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AVANT-PROPOS

Cette thèse de doctorat a été réalisée en co-tutelle entre l'Université du Québec en Abitibi-Témiscamingue et l'Université Paul Sabatier Toulouse 3. La thèse est présentée sous forme d'articles scientifiques et se compose de trois chapitres ainsi que d'une introduction et d'une conclusion générale. Le cœur de la thèse est formé de trois chapitres dont chacun des manuscrits est publié, accepté ou en préparation pour publication dans des revues à comité de lecture.

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LISTE DES ABRÉVIATIONS, DES SIGLES ET DES ACRONYMES

ADN/DNA : acide désoxyribonucléique / desoxyribonucleic acid

Al : aluminium

ANOVA : analyse de la variance

BF : balsam fir / sapin baumier

BS : black spruce / épinette noire

BSE : black spruce + ericaceous srub / épinette noire + plante éricacée

BSM : black spruce, mineral soil / épinette noire, sol minéral

BSO : black spruce, organic soil / épinette noire, sol organique

C : carbone

Ca : calcium

$CaCl_2$: Chlorure de calcium

CMN : common mycorrhizal network / réseau mycorhizien commun

ECEC : cationic exchange capacity / capacité d'échange cationique effective

ECM : ectomycorrhiza / ectomycorhize

ERM : ericoid mycorrhiza / mycorhize éricoïde

Fe : fer

Fwd : forward

ITS : internal transcibed spacer / espaceur intergénique transcrit

K : potassium

Mg : magnésium

Mn : manganèse

N: azote

NMDS : non-metric multi dimensionnal scaling / analyse multivariée non-métrique

OTU : operational taxonomic unit / unité taxonomique opérationnel

P : phosphore

p : P-value (valeur P)

pH : potentiel d’hydrogène

pb : paire de base

PCR : polymerase chain reaction / réaction en chaîne par polymérase

PERMANOVA : permutational analyse of variance / analyse de la variance avec permutation

Rev : reverse

S : soufre

TA : trembling aspen / peuplier faux-tremble

TAM : trembling aspen, mineral soil / peuplier faux-tremble, sol minéral

TAO : trembling aspen, organic soil / peuplier faux-tremble, sol organique

LISTE DES SYMBOLES ET UNITÉS

% : percent / pourcentage

°C : Celsius degree / degré Celsius

°N : north latitude degree / degré de latitude Nord

µL : microliter / microlitre

cm : centimeter / centimètre

ha : hectare

km : kilometer / kilomètre

m : meter/mètre

min : minute

mg : milligram / milligramme

mm : millimeter / millimètre

ng : nanogram / nanogramme

sec : seconde

RÉSUMÉ

Au Québec, le 49° de latitude nord représente la frontière entre d'une part la forêt mixte dominée par le sapin baumier et le bouleau et d'autre par la forêt boréale dominée par l'épinette noire. Cette frontière tend à migrer vers le nord avec la migration du sapin. Dans les plaines argileuses de l'Abitibi-Témiscamingue qui se trouvent à cette latitude, le sapin possède localement une meilleure capacité d'établissement sous les couverts dominés par le peuplier faux-tremble en comparaison à ceux dominés par l'épinette noire. Les conditions climatiques et édaphiques sont similaires dans les deux types de peuplement, mais les conditions biotiques diffèrent. Le sous-bois sous épinette est dominé par les mousses et des arbustes de la famille des éricacées, tandis que le sous-bois associé aux peuplements de peupliers présente une richesse spécifique plus élevée, plus particulièrement en espèces arbustives et herbacées. Les communautés végétales des strates arborées et de sous-bois sont connues pour affecter les communautés fongiques du sol et notamment les communautés mycorhiziennes. Or, ces dernières pourraient expliquer les différences d'établissement du sapin observées entre les deux types de peuplement. En effet, les mycorhizes sont des symbioses à bénéfices réciproques entre des champignons et les racines des arbres et elles sont particulièrement importantes pour la nutrition des plantes en forêt boréale. Cependant, il y a très peu d'informations sur les mycorhizes dans le système boréal québécois relativement à la Scandinavie ou l'Alaska. Dans ce projet, nous avons testé 1) si les communautés de champignons du sol sont différentes entre les peuplements de peuplier et d'épinette, 2) si les sapins s'associent avec un plus grand nombre d'espèces de champignons, mais aussi à des espèces différentes sous les peupliers et 3) si les symbioses mises en place sous les peupliers sont plus efficaces que celles sous les épinettes pour la nutrition du sapin.

Le séquençage haut débit de l'ADN des champignons du sol a permis de détecter une forte diversité fongique et de mettre en évidence des différences dans la composition des communautés fongiques du peuplier et de l'épinette, aussi bien pour les champignons décomposeurs que pour les champignons mycorhiziens. Pendant deux années, soixante jeunes plants de sapins ont été suivis sur le terrain afin de relier la croissance et le taux de nutriments dans les aiguilles (deux estimateurs de la vigueur) au taux de mycorhization et à la diversité fongique. L'analyse a révélé que le taux de nutriments dans les aiguilles du sapin était supérieur sous les peupliers par rapport au sapin poussant sous les épinettes à proximité de plantes éricacées. De plus, la présence des plantes éricacées était corrélée à des changements de la communauté fongique mycorhizienne associée aux racines du sapin, ainsi qu'à une diminution du contenu en azote dans les aiguilles. Des expériences ont également été menées en chambre de croissance afin de déterminer si la mycorhization avait un impact sur la germination, la survie, la nutrition et la croissance des jeunes plantules. Pour ce faire, des sapins ont été semés dans des sols organiques et minéraux provenant des différents types de peuplement et la moitié a été stérilisée afin d'éliminer les microorganismes. Les résultats obtenus après trois saisons de croissance ont permis de détecter un effet de l'identité des microorganismes du sol plutôt qu'un effet du taux de mycorhization sur la nutrition et la croissance du sapin. De plus, les sapins poussant dans les sols récoltés sous épinette mais à distance des éricacées ont eu une meilleure nutrition azotée que dans les sols prélevés sous peuplier. Une expérience sur une plus longue durée et un dispositif expérimental plus complexe intégrant la possibilité de formation de réseaux mycorhiziens fonctionnels entre plantes sont à envisager afin de tester l'importance des interactions sur l'établissement du sapin et sa nutrition à plus long terme.

Globalement, les résultats de la thèse nous indiquent que les différences observées d'abondance et de croissance du sapin sous le peuplier et l'épinette sont la résultante d'interactions complexes, où les communautés mycorhiziennes et les communautés

végétales dominantes jouent un rôle. Les différences des communautés fongiques du sol et de nutriments dans le sol organique observées entre les deux peuplements pourraient expliquer la meilleure nutrition du sapin sous les peupliers. D'autre part, la présence d'une communauté ectomycorrhizienne non compatible et/ou peu favorable aux sapins à proximité des éricacées pourrait expliquer en partie les problèmes de croissance et de nutrition sous les épinettes. Ainsi, la facilitation du sapin par le peuplier est partiellement expliquée par les communautés fongiques du sol. Enfin, nos données confirment que les champignons du sol (dont les ectomycorhiziens) sont à prendre en compte dans les modèles de migration du sapin vers le nord en réponse au changement climatique.

Mots clefs : ectomycorhizes, épinette noire, peuplier faux-tremble, sapin baumier, sol, séquençage haut débit

CHAPITRE 0

INTRODUCTION GÉNÉRALE

0.1 Échanges en sous-sol

0.1.1 Le sol comme lieu de vie

D’un point de vue physique, le sol est l’interface entre l’atmosphère et la lithosphère, c’est littéralement tout ce qui se trouve entre l’air et la roche mère. Il est à la fois composé de matière organique et inorganique; c’est un mélange entre la roche mère dégradée, la matière organique qui s’y accumule et les organismes qui y vivent. Et ce sont ces derniers qui nous intéressent particulièrement: une grande partie de la diversité des écosystèmes terrestres se retrouve dans le sol mais reste peu connue (André *et al.*, 2002; Bardgett et van der Putten, 2014; Tiedje *et al.*, 1999). Une multitude d’organismes, appartenant à des groupes phylogénétiques divers, s’activent et tiennent, à l’abri des regards, des rôles fondamentaux pour les écosystèmes forestiers.

Les organismes du sol ont des rôles divers. Ils peuvent être décomposeurs, c’est-à-dire dégrader les matières organiques mortes et ainsi en rendre les nutriments accessibles aux plantes (Miki *et al.*, 2010). On trouve une grande diversité de forme chez les décomposeurs (nématodes, collemboles et insectes, bactéries et champignons) qui est associée à une grande diversité de fonctions: certains se nourrissent de fèces, d’autres préfèrent le bois mort, ou l’odeur d’un cadavre en décomposition (Gessner *et al.*, 2010; Wardle *et al.*, 2006). D’autres organismes interagissent avec les racines des plantes: les symbiotes (bactériens ou fongiques)

organisent des échanges souterrain avec les plantes. En échangeant des nutriments contre des sucres les symbiotes du sol se positionnent comme des agents essentiels de la chaîne trophique (Ke *et al.*, 2015; Wall et Moore, 1999). Dans le sol se trouvent également des parasites, des herbivores, des endophytes et encore d'autres dont on ignore encore le rôle. Dans le sol cohabitent tous les règnes et tous les domaines du vivant, c'est l'essence même du sol : accueillir en son sein, une diversité d'organismes. Parmi eux, le manque d'information quant à leur rôle dans les écosystèmes dans un contexte de changement climatique, la grande diversité de leurs modes de vie ainsi que l'originalité de leur forme confèrent aux champignons de quoi attiser la curiosité de tout un chacun, et c'est par ce prisme que j'ai choisi d'étudier les sols, ou du moins, leur diversité biologique et leur capacité à échanger avec la biosphère et l'atmosphère.

0.1.2 Les champignons : un groupe d'organismes diversifié et méconnu

Les champignons au sens large forment un groupe polyphylétique au sein du domaine des Eukaryotes. Les cellules de ces organismes se caractérisent par la présence d'une paroi (composée de chitine et de glucose) autour de la membrane cytoplasmique et par l'hétérotrophie pour le carbone (et donc l'absence de plaste dans la cellule). Le thalle (ou appareil végétatif) peut être uni- (levures) ou pluricellulaire (champignons filamenteux, dans ce cas, le thalle est appelé mycélium) (Webster et Weber, 2007).

Les champignons au sens strict (ou *Fungi*) désignent un groupe monophylétique comprenant les Ascomycètes, les Basidiomycètes, les Zygomycètes et les Chytridiomycètes. Les deux derniers groupes sont polyphylétiques et pour cette raison les classifications les plus récentes abandonnent ces deux dernières divisions. Ainsi les Zygomycètes sont remplacés par différentes lignées monophylétiques, par ex. les Gloméromycètes et les Mucoromycètes (*incertae sedis*) pour lesquels la position phylogénétique reste encore incertaine (Spatafora *et al.*, 2016). Le groupe des champignons est assez divers puisqu'on estime le nombre d'espèces de 2.2

millions jusqu'à 3.8 millions dont 3 à 8 % sont décrites (Hawksworth et Lücking, 2017), mais ce chiffre a souvent fluctué et peut donc prêter à débat (Hawksworth, 2001; Mueller et Schmit, 2007; Schmit et Mueller, 2007; Taylor *et al.*, 2013).

Une grande part de leur diversité reste donc méconnue. Le mycologue qui voudrait décrire avec précision la totalité (ou quasi-totalité) des espèces de champignons présentes dans les sols se heurte à plusieurs obstacles. Premièrement, la description taxonomique des macromycètes est basée sur le carpophore, l'organe de reproduction sexuée des Asco- et Basidiomycètes. Un inventaire basé sur les carpophores nécessiterait des inventaires quasi journaliers sur le terrain pour ne pas manquer des espèces, et les carpophores souterrains restent invisibles. De plus, il a déjà été démontré que l'abondance des carpophores ne reflète pas l'abondance des champignons en sous-sol (Gardes et Bruns, 1996; Horton et Bruns, 2001). Deuxièmement, les champignons dans les groupes des Chytridio- et Zygomycètes ne forment pas de carpophores, ce qui rend l'identification des espèces encore plus difficile à partir des caractères morphologiques. Enfin, les Gloméromycètes ont perdu la capacité de se reproduire sexuellement (Webster et Weber, 2007).

L'essor de la génétique moderne et en particulier du séquençage haut débit de l'ADN a permis de résoudre, en partie, ce problème d'identification. La technique de métabarcoding d'échantillons environnementaux (sol, eau, bois mort, fèces, etc.) consiste en l'extraction de l'ADN de ces échantillons suivi de l'amplification et du séquençage haut débit (Mardis, 2008) d'un marqueur ADN utilisé comme un code-barre (Chmolowska, 2013; Geremia et Zinger, 2013; Taberlet *et al.*, 2012). Pour les champignons, l'utilisation de la région de l'espaceur intergénique transcrit (ITS) est utilisée comme code-barre universel (Nilsson *et al.*, 2009). Le séquençage haut débit permet l'obtention d'un grand nombre de séquences qui sont ensuite triées et regroupées en unités taxonomiques opérationnelles (OTUs) selon un seuil de similarité des séquences spécifique à chaque groupe d'organisme (97 % de similarité

pour le groupe des champignons, Nilsson *et al.*, 2008). Les OTUs, ne sont pas des espèces à proprement parler (car non décrites taxonomiquement), mais un proxy d'espèces ou espèces moléculaires. Ces techniques ont permis de mettre à jour l'importante biodiversité retrouvée dans de nombreux écosystèmes, notamment les écosystèmes boréaux (Taylor *et al.*, 2013).

Les champignons présentent donc une diversité d'espèces, mais aussi une diversité de modes de d'acquisition du carbone. En effet, pratiquement tous les modes d'acquisition du carbone sont représentés chez les champignons: décomposeurs, parasites, symbiotes et endophytes. La structure commune formée par la symbiose entre certaines espèces de champignon et la racine d'une plante est appelée mycorhize, leur présence pourrait avoir plusieurs impacts sur les écosystèmes terrestres.

0.1.3 Symbiose mycorhizienne : quels bénéfices pour la plante?

La symbiose mycorhizienne matérialise un mutualisme entre les racines des plantes et certains champignons appartenant aux groupes des Asco-, Basidio-, Mucoro- et Gloméromycètes. Les échanges vont dans les deux sens : les plantes reçoivent des minéraux en échange de sucres issus de la photosynthèse. Les échanges entre les champignons et les plantes sont importants en termes de carbone et de nutriments (N, P, K...), et la présence des champignons mycorhiziens sur les racines des plantes a souvent un effet positif sur leur croissance et leur nutrition. En fonction de l'espèce et des conditions environnementales locales, les champignons mycorhiziens apportent une part plus ou moins importante d'azote à la plante (jusqu'à 80%) qu'ils puisent dans le sol sous forme minérale (nitrate ou ammonium) ou organique (acides aminés, peptides et polypeptides). Les plantes ne profitent pas seulement des échanges nutritifs, les champignons peuvent également contribuer à la protection des racines contre des pathogènes (en produisant des antibiotiques) et la résistance des plantes à des périodes de sécheresse (en augmentant la surface d'exploration). Il existe sept

grands groupes de mycorhizes : les mycorhizes à arbuscules (Angio- et Gymnospermes, Bryophytes, Pteridophytes), les ectomycorhizes (Angio- et Gymnospermes), les ectendomycorhizes (Angio- et Gymnospermes), les mycorhizes arbutoïdes (Ericales), les mycorhizes monotropoïdes (Monotropideae), les mycorhizes ericoïdes (Ericales, Bryophytes) et enfin les mycorhizes orchidoïde (Orchidales). Les champignons mycorhiziens sont omniprésents dans les écosystèmes puisque l'on estime qu'environ 90% des espèces de plantes terrestres en bénéficient (Brundrett et Tedersoo, 2018). En général, une espèce (ou un groupe) de champignon est capable de s'associer avec plusieurs espèces (ou groupes) de plantes et inversement, bien qu'il existe des cas de spécificité entre les deux partenaires (Bruns *et al.*, 2002; Molina *et al.*, 1992; Smith et Read, 2008).

Les effets bénéfiques des symbioses sont nombreux et vont de ceux associés aux individus à des bénéfices pour l'écosystème au complet. Au niveau individuel, la présence de mycorhizes qui permet une bonne exploitation des ressources, est indispensable à la croissance et la survie d'un grand nombre de plantes (Molina *et al.*, 1992; Smith et Read, 2008). Le nombre d'espèces mycorhiziennes associées à une plante peut avoir un effet bénéfique sur sa croissance grâce à leur spécialisation pour l'acquisition de nutriments sous différentes formes, leur capacité à explorer le sol ou à protéger contre les parasites. C'est le cas pour la symbiose à arbuscules (van der Heijden *et al.*, 1998). Cette relation est plus complexe dans le cas des ectomycorhizes, puisqu'elle est dépendante de l'espèce végétale et des caractéristiques du sol (Jonsson *et al.*, 2001; Sousa *et al.*, 2015). La symbiose mycorhizienne a également un rôle sur la dynamique des communautés végétales puisqu'elle module les relations de compétition et de facilitation entre les plantes (Booth et Hoeksema, 2009; Perry *et al.*, 1989; van der Heijden et Horton, 2009).

La présence de champignons mycorhiziens dans le sol peut mener à la mise en place d'un réseau mycorhizien commun (CMN) entre plantes et champignons. Un réseau

mycorhizien commun est défini comme des échanges nutritifs entre deux plantes via un mycélium commun (Simard *et al.*, 1997, 2012). La mise en place d'un CMN peut se faire entre des plantes de la même espèce ou d'espèces différentes (Richard *et al.*, 2009; Selosse *et al.*, 2006; van der Heijden et Horton, 2009). La présence d'un CMN apporte plusieurs bénéfices aux plantes grâce au transfert des nutriments : aide à l'établissement des plantules, transfert de nutriments entre végétaux et modulation de la compétition (Simard et Durall, 2004). Dans le cas de l'aide à l'établissement des plantules dans les écosystèmes forestiers, des effets bénéfiques d'un CMN ont été démontrés dans 7 cas sur 10 (van der Heijden et Horton, 2009). Une étude en particulier (Nara et Hogetsu, 2004) a démontré que les saules (espèce de succession primaire) favorisent l'établissement des bouleaux et mélèzes (espèces de succession secondaire) via un CMN. Un CMN peut néanmoins entraîner un effet négatif sur l'établissement des plantules par exemple, quand le partenaire mycorhizien alloue plus de ressources à une plante plus mature ou bénéficiant de plus de lumière (Kytöviita *et al.*, 2003; Weremijewicz *et al.*, 2016).

0.2 Les mycorhizes en forêt boréale

La forêt boréale est une vaste étendue forestière discontinue qui couvre une grande partie du Canada et de l'Alaska en Amérique du Nord, La Fennoscandinavie en Europe, une grande partie de la Russie en Eurasie et une partie de la Chine en Asie. Au niveau climatique, la forêt boréale se caractérise par un hiver long, froid et enneigé. Principalement composée de genres de conifères de la famille des Pinaceae (*Picea*, *Pinus*, *Larix* et *Abies*), elle abrite également plusieurs espèces feuillues adaptées aux conditions climatiques typiques des écosystèmes nordiques (principalement dans les familles Betulaceae, Fabaceae et Salicaceae) (Brandt *et al.*, 2013). Le sol des forêts boréales est généralement à tendance acide, humide et pauvre en nutriments sous forme minérale. Dans ces conditions extrêmes, la végétation a un allié de taille : les champignons du sol. D'une part, les champignons saprophytes y

sont les principaux acteurs de la décomposition et donc du cycle de nutriments (Thormann, 2006a). D'autre part, les champignons mycorhiziens favorisent une bonne nutrition via d'importants échanges entre végétation et champignons (Read *et al.*, 2004). La diversité des communautés fongiques est forte en forêt boréale, et les analyses par métabarcoding démontrent qu'une grande part de cette diversité reste peu connue (Taylor *et al.*, 2010, 2013).

Deux types mycorhiziens sont principalement présents en forêt boréale: les ectomycorhizes (ECM) qui s'associent avec la plupart des espèces de la strate arborée et les mycorhizes éricoïdes (ERM) qui s'associent avec les plantes de la famille des Ericaceae. On trouve également des mycorhizes à arbuscules associées à quelques plantes de sous-bois et arbustes (Betulaceae), mais leur importance en terme de biomasse fongique reste plus faible (Read *et al.*, 2004).

Ce qu'on appelle ECM est la structure commune formée par un champignon de la famille des Asco- ou Basidiomycètes avec un apex racinaire d'une plante de la famille des Angio- ou Gymnospermes. Dans le cas des ECM, les cellules du champignon ne pénètrent pas dans les cellules de la racine, elles forment un manteau mycélien enrobant cette dernière. Le mycélium s'immisce entre les cellules de la plante de la racine pour former le réseau de Hartig. C'est là qu'ont lieu les échanges entre la plante et le champignon. Le manteau est relié au milieu extérieur via l'extension des hyphes extramatricielles, qui puisent les nutriments dans le sol. Les champignons ECM sont capables de puiser dans le sol des nitrates et ammonium mais aussi des nutriments sous formes organiques soit par ingestion directe (acides aminés et petits peptides) soit par dégradation enzymatique (polypeptides et protéines) (Smith et Read, 2008). Un arbre est capable de s'associer avec des dizaines d'espèces ECM tout comme les champignons ECM sont capables de s'associer avec plusieurs espèces d'arbres, bien qu'il existe des cas de spécificité de l'un ou l'autre des partenaires (Horton et Bruns, 2001). L'ensemble des espèces ECM associé à une

plante dans un milieu donné est appelé communauté ectomycorhizienne. De nombreuses études ont démontré que l'espèce dominant la canopée était corrélé àla composition des communautés ECM dans le sol (DeBellis *et al.*, 2006; Ishida *et al.*, 2007; Taylor *et al.*, 2010; Urbanová *et al.*, 2015). Les champignons ECM retrouvés dans le sol des forêts boréales appartiennent aussi bien aux familles Asco- et Basidiomycètes. Les familles ECM Thelephoraceae, Russulaceae, Cortinariacae et Atheliaceae (toutes appartenant à la division des Basidiomycètes) semblent y être parmi les plus abondantes, mais les conditions de sols et la composition des communautés végétales ont un impact sur l'abondance des familles ECM (via leur préférences écologiques) (Kernaghan *et al.*, 2003; Taylor *et al.*, 2013; Toljander *et al.*, 2006).

La plupart des plantes de la strate arborée est ectomycorhizienne en forêt boréale. L'association avec son partenaire mycorhizien assure à la plante une bonne nutrition en termes de N, P, K, Mg, Ca etc. La mise en place de la symbiose permet donc d'assurer une bonne croissance des arbres et de maintenir une bonne productivité (Qu *et al.*, 2010; Thormann, 2006b). En forêt boréale, l'azote est souvent l'élément limitant le plus la croissance des arbres (Högberg *et al.*, 2017; Tamm, 2012), et la présence des champignons ectomycorhiziens dans leurs racines devrait améliorer leur nutrition azotée via les échanges entre les deux partenaires (Smith et Read, 2008). Cependant, cette relation est dépendante de la fertilité du sol, puisque dans les sols peu fertiles des forêts boréales, une augmentation de l'allocation en C de la plante au champignon résulte en une rétention du N par le champignon au détriment de la nutrition de la plante ce qui pourrait dans certains cas aggraver la situation (Franklin *et al.*, 2014; Hasselquist *et al.*, 2015; Näsholm *et al.*, 2013). En plus de ces effets sur la nutrition des plantes, la présence de champignons ECM dans le sol des forêts boréales est impliquée dans le stockage du carbone et le cycle de l'azote (via une accumulation de la matière organique par le bas) exerçant ainsi des rétroactions sur la

nutrition des plantes (Clemmensen *et al.*, 2013; Kyaschenko *et al.*, 2019; Lindahl *et al.*, 2002; Parker *et al.*, 2017).

La symbiose pourrait avoir un rôle important dans la dynamique forestière puisqu'il a été démontré que les communautés de champignons ECM sont dépendantes du stade de succession forestière, il existe donc une rétroaction entre communauté de champignon ECM et dynamique forestière (Peay *et al.*, 2011; Smith *et al.*, 2002; Taudiere *et al.*, 2015). Certaines espèces de champignons sont plus fortement associées à des stades forestiers de début de succession et d'autres à des stades plus matures (Last *et al.*, 1983; Molina *et al.*, 1992). L'accumulation d'espèces ECM et le changement de composition des communautés au fil du temps (Twieg *et al.*, 2007), supposent également que les espèces ECM sont adaptées à un stade forestier particulier.

Les ERM représentent la structure commune formée par des champignons de la famille des Ascomycètes et certaines plantes du groupe des Ericales. Contrairement au cas des ECM, le mycélium du champignon ERM pénètre à l'intérieur des cellules des racines pour former une structure de type arbusculaire (Smith et Read, 2008). Les champignons ERM sont moins étudiés que leurs homologues ECM et peu d'espèces ERM sont décrites. Il faut aussi noter que les familles Helotiaceae et Sebacinaceae comptent parmi elles des champignons ERM (Kohout, 2017; Walker *et al.*, 2011). Un effet négatif de la présence de plantes éricacées à ERM sur l'établissement de plantules de conifères a déjà été détecté en forêt boréale (Mallik, 2003; Mallik *et al.*, 2016; Zackrisson *et al.*, 1997). L'effet négatif des éricacées à ERM sur les plantules est souvent attribué à la libération de composés phénoliques nocifs par celles-ci dans le sol (Carballeira, 1980; Gallet, 1994; Mallik, 2003; Peterson, 1965). Cet effet négatif pourrait, entre autres, être renforcé par la présence d'ERM, car il a été observé des changements des communautés ECM associées aux plantes se trouvant à

proximité d'Ericaceae à ERM (Kohout *et al.*, 2011; Walker *et al.*, 1999; Yamasaki *et al.*, 1998, Kennedy et al. 2018).

0.3 La forêt boréale du Québec : un système simple pour étudier le rôle des ectomycorhizes sur l'établissement d'une espèce

0.3.1 La forêt boréale au Québec

Au Québec, la forêt boréale se décompose en trois sous-domaines. La sapinière à bouleau ou forêt boréale mixte est composée de diverses espèces de conifères (*Abies balsamea [L.] Miller*, *Pinus banksiana* Lambert, *Picea mariana* [Miller] *B.S.P.*, *Picea glauca* (Moench) Voss et *Thuya occidentalis* L.) et de feuillus (*Populus tremuloides Michaux*, *Betula papyrifera Marshall*, *Betula alleghaniensis* Britton) avec une relative dominance du sapin baumier et du bouleau. Le sous-bois y est composé d'une multitude d'espèces, comprenant quelques bryophytes, mais surtout des arbustes dans les familles Betulaceae et Ericaceae. La sapinière à bouleau s'étend jusqu'au 49°N parallèle. À partir du 49°N et jusqu'au 52°N, la pessière à mousse devient l'écosystème dominant. La strate arborée y est principalement composée d'épinette noire (*Picea mariana* [Miller] *B.S.P.)* ou de pin gris (*Pinus banksiana* Lambert, en fonction du dépôt dominant) et le sous-bois est composé d'une épaisse couche muscinale et d'arbustes de la famille des Ericaceae. Enfin à partir du 52°N parallèle et jusqu'à la steppe arctique se trouve la pessière à lichen, là encore les épinettes noires et les pins gris sont dominants dans la strate arborée, mais le sous-bois est composé de lichens (Robitaille et Saucier, 1996).

Au Québec, la forêt boréale apporte de nombreux bénéfices économiques, culturels et environnementaux (Gauthier *et al.*, 2015). L'aménagement de la forêt boréale pour en tirer du bois d'œuvre ou de la pâte à papier représente une part importante de l'économie de la province. Cet écosystème fait donc face à deux perturbations d'origine anthropique représentant des risques différents : les changements

climatiques et l'aménagement forestier. Les contraintes appliquées sur la foret boréale couplée à son importance économique forcent les forestiers à gérer de façon durable cette ressource. Depuis 2013, le Québec doit intégrer l'aménagement forestier écosystémique dans sa gestion des écosystèmes forestiers. L'un des principaux enjeux de l'aménagement écosystémique est de diminuer l'écart entre forêt naturelle et forêt aménagée en se calquant sur les effets des perturbations d'origine naturelle (Gauthier et Vaillancourt, 2008).

0.3.2 Le sapin baumier et sa migration au nord : une opportunité pour tester le rôle des interactions biotiques

Pour étudier l'effet des symbiotes mycorhiziens sur l'établissement d'une espèce en forêt, il faut un système plutôt simple pour pouvoir écarter les effets abiotiques, c'est-à-dire qui ne sont pas la conséquence de la présence d'êtres vivants. Le sapin baumier (*Abies balsamea* [L.] Miller*)* et sa récente histoire biogéographique nous offrent un modèle simple pour quantifier le rôle des mycorhizes sur son établissement, sa croissance et sa nutrition. Le sapin baumier est une espèce de conifère de la famille des Pinaceae. Cette espèce originaire de l'Amérique du Nord a une aire de répartition qui s'étend jusqu'au 58°N parallèle, cependant il ne forme des peuplements purs et denses qu'au sud du 49°N parallèle. Depuis quelque temps et en réponse aux changements climatiques, il semble que cette espèce soit de plus en plus abondante au nord du 49°N, dans la pessière à mousse (Messaoud *et al.*, 2007a).

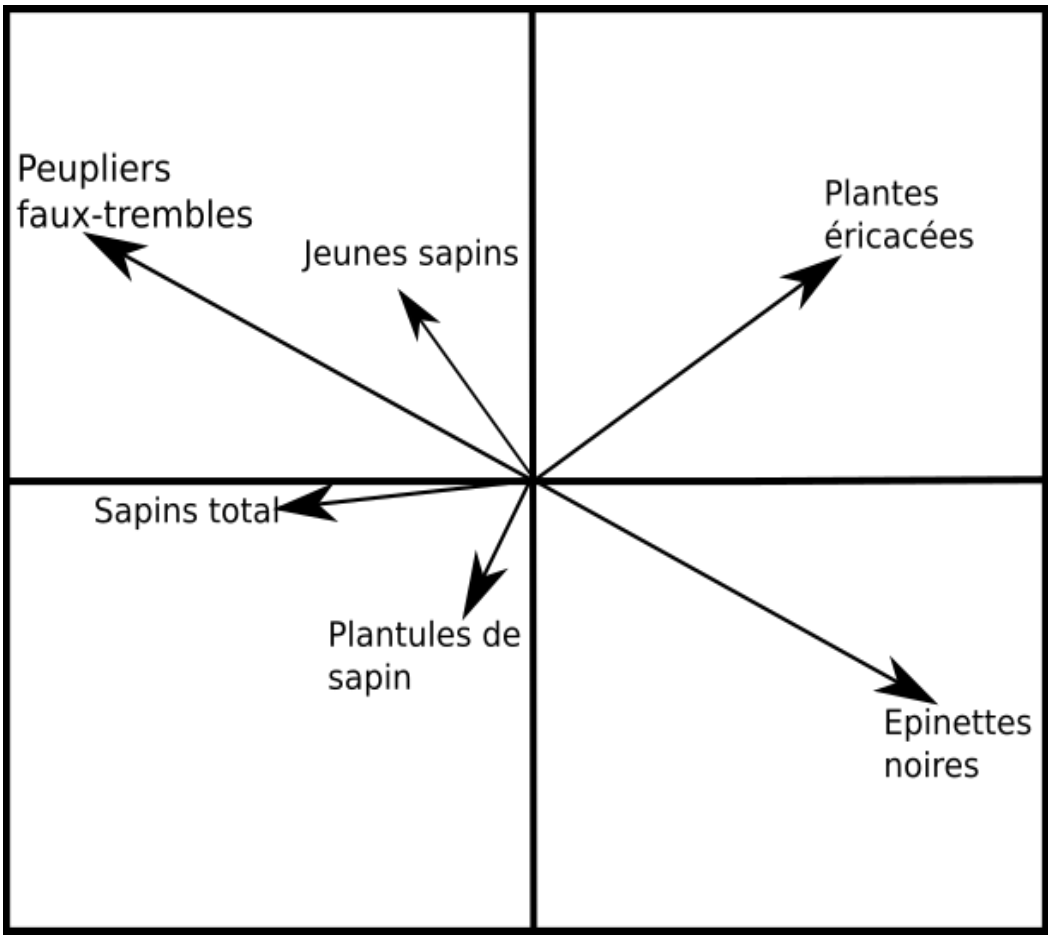

Figure 0.1 Analyse de redondance de l'abondance de la régénération du sapin baumier en pessière à mousse (modifié d'après Arbour et Bergeron, 2011)

À l'écotone entre la sapinière à bouleau blanc et la pessière à mousse (au niveau du 49°N parallèle), dans les plaines argileuses de l'Abitibi-Témiscamingue et du Nord-du-Québec on trouve des peuplements de densité et de tailles différentes dominés par le peuplier faux-tremble (*Populus tremuloides* Michaux*)* dans une matrice forestière dominée par l'épinette noire. Ces deux types de peuplements poussent dans les mêmes conditions édaphiques (sol minéral) et climatiques (Cavard *et al.*, 2011; Légaré *et al.*, 2005). Les communautés végétales du sous-bois sont très différentes entre les deux peuplements. Alors qu'elles sont principalement composées de

bryophytes et de plantes éricacées sous les épinettes, les communautés végétales sont plus diverses sous les peupliers faux-trembles (Légaré *et al.*, 2001). La couche organique du sol a des caractéristiques différentes entre les deux types de peuplements puisqu'il est plus riche en nutriments et moins acide sous les peupliers que sous les épinettes (Cavard *et al.*, 2011). D'un point de vue de la dispersion, le sapin a la possibilité de s'installer dans les deux peuplements (Messaoud *et al.*, 2007b). Or, le sapin baumier a une meilleure croissance et un meilleur établissement sous les peupliers que sous les épinettes, et ce pour des conditions climatiques semblables (Arbour et Bergeron, 2011) (Figure 0.1, modifiée d'après Arbour et Bergeron, 2011). Cette situation est donc idéale pour déterminer le rôle des interactions biotiques dans l'établissement et la croissance d'une espèce.

Des différences dans les caractéristiques du sol et dans l'identité de l'espèce dominant la canopée peuvent amener à des taux de mycorhization différents et des différences de composition des communautés fongiques, particulièrement ECM. Sachant que les effets bénéfiques de la symbiose mycorhizienne sont dépendants du nombre d'interactions et de l'identité des espèces fongiques, l'hypothèse principale de cette thèse est que le taux de mycorhization (pourcentage d'apex racinaire mycorhizés) et les communautés fongiques diffèrent entre peuplements de peupliers et d'épinettes et que ces modifications ont un impact sur la croissance et la nutrition du sapin.

0.4 Objectifs de la thèse

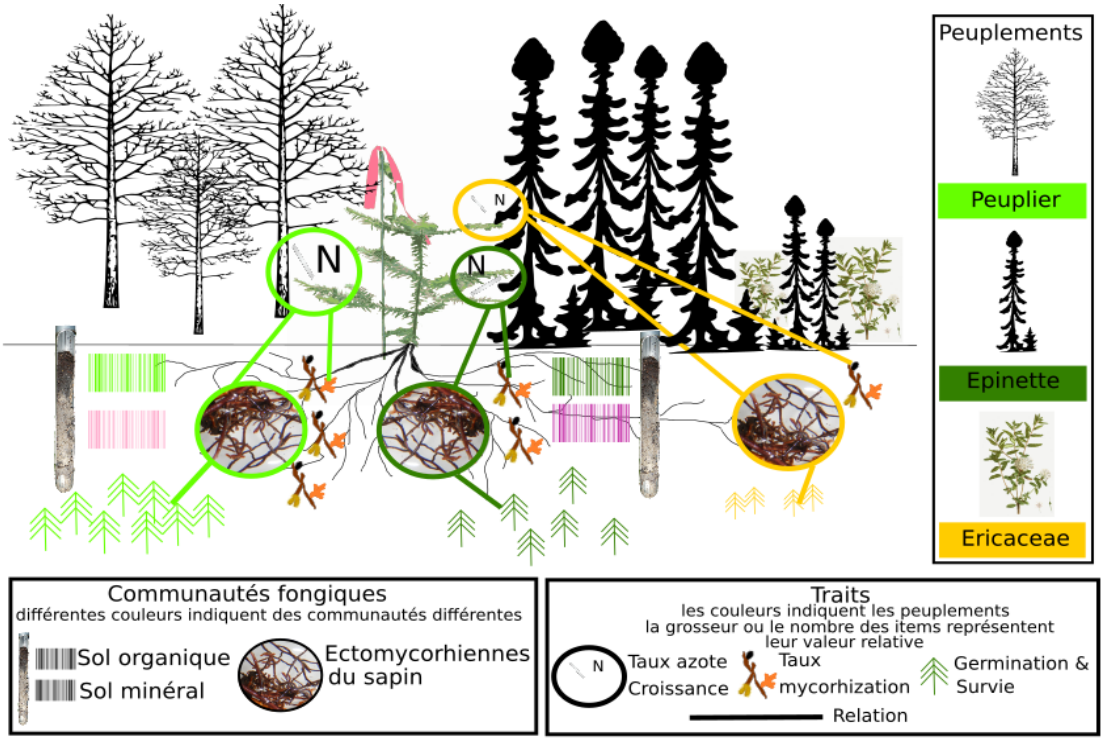

Figure 0.2 Résumé des hypothèses de la thèse

0.4.1 Peuplements forestiers et communautés fongiques

Le premier objectif de la thèse était de d'identifier et décrire les communautés fongiques dans les sols des peuplements d'épinettes noires et de peupliers faux-trembles. Les sites sélectionnés (Annexe A, Figure A.1) pour cette étude ont fait l'objet d'études sur la communauté végétale, et le cycle des nutriments, mais pas sur la composition des communautés fongiques. La première étape a donc été de faire un état des lieux des communautés fongiques du sol dans les horizons organiques et minéraux de ces deux types de peuplements. Pour cela, nous avons utilisé les méthodes de séquençage à haut débit de l'ADN du sol. L'hypothèse principale de cet objectif était que les communautés fongiques sont différentes entre les peuplements et

les horizons du sol (Figure 0.2). Cet objectif, les hypothèses spécifiques qui y sont associées et ses résultats sont présentés dans le Chapitre 1 de la thèse.

0.4.2 Effet de la symbiose ectomycorhizienne sur la nutrition et la croissance du sapin baumier

Le second objectif de la thèse était de faire le lien entre la mycorhization et la croissance des arbres. L'hypothèse principale de cet objectif était que le taux de mycorhization (pourcentage d'apex racinaire mycorhizés) était plus grand sous peuplier et plus faible à proximité des éricacées, que les communautés ECM étaient différentes en fonction de la communauté végétale et que ces effets sur les ECM se répercutaient sur la croissance et la nutrition du sapin (Figure 0.2). Nous avons relié le taux de mycorhization et les communautés ECM à la croissance et la nutrition (concentration en azote dans les aiguilles), le tout en milieu naturel. Pour cela, nous avons sélectionné de jeunes sapins baumiers sur le terrain et avons pris ces mesures sur chacun d'entre eux. Des analyses de pistes ont par la suite été réalisées pour relier les différentes mesures acquises. Cet objectifs et les résultats qui lui sont associés sont présentés dans le Chapitre 2 de la thèse.

0.4.3 Effet de l'origine des sols et leur stérilisation sur la germination et la croissance du sapin baumier

Le dernier objectif de la thèse était de tester si les communautés microbiennes du sol ont un impact sur l'établissement (germination, croissance, nutrition et survie durant les trois premières années) du sapin dans les différents types de peuplement. Les hypothèses principales de cet objectif étaient que le taux de mycorhization est positivement corrélé à la croissance, qu'il est supérieur sous peuplier et plus faible à proximité des éricacées, et que l'absence de mycorhizes sur les sapins augmente sa mortalité et diminue sa croissance (Figure 0.2). Pour cela des sols ont été prélevés dans les deux types de peuplement et à proximité de plantes éricacées à ERM. La

moitié a été stérilisée afin d'en supprimer tous les organismes. Des graines de sapin ont ensuite été mises à germer dans ces sols en chambre de croissance durant trois saisons de croissance. Les taux de germination et mycorhization, la croissance et le taux d'azote dans les aiguilles ont été mesurés afin de les relier aux différents traitements (origine du sol et stérilisation). Les résultats sont présentés dans le Chapitre 3 de la thèse.

CHAPITRE I

IMPACT OF LOCAL FOREST COMPOSITION ON SOIL FUNGAL COMMUNITIES IN A MIXED BOREAL FOREST

(IMPACT DE LA COMPOSITION FORESTIÈRE LOCALE SUR LES COMMUNAUTÉS FONGIQUES DU SOL DANS UNE FORÊT BORÉALE MIXTE)

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1.1 Abstract

Aims: While fungi are key drivers of the carbon cycle and obligate symbionts of trees, the link between plant-fungal interactions and landscape vegetation changes has been largely overlooked. Our aim was to test whether a local difference in dominant tree species would shape the composition of soil fungi communities.

Methods: Fungal communities were described using next-generation DNA sequencing. Composite soil samples were collected in four paired sites (represented by one pure aspen-dominated stand and one pure spruce-dominated stand) and soil nutriments were measured.

Results: Of the more than 1119 OTUs, 31.6% were Ascomycota while 27.8% were Basidiomycota, 15% were ectomycorrhizal whereas 19.7% were saprotrophic fungi. Communities displayed high species turnover among forest types rather than differences in species richness. Among tested predictors, the dominant tree species explained around 11 % of fungal community variation. pH and soil nutrients were also strong predictors of fungal communities.

Conclusions: Our study revealed strong correlations between dominant tree species and fungal communities at a local scale and raised questions regarding the impact of fungal communities on forest soil nutrient dynamics.

Key-words: black spruce-feather moss, fungal diversity, soil ecology, *Picea mariana*, *Populus tremuloides*, NGS

1.2 Résumé

Bien que les champignons soient des acteurs importants du cycle du carbone et les symbiotes obligatoires de nombreux arbres, les liens entre les interactions plante-champignon et les changements de végétation au niveau du paysage ont été peu

étudiés. Notre but était de tester si une différence locale dans les communautés végétales dominantes avait un effet sur la composition des communautés fongiques du sol.
Pour ce faire, les communautés végétales ont été décrites avec les méthodes de séquençage haut débit de l'ADN. Des échantillons de sol ont été collectés dans quatre paires de sites (chaque site étant représenté par un peuplement pur d'épinettes noires et un peuplement pur de peupliers faux-trembles), les nutriments du sol y ont été mesurés.

Sur les 1119 OTUs détectées, 31.6% étaient des Ascomycètes, 27.8% des Basidiomycètes, 15% étaient ectomycorhiziennes et 19.7% saprophytes. Les communautés ont montré une différence de diversité bêta entre les peuplements forestiers plutôt qu'une différence de richesse spécifique. Parmi les indicateurs testés, l'identité de l'espèce dominante expliquait environ 11% de la variation des communautés fongiques, le pH et les nutriments du sol étaient aussi des indicateurs significatifs des communautés fongiques.

Notre étude révèle une corrélation forte entre espèces dominant la canopée et communautés fongiques à une échelle locale et soulève la question de l'impact des communautés fongiques sur la dynamique des nutriments dans le sol forestier.

Mots-clefs : pessière à mousse, diversité fongique, écologie du sol, *Picea mariana*, *Populus tremuloides*, NGS

1.3 Introduction

The boreal forest covers 1889.9 million hectares of the Holarctic land surface and is among the largest forest ecosystems in the world (Brandt *et al.*, 2013). Dominated by coniferous species, this ecosystem contributes to human benefits through wood

production and ecosystem services, such as carbon storage (Gauthier *et al.*, 2015). Indeed, 33 % of the Earth's total carbon is stored in the boreal forests of the Northern Hemisphere, including about 60 % in their soils (Pan *et al.*, 2011). In the province of Quebec, the boreal forest covers about 56 million hectares, and extends over a 10° latitudinal gradient. It is comprised of four bioclimatic domains, including the spruce-feather moss domain, which is dominated by black spruce (*Picea mariana* [Miller] B.S.P.) (Robitaille and Saucier, 1996) and further characterised by an understorey that is composed of mosses and ericaceous shrubs (Fenton and Bergeron, 2006). Other important tree species in this domain include jack pine (*Pinus banksiana* Lambert) and balsam fir (*Abies balsamea* [L.] Miller), together with broadleaf trees such as trembling aspen (*Populus tremuloides* Michaux) and paper birch (*Betula papyrifera* Marshall). The dominance of black spruce leads to soil acidification and exerts negative effects on nutrient access by other plants (Simard *et al.*, 2007). In the absence of fire, black spruce-feather moss forests are prone to organic matter accumulation and, consequently, a thick forest floor often develops (Légaré *et al.*, 2005). Within the black spruce forest, patches of varying sizes of locally dominant trembling aspen are often found. Attributable to pre-fire dominance of each species, side by side natural stands of black spruce and trembling aspen are often found, originating from the same fire event (Gagnon 1989). Trembling aspen develops under the same general mineral soil and site conditions as black spruce, and occurs in the southernmost part of the spruce-feather moss domain. In these hardwood forests, the tree litter is easily decomposable and results in a higher soil pH and a faster nutrient turnover as compared to nearby spruce-dominated stands (Cavard *et al.*, 2011; Légaré *et al.*, 2005). A decrease in litter accumulation also occurs and the moss cover is repressed. In contrast to the spruce forests, aspen forests are characterised by an understorey vegetation that is dominated by herbaceous species, ericaceous shrubs, and ectomycorrhizal (ECM) tree saplings (e.g., species in the genera *Alnus* and *Abies*), with a comparatively higher plant species richness (Cavard *et al.*, 2011; Légaré *et al.*, 2005). According to recent modelling studies, the proportion of

trembling aspen stands in the spruce-feather moss domain is likely to increase, due to climate change (Drobyshev *et al.*, 2013) and to current forest management practices that are being implemented in Quebec (Laquerre *et al.*, 2011). Messaoud *et al.*, (2007a) predicted that the ecotone between the northern spruce-feather moss and the southern fir-paper birch (dominated by balsam fir) domains could move northward. This migration may affect ecosystem functioning. Indeed, Légaré *et al.*, (2005) showed that black spruce stands were more productive when mixed with a small proportion of aspen than in pure stands. Moreover, facilitation processes between aspen and balsam fir in black spruce-feather moss forests were demonstrated by Arbour and Bergeron, (2011), showing that aspen stands were more easily colonised by other tree species than are spruce stands.

Changing forest dominance may impact ecosystem functioning, and especially micro-organisms interacting with plants such as Fungi. More generally, forest soils host numerous fungal species that play important roles for soil functioning, organic matter decomposition, and plant nutrition (Bardgett and Wardle, 2010). As primary decomposers and plant mutualists, soil fungi are key drivers of the carbon cycle in the boreal ecosystem (Lindahl *et al.*, 2002; Thormann, 2006a), and they represent a major carbon stock in the absence of disturbances (Clemmensen *et al.*, 2013). Saprotrophic fungi play a significant role in organic matter decomposition and nutrient turnover in boreal forests (Thormann, 2006a) and their identity and community composition are shaped by litter type and decomposition stage (Foudyl-Bey *et al.*, 2016; Treseder *et al.*, 2014). Decomposition of organic matter by saprotrophic fungi has indirect effect on tree growth by providing mineral nutrients, and their relative abundance has been shown to be greater in N-rich compared to N-poor soil in boreal forests (Kyaschenko *et al.*, 2017).

Another important guild of fungi in the boreal forest is represented by ectomycorrhizal (ECM) fungi, which form symbiotic relationships with tree roots

(Taylor *et al.*, 2010; Tedersoo *et al.*, 2014). Because boreal forest soils develop under low temperatures and are poor in mineralised forms of nutrients, ECM fungi are essential for plant nutrition in these N-poor conditions, as they provide organic forms of nutrients to plants, which are as important as mineral forms in ECM-dominated ecosystems (Read *et al.*, 2004). ECM fungal communities often follow tree dynamics and their composition is related to forest succession (Smith *et al.*, 2002; Twieg *et al.*, 2007). Some ECM fungal species preferentially associate with pioneer tree species (Molina *et al.*, 1992; Taudiere *et al.*, 2015), such as aspen and birch in the boreal forest (Bent *et al.*, 2011). Moreover, ECM fungi can facilitate the establishment of late-succession trees (Booth, 2004; Nara and Hogetsu, 2004). Ectomycorrhizal fungi are thus major actors in forest dynamics and may also affect host tolerance to climate change (Fernandez *et al.*, 2016; Mucha *et al.*, 2018).

Our aim was to investigate if and how a local difference in dominant tree species shaped the composition of soil fungal communities at the ecotone between spruce-feather moss and balsam fir-paper birch domains. We conducted this study in the Clay Belt of northwestern Quebec, which is located in the southern part of the spruce-feather moss domain where intermingled forests of trembling aspen are frequently found. Fungal communities were characterised by next-generation sequencing, and soil analyses were performed for each vegetation type and soil horizon to explore their effects on fungal richness, composition and functional diversity. We hypothesised that: 1) the occurrence of trembling aspen close to black spruce forests will increase richness and change composition of the soil fungal communities and the different fungal guilds (particularly ECM fungi) in both the organic and mineral soil layers, 2) dominant tree species identity will affect soil fungal communities through soil characteristics and particularly pH, 3) high vascular plant richness and abundance of multiple ECM hosts in trembling aspen forests will favour fungal species richness and diversity of ECM fungi.

1.4 Materiel and Methods

1.4.1 Study sites

Study sites were located in the black spruce-feather moss forests of western Quebec at the border of the Abitibi-Témiscamingue and Nord-du-Québec administrative regions (Annexe A, Figure A.1). This area is part of the Clay Belt of northern Quebec and Ontario. Selected sites were unmanaged but were surrounded by managed and harvested forest stands. The sites originated from the same fire that occurred in the area ca. 1916 (Légaré *et al.*, 2005). The nearest meteorological station, which is located in La Sarre (QC), recorded average annual temperatures of 0.7 °C and average annual precipitations totalling 889.8 mm (Environment Canada 2017).

Four paired sites on the same geological parent material were selected across a 36 km² area, based upon their similar conditions with respect to canopy, understorey vegetation, tree density, stand age, substrate and topography and their representativeness regarding unmanaged forest in the region (Annexe A, Table A.1, described in Cavard *et al.*, 2011). We chose sites within mature forests as age could affect fungal communities. The paired design allowed us to account for variations between sites. Each site consisted of a pure (>75 % of canopy cover) stand dominated by trembling aspen (TA), adjacent to a pure stand dominated by black spruce (BS). Each paired stand originated from the same fire ca. 1916. Distance between stands within a site ranged from 0.1 to 2.3 km (Annexe 1, Figure A.1).

1.4.2 Soil sampling and processing

Soil cores were collected over one week in the middle of the growing season, July 2015. In each stand (representing 0.25 ha), five 50- metre transects were established 10 m apart from one another, following the protocol of Taylor *et al.*, (2013). Soil cores 1.8 cm in diameter and 20 cm deep were collected every 5 m along the five

transects, resulting in a total of 10 cores per transect and 50 cores per stand. Only four transects could be installed in the TA stand in site 3 due to its smaller area, but instead we collected 11 cores per transect.

For each core, the top litter layer (dead leaves, needles and living mosses) was removed and samples of the organic and mineral horizons were separated in the field. Large roots and woody debris were removed from each sample. Samples were pooled per horizon and per transect, resulting in 2 samples per transect and 10 samples per stand. Composite soil samples for DNA analysis were stored at -20 °C by the end of the day and stored at -80 °C in the laboratory by the end of the week. Composite soil samples for chemical analysis were forced air-dried at 24 °C for 13 days and then finely ground in a ball mill for 1 min at 30 Hz (Mixer Mill MM 301, Retsch GMbH, Haan, Germany).

1.4.3 Soil Analyses

Soil samples were sent to the Laurentian Forestry Centre, Canadian Forest Service (Quebec, QC, Canada) for chemical analyses. Percentage C, N and S were obtained with a Leco TruMac CNS mass spectrometer (LECO Corporation, St. Joseph, MI, USA). Analyses of bulk soil pH in water and aqueous $CaCl_2$ were done following the method described by Carter and Gregorich (2008) using a Thermo Scientific Orion 2 pH meter (Thermo Fisher Scientific, Waltham, MA, USA). Finally, exchangeable cations were quantified following extraction in Mehlich-3 solution (Mehlich 1984), following the protocol outlined by Carter and Gregorich (2008). They were summed to produce an estimate of ECEC (Effective Cation Exchange Capacity). The element concentrations were determined by ICP spectroscopy (Perkin-Elmer Optima 7300 DV, Perkin-Elmer, Waltham, MA). Results of soil analyses (mean per site, stand and soil horizon) are given in Table S1.

1.4.4 DNA extraction and amplification

DNA extractions were carried out on 250 mg of thawed soil from each composite sample using PowerSoil DNA extraction kit (MoBio, Carlsbad, CA, USA). Only a small amount of soil was available for 13 of the samples and, in these cases, genomic DNA was extracted from at least 80 mg of soil. All soil DNA extracts were normalised to 4 ng/µL. DNA concentrations were assessed using fluorometric quantitation with a Qubit 2.0 (Invitrogen-Life Technologies, Carlsbad, CA, USA). DNA was also extracted from water that was used to wash field and laboratory materials. The fungal ITS1 region was amplified using Fwd: ITS5 GGAAGTAAAAGTCGTAACAAGG (White et al. 1990) and a modified version of Rev: 5.8S_Fungi CAAGAGATCCGTTGTTGAAAGTK, which improves taxonomic resolution and specificity to the fungal kingdom (Epp *et al.*, 2012). Each primer was synthesised with a tag of three degenerated nucleotides and eight variable nucleotides. Each DNA extract was amplified with a unique combination of tagged primers. Two PCR replicates were performed under the following conditions: the mixture was denatured at 95 °C for 10 min, followed by 35 cycles of 30 sec at 95 °C, 30 sec at 55 °C and 1 min at 72 °C; followed by a final step of 7 min at 72 °C; at the end of the process, the amplified samples were stored at 10 °C. Our PCR design included 13 negative controls containing only PCR mix without DNA extract. The number of PCR cycles and the choice of DNA concentration were determined after running several PCR tests. All PCR reactions were pooled and sent to the GENOTOUL sequencing platform (http://www.genotoul.fr/en/, Toulouse, France) for Illumina sequencing with the TruSeq Nano PCR-free kit and were loaded on an Illumina Miseq next generation sequencer. Sequencing was conducted using the paired-end sequencing technology (2x250 pb) with the chemistry V2.

1.4.5 Bioinformatics and sequence analysis

Based on the occurrence of sequences among samples, an abundance matrix was produced with *OBITool* package (Boyer *et al.*, 2016) and R script (R Core Team 2015, version 3.2.3, 2017). We first performed read-pairing assembly, read attribution to samples and read dereplication. We removed low-quality sequences that were shorter than expected (less than 50 bp) and which contained ambiguous nucleotides (based on the qphred scores, all over 50), displayed low score paired-end alignments (paired-end alignment score less than 50), or corresponded to singletons (sequences represented by one single read). This cleaning step allowed us to remove chimeras from the dataset. Clustering at 97 % identity (Nilsson *et al.*, 2008) was performed with the *OBITool Sumaclust* using the raw number of mismatches (deletions account as mismatches) as a measure of sequence dissimilarity. Subsequent taxonomic identification was performed primarily with BLAST (Altschul *et al.*, 1990) against the Genbank (genbank.com) extracted database (March 2016). The most similar sequence was reported for each OTU (Operational Taxonomic Unit). In order to assign OTUs to a taxonomic rank we used two methods and chose the one that gave the better assignment rate; the first method was to assign OTUs to UNITE Species Hypothesis (Kõljalg *et al.*, 2013) using PlutoF workbench (Abarenkov *et al.*, 2010). In a second step, we also used the *OBITool Ecotag* function, which analysed our sequences with Genbank sequences, formed clusters of most similar sequences, and assigned sequences to the closest ancestor that was shared with the most similar sequences in a cluster. The *Ecotag* function does not rely upon a threshold and OTUs can be assigned to a species or a higher taxonomic level, if the most similar sequences are taxonomically distant. With the first method, 38% of OTUs were assigned to a Species Hypothesis whereas up to 50% of OTUs were at least assigned to the family level with the ecotag function. Based on these results, the ecotag assignment was retained for further analyses.

The sequence dataset was transformed into an abundance matrix with R script by summing read abundances for sequences belonging to the same OTU. OTUs were removed from the dataset when the read count was maximal in negative extraction controls, water controls and PCR blanks. PCR replicates of the same sample were summed and finally, OTUs were assigned to a trophic status (i.e., saprotroph, ECM, plant parasite, other or unknown) based upon *FUNguild* software outputs (Nguyen *et al.*, 2016) and our knowledge regarding the ecology of ECM fungal genera. DNA sequence data are available on Dryad repository.

1.4.6 Statistical analysis

All statistical analyses were performed within R (R Core Team, version 3.2.3, 2017). R code is available on Dryad repository. Chemical content means were compared using an one-way ANOVA followed by Tukey post hoc tests. Species accumulation curves were computed for our 4 sample types (Annexe A, Figure A.2): black spruce organic (BS-O) and mineral (BS-M) layers, trembling aspen organic (TA-O) and mineral (TA-M) layers. Community analyses were performed on three different datasets: total, ECM and saprotrophic communities. Species richness was measured by the observed number of OTUs per sample and by the estimated richness index for the whole stand, i.e., Chao 1 (Chao and Lee, 1992). Given that the difference in species richness was compared at the patch level (4 sites * 2 dominant trees * 2 soil layers = 16 diversity estimates), non-parametric tests were used. A Mann Whitney test was used to test differences of richness estimates between BS- and TA-dominated stands. A second Mann Whitney test was performed to test differences in richness estimates between organic and mineral layers in each forest type.

The community data were used to calculate a dissimilarity matrix based on Bray-Curtis index. Analyses of the dissimilarity index were performed on three different datasets: total, ECM and saprotrophic communities. The effect of both qualitative (dominant tree species, site and soil horizon) and quantitative factors (soil chemical

analysis) on community composition were tested with permutational multivariate analysis of variance, i.e., PERMANOVA on dissimilarity matrix (*Adonis* function, *vegan* package in R, Oksanen *et al.*, 2017). Given that soil layer was nested within stands and stands nested within sites, the PERMANOVA was nested for these factors (site/stand/soil layer), and thus took into account the nested design of the sampling. Soil variables were included in the model in the following order: pH, C, N, P, K, Ca, ECEC, Mg, Mn, Al, Fe, C/N ratio and backward elimination of non-significant predictors from the saturated model was performed. The variance of Bray-Curtis distances between the combinations of dominant tree species and soil horizon was compared through a multivariate analogue of Levene's test (*betadisper* function, *vegan* package in R, Oksanen *et al.*, 2017). Finally, non-metric multidimensional scaling (NMDS) was implemented in order to visualise the effects of the dominant tree species and soil horizon on fungal communities. We used a two-dimension NMDS solution, as stress scores were always less than 0.2, which is acceptable for a large matrix. We tested correlations between soil characteristics and the NMDS space through *envfit* (in *vegan* package), and between OTU-richness of fungal families and ECM NMDS space. When *P*-values were significant ($P < 0.05$), *envfit* vectors of the soil characteristics were plotted on the NMDS.

1.5 Results

1.5.1 Soil chemical properties

Soil pH was lower in BS-dominated compared to TA-dominated ($P < 0.01$) stands, and was also lower in BS-O than in BS-M ($P < 0.01$). Soil pH mean values (± standard errors) per forest type were respectively 4.87 (± 0.47), 4 (± 0.26), 5.1 (± 0.24) and 5 (± 0.29) for BS-M, BS-O, TA-M and TA-O. Soil concentrations of N, S, P, K, Mg, C, Fe, Ca, Na and Mn were lower in the mineral soil than in the organic layer of both forest types, whereas CEC was higher in the mineral layer ($P < 0.01$). Nutrient concentrations of N, S, P, K, Mg and Mn were lower in BS-O than in TA

stands ($P < 0.01$). C and Fe concentrations were higher in BS-O compared to TA-O stands ($P < 0.01$). Soil Ca concentration was lower in BS than TA stands. Finally, C/N ratios were greater in BS-O than in the other soil types.

1.5.2 NGS data characteristics, community composition and taxonomic diversity

A total of 1 629 297 raw sequences were initially obtained from the 78 soil samples in addition to the 60 samples not included in this study. After quality filtering and removal of singletons, the dataset was reduced to 1 505 545 reads (of which 860 889 originated from composite soil samples used in this study), with an average length of 224.25 nucleotides. The resulting sequences were clustered into 1257 operational taxonomic units (OTUs) with a 97 % threshold. Of these, we removed 25 OTUs for which read count was the highest in negative controls and PCR blanks, and 103 that were non-fungal or unidentifiable OTUs (identified as Eukaryota, plant or insect), which left 1119 fungal OTUs in the final curated dataset (858 511 reads) for downstream analyses.

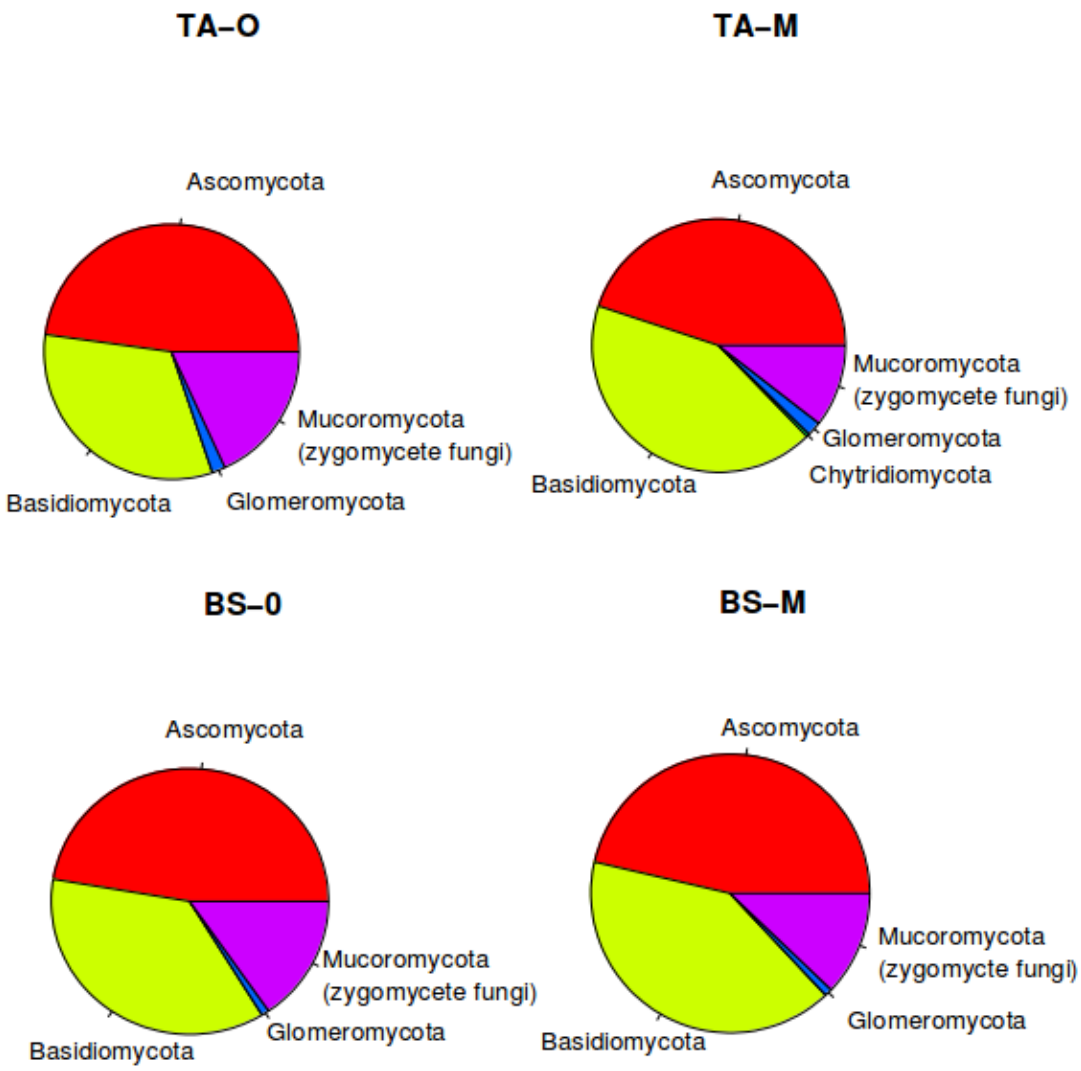

Figure 1.1 Number of OTUs per fungal phylum for black spruce organic layer (BS-O), black spruce mineral layer (BS-M), trembling aspen organic layer (TA-O) and trembling aspen mineral layer (TA-M) subsamples.

Ascomycota represented the dominant phylum (31.36 % of OTUs, 48.5 % of reads, see Annexe A, Figure A.3 for proportions of Ascomycota's order per sample type), while the Basidiomycota accounted for 27.8 % of OTUs and only 1.2 % of reads (see Annexe A, Figure A.4 for proportions of Basidiomycota's genus per sample type). The number of OTUs belonging to the Ascomycota and Basidiomycota were higher in mineral than organic samples ($P < 0.05$, Mann Whitney Test). On the basis of assignment results, all the zygomycete fungi belonged to the phylum Mucoromycota (Spatafora *et al.* 2016), and we used either zygomycete fungi or Mucoromycota, indistinctly, to qualify these specific fungi. They were the second most abundant phylum, with 43.5 % of reads and 9.3 % of OTUs. The number of Mucoromycota OTUs was higher in TA than in BS stands ($P < 0.01$, Mann Whitney Test). Few sequences belonging to Glomeromycota (1.07 % of OTUs, 140 reads) and Chytridiomycota (0.18 % of OTUs, 6 reads) were retrieved. Proportions of each phylum by sample types are available in Figure 1.1. Taxonomic assignments through the *OBITool ecotag* function was less precise (i.e., at the genus or family level, rather than species) than BLAST assignment. However, e-values for BLAST were often very low and so, not as accurate as expected, and an assignment to the family level seemed to be more reliable than the BLAST assignment. Based upon assignments through *OBITool ecotag* function, 36.1 % of the OTUs (comprising 64.8 % of the reads) were identified to the species-level, another 10.3 % (5.4 % of reads) were to genus, and a further 18.4 % of OTUs (16.9 % of reads) were identified to family-level.

The most abundant OTUs were tree pathogens, root endophytes and decomposers: *Coniochaeta mutabilis* (22.9 % reads, 97.5 % similarity with HQ157861), *Umbelopsis ramanniana* (12.5% reads, 97.6 % similarity with KF765446), *Umbelopsis* sp. II (10 % of reads, 97.9 % similarity with HQ157863), *Umbelopsis* sp. I (7 % of reads, 95.4 % similarity with KC007256), and *Trichoderma asperellum* (5 % of reads, 98.6 % similarity with JQ272391). When OTUs were assigned to

functional categories (trophic status) using FUNGuild, our results showed that the majority of taxa (389 OTUs) were either ECM fungi (15 % of OTUs, 1.9 % of reads) or saprotrophic fungi (decomposers) (19.7 % of OTUs, 29.8 % of reads). Only 5.6 % of OTUs were parasitic, and the remaining OTUs were not assigned to a trophic status (59.7 %). In total, 102 families and 129 genera of fungi were detected. Among the ECM fungi, *Russula* (14 OTUs), *Cortinarius* (14 OTUs), *Inocybe* (13 OTUs) and *Piloderma* (6 OTUs) were the most OTU-rich genera. Among the saprotrophic fungi, *Coniochaeta* (30 OTUs), *Penicillium* (13 OTUs), *Sphaerosporella* (11 OTUs) and *Archaeorhizomyces* (10 OTUs) were the most OTU-rich genera. A Venn diagram was used to visualise unique and shared OTUs; among 1119 OTUs, 557 were unique to one forest type (BS *vs* TA) and 565 were unique to one soil layer (Annexe A, Figure A.5).

1.5.3 Species richness and abundance

The average number of OTUs per sample was 119 ± 40 (10 630 reads ± 4431); the minimum and maximum number of OTUs were 45 (1418 reads) and 232 (19 050 reads), respectively. The estimate of the Chao diversity index suggested that between 61 and 75 % of the species were observed (Table 1). The accumulation curves of OTU richness showed a tendency to level off for all sample types, indicating that our sampling design was almost sufficient to describe the whole community, even when only 4 transects were sampled (Annexe A, A.2). Fungal species richness did not differ between the two forest types ($P = 0.098$), and BS-O hosted fewer OTUs (89.25 ± 21.98) than BS-M (129.25 ± 18.81; $P < 0.01$).

Table 1.1 Proportion of fungal guild per sample type based on the number of OTUs.

Dominant tree	Black spruce		Trembling aspen	
Soil layer	Mineral	Organic	Mineral	Organic
Observed richness	607	461	761	620
Estimated Chao (standard error)	896.8 (50.5)	691.7 (43.8)	958.7 (39.6)	833 (35.6)
Observed ECM richness	83	50	107	68
Estimated Chao ECM	128.1 (19.5)	82 (17.1)	162.1 (20.3)	116.7 (21.6)
Observed saprophyte richness	125	134	128	127
Estimated Chao saprophyte	180.4 (20.1)	185.3 (18.7)	168.6 (15.3)	184.8 (24.8)
% ECM	13.7	10.8	14.9	11
% Parasites	5	5.5	5.4	5.7
% Saprophytes	20.6	29.1	17.9	20.5
% Unknown guild	45.4	47.9	44	46.7
% Unique to sample type	16.8	12.7	19	15.4

The proportion of each trophic mode varied little among sample types (see Table 1.1). The average number of fungal OTUs that were recovered per sample was 9.5 ± 6.3 (mean ± SD) for ECM fungi and 26.1 ± 6.5 for saprotrophic fungi. Species accumulation curves for ECM fungi showed a tendency to level off, whereas the curve for saprotrophic fungi did not approach an asymptote (data not shown). In order

to detect whether diversity of saprotrophic and ECM fungi responded similarly to stand characteristics, these groups were analysed independently. ECM fungal species richness did not differ between forest types ($P = 0.82$), but mineral soil layers always hosted almost twice as many ECM OTUs than did the organic layers (BS, $P < 0.01$; TA, $P = 0.028$). Saprotrophic species richness did not differ between forest types and between soil layers ($P > 0.05$).

1.5.4 Fungal community composition across forest types and soil layers

In line with our first hypothesis, dominant tree species and soil layer had a direct and significant effect on total fungal community composition, and a strong indirect effect through soil pH and soil C concentration. Together, these factors explained 56.15 % of the variability, while 75 % of the variability was explained when other soil variables were included in the model (N, P, K, Ca, CEC, Mg, Mn, Al, Fe and C/N ratio; Table 2). The NMDS (Figure 1.2a) strongly supported results of the PERMANOVA and showed differences among the four sample types. According to *envfit* test, all soil parameters had a significant association with NMDS space. Variance of the community composition was similar for the two forest types, and always greater in the organic than in the mineral soil layer (Figure 1.3a, $P < 0.001$).

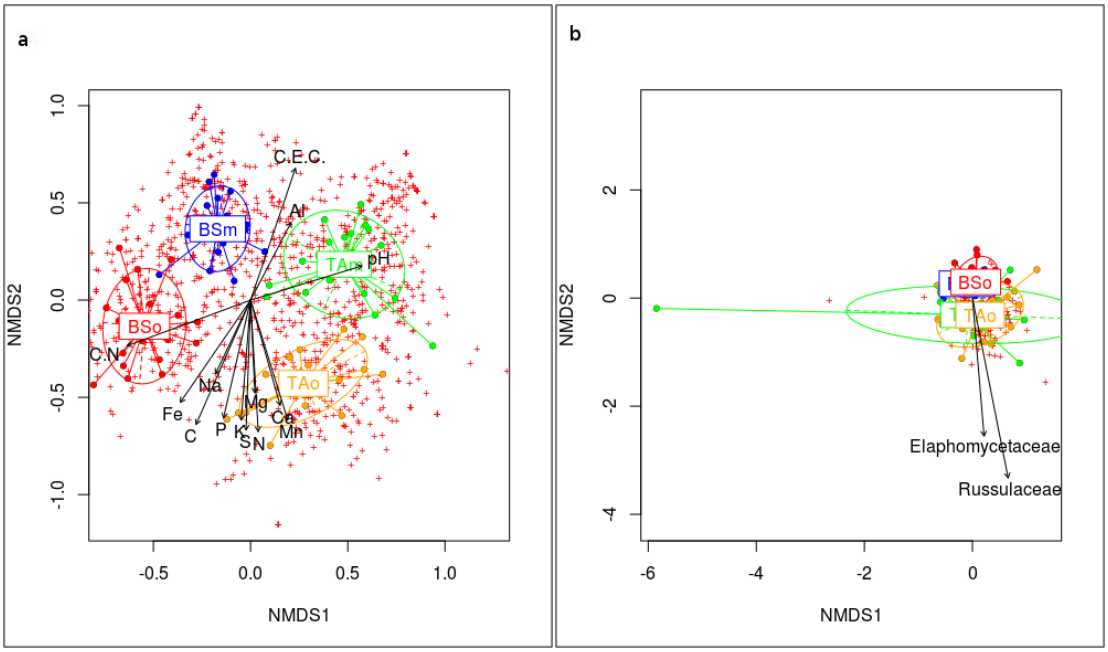

Figure 1.2 Non-metric multidimensional scaling (NMDS) plots representing similarity of total (a) and ectomycorrhizal (b) fungal communities from black spruce organic layer (BS-O, red), black spruce mineral layer (BS-M, blue), trembling aspen organic layer (TA-O, orange) and trembling aspen mineral layer (TA-M, green). Factors significantly explaining sample distribution among sites are represented by arrows. In order to clarify plots, soil factors are only presented for the total community and the influence of family abundance per site is presented only for ectomycorrhizal fungi.

Table 1.2 results of the PERMANOVA for the global, ECM and saprophytic communities, signif code : *** P < 0.001 ; ** P < 0.01 ; * P < 0.05 ; . P < 0.1

Data	Complete dataset		ECM fungi		Saprophytic fungi	
Variable	R2	Signif code	R2	Signif code	R2	Signif code
Site	0.06705	***	0.09656	***	0.12618	***
PH	0.14745	***	0.04559	***	0.02771	**
C total	0.20995	***	0.04376	***	-	-
N total	0.02892	***	0.04376	***	-	-
P	0.01226	**	0.01793	*	-	-
K	0.02639	***	0.1549	.	-	-
Ca	0.01244	*	-	-	-	-
C.E.C.	0.01699	**	0.01401	.	-	-
Mg	0.01156	*	-	-	-	-
Mn	0.01033	*	-	-	-	-
Al	0.00993	*	-	-	-	-
Fe	0.00958	*	0.01848	*	-	-
C/N	0.01132	*	-	-	-	-
Site:dominant tree	0.10649	***	0.10949	***	0.11589	***
Site:dominant tree:soil layer	0.09699	***	0.10777	**	0.12110	*
Residuals	0.22236	-	0.51011	-	0.60912	-

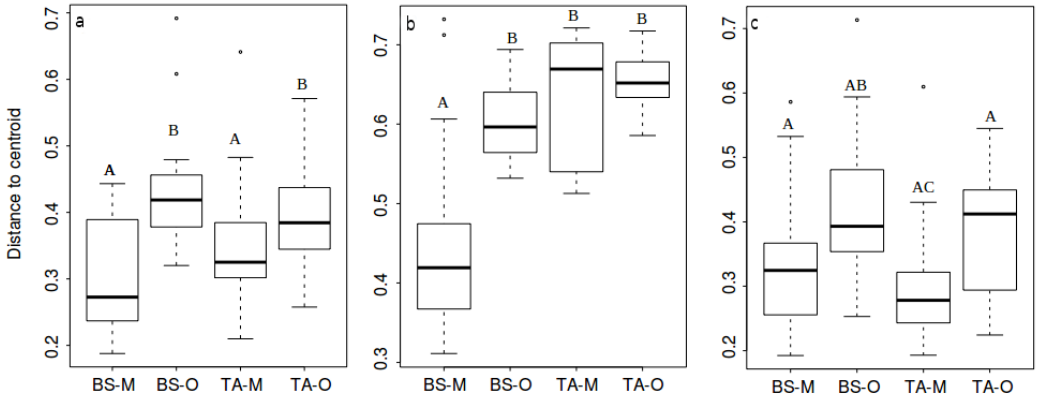

Figure 1.3 Boxplot showing distance to the centroid and therefore the variance of Bray-Curtis distances within each sample type (BS-M = Black spruce mineral layer, BS-O = Black spruce organic layer, TA-M = Trembling aspen mineral layer, TA-O = Trembling aspen organic layer) and for total (a), ectomycorrhizal (b) and saprotrophic (c) fungal communities. Bar with the same letter within a graph are not significantly different.

Considering only ECM fungi, dominant tree species, soil layer and site all had a significant effect on community composition (10.9 %, 10.8 % and 9.6 % of variance explained, respectively: Table 1.2). Soil pH and C concentrations also significantly affected ECM community composition but they explained less than 5 % of the variation (Table 1.2, Figure 1.2b). Soil N, P, K, Fe and ECEC also explained a small part of ECM community composition. Community compositions were more variable under TA than under BS (average distances to centroids are respectively 0.56 and 0.45; $P < 0.001$; Figure 1.3b), regardless of soil layer. NMDS confirmed the PERMANOVA results, as sampling points were grouped by dominant tree species. Moreover, the distributions of Russulaceae and Eurotiales (Elaphomycetaceae) were

correlated with changes in communities. Both were represented by more OTUs under TA as compared to BS.

The best predictors of saprotrophic community composition were soil layer and dominant tree species (12.1 % and 11.6 % of variance explained, respectively; Table 2). Soil pH also had a moderate effect on saprotrophic fungal community composition, while the other chemical measurements had no significant effect on saprotrophic community. Communities were more variable under BS than under TA ($P < 0.001$, Figure 1.3), especially in the organic compared to the mineral layer ($P < 0.01$).

To summarise, much of the community variance was explained by measured variables for the total (i.e., all OTUs; around 77 % of explained variability), ECM (around 49 %) and saprotrophic (around 39 %) communities, and the dominant tree species had a driving effect for all communities.

1.6 Discussion

1.6.1 A high level of diversity revealed by NGS in the boreal forest

The use of NGS revealed high fungal diversity (1119 OTUs) for a forested sampling area of only about 2 ha, with 63.9 % of unknown fungal species. Other studies have also detected a high diversity of fungi at higher latitudes (Tedersoo *et al.*, 2014), including in boreal forests (Shi *et al.*, 2014; Taylor *et al.*, 2013). Taylor *et al.*, (2013) further found that numerous OTUs could not be taxonomically assigned to the species level (72 % of OTUs). Compared with previous studies in the boreal forest, our study differed in the abundance of each phylum, but not in the number of OTUs. In mature mixed black spruce forest of Alaska sampled in mid-season (August and September), Basidiomycota represented 39.6 % of OTUs and 48 % of reads (Taylor *et al.*, 2013), whereas they represented 27.8 % of OTUs and 1.2 % of reads in our study. Such a

difference is difficult to interpret as these results arise from different DNA-extractions, PCR and sequencing protocols that can slightly bias the resulting community. Nevertheless they likely reflect the lower abundance of ECM fungi in our system. Moreover, we detected a high abundance of Mucoromycota (43.5 %), whereas they only represented < 1 % in Taylor *et al.*, (2013) and 17.3 % in Santalahti *et al.*, (2016) who sampled pine forests in Finland. A recent study carried out in the Clay Belt of Abitibi-Témiscamingue (around 100 km southwest of our study sites) also revealed a high abundance of Mucoromycota in the wood and leaf litter of TA (up to 35% of sequences) and jack pine (up to 51.4% of sequences), and especially in the highly degraded organic matter layer (Foudyl-Bey *et al.*, 2016). Given these results, the high abundance of Mucoromycota that we found seems to be standard in the region and we are confident that this result does not arise from a methodological bias. Study sites in Alaska were generally drier than study sites in the Abitibi region (annual precipitations are around 286mm in Alaskan sites whereas they are 890mm in our sites, including snow melt). Zygomycote fungi are described as opportunistic "snow molds" in alpine forest-tundra ecotone (Schmidt *et al.*, 2008), which could be an adaptation of this ecological group of fungi to soils with extended periods of thick snow cover. Adding reference sequences from fruiting bodies and specimens that were collected in the boreal forest might help identify more sequences than with NGS data only, and to serve to link those environmental sequences with fungal ecology, as suggested by Truong *et al.* (2017).

1.6.2 Dominant trees shape fungal communities

Dominant tree species affected total, ECM and saprotrophic communities in the two soil layers, and this effect resulted in a change in community composition rather than variation of species richness. Interestingly, the soil layer and the dominant tree explained a similar proportion of the variance (ca.10 % each) for all fungi and all fungal guilds. Although other studies already found differences in fungal

communities under conifers and hardwood trees, few studies have compared different forests of similar age, even though stand age may also shape fungal communities (Molina *et al.*, 1992; Twieg *et al.*, 2007). Soil parameters, particularly C content and pH, were highly correlated with total fungal communities (21% and 14.7% of the variance explained, respectively). Curiously, the effect of soil pH and C content was weaker on ECM (4.4 % and 4.6 %, respectively) and saprotrophic communities (only soil pH had a significant effect and explained only 2.8 % of the variance) than on the total community. As parent soil material was identical on all our sites, differences in soil chemistry could be attributed to biotic activities, as proposed by Légaré *et al.*, (2005). Nutrient concentrations were always lower in mineral and BS soils and could thus have been altered by plant community composition. Nutrient concentrations may also be altered by fungal communities and in turn affect plant communities by facilitating the post-fire establishment success of one species. The strong correlation we detected between fungal communities' composition and soil nutrient concentrations suggests that these two soil components are firmly linked in boreal forests. Soil nutrient concentrations and fungal communities together probably exert a positive feedback on the stand-level dominance of TA or BS, as was already demonstrated in ECM-dominated temperate forest ecosystems (Bennett *et al.*, 2017).

1.6.3 Community composition and variability are distinct for ECM and saprotrophic fungi

TA stands hosted almost twice as many exclusive OTUs than did BS, showing that these patches, despite their small size and discontinuity within the coniferous landscape, hosted a more diverse fungal community than BS. On one hand, TA stands were not clearly disconnected from BS stands and had 41 % of their fungal species in common. On the other hand, only a few ECM fungi were shared between the two forest types (15 OTUs were common to all sample types, representing 14.3 % and 12 % of ECM OTUs that were associated with BS and TA, respectively); TA hosted

more stand-specific ECM fungi than BS (61 and 41 exclusive OTUs, respectively). ECM communities under TA were also particularly variable among forest patches, and such beta diversity could be related to the great number and diversity of seedlings and saplings of other ECM species in the understorey of TA stands (Ishida *et al.*, 2007; Tedersoo *et al.*, 2012). Indeed, TA stands were colonised by *Abies balsamea*, *Pinus banksiana*, *Picea mariana* and *Alnus incana* subsp. *rugosa* (Du Roi) R.T. Clausen (= *A. rugosa* Du Roi), while the understorey of BS was sparse and poor in other ECM tree species (Légaré *et al.*, 2005; authors Pers. Obs.). Several studies have demonstrated that ECM networks displayed modularity or anti-nestedness patterns, indicating that the presence of several ECM hosts was correlated with a high degree of variation in community composition (Bahram *et al.*, 2014; Taudiere *et al.*, 2015). In the organic layer, although Ascomycota and saprotrophic fungi were not more diverse than in the mineral layer, the saprotrophic community composition was more variable than in the mineral soil. Variability of saprotrophic communities that are associated with leaf and wood litter has already been observed in Quebec's boreal forest, and has been correlated with different decomposition stages and litter identity (Foudyl-Bey *et al.*, 2016). In our study, the saprotrophic community was more variable under BS than under TA; BS is characterized by low diversity and by the presence of a thick moss layer. Mosses can host several fungi that do not necessarily form symbioses; rather, they degrade and inhabit dead parts of mosses. Moss-fungal interactions are known to be complex (Davey and Currah, 2006) and relatively host-specific (Davey *et al.*, 2017; Hirose *et al.*, 2016). As the moss community is very diverse under BS in this area (Fenton and Bergeron, 2008), it would be interesting to investigate moss-fungal interactions and test whether these could explain some of the variability that was observed under BS stands.

1.6.4 Conclusion

Delineation of the boundary between balsam fir-paper birch and black spruce-feather moss domains of the boreal forest and its possible climate and land-use induced northward migration has been hitherto only based upon plant species distributions and modelling (Messaoud *et al.*, 2007a,b). Considering the strong links that we detected between dominant tree cover and the distribution of numerous fungi that are exclusively associated with certain plant species, the question regarding their ecological roles and possible feedbacks on forest dynamics emerges. The known differences in nutrient turnover between TA and BS stands could be correlated with the shift in saprotrophic species composition that we observed between the two forest types. In this context, a change in saprotrophic community may be associated with a change in forest stand productivity. Moreover, the high turnover that was observed for ECM fungi under TA stands could partly be explained by the large amount of ECM-trees of other species in TA stands and potentially points to a large pool of compatible fungal species for migrating tree species, such as balsam fir. In a context of climate change, the increasing proportion of TA in the spruce-feather moss forest may be partly amplified by a positive plant-fungal-soil feedback in TA-dominated stands. The future of the boreal forest may be tightly linked with its fungal communities, and future studies at the limit of boreal forest domains would be particularly useful for understanding the role of fungi in poleward tree migration.

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CHAPITRE II

FACILITATION OF BALSAM FIR BY TREMBLING ASPEN IN THE BOREAL FOREST: DO ECTOMYCORRHIZAL COMMUNITIES MATTER?

(FACILITATION DU SAPIN BAUMIER PAR LE PEUPLIER FAUX-TREMBLE EN FORÊT BORÉALE : UNE ACTION DES COMMUNAUTÉS ECTOMYCORHIZIENNES?)

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2.1 Abstract

Succession is generally well described above-ground in the boreal forest, and several studies have demonstrated the role of interspecific facilitation in tree species establishment. However the role of mycorrhizal communities for tree establishment and interspecific facilitation, has been little explored. At the ecotone between the mixed boreal forest, dominated by balsam fir and hardwood species, and the boreal forest, dominated by black spruce, several stands of trembling aspen can be found, surrounded by black spruce forest. Regeneration of balsam fir seems to have increased in the recent decades within the boreal forest, and it seems better adapted to grow in trembling aspen stands than in black spruce stands, even when located in similar abiotic conditions. As black spruce stands are also covered by ericaceous shrubs, we investigated if differences in soil fungal communities and ericaceous shrubs abundance could explain the differences observed in balsam fir growth and nutrition. We conducted a study centered on individual saplings to link growth and foliar nutrient concentrations to local vegetation cover, mycorrhization rate and mycorrhizal communities associated with balsam fir roots. We found that foliar nutrient concentrations and ramification index (colonization by mycorrhiza per length of root) were greater in trembling aspen stands and were positively correlated to apical and lateral growth of balsam fir saplings. In black spruce stands, the presence of ericaceous shrubs near balsam fir saplings affected ectomycorrhizal communities associated with tree roots which in turn negatively correlated with N foliar concentrations. Our results reveal that fungal communities observed under aspen are drivers of balsam fir early growth and nutrition in boreal forest stands and may facilitate ecotone migration in a context of climate change.

Keywords: *Abies balsamea*, boreal forest, ectomycorrhiza, ericaceous shrubs, facilitation, *Picea mariana*, *Populus tremuloides*

2.2 Résumé

La succession végétale est généralement bien décrite en forêt boréale et de nombreuses études ont démontré un rôle de la facilitation interspécifique dans l'établissement des arbres. Cependant, le rôle des communautés mycorhiziennes dans l'établissement et la facilitation interspécifique entre arbres est moins connu. Des peuplements dominés par le peuplier faux-tremble et entourés par des épinettes noires sont fréquemment trouvés à l'écotone entre d'une part la forêt boréale mixte, dominée par le sapin baumier et des espèces de feuillus, et d'autre part la forêt boréale, dominée par l'épinette noire. La régénération du sapin baumier en forêt boréale semble être en augmentation depuis quelques dizaines d'années, et sa croissance est meilleure sous les peupliers faux-trembles que sous les épinettes noires, pour des conditions abiotiques similaires. Les peuplements d'épinettes noires abritant des plantes éricacées en sous-bois, nous avons exploré si les différences dans leur abondance et dans les communautés fongiques pouvaient expliquer les différences observées dans la croissance et la nutrition du sapin baumier. Nous avons mené une étude individu-centré sur des jeunes plants de sapin baumier pour relier la croissance et la concentration de nutriments dans les aiguilles aux communautés végétales locales, au taux de mycorhization et aux communautés ectomycorhiziennes associées à ses racines. Nous avons déterminé que la concentration en nutriments dans les aiguilles et l'indice de ramification (nombre d'apex racinaires mycorhizés par cm de racine) étaient plus importants dans les peuplements de peupliers et qu'ils étaient positivement corrélés à la croissance latérale et apicale du sapin baumier. Dans les peuplements d'épinette noire, la présence des plantes éricacées à proximité du sapin baumier était corrélée à une modification des communautés ectomycorhiziennes associées aux racines du sapin ce qui a eu un effet négatif sur la concentration foliaire en N. Nos résultats indiquent que les communautés mycorhiziennes observées sous les peupliers sont des acteurs importants de la croissance et de la nutrition du sapin

baumier en forêt boréale et pourraient faciliter la migration de l'écotone dans un contexte de changement climatique.

Mots-clefs: *Abies balsamea*, forêt boréale, ectomycorhize, Ericacées, facilitation, *Picea mariana*, *Populus tremuloides*

2.3 Introduction

Tree establishment, growth and survival in a new area are primarily dependent upon seed availability and local environmental conditions. For a given tree species, its capacity to establish under new biotic conditions is predominantly driven by either facilitation or competitive processes (Callaway and Walker, 1997). Within a multi-species environment, species can compete for resources such as light, nutrients or water. At the same time, survival of one species can be facilitated by another species providing protection against predators or extreme climate events (Stachowicz, 2001), or by the presence of their mycorrhizal symbionts which could result in mycorrhizal networks (Pickles and Simard, 2017; Simard *et al.*, 2015), or exchanges of resources (Brooker *et al.*, 2008; Teste *et al.*, 2009). Among the consequences of climate change, changes in species distribution are already observed and could result in new or modified species interactions. Changes in distribution range of species are particularly visible at ecotones between forested ecosystems (Barbeta and Peñuelas, 2017; Messaoud *et al.*, 2007a) or at the northern tree line (Harsch *et al.*, 2009; Ratcliffe *et al.*, 2017).

In Québec (Canada), the 49th parallel represents the ecotone between the southern balsam fir-paper birch (mixedwood boreal forest) and the northern black spruce-feather moss (boreal forest) bioclimatic domains. It has been suggested that this ecotone is likely to migrate northward with climate change (Messaoud *et al.*, 2007a). South of this ecotone, forest stands are mixed and dominated by *Abies balsamea* [L.] Mill. (balsam fir-BF), *Picea glauca* [Moench] Vosswhite spruce (white spruce),

Populus tremuloides Michx. (trembling aspen-TA) and *Betula papyrifera* Marsh. (paper birch), whereas *Picea mariana* [Mill.] BSP (black spruce-BS) and *Pinus banksiana* Lamb. (Jack pine) dominate north of the ecotone (Robitaille and Saucier, 1996). In the southern part of the black spruce-feather moss domain, small stands of trembling aspen occur, in which trembling aspen can be dominant (more than 75 % of the canopy cover) or mixed with black spruce (between 25 % and 75 % of the canopy cover; Cavard *et al.*, 2011; Légaré *et al.*, 2005). Following classical forest dynamics in these forests, TA would eventually be replaced by BS in the absence of disturbance (Belleau *et al.*, 2011; Lecomte and Bergeron, 2005), thereby maintaining BS dominance in the area. In areas where balsam fir establishes under BS- and TA-dominated stands, a greater abundance of more vigorous saplings has been observed under TA (Arbour and Bergeron, 2011). As those two types of stands are very close geographically and grow under similar climate, slope and substrates (Légaré *et al.*, 2005, Cavard *et al.*, 2011), the idea of an abiotic determinism could be rejected. On the contrary, aspen-dominated and spruce-dominated stands have different understorey plant and fungal communities, and soil organic contents (Cavard *et al.*, 2011; Légaré *et al.*, 2005; Nagati *et al.*, 2018), suggesting that biotic factors could explain differences in balsam fir growth. Indeed several mechanisms resulting from microbe-mediated interactions could enhance or reduce BF nutrition and growth, and may be involved in plant-plant interactions in the boreal forest.

Among the fungi that interact with tree roots, including BF roots, ectomycorrhizal fungi (EMF) are particularly abundant in boreal soils (Taylor *et al.*, 2013). These fungi improve tree growth and foliar nutrient status in ecosystems where nutrients are mainly found in organic forms, as in boreal forests (Franklin *et al.*, 2014; Inselsbacher and Näsholm, 2012; Smith and Read, 2008). At a worldwide scale, closely related host species tend to share more similar EMF communities than more phylogenetically distant host species (Tedersoo *et al.*, 2013), which may result in greater similarity between BF and BS fungal communities. However, at the local

scale, cases of facilitation through mycorrhizal symbioses have rather been detected between phylogenetically distinct plants (Dickie *et al.*, 2006; Nara and Hogetsu, 2004; van der Heijden and Horton, 2009). Mycorrhizal fungi could also shape plant-plant interactions through indirect interactions with plants (Bever *et al.*, 2010). The understorey vegetation under TA is dominated by various ectomycorrhizal tree saplings, endomycorrhizal (AM) shrubs and herbaceous species (Légaré *et al*., 2005; Cavard *et al*., 2011) while BS stands understorey is covered by a thick layer of bryophytes and ericaceous shrubs. Limitations to tree establishment and growth for EMF tree species that are adjacent to ericaceous shrubs, which are associated with ericoid fungi, have been documented many times in various ecosystems (*e.g*., Mallik, 2003; Peterson, 1965; Walker *et al.*, 1999; Yamasaki *et al.*, 1998; Zackrisson *et al.*, 1997). Although the mechanisms explaining such regeneration failures of EMF plant species near ericoid shrubs are not clearly understood (Collier and Bidartondo, 2009; Gallet, 1994; Peterson, 1965; Richard *et al.*, 2009), potential alterations to BF mycorrhizal colonization and mycorrhizal networks cannot be excluded in our system that would explain BF growth differences between TA- and BS-dominated stands.

Our aim was to disentangle the processes controlling BF early growth and nutrition at this boreal ecotone. To achieve this goal, we monitored growth parameters of individual BF saplings for two consecutive years and investigated their EMF communities and their foliar nutrient concentrations under BS and TA stands. We hypothesized that 1) local conditions under TA would lead to greater mycorrhization rates and different EMF communities than under BS and 2) the presence of ericaceous shrubs near BF saplings would reduce growth and mineral nutrition through their effects on EMF symbioses.

2.4 Material and methods

2.4.1 Site description and fir sapling selection

The study was located across a 36-km^2 area within the Clay Belt of northern Québec and Ontario (Canada). Four paired and unmanaged sites within the black spruce-feather moss domain were selected for study (Table 3). Each pair of stands contained one that was dominated by TA, while the other one was dominated by BS (for more information on sites, see Nagati *et al*., 2018). At each site, 15 BF saplings that were 25-75 cm tall and separated from one another by at least 5 m were selected: five in TA stands; five in BS stands at least 4m away from an ericaceous shrub; and five in BS stands at a maximum distance of 3m from an ericaceous shrub (BSE, Labrador tea (*Rhododendron groenlandicum* [Oeder] Kron & Judd) or sheep-laurel (*Kalmia angustifolia* L.)). Our sampling design permitted to sample 15 saplings per site (10 in BS stands and 5 in TA stands), resulting in 60 saplings.

Table 2.1 GPS coordinates of sites (BS= black spruce – *Picea Mariana*, TA = trembling aspen – *Populus Tremuloides*)

Site	Stand	Latitude	Longitude
1	BS	49.19061	-78.82797
	TA	49.18942	-78.82817
2	BS	49.19367	-78.83556
	TA	49.19375	-78.8345
3	BS	49.196695	-78.842092
	TA	49.196422	-78.84237
4	BS	49.168972	-78.885194
	TA	49.180417	-78.883611

2.4.2 Balsam fir measurements

Annual apical growth (cm) was measured in August 2015 and 2016 with calipers and the two measures were summed. Annual lateral growth was measured on two randomly selected branches per sapling in August 2015 and 2016 with calipers, averaged by year, and then summed. In 2016, the basal diameter of stems (cm) was measured with calipers. Fir needles were harvested in August 2016, forced air-dried at 30 °C for 12 h, and sent to the Laurentian Forestry Centre, Canadian Forest Service (Quebec, QC) for chemical analyses. Percentage foliar C and N were measured with a

Leco TruMac CNS mass spectrometer (LECO Corporation, St. Joseph, MI, USA). Minor and major cation concentrations (g/kg of needles) were obtained by ICP-OES with a Perkin-Elmer Optima 7300 DV (Waltham, MA, USA), following the method proposed by Kalra (1998).

2.4.3 Characterization of local biotic environment

Given that the main goal of our study was to determine whether biotic interactions affected BF growth and foliar nutritional status, we characterized their local biotic environment. Forest composition within a 3 m radius around each balsam fir sapling was evaluated by measuring the percentages of TA, BS and BF relative to their DBH (diameter at breast height, 1.3 m). Only trees > 5 cm DBH were taken into account. These data allowed to calculate the percent of each tree species (TA, BS and BF) in the canopy around each BF sapling.

2.4.4 Mycorrhizal root tip counts, mycorrhizal DNA extraction, amplification and sequencing

Mycorrhizal communities were assessed for each sapling to test their effect on individual BF growth and foliar nutrient status. The root system of each sapling was gently excavated in August 2016 and washed to preserve mycorrhizal root tips. Mycorrhizal root tip counts were performed the same day that the roots were extracted. For each sapling, we randomly selected three 10-cm root fragments for live mycorrhizal root tip counts under a magnifying glass to calculate mycorrhization rate (number of EMF root tips/total number of root tips) and ramification index (number of EMF root tips per 10 cm root). For each fir sapling, 50 mycorrhizal root tips were sampled and stored in CTAB 2X at -20 °C until DNA extraction. Root tips were pooled by tree sapling and manually ground with a pestle before DNA extraction. DNA was extracted with a PowerSoil DNA extraction kit (MoBio, Carlsbad, CA, USA) following the manufacturer’s instructions. A negative blank extraction

(extraction without any material) was performed for every set of 23 extractions. In addition, a tool control was performed on tools that were used for field and laboratory work by extracting DNA from distilled water that was used to wash tools. DNA amplification was performed using the method described in Nagati *et al*. (2018). Briefly, the fungal ITS1 region (Forward: ITS5 GGAAGTAAAAGTCGTAACAAGG, White *et al.*, 1990; and a modified version of Reverse: 5.8S_Fungi CAAGAGATCCGTTGTTGAAAGTK, Epp *et al.*, 2012) was amplified for 35 PCR cycles. PCR samples were sent to GENETOUL GetPlaGe (Toulouse, France) for sequencing on an Illumina MiSeq platform with the TruSeq Nano PCR-free kit. Sequencing was conducted using the paired-end sequencing technology (2 x 250pb) with the chemistry V2.

2.4.5 Bioinformatics and sequence analysis

An abundance matrix was constructed with *OBITool* packages (Boyer *et al.*, 2016) and R script (R Core Team 2018, version 3.2.3, 2018) based upon the occurrence of sequences among samples. We performed read-pairing assembly, read attribution to samples, read dereplication, and removal of low-quality sequences (shorter than expected, containing ambiguous nucleotides, displaying low score paired-end alignments, or corresponding to singletons). *OBITool Sumaclust* was used to cluster sequences as OTUs (Operational Taxonomic Units) at a 97 % identity threshold (Nilsson *et al.*, 2008). Taxonomic assignment was performed with the *OBITool Ecotag* function against the GenBank extracted database (ftp://ftp.ncbi.nih.gov/genbank/). As was the case for our previous dataset that was collected in the same geographic area (Nagati *et al.*, 2018), taxonomic assignments were more accurate with Genbank than with the UNITE public database (https://unite.ut.ee/). Trophic guild assignment was based upon FUNguild software outputs (Nguyen *et al.*, 2016). Sequences belonging to the same OTU were then summed by sample. Lastly, we removed OTUs that were dominant (with the highest

read count) in negative or tool extraction and amplification controls, OTUs not belonging to fungi, or OTUs with coarse-resolution taxonomic assignments (*i.e.*, assigned to Eukaryota).

2.4.6 Statistical analyses

Statistical analyses were performed in R (R Core Team, 2018). Data are available at Dryad repository (doi:10.5061/dryad.914j5m0). Our main goal was to compare individual traits and fungal communities among sapling types (BS, BSE, TA). Abundance of each OTU was used to avoid giving too much importance to rare OTUs, as recommended for fungal ITS (Unterseher et al., 2011; Lindahl et al., 2013). Ectomycorrhizal species richness, Shannon index (*H'*), ramification index and mycorrhization rate were each calculated and compared according to one-way ANOVA (three levels), followed by Tukey *post-hoc* tests of the means. Foliar nutrient status among saplings growing under BS, BSE, and TA, were compared for each nutrient (N, P, K, Ca and Mg) with non-parametric Kruskall-Wallis tests, given that the data were not normally distributed, followed by Dunn's *post-hoc* tests with Bonferroni corrections. As well, sapling growth between the three modalities was compared with Kruskall-Wallis tests and Dunn's *post hoc* tests with Bonferonni corrections.

Differences in abundance of each ectomycorrhizal family represented in root tip samples were tested between sapling types with Kruskall-Wallis tests, followed by Dunn *post-hoc* tests with Bonferroni corrections. We only tested differences in abundance at the family level, given that about 30 % of OTUs could not be assigned to a genus. Differences in ectomycorrhizal community structure between the roots of the three sapling types were visually described with Non-Metric Multidimensional Scaling (NMDS, *vegan* package in R; Oksanen *et al.*, 2017) and coordinates of scores on the first two axes of NMDS were extracted for further analyses. Correlation between the NMDS space and individual measures of fir saplings (nutrient

concentrations and growth) were tested with envfit (vegan package). Envfit vectors of individual measures were plotted on the NMDS space when p-values where significant ($p < 0.05$). Differences in ectomycorrhizal community structure between sites, dominant plant communities (BS, BSE and TA) were tested with PERMANOVA (*Adonis* function, *vegan* package; Oksanen *et al.* 2017) with nested factors (site / plant community).

2.4.7 Path analysis

To test direct and indirect effects of the local biotic environment on growth (for this analysis, growth measures from 2015 and 2016 were summed) and foliar N concentrations, we compared three hypotheses by fitting structural equation models (SEM) to the data (with lavaan package; Rosseel *et al.*, 2018). We included only foliar N concentrations in our model, as it was the only nutrient concentration available for all saplings. We constructed three SEMs to represent three *a priori* hypotheses. Each of the hypotheses is rooted in current knowledge regarding the processes that have been described in the literature, and which are described below. All models were fitted to centered and reduced data.

Complete model Mod1: Considering direct links, we formulated five hypotheses. The first was that foliar N concentration was positively correlated with growth (Pallardy and Kozlowski, 2008). The second one was that EMF abundance and communities were correlated with growth and foliar N concentrations (Smith and Read, 2008). The third hypothesis was that BS stands and the percent of BS near fir saplings were negatively correlated with BF growth and foliar N concentrations (Arbour and Bergeron, 2011). The fourth hypothesis was that the presence of ericaceous shrubs near saplings was negatively correlated with BF growth and nutrition (Mallik, 2003; Peterson, 1965; Yamasaki *et al.*, 1998). Finally, we hypothesized that the percentage of conspecific mature trees near BF saplings was positively correlated with their growth and foliar N, given that local conditions have permitted their growth and

survival. Lateral and apical growth were added as co-variables in the model and together co-varied with basal diameter of BF saplings.

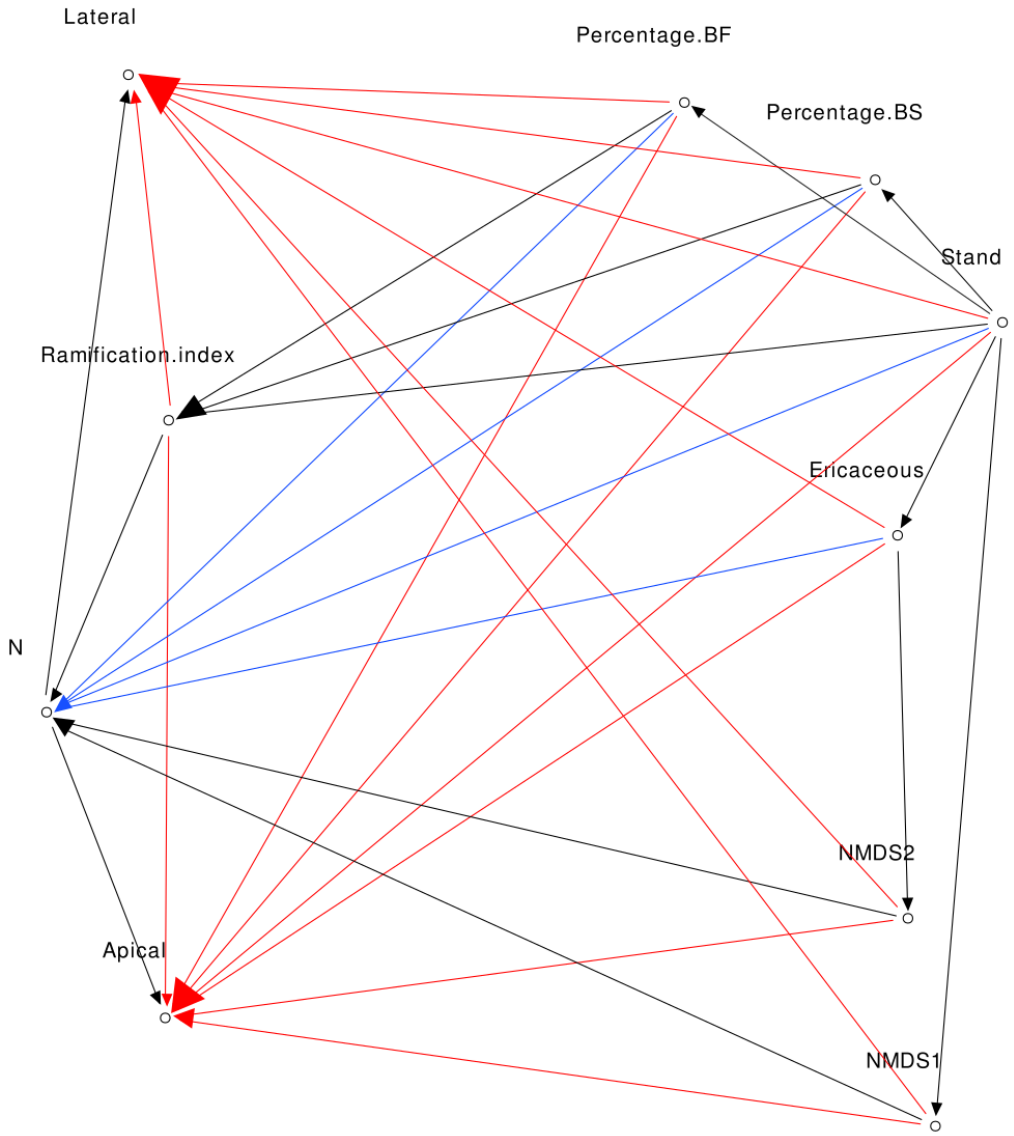

Figure 2.1 Direct acyclic graph of the complete model Mod1 (red, blue and black arrows), Mod2 (blue and black arrows) and Mod3 (black arrows).

For this model, we also formulated the following indirect linkage hypotheses: the relative percentage of BF was correlated with stand type (Arbour and Bergeron, 2011). The relative percentage of BS was correlated with the stand (Cavard *et al.*, 2011). The presence of ericaceous shrubs was correlated with the stand type (BF saplings in TA stands were never next to ericaceous shrubs). The ramification index was correlated with the stand (see Figure 2.3), and percent of BF (the presence of mature trees near BF saplings increased the probability of encountering EMF partners). The NMDS first axis was correlated with the stand and the second axis negatively with the presence of ericaceous shrubs (see Figure 2.5 and PERMANOVA results). The complete model is presented in Figure 2.1.

Nutrient model Mod2: the indirect links are the same as for Mod1 but in this model we assumed that foliar N concentration was the only variable that was correlated to growth.

Fungi centered model Mod3: the links are the same as for Mod2, but in this model we assumed that only variables linked to EMF were correlated with foliar N concentrations. In this model, NMDS first and second axes and ramification index were the only variables directly linked to N concentrations.

To ensure that our models respected independence between non-linked variables, we performed Fisher's C test (*ggm* package, Marchetti *et al.*, 2015). Models with *p*-values greater than 0.05 are considered to have respected claims of independence (Shipley, 2000). A model was considered to be representative of the population if the *p*-value of the Chi-square test was greater than 0.05. For each model, we calculated the Comparative Fit Index (CFI) and the Tucker-Lewis Index (TLI) to evaluate how each model fits to the data. Values greater than 0.95 were considered to be good fits (Hu et Bentler, 1999). Each structural equation model with Fisher's C test and Chi-Square *p*-values > 0.05, CFI > 0.95, and TLI > 0.95 could describe the data well, we used these values as a primary filter to ensure that models fit well the data and are

representative of the population. After applying this filter our aim was to select the best model based on AIC criterion, however only Mod1 passed the primary filter and was de facto selected.

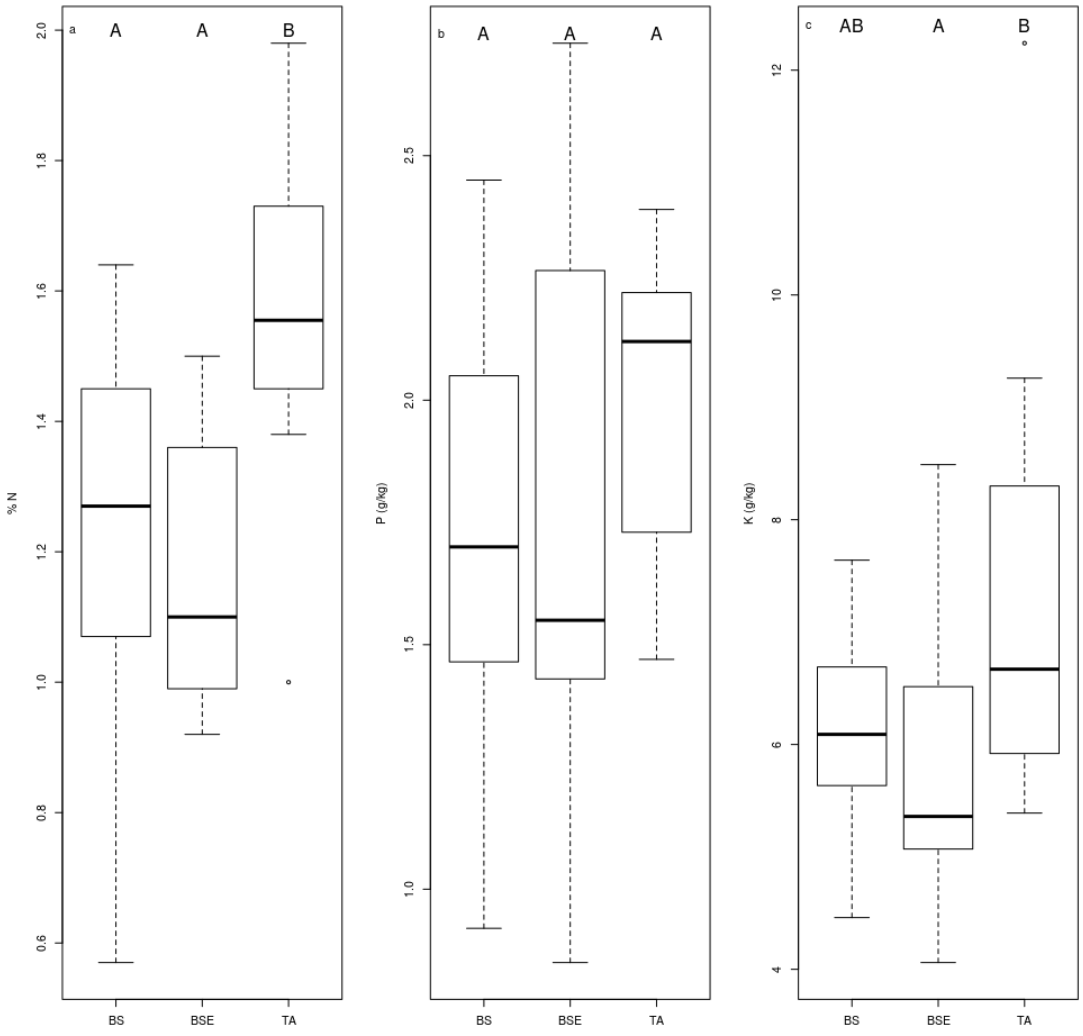

Figure 2.2 Boxplot chart representing foliar a) N, b) P and c) K concentrations of BF needles sampled in different stands (BS = black spruce, BSE = black spruce plus ericaceous shrubs, TA = trembling aspen). Different letters indicate differences between modalities.

2.5 Results

2.5.1 Fir sapling traits and differences between stands

Given that 6 of the 60 BF saplings were missing after the first year of fieldwork, the results presented here are based on 18 saplings for each sapling type (*i.e.*, 54 saplings). For 11 saplings, the quantity of needles was insufficient to measure minor and major cations, thereby further reducing the sample size.

Mean foliar N concentrations of BF saplings were highest in TA stands compared to BS and BSE stands ($p < 0.05$, Figure 2.2a). Mean foliar K concentration was higher in TA than in BSE stands ($p < 0.05$, Figure 2.2c). Foliar P (Figure 2.2b), Ca and Mg did not vary between sapling types ($p > 0.05$). No significant differences were found in root tip EMF richness and Shannon index among sapling types (Figure 2.3a, $p > 0.05$). Mean lateral growth of fir saplings did not differ between stands in 2015 and 2016. Apical growth was greater in TA than in BSE stands in 2015, while no difference was detected for apical growth in 2016. Summed lateral and apical growth did not differ between stands. Ramification index of BF roots was higher in TA than in other stands (Figure 2.3b, $p < 0.05$).

Table 2.2 Results of PERMANOVA conducted on EMF community of balsam fir saplings

	Df	F model	R2	P-value
Site	3	1.1749	0.06437	0.104
Site:Plant community	8	1.1852	0.16855	0.035
Residuals	42	-	0.76708	-

2.5.2 Ectomycorrhizal community

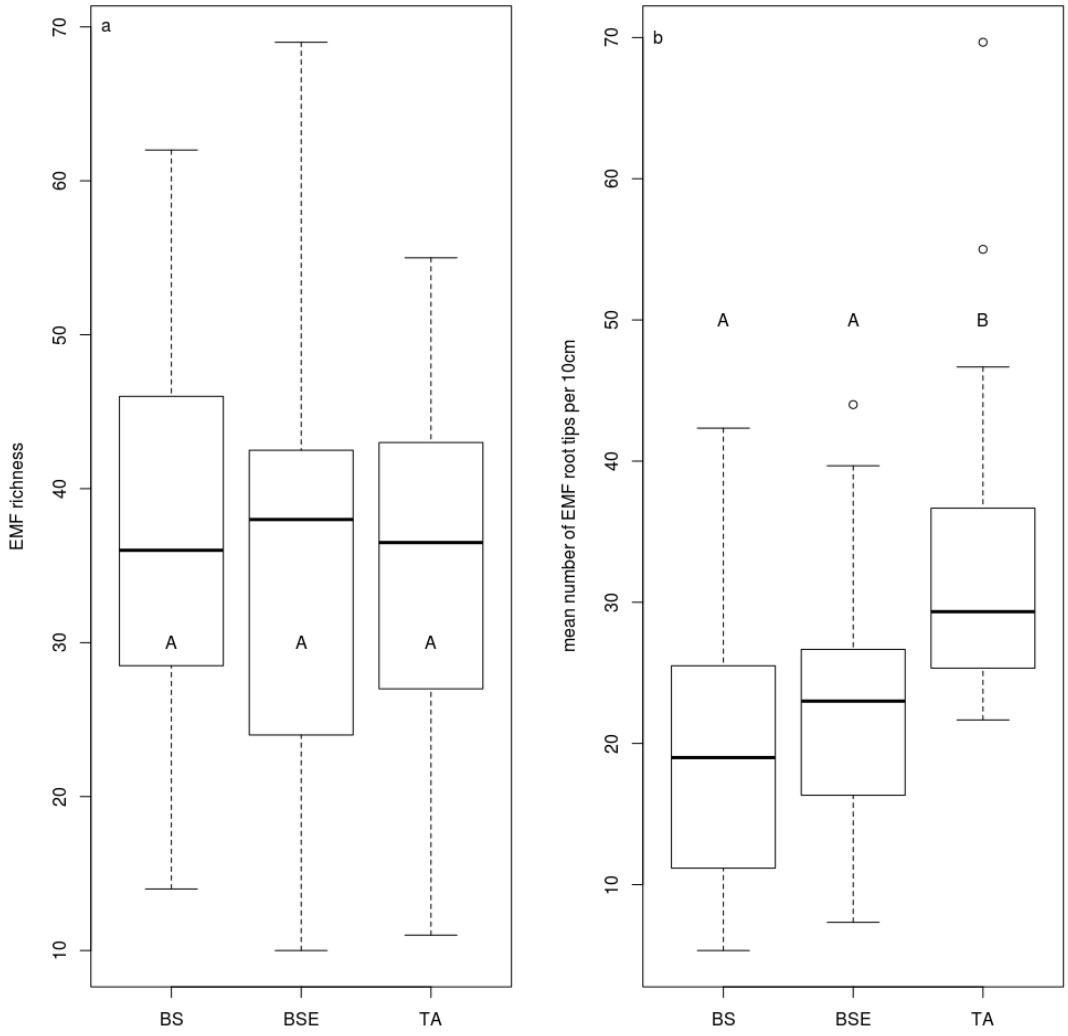

Figure 2.3 Boxplot charts showing a) EMF richness and b) number of EMF root tips per 10 cm roots. BS = black spruce, BSE = black spruce plus ericaceous shrubs, TA = trembling aspen. Different letters indicate differences between means.

A total of 400 EMF OTUs (655,495 reads) were found in EMF root tips from BF saplings, representing 19.5% of OTUs and 59.7% of reads, with an average of 35.4 OTUs and 12,056.8 reads per sample. Variation in EMF root tip communities was explained by plant community (p-value = 0.035, F = 1.1852 , R^2 = 0.169), but no effect of site was detected (Table 2). Clavulinaceae were only found under TA. Further, the abundance of Cortinariaceae was higher under BS compared to TA stands, that of Gloniaceae was higher under TA than under BSE, and that of Helotiaceae was higher under BSE than under TA. Finally, abundance of Inocybaceae and Thelephoraceae were greater under TA than under BS and BSE (Kruskal-Wallis and Dunn *post-hoc* tests, $p < 0.025$, Figure 2.4). According to the NMDS, EMF communities on root tips from the TA stands were distinct from those under BS and BSE, while communities in BS and BSE overlapped (Figure 2.5). *Envfit* test demonstrated that N concentration were significantly associated with NMDS structure ($p < 0.05$, Figure 2.5).

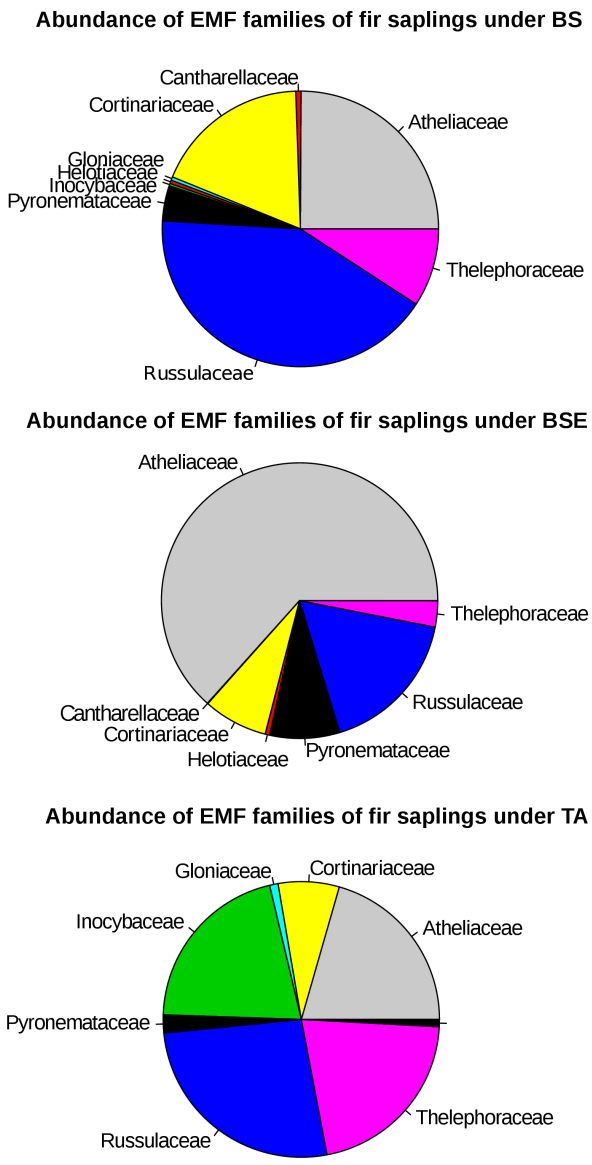

Figure 2.4 Pie chart representing percent of EMF families per stand type forroot tips samples of BF saplings (based on the abundance of reads). BS,black spruce; BSE, black spruce plus ericaceous shrub; TA, trembling aspen.

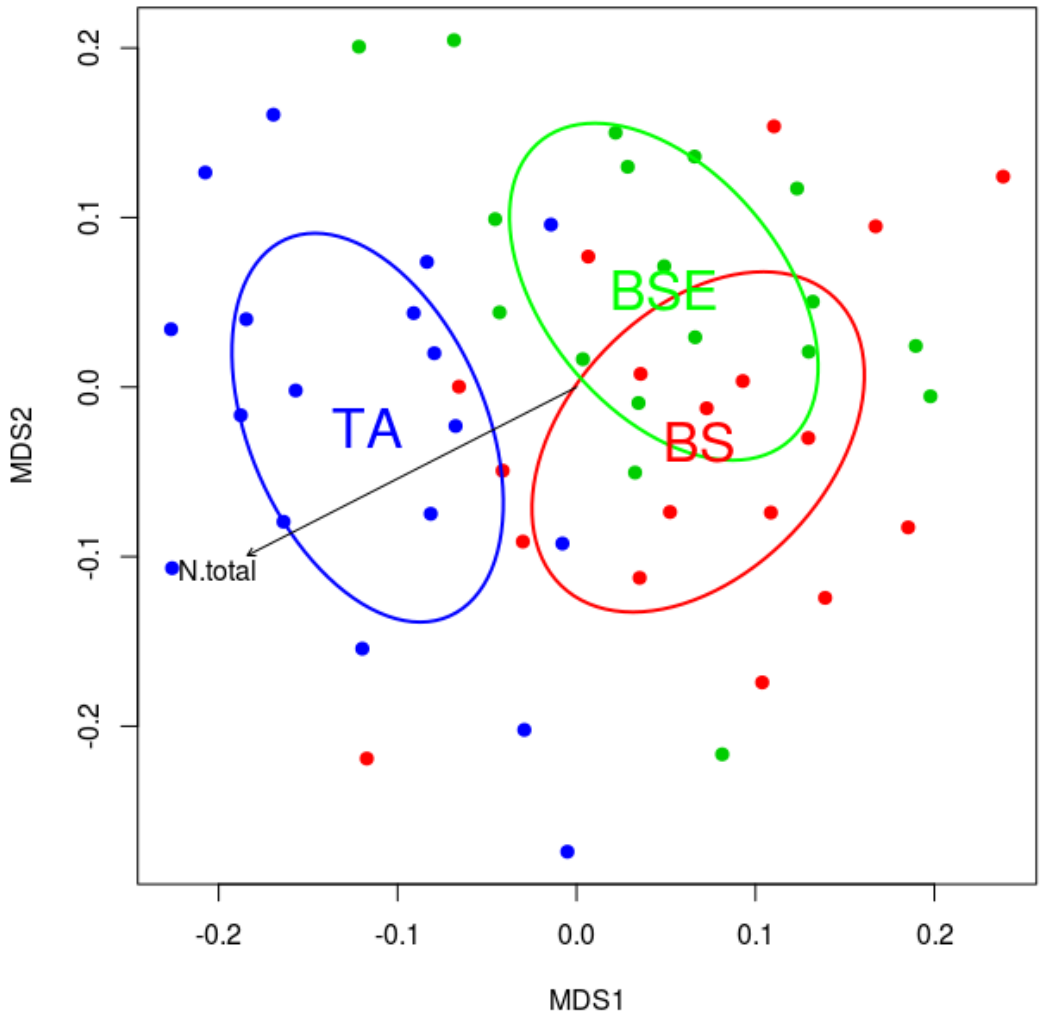

Figure 2.5 Non-metric multidimensional scaling (NMDS) plots representing similarity between EMF communities of BF root tips in black spruce (BS, red), black spruce + ericaceous shrubs (BSE, green) and trembling aspen (TA, blue) stands. Arrow represents the correlation between N concentration in fir needle and NMDS space.

2.5.3 Path analysis

The *p*-values of the Fisher's C tests were greater than 0.05 for the three models, indicating that all models could be accepted. The *p*-values of the associated Chi-Square tests ranged between 0.019 and 0.401. CFI ranged between 0.930 and 0.997, while those for TLI ranged between 0.901 and 0.991 (Table 5). Given these results, the interaction model Mod1 was the only one passing the primary filter. Here, we present direct and indirect links for which *p*-values were significant at $p < 0.05$ (for a summary of the parameter estimates, see Annexe B Table B.1). Apical and lateral growth were directly correlated with foliar N concentrations (path coefficients were 0.547 and 0.609, respectively) and the ramification index (0.253 and 0.254). Of interest, foliar N concentrations were negatively correlated with NMDS second axis (-0.226) and the percentage of BS (-0.469). The ramification index was negatively correlated with BS stand (-1.573). NMDS first axis was positively correlated with BS stands (1.499), while the second axis was positively correlated with the presence of ericaceous shrubs (0.773). The presence of ericaceous shrubs, in turn, was positively correlated with BS stands (0.500). Percentage of BS was positively correlated with BS stands (1.708), while the percentage of BF was negatively correlated with BS stands (-1.306). Significant direct and indirect links between variables are presented in Figure 2.6. All significant and non-significant parameter estimates are presented in Annexe B, Table B.1.

Table 2.3 Statistics of each structural equation models

Model	P-value (Chisq)	CFI	TLI
Mod1	0.401	0.997	0.991
Mod2	0.103	0.962	0.941
Mod3	0.019	0.930	0.901

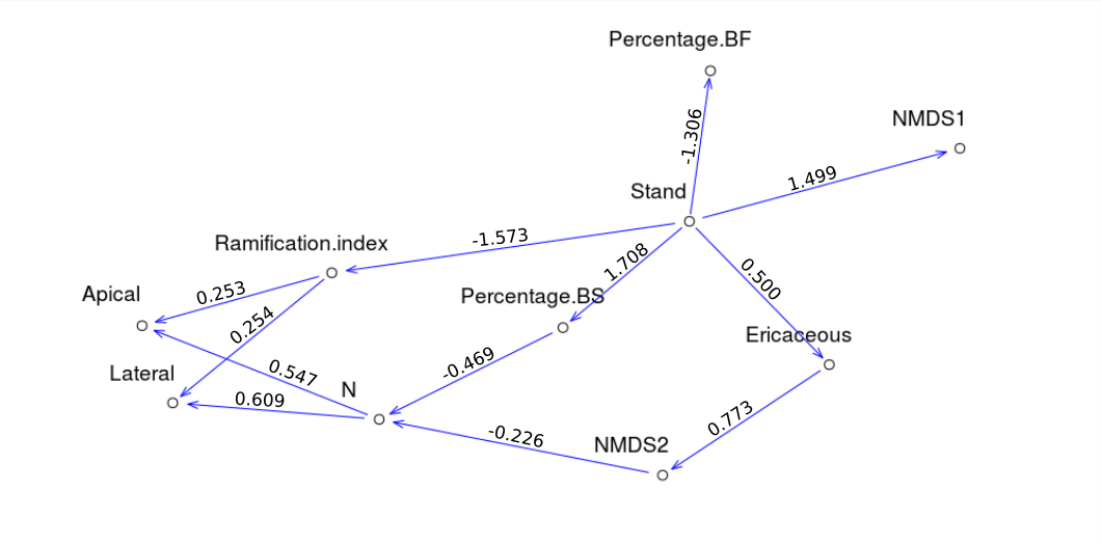

Figure 2.6 Direct acyclic graphs corresponding to Mod1, only significant links between variables are shown, path coefficients are indicated above each arrow.

2.6 Discussion

The role of biotic interactions was investigated here to explain observed growth differences in balsam fir saplings growing at a northern ecotone of the boreal forest. We hypothesized that biotic interactions could explain the higher nutrition under aspen than under spruce. Our results suggest that facilitation through ectomycorrhizal fungi, together with competition with ericaceous shrubs under BS, are significant drivers of BF growth and nutrition.

As soil EMF communities were strongly divergent between TA and BS-dominated stands (Nagati *et al.*, 2018), we hypothesized that young BF would associate with distinct EMF in the two stands, and that BF would have greater mycorrhization rates under TA stands. Contrary to our hypothesis, observation of BF root tips revealed EM root tips in all conditions, with similar mycorrhization rate. Sequencing of EM root tips confirmed our observation as species richness was not different between stands. We rather detected differences in root architecture and community composition.

Indeed, we observed a higher ramification index under TA than under BS stands, probably leading to enhanced soil exploration for ressources. Our path analyses suggested that soil conditions in TA stands led to a higher ramification index, which in turn improved BF early growth, likely by enhancing foliar nutrient concentrations. Moreover we detected that percent BS around saplings was negatively correlated to sapling N content, this is probably linked to the greater N availability in TA than in BS stands (Nagati et al., 2018). As demonstrated by path analysis, N availability (reflected by the stand and so on, by the percent of BS) was not the only factor affecting sapling needle N content and EMF community structure was also correlated to N nutrition. There are therefore complex interactions leading to a better nutrition of young BF, and path analyses highlighted the importance of both stand dominance and EMF community.

Between stands, communities of EMF on BF roots were not distinct in species richness but rather in composition, and for example Clavulinaceae were only detected under TA stands. Based on soil sequencing, our previous study also revealed a difference of composition but not species richness between stands (Nagati et al., 2018). More generally, changes in plant dominance shaped EMF community structure rather than the species richness (Tedersoo *et al.*, 2012). Such a change in EMF community could have strong functional consequences. Based on our results, changes in community composition were correlated with changes in N concentration. We detected a higher abundance of Inocybaceae and Thelephoraceae in BF roots tips in TA than in BSE and BS stands, and Helotiaceae were more abundant in BSE than TA stand. These differences of EMF abundance between stands could partly explain differences in N uptake by BF saplings. Depending on site conditions, different EMF species or assemblage could differently uptake nutrients in soils and transfer these nutrients to plants (Buée *et al.*, 2007; Jonsson *et al.*, 2001). This result suggests that some fungi are more efficient provider of N to young BF. Nara (2006) experiments illustrated how variable can be the result of EM interactions with distinct EMF fungi.

By artificially connecting two different tree species through a common mycorrhizal network from different EMF species, Nara (2006) showed that N nutrition could be 6 times higher when trees were connected by *Hebeloma mesophaeum* as compared to trees connected by *Laccaria amethystina*. As BF needles were generally richer in N under TA, the question remains if this benefit under TA could be due to N transfer through a common mycorrhizal network (Simard *et al.*, 2012) involving BF and TA, or only to better soil conditions under TA. We did not sequence TA roots, to detect possible shared fungi and experimental manipulations may be necessary to test whether TA could bring a direct benefit to young BF saplings. As suggested by the models of Bever *et al.* (2010), the facilitation of BF by TA may not only be explained by shared fungi, but simply by changes in EMF abundance, which we detected between the two stands.could explain the lower N nutrition of BF near ericaceous shrubs. and could be more efficient to provide nutrients to ericaceous shrubs than to BF saplings These speciesycorrhiza as well as ericoid mycorrhiza (Vrålstad et al., 2000; Villarreal-Ruiz et al., 2004; Grelet et al., 2010).Several studies have demonstrated that some fungi in this family could have a dual-mode of root colonization and forms ectomThe higher abundance of Helotiaceae associated with BF roots near ericaceous shrubs is also interesting.

The lower nutrient concentrations found in fir needles (N and K) under BSE stands compared to TA stands (Figure 2.2), suggest a negative effect of black spruce and ericaceous shrubs association on BF seedlings. These results are consistent with previous studies where ericaceous shrubs that are associated with non-EMF fungi may compete with EMF-associated trees (Mallik, 2003; Walker *et al.*, 1999; Yamasaki *et al.*, 1998). Ericoid mycorrhizal fungi are particularly competitive for recalcitrant organic matter (ROM) decomposition and uptake of N and P (Read, 1996) and probably have the ability to take up nutrient from ROM found around ericaceous shrubs (Joanisse *et al.*, 2018) contrarily to BF-associated EMF communities. In hardwood forests of the southern Appalachians, the presence of *Rhododendron*

maximum L. reduced the ramification index of eastern hemlock (*Tsuga canadensis* [L.] Carrière) saplings four-fold (Walker *et al.*, 1999). In our study, the presence of ericaceous shrubs (associated with ericoid fungi) close to fir saplings affected their root EMF communities rather than reduced the ramification index. Numerous studies have already shown how ericaceous shrubs affected EMF communities associated with trees, such as red oak (*Quercus rubra* L.), hemlock (Walker *et al*., 1999), pine (*Pinus strobus* L., *P. sylvestris* L.) (Kohout *et al.*, 2011), and black spruce (Kennedy *et al.*, 2018; Yamasaki *et al.*, 1998). In the boreal forest, ericaceous shrubs not only compete with fir growth and nutrition, but also modify forest dynamics and lead to thick accumulations of soil organic matter and soil acidification. This phenomenon, is a recurrent problem within boreal forests that are situated in the Ontario-Quebec Clay Belt and is often associated with the presence of ericaceous shrubs and Sphagnum mosses (Fenton *et al.*, 2005) and a loss of forest productivity (Simard *et al.*, 2007). The thickness of the soil organic layer is correlated with a decrease in TA establishment and growth within the black spruce-feather moss domain (Lafleur *et al.*, 2015), together with a decrease in black spruce establishment in sites that are dominated by *Kalmia angustifolia* L. (Mallik and Kravchenko, 2018). Our results suggest that forest regeneration failure in areas with high ericaceous shrub abundance could be explained by their effect on EMF communities, and reciprocally, invite to consider below-ground interactions to avoid or limit regeneration failure.

Whilst our study focused on fir growth, we revealed stronger differences in foliar N concentrations and ramification indices between stands. Indeed difference in annual growth between stands was detected only in 2015. Annual growth of trees is relatively slow in the boreal forest and is correlated with the length of the growing season, which can differ from one year to the next (Jarvis and Linder, 2000). Differences in early growth could thus be difficult to detect over a short period of time and would be probably more pronounced when studying several years of growth (*see* Arbour and Bergeron, 2011). Measuring fitness is always difficult for young

trees over a short period of study and measures of foliar nutrient concentrations were more useful to detect differences between stands, and reflected the benefits of EMF symbiosis. Foliar nutrient concentrations were generally greater under TA compared to BS and correlated with growth, which confirmed our hypothesis. This result could be linked to the greater availability of nutrients in TA stands than in BS stands (Cavard *et al.*, 2011; Nagati *et al.*, 2018).

A major goal of forest ecology today is to determine ecosystem trajectories in a context of climate change. In the case of the balsam fir-black spruce forest ecotone, it appears that trembling aspen stands would provide a favorable niche for fir establishment and growth. As demonstrated by Arbour and Bergeron (2011), the higher abundance of balsam fir in TA than BS stands is more pronounced for saplings than seedlings. This result translates a lower mortality and better growth of balsam fir in TA stands which leads to a greater abundance of mature and reproductive trees in these stands. This in turn, may result in an increase of mixed forests and deep changes in ecosystem functioning. The distributional ranges of numerous tree species are likely to change within the context of climate change (Bellard *et al.*, 2012; *I*PCC. Climate Change 2014; Iverson eand McKenzie, 2013). Migration has already begun for many tree species in North America (Brandt, 2009; Woodall *et al.*, 2009). Their geographic ranges have been extending northward rapidly (up to 100 km/century; Woodall *et al*., 2009). Our results suggest that the climatic niche could not alone explain species abilities to establish and that the mutualistic niche (sensus Peay, 2016) have to be explored to ensure a better comprehension of tree migration processes.

2.7 Acknowledgements

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CHAPITRE III

IMPACT OF SOIL ORIGIN AND STERILIZATION ON GERMINATION AND EARLY GROWTH OF BALSAM FIR

(IMPACT DE L'ORIGINE DU SOL ET DE SA STÉRILISATION SUR LA GERMINATION ET LA CROISSANCE DU SAPIN BAUMIER)

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En préparation

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3.1 Abstract

Studying tree regeneration brings clues to understand ecosystems dynamic, particularly in a context of climate change. The presence of young balsam fir is increasingly observed in the black spruce domain of the boreal forest, due to its northern migration. Balsam fir regeneration is not homogeneous, and seedlings established under aspen-dominated stands are usually more vigorous than those established in adjacent black spruce-dominated stands. Although abiotic conditions are similar between the two stand types, composition of the understorey differs, with notably more ericaceous shrubs in spruce stands and different soil fungal communities. A first study revealed that balsam firs associated with different ectomycorrhizal communities depending on stand type and the presence of ericaceous shrubs, that in turn, negatively affected fir sapling N nutrition. The goal of this study was to determine if the difference in ectomycorrhizal communities between stand types affected germination, survival, growth and nutrition of balsam fir seedlings. Balsam fir seeds were sowed and grown for three growing seasons in organic and mineral soils sampled in the two stand types and near *Rhododendron groenlandicum* shrubs. Half of soils were sterilized in order to disentangle abiotic and biotic soil conditions. Soil sterilization had a negative effect on balsam fir N nutrition. However, mycorrhization rate was not correlated to growth nor to nutrient concentration of seedlings. Both germination rates and nutrition were greater in soils collected from spruce stands. Survival and germination rates were greater in organic than in mineral soils, possibly because of the heavy clay content in mineral soils that are prone to water logging. This study showed that mycorrhization rate did not affected balsam fir seedlings nutrition and early growth contrary to mycorrhizal fungal communities. Considering balsam fir nutrition, observations were contrasted between field measures and growth chamber experiment and common mycorrhizal networks could partly explain the better nutrition of balsam fir in aspen stands as compared to spruce stands under natural conditions.

Keywords: *Abies balsamea*, organic soil, mineral soil, ectomycorrhiza, *Rhododendron groenlandicum, Picea mariana, Populus tremuloides*

3.2 Résumé

Étudier la régénération des arbres aide à comprendre la dynamique des écosystèmes, particulièrement dans un contexte de changement climatique. Les jeunes sapins baumiers sont de plus en plus observés dans la forêt boréale, à cause de sa migration vers le nord. Sa régénération n'est pas homogène, et les sapins baumiers sont généralement plus vigoureux dans les peuplements dominés par le peuplier faux-tremble que dans les peuplements adjacents dominés par l'épinette noires. Les conditions abiotiques de ces peuplements sont semblables, mais la composition du sous-bois y diffère, avec notamment une plus grande présence d'espèces éricacées dans les peuplements d'épinette, et des communautés fongiques du sol différentes. Ces deux types de peuplements abritent des communautés mycorhiziennes différentes, et une première étude a permis de démontrer que le sapin baumier s'associait à une communauté différente en fonction du peuplement dominant et de la présence d'éricacées. La modification des communautés ectomycorhiziennes à proximité des éricacées a un effet négatif sur la concentration en azote des aiguilles de semis de sapins. Le but de cette étude était de tester si ces différences de communautés ont aussi un impact sur la germination, la survie, la croissance et la nutrition de jeunes plantules. Des graines de sapins ont été mises à germer et un suivi de croissance a été effectué pendant trois saisons dans des sols prélevés dans les deux types de peuplements et à proximité de *Rhododendron groenlandicum*. La moitié des sols a été stérilisée afin de séparer les effets biotiques et abiotiques du sol. La stérilisation n'a impacté négativement que la nutrition azotée des semis. De plus, le taux de mycorhization n'a eu d'effet ni sur la croissance ni sur la concentration en azote dans les aiguilles. La germination et la nutrition étaient meilleures dans les sols prélevés sous les épinettes. La survie et la germination étaient meilleures dans les sols

organiques que minéraux, possiblement à cause de la saturation en eau des sols minéraux essentiellement composés d'argile lourde, susceptibles à l'ennoiement. Les résultats de cette étude nous indiquent que le taux de mycorhization n'a pas eu d'effet sur la croissance et la nutrition des semis de sapin baumier, contrairement aux changements dans les communautés fongiques et posent la question de la présence d'un réseau mycorhizien commun pouvant potentiellement expliquer la meilleure nutrition du sapin à proximité des peupliers observée en conditions naturelles par rapport aux peuplements d'épinettes.

Mots-clefs : *Abies balsamea*, sol organique, sol minéral, ectomycorhize, *Rhododendron groenlandicum, Picea mariana, Populus tremuloides*

3.3 Introduction

Tree establishment in a new area depends on abiotic and biotic conditions and is successful when seeds are able to disperse, germinate and grow. All those steps can be controlled by abiotic constraints, the amount of space available and biotic interactions that can locally modulate tree abilities to establish and grow (Peay, 2016).

In Quebec (Canada), the boreal black spruce-feathermoss domain is convenient to assess the effects of biotic interactions on balsam firs (*Abies balsamea* [L.] Miller) establishment because it is dominated by a single tree species, black spruce (*Picea mariana* [Miller] B.S.P.; Robitaille and Saucier, 1996). Due to climate change and forest management practices, stands dominated by trembling aspen (*Populus tremuloides* Michaux) within the black spruce-feathermoss domain are increasing in size and abundance (Laquerre *et al.*, 2009). Balsam fir is a tree species that is expanding at the leading edge of its continuous range (leading edge is defined as the northern front of migration) within the boreal black spruce forest (Messaoud *et al.*, 2007), and which appears to establish and growth better in trembling aspen-

dominated than in black spruce-dominated stands (Arbour and Bergeron, 2011). These two types of stand host the same climatic and edaphic conditions but contrast in understorey vegetation and soil fungal communities composition (Cavard *et al.*, 2011; Légaré *et al.*, 2001); of interest, ectomycorrhizal communities have different structures between the two stands (Nagati *et al.*, 2018). The latest are known to impact tree growth and nutrition (Smith and Read, 2008). Cases of facilitation between different tree species through sharing of mycorrhizal species have been demonstrated several times (Booth and Hoeksema, 2009; Dickie *et al.*, 2006; Nara and Hogetsu, 2004; Richard *et al.*, 2009; van der Heijden and Horton, 2009) and a previous study (Nagati *et al.*, 2019) also tended to demonstrate a link between mycorrhizal communities structure and balsam fir N nutrition.

It is rather difficult to disentangle biotic and abiotic factors affecting tree growth and nutrition in the field, and most studies on mycorrhizal communities are descriptive and do not link fungal communities with ecosystem processes (Högberg *et al.*, 2007; Nagati *et al.*, 2018; Tedersoo *et al.*, 2014; Toljander *et al.*, 2006). By focusing on mycorrhizal communities at the stand level, studies give little information about their direct effects on trees or give only correlative or speculative links between mycorrhizal fungi and ecosystem functioning. Causal links between mycorrhizal fungi and plant establishment (Nara, 2006; Newbery *et al.*, 2000), growth (Dickie *et al.*, 2002; Teste and Simard, 2008) or survival (Booth and Hoeksema, 2009; Teste *et al.*, 2009) are only given by experimental investigation.

In this context, experiments are needed to infer importance of mycorrhizal associations on germination, early growth and nutrition of balsam fir at its leading edge. A previous study on growth and nutrition of balsam fir saplings within the two stand types showed that fungal communities and root development (number of root apex) partly explained the better nutrition of balsam fir observed under trembling aspen than under black spruce (Nagati et al., 2019). In black spruce-dominated

stands, the presence of ericaceous shrubs has also been pointed out several times to explain coniferous regeneration failures (Mallik, 2003; Mallik and Pellissier, 2000; Zackrisson *et al.*, 1997). The presence of ericaceous shrubs could modify the mycorrhization rate and structure of mycorrhizal communities for neighbor trees (Kennedy *et al.*, 2018; Nagati *et al.*, 2019; Walker *et al.*, 1999; Yamasaki *et al.*, 1998). In the field, we found better balsam fir nutrition when growing in trembling aspen-dominated stands, intermediate in black spruce stands and lowest near ericaceous shrubs. The presence of ericaceous shrubs was shown to negatively impact balsam fir nutrition through its effect on ectomycorrhizal community structure(Nagati *et al.*, 2019). To exclude any effect of abiotic conditions, a growth chamber experiment was conducted. Balsam fir seeds were sown and grown in mineral and organic soil layers harvested in aspen-dominated and spruce-dominated stands as well as under ericaceous shrubs in spruce-dominated stands. Half of the soils were sterilized in order to disentangle biotic and abiotic factors. Our hypotheses were that 1) germination rate will be independent of soil biotic and abiotic factors 2) seedlings growing in sterilized soils will have lower nutrient concentrations and growth than seedlings in non-sterilized soils, and 3) seedling mycorrhization rates, nutrition and growth will be greater in soils from aspen-dominated stands than in soils collected near ericaceous shrubs.

3.4 Material and methods

3.4.1 Soil sampling and sterilization

The site where soil were collected was located in the black spruce-feather moss forest domain on the border of Abitibi-Témiscamingue and Nord du Québec region (site 4 in Nagati et al. 2018). This area is a part of the Clay Belt of northern Québec and Ontario, which resulted from deposits left by the proglacial lakes Barlow and Ojibway at the time of their maximum expenses (Veillette, 1994). Between 40 and 45% of the soil particles are clay within the site (Cavard *et al.*, 2011). Soils were

collected in August 2016 in one stand dominated by trembling aspen (representing more than 75% of the canopy cover) and one stand dominated by black spruce (representing more than 75% of the canopy cover). Within the black spruce-dominated stand, soils were collected far from any or directly under *Rhododendron* shrubs, resulting in three soil origins: trembling aspen (TA), black spruce (BS) and *Rhododendron groenlandicum* (BSE). For each soil origin we collected 10L of organic soil and 10L of mineral soil. Half of the soil collected in each stand and soil layer was sterilized. As our primary goal was to avoid plant colonisation by fungal species, soil were microwaved three time for 5 minutes at maximum power (1000 watts) (Trevors, 1996).

3.4.2 Germination Process

Balsam fir seeds were bought from a garden center. To initiate germination, seeds were first immersed in cold freshwater for 24h (tap was let opened to ensure water flow at 0.5L/min). Seeds were then left to dry in the open air for 48 hours. Seeds were stratified by putting them at 3°C in a polyethylene bag for 28 days and mixed each week to ensure oxygenation. At the end of the stratification process, seeds were ready for germination. For each soil treatment (soil origin by sterilization) we put 10 seeds in 10 replicate pots (100 seeds by soil treatment) at 0.5 cm depth for two months in a growth chamber (Conviron model CG-108, Winnipeg, Canada). The following conditions were set for germination period: 16h photoperiod with a photosynthetically active radiation at pot level of 450 $\mu mol\ m^{-2}\ s^{-1}$, day/night temperature 20/10°C and relative humidity 80%. After two months, we counted the number of germinated seeds by pots to calculate germination rate. One randomly selected seedling per pot was kept for the next part of the experiment. The selected seedlings were grown under these conditions for one supplementary month corresponding to the first growth phase.

3.4.3 Growth

In addition to the first growth phase, two additional growing seasons were simulated, each preceded by a dormancy period. Each growing season lasted 3 months under the same conditions as for the germination phase. Each dormancy phase lasted 2 months and the following conditions were set: 4h photoperiod with photosynthetically active radiation at pot level of 450 µmol m^{-2} s^{-1}, temperature 4°C and humidity 80%. At the beginning of the second-growth phase, soils were fertilized with 20/20/20 (N/P/K) fertilizer at a 2 g/L concentration. Each pot received around 0.5L of fertilized water.

3.4.4 Measurements

The number of mycorrhizal and non-mycorrhizal root apex was counted for each seedling under a magnifying glass. Stem height and basal diameter (cm) were measured with a caliper before seedlings were separated into shoots and roots and separately weighed. Stem and root tissues were dried in a forced-air drying oven at 40°C for 48h and dry mass recorded. Needles were finely ground with a ball mill for 1 min at 30 Hz (Mixer Mill MM 301, Retsch GMbH, Haan, Germany) and sent to the UC Davis Stable Isotope Facility to determine total N concentrations using a PDZ Europa ANCA-GSL elemental analyzer.

3.4.5 Statistical analyses

Statistical analyses were done with R software (version 3.5.2, R development core team, 2017). Differences in germination, mycorrhization rate, basal diameter, root dry mass and needle N concentrations between treatments were tested with a Kruskall-Wallis test followed by a Dunn post hoc test, as data were not normally distributed (log and square root transformation were insufficient to reach normality). Differences in survival rates between treatments at the end of the experiment were tested with a Chi-square test. Multi-factorial ANOVA were performed for height, log (dry mass) and log (root:shoot ratio) with stand type, soil layer and sterilization as dependent

variables. Interactions between stand type, soil layer and sterilization were also included in ANOVA. When significant, ANOVA were followed by Dunn post hoc tests. Correlation between mycorrhization rate and height, dry mass, root:shoot ratio and N concentration were tested with a Spearman rank correlation, as data were not normally distributed.

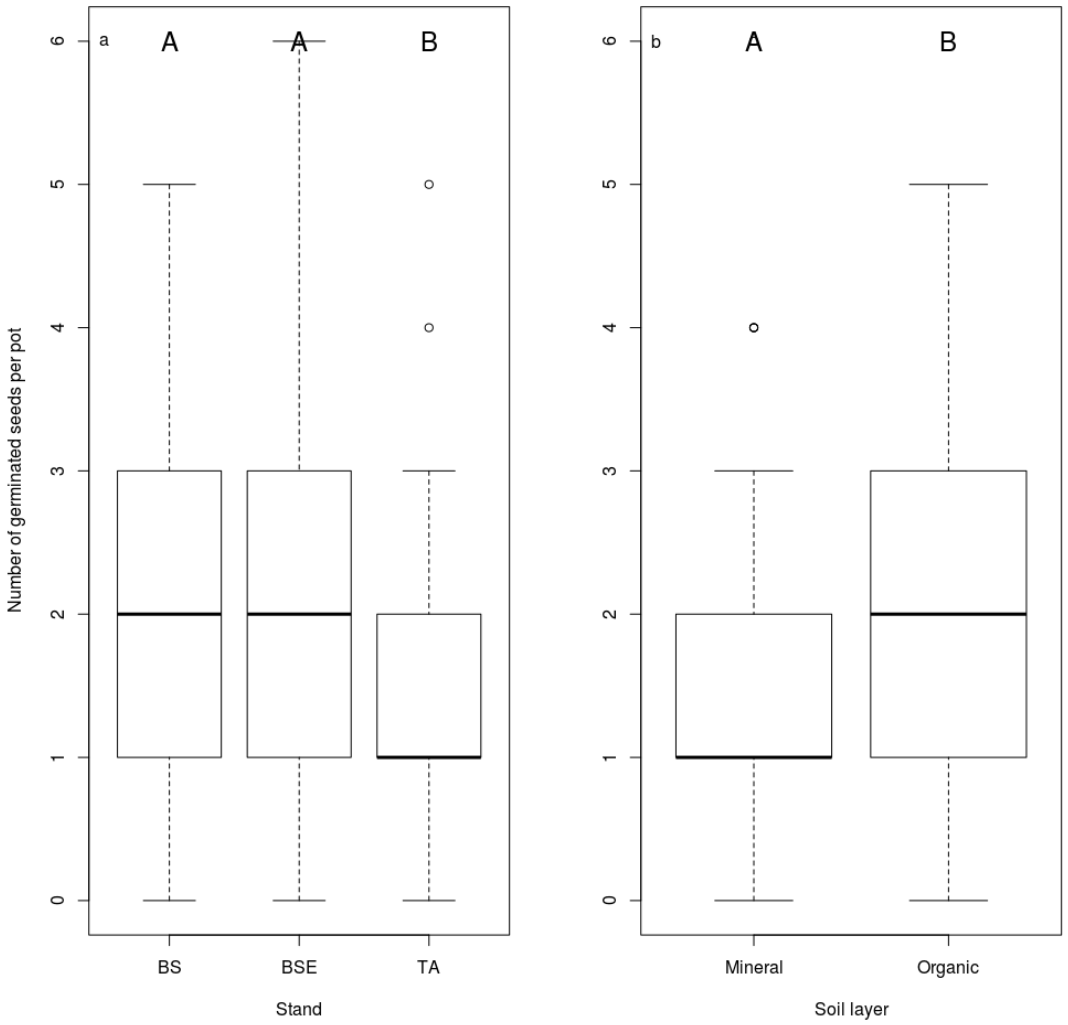

Figure 3.1 Germination rate of balsam fir seeds between a) different stand types and b) different soil layers. BS = black spruce stands, TA = trembling aspen stands, BSE = Rhododendron groenlandicum stands. Different letters indicate significant differences between conditions.

3.5 Results

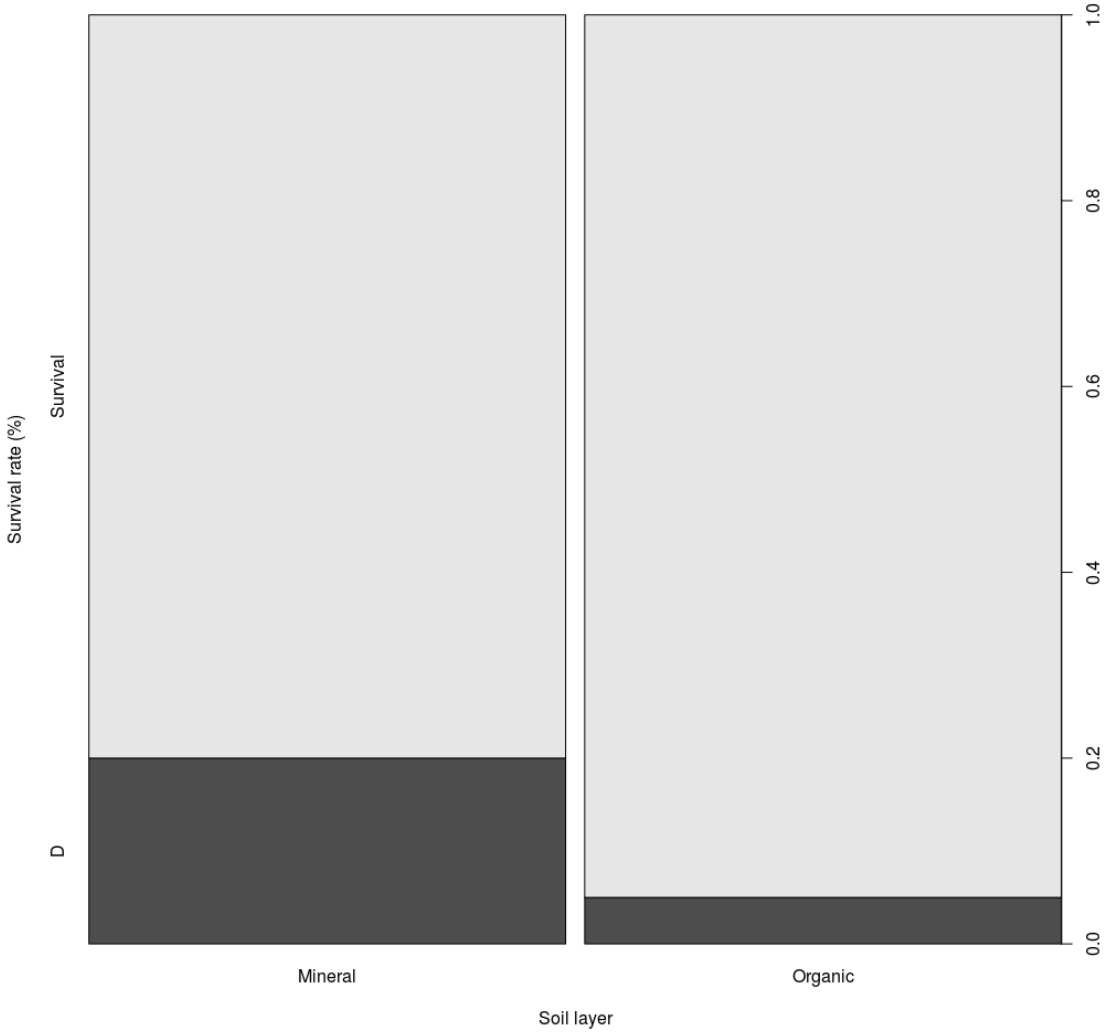

Figure 3.2 Survival of balsam fir seedlings after three growing seasons in organic and mineral soil layers. D = dead seedlings.

Germination was greater in soil from BS and BSE stands compared to soils from trembling aspen-dominated stands (Figure 3.1a, $p<0.025$). Balsam fir germination

was also better in organic than in mineral soil, independently of sterilization treatment but not interactions among the sterilization treatments, soil lauer and forest types were detected (Figure 3.1b, $p<0.025$). Survival of seedlings at the end of the experiment was better in organic than in mineral soils (Figure 3.2, $p<0.05$) and was neither affected by soil origin nor sterilization, or interactions between variables. Mycorrhization rate was better in non-sterilized soil than in sterilized soils, although mycorrhizal apex were present in all treatments (Figure 3.3a, $p<0.025$). Mycorrhization rate was greater in TA and BS soils compared to BSE soils, no correlations between variables were detected (Figure 3.3b, $p<0.025$). No correlation was detected between mycorrhization rate and seedling height, total dry mass, root:shoot ratio and N foliar concentration, $P>0.05$).

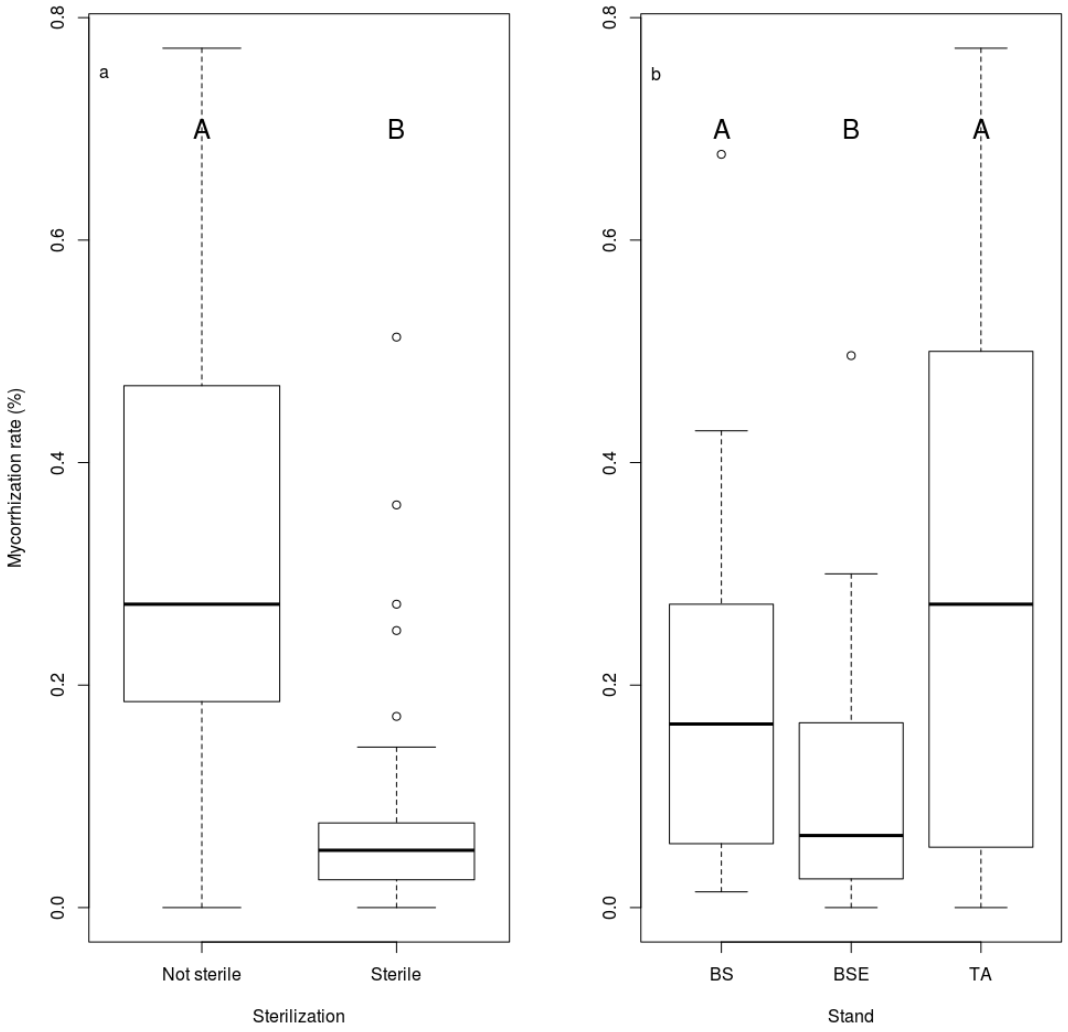

Figure 3.3 Mycorrhization rate of balsam fir seedling after three growing seasons for a) different stand types and b) different sterilization treatments. BS = black spruce-dominated stands, TA = trembling aspen-dominated stands, BSE = Rhododendron groenlandicum stands. Different letters indicate significant differences between conditions.

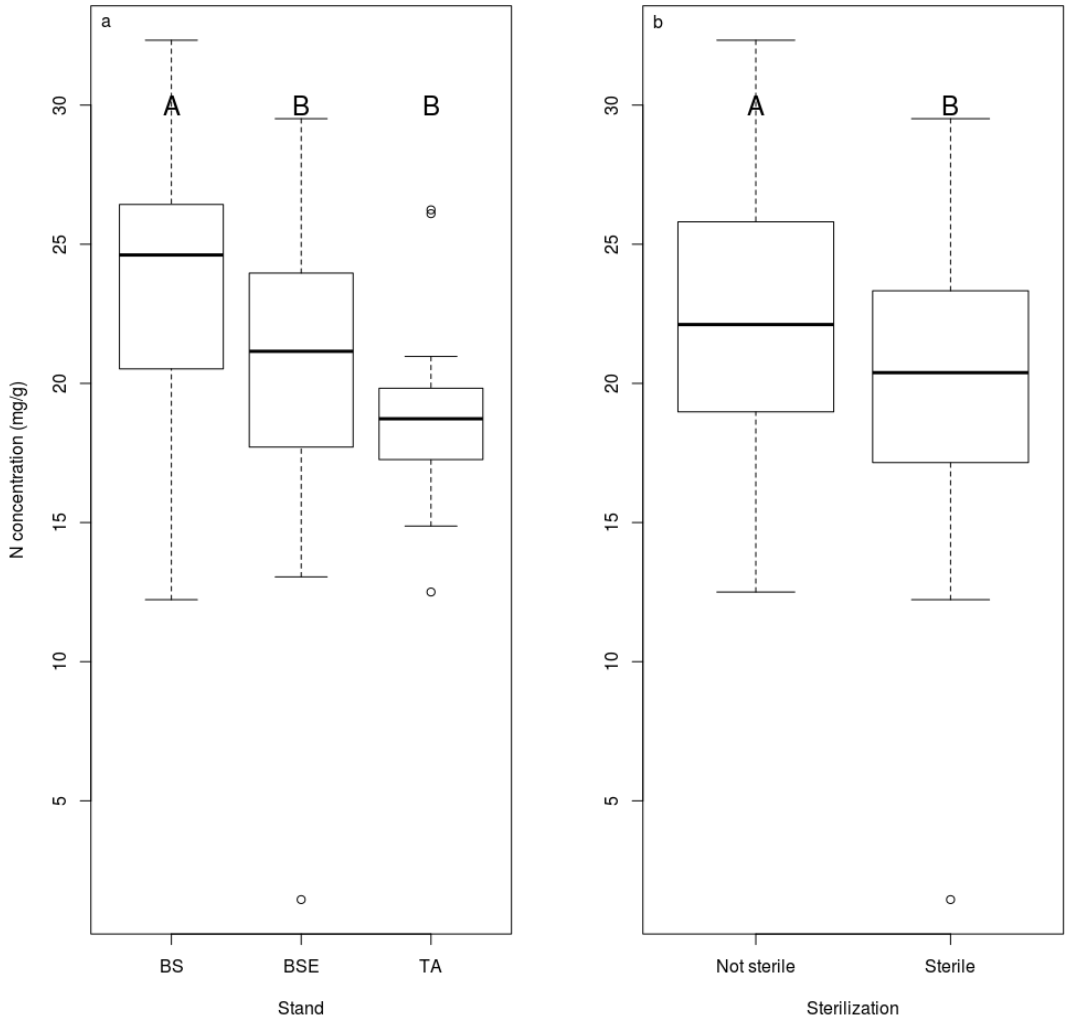

Figure 3.4 Concentration of balsam fir seedling needles after three growing seasons for a) different stand types and b) different sterilization treatments. BS = black spruce stands, TA = trembling aspen stands, BSE = Rhododendron groenlandicum stands. Different letters indicate significant differences between conditions.

Foliar N concentration was higher for seedlings in BS than in BSE and TA soils (Figure 3.4a, Dunn post hoc test, $p<0.025$). Seedlings in sterilized soils had lower needles N concentrations (Figure 3.4b, $p<0.025$).

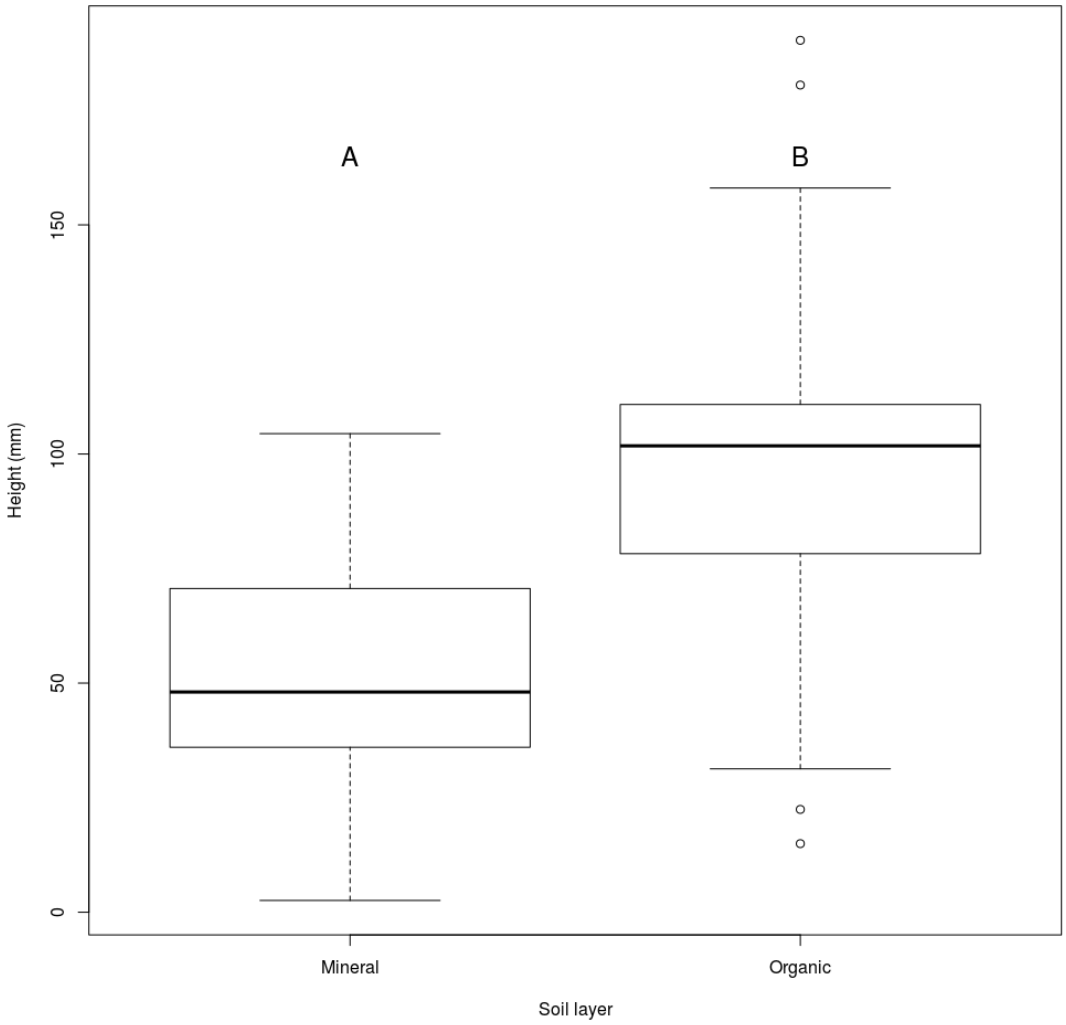

Figure 3.5 Total height of balsam fir seedling after three growing seasons for the mineral and organic soil layers, all stand types pooled. Different letters indicate significant differences between conditions.

Mean seedling height and basal diameter were greater in organic soils (Figure 3.5, Dunn post hoc test, $p<0.025$, ANOVA, $p<0.05$). There was a significant interaction between stand type and soil layer for dry mass of seedlings, showing that dry mass was not affected by soil layer when seedlings were grown in BS soils but that dry mass was greater in organic than in mineral soil when seedlings were grown in BSE and TA soils. (Figure 3.6, $p<0.05$). Dry mass was the lowest in TA and BSE mineral soils (Figure 3.6). Root dry mass was greater in organic than in mineral soil (Figure 3.7, $p<0.05$).

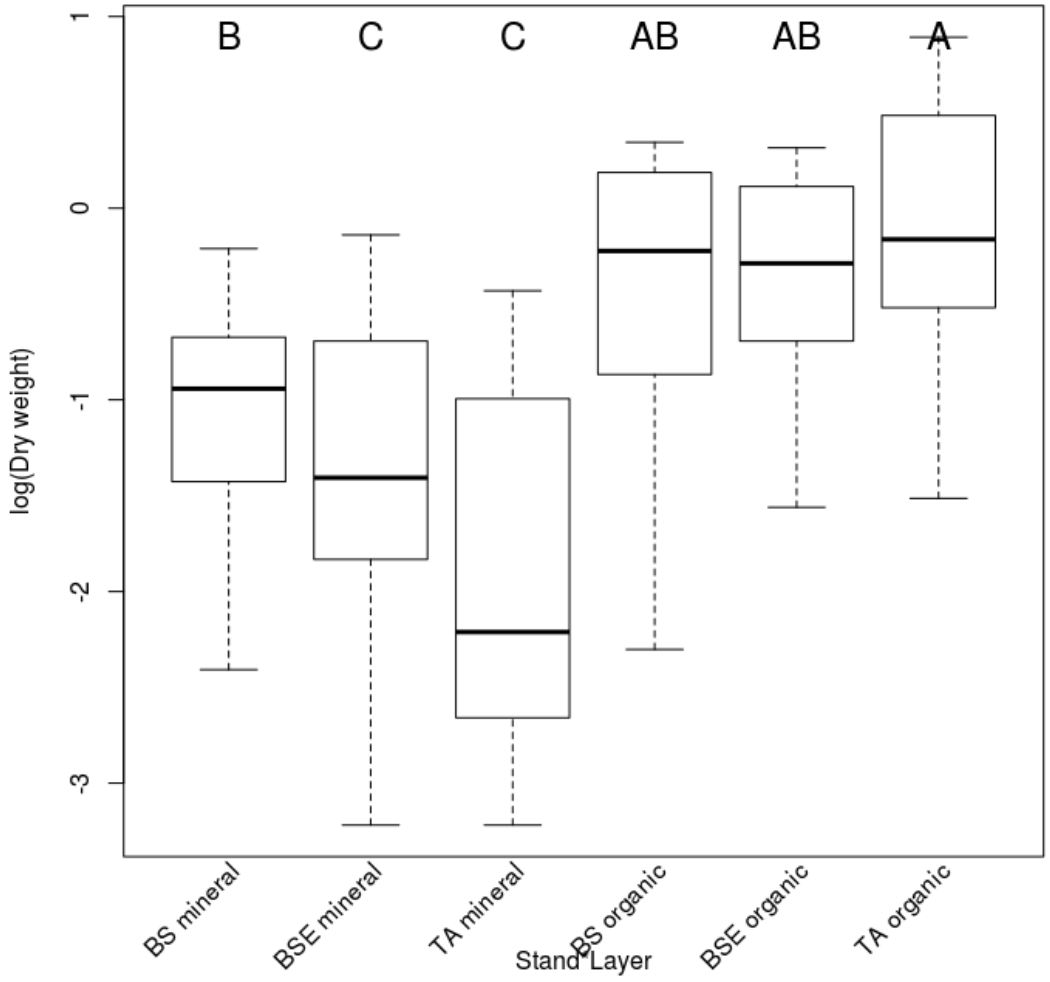

Figure 3.6 Total dry mass of balsam fir seedlings after three growing seasons showing the interaction between stand type and soil layer. BS = black spruce stands, TA = trembling aspen stands, BSE = Rhododendron groenlandicum stands. Different letters indicate significant differences between conditions.

Root:shoot ratios of seedlings were affected by a stand type by soil layer interaction; ratios were similar among all stand types in organic soils while root shoot ratios were

lower in BSE mineral soils. Ratio were higher in mineral than in organic soil expect for BSE stands for wich ratios were similar between organic and mineral soil (Figure 3.8, $p<0.05$).

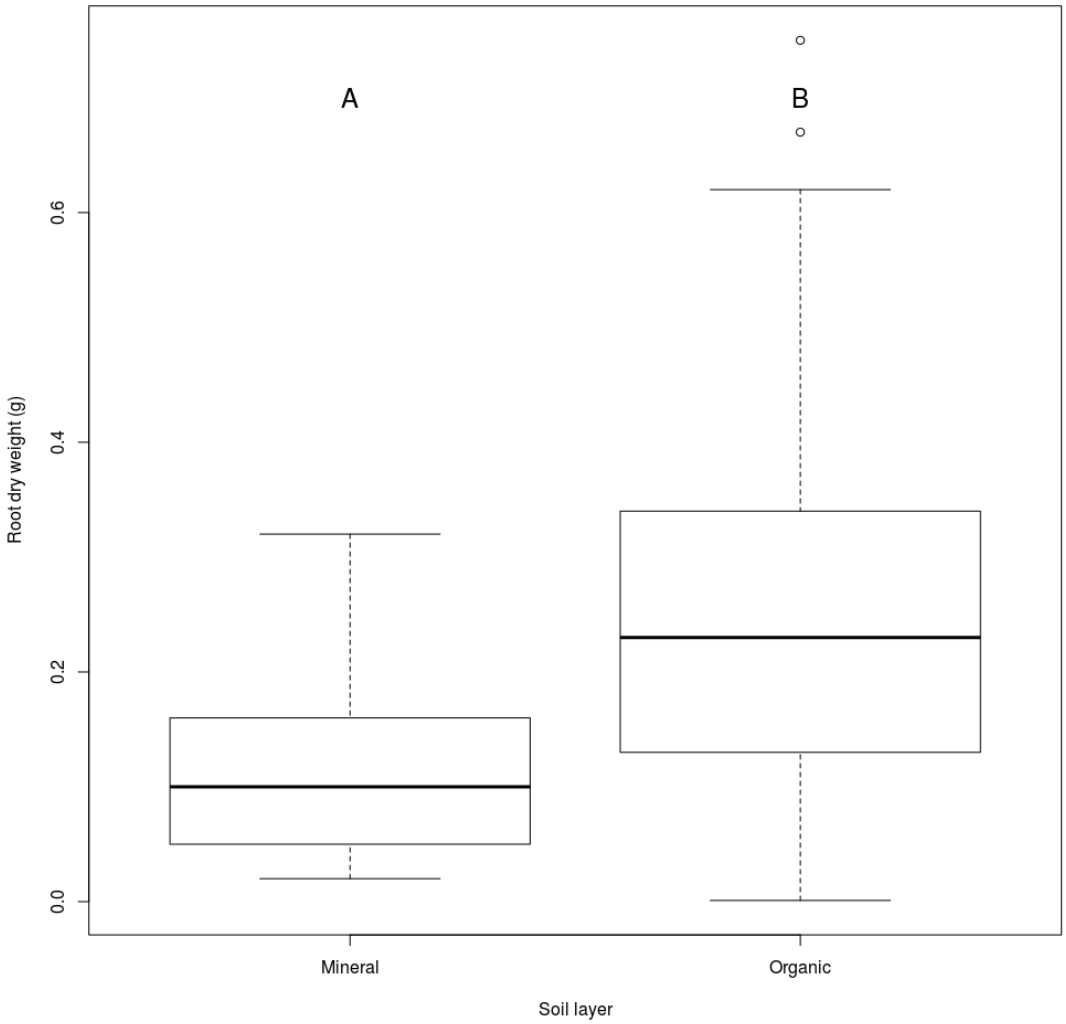

Figure 3.7 Mean root dry mass of balsam fir seedlings after three growing seasons for the mineral and organic soil layers, all stand types pooled. Different letters indicate significant differences between conditions.

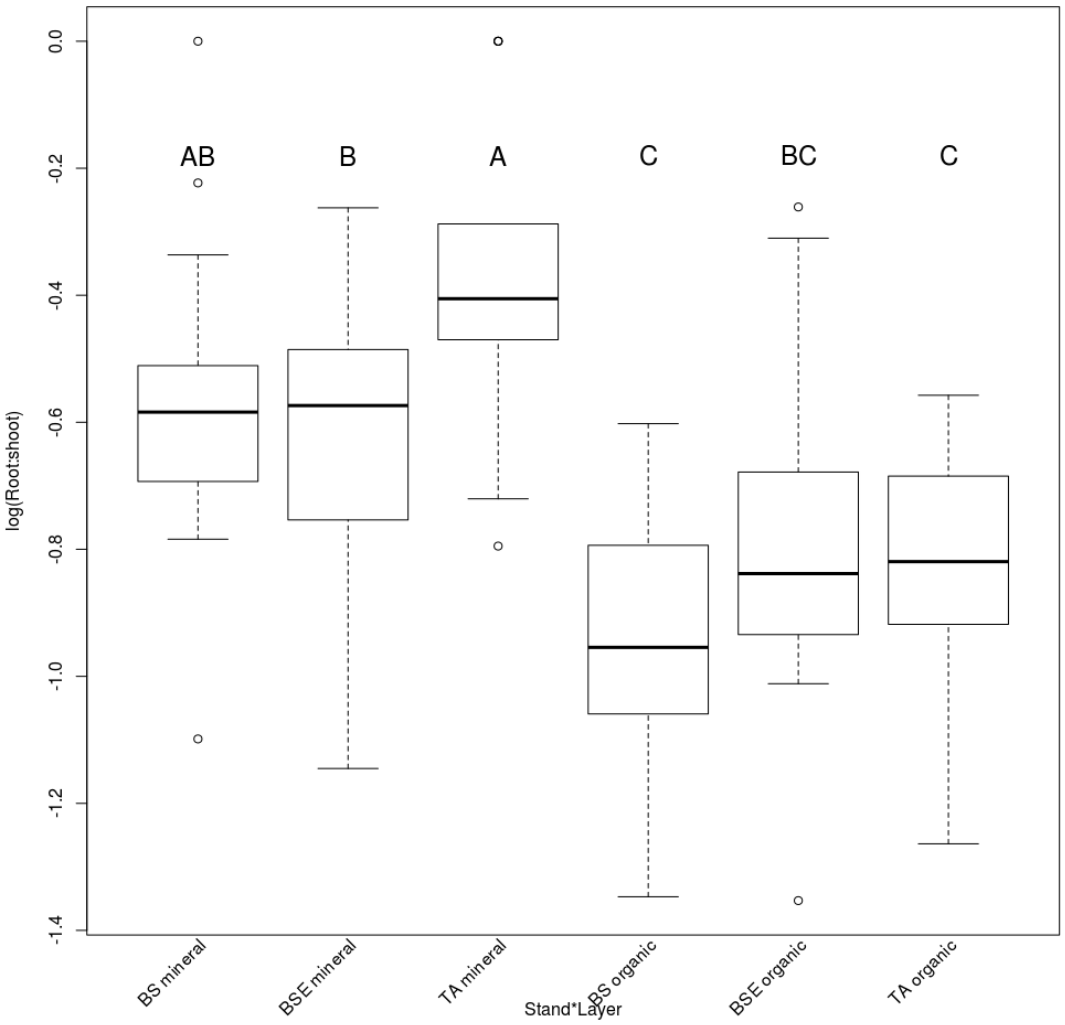

Figure 3.8 Root:shoot ratios of balsam fir seedlings after three growing seasons for different stand types by soil layers interactions.

3.6 Discussion

We conducted this experiment in order to detect a potential effect of ectomycorrhizal interactions that could explain the better establishment and growth of balsam fir seedlings observed under TA than BS stands. Mycorrhiza are involved in plant N

nutrition since they are able to collect N in mineral and organic forms in soils and transfer it to associated plants (Smith and Read, 2008). Mycorrhization rate as well as mycorrhizal community structure are determinant factors of N absorption by plants in boreal forests where nutrients are mainly found in organic forms (Read *et al.*, 2004). We detected greater mycorrhization rates in unsterilized soils, although seedlings in sterilized soils were also mycorrhized (Figure 3.3). Colonization of seedlings by mycorrhizal fungi in sterilized soil could have come from airborne spores within the growth chamber (Stottlemyer *et al.*, 2008). This contamination by spores probably yielded few ectomycorrhizal species representing the ectomycorrhizal communities present around the experimental zone (Amos, QC, Canada, around 100 km south-west from study sites) rather than the ectomycorrhizal communities from the studied zone (Authier-Nord, QC, Canada), as most of spores do not disperse long distance (Peay *et al.*, 2010). Mycorrhization rate was higher in unsterilized soil but not correlated to size of seedlings nor to needle N concentrations, indicating that the number of mycorrhizal root tips had no effect on growth of the seedlings during the first three years of growth. As mycorrhization in sterilized soils might be the result of local contamination by a few number of species, mycorrhizal communities were probably low diverse in sterilized than in non-sterilized soils. Needle N concentrations were greater in unsterilized soils (Figure 3.4b) and the divergence in mycorrhizal diversity could explain this difference. Several studies have demonstrated an effect of ectomycorrhizal community structure and diversity on N nutrition (Leberecht *et al.*, 2015; Nguyen *et al.*, 2017). Contrary to our hypotheses, N concentration in fir needles was higher in BS than in BSE and TA stands (Figure 3.4). In the field, we found greater needle N concentrations in balsam fir saplings growing in TA-dominated stands compared to BSE stands (Nagati et al., 2019) which was linked to ectomycorrhizal community divergence between these two stand types. Therefore, direct biotic interactions between TA and balsam fir may explain the difference between the field and our experiment. Among those interactions, we could hypothesize that there are common mycorrhizal networks (Simard *et al.*, 2012)

between balsam fir and TA that would increase balsam fir N uptake near trembling aspen but not in other conditions. Positive effects of the presence of a common mycorrhizal network on growth of coniferous seedlings have been detected elsewhere (Booth, 2004; Booth and Hoeksema, 2009), including facilitation between a coniferous species and a hardwood species (Nara, 2006; Nara and Hogetsu, 2004). Further analysis and experimental tests would have to be done to confirm and demonstrate the existence of common mycorrhizal networks between aspen and balsam fir.

We had hypothesized that germination rate would be independent from soil origin, layer or sterilization, and this hypothesis was invalidated, as stand type and soil layer significantly affected germination rate. Seeds germinated in larger numbers in organic soils and in soils from BS and BSE stands (Figure 3.1). The presence of mosses and particularly *Pleurozium spp.* in the organic layer of BS and BSE stands represent a good seedbed for balsam fir seeds contrary to the broadleaf litter (McLaren and Janke, 1996; Parent *et al.*, 2003). Survival was also greater in our organic soils (Figure 3.2), contrarily to McLaren and Janke (1996) that observed higher mortality on broadleaf litter after the first year of germination, probably due to a lower humidity in these soil if compared to coniferous soil. Our results differ perhaps due to the high humidity level that was maintained in the growth chamber during the experiment.

Lesser germination and survival of balsam fir in mineral compared to organic soils could be explained by the heavy clay texture of mineral soils collected for this study. Clay soils are prone to compaction that lead to drought during dry periods and to waterlogging during long wet conditions. Considering the relative humidity within the chamber and the frequent waterings during the experiment to avoid organic soil dessication, seeds and seedlings could have suffered from waterlogging in mineral soil layers. Germination and survival of tree seeds are often lower in waterlogged

soils due to anoxic conditions (Mukassabi *et al.*, 2012; Pérez-Ramos and Marañón, 2009; Sanderson and Armstrong, 1978; Xu *et al.*, 1997). We detected a lower development of seedling roots and a higher root:shoot ratio in mineral than in organic soils (Figures 3.7&3.8) which could have been also caused by waterlogging (Levan and Riha, 1986). Height and total dry mass of seedlings were greater in organic than in mineral soils, which has also been demonstrated for black spruce seedlings growing in the clay-belt of Abitibi-Témiscamingue region due to waterlogging caused by soil compaction (Lavoie *et al.*, 2007a, 2007b).

To conclude, our experiment suggests a strong influence of soil layer but not of mycorrhization rates on balsam fir, germination, survival and early growth. Germination, survival and growth were better in organic compared to in mineral soils and probably represent the response of BF seedlings to waterlogging conditions that were generated in compacted clay soil. Our results also tend to demonstrate that germination and early growth are not factors affecting the lower abundance of balsam fir in BS compared to TA stands. Moreover, mycorrhizal interactions could not be invoked to explain abundance even if they could play a role in nutrition. Low regeneration and growth of balsam fir in BS and BSE stands may be caused by nutritional or survival failure at sapling instead of seedling stage during balsam fir development.

3.7 Acknowledgements

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CHAPITRE IV

CONCLUSION GÉNÉRALE

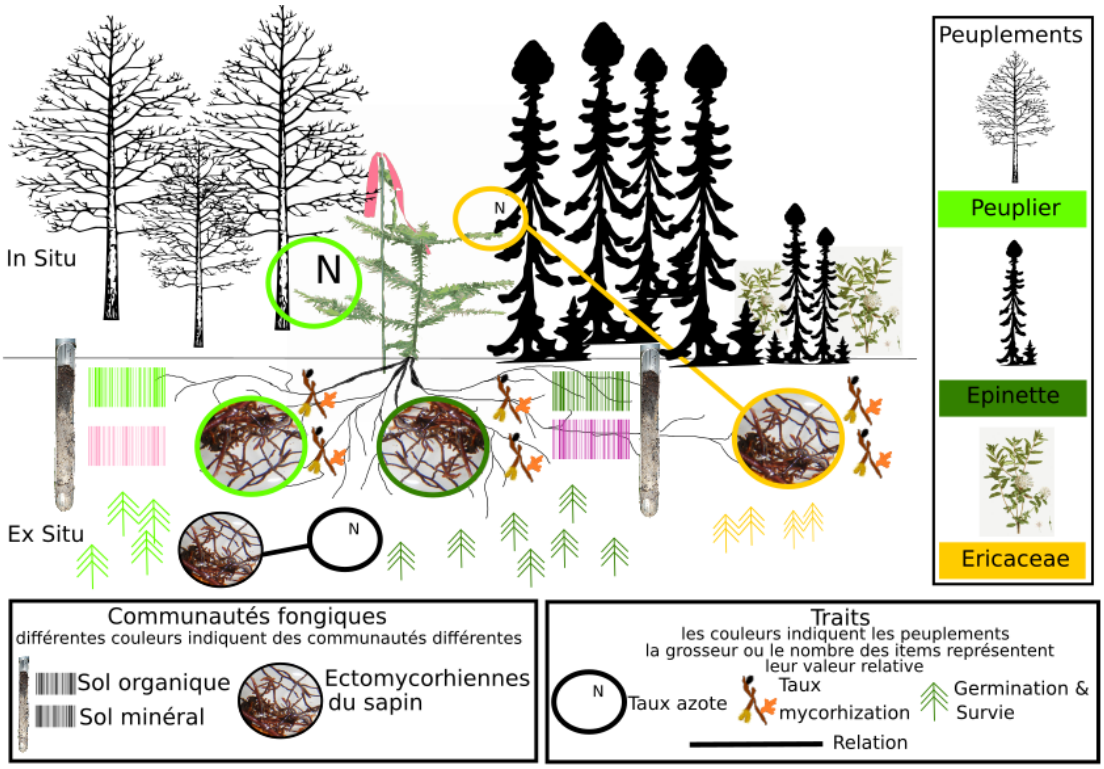

Figure 4.1 Résumé des principaux résultats de la thèse

L'objectif principal de la thèse était de déterminer si les champignons ectomycorhiziens jouaient un rôle dans la facilitation observée entre le sapin baumier et le peuplier faux-tremble. Nos données suggèrent que le taux de mycorhization n'est pas corrélé à la croissance et la nutrition des jeunes plantules, mais que les communautés microbiennes ont un rôle sur la nutrition (Chapitre 3). Pour les plants à un stade de développement plus avancé, nous avons démontré que c'était la

composition des communautés ectomycorhiziennes plutôt que la richesse ou le taux de mycorhization qui a joué un rôle sur la nutrition azotée (Chapitre 2). La Figure 4.1 présente les hypothèses validées grâce aux expériences menées durant la thèse, nous avons détecté des différences dans les communautés fongiques du sol et dans les communautés ectomycorhiziennes associées au sapin entre les peuplements, mais il n'y avait pas de différence significative des taux de mycorhization. La nutrition azotée était corrélée aux modifications des communautés ECM à proximité des éricacées, mais pas au taux de mycorhization. Les analyses de séquençage haut débit du sol et des mycorhizes menées durant ces travaux de doctorat ont permis de révéler une très forte diversité d'espèces fongiques dans les sols des forêts boréales et des différences de communautés liées aux espèces d'arbres dominants et aux horizons du sol.

4.1 Contributions à l'avancement des connaissances

4.1.1 Forte diversité fongique dans les forêts boréales

Les grands patrons de biodiversité décrivent une diversité maximum à l'équateur et qui diminue en se rapprochant des pôles. Si ces patrons sont confirmés pour les plantes vasculaires (Kier *et al.*, 2005; Kreft et Jetz, 2007) et les vertébrés terrestres (Jenkins *et al.*, 2013) d'autres organismes ne les suivent pas. Cela semble être le cas pour les ECM dont la richesse spécifique est plus élevée en zone boréale et tempérée (Tedersoo *et al.*, 2014). L'analyse par séquençage haut débit des sols en pessière à mousse réalisée durant ce doctorat (Chapitres 1 et 2) permet de conforter l'idée d'une forte diversité fongique (toutes fonctions confondues) en forêt boréale. Dans les sites étudiés, les communautés fongiques n'étaient toutefois pas dominées par les ECM mais par les saprophytes. Cette observation contraste avec les résultats de Taylor *et al.*, (2013) qui montraient une dominance des ECM dans les forêts boréales de l'Ouest canadien, et pourrait en partie être reliée aux différences de conditions abiotiques entre les deux sites d'études, tel que l'humidité et le pH du sol. Un point

important soulevé par l'analyse des communautés fongiques du sol est le manque de référence pour un grand nombre d'OTUs pour lesquelles il a été impossible de faire une identification taxonomique et donc fonctionnelle. Ce constat démontre à quel point la diversité des champignons est mal référencée en forêt boréale. Des analyses couplées de barcoding et de description taxonomique des carpophores dans les zones d'études couvertes par le métabarcoding pourraient aider à régler ce problème (Truong *et al.*, 2017).

L'espèce végétale dominant la canopée a eu un effet fort, non pas sur la diversité, mais sur la composition en espèces, quel que soit le groupe fonctionnel (saprophyte ou ectomycorhizien). Ainsi, aussi bien les ECM (Chapitres 1 et 2) que les saprophytes (Chapitre 1) forment des communautés qui diffèrent entre les peuplements de peuplier faux-tremble et d'épinette. Concernant les ECM, les Russulaceae et les Elaphomycetaceae étaient plus abondants dans les sols prélevés sous peupliers que dans les sols prélevés sous les épinettes noires (Chapitre 1). Les Russulaceae semblent s'associer préférentiellement aux feuillus plutôt qu'aux conifères en forêt boréale (Kernaghan *et al.*, 2003), ce qui pourrait expliquer leur prévalence sous les peupliers dans notre étude. L'épinette noire et le peuplier faux-tremble sont des espèces de stades de succession forestière différents (Bergeron, 2000). Les différences observées au niveau des communautés fongiques appuient les travaux antérieurs démontrant que celles-ci sont dépendantes du stade de succession forestier et qu'elles pourraient avoir une influence sur les rétroactions entre sol et dynamique forestière (Bennett *et al.*, 2017; Kardol *et al.*, 2007.; Ke *et al.*, 2015; Twieg *et al.*, 2007).

4.1.2 Lien entre ectomycorhize et établissement du sapin

L'hypothèse communément admise d'une corrélation positive entre la présence de mycorhizes dans les racines et la croissance et/ou nutrition a été démontrée de nombreuses fois (Klironomos *et al.*, 2000; Rygiewicz *et al.*, 1984; Smith et Read,

2008; Vogelsang *et al.*, 2006). La stérilisation de sols n'ayant pas duré tout le long de l'expérience dans les expériences en chambre de croissance et tous les sapins récoltés sur le terrain étant mycorhizés, nous n'avons pas de référence sur la croissance du sapin sans mycorhizes et on ne peut pas exclure une moins bonne survie des sapins en l'absence totale de mycorhize. Les taux de mycorhization n'étaient pas différents pour les sapins ayant poussé sous épinette, sous peuplier ou à proximité de plantes éricacées sur le terrain (Chapitre 2). Une certaine tendance vers un taux de mycorhization plus élevé sous les peupliers dans les expériences en serre a été détectée, sans qu'il soit toutefois possible de le relier aux paramètres de croissance et nutrition mesurés (Chapitre 3). Les données du Chapitre 2 indiquent que le sapin baumier s'associe à des partenaires mycorhiziens quel que soit le peuplement, et que la richesse spécifique en ECM ne diffère pas. Les données de la thèse suggèrent que le taux de mycorhization n'est pas un bon marqueur de l'efficience de la mycorhization en forêt boréale, et que l'identité des mycorhizes et la structure de leur communauté ont un plus fort impact sur la croissance et l'absorption des nutriments.

L'utilisation couplée d'analyse de piste et de métabarcoding a permis de relier l'état physiologique du sapin avec les communautés ECM qui y sont associées (Chapitre 2). Le sapin s'associait avec des mycorhizes quel que soit le type de communauté végétale, mais la composition en espèces diffèrait en fonction de ces dernières. Les familles ECM Inocybaceae, Thelephoraceae, Gloniaceae et Clavulinaceae étaient plus fortement associées au sapin dans les peuplements de peupliers, les Cortinariaceae sont plus présents sous couvert d'épinette, et enfin, la proximité aux plantes éricacées augmentait l'abondance des Helotiaceae associées aux racines des sapins (Chapitre 2). La variabilité des communautés ECM associées au sapin était expliquée à 16% par les communautés végétales, le reste de la variation étant probablement due à des différences entre les microsites et des processus neutres. Dans le cas de la proximité à une plante éricacée, la modification de la communauté ECM s'est avérée reliée à une moins bonne nutrition azotée pour les sapins suivis sur le terrain. L'effet de la

stérilisation, mais non du taux de mycorhization, sur la concentration en azote démontrée dans le Chapitre 3 tend à démontrer l'effet d'un changement de communauté fongique plutôt que du taux de mycorhization. Les mycorhizes détectées sur les sapins dans les sols stérilisés provennaient probablement d'une contamination par quelques espèces mycorhiziennes (Stottlemyer *et al.*, 2008), ce qui va dans le sens d'un effet de la modification des communautés sur la nutrition azotée. Dans ce contexte, il est possible que quelques espèces et/ou la présence d'un nombre minimum d'espèces ECM soient nécessaires pour améliorer la croissance du sapin. De plus, la différence entre sol stérilisé et sol non stérilisé pourrait aussi être le fait de la présence d'autres espèces (bactériennes ou fongiques) présentes dans le sol puisque la stérilisation n'a pas ciblé les ECM en particulier. L'utilisation d'autres méthodes de stérilisation (notamment l'utilisation de fongicide) aurait pu donner des réponses plus précises.

Les effets négatifs des éricacées à ERM ont déjà été démontrés plusieurs fois, sans que les processus mis en cause ne soient déterminés (Mallik, 2003; Mallik et Pellissier, 2000; Peterson, 1965; Zackrisson *et al.*, 1997). Dans notre étude, la modification des communautés ECM associées au sapin à proximité des éricacées à ERM a été démontrée, comme ce fut déjà le cas dans plusieurs écosystèmes (Kennedy *et al.*, 2018; Kohout *et al.*, 2011; Yamasaki *et al.*, 1998). La thèse apporte cependant une nouveauté, soit que les modifications de la composition des communautés ECM à proximité des éricacées à ERM ont eu un effet négatif sur la nutrition azotée des sapins. Cela pourrait expliquer en partie les problèmes de régénération des plantes détectés dans plusieurs écosystèmes en présence de plantes éricacées. Les données du Chapitre 3 suggèrent toutefois que les ECM seules ne peuvent être mises en cause. En effet, en serre, les sapins poussant dans le sol récolté à proximité des éricacées à ERM montraient une moins bonne nutrition azotée que ceux poussant dans le sol récolté à proximité des épinettes, et ce quel que soit la condition de stérilisation. Cela pourrait être expliqué par la présence de matière

organique récalcitrante dans ces sols (Joanisse *et al.*, 2018) ou par la présence de composés phénoliques apportés par les racines des éricacées et nocifs pour les autres plantes (Carballeira, 1980; Gallet, 1994; Mallik *et al.*, 2016).

Au vu des résultats contrastés entre les Chapitres 2 et 3 de la thèse, une hypothèse émerge. La nutrition azotée des sapins (qui est positivement corrélée à la croissance) était moins bonne à proximité des plantes éricacées que sous peuplier sur le terrain et à l'inverse, elle était meilleure sous épinette que sous peuplier ou à proximité d'éricacées dans les expériences en chambre de croissance. Il semblerait donc que ce ne soit pas seulement la présence ou l'abondance de tel ou tel partenaire mycorhizien qui soit directement en jeu (reflétée par une absence de lien entre concentrations en N et origine du sol dans le Chapitre 3), mais une interaction plus directe avec les plantes, et possiblement la présence d'un réseau mycorhizien commun (Selosse *et al.*, 2006; Simard *et al.*, 2012, 2015). Il a déjà été démontré que la présence d'un réseau mycélien commun entre une espèce de succession primaire favorise le développement d'une espèce de succession secondaire (Nara, 2006; Nara et Hogetsu, 2004). Le peuplier étant une espèce de début de succession et le sapin de fin de succession dans le domaine de la sapinière à bouleau (Bergeron, 2000). La présence d'un réseau mycélien commun entre les deux espèces et son effet potentiel sur la dynamique forestière des sites à l'étude ne peuvent pas être exclus. Nous avons constaté une plus forte abondance de Thelephoraceae associée aux racines de sapin sous couvert de peuplier (Chapitre 2), or, cette famille est généralement associée aux peupliers en forêt boréale (Kernaghan *et al.*, 2003), leur présence dans les racines du sapin pourrait donc résulter de la présence d'un réseau mycélien commun avec le peuplier faux-tremble. À l'inverse, il a déjà été démontré un effet négatif de la présence d'un réseau mycélien commun sur l'établissement d'une espèce, bien que ces effets négatifs soient plus communs pour les mycorhizes à arbuscules (van der Heijden et Horton, 2009). Certains champignons de la famille des Helotiaceae (qui sont plus associés aux racines du sapin en présence des éricacées, Chapitre 2), et plus

particulièrement le groupe *Rhizoscyphus ericae*, pourraient être capables de former à la fois des ECM et des ERM (Grelet *et al.*, 2010; Villarreal-Ruiz *et al.*, 2004; Vrålstad *et al.*, 2000), ce qui est aussi le cas pour d'autres espèces de champignons (Bergero *et al.*, 2000; Chambers *et al.*, 2008; Vohník *et al.*, 2016). Ces espèces pourraient relier ces deux types de plantes via un CMN, mais cela n'a, à ma connaissance, jamais été factuellement démontré et les études susmentionnées révèlent en général une colonisation de la racine par le champignon et la mise en place d'une structure ressemblant à une ectomycorhize.

Les résultats du Chapitre 3 contrastent beaucoup avec nos hypothèses de départ, et démontrent que la quantité de mycorhizes formées sur les racines n'est que peu impliquée dans les problèmes de régénération des plantules de sapin, mais que l'identité des microorganismes du sol, et notamment des ECM, à un rôle sur la nutrition des plantules et des jeunes individus. Ces expériences ont permis de constater que la provenance du sol importe peu pour la survie et la croissance du sapin durant les trois premières années de sa vie. Ce qui est en accord avec les résultats de Arbour et Bergeron (2011) qui montrent que ce sont surtout les jeunes arbres (en opposition aux plantules) qui sont moins abondants sous épinette que sous peuplier.

4.2 Perspectives de recherche

Nos travaux amènent des réponses sur le rôle des champignons dans l'établissement du sapin en forêt boréale, mais font également émerger d'autres questions. Nos données ont permis de mettre à jour la forte diversité fongique dans les forêts de l'Abitibi-Témiscamingue avec une grande influence des communautés végétales sur leur composition. Le pH plus bas dans les peuplements d'épinette noire (Chapitre 1) laisse à penser la diminution du ratio champignon/bactéries, comme cela a déjà été démontré dans les écosystèmes forestiers (Bååth et Anderson, 2003; Blagodatskaya et Anderson, 1998). La diminution du pH et donc du ratio champignon/bactéries

diminue la minéralisation en forêt boréale (Högberg et al. 2007) et donc changer les quantités de nitrate et d'ammonium dans les deux types de peuplements. Dans ce contexte, une étude sur les formes d'azote présentes dans les sols et leur lien avec les communautés fongiques du sol et les communautés ECM associées au sapin est à envisager. De plus nous n'avons pousser les investigations pour savoir si le sapin baumier utilisait les mêmes sources d'azote dans les deux peuplements. Au vu des différences contrastées, de pH, de ratio C/N et de communauté fongique entre les deux peuplements, la quantité de nitrate pourrait être supérieure sous les peupliers et impacter positivement la croissance du sapin. Finalement, les champignons ECM pourraient transférer moins d'azote (pour la même quantité de carbone) à leur partenaire végétal lorsqu'ils le puisent sous forme organique dans le sol et donc les échanges seraient moins intéressants pour la plante (Smith et Read, 2008; Kyaschenko et al. 2019). L'azote se trouvant principalement sous forme organique sous les épinettes, la symbiose ECM pourrait être moins efficace pour le sapin dans ces conditions, mais cela devrait être démontré expérimentalement.

La présence de peuplements mixtes (Cavard *et al.*, 2011) à proximité des peuplements étudiés dans les Chapitres 1 et 2 pose la question de l'établissement du sapin et de la composition des communautés fongiques dans ces peuplements mixtes. Les peuplements mixtes représentent de meilleurs sites d'établissement pour le sapin (Messaoud *et al.*, 2019). Des données préliminaires comparant les peuplements en sapinière à ceux en pessière (Annexe C, Figure C.1) tendent à démontrer que les communautés ECM sous peuplier ont une composition qui se trouve entre celle sous épinette et celle en sapinière. La sapinière, quant à elle, semble avoir des communautés fongiques à part. Si les communautés dans les peuplements mixtes sont davantage similaires à celles trouvées en sapinière qu'à celles des peuplements purs (épinette ou peuplier) en pessière, alors le sapin baumier aurait un terrain biotique plus favorable à son établissement dans les peuplements mixtes. Une comparaison

des communautés fongiques du sol entre les différents peuplements en pessière et en sapinière est à envisager.

Une hypothèse qui émerge de ces travaux est la possibilité d'un CMN entre les sapins et les peupliers d'un côté et entre les sapins et les éricacées de l'autre. La présence d'un CMN pourrait être testée à la fois *in situ* et *ex situ*, et ces deux tests seraient complémentaires. Le dispositif expérimental sur le terrain proposé par Booth (2004) et Booth et Hoeksema (2009) permettrait de distinguer les effets de la présence seule des ECM de ceux de la présence d'un CMN ou de la présence des racines des plantes alentour. Dans ces expériences, des plantules d'un an sont plantées sur le terrain dans des cylindres en métal dont le fond est fermé et les parois percées de trous laissant passer les racines, il existe aussi une version avec des parois sans orifice. Avec ces deux versions, on teste donc l'effet de la présence ou de l'absence des racines des autres individus sur la vigueur des plantules. Pour les cylindres troués, il est possible d'appliquer une grille laissant passer le mycélium, mais pas les racines, on peut ainsi distinguer l'effet des racines de celui d'un CMN. Enfin, il est possible de trancher régulièrement le sol autour des cylindres afin de détruire le potentiel CMN et ainsi de distinguer son effet de celui des mycorhizes seules. De telles expériences ne sont pas très compliquées à mettre en place et apportent une quantité de réponses, leur mise en place serait un atout pour mieux comprendre le rôle des mycorhizes dans les forêts boréales. D'un autre côté, il serait tout à fait possible de refaire les expériences présentées en Chapitre 3 en ajoutant une condition, soit la présence ou l'absence de la plante dominant chaque type de peuplement. L'inoculation de certaines souches ECM sur les sapins serait également à envisager pour tester l'effet de la diversité spécifique et/ou de l'identité des espèces ECM. Finalement, il serait intéressant de tester plusieurs types de stérilisation pour exclure l'effet des autres microorganismes du sol sur l'établissement du sapin.

4.3 Quelles conséquences pour l'aménagement forestier?

4.3.1 Besoin d'un état de référence de la diversité des sols pour l'aménagement forestier écosystémique

La description de la strate arborée et du sous-bois est souvent réalisée dans les inventaires forestiers, ce qui n'est pas le cas des microorganismes du sol. Les résultats du Chapitre 1 démontrent que les communautés fongiques peuvent être très différentes d'un peuplement à l'autre, et ceci à des fines échelles spatiales. Des études ont montré à quel point les coupes peuvent modifier la structure des communautés fongiques, et particulièrement ECM, en forêt boréale (Goldmann *et al.*, 2015; Teste *et al.*, 2012; Varenius *et al.*, 2016; Vašutová *et al.*, 2018) et ainsi affecter le reboisement. Quant aux communautés de saprophytes, elles jouent un rôle majeur dans le cycle des nutriments en forêt boréale (Thormann, 2006b). De plus, les champignons sont impliqués dans le stockage du carbone dans le sol (Cairney, 2012; Clemmensen *et al.*, 2013, 2015; Courty *et al.*, 2010). Au vu des résultats du Chapitre 2 et des données fournies par la littérature sur le sujet (références dans le texte), le rôle des champignons ECM dans la dynamique forestière naturelle semble évident. Pour ne pas exclure la biodiversité des sols de l'aménagement forestier durable, des états de référence de la forêt naturelle devraient être mis en place et les champignons pris en compte dans les politiques d'aménagement (Suz *et al.*, 2015). En effet, connaître en profondeur cette biodiversité et les effets de l'aménagement actuel sur cette dernière permettrait de réduire les écarts entre forêt aménagée et forêt naturelle, ce qui est le fondement de l'aménagement écosystémique. Pour cela, les techniques de séquençage haut débit de l'ADN du sol devraient être mises en place dans les placettes d'inventaires permanents, ce qui permettrait en plus d'avoir un état de référence, d'avoir un suivi temporel et d'incrémenter les bases de données de séquences. Enfin, les différentes techniques d'aménagement ont des impacts différents sur les communautés fongiques. Savoir comment réagissent ces dernières aux méthodes

utilisées au Québec serait un atout pour réaliser au mieux l'aménagement forestier écosystémique.

4.3.2 Les éricacées comme frein à la productivité

L'un des points importants soulevés par la thèse est l'effet négatif des éricacées à ERM sur la nutrition des jeunes sapins. Que ce soit sur le terrain ou en serre (Chapitres 2 et 3 de la thèse), la présence des éricacées a eu un impact négatif sur la nutrition des sapins. Les résultats de cette étude pourraient être élargis à d'autres espèces que le sapin baumier, en effet, une modification des communautés ECM associée à l'épinette noire a déjà été détectée à proximité de *Kalmia* (Mallik *et al.*, 2016; Yamasaki *et al.*, 1998). La perte de productivité associée à la présence d'éricacées (Joanisse *et al.*, 2018; Mallik, 2003; Mallik *et al.*, 2016) est à nouveau appuyée par les résultats de cette étude. Les aménagistes forestiers devraient donc prendre en considération la présence ou l'absence de ces plantes lors des activités de reboisement.

Finalement, sans faire de proposition d'aménagement précise (la thèse n'étant pas tournée directement pour répondre à ces problématiques), nous incitons les acteurs de l'aménagement forestier à se tourner vers une recherche leur permettant une meilleure connaissance et compréhension des communautés fongiques du sol et de leurs interactions avec le sol et les plantes. Une meilleure connaissance apportera une vue plus globale sur les rétroactions entre plantes, sol et champignons, et ainsi ces derniers pourraient être pris en compte dans les directives d'aménagement forestier au Québec.

ANNEXE A

MATÉRIEL SUPPLÉMENTAIRE DU CHAPITRE I

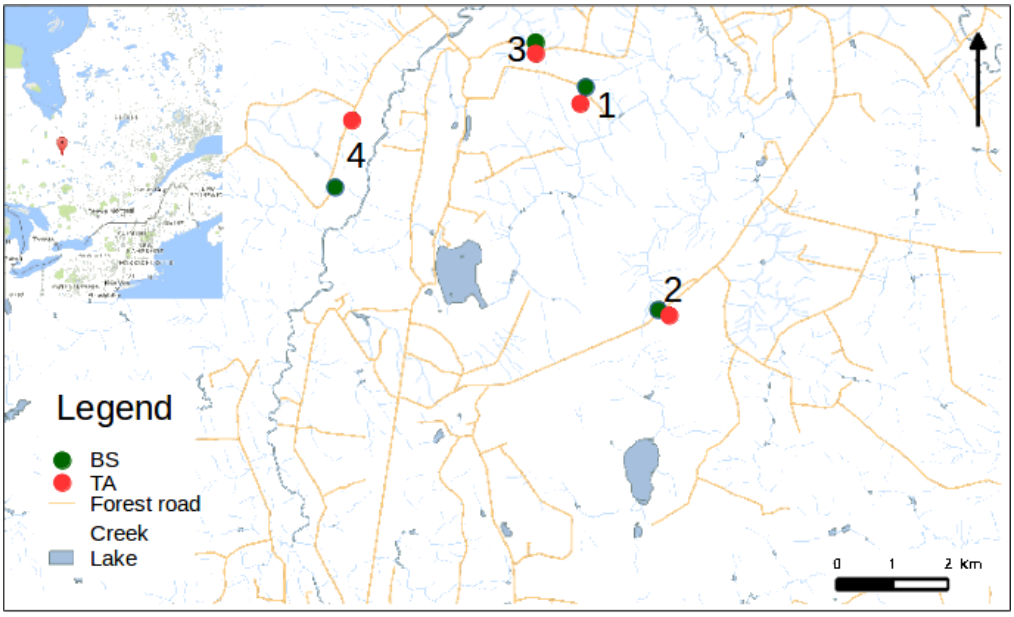

Figure A.1 Location of study sites.

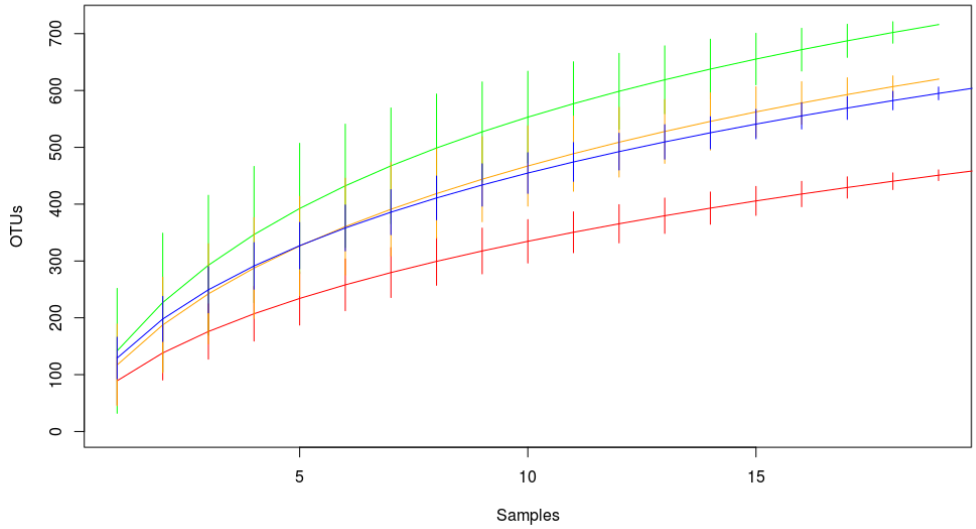

Figure A.2 OTU accumulation curves of fungal communities from black spruce organic layer (red), black spruce mineral layer (blue), trembling aspen organic layer (orange) and trembling aspen mineral layer (green), vertical bars represent standard errors of the mean.

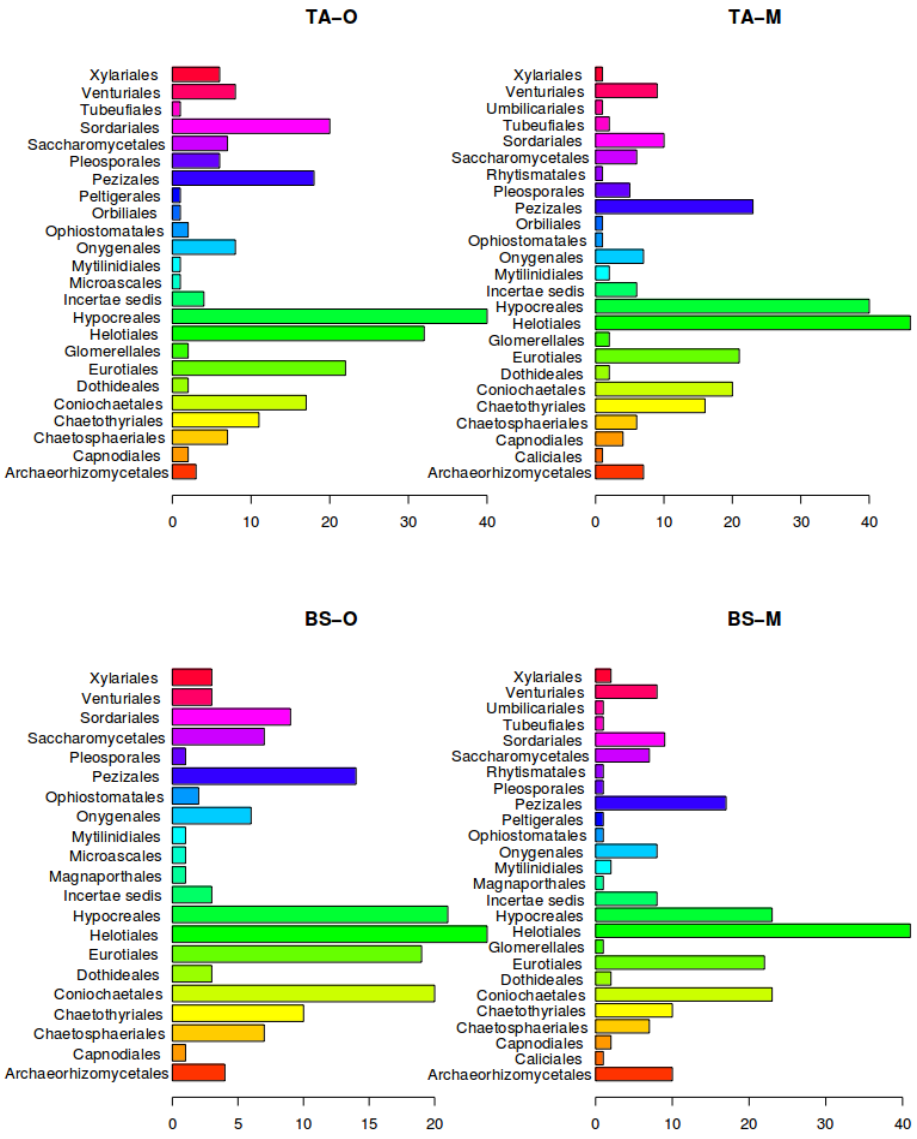

Figure A.3: Number of OTUs by genera of Ascomycota and by sample type (TA-M = trembling aspen mineral layer, TA-O = trembling aspen organic layer, BS-M = black spruce mineral layer and BS-O = black spruce organic layer).

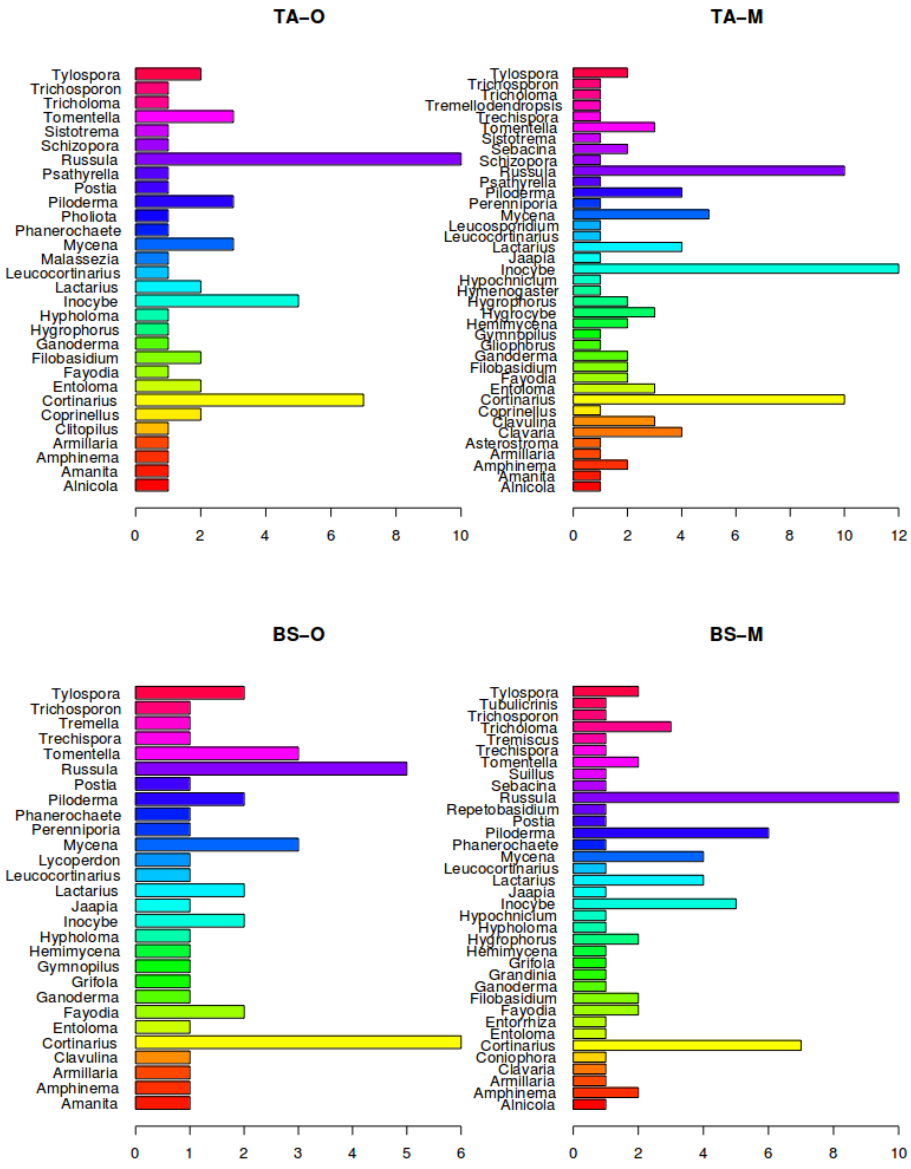

Figure A.4: Number of OTUs by genera of Basidiomycota and by sample type (TA-M = trembling aspen mineral layer, TA-O = trembling aspen organic layer, BS-M = black spruce mineral layer and BS-O = black spruce organic layer).

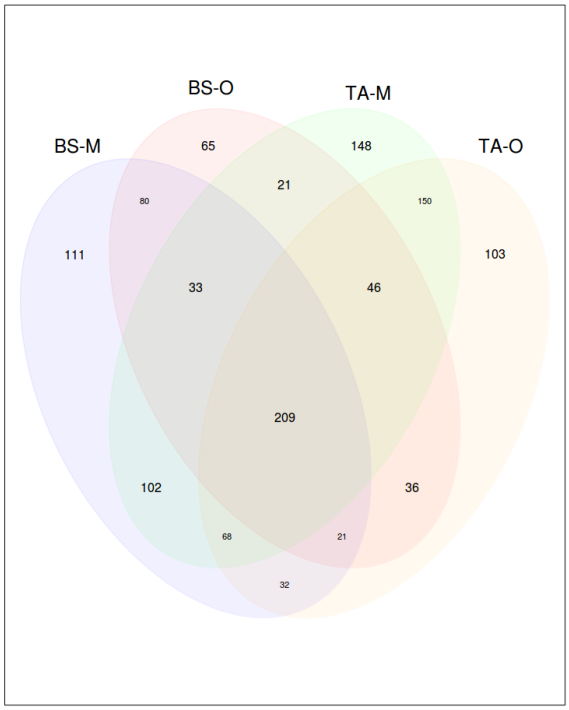

Figure A.5 Venn diagram representing shared fungal OTUs between black spruce organic layer (BS-O), black spruce mineral layer (BS-M), trembling aspen organic layer (TA-O) and trembling aspen mineral layer (TA-M).

Stand/Site	Lat/Long	Soil layer	C %	N %	S %	pH water	PH $CaCl_2$	P mg/kg	K cmol/kg	Ca cmol/kg	Mg cmol/kg	Mn cmol/kg	Al cmol/kg	Fe cmol/kg	Na cmol/kg	ECEC cmol/kg
BS 1	49.19061/-78.82797	Mineral	3.24	0.13	0.02	4.55	3.83	6.05	0.14	0.39	0.30	0.02	15.23	2.27	0.04	18.39
		Organic	32.08	0.60	0.09	3.79	3.12	78.95	0.75	2.91	1.71	0.14	11.15	4.03	0.10	20.78
TA 1	49.18942/-78.82817	Mineral	6.21	0.34	0.04	4.91	4.10	5.13	0.56	2.76	1.16	0.05	19.92	2.25	0.06	26.75
		Organic	35.46	1.66	0.20	5.08	4.41	129.01	1.51	17.48	4.53	0.78	10.04	3.56	0.12	38.01
BS 2	49.19367/-78.83556	Mineral	6.01	0.24	0.02	4.60	3.82	4.27	0.12	0.79	0.48	0.00	14.65	2.14	0.04	18.22
		Organic	40.21	0.84	0.12	4.01	3.23	70.10	1.11	4.67	3.17	0.12	7.90	4.51	0.17	21.66
TA 2	49.19375/-78.8345	Mineral	3.95	0.23	0.04	5.44	4.60	3.60	0.26	7.97	3.29	0.07	13.55	1.89	0.09	27.11
		Organic	30.84	1.33	0.17	5.14	4.54	69.08	0.78	20.47	6.18	0.61	6.93	3.74	0.16	38.88
BS 3	49.196695/-78.842092	Mineral	5.56	0.26	0.04	5.61	4.72	13.60	0.26	7.40	3.10	0.07	10.84	2.07	0.10	23.84
		Organic	42.53	0.86	0.13	4.33	3.64	116.57	0.97	14.00	5.55	0.18	3.48	4.40	0.16	28.74
TA 3	49.196422/-78.84237	Mineral	2.87	0.14	0.02	4.88	4.07	15.81	0.19	0.99	0.45	0.03	17.41	2.35	0.03	21.44
		Organic	40.55	1.43	0.18	4.57	4.01	245.60	2.26	17.76	4.30	0.57	6.28	3.56	0.10	34.83
BS 4	49.168972/-78.885194	Mineral	5.02	0.21	0.04	4.73	3.88	9.11	0.22	0.95	0.73	0.03	20.81	2.47	0.09	25.29
		Organic	41.53	0.74	0.11	3.89	3.18	136.23	1.66	4.79	3.61	0.17	3.48	4.47	0.16	18.33
TA 4	49.180417/-78.883611	Mineral	2.64	0.11	0.03	5.12	4.29	8.50	0.57	3.12	1.69	0.09	15.94	1.88	0.08	23.36
		Organic	32.77	1.48	0.18	5.12	4.50	146.02	1.75	20.16	5.52	0.58	4.23	3.26	0.12	35.63

Table A.1 Sites description and soil characteristics per sample type, BS= black spruce, TA= trembling aspen.

ANNEXE B

MATÉRIEL SUPPLÉMENTAIRE DU CHAPITRE II

Table B.1 Summary of parameters estimates of Mod1

Regressions:

		Estimate	Std.Err	z-value	P(>|z|)
Apical	~				
N.total		0.547	0.128	4.284	0
Stand		-0.208	0.441	-0.472	0.637
Ericaceous		0.077	0.227	0.338	0.735
Percentage BS		0.256	0.167	1.534	0.125
Percentage BF		-0.146	0.113	-1.289	0.197
Ramification index		0.253	0.104	2.425	0.015
NMDS1		0.163	0.129	1.269	0.205
NMDS2		-0.138	0.098	-1.404	0.16
Lateral	~				
N.total		0.609	0.126	4.828	0
Stand		0.138	0.435	0.316	0.752
Ericaceous		0.11	0.224	0.49	0.624
Percentage BS		0.272	0.165	1.649	0.099
Percentage BF		0.017	0.112	0.154	0.878
Ramification index		0.254	0.103	2.472	0.013
NMDS1		0.073	0.127	0.572	0.567
NMDS2		-0.044	0.097	-0.451	0.652
N.total	~				
Stand		0.049	0.469	0.105	0.916
Ericaceous		-0.183	0.24	-0.763	0.446
Percentage BS		-0.469	0.166	-2.827	0.005
Percentage BF		-0.111	0.12	-0.925	0.355
Ramification index		0.209	0.107	1.947	0.051
NMDS1		-0.226	0.134	-1.692	0.091
NMDS2		-0.211	0.101	-2.096	0.036
Ramification index	~				
Stand		-1.573	0.473	-3.327	0.001
Percentage BS		0.371	0.204	1.816	0.069
Percentage BF		-0.023	0.152	-0.153	0.879
NMDS1	~				
Stand		1.499	0.201	7.474	0
NMDS2	~				
Ericaceous		0.773	0.266	2.907	0.004

Table B.1 (suite) Summary of parameters estimates of Mod1

Ericaceous	~				
Stand		0.5	0.118	4.243	0
Percentage BS	~				
Stand		1.708	0.167	10.254	0
Percentage BF	~				
Stand		-1.306	0.224	-5.825	0

Covariances:

		Estimate	Std.Err	z-value	P(>|z|)
.Apical	~~				
.Lateral		0.436	0.1	4.349	0
Basal diameter		0.429	0.12	3.593	0
.Lateral	~~				
Basal diameter		0.432	0.119	3.625	0

Variances:

	Estimate	Std.Err	z-value	P(>|z|)
.Apical	0.598	0.115	5.196	0
.Lateral	0.59	0.114	5.196	0
.N.total	0.466	0.09	5.196	0
.Ramification index	0.75	0.144	5.196	0
.NMDS1	0.482	0.093	5.196	0
.NMDS2	0.849	0.163	5.196	0
.Ericaceous	0.167	0.032	5.196	0
.Percentage BS	0.333	0.064	5.196	0
.Percentage BF	0.603	0.116	5.196	0
Colar diameter	0.981	0.189	5.196	0

R-Square:

	Estimate
Apical	0.374
Lateral	0.366
N.total	0.544
Ramification index	0.235
NMDS1	0.508
NMDS2	0.135
Ericaceous	0.25
Percentage BS	0.661
Percentage BF	0.386

ANNEXE C

MATÉRIEL SUPPLÉMENTAIRE DE LA THÈSE

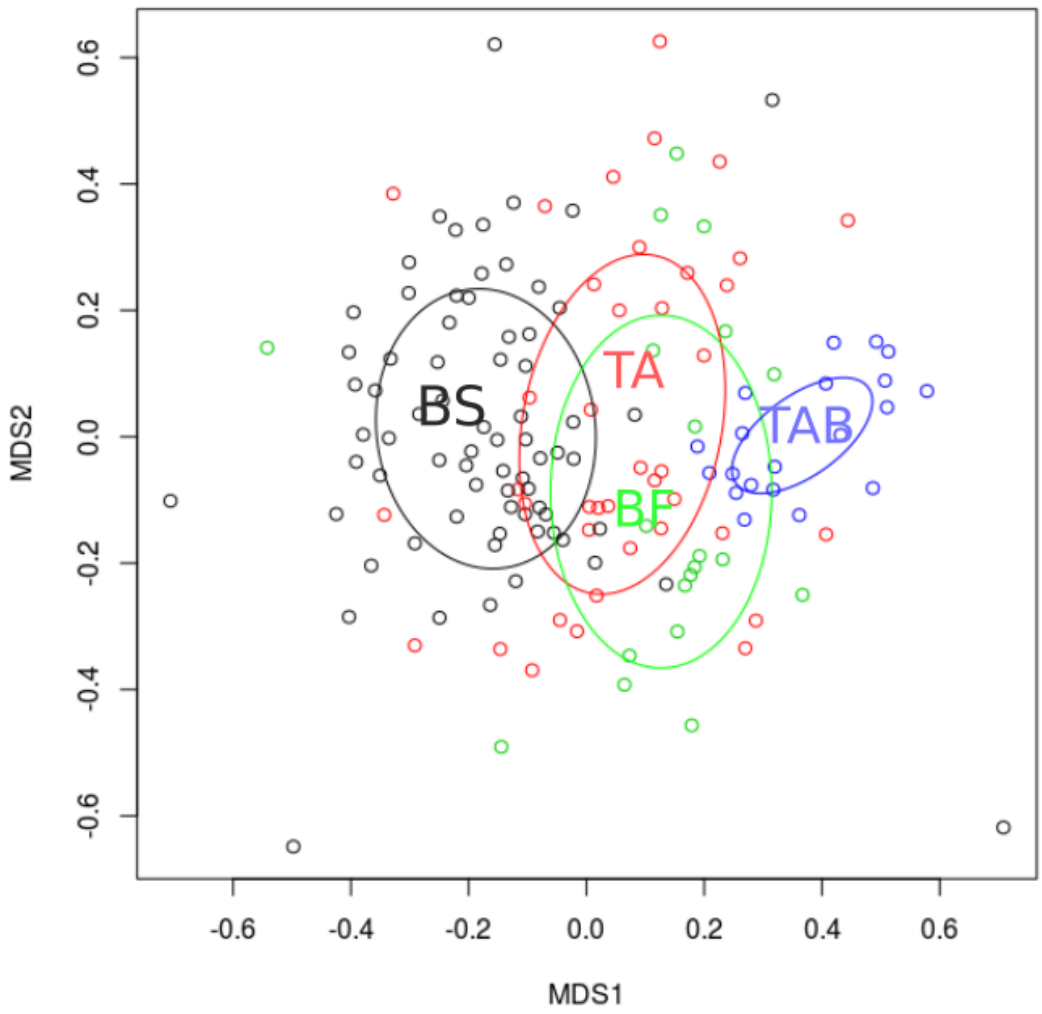

Figure C.1 Analyse multivariée non-métrique des communautés ectomycorhiziennes de sol décrites par métabarcoding pour les peuplements d'épinette noire en pessière à mousse (BS), de peuplier faux-tremble en pessière à mousse (TA), de sapin baumier en sapinière (BF) et peuplier faux-tremble en sapinière (TAB)

BIBLIOGRAPHIE GÉNÉRALE

Abarenkov, K., Tedersoo, L., Nilsson, R. H., Vellak, K., Saar, I., Veldre, V., … Kõljalg, U. (2010). Plutof—a web based workbench for ecological and taxonomic research, with an online implementation for fungal its sequences. E*volutionary Bioinformatics*, *6*, ebo.s6271. doi: 10.4137/ebo.s6271

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. et Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403-410. doi: 10.1016/S0022-2836(05)80360-2

André, H. M., Ducarme, X. et Lebrun, P. (2002). Soil biodiversity: myth, reality or conning? *Oikos*, *96*(1), 3-24. doi: 10.1034/j.1600-0706.2002.11216.x

Arbour, M.-L. et Bergeron, Y. (2011). Effect of increased *Populus* cover on *Abies* regeneration in the *Picea*-feathermoss boreal forest. *Journal of Vegetation Science*, *22*(6), 1132-1142. doi: 10.1111/j.1654-1103.2011.01314.x

Bååth, E. et Anderson, T.-H. (2003). Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biology and Biochemistry*, *35* (7), 955-963. doi: 10.1016/S0038-0717(03)00154-8

Bahram, M., Harend, H. et Tedersoo, L. (2014). Network perspectives of ectomycorrhizal associations. *Fungal Ecology*, *7*, 70-77. doi: 10.1016/j.funeco.2013.10.003

Barbeta, A. et Peñuelas, J. (2017). Increasing carbon discrimination rates and depth of water uptake favor the growth of Mediterranean evergreen trees in the ecotone with temperate deciduous forests. *Global Change Biology*, *23*(12), 5054-5068. doi: 10.1111/gcb.13770

Bardgett, R. D. et van der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. *Nature*, *515*(7528), 505-511. doi: 10.1038/nature13855

Bardgett, R. D. et Wardle, D. A. (2010). Aboveground-belowground linkages: biotic interactions, ecosystem processes, and global change. Oxford; New York : Oxford University Press. Récupéré de Open WorldCat : http://public.eblib.com/choice/publicfullrecord.aspx?p=3053826

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. et Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. *Ecology Letters*, *15*(4), 365-377. doi: 10.1111/j.1461-0248.2011.01736.x

Belleau, A., Leduc, A., Lecomte, N. et Bergeron, Y. (2011). Forest succession rate and pathways on different surface deposit types in the boreal forest of northwestern Quebec. *Écoscience*, *18*(4), 329-340. doi: 10.2980/18-4-3393

Bennett, J. A., Maherali, H., Reinhart, K. O., Lekberg, Y., Hart, M. M. et Klironomos, J. (2017). Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science*, *355*(6321), 181-184. doi: 10.1126/science.aai8212

Bent, E., Kiekel, P., Brenton, R. et Taylor, D. L. (2011). Root-associated ectomycorrhizal fungi shared by various boreal forest seedlings naturally regenerating after a fire in interior alaska and correlation of different fungi with host growth responses. *Applied and Environmental Microbiology*, *77*(10), 3351-3359. doi: 10.1128/AEM.02575-10

Bergero, R., Perotto, S., Girlanda, M., Vidano, G. et Luppi, A. M. (2000). Ericoid mycorrhizal fungi are common root associates of a Mediterranean ectomycorrhizal plant (*Quercus ilex*). *Molecular Ecology*, *9*(10), 1639-1649. doi: 10.1046/j.1365-294x.2000.01059.x

Bergeron, Y. (2000). Species and stand dynamics in the mixed woods of quebec's southern boreal forest. *Ecology*, *81*(6), 1500-1516. doi: 10.1890/0012-9658(2000)081[1500:SASDIT]2.0.CO;2

Bever, J. D., Dickie, I. A., Facelli, E., Facelli, J. M., Klironomos, J., Moora, M., … Zobel, M. (2010). Rooting theories of plant community ecology in microbial interactions. *Trends in Ecology & Evolution*, *25*(8), 468-478. doi: 10.1016/j.tree.2010.05.004

Blagodatskaya, E. V. et Anderson, T.-H. (1998). Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and qCO2 of microbial communities in forest soils. *Soil Biology and Biochemistry*, *30* (10), 1269-1274. doi: 10.1016/S0038-0717(98)00050-9

Booth, M. G. (2004). Mycorrhizal networks mediate overstorey-understorey competition in a temperate forest: Mycorrhizal networks and plant competition. *Ecology Letters*, *7*(7), 538-546. doi: 10.1111/j.1461-0248.2004.00605.x

Booth, M. G. et Hoeksema, J. D. (2009). Mycorrhizal networks counteract competitive effects of canopy trees on seedling survival. *Ecology*, *91*(8), 2294-2302. doi: 10.1890/09-1139.1

Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P. et Coissac, E. (2016). OBITOOLS : a UNIX -inspired software package for DNA metabarcoding. *Molecular Ecology Resources*, *16*(1), 176-182. doi: 10.1111/1755-0998.12428

Brandt, J. P. (2009). The extent of the North American boreal zone. *Environmental Reviews*, *17*(NA), 101-161. doi: 10.1139/A09-004

Brandt, J. P., Flannigan, M. D., Maynard, D. G., Thompson, I. D. et 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

Brooker, R. W., Maestre, F. T., Callaway, R. M., Lortie, C. L., Cavieres, L. A., Kunstler, G., … Michalet, R. (2008). Facilitation in plant communities: the past, the present, and the future. *Journal of Ecology*, *96*(1), 18-34. doi: 10.1111/j.1365-2745.2007.01295.x

Brundrett, M. C. et Tedersoo, L. (2018). Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist*, *220*(4), 1108-1115. doi: 10.1111/nph.14976

Bruns, T. D., Bidartondo, M. I. et Taylor, D. L. (2002). Host specificity in ectomycorrhizal communities: what do the exceptions tell us? *Integrative and Comparative Biology*, *42*(2), 352-359. doi: 10.1093/icb/42.2.352

Buée, M., Courty, P. E., Mignot, D. et Garbaye, J. (2007). Soil niche effect on species diversity and catabolic activities in an ectomycorrhizal fungal community. *Soil Biology and Biochemistry*, *39*(8), 1947-1955. doi: 10.1016/j.soilbio.2007.02.016

Cairney, J. W. G. (2012). Extramatrical mycelia of ectomycorrhizal fungi as moderators of carbon dynamics in forest soil. *Soil Biology and Biochemistry*, *47*, 198-208. doi: 10.1016/j.soilbio.2011.12.029

Callaway, R. M. et Walker, L. R. (1997). Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology*, *78*(7), 1958-1965. doi: 10.1890/0012-9658(1997)078[1958:CAFASA]2.0.CO;2

Carballeira, A. (1980). Phenolic inhibitors in *Erica australis* L. and in associated soil. *Journal of Chemical Ecology*, *6*(3), 593-596. doi: 10.1007/BF00987671

Carter, M. R. et Gregorich, E. G. (dir.). (2008). Soil sampling and methods of analysis (2nd ed). [Pinawa, Manitoba] : Boca Raton, FL : Canadian Society of Soil Science ; CRC Press. Récupéré de Library of Congress ISBN. (S593 .S7425 2008)

Cavard, X., Bergeron, Y., Chen, H. Y. H. et 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

Chambers, S. M., Curlevski, N. J. A. et Cairney, J. W. G. (2008). Ericoid mycorrhizal fungi are common root inhabitants of non-Ericaceae plants in a south-eastern Australian sclerophyll forest. *FEMS Microbiology Ecology*, *65*(2), 263-270. doi: 10.1111/j.1574-6941.2008.00481.x

Chao, A. et Lee, S.-M. (1992). Estimating the number of classes via sample coverage. *Journal of the American Statistical Association*, *87*(417), 210-217. doi: 10.1080/01621459.1992.10475194

Chmolowska, D. (2013). A practical introduction to microbial community sequencing. *Central European Journal of Biology*, *8*(5), 399-409. doi: 10.2478/s11535-013-0155-8

Clemmensen, K. E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., … Lindahl, B. D. (2013). Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science*, *339*(6127), 1615-1618. doi: 10.1126/science.1231923

Clemmensen, Karina E., Finlay, R. D., Dahlberg, A., Stenlid, J., Wardle, D. A. et Lindahl, B. D. (2015). Carbon sequestration is related to mycorrhizal fungal community shifts during long-term succession in boreal forests. *New Phytologist*, *205*(4), 1525-1536. doi: 10.1111/nph.13208

Collier, F. A. et Bidartondo, M. I. (2009). Waiting for fungi: the ectomycorrhizal invasion of lowland heathlands. *Journal of Ecology*, *97*(5), 950-963. doi: 10.1111/j.1365-2745.2009.01544.x

Courty, P.-E., Buée, M., Diedhiou, A. G., Frey-Klett, P., Le Tacon, F., Rineau, F., … Garbaye, J. (2010). The role of ectomycorrhizal communities in forest

ecosystem processes: New perspectives and emerging concepts. *Soil Biology and Biochemistry*, *42*(5), 679-698. doi: 10.1016/j.soilbio.2009.12.006

Davey, M. L. et Currah, R. S. (2006). Interactions between mosses (Bryophyta) and fungi. *Canadian Journal of Botany*, *84*(10), 1509-1519. doi: 10.1139/b06-120

Davey, M. L., Skogen, M. J., Heegaard, E., Halvorsen, R., Kauserud, H. et Ohlson, M. (2017). Host and tissue variations overshadow the response of boreal moss-associated fungal communities to increased nitrogen load. *Molecular Ecology*, *26*(2), 571-588. doi: 10.1111/mec.13938

DeBellis, T., Kernaghan, G., Bradley, R. et Widden, P. (2006). Relationships between stand composition and ectomycorrhizal community structure in boreal mixed-wood forests. *Microbial Ecology*, *52*(1), 114-126. doi: 10.1007/s00248-006-9038-8

Dickie, I. A., Oleksyn, J., Reich, P. B., Karolewski, P., Zytkowiak, R., Jagodzinski, A. M. et Turzanska, E. (2006). Soil modification by different tree species influences the extent of seedling ectomycorrhizal infection. *Mycorrhiza*, *16*(2), 73-79. doi: 10.1007/s00572-005-0013-x

Dickie, Ian A., Koide, R. T. et Steiner, K. C. (2002). Influences of established trees on mycorrhizas, nutrition, and growth of *Quercus rubra* seedlings. *Ecological Monographs*, *72*(4), 505-521. doi: 10.1890/0012-9615(2002)072[0505:IOETOM]2.0.CO;2

Drobyshev, I., Gewehr, S., Berninger, F. et Bergeron, Y. (2013). Species specific growth responses of black spruce and trembling aspen may enhance resilience of boreal forest to climate change. *Journal of Ecology*, *101*(1), 231-242. doi: 10.1111/1365-2745.12007

Environment Canada. Canadian climate normals 1971-2000. Environment Canada, National Meteorological Service, Downsview, ON. Accessed November 2017. http://climate.weatheroffice.gc.ca/climate_normals/index_e.html.

Epp, L. S., Boessenkool, S., Bellemain, E. P., Haile, J., Esposito, A., Riaz, T., … Brochmann, C. (2012). New environmental metabarcodes for analysing soil DNA: potential for studying past and present ecosystems. *Molecular Ecology*, *21*(8), 1821-1833. doi: 10.1111/j.1365-294X.2012.05537.x

Fenton, N. J. et Bergeron, Y. (2006). Facilitative succession in a boreal bryophyte community driven by changes in available moisture and light. *Journal of Vegetation Science*, *17*(1), 65-76. doi: 10.1111/j.1654-1103.2006.tb02424.x

Fenton, N. J. et Bergeron, Y. (2008). Does time or habitat make old-growth forests species rich? Bryophyte richness in boreal *Picea mariana* forests. *Biological Conservation*, *141*(5), 1389-1399. doi: 10.1016/j.biocon.2008.03.019

Fenton, N., Lecomte, N., Légaré, S. et Bergeron, Y. (2005). Paludification in black spruce (*Picea mariana*) forests of eastern canada: potential factors and management implications. *Forest Ecology and Management*, *213*(1–3), 151-159. doi: 10.1016/j.foreco.2005.03.017

Fernandez Christopher W., Nguyen Nhu H., Stefanski Artur, Han Ying, Hobbie Sarah E., Montgomery Rebecca A., … Kennedy Peter G. (2016). Ectomycorrhizal fungal response to warming is linked to poor host performance at the boreal-temperate ecotone. *Global Change Biology*, *23*(4), 1598-1609. doi: 10.1111/gcb.13510

Foudyl-Bey, S., Brais, S. et Drouin, P. (2016). Litter heterogeneity modulates fungal activity, C mineralization and N retention in the boreal forest floor. *Soil Biology and Biochemistry*, *100* (Supplement C), 264-275. doi: 10.1016/j.soilbio.2016.06.017

Franklin, O., Näsholm, T., Högberg, P. et Högberg, M. N. (2014). Forests trapped in nitrogen limitation - an ecological market perspective on ectomycorrhizal symbiosis. *New Phytologist*, *203*(2), 657-666. doi: 10.1111/nph.12840

Gagnon, R. (1989). Maintien après feu de limites abruptes entre des peuplements d'épinettes noires (*Picea mariana*) et des formations de feuillus intolérants (*Populus tremuloides* et *Betula papyrifera*) dans la région du Saguenay–Lac-Saint-Jean (Québec). *Nat. Can*, *116*, 117-124.

Gallet, C. (1994). Allelopathic potential in bilberry-spruce forests: influence of phenolic compounds on spruce seedlings. *Journal of Chemical Ecology*, *20*(5), 1009-1024. doi: 10.1007/BF02059738

Gardes, M. et Bruns, T. D. (1996). Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views. *Canadian Journal of Botany*, *74*(10), 1572-1583. doi: 10.1139/b96-190

Gauthier, S., Bernier, P., Kuuluvainen, T., Shvidenko, A. Z. et Schepaschenko, D. G. (2015). Boreal forest health and global change. *Science*, *349*(6250), 819-822. doi: 10.1126/science.aaa9092

Gauthier, Sylvie et Vaillancourt, M.-A. (2008). *Aménagement écosystémique en forêt boréale*. (s. l.) : PUQ. [Google-Books-ID: UbIyIAP9EVwC]. Récupéré de Google Books.

Geremia, R. A. et Zinger, L. (2013). Molecular Fingerprinting of Fungal Communities in Soil. Dans V. K. Gupta, M. G. Tuohy, M. Ayyachamy, K. M. Turner et A. O'Donovan (dir.), *Laboratory Protocols in Fungal Biology* (p. 349-356). New York, NY : Springer New York. Récupéré de CrossRef : http://link.springer.com/10.1007/978-1-4614-2356-0_31

Gessner, M. O., Swan, C. M., Dang, C. K., McKie, B. G., Bardgett, R. D., Wall, D. H. et Hättenschwiler, S. (2010). Diversity meets decomposition. *Trends in Ecology & Evolution*, *25*(6), 372-380. doi: 10.1016/j.tree.2010.01.010

Goldmann, K., Schöning, I., Buscot, F. et Wubet, T. (2015). Forest management type influences diversity and community composition of soil fungi across temperate forest ecosystems. *Frontiers in Microbiology*, *6*. doi: 10.3389/fmicb.2015.01300

Grelet, G.-A., Johnson, D., Vrålstad, T., Alexander, I. J. et Anderson, I. C. (2010). New insights into the mycorrhizal *Rhizoscyphus ericae* aggregate: spatial structure and co-colonization of ectomycorrhizal and ericoid roots. *New Phytologist*, *188*(1), 210-222. doi: 10.1111/j.1469-8137.2010.03353.x

Harsch, M. A., Hulme, P. E., McGlone, M. S. et Duncan, R. P. (2009). Are treelines advancing? A global meta-analysis of treeline response to climate warming. *Ecology Letters*, *12*(10), 1040-1049. doi: 10.1111/j.1461-0248.2009.01355.x

Hasselquist, N. J., Metcalfe, D. B., Inselsbacher, E., Stangl, Z., Oren, R., Näsholm, T. et Högberg, P. (2015). Greater carbon allocation to mycorrhizal fungi reduces tree nitrogen uptake in a boreal forest. *Ecology*, *0*(ja). Doi: 10.1890/15-1222

Hawksworth, D. L. et Lücking, R. (2017). Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiology spectrum*, *5*(4). doi: 10.1128/microbiolspec.FUNK-0052-2016

Hawksworth, David L. (2001). The magnitude of fungal diversity: the 1·5 million species estimate revisited. *Mycological Research*, *105*(12), 1422-1432. doi: 10.1017/S0953756201004725

Hirose, D., Hobara, S., Matsuoka, S., Kato, K., Tanabe, Y., Uchida, M., … Osono, T. (2016). Diversity and community assembly of moss-associated fungi in ice-

free coastal outcrops of continental Antarctica. *Fungal Ecology*, *24*, 94-101. doi: 10.1016/j.funeco.2016.09.005

Högberg, M. N., Chen, Y. et Högberg, P. (2007a). Gross nitrogen mineralisation and fungi-to-bacteria ratios are negatively correlated in boreal forests. *Biology and Fertility of Soils*, *44* (2), 363-366. doi: 10.1007/s00374-007-0215-9

Högberg, M. N., Högberg, P. et Myrold, D. D. (2007). Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? *Oecologia*, *150*(4), 590-601. doi: 10.1007/s00442-006-0562-5

Högberg, P., Näsholm, T., Franklin, O. et Högberg, M. N. (2017). Tamm Review: On the nature of the nitrogen limitation to plant growth in Fennoscandian boreal forests. *Forest Ecology and Management*, *403*, 161-185. doi: 10.1016/j.foreco.2017.04.045

Horton, T. R. et Bruns, T. D. (2001). The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology*, *10*(8), 1855–1871. doi: 10.1046/j.0962-1083.2001.01333.x

Hu, L. et 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

Inselsbacher, E. et Näsholm, T. (2012). The below-ground perspective of forest plants: soil provides mainly organic nitrogen for plants and mycorrhizal fungi. *New Phytologist*, *195*(2), 329-334. doi: 10.1111/j.1469-8137.2012.04169.x

IPCC. Climate Change 2014: Impacts, Adaptation, & Vulnerability - Summary for Policymakers. (2014). Dans *GlobalChange.gov*. Récupéré de http://www.globalchange.gov/browse/reports/ipcc-climate-change-2014-impacts-adaptation-vulnerability-summary-policymakers

Ishida, T. A., Nara, K. et Hogetsu, T. (2007). Host effects on ectomycorrhizal fungal communities: insight from eight host species in mixed conifer-broadleaf forests. *New Phytologist*, *174*(2), 430-440. doi: 10.1111/j.1469-8137.2007.02016.x

Iverson, L. R. et McKenzie, D. (2013). Tree-species range shifts in a changing climate: detecting, modeling, assisting. *Landscape Ecology*, *28*(5), 879-889. doi: 10.1007/s10980-013-9885-x

Jarvis, P. et Linder, S. (2000). Botany: constraints to growth of boreal forests. *Nature*, *405*(6789), 904-905. doi: 10.1038/35016154

Jenkins, C. N., Pimm, S. L. et Joppa, L. N. (2013). Global patterns of terrestrial vertebrate diversity and conservation. *Proceedings of the National Academy of Sciences*, *110*(28), E2602-E2610. doi: 10.1073/pnas.1302251110

Joanisse, G. D., Bradley, R. L. et Preston, C. M. (2018). The spread of *Kalmia angustifolia* on black spruce forest cutovers contributes to the spatial heterogeneity of soil resources. *PLOS ONE*, *13*(6), e0198860. doi: 10.1371/journal.pone.0198860

Jonsson, L. M., Nilsson, M.-C., Wardle, D. A. et Zackrisson, O. (2001). Context dependent effects of ectomycorrhizal species richness on tree seedling productivity. *Oikos*, *93*(3), 353-364. doi: 10.1034/j.1600-0706.2001.930301.x

Kalra, Y. P. et Soil and Plant Analysis Council (dir.). (1998). *Handbook of reference methods for plant analysis.* Boca Raton : CRC Press. Récupéré de Library of Congress ISBN. (QK865 .H26 1998)

Kardol, P., Cornips, N. J., Kempen, M. M. L. van, Bakx-Schotman, J. M. T. et Putten, W. H. van der. (2007). Microbe-mediated plant–soil feedback causes historical contingency effects in plant community assembly. *Ecological Monographs*, *77*(2), 147-162. doi: 10.1890/06-0502

Ke, P.-J., Miki, T. et Ding, T.-S. (2015). The soil microbial community predicts the importance of plant traits in plant-soil feedback. *New Phytologist*, *206*(1), 329-341. doi: 10.1111/nph.13215

Kennedy, P. G., Mielke, L. A. et Nguyen, N. H. (2018). Ecological responses to forest age, habitat, and host vary by mycorrhizal type in boreal peatlands. *Mycorrhiza*, *28*(3), 315-328. doi: 10.1007/s00572-018-0821-4

Kernaghan, G., Widden, P., Bergeron, Y., Légaré, S. et Paré, D. (2003). Biotic and abiotic factors affecting ectomycorrhizal diversity in boreal mixed-woods. *Oikos*, *102*(3), 497-504. doi: 10.1034/j.1600-0706.2003.12415.x

Kier, G., Mutke, J., Dinerstein, E., Ricketts, T. H., Küper, W., Kreft, H. et Barthlott, W. (2005). Global patterns of plant diversity and floristic knowledge. *Journal of Biogeography*, *32*(7), 1107-1116. doi: 10.1111/j.1365-2699.2005.01272.x

Klironomos, J. N., McCune, J., Hart, M. et Neville, J. (2000). The influence of arbuscular mycorrhizae on the relationship between plant diversity and

productivity. *Ecology Letters*, *3*(2), 137-141. doi: 10.1046/j.1461-0248.2000.00131.x

Kohout, P. (2017). Biogeography of ericoid mycorrhiza. Ecological studies. Dans L. Tedersoo (dir.), *Biogeography of Mycorrhizal Symbiosis* (p. 179-193). Cham : Springer International Publishing. Doi: 10.1007/978-3-319-56363-3₉

Kohout, P., Sýkorová, Z., Bahram, M., Hadincová, V., Albrechtová, J., Tedersoo, L. et Vohník, M. (2011). Ericaceous dwarf shrubs affect ectomycorrhizal fungal community of the invasive *Pinus strobus* and native *Pinus sylvestris* in a pot experiment. *Mycorrhiza*, *21*(5), 403-412. doi: 10.1007/s00572-010-0350-2

Kõljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F. S., Bahram, M., … Larsson, K.-H. (2013). Towards a unified paradigm for sequence-based identification of fungi. *Molecular Ecology*, *22*(21), 5271-5277. doi: 10.1111/mec.12481

Kreft, H. et Jetz, W. (2007). Global patterns and determinants of vascular plant diversity. *Proceedings of the National Academy of Sciences*, *104*(14), 5925-5930. doi: 10.1073/pnas.0608361104

Kyaschenko, J., Clemmensen, K. E., Karltun, E. et Lindahl, B. D. (2017). Below-ground organic matter accumulation along a boreal forest fertility gradient relates to guild interaction within fungal communities. *Ecology Letters*, *20*(12), 1546-1555. doi: 10.1111/ele.12862

Kyaschenko, J., Ovaskainen, O., Ekblad, A., Hagenbo, A., Karltun, E., Clemmensen, K. E. et Lindahl, B. D. (2019). Soil fertility in boreal forest relates to root-driven nitrogen retention and carbon sequestration in the mor layer. *New Phytologist*, *221*(3), 1492-1502. doi: 10.1111/nph.15454

Kytöviita, M.-M., Vestberg, M. et Tuomi, J. (2003). A test of mutual aid in common mycorrhizal networks: established vegetation negates benefit in seedlings. *Ecology*,*84*(4),898-906.doi:10.1890/00129658(2003)084[0898:ATOMAI]2.0.CO;2

Lafleur, B., Cazal, A., Leduc, A. et Bergeron, Y. (2015). Soil organic layer thickness influences the establishment and growth of trembling aspen (*Populus tremuloides*) in boreal forests. *Forest Ecology and Management*, *347*, 209-216. doi: 10.1016/j.foreco.2015.03.031

Laquerre, S., Harvey, B. D. et Leduc, A. (2011). Spatial analysis of response of trembling aspen patches to clearcutting in black spruce-dominated stands. *The Forestry Chronicle*, *87*(1), 77-85. doi: 10.5558/tfc87077-1

Laquerre, S., Leduc, A. et Harvey, B. D. (2009). Augmentation du couvert en peuplier faux-tremble dans les pessières noires du nord-ouest du québec après coupe totale. *Ecoscience*, *16*(4), 483-491. doi: 10.2980/16-4-3252

Last, F. T., Mason, P. A., Wilson, J. et Deacon, J. W. (1983). Fine roots and sheathing mycorrhizas: their formation, function and dynamics. Developments in Plant and Soil Sciences. Dans D. Atkinson, K. K. S. Bhat, M. P. Coutts, P. A. Mason et D. J. Read (dir.), *Tree Root Systems and Their Mycorrhizas* (p. 9-21). Springer Netherlands. Doi: 10.1007/978-94-009-6833-2$_2$

Lavoie, M., Paré, D. et Bergeron, Y. (2007a). Quality of growth substrates of post-disturbed lowland black spruce sites for black spruce (*Picea mariana*) seedling growth. *New Forests*, *33*(2), 207-216. doi: 10.1007/s11056-006-9024-5

Lavoie, M., Paré, D. et Bergeron, Y. (2007b). Relationships between microsite type and the growth and nutrition of young black spruce on post-disturbed lowland black spruce sites in eastern Canada. *Canadian Journal of Forest Research*, *37*(1), 62-73. doi: 10.1139/x06-196

Leberecht, M., Dannenmann, M., Gschwendtner, S., Bilela, S., Meier, R., Simon, J., … Polle, A. (2015). Ectomycorrhizal communities on the roots of two beech (*Fagus sylvatica*) populations from contrasting climates differ in nitrogen acquisition in a common environment. *Appl. Environ. Microbiol.*, *81*(17), 5957-5967. doi: 10.1128/AEM.01481-15

Lecomte, N. et Bergeron, Y. (2005). Successional pathways on different surficial deposits in the coniferous boreal forest of the Quebec Clay Belt. *Canadian Journal of Forest Research*, *35*(8), 1984-1995. doi: 10.1139/x05-114

Légaré, S., Bergeron, Y., Leduc, A. et Paré, D. (2001). Comparison of the understory vegetation in boreal forest types of southwest Quebec. *Canadian Journal of Botany*, *79*(9), 1019-1027. doi: 10.1139/b01-076

Légaré, S., Paré, D. et Bergeron, Y. (2005). Influence of aspen on forest floor properties in black spruce-dominated stands. *Plant and Soil*, *275*(1-2), 207-220. doi: 10.1007/s11104-005-1482-6

Levan, M. A. et Riha, S. J. (1986). Response of root systems of northern conifer transplants to flooding. *Canadian Journal of Forest Research*, *16*(1), 42-46. doi: 10.1139/x86-008

Lindahl, B. O., Taylor, A. F. S. et Finlay, R. D. (2002). Defining nutritional constraints on carbon cycling in boreal forests – towards a less `phytocentric' perspective. *Plant and Soil*, *242*(1), 123-135. doi: 10.1023/A:1019650226585

Lindahl, B. D., Nilsson, R. H., Tedersoo, L., Abarenkov, K., Carlsen, T., Kjøller, R., et al. (2013). Fungal community analysis by high-throughput sequencing of amplified markers – a user's guide. *New Phytologist* 199, 288–299. doi:10.1111/nph.12243.

Mallik, A. et Kravchenko, D. (2018). Recruitment and ontogenic patterns of stunting and growth release of black spruce (*Picea mariana*) in post-fire *Kalmia* heaths. *Forest Ecology and Management*, *407*, 135-144. doi: 10.1016/j.foreco.2017.09.068

Mallik, A. U. (2003). Conifer regeneration problems in boreal and temperate forests with ericaceous understory: role of disturbance, seedbed limitation, and keytsone species change. *Critical Reviews in Plant Sciences*, *22*(3-4), 341-366. doi: 10.1080/713610860

Mallik, A. U. et Pellissier, F. (2000). Effects of *Vaccinium myrtillus* on spruce regeneration: testing the notion of coevolutionary significance of allelopathy. *Journal of Chemical Ecology*, *26*(9), 2197-2209. doi: 10.1023/A:1005528701927

Mallik, Azim U., Biswas, S. R. et Collier, L. C. S. (2016). Belowground interactions between *Kalmia angustifolia* and *Picea mariana*: roles of competition, root exudates and ectomycorrhizal association. *Plant and Soil*, *403*(1), 471-483. doi: 10.1007/s11104-016-2819-z

Marchetti, G. M., Drton, M. et Sadeghi, K. (2015). ggm: Functions for graphical Markov models (version 2.3). Récupéré de R-Packages : https://CRAN.R-project.org/package=ggm

Mardis, E. R. (2008). Next-Generation DNA Sequencing Methods. *Annual Review of Genomics and Human Genetics*, *9*(1), 387-402. doi: 10.1146/annurev.genom.9.081307.164359

McLaren, B. E. et Janke, R. A. (1996). Seedbed and canopy cover effects on balsam fir seedling establishment in Isle Royale National Park. *Canadian Journal of Forest Research*, *26*(5), 782-793. doi: 10.1139/x26-088

Messaoud, Y., Bergeron, Y. et Leduc, A. (2007a). Ecological factors explaining the location of the boundary between the mixedwood and coniferous bioclimatic zones in the boreal biome of eastern North America. *Global Ecology and Biogeography*, *16*(1), 90-102. doi: 10.1111/j.1466-8238.2006.00277.x

Messaoud, Y., Bergeron, Y. et Asselin, H. (2007b). Reproductive potential of balsam fir (*Abies balsamea*), white spruce (*Picea glauca*), and black spruce (*P. mariana*) at the ecotone between mixedwood and coniferous forests in the boreal zone of western Quebec. *American Journal of Botany*, *94*(5), 746-754. doi: 10.3732/ajb.94.5.746

Messaoud, Y., Goudiaby, V. et Bergeron, Y. (2019). Persistence of balsam fir and black spruce populations in the mixedwood and coniferous bioclimatic domain of eastern North America. *Ecology and Evolution*, *9*(9), 5118-5132. doi: 10.1002/ece3.5069

Miki, T., Ushio, M., Fukui, S. et Kondoh, M. (2010). Functional diversity of microbial decomposers facilitates plant coexistence in a plant–microbe–soil feedback model. *Proceedings of the National Academy of Sciences*, *107*(32), 14251-14256. doi: 10.1073/pnas.0914281107

Molina, R., Massicotte, H. et Trappe, J. M. (1992). Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications. Récupéré de agris.fao.org : http://agris.fao.org/agris-search/search.do?recordID=GB9404954

Mucha, J., Peay, K. G., Smith, D. P., Reich, P. B., Stefański, A. et Hobbie, S. E. (2018). Effect of simulated climate warming on the ectomycorrhizal fungal community of boreal and temperate host species growing near their shared ecotonal range limits. *Microbial Ecology*, *75*(2), 348-363. doi: 10.1007/s00248-017-1044-5

Mueller, G. M. et Schmit, J. P. (2007). Fungal biodiversity: what do we know? What can we predict? *Biodiversity and Conservation*, *16*(1), 1-5. doi: 10.1007/s10531-006-9117-7

Mukassabi, T. A., Polwart, A., Coleshaw, T. et Thomas, P. A. (2012). How long can young Scots pine seedlings survive waterlogging? *Trees*, *26*(5), 1641-1649. doi: 10.1007/s00468-012-0740-5

Nagati, M., Roy, M., Manzi, S., Richard, F., Desrochers, A., Gardes, M. et Bergeron, Y. (2018). Impact of local forest composition on soil fungal communities in a mixed boreal forest. *Plant and Soil*, *432*(1), 345-357. doi: 10.1007/s11104-018-3806-3

Nara, K. (2006). Ectomycorrhizal networks and seedling establishment during early primary succession. *New Phytologist*, *169*(1), 169-178. doi: 10.1111/j.1469-8137.2005.01545.x

Nara, K. et Hogetsu, T. (2004). Ectomycorrhizal fungi on established shrubs facilitate subsequent seedling establishment of successional plant species. *Ecology*, *85*(6), 1700-1707. doi: 10.1890/03-0373

Näsholm, T., Högberg, P., Franklin, O., Metcalfe, D., Keel, S. G., Campbell, C., … Högberg, M. N. (2013). Are ectomycorrhizal fungi alleviating or aggravating nitrogen limitation of tree growth in boreal forests? *New Phytologist*, *198*(1), 214-221. doi: 10.1111/nph.12139

Newbery, D. M., Alexander, I. J. et Rother, J. A. (2000). Does proximity to conspecific adults influence the establishment of ectomycorrhizal trees in rain forest? *New Phytologist*, *147*(2), 401-409. doi: 10.1046/j.1469-8137.2000.00698.x

Nguyen, D. Q., Pena, R. et Polle, A. (2017). Impact of ectomycorrhizal community composition and soil treatment on inorganic nitrogen nutrition and performance of beech (*Fagus sylvatica* L.) provenances. *Trees*, *31*(6), 1891-1904. doi: 10.1007/s00468-017-1594-7

Nguyen, N. H., Song, Z., Bates, S. T., Branco, S., Tedersoo, L., Menke, J., … Kennedy, P. G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, *20*, 241-248. doi: 10.1016/j.funeco.2015.06.006

Nilsson, R. Henrik, Kristiansson, E., Ryberg, M., Hallenberg, N. et Larsson, K.-H. (2008). Intraspecific ITS variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evolutionary Bioinformatics Online*, *4*, 193-201. Récupéré de PubMed Central.

Nilsson, Rolf Henrik, Ryberg, M., Abarenkov, K., Sjökvist, E. et Kristiansson, E. (2009). The ITS region as a target for characterization of fungal communities using emerging sequencing technologies. *FEMS Microbiology Letters*, *296*(1), 97-101. doi: 10.1111/j.1574-6968.2009.01618.x

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2017). *vegan: Community Ecology Package* (version 2.4-5). Récupéré de R-Packages : https://cran.r-project.org/web/packages/vegan/index.html

Pallardy, S. G. et Kozlowski, T. T. (2008). Physiology of woody plants (3rd ed). Amsterdam ; Boston : Elsevier. Récupéré de Library of Congress ISBN. (QK711.2 .K72 2008)

Pan, Y., Birdsey, R. A., Fang, J., Houghton, R., Kauppi, P. E., Kurz, W. A., … Hayes, D. (2011). A large and persistent carbon sink in the world's forests. *Science*, *333*(6045), 988-993. doi: 10.1126/science.1201609

Parent, S., Simard, M.-J., Morin, H. et Messier, C. (2003). Establishment and dynamics of the balsam fir seedling bank in old forests of northeastern Quebec. *Canadian Journal of Forest Research*, *33*(4), 597-603. doi: 10.1139/x02-194

Parker, T. C., Sadowsky, J., Dunleavy, H., Subke, J.-A., Frey, S. D. et Wookey, P. A. (2017). Slowed biogeochemical cycling in sub-arctic birch forest linked to reduced mycorrhizal growth and community change after a defoliation event. *Ecosystems*, *20*(2), 316-330. doi: 10.1007/s10021-016-0026-7

Peay, K. G. (2016). The mutualistic niche: mycorrhizal symbiosis and community dynamics. *Annual Review of Ecology, Evolution, and Systematics*, *47*(1), 143-164. doi: 10.1146/annurev-ecolsys-121415-032100

Peay, K. G., Garbelotto, M. et Bruns, T. D. (2010). Evidence of dispersal limitation in soil microorganisms: isolation reduces species richness on mycorrhizal tree islands. *Ecology*, *91*(12), 3631-3640. doi: 10.1890/09-2237.1

Peay, K. G., Kennedy, P. G. et Bruns, T. D. (2011). Rethinking ectomycorrhizal succession: are root density and hyphal exploration types drivers of spatial and temporal zonation? *Fungal Ecology*, *4*(3), 233-240. doi: 10.1016/j.funeco.2010.09.010

Pérez-Ramos, I. M. et Marañón, T. (2009). Effects of waterlogging on seed germination of three Mediterranean oak species: ecological implications. *Acta Oecologica*, *35*(3), 422-428. doi: 10.1016/j.actao.2009.01.007

Perry, D. A., Margolis, H., Choquette, C., Molina, R. et Trappe, J. M. (1989). Ectomycorrhizal mediation of competition between coniferous tree species. *New Phytologist*, *112*(4), 501-511. doi: 10.1111/j.1469-8137.1989.tb00344.x

Peterson, E. B. (1965). Inhibition of black spruce primary roots by a water-soluble substance in *Kalmia angustifolia*. *Forest Science*, *11*(4), 473-479. Récupéré de IngentaConnect.

Pickles, B. J. et Simard, S. W. (2017). Mycorrhizal networks and forest resilience to drought. Dans *Mycorrhizal Mediation of Soil* (p. 319-339). Elsevier. doi: 10.1016/B978-0-12-804312-7.00018-8

Qu, L., Makoto, K., Choi, D. S., Quoreshi, A. M. et Koike, T. (2010). The role of ectomycorrhiza in boreal forest ecosystem. Dans A. Osawa, O. A. Zyryanova, Y. Matsuura, T. Kajimoto et R. W. Wein (dir.), *Permafrost Ecosystems* (vol. 209, p. 413-425). Dordrecht : Springer Netherlands. Doi: 10.1007/978-1-4020-9693-8_21

R Core Team. 2017. R: *A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.. https://www.R-project.org/.

Ratcliffe, J. L., Creevy, A., Andersen, R., Zarov, E., Gaffney, P. P. J., Taggart, M. A., … Payne, R. J. (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

Read, D. J. (1996). The structure and function of the ericoid mycorrhizal root. *Annals of Botany*, *77*(4), 365-374. doi: 10.1006/anbo.1996.0044

Read, D. J, Leake, J. R. et Perez-Moreno, J. (2004). Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. *Canadian Journal of Botany*, *82*(8), 1243-1263. doi: 10.1139/b04-123

Richard, F., Selosse, M.-A. et Gardes, M. (2009). Facilitated establishment of *Quercus ilex* in shrub-dominated communities within a Mediterranean ecosystem: do mycorrhizal partners matter? *FEMS Microbiology Ecology*, *68*(1), 14-24. doi: 10.1111/j.1574-6941.2009.00646.x

Robitaille, A. et Saucier, J.-P. (1996). Land district, ecophysiographic units and areas: the landscape mapping of the ministère des ressources naturelles du Québec. [Land District, Ecophysiographic Units and Areas]. Dans *Global to Local:*

Ecological Land Classification (p. 127-148). Springer, Dordrecht. Doi: 10.1007/978-94-009-1653-1_12

Rosseel, Y., Oberski, D., Byrnes, J., Vanbrabant, L., Savalei, V., Merkle, E., … Jorgensen, T. D. (2018). *lavaan: Latent Variable Analysis* (version 0.6-3). Récupéré de R-Packages : https://CRAN.R-project.org/package=lavaan

Rygiewicz, P. T., Bledsoe, C. S. et Zasoski, R. J. (1984). Effects of ectomycorrhizae and solution pH on [15N]ammonium uptake by coniferous seedlings. *Canadian Journal of Forest Research*, *14*(6), 885-892. doi: 10.1139/x84-158

Sanderson, P. L. et Armstrong, W. (1978). Soil waterlogging, root rot and conifer windthrow: oxygen deficiency or phytoxicity? *Plant and Soil*, *49*(1), 185-190. doi: 10.1007/BF02149920

Santalahti, M., Sun, H., Jumpponen, A., Pennanen, T. et Heinonsalo, J. (2016). Vertical and seasonal dynamics of fungal communities in boreal Scots pine forest soil. *FEMS Microbiology Ecology*, *92*(11), fiw170. doi: 10.1093/femsec/fiw170

Schmidt, S. K., Wilson, K. L., Meyer, A. F., Gebauer, M. M. et King, A. J. (2008). Phylogeny and ecophysiology of opportunistic “snow molds” from a subalpine forest ecosystem. *Microbial Ecology*, *56*(4), 681-687. doi: 10.1007/s00248-008-9387-6

Schmit, J. P. et Mueller, G. M. (2007). An estimate of the lower limit of global fungal diversity. *Biodiversity and Conservation*, *16*(1), 99-111. doi: 10.1007/s10531-006-9129-3

Selosse, M.-A., Richard, F., He, X. et Simard, S. W. (2006). Mycorrhizal networks: des liaisons dangereuses? *Trends in Ecology & Evolution*, *21*(11), 621-628. doi: 10.1016/j.tree.2006.07.003

Shi, L.-L., Mortimer, P. E., Slik, J. W. F., Zou, X.-M., Xu, J., Feng, W.-T. et Qiao, L. (2014). Variation in forest soil fungal diversity along a latitudinal gradient. *Fungal Diversity*, *64*(1), 305-315. doi: 10.1007/s13225-013-0270-5

Shipley, B. (2000). A new inferential test for path models based on directed acyclic graphs. *Structural Equation Modeling: A Multidisciplinary Journal*, *7*(2), 206-218. doi: 10.1207/S15328007SEM0702₄

Simard, M., Lecomte, N., Bergeron, Y., Bernier, P. Y. et Paré, D. (2007). Forest productivity decline caused by successional paludification of boreal soils. *Ecological Applications*, *17*(6), 1619-1637. doi: 10.1890/06-1795.1

Simard, S., Asay, A., Beiler, K., Bingham, M., Deslippe, J., He, X., … Teste, F. (2015). Resource transfer between plants through ectomycorrhizal fungal networks. Dans T. R. Horton (dir.), *Mycorrhizal Networks* (vol. 224, p. 133-176). Dordrecht : Springer Netherlands. Doi: 10.1007/978-94-017-7395-95

Simard, S. W., Beiler, K. J., Bingham, M. A., Deslippe, J. R., Philip, L. J. et Teste, F. P. (2012). Mycorrhizal networks: mechanisms, ecology and modelling. *Fungal Biology Reviews*, *26*(1), 39-60. doi: 10.1016/j.fbr.2012.01.001

Simard, S. W. et Durall, D. M. (2004). Mycorrhizal networks: a review of their extent, function, and importance. *Canadian Journal of Botany*, *82*(8), 1140-1165. doi: 10.1139/b04-116

Simard, S. W., Perry, D. A., Jones, M. D., Myrold, D. D., Durall, D. M. et Molina, R. (1997). Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature*, *388*(6642), 579-582. doi: 10.1038/41557

Smith, J. E., Molina, R., Huso, M. M., Luoma, D. L., McKay, D., Castellano, M. A., … Valachovic, Y. (2002). Species richness, abundance, and composition of hypogeous and epigeous ectomycorrhizal fungal sporocarps in young, rotation-age, and old-growth stands of Douglas-fir (*Pseudotsuga menziesii*) in the Cascade Range of Oregon, U.S.A. *Canadian Journal of Botany*, *80*(2), 186-204. doi: 10.1139/b02-003

Smith, S. E. et Read, D. J. (2008). Mycorrhizal symbiosis. San Diego [etc.] : Academic Press. Récupéré de Open WorldCat.

Sousa, N. R., Franco, A. R., Ramos, M. A., Oliveira, R. S. et Castro, P. M. L. (2015). The response of *Betula pubescens* to inoculation with an ectomycorrhizal fungus and a plant growth promoting bacterium is substrate-dependent. *Ecological Engineering*, *81*, 439-443. doi: 10.1016/j.ecoleng.2015.04.024

Spatafora, J. W., Chang, Y., Benny, G. L., Lazarus, K., Smith, M. E., Berbee, M. L., … Stajich, J. E. (2016). A phylum-level phylogenetic classification of zygomycete fungi based on genome-scale data. *Mycologia*, *108*(5), 1028-1046. doi: 10.3852/16-042

Stachowicz, J. J. (2001). Mutualism, Facilitation, and the Structure of Ecological Communities. *BioScience*, *51*(3), 235. doi: 10.1641/0006-3568(2001)051[0235:MFATSO]2.0.CO;2

Stottlemyer, A. D., Wang, G. G., Wells, C. E., Stottlemyer, D. W. et Waldrop, T. A. (2008). Reducing airborne ectomycorrhizal fungi and growing non-mycorrhizal loblolly pine (*Pinus taeda* L.) seedlings in a greenhouse. *Mycorrhiza*, *18*(5), 269-275. doi: 10.1007/s00572-008-0176-3

Suz, L. M., Barsoum, N., Benham, S., Cheffings, C., Cox, F., Hackett, L., … Bidartondo, M. I. (2015). Monitoring ectomycorrhizal fungi at large scales for science, forest management, fungal conservation and environmental policy. *Annals of Forest Science*, *72*(7), 877-885. doi: 10.1007/s13595-014-0447-4

Taberlet, P., Coissac, E., Pompanon, F., Brochmann, C. et Willerslev, E. (2012). Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology*, *21*(8), 2045-2050. doi: 10.1111/j.1365-294X.2012.05470.x

Tamm, C. O. (2012). Nitrogen in terrestrial ecosystems: questions of productivity, vegetational changes, and ecosystem stability. (s. l.) : Springer Science & Business Media. [Google-Books-ID: EOfrCAAAQBAJ]. Récupéré de Google Books.

Taudiere, A., Munoz, F., Lesne, A., Monnet, A.-C., Bellanger, J.-M., Selosse, M.-A., … Richard, F. (2015). Beyond ectomycorrhizal bipartite networks: projected networks demonstrate contrasted patterns between early- and late-successional plants in Corsica. *Frontiers in Plant Science*, *6*. doi: 10.3389/fpls.2015.00881

Taylor, D. L., Herriott, I. C., Stone, K. E., McFarland, J. W., Booth, M. G. et Leigh, M. B. (2010). Structure and resilience of fungal communities in Alaskan boreal forest soils This article is one of a selection of papers from The Dynamics of Change in Alaska's Boreal Forests: Resilience and Vulnerability in Response to Climate Warming. *Canadian Journal of Forest Research*, *40*(7), 1288-1301. doi: 10.1139/X10-081

Taylor, D. L., Hollingsworth, T. N., McFarland, J. W., Lennon, N. J., Nusbaum, C. et Ruess, R. W. (2013). A first comprehensive census of fungi in soil reveals both hyperdiversity and fine-scale niche partitioning. *Ecological Monographs*, *84*(1), 3-20. doi: 10.1890/12-1693.1

Tedersoo, L., Bahram, M., Põlme, S., Kõljalg, U., Yorou, N. S., Wijesundera, R., … Abarenkov, K. (2014). Global diversity and geography of soil fungi. *Science*, *346*(6213), 1256688. doi: 10.1126/science.1256688

Tedersoo, L., Bahram, M., Toots, M., DiéDhiou, A. G., Henkel, T. W., KjøLler, R., … KõLjalg, U. (2012). Towards global patterns in the diversity and community structure of ectomycorrhizal fungi. *Molecular Ecology*, *21*(17), 4160-4170. doi: 10.1111/j.1365-294X.2012.05602.x

Tedersoo, L., Mett, M., Ishida, T. A. et Bahram, M. (2013). Phylogenetic relationships among host plants explain differences in fungal species richness and community composition in ectomycorrhizal symbiosis. *New Phytologist*, *199*(3), 822-831. doi: 10.1111/nph.12328

Teste, F. P., Lieffers, V. J. et Strelkov, S. E. (2012). Ectomycorrhizal community responses to intensive forest management: thinning alters impacts of fertilization. *Plant and Soil*, *360*(1-2), 333-347. doi: 10.1007/s11104-012-1231-6

Teste, F. P. et Simard, S. W. (2008). Mycorrhizal networks and distance from mature trees alter patterns of competition and facilitation in dry Douglas-fir forests. *Oecologia*, *158*(2), 193-203. doi: 10.1007/s00442-008-1136-5

Teste, F. P., Simard, S. W. et Durall, D. M. (2009). Role of mycorrhizal networks and tree proximity in ectomycorrhizal colonization of planted seedlings. *Fungal Ecology*, *2*(1), 21-30. doi: 10.1016/j.funeco.2008.11.003

Thormann, M. N. (2006a). Diversity and function of fungi in peatlands: a carbon cycling perspective. *Canadian Journal of Soil Science*, *86* (Special Issue), 281-293. doi: 10.4141/S05-082

Thormann, M. N. (2006b). The role of fungi in boreal peatlands. Dans R. K. Wieder et D. H. Vitt (dir.), *Boreal Peatland Ecosystems* (vol. 188, p. 101-123). Springer Berlin Heidelberg. Doi: 10.1007/978-3-540-31913-9$_6$

Tiedje, J. M., Asuming-Brempong, S., Nüsslein, K., Marsh, T. L. et Flynn, S. J. (1999). Opening the black box of soil microbial diversity. *Applied Soil Ecology*, *13*(2), 109-122. doi: 10.1016/S0929-1393(99)00026-8

Toljander, J. F., Eberhardt, U., Toljander, Y. K., Paul, L. R. et Taylor, A. F. S. (2006). Species composition of an ectomycorrhizal fungal community along a local nutrient gradient in a boreal forest. *New Phytologist*, *170*(4), 873-884. doi: 10.1111/j.1469-8137.2006.01718.x

Treseder, K. K., Bent, E., Borneman, J. et McGuire, K. L. (2014). Shifts in fungal communities during decomposition of boreal forest litter. *Fungal Ecology*, *10*(Supplement C), 58-69. doi: 10.1016/j.funeco.2013.02.002

Trevors, J. T. (1996). Sterilization and inhibition of microbial activity in soil. *Journal of Microbiological Methods*, *26* (1), 53-59. doi: 10.1016/0167-7012(96)00843-3

Truong, C., Mujic, A. B., Healy, R., Kuhar, F., Furci, G., Torres, D., … Smith, M. E. (2017). How to know the fungi: combining field inventories and DNA-barcoding to document fungal diversity. *New Phytologist*, *214*(3), 913-919. doi: 10.1111/nph.14509

Twieg, B. D., Durall, D. M. et Simard, S. W. (2007). Ectomycorrhizal fungal succession in mixed temperate forests. *New Phytologist*, *176*(2), 437–447. doi: 10.1111/j.1469-8137.2007.02173.x

Unterseher, M., Jumpponen, A., Öpik, M., Tedersoo, L., Moora, M., Dormann, C. F., et al. (2011). Species abundance distributions and richness estimations in fungal metagenomics – lessons learned from community ecology. *Molecular Ecology* 20, 275–285. doi:10.1111/j.1365-294X.2010.04948.x.

Urbanová, M., Šnajdr, J. et Baldrian, P. (2015). Composition of fungal and bacterial communities in forest litter and soil is largely determined by dominant trees. *Soil Biology and Biochemistry*, *84*, 53-64. doi: 10.1016/j.soilbio.2015.02.011

van der Heijden, M. G. A. et Horton, T. R. (2009). Socialism in soil? The importance of mycorrhizal fungal networks for facilitation in natural ecosystems. *Journal of Ecology*, *97*(6), 1139-1150. doi: 10.1111/j.1365-2745.2009.01570.x

van der Heijden, M. G. A., Klironomos, J. N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., … Sanders, I. R. (1998). Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*, *396*(6706), 69-72. doi: 10.1038/23932

Varenius, K., Kårén, O., Lindahl, B. et Dahlberg, A. (2016). Long-term effects of tree harvesting on ectomycorrhizal fungal communities in boreal Scots pine forests. *Forest Ecology and Management*, *380*(Supplement C), 41-49. doi: 10.1016/j.foreco.2016.08.006

Vašutová, M., Edwards-Jonášová, M., Veselá, P., Effenberková, L., Fleischer, P. et Cudlín, P. (2018). Management regime is the most important factor

influencing ectomycorrhizal species community in Norway spruce forests after windthrow. *Mycorrhiza*, *28*(3), 221-233. doi: 10.1007/s00572-018-0820-5

Veillette, J. J. (1994). Evolution and paleohydrology of glacial lakes barlow and ojibway. *Quaternary Science Reviews*, *13*(9), 945-971. doi: 10.1016/0277-3791(94)90010-8

Villarreal-Ruiz, L., Anderson, I. C. et Alexander, I. J. (2004). Interaction between an isolate from the *Hymenoscyphus ericae* aggregate and roots of *Pinus* and *Vaccinium*. *New Phytologist*, 183-192. doi: 10.1111/j.1469-8137.2004.01167.x@10.1002/(ISSN)1469-8137(CAT)

Vogelsang, K. M., Reynolds, H. L. et Bever, J. D. (2006). Mycorrhizal fungal identity and richness determine the diversity and productivity of a tallgrass prairie system. *New Phytologist*, *172*(3), 554-562. doi: 10.1111/j.1469-8137.2006.01854.x

Vohník, M., Pánek, M., Fehrer, J. et Selosse, M.-A. (2016). Experimental evidence of ericoid mycorrhizal potential within Serendipitaceae (Sebacinales). *Mycorrhiza*, *26*(8), 831-846. doi: 10.1007/s00572-016-0717-0

Vrålstad, T., Fossheim, T. et Schumacher, T. (2000). *Piceirhiza bicolorata*- the ectomycorrhizal expression of the *Hymenoscyphus ericae* aggregate? *New Phytologist*, *145*(3), 549-563. doi: 10.1046/j.1469-8137.2000.00605.x

Walker, J. F., Jr, O. K. M., Lei, T., Semones, S., Nilsen, E. et Clinton, B. D. (1999). Suppression of ectomycorrhizae on canopy tree seedlings in *Rhododendron maximum* L. (Ericaceae) thickets in the southern Appalachians. *Mycorrhiza*, *9*(1), 49-56. doi: 10.1007/s005720050262

Walker, John F., Aldrich-Wolfe, L., Riffel, A., Barbare, H., Simpson, N. B., Trowbridge, J. et Jumpponen, A. (2011). Diverse Helotiales associated with the roots of three species of arctic Ericaceae provide no evidence for host specificity. *New Phytologist*, *191*(2), 515-527. doi: 10.1111/j.1469-8137.2011.03703.x

Wall, D. H. et Moore, J. C. (1999). Interactions underground: soil biodiversity, mutualism, and ecosystem processes. *BioScience*, *49*(2), 109-117. doi: 10.2307/1313536

Wardle, D. A., Yeates, G. W., Barker, G. M. et Bonner, K. I. (2006). The influence of plant litter diversity on decomposer abundance and diversity. *Soil Biology and Biochemistry*, *38*(5), 1052-1062. doi: 10.1016/j.soilbio.2005.09.003

Webster, J. et Weber, R. (2007). Introduction to Fungi. (2007) : Cambridge University Press. [Google-Books-ID: SApIn7IEnucC]. Récupéré de Google Books.

Weremijewicz, J., Sternberg, L. da S. L. O. et Janos, D. P. (2016). Common mycorrhizal networks amplify competition by preferential mineral nutrient allocation to large host plants. *New Phytologist*, *212*(2), 461-471. doi: 10.1111/nph.14041

White, T., Bruns, T., Lee, S., Taylor, J., A Innis, M., H Gelfand, D. et Sninsky, J. (1990). Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics (vol. 31). (s. l. n. é.). Récupéré de ResearchGate.

Woodall, C. W., Oswalt, C. M., Westfall, J. A., Perry, C. H., Nelson, M. D. et Finley, A. O. (2009). An indicator of tree migration in forests of the eastern United States. *Forest Ecology and Management*, *257*(5), 1434-1444. doi: 10.1016/j.foreco.2008.12.013

Xu, Y.-J., Röhrig, E. et Fölster, H. (1997). Reaction of root systems of grand fir (*Abies grandis* Lindl.) and Norway spruce (*Picea abies* Karst.) to seasonal waterlogging. *Forest Ecology and Management*, *93*(1), 9-19. doi: 10.1016/S0378-1127(96)03951-5

Yamasaki, S. H., Fyles, J. W., Egger, K. N. et Titus, B. D. (1998). The effect of *Kalmia angustifolia* on the growth, nutrition, and ectomycorrhizal symbiont community of black spruce. *Forest Ecology and Management*, *105*(1–3), 197-207. doi: 10.1016/S0378-1127(97)00285-5

Zackrisson, O., Nilsson, M.-C., Dahlberg, A. et Jäderlund, A. (1997). Interference mechanisms in conifer-ericaceae-feathermoss communities. *Oikos*, *78*(2), 209-220. doi: 10.2307/3546287(2), 209-220. doi: 10.2307/3546287