deciduous 24 mixedwood 6 wetland	21 coniferous 5 deciduous 19 mixedwood 4 wetland	9 coniferous 27 deciduous 17 mixedwood 4 wetland	22 coniferous 9 deciduous 12 mixedwood 6 wetland	32 coniferous 2 deciduous 6 mixedwood 16 wetland	123 coniferous 53 deciduous 84 mixedwood 49 wetlands
Total number of plots	42	56	49	56	49	56	308

Table A3.2 Fixed effect variables and interactions included in each model after the forward selection method.

Species	Frequency models	Abundance models
Coptis trifolia	Variables: Distance from mine Ecosystem type Interaction: Ecosystem type: distance	Variables: Distance from mine Ecosystem type Mine stage Interaction: -
Cornus canadensis	Variables: Distance from mine Ecosystem type Mine stage Interaction: Mine stage: distance	Variables: Distance from mine Ecosystem type Mine stage Interactions: Mine stage: distance Ecosystem type: mine stage
Linnaea borealis	Variables: Distance from mine Ecosystem type Mine stage Interactions: Ecosystem type: distance Mine stage: distance	Variable: Distance from mine Interaction: -
Lysimachia borealis	Variables: Distance from mine Ecosystem type Mine stage Interaction: Ecosystem type: distance	Variables: Distance from mine Ecosystem type Mine stage Interaction: Ecosystem type: mine stage
Maianthemum canadense	Variables: Distance from mine Ecosystem type Mine stage Interactions: Ecosystem type: mine stage	Variables: Distance from mine Ecosystem type Mine stage Interactions: Mine stage: distance Ecosystem type: distance

This study aimed to improve our understanding of the offsite impacts of mines on understorey plant communities in the boreal forest. Specifically, our objective was to investigate the offsite impacts of mining on five common understorey plant species: *Coptis trifolia* (L.) Salisb., *Cornus canadensis* L., *Linnaea borealis* L., *Lysimachia borealis* (Raf.) U.Manns & Anderb., and *Maianthemum canadense* Desf. To do so, we assessed the frequency and the abundance of these species surrounding six mines in Abitibi-Témiscamingue and Nord-du-Québec (Québec). Because the loss of canopy cover, soil contamination, and dust deposition generated by the mines are expected to be greater at shorter distances, we expected that both frequency and abundance of all five species would increase with distance from the mine sites.

A3.4 Materials and methods

A3.4.1 Study area

The study was conducted on six gold mines in western Québec (Figure A3.1): Casa Berardi (Hecla Québec Inc.) and Joutel (Agnico EagleMines Ltd.), both located in the Picea mariana–moss bioclimatic domain and in the Nord-du-Québec administrative region; Akasaba (Agnico Eagle Mines Ltd.), Canadian Malartic (Canadian Malartic Corp.), Lapa (Agnico Eagle Mines Ltd.), and LaRonde (Agnico Eagle Mines Ltd.), all located in the Abies balsamea–Betula papyrifera bioclimatic domain and in the Abitibi-Témiscamingue administrative region (Saucier et al. 1998). At the time of the study, three of the mines were operating (Canadian Malartic, since 2005; Casa Berardi, since 1988; LaRonde, since 1988), and the three other mines were non-operating (Akasaba, establishing; Lapa, closed since 2018; Joutel, closed since 1998).Most of themines extracted other commodities in addition to the gold, such as copper (in Akasaba, LaRonde, and Joutel), silver (in Canadian Malartic, Casa Berardi, and LaRonde), and zinc (in LaRonde).

A3.4.2 Plant species

Plant species were selected based on the fact they are all widespread in the boreal forest understorey. All five species are common in coniferous, mixedwood, and deciduous stands, except for *Coptis trifolia*, which is mostly associated with coniferous stands (Carleton and Maycock 1980; Howard 1993). All of the species also occasionally occur in wetlands (Taft and Solecki 1990; Sullivan 1992; Howard 1993; Pavek 1993; Gucker 2012). *Coptis trifolia* and *Lysimachia borealis* are mainly associated with low light conditions and mature forests, while *Cornus canadensis*, *Linnaea borealis*, and *M. canadense* tolerate a wide spectrum of light intensity and can be found in every successional stage, although *M. canadense* is predominantly found in late successional forests (Howard 1993; Pavek 1993; Howard and Lee 2002; Moola and Vasseur 2004; Gucker 2012). All species occurred around everymine studied.

A3.4.3 Sampling design

Six to eight transects were established perpendicular to each mine's periphery through different types of ecosystems (coniferous, mixedwood, deciduous stands, and wetlands). Along each transect, seven plots, each 3 m in radius, were positioned at different distances from the mine's edge (0, 20, 50, 100, 200, 500 and 1000m) and three 1 m^2 quadrats were placed randomly within each plot. In total, 123 plots were in coniferous stands, 84 were in mixed wood stands, 53 were in deciduous stands, and 49 were in wetlands (Table A3.1). The presence or absence of each species was recorded in every 3 m radius plot. A presence record in any one of those plots was considered as one event of frequency. Abundances of the five species were assessed by visually estimating their percent cover in each 1 m^2 quadrat. Sampling around all six mines was completed between June and August 2019.

Table A3.3 Effects of distance from mine, mine stage (non-operating compared to operating) and interactions between distance from mine and mine stage on the frequency and abundance of *Coptis trifolia*, *Cornus canadensis*, *Linnaea borealis*, *Lysimachia borealis* and *Maianthemum canadense*.

Specie		Effect of distance from the mine	Effect of the mine stage (non-operating)	Effect of the interactions between distance from mine and mine stage (non-operating)
Coptis trifolia	Frequency[a]	1.705 (p=0.008*)	-	-
	Abundance[b]	0.110 (p=0.06)	-0.088 (p=0.463)	-
Cornus canadensis	Frequency	-0.364 (p=0.47)	-1.174(p=0.004*)	2.705 (p=0.0008*)
	Abundance	0.057 (p=0.78)	-0.340 (p=0.26)	0.625 (p=0.049*)
Linnaea borealis	Frequency	0.618 (p=0.48)	-0.514 (p=0.42)	-0.693 (p=0.51)
	Abundance	-0.113 (p=0.13)	-	-
Lysmiachia borealis	Frequency	-1.372 (p=0.06)	-0.704 (p=0.32)	-
	Abundance	0.073 (p=0.29)	-0.072 (p=0.66)	-
Maianthemum canadense	Frequency	0.803 (p=0.04*)	-0.989 (p=0.36)	-
	Abundance	0.202 (p=0.50)	-0.071 (p=0.87)	-0.163 (p=0.58)

========Note: An "*" indicates a significant effect ($p < 0.05$). A "-" means that the variable was not included in the model. (a) Frequencies were analyzed with generalised linear mixed-effects models with a binomial distribution (0 corresponding to absence of the species and 1 to its presence). (b) Abundances were log-transformed and analyzed with linear mixed-effects models.

A3.5 Statistical analysis

The frequency of each species was analyzed with a generalised linear mixed-effects modes with a binomial distribution using the glmer function of the lme4 package in R version 3.5.1 (Bates et al. 2015; RCoreTeam2018). Abundances were log-transformed and analyzed for each species with a linear mixed-effects model using the lme function of the nlme package in R version 3.5.1 (R Core Team 2018; Pinheiro et al. 2019). Mine sites were included as a random effect variable for both frequency and abundances models. Fixed-effect variables were selected using the forward selection method with the following variables and their interactions: distance from mine, ecosystem type, and mine stage (operating or non-operating). Distance from mine was always included. Only variables and interactions that had a statistically significant effect or lowered the p value of the effect of distance from mine were conserved. Table A3.2 shows variables and interactions included in the models following this method. The ecosystem type variable acted as a control variable in the models since the study focused on the effects of mining. Therefore, only the effect of distance from mine, mine stage, and the interaction between distance from mine and mine stage were analyzed.

A3.6 Results and discussion

Species response to mining activity

Of the five species, *Coptis trifolia* and *M. canadense* appear to have been negatively influenced by mines. Their frequency increased with distance from the mines (p = 0.008 and p = 0.040, respectively) (see Table A3.3). This pattern was observed around both operating and nonoperating mines. The effect was more significant for *Coptis trifolia*, which was observed in less than 16% of the plots between 0m and 50 m of distance, while at distances of 500 m and 1000 m, frequencies were 30% and 27%, respectively (see Figure A3.2). For *M. canadense*, the effect seemed more significant at the edge of mine, where the species was observed in 27% of the plots, while it was observed in 50% of the plots at a distance of 1000 m. From 50 m to 500 m away from the mines, frequencies fluctuated between 34% and 39% (see Figure A3.2). Other studies have also shown that these species are strongly affected by disturbance, although they focused on the effects of clear-cut harvesting (Moola and Vasseur 2004; Roberts and Gilliam 1995; Aikens et al. 2007; Zenner and Berger 2008). *Coptis trifolia* and *M. canadense* might have been influenced by the loss of canopy cover induced by the mines' edge effect because both species are mostly associated with mid-late to late-seral forests (Howard and Lee 2002; Moola and Vasseur 2004). Another potential cause of the decrease of *Coptis trifolia* and *M. canadense* next to mining sites might have been the stress induced by dust deposition and the subsequent soil contamination generated by the mines. Although, to our knowledge, no study has specifically investigated the effects of mines or metal contamination on these species, it is known that dust deposition can reduce plant photosynthetic rate, stomatal conductance, and transpiration rate (Li et al. 2018), and that metal contamination can extend several kilometers away from its source and modify vegetal community composition (Escarré et al. 2011). *Coptis trifolia* and *M. canadense* could have been disadvantaged by those stresses, and other species not studied in this research could have been similarly affected.

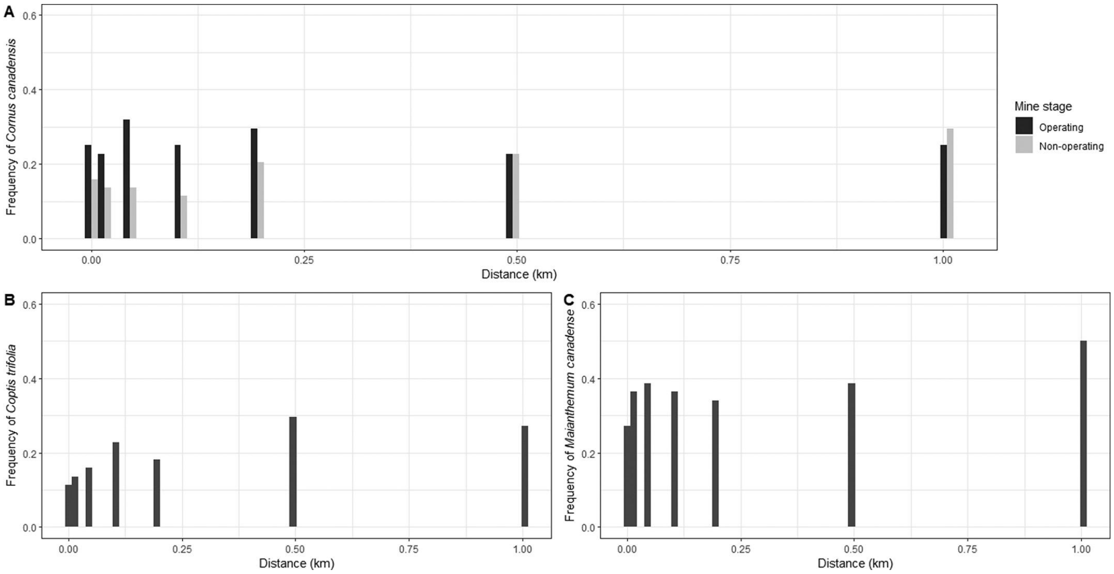

Figure A3.2. Normalized frequencies at 0 km, 0.02 km, 0.05 km, 0.1 km, 0.2 km, 0.5 km, and 1 km from mines for (A) *Cornus canadense*, according to mine stage, (B) *Coptis trifolia*, and (C) *Maianthemum canadense*.

Conversely, *Cornus canadensis* appeared to favour mining activity. The species was significantly more frequent around operating mines than around non-operating mines (p = 0.004). Moreover, significant differences in the effect of distance on frequency (p = 0.0008) and abundance (p = 0.049) were observed between operating and nonoperating mines (see Table A3.3). In fact, *Cornus canadensis* was negatively influenced around non-operating mines, while it was not influenced around operating mines (see Figure A3.2). These results suggest that *Cornus canadensis* is tolerant to the stress induced by mining activity. Similarly, previous studies have shown that this species persisted following a disturbance (Moola and Vasseur 2004) and some research even showed that its abundance increased (Chávez andMacdonald 2010). *Cornus canadensis*' wide plasticity and its ability to adapt to different soil and light conditions might allow it to exploit the niche left by species that are more sensitive to environmental changes (Kumordzi et al. 2019). The reduced frequency and abundance observed next to non-operating mines compared to operating ones suggests that itmay have been shaded out by vegetation regeneration after mine closure. Canopy cover loss would therefore be an important variable advantaging *Cornus canadensis*. The combination of soil contamination and shade might explain the low frequencies observed near the non-operating mines. With respect to *Linnaea borealis* and *Lysimachia canadensis*, specimen frequencies and abundances did not have a statistically significant relationship with distance from both operating and non-operating mines.

A3.7 Extent of the observed effects

Effects of mining activity on vegetation were observed from 0.1 km to 0.2 km away from the mine sites, although this differed slightly between species. Considering frequencies of *Coptis trifolia*, offsite effects of mining seemed to extend 0.2 km away from the mine sites. In fact, *Coptis trifolia* reached its highest frequencies at 0.5 km and 1.0 km from the mines, while its lowest frequencies were observed at 0, 0.02, and 0.05 km from the mines (see Figure A3.2). A similar pattern was observed with *Cornus canadensis* around non-operating mines, where frequencies where considerably lower at 0, 0.02, 0.05, and 0.1 km, and slightly lower at 0.2 km from the mines. For *M. canadense*, no clear threshold distance was detected, although minimum frequency was observed at the edge of the mine and maximum frequency was observed at 1.0 km away. Further studies should be conducted to evaluate the extent of soil contamination, dust deposition, and loss of canopy cover around mines in boreal forests. Our study suggests the possible presence of offsite impacts of mines on plant communities. The stresses and changes commonly induced by mining activity may lead to a decrease of sensitive species and to an increase of stress tolerant species in the surrounding communities. Further studies considering enigmatic impacts should be made to develop our understanding of the environmental footprint of mining activity.

REFERENCES

Aikens, M.L., Ellum, D., McKenna, J.J., Kelty, M.J., and Ashton, M.S. 2007. The effects of disturbance intensity on „„„„„„„„„„„„„„„„„„„„stemporal and spatial pattern of herb colonization in a southern New England mixed-oak forest. For. Ecol. Manage. 252: 144–158. doi:10.1016/j.foreco.2007.06.031.

Bates, D., Mächler, M., Bolker, B., and Walker, S. 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Soft. 67(1): 1–48. doi:10.18637/jss.v067.i01.

Carleton, T.J., and Maycock, P.F. 1980. Vegetation of the boreal forest south of James Bay: Non-centered component analysis of the vascular flora. Ecology. 61(5): 1199–1212. doi:10.2307/1936838.

Chávez, V., and Macdonald, S.E. 2010. Understory species interactions in mature boreal mixedwood forests. Botany. 88: 912–922. doi:10.1139/B10-062.

Csavina, J., Field, J., Taylor, M.P., Gao, S., Landázuri, A., Betterton, E.A., and Sáez, A.E. 2012. A review on the importance of metals and metalloids in atmospheric dust and aerosol from mining operations. Sci. Total Environ. 433: 58–73. doi:10.1016/j.scitotenv.2012.06.013. PMID:22766428.

Escarré, J., Lefèbvre, C., Raboyeau, S., Dossantos, A., Gruber, W., Cleyet Marel, J.C., et al. 2011. Heavy metal concentration survey in soils and plants of the Les Malines Mining District (Southern France): implications for soil restoration. Water. Air. Soil Pollut. 216: 485–504. doi:10.1007/s11270-010-0547-1.

Ghose, M.K., and Majee, S.R. 2000. Assessment of Dust Generation Due to Opencast Coal Mining – An Indian Case Study. Environ. Monit. Assess. 61: 257–263. doi:10.1023/A:1006127407401.

Gould, S.F. 2011. Does post-mining rehabilitation restore habitat equivalent to that removed by mining? A case study from the monsoonal tropics of northern Australia. Wildl. Res. 38: 482–490. doi:10.1071/WR11019.

Gucker, C.L. 2012. Cornus canadensis. In: Fire Effects Information System. U.S. Department of Agriculture Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. Available at https://www.fs.fed.us/database/feis/plants/forb/ corcan/all.html#93 [accessed 2 December 2020].

Hooper, D.U., Chapin, F.S., Ewel, J.J., Hector, A., Inchausti, P., Lavorel, S., et al. 2005. Effects of Biodiversity on Ecosystem Functioning: A Consensus of Current Knowledge. Ecol. Monogr. 75(1): 3–35. doi:10.1890/04-0922.

Howard, J.L. 1993. Linnaea borealis. In: Fire Effects Information System. U.S. Department of Agriculture Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. Available at https://www.fs.fed.us/database/feis/plants/forb/linbor/all.html [accessed 2 December 2020].

Howard, L.F., and Lee, T.D. 2002. Upland old-field succession in south eastern New Hampshire. J. Torrey Bot. Soc. 129(1): 60–76.doi:10.2307/3088683.

Johnson, C.D., and Skousen, J.G. 1995. Mine soil Properties of 15 Abandoned Mine Land Sites in West Virginia. J. Environ. Qual. 24: 635–643. doi:10.2134/jeq1995.00472425002400040014x.

Kumordzi, B.B., Aubin, I., Cardou, F., Shipley, B., Violle, C., Johnstone, J., et al. 2019. Geographic scale and disturbance influence intraspecific trait variability in leaves and roots of North American understorey plants. Funct. Ecol. 33: 1771–1784. doi:10.1111/1365-2435.13402.

Li, Y., Zhao, N., Cao, Y., and Yang, J. 2018. Effects of coal dust deposition on the physiological properties in an open pit coal mine. Acta Ecol. Sin. 38: 8129–8138.

Ministère de l'Energie et des Ressources naturelles. 2018. Découpages administratifs. Québec. Available at https://www.donneesquebec.ca/recherche/dataset/decoupages-administratifs [accessed 24 January 2021].

Ministère de l'Energie et des Ressources naturelles. 2019. Géobase du réseau hydrographique du Québec (GRHQ). Québec. Available at https://www.donneesquebec.ca/recherche/dataset/grhq# [accessed 24 January 2021].

Ministère des Forêts, de la Faune et des Parcs. 2016. Classification écologique du territoire québécois. Québec. Available at https://www.donneesquebec.ca/recherche/dataset/systeme-hierarchique-de-classification-ecologique-du-territoire [accessed 24 January 2021].

Moola, F.M., and Vasseur, L. 2004. Recovery of late-seral vascular plants in a chronosequence of post-clearcut forest stands in coastal Nova Scotia, Canada. Plant Ecol. 172: 183–197. doi:10.1023/B:VEGE.0000026326.09137.06.

Nascimento, H.E.M., and Laurance, W.F. 2004. Biomass dynamics in Amazonian forest fragments. Ecol. Appl. 14(sp4): 127–138. doi:10.1890/01-6003.

Pavek, D.S. 1993. Maianthemum canadense. In: Fire Effects Information System. U.S. Department of Agriculture Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. Available at https://www.fs.fed.us/database/feis/plants/forb/maican/all.html#72 [accessed 2 December 2020]

Pinheiro, J., Bates, D., DebRoy, S., and Sarkar, D. 2019. nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-143.

QGIS Development Team. 2020. QGIS Geographic Information System. Open Source Geospatial Foundation Project. Available at http://qgis.osgeo.org.

R Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing,

Vienna, Austria. Raiter, K.G., Possingham, H.P., Prober, S.M., and Hobbs, R.J. 2014. Under the radar: mitigating enigmatic ecological impacts. Trends Ecol. Evol. 29(11): 635–644. doi:10.1016/j.tree.2014.09.003. PMID:25269749.

Roberts, M.R., and Gilliam, F.S. 1995. Disturbance effects on herbaceous layer vegetation and soil nutrients in Populus forests of northern lower Michigan. J. Veg. Sci. 6: 903–912. doi:10.2307/3236405.

Saucier, J.-P., Bergeron, J.-F., Grondin, P., and Robitaille, A. 1998. Les régions écologiques du Québec (3e version): un des éléments du système hiérarchique de classification écologique du territoire mis au point par le ministère des Ressources naturelles du Québec. L'Aubelle. 124: S1–S12.

Simmons, J.A., Currie, W.S., Eshleman, K.N., Kuers, K., Monteleone, S., Negley, T.L., et al. 2008. Forest to reclaimed mine land use change leads to altered ecosystem structure and function. Ecol. Appl. 18(1): 104–118. doi:10.1890/07-1117.1.PMID:18372559.

Statistics Canada. 2011. Province and Territory Cartographic Boundaries Files – 2011 Census (cartographic boundary file, cgb_lgc_canada_shp_fr). Available at https://open.canada.ca/data/en/dataset/bab06e04-e6d0-41f1-a595-6cff4d71bedf [accessed 23 January 2021].

Sullivan, J. 1992. Coptis trifolia. In: Fire Effects Information System. U.S. Department of Agriculture Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory. Available at https://www.fs.fed.us/database/feis/plants/forb/coptri/all.html#41 [accessed 2 December 2020].

Taft, J.B., and Solecki, M.K. 1990. Vascular flora of the wetland and prairie communities of Gavin Bog and Prairie Nature Preserve, Lake County, Illinois. Rhodora. 92(871): 142–165.

Vepakomma, U., Kneeshaw, D.D., and De Grandpré, L. 2018. Influence of Natural and Anthropogenic Linear Canopy Openings on Forest Structural Patterns Investigated Using LiDAR. Forests. 9(9): 540. doi:10.3390/f9090540.

Zenner, E.K., and Berger, A.L. 2008. Influence of skidder traffic and canopy removal intensities on the ground flora in a clearcut-with-reserves northern hardwood stand in Minnesota, USA. For. Ecol. Manage. 256(10): 1785–1794. doi:10.1016/j.foreco.2008.05.030.

Zhuang, P., McBride, M.B., Xia, H., Li, N., and Li, Z. 2009. Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. Sci. Total Environ. 407(5): 1551–1561. doi:10.1016/j.scitotenv.2008.10.061. PMID:19068266.

BIBLIOGRAPHIE

Ab Rahman, S.F.S., Singh, E., Pieterse, C.M. and Schenk, P.M. 2018. Emerging microbial biocontrol strategies for plant pathogens. *Plant Science*, 267, 102-111. doi: 10.1016/j.plantsci.2017.11.012

Abril, A.B., Torres, P.A. and Bucher, E.H. 2005. The importance of phyllosphere microbial populations in nitrogen cycling in the Chaco semi-arid woodland. *Journal of tropical ecology*, 21(1), 103-107. doi:10.1017/S0266467404001981

Adesipo, A.A., Akinbiola, S., Awotoye, O.O., Salami, A.T. and Freese, D. 2020. Impact of mining on the floristic association of gold mined sites in Southwest Nigeria. *BMC ecology,* 20(9), 1-13. doi:10.1186/s12898-020-00276-9

Andersen, L. H., Skærbæk, A. S. K., Sørensen, T. B., Knudsen, J. S., Pertoldi, C., Bahrndorff, S., & Bruhn, D. 2020. Turnover and change in plant species composition in a shielded salt marsh following variation in precipitation and temperature. *Journal of Vegetation Science*, 31(3), 465-475. Doi: doi.org/10.1111/jvs.12863.

Adriaensen, K., Vralstad, T., Noben, J.P., Vangronsveld, J. and Colpaert, J.V., 2005. Copper-adapted Suillus luteus, a symbiotic solution for pines colonizing Cu mine spoils. *Applied and Environmental Microbiology*, 71(11): 7279-7284. doi: 10.1128/AEM.71.11.7279-7284.2005

Allison, S.D. and Martiny, J.B., 2008. Resistance, resilience, and redundancy in microbial communities. *The Proceedings of the National Academy of Sciences*, 105(supplement_1), 11512-11519. doi: 10.1073/pnas.0801925105.

Al-Amin, A., Parvin, F., Chakraborty, J. and Kim, Y.-I. 2021. Cyanobacteria mediated heavy metal removal: a review on mechanism, biosynthesis, and removal capability. *Environmental Technology Reviews*, 10(1), 44-57. doi:10.1080/21622515.2020.1869323

Anderson, M.J. 2005. PERMANOVA: A FORTRAN Computer Program for Permutational Multivariate Analysis of Variance. Department of Statistics, University of Auckland, New Zealand.

Anderson, C.M., Field, C.B. and Mach, K.J. 2017. Forest offsets partner climate-change mitigation with conservation. *Frontiers in Ecology and the Environment*, 15(7), 359-365. doi: 10.1002/fee.1515

Arroniz-Crespo, M., Perez-Ortega, S., De los Rios, A., Green, T.A., Ochoa-Hueso, R., Casermeiro, M.Á., De La Cruz, M.T., Pintado, A., Palacios, D. and Rozzi, R. 2014. Bryophyte-cyanobacteria associations during primary succession in recently deglaciated areas of Tierra del Fuego (Chile). *PloS one*, 9(5), e96081. doi:10.1371/journal.pone.0096081

Arun, K.D., Sabarinathan, K.G., Gomathy, M., Kannan, R. and Balachandar, D. 2020. Mitigation of drought stress in rice crop with plant growth-promoting abiotic stress-tolerant rice phyllosphere bacteria. *Journal of Basic Microbiology*, 60(9), 768-786. doi:10.1002/jobm.202000011

Augusto, L., Ranger, J., Binkley, D. and Rothe, A. 2002. Impact of several common tree species of European temperate forests on soil fertility. *Annals of Forest Science*, 59(3), 233-253. doi:1051/forest:2002020

Balabanova, B., Stafilov, T., Šajn, R. and Andonovska, K.B. 2017. Quantitative assessment of metal elements using moss species as biomonitors in downwind area of lead-zinc mine. *Journal of Environmental Science and Health, Part A*, 52(3), 290-301. doi:10.1080/10934529.2016.1253403

Barbé, M., Bouchard, M. and Fenton, N.J. 2020. Examining boreal forest resilience to temperature variability using bryophytes: forest type matters. *Ecosphere*, 11(8), e03232. doi:10.1002/ecs2.3232

Barbier, S., Gosselin, F. and Balandier, P. 2008. Influence of tree species on understory vegetation diversity and mechanisms involved—a critical review for temperate and boreal forests. *Forest Ecology and Management*, 254(1), 1-15. doi:10.1016/j.foreco.2007.09.038

Barrett, S.E. and Watmough, S.A. 2015. Factors controlling peat chemistry and vegetation composition in Sudbury peatlands after 30 years of pollution emission reductions. *Environmental Pollution*, 206, 122-132. doi:10.1016/j.envpol.2015.06.021

Bartels, S.F., Caners, R.T., Ogilvie, J., White, B. and Macdonald, S.E. 2018a. Relating bryophyte assemblages to a remotely sensed depth-to-water index in boreal forests. *Frontiers in plant science*, 9, 858. doi: 10.3389/fpls.2018.00858

Bartels, S.F., Gendreau-Berthiaume, B. and Macdonald, S.E. 2019. The impact of atmospheric acid deposition on tree growth and forest understory vegetation in the Athabasca Oil Sands Region. *Science of The Total Environment*, 696, 133877. doi: 10.1016/j.scitotenv.2019.133877

Bartels, S.F., Macdonald, S.E., Johnson, D., Caners, R.T. and Spence, J.R. 2018b. Bryophyte abundance, diversity and composition after retention harvest in boreal mixedwood forest. *Journal of Applied Ecology*, 55(2), 947-957. doi: 10.1111/1365-2664.12999

Barton, K. and Barton, M.K. 2015. Package 'MuMIn', R package version 1.15.1 (2015-07-02).

Bashir, I., War, A.F., Rafiq, I., Reshi, Z.A., Rashid, I. and Shouche, Y.S. 2021. Phyllosphere microbiome: Diversity and functions. *Microbiological Research*, 254, e126888. doi: 10.1016/j.micres.2021.126888

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R.H.B., Singmann, H., Dai, B., Scheipl, F., Grothendieck, G. and Green, P. 2018. Package 'lme4'. Version 1.1-17 (2018-04-03).

Bech, J., Duran, P., Roca, N., Poma, W., Sánchez, I., Barceló, J., Boluda, R., Roca-Pérez, L. and Poschenrieder, C. 2012. Shoot accumulation of several trace elements in native plant species from contaminated soils in the Peruvian Andes. *Journal of Geochemical Exploration*, 113, 106-111. doi:10.1016/j.gexplo.2011.04.007

Benítez, Á., Prieto, M. and Aragón, G. 2015. Large trees and dense canopies: key factors for maintaining high epiphytic diversity on trunk bases (bryophytes and lichens) in tropical montane forests. *Forestry: An International Journal of Forest Research*, 88(5), 521-527. doi: doi.org/10.1093/forestry/cpv022

Benjamini, Y. and Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, 57(1), 289-300. doi: 10.1111/j.2517-6161.1995.tb02031.x

Bergeron, O., Margolis, H. and Coursolle, C. 2009. Forest floor carbon exchange of a boreal black spruce forest in eastern North America. *Biogeosciences*, 6(9), 1849-1864. doi:10.5194/bg-6-1849-2009

Bergeron, Y., Gauthier, S., Flannigan, M. and Kafka, V. 2004. Fire regimes at the transition between mixedwood and coniferous boreal forest in northwestern Quebec. *Ecology*, 85(7), 1916-1932. doi:10.1890/02-0716

Betancourt, O., Narváez, A. and Roulet, M. 2005. Small-scale gold mining in the puyango river basin, Southern Ecuador: A study of environmental impacts and human exposures. *EcoHealth*, 2, 323-332. doi: 10.1007/s10393-005-8462-4

Bisbee, K.E., Gower, S.T., Norman, J.M. and Nordheim, E.V. 2001. Environmental controls on ground cover species composition and productivity in a boreal black spruce forest. *Oecologia*, 129(2), 261-270. doi: 10.1007/s004420100719

Blackmore, S., Vriens, B., Sorensen, M., Power, I.M., Smith, L., Hallam, S.J., Mayer, K.U. and Beckie, R.D. 2018. Microbial and geochemical controls on waste rock weathering and drainage quality. *Science of The Total Environment*, 640, 1004-1014. doi: 10.1016/j.scitotenv.2018.05.374

Blagnytė, R. and Paliulis, D. 2010. Research into heavy metals pollution of atmosphere applying moss as bioindicator: a literature review. *Environmental Research, Engineering and Management*, 54(4), 26-33. URL: https://www.erem.ktu.lt/index.php/erem/article/view/93

Böhmer, M., Ozdín, D., Račko, M., Lichvár, M., Budiš, J. and Szemes, T. 2020. Identification of Bacterial and Fungal Communities in the Roots of Orchids and Surrounding Soil in Heavy Metal Contaminated Area of Mining Heaps. *Applied Sciences*, 10(20), 7367. doi:10.3390/app10207367

Boisvert-Marsh, L. and de Blois, S. 2021. Unravelling potential northward migration pathways for tree species under climate change. *Journal of Biogeography*, 48(5), 1088-1100. doi: 10.1111/jbi.14060

Boisvert, R., Yin, X.B. and Fenton, N.J. 2021. Offsite effects of mining on the frequency and abundance of five understorey plant species in western Quebec (Canada). *Botany*, 99(7), 449-455. doi:10.1139/cjb-2020-0158

Bokulich, N.A., Subramanian, S., Faith, J.J., Gevers, D., Gordon, J.I., Knight, R., Mills, D.A. and Caporaso, J.G., 2013. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature methods*, 10(1), 57-59. doi: 10.1038/nmeth.2276

Boojar, M. and Tavakkoli, Z. 2011. Antioxidative responses and metal accumulation in invasive plant species growing on mine tailings in Zanjan, Iran. *Pedosphere*, 21(6), 802-812. doi:10.1016/S1002-0160(11)60184-7

Boyce, J.S. 2002. Invasive species–an emerging issue for mining and reclamation. Paper was presented at the 2002 National Meeting of American Society of Mining and Reclamation, Lexington, KY, June 9-13, 2002. Published by ASMR, 3134 Montavesta Rd.,Lexington, KY 40502

Bradshaw, C.J. and Warkentin, I.G. 2015. Global estimates of boreal forest carbon stocks and flux. *Global and Planetary Change*, 128, 24-30. doi:10.1016/j.gloplacha.2015.02.004

Brandt, J.P., Flannigan, M.D., Maynard, D.G., Thompson, I.D. and Volney, W.J.A. 2013. An introduction to Canada's boreal zone: ecosystem processes, health, sustainability, and environmental issues. *Environmental Reviews*, 21(4), 207-226. doi:10.1139/er-2013-0040

Brassard, B.W., Chen, H.Y., Wang, J.R. and Duinker, P.N. 2008. Effects of time since stand-replacing fire and overstory composition on live-tree structural diversity in the boreal forest of central Canada. *Canadian Journal of Forest Research*, 38(1), 52-62. doi:10.1139/X07-125

Bringel, F. and Couée, I., 2015. Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. *Frontiers in microbiology*, 6: 486. doi: 10.3389/fmicb.2015.00486.

Brooks, M.E., Kristensen, K., van Benthem, K.J., Magnusson, A., Berg, C.W., Nielsen, A., Skaug, H.J., Machler, M. and Bolker, B.M. 2017. glmmTMB Balances Speed and

Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *R Journal*, 9(2), 378-400. doi:10.3929/ethz-b-000240890

Brudvig, L.A., Damschen, E.I., Tewksbury, J.J., Haddad, N.M. and Levey, D.J. 2009. Landscape connectivity promotes plant biodiversity spillover into non-target habitats. *Proceedings of the National Academy of Sciences*, 106(23), 9328-9332. doi:10.1073/pnas.080965810

Buzzini, P., Turchetti, B. and Yurkov, A. 2018. Extremophilic yeasts: the toughest yeasts around? *Yeast*, 35(8), 487-497. doi:10.1002/yea.3314

Calinger, K., Calhoon, E., Chang, H.C., Whitacre, J., Wenzel, J., Comita, L. and Queenborough, S. 2015. Historic mining and agriculture as indicators of occurrence and abundance of widespread invasive plant species. *PloS one*, 10(6), e0128161. doi:10.1371/journal.pone.0128161

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J. and Holmes, S.P. 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, 13(7), 581-583. doi:0.1038/nmeth.3869

Caners, R.T., Macdonald, S.E. and Belland, R.J. 2013. Bryophyte assemblage structure after partial harvesting in boreal mixedwood forest depends on residual canopy abundance and composition. *Forest Ecology and Management*, 289, 489-500. doi:10.1016/j.foreco.2012.09.044

Canto, A. and Herrera, C.M. 2012. Micro-organisms behind the pollination scenes: microbial imprint on floral nectar sugar variation in a tropical plant community. *Annals of Botany*, 110(6), 1173-1183. doi:10.1093/aob/mcs183

Cavard, X., Bergeron, Y., Chen, H.Y. and Paré, D. 2011. Effect of forest canopy composition on soil nutrients and dynamics of the understorey: mixed canopies serve neither vascular nor bryophyte strata. *Journal of Vegetation Science*, 22(6), 1105-1119. doi: 10.1111/j.1654-1103.2011.01311.x

Cayuela, L., Granzow-de la Cerda, Í., Albuquerque, F.S. and Golicher, D.J. 2012. Taxonstand: An R package for species names standardisation in vegetation databases. *Methods in Ecology and Evolution*, 3(6), 1078-1083. doi:10.1111/j.2041-210X.2012.00232.x

Ceschin, S., Aleffi, M., Bisceglie, S., Savo, V. and Zuccarello, V. 2012. Aquatic bryophytes as ecological indicators of the water quality status in the Tiber River basin (Italy). *Ecological indicators*, 14(1), 74-81. doi:10.1016/j.ecolind.2011.08.020

Chávez, V. and Macdonald, S. 2010. The influence of canopy patch mosaics on understory plant community composition in boreal mixedwood forest. *Forest Ecology and Management*, 259(6), 1067-1075. doi:10.1016/j.foreco.2009.12.013

Chávez, V. and Macdonald, S.E. 2012. Partitioning vascular understory diversity in mixedwood boreal forests: The importance of mixed canopies for diversity conservation. *Forest Ecology and Management*, 271, 19-26. doi:10.1016/j.foreco.2011.12.038

Chen, L., Reeve, J., Zhang, L., Huang, S., Wang, X. and Chen, J. 2018b. GMPR: A robust normalization method for zero-inflated count data with application to microbiome sequencing data. *PeerJ*, 6, e4600. doi:10.7717/peerj.4600

Chen, H. Y., Biswas, S. R., Sobey, T. M., Brassard, B. W., & Bartels, S. F. 2018a. Reclamation strategies for mined forest soils and overstorey drive understorey vegetation. *Journal of applied ecology*, 55(2), 926-936. doi: 10.1111/1365-2664.13018

Chen, W., Leblanc, S.G., White, H.P., Prevost, C., Milakovic, B., Rock, C., Sharam, G., O'Keefe, H., Corey, L. and Croft, B. 2017. Does dust from arctic mines affect caribou forage? *Journal of Environmental Protection*, 8(03), 258. doi:10.4236/jep.2017.83020

Chester, M.V., Markolf, S. and Allenby, B. 2019. Infrastructure and the environment in the Anthropocene. *Journal of Industrial Ecology*, 23(5), 1006-1015. doi:10.1111/jiec.12848

Christou, A., Theologides, C.P., Costa, C., Kalavrouziotis, I.K. and Varnavas, S.P. 2017. Assessment of toxic heavy metals concentrations in soils and wild and cultivated plant species in Limni abandoned copper mining site, Cyprus. *Journal of Geochemical Exploration*, 178, 16-22. doi:10.1016/j.gexplo.2017.03.012

Chung, A.P., Coimbra, C., Farias, P., Francisco, R., Branco, R., Simão, F.V., Gomes, E., Pereira, A., Vila, M.C. and Fiúza, A. 2019. Tailings microbial community profile and prediction of its functionality in basins of tungsten mine. *Scientific Reports*, 9, 19596. doi:10.1038/s41598-019-55706-6

Cole, H.A., Newmaster, S.G., Bell, F.W., Pitt, D. and Stinson, A. 2008. Influence of microhabitat on bryophyte diversity in Ontario mixedwood boreal forest. *Canadian Journal of Forest Research*, 38(7), 1867-1876. doi:10.1139/X08-03

Compant, S., Samad, A., Faist, H. and Sessitsch, A. 2019. A review on the plant microbiome: ecology, functions, and emerging trends in microbial application. *Journal of advanced research*, 19, 29-37. doi:10.1016/j.jare.2019.03.004

Coors, A. and De Meester, L. 2008. Synergistic, antagonistic and additive effects of multiple stressors: predation threat, parasitism and pesticide exposure in *Daphnia magna*. *Journal of Applied Ecology*, 45(6), 1820-1828. doi:10.1111/j.1365-2664.2008.01566.x

Corlett, R.T. 2015. The Anthropocene concept in ecology and conservation. *Trends in Ecology & Evolution*, 30(1), 36-41. doi:10.1016/j.tree.2014.10.007

Corriveau, M., Jamieson, H., Parsons, M., Campbell, J. and Lanzirotti, A. 2011. Direct characterization of airborne particles associated with arsenic-rich mine tailings: Particle size, mineralogy and texture. *Applied Geochemistry*, 26(9-10), 1639-1648. doi:10.1016/j.apgeochem.2011.04.021

Craine, J.M. and Dybzinski, R. 2013. Mechanisms of plant competition for nutrients, water and light. *Functional Ecology*, 27(4), 833-840. doi:10.1111/1365-2435.12081

Csavina, J., Field, J., Taylor, M.P., Gao, S., Landázuri, A., Betterton, E.A. and Sáez, A.E. 2012. A review on the importance of metals and metalloids in atmospheric dust and aerosol from mining operations. *Science of The Total Environment*, 433, 58-73. doi:10.1016/j.scitotenv.2012.06.013

Cui, J., Xie, Y., Sun, T., Chen, L. and Zhang, W. 2021. Deciphering and engineering photosynthetic cyanobacteria for heavy metal bioremediation. *Science of The Total Environment*, 761, 144111. doi: 10.1016/j.scitotenv.2020.144111

Cusack, D.F., Karpman, J., Ashdown, D., Cao, Q., Ciochina, M., Halterman, S., Lydon, S. and Neupane, A. 2016. Global change effects on humid tropical forests: Evidence for biogeochemical and biodiversity shifts at an ecosystem scale. *Reviews of Geophysics*, 54(3), 523-610. doi:10.1002/2015RG000510

Cutler, N.A., Arróniz-Crespo, M., Street, L.E., Jones, D.L., Chaput, D.L. and DeLuca, T.H. 2017. Long-term recovery of microbial communities in the boreal bryosphere following fire disturbance. *Microbial ecology*, 73(1), 75-90. doi:10.1007/s00248-016-0832-7

Dai, Y.-H. 2002. Convergence properties of the BFGS algoritm. *SIAM Journal on Optimization*, 13(3), 693-701. doi:10.1137/S1052623401383455

Danneyrolles, V., Dupuis, S., Fortin, G., Leroyer, M., de Römer, A., Terrail, R., Vellend, M., Boucher, Y., Laflamme, J. and Bergeron, Y. 2019. Stronger influence of anthropogenic disturbance than climate change on century-scale compositional changes in northern forests. *Nature communications*, 10(1), 1-7. doi:10.1038/s41467-019-09265-z

Davis, N.M., Proctor, D.M., Holmes, S.P., Relman, D.A. and Callahan, B.J. 2018. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*, 6(1), 226. doi:10.1186/s40168-018-0605-2

De Caceres, M., Jansen, F. and De Caceres, M.M. 2016. Package ‘indicspecies’, R package version 1.7.6 (2016-08-29).

De Jalón, S.G., Burgess, P., Yuste, J.C., Moreno, G., Graves, A., Palma, J., Crous-Duran, J., Kay, S. and Chiabai, A. 2019. Dry deposition of air pollutants on trees at regional scale: A case study in the Basque Country. *Agricultural and Forest Meteorology*, 278, 107648. doi:10.1016/j.agrformet.2019.107648

DeLuca, T.H., Zackrisson, O., Nilsson, M.C. and Sellstedt, A. 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature, 419, 917-920. doi:10.1038/nature01051

Dhar, A., Comeau, P.G., Karst, J., Pinno, B.D., Chang, S.X., Naeth, A.M., Vassov, R. and Bampfylde, C. 2018. Plant community development following reclamation of oil sands mine sites in the boreal forest: a review. *Environmental reviews*, 26(3), 286-298. doi:10.1139/er-2017-0091

Dimitriu, P.A. and Grayston, S.J. 2010. Relationship between soil properties and patterns of bacterial β-diversity across reclaimed and natural boreal forest soils. *Microbial ecology*, 59, 563-573. doi:10.1007/s00248-009-9590-0

Dochinger, L.S. 1980. Interception of airborne particles by tree plantings. *Journal of Environmental Quality*, 9(2), 265-268. doi:10.2134/jeq1980.00472425000900020020x

Dražina, T., Špoljar, M., Primc, B. and Habdija, I. 2016. Distribution of rotifers and other meiofauna in the bryophytes and hyporheic zone of a karst hydrosystem–an example of a nested community. *Marine and Freshwater Research*, 68(1), 43-52. doi:10.1071/MF14291

Duarte, M., Sousa-Lima, R., Young, R., Farina, A., Vasconcelos, M., Rodrigues, M. and Pieretti, N. 2015. The impact of noise from open-cast mining on Atlantic forest biophony. *Biological Conservation*, 191, 623-631. doi:10.1016/j.biocon.2015.08.006

Dyderski, M.K. and Jagodziński, A.M. 2018. Low impact of disturbance on ecological success of invasive tree and shrub species in temperate forests. *Plant Ecology*, 219, 1369-1380. doi:10.1007/s11258-018-0885-4

Dyer, S.J., O'Neill, J.P., Wasel, S.M. and Boutin, S. 2001. Avoidance of industrial development by woodland caribou. *The Journal of Wildlife Management*, 531-542. doi:10.2307/3803106

Edge, T.A., Baird, D.J., Bilodeau, G., Gagné, N., Greer, C., Konkin, D., Newton, G., Séguin, A., Beaudette, L. and Bilkhu, S. 2020. The Ecobiomics project: Advancing metagenomics assessment of soil health and freshwater quality in Canada. *Science of The Total Environment*, 710, 135906. doi:10.1016/j.scitotenv.2019.135906

Ekka, N. and Behera, N. 2011. Species composition and diversity of vegetation developing on an age series of coal mine spoil in an open cast coal field in Orissa, India. *Tropical Ecology*, 52, 337-343.URL: http://tropecol.com/pdf/open/PDF_52_3/Jour-12.pdf

Ellis, E.C., Antill, E.C. and Kreft, H. 2012. All is not loss: plant biodiversity in the Anthropocene. *PloS one*, 7(1), e30535. doi:10.1371/journal.pone.0030535

Ellison, A.M. and Gotelli, N.J. 2001. Evolutionary ecology of carnivorous plants. *Trends in Ecology and Evolution*, 16(11), 623-629. doi:10.1016/S0169-5347(01)02269-8

Elmayel, I., Esbri, J.M., Efren, G.O., Garcia-Noguero, E.M., Elouear, Z., Jalel, B., Farieri, A., Roqueni, N., Cienfuegos, P. and Higueras, P. 2020. Evolution of the Speciation and Mobility of Pb, Zn and Cd in Relation to Transport Processes in a Mining Environment. *International Journal of Environmental Research and Public Health*, 17(14), 4912. doi:10.3390/ijerph17144912

Environmnet Canada. 2021. Normales climatiques canadiennes. https://climat.meteo.gc.ca/climate_normals/. Consulted : 14/01/2021

Eugster, W., Rouse, W.R., Pielke Sr, R.A., Mcfadden, J.P., Baldocchi, D.D., Kittel, T.G.F., Chapin III, F.S., Liston, G.E., Vidale, P.L. and Vaganov, E. 2000. Land–atmosphere energy exchange in Arctic tundra and boreal forest: available data and feedbacks to climate. *Global change biology*, 6(S1), 84-115. doi:10.1046/j.1365-2486.2000.06015.x

Farmer, A.M. 1993. The effects of dust on vegetation--a review. *Environmental Pollution*, 79(1), 63-75. doi:10.1016/0269-7491(93)90179-R

Faubert, J. 2012. Flore des bryophytes du Québec-Labrador, Société québécoise de bryologie Saint-Valérien, QU, CA.

Feng, Y., Wang, J., Bai, Z. and Reading, L. 2019. Effects of surface coal mining and land reclamation on soil properties: A review. *Earth-Science Reviews*, 191, 12-25. doi:10.1016/j.earscirev.2019.02.015

Festin, E.S., Tigabu, M., Chileshe, M.N., Syampungani, S. and Oden, P.C. 2019. Progresses in restoration of post-mining landscape in Africa. *Journal of Forestry Research*, 30, 381-396. doi:10.1007/s11676-018-0621-x

Fox J., Weisberg D., Bates M. 2012. Package ‘car’. Vienna: R Foundation for Statistical Computing. https://cran.r-project.org/web/packages/car/index.html

Freedman, B. and Hutchinson, T. 1980. Effects of smelter pollutants on forest leaf litter decomposition near a nickel–copper smelter at Sudbury, Ontario. *Canadian Journal of Botany*, 58, 1722-1736. doi:10.1139/b80-200

Frouz, J., Keplin, B., Pižl, V., Tajovský, K., Starý, J., Lukešová, A., Nováková, A., Balı́k, V.r., Háněl, L. and Materna, J. 2001. Soil biota and upper soil layer development in two contrasting post-mining chronosequences. *Ecological Engineering*, 17(2-3), 275-284. doi:10.1016/S0925-8574(00)00144-0

Fürnkranz, M., Wanek, W., Richter, A., Abell, G., Rasche, F. and Sessitsch, A. 2008. Nitrogen fixation by phyllosphere bacteria associated with higher plants and their colonizing epiphytes of a tropical lowland rainforest of Costa Rica. The ISME journal, 2, 561-570. doi:10.1038/ismej.2008.14

Gagnon, V., Rodrigue-Morin, M., Tremblay, J., Wasserscheid, J., Champagne, J., Bellenger, J.-P., Greer, C.W. and Roy, S. 2020. Vegetation drives the structure of active microbial communities on an acidogenic mine tailings deposit. *PeerJ*, 8, e10109. doi:10.7717/peerj.10109

Galbally, I.E. and Kirstine, W. 2002. The production of methanol by flowering plants and the global cycle of methanol. *Journal of Atmospheric Chemistry*, 43, 195-229. doi:10.1023/A:1020684815474

Gauthier, S., Bernier, P., Kuuluvainen, T., Shvidenko, A. and Schepaschenko, D. 2015. Boreal forest health and global change. *Science*, 349(6250), 819-822. doi:10.1126/science.aaa9092

Gerdol, R., Marchesini, R., Iacumin, P. and Brancaleoni, L. 2014. Monitoring temporal trends of air pollution in an urban area using mosses and lichens as biomonitors. *Chemosphere*, 108, 388-395. doi:10.1016/j.chemosphere.2014.02.035

Gignac, L.D. 2001. Bryophytes as indicators of climate change. *The bryologist*, 104(3), 410-420. URL:https://www.jstor.org/stable/3244774

Gignac, L.D. and Beckett, P.J. 1986. The effect of smelting operations on peatlands near Sudbury, Ontario, Canada. *Canadian Journal of Botany*, 64, 1138-1147. doi:10.1139/b86-157

Giller, K.E., Witter, E. and Mcgrath, S.P. 1998. Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. *Soil biology and biochemistry*, 30(10-11), 1389-1414. doi:10.1016/S0038-0717(97)00270-8

Gillings, M.M., Fry, K.L., Morrison, A.L. and Taylor, M.P. 2022. Spatial distribution and composition of mine dispersed trace metals in residential soil and house dust:

Implications for exposure assessment and human health. *Environmental Pollution*, 293, 118462. doi:10.1016/j.envpol.2021.118462

Glina, B., Gajewski, P., Mendyk, Ł., Zawieja, B. and Kaczmarek, Z. 2019. Recent changes in soil properties and carbon stocks in fen peatlands adjacent to open-pit lignite mines. *Land Degradation & Development*, 30(18), 2371-2380. doi:10.1002/ldr.3428

Goodenough, K.M., Wall, F. and Merriman, D. 2018. The rare earth elements: demand, global resources, and challenges for resourcing future generations. *Natural Resources Research*, 27, 201-216. doi:10.1007/s11053-017-9336-5

Gorman, M.R. and Dzombak, D.A. 2018. A review of sustainable mining and resource management: Transitioning from the life cycle of the mine to the life cycle of the mineral. *Resources, Conservation and Recycling*, 137, 281-291. doi:10.1016/j.resconrec.2018.06.001

Gould, S.F. 2012. Comparison of post-mining rehabilitation with reference ecosystems in monsoonal eucalypt woodlands, northern Australia. *Restoration Ecology*, 20(2), 250-259. doi:10.1111/j.1526-100X.2010.00757.x

Grasby, S.E., Richards, B.C., Sharp, C.E., Brady, A.L., Jones, G.M. and Dunfield, P.F. 2013. The paint pots, kootenay national park, canada—a natural acid spring analogue for Mars. *Canadian Journal of Earth Sciences*, 50(1), 94-108. doi:10.1139/e2012-060

Gray, L. and He, F. 2009. Spatial point-pattern analysis for detecting density-dependent competition in a boreal chronosequence of Alberta. *Forest Ecology and Management*, 259(1), 98-106. doi:10.1016/j.foreco.2009.09.048

Guo, H., Nasir, M., Lv, J., Dai, Y. and Gao, J. 2017. Understanding the variation of microbial community in heavy metals contaminated soil using high throughput sequencing. *Ecotoxicology and Environmental Safety*, 144, 300-306. doi:10.1016/j.ecoenv.2017.06.048

Guo, Q., Li, N. and Xie, S. 2019. Heavy metal spill influences bacterial communities in freshwater sediments. *Archives of microbiology*, 201, 847-854. doi:10.1007/s00203-019-01650-y

Hart, S.A. and Chen, H.Y. 2008. Fire, logging, and overstory affect understory abundance, diversity, and composition in boreal forest. *Ecological Monographs*, 78(1), 123-140. doi:10.1890/06-2140.1

Hart, S.A. and Chen, H.Y.H. 2006. Understory vegetation dynamics of North American boreal forests. *Critical Reviews in Plant Sciences*, 25(4), 381-397. doi:10.1080/07352680600819286

Haughian, S.R. 2018. Short-term effects of alternative thinning treatments on the richness, abundance and composition of epixylic bryophytes, lichens, and vascular plants in conifer plantations at microhabitat and stand scales. *Forest Ecology and Management*, 415, 106-117. doi:10.1016/j.foreco.2018.02.019

Haughian, S.R. and Frego, K.A. 2017. Does CWD mediate microclimate for epixylic vegetation in boreal forest understories? A test of the moisture-capacitor hypothesis. *Forest Ecology and Management*, 389, 341-351. doi:10.1016/j.foreco.2017.01.011

Helmisaari, H.-S., Derome, J., Fritze, H., Nieminen, T., Palmgren, K., Salemaa, M. and Vanha-Majamaa, I. 1995. Copper in Scots pine forests around a heavy-metal smelter in south-western Finland. *Water, air, and soil pollution*, 85, 1727-1732. doi:10.1007/BF00477229

Hervé, M. 2017. RVAideMemoire: Diverse basic statistical and graphical functions, R package version 0.9-63 (2017-02-24).

Hessburg, P.F., Prichard, S.J., Hagmann, R.K., Povak, N.A. and Lake, F.K. 2021. Wildfire and climate change adaptation of western North American forests: a case for intentional management. *Ecological applications*, 31(8), e02432. doi:10.1002/eap.2432

Hirota, M., Holmgren, M., Van Nes, E.H. and Scheffer, M. 2011. Global resilience of tropical forest and savanna to critical transitions. *Science*, 334(6053), 232-235. doi:10.1126/science.1210657

Ho, N.T., Li, F., Wang, S. and Kuhn, L. 2019. metamicrobiomeR: an R package for analysis of microbiome relative abundance data using zero-inflated beta GAMLSS and meta-analysis across studies using random effects models. *Bmc Bioinformatics*, 20(1), 1-15. doi:10.1186/s12859-019-2744-2

Holl, K.D. 2002. Long-term vegetation recovery on reclaimed coal surface mines in the eastern USA. *Journal of Applied Ecology*, 39(6), 960-970. doi:10.1046/j.1365-2664.2002.00767.x

Holland-Moritz, H., Stuart, J.E.M., Lewis, L.R., Miller, S.N., Mack, M.C., Ponciano, J.M., McDaniel, S.F. and Fierer, N. 2021. The bacterial communities of Alaskan mosses and their contributions to N_2-fixation. *Microbiome*, 9, 53. doi:10.1186/s40168-021-01001-4

Holland-Moritz, H., Stuart, J., Lewis, L.R., Miller, S., Mack, M.C., McDaniel, S.F. and Fierer, N. 2018. Novel bacterial lineages associated with boreal moss species. *Environmental microbiology*, 20(7), 2625-2638. doi:10.1111/1462-2920.14288

Horodecki, P. and Jagodziński, A.M. 2017. Tree species effects on litter decomposition in pure stands on afforested post-mining sites. *Forest Ecology and Management*, 406, 1-11. doi:10.1016/j.foreco.2017.09.059

Horstkotte, T. and Moen, J. 2019. Successional pathways of terrestrial lichens in changing Swedish boreal forests. *Forest Ecology and Management*, 453, 117572. doi:10.1016/j.foreco.2019.117572

Howson, T., Chapman, P.J., Shah, N., Anderson, R. and Holden, J. 2021. The effect of forest-to-bog restoration on the hydrological functioning of raised and blanket bogs. *Ecohydrology*, 14(7), e2334. doi:10.1002/eco.2334

Hoyo, Y. and Tsuyuzaki, S. 2014. Habitat differentiation between *Drosera anglica* and *D. rotundifolia* in a post-mined peatland, Northern Japan. *Wetlands*, 34, 943-953. doi:10.1007/s13157-014-0555-9

Hu, L.t. and Bentler, P.M. 1999. Cutoff criteria for fit indexes in covariance structure analysis: Conventional criteria versus new alternatives. *Structural equation modeling: a multidisciplinary journal*, 6(1), 1-55. doi:10.1080/10705519909540118

Iguchi, H., Sato, I., Sakakibara, M., Yurimoto, H. and Sakai, Y. 2012. Distribution of methanotrophs in the phyllosphere. *Bioscience, Biotechnology, and Biochemistry*, 76(8), 1580-1583. doi:10.1271/bbb.120281

Jactel, H., Bauhus, J., Boberg, J., Bonal, D., Castagneyrol, B., Gardiner, B., Gonzalez-Olabarria, J.R., Koricheva, J., Meurisse, N. and Brockerhoff, E.G. 2017. Tree diversity drives forest stand resistance to natural disturbances. *Current Forestry Reports*, 3(3), 223-243. doi:10.1007/s40725-017-0064-1

Jakobsson, S., Bernes, C., Bullock, J.M., Verheyen, K. and Lindborg, R. 2018. How does roadside vegetation management affect the diversity of vascular plants and invertebrates? A systematic review. *Environmental Evidence*, 7(1), 1-14. doi:10.1186/s13750-018-0129-z

Jean, M., Alexander, H.D., Mack, M.C. and Johnstone, J.F. 2017. Patterns of bryophyte succession in a 160-year chronosequence in deciduous and coniferous forests of boreal Alaska. *Canadian Journal of Forest Research*, 47(8), 1021-1032. doi:10.1139/cjfr-2017-0013

Jean, M., Fenton, N.J., Bergeron, Y. and Nilsson, M.-C. 2021. *Sphagnum* and feather moss-associated N_2 fixation along a 724-year chronosequence in eastern boreal Canada. *Plant Ecology*, 222, 1007-1022. doi:10.1007/s11258-021-01157-x

Jean, M., Holland-Moritz, H., Melvin, A.M., Johnstone, J.F. and Mack, M.C. 2020a. Experimental assessment of tree canopy and leaf litter controls on the microbiome and nitrogen fixation rates of two boreal mosses. *New Phytologist*, 227(5), 1335-1349. doi:10.1111/nph.16611

Jean, M., Melvin, A.M., Mack, M.C. and Johnstone, J.F. 2020b. Broadleaf litter controls feather moss growth in black spruce and birch forests of interior Alaska. *Ecosystems*, 23(1), 18-33. doi:10.1007/s10021-019-00384-8

Ji, M., Greening, C., Vanwonterghem, I., Carere, C.R., Bay, S.K., Steen, J.A., Montgomery, K., Lines, T., Beardall, J., van Dorst, J., Snape, I., Stott, M.B., Hugenholtz, P. and Ferrari, B.C. 2017. Atmospheric trace gases support primary production in Antarctic desert surface soil. *Nature*, 552, 400–403. doi:10.1038/nature25014

Jo, I., Fridley, J.D. and Frank, D.A. 2015. Linking above-and belowground resource use strategies for native and invasive species of temperate deciduous forests. *Biological Invasions*, 17, 1545-1554. doi:10.1007/s10530-014-0814-y

Jönsson, M.T. and Jonsson, B.G. 2007. Assessing coarse woody debris in Swedish woodland key habitats: Implications for conservation and management. *Forest Ecology and Management*, 242(2-3), 363-373. doi:10.1016/j.foreco.2007.01.054

Kane, J.L., Morrissey, E.M., Skousen, J.G. and Freedman, Z.B. 2020. Soil microbial succession following surface mining is governed primarily by deterministic factors. *FEMS Microbiology Ecology*, 96(11), fiaa114. doi:10.1093/femsec/fiaa114

Karimi, B., Maron, P.A., Chemidlin-Prevost Boure, N., Bernard, N., Gilbert, D. and Ranjard, L. 2017. Microbial diversity and ecological networks as indicators of environmental quality. *Environmental Chemistry Letters*, 15, 265-281. doi:10.1007/s10311-017-0614-6

Kariuki, M., Kooyman, R.M., Smith, R.G.B., Wardell-Johnson, G. and Vanclay, J.K. 2006. Regeneration changes in tree species abundance, diversity and structure in logged and unlogged subtropical rainforest over a 36-year period. *Forest Ecology and Management*, 236(2-3), 162-176. doi:10.1016/j.foreco.2006.09.021

Kartikeya B. 2017. Package ‘stats’, R package version 3.5.0 (2017-10-3).

Kavehei, A., Gore, D.B., Chariton, A.A. and Hose, G.C. 2021. Impact assessment of ephemeral discharge of contamination downstream of two legacy base metal mines using environmental DNA. *Journal of Hazardous Materials*, 419, 126483. doi:10.1016/j.jhazmat.2021.126483

Kembel, S.W. and Dale, M.R. 2006. Within-stand spatial structure and relation of boreal canopy and understorey vegetation. *Journal of Vegetation Science*, 17(6), 783-790. doi:10.1111/j.1654-1103.2006.tb02501.x

Kembel, S.W., O’Connor, T.K., Arnold, H.K., Hubbell, S.P., Wright, S.J. and Green, J.L. 2014. Relationships between phyllosphere bacterial communities and plant functional traits in a neotropical forest. *Proceedings of the National Academy of Sciences*, 111(38), 13715-13720. doi:10.1073/pnas.1216057111

Kelly, J.J., Häggblom, M.M. and Tate, R.L., 2003. Effects of heavy metal contamination and remediation on soil microbial communities in the vicinity of a zinc smelter as indicated by analysis of microbial community phospholipid fatty acid profiles. *Biology and Fertility of Soils*, 38(2): 65-71. doi: 10.1007/s00374-003-0642-1

Király, I. and Ódor, P. 2010. The effect of stand structure and tree species composition on epiphytic bryophytes in mixed deciduous–coniferous forests of Western Hungary. *Biological Conservation*, 143(9), 2063-2069. doi:10.1016/j.biocon.2010.05.014

Kirk, D.A., Hebert, K. and Goldsmith, F.B. 2019. Grazing effects on woody and herbaceous plant biodiversity on a limestone mountain in northern Tunisia. *PeerJ*, 7, e7296. doi:10.7717/peerj.7296

Kłos, A., Ziembik, Z., Rajfur, M., Dołhańczuk-Śródka, A., Bochenek, Z., Bjerke, J.W., Tømmervik, H., Zagajewski, B., Ziółkowski, D., Jerz, D., Zielińska, M., Krems, P., Godyń, P., Marciniak, M., Świsłowski, P. 2018. Using moss and lichens in biomonitoring of heavy-metal contamination of forest areas in southern and north-eastern Poland. *Science of the Total Environment*, 627, 438-449. doi:10.1016/j.scitotenv.2018.01.211

Koljalg, U., Larsson, K.H., Abarenkov, K., Nilsson, R.H., Alexander, I.J., Eberhardt, U., Erland, S., Hoiland, K., Kjoller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A.F., Tedersoo, L., Vralstad, T. and Ursing, B.M. 2005. UNITE: a database providing web-based methods for the molecular identification of ectomycorrhizal fungi. *New Phytologist*, 166(3), 1063-1068. doi:10.1111/j.1469-8137.2005.01376.x

Korzeniowska, J. 2021. *Polytrichum formosum* and *Vaccinium myrtillus* as Phytoindicators of Pollutants from Long-Range Emissions of Environmentally Important Protected Areas (The Tatra National Park, the Central Western Carpathians, Poland). doi: 10.5772/intechopen.99962

Kumar, P., Chen, H.Y., Searle, E.B. and Shahi, C. 2018. Dynamics of understorey biomass, production and turnover associated with long-term overstorey succession in boreal forest of Canada. *Forest Ecology and Management*, 427, 152-161. doi:10.1016/j.foreco.2018.05.066

Labay, K.B., Bellora, M., Schulz, J., DeYoung, K., RR, I., Mauk, D. and JL San Juan, C. 2017. Global Distribution of Selected Mines, Deposits, and Districts of Critical Minerals. doi:10.5066/F7GH9GQR

Lachance, D., Lavoie, C. and Desrochers, A. 2005. The impact of peatland afforestation on plant and bird diversity in southeastern Québec. *Ecoscience*, 12(2), 161-171. doi:10.2980/i1195-6860-12-2-161.1

Lajoie, G., Maglione, R. and Kembel, S.W. 2020. Adaptive matching between phyllosphere bacteria and their tree hosts in a neotropical forest. *Microbiome*, 8, 70. doi:10.1186/s40168-020-00844-7

Lajoie, G. and Kembel, S.W., 2021. Host neighborhood shapes bacterial community assembly and specialization on tree species across a latitudinal gradient. *Ecological Monographs*, 91(2), e01443. doi:10.1002/ecm.1443

Langsrud, Ø. 2003. ANOVA for unbalanced data: Use Type II instead of Type III sums of squares. *Statistics and Computing*, 13, 163-167. doi:10.1023/A:1023260610025

Lavoie, C. 2010. Should we care about purple loosestrife? The history of an invasive plant in North America. *Biological Invasions*, 12, 1967-1999. doi:10.1007/s10530-009-9600-7

Lavoie, M., Paré, D. and Bergeron, Y. 2005. Impact of global change and forest management on carbon sequestration in northern forested peatlands. *Environmental Reviews*, 13(4), 199-240. doi:10.1139/a05-014

Légaré, S., Paré, D. and Bergeron, Y. 2005. Influence of aspen on forest floor properties in black spruce-dominated stands. *Plant Soil*, 275, 207-220. doi:10.1007/s11104-005-1482-6

Legendre, P., Borcard, D. and Peres-Neto, P.R. 2005. Analyzing beta diversity: partitioning the spatial variation of community composition data. *Ecological monographs*, 75(4), 435-450. doi:10.1890/05-0549

Lemke, D., Schweitzer, C., Tazisong, I., Wang, Y. and Brown, J. 2013. Invasion of a mined landscape: what habitat characteristics are influencing the occurrence of invasive plants? *International Journal of Mining, Reclamation and Environment*, 27(4), 275-293. doi:10.1080/17480930.2012.699215

Lenth, R., Singmann, H., Love, J., Buerkner, P. and Herve, M. 2019. Package 'emmeans', R package version 1.3.5.1 (2019-06-26).

Leveau, J.H. 2019. A brief from the leaf: latest research to inform our understanding of the phyllosphere microbiome. *Current Opinion in Microbiology*, 49, 41-49. doi:10.1016/j.mib.2019.10.002

Li, L., Liu, Z., Zhang, M., Meng, D., Liu, X., Wang, P., Li, X., Jiang, Z., Zhong, S. and Jiang, C. 2020. Insights into the metabolism and evolution of the genus *Acidiphilium*, a typical acidophile in acid mine drainage. *Msystems*, 5(6), e00867-00820. doi:10.1128/mSystems.00867-20

Li, X., Meng, D., Li, J., Yin, H., Liu, H., Liu, X., Cheng, C., Xiao, Y., Liu, Z. and Yan, M. 2017. Response of soil microbial communities and microbial interactions to long-term heavy metal contamination. *Environmental Pollution*, 231, 908-917. doi:10.1016/j.envpol.2017.08.057

Li, Z., Ma, Z., van der Kuijp, T.J., Yuan, Z. and Huang, L. 2014. A review of soil heavy metal pollution from mines in China: pollution and health risk assessment. *Science of The Total Environment*, 468, 843-853. doi:10.1016/j.scitotenv.2013.08.090

Limpitlaw, D. 2004. Mine closure as a framework for sustainable development, paper presented at a Sustainable Development Practices on Mine Sites Conference, University of the Witwatersrand, Johannesburg 8-10 March.

Łuców, D., Lamentowicz, M., Obremska, M., Arkhipova, M., Kittel, P., Łokas, E., Mazurkevich, A., Mróz, T., Tjallingii, R. and Słowiński, M. 2020. Disturbance and resilience of a *Sphagnum* peatland in western Russia (Western Dvina Lakeland) during the last 300 years: A multiproxy, high-resolution study. *The Holocene*, 30(11), 1552-1566. doi:10.1177/0959683620941064

Lüdecke, D. and Lüdecke, M.D. 2019. Package 'sjstats', R package version 0.17.3 (2019-01-04).

Macdonald, C.A., Clark, I.M., Zhao, F.-J., Hirsch, P.R., Singh, B.K. and McGrath, S.P. 2011. Long-term impacts of zinc and copper enriched sewage sludge additions on bacterial, archaeal and fungal communities in arable and grassland soils. *Soil Biology and Biochemistry*, 43(5), 932-941. doi:10.1016/j.soilbio.2011.01.004

Mack, M.C., Walker, X.J., Johnstone, J.F., Alexander, H.D., Melvin, A.M., Jean, M. and Miller, S.N. 2021. Carbon loss from boreal forest wildfires offset by increased dominance of deciduous trees. *Science*, 372(6539), 280-283. doi:10.1126/science.abf3903

Mahapatra, B., Dhal, N.K., Dash, A.K., Panda, B.P., Panigrahi, K.C.S. and Pradhan, A. 2019. Perspective of mitigating atmospheric heavy metal pollution: using mosses as biomonitoring and indicator organism. *Environmental Science and Pollution Research*,26, 29620-29638. doi:10.1007/s11356-019-06270-z

Maleki, M., Ghorbanpour, M. and Kariman, K., 2017. Physiological and antioxidative responses of medicinal plants exposed to heavy metals stress. *Plant Gene*, 11, 247-254. doi: 10.1016/j.plgene.2017.04.006

Mancuso, C.P., Lee, H., Abreu, C.I., Gore, J. and Khalil, A.S. 2021. Environmental fluctuations reshape an unexpected diversity-disturbance relationship in a microbial community. *Elife*, 10, e67175. doi:10.7554/eLife.67175

Márialigeti, S., Németh, B., Tinya, F. and Ódor, P. 2009. The effects of stand structure on ground-floor bryophyte assemblages in temperate mixed forests. *Biodiversity and Conservation*, 18, 2223-2241. doi: 10.1007/s10531-009-9586-6

Martineau, C., Beguin, J., Séguin, A. and Paré, D. 2020. Cumulative Effects of Disturbances on Soil Nutrients: Predominance of Antagonistic Short-Term Responses to the Salvage Logging of Insect-Killed Stands. *Ecosystems*, 23, 812-827. doi:10.1007/s10021-019-00432-3

Martins, W.B.R., Lima, M.D.R., Junior, U.d.O.B., Amorim, L.S.V.-B., de Assis Oliveira, F. and Schwartz, G. 2020. Ecological methods and indicators for recovering and monitoring ecosystems after mining: A global literature review. *Ecological Engineering*, 145, 105707. doi:10.1016/j.ecoleng.2019.105707

Mathakutha, R., Steyn, C., le Roux, P.C., Blom, I.J., Chown, S.L., Daru, B.H., Ripley, B.S., Louw, A. and Greve, M. 2019. Invasive species differ in key functional traits from native and non-invasive alien plant species. *Journal of Vegetation Science*, 30(5), 994-1006. doi:10.1111/jvs.12772

Mattielli, N., Petit, J.C., Deboudt, K., Flament, P., Perdrix, E., Taillez, A., Rimetz-Planchon, J. and Weis, D. 2009. Zn isotope study of atmospheric emissions and dry depositions within a 5 km radius of a Pb–Zn refinery. *Atmospheric Environment*, 43(6), 1265-1272. doi:10.1016/j.atmosenv.2008.11.030

Maus, V., Giljum, S., Gutschlhofer, J., da Silva, D.M., Probst, M., Gass, S.L., Luckeneder, S., Lieber, M. and McCallum, I. 2020. A global-scale data set of mining areas. *Scientific data*, 7(1), 1-13. doi:10.1038/s41597-020-00624-w

Maynard, D., Paré, D., Thiffault, E., Lafleur, B., Hogg, K. and Kishchuk, B. 2014. How do natural disturbances and human activities affect soils and tree nutrition and growth in the Canadian boreal forest? *Environmental Reviews*, 22(2), 161-178. doi:10.1139/er-2013-0057

McMahon, G. and Moreira, S. 2014. The Contribution of the Mining Sector to Socioeconomic and Human Development. Extractive industries for development series; no. 30. World Bank, Washington, DC. © World Bank. https://openknowledge.worldbank.org/handle/10986/18660 License: CC BY 3.0 IGO.

McMurdie, P.J. and Holmes, S. 2013. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, 8(4), e61217. doi:10.1371/journal.pone.0061217

Mekonnen, Z.A., Riley, W.J., Randerson, J.T., Grant, R.F. and Rogers, B.M. 2019. Expansion of high-latitude deciduous forests driven by interactions between climate warming and fire. *Nature plants*, 5, 952-958. doi:10.1038/s41477-019-0495-8

Menkis, A., Burokienė, D., Gaitnieks, T., Uotila, A., Johannesson, H., Rosling, A., Finlay, R.D., Stenlid, J. and Vasaitis, R. 2012. Occurrence and impact of the root-rot biocontrol agent *Phlebiopsis gigantea* on soil fungal communities in *Picea abies* forests of northern Europe. *FEMS microbiology ecology*, 81(2), 438-445. doi:10.1111/j.1574-6941.2012.01366.x

Metzger, K., Coughenour, M., Reich, R. and Boone, R. 2005. Effects of seasonal grazing on plant species diversity and vegetation structure in a semi-arid ecosystem. *Journal of Arid Environments*, 61(1), 147-160. doi: 10.1016/j.jaridenv.2004.07.019

Meyer, K.M., Porch, R., Muscettola, I.E., Vasconcelos, A.L.S., Sherman, J.K., Metcalf, C.J.E., Lindow, S.E. and Koskella, B., 2022. Plant neighborhood shapes diversity and reduces interspecific variation of the phyllosphere microbiome. *The ISME Journal*, 16(5), 1376-1387. doi: 10.1038/s41396-021-01184-6.

Mijnendonckx, K., Provoost, A., Ott, C., Venkateswaran, K., Mahillon, J., Leys, N. and Van Houdt, R. 2013. Characterization of the survival ability of *Cupriavidus metallidurans* and *Ralstonia pickettii* from space-related environments. Microbial ecology 65(2), 347-360. doi:10.1007/s00248-012-0139-2

Mullan-Boudreau, G., Belland, R., Devito, K., Noernberg, T., Pelletier, R. and Shotyk, W. 2017. *Sphagnum* moss as an indicator of contemporary rates of atmospheric dust deposition in the Athabasca Bituminous Sands region. *Environmental Science & Technology*, 51(13), 7422-7431. doi:10.1021/acs.est.6b06195

Musetta-Lambert, J. L., Enanga, E. M., Teichert, S., Creed, I. F., Kidd, K. A., Kreutzweiser, D. P., and Sibley, P. K. 2019. Industrial innovation and infrastructure as drivers of change in the Canadian boreal zone. *Environmental Reviews*, *27*(3), 275-294. doi:10.1139/er-2018-0056

Murguia, D.I., Bringezu, S. and Schaldach, R. 2016. Global direct pressures on biodiversity by large-scale metal mining: Spatial distribution and implications for conservation. *Journal of Environmental Management*, 180, 409-420. doi:10.1016/j.jenvman.2016.05.040

Näsholm, T., Ekblad, A., Nordin, A., Giesler, R., Högberg, M. and Högberg, P. 1998. Boreal forest plants take up organic nitrogen. *Nature*, 392, 914-916. doi:10.1038/31921

Newmaster, S.G., Belland, R.J., Arsenault, A., Vitt, D.H. and Stephens, T.R. 2005. The ones we left behind: comparing plot sampling and floristic habitat sampling for estimating bryophyte diversity. *Diversity and distributions*, 11(1), 57-72. doi:10.1111/j.1366-9516.2005.00123.x

Nguyen, T., Yu, X., Zhang, Z., Liu, M. and Liu, X. 2015. Relationship between types of urban forest and $PM_{2.5}$ capture at three growth stages of leaves. *Journal of Environmental Sciences*, 27, 33-41. doi:10.1016/j.jes.2014.04.019

Nilsson, M.-C. and Wardle, D.A. 2005. Understory vegetation as a forest ecosystem driver: evidence from the northern Swedish boreal forest. *Frontiers in Ecology and the Environment*, 3(8), 421-428. doi:10.1890/1540-9295(2005)003[0421:UVAAFE]2.0.CO;2

Norgate, T. and Haque, N. 2010. Energy and greenhouse gas impacts of mining and mineral processing operations. *Journal of Cleaner Production*, 18(3), 266-274. doi:10.1016/j.jclepro.2009.09.020

Norman, M.A., Koch, J.M., Grant, C.D., Morald, T.K. and Ward, S.C. 2006. Vegetation succession after bauxite mining in Western Australia. *Restoration Ecology*, 14(2), 278-288. doi:10.1111/j.1526-100X.2006.00130.x

Ochieng, G.M., Seanego, E.S. and Nkwonta, O.I. 2010. Impacts of mining on water resources in South Africa: A review. *Scientific Research and Essays*, 5(22), 3351-3357. doi:10.5897/SRE.9000572

Odell, S.D., Bebbington, A. and Frey, K.E. 2018. Mining and climate change: A review and framework for analysis. *The extractive industries and society*, 5(1), 201-214. doi:10.1016/j.exis.2017.12.004

Oechel, W. and Van Cleve, K. 1986. The role of bryophytes in nutrient cycling in the taiga. Forest ecosystems in the Alaskan taiga, 121-137. Springer, New York, NY. doi:10.1007/978-1-4612-4902-3_9

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R., Simpson, G.L., Solymos, P., Stevens, M.H.H. and Wagner, H. 2013. Package 'vegan', R package version 2.0-7 (2013-03-19).

Oldén, A., Ovaskainen, O., Kotiaho, J.S., Laaka-Lindberg, S. and Halme, P. 2014. Bryophyte species richness on retention aspens recovers in time but community structure does not. *PLoS One*, 9(4), e93786. doi:10.1371/journal.pone.0093786

Onianwa, P. 2001. Monitoring atmospheric metal pollution: a review of the use of mosses as indicators. *Environmental Monitoring and Assessment*, 71, 13-50. doi:10.1023/A:1011660727479

Page, S.E. and Baird, A. 2016. Peatlands and global change: response and resilience. *Annual Review of Environment and Resources*, 41, 35-57. doi:10.1146/annurev-environ-110615-085520

Pakarinen, A., Fritze, H., Timonen, S., Kivijärvi, P. and Velmala, S. 2021. Boreal soil microbial diversity and seed onion mycorrhizal colonization is unaffected by preceding one season crop cultivation. *European Journal of Soil Biology*, 105, 103335. doi:10.1016/j.ejsobi.2021.103335

Paquette, M., Boudreault, C., Fenton, N., Pothier, D. and Bergeron, Y. 2016. Bryophyte species assemblages in fire and clear-cut origin boreal forests. *Forest Ecology and Management*, 359, 99-108. doi:10.1016/j.foreco.2015.09.031

Parada, A.E., Needham, D.M. and Fuhrman, J.A. 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. *Environmental microbiology*, 18(5), 1403-1414. doi:10.1111/1462-2920.13023

Parody, J.M., Cuthbert, F.J. and Decker, E.H. 2001. The effect of 50 years of landscape change on species richness and community composition. Global Ecology and Biogeography 10(3), 305-313. doi:

Patra, A.K., Gautam, S. and Kumar, P. 2016. Emissions and human health impact of particulate matter from surface mining operation—A review. *Environmental Technology & Innovation*, 5, 233-249. doi:10.1016/j.eti.2016.04.002

Pawlowski, J., Bonin, A., Boyer, F., Cordier, T. and Taberlet, P. 2021. Environmental DNA for biomonitoring. *Molecular Ecology*, 30(13), 2931. doi:10.1111/mec.16023

Peckham, S.D., Ahl, D.E. and Gower, S.T. 2009. Bryophyte cover estimation in a boreal black spruce forest using airborne lidar and multispectral sensors. *Remote Sensing of Environment*, 113(6), 1127-1132. doi:10.1016/j.rse.2009.02.008

Peng, C., Ma, Z., Lei, X., Zhu, Q., Chen, H., Wang, W., Liu, S., Li, W., Fang, X. and Zhou, X. 2011. A drought-induced pervasive increase in tree mortality across Canada's boreal forests. *Nature climate change*, 1, 467-471. doi:10.1038/nclimate1293

Peng, X., Li, G. and Liu, Z. 2016. Zero-inflated beta regression for differential abundance analysis with metagenomics data. *Journal of Computational Biology*, 23(2), 102-110. doi:10.1089/cmb.2015.0157

Peñuelas, J. and Terradas, J. 2014. The foliar microbiome. *Trends in plant science*, 19(5), 278-280. doi:10.1016/j.tplants.2013.12.007

Perini, L., Gostinčar, C. and Gunde-Cimerman, N. 2019. Fungal and bacterial diversity of Svalbard subglacial ice. *Scientific Reports*, 9, 20230. doi:10.1038/s41598-019-56290-5

Perreault, R. and Laforest-Lapointe, I. 2022. Plant-microbe interactions in the phyllosphere: facing challenges of the anthropocene. *ISME J*, 16, 339–345. doi:10.1038/s41396-021-01109-3

Petschinger, K., Adlassnig, W., Sabovljevic, M.S. and Lang, I. 2021. Lamina cell shape and cell wall thickness are useful indicators for metal tolerance—An example in bryophytes. *Plants*, 10(2), 274. doi:10.3390/plants10020274

Pollastrini, M., Nogales, A.G., Benavides, R., Bonal, D., Finer, L., Fotelli, M., Gessler, A., Grossiord, C., Radoglou, K. and Strasser, R.J. 2017. Tree diversity affects chlorophyll a fluorescence and other leaf traits of tree species in a boreal forest. *Tree physiology*, 37(2), 199-208. doi:10.1093/treephys/tpw132

Printarakul, N. and Meeinkuirt, W., 2022. The bryophyte community as bioindicator of heavy metals in a waterfall outflow. *Scientific reports*, 12, 6942. Doi: 10.1038/s41598-022-10980-9

Punia, A. 2021. Role of temperature, wind, and precipitation in heavy metal contamination at copper mines: a review. Environ. *Environmental Science and Pollution Research*, 28, 4056-4072. doi:10.1007/s11356-020-11580-8

Putkinen, A., Larmola, T., Tuomivirta, T., Siljanen, H.M., Bodrossy, L., Tuittila, E.-S. and Fritze, H. 2012. Water dispersal of methanotrophic bacteria maintains functional methane oxidation in *Sphagnum* mosses. *Frontiers in Microbiology*, 3, article 15. doi:10.3389/fmicb.2012.00015

Qian, H., Klinka, K., Økland, R.H., Krestov, P. and Kayahara, G.J. 2003. Understorey vegetation in boreal *Picea mariana* and *Populus tremuloides* stands in British Columbia. *Journal of Vegetation Science*, 14(2), 173-184. doi:10.1111/j.1654-1103.2003.tb02142.x

Qu, Z.L., Liu, B., Ma, Y., Xu, J. and Sun, H. 2020. The response of the soil bacterial community and function to forest succession caused by forest disease. *Functional Ecology*, 34(12), 2548-2559. doi:10.1111/1365-2435.13665

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. and Glockner, F.O. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, 41(D1), D590-596. doi:10.1093/nar/gks1219

Raiter, K.G., Possingham, H.P., Prober, S.M. and Hobbs, R.J. 2014. Under the radar: mitigating enigmatic ecological impacts. *Trends in Ecology & Evolution*, 29(11), 635-644. doi:10.1016/j.tree.2014.09.003

Ratcliffe, J.L., Creevy, A., Andersen, R., Zarov, E., Gaffney, P.P., Taggart, M.A., Mazei, Y., Tsyganov, A.N., Rowson, J.G. and Lapshina, E.D. 2017. Ecological and environmental transition across the forested-to-open bog ecotone in a west Siberian peatland. *Science of The Total Environment*, 607-608, 816-828. doi:10.1016/j.scitotenv.2017.06.276

Reith, F., Etschmann, B., Grosse, C., Moors, H., Benotmane, M.A., Monsieurs, P., Grass, G., Doonan, C., Vogt, S. and Lai, B. 2009. Mechanisms of gold biomineralization in the bacterium *Cupriavidus metallidurans*. *Proceedings of the National Academy of Sciences*, 106(42), 17757-17762. doi:10.1073/pnas.09045831

Rodríguez-Rodríguez, J.C., Bergeron, Y., Kembel, S.W. and Fenton, N.J. 2022. Dominance of coniferous and broadleaved trees drives bacterial associations with boreal feather mosses. *Environmental Microbiology*, Early View. doi:10.1111/1462-2920.16013

Rosseel, Y. 2012. Lavaan: An R package for structural equation modeling. *Journal of Statistical Software*, 48(2), 1-36. doi:10.18637/jss.v048.i02

Rousk, K., Jones, D.L. and DeLuca, T.H. 2013. Moss-cyanobacteria associations as biogenic sources of nitrogen in boreal forest ecosystems. *Frontiers in Microbiology*, 4, 150. doi:10.3389/fmicb.2013.00150

Royo, A.A. and Carson, W.P. 2006. On the formation of dense understory layers in forests worldwide: consequences and implications for forest dynamics, biodiversity, and succession. *Canadian Journal of Forest Research*, 36(6), 1345-1362. doi:10.1139/x06-025

Rydgren, K., Økland, R.H. and Hestmark, G. 2004. Disturbance severity and community resilience in a boreal forest. *Ecology*, 85(7), 1906-1915. doi:10.1890/03-0276

Rydin, H. and Jeglum, J.K. 2013. The Biology of Peatlands, Oxford University Press.

Saetre, P., Saetre, L.S., Brandtberg, P.-O., Lundkvist, H. and Bengtsson, J. 1997. Ground vegetation composition and heterogeneity in pure Norway spruce and mixed Norway spruce-birch stands. *Canadian Journal of Forest Research*, 27(12), 2034-2042. doi:10.1139/x97-177

Saleem, M., Meckes, N., Pervaiz, Z.H. and Traw, M.B. 2017. Microbial Interactions in the Phyllosphere Increase Plant Performance under Herbivore Biotic Stress. *Frontiers in Microbiology*, 8, 41. doi:10.3389/fmicb.2017.00041

Salemaa, M., Derome, J., Helmisaari, H.S., Nieminen, T. and Vanha-Majamaa, I. 2004. Element accumulation in boreal bryophytes, lichens and vascular plants exposed to heavy metal and sulfur deposition in Finland. *Science of The Total Environment*, 324(1-3), 141-160. doi:10.1016/j.scitotenv.2003.10.025

Salemaa, M., Vanha-Majamaa, I. and Derome, J. 2001. Understorey vegetation along a heavy-metal pollution gradient in SW Finland. *Environmental Pollution*, 112(3), 339-350. doi:10.1016/S0269-7491(00)00150-0

Sánchez-Pinillos, M., Leduc, A., Ameztegui, A., Kneeshaw, D., Lloret, F. and Coll, L. 2019. Resistance, resilience or change: post-disturbance dynamics of boreal forests after insect outbreaks. *Ecosystems*, 22, 1886-1901. doi:10.1007/s10021-019-00378-6

Sase, H., Matsuda, K., Visaratana, T., Garivait, H., Yamashita, N., Kietvuttinon, B., Hongthong, B., Luangjame, J., Khummongkol, P. and Shindo, J. 2012. Deposition process of sulfate and elemental carbon in Japanese and Thai forests. *Asian Journal of Atmospheric Environment*, 6(4), 246-258. doi:10.5572/ajae.2012.6.4.246

Scheffers, B.R., Edwards, D.P., Diesmos, A., Williams, S.E. and Evans, T.A. 2014. Microhabitats reduce animal's exposure to climate extremes. *Global change biology*, 20(2), 495-503. doi:10.1111/gcb.12439

Schumacher, E., Kueffer, C., Edwards, P.J. and Dietz, H. 2009. Influence of light and nutrient conditions on seedling growth of native and invasive trees in the Seychelles. *Biological Invasions*, 11(8), 1941-1954. doi:10.1007/s10530-008-9371-6

Seddon, N., Mace, G.M., Naeem, S., Tobias, J.A., Pigot, A.L., Cavanagh, R., Mouillot, D., Vause, J. and Walpole, M. 2016. Biodiversity in the Anthropocene: prospects and policy. *Proceedings of the Royal Society B: Biological Sciences*, 283(1844), 20162094. doi:10.1098/rspb.2016.2094

Sharma SK, Ramesh A, Sharma MP, Joshi OP, Govaerts B, Steenwerth KL, Karlcn DL. 2010. Microbial community structure and diversity as indicators for evaluating soil quality. In: Lichtfouse E, ed. *Biodiversity, biofuels, agroforestry and conservation agriculture*. Dordrecht: Springer. 317-358

Sivakumar, N., Sathishkumar, R., Selvakumar, G., Shyamkumar, R. and Arjunekumar, K., 2020. Phyllospheric microbiomes: diversity, ecological significance, and biotechnological applications. *Plant microbiomes for sustainable agriculture*, 25: 113-172. doi: 10.1007/978-3-030-38453-1_5

Sonter, L.J., Ali, S.H. and Watson, J.E.M. 2018. Mining and biodiversity: key issues and research needs in conservation science. *Proceedings of the Royal Society B*, 285, 20181926. doi:10.1098/rspb.2018.1926

Sonter, L.J., Dade, M.C., Watson, J.E.M. and Valenta, R.K. 2020. Renewable energy production will exacerbate mining threats to biodiversity. *Nature Communications*, 11(1), 4174. doi:10.1038/s41467-020-17928-5

Spratt, A.K., and Weider, R.K.. 1988. Growth responses and iron uptake in *Sphagnum* plants and their relation to acid mine drainage. In: Vol. 1: *Mine water and mine waste. Information Circ. IC-9183*. Proceedings of the International Land Reclamation and Mine Drainage Conference and the Third International Conference on the Abatement of Acidic Drainage, Pittsburgh, PA. 19–21 Apr. 1988. US Dep. of Interior, Bureau of Mines, Washington, DC. p. 279– 286.

Stanković, J.D., Sabovljević, A.D. and Sabovljević, M.S. 2018. Bryophytes and heavy metals: a review. *Acta Botanica Croatica*, 77(2), 109-118. doi:10.2478/botcro-2018-0014

Stewart, K. J., & Mallik, A. U. 2006. Bryophyte responses to microclimatic edge effects across riparian buffers. *Ecological applications*, 16(4), 1474-1486. doi: 10.1890/1051-0761(2006)016[1474:BRTMEE]2.0.CO;2

Steiger, J.H. 2007. Understanding the limitations of global fit assessment in structural equation modeling. *Personality and Individual differences*, 42(5), 893-898. doi:10.1016/j.paid.2006.09.017

Street, L.E., Subke, J.A., Sommerkorn, M., Sloan, V., Ducrotoy, H., Phoenix, G.K. and Williams, M., 2013. The role of mosses in carbon uptake and partitioning in arctic vegetation. *New Phytologist*, 199(1): 163-175. doi: 10.1111/nph.12285.

Stone, B.W.G., Weingarten, E.A. and Jackson, C.R. 2018. The Role of the Phyllosphere Microbiome in Plant Health and Function. *Annual Plant Reviews*, 1(2), 533-556. doi:10.1002/9781119312994.apr0614

Storch, D., Šímová, I., Smyčka, J., Bohdalková, E., Toszogyova, A. and Okie, J.G. 2021. Biodiversity dynamics in the Anthropocene: how human activities change equilibria of species richness. *Ecography*, 2022: e05778. doi:10.1111/ecog.05778

Strojan, C.L. 1978. Forest leaf litter decomposition in the vicinity of a zinc smelter. *Oecologia*, 32, 203-212. doi:10.1007/BF00366072

Strömgren, M. and Mjöfors, K. 2012. Soil-CO_2 flux after patch scarification, harrowing and stump harvest in a hemi-boreal forest. *Scandinavian Journal of Forest Research*, 27(8), 754-761. doi:10.1080/02827581.2012.723741

Su, X., Wang, M., Huang, Z., Fu, S. and Chen, H.Y. 2019. Forest understorey vegetation: Colonization and the availability and heterogeneity of resources. *Forests*, 10(11), 944. doi:10.3390/f10110944

Szczepaniak, K. and Biziuk, M. 2003. Aspects of the biomonitoring studies using mosses and lichens as indicators of metal pollution. *Environmental research*, 93(3), 221-230. doi:10.1016/S0013-9351(03)00141-5

Tang, S., Zheng, C., Chen, M., Du, W. and Xu, X. 2020. Geobiochemistry characteristics of rare earth elements in soil and ground water: a case study in Baotou, China. *Scientific Reports*, 10, 11740. doi:10.1038/s41598-020-68661-4

Thavamani, P., Samkumar, R.A., Satheesh, V., Subashchandrabose, S.R., Ramadass, K., Naidu, R., Venkateswarlu, K. and Megharaj, M. 2017. Microbes from mined sites: Harnessing their potential for reclamation of derelict mine sites. *Environmental Pollution*, 230, 495-505. doi:10.1016/j.envpol.2017.06.056

Tinya, F., Márialigeti, S., Király, I., Németh, B. and Odor, P. 2009. The effect of light conditions on herbs, bryophytes and seedlings of temperate mixed forests in Őrség, Western Hungary. *Plant Ecology*, 204, 69-81. doi:10.1007/s11258-008-9566-z

Tolvanen, A., Eilu, P., Juutinen, A., Kangas, K., Kivinen, M., Markovaara-Koivisto, M., Naskali, A., Salokannel, V., Tuulentie, S. and Similä, J. 2019. Mining in the Arctic environment – A review from ecological, socioeconomic and legal perspectives. *Journal of environmental management*, 233, 832-844. doi:10.1016/j.jenvman.2018.11.124

Trivedi, P., Leach, J.E., Tringe, S.G., Sa, T. and Singh, B.K. 2020. Plant–microbiome interactions: from community assembly to plant health. *Nature reviews microbiology*, 18, 607-621. doi:10.1038/s41579-020-0412-1

Turetsky, M.R., Benscoter, B., Page, S., Rein, G., Van Der Werf, G.R. and Watts, A. 2015. Global vulnerability of peatlands to fire and carbon loss. *Nature Geoscience*, 8, 11-14. doi:10.1038/ngeo2325

Turetsky, M.R., Mack, M.C., Hollingsworth, T.N. and Harden, J.W. 2010. The role of mosses in ecosystem succession and function in Alaska's boreal forest. *Canadian Journal of Forest Research*, 40(7), 1237-1264. doi:10.1139/X10-07

Uprety, Y., Asselin, H., Dhakal, A. and Julien, N. 2012. Traditional use of medicinal plants in the boreal forest of Canada: review and perspectives. *Journal of Ethnobiology and Ethnomedicine*, 8, article 7. doi:10.1186/1746-4269-8-7

van der Velde, Y., Temme, A.J., Nijp, J.J., Braakhekke, M.C., van Voorn, G.A., Dekker, S.C., Dolman, A.J., Wallinga, J., Devito, K.J. and Kettridge, N. 2021. Emerging forest–peatland bistability and resilience of European peatland carbon stores. *Proceedings of the National Academy of Sciences*, 118(38), e2101742118. doi:10.1073/pnas.2101742118

Van Houdt, R., Provoost, A., Van Assche, A., Leys, N., Lievens, B., Mijnendonckx, K. and Monsieurs, P. 2018. *Cupriavidus metallidurans* strains with different mobilomes and from distinct environments have comparable phenomes. *Genes*, 9(10), 507. doi:10.3390/genes9100507

Van Nevel, L., Mertens, J., Staelens, J., De Schrijver, A., Tack, F.M., De Neve, S., Meers, E. and Verheyen, K. 2011. Elevated Cd and Zn uptake by aspen limits the phytostabilization potential compared to five other tree species. *Ecological Engineering*, 37(7), 1072-1080. doi:10.1016/j.ecoleng.2010.07.010

Venier, L., Thompson, I., Fleming, R., Malcolm, J., Aubin, I., Trofymow, J., Langor, D., Sturrock, R., Patry, C. and Outerbridge, R. 2014. Effects of natural resource development on the terrestrial biodiversity of Canadian boreal forests. *Environmental reviews*, 22(4), 457-490. doi:10.1139/er-2013-0075

Vorholt, J.A. 2012. Microbial life in the phyllosphere. *Nature Reviews Microbiology*, 10(12), 828-840. doi:10.1038/nrmicro2910

Wang, S. and Mulligan, C.N. 2006. Occurrence of arsenic contamination in Canada: sources, behavior and distribution. *Science of The Total Environment*, 366(2-3), 701-721. doi: 10.1016/j.scitotenv.2005.09.005

Wang, S., Yang, Y., Luo, Y. and Rivera, A., 2013. Spatial and seasonal variations in evapotranspiration over Canada's landmass. *Hydrology and Earth System Sciences*, 17(9): 3561-3575. doi:10.5194/hess-17-3561-2013.

Wardle, D.A., Jonsson, M., Bansal, S., Bardgett, R.D., Gundale, M.J. and Metcalfe, D.B. 2012. Linking vegetation change, carbon sequestration and biodiversity: insights from island ecosystems in a long-term natural experiment. *Journal of Ecology*, 100(1), 16-30. doi:10.1111/j.1365-2745.2011.01907.x

Watari, T., Nansai, K. and Nakajima, K. 2021. Major metals demand, supply, and environmental impacts to 2100: A critical review. *Resources, Conservation and Recycling*, 164, 105107. doi:10.1016/j.resconrec.2020.105107

Watkinson, A.D., Virgl, J., Miller, V.S., Naeth, M.A., Kim, J., Serben, K., Shapka, C. and Sinclair, S. 2021. Effects of dust deposition from diamond mining on subarctic plant communities and barren-ground caribou forage. *Journal of Environmental Quality*. doi:10.1002/jeq2.20251

Weir, J.N., Mahoney, S.P., McLaren, B. and Ferguson, S.H. 2007. Effects of mine development on woodland caribou *Rangifer tarandus* distribution. *Wildlife Biology*, 13(1), 66-74. doi:10.2981/0909-6396(2007)13[66:EOMDOW]2.0.CO;2

Weiss, S., Xu, Z.Z., Peddada, S., Amir, A., Bittinger, K., Gonzalez, A., Lozupone, C., Zaneveld, J.R., Vazquez-Baeza, Y., Birmingham, A., Hyde, E.R. and Knight, R. 2017. Normalization and microbial differential abundance strategies depend upon data characteristics. *Microbiome*, 5, 27. doi:10.1186/s40168-017-0237-y

Wells, J., Roberts, D., Lee, P., Cheng, R. and Darveau, M. 2010. A forest of blue–Canada’s boreal forest: the world’s waterkeeper. International Boreal Conservation Campaign, Seattle, Wash.

Wells, J.V., Dawson, N., Culver, N., Reid, F.A. and Morgan Siegers, S. 2020. The state of conservation in North America’s boreal forest: issues and opportunities. *Frontiers in Forests and Global Change*, 3, article 90. doi:10.3389/ffgc.2020.00090

Wilsey, B., & Stirling, G. 2007. Species richness and evenness respond in a different manner to propagule density in developing prairie microcosm communities. *Plant ecology*, 190(2), 259-273. doi: 10.1007/s11258-006-9206-4

Wickham, H. 2011. The split-apply-combine strategy for data analysis. *Journal of Statistical Software*, 40(1), 1-29. doi:10.18637/jss.v040.i01

Wickham, H. and Chang, W. 2015. ggplot2: elegant graphics for data analysis. Springer-Verlag, New York (2016). Package 'ggplot2', R package version 3.3.6 (2022-05-03).

Williams-Mounsey, J., Grayson, R., Crowle, A. and Holden, J. 2021. A review of the effects of vehicular access roads on peatland ecohydrological processes. *Earth-Science Reviews*, 214, 103528. doi:10.1016/j.earscirev.2021.103528

Wu, B., Luo, H., Wang, X., Liu, H., Peng, H., Sheng, M., Xu, F. and Xu, H. 2022. Effects of environmental factors on soil bacterial community structure and diversity in different contaminated districts of Southwest China mine tailings. *Science of The Total Environment*, 802, 149899. doi:10.1016/j.scitotenv.2021.149899

Wu, B., Peng, H., Sheng, M., Luo, H., Wang, X., Zhang, R., Xu, F. and Xu, H. 2021. Evaluation of phytoremediation potential of native dominant plants and spatial distribution of heavy metals in abandoned mining area in Southwest China. *Ecotoxicology and Environmental* Safety, 220, 112368. doi:10.1016/j.ecoenv.2021.112368

Xia, Z., Zheng, Y., Stelling, J.M., Loisel, J., Huang, Y. and Yu, Z. 2020. Environmental controls on the carbon and water (H and O) isotopes in peatland *Sphagnum* mosses. *Geochimica et Cosmochimica Acta*, 277, 265-284. doi:10.1016/j.gca.2020.03.034

Xu, N., Qu, Q., Zhang, Z., Yuan, W., Cui, H., Shen, Y., Lin, W., Lu, T. and Qian, H. 2020. Effects of residual S-metolachlor in soil on the phyllosphere microbial communities of wheat (*Triticum aestivum* L.). *Science of The Total Environment*, 748, 141342. doi:10.1016/j.scitotenv.2020.141342

Yan, C., Wang, F., Geng, H., Liu, H., Pu, S., Tian, Z., Chen, H., Zhou, B., Yuan, R. and Yao, J. 2020. Integrating high-throughput sequencing and metagenome analysis to reveal the characteristic and resistance mechanism of microbial community in metal contaminated sediments. *Science of The Total Environment*, 707, 136116. doi:10.1016/j.scitotenv.2019.136116

Yin, X., Martineau, C., & Fenton, N. J. 2022. Synergistic effects in mine offsite landscapes: Predicted ecosystem shifts could exacerbate mining effects on bryophyte community structure. *Ecological Indicators*. 144, 109555. doi: 10.1016/j.ecolind.2022.109555

Yin, X.B., Martineau, C., Demers, I., Basiliko, N. and Fenton, N.J. 2021. The potential environmental risks associated with the development of rare earth element production in Canada. *Environmental Reviews*, 29(3), 354-377. doi:10.1139/er-2020-0115

Zeng, X.Y., Li, S.W., Leng, Y. and Kang, X.H., 2020. Structural and functional responses of bacterial and fungal communities to multiple heavy metal exposure in arid loess. *Science of The Total Environment*. 723: 138081. doi: 10.1016/j.scitotenv.2020.138081.

Zhang, G., Yuan, X. and Wang, K. 2019. Biodiversity and temporal patterns of macrozoobenthos in a coal mining subsidence area in North China. *PeerJ*, 7, e6456. doi:10.7717/peerj.6456

Zhang, M., Sun, Q., Chen, P., Wei, X. and Wang, B. 2022. How microorganisms tell the truth of potentially toxic elements pollution in environment. *Journal of Hazardous Materials*, 431, 128456. doi:10.1016/j.jhazmat.2022.128456

Zhao, X., Huang, J., Zhu, X., Chai, J. and Ji, X. 2020a. Ecological effects of heavy metal pollution on soil microbial community structure and diversity on both sides of a river around a mining area. *International Journal of Environmental Research and Public Health*, 17(16), 5680. doi:10.3390/ijerph17165680

Zhao, X., Sun, Y., Huang, J., Wang, H. and Tang, D. 2020b. Effects of soil heavy metal pollution on microbial activities and community diversity in different land use types in mining areas. *Environmental Science and Pollution Research*, 27, 20215-20226. doi:10.1007/s11356-020-08538-1

Zheng, L., Li, Y., Shang, W., Dong, X., Tang, Q. and Cheng, H. 2019. The inhibitory effect of cadmium and/or mercury on soil enzyme activity, basal respiration, and microbial community structure in coal mine–affected agricultural soil. *Annals of Microbiology*, 69(8), 849-859. doi:10.1007/s13213-019-01478-3

Zhou, B., Li, Z. and Chen, C. 2017. Global potential of rare earth resources and rare earth demand from clean technologies. *Minerals*, 7(11), 203. doi:10.3390/min7110203

Žibret, G., Gosar, M., Miler, M. and Alijagić, J. 2018. Impacts of mining and smelting activities on environment and landscape degradation—Slovenian case studies. *Land Degradation & Development*, 29(12), 4457-4470. doi:10.1002/ldr.3198

Zielińska, K.M., Kiedrzyński, M., Grzyl, A. and Rewicz, A. 2016. Forest roadsides harbour less competitive habitats for a relict mountain plant (*Pulsatilla vernalis*) in lowlands. *Scientific Reports*, 6, 31913. doi:https://doi.org/10.1038/srep31913

Zou, L., Lu, Y., Dai, Y., Khan, M.I., Gustave, W., Nie, J., Liao, Y., Tang, X., Shi, J. and Xu, J. 2021. Spatial variation in microbial community in response to As and Pb contamination in paddy soils near a Pb-Zn mining site. *Frontiers in Environmental Science*, 9, 138. doi:10.3389/fenvs.2021.63