UNIVERSITÉ DU QUÉBEC EN ABITIBI-TÉMISCAMINGUE

DOMINANCE BY CONIFEROUS VERSUS BROADLEAF DECIDUOUS TREES IN BOREAL FORESTS AS A DRIVER OF UNDERSTORY VEGETATION AND ASSOCIATED MICROORGANISMS

PHD THESIS

PRESENTED

AS A PARTIAL REQUIREMENT

PHD IN ENVIRONMENTAL SCIENCES

BY

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UNIVERSITÉ DU QUÉBEC EN ABITIBI-TÉMISCAMINGUE

LA DOMINANCE DES ARBRES DANS LES FORÊTS DE CONIFÈRES ET DE FEUILLUS COMME FACTEUR DÉTERMINANT DES PLANTES DE SOUS-BOIS ET DES MICROORGANISMES ASSOCIÉS DANS LA FORÊT BORÉALE

THÈSE

PRÉSENTÉ

COMME EXIGENCE PARTIELLE

DOCTORAT EN SCIENCES DE L'ENVIRONNEMENT

PAR

JUANITA CAROLINA RODRIGUEZ RODRIGUEZ

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L'image d'un étudiant de doctorat est souvent l'individu seul encabané en face de son ordinateur à travailler sur son projet de recherche pendant plusieurs années. La réalité c'est que tout au long du doctorat j'ai été accompagnée par plusieurs personnes sans lesquelles je n'y serais jamais arrivée. C'est ici que j'aimerais remercier toutes ces personnes avec qui j'ai partagé des précieux moments pendant ces dernières années. Ces personnes ont contribué à mes connaissances et à ma formation académique, m'ont accompagnée dans plusieurs moments et m'ont aussi fait évoluer en tant que personne. Cette aventure du doctorat m'a apporté beaucoup plus que ce que je m'attendais. Je n'ai pas seulement trouvé un excellent groupe de recherche sur le sujet qui m'intéresse, mais aussi une bonne ambiance académique, avec des personnes très accueillantes et amicales et un groupe d'étudiants québécois et internationaux qui ont enrichi mon expérience à l'université. Les fonctionnaires et professeurs de l'UQAT, toujours dynamiques, nous donnent la possibilité de participer à plusieurs activités académiques et ludiques et ils offrent un soutien constant dans toutes les étapes du doctorat. C'est pourquoi je remercie cette grande famille de l'IRF-UQAT, du CEF, du KembelLab et du Laboratoire de Bryologie!

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Tout commence par une idée, quelque chose qui te passionne et qui devient un rêve. Si tu gardes cette idée en tête et si tu as le courage de persévérer, tu es capable d'en faire une réalité.

DÉDICACE

À ma mère Esperanza Rodríguez Díaz,

pour son soutien inconditionnel et son amour infini,

qui a fait de moi ce que je suis aujourd'hui

A mi mamá Esperanza Rodríguez Díaz,

por su apoyo incondicional y su infinito amor

quien hizo de mi todo lo que soy ahora

À mon père Jorge Ricardo Rodríguez de Jammes du Mourier,

pour être présent dans ma vie et toujours veiller sur moi

A mi padre Jorge Ricardo Rodríguez de Jammes du Mourier,

Por estar presente en mi vida, siempre pendiente de mi

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et qui a été à mon côté en me soutenant toutes ces années

A mi pareja Mathieu Surprenant,

quien me ha hecho sentir siempre en casa

y ha estado a mi lado apoyándome todos estos años

AVANT-PROPOS

Cette thèse de doctorat a été réalisée à Rouyn-Noranda, à l'Université du Québec en Abitibi-Témiscamingue (UQAT). La thèse est composée d'une introduction générale, de trois chapitres sous la forme d'articles scientifiques en anglais et d'une conclusion générale. Les trois articles sont en processus de publication dans des revues à comité de lecture : Le Chapitre 1 a été soumis au journal *Ecological Monographs* (facteur d'impact : 10,32), il a été révisé par des pairs et il a été soumis de nouveau au même journal. Le Chapitre 2 a été soumis au journal *Plant and Soil* (facteur d'impact : 4,19) avec une version dans un service le *preprint* (serveur de préimpressions en ligne sans révision par de pairs) *Research Square*. Le Chapitre 3 a été rendu publique dans *bioRxiv* (un autre service de *preprint* et après il a été publié dans le journal *Environmental Microbiology* (facteur d'impact : 5,49).

Chapitre 1. Rodríguez-Rodríguez J.C., Fenton N.J., Kembel S.W., Mestre E., Jean M. Bergeron Y. Drivers of contrasting boreal understory vegetation in coniferous and broadleaf deciduous alternative states. *Ecological Monographs – En révision*.

Chapitre 2. Rodríguez-Rodríguez J.C., Bergeron Y., Fenton N.J., Kembel S.W. (*soumis*). Tree dominance shapes soil and tree phyllosphere microbial communities in coniferous and broadleaf deciduous forests. *Soumis au journal Plant and Soil. Research Square* DOI: https://doi.org/10.21203/rs.3.rs-2238260/v1

Chapitre 3. Rodríguez-Rodríguez, J.C., Bergeron, Y., Kembel, S.W. and Fenton, N.J. 2022. Dominance of coniferous and broadleaved trees drives bacterial associations with boreal feather mosses. *Environmental Microbiology* **24**(8) :3517–3528. DOI : https://doi.org/10.1111/1462-2920.16013

La collecte des données, le travail de terrain et de laboratoire, les analyses statistiques et bioinformatiques, l'interprétation des résultats et l'écriture des manuscrits ont été réalisés par JCRR pour les trois chapitres de la thèse. Ma directrice de thèse Nicole Fenton (NF) et mes co-directeurs Yves Bergeron (YB) et Steven Kembel (SK) ont contribué de plusieurs façons tout au long du projet dans leurs champs d'expertises, et leur place dans les listes des auteurs sont fonction de leur implication respective dans la réalisation de chacun des chapitres. Il est important de souligner que le projet du Chapitre 1 a commencé en 2013, avant que je commence mon doctorat. Ce chapitre a commencé sous la direction du laboratoire de YB a été mis en place sur le terrain en 2013 par NF. Ensuite, l'étudiant Evick Mestre (EM) a participé en 2014 et 2015 dans le cadre de son projet de baccalauréat, et il a aussi dernièrement contribué aux analyses statistiques et a participé dans l'amélioration finale de l'article. Ce premier projet m'a été attribué pour mon premier chapitre de thèse et j'ai participé dans la collecte des données en 2017 et 2018. Mélanie Jean (MJ) a présenté un soutien aux analyses statistiques, a apporté un œil éclairé sur l'interprétation des résultats et a également aidé à l'amélioration du manuscrit. Les analyses bioinformatiques et statistiques des données de séquençage des Chapitres 2 et 3 ont été réalisés par JCRR avec l'orientation et le support constant de SK. L'interprétation des résultats et à la rédaction de chacun des chapitres a été réalisé par JCRR avec un fort soutien de NF et SK. Finalement, tous les co-auteurs ont participé dans la révision des versions finale des articles et ont donné son approbation finale pour les publier.

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1F	Single-litter treatment / Traitement de litière en simple
2F	Double-litter treatment / Traitement de litière en double
Al	Aluminium
ANOVA	Analysis of variance / Analyse de variance
ASV	Amplicon sequence variant / Variant de séquence d'amplicon
B/den	Denominator of species abundance losses (B) / Dénominateur de pertes de l'abondance des espèces (B)
B-C plots	Species abundance losses (B) and gains (C) plots / Pertes (B) et gains (C) de l'abondance des espèces
BD_{total}	Total β-diversity / Diversité beta totale
BFGS	Broyden-Fletcher-Goldfarb-Shanno algorithm / algorithme
BS	Black spruce / Épinette noire / *Picea mariana* (Mill.) Britton, Sterns & Poggenb.
C : N	Carbon : Nitrogen ratio / Rapport carbone : azote
C	Carbon / Carbone
C	Control / Témoin
C	Knights plume feather moss (*Ptilium crista-castrensis* (Hedw.) De Not.) / Hypne plumeuse
C/den	Denominator of species abundance gains (C) / Dénominateur de gains de l'abondance des espèces (C)
Ca	Calcium
CEC	Cation exchange capacity / Capacité d'échange de cations
CTAB	Cetrimonium bromide / Bromure de cétyltriméthylammonium / ([(C16H33)N(CH3)3]
DESeq2	Differential Abundance for Microbiome Data analysis / Analyse d'abondance différentielle pour des données de microbiomes

Df	Degrees of freedom / Degrées de liberté
Df_{total}	Total degrees of freedom / Degrées de liberté xxxabrad
DMSO	Dimethyl sulfoxide / Diméthylsulfoxyde
DNA / ADN	Desoxyribonucleic acid / Acide désoxyribonucléique
dNTPs	Deoxyribose nucleotide triphosphate / Désoxyribose xxxabradorxxxé triphosphate
EDTA	Ethylenediaminetetraacetic acid / Acide éthylènediaminetétraacétique
F	F statistic / F statistique
F	Forward / Avant
Fe	Iron / Fer
FT	Forest type / Type de forêt
glmmTMB	Generalized linear mixed model using Template Model Builder / Modèle mixte linéaire généralisé à l'aide d'un générateur de modèle
H	Host species / Espèce hôte
H	Hydrogen / Hydrogène
HCL	Hydrochloric acid / Acide chlorhydrique
ICP-OES	Inductively coupled plasma – optical emission spectrometry / Spectrométrie d'émission optique avec plasma induit par haute fréquence.
ITS	Internal transcibed spacer / Espaceur intergénique transcrit
K	Potassium
LCBD	Local Contributions to Beta Diversity / Contributions locales à la diversité beta
Li	Light treatment / Traitement de lumière
lm	Linear model / Modèle linéaire
LMEMs	Linear mixed-effects model / Modèle mixtes linéaires
Mg	Magnesium / Magnésium
Mn	Manganese / Manganèse
N : P	Nitrogen : Phosphorus ratio / Rapport azote : phosphore

N	Nitrogen / Azote
Na	Sodium
NCBI	National Center for Biotechnology Information / National Center for Biotechnology Information
NMDS	Non-metric multi dimensionnal scaling / Analyse multivariée non-métrique
NPK	Nitrogen-Phosphorus-Potassium / Azote-Phosphore-Potassium
Nu	Nutrients treatment / Traitement de nutriments
OTU	Operational taxonomic units / Unité taxonomique opérationnelle
P : Al	Phosphorus : Aluminium ratio / Rapport phosphore : aluminium
P : Ca	Phosphorus : Calcium ratio / Rapport phosphore : calcium
P	Phosphorus / Phosphore
P	P-value / Valeur P
P_{adj}	P adjusted value / Valeur ajusté de P
PCA	Principal component analysis / Analyse des composantes principales
PCoA	Principal coordinate analysis / Analyse en coordonnées principales
PCR	Polymerase chain reaction / Réaction en chaîne par polymérase
PERMANOVA	Permutational multivariate analysis of variance / Analyse de la variance avec permutation
R	Reverse
R^2	R squared – Coefficient of determination / R carrée – coefficient de détermination
RDP	Ribosomal Database Project / Projet de base de données ribosomale
RefSeq	Reference sequences / Séquences de référence
rRNA	Ribosomal Ribonucleic Acid / Acide ribonucléique ribosomique
S	Red-stemmed feathermoss (*Pleurozium schreberi* (Willd. Ex Brid.) Mitt.) / Hypne de Schreber
S	Sulfur / Sulfure

SCBD	Species Contributions to Beta Diversity / Contributions des espèces à la diversité beta
Sig.	Significant / Significatif
SMP	Shoemaker-McLean-Pratt
SRA	Sequence Read Archive / Archive de lecture de séquence
SS_{total}	Total sum-of-squares / Somme des carrés totale
T	Treatment / Traitement
TA	Trembling aspen / Peuplier faux-tremble / *Populus tremuloides* Michx.
TBI	Temporal Beta diversity Index / Indice de la diversité beta temporelle
Ti	Transplants-in treatment / Traitement de transplantation « intra-site »
To	Transplants-out treatment / Traitement de transplantation « inter-site »
Tris	Tris(hydroxymethyl)aminomethane / Tris(hydroxyméthyl)aminométhane (HOCH2)3CNH2)
UV	Ultraviolet
V/V dilution	Volume/Volume dilution
VWC	Volumetric water content / Teneur volumétrique en eau
z	z score statistic / Score z statistique

Organisations and people

UQAT	Université du Québec en Abitibi-Témiscamingue / University of Quebec in Abitibi-Témiscamingue
UQAM	Université du Québec à Montréal / University of Quebec in Montreal
IRF	Forest research institut / Institut de recherche sur les forêts
CEF	Forest research center / Centre d'études de la forêt
CERMO-FC	Center of Excellence in Research on Orphan Diseases – Fondation Courtois / Centre d'Excellence en Recherche sur les Maladies Orphelines – Fondation Courtois
FRQNT	Fonds de recherche du Québec – Nature et Technologies / Nature and Technologies

EM	Evik Mestre
JCRR	Juanita C. Rodríguez Rodríguez
MJ	Mélanie Jean
NJF	Nicole J. Fenton
SK	Steven W. Kembel
YB	Yves Bergeron

Understory species

AME	*Amelanchier* spp.
ARN	*Aralia nudicaulis*
ASA	*Aster acuminatus*
ASM	*Aster macrophyllus*
AUC	*Alnus crispa*
AUR	*Alnus rugosa*
BOP	*Betula papyrifera*
CAX	*Carex* spp.
CHL	*Leucanthemum vulgare*
CIA	*Circaea alpina*
CLB	*Clintonia borealis*
COL	*Comandra xxxiiiabrad*
CON	*Cornus canadensis*
COT	*Coptis trifolia*
COV	*Corylus* spp.
DIE	*Diervilla lonicera*
DIP	*Dicranum polysetum*
DRD	*Dryopteris disjuncta*
DRS	*Dryopteris spinulosa*
EPA	*Epilobium angustifolium*
EPN	*Picea mariana* (seedlings / semis)

EQP	*Equisetum pratense*
FRA	*Fragaria* spp.
GAA	*Galium asprellum*
GAH	*Gaultheria hispidula*
GOR	*Goodyera repens*
HIA	*Hieracium aurantiacum*
HIA	*Hieracium aurantiacum*
HYS	*Hylocomium splendens*
KAA	*Kalmia angustifolia*
LEG	*Rhododendron groenlandicum*
LIB	*Linnaea borealis*
LON	*Lonicera canadensis*
LYA	*Lycopodium annotinum*
LYC	*Lycopodium clavatum*
LYO	*Lycopodium obscurum*
LYS	*Lycopodium* spp.
MAC	*Maianthemum canadense*
MEP	*Mertensia paniculata*
MIN	*Mitella nuda*
OXM	*Oxalis montana*
PES	*Petasites palmatus*
PET	*Populus tremuloides* (seedlings / semis)
PLG	*Plagiomnium* spp.
PLS	*Pleurozium schreberi*
POA	*Poa* spp.
POC	*Polytrichum commune*
PTC	*Ptilium crista-castrensis*
PTI	*Ptilidium ciliare*
PYE	*Pyrola elliptica*

PYR	*Pyrola secunda*
RIG	*Ribes glandulosum*
RIT	*Ribes triste*
ROA	*Rosa acicularis*
RUI	*Rubus idaeus*
RUP	*Rubus pubescens*
RYT	*Rhytidiadelphus triquetrus*
SAB	*Abies balsamea*
SMT	*Smilacina trifolia*
SOM	*Solidago macrophylla*
SPS	*Sphagnum* spp.
STA	*Streptopus amplexifolius*
TRB	*Trientalis borealis*
TUD	*Thuidium delicatulum*
VAA	*Vaccinium angustifolium*
VAM	*Vaccinium myrtilloides*
VEJ	*Vicia cracca*
VIE	*Viburnum edule*
VIS	*Viola* spp.

LISTE DES SYMBOLES ET DES UNITÉS

%	Percent / Pourcentage
<	Less than / Inférieur à
>	Greater than / Supérieur à
≤	Less or equal than / Inférieur ou égal à
≥	Greater or equal than / Supérieur ou égal à
°C	Celsius degree / Degré Celsius
°N	North latitude degree / Degré de latitude Nord
°W	West longitude degree / Degré de longitude ouest
cm	Centimeter / Centimètre
g	Gram / Gramme
ha	Hectare
km	Kilometer / Kilomètre
m	Meter / Mètre
meq	Milliequivalents / Milliéquivalent
mg	Milligram / Milligramme
min	Minute
mm	Millimeter / Millimètre
s	Second / Seconde
yr	year
α	Alpha
β	Beta
γ	Gamma
µ	Micrometers / Micromètres
µL	Microliter / Microlitre
µM	Micromolar / Micromolaire

ABSTRACT

Boreal forests are increasingly changing in tree dominance from coniferous to broadleaf deciduous forests, due to natural fires, human land use and climate change. In the boreal forest of northwestern Quebec, coniferous forests dominated by black spruce trees (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) experience an expansion of broadleaf forests, producing areas dominated by trembling aspen trees (*Populus tremuloides* Michx.). These two forest types can be considered as two alternative stable states, with similar permanent abiotic conditions originating from the same disturbance trigger, and only differing in tree dominance. Alternative stable states defined by tree dominance shape local conditions for plant and microbial communities in the understory, via their effects on environmental factors, such as litter deposition, light conditions, and soil nutrient availability. Consequently, increasing changes in tree dominance in boreal forests may differentially affect understory vegetation and associated microbial communities. The general objective of this thesis was to understand how tree dominance of coniferous and broadleaf deciduous forests influenced understory vegetation and associated microbial communities in the boreal forest. This was analyzed in three chapters. In Chapter 1, we analyzed the main factors related with tree dominance driving changes in understory vegetation between forest types. We performed a 5-year *in situ* experiment using alternative stable states as a guiding theoretical framework including two approaches: 1) the ecosystem approach, manipulating environmental conditions of light, litter and nutrients; and 2) the community approach, exchanging understory communities between forest types. In Chapter 2, we evaluated the relative importance of factors related to tree dominance shaping microbial community structure. First, we analyzed differences in bacterial communities between microhabitats (soil microbiome vs. tree phyllosphere) and forest types (black spruce vs. trembling aspen forests). Secondly, we analyzed if bacterial and fungal communities were affected by changes in factors associated with tree dominance, including shifts in litter deposition and in understory transplantations between forest types, and correlated them with abiotic and biotic factors. Finally, in Chapter 3, we used genetic sequencing to identify phyllosphere bacteria associated with the two most abundant feather mosses in both forest types, in order to determine if moss-host species or tree dominance drive bacterial diversity in the moss phyllosphere. Results of Chapter 1 indicate that the plant understory composition in trembling aspen stands was both resistant to shifts in local conditions and resilient in the alternative state dominated by black spruce. In contrast, the abundance of mosses and ericaceous plants that typically compose black spruce stands was negatively affected by a physical effect of broadleaf addition and they were less resilient in trembling aspen stands as they were invaded by local plants over time. Despite the fact that shifts in litter deposition and in understory vegetation exchanged between forest types were important field manipulations that altered understory vegetation after 5

years, in the results of Chapter 2 we did not observe a significant effect of treatments on the soil microbial α-diversity, relative abundance, and community composition. These bacterial and fungal communities in the mineral soil were resilient to the aboveground changes made by the treatments and were only shaped by the underlying effects of forest type. While microbial communities were influenced by forest type, they were also highly specific to their microhabitat, *i.e.* in the tree phyllosphere or soil microbiome of each forest type. Finally, in Chapter 3, the overall composition of moss phyllosphere bacterial communities was defined by the interaction of both forest type and host species, although the abundance of most bacterial phyla was most strongly influenced by forest type. The moss phyllosphere in trembling aspen forests harboured a higher relative abundance of diazotrophic bacteria, including ecologically important cyanobacteria, compared to black spruce forests. Our project advanced the understanding of two representative forest types in the boreal area, by highlighting the effect of tree dominance of black spruce and trembling aspen forests on understory vegetation (Chapter 1), tree phyllosphere and soil microbial communities (Chapter 2), and moss bacterial phyllosphere (Chapter 3) in the boreal forest of eastern Canada. Forests dominated by trembling aspen trees harbour an abundant and more resilient plant understory and a more diverse soil microbial community than black spruce forests, which is useful in forest management to better cope with increasing changes in the boreal biome.

Keywords : Alternative stable states, Bacteria, Bryophytes, Fungi, *Picea mariana* (black spruce), Plant ecology, Plant-microbial interactions, *Populus tremuloides* (Trembling aspen), Resilience, Resistance, Soil microbiome, Tree-canopy dominance, Tree phyllosphere, Understory vegetation.

RÉSUMÉ

La dominance de la canopée du biome boréal change des forêts de conifères à des forêts de feuillus, à cause des feux naturels, de l'utilisation des terres par l'homme et du changement climatique. Dans la forêt boréale du nord-ouest du Québec, les forêts de conifères dominées par l'épinette noire (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) ont été en partie remplacées par le peuplier faux-tremble (*Populus tremuloides* Michx.). Ces deux types de forêts peuvent être considérés comme deux états stables alternatifs, présentant des conditions abiotiques permanentes similaires, provenant du même déclencheur de perturbation, et ne différant que par la dominance de la canopée. Les états stables alternatifs définis par la dominance de la canopée façonnent les conditions locales pour les communautés végétales et microbiennes du sous-bois, par ses effets sur les facteurs environnementaux, tels que l'apport en litière, les conditions de lumière et la disponibilité des nutriments dans le sol. Par conséquent, les changements croissants de la dominance de la canopée dans les forêts boréales peuvent affecter différemment la végétation du sous-bois et les communautés microbiennes associées. L'objectif général de cette thèse était de comprendre comment la dominance de la canopée des forêts de conifères et de feuillus influence la végétation de sous-bois et les communautés microbiennes associées dans la forêt boréale. Cet objectif a été réalisé dans trois chapitres. Dans le Chapitre 1, nous avons analysé les principaux facteurs liés à la dominance des arbres qui entraînent des changements dans la végétation de sous-bois entre les types de forêts. Nous avons réalisé une expérience *in situ* de 5 ans en utilisant des états stables alternatifs comme cadre théorique comprenant deux approches : 1) l'approche écosystémique, en manipulant les conditions environnementales de lumière, de litière et de nutriments ; et 2) l'approche des communautés (transplantation réciproque), en échangeant les communautés de sous-bois entre les types de forêts. Dans le Chapitre 2, nous avons évalué l'importance relative des facteurs liés à la dominance des arbres qui façonnent la structure des communautés microbiennes. Premièrement, nous avons analysé les différences dans les communautés bactériennes entre les microhabitats (microbiome du sol *vs* phyllosphere des arbres) et les types de forêts (forêts d'épinette noire *vs* forêts de peuplier faux-tremble). Ensuite, nous avons analysé si les communautés bactériennes et fongiques étaient affectées par des changements dans les facteurs associés à la dominance des arbres, y compris les changements dans l'apport en litière et dans les transplantations du sous-bois entre les types de forêts, et nous les avons corrélés avec des facteurs abiotiques et biotiques. Enfin, dans le Chapitre 3, nous avons identifié par séquençage génétique la phyllosphere des deux mousses hypnacées les plus abondantes dans les deux types de forêts, afin de déterminer si les espèces hôtes des mousses ou la dominance des arbres déterminent la diversité bactérienne dans la phyllosphere des mousses. Les résultats du Chapitre 1 indiquent que la composition des plantes du sous-bois dans les forêts des peupliers faux-tremble était à la fois résistante aux changements

des conditions locales et résiliente dans l'état alternatif dominé par l'épinettes noires. En revanche, l'abondance des mousses et des plantes éricacées qui composent généralement les peuplements d'épinette noire a été affectée négativement par un effet physique de l'apport en litière du tremble et elles étaient moins résistantes dans les peuplements de peuplier faux-tremble envahis par des plantes locales au cours du temps. Malgré le fait que les changements dans l'apport en litière et le sous-bois échangés entre les types de forêts étaient des manipulations assez importantes qui ont modifié la végétation du sous-bois après 5 ans, dans le Chapitre 2 nous n'avons pas observé d'impact significatif des traitements sur la diversité alpha, l'abondance et la composition des xlabradorxlés microbiennes du sol. Ces communautés bactériennes et fongiques dans le sol minéral étaient résilients aux changements causées par les traitements bien que contrôlées par les effets hérités du type de forêt. Si les communautés microbiennes étaient influencées par le type de forêt, elles étaient également très spécifiques à leur microhabitat dans la phyllosphere des arbres ou dans le microbiome du sol dans chaque forêt. Enfin, dans le Chapitre 3, la composition globale de la phyllosphere des mousses a été définie par l'interaction du type de forêt et les espèces hôtes, bien que la plupart des *phyla* bactériens aient été déterminés par un fort effet du type de forêt. La phyllosphere des mousses dans les forêts de peuplier faux-tremble abritait une plus grande abondance relative de bactéries diazotrophes, y compris des cyanobactéries très importantes sur le plan écologique, par rapport aux forêts d'épinettes noires. Notre projet a permis de mieux comprendre les deux types de forêts les plus représentatifs de la région boréale, en mettant en évidence l'effet de la dominance des arbres des forêts d'épinette noire et de peuplier faux-tremble sur la végétation du sous-bois (Chapitre 1), la phyllosphere des arbres et les communautés microbiennes du sol (Chapitre 2), et la phyllosphere des bactéries des mousses (Chapitre 3) dans la forêt boréale de l'est du Canada. Les forêts dominées par le peuplier faux-tremble présentent une grande abondance et diversité de plantes et un sous-bois plus résilient que le sous-bois dominé par des épinettes noires, ainsi qu'une communauté microbienne du sol aussi plus diversifiée, ce qui serait une avantage pour la gestion forestière dans le contexte des changements croissants dans l'écosystème boréal.

Mots clés : Bactéries, Bryophytes, Champignons, Dominance de la canopée, Écologie végétale, États stables alternatifs, Interactions plantes-microorganismes, Microbiome du sol, Phyllosphère des arbres, *Picea mariana* (épinette noire), *Populus tremuloides* (peuplier faux-tremble), Résilience, Résistance, Végétation de sous-bois.

CHAPITRE 0

GENERAL INTRODUCTION

0.1 Tree dominance of coniferous and broadleaf deciduous boreal forests

The boreal forest is a social-ecological system that represents ~30 % of the world's forest area and is present mainly in Canada, Alaska, Russia, and northern Europe (Burton et al., 2010; Gauthier et al. 2015). The globally significant ecosystem services of the boreal forest are related to climate and water regulation, it is a wildlife habitat, and it offers several recreational and economic activities (Chapin et al. 2010; Gauthier et al. 2015). The extensive boreal biome dominated by forests and lakes has a low human population density compared to other biomes, but it experiences important pressures related to human activities that extract wood and minerals for numerous economic products worldwide (Gauthier et al. 2015). All these disturbances (*e.g.* clear cuts, open-pit mining, natural fires) can trigger changes in ecosystem drivers, such as tree species dominance, which affect the stability of the system and can cause the community to switch from one state to an alternative state (Beisner et al. 2003; Chapin et al. 2010; Fenton 2016).

Alternative stable states are used as a conceptual framework to explain different major vegetation patterns or ecosystem drivers, in sites with similar substrate and climate conditions (Beisner et al. 2003; Pausas and Bond 2020). These alternative states can rapidly switch to another state with a disturbance trigger (Pausas and Bond 2020). Tree dominance of coniferous versus broadleaf deciduous trees among similar sites has been considered as two alternative stable states (Johnstone et al. 2010a, Johnstone et al. 2020, Baltzer et al. 2021). The conceptual framework of alternative stable states also facilitates the analysis of the resistance and resilience of different ecosystems to local and global changes (Beisner et al. 2003; Chapin et al. 2010). While resistance is defined

as the capacity of a system to stay essentially unchanged despite disturbances (Grimm and Wissel 1997), resilience is a measure of persistence of the system that absorbs a change without dramatically altering population relationships, fundamental functions and feedbacks (Chapin et al. 2010; Holling 1973). Depending on the resistance and resilience of the system, a disturbance trigger can generate alternative states in sites with similar topoedaphic conditions and species pools by producing changes in the abundance of dominant tree species (Chapin et al. 2010). If a system is neither resistant, not resilient, a change to an alternative state can be produced.

The resilience of the widespread black spruce forests (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) in the boreal system is threatened by changes in climate and wildfire regimes, which can impair black spruce seed production, generate a reduction of the soil organic layer and a more open canopy (Baltzer et al. 2021). All these factors, together with other important disturbances related with human land colonization, agriculture, forestry, and mining in eastern Canada, have favored the increasing abundance of broadleaf deciduous forests, such as trembling aspen stands (*Populus tremuloides* Michx.) (Laquerre et al. 2009; Marchais et al. 2020). These changes in tree dominance can also produce shifts in local environmental conditions and in the understory community composition (Augusto et al. 2015; Barbier et al. 2008; Urbanová et al. 2015).

Tree dominance of coniferous black spruce trees compared to the broadleaf deciduous trembling aspen trees, define local environmental conditions (Augusto et al. 2015) and shape the composition and dynamics of understory plants (Barbier et al. 2008; Bartels and Chen 2013; Qian et al. 2003) and soil microbial communities (Hannam et al. 2006; Norris et al. 2016; Urbanová et al. 2015) (Figure 0.1). Black spruce trees modify forest floor conditions through the low-nutrient needleleaf litter that acidifies the soil, and decreases soil temperature and decomposition rates (Laganière et al. 2010). These conditions lead to soils with slow nutrient cycling and low forest productivity (Légaré

et al. 2004). In contrast, the nutrient-rich deciduous broadleaf litter of trembling aspen trees promotes a higher soil pH, temperature and moisture that leads to fast nutrient cycling and decomposition rates inducing more productive forests (Cavard et al. 2011; Laganière et al. 2010). Forest type also modifies light transmission to the understory; in trembling aspen forests more light is transmitted to the understory than in black spruce forests (Messier et al. 1998). The understory vegetation of black spruce forests is frequently dominated by feather-moss species, such as *Pleurozium schreberi* (Brid.) Mitt. And *Ptilium crista-castrensis* (Hedw.), that produce soils with a thick organic layer (Fenton and Bergeron 2006). Also, the understory composition is characterized by the presence of ericaceous plants, such as blueberries (*Vaccinium angustifolium* Aiton and *Vaccinium myrtillus* L.) and Labrador tea (*Rhododendron groenlandicum* (Oeder) Kron & Judd) (Légaré et al. 2001; Nilsson and Wardle 2005). In contrast to the homogeneous black spruce stands, trembling aspen forests have a heterogeneous understory with a high diversity and abundance of herbs and shrubs and a limited bryophyte abundance (Bartels and Chen 2013; Cavard et al. 2011). Although factors related with each forest type, including changes in light, litter deposition, nutrient status and understory vegetation have been extensively studied, there are few *in situ* studies evaluating shifts in these parameters with a holistic view of canopy-related factors affecting understory communities in the boreal forest. In this sense, multi-taxon analysis is essential to understand global effects on communities, particularly for biodiversity conservation, forest management and climate change strategies (Jokela et al. 2018).

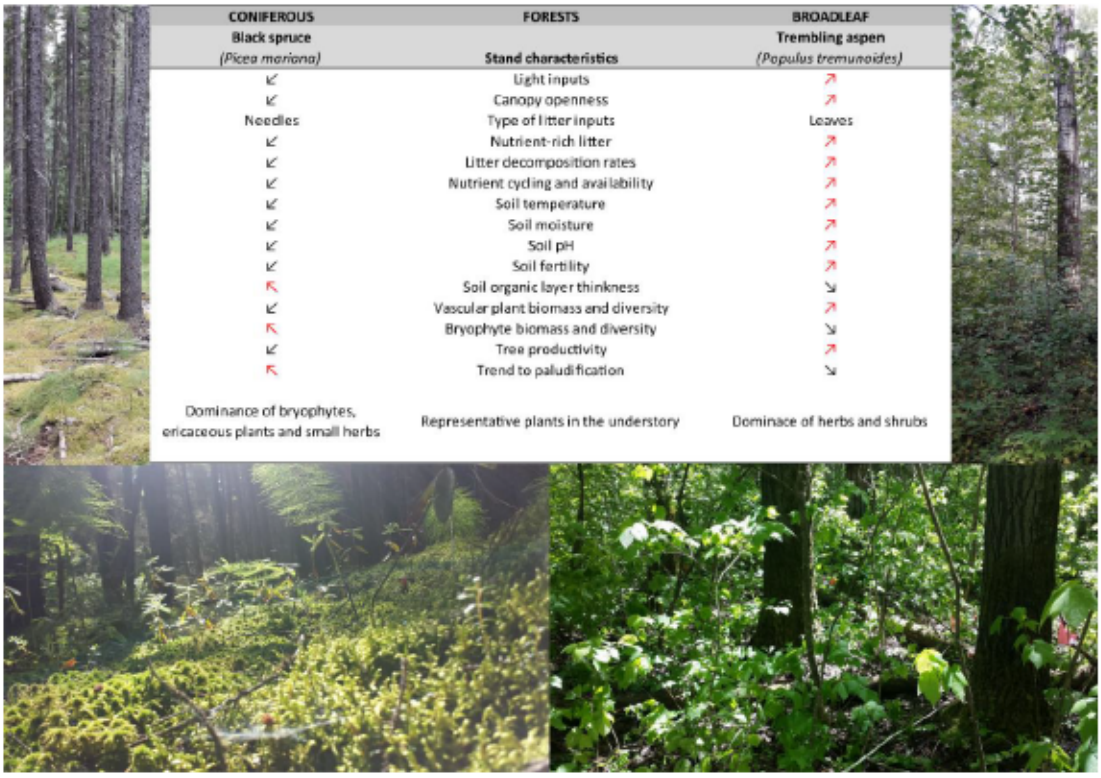

Figure 0.1. Trends in local environmental conditions and understory community composition of forests dominated by the coniferous black spruce forests and dominated by the broadleaf deciduous trembling aspen forests. References : Messier et al., 1998; Lamarche et al., 2007; Cavard et al., 2011; Laganière et al., 2011; Bartels & Chen, 2013; Augusto et al., 2015.

0.2 Aboveground-belowground interactions

Natural systems should be analyzed by considering their different components, including overstory and understory vegetation, plant-associated microorganisms and the soil microbiome, as these components have constant feedback loops affecting one another. The microbiome is defined as the assemblage of microorganisms existing in or associated with a habitat (Shade and Handelsman 2012). Microorganisms are important drivers of soil dynamics, as they decompose soil nutrients beginning the trophic chain through protozoans, nematodes and microarthropods that feed on bacteria and fungi and expulse all those nutrients in soluble forms available to plants (Coleman et al. 2017; Ingham et al. 1985; Van Der Heijden et al. 2008). This is a stunning self

regulation of nutrient and microbial dynamics that influences both aboveground and belowground composition. Dominant trees influence soil dynamics and microbial community composition through their impacts on litter quality and by changing soil physicochemical conditions in the forest floor (Hannam et al. 2006; Lindo and Visser 2003; Urbanová et al. 2015). Depending on the dominance of coniferous and broadleaf deciduous trees, the relative abundance of bacteria and fungi vary as each microbial group carries out different ecological functions in the forest floor (Augusto et al. 2015; Van Der Heijden et al. 2008). Forest floors with a dominance of fungal communities are associated with slow nutrient cycling, low nutrient availability, more organic matter, and low leaf quality, which all produce a low net primary productivity and promote the presence of slow growing plant species (Van Der Heijden et al. 2008; Wardle et al. 2004). In contrast, forests dominated by bacterial communities are associated with fast nutrient cycling and availability, limited soil organic matter abundance and high litter quality, which produces a high net primary productivity and promote the presence of fast-growing plants (Van Der Heijden et al. 2008; Wardle et al. 2004). Although forest type seems to influence microbial community structure, it is not clear to what extent the different factors associated with tree dominance of coniferous and broadleaf deciduous trees affect soil microbial composition.

Plant-microbial associations contribute to plant growth and adaptation to harsh environments (Gaiero et al., 2013; Ritpitakphong et al., 2016), driving plant diversity and productivity (Van Der Heijden et al. 2008). More than 20,000 plant species around the world are dependent on symbiotic microorganisms for their development and survival, especially in nutrient poor environments such as boreal forests (Van Der Heijden et al., 2008). Plants are associated with microorganisms in different microhabitats, not only belowground (soil and root microbiomes), but also in their phyllosphere. The phyllosphere refers to the microbial habitat of aerial plant surfaces including leaves (Vorholt 2012). It has been suggested that the phyllosphere is mainly dominated by bacterial communities, while filamentous fungal communities are

considered to be transient inhabitants of leaf surfaces as spores that are further developed in other microhabitats (Andrews and Harris 2000; Lindow and Brandl 2003). Leaf surfaces are relatively harsh environments exposed to ultraviolet radiation, low nutrient availability and constant fluctuations in temperature and moisture during a single day and across seasons (Leveau 2006). Despite these harsh conditions, the phyllosphere has a dense bacterial community of 106 to 107 cells / cm^2 of leaf surface or up to 108 cells / g of leaf material (Remus-Emsermann et al. 2014; Vorholt 2012). The composition of phyllosphere microbial communities is related to the physicochemistry of host leaves, which leads to a high inter-specific variability, more than intra-specificity or across different environments (Laforest-Lapointe et al. 2016; Redford et al. 2010; Schlechter et al. 2019). Hence, phylogenetically different trees have distinct phyllosphere communities (Kembel et al. 2014; Lajoie and Kembel 2021a; Redford et al. 2010), which likely affects belowground nutrient dynamics through litter inputs (Augusto et al. 2015; Prescott and Grayston 2013). Several studies have evaluated the co-occurrence between the microhabitats of litter and soil microbial communities (Hannam et al. 2007; Prescott and Grayston 2013; Urbanová et al. 2015). However, litter on the forest floor has already begun the decomposition processes and the microbial community structure in litter might be different from the original inputs of tree leaves. Thus, there is a knowledge gap in understanding differences in microbial community structure between microhabitats and the possible influence of tree phyllosphere on soil microbial community composition. Considering the importance of plant-microbial interactions in boreal forests, a fundamental question in microbial ecology is what drivers shape microbial communities (Vorholt 2012) and furthermore, how microbial composition varies among microhabitats and forest types.

0.3 Moss-bacterial associations

Similar to the tree phyllosphere, microbial communities in the moss phyllosphere seem to be mainly host specific (Bragina et al. 2012; Holland-Moritz et al. 2018; Opelt et al.

2007). However, moss-associated microbial composition seems to be also affected by diverse environmental conditions associated with different forest types, collection sites or elevation gradients (Davey et al. 2013; Holland-Moritz et al. 2018; Jean et al. 2020; Tang et al. 2016). Few studies have explored the moss phyllosphere, despite the high diversity and abundance of bryophytes in the boreal biome, particularly in coniferous forests frequently dominated by feather mosses, such as *Pleurozium schreberi* and *Ptilium crista-castrensis* (Fenton and Bergeron 2006; Nilsson and Wardle 2005). These moss-microbial associations are important in boreal forests, as they contribute to methane oxidation (Kip et al., 2010), and to carbon and nitrogen cycling (DeLuca et al., 2002; Turetsky, 2003). As boreal forests are limited in nitrogen (Högberg et al. 2017), the association of mosses with diazotrophic bacteria, such as *Cyanobacteria*, play a key ecological role, significantly contributing to nitrogen inputs by fixing up to 7 kg N ha-1 yr-1 in boreal ecosystems (DeLuca et al., 2007; Lindo et al., 2013; Rousk et al., 2013a). Despite their important ecological functions, the studies about the moss phyllosphere have been limited in scope, as most studies have focused on homogeneous and nutrient-poor coniferous forests, while heterogeneous and nutrient-rich broadleaf forests have been less studied. If the microbial community composition is indeed influenced by forest type, moss-bacterial interactions could be affected by increasing changes in tree dominance from coniferous to broadleaf deciduous trees in the boreal system.

0.4 Identifying the unseen: DNA sequencing of environmental samples

Studies of plant-microbial interactions are now possible due to relatively recent advances in sequencing techniques. The possibility to sequence DNA from environmental samples has opened the door for biodiversity screening of communities in different environments (Epp et al. 2012). We use conserved regions, such as rRNA gene regions of 16S in bacteria and ITS in fungi, that also have high variability to taxonomically differentiate the microbial groups within the amplicon sequence (Edgar

2018). High-throughput sequencing represents a useful approach for identification of microorganisms in forest ecosystems, even though it presents certain biases and still has challenges, such as the generation of sequencing errors (Nilsson et al. 2019). In the bioinformatic analysis process, amplicon reads of the targeted sequence are filtered, clustered and compared to a reference database for taxonomic assignment (Callahan et al. 2016a). The high-resolution Amplicon Sequence Variants (ASVs) approach is a recent approach that distinguishes sequence variants differing in even just one nucleotide and infer the biological sequences in the sample, taking into account amplification and sequencing errors (Callahan et al. 2017). This ASV approach is an analogue to the previous approach of Operational Taxonomic Units (OTUs), in which sequences are clustered by reads differing in a fixed percentage of sequence dissimilarity (often 3%) but the biological variation outside the reference database is not captured (Callahan et al. 2017). With the ASV approach, the clustering process produces a table with the number of times each exact ASV was observed in each sample, which can be directly analyzed and then taxonomically assigned based on a reference databased. Hence, we used the ASVs approach in our study to identify microbial communities in the boreal forest.

0.5 Objectives and thesis structure

Considering the increasing changes in tree dominance from coniferous to broadleaf deciduous forests, the main objective of my thesis was to understand how tree dominance of black spruce and trembling aspen forests influence the composition of plants and associated microbial communities in the understory. This thesis is presented in three chapters:

0.5.1 Chapter 1

General objective: Analyze the main factors that induce shifts between alternative stable states defined by tree-canopy dominance of black spruce and trembling aspen

forests. With a 5-year in situ experiment and based on the theoretical framework of alternative stable states described by Beisner et al. (2003), we manipulated both local environmental conditions using an "ecosystem approach" and understory communities using a "community approach".

Objective 1 – Ecosystem approach: Analyze effects of shifts in environmental drivers associated with tree-canopy dominance (light conditions, nutrient status, and litter deposition) on plant understory composition. Hypothesis: Tree-canopy dominance will alter understory vegetation composition primarily through litter deposition because litter significantly influences decomposition and nutrient mineralization processes (Laganière et al. 2010, Rodríguez-Calcerrada et al. 2011, Chen et al. 2017).

Objective 2 – Community approach: Analyze shifts in understory plant communities exchanged between forest types, to determine if understory vegetation was resistant to a new tree-canopy dominance through time and if the forest was resilient to the small-scale disturbance of the transplantation. Hypothesis: Understory plant communities are expected to react depending on their resistance capacity to stay essentially unchanged despite disturbances, and the forest understory resilience to absorbs the disturbance without dramatically altering their species composition.

0.5.2 Chapter 2

General objective: Analyze differences in soil microbial communities in coniferous and broadleaf deciduous forests and the factors driving these.

Objective 1: Analyze differences in bacterial communities between microhabitats (soil microbiome vs. tree phyllosphere) and forest types (black spruce vs. trembling aspen forests). Hypothesis: Microbial communities will be different between forest types, but within forest types, bacteria will co-occur between both microhabitats, because we

expect a sharing of bacterial communities from tree phyllosphere to soil microbiome through litter fall.

Objective 2: Analyze if bacterial and fungal communities were affected by changes in factors associated with tree dominance, including shifts in litter deposition and understory transplantations between forest types, and correlated them with abiotic and biotic factors. On the one hand, shifts in litter deposition consisted in adding needleleaves in trembling aspen forests and broadleaves in black spruce forests. On the other hand, we transplanted complete plots of understory vegetation from black spruce to trembling aspen forests, and vice-versa and analyzed their microbial composition after 5 years. Hypothesis: Factors associated with tree dominance will produce a microbial community composition different than the natural conditions (control) and more similar to the opposite forest type and will be correlated with abiotic and biotic factors in each forest type.

0.5.3 Chapter 3

Objective: Determine if host species or environmental conditions defined by tree-canopy dominance drive bacterial diversity in the moss phyllosphere. We used 16S rRNA gene amplicon sequencing to quantify changes in moss-associated bacterial communities as a function of host species (*Pleurozium schreberi* and *Ptilium crista-castrensis*) and forest type (coniferous black spruce versus deciduous broadleaf trembling aspen). Hypothesis: Host species will have the greatest effect on bacterial community composition while forest type will have a secondary effect.

0.5.4 Study area

The study area was the same for all three chapters and is located in the Eastern Boreal Shield of Canada, in the spruce-moss bioclimatic domain of the Clay Belt in western Quebec (Bergeron et al. 1996), created by proglacial Lakes Barlow and Ojibway from

the maximum expansion during the Wisconsinan glaciation (Vincent and Hardy 1977, Veillette 1994). Tree species composition is characterized by a dominance of black spruce and trembling aspen trees, but other important tree species in this bioclimatic domain include jack pine (*Pinus banksiana* Lambert), balsam fir (*Abies balsamea* (L.) Miller) and paper birch (*Betula papyrifera* Marshall) (Saucier 1994). We selected three study sites that were specifically located on the border of Abitibi-Témiscamingue and Nord-du-Québec regions (Site A: 49°11'46'' N – 78°50'33'' W; Site B: 49°09'20''N – 78°47'56''W and Site C: 49°09'39''N – 78°47'55''W); Sites B and C were 0.5 km apart and Site A was 5.3 km away. (Figure 0.2) and were selected based on previous studies (Cavard et al. 2011; Légaré et al. 2005). Each site has adjacent stands dominated by black spruce (≥ 75% of *Picea mariana* canopy cover) and trembling aspen (≥ 75% of *Populus tremuloides* canopy cover) (Cavard et al. 2011), is about 1 ha in size and they are separated by between 34 to 115 m. Regardless of their canopy composition, sites had comparable abiotic conditions of surface deposit, slope, drainage, moderately dry, clay-dominated subhygric soils, and flat topography, and were originated from the same wildfire ca. 1916 (Bergeron et al. 2004; Laganière et al. 2011; Légaré et al. 2005). Therefore, tree composition was the only significant source of variation between sites. Within each forest type at each site, three blocks separated by 16 to 49 m were used for the different analyses as explained in each chapter of the thesis. This study area is characterized by short summers, with a growing season of about 150 days and a moderately dry, cool climate, with average annual temperatures of 0.7 °C and average annual precipitation of 889.8 mm, registered in the nearest meteorological station in La Sarre (QC) (Environment Canada 2017) (Laquerre et al. 2009).

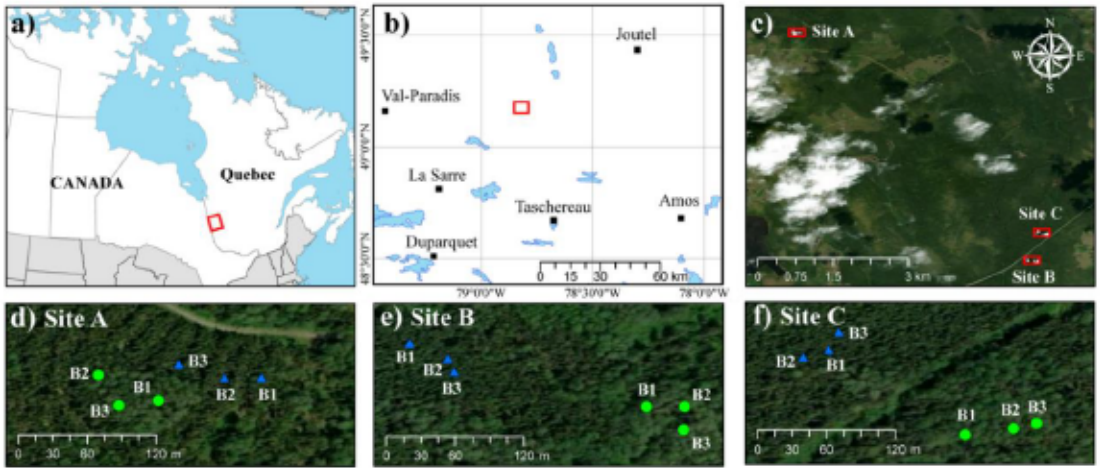

Figure 0.2 Location of the study sites in the boreal forest of eastern Canada (a), within western Quebec above the parallel 49ºN (b). Sites B and C are 0.5 km apart and Site A is 5.3 km away (c). Each site has adjacent stands dominated by black spruce (as blue triangles) and trembling aspen (as green circles), separated from 34 to 115 m; and within each forest type, three blocks (B1, B2 and B3) were placed separated from 16 to 49 m (d, e, f).

INTRODUCTION GÉNÉRALE

0.6 Dominance des arbres dans les forêts boréales de conifères et de feuillus

La forêt boréale est un système socio-écologique qui représente ~30 % de la superficie forestière mondiale, et elle est présente principalement au Canada, en Alaska, en Russie, et en Europe du Nord (Burton et al., 2010 ; Gauthier et al. 2015). Les services écosystémiques de la forêt boréale, d'importance mondiale, sont liés à la régulation du climat et de l'eau, est un habitat faunique, et offrent plusieurs activités récréatives et économiques (Chapin et al. 2010 ; Gauthier et al. 2015). Le vaste biome boréal dominé par des forêts et des lacs a une faible densité de population humaine par rapport aux autres biomes, mais il subit d'importantes pressions liées aux activités humaines qui extraient du bois et des minéraux pour de nombreux produits économiques importants dans le monde (Gauthier et al. 2015). Toutes ces perturbations (par exemple, les coupes à blanc, l'exploitation minière à ciel ouvert, les feux naturels) peuvent déclencher des changements dans les facteurs qui influencent significativement la forêt, tels que la dominance des arbres, ce qui affecte la stabilité du système et peuvent induire la formation de plusieurs états alternatifs stables (Beisner et al. 2003 ; Chapin et al. 2010 ; Fenton 2016).

Les états stables alternatifs sont utilisés comme cadre conceptuel pour expliquer les différences dans la végétation et les facteurs contrôlant les écosystèmes et cela, dans des sites présentant des conditions de substrat et de climat similaires (Beisner et al. 2003 ; Pausas et Bond 2020). Ces états stables alternatifs peuvent rapidement passer à un autre état lorsqu'une forte perturbation est déclenchée (Pausas et Bond 2020). La dominance des conifères par rapport aux feuillus dans des sites similaires a été considérée comme deux états stables alternatifs (Johnstone et al. 2010a, Johnstone et al. 2020, Baltzer et al. 2021). Le cadre conceptuel des états stables alternatifs facilite également l'analyse de la résistance et de la résilience des différents écosystèmes aux

changements locaux et globaux (Beisner et al. 2003 ; Chapin et al. 2010). Alors que la résistance est définie comme la capacité d'un système à rester essentiellement inchangé malgré les perturbations (Grimm et Wissel 1997), la résilience est une mesure de la persistance du système qui absorbe un changement sans altérer de façon significative les relations entre les populations, les fonctions fondamentales et les rétroactions (Chapin et al. 2010 ; Holling 1973). Selon la résistance et la résilience du système, un déclencheur de perturbation peut générer des états alternatifs dans des sites présentant des conditions topoédaphiques et des pools d'espèces similaires en produisant des changements dans l'abondance des espèces arborescentes dominantes (Chapin et al. 2010). Si un système n'est ni résistant, ni résilient, un changement vers un état alternatif peut être produit.

La résilience des forêts d'épinette noire (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.), une espèce très répandue dans le système boréal, est menacée par les changements climatiques et les régimes d'incendies de forêts, qui peuvent nuire à la production de graines d'épinettes noires, générer une réduction de la couche organique du sol et une canopée plus ouverte (Baltzer et al. 2021). Tous ces facteurs, ainsi que d'autres perturbations importantes liées à l'occupation humaine, à l'agriculture, à la foresterie et à l'exploitation minière dans l'est du Canada, ont favorisé l'abondance croissante des forêts de feuillus à feuilles caduques, comme le peuplier faux-tremble (*Populus tremuloides* Michx.) dans l'est du Canada (Laquerre et al. 2009 ; Marchais et al. 2020). Ces changements dans la dominance des arbres peuvent également entraîner des modifications des conditions environnementales locales et de la composition de la communauté de sous-bois (Augusto et al. 2015 ; Barbier et al. 2008 ; Urbanová et al. 2015).

La dominance des conifères, comme l'épinette noire, comparée à la dominance de feuillus, comme le peuplier faux-tremble, contrôle les conditions environnementales locales (Augusto et al. 2015) et façonne la composition et la dynamique des plantes de

sous-bois (Barbier et al. 2008 ; Bartels et Chen 2013 ; Qian et al. 2003) et des communautés microbiennes du sol (Hannam et al. 2006 ; Norris et al. 2016 ; Urbanová et al. 2015) (Figure 0.1). Les épinettes noires modifient les conditions du sol forestier par le biais de la litière d'aiguilles à faible teneur en nutriments qui acidifie le sol, diminue la température du sol et les taux de décomposition (Laganière et al. 2010). Ces conditions conduisent à des sols avec un faible cycle des nutriments et une faible productivité forestière (Légaré et al. 2004). En revanche, la litière de feuillus est riche en nutriments et elle favorise un pH, une température et une humidité du sol plus élevés, ce qui conduit à un cycle plus efficace des nutriments, et à des taux de décomposition élevés produisant des forêts plus productives (Cavard et al. 2011 ; Laganière et al. 2010). Le type de forêt modifie également la transmission de la lumière au sous- bois. Dans les forêts de peupliers faux-trembles, les plantes du sous-bois reçoivent une transmission lumineuse plus élevée que dans les forêts d'épinette noire (Messier et al. 1998). La végétation du sous-bois des forêts d'épinettes noires est fréquemment dominée par des espèces de mousses hypnacées, comme *Pleurozium schreberi* (Brid.) Mitt. Et *Ptilium crista-castrensis* (Hedw.), qui produisent des sols avec une épaisse couche organique (Fenton et Bergeron 2006). De plus, la composition du sous-étage est caractérisée par la présence d'éricacées, comme les bleuets (*Vaccinium angustifolium* Aiton et *Vaccinium myrtillus* L.) et le thé du Labrador (*Rhododendron groenlandicum* (Oeder) Kron & Judd) (Légaré et al. 2001 ; Nilsson et Wardle 2005). Contrairement aux peuplements homogènes d'épinettes noires, les forêts de peupliers faux-tremble ont un sous-bois hétérogène avec une grande diversité et abondance d'herbes et d'arbustes et une abondance limitée de bryophytes (Bartels et Chen 2013 ; Cavard et al. 2011). Bien que les facteurs liés à chaque type de forêt, y compris les changements dans la lumière, le dépôt de litière, le statut nutritif et la végétation de sous-bois, aient été largement étudiés, il existe peu d'études *in situ* évaluant les changements dans ces paramètres avec une vision holistique des facteurs liés à la canopée qui affectent les communautés de sous-bois dans la forêt boréale. En ce sens, l'analyse multi-taxons est essentielle pour comprendre les effets globaux sur les

communautés, notamment pour la conservation de la biodiversité, la gestion forestière et les stratégies de changement climatique (Jokela et al. 2018).

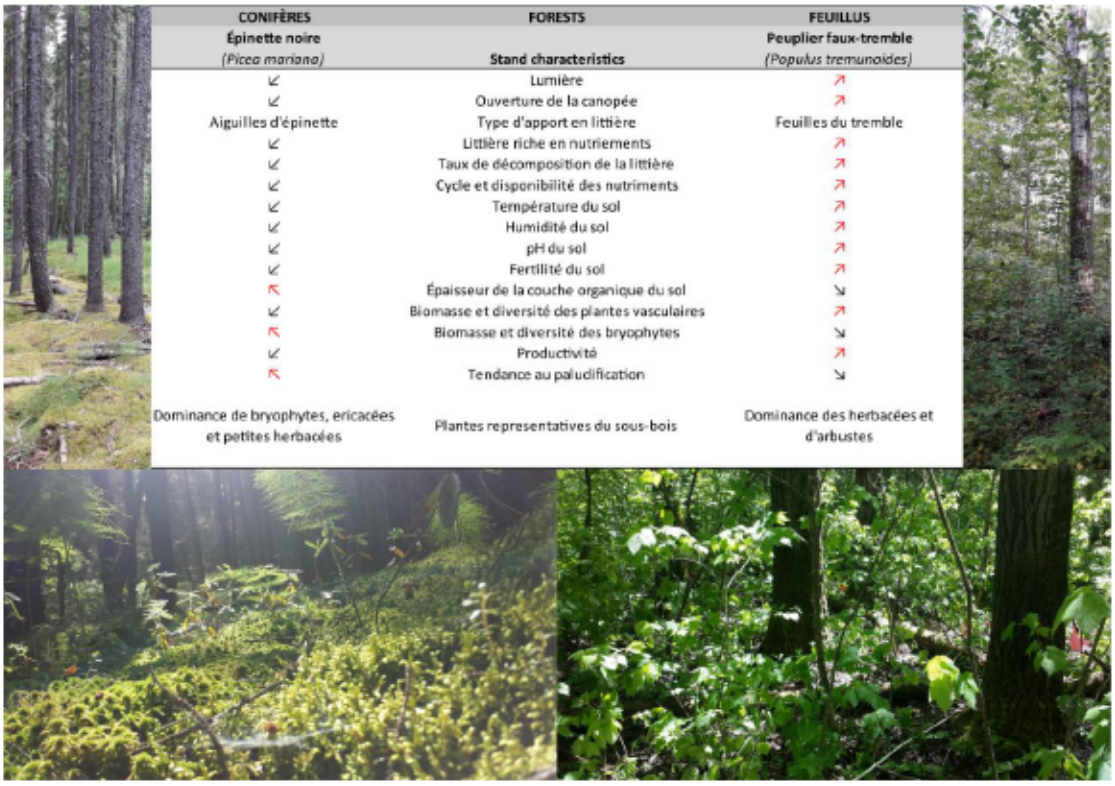

Figure 0.1 Tendances des conditions environnementales locales et de la composition de la communauté de sous-étage des forêts de conifères dominées par des épinettes noires et des forêts de feuillus dominées par des peupliers faux-trembles. Références : Messier et al., 1998; Lamarche et al., 2007; Cavard et al., 2011; Laganière et al., 2011; Bartels & Chen, 2013; Augusto et al., 2015

0.7 Interactions entre les communautés des plantes et du sol

Les systèmes naturels doivent être analysés en tenant compte de leurs différentes composantes, notamment la composition de la canopée, la composition d'espèces dans le sous-bois, les microorganismes associés aux plantes et le microbiome du sol, car ils forment des boucles de rétroaction constantes qui s'influencent mutuellement. Le microbiome est défini comme l'assemblage de microorganismes existant dans un habitat ou associés à celui-ci (Shade et Handelsman 2012). Les microorganismes sont

des moteurs importants de la dynamique des sols, car ils décomposent la matière organique et rendent disponibles les nutriments du sol à travers la chaîne trophique qui inclue les protozoaires, les nématodes et les microarthropodes qui se nourrissent de bactéries et de champignons et expulsent tous ces nutriments sous des formes solubles disponibles pour les plantes (Coleman et al. 2017 ; Ingham et al. 1985 ; Van Der Heijden et al. 2008). Il s'agit d'une autorégulation surprenante des dynamiques de nutriments et de microorganismes qui influencent là composition d'espèces sur et dans le sol. Les arbres dominants influencent la dynamique du sol et la composition des communautés microbiennes par leur impact sur la qualité de la litière et en modifiant les conditions physico-chimiques du sol forestier (Hannam et al. 2006 ; Lindo et Visser 2003 ; Urbanová et al. 2015). En fonction de la dominance des conifères ou des feuillus, l'abondance relative des bactéries et des champignons varie car chaque groupe microbien remplit des fonctions écologiques différentes dans le sol forestier (Augusto et al. 2015 ; Van Der Heijden et al. 2008). Les sols forestiers où dominent les communautés fongiques sont associés à un cycle lent des nutriments, une faible disponibilité des nutriments, une plus grande quantité de matière organique et une faible qualité des feuilles, qui produisent tous une faible productivité primaire nette et favorisent la présence d'espèces végétales à croissance lente (Van Der Heijden et al. 2008 ; Wardle et al. 2004). En revanche, les forêts dominées par des communautés bactériennes sont associées à un cycle et une disponibilité rapides des nutriments, à une abondance limitée de la matière organique du sol et à une qualité élevée de la litière, ce qui produit une productivité primaire nette élevée et favorise la présence de plantes à croissance rapide (Van Der Heijden et al. 2008 ; Wardle et al. 2004). Bien que le type de forêt semble influencer la structure de la communauté microbienne, il n'est pas clair dans quelle mesure les différents facteurs associés à la dominance des conifères ou de feuillus affectent la composition microbienne du sol.

Les associations plantes-microbes contribuent à la croissance des plantes et à leur adaptation à des environnements difficiles (Gaiero et al., 2013 ; Ritpitakphong et al.,

2016), ce qui favorise la diversité et la productivité des plantes (Van Der Heijden et al., 2008). Plus de 20 000 espèces végétales dans le monde dépendent de microorganismes symbiotiques pour leur développement et leur survie, en particulier dans des environnements pauvres en nutriments comme les forêts boréales (Van Der Heijden et al., 2008). Les plantes sont associées à des microorganismes dans différents microhabitats, non seulement dans le sol (microbiomes du sol et des racines), mais aussi dans leur phyllosphère. La phyllosphère désigne l'habitat microbien des surfaces aériennes des plantes, y compris les feuilles (Vorholt 2012). Il a été suggéré que la phyllosphère est principalement dominée par des communautés bactériennes, tandis que les communautés fongiques filamenteuses sont considérées comme des habitants transitoires de la surface des feuilles sous forme de spores qui se développent ensuite dans d'autres microhabitats (Andrews et Harris 2000 ; Lindow et Brandl 2003). Les surfaces foliaires sont des environnements relativement difficiles, exposés aux rayons ultraviolets, à une faible disponibilité en nutriments et à des fluctuations constantes de la température et de l'humidité au cours d'une même journée et au fil des saisons (Leveau 2006). Malgré ces conditions difficiles, la phyllosphère possède une communauté bactérienne dense de 106 à 107 cellules / cm^2 de surface foliaire ou jusqu'à 108 cellules / g de matériel foliaire (Remus-Emsermann et al. 2014 ; Vorholt 2012). La composition des communautés microbiennes de la phyllosphère est liée à la physicochimie des feuilles hôtes, ce qui entraîne une grande variabilité interspécifique, plus qu'intraspécifique ou entre différents environnements (Laforest-Lapointe et al. 2016 ; Redford et al. 2010 ; Schlechter et al. 2019). Par conséquent, des arbres phylogénétiquement différents ont des communautés phyllosphériques distinctes (Kembel et al. 2014 ; Lajoie et Kembel 2021a ; Redford et al. 2010), qui affectent probablement la dynamique des nutriments souterrains par le biais des apports de litière (Augusto et al. 2015 ; Prescott et Grayston 2013). Plusieurs études ont évalué la co-occurrence entre les microhabitats de la litière et les communautés microbiennes du sol (Hannam et al. 2007 ; Prescott et Grayston 2013 ; Urbanová et al. 2015). Cependant, la litière sur le sol forestier a déjà commencé les processus de décomposition et la

structure de la communauté microbienne dans la litière pourrait être différente des apports originaux de feuilles de chaque type d'arbre. Il existe donc un manque de connaissances pour comprendre les différences de structure des communautés microbiennes entre les microhabitats et l'influence possible de la phyllosphère des arbres sur la composition des communautés microbiennes du sol. Compte tenu de l'importance des interactions plantes-microbes dans les forêts boréales, une question fondamentale en écologie microbienne est de savoir quels facteurs façonnent les communautés microbiennes (Vorholt 2012) et, en outre, comment la composition microbienne varie entre les microhabitats et les types de forêts.

0.8 Associations mousses-bactéries

De façon similaire à la phyllosphère des arbres, les communautés microbiennes de la phyllosphère des mousses semblent être principalement spécifiques à l'hôte (Bragina et al. 2012 ; Holland-Moritz et al. 2018 ; Opelt et al. 2007). Cependant, la composition microbienne associée aux mousses semble également être affectée par diverses conditions environnementales associées à différents types de forêts, sites de collecte ou gradients d'altitude (Davey et al. 2013 ; Holland-Moritz et al. 2018 ; Jean et al. 2020 ; Tang et al. 2016). Peu d'études ont exploré la phyllosphère des mousses, malgré la grande diversité et l'abondance des bryophytes dans le biome boréal, en particulier dans les forêts de conifères fréquemment dominées par les mousses hypnacées, telles que *Pleurozium schreberi* et *Ptilium scista-castrensis* (Fenton et Bergeron 2006 ; Nilsson et Wardle 2005). Ces associations mousses-microbes sont importantes dans les forêts boréales, car elles contribuent à l'oxydation du méthane (Kip et al., 2010) et aux cycles du carbone et de l'azote (DeLuca et al., 2002 ; Turetsky, 2003). Les forêts boréales étant limitées en azote (Högberg et al. 2017), l'association des mousses avec des bactéries diazotrophes, telles que les cyanobactéries, joue un rôle écologique clé en contribuant de manière significative aux apports d'azote en fixant jusqu'à 7 kg N ha^{-1} an^{-1} dans les écosystèmes boréaux (DeLuca et al., 2007 ; Lindo et al., 2013 ; Rousk et

al., 2013a). Malgré leurs importantes fonctions écologiques, les études existantes sur la phyllosphère des mousses ont été limitées dans leur portée, car la plupart des études se sont concentrées sur les forêts de conifères homogènes et pauvres en nutriments, tandis que les forêts de feuillus hétérogènes et riches en nutriments ont été moins étudiées. Si la composition de la communauté microbienne est effectivement influencée par le type de forêt, les interactions mousses-bactéries pourraient être affectées par les changements croissants de la dominance des arbres, des conifères aux feuillus dans le système boréal.

0.9 Identifier l'invisible : Séquençage d'ADN d'échantillons environnementaux

Les études des interactions plantes-microorganismes sont désormais possibles grâce aux progrès relativement récents des techniques de séquençage. La possibilité de séquencer l'ADN à partir d'échantillons environnementaux a ouvert la porte à l'étude de la biodiversité des communautés microbiennes dans différents environnements (Epp et al. 2012). L'utilisation des régions conservées, telles que les régions du gène de l'ARNr 16S chez les bactéries et l'ITS chez les champignons, qui présentent également une grande variabilité permet de différencier taxonomiquement les groupes microbiens au sein de la séquence d'amplicon (Edgar 2018). Le séquençage à haut débit représente une approche utile pour l'identification des microorganismes dans les écosystèmes forestiers, même s'il présente certains biais et comporte encore des défis, tels que la génération d'erreurs de séquençage (Nilsson et al. 2019). Dans le processus d'analyse bioinformatique, les lectures d'amplicon de la séquence ciblée sont filtrées, regroupées et comparées à une base de données de référence pour l'identification taxonomique (Callahan et al. 2016a). L'approche récente des variants de séquence d'amplicon (ASV) permet une haute résolution; il distingue même les variants de séquence différant que par un seul nucléotide et il déduit les séquences biologiques de l'échantillon en tenant compte des erreurs d'amplification et de séquençage (Callahan et al. 2017). Cette approche d'utiliser des ASVs est analogue à l'approche des unités

taxonomiques opérationnelles (OTU) qui a été fréquemment utilisé, dans laquelle les séquences sont regroupées par des lectures différants par un pourcentage fixe de dissimilarité de séquence (souvent 3%), mais la variation biologique en dehors de la base de données de référence n'est pas capturée (Callahan et al. 2017). Avec l'approche avec des ASVs, le processus de groupement produit un tableau avec le nombre de fois où chaque ASV exact a été observé dans chaque échantillon, qui peut être directement analysé et ensuite assigné taxonomiquement à l'aide d'une base de données de référence. Nous avons donc utilisé l'approche des ASVs dans notre étude pour identifier les communautés microbiennes de la forêt boréale.

0.10 Objectifs et structure de la thèse

Compte tenu des changements croissants dans la dominance des arbres, des forêts de conifères aux forêts de feuillus, l'objectif principal de cette thèse était de comprendre comment la dominance des arbres des forêts d'épinettes noires et de peupliers faux-trembles influence la composition des plantes et des communautés microbiennes associées dans le sous-bois. Ainsi, cette thèse est présentée en trois chapitres :

0.10.1 Chapitre 1

Objectif général : Analyser les effets de la variation des facteurs principaux qui induisent les changements entre les états stables alternatifs définis par la dominance de la canopée des forêts d'épinettes noires et de peupliers faux-trembles. Grâce à une expérience *in situ* de 5 ans et sur la base du cadre théorique des états stables alternatifs décrit par Beisner et al. (2003), nous avons manipulé à la fois les conditions environnementales locales en utilisant une « approche écosystémique » et les communautés du sous-bois en utilisant une « approche des communautés » (transplantation réciproque).

Objectif 1 – Approche écosystémique : Analyser la variation des changements dans les facteurs environnementaux associés à la dominance de la canopée (conditions de lumière, statut nutritif, et apport en litière) sur la composition du sous-bois. Hypothèse : Le type de forêt modifiera la composition de la végétation du sous-bois principalement par l'apport de litière, car la litière influence de manière significative les processus de décomposition et de minéralisation des nutriments (Laganière et al. 2010, Rodríguez-Calcerrada et al. 2011, Chen et al. 2017).

Objectif 2 – Approche des communautés (transplantation réciproque) : Analyser les changements dans les communautés végétales de sous-bois échangées entre les types de forêts, afin de déterminer si la végétation de sous-bois était résistante à un changement de la canopée au cours du temps et si la forêt était résiliente à la perturbation à petite échelle de la transplantation. Hypothèse : Les communautés végétales du sous-bois répondront différemment en fonction de leur résistance aux changements des facteurs liés à la dominance de la canopée, et en fonction de la résilience du sous-bois de chaque forêt à ne pas modifier radicalement sa composition d'espèces face à la perturbation de la transplantation réciproque.

0.10.2 Chapitre 2

Objectif général : Analyser les différences entre les communautés microbiennes du sol dans les forêts de conifères et de feuillus et les facteurs qui les déterminent.

Objectif 1 : Analyser les différences dans les communautés bactériennes entre les microhabitats (microbiome du sol *vs*. phyllosphère des arbres) et les types de forêts (forêts d'épinette noire *vs*. forêts de peuplier faux-tremble). Hypothèse : Les communautés microbiennes seront différentes entre les types de forêts, mais pour chaque types de forêt, les bactéries seront co-occurrentes entre les deux microhabitats, car nous nous attendons à un partage des communautés bactériennes de la phyllosphère de l'arbre au microbiome du sol à travers la chute de la litière.

Objectif 2 : Analyser si les communautés bactériennes et fongiques ont été affectées par des changements dans les facteurs associés à la dominance des arbres, y compris les changements dans l'apport en litière et les transplantations de sous-bois entre les types de forêts, et les corréler avec les facteurs abiotiques et biotiques. D'une part, les changements dans l'apport en litière consistaient à ajouter des aiguilles dans les forêts de peupliers faux-trembles et des feuilles dans les forêts d'épinettes noires. D'autre part, nous avons transplanté des parcelles complètes de végétation de sous-bois d'épinettes noires vers des forêts de peupliers faux-trembles, et vice-versa, et analysé leur composition microbienne après 5 ans. Hypothèse : Les facteurs associés à la dominance des arbres produiront une composition de la communauté microbienne différente des conditions naturelles (témoin) mais plus similaire au type de forêt opposé, et seront corrélés avec des facteurs abiotiques et biotiques dans chaque type de forêt.

0.10.3 Chapitre 3

Objectif : Déterminer si les espèces hôtes ou les conditions environnementales définies par la dominance de la canopée déterminent la diversité bactérienne dans la phyllosphère des mousses. Nous avons utilisé le séquençage de l'amplicon du gène de l'ARNr 16S pour quantifier les changements dans les communautés bactériennes de la phyllosphère des mousses en fonction de l'espèce hôte (*Pleurozium schreberi* et *Ptilium crista-castrensis*) et du type de forêt (épinette noire *vs.* peuplier faux-tremble). Hypothèse : L'espèce hôte aura le plus grand effet sur la composition de la communauté bactérienne tandis que le type de forêt aura un effet secondaire.

0.10.4 Zone d'étude

La zone d'étude est la même pour les trois chapitres. Elle est située dans l'est du Bouclier boréal du Canada, dans le domaine bioclimatique de la pessière à mousse de la ceinture argileuse de l'ouest du Québec (Bergeron et al. 1996), créée par le lac

proglaciaire Ojibway à partir de l'expansion maximale pendant la glaciation du Wisconsin (Vincent et Hardy 1977, Veillette 1994). La composition de la canopée est caractérisée par une dominance de l'épinette noire et du peuplier faux-tremble. Les autres espèces importantes dans ce domaine bioclimatique comprennent le pin gris (*Pinus banksiana* Lambert), le sapin baumier (*Abies balsamea* (L.) Miller) et le bouleau à papier (*Betula papyrifera* Marshall) (Saucier 1994). Sur la base d'études antérieurs (Cavard et al. 2011 ; Légaré et al. 2005), nous avons sélectionné trois sites d'étude spécifiquement situés à la limite des régions de l'Abitibi-Témiscamingue et du Nord-du-Québec (Site A : 49°11'46'' N – 78°50'33'' W ; Site B : 49°09'20''N – 78°47'56''W et Site C : 49°09'39''N – 78°47'55''W) ; les sites B et C étaient distants de 0,5 km et le site A de 5,3 km. (Figure 0.2). Chaque site posséde des peuplements adjacents dominés soit par des épinettes noires (≥ 75 % de la surface terrière de *Picea mariana*) et soit par des peupliers faux-trembles (≥ 75 % de la surface terrière de *Populus tremuloides*) (Cavard et al. 2011). Chaque pair de peuplements a une superficie d'environ 1 ha et sont séparés par une distance variant de 34 à 115 m. Indépendamment de la composition de leur canopée, les sites présentaient des conditions abiotiques comparables quant aux dépôt de surface, de pente et, de drainage. Les sols présentent un drainage subhygriques modérément secs avec une texture argileuse et une topographie plane. Les sites provenaient du même incendie de forêt *ca.* 1916 (Bergeron et al. 2004 ; Laganière et al. 2011 ; Légaré et al. 2005). Par conséquent, la composition des arbres était la seule source significative de variation entre les sites. Au sein de chaque type de forêt, trois blocs séparés entre 16 et 49 m ont été utilisés pour les différentes analyses pour chaque chapitre de la thèse. Cette aire d'étude est caractérisée par des étés courts, avec une saison de croissance d'environ 150 jours et un climat modérément sec et frais, avec des températures annuelles moyennes de 0,7 °C et des précipitations annuelles moyennes de 889,8 mm, enregistrées dans la station météorologique la plus proche à La Sarre (QC) (Environnement Canada 2017) (Laquerre et al. 2009).

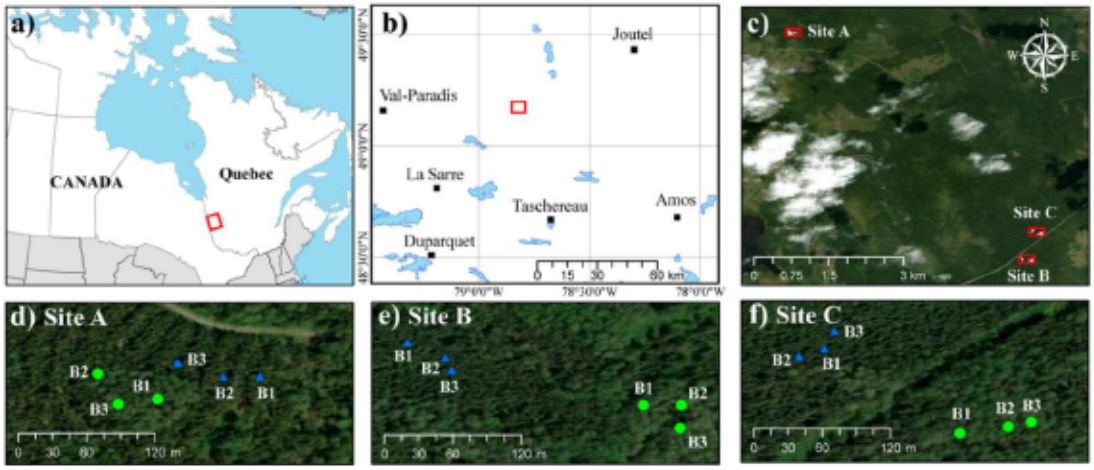

Figure 0.2 Localisation des sites d'étude dans la forêt boréale de l'est du Canada (a), dans l'ouest du Québec au-dessus du parallèle 49ºN (b). Les sites B et C sont distants de 0,5 km et le site A de 5,3 km (c). Chaque site comporte des peuplements adjacents dominés par l'épinette noire (triangles bleus) et le peuplier faux-tremble (cercles verts), séparés de 34 à 115 m ; et à l'intérieur de chaque type de forêt, trois blocs (B1, B2 et B3) ont été placés séparés de 16 à 49 m (d, e, f).

CHAPITRE 1

DRIVERS OF CONTRASTING BOREAL UNDERSTORY VEGETATION IN CONIFEROUS AND BROADLEAF DECIDUOUS ALTERNATIVE STATES

FACTEURS QUI CONTROLENT DE LA VÉGÉTATION BORÉALE DU SOUS-BOIS DANS LES ÉTATS ALTERNATIFS DE FORÊTS DES CONIFÈRES ET DES FEUILLUS

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1.1 Abstract

Alternative stable states defined by tree-canopy dominance generate different ecosystem functions and shape habitat conditions for the understory vegetation. One example in the boreal forest is the alternation between broadleaf deciduous and coniferous forests. Disturbances related to natural fires and human land uses have produced changes in tree-canopy dominance in the boreal area from coniferous to broadleaved forests, affecting understory community dynamics and their related ecosystem processes and functions. To analyze the factors driving changes in understory vegetation and its resistance to shifts between alternative states, we compared the effects of changes in the system between two contrasting boreal forest types (black spruce vs. trembling aspen). We performed a 5-year *in situ* experiment using alternative stable states as a theoretical framework including two approaches: 1) the ecosystem approach, manipulating environmental conditions of light conditions, litter type and nutrient status in each forest type to determine the main mechanisms associated with tree-canopy dominance that affect the diversity and composition of understory communities; and 2) the community approach, physically exchanging understory communities between alternative states, to determine their resistance under a new tree-canopy dominance through time, as well as the resilience of the forest understory after a small-scale disturbance. Results indicate that the understory vegetation of trembling aspen forests were resistant through time both after changes in local conditions in the ecosystem approach and in the new black spruce-dominated alternative state in the community approach. In contrast, mosses and ericaceous plants that typically dominate the forest floor of black spruce forests were negatively affected by the physical effect of broadleaf litter addition in our ecosystem approach and they were not resistant when transplanted to trembling aspen forests in the community approach, as they decreased in abundance and were invaded by aspen understory community species over time. The understory vegetation is a key forest ecosystem driver that shape the future overstory, can contribute to maintain the resilience of the

boreal system and help to preserve their ecosystem services, which is a key aspect to consider in forest management faced with the effects of climate change.

Key words: Alternative stable states, *Picea mariana* (black spruce), *Populus tremuloides* (Trembling aspen), Understory vegetation, Tree-canopy dominance, Community ecology, Bryophytes, Ericaceous species, Herbs, Resilience, Resistance.

1.2 Résumé

Les états alternatifs stables définis par la dominance des arbres génèrent des fonctions écosystémiques différentes et façonnent les conditions d'habitat de la végétation de sous-bois. Un exemple dans la forêt boréale est l'alternance entre les forêts de feuillus et de conifères. Les perturbations liées aux incendies naturels et à la l'utilisation des terres ont entraîné des changements dans la dominance de la canopée dans la forêt boréale, des forêts de conifères aux forêts de feuillus, ce qui a affecté la dynamique des communautés de sous-bois et les processus et fonctions écosystémiques associées. Afin d'analyser les facteurs à l'origine des changements dans la végétation de sous-bois et sa résistance aux changements d'états alternatifs, nous avons comparé les effets des changements dans le système entre deux types de forêts boréales (épinette noire vs peuplier faux-tremble). Nous avons réalisé une expérience *in situ* pendant 5 ans en utilisant des états stables alternatifs comme cadre théorique comprenant deux approches : 1) l'approche écosystémique, en manipulant les conditions environnementales des conditions de lumière, du type de litière et le statut de nutriments pour déterminer les principaux mécanismes associés à la dominance de la canopée qui affectent la diversité et la composition des communautés du sous-bois; et 2) l'approche de transplantations de communautés de sous-bois, en les échangeant physiquement entre les états alternatifs, pour déterminer leur résistance à une nouvelle dominance de la canopée des arbres au fil du temps, ainsi que la résilience du sous-bois de la forêt après une perturbation à petite échelle. Les résultats indiquent que la végétation de sous-bois des forêts de peupliers faux-trembles était résistante au cours du temps, à la fois dans l'approche écosystémique après des changements dans les conditions locales et dans l'approche de transplantations dans le nouvel état alternatif dominé par l'épinette noire. Par contre, les mousses et les éricacées qui dominent généralement le sous-bois des forêts d'épinettes noires ont été affectées négativement par l'effet physique de l'ajout de litière de feuillus dans notre approche écosystémique et elles ont montré une faible résistance lorsqu'elles ont été transplantées dans des forêts

de peuplier faux-tremble, car elles ont été envahies par les espèces de la communauté de peupliers faux-tremble au fil du temps. La végétation de sous-bois est un élément clé de l'écosystème forestier qui façonne la future composition de la canopée, peut contribuer à maintenir la résilience du système boréal et aider à préserver ses services écosystémiques, ce qui est un aspect essentiel à prendre en compte dans la gestion forestière face aux effets du changement climatique.

Mots clés : États alternatifs stables, *Picea mariana* (épinette noire), *Populus tremuloides* (Peuplier faux-tremble), Végétation de sous-bois, Dominance de la canopée, Écologie des communautés, Bryophytes, Éricacées, Herbes, Résilience, Résistance.

1.3 Introduction

The boreal forest is a social-ecological system that experiences significant anthropogenic and natural disturbances influencing forest dynamics, structure, functioning and feedbacks (Chapin et al. 2010). These forests represent 30 % of the world's forest area and provide globally significant ecosystem services, such as climate and water regulation, carbon storage, wood production and derivates, fishing, hunting and other recreational and economic activities (Gauthier et al., 2015). Plant communities in the boreal forest are considered to be in a dynamic equilibrium within stable states defined by their physical environment that act as drivers of community composition (Beisner et al. 2003). A disturbance trigger (*e.g.* severe fire, clear cut) can generate alternative states in sites with similar topoedaphic conditions and species pools by producing changes in the structure and composition of keystone species (such as dominant tree species) (Chapin et al. 2010; Seidl and Turner 2022). The highly debated concept of alternative stable states is presented by Beisner et al. (2003) as a conceptual framework with a ball-in-cup diagram (Figure 1.8) that was used in this study to facilitate the exploration of alternative stable states in real communities. The boreal forest can be used as a model in which to analyze alternative stable states, with areas dominated by coniferous and deciduous broad-leaved trees, in which the understory conditions and plant communities are remarkably different despite common soil and topographic conditions (Johnstone et al. 2010a, Johnstone et al. 2020, Baltzer et al. 2021).

In North America, black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) is a dominant forest type that contributes to carbon sequestration, is an important wildlife habitat (*e.g.* for Caribou – *Rangifer tarandus*), is shaped by boreal fire dynamics, and black spruce is one of the most economically important tree species for wood industries (Hins et al. 2009, Baltzer et al. 2021). However, changes in boreal forest composition have been observed over the last decade (Gauthier et al. 2015) and are mainly due to

changes in natural fire regimes associated with climate change and anthropogenic causes (land logging, clearing, slash and burn activities related with human land colonization, agriculture, forestry, and mining) (Laquerre et al. 2009, Marchais et al. 2020, Baltzer et al. 2021). Specifically, spruce-moss forests have changed in tree-canopy dominance to broadleaved forests via the expansion of deciduous broadleaf trees, such as aspen and birch species, depending on biogeographic zone in boreal North America (Johnstone et al. 2020, Baltzer et al. 2021, Mack et al. 2021). The boreal black spruce forests in western Québec have been experiencing an expansion of trembling aspen trees (*Populus tremuloides* Michx.), resulting areas dominated by or with mixed black spruce and trembling aspen trees (Grondin et al. 2003, Marchais et al. 2022). Trembling aspen can regenerate in the high-light post-disturbance environments much faster than black spruce, particularly by root suckering, producing forests dominated by these deciduous trees (Laquerre et al. 2011). However, the fire-born mixed stands of black spruce - trembling aspen have frequently a successional replacement towards black spruce dominance in the absence of a major disturbance, with an accumulation of a soil organic layer that limits suckering and seedling establishment (Bergeron et al. 2004; Laquerre et al. 2011). Even so, multiple pathways can be generated after disturbance depending on landscape composition, regeneration conditions in the understory (*e.g.* species pools, soil organic layer thickness, parent material, etc.), climate and fire regimes (Johnstone et al. 2010a; Bergeron et al. 2014; Baltzer et al. 2021). When two areas regenerate differently after fire, one as a black spruce-dominated and the other as trembling aspen-dominated stand, each pure forest type can remain stable over time; however, mixed stands can show an aspen dominated canopy with a black spruce understory (Boucher et al. 2014; Bergeron et al. 2014). Under short fire cycles, pure and mixed stands tend to be maintained as stable states, but spruce can replace aspen under long fire interval cycles (Lecompte and Bergeron 2005). Post fire shifts from spruce to aspen could also occur if fire intervals are too short to allow for sufficient spruce cones production (Splawinski et al. 2019), or if fire severity does not provide good germination seedbeds for spruce (Greene et al. 2007;

Baltzer et al. 2021). At the landscape level, spruce, aspen and mixed stands can cooccur as alternative stable states in proportions linked to the natural fire regimes. In Quebec, natural fire disturbance cycles consisted in severe stand-replacing crown fires of around 100 years before 1850, and lately these cycles have increased to around 400 years (Bergeron et al. 2004). However, fire frequency might increase with climate change in boreal forests, producing more frequent changes in tree-canopy composition (Boulanger et al. 2014).

Tree-canopy composition is a strong filter on understory plant communities (vascular plants and bryophytes) via its effects on environmental factors, such as litter deposition (Laganière et al. 2010), light (Bartemucci et al. 2006) and soil nutrient availability (Laganière et al. 2011). Compared to stands dominated by black spruce, trembling aspen stands have higher light intensity in the understory (Messier et al. 1998) and their broadleaf litter decomposes rapidly compared to black spruce needles (Laganière et al. 2010). Therefore, the presence of trembling aspen in the canopy changes local conditions by promoting a faster rate of nutrient cycling and greater nutrient availability (Légaré et al. 2005) and consequently, these stands have thinner organic layers over the mineral soil than black spruce stands (Fenton et al., 2005). Understory vegetation in black spruce forests is dominated by a thick layer of mosses (mainly the feather mosses *Pleurozium schreberi* and *Ptilium crista-castrensis*) and ericaceous plants, such as *Vaccinum* species, *Gaultheria hispidula,* and *Rhododendron groenlandicum*, whereas there is a high diversity and abundance of herbs and shrubs that dominate the understory of trembling aspen forests, such as *Aralia nudicaulis, Clintonia borealis, Cornus canadensis, Viburnum edule* and *Rubus species* (Cavard et al. 2011, Table S 3.1). Considering the bilateral influence and feedbacks between overstory and understory vegetation and the contrasting plant composition despite sharing similar local conditions, these two forest systems are considered in this study as alternative stable states.

In these two contrasting forest types, we explored the main factors that induce changes in understory vegetation between alternative stable states defined by tree-canopy dominance. We used the theoretical framework of alternative stable states described by Beisner et al. (2003). This framework establishes that, when two alternative stable states exist at the same time in a matrix (Figure 1.8-a1), plant understory communities are pulled to one state or the other, which act as basins of attraction (Figure 1.8-a2). To explore effects of changes in the system on understory vegetation, we manipulated in situ local environmental conditions and understory communities using the ecosystem and the community approaches (Beisner et al. 2003). In the ecosystem approach (Objective 1, Figure 1.8-b1), we experimentally manipulated the environmental drivers associated with tree-canopy dominance (light conditions, nutrient status, and type of litter deposition) that have the greatest effect on understory vegetation. Tree-canopy dominance was expected to alter understory vegetation composition primarily through type of litter deposition, because litter type significantly influences decomposition and nutrient mineralization processes (Laganière et al. 2010, Rodríguez-Calcerrada et al. 2011, Chen et al. 2017). In the community approach (Objective 2, Figure 1.8-b2), the environment was considered fixed, and the understory communities were transferred from one stable state to another (*e.g.* black spruce understory transplanted to trembling aspen understory, and vice-versa). We then evaluated changes in understory plant communities in the two stable states to determine if understory vegetation was resistant to a new tree-canopy dominance through time and if the forest was resilient to the small-scale disturbance of the transplantation. Understory plant communities were expected to react depending on their resistance to changes in their habitat and on their resilience of the host forest understory to a small disturbance of transplanted vegetation. The resistance is defined as the capacity to stay essentially unchanged despite disturbances (Grimm and Wissel 1997), and resilience is considered here as a measure of persistence of the system that absorbs a change (*e.g.* transplantations) without dramatically altering population relationships or state variables (Holling 1973). If the understory composition is resilient to the change in community it implies that it

is able to survive in the conditions created by the opposite tree canopy, and limitations must be due to some earlier limitation. However, if the understory composition is not resilient to the change in community, this implies that the current conditions of the opposite tree canopy alone are sufficient to limit the spatial distribution of the community. Therefore, this study used two boreal forest types to analyse in situ changes in natural systems and to determine how attributes of alternative stable states influence plant communities. Considering the globally important ecosystem services of the boreal forest (climate regulation, disturbance regime, food, fiber, water, etc.), insights in factors producing shifts between alternative stable states can give insights on forest management and to predict future habitat changes related with global warming.

1.4 Methods

1.4.1 Study area and experimental design

The study area (Figure 1.1) was in the eastern Canadian boreal forest and is part of the spruce-moss forest bioclimatic domain (Bergeron et al. 1996) located in the Clay Belt of Quebec and Ontario, created by proglacial Lake Ojibway from the maximum expansion during the Wisconsin glaciation (Vincent and Hardy 1977, Veillette 1994). Three similar sites (Site A: *49°11'46" N - 78°50'33" W;* Site B: *49°09'20" N - 78°47'56" W* and Site C: *49°09'39"N - 78°47'55" W*) were selected because they had comparable abiotic conditions of surface deposit, slope, drainage, clay-dominated subhygric soils and topography regardless of their tree-canopy composition (Légaré et al. 2005; Cavard et al. 2011). Sites B and C were 0.5 km apart and Site A was 5.3 km away from B and C. These sites were initiated after a fire disturbance in ca. 1916 (Bergeron et al. 2004), which produced adjacent stands dominated by black spruce and trembling aspen. Thus, each of the three sites had an area dominated by black spruce (≥ 75 % of *Picea mariana* canopy cover) and an area dominated by trembling aspen (≥ 75 % of *Populus tremuloides* canopy cover). Stands were about 1 ha in size and were separated by 34 to

115 m. In order to capture the variation within sites, we randomly placed three blocks in each stand (forest type), separated by 16 to 49 m. In each block, seven 1 m^2 permanent plots were randomly installed (one plot per treatment in each block). A total of seven treatments (including control) were chosen to artificially reproduce some of the environmental conditions from one forest type in the other (simulating conditions of black spruce in trembling aspen stands and vice-versa) and to analyze the overall effects of transplantation of the understory vegetation on the other tree-canopy composition (Figure 1.1). The Control treatment (Figure S 3.1-c) corresponded to unmanipulated plots and was used to analyze changes in each forest type community in stable states for both the ecosystem and the community approaches. Therefore, the nested block experimental design consisted of 3 sites x 2 forest types x 3 blocks x 7 treatments (1 control plot + 4 treatments of the ecosystem approach + 2 treatments of the community approach), for a total of 126 plots. This experiment started in November 2013 and data was collected in the permanent plots every year between 2014 and 2018.

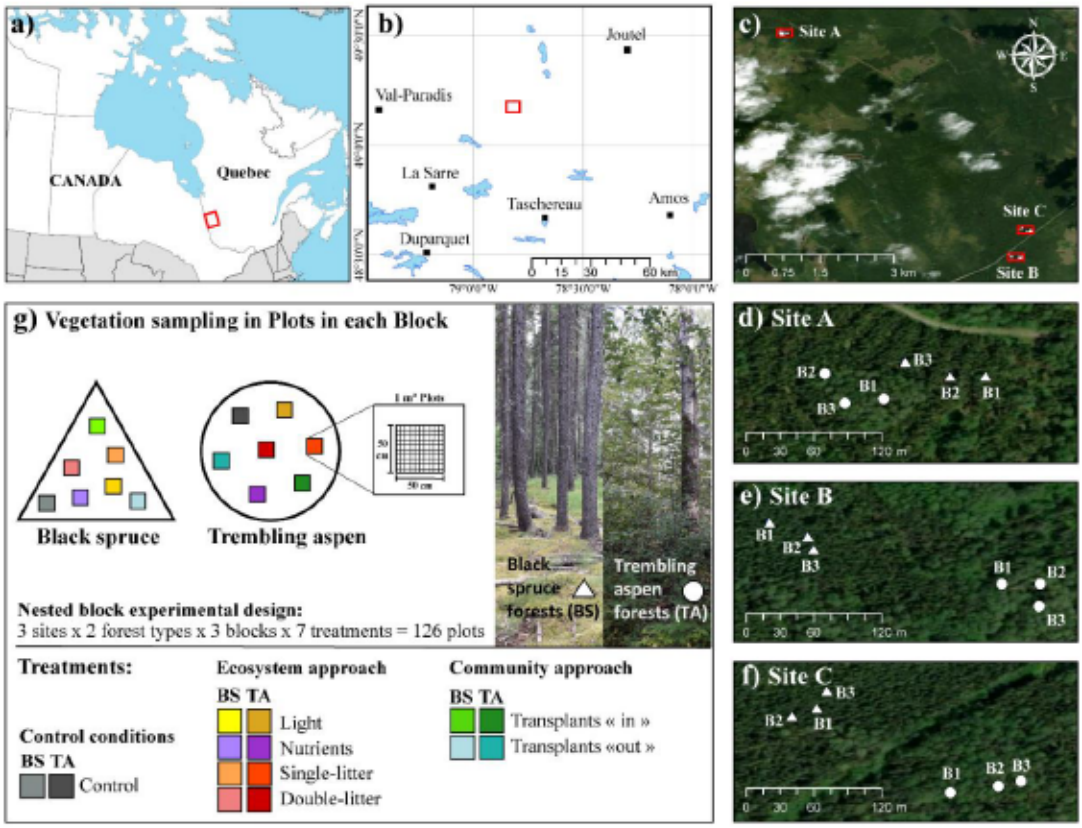


Figure 1.1 Experimental design for overstory – understory relationships. a) The study area is located in the boreal forest of eastern Canada; b) above the parallel 49ºN in western Quebec. c) The experiment was conducted in three sites A, B and C (Sites B and C are 0.5 km apart and Site A is 5.3 km away). d, e, f) Each site has adjacent stands (separated from 34 to 115 m) dominated by black spruce (BS, as triangles) and trembling aspen (TA, as circles), in which we placed 3 blocks (B1, B2, B3) separated from 16 to 49 m. g). In each block, one plot per treatment was placed to obtain a nested block experimental design with a total of 126 plots = 3 sites x 2 forest types x 3 blocks x 7 treatments (1 control + 4 treatments of the community approach + 2 treatments of the community approach). Vegetation abundance in all treatments was measured every year from 2013 to 2018 by using a 50 x 50 cm grid placed in the middle of each plot. Treatments in different colors correspond to *Control* conditions (C, in grey) and to treatments of the ecosystem approach: *Light* (Li, in yellow), *Nutrients* (Nu, in purple), *Single-litter* (1F, in orange) and *Double-litter* (2F, in red); and of the community approach: *Transplants-out* (To, in blue), *Transplants-in* (Ti, in green). Treatments in black spruce forests in light colors and in trembling aspen forests in dark colors.

1.4.2 Treatments for ecosystem approach

The first objective (ecosystem approach, Figure 1.8-b1) assessed what mechanisms associated with tree-canopy dominance affect the diversity and composition of understory vegetation. Therefore, we modified environmental parameters (Figure 1.8-c1,2,3) that are believed to be the main drivers of effects of tree dominance on understory plant communities: available light, nutrient status and litter type. To manipulate these drivers, we used treatments that differed in each forest type: *Light, Nutrients*, *Single-litter* and *Double-litter* (Figure S 3.1-a).

1.4.2.1 *Light* (Li) treatment.

Since tree-canopy dominance modulates light conditions, which affect understory vegetation (Sercu et al. 2017), we manipulated light conditions with the intention of simulating the light conditions of black spruce forests in trembling aspen forests, and vice-versa, to determine if light conditions alone could cause changes in the understory vegetation. The understory of deciduous broadleaved forests has higher light availability than coniferous forests (Canham et al. 1994, Messier et al. 1998, Légaré et al. 2001). Therefore, in trembling aspen forests we placed a shade cover over the 1 m^2 plot at 1 m high on every *Light* plot to reduce light inputs in these treatment-plots. These shade covers filtered approximately 60 % of light and were installed every spring and removed in the fall every year, as snow covers all soil and vegetation in the winter. In contrast, in black spruce forests, the *Light* treatment-plot was placed in a natural canopy opening that received more light than in the rest of the stand.

1.4.2.2 *Nutrients* (Nu) treatment

The characteristics of leaf litter can influence understory vegetation via the creation of a physical barrier to light and plant growth or via changes in chemical composition of the surface organic soil layers or by allelopathy (Startsev et al. 2008). Black spruce needles are more recalcitrant to decomposition and form an acidic layer of humus rich

in lignin, in contrast to trembling aspen litter that is decomposed more easily and is rich in nutrients (Laganière et al. 2010). Therefore, to separate the physical and chemical effects of litter, we manipulated the nutrient status of each understory via the *Nutrient* that treatment was applied once each spring. A fertilizer Nitrogen-Phosphorus-Potassium (30 g of NPK 21:2:4) was applied in black spruce plots and an acidifier (33.33 g of Botanix, soil acidifier 14 % Sulphur, 8 % Aluminium, neutralizing value 37 %, equivalent in hydrochloric acid) in aspen plots, to simulate soil conditions under each forest type. The amount applied the first year was artificially high by error (1 kg of NPK and 333 g of Botanix in 2014), but this was corrected starting in 2015.

1.4.2.3 *Single-litter* (1F) and *Double-litter* (2F) treatments

To analyze the physical effect of litter on understory vegetation, we manipulated the deposition of litter in each forest type every year since 2013. Broadleaves were collected in the autumn with 2 m^2 sheets placed on the soil and needle leaves were collected with 50 cm x 100 cm boxes with a net to retain needles during winter. We added the equivalent of the amount of litter that fell each year in a 1 m^2 area in a single layer of leaves (*Single-litter* treatment) and a double layer of leaves (*Double-litter* treatment) on each plot from the opposite forest type (broadleaved litter on black spruce stands and needleleaf litter on trembling aspen stands). This manipulation was in addition to the naturally falling litter of each forest type.

1.4.3 Treatments for community approach

The second objective evaluated shifts in understory communities (community approach, Figure 1.8-b2), to analyze the resilience of understory vegetation under a new canopy dominance through time. Thus, the treatments *Transplants-out* (Figure 1.8-c4) and *Transplants-in* were designed to analyze a global effect of tree-canopy composition on understory vegetation (Figure S 3.1-c).

1.4.3.1 *Transplants-out* (To) treatment

Transplants-out consisted of transplants harvested from one forest type and transplanted to the other forest type (inter-population transplants). Therefore, understory vegetation from black spruce was transferred under trembling aspen stands, and vice-versa. Each transplant consisted of a $1m^2$ surface of understory vegetation and approximately 30 cm of soil depth that was rolled on itself in a sheet to be transferred to the corresponding plot at the beginning of the study in November 2013.

1.4.3.2 *Transplants-in* (Ti) treatment

To distinguish the effect that the transplantation itself can have on understory plants, *Transplants-in* (intra-population transplants) were used as controls of the transplantation so transplants were moved from one forest type to the same forest type. For example, vegetation from a black spruce stand was taken and installed under another black spruce stand following the same methods as for the *Transplants-out* treatments.

1.4.4 Vegetation sampling

In each permanent plot, a grid was placed in the center, leaving a 25 cm buffer area around it to avoid edge effects (Figure 1.1). This 50 x 50 cm grid was divided every 5 cm (total of one hundred 25 cm^2 squares per grid) and was used to calculate the percent cover of each understory plant species present in the grid in each plot. As we used permanent plots, species identification was done by observation of plants in each plot without collecting or disturbing plants inside the plot and collecting plants around the plot if necessary. Therefore, some species of bryophytes that required microscopic observations could not be precisely identified. Species abbreviations and assignment of functional groups (Table S 3.1) were based on a guide for our study region (Blouin and Berger 2002) and taxonomic names were updated with the database Canadensys (www.canadensys.net). We selected the groups based on their function rather than their

taxonomical association. Thus, some groups were separated into more specific categories considering their differences in function. First, the "*Sphagnum*" group was not included in "Bryophytes" *sensu stricto* because it is frequently considered as an independent group based on their distinctive morphology, taxonomy and functional traits and because the presence of *Sphagnum* species could indicate paludification processes in black spruce stands with more humid conditions and lower decomposition rates (Fenton and Bergeron 2006, 2008). Secondly, "*Ericaceae"* was separated from "Shrubs" because they are indicating the acidic understory conditions characteristic of black spruce forests, while Herbs group included several other species present in trembling aspen forests. *Pteridophyta* has also different taxonomical and ecological functions than other herbs. Finally, "Trees" corresponded to seedlings or young plants that were separated from other species in the understory because of their different influence on the forest once they grow to maturity compared to shrubs.

1.4.5 Environmental variables

Abiotic factors such as temperature, soil moisture and overstory density were measured for each treatment. Temperature was monitored by using i-Buttons® (DS1922L), individually packed in 2 Ziploc bags for protection, installed in the buffer zone of every plot at 5-10 cm under surface soil. All 126 i-Buttons measured temperature every six hours, from October 2013 to August 2018. At the beginning of spring and summer, i-Buttons were collected to download data and confirm correct functioning, before being returned the following day. Soil moisture was measured with a sensor (FIELDSCOUT, TDR 300 soil moisture meter, Spectrum Technologies, Inc.) in four spots in the buffer zone of each plot (to get an average per plot), once per year during the vegetation sampling period in summer, after a 48 h period without rain to avoid a saturated relative humidity. Light was measured in August 2018 by the device Sunfleck Ceptometer (Decagon, USA) providing values in µmol m^2s^{-1} units. We also used a spherical convex

densiometer (No. 43887/8, Forestry Suppliers, Inc.) in 2017 to calculate the percentage of overstory density in the different plots.

The organic soil layer was thicker in black spruce (10 cm in average) than in trembling aspen forests (5 cm in average) and is underlain by a clay layer (representative of the clay belt of Québec and Ontario). Samples of the interface between the organic and the mineral soil horizons were sampled (just before the unmodified clay layer, with a consistent texture that sticks together) were taken during the same week as the light measurements (August 2018). From each treatment plot, four soil samples were taken leaving a buffer area and were combined in a plastic bag. Soils were sieved to 2 mm, dried out at room temperature, and stored in plastic bags for physicochemical analysis at the "Station de recherche agroalimentaire de l'Abitibi-Témiscamingue". Total carbon and nitrogen were obtained by total combustion and gas detection with a Thermal conductivity detector with the vario MAX cube analyzer. A dilution 1:1 V/V with water was used to calculate pH and the Shoemaker-McLean-Pratt (SMP) to calculate pH-buffer. Minerals were measured by the Mehlich III extraction method and analyzed with the ICP-OES (Inductively coupled plasma - optical emission spectrometry) and the cation exchange capacity (CEC) was calculated (CEC = meq / 100 g).

1.4.6 Statistical analysis

In this *in situ* study, we analysed how treatments of the ecosystem approach (changes in environmental parameters) and the community approach (shifts in understory communities between forest types) affected the diversity and community composition of understory plant species (vascular plants and bryophytes) in each forest type and how they changed through time (2013 to 2018), as well as the influence of local environmental conditions. All statistical analysis were performed using R software, version 3.6.0 (R Core Development Team 2019), and significant differences were defined with a $P < 0.05$. The understory vegetation was separated into the previously

described functional groups (Bryophytes, *Sphagnaceae*, *Ericaceae, Pteridophyta*, Graminoids, Herbs, and Shrubs, and Trees). We then presented the data of control plots in 2018 as boxplots for each functional group and forest type, using *ggplot2* package, version 3.3.5 (Wickham et al. 2016), including *post hoc* contrasts of the linear model of the functional group for each forest type separately, using the package *emmeans* version 1.6.2-1 (Lenth et al. 2021). Also, the average abundance per functional group was also calculated for the effect of the different treatments in 2018 (Table S 3.1).

1.4.6.1 Alpha diversity

Alpha diversity (local diversity) was calculated for each treatment in each forest type, from 2013 to 2018, using the Shannon index with the *vegan* package, version 2.5-7 (Oksanen et al. 2020). Differences in α-diversity among forest types, treatments and time were compared with a linear mixed effect model in which we included the interaction of Time, Forest type and Treatment, using Sites and Blocks as nested random factors with the *lme4* package, version 1.1-27.1 (Bates et al. 2015). All treatments were included together in the model to analyze the general trends of Time, forest type and treatment, but in the *post hoc* estimated marginal means comparisons, we only compared independently in each forest type and year, the Control versus each Treatment, with the *emmeans* package, version 1.6.2-1 (Lenth et al. 2021).

1.4.6.2 Partitioning beta diversity

Beta diversity (among-sample differentiation) is the variation in species composition among sites in an area of interest (Legendre and Legendre 2012). A non-directional approach was used to study the variation in understory community composition from the different treatments under each forest type. As explained in Legendre and De Cáceres (2013), the Hellinger-transformed data was used to calculate the total sum-of-squares (SS_{total}) in the community composition table (Euclidean distance) and the total β-diversity (BD_{total}) with values between zero (all sites with exact same species

composition) and one (all sites have completely different species composition). BD_{total} is obtained by dividing SS_{total} by n–1 (Legendre and De Cáceres 2013). Also, SS_{total} can be partitioned into contributions of single sites (LCBD = Local Contributions to Beta Diversity) and contributions of individual species (SCBD = Species Contributions to Beta Diversity). Calculating LCBD values, we analyzed the relative contribution (ecological uniqueness) of a plot to β-diversity in terms of community composition and it was visually presented for each forest type and each approach. The LCBD values are also specified using the $P_{adjusted}$. Likewise, with SCBD values we get the contribution of a species to the overall β-diversity in the data set. Therefore, we examined the local and species contributions to the overall β-diversity of the understory vegetation of the study, using the last year data in 2018 to have the cumulative effect of a five years of treatment conditions. This analysis was carried out using the *adespatial* package, version 0.3-14 (Dray et al. 2021).

1.4.6.3 Species turnover (TBI analysis)

Understory species turnover was also evaluated along a temporal gradient by comparing 2013 and 2018 data using the Temporal Beta diversity Index (TBI). Temporal β-diversity is defined as the variation in community composition across time (species turnover) and is based on the statistical method described in Legendre (2019) and using the *adespatial* package, version 0.3-14 (Dray et al. 2021). The TBI was computed for each treatment-plot, to measure the change in understory species composition along a temporal gradient (between the first survey in 2013 and second in 2018), using the percentage difference $D_{\%diff}$ (also known as Bray-Curtis) as dissimilarity coefficient for species abundance. The objective of the TBI analysis was to identify sites where understory species abundance changed in exceptional ways from 2013 to 2018. TBI dissimilarities were partitioned into losses (B) and gains (C) of species from 2013 to 2018 independently for each site. Then, B-C plots were created based on the scaled B and C, to illustrate if temporal changes at the studied sites are

dominated by species abundance losses or gains, for all treatments in each forest type at the interval from 2013 to 2018 (Legendre 2019). Treatments of both the ecosystem and community approach were grouped together to facilitate the analysis of species abundance loss and gains from all sites and visually compare each treatment to the controls. The summary statistics was based on the mean of each component and represent the overall change across all sites. Finally, a test of significance (paired t-test) of the difference between the vectors of species gains and losses was used to know which sites were TBI-significant, based on the P-adjusted for multiple tests (Holm correction, $P_{adj} < 0.05$).

1.4.6.4 Community composition analysis

The understory plant communities were analyzed both at the species level and using eight functional groups: Bryophytes, *Sphagnaceae*, *Ericaceae, Pteridophyta*, Graminoids, Herbs, and Shrubs, and Trees. To illustrate how understory communities were affected by treatments in both forest types, we carried out a principal component analysis (PCA) ordination (Euclidean distance) for both the ecosystem approach (treatments 1F, 2F, Li, Nu and C) and the community approach (treatments To, Ti and C). The ordinations were based on Hellinger transformed data of species composition calculated with the *prcomp* function of the *Stats* package (R Core Development Team 2019). To highlight the changes from 2013 to 2018, the centroids of each year from the Site/Block nested design for each treatment in each forest type were illustrated in the ordination. The most influential understory species were shown with the *factoextra* package (Kassambara and Mundt 2020). Furthermore, to test for significant differences among factors (Forest type, Year, Treatment) and their interactions, a PERMANOVA analysis carried out using with the *adonis2* function of the *vegan* package (Oksanen et al. 2020), using Site and Block as random factors.

We analyzed the community composition separately for each forest type for each approach to observe differences of treatments compared to the controls. We performed

a Principal Component Analysis (PCoA) of the understory species abundance in 2013 and 2018 based on the Bray-Curtis distance with the Cailliez correction to correct negative eigenvalues (Cailliez 1983) with *Stats* package (R Core Development Team 2019). The ordination figures were constructed using the *vegan* package (Oksanen et al. 2020). A separate PERMANOVA was carried out to analyze differences in species composition between the Treatment and Year for each approach within each forest type. These PERMANOVAs used Bray-Curtis distance of understory vegetation abundance, using Site and Block as random factors, with the *vegan* package (Oksanen et al. 2020). With the same vegan package, we also analyzed the multivariate homogeneity of group dispersions (variances) with *betadisper* function based on Bray-Curtis distances and applied a permutational ANOVA (PERMUTEST), including Sites and Blocks as random factors to obtain *post hoc* contrasts between groups in each forest type and over time. Finally, changes in each functional group's abundance through time were plotted using the *ggplot2* package (Wickham et al. 2016), using the linear model method with a smoothing parameter (cubic polynomial), for each treatment of the ecosystem and the community approach.

1.4.6.5 Environmental data analysis

We calculated the average and standard deviation per forest type of the local environmental conditions (Overstory density, light inputs, soil moisture and soil temperature). We compared each environmental variable between forest types, treatments, and the interaction of both, using Site as a random factor with linear mixed-effect ANOVA with the *lme4* package, version 1.1-27.1 (Bates et al. 2015) and the *Stats* package (R Core Development Team 2019), respectively. The, we used the estimated marginal means (*emmeans,* $P < 0.05$) using the package *emmeans* version 1.6.2-1 (Lenth et al. 2021), based on the previous model for *post hoc* comparisons for each forest type separately. Likewise, the treatments of *Single-litter, Transplants-in, Transplants-out and Control* were chosen to analyze soil physicochemical properties

in 2018, as they correspond to another part of the project to further analyze soil microbial communities in those contrasting treatments (Rodríguez-Rodríguez et al. 2022b). The same statistical analyses were applied for soil physicochemical properties as for the local environmental conditions.

1.5 Results

1.5.1 General understory plant species and local environmental conditions

We found a minimum of 65 plant taxa in our plots separated to the different functional groups: Bryophytes (8), *Sphagnaceae* (1, non-identified *Sphagnum* species), *Ericaceae* (5), *Pteridophyta* (7), Graminoids (2) Herbs (27) and Shrubs (11), and Trees (4, mostly tree seedlings) (Table S 3.1). The average abundance of functional groups differed between forest types in the *Control* plots in 2018 (Figure 1.2). In control conditions, the dominant functional groups in black spruce forests are bryophytes, forming a thick layer of feather mosses (*Pleurozium schreberi* and *Ptilium crista-castrensis)*, ericaceous plants, *Equisetum pratense* and some small herbs such as *Cornus canadensis, Carex* spp. and *Linnaea borealis*. In contrast, the aspen understories were dominated by a variety of herbs, pteridophytes and shrubs in the understory, such as *Clintonia borealis, Rubus pubescens, Spinulum annotinum* and *Aralia nudicaulis*.

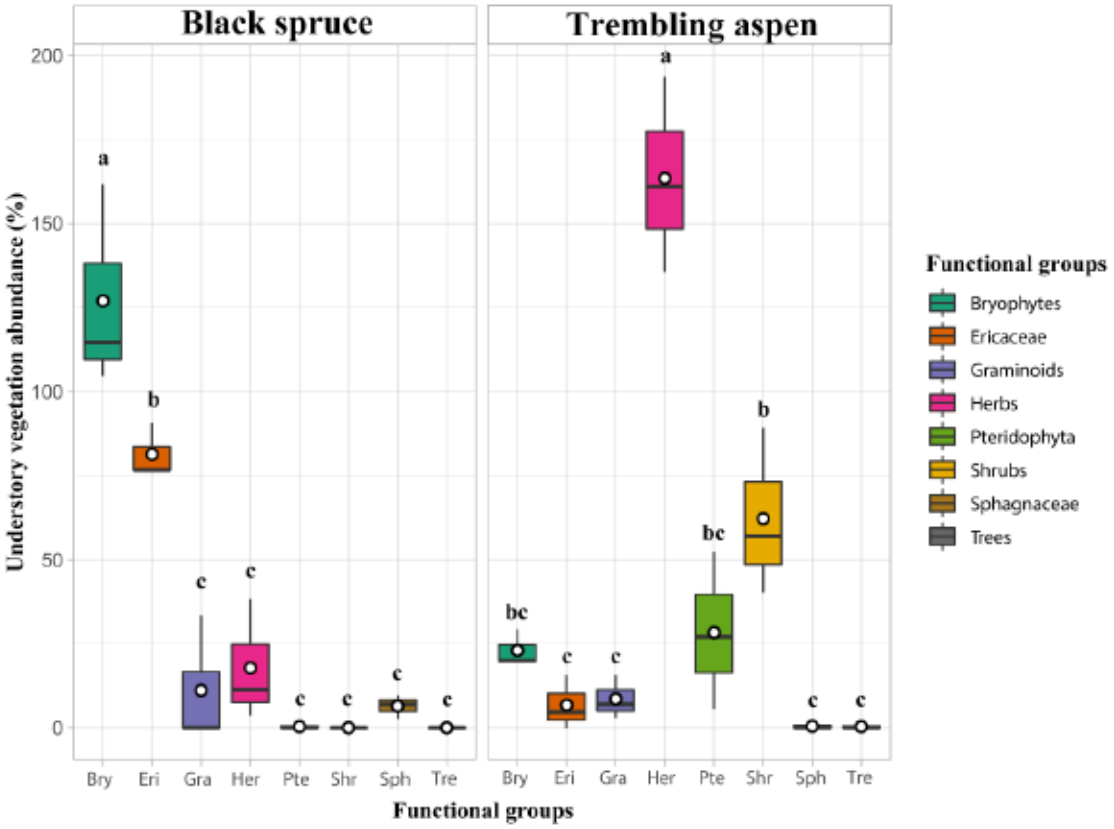


Figure 1.2 Abundance of understory vegetation of black spruce and trembling aspen forests divided into functional groups: Bryophytes (turquoise), *Ericaceae* (orange), Graminoids (violet), Herbs (pink), *Pteridophyta* (green), Shrubs (yellow), *Sphagnaceae* (brown) and Trees (gray). Boxplots include the median (black horizontal lines) and mean (white circles) of sites for each functional group in control plots in 2018, among forest types. Letters correspond to contrasts of means between functional groups per forest type (*emmeans*, $P < 0.05$).

Local environmental conditions differed among forest types, treatments, and their interaction (Table 1.1). This was the case for overstory density (ANOVA, interaction $F_{6,110} = 5.56$, $P < 0.0001$) and available light (ANOVA, interaction $F_{6,110} = 3.812$, $P < 0.002$), both of which where generally higher in black spruce than in trembling aspen forests and were significantly different compared to the *Control* (*emmeans*, $P < 0.05$, Table 1.1). Soil moisture was significantly higher in black spruce than in trembling aspen forests (ANOVA, $F_{1,110} = 4.242$, $P = 0.042$) and treatments (ANOVA, $F_{6,110} = 2.253$, $P = 0.043$), but their interaction was not significant. However, *post hoc* contrasts

comparing of soil moisture in treatments to the *Contol* were not significantly different (*emmeans*, $P < 0.05$, Table 1.1). There were no significant differences in soil temperature in any of the comparisons (ANOVA, $P > 0.05$). Finally, the analysis of soil physicochemical properties in the selected treatment plots demonstrated that several elements were significantly different between forest types (Table 1.2, in orange), but in each forest type, treatments were not significantly different compared to the control.

Table 1.1 Local environmental conditions between treatments and forest types in 2018. *Notes:* Local environmental conditions (Overstory density, available light, soil moisture and soil temperature) for each forest type (Black spruce – BS, Trembling aspen - TA) and Treatments: Control conditions (C, in grey), Light (Li, in yellow), Nutrients (Nu, in purple), Single-litter (1F, in orange), Double-litter (2F, in red), Transplants-out (To, in blue), Transplants-in (Ti, in green). Data represent the average and standard deviation for sites and blocks in 2018. Differences between the *Control* to each Treatment were calculated per Forest type (letters), based on the estimated marginal means (*emmeans*) of the linear mixed-effect model using Site and Block as nested random factors ($P < 0.05$). VWC (Volumetric water content). Notice that the *Light* values in this table correspond to the treatment-plots in black spruce placed in more canopy-opened areas, whereas the *Light* treatment-plots in trembling aspen had placed a shade cover over the plot (light inputs in trembling aspen plots without the shade cover was 51 µmol m^2s^{-1} ± 29 SD, on average).

Canopy	Treatment	Overstory Density		Light (µmol m^2s^{-1})		Soil Humidity (VWC)		Soil Temperature (°C)	
BS	C	94.16 4.04	b	51.53 25.42	a	15.26 9.09	a	10.88 0.59	a
	1F	94.66 2.15	b	46.50 13.50	a	13.00 5.24	a	10.92 0.57	a
	2F	95.55 1.77	b	49.67 21.46	a	12.76 7.66	a	10.96 0.47	a
	Li	86.05 8.34	a	70.81 37.10	a	21.55 11.98	a	10.64 0.57	a
	Nu	94.94 2.70	b	49.58 17.46	a	11.21 5.07	a	10.97 0.50	a
	Ti	95.35 1.96	b	48.56 17.71	a	16.31 10.77	a	10.75 0.68	a
	To	95.29 2.65	b	48.64 16.43	a	15.88 11.03	a	11.30 0.68	a
TA	C	94.71 2.76	a	47.72 16.12	b	12.41 3.10	a	11.19 0.91	a
	1F	95.72 2.38	a	45.83 22.07	b	10.36 2.19	a	10.99 0.64	a
	2F	94.77 3.13	a	47.67 28.89	b	10.26 3.62	a	11.05 0.70	a
	Li	96.65 2.46	a	20.56 10.97	a	13.22 2.29	a	10.87 1.03	a
	Nu	95.52 3.56	a	44.25 23.55	b	12.23 3.34	a	10.87 0.76	a
	Ti	96.24 2.35	a	47.64 28.12	b	14.57 3.57	a	10.84 1.22	a
	To	95.29 3.76	a	47.39 19.16	b	15.74 5.24	a	10.79 1.09	a

Table 1.2 Soil physicochemical properties between treatments and forest types in 2018. *Notes:* Soil physicochemical properties for each forest type (Black spruce – BS, Trembling aspen - TA) and treatment: *Control* conditions (C, in grey), *Single-litter* (1F, in orange), *Transplants-in* (Ti, in green) and *Transplants-out* (To, in blue). Data represent the average and standard deviation (in grey numbers) for sites and blocks in 2018. Variables: pH 1 (1:1 refers to V/V dilution with water), pH 2 (pH-buffer based on the Shoemaker-McLean-Pratt method - SMP), elements N, C, K, Mg, Ca, Na and H as percentage, CEC (Cation Exchange Capacity in meq*100 g^{-1}), ratios of C:N, N:P and P:Al, elements P, Al, Mn, Fe and S in mg g^{-1} units. Differences among forest types and treatments were obtained with a linear mixed-effect ANOVA using Site and Block as nested random factor ($P < 0.05$). *Significant differences between forest types (no significant differences between treatments for any variable).

		Soil pH		Soil chemical properties															
		pH 1*	pH 2	N*	C*	K	Mg	Ca*	Na*	H*	CEC*	C:N*	N:P*	P:Al*	P:Ca	P*	Al*	Mn*	Fe*	S*
BS	C	4.06	4.94	0.79	26.18	2.14	10.21	19.23	0.47	0.68	38.29	34.18	331.06	0.02	0.08	0.03	1.76	0.02	0.46	0.03
		0.66	0.56	0.16	3.76	0.58	6.05	16.56	0.14	0.23	8.95	8.20	167.61	0.01	0.11	0.01	0.41	0.02	0.06	0.01
	1F	4.01	4.98	0.72	24.15	2.27	9.93	18.15	0.43	0.69	37.97	35.26	267.59	0.02	0.09	0.03	1.70	0.02	0.44	0.03
		0.59	0.60	0.28	7.70	1.05	7.49	18.14	0.16	0.25	11.17	8.10	166.45	0.02	0.12	0.01	0.46	0.01	0.07	0.01
	Ti	4.01	4.93	0.78	23.71	2.25	9.83	18.72	0.43	0.69	37.44	32.30	342.05	0.02	0.04	0.03	1.72	0.03	0.43	0.03
		0.38	0.44	0.23	4.67	0.82	4.50	13.04	0.19	0.17	9.09	9.26	244.68	0.01	0.05	0.01	0.37	0.03	0.06	0.01
	To	4.02	4.99	0.78	25.43	2.42	10.80	18.84	0.45	0.68	37.40	33.11	287.68	0.02	0.03	0.03	1.70	0.02	0.45	0.03
		0.48	0.39	0.19	6.36	0.93	5.46	12.42	0.15	0.18	8.98	6.94	166.34	0.02	0.02	0.01	0.37	0.01	0.07	0.01
TA	C	4.52	5.31	0.61	15.38	2.20	10.10	30.36	0.26	0.57	37.09	26.11	218.37	0.04	0.04	0.05	1.29	0.05	0.34	0.03
		0.41	0.35	0.17	3.31	0.49	4.76	11.73	0.05	0.17	7.96	5.87	163.53	0.03	0.05	0.04	0.16	0.04	0.06	0.01
	1F	4.55	5.55	0.58	13.53	2.28	11.60	34.55	0.28	0.51	36.32	23.77	221.55	0.04	0.03	0.04	1.20	0.05	0.33	0.03
		0.33	0.27	0.23	6.03	0.35	5.01	10.27	0.07	0.15	9.94	6.36	142.77	0.03	0.04	0.03	0.22	0.03	0.05	0.01
	Ti	4.58	5.38	0.55	12.16	2.36	10.02	30.16	0.28	0.57	35.55	22.54	213.44	0.02	0.02	0.03	1.37	0.06	0.33	0.02
		0.28	0.43	0.21	4.11	0.78	4.33	11.65	0.09	0.16	11.06	2.06	118.81	0.01	0.02	0.01	0.27	0.04	0.04	0.01
	To	4.63	5.41	0.51	12.45	2.38	10.08	27.84	0.29	0.60	34.10	24.08	228.45	0.03	0.05	0.04	1.36	0.04	0.34	0.02
		0.28	0.39	0.16	4.19	0.60	4.00	10.17	0.04	0.14	11.09	2.88	145.71	0.03	0.10	0.03	0.13	0.03	0.05	0.01

1.5.2 Alpha diversity of understory communities

Alpha diversity (Shannon index) varied over time between forest types and treatments (Figure 1.3, Table S 3.2). We found a significant interaction in α-diversity between Time, Forest type and Treatment (ANOVA, $F_{30,664} = 1.565$, $P < 0.0291$, Table S 3.2). The significant interaction term was driven by the *Transplant-out* plots that were significantly more diverse every year in the black spruce stands compared to the *Control* (*emmeans*, P < 0.0001, Table S 3.3). In aspen stands the *Transplant-out* plots were significantly less diverse in 2013 but these plots gradually became similar to the other plots over time. Other than this interaction, α-diversity was lower in black spruce stands than in trembling aspen stands. The control of the transplantation (*Transplants-in)* was not significantly different from the *Control* in any year (Table S 3.3) in black spruce stands. All other treatments did not significantly differ from the *Control*, except *Single-litter* in 2015 (*emmeans*, P < 0.0319, Table S 3.3) and *Nutrients* in 2017 (*emmeans*, P < 0.0264, Table S 3.3), but in no other year, indicating no general trend of effects of changes in light, litter or nutrients in plant understory alpha diversity. In trembling aspen forests, no other treatment was significantly different from the control, showing no effect on plant understory alpha diversity of changes in light, litter or nutrient conditions in trembling aspen forests.

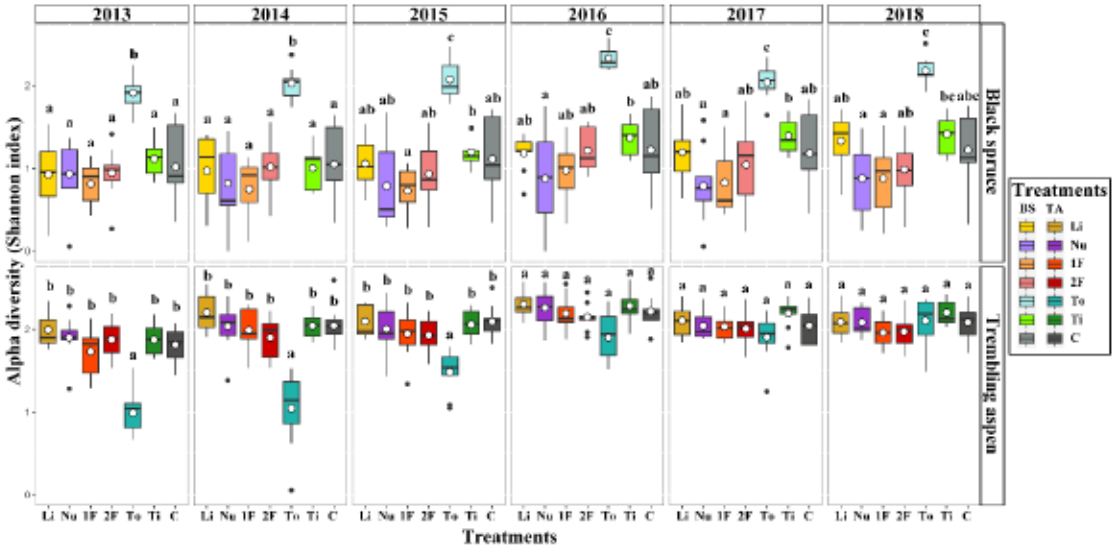


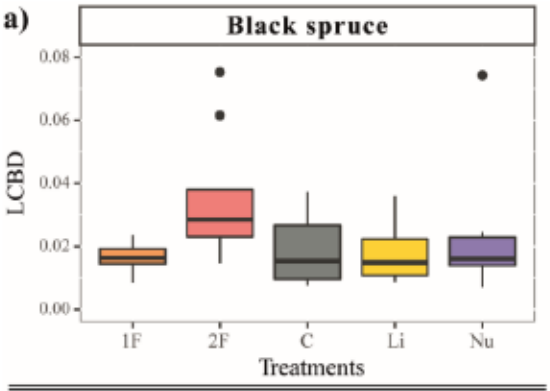
Figure 1.3 Alpha diversity (Shannon index) of understory vegetation in 2018 among treatments in each forest type (Black spruce – BS and Trembling aspen – TA), over time (2013-2018) from the nested Site/Block experimental design. Treatments correspond to *Light* (Li, in yellow), *Nutrients* (Nu, in purple), *Single-litter* (1F, in orange) and *Double-litter* (2F, in red), *Transplants-out* (To, in blue), *Transplants-in* (Ti, in green) and *Control* conditions (C, in grey). Treatments in black spruce forests in light colors and in trembling aspen forests in dark colors. Plot-level alpha diversity represented in the boxplots include the median (black horizontal lines), the mean (white cilcles), and the *emmeans* contrasts (in letters, $P < 0.05$), comparing control versus each treatment in each Forest type and per Year (Table S 3.3).

1.5.3 Partitioning beta diversity of understory communities

We analysed changes in β-diversity of understory composition among treatments in each forest type by partitioning β-diversity (Figure 1.4). Total β-diversity (BD_{total}) was 0.4 in black spruce and 0.5 in trembling aspen forests, but they are not directly comparable because these were calculated independently for each forest type. The total sum-of-squares (SS_{total}) statistic was partitioned into local contributions to β-diversity (LCBD) of single plots and to species contributions of β-diversity (SCBD), for each forest type for the ecosystem (Figure 1.4-a,b) and community approaches (Figure 1.4-c,d). The larger the LCBD values, the stronger the difference in species composition (high uniqueness) at those plots (Legendre and De Cáceres 2013). Hence, plots from

the ecosystem approach in black spruce forests (Figure 1.4-a), corresponding to two plots of *Double-litter* and *Nutrient* treatment, have a unique community composition that significantly affected the mean β-diversity ($P_{adj} < 0.05$). However, in the case of *Double-litter* treatment, the high LCBD value corresponded to a small number of species as litter reduced the abundance of mosses over time (Figure S 3.2). No other plot in any of the approaches or forest types was significantly unique according to the $P_{adj} < 0.05$. Finally, the species that contribute the most to β-diversity according to the SCBD values are presented in a decreasing order of contribution, for forests for both approaches in black spruce (Figure 1.4-a,d) and trembling aspen (Figure 1.4-b,c), with the corresponding abbreviations of understory plant species (Table S 3.1).

a)



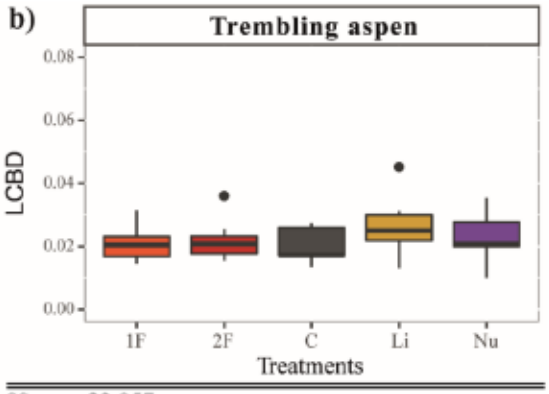
SS_{total} = 18.129

BD_{total} = 0.412

SCBD

Pleurozium schreberi, Ptilium crista-castrensis, Glaultheria hispidula, Cornus canadensis, Carex spp., Equisetum pratense, Ptilidium ciliare, Rhododendron groenlandicum, Polytrichum commune, Vaccinum angustifolium, Sphagnum spp., Vaccinum myrtilloides, Maianthemum trifolium, Dicranum polysetum.

b)



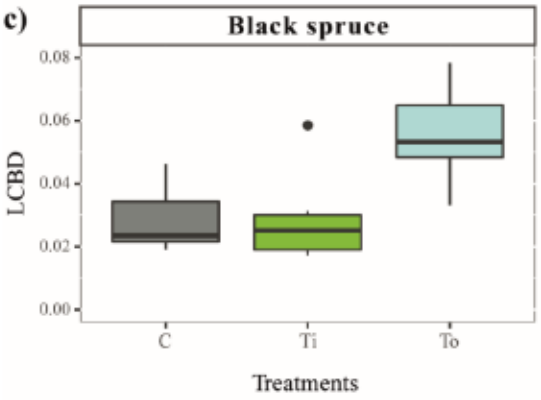
SS_{total} = 22.957

BD_{total} = 0.522

SCBD

Cornus canadensis, Spinulum annotinum, Rubus pubescens, Clintonia borealis, Aralia nudicaulis, Viburnum edule, Vaccinum myrtilloides, Maianthemum canadense, Linnaea borealis, Poa spp., Oxalis montana, Viola spp., Pleurozium schreberi, Vaccinum angustifolium, Ribes glandulosum, Galium asprellum, Coptis trifolia, Ptilium crista-castrensis, Petasites frigidus var. palmatus, Fragaria spp.

c)



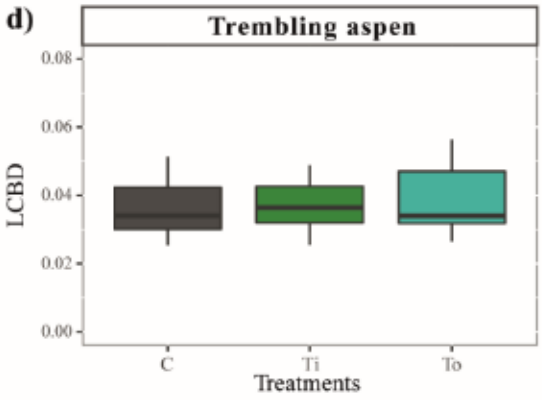
SS_{total} = 13,941

BD_{total} = 0,536

SCBD

Glaultheria hispidula, Pleurozium schreberi, Ptilium crista-castrensis, Cornus canadensis, Rubus pubescens, Viola spp., Carex spp., Linnaea borealis, Poa spp., Polytrichum commune, Vaccinum myrtilloides, Maianthemum canadense, Dicranum polysetum, Spinulum annotinum, Sphagnum spp., Leucanthemum vulgare, Ptilidium ciliare, Oxalis montana.

d)



SS_{total} = 12,909

BD_{total} = 0,496

SCBD

Rubus pubescens, Spinulum annotinum, Clintonia borealis, Glaultheria hispidula, Linnaea borealis, Pleurozium schreberi, Ptilium crista-castrensis, Cornus canadensis, Poa spp., Viola spp., Aralia nudicaulis, Carex spp., Viburnum edule, Maianthemum canadense, Petasites frigidus var. palmatus, Rubus ideaeus, Ribes glandulosum, Oxalis montana, Vaccinum myrtilloides, Populus tremuloides, Galium asprellum, Lysimachia borealis, Coptis trifolia, Plagiomnium spp., Ptilidium ciliare.

Figure 1.4 Partitioning of β-diversity into local contributions of single plots (LCBD = Local Contributions to Beta Diversity) for treatments in each forest type (Black spruce and Trembling aspen) of (a, b) the ecosystem approach corresponding to *Light* (Li, in yellow), *Nutrients* (Nu, in purple), *Single-litter* (1F, in orange) and *Double-litter* (2F, in red), and for (c, d) the community approach corresponding to *Transplants-out* (To, in blue), *Transplants-in* (Ti, in green), as well as *Control* conditions (C, in grey) for both approaches. Treatments in black spruce forests in light colors and in trembling aspen forests in dark colors. Tables below each figure present the total sum-of-squares (SS_{total}), total β-diversity (BD_{total}) and the contributions (decreasing order) of individual species (SCBD = Species Contributions to Beta Diversity), for each forest type in each approach. Data correspond to 2018, n = 9, from the combination of Site (A, B, C) and Blocks (1, 2, 3) in each forest type.

1.5.4 Species turnover (TBI analysis)

The turnover of the understory community composition was analyzed by the temporal β-diversity index (TBI) to observe changes in understory community composition along a temporal gradient (2013 and 2018). The B-C plots in black spruce forests (Figure 1.5-a) exhibited a non-significant negative change of the average of species abundance losses (green line over red line) (t-test, t-value: -0.988, $P = 0.325$), given the variability of species gains and losses across the different treatments. In contrast, trembling aspen forests (Figure 1.5-b) had on average species abundance gains (red line over green line) with a significant positive change (t-test, t-value: 9.935, $P < 0.0001$), meaning that most of the treatments gained in species abundance over time.

In black spruce forests, the treatments *Single-litter* and *Double-litter* resulted in species losses for all plots with a high temporal β-diversity, whereas the *Nutrient* treatments followed the same trend of species losses but with more variability between plots and a low temporal β-diversity. In contrast, *Light* and *Control* resulted in species gains with a high variability between plots and a low temporal β-diversity, whereas *Transplants-out* and *Transplants-in* treatments resulted in species gains for all plots with a high temporal β-diversity. In trembling aspen forests, most plots gained species in average, and only seven plots (corresponding to some treatments from the ecosystem approach) presented species losses. Therefore, changes in species abundance through time in the

ecosystem approach varied in response to treatments in both forest types (albeit particularly in black spruce forests), whereas increases in species abundance were observed in both forest types for the treatments of the community approach.

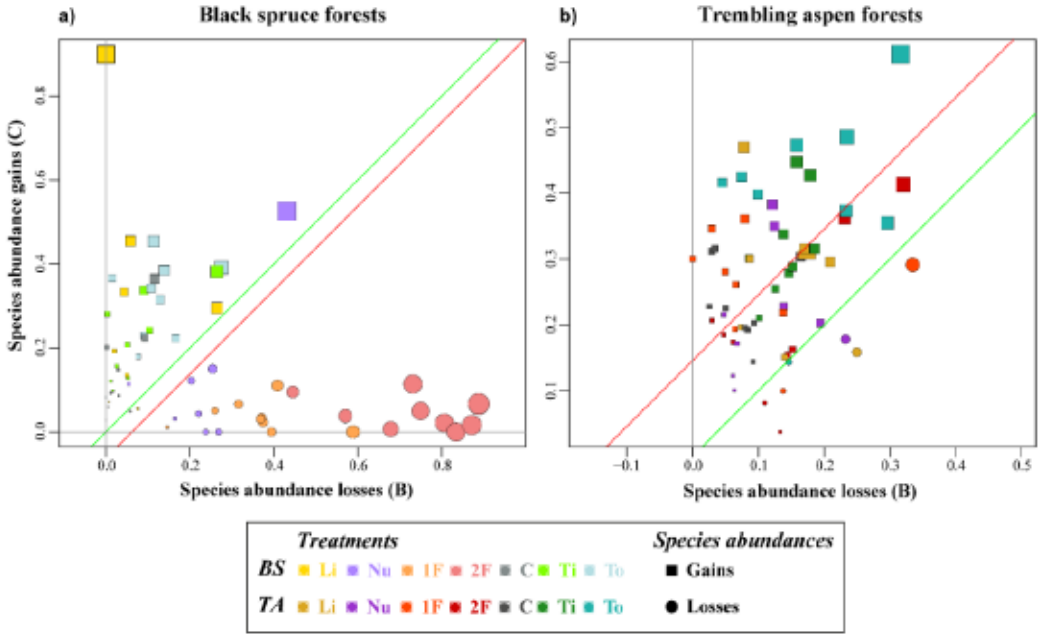


Figure 1.5 Analysis of Temporal Beta-diversity Index (TBI) of understory communities in black spruce (left panel) and trembling aspen (right panel) stands. Figures correspond to B-C plots of understory vegetation comparing all treatments from 2013 to 2018, where 63 plots per forest type were plotted using losses (B/den statistics) and gains (C/den statistics) computed from the abundance data of understory species. These sites correspond to the combination of sampling design of Sites (A,B,C), Blocks (1, 2, 3) and seven Treatments: *Control* conditions (C, in grey), to treatments of the ecosystem approach: *Light* (Li, in yellow), *Nutrients* (Nu, in purple), *Single-litter* (1F, in orange) and *Double-litter* (2F, in red), and of the community approach: *Transplants-out* (To, in blue), *Transplants-in* (Ti, in green). Treatments in black spruce forests in light colors and in trembling aspen forests in dark colors. Notice also that axes are different to allow clearer data visualisation. The position of green line respective to red line indicates an average of species losses (green line above red line, as in left panel) or species gains (green line below red line, as in right panel) across the sites. Distinctive symbols are used for the sites dominated by gains (squares) and by losses (circles). Symbols sizes represent the values of the D = (B+C) statistics: larger points found in the upper-right corner of each plot represent a higher temporal β-diversity than the smaller points in the lower-left corner.

1.5.5 Understory community composition

The ecosystem approach ordination (Figure 1.6-a), clearly separated both forest types along the first axis (38 % of variance), while the second axis is explained by changes over time (2013-2018) for the different treatments (10 % of variance), as the interaction of Time and Forest type was significant (PERMANOVA, $R^2 = 0.009$, $F_{1,179} = 2.274$, $P = 0.038$, Table S 3.4). These results show that understory vegetation differed between forest types and changed over time. Treatments in black spruce forests (on the left side of the ordination) show a correlation with the typical understory species of these stands, such as *Pleurozium schreberi* (PLS), *Gaultheria hispidula* (GAH), *Sphagnum* spp. (SPS), *Ptilium crista-castrensis* (PTC), *Rhododendron groenlandicum* (LEG), *Vaccinium myrtilloides* (VAM) and *Equisetum pratense* (EQP). Understory communities were affected by *Double-leaves* over time associated with a change along the first axis. In contrast, treatments in trembling aspen forests (on the right side of the ordination) where associated with dominant herbs and shrubs in the understory, such as *Rubus pubescens* (RUB), *Spinulum annotinum* (LYA), *Clintonia borealis* (CLB), *Viburnum edule* (VIE), *Aralia nudicaulis* (ARN), *Ribes glandulosum* (RIG), *Alnus incana subsp. rugosa* (AUR) and *Poa* spp. (POA). The *Light* treatment showed the most variation over time along the second axis.

In the community approach ordination (Figure 1.6-b), forest types separated along the first axis (38 % of variance), while the second axis was explained by changes over time for the different treatments (9 % of variance). Significant differences were found for the interaction between Year, Forest type and Treatment (PERMANOVA, $R^2 = 0.034$, $F_{2,107} = 2.763$, $P = 0.004$, Table S 3.4), indicating that treatments differed over time in their effects on community composition depending on the forest type. The understory vegetation of the *Transplants-out* treatment transplanted from black spruce to trembling aspen stands, was initially dominated by feather mosses, such as *Pleurozium schreberi* (PLS) and *Ptilium crista-castrensis* (PTC), as well as ericaceous plants, such

as *Gaultheria hispidula* (GAH). Over time, understory vegetation shifted to more resemble that of the host trembling aspen forest (right side of the ordination). In contrast, in 2013, the *Transplants-out* treatment (from trembling aspen to black spruce forests), presented a diverse understory vegetation typical of trembling aspen forests and remained similar until 2018. The transplantation controls (*Transplants-in* treatments) from each forest type, indicated that the transplantation itself did not affect understory vegetation composition, because their understory composition was similar to their *Control* in each forest type.

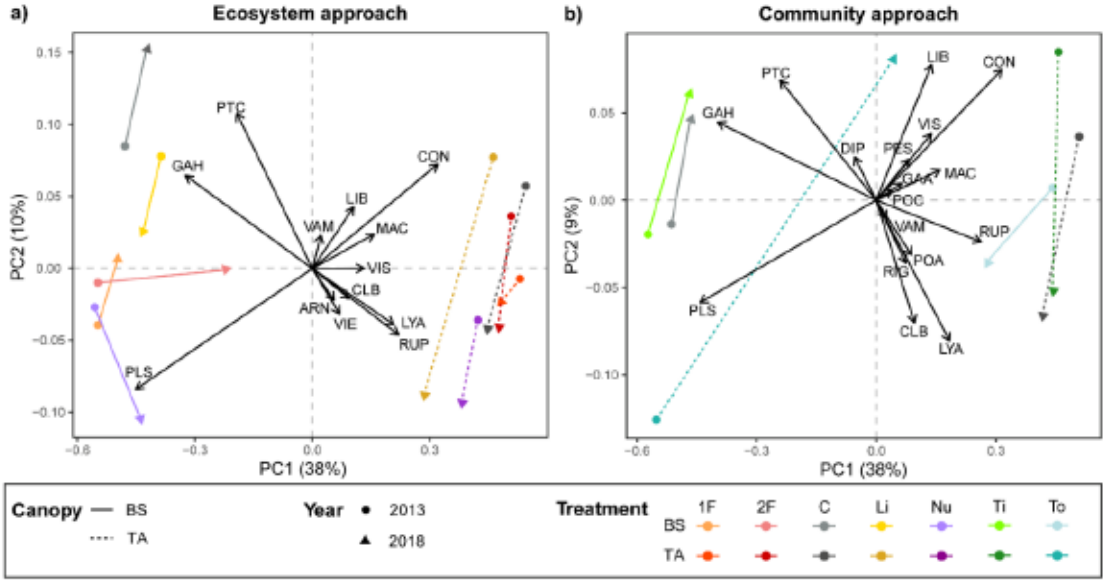


Figure 1.6 Principal Component Analysis (PCA) for the Objective 1 (a - Ecosystem approach) and the Objective 2 (b - Community approach) illustrating community change between 2013 and 2018. Points correspond to the centroids per treatment (means of 9 replicate plots from nested design of 3 Sites x 3 Blocks) of the Hellinger-transformed abundance of understory species from the beginning (2013, circles) and the end of the experiment (2018, triangles), united by lines indicating change over time. Solid lines correspond to black spruce stands (BS) and dotted lines correspond to trembling aspen stands (TA). Colors correspond to treatments of the ecosystem approach: *Single-litter* (1F, in orange), *Double-litter* (2F, in red), *Control* (C, in grey), *Light* (Li, in yellow) and *Nutrients* (Nu, in purple) and of treatments of the community approach: *Control* (C, in grey), *Transplants-in* (Ti, in green) and *Transplants-out* (To, in blue). Treatments in black spruce forests in light colors and in trembling aspen

forests in dark colors. Species ordination is in the middle of the panel, with only the main species to allow a clear visualization. Notice that the scales are different.

The abundance of the different functional groups (Bryophytes, *Sphagnaceae*, *Ericaceae*, *Pteridophyta,* Graminoids, Herbs, Shrubs and Trees, in Table S 3.1) in the different treatments of both the ecosystem and community approaches changed during the five years of the experiment (Figure S 3.2, Figure S 3.3, respectively). In the ecosystem approach, in black spruce forests, the largest changes in abundance were found for bryophytes and ericaceous plants in the *Litter* (1F and 2F) and *Nutrients* treatments. In trembling aspen forests, only the *Light* treatment resulted in a slight decrease in *Pteridophyta* and Shrub abundance and a slight increase in Bryophyte abundance. In the community approach, the *Transplants-out* treatments resulted in the greatest change in abundance in both forests compared to their controls (C and Ti).

As black spruce and trembling aspen forests formed distinctive groups in the general ordination (Figure 1.6), we further analyzed their 2013 and 2018 understory community composition, separately using the ecosystem (Figure 1.7-a,b) and community (Figure 1.7-c,d) approaches over time. Factors of Treatment, Year and their interaction were tested (Table 1.3), as well as the differences of their variances (PERMUTEST, *betadisper*, $P < 0.05$, (Table 1.4). The understory species that significantly influenced the community composition are represented (Envfit, $P < 0.05$). In the ecosystem approach in black spruce forests, the interaction between Treatment and Year was significant (PERMANOVA, $R^2 = 0.108$, $F_{1,89} = 2.975$, $P = 0.0001$, Table 1.3). *Single-litter* and *Double-litter* treatments differed the most among all treatments, compared to the *Control*, but only the variances of *Double-litter* differed significantly between Year and compared to the *Control* (PERMUTEST, *betadisper,* $P < 0.05$, (Table 1.4). In trembling aspen forests, Treatment had a significant effect (PERMANOVA, $R^2 = 0.042$, $F_{1,89} = 0.905$, $P = 0.0131$, Table 1.3) on the community composition, as well as Year (PERMANOVA, $R^2 = 0.028$, $F_{1,89} = 2.496$, $P = 0.0026$,

Table 1.3), but not their interaction. However, no contrasts among treatments were significantly different (PERMUTEST, *betadisper,* $P > 0.05$, Table 1.4). Thus, the community composition changed over time and among treatments, but not significantly when comparing among groups of treatments.

For the community approach in black spruce forests, only Treatment was significant (PERMANOVA, $R^2 = 0.433$, $F_{1,53} = 19.971$, $P < 0.0001$, Table 1.3), as the transplantations from trembling aspen forests remained similar after five years in black spruce forests, *Transplants-out* being significantly different compared to the *Control* and *Transplants-in* in both 2013 and 2018 (PERMUTEST, *betadisper,* $P < 0.05$, Table 1.4), whereas both controls (C and Ti) were not significantly different with each other, indicating no effect of the transplantation itself (PERMUTEST, *betadisper,* $P > 0.05$, Table 1.4). In contrast, in the community approach in trembling aspen forests, the interaction of both Year and Treatment was significant (PERMANOVA, $R^2 = 0.079$, $F_{1,53} = 3.221$, $P = 0.0025$, Table 1.3) indicating that the effect of the treatments changed over time, particularly for the transplantations from black spruce to trembling aspen stands, which were significantly different in 2013 compared to both *Control* and *Transplants-in* (PERMUTEST, *betadisper,* $P < 0.05$, Table 1.4), but not significantly different in 2018 (PERMUTEST, *betadisper,* $P > 0.05$, Table 1.4).

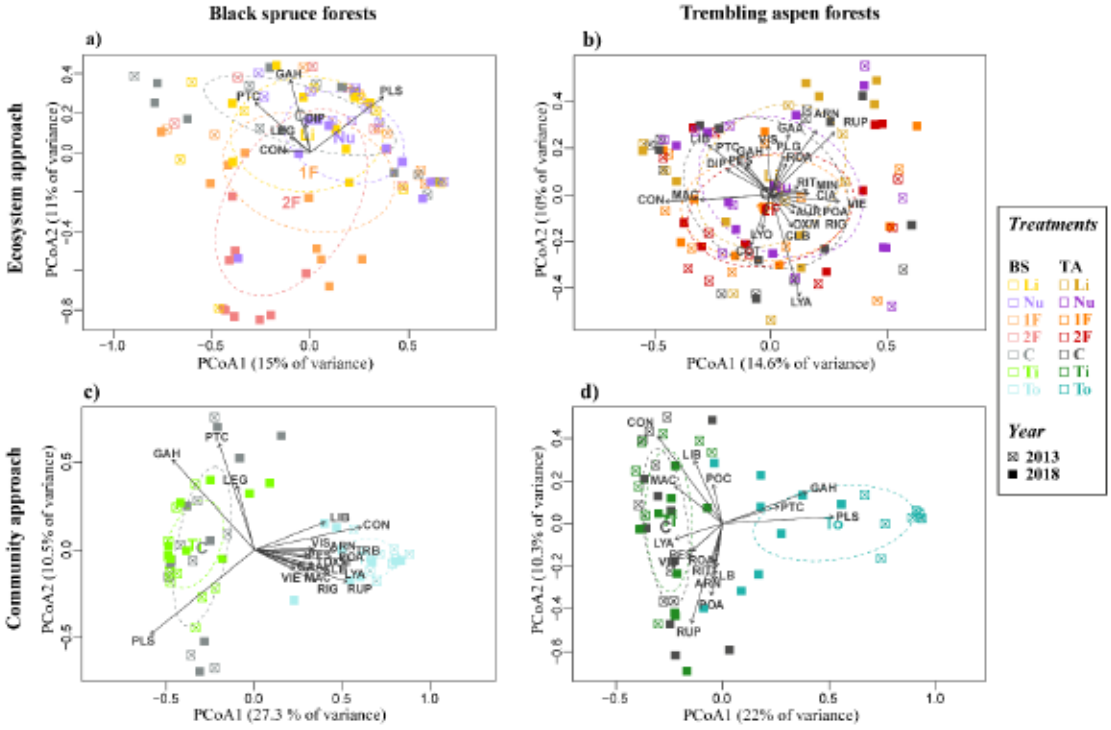


Figure 1.7 Principal Coordinate Analysis (PCoA) based on the Bray-Curtis distance with Cailliez correction of the understory species abundance in 2013 (crossed squares) and 2018 (filled squares), with their corresponding percentage of variances in each axis. Treatments of the ecosystem approach in black spruce (a) and trembling aspen forests (b) correspond to *Light* (Li, in yellow), *Nutrients* (Nu, in purple), *Single-litter* (1F, in orange), *Double-litter* (2F, in red) and *Control* (C, in grey), with the corresponding centroids (standard deviation). Treatments of the community approach in black spruce (c) and trembling aspen forests (d) correspond to *Control* (C, in grey), *Transplants-in* (Ti, in green) and *Transplants-out* (To, in blue). Treatments in black spruce forests in light colors and in trembling aspen forests in dark colors. Arrows correspond to the most important understory species defining the community composition (Envfit, $P < 0.05$). Notice that scales are different.

Table 1.3 Differences in composition of understory plant communities among treatments and forest types. *Notes:* Differences in composition of understory vegetation communities for each objective (ecosystem and community approaches) in each forest type (BS – Black spruce and TA – Trembling aspen) for the variables Treatment, Year and the Interaction of both as indicated by PERMANOVA based on the Hellinger-transformed data (Bray-Curtis distance) of understory vegetation abundance, using Site and Block as a random factors.

Objective	Forest type	Variable	Df	Df_{total}	Sum of squares	R^2	*F*	*P*
Ecosystem approach	BS	Treatment	4	89	1.8968	0.11968	3.3087	0.0001***
		Year	1	89	0.7806	0.04926	5.4468	0.0001***
		Interaction	**4**	**89**	**1.7056**	**0.10762**	**2.9752**	**0.0001*****
	TA	**Treatment**	**4**	**89**	**0.8184**	**0.04161**	**0.9045**	**0.0131***
		Year	**1**	**89**	**0.5425**	**0.02758**	**2.3979**	**0.0026*****
		Interaction	4	**89**	0.2085	0.0106	0.2304	1,0000
Community approach	BS	**Treatment**	**2**	**53**	**5.5612**	**0.43283**	**19.9709**	**0.0001*****
		Year	1	53	0.1967	0.01531	1.4131	0.2061
		Interaction	2	53	0.4072	0.0317	1.4625	0.1758
	TA	Treatment	2	**53**	3.9712	0.29006	11.8644	0.0001***
		Year	1	53	0.6084	0.04444	3.6356	0.0063**
		Interaction	**2**	53	**1.078**	**0.07874**	**3.2207**	**0.0025****

Table 1.4 Homogeneity of multivariance dispersion of understory plant abundance among treatments and years for each forest type. *Notes:* Homogeneity of multivariance dispersion (PERMUTEST, function *betadisper*) to compare groups of Year (2013-2018) and Forest types (BS – Black spruce, TA – Trembling aspen) among Treatments of the: Ecosystem approach: *Light* (Li, in yellow), *Nutrients* (Nu, in purple), *Single-litter* (1F, in orange) and *Double-litter* (2F, in red); and Community approach: *Transplants-out* (To, in blue), *Transplants-in* (Ti, in green); as well as *Control* conditions (C, in grey) for both approaches. Analysis based on Bray-Curtis distances, including Sites and Blocks as random factors. Significant values are in bold ($P < 0.05$) and meaningful contrasts are underlined.

Ecosystem approach	Contrast			2013					2018				
				1F	2F	C	Li	Nu	1F	2F	C	Li	Nu
BS	2013	1F		0.965	0.677	0.243	0.582	0.165	**0.002**	0.314	0.537	0.714
		2F	0.955		0.600	0.222	0.601	0.097	**0.002**	0.237	0.469	0.683
		C	0.689	0.605		0.359	0.263	0.217	**0.001**	0.435	0.781	0.923
		Li	0.239	0.195	0.335		0.089	0.907	0.118	0.718	0.486	0.491
		Nu	0.594	0.582	0.241	0.083		**0.020**	**0.002**	0.075	0.180	0.399
	2018	1F		0.091	0.217	0.895	**0.012**		**0.011**	0.728	0.387	0.463
		2F	**0.002**	**0.000**	**0.001**	0.113	**0.000**	**0.012**		**0.011**	**0.003**	**0.010**
		C	0.301	0.229	0.444	0.713	0.067	0.730	**0.013**		0.638	0.658
		Li	0.523	0.438	0.768	0.462	0.158	0.379	**0.003**	0.641		0.910
		Nu	0.702	0.652	0.923	0.487	0.402	0.462	**0.023**	0.648	0.911	
TA	2013	1F		0.782	0.739	0.770	0.435	0.888	0.724	0.900	0.378	0.793
		2F	0.777		0.963	0.523	0.230	0.914	0.479	0.847	0.198	0.557
		C	0.751	0.957		0.506	0.262	0.862	0.484	0.832	0.216	0.540
		Li	0.783	0.517	0.507		0.575	0.694	0.944	0.617	0.476	0.997

Table 1.4: continued

			Nu	0.449	0.252	0.255	0.567		0.353	0.592	0.299	0.878	0.624
		2018	1F	0.890	0.904	0.872	0.674	0.379		0.618	0.983	0.307	0.689
			2F	0.744	0.483	0.476	0.950	0.610	0.639		0.573	0.500	0.971
			C	0.890	0.858	0.823	0.621	0.306	0.982	0.582		0.233	0.645
			Li	0.375	0.200	0.205	0.471	0.884	0.316	0.511	0.245		0.517
			Nu	0.798	0.566	0.552	0.994	0.612	0.698	0.962	0.663	0.521	

Community approach	Contrast			2013			2018		
				C	Ti	To	C	Ti	To
	BS	2013	C		0.096	**0.028**	0.446	0.742	**0.005**
			Ti	0.086		**0.001**	**0.040**	0.324	**0.001**
			To	**0.016**	**0.000**		0.222	**0.044**	0.094
		2018	C	0.468	**0.028**	0.201		0.352	**0.039**
			Ti	0.766	0.298	**0.029**	0.371		**0.011**
			To	**0.002**	**0.000**	0.092	**0.045**	**0.006**	
	TA	2013	C		0.430	**0.003**	0.812	0.868	0.796
			Ti	0.411		**0.001**	0.250	0.204	0.618
			To	**0.002**	**0.003**		**0.001**	**0.001**	**0.001**
		2018	C	0.822	0.236	**0.001**		0.931	0.620
			Ti	0.863	0.200	**0.000**	0.933		0.642
			To	0.800	0.598	**0.003**	0.619	0.636	

1.6 Discussion

1.6.1 Different tree-canopy dominance, different plant understory

In our study, two adjacent stands in each site were very similar in permanent abiotic conditions but formed two alternative stable states defined by tree-canopy dominance, each with a different composition of understory vegetation. The composition of the understory vegetation was clearly associated with the tree-canopy composition, as shown in other studies (Qian et al. 2003, Barbier et al. 2008, Cavard et al. 2011). Understory plant α-diversity (Shannon index) was higher in trembling aspen compared to black spruce forests. This high diversity may be due to environmental conditions of trembling aspen stands where there is more available light across the season (Messier et al. 1998), greater nutrient availability, and higher inputs of more nutrient-rich easily decomposed litter than that found in black spruce stands (Laganière et al. 2010, Cavard et al. 2011). In contrast, forests dominated by black spruce normally have low nutrient availability, low light availability, and acidic soils that favour the establishment of feather mosses and ericaceous plants (Qian et al. 2003, Fenton et al. 2005). However, while the homogeneous understory of black spruce forests was dominated by feather mosses, bryophyte species richness was higher in trembling aspen forests, which could be due to more micro-habitat diversity in these heterogeneous forests.

1.6.2 Ecosystem approach

The effects on the understory communities of the evaluated mechanisms associated with each tree-canopy dominance (light availability, nutrient status, and type of litter deposition) were different in each forest type compared to their controls and changed with time, from 2013 to 2018. In our initial hypothesis, we expected litter type to be the dominant factor driving the composition of understory vegetation in both forest types. This hypothesis was confirmed in the black spruce understory as broadleaf litter changed the understory composition more than either the *Nutrients* or *Light* treatments. However, in the trembling aspen understory, black spruce litter did not affect

understory composition, which was also true for all the other factors of the ecosystem approach.

The negative effect of broadleaf litter on bryophyte abundance observed in black spruce stands was in line with previous studies on feather mosses, in which broadleaf litter affected feather-moss biomass, growth, survival and reproductive potential (Startsev et al. 2008; Jean et al. 2020). Decreased bryophyte abundance over time was probably due to the physical effect of broadleaf litter by shading or crushing of the mosses (Jean et al. 2020), whereas the short and narrow coniferous needles allowed mosses to grow around them. Previous studies have suggested a negative chemical effect of broadleaf litter on mosses due to a combined effect of allelopathic compounds (*i.e.* phenols), more soluble sugars and more nitrogen in broadleaf litter than in coniferous needleleaf litter (Startsev et al. 2008, Laganière et al. 2010) that may negatively affect mosses; however, these kind of compounds were not evaluated in our experimental nutrient additions.

In black spruce forests, the acidic soil conditions induced by needleleaf litter decomposition produced favorable conditions for feather mosses and ericaceous plants, limiting the establishment of other species in the understory (Qian et al. 2003, Fenton et al. 2005). The thick layer of live and dead feather mosses in coniferous forests maintains very low decomposition rates and enhances moisture retention, reducing black spruce seedlings establishment and growth, and favor paludification processes in the long term (Thiffault et al. 2013). We observed that, over time, bryophytes and ericaceous plants were negatively affected by both the physical effects of broadleaf litter and nutrients additions. However, nutrient additions were too high at the beginning of the experiment, and after reducing the nutrient concentration in 2015, bryophytes recovered over time, contrary to the ericaceous plants.

We were expecting higher available light levels in trembling aspen stands than in black spruce stands, based on previous work at the same sites (Légaré et al. 2001). However, natural light inputs in black spruce and trembling aspen forests were similar when measured in 2018, possibly because the stands were opening up as time passed due to individual tree death. Even though the *Light* treatments did not simulate the conditions of the alternate forest type, the changes in light conditions obtained still affected plant understory communities. The *Light* availability treatments in trembling aspen forests were about 2.3 times darker than natural conditions, transmitting an average of 40% of the ambient light. This reduction led to slight changes in understory diversity and community composition over time, allowing shade-tolerant species such as bryophytes and herbs that were already present in the stand to expand in those plots, while pteridophytes and shrubs abundance decreased. In contrast, *Light* availability treatments in black spruce forests were placed in more open areas, with about 1.4 times more light than ambient conditions, but the diversity and composition of understory vegetation remained more similar to those of the black spruce control plots.

1.6.3 Community approach

We evaluated the resistance of understory species transplanted to a new tree-canopy dominance and the resilience of the host forest understory to the small-scale disturbance of the transplantation, by analyzing changes in understory composition. Our second hypothesis was that transplanted understory plant composition would change over time towards the vegetation of the host stand. In black spruce forests we rejected this hypothesis because the transplanted understory vegetation from trembling aspen stands did not change in diversity and community composition towards the vegetation of the host stand, but instead, it resisted and showed little change after five years. In contrast, the hypothesis was accepted in trembling aspen stands, because the diversity and composition of understory vegetation transplanted from black spruce stands changed to resemble the vegetation of the host trembling aspen stand, indicating

that transplants from black spruce were not resistant over time. The changes in transplanted mosses and ericaceous plants from black spruce to trembling aspen forests could be linked to the broadleaf litter inputs that negatively affect mosses, as observed in our study and in previous experiments (Startsev et al. 2008; Jean et al. 2020).

The contrasting results of the community approach in each forest type depended on both forest resilience to a disturbance (transplantation of "foreign" plants), as well as the intrinsic resistance of species to establish in a new habitat. On the one hand, black spruce forests were not resilient enough to absorb the disturbance produced by transplanted plants and its understory plants transplanted to trembling aspen forests were not resistant enough to adapt and maintain their abundance over time. On the other hand, trembling aspen forests were resilient to a disturbance produced by the transplanted plants, because local plants colonized transplants from black spruce over time, and their understory vegetation transplanted to the coniferous forests was resistant over time. Black spruce forest understories have a thick and acid organic layer usually dominated by feather mosses (*Ptilium crista-castrensis* and *Pleurozium schreberi*), *Galium triflorum* and *Rhododendron groenlandicum* that limit understory vascular plant diversity. However, the resistant transplanted plants from trembling aspen to black spruce forests included several small herbs, such as *Oxalis montana*, *Clintonia borealis* and *Aralia nudicaulis*, and some shrubs, such as *Rubus pubescens* and *Viburnum edule*, that normally colonize nutrient-rich environments (Blouin and Berger 2002). Since the study sites were about 100 years after fire, the black spruce canopy was relatively open, with light inputs equivalent to those of trembling aspen forests, which may have allowed the transplanted vascular plants to thrive, contrary to younger canopy-closed stands. Furthermore, the underlying soil physiochemistry could have been enriched by the presence of a more diverse understory composition in transplantations plots from trembling aspen forests but there was no major change in the mineral soil nutrient availability between treatments within the same forest type.

1.6.4 Effects of treatments in each forest type

Based on the experimental approaches proposed by Beisner et al. (2003), we present the effects of shifts between two alternative stable states on plant understory communities (Figure 1.8). Changes in the environmental conditions (Figure 1.8-C, ecosystem approach) of light availability, nutrient status and litter type in each forest type intended to simulate the conditions of the alternative state. We observed that understory communities in trembling aspen forests were resistant to changes in the evaluated canopy-related factors. In contrast, understory vegetation in black spruce forests was resistant to changes in light availability and nutrient status, but plant composition changed with broadleaf litter addition.

Shifts in understory communities (Figure 1.8-D, community approach) showed that trembling aspen understory was resistant and successfully established in the black spruce forest stable state. In contrast, understory communities from black spruce forests were not resistant in the trembling aspen stable state, since over time understory community structure became more similar to that of the host trembling aspen forest. Likewise, black spruce forests were not resilient in response to the disturbance produced by the transplanted vegetation from trembling aspen stands. The transplanted understory successfully established and even colonized outside the plots (*personal observations*). In contrast, trembling aspen forests were resilient upon changes in their system, because they were able to absorb the small disturbance of the transplanted understory vegetation and return over time to a similar community composition as in the control plots. Considering that forest resilience is related with a higher biodiversity via the creation of a range of habitats and resources at different scales (Oliver et al. 2015), the heterogeneous and diverse trembling aspen forest offered more nutrient-rich soils and various microconditions than the homogenous and less diverse black spruce forests. Therefore, trembling aspen forests and its understory have the capacity to contribute to the stability and resilience of the system to a disturbance in boreal forests.

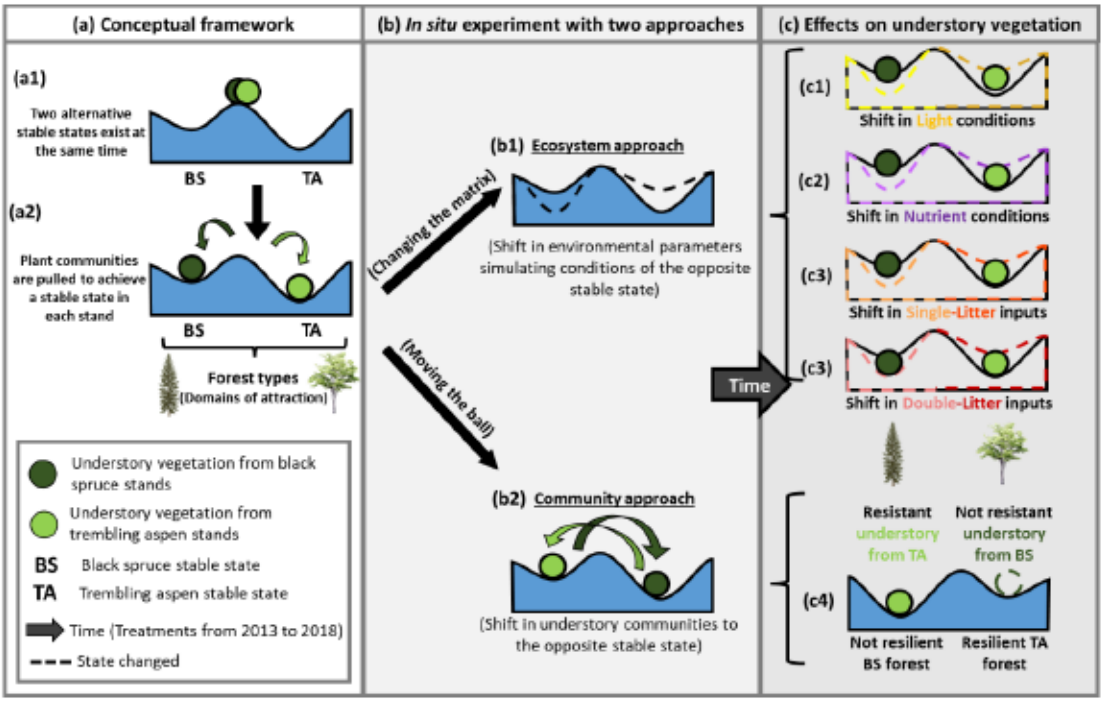


Figure 1.8 Ball-in-cup diagram that represent (a) two alternative stable states that exist at the same time in a matrix (in blue), which are defined by canopy dominance of black spruce (BS, left side) and trembling aspen (TA, right side). Balls represent understory plant communities adapted in black spruce (dark green) or in trembling aspen (light green) stable states. Stable states exist in the matrix and attract a particular ball (understory plant communities) to its respective stable state. (b) We analyzed two possible alternatives for changes the system: (b1) the "Ecosystem approach", with changes in parameters of the matrix, and (b2) the "Community approach", with transplantations of understory vegetation exchanged between forest types (the matrix is fixed). (c) As results we present how understory vegetation is affected by changes in parameters of the matrix corresponding to (c1) Light, (c2) Nutrient and (c3) and Litter (Single-litter and Double-litter) conditions; and (b-2) shifts in communities with transplantations exchanged between forest types ("Transplants-out"). Both approaches were compared to a control with the unchanged initial conditions and a control of the transplantation ("Transplants-in"). Inspired from Beisner et al. (2003).

1.6.5 Implications for forest management

Forest management is frequently focused on trees, but the understory vegetation is a key forest ecosystem driver that shapes future overstory by filtering seedling establishment, affecting belowground processes (*e.g.* decomposition and nutrient mineralisation) (Nilsson and Wardle 2005), and maintaining the stability of the system.

To maintain the stability of the boreal system, forest management needs to consider the key sources of ecological resilience, which includes resource supply, disturbance regimes and stochastic events, ecosystem feedbacks, and species and functional diversity (Chapin et al. 2006; Johnstone et al. 2010a; Oliver et al. 2015). Thus, the maintenance of species diversity, community structure and associated ecological functions of coniferous and broadleaf deciduous forests in both the overstory and the understory is one of the key aspects to maintain the ecosystem resilience face to global changes.

Our results indicate that while trembling aspen understory was both resistant and resilient, black spruce understory was neither. Black spruce forests are a highly important ecosystem to conserve, because it provides numerous ecosystem services, such as wildlife habitat (Hins et al. 2009), diverse bryophyte communities (Bergeron and Fenton 2012, Barbé et al. 2020), carbon sequestration in soil organic layers, and are of high importance for timber production (Baltzer et al. 2021, Mack et al. 2021). Consequently, forest management practices should be tailored to obtain the desired forest composition depending on the management objectives. If, in a given sector, black spruce forests are under-represented or the management goal is to maintain or increase their area, silvicultural actions need to be taken to ensure the regeneration of dense black spruce stands. In contrast, because of their resistance and resilience and ability to invade black spruce stands, no specific actions need to be taken to encourage the development of trembling aspen stands. Trembling aspen stands on the landscape can contribute to local and regional biodiversity, soil carbon storage (Gauthier et al. 2015, Oliver et al. 2015, Mack et al. 2021), and in mixed stands, can promote stand productivity (Légaré et al. 2005). Maintaining a balance between black spruce and trembling aspen on the landscape could likely contribute to the resilience of the boreal system and preserve their ecosystem services (Gauthier et al. 2015, Oliver et al. 2015).

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2015. MJ made valuable contributions to the manuscript. All authors edited the manuscript and gave final approval for publication.

CHAPITRE 2

TREE DOMINANCE SHAPES SOIL AND TREE PHYLLOSPHERE MICROBIAL COMMUNITIES IN CONIFEROUS AND BROADLEAF DECIDUOUS BOREAL FORESTS

LA DOMINANCE DES ARBRES FAÇONNE LES COMMUNAUTÉS MICROBIENNES DU SOL ET DE LA PHYLLOSPHERE DES ARBRES DANS LES FORÊTS DE CONIFÈRES ET DE FEUILLUS BORÉAUX

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2.1 Abstract

Boreal forests have been changing in tree dominance from coniferous to broadleaf deciduous forests, due to natural and anthropogenic causes. These changes in tree dominance produce shifts in litter inputs and plant understory composition. The impact of changes in canopy-associated factors on belowground microbial communities remain poorly understood. The objective of this study was to better understand how abiotic and biotic factors in black spruce and trembling aspen forests shape soil microbial community structure. First, we analyzed differences in microbial communities between microhabitats (tree phyllosphere vs. soil microbiome) and forest types (black spruce vs. trembling aspen). Second, we analyzed how shifts in factors related to each forest type (litter deposition and understory vegetation) affected soil microbial community composition. The identification of microbial communities with high throughput sequencing revealed high microhabitat specificity of bacterial communities interacting with forest type. Shifts in litter deposition and understory vegetation between forest types did not influence microbial community composition, but the legacy effects of each forest type defined soil bacterial and fungal communities. Fungal community composition was more strongly influenced by forest type compared with bacterial communities, and both were correlated with several soil physicochemical properties that differed among forest types. This study expands our knowledge of the microbial composition of tree phyllosphere and soil microbial communities in black spruce and trembling aspen forests and their correlation with abiotic and biotic factors in each forest type. Our study demonstrates the resistance of microorganisms to variation in canopy-related factors and the importance of legacy effects of forest type in defining soil microbial community composition.

Key words: Aboveground-belowground interactions, tree dominance, tree phyllosphere, soil microbiome, *Picea mariana* (black spruce), *Populus tremuloides*

(Trembling aspen), boreal forest, plant ecology, coniferous forests, broadleaved deciduous forests, fungi, bacteria, understory vegetation.

2.2 Résumé

Les forêts boréales ont connu des changements dans la dominance des arbres, passant des conifères aux forêts de feuillus à feuilles caduques, en raison de causes naturelles et anthropiques. Ces changements dans la dominance des arbres entraînent des modifications des apports de litière et de la composition du sous-bois végétal. L'impact des changements des facteurs associés à la canopée sur les communautés microbiennes souterraines reste mal compris. L'objectif de cette étude était de mieux comprendre comment les facteurs abiotiques et biotiques dans les forêts d'épinettes noires et de peupliers faux-trembles façonnent la structure des communautés microbiennes du sol. Tout d'abord, nous avons analysé les différences dans les communautés microbiennes entre les microhabitats (phyllosphere des arbres vs. microbiome du sol) et les types de forêts (épinette noire vs. peuplier faux-tremble). Ensuite, nous avons analysé comment les variations des facteurs liés à chaque type de forêt (dépôt de litière et végétation de sous-bois) affectaient la composition des communautés microbiennes du sol. L'identification des communautés microbiennes par séquençage à haut débit a révélé une grande spécificité des microhabitats des communautés bactériennes en interaction avec le type de forêt. Les changements de dépôt de litière et de végétation de sous-bois entre les types de forêts n'ont pas influencé la composition des communautés microbiennes, mais les effets hérités de chaque type de forêt ont défini les communautés bactériennes et fongiques du sol. La composition des communautés fongiques a été plus fortement influencée par le type de forêt que les communautés bactériennes, et les deux étaient corrélées à plusieurs propriétés physico-chimiques du sol qui différaient entre les types de forêt. Cette étude élargit nos connaissances sur la composition microbienne de la phyllosphère des arbres et des communautés microbiennes du sol dans les forêts d'épinettes noires et de peupliers faux-trembles et sur leur corrélation avec les facteurs abiotiques et biotiques de chaque type de forêt. Notre étude démontre la résistance des microorganismes à la variation des facteurs liés

à la canopée et l'importance des effets hérités du type de forêt dans la définition de la composition des communautés microbiennes du sol.

Mots clés : Interactions au-dessus du sol et sous-sol, dominance des arbres, phyllosphère des arbres, microbiome du sol, *Picea mariana* (épinette noire), *Populus tremuloides* (peuplier faux-tremble), forêt boréale, écologie végétale, forêts de conifères, forêts feuillus, champignons, bactéries, végétation de sous-bois.

2.3 Introduction

Aboveground-belowground interactions and their feedback loops shape community composition and ecological functions in forest ecosystems (Augusto et al. 2015; Kardol et al. 2018; Nilsson and Wardle 2005). Several studies have demonstrated that tree composition defines local environmental conditions (Augusto et al. 2015; Cavard et al. 2011), understory plant communities (Barbier et al. 2008; Bartels and Chen 2013; Qian et al. 2003), microbial communities in litter (Prescott and Grayston 2013; Urbanová et al. 2015) and soil microbiome composition within forests (Ghotsa Mekontchou et al. 2022; Hannam et al. 2006; Urbanová et al. 2015). The soil microbiome and plant understory communities both affect tree seedling regeneration, decomposition, and nutrient cycling, but in turn, their community composition is shaped by different factors related to tree dominance, such as different kinds of litter deposition, as well as changes in local abiotic conditions, such as light, temperature, and soil moisture and nutrient composition (Laganière et al. 2010; Nilsson and Wardle 2005; Sercu et al. 2017).

Litter deposition is one of the most important factors influencing the soil microbial community, as it changes soil nutrient inputs and dynamics in the understory (Chen et al. 2017; Laganière et al. 2010). One example is the difference between coniferous needleleaf versus broadleaf deciduous tree litter that has different chemical compositions and decomposition rates (Laganière et al. 2010; Prescott et al. 2000). For example, aspen litter contains more N, P, K, Ca and Mg and decomposes faster than spruce needleleaf litter (Laganière et al. 2010; Prescott et al. 2000). A different soil microbial community structure and a higher microbial biomass and community composition in aspen soils compared to spruce soils has been associated to differences in litter quality, plant understory composition and forest floor physicochemical properties (Hannam et al. 2006; Lamarche et al. 2004; Nagati et al. 2018; Prescott and Grayston 2013). Also, phylogenetically different trees have distinct associated phyllosphere microbiomes (i.e., microorganisms living on leaves) (Kembel et al. 2014;

Laforest-Lapointe et al. 2016), which can affect belowground nutrient dynamics through litter inputs. Although there have been studies comparing litter microbial and soil microbial community composition (Hannam et al. 2007; Prescott and Grayston 2013; Urbanová et al. 2015), we have less information on the possible influence of microbial communities from the phyllosphere of dominant trees to the soil microbiome, or how canopy-related factors affect soil microbial community structure, particularly in coniferous and broadleaf deciduous forests.

The boreal forest has been changing in tree dominance from coniferous to broadleaf deciduous forests due to natural and anthropogenic disturbances (Baltzer et al. 2021; Laquerre et al. 2009; Marchais et al. 2020). These changes in turn affect understory composition and dynamics (Barbier et al. 2008). In the natural boreal forests of eastern Canada, the dominant black spruce forests (*Picea mariana* (Miller) Britton, Sterns & Poggenburgh) have a homogeneous understory with a relatively thick organic layer dominated by feather mosses and ericaceous plants (Légaré et al. 2001; Nilsson and Wardle 2005). These forests are characterized by acid and nutrient-poor soils with coniferous leaf litter inputs that are difficult to decompose (Laganière et al. 2010), which affects the soil microbial community composition (Lamarche et al. 2004). In contrast, the increasingly abundant trembling aspen forests (*Populus tremuloides* Michx.) have nutrient-rich litter inputs that promote nutrient cycling and decomposition (Cavard et al. 2011; Laganière et al. 2010), which leads to a higher abundance of microbial communities than in coniferous forests (Hannam et al. 2006). These deciduous forests have a heterogeneous understory composed by a high diversity of shrubs, herbs and bryophyte species (Cavard et al. 2011; Laganière et al. 2011; Qian et al. 2003). Hence, these increasing changes in the boreal tree dominance are likely to have a strong effect on understory dynamics, which is essential to better understand in forest management.

Litter deposition and understory plants main factors associated with tree dominance of coniferous and broadleaf deciduous trees that shape the understory composition (Rodríguez-Rodríguez et al. 2022a,b). We analyzed factors shaping the soil microbial community structure in coniferous and broadleaf deciduous forests. First, we analysed differences in bacterial communities between microhabitats (soil microbiome vs. tree phyllosphere) and forest types (black spruce vs. trembling aspen forests). We hypothesized that microbial communities will be different between forest types, but within forest types, bacteria will co-occur between both microhabitats, because we expect a sharing of bacterial communities from tree phyllosphere to soil microbiome through litter fall. Secondly, we analyzed if bacterial and fungal communities were affected by changes in factors associated with tree dominance, including shifts in litter deposition and understory transplantations between forest types, and correlated them with abiotic and biotic factors. On the one hand, shifts in litter deposition consisted in adding needle leaves in trembling aspen forests and broad leaves in blacks spruce forests. On the other hand, we transplanted complete plots of understory vegetation from black spruce to trembling aspen forests, and vice-versa and analyzed their microbial composition after 5 years. We hypothesized that factors associated with tree dominance will produce a microbial community composition different than the natural conditions (control) but more similar to the opposite forest type, and will be correlated with abiotic and biotic factors in each forest type.

2.4 Methods

2.4.1 Study area and experimental design

The study area corresponds to the spruce-moss forest domain of the Clay Belt in western Quebec (Site A: 49°11'46" N - 78°50'33" W; Site B: 49°09'20"N - 78°47'56"W and Site C: 49°09'39"N - 78°47'55"W), located in the Boreal Shield of Canada. Three similar sites (Figure 2.1), approximatively between 0.3 and 2.3 km apart, were selected with adjacent stands of around dominant compositions of black spruce ($\geq$ 75 of Picea

mariana canopy cover) and trembling aspen (≥ 75% of Populus tremuloides canopy cover) forests. Each stand was approximatively 1 ha in size. These sites are natural forests that originated from the same wildfire ca. 1916 (Bergeron et al. 2004; Légaré et al. 2005) and have comparable abiotic conditions, such as surface deposit, slope, drainage, soil type and topography, regardless of their canopy composition, as has been described in previous studies (Cavard et al. 2011; Laganière et al. 2010; Légaré et al. 2005). These subhygric soils are composed of a fine-textured parent material dominated by clay (more than 50 %) (Cavard et al. 2011).

For a complete random block design, six blocks were placed in each site, three in black spruce and three in trembling aspen stands, each block separated by at least three meters. Therefore, our study corresponds to a nested block experimental design (Figure 2.1), in which we collected a total of 54 leaf samples (3 sites x 2 forest types x 3 blocks x 3 leaf samples), and 72 plots (3 sites x 2 forest types x 3 blocks x 4 treatments) in which we collected soil samples and determined understory vegetation composition.

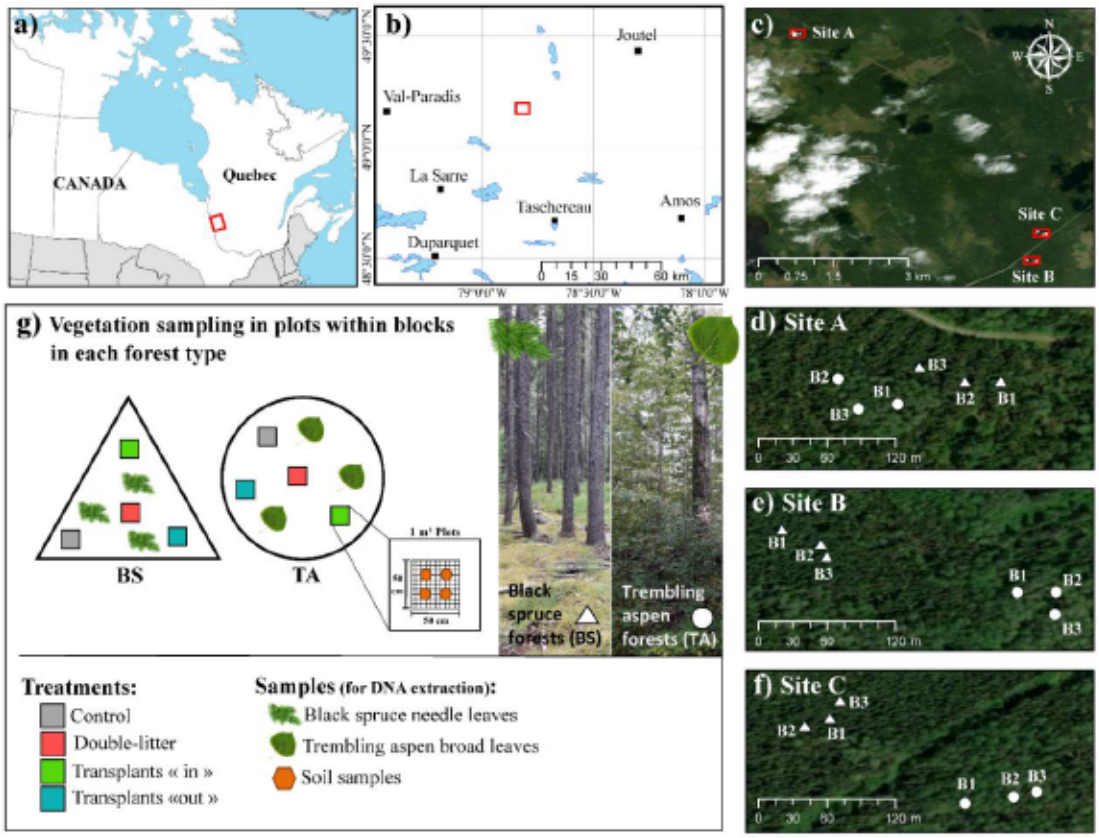

Figure 2.1 The study area is located in a) the boreal forest of eastern Canada, b) within western Quebec above the parallel 49ºN. c) The sampling design comprises three sites A, B and C (Sites B and C are 0.5 km apart and Site A is 5.3 km away). d, e, f) Each site has adjacent stands dominated by black spruce (as triangles) and trembling aspen (as circles), separated from 34 to 115 m. Within each forest type, three blocks (B1, B2 and B3) were placed separated from 16 to 49 m. (Nested block experimental design: 3 Sites x 2 Forest types x 3 Blocks). g) In each block, leaves and soil samples were taken. To obtain the tree phyllosphere, three samples per block of black spruce needles and trembling aspen broadleaves were collected. To obtain soil microbiome, soil samples were taken in each treatment plot inside the grid (four sub-samples in each plot mixed together corresponding to one soil sample from each plot). Treatment plots in different colors correspond to Control conditions (C, in grey), Litter (F, in orange), Transplants-in (Ti, in green) and Transplants-out (To, in blue). Vegetation abundance was registered using a 50 x 50 cm grid placed in the middle of each plot.

Within each block, four 1 m^2 treatment-plots were randomly installed. Treatments corresponded to the 1) *Control* (C), as unmanipulated plots. ; 2) *Litter* addition (F), in

which we exchanged litter inputs between forest types (*i.e.* adding needleleaf litter in plots in trembling aspen stands and broadleaf litter in plots in black spruce stands); 3) *Transplants-out* (To), corresponding to transplantations of understory vegetation from the opposite forest type (*i.e.* black spruce understory in trembling aspen stands and vice-versa) and 4) *Transplants-in* (Ti), corresponding to transplantations of understory vegetation from the same forest type, to distinguish the effect that the transplantation itself can have on the understory vegetation and soil microbial communities (i.e. black spruce understory in another black spruce stand and similarly for trembling aspen understory). These treatments were stablished in 2013 and were maintained over 5 years until 2018, as a part of the previous study in which the details of the complete *in situ* experiment have been fully described (Rodríguez-Rodríguez et al. 2022a). We chose these four treatments among others, as they were the two most important factors associated with tree dominance and had the stronger effect on plant understory composition (Rodríguez-Rodríguez et al. 2022a). With these shifts in litter deposition and in understory vegetation we could analyze the cumulative 5 years-effect of treatments on soil microbial communities among forest types.

To collect broadleaf litter for the litter treatment, we used 2 m^2 sheets placed on the ground, every year at the beginning of autumn, and the collected leaves were added to the plots. To collect needleleaf litter, 50 cm x 100 cm litter traps were placed in black spruce forests to retain the litter during winter and the collected needles were added to the plots in spring. We added the equivalent of the amount of litter that fell that year in an approximately 1 m^2 area in a single layer of needleleaf litter or broadleaf litter on each plot from the opposite forest type (broadleaf litter on black spruce stands and needleleaf litter on trembling aspen stands). Regarding the transplantations treatments (transplants-in and transplants-out), each transplant consisted of a 1m^2 surface of understory vegetation and approximately 30 cm of soil depth that was rolled on itself in a sheet and transferred to the corresponding plot in November 2013. The

Transplants-in (Ti) was a treatment control to ensure changes in microbial communities were not due to the shock the transplantation itself.

2.4.2 Sampling and DNA extraction

2.4.2.1 Leaf sampling and DNA extraction

For leaf sampling, three trees within each block in the corresponding forest type (black spruce or trembling aspen) were randomly selected at least 2 meters apart and leaves (needle leaves or broad leaves) were collected by shooting the very high branches of the bottom third of the crown with a 12 mm caliber rifle and the leaves were caught before they reached the soil to avoid possible contamination of the leaf phyllosphere with the soil microbiome. Between 50 – 100 g of leaf mass per sample was collected in a single day in September 2018, and the samples were stored in sterile roll bags, in a cooler on ice at approximately 4 °C and then transferred to a -20 °C freezer before phyllosphere DNA extraction. Epiphytic microorganisms in the tree phyllosphere were extracted from the 54 samples, following a modified version of existing protocols (Kembel et al. 2014). We added 100 ml of washing solution (1:50 diluted Redford Buffer: 1 M Tris·HCl, 0.5 M Na EDTA, and 1.2 % CTAB) (Kadivar and Stapleton 2003) into the sterile bags of each sample which were shaken by hand for 5 minutes to wash epiphytic microbes from leaves. The fluid was transferred to 50ml Falcon tubes that were centrifuged (4.000 × g, 20 min, 4 °C) and the supernatant was discarded by pipetting. The pellet with microbial cells was resuspended with 500 µL PowerSoil bead solution and up to 1ml was returned to the Powerbead tube and the DNA extraction was performed according to the manufacturer's protocol of the PowerSoil DNA extraction Kit (QIAGEN). One negative extraction control per kit was processed and sequenced following the same extraction conditions. Finally, all samples were stored at -80 °C prior to sequencing.

2.4.2.2 Soil sampling and DNA extraction

From each treatment plot, four soil samples were taken leaving a buffer area (as shown in Figure 2.1) and were combined in a sterile bag and kept in a cooler on ice at approximately 4 °C and then transferred to a -20 °C freezer. Soil samples corresponded to the interface between the organic and the mineral soil horizon, taking the last organic layer just before the clay layer, with a consistent texture that sticks together. The organic soil layer was thicker in black spruce (10 cm in average) than in trembling aspen forests (5 cm in average) and is underlain by a clay layer (representative of the clay belt of Québec and Ontario). Soils were collected in the same week in August 2018 and were sieved to 2mm. We then added 0.25 g of soil into Powerbead tubes and which were then stored at -20 °C before microbial DNA extraction. The rest of the soil material was dried at room temperature and stored in plastic bags for physicochemical analysis. We used the PowerSoil DNA Kit (QIAGEN) for microbial DNA extraction following the manufacturer's protocol and a negative control of each extraction kit was processed and sequenced following the same conditions as the rest of the samples. Samples were stored at -80 °C prior to sequencing.

2.4.2.3 Vegetation sampling

Considering that this project corresponded to a long-term study started in 2013 (Rodríguez-Rodríguez et al. 2022a), we used the data of understory vegetation abundance from control plots in 2018 from each forest type. These percentage abundances were calculated from counts of all species (vascular and non-vascular plants) present in a 50 x 50 cm grid placed in the center of each 1 m^2-plot, leaving around 25 cm of buffer area to avoid edge effects (Figure 2.1). Species taxonomy was based on the Canadensys database (http://www.canadensys.net/) and abbreviations were assigned based on a guide for our study region (Blouin and Berger 2002).

2.4.3 Microbial DNA sequencing

2.4.3.1 Soil DNA samples

To identify soil bacterial communities, we used a one-step PCR protocol to produce amplicon libraries containing the specific sequence for amplicon, dual indexes and all the motifs (Nextera adapters) needed for Miseq sequencing. The PCRs were performed in a 25 µL mixture containing 1 µL of 1/10 diluted DNA extract, 0.2 µM of each forward and reverse primers, 0.5 U of Phusion Hot Start II High-Fidelity DNA Polymerase (ThermoFisher), 1X of Phusion HF Buffer, 0.2mM of dNTPs, 3% DMSO following the thermal cycling conditions at 98 °C (30 s and 15 s), 64°C (30 s x 33), 72°C (30 s and 10 min) and 8 °C and hold. Soil DNA was PCR-amplified with the chloroplast-excluding 16S primers 799F/1115R (799F: AACMGGATTAGATACCCKG - 1115R: AGGGTTGCGCTCGTTG) from the region V5-V6 of the bacterial 16S rRNA gene (Parada et al., 2016). In order to identify potential contaminants and verify sequencing run quality we used negative and positive controls for both PCR amplification and sequencing. As negative controls we used sterile water instead of DNA, and as positive controls we used the ZymoBIOMICS™ Microbial Community DNA Standard (Zymoresearch). A total of 77 samples were sequenced (72 samples of soil microbiome, two negative extraction controls from each extraction kit, one negative PCR control, one negative extraction-PCR control and one positive PCR control), to identify soil bacteria and obtain the relative abundance of each taxon with respect to other identified bacterial taxa. Likewise, soil fungal communities were identified using the same PCR conditions explained above, with the exception that the initial mixture contained 1 µL of DNA extract (not 1/10 diluted) and we used the nuclear fungal ribosomal Internal Transcribed Spacer (ITS) region with the primers ITS1F/ITS2 (ITS1F: CTTGGTCATTTAGAGGAAGTAA - ITS2: GCTGCGTTCTTCATCGATGC).

2.4.3.2 Leaf DNA samples

To identify the epiphytic bacterial communities, we used the 16S primers 799F/1115R and the same PCR conditions as for soil DNA samples, except that the initial mixture contained 1 µL of DNA extract (not 1/10 diluted). We used sterile water instead of DNA as negative control and there was no positive control. Therefore, a total of 57 samples were sequenced (54 samples of soil microbiome, two negative extraction controls from each extraction kit and one negative PCR control).

2.4.3.3 Sequencing

After amplification, purification and normalization, samples to identify bacterial communities from both soils and leaves were sequenced in a single run, whereas samples to identify fungal communities were sequenced in a different run, with the same conditions. DNA libraries were sequenced using MiSeq paired-end 2 x 300 base pair, v3 Kit (600-cycles, Illumina reference MS-102-3003) at the UQAM CERMO-FC Genomics Platform.

2.4.4 Environmental data and soil physicochemical analysis

Abiotic factors were measured in each treatment-plot in 2018. A spherical convex densiometer (No. 43887/8, Forestry Suppliers, Inc.) was used to calculate the percentage of overstory density in each plot, to obtain an average of four measures by plot. Light inputs obtained in µmol m^2s^{-1} units were measured in August 2018 using the device Sunfleck Ceptometer (Decagon, USA), by making an average of four measures by plot. Soil moisture was measured during one day in summer, after a 48 h period without rain to avoid a saturated relative humidity. We placed a sensor (FIELDSCOUT, TDR 300 soil moisture meter, Spectrum Technologies, Inc.), in four spots in the buffer zone of each plot to calculate an average of soil moisture per plot. To measure the soil temperature, i-Buttons® (DS1922L) were individually packed in 2 Ziploc bags for protection, configured to register the temperature every six hours in

summer (May to August 2018), and placed in the buffer zone of every plot at a depth of 5-10 cm under the soil surface.

From the collected soil samples, physicochemical analysis were performed at the "Station de recherche agroalimentaire de l'Abitibi-Témiscamingue". We obtained total carbon and nitrogen by total combustion and gas detection with a Thermal conductivity detector using the vario MAX cube analyzer. The pH was calculated in two different ways: 1) pH-H_2O with a dilution 1:1 V/V with water, and 2) pH-buffer with the Shoemaker-McLean-Pratt (SMP) method. The Mehlich III extraction method was used to measure soil minerals, that were analyzed with the Inductively coupled plasma - optical emission spectrometry (ICP-OES); and finally, we calculated the cation exchange capacity (CEC = meq / 100 g).

2.4.5 Bioinformatic analysis of microbial communities

2.4.5.1 Bacterial communities in soils and leaves

We analyzed the complete bacterial composition of the soil microbiome and the tree phyllosphere that were sequenced in the same run using the 16S bacterial primers to quantify variation in bacterial taxa in each forest type. A total of 5,555,443 reads were produced by high-throughput Illumina sequencing. We used the amplicon sequence variant (ASV) approach in the DADA2 package version 1.6 (Callahan et al. 2016a; Callahan et al. 2016b) in R software, version 3.6.0 (R Core Development Team 2019) for the bioinformatic analysis, following the DADA2 sequence processing workflow (Callahan et al. 2016a) with default parameters except as noted. We trimmed the first 20 nucleotides from the beginning of both forward and reverse reads to eliminate primers. We truncated the reads where quality decreased sharply at positions 270 (forward reads) and 205 (reverse reads) with maxEE setting of 2 and we used the pseudo-pooling approach to infer amplicon sequence variants (ASVs). Then, we merged forward and reverse reads, obtaining a total of 20,326 ASVs. The chimeric

ASVs were removed using the consensus method, obtaining 11274 non-chimeric ASVs that were used for subsequent analyses. We used the RDP Naive Bayesian Classifier algorithm method (Wang et al. 2007) with the SILVA version 132 database (www.arb-silva.de) for taxonomic assignment from phylum to genus, and the RefSeq + RDP database (NCBI RefSeq 16S rRNA database supplemented by RDP) (Sousa 2019) to assign species-level taxonomy, to obtain more accurate species assignments. The phyloseq R package (McMurdie and Holmes 2013) was used to analyze the taxonomically annotated ASV data, containing 11,274 ASVs from 132 samples (52 from tree phyllosphere, 72 from soil microbiome and a total of 6 controls) with a total of 2,919,227 sequences.

We filtered out ASVs annotated as originating from plant chloroplasts, leaving only sequences corresponding to the kingdom Bacteria for a total of 8782 taxa and 2,622,199 sequences. The compositions of both positive and negative control samples were completely different from the evaluated samples according to a PCA ordination and all negative controls contained few sequences (between 0 and 192 sequences). Hence, we excluded all positive and negative controls prior rarefaction. The rarest ASVs were excluded, keeping ASVs with more than one sequence per sample in and with at least 10 sequences per ASV. All ASVs that occurred in at least two samples and with a minimum total abundance of 10 sequences were selected. This led to the exclusion of 4,626 ASVs. A threshold of 7300 sequences per sample was chosen to randomly rarefy the data for subsequent analysis, as this rarefaction cutoff was sufficient to capture the vast majority of ASVs in samples (Figure S 3.4), leaving a total of 912,500 sequences from 4156 ASVs in 125 samples after rarefaction.

2.4.5.2 Fungal communities in soils

The complete fungal composition of the soil microbiome was sequenced using the ITS primers with regions ITS1F/ITS2 (ITS1F: CTTGGTCATTTAGAGGAAGTAA - ITS2: GCTGCGTTCTTCATCGATG) (Gardes and Bruns 1993; White et al. 1990), to

quantify variation in fungal taxa in each forest type. A total of 2,491,414 reads were produced by high-throughput Illumina sequencing. As for bacterial sequences, we used the ASV approach with DADA2 package version 1.6 (Callahan et al. 2016a; Callahan et al. 2016b) and sequence processing workflow in R software for the bioinformatic analysis (Callahan et al. 2016a), with default parameters except as noted. We trimmed the first 20 nucleotides from the beginning of both forward and reverse reads to eliminate primers. We truncated the reads where quality decreased sharply at positions 270 (forward reads) and 240 (reverse reads) with maxEE setting of 2 and we used the pseudo-pooling approach to infer amplicon sequence variants (ASVs). Then, we merged forward and reverse reads, obtaining a total of 3,679 ASVs. Chimeric ASVs were removed using the consensus method, obtaining 2,757 non-chimeric ASVs that were used for subsequent analyses. We determined the taxonomic identity of each ASV using the UNITE QIIME database for fungi, version 8.3 (Abarenkov et al. 2021). The phyloseq R package (McMurdie and Holmes 2013) was used to analyze the taxonomically annotated ASV data, containing 2,757 ASVs from 74 samples (72 from soil microbiome and 2 controls) with a total of 1,580,209 sequences.

Both negative controls of PCR and kit DNA extraction contained few sequences (5 and 9 sequences, respectively) and their composition were completely different from the evaluated samples according to a PCA ordination. Hence, we filtered out both controls prior rarefaction. The rarest ASVs were excluded, keeping ASVs with more than one sequence per sample in and with at least 10 sequences per ASV. This led to the exclusion of 1,655 ASVs. A threshold of 2902 sequences per sample was chosen to randomly rarefy the data for subsequent analysis, as this rarefaction cutoff was sufficient to capture the vast majority of ASVs in samples (Figure S 3.5), leaving a total of 206,042 sequences from 1076 ASVs in 71 samples after rarefaction.

2.4.6 Statistical analysis

This study analyses the bacterial and fungal communities in soils of black spruce and trembling aspen forests, and we further analyzed the possible link between the bacterial phyllosphere of each tree species to their soil bacterial microbiome. We analyzed differences in the bacterial diversity, composition, and relative abundance between microhabitats (soil microbiome and tree phyllosphere) and forest types (black spruce and trembling aspen forests). All statistical analysis were performed using R software, version 3.6.0 (R Core Development Team 2019) and figures were made using the *ggplot2* package, version 3.3.5 (Wickham 2016), unless otherwise specified, and then modified in Adobe Illustrator (version 26.3.1) for a better data visualization. Differences in bacterial communities between microhabitats (phyllosphere vs. soil microbiome) and forest types (black spruce - BS vs. trembling aspen - TA) were analyzed first by randomly taking 20 subsamples per microhabitat in each forest type to use an equally amount of samples to compare and to represent the co-occurrence of bacterial ASVs in a Venn diagram, using *ps_venn* function (Russel 2021). The bacterial ASVs Shannon diversity (sample-level α-diversity) between tree phyllosphere and soil microbiome in black spruce and trembling aspen forests was calculated with the *vegan* package, version 2.5-7 (Oksanen et al. 2020) based on the rarefied relative abundances. Differences in Shannon diversity among microhabitats and forest types were tested with an ANOVA of a mixed effect model in which we included the interaction of microhabitat and forest type, using Sites and Blocks as random factors, using the *stats* package, version 4.1 (R Core Development Team 2019) and the *lme4* package, version 1.1-27.1 (Bates et al. 2015). From the model, we also performed *post hoc* pairwise Tukey comparisons between microhabitat and forest type with the *emmeans* package, version 1.6.2-1 (Lenth et al. 2021). Finally, the Shannon diversity among microhabitats and forest types is presented as boxplots, including both the median and mean.

The bacterial community composition among microhabitats (tree phyllosphere and soil microbiome) and forest types (black spruce and trembling aspen) was analysed using a Non-Metric Multidimensional Scaling (NMDS), based on the ASVs relative abundance data previously rarefied, Hellinger transformed and with Bray-Curtis distance, using the *vegan* package (version 2.5-7), with bacterial phyla abundances added *a posteriori* to the ordination. In order to test for differences in bacterial community composition explained by microhabitat and forest type, a PERMANOVA analysis carried out using the *adonis2* function of vegan package (Oksanen et al. 2020), including Site and Block as random factors.

After separating the analysis of tree phyllosphere and soil microbiome, we analyzed the relative abundance of ASVs assigned to bacterial phyla in the phyllosphere of black spruce needles and trembling aspen leaves in a stacked barplot plot using a linear model of $\log_{10}$-transformed bacterial relative abundances (function *lm* of *stats4* package, version 4.1.0), with a cut-off of adjusted $P < 0.05$ using the method of Benjamini and Hochberg (1995) to correct for multiple hypothesis testing.

In order to analyze the effect of treatments on soil bacterial and fungal communities, we explored in detail the Shannon diversity, relative abundance and community composition between treatments. Similarly as before, the Shannon diversity (sample-level α-diversity) of soil bacterial and fungal communities were calculated for the different treatments and among forest types with the *vegan* package (Oksanen et al. 2020) based on the rarefied relative abundances. Differences in Shannon diversity were tested with an ANOVA of a mixed effect model in which we included the interaction of forest type and treatments, using Sites and Blocks as random factors, using the *stats* package, version 4.1 (R Core Development Team 2019) and the *lme4* package, version 1.1-27.1 (Bates et al. 2015). From the model, *post hoc* pairwise Tukey comparisons between microhabitat and forest type were performed with the *emmeans* package,

version 1.6.2-1 (Lenth et al. 2021). Finally, the Shannon diversity among microhabitats and forest types is presented as boxplots, including both the median and mean.

The relative ASVs abundance of the assigned bacterial and fungal phyla in soils were compared among treatments and forest types using a linear model of $\log_{10}$-transformed ASV relative abundances (stats4 package), with a cut-off of adjusted $P < 0.05$ using the method of Benjamini and Hochberg (1995) to correct for multiple hypothesis testing. The soil microbial community composition among treatments and forest types were independently presented for bacteria and fungi in a NMDS, based on the rarefied ASVs relative abundance data Hellinger transformed and with Bray-Curtis distance, using the *vegan* package (version 2.5-7). The ordination was presented three times, adding *a posteriori* with the *envfit* function ($P < 0.05$, vegan package), the microbial phyla, the abiotic factors and understory vegetation. The goodness of fit of environmental variables and soil physicochemical properties was tested from each NMDS ordination of bacterial and fungal communities, based on 999 random permutations. Also, in order to test for differences in their microbial community composition, we performed again for these ordinations a PERMANOVA analysis, including Site and Block as random factors.

Finally, we evaluated the relative importance of forest type, treatments, abiotic and biotic factors (understory vegetation) affecting soil bacterial and fungal community structure. We tested for differences in the abiotic factors including both environmental properties (soil temperature, light, overstory density and soil moisture) and soil physicochemical properties between treatments, forest types and their interaction, with a linear mixed-effect ANOVA with the nlme package (Pinheiro et al. 2021) and the Stats package (R Core Development Team 2019), respectively, using Site as a random factor. iButtons from certain plots did not work so we did data imputation for the missing values, using the averaged temperature from each Site in each forest type.

2.5 Results

2.5.1 Differences in bacterial communities between microhabitats and forest types

The identified bacterial ASVs varied depending on the microhabitat (tree phyllosphere *vs.* soil microbiome) and forest type (black spruce *vs*. trembling aspen). The bacterial Shannon diversity (sample-level α-diversity, Figure 2.2), based on all identified ASVs, was significantly influenced by the interaction of both microhabitat (phyllosphere and soil microbiome) and forest type (black spruce and trembling aspen) (ANOVA, $F_{1,121} = 45.744$, $P < 0.0001$, Table S 3.5). All *post hoc* comparisons of Shannon diversity among categories were significantly different (Tukey tests, All $P > 0.001$, Table S 3.6), with a higher Shannon diversity in soils than in tree phyllosphere, a higher Shannon diversity in the phyllosphere of black spruce needles than in trembling aspen leaves, and the opposite pattern in the soil bacterial community with a Shannon diversity higher in trembling aspen than in black spruce soils.

The Venn diagram, based on 20 samples randomly taken for each microhabitat and forest type, show the cooccurrence of bacterial ASVs between in the leaves phyllosphere and soil microbiome of black spruce and trembling aspen (Figure 2.2b-h). A 65 % of bacterial ASVs (2666 ASVs) was shared between black spruce and trembling aspen for both microhabitats (Figure 2.2c), whereas 12 % (476 ASVs) cooccurred between the phyllosphere and the soil microbiome for both forest types (Figure 2.2f). In both black spruce (Figure 2.2d) and trembling aspen forests (Figure 2.2e), the soil microbiome had a higher percentage of about ~70 % of bacterial ASVs (2355 ASVs), compared to its phyllosphere (~22 %, ≥748 ASVs). Therefore, a small proportion of ASVs (~7-9 %) was shared between the tree leaves and the soil in both forest types. Comparing between forest types for each microhabitat, the needle leaves phyllosphere shared 28 % of bacterial ASVs (452 ASVs) with broad leaves (Figure 2.2g). In contrast, the soil microbiome shared 77 % of bacterial ASVs (2300 ASVs) between black spruce and trembling aspen dominated forests (Figure 2.2h). Thus, a

higher proportion of soils ASVs were shared between forest types than phyllosphere ASVs between leaf types.

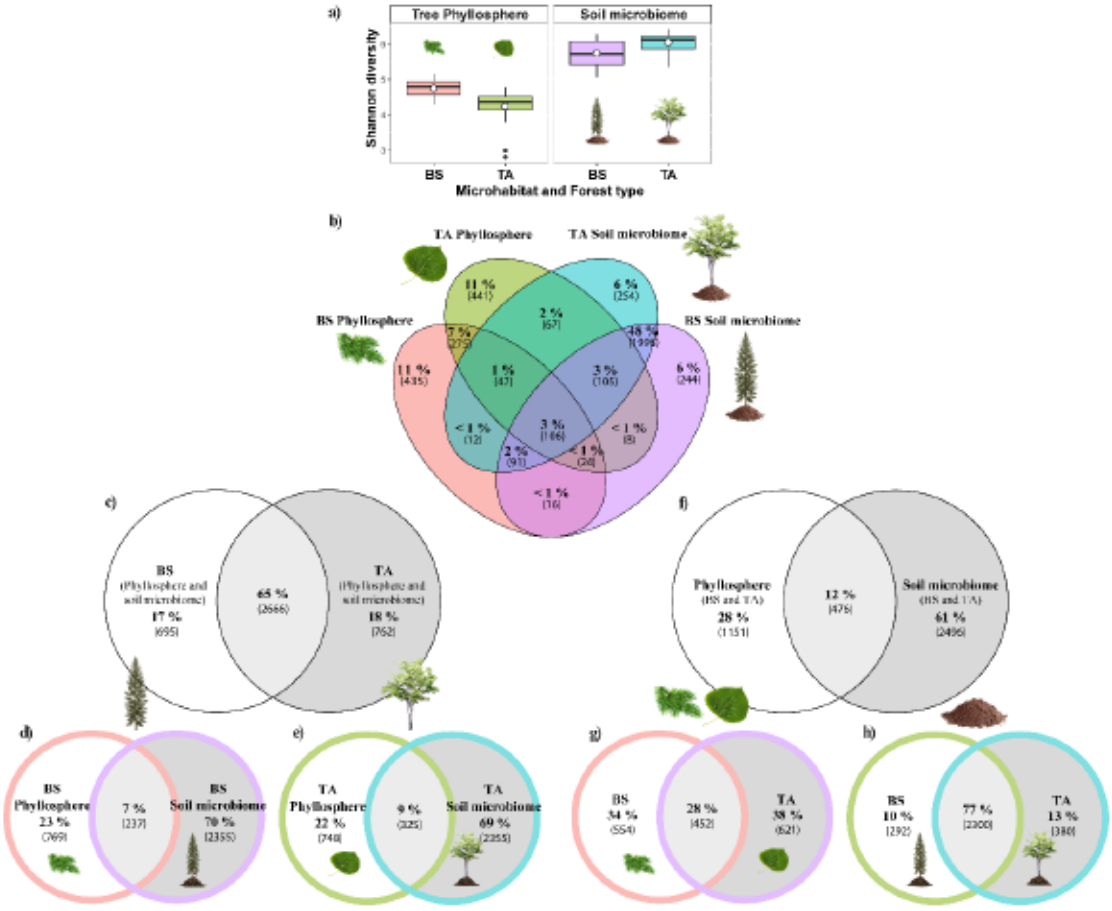

Figure 2.2 Differences in bacterial communities between microhabitats (phyllosphere vs. soil microbiome) and forest types (black spruce - BS vs. trembling aspen - TA). a) Bacterial Shannon diversity of tree phyllosphere and soil microbiome between black spruce (brown) and trembling aspen (green) forests, based on all identified ASVs. White points represent the mean and black lines the median. b-h) Venn diagrams representing co-occurrence of bacterial ASVs in the phyllosphere and soil microbiome of black spruce and trembling aspen forests, based on 20 randomly chosen samples from each microhabitat. Values indicate percentage of shared ASVs (values in parentheses are total number of ASVs). The different Venn diagrams represent all comparisons between microhabitat and forest types (b), between BS and TA for both microhabitats (c), the black spruce phyllosphere and soil microbiome (d), the trembling aspen phyllosphere and soil microbiome (c), the phyllosphere and soil microbiome of

both forest types (d), the phyllosphere in black spruce and trembling aspen (e), and the microbiome in black spruce and trembling aspen (f).

The bacterial community composition was different depending on microhabitat, differing between soil microbiome and tree phyllosphere (Figure 2.3Figure 2.3, first axis), and between forest types (Figure 2.3, second axis), with a significant interaction between microhabitat and forest type (PERMANOVA, $R^2 = 0.125$, $F_{1,124} = 35.573$, $P < 0.0001$, Table S 3.7). The differences in bacterial phyllosphere communities between forest types were more distinct than those in soils, and the relative abundance of ASVs assigned to the different bacterial phyla in tree phyllosphere varied between forest types (Figure 2.4).

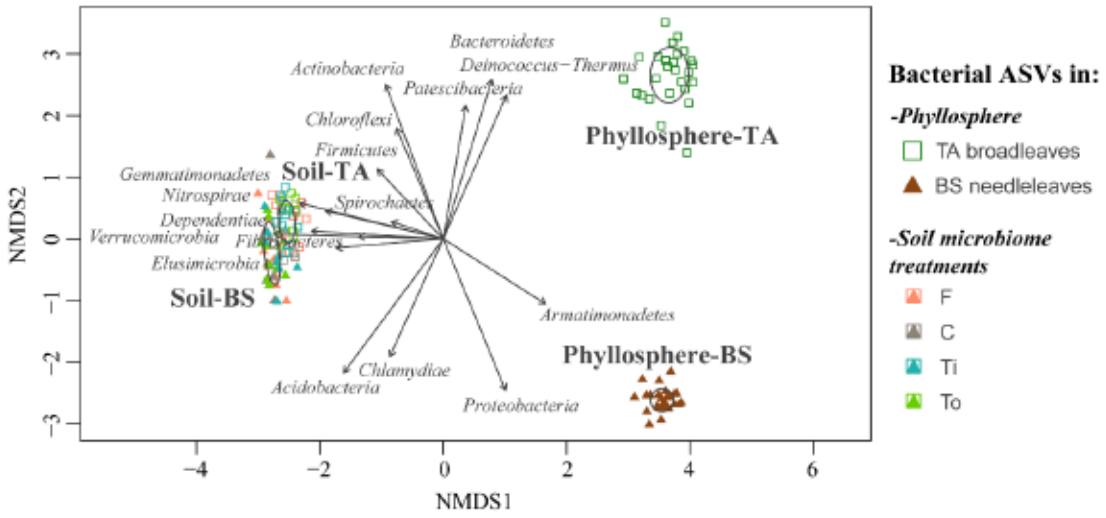

Figure 2.3 Two-dimensional non-metric multidimensional scaling (NMDS) of the relative abundance of bacterial ASVs (Amplicon Sequence Variants) in: 1) Phyllosphere of black spruce (BS - brown filled triangles) and trembling aspen (TA - green empty squares) and in 2) Soil microbiome from each forest type (BS in filled triangles and TA in empty squares), among the different treatments in colors: Control conditions (C, in grey), Litter addition (F, in orange), Transplants-in (Ti, in green) and Transplants-out (To, in blue). Points correspond to 72 sampling units of soil microbiome (n = 9 per forest type and treatment) and 54 sampling units of phyllosphere (n = 27 per forest type). Ellipses in grey correspond to standard deviation of ordination scores for samples according to the bacterial microhabitat (Soil microbiome or Phyllosphere) and forest type (BS or TA). Arrows indicate the correlation between sample level values and ordination axes scores for bacterial phyla added a posteriori in

the ordination (displayed phyla of maximum estimated $P < 0.05$). Ordination based on the Bray-Curtis distance of the rarefied and Hellinger transformed ASV relative abundances.

The relative abundance of ASVs from the tree phyllosphere assigned to bacterial phyla between forest types (Figure 2.4 and Table S 3.8) show that the most abundant bacteria in black spruce needles were *Proteobacteria, Acidobacteria* and *Actinobacteria*, whereas in trembling aspen leaves the most abundant phyla were *Actinobacteria, Proteobacteria* and *Bacteroidetes*. Also, *Planctomycetes* and *Verrucomicrobia* were only present on black spruce leaves, whereas *Deinococcus-Thermus, Fusobacteria* and *Gemmatimonadetes* were only present in trembling aspen leaves. All bacteria were significantly different between forest types (ANOVA, all Benjamini–Hochberg-adjusted $P < 0.05$), except *Armatimonadetes*, *Verrucomicrobia* and *Fusobacteria*. However, the more abundant phyla differentiating the most in relative abundance between forest types were *Proteobacteria* and *Acidobacteria* being more abundant in black spruce leaves, whereas *Actinobacteria* and *Bacteroidetes* were more abundant in trembling aspen leaves.

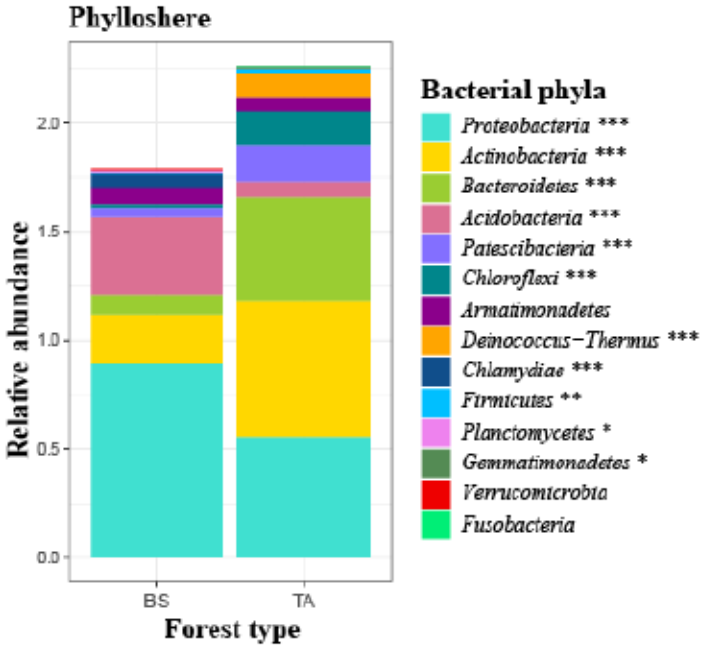

Figure 2.4 Differences in relative abundance (%) of ASVs assigned to bacterial phyla (in colors) among the forest types dominated by black spruce (BS) and trembling aspen (TA). Significant differences (ANOVA, all Benjamini–Hochberg-adjusted $P < 0.0001$***, $P < 0.001$**, $P < 0.05$*, Table S 3.8) between forest types.

2.5.2 Effects of shifts in factors associated with tree dominance on soil bacteria and fungi

We evaluated the effect of shifts in factors associated with tree dominance, including treatments of litter inputs and understory transplantations, on bacterial and fungal Shannon diversity, relative abundance and community composition in black spruce and trembling aspen forests and how they are correlated with abiotic and biotic factors in each forest type.

Soil bacterial Shannon diversity (Figure 2.5Figure 2.5a) was significantly different between forest types (ANOVA, $F_{1,62} = 18.139$, $P < 0.0001$, Table S 3.9), being higher in trembling aspen (*emmeans* of 6.05 ± 0.067 SD) than in black spruce forests (*emmeans* of 5.73 ± 0.067 SD), but did not differ significantly between treatments. In contrast, fungal Shannon diversity (Figure 2.5Figure 2.5b) was not significantly

different between forest types, nor treatments (ANOVA, All $P > 0.05$, Table S 3.9). Furthermore, bacterial communities were more diverse than fungal communities in soils of both forest types, according to the Shannon index (Figure 2.5a,b).

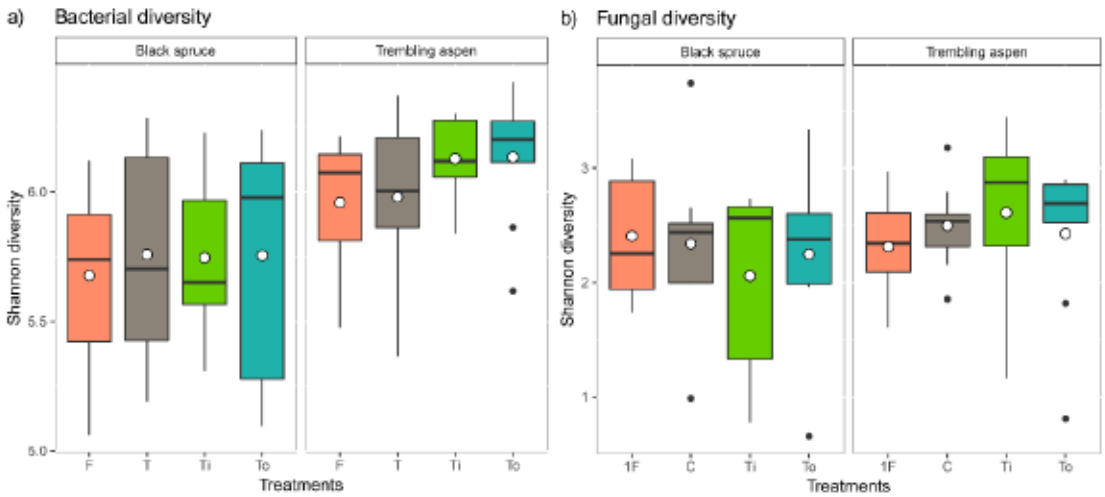

Figure 2.5 Shannon diversity of soil microbial communities of bacteria (a) and fungi (b) in black spruce and trembling aspen forests among the treatments of Litter addition (F, in orange), Control conditions (C, in grey), Transplants-in (Ti, in green) and Transplants-out (To, in blue). White points represent the mean and black lines the median. Notice both panels have different scales.

The relative abundance of ASVs assigned to soil bacterial and fungal phyla was similar between treatments and some phyla differed between forest types (Figure 2.6). The more relative abundant ASVs assigned to bacterial phyla (Figure 2.6a) were *Actinobacteria, Proteobacteria* and *Acidobacteria,* followed by *Chloroflexi* and *Bacteroidetes*. At the phyla level, all bacteria were similar in relative abundance among treatments, whereas 12 phyla were significantly different between forest types (ANOVA, all Benjamini–Hochberg-adjusted $P < 0.05$, Figure 2.6a). Other phyla, including *Fusobacteria*, *Deinococcus-Thermus* and *Spirochaetes*, had a very low relative abundance to find significant differences between treatments or forest types. Furthermore, comparing the tree phyllosphere (Figure 2.4) to the soil microbiome (Figure 2.6a), most of the identified bacterial ASVs co-occurred in both, except for

Dependentiae, Nitrospirae, Fibrobacteres, and *Elusimicrobia*, which where only present in soils.

The most relative abundant ASVs assigned to fungal phyla (Figure 2.6b) were *Basidiomycota, Ascomycota* and *Mortierellomycota*, with a relative abundance similar between treatments. However, in black spruce soils, *Chytridiomycota* and *Zoopagomycota* were only present in treatment To, whereas in trembling aspen soils, *Deinococcus-Thermus* was only present in treatment F, and *Spirochaetes* was absent in the control. We found significant differences in ASVs relative abundance between forest types for the assigned phyla *Mortierellomycota* and *Murocomycota* (ANOVA, all Benjamini–Hochberg-adjusted $P < 0.05$, Figure 2.6b and Table S 3.7). The other phyla (*Chytridiomycota, Zoopagomycota* and *Deinococcus-Thermus*) had a very low relative abundance to find significant differences between treatments or forest types.

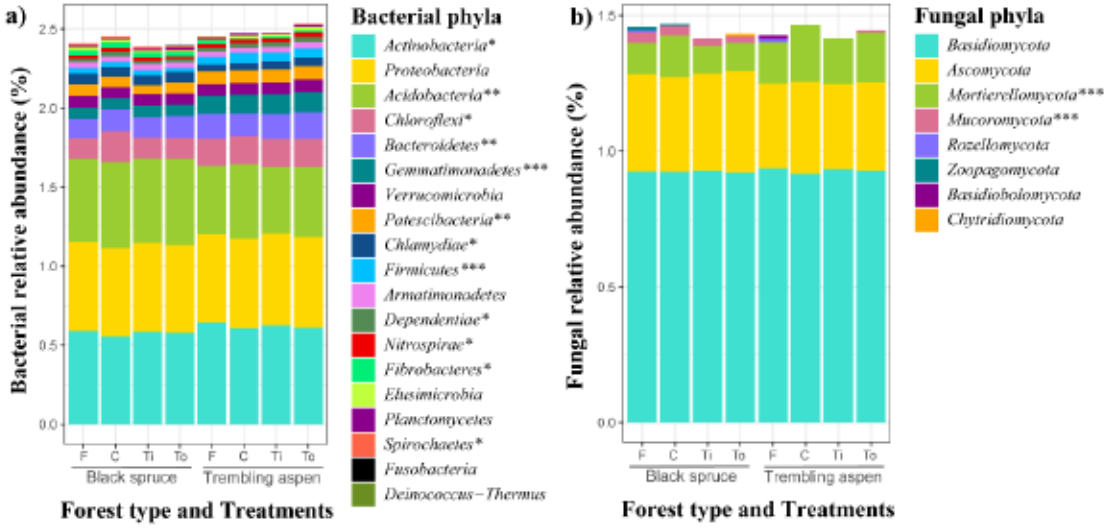

Figure 2.6 Differences in relative abundance of ASVs assigned to phyla (in colors) from soil microbiome: a) Bacterial phyla and b) Fungal phyla; for each forest type (black spruce and trembling aspen) and among treatments: Control conditions (C), Litter addition (F), Transplants-in (Ti) and Transplants-out (To). Significant differences between forest types (ANOVA, all Benjamini–Hochberg-adjusted P < 0.0001***, P < 0.001**, P < 0.05*, Table S 3.7), but not among treatments or the interaction.

The soil microbial community composition was analyzed among treatments in black spruce and trembling aspen forests. We analyzed independently the soil bacterial (Figure 2.7) and fungal (Figure 2.8) community composition and their correlation with abiotic factors and understory plant communities in each forest type. We found significant differences between forest types of different abiotic factors, including environmental conditions (*i.e.* light, canopy cover, soil moisture, soil temperature) and soil physicochemical properties between forest types (All ANOVA, $P < 0.05$, Table S 3.10), but any significant differences among treatments (All comparisons ANOVA, $P > 0.05$). The different factors were correlated with the soil microbial community composition (Table S 3.11).

Soil bacterial community composition (Figure 2.7a) was grouped by forest type in the NMDS ordination but not among treatments, which is statistically supported by significant differences between forest types (PERMANOVA, $R^2 = 0.131$, $F_{1,71} = 10.195$, $P < 0.0001$), but no effect of treatment or their interaction (Table S 3.12). The first axis of the NMDS was correlated with a gradient in cations, including H, Ca and soil pH, but most of elements were significantly correlated with each forest type (Figure 2.7b and Table S 3.11). Black spruce soils were significantly correlated with higher concentration N, C, Al, Fe, H, S, CEC and C : N and N : P ratios, as well as higher concentration of soil moisture and light inputs than trembling aspen soils (Envfit , $P < 0.05$, Table S 3.11). In contrast, trembling aspen soils were significantly correlated with soil pH, Ca, Mn and P : Ca ratio than black spruce soils (Envfit , $P < 0.05$, Table S 3.11). Finally, the composition of understory vegetation (Figure 2.7c) was also significantly correlated with each forest type (Envfit, $P < 0.05$; Table S 3.11). Black spruce forests were correlated with moss species such as *Pleurozium schreberi* (PLS), *Ptilium crista-castrensis* (PTC), *Dicranum polysetum* (DIP) and *Sphagnum* spp. (SPS), as well as small plants including *Gaultheria hispidula* (GAH) and *Geocaulon lividum* (COL). In contrast, trembling aspen stands were associated with several herbs and shrubs in the understory, including *Aralia nudicaulis* (ARN), *Viola* spp. (VIS),

Viburnum edule (VIE), *Lysimachia borealis* (TRB), *Spinulum annotinum* (LYA), *Rubus idaeus* (RUI), *Petasites frigidus var. palmatus* (PES), *Rubus pubescens* (RUP), *Cornus canadensis* (CON) and *Mitella nuda* (MAC). Therefore, forest type shapes soil bacterial communities, soil physicochemical properties and plant understory vegetation.

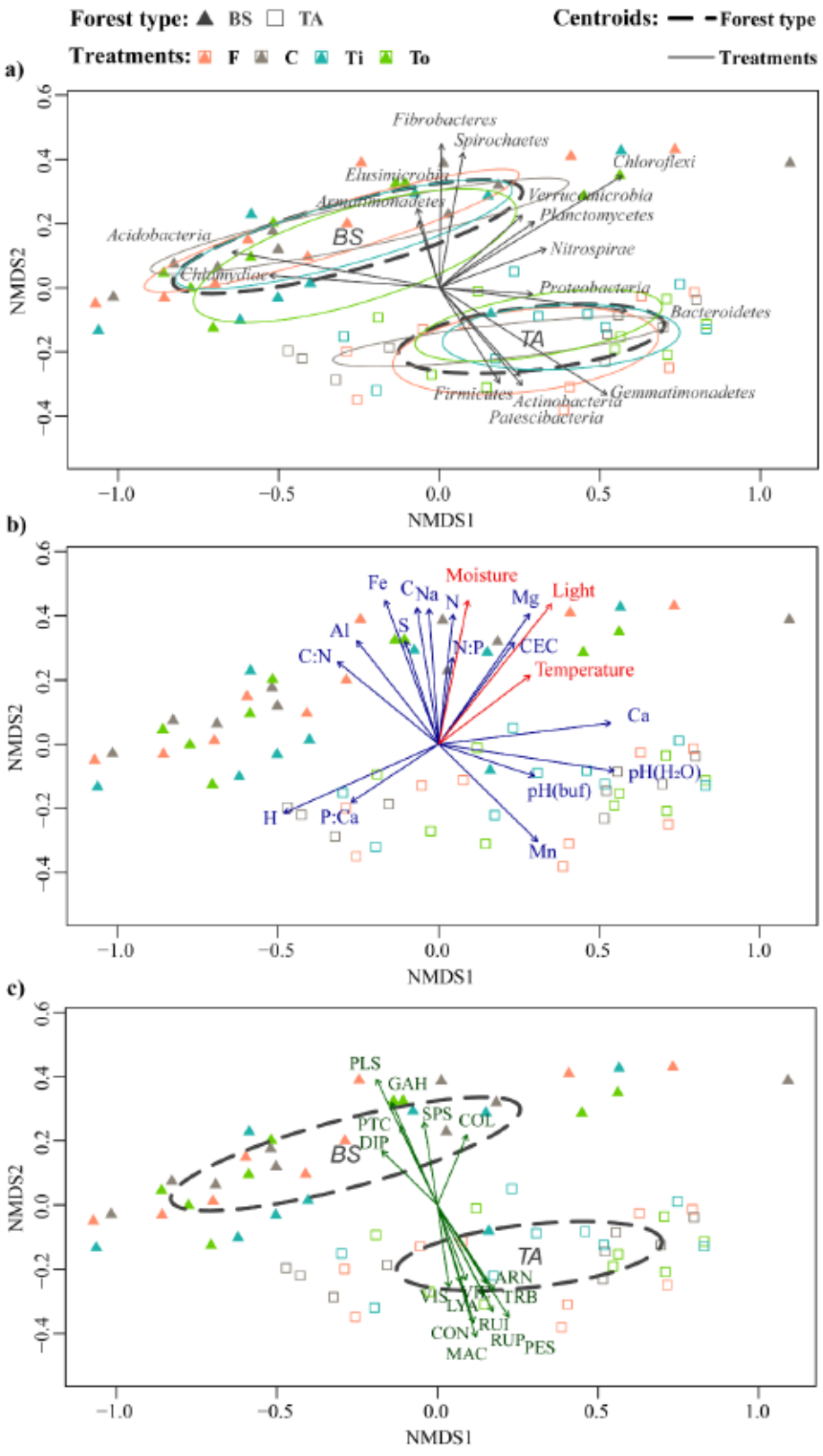
Forest type: BS TA
Centroids: Forest type
Treatments: F C Ti To
Treatments
a)
Fibrobacteres
Spirochaetes
Chloroflexi
Elusimicrobia
Verrucomicrobia
Armatimonadetes
Planctomycetes
Acidobacteria
BS
Nitrospirae
Chlamydiae
Proteobacteria
Bacteroidetes
TA
Firmicutes
Actinobacteria
Gemmatimonadetes
Patescibacteria
NMDS2
NMDS1
b)
Fe
C
Na
N
Moisture
Mg
Light
Al
S
N:P
CEC
C:N
Temperature
Ca
pH(buf)
pH(H2O)
H
P:Ca
Mn
c)
PLS
GAH
SPS
COL
PTC
DIP
BS
TA
ARN
VIS
TRB
LYA
CON
RUI
RUP
PES
MAC

Figure 2.7 Two-dimensional non-metric multidimensional scaling (NMDS) of the relative abundance of bacterial ASVs (Amplicon Sequence Variants) in the soil microbiome from the different treatments in colors: *Control* conditions (C, in grey), *Litter* addition (F, in orange), *Transplants-in* (Ti, in green) and *Transplants-out* (To, in blue), in black spruce (BS, as triangles) and trembling aspen (TA, as squares). Points corresponding to soil microbiome correspond to a total of 72 sampling units (n = 9 per forest type and treatment) and points corresponding to phyllosphere correspond to a total of 54 sampling units (n = 27 per forest type). Ellipses in grey correspond to standard deviation of ordination scores for samples according to the bacterial microhabitat (Soil or Phyllosphere) and forest type (BS or TA). Arrows indicate the correlation between sample level values and ordination axes scores for bacterial phyla added a posteriori in the ordination (displayed phyla of maximum estimated $P < 0.05$). Ordination based on the Bray-Curtis distance of the rarefied and Hellinger transformed ASVs relative abundances.

Soil fungal community composition (Figure 2.8a) was significantly different between forest types in the first axis of the NMDS ordination (PERMANOVA, $R^2 = 0.082$, $F_{1,70} = 6.105$, $P < 0.0001$), but not among treatments or their interaction (Table S 3.12). The second axis of the NMDS ordination was correlated with soil moisture and driven by the presence of *Mucoromycota* and *Ascomycota*, which were only present in the transplants from black spruce to trembling aspen forests. Abiotic factors were significantly correlated with forest type in the first axis (Figure 2.8b), with higher concentration of N, C, Na, H, Al, Fe and C : N ratio in black spruce soils (Envfit , $P < 0.05$, Table S 3.11), whereas a higher concentration of Ca, Mn and soil pH in trembling aspen soils (Envfit , $P < 0.05$, Table S 3.11). Finally, understory vegetation (Figure 2.8c) was also significantly correlated with each forest type (Envfit, $P < 0.05$, Table S 3.11). Black spruce forests were correlated with moss species, such as *Pleurozium schreberi* (PLS), *Ptilium crista-castrensis* (PTC), *Dicranum polysetum* (DIP) and *Sphagnum* spp. (SPS), as well as small plants including *Gaultheria hispidula* (GAH) and *Kalmia angustifolia* (KAA). In contrast, trembling aspen stands were associated with several herbs and shrubs in the understory, including *Rubus pubescens* (RUP), *Petasites frigidus var. palmatus* (PES), *Mitella nuda* (MAC), *Galium asprellum* (GAA), *Aralia nudicaulis* (ARN), *Rubus idaeus* (RUI), *Lysimachia borealis* (TRB),

Cornus canadensis (CON), *Poa* spp. (POA), *Linnaea borealis* (LIB), *Viburnum edule* (VIE), *Viola* spp. (VIS), *Spinulum annotinum* (LYA) and *Clintonia borealis* (CLB). Therefore, forest type was the principal factor shaping soil fungal communities, soil physicochemical properties and plant understory vegetation.

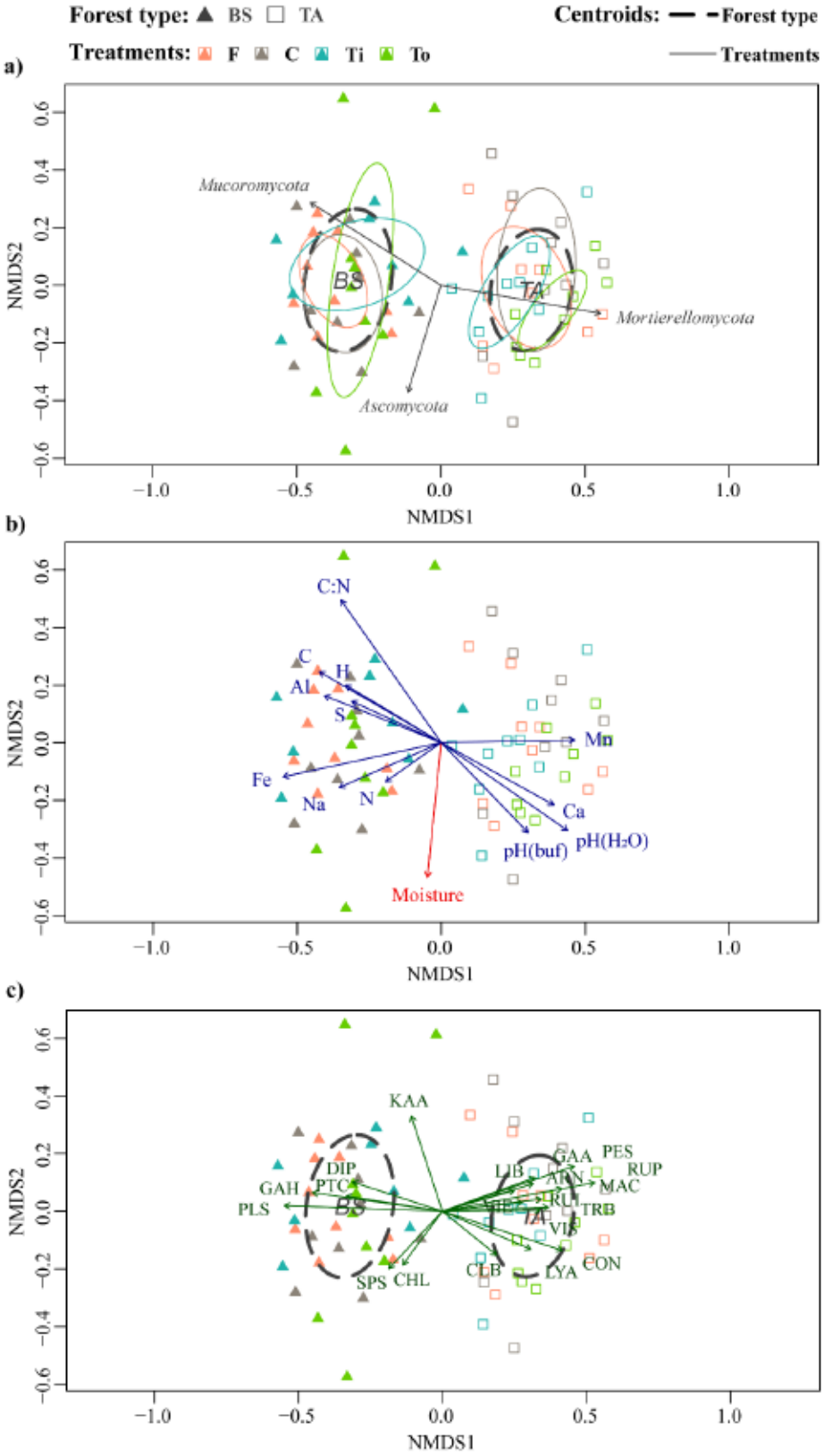
Forest type: BS TA
Centroids: Forest type
Treatments: F C Ti To
Treatments
a)
Mucoromycota
BS
TA
Mortierellomycota
Ascomycota
NMDS2
NMDS1
b)
C:N
C
H
Al
S
Mn
Fe
Na
N
Ca
pH(buf)
pH(H2O)
Moisture
NMDS2
NMDS1
c)
KAA
GAA
PES
DIP
LIB
ARN
RUP
GAH
PTC
MAC
PLS
BS
TRB
TA
VIB
CLB
LYA
CON
SPS
CHL
NMDS2
NMDS1

Figure 2.8 Two-dimensional non-metric multidimensional scaling (NMDS) of the relative abundance of fungal ASVs (Amplicon Sequence Variants) in the soil microbiome from the different treatments in colors: Control conditions (C, in grey), Litter addition (F, in orange), Transplants-in (Ti, in green) and Transplants-out (To, in blue), in black spruce (BS, as triangles) and trembling aspen (TA, as squares). Points corresponding to soil microbiome correspond to a total of 72 sampling units (n = 9 per forest type and treatment) and points corresponding to phyllosphere correspond to a total of 54 sampling units (n = 27 per forest type). Ellipses in grey correspond to standard deviation of ordination scores for samples according to the bacterial microhabitat (Soil or Phyllosphere) and forest type (BS or TA). Arrows indicate the correlation between sample level values and ordination axes scores for bacterial phyla added a posteriori in the ordination (displayed phyla of maximum estimated $P < 0.05$). Ordination based on the Bray-Curtis distance of the rarefied and Hellinger transformed ASVs relative abundances.

2.6 Discussion

2.6.1 Soil bacterial communities differed between microhabitats and forest types

We analyzed the variation in soil bacterial communities between microhabitats of tree phyllosphere vs. soil microbiome, and between forest types of black spruce vs. trembling aspen forests. We found that the diversity and community composition of bacterial communities were influenced by both microhabitat and forest type. Comparing between microhabitats, we found a higher sample-level diversity (Shannon α-diversity) of bacteria in soil microbiome than in tree phyllosphere for both forest types. More than the half of identified bacterial ASVs were exclusively identified in soil microbiomes (~61 % of ASVs) from both forest types, whereas only ~28 % of ASVs were exclusively found in in the phyllosphere of black spruce and trembling aspen trees. Comparing between forest types, black spruce needle leaves had a higher Shannon diversity in their phyllosphere of trembling aspen broad leaves, whereas in soils the bacterial Shannon diversity was higher in trembling aspen than in black spruce forests.

We were expecting a bacterial sharing (co-occurrence) from tree phyllosphere to the soil microbiome in each forest type. However, our results indicate a high microhabitat

specificity within forest types, as only a small percentage (6-7%) of ASVs co-occurred between tree phyllosphere and soil microbiome in both forest types. Although we found major differences in ASVs composition between microhabitats, at the phylum level we observed that the most relatively abundant groups present in soil microbiomes (*Actinobacteria, Proteobacteria, Acidobacteria, Cloroflexi* and *Bacteroidetes*) co-occurred with tree phyllosphere of black spruce and trembling aspen trees, indicating a prevalence of these abundant bacterial phyla in the evaluated boreal forests. Moreover, *Proteobacteria* and *Acidobacteria* were dominant phyla in black spruce needle-leaves, whereas *Actinobacteria, Proteobacteria* and *Bacteroidetes* were dominant in trembling aspen broad leaves, groups that have also been previously associated with the phyllosphere of different dominant trees (Khlifa et al. 2021; Laforest-Lapointe et al. 2016; Mason et al. 2015).

Bacterial community composition (β-diversity) was also structured by both microhabitat and forest type. Bacterial communities in soils were less variable between forest types than the tree phyllosphere, as needleleaf bacterial composition was very different compared to broad leaves. Our results agree with some European studies that found differences in microbial composition among a variety of microhabitats and among deciduous and broadleaf forests. For example, there has been reported differences among spruce needle leaves, roots and soil microbiome (Haas et al. 2018), among litter, roots, rhizosphere and soil of spruce forests (Starke et al. 2021), and among litter and soil microbial communities differing also between coniferous and broadleaf deciduous forests (Urbanová et al. 2015). Soil microbial communities are stable and resistant to local aboveground changes (Hannam et al. 2007), which explain the relatively similar soil microbial community composition within forest types. In contrast to soil microbiomes, bacterial epiphytic communities are exposed to harsh environmental conditions in the tree phyllosphere, such as ultraviolet radiation, low nutrient availability and constant fluctuations in temperature and moisture during a single day (Leveau 2006; Schlechter et al. 2019). Even the bacterial phyllosphere

composition of small moss leaves in the understory is affected by forest type (Rodríguez-Rodríguez et al. 2022c). Furthermore, coniferous trees and broadleaf deciduous tress have a different physiological structure, chemical composition, and other distinct plant traits, that shape their bacterial community composition (Laforest-Lapointe et al. 2016; Lajoie and Kembel 2021a; Leveau 2006). All these factors could explain the higher differences in community composition between phyllosphere of black spruce and trembling aspen, than between soil microbiomes in each forest type.

2.6.2 Factors associated with tree dominance affecting soil microbial communities

We analyzed if soil bacterial and fungal communities were affected by shifts in factors associated with black spruce and trembling aspen forests, including shifts in litter deposition and understory transplantations, and how the microbial composition was correlated with abiotic and biotic conditions of each forest type. We were expecting that treatments of shifts in litter deposition and understory vegetation exchanged between forest types would affect soil bacterial and fungal communities in both forest types. Despite that these same treatments were quite important field manipulations that altered understory vegetation after 5 years (Rodríguez-Rodríguez et al. 2022a), neither treatment had a significant impact on the soil bacterial and fungal Shannon diversity, relative abundance or community composition, but they were resilient to the aboveground changes made by treatments and only shaped by underlying effects of forest type. These results were surprising because litter deposition is likely one of the most important canopy-related factors affecting soil microbial communities, as it is the raw material to be decomposed and contribute with different kinds of nutrients, affecting soil pH and microbial activities (Haas et al. 2018; Laganière et al. 2010). The evaluated treatments were permanent plots, which have been submitted to leaves inputs Also, in a previous study, the transplanted understory vegetation from trembling aspen to black spruce forests remained similar to the original transplanted vegetation after 5 years, instead of becoming similar to the host stand (Rodríguez-Rodríguez et al.

2022a). Consequently, we were expecting a similar pattern for the soil microbial composition, being different as the host stand microbial composition, due to the important effect of understory plants on soil conditions and microbial communities. However, we did not detect effects on soil microbial communities, but they seem to be resistant to shifts in both litter deposition and understory vegetation, which show the importance of legacy effects of each forest type in defining microbial communities. Perhaps we could have observed a stronger effect in soil horizon above the evaluated soil layer, which corresponded to the last organic layer at the transition to the mineral soil. Also, since decomposition could be a long process, perhaps the litter inputs each year could need more time to achieve significant changes in the evaluated soil horizon. However, since these were 5-years permanent plots, we were expecting to observe an effect as it was observed for the plant understory composition (Rodríguez-Rodríguez et al. 2022a). However, our results agree with previous studies comparing spruce and aspen forests, in which shifts in litter inputs and in environmental conditions (fine root biomass, soil moisture, pH, nitrate concentrations and mesofauna abundance) did not significantly affected soil microbial communities but instead, there was a strong legacy effect of the forest type (Hannam et al. 2007; Norris et al. 2016). However, a substantial disruption in the forest floor, such as forest harvesting, can have a strong negative effect on soil microbial communities, specially for fungal more than bacterial communities, even after 15 years (Hartmann et al. 2012). Therefore, microbial communities seem to be resistant to aboveground changes, such as shifts in litter type and understory vegetation, but stronger disturbances in the forest floor (e.g. forest harvesting) or changes in tree composition can affect microbial communities.

The strong influence of forest type on soil microbial community composition found in our study agree with previous studies between coniferous and broadleaf deciduous forests (Hannam et al. 2006; Nagati et al. 2018; Norris et al. 2016; Urbanová et al. 2015). We observed that forest type had a stronger effect on fungal than bacterial communities in soil, which seems to be also a trend in other studies (Chen et al. 2019;

Urbanová et al. 2015). Tree dominance of these forest types influence local conditions in the understory by affecting soil nutrient dynamics and decomposition processes (Augusto et al. 2015). Despite that the study sites had adjacent stands dominated by each forest type, with comparable soil abiotic conditions and topography, we found differences in soil physicochemical properties between forest types that were correlated with a different microbial community structure. Our results of the soil pH and chemical concentrations for both forest types agree with results of Nagati et al. (2020) from the same study sites. Forest type of broadleaf and coniferous trees, soil pH and base cation content have been considered to be main factors associated with differences in soil microbial communities (Prescott and Grayston 2013). Therefore, the influence of different factors associated with tree dominance, such as soil physicochemical properties, affects the composition of both bacterial and fungal communities.

We found differences of our results among microbial groups. Soil fungi had a lower sample-level diversity (Shannon α-diversity) than soil bacteria, a similar pattern reported in Urbanová et al. (2015) for European soils associated with different coniferous and broadleaf trees. Only three fungal phyla significantly influenced the community composition (*i.e. Ascomycota, Mortierellomycota* and *Mucoromycota*), whereas several groups of the soil bacterial community composition were significantly associated with each forest type, which included the relative abundant *Actinobacteria, Proteobacteria* and *Acidobacteria*. The differences in bacterial and fungal communities can be an indicator of forest conditions, as each microbial group carries out different ecological functions and affects the physicochemical conditions in the forest floor (Augusto et al. 2015; Van Der Heijden et al. 2008). However, the presence of the identified microbial taxa could be further analyzed in other studies to know how these microbial groups vary across seasons and which ecological functions they carry out in these boreal ecosystems, for example through metaproteomics, as it has been recently evaluated for temperate coniferous forests (Starke et al. 2021).

The relative importance of the evaluated factors associated with tree dominance, including forest type, treatments (shifts in litter deposition and plant understory), abiotic factors (local environmental conditions and physicochemical properties) and biotic factors (plant understory composition) was different for bacteria and fungal communities. Forest type was important in defining the bacterial community composition and soil physicochemical properties were also explaining part of the variance of soil bacterial communities. Several soil characteristics, including soil pH, organic carbon, soil (O2) and redox status are considered important factors in structuring soil bacterial communities (Fierer 2017; Rousk et al. 2010). Our results indicate that soil pH, Ca and H seem to be associated with the main variation in the bacterial composition. The higher relative abundance of *Acidobacteria* in the more acid black spruce soils compared to trembling aspen soils are consistent with other studies highlighting the presence of this phyla in low-pH soils (Rousk et al. 2010; Urbanová et al. 2015). Several elements were also strongly associated with the bacterial community composition and differed in concentration between forest types. In black spruce soils, we found higher levels of N, C, Al, Fe, H, S, CEC and C : N and N : P ratios, as well as higher levels of soil moisture and light inputs. In contrast, trembling aspen soils had higher levels of pH, Ca, Mn and P : Ca ratio. Finally, the understory vegetation was also correlated with the bacterial community composition in each forest type, with several moss species and ericaceous plants in black spruce forests, whereas a high diversity of herbs and shrubs in trembling aspen forests, as previously reported (Rodríguez-Rodríguez et al. 2022a). However, it is the combined effect of all factors that explain 22% of the variation of soil bacterial communities, and the half of the variation was explained by the evaluated factors.

Regarding the soil fungal community composition, forest type had the strongest effect, followed by abiotic factors and understory vegetation. This agree with previous studies in which the effect of tree composition was more important than soil properties, explaining a large proportion of the fungal community structure (Urbanová et al. 2015).

Also, in previous articles from the same study sites, the fungal composition varied between black spruce and trembling aspen forests (Ghotsa Mekontchou et al. 2022; Nagati et al. 2018). While the variability in the fungal community composition was mainly explained by forest type, soil moisture and the composition of either *Mucoromycota* or *Ascomycota* seem influence the variation of fungal communities. Both soil chemical properties and understory vegetation were associated with the fungal community composition in each forest type. In black spruce forests, we found higher levels of N, C, Na, H, Al, Fe and C : N ratio with a plant composition of mosses and ericaceous plants, whereas in trembling aspen forests, the levels of pH, Ca and Mn were higher and the understory composition was composed by diverse herbs and shrubs. However, 72% of the variation of fungal communities was not explained by the evaluated factors but remained to be elucidated.

As a conclusion, this study expands our knowledge of the microbial composition of tree phyllosphere and soil microbial communities in black spruce and trembling aspen forests and the correlation with abiotic and biotic factors in each forest type. Our study demonstrates a legacy effect of forest type in shaping soil microbial communities that were resistant to aboveground changes of litter inputs and understory vegetation. Considering the strong effect of forest type in defining the soil microbial community composition, it is to be further analyzed how increasing changes in tree dominance of different regions in the boreal system (Danneyrolles et al. 2019; Mack et al. 2021; Marchais et al. 2020) may affect soil microbial communities and their associated ecological functions.

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CHAPITRE 3

DOMINANCE OF CONIFEROUS AND BROADLEAVED TREES DRIVES BACTERIAL ASSOCIATIONS WITH BOREAL FEATHER MOSSES

LA DOMINANCE DES CONIFÈRES ET DES FEUILLUS CONTRÔLE LES ASSOCIATIONS BACTÉRIENNES AVEC LES MOUSSES HYPNACÉES BORÉALES

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3.1 Abstract

The composition of ecologically important moss-associated bacterial communities seems to be mainly driven by host species but may also be shaped by environmental conditions related with tree dominance. The moss phyllosphere has been studied in coniferous forests while broadleaf forests remain understudied. To determine if host species or environmental conditions defined by tree dominance drives the bacterial diversity in the moss phyllosphere, we used 16S rRNA gene amplicon sequencing to quantify changes in bacterial communities as a function of host species (*Pleurozium schreberi* and *Ptilium crista-castrensis*) and forest type (coniferous black spruce versus deciduous broadleaf trembling aspen) in eastern Canada. The overall composition of moss phyllosphere was defined by the interaction of both factors, though most of bacterial phyla were determined by a strong effect of forest type. Bacterial α-diversity was highest in spruce forests, while there was greater turnover (β-diversity) and higher γ-diversity in aspen forests. Unexpectedly, Cyanobacteria were much more relatively abundant in aspen than in spruce forests, with the cyanobacteria family *Nostocaceae* differing the most between forest types. Our results advance the understanding of moss-associated microbial communities among coniferous and broadleaf deciduous forests, which are important with the increasing changes in tree dominance in the boreal system.

Key words: Boreal forest, bryophytes, microbial ecology, microbiome, phyllosphere, *Picea mariana* (black spruce), plant-microbial interactions, *Populus tremuloides* (Trembling aspen).

3.2 Résumé

La composition des communautés bactériennes associées aux mousses est importante sur le plan écologique et semble être principalement déterminée par les espèces hôtes, bien qu'elle peut également être contrôlée par les conditions environnementales liées à la dominance de la canopée des arbres. La phyllosphère des mousses a surtout été étudiée dans les forêts de conifères alors que les forêts de feuillus restent peu étudiées. Pour déterminer si l'espèce hôte ou les conditions environnementales définies par la dominance de la canopée des arbres déterminent la diversité bactérienne dans la phyllosphère des mousses, nous avons utilisé le séquençage des amplicons du gène de l'ARNr 16S pour quantifier les changements dans les communautés bactériennes en fonction de l'espèce hôte (*Pleurozium schreberi et Ptilium crista-castrensis*) et du type de forêt (épinette noire et peuplier faux-tremble) dans l'est du Canada. La composition globale de la phyllosphère des mousses a été définie par l'interaction de ces deux facteurs, bien que la plupart des phyla bactériens aient été déterminés par un fort effet du type de forêt. La diversité alpha de bactéries était plus élevée dans les forêts d'épinettes noires, tandis qu'il y avait un plus grand renouvellement d'espèces (diversité beta) et une diversité gamma plus élevée dans les forêts de peupliers faux-trembles. Contrairement aux attentes, les cyanobactéries étaient beaucoup plus abondantes dans les forêts de peupliers faux-trembles que dans les forêts d'épinettes noires, la famille de cyanobactéries *Nostocaceae* différant le plus entre les deux types de forêts. Nos résultats permettent de mieux comprendre les communautés microbiennes associées aux mousses dans les forêts de conifères et de feuillus, ce qui est important compte tenu des changements croissants de la dominance des arbres dans le système boréal.

Mots clés : Forêt boréale, bryophytes, écologie microbienne, microbiome, phyllosphere, *Picea mariana* (Épinette noire), interactions plantes-microorganismes, *Populus tremuloides* (Peuplier faux-tremble).

3.3 Introduction

Mosses are an important part of the boreal forest, in terms of cover area, biomass and diversity, particularly in coniferous forests (Nilsson and Wardle, 2005; Lindo et al., 2013). Studies have demonstrated the ecosystem functions of bryophytes (Lindo and Gonzalez, 2010), which contribute to the resilience of boreal and arctic ecosystems (Turetsky et al., 2012), to ecosystem succession (Turetsky et al., 2010), to methane oxidation by moss-associated bacteria (Kip et al., 2010), and to carbon and nitrogen cycling (DeLuca et al., 2002; Turetsky, 2003). Bryophytes harbour a variety of bacterial taxa in their phyllosphere (Holland-Moritz et al., 2018). The phyllosphere refers to the microbial habitat present in aboveground plant surfaces mainly dominated by leaves of vascular plants (Vorholt, 2012), equivalent to the whole gametophyte (leaves and stem) in mosses. A dominant role of the moss phyllosphere microorganisms is their significant contribution to nitrogen (N) inputs by associated diazotrophic bacteria (Rousk et al., 2013a), which fix up to 7 kg N ha^{-1} yr^{-1} in boreal ecosystems (DeLuca et al., 2007; Lindo et al., 2013). There are numerous diazotrophic bacterial taxa (Holland-Moritz et al., 2021), but among these, the Cyanobacteria are the most studied and diverse group, with all members of the phylum being diazotrophs, and are strongly associated with bryophytes (Adams and Duggan, 2008; Rousk et al., 2013a).

Bacterial community composition in the moss phyllosphere seems to be mainly driven by host species (Opelt et al., 2007; Bragina et al., 2012; Holland-Moritz et al., 2021). In particular, moss-associated cyanobacteria as well as their N_2-fixation rates seem to be host-specific (Holland-Moritz et al., 2018; Stuart et al., 2020), and do not seem to vary with environmental conditions, such as nutrient availability, soil moisture, or light availability (Ininbergs et al., 2011). However, other studies have suggested that bacterial community composition can vary among forest types for a given moss species (Wang et al., 2018; Jean et al., 2020; Holland-Moritz et al., 2021), as does bacterial diversity in other habitats including soil, litter and the tree phyllosphere (Redford et al.,

2010; Kembel et al., 2014; Urbanová et al., 2015). Furthermore, moss-associated bacterial communities can be shaped by different environmental conditions defined by the tree dominance that influences the availability of nutrients, such as nitrogen. For example, *Pleurozium schreberi* is colonized by cyanobacteria in forests with low N deposition (DeLuca et al., 2007; Ackermann et al., 2012; Rousk et al., 2013b) and even relatively low rates of N deposition can supress N_2-fixation (Salemaa et al., 2019). Also, when N is limited, feather mosses secrete species-specific chemo-attractants to induce the association with cyanobacteria in order to fulfill their N requirements (Bay et al., 2013). In this sense, the presumed low-N black spruce stands seem to favor cyanobacterial-moss associations (DeLuca et al., 2008; Bay et al., 2013; Salemaa et al., 2019), whereas the deciduous broadleaf litter of nutrient rich trembling aspen stands seems to have a negative effect on cyanobacteria (Jean et al., 2020). However, comparisons among forest types have been limited in scope and geography, as most studies have focused on in homogeneous and nutrient-poor coniferous forests, while heterogeneous and nutrient-rich broadleaf forests have been less studied. Therefore, moss associated bacterial communities seem to be driven by both host species and environmental conditions related to forest type (Jean et al., 2020; Holland-Moritz et al., 2021).

In recent years, the boreal system dominated by coniferous forests has been changing with an increasing proportion of broadleaf forests due to various disturbances including natural fires and human activities (Danneyrolles et al., 2019; Marchais et al., 2020; Mack et al., 2021). Likewise, effects of global warming that could influence shifts in tree composition from coniferous to broadleaved forests (Boisvert-Marsh et al., 2014) could also affect nitrogen fixation rates by microorganisms associated with bryophytes because of possible changes in temperature, light and humidity (Gundale et al., 2012; Whiteley and Gonzalez, 2016; Salemaa et al., 2019). The coniferous boreal landscape in Quebec is frequently dominated by black spruce trees (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.), with some areas dominated by broadleaf trees such as

trembling aspen (*Populus tremuloides* Michx.). Black spruce stands have a thick layer of bryophytes dominated by the feather mosses *Pleurozium schreberi* (Willd. ex Brid.) Mitt. and *Ptilium crista-castrensis* (Hedw.) De Not., and a few vascular plants (such as ericaceous plants and small herbs), which result in acid soils with low decomposition rates and N-limited conditions (Barbier et al., 2008; Cavard et al., 2011; Högberg et al., 2017). In contrast, trembling aspen stands have a more diverse understory with several shrubs, herbs and bryophytes that promote nutrient cycling (Légaré et al., 2001; Cavard et al., 2011). Consequently, coniferous and broadleaf forests not only differ in nutrient availability but also in local environmental conditions, including light, soil moisture and the composition of understory vegetation (Barbier et al., 2008; Cavard et al., 2011), which should in turn influence the microconditions experienced by bryophytes and their associated microbial communities.

Considering that changes in tree composition could affect moss-associated microbial communities and particularly the epiphytic bacteria being exposed to habitat changes, we quantified differences in microbial communities as a function of host species (*Pleurozium schreberi* and *Ptilium crista-castrensis*) and forest type (black spruce and trembling aspen) in boreal forests of eastern Canada. These two feather mosses are the most abundant bryophytes in both forest types and they co-occur at small spatial scales in the understory which make them interesting to measure both microbial host specificity and forest type effects. We hypothesized that host species will have the greatest effect of bacterial community composition while forest type will have a secondary effect.

3.4 Methods

3.4.1 Study area and sampling design

The three study sites were located in the Eastern Boreal Shield of Canada, in the spruce-moss forest domain of the Clay Belt in western Quebec (Figure 3.1, ArcGIS v.10.5 -

ESRI, 2016). All three sites (Site A: *49°11'46" N - 78°50'33" W*; Site B: *49°09'20" N - 78°47'56" W* and Site C: *49°09'39' 'N - 78°47'55" W*) had comparable abiotic conditions (surface deposit, gentle slope, moderate drainage and soil type) as described in previous studies (Légaré et al., 2005; Laganière et al., 2010; Cavard et al., 2011). Sites B and C were 0.5 km apart and Site A was 5.3 km away. These forests were initiated by the same wildfire in 1916 (Bergeron et al., 2004; Légaré et al., 2005). This disturbance produced in each site two adjacent stands with a different tree dominance of black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) and *trembling aspen* (*Populus tremuloides* Michx.), each representing ≥ 75 % of their canopy cover. Stands were around 1 ha in size and were separated from 43 to 121 m. Hence, each site had both forest types and within each forest type we placed two blocks separated from 16 to 41 m. Within each block, six spots with at least 1 m apart with abundant feather-mosses (*Pleurozium schreberi* (Willd. ex Brid.) Mitt. and *Ptilium crista-castrensis* (Hedw.) De Not.) were randomly chosen to collect moss monospecific samples for phyllosphere extraction, with a nested block experimental design (3 sites * 2 forest types * 2 blocks *2 moss species * 6 samples, for a total of 144 samples, Figure 3.1). We selected these two feather mosses as they are the most abundant in both forest types, co-occur in the understory and were distributed in generally monospecific colonies than ranged in size from 400 cm^2 to several m^2, which makes it possible to measure both microbial host specificity and forest type effects. We focused on the epiphytic bacteria associated with the moss phyllosphere, sampling the top 3 cm of each feather-moss shoot to avoid decomposing parts mixed with soil and collect approximately the same biomass for each sample. Large debris and other plants were manually removed and a total of 15 shoots of mosses from a single sample were placed into sterile falcon tubes (Holland-Moritz et al., 2018). Samples were collected within one week, in July 2018. Samples were immediately stored in a cooler on ice at approximately 4 ºC and then transferred to a -20 ºC freezer before further analysis (Holland-Moritz et al., 2018).

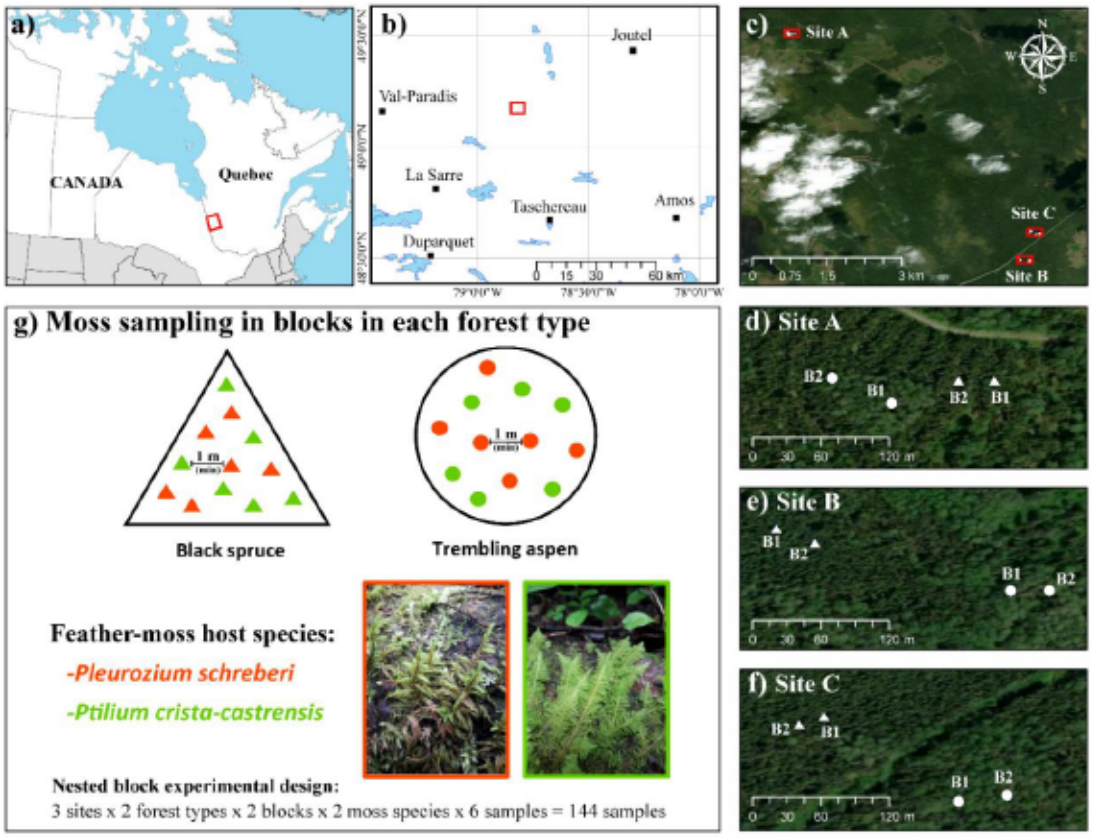

Figure 3.1 The study area is located in a) the boreal forest of eastern Canada, b) within western Quebec above the parallel 49ºN. c) The sampling design comprise three sites A, B and C (Sites B and C are 0.5 km apart and Site A is 5.3 km away). d, e, f) Each site has adjacent stands dominated by black spruce (as triangles) and trembling aspen (as circles), separated from 43 to 121 m; and within each forest type, two blocks (B1 and B2) were placed separated from 16 to 41 m. g) Six samples of each feather moss (*Pleurozium schreberi*, in orange and *Ptilium crista-castrensis*, in green) were randomly taken at least 1 m apart, for bacterial DNA extraction.

3.4.2 Sample preparation, DNA extraction and sequencing

Epiphytic microorganisms in the phyllosphere were extracted from the moss leaves of the 144 samples, following a modified version of existing protocols (Kembel et al., 2014). We targeted epiphytic bacteria to allow the use of universal 16S primers that amplify chloroplast DNA and thus make it difficult to obtain sufficient bacterial sequences if we were extracting endophytic bacteria from whole shoot samples, which

would contain large quantities of chloroplast and thus host plant DNA, as well as to make it possible to compare our results with data of a broader project using the same protocols to quantify soil and vascular plant-associated microbes. Therefore, each sample was mixed with 25 ml of washing solution (1:50 diluted Redford Buffer: 1 M Tris·HCl, 0.5 M Na EDTA, and 1.2 % CTAB) (Kadivar and Stapleton, 2003) and agitated for 5 min to extract the epiphytic microbes. Then, mosses were carefully removed with sterile metal forceps. Samples were centrifuged (3.900 × g, 5 min, 4 °C) and the supernatant was discarded by pipetting. The pellet with microbial cells was resuspended in 500 µL PowerSoil bead solution and up to 1ml was placed in the Powerbead tube to continue with DNA extraction with the PowerSoil DNA Kit (QIAGEN). During the DNA extraction, one negative extraction control per kit was processed and sequenced following exactly the same extraction conditions. Samples were stored at -80 °C prior to sequencing.

Samples were PCR-amplified with the universal bacterial 16S primers 515F/926R (515F: GTGYCAGCMGCCGCGGTAA - 926R: CCGYCAATTYMTTTRAGTTT) from the region V4-V5 of the bacterial 16S rRNA gene (Parada et al., 2016), a bacterial taxonomic barcode gene present in all bacteria (Callahan et al., 2016b). This primer was chosen in order to identify all bacterial DNA, including cyanobacteria. The one-step PCR contained the specific sequence for amplification, dual indexes and motifs (Nextera adapters) required for Miseq sequencing. The PCR was performed in a 25 µL mixture containing 1 µL of DNA extract, 0.2 µM of each primer, 0.5 U of Phusion Hot Start II High-Fidelity DNA Polymerase (ThermoFisher), 1X of Phusion HF Buffer, 0.2mM of dNTPs, 3% DMSO following the thermal cycling conditions at 98 °C (30 s and 15 s x 30), 50°C (30 s), 72°C (30 s and 10 min) and 4 °C and hold; the resulting amplicons contained all the motifs needed for sequencing and thus there was no need for a second barcoding step. After amplification, purification and normalization, samples were sequenced in a single run with the MiSeq paired-end 2 x 300 base pair, v3 Kit (600-cycles, Illumina reference MS-102-3003) at the UQAM CERMO-FC

Genomics Platform. Positive controls (ZymoBIOMICS™ Microbial Community Standard) and negative controls (DNA replaced by sterile water) were included for both PCR amplification and sequencing, in order to identify potential contaminants and verify sequencing run quality. A total of 149 samples were sequenced (144 samples of moss phyllosphere, 3 negative extraction controls, one negative and one positive PCR control), to identify bacteria and the relative abundance of each taxon with respect to other identified bacterial taxa.

3.4.3 Bioinformatic analysis of bacterial communities

We analyzed the complete bacterial composition in the moss phyllosphere based on the universal primer to quantify variation in the different bacterial taxa associated with feather mosses that inhabit each forest type. High-throughput Illumina sequencing produced a total of 4,297,585 reads. The bioinformatic analysis of microbial community data was carried out using the amplicon sequence variant (ASV) approach in the DADA2 package version 1.6 (Callahan et al., 2016a; Callahan et al., 2016b) in R software, version 3.6.0 (R Core Development Team, 2019).

We followed the DADA2 sequence processing workflow (Callahan et al., 2016b) with default parameters except as noted. In the trimming and filtering process, the first 20 nucleotides from the beginning of both forward and reverse reads were trimmed to eliminate primers. Reads were truncated where quality decreased sharply at positions 270 (forward reads) and 220 (reverse reads) with maxEE setting of 2. We inferred amplicon sequence variants (ASVs) using a pseudo-pooling approach. Then, we merged forward and reverse reads, obtaining a total of 30,463 ASVs. We then removed chimeric ASVs using the consensus method, resulting in 10,870 non-chimeric ASVs that were used for subsequent analyses. We assigned ASV taxonomic identity from phylum to genus using the RDP Naive Bayesian Classifier algorithm method (Wang et al., 2007) with the SILVA version 132 database (www.arb-silva.de), and assigned species-level taxonomy with the RefSeq + RDP database (NCBI RefSeq 16S rRNA

database supplemented by RDP, Sousa, 2019) to obtain more accurate species assignment.

Taxonomically annotated ASV data were analyzed with the phyloseq R package (McMurdie and Holmes, 2013). The ASVs initial table contained 10,870 ASVs from 149 samples (144 from mosses and 5 controls) with a total of 1,423,140 sequences. ASVs annotated as originating from plant chloroplasts were filtered out leaving only sequences annotated as belonging to the kingdom Bacteria for a total of 9409 taxa and 1,177,237 sequences. The composition of positive control samples was completely different from the evaluated samples according to a PCA ordination. Furthermore, none of the negative controls contained more than 129 sequences and were also different from all samples in the ordination. Therefore, all positive and negative control samples were excluded prior to rarefaction.

We selected all ASVs that occurred in at least 2 samples and with a minimum total abundance of 10 sequences, regardless of sample origin. This led to the exclusion of 5695 ASVs. We selected samples with at least 1920 sequences for subsequent analysis, and we randomly rarefied to 1920 sequences per sample. threshold was chosen to maximize the number of samples included in the analysis. This rarefaction cutoff was sufficient to capture the vast majority of ASVs in samples (Figure S 3.6), leaving a total of 276,480 sequences from 3694 ASVs in 144 samples after rarefaction.

3.4.4 Statistical analysis

Statistical analysis were conducted using R version 3.6.0 (R Core Development Team, 2019), with data visualization using ggplot package, version 3.3.0 (Wickham et al., 2016). The nested design of the experiment was taken into account by using mixed models throughout. Differences were considered statistically significant for all tests if $P < 0.05$. To evaluate differences in the structure of both feather-moss phyllosphere communities between forest types, we performed a non-metric multidimensional

scaling (NMDS) of bacterial ASV relative abundances based on rarefied data that were Hellinger transformed and based on the Bray-Curtis dissimilarities using the vegan package (version 2.5-7). To evaluate the effect of forest type and host species on the total multidimensional variation, we performed a permutational multivariate analysis of variance (PERMANOVA) on Bray-Curtis dissimilarities based on rarefied and Hellinger-transformed data and using sites as strata variable and 9999 permutations. We also used a β-dispersion test (multivariate homogeneity of groups dispersions from vegan package) on the Bray-Curtis dissimilarities of ASVs to assess differences in β-diversity between forest types. Furthermore, to determine if there were differences in α-diversity of phyllosphere communities among forest types, we performed a linear mixed-effects model (nlme package, version 3.1-147) of bacterial relative abundances between forest types and host species, selecting the Shannon index with a $P < 0.05$, with "Site" and "Block" as random variables, to determine significant differences by the anova function (stats package, version 3.6.0). We also calculated the total ASV richness (γ-diversity) for each forest type.

We also analyzed the relative abundance of each bacterial phyla between forest types and host species, based on the rarefied data, with a generalized linear mixed model using Template Model Builder (glmmTMB package, version 1.1.2.3; Brooks et al., 2017) with a negative binomial family (quadratic parameterization) (Hardin and Hilbe, 2018). When a model for a phyla failed to converge, we used the glmmTMB optimization (glmmTMBControl function) with BFGS method (Broyden–Fletcher–Goldfarb–Shanno algorithm) for optimization from the same package. From each model per phyla, significant differences of all contrasts of forest types and host species were tested using emmeans package, version 1.6.2-1 with pairwise Tukey test ($P < 0.0001$***, $P < 0.001$**, $P < 0.05$*). Furthermore, the overall relative abundance of bacterial phyla in forest types and on moss species were analyzed based on the rarefied data and were presented separately to evaluate significant differences using a linear model of log10 of bacterial relative abundances (function lm of stats4 package, version

3.6.0), with a cut-off of adjusted $P < 0.05$ using the method of Benjamini and Hochberg (1995) to correct for multiple hypothesis testing. Finally, in order to identify the bacterial ASVs differentially associated with each forest type, we performed an analysis of Differential Abundance for Microbiome Data (DESeq2) (Love et al., 2014), based on the pseudocount-transformed non-rarefied ASV abundances (McMurdie and Holmes, 2014). In a ggplot2 figure, the DESeq2 results are sorted by the by the average log2-fold change of ASVs relative abundance that are significantly different (Benjamini–Hochberg-adjusted $P < 0.05$) between trembling aspen (positive log-fold change values) and black spruce forests (negative log-fold change values), and that are grouped by family on the x-axis and colored by phylum.

3.5 Results

The moss-associated bacterial community composition based on the ASV relative abundance (Figure 3.2 and Table 3.1) was significantly affected by the interaction of forest type and host species (PERMANOVA, $R^2 = 0.0145$, $P = 0.0038$). However, when analyzing differences in relative abundance of ASVs assigned to the different phyla (Figure 3.3 and Table S 3.13), only *Actinobacteria* and *Proteobacteria* had a significant interaction between forest type and host species. In contrast, most of phyla were affected by forest type or had very low relative abundances. *Acidobacteria* and *Verrucomicrobia* were only significantly influenced by forest type, whereas *Armatimonadetes, Bacteroidetes, Cyanobacteria* and *Planctomycetes* were significantly influenced by both forest type and host species independently. None was exclusively determined by host species and the phyla *Chlamidiae, Chloroflexi, Deinococcus-Thermus, Dependentiae, Firmicutes, Patescibacteria* and *Tenericutes* had very low relative abundances in both feather mosses and forest types. Finally, the most relative abundant ASVs assigned to phyla were *Proteobacteria* and *Acidobacteria*, which were higher in black spruce than in trembling aspen stands, as well as *Bacterioidetes* and *Cyanobacteria*, which were less abundant in black spruce

than in trembling stands. Thus, we found significant differences between forest types and host species varying depending on bacterial phyla (Figure 3.3 and Table S 3.14 - Tukey contrasts, $P < 0.05$). Most of the bacterial phyla followed the same direction pattern of differences between forest types for both mosses. Only *Actinobacteria* presented a different direction of the pattern, with a higher relative abundance of bacteria associated with *P. crista-castrensis* in black spruce than in trembling aspen forests, whereas the opposite pattern direction for bacteria associated with *P. schreberi*, with higher abundance in trembling aspen than in black spruce forests. In terms of the overall relative abundance of bacterial phyla associated with both feather mosses (Figure S 3.7-a), black spruce forests had a higher relative abundance of *Proteobacteria* and *Actinobacteria* than trembling aspen forests, whereas the relative abundance of *Bacteroidetes* and *Cyanobacteria* was higher in trembling aspen than in black spruce forests. These four bacterial groups were the most relative abundant in the moss phyllosphere of both boreal forest types (Figure S 3.7-b).

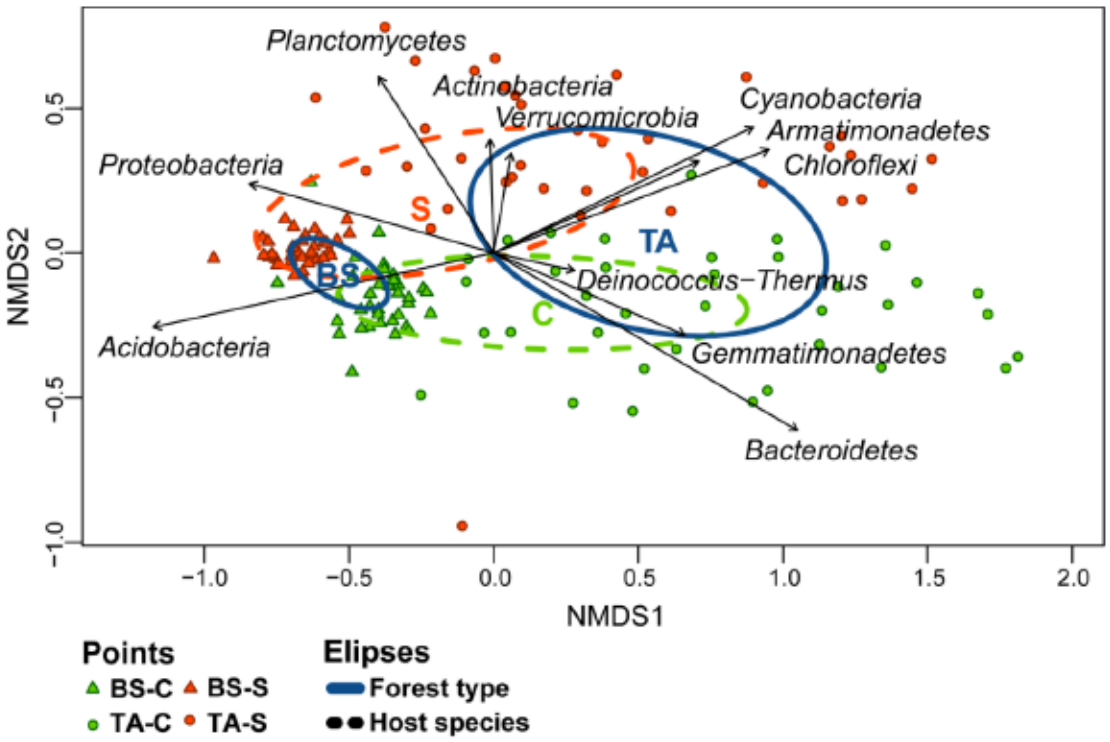

Figure 3.2 Two-dimensional non-metric multidimensional scaling (NMDS) of relative abundance of feather-moss phyllosphere bacterial ASVs (amplicon sequence variants) in each forest type dominated by black spruce (BS as triangles) or trembling aspen (TA as circles), using Bray-Curtis dissimilarities. Colors correspond to host species of *Pleurozium schreberi* (S in orange) and *Ptilium crista-castrensis* (C in green). Points correspond to a total of 144 sampling units (n = 36 per forest type and moss species). Ellipses correspond to standard deviation of ordination scores for samples according to forest type (BS or TA, as solid lines in blue) and host species (C in green or S in orange, as dashed lines). Arrows indicate the correlation between sample level relative abundances and ordination axes scores for bacterial phyla added a posteriori to the ordination (only phyla with $P < 0.05$ were included).

Table 3.1 Relative importance of forest type (black spruce and trembling aspen) and host species (*Pleurozium schreberi* and *Ptilium crista-castrensis*) as factors affecting moss-associated bacterial communities. PERMANOVA results on Bray-Curtis dissimilarities of the Hellinger transformed bacterial relative abundances, using Site as random factor. DF, degrees of freedom; DF_{den}, denominator degrees of freedom. Statistically significant values are indicated in bold text.

	DF	DF_{den}	Size effect	*F*	R^2	*P*
Forest type	1	143	5.843	31.7361	0.1705	**0.0001** ***
Host species	1	143	2.158	11.7196	0.0630	**0.0001** ***
Interaction	1	143	0.498	2.7031	0.0145	**0.0038** **
Residual	140	143	25.776		0.7521	

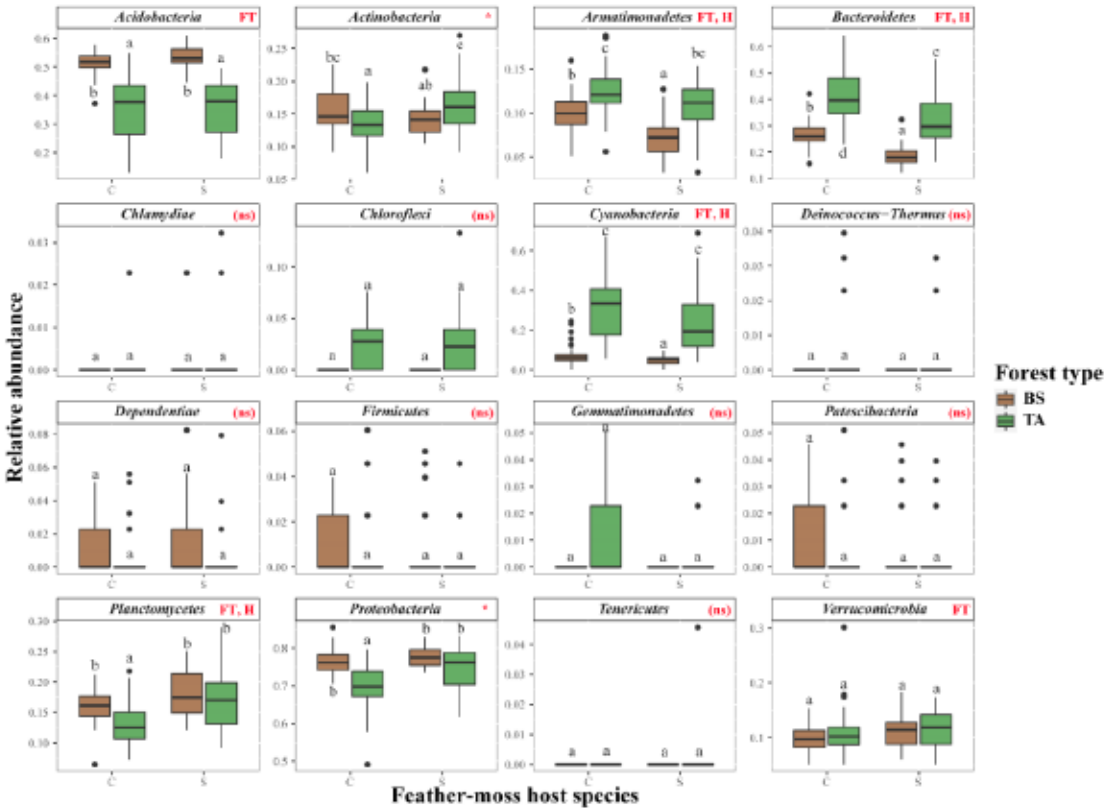

Figure 3.3 Differences in relative abundance of each bacterial phyla between forest types (black spruce – BS and trembling aspen – TA) and host species (*Pleurozium schreberi* – S and *Ptilium crista-castrensis* – C). Analysis of relative abundances of each phyla are based on rarefied data and the generalized linear mixed model using Template Model Builder (glmmTMB) with a negative binomial family (quadratic parameterization). From each model, significant differences between forest types (FT),

host species (H), the interaction of both factors (I) or not significant differences (ns) are indicated in red for each phylum (Table S 3.13). Significant differences of contrasts for each phyllum (emmeans, pairwise Tukey test, $P < 0.05$) as letters (Table S 3.14).

Also, the β-diversity of moss-associated bacterial communities corresponding to the dispersion of ASVs in the ordination was different between forest types (β-dispersion test, $F = 209.28$, $P < 0.001$), with a higher compositional difference among samples within trembling aspen (β-dispersion test, average distance to median = 0.528) than within black spruce (β-dispersion test, average distance to median = 0.399), indicating that mosses in black spruce forests hosted more homogeneous bacterial communities than those in trembling aspen forests. Furthermore, the α-diversity of the bacterial communities was significantly different between forest types (ANOVA of the linear mixed model of bacterial relative abundances based on Shannon index, $F = 9.08$, $P < 0.005$), as they were slightly higher in black spruce than in trembling aspen forests (Tukey test, $P < 0.005$; lsmeans = 5.38 and 5.21, respectively) (Tukey test, $P < 0.005$). In contrast, host species (ANOVA of the linear mixed model of bacterial relative abundances based on Shannon index, $F = 0.41$, $P = 0.5253$) and the interaction (ANOVA of the linear mixed model of bacterial relative abundances based on Shannon index, $F = 2.50$, $P = 0.1160$) did not have effects on bacterial diversity. Finally, the γ-diversity was higher in trembling aspen (3340 ASVs) than in black spruce stands (2436 ASVs).

We identified numerous bacterial ASVs that were differentially abundant in trembling aspen versus black spruce forests (Figure 3.4). The ASVs that were most strongly associated with trembling aspen forests were identified as belonging to the *Nostocaceae* family of *Cyanobacteria* and to *Chitinophagaceae* family of *Bacterioidetes* (DESeq2, all Benjamini–Hochberg-adjusted $P < 0.05$). In contrast, ASVs belonging to *Acidobacteriaceae* (from *Actinobacteria* phylum) and *Acetobacteriaceae* (from *Proteobacteria* phylum) had a stronger association with black

spruce forests, among which diazotrophic groups has been identified (Maier et al., 2018; Holland-Moritz et al., 2021).

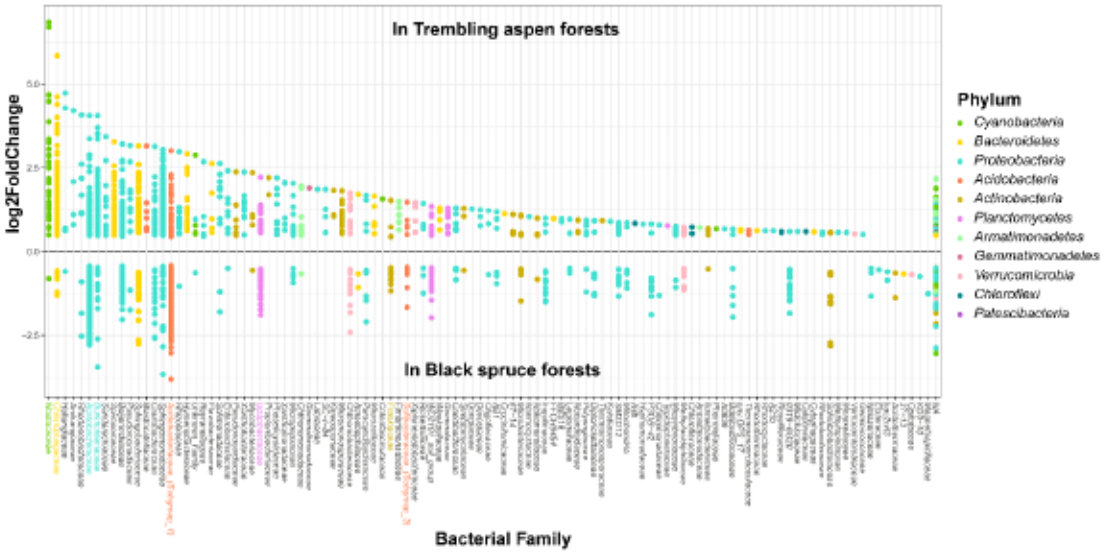

Figure 3.4 Differentially abundant ASVs identified using DESeq2 analysis of bacterial communities associated with feather mosses in trembling aspen compared to black spruce stands based on an analysis of pseudocount-transformed non-rarefied ASV (amplicon sequence variant) abundances. ASVs are grouped by taxonomic family. Only taxa with a significantly differential abundance between forest types are shown (Benjamini–Hochberg-adjusted $P < 0.05$). Points correspond to ASVs that are sorted by the average log2-fold change in relative abundance of ASVs grouped by family on the x-axis and colored by phylum. In the y-axis, positive log2-fold change values correspond to ASVs associated with trembling aspen stands whereas negative values correspond ASVs associated with black spruce stands. Colored names correspond to phyla known to contain diazotrophic bacteria (Maier et al., 2018; Holland-Moritz et al., 2021).

3.6 Discussion

The overall community composition of bacteria living in the moss phyllosphere was determined by the interaction of both forest type and host species, leading us to reject our first hypothesis that moss host species would be the most important factor affecting the community composition of moss-associated bacteria. Our results indicate that the bacterial phyla with high relative abundance (*i.e. Acidobacteria, Bacteroidetes* and

Cyanobacteria) and those with a lower relative abundance (*i.e. Armatimonadetes, Planctomycetes* and *Verrucomicrobia*) differed between forest types for the same host species, and only Actinobacteria and Proteobacteria had an interaction between both forest type and host species. Therefore, differences between black spruce coniferous forest and trembling aspen deciduous forests were a strong factor in determining moss phyllosphere composition. We also found a mixed effect of forest type on bacterial diversity as α-diversity was higher in black spruce than in trembling aspen stands, whereas there was a greater species turnover (β-diversity) and higher γ-diversity in trembling aspen than in black spruce forests.

3.6.1 Host species vs. forest type as factors affecting moss phyllosphere

In previous studies, moss-phyllosphere bacterial communities were found to be mainly structured by host species (Opelt et al., 2007; Bragina et al., 2012; Holland-Moritz et al., 2021). In contrast, we found that forest type is a major factor in determining the composition of moss-associated bacterial communities. The host specificity of phyllosphere bacterial composition in vascular plants is likely related to leaf traits (*e.g.* leaf physicochemistry) (Schlechter et al., 2019; Lajoie et al., 2020) and recently, interspecific variations in moss traits have also been demonstrated to affect cyanobacterial coloization in feather mosses (Liu and Rousk, 2022). However, since we only examined two moss species, it is not possible to determine the importance of functional traits in shaping bacterial epiphyte associations with different moss species. Furthermore, while differences in the intrinsic physicochemistry of moss leaves could be an explanation for host species as an important factor shaping microbial communities, moss chemical composition and growth are also highly influenced by the surrounding environmental conditions regardless of host species (Gałuszka, 2007; Klavina et al., 2018; Liu and Rousk, 2022). Hence, future studies could test if there is an influence of tree dominance on the moss chemical composition that could explain differences in moss the phyllosphere between coniferous and broadleaf deciduous

forests. In any case, our results suggest that forest type is a main factor shaping bacterial community composition when considering highly contrasting environmental conditions such as those defined by deciduous vs. coniferous dominated forests. However, moss host species can be a main factor defining bacterial communities when comparing more similar conditions, that is in the same forest type or in similar forests.

3.6.2 Differences in bacterial diversity between forest types

Bacterial community composition of the moss phyllosphere differed between forest types, with a higher sample-level diversity (Shannon α-diversity) in black spruce than in trembling aspen forests, but a higher variability in bacterial community composition (β-diversity) and overall diversity (γ-diversity) of bacterial ASVs in trembling aspen compared to black spruce forests. Also, the dominant groups in black spruce forests were ASVs assigned to *Proteobacteria* and *Acidobacteria*, while *Bacteroidetes* and *Cyanobacteria* were more dominant in trembling aspen forests. These findings are likely due to differences between forest types that shape local environmental conditions and communities in the understory. Moss-associated bacteria could be influenced by neighboring bacterial communities from soil microbiomes or the phyllosphere of other plant species (Lajoie and Kembel, 2021b) given the diverse vascular plant composition in the heterogeneous trembling aspen forest, which was more variable than in homogeneous and moss-dominated black spruce forests. Although both forest types had the same landscape features across all study sites (*i.e.* surface deposit, soil type, slope, etc.) (Légaré et al., 2005; Laganière et al., 2011), numerous factors related to tree dominance might influence the moss-associated bacterial communities, including light inputs of the different forest strata, litter deposition in the understory (Laganière et al., 2010), and differences in nutrient composition from organic soil layers (Cavard et al., 2011). While it is likely that these contrasting environmental conditions drive the differences in bacterial diversity between forest types, further experimental studies will

be needed to identify the specific mechanisms driving differences in moss-bacteria associations in these forest types.

Finally, we remarked that *Cyanobacteria* were significantly more abundant in trembling aspen stands, and *Nostocaceae* was the family that differed the most in relative abundance between broadleaf and coniferous forests, contrary to our expectations. Thus, our results are contrasting to those of Jean et al. (2020) in Alaska's boreal forests, who found that *Cyanobacteria* abundances and related N_2-fixation rates were higher in coniferous forests than in broadleaf forests dominated by *Betula neoalaskana*. However, differences in extraction methods of total bacterial composition (endophytes and epiphytes) compared to our extraction of only the epiphytes could partially explain these differences. Furthermore, the floristic composition in the understory of deciduous Alaskan forests differs from trembling aspen forests in Quebec. However, we did find that several bacterial families known to contain diazotrophic bacteria (*i.e. Acetobacteraceae, Burkholderiaceae, Acidobacteriaceae, Isophaeraceae, Frankiaceae* and *Solibacteraceae*) (Maier et al., 2018; Holland-Moritz et al., 2021) were present in both forest types, which suggest that these taxa could potentially be carrying out N_2-fixation in these forests even in the absence of *Cyanobacteria*. For example. *Chitinophagaceae*, a taxon that was strongly associated with trembling aspen forests, is a cellulose and chitin-degrading taxon, containing numerous diazotrophic species found in moss-dominated biocrusts (Maier et al., 2018), suggesting the potential for N_2-fixation and degradation of complex carbon compounds by the moss phyllosphere in these forests, possibly contributing with the degradation of vascular plants litter in these diverse understories. However, since we did not directly measure N_2-fixation, we can only speculate based on knowledge of the ecology and diazotrophic nature of these taxa. It is possible that the environmental conditions in trembling aspen forests, such as higher light inputs reaching the understory, could also promote the presence of cyanobacteria in trembling aspen stands compared with black spruce forests. However, future studies will be

required to quantify the relative importance of *Cyanobacteria* and other diazotrophic bacteria for N-fixation rates across seasons (Warshan et al., 2016) for different forest types, as a function of nutrient availabilities and to understand spatio-temporal dynamics of these microbial populations.

In conclusion, our results contribute to better understand moss-associated microbial communities among coniferous and broadleaf deciduous forests. The strong effect of forest type on moss-associated bacteria, the significant abundance of bryophytes in boreal forests (particularly the ubiquitous feather mosses *P. schreberi* and *P. crista-castrensis*), and the important ecological roles of moss-associated bacteria highlight the importance of changes in tree composition. With increasing changes in tree composition in the boreal system, due to natural and anthropogenic causes (natural fires, land colonization, mining and forestry) (Danneyrolles et al., 2019; Marchais et al., 2020; Mack et al., 2021), it is important to determine whether the same trends are found in other forest types and regions. Also, studies of moss-phyllosphere associations should further explore composition and functional roles to understand management implications of differences in moss-associated microbial communities related to forest type.

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CHAPITRE 4

GENERAL CONCLUSION

The general objective of this thesis was to understand how shifts between alternative stable states of coniferous and broadleaf deciduous forests influence the composition of plants and associated microbial communities in the understory. This was achieved by evaluating to what extent tree dominance of black spruce and trembling aspen forests and their associated factors affected understory plants and microbial communities in boreal forests of eastern Canada. Our study confirms that the tree dominance of coniferous and broadleaf deciduous forests affects local conditions in the understory and drives the composition of understory vegetation (Chapter 1), the tree phyllosphere and soil microbial communities (Chapter 2), and the moss bacterial phyllosphere (Chapter 3) in the boreal forest of eastern Canada.

In Chapter 1 we discussed the resistance and resilience of shifts in factors related to tree dominance on plant understory communities. We demonstrated that the understory plants of deciduous forests were resistant to changes in all evaluated factors related to tree dominance, including shifts in light, nutrients and litter deposition. Trembling aspen forests were resilient to local changes, as shown by the fact that transplanted black spruce vegetation was not able to establish, and the surrounding understory vegetation colonized it over time. In contrast, the understory vegetation of coniferous forests was resistant to changes in light availability and nutrient status, but the addition of trembling aspen litter decreased moss abundance in black spruce forests. Black spruce forests were not resilient in response to the disturbance produced by the transplanted vegetation from trembling aspen stands, but instead, the resistant plants from trembling aspen forests successfully established in the black spruce understory and further colonized outside the plots and limited moss abundance. The high plant diversity of trembling aspen forests and the nutrient-rich broadleaf litter inputs,

compared to black spruce forests and the low nutrient needleleaf litter, led to the creation of different microhabitats and resources in the understory, factors contributing to forest resilience (Oliver et al. 2015). In the context of increasing shifts in dominance from coniferous to broadleaf trees in boreal forests as a result of global change, our results highlight the vulnerability of black spruce forests to change via their understory vegetation compared to the resistance and resilience of trembling aspen forests.

In Chapters 2 and 3 we advanced the understanding of the occurrence and distribution of microbial communities in different microhabitats, including the tree phyllosphere of black spruce needles and trembling aspen leaves, the mineral soil microbiome and the phyllosphere of two of the most abundant feather mosses in the eastern boreal forest of Canada. We also demonstrated how these microbial communities in all evaluated microhabitats changed between forest types. While in Chapter 1 we observed differences in the resistance of understory plants to shifts in factors related to tree dominance between forest types, the results of Chapter 2 demonstrate that bacterial and fungal communities were not affected by aboveground changes in plant understory composition or shifts in litter inputs, but the legacy effect of each forest type defined soil microbial community composition. Although we found changes in microbial community composition among forest types, almost half of the identified bacterial ASVs were shared between them, contrary to the tree phyllosphere that showed a higher specificity for broadleaves or needleleaves. We also observed a high microhabitat specificity of microbial communities, as only a small proportion of bacterial ASVs were shared between the tree phyllosphere and the soil.

Finally, in Chapter 3 we demonstrated that the overall composition of the moss phyllosphere was defined by the interaction of host species and forest type. Forest type had a strong effect on the moss phyllosphere, as most bacterial phyla varied in relative abundance between black spruce and trembling aspen stands. Considering the important ecological functions of mosses and associated microbial communities in

nutrient limited boreal forests, changes in tree dominance are likely to affect the composition of moss-associated bacteria in boreal forests, such as N_2-fixation. Also, ecologically important diazotrophic bacteria such as *Cyanobacteria* not only differed in relative abundance between forest types, but they were more relatively abundant in trembling aspen than in black spruce forests, demonstrating a different trend in our study for differences between coniferous and broadleaf deciduous forests, compared with previous studies in Alaska.

This research project improved the knowledge available about of black spruce and trembling aspen forests and their biological composition, but it also had certain limitations and generated further questions and research insights for future studies. Our study highlights that the analysis of different groups of species in the understory, in different forest types, such as deciduous and coniferous forests, is important to be considered to understand variation in boreal forest community composition and its responses to environmental changes that could affect associated ecological functions. Science-based decisions on forest management strategies could take into account not only shifts in dominant tree species, but also in plant understory composition and their associated microbial communities, considering the significant impact of human activities and climate change in boreal forests. In order to conserve the worldwide ecological services offered by the boreal forest, it is essential to have an adequate forest management plan, based on the knowledge of the ecology of different groups of species, their interactions and their associated ecological functions. Currently, ecosystem-based forest management in Quebec seeks to reduce the gaps between the managed forest and the natural forest, with the goal of maintaining the integrity of the ecosystem with all of its components, functions and processes that are essential for sustainable management (Bergeron et al., 2004; Jetté et al., 2013). Nonetheless, limited knowledge about important species in the boreal ecosystem can lead us to make assumptions for forest management without having more in-depth knowledge on the system (Chipman & Johnson, 2002; Kumar et al., 2017). For example, understory

vegetation and its associations with microbial communities in the phyllosphere and in the soil are an important part of a forest's biodiversity and play a critical role in forest productivity and ecosystem functions (Chen et al., 2004; Gilliam 2007). Specifically in nutrient-poor areas, symbiotic microorganisms are primarily responsible for the uptake of atmospheric elements that benefit plant growth (Van Der Heijden et al., 2008). These associations have benefits not only related to plant nutrition, but also for adaptation to hostile environments and defense against pathogens (Ritpitakphong et al., 2016). However, forest management frequently focuses on tree composition ignoring all these other important components. In this thesis, we cannot directly address how to improve forest management of the boreal forest, but we contributed to the knowledge of the boreal forest by using black spruce and trembling aspen forests of eastern Quebec as a model system, and this knowledge can be used to inform management decisions.

Our results of Chapter 1 highlight the resistance and resilience of the trembling aspen system and its plant and microbial diversity, in contrast to black spruce forests. This suggests that management efforts could be focused on preserving certain components of black spruce forests, to conserve their biodiversity but also to think about strategies to maintain forest productivity and perhaps take advantage of the presence of a small percentage of trembling aspen trees to improve understory conditions in mixed forests, as previously suggested (Légaré et al., 2005; Ghotsha et al., 2022). While black spruce forests provide numerous ecosystem services, such as wildlife habitat, short-term carbon sequestration and wood production, they can be also compromised over time by paludification processes that lead to the accumulation of a very humid soil organic layer mainly composed of *Sphagnum* species, which reduces tree regeneration and productivity (Fenton et al. 2005). In forests with a marked trend to paludification processes such as our study sites, a forest management plan focused on mixed-canopy forest, with an increasing broadleaved component in coniferous forests could limit moss abundance and promote nutrient cycling that leads to a more diverse understory, increasing productivity, limiting paludification, favouring forest resilience and a better

black spruce growth for timber production, as previously suggested (Chavardès et al. 2021; Légaré et al. 2005).

Our project is framed in a context of increasing changes in the boreal forests from coniferous to broadleaf deciduous forests, but we are limited to studying black spruce and trembling aspen forests in a small area in western Quebec as a model system to better understand changes in the boreal forest. While we can analyse shifts in factors between forest types, and the resilience and resistance of understory species to changes in their habitat, we cannot draw conclusions about the resilience and resistance of the entire boreal forest. With increasing changes in tree composition in the boreal system (Danneyrolles et al., 2019; Marchais et al., 2020; Mack et al., 2021), it is important to determine whether the same trends we observed are also observed in other forest types and boreal regions.

We used the theoretical framework of alternative stable states to facilitate the analysis and better explain the processes between two forest types representative of the Canadian boreal forests. Coniferous and deciduous broadleaf forests have also been considered as alternative stable states in other studies (Johnstone et al. 2010a, Johnstone et al. 2020, Baltzer et al. 2021), but it is important to consider that forests are not static but dynamic, they are always in constant change and face disturbance pressures that could induce a transition to another state. We considered tree dominance as a factor defining two alternative states in our study sites, because they were very similar in abiotic conditions, originated from the same disturbance trigger (a fire in 1916) and only differed in tree dominance. These study sites are useful as study models and have been used to test several hypotheses, not only in this project but also in previous studies referenced throughout the thesis (studies from Bergeron, Légaré, Laganière, Cavard, Nagati, Ghotsa, and other collaborators, among others). Therefore, these sites have been studied at different levels and give us the potential to go deeper

in the ecological analysis of black spruce and trembling aspen forests, their biodiversity, and forest dynamics.

The analysis of microbial communities in Chapters 2 and 3 highlight the high microhabitat specificity of bacterial communities, the resilience of soil bacterial and fungal communities to local changes in their habitat, and the strong influence of tree dominance on their community composition. This suggests that changes in canopy composition could affect microbial communities and associated ecological processes (Lamarche et al., 2007). Thus, the study of plant-microorganism interactions is essential to better understand the dynamics of boreal ecosystems. With increasing advances in high-throughput sequencing, we are able to identify microorganisms present in different microhabitats and host species in Chapters 2 and 3. However, this sequencing approach has still certain limitations important to discuss here. First, taxonomic assignment of the identified ASVs is based on currently available databases, which include a limited number of identified species and are biased towards taxa from certain microhabitats, such as the human microbiota. Based on these limited databases, the identified ASVs are assigned to different taxonomical levels, from phyla to species. However, identification at finer taxonomic levels is difficult; most ASVs could not be identified to the genus or species level. We overcame this drawback by focusing our analyses mainly on the ASV composition and on the taxonomic annotations at the phylum level, avoiding analyses at other less accurate taxonomic levels. Second, a link between the identified microorganisms and their ecological functions would be interesting to further evaluate and to better understand the impacts of changes in tree dominance in boreal forests on microbial function. It is possible to link the taxonomically assigned ASVs to their ecological functions through tools such as PICRUSt for bacteria (Langille et al. 2013), and FUNGUILD for fungi (Nguyen et al. 2016). However, we did not used this approach because our sequencing results only indicate that the identified microbial ASVs were present in the samples, but it does not necessarily mean they were living organisms or were actively playing those associated

ecological functions. Also, the functions associated in these databases are also limited and come from frequently studied microhabitats and hosts, but they could be playing different roles in less studied hosts, such as bryophytes. Metagenomics, metatranscriptomics, and proteomics are alternative approaches to obtain both taxonomic and functional information to further understand the ecological functions of the studied microorganisms (Starke et al. 2021). However, these approaches are quite expensive and time demanding. While our results highlight the differences in microbial community composition between microhabitats and forest types, further studies will be needed to link microbial taxa with their ecological functions. Even though high-throughput sequencing still has several limitations, it still provides valuable data that can answer important biological questions and help us to improve our knowledge of microbial communities, which are otherwise impossible to analyze with culture-based techniques that miss the majority of micobial diversity.

The link between moss phyllosphere and ecological functions has been evaluated particularly for N_2-fixation by cyanobacteria, as it is one of the main ecological functions that has been studied in the boreal forest, notably by research groups in Northern Europe (DeLuca et al. 2002; Lindo et al. 2013; Rousk et al. 2013). Contrary to our expectation, our results show a higher relative abundance of cyanobacteria in trembling aspen than in black spruce forests. We did not directly measure N_2-fixation activity by moss-associated cyanobacteria because of time and logistical limitations; it was not the focus of our research objectives and it has been previously studied (Lindo et al. 2013; Liu and Rousk 2022). Other studies have already correlated the presence of cyanobacteria with N_2-fixation (DeLuca et al., 2007; Rousk et al., 2013) and *Nostocaceae* is a confirmed N_2-fixing taxon associated with feather mosses (Holland-Moritz et al., 2021). Therefore, our results focused on differences in bacterial communities (including diazotrophic bacteria) between forest types and we can only speculate based on previous studies about the correlation between cyanobacterial abundance and ecological functions in deciduous forests. Future studies could also

integrate the endophytic community of leaves and mosses, to compare with the epiphytic communities we evaluated in our study. Furthermore, a link between microbial communities, ecological functions and differences on leaf traits between forest types could be analyzed for different moss species (Liu and Rousk 2022). While our results on moss phyllosphere composition come from natural forests dominated by black spruce or by trembling aspen trees, we could further explore possible differences compared with mixed forests and their associated ecological functions.

Our project advanced our understanding of two dominant forest types in the boreal biome, by comparing shifts in coniferous black spruce forests versus broadleaf deciduous trembling aspen forests and the different associated factors influencing the plant understory and associated microbial communities. We highlight that the biological diversity should not be studied and understood as independent components, but instead, it should be considered as a whole, taking into account the symbioses and intrinsic interactions among the organisms that depend on each other to survive, to reproduce over time, and to respond synergically faced with changes in their habitat.

CONCLUSION GÉNÉRALE

L'objectif général de cette thèse était de comprendre comment les changements entre les états stables alternatifs des forêts de conifères et de feuillus influencent la composition des plantes et des communautés microbiennes associées dans le sous-bois. Cet objectif a été atteint en évaluant dans quelle mesure la dominance des arbres dans les forêts d'épinette noire et de peuplier faux-tremble et leurs caractéristiques associées ont affecté les plantes et les communautés microbiennes de sous-bois dans les forêts boréales de l'est du Canada. Notre étude confirme que la composition des arbres dominantes dans les forêts de conifères et de feuillus de la forêt boréale de l'est du Canada affecte les conditions locales dans le sous-bois et détermine la composition de la végétation du sous-bois (chapitre 1), la phyllosphère des arbres et les communautés microbiennes du sol (chapitre 2), ainsi que la phyllosphère bactérienne des mousses (chapitre 3).

Dans le chapitre 1, nous avons discuté de la résistance et de la résilience des changements de facteurs liés à la dominance des arbres sur les communautés végétales de sous-bois. Nous avons démontré que les plantes de sous-bois des forêts feiullues étaient résistantes aux changements de tous les facteurs évalués liés à la composition arborescente, y compris les changements de lumière, de nutriments et de l'apport en litière d'aiguilles d'épinette. Les forêts de peuplier faux-tremble étaient résistantes aux changements locaux, car comme le montre le fait que la végétation du sous-bois des peuplements d'épinette noire transplantés dans les forêts des peupliers ne s'est pas établie au cour du temps, mais elle a été colonisé par la végétation du sous-bois environnant. En revanche, la végétation de sous-bois des forêts de conifères était résistante aux changements de lumière et de nutriments, mais l'ajout de la litière de peuplier faux-tremble a diminué l'abondance des mousses dans les forêts d'épinettes noires. Les forêts d'épinette noire n'ont pas été résilientes en réponse à la perturbation

produite par la végétation transplantée des peuplements de peupliers faux-tremble, mais au contraire, les plantes résistantes des peupliers faux-tremble ont réussi à s'établir dans le sous-bois d'épinettes noires et à coloniser davantage l'extérieur des parcelles, ce qui a limité l'abondance des mousses. La grande diversité végétale des forêts de peupliers faux-trembles et l'apport de litière de feuillus riche en nutriments, par rapport aux forêts d'épinette noire et à la litière d'aiguilles pauvre en nutriments, ont conduit à la création de microhabitats et de ressources différentes dans le sous-bois, facteurs contribuant à la résilience des forêts (Oliver et al. 2015). Dans le contexte des changements climatiques, pressions naturelles et anthropiques dans la forêt boréale, qui produisent une diminution de la dominance de dominance des conifères et une augmentation des forêts feuillus, nos résultats mettent en évidence la vulnérabilité des forêts d'épinettes noires via sa végétation du sous-bois par rapport à la résistance et la résilience des forêts de peupliers faux-trembles.

Dans les chapitres 2 et 3, nous avons contribué à la compréhension de la distribution des communautés microbiennes dans différents microhabitats, notamment la phyllosphère des aiguilles d'épinettes noires et des feuilles de peupliers faux-trembles, le microbiome du sol minéral et la phyllosphère de deux espèces de mousses les plus abondantes dans la forêt boréale de l'est du Canada. Nous avons également démontré comment ces communautés microbiennes dans tous les microhabitats évalués ont changé entre les types de forêts. Alors que dans le chapitre 1, nous avons observé des différences dans la résistance des plantes de sous-bois à des changements dans les facteurs liés à la dominance des arbres entre les types de forêts, les résultats du chapitre 2 démontrent que les communautés bactériennes et fongiques n'ont pas été affectées par les changements en surface dans la composition des plantes de sous-bois ou par les changements dans les apports de litière, mais que l'effet de l'héritage en composition et conditions de sol dans chaque type de forêt a défini la composition de la communauté microbienne du sol. Bien que nous ayons constaté des changements dans la composition de la communauté microbienne entre les types de forêt, près de la moitié

des ASV bactériens identifiés étaient partagés entre eux, contrairement à la phyllosphère des arbres qui a montré une plus grande spécificité pour les feuilles du peuplier ou les aiguilles d'épinette. Nous avons également observé une forte spécificité de microhabitat des communautés microbiennes, car seule une petite proportion des ASV bactériennes était partagée entre la phyllosphère des arbres et le sol.

Enfin, dans le chapitre 3, nous avons démontré que la composition globale de la phyllosphère des mousses était définie par l'interaction entre deux facteurs, notamment l'espèce hôte et le type de forêt. Nous avons toutefois souligné le fort effet du type de forêt en tant que facteur déterminant des changements dans l'abondance relative de la plupart des phyla bactériens. Compte tenu des fonctions écologiques importantes des mousses et des communautés microbiennes associées dans les forêts boréales limitées en nutriments, les changements dans la dominance des arbres sont susceptibles d'affecter la composition des bactéries associées aux mousses et la fixation d'azote dans les forêts boréales. De plus, les bactéries diazotrophes écologiquement importantes, telles que les cyanobactéries, non seulement différaient en abondance relative entre les types de forêts, mais elles étaient relativement plus abondantes dans les forêts de peuplier faux-tremble que dans les forêts d'épinette noire, ce qui démontre une nouvelle tendance dans notre étude, différente à celle observé dans des forêts de conifères et de feuillus en Alaska.

Ce projet de recherche a permis d'améliorer les connaissances sur les forêts d'épinettes noires et de peupliers faux-trembles et leur composition biologique, mais il présentait également certaines limites et il génère d'autres questions et pistes de recherche pour des études futures. Notre étude souligne que l'analyse de différents groupes d'espèces dans différents types de forêts est importante à considérer pour comprendre la variation de la composition de la communauté de la forêt boréale et ses réponses aux changements environnementaux qui pourraient affecter les fonctions écologiques associées. Compte tenu de l'impact significatif des activités humaines et du

changement climatique dans les forêts boréales, les décisions basées sur les connaissances scientifiques pour des stratégies en gestion forestière devraient tenir compte non seulement des changements dans les espèces arborescentes dominantes, mais aussi de la composition du sous-bois végétal et des communautés microbiennes associées. Afin de conserver les services écologiques mondiaux offerts par la forêt boréale, il est essentiel de disposer d'un plan d'aménagement forestier adéquat, basé sur la connaissance de l'écologie des différents groupes d'espèces, de leurs interactions et des fonctions écologiques qui leur sont associées. Actuellement, l'aménagement forestier écosystémique au Québec vise à réduire les écarts entre la forêt aménagée et la forêt naturelle, dans le but de maintenir l'intégrité de l'écosystème avec toutes ses composantes, fonctions et processus essentiels à l'aménagement durable (Bergeron et al., 2004 ; Jetté et al., 2013). Néanmoins, des connaissances limitées sur les espèces importantes de l'écosystème boréal peuvent limiter de manière significative notre connaissance de l'ensemble de l'écosystème, conduisant à la nécessité de formuler des hypothèses pour la gestion forestière sans avoir des connaissances plus approfondies sur le système (Chipman & Johnson, 2002 ; Kumar et al., 2017). Par exemple, la végétation de sous-bois et ses associations avec les communautés microbiennes dans leur phyllosphère et dans le sol, sont une partie importante de la biodiversité d'une forêt et jouent un rôle critique dans la productivité de la forêt et les fonctions de l'écosystème (Chen et al., 2004 ; Gilliam 2007). En particulier dans les zones pauvres en nutriments, les micro-organismes symbiotiques sont principalement responsables de l'absorption d'éléments atmosphériques qui profitent à la croissance des plantes (Van Der Heijden et al., 2008). Ces associations présentent des avantages non seulement liés à la nutrition des plantes, mais aussi à l'adaptation à des environnements hostiles et à la défense contre les agents pathogènes (Ritpitakphong et al., 2016). Cependant, la gestion forestière se concentre fréquemment sur la composition des arbres en ignorant les autres composantes importantes. Dans cette thèse, nous ne pouvons pas aborder directement comment améliorer la gestion forestière de la forêt boréale, mais nous

avons contribué à la connaissance de la forêt boréale en utilisant les forêts d'épinettes noires et de peupliers faux-trembles de l'est du Québec comme système modèle.

Nos résultats du Chapitre 1 mettent en évidence la résistance et la résilience du système du peuplier faux-tremble et sa diversité végétale et microbienne, contrairement aux forêts d'épinette noire. Cela suggère que les efforts de gestion pourraient être axés sur la préservation de certaines composantes des forêts d'épinette noire, afin de conserver leur biodiversité, mais aussi de réfléchir à des stratégies pour maintenir la productivité de la forêt et peut-être de tirer parti de la présence d'un petit pourcentage de peupliers faux-trembles pour améliorer les conditions du sous-bois dans les forêts mixtes, comme cela a été suggéré précédemment (Légaré et al., 2005 ; Ghotsha et al., 2022). Si les forêts d'épinettes noires fournissent de nombreux services écosystémiques, tels que l'habitat de la faune, la séquestration du carbone à court terme et la production de bois, elles peuvent également être compromises au fil du temps par des processus de paludification qui produisent l'accumulation d'une couche organique de sol très humide composée principalement d'espèces de *Sphagnum*, ce qui réduit la régénération et la productivité des arbres (Fenton et al., 2005). Dans les forêts ayant une tendance marquée aux processus de paludification comme nos sites d'étude, une gestion forestière axée sur les forêts mixtes, avec une augmentation des feuillus dans les forêts de conifères, pourrait limiter l'abondance des mousses et promouvoir le cycle des nutriments qui conduit à un sous-étage plus diversifié, augmentant la productivité, limitant la paludification, favorisant la résilience de la forêt et une meilleure croissance d'épinettes noires pour la production de bois, comme suggéré précédemment (Chavardès et al. 2021 ; Légaré et al. 2005).

Notre projet s'inscrit dans un contexte de changement progressif de la dominance de la canopée dans le biome boréal, passant de forêts de conifères à feuillus, mais nous sommes limités à l'étude des forêts d'épinettes noires et de peupliers faux-trembles dans une petite zone de l'ouest du Québec comme système modèle pour mieux comprendre

les changements dans la forêt boréale. Bien que nous puissions analyser les changements de facteurs entre les types de forêts, ainsi que la résilience et la résistance des espèces du sous-bois aux changements dans leur habitat, nous ne pouvons pas tirer de conclusions sur la résilience et la résistance de l'ensemble de la forêt boréale. Avec les changements croissants de la composition arborescente dans le système boréal (Danneyrolles et al., 2019 ; Marchais et al., 2020 ; Mack et al., 2021), il est important de déterminer si les mêmes tendances dans nos résultats sont également observées dans d'autres types de forêts et dans d'autres régions boréales.

Nous avons utilisé le cadre théorique des états stables alternatifs pour faciliter l'analyse et mieux expliquer les processus entre deux types de forêts représentatifs des forêts boréales canadiennes. Les forêts de conifères et de feuillus ont également été considérées comme des états alternatifs stables dans d'autres études (Johnstone et al. 2010a, Johnstone et al. 2020, Baltzer et al. 2021), mais il est important de considérer que les forêts ne sont pas statiques mais dynamiques, elles sont toujours en changement constant et font face à des pressions de perturbation qui pourraient induire une transition vers un autre état. Nous avons considéré la dominance des arbres comme un facteur définissant deux états alternatifs dans nos sites d'étude, car ils étaient très similaires dans les conditions abiotiques permanentes, provenaient du même déclencheur de perturbation (un incendie en 1916) et ne différaient que par la dominance des arbres. Ces sites d'étude sont utiles en tant que modèles d'étude et ont été utilisés pour tester plusieurs hypothèses, non seulement dans ce projet mais aussi dans des études antérieures référencées tout au long de la thèse (études de Bergeron, Légaré, Laganière, Cavard, Nagati, Ghotsa, et autres collaborateurs, entre autres). Par conséquent, ces sites d'étude ont été étudiés à différents niveaux et ont le potentiel d'approfondir l'analyse écologique des forêts d'épinette noire et de peuplier faux-tremble, leur biodiversité et la dynamique forestière.

L'analyse des communautés microbiennes dans les chapitres 2 et 3 souligne la grande spécificité des microhabitats pour les communautés bactériennes, la résilience des communautés bactériennes et fongiques du sol aux changements locaux de leur habitat et la forte influence de la dominance des arbres sur la composition de leur communauté. Cela suggère que les changements dans la composition de la canopée pourraient affecter les communautés microbiennes et les processus écologiques associés (Lamarche et al., 2007). Ainsi, l'étude des interactions plantes-microorganismes est essentielle pour mieux comprendre la dynamique des écosystèmes boréaux. Grâce aux progrès croissants du séquençage à haut débit, nous sommes en mesure d'identifier les microorganismes présents dans les différents microhabitats et espèces hôtes des chapitres 2 et 3. Cependant, cette approche de séquençage présente encore certaines limites qu'il est important de discuter ici. Tout d'abord, l'attribution taxonomique aux ASV identifiés est basée sur les bases de données actuellement disponibles, qui comprennent un nombre limité d'espèces identifiées et sont biaisés vers des taxons provenant certains microhabitats, comme le microbiome humain. À partir de ces bases de données limitées, les ASVs de séquençage identifiés sont attribués à différents niveaux taxonomiques, du phyla à l'espèce. Cependant, l'identification à un niveau taxonomique plus fin est difficile; la plupart des ASV n'ont pas pu être identifiés au niveau du genre ou de l'espèce. Nous avons surmonté cet inconvénient en concentrant notre analyse principalement sur la composition des ASVs et sur les annotations taxonomiques au niveau des phyla, en évitant l'analyse avec d'autres niveaux taxonomiques moins précis. Deuxièmement, il serait intéressant d'établir un lien entre les micro-organismes identifiés et leurs fonctions écologiques afin d'évaluer et de mieux comprendre les impacts des changements de la dominance des arbres dans les forêts boréales sur leur fonction ecologique. Il est possible de lier les ASVs taxonomiquement assignés à leurs fonctions écologiques grâce à des outils tels que PICRUSt pour les bactéries (Langille et al. 2013), et FUNGUILD pour les champignons (Nguyen et al. 2016). Cependant, nous n'avons pas utilisé cette approche car nos résultats de séquençage indiquent seulement que les ASVs microbiens

identifiés étaient présents dans les échantillons, mais cela ne signifie pas nécessairement qu'ils étaient des organismes vivants ou qu'ils réalisaient les fonctions écologiques associées. De plus, les fonctions associées dans ces bases de données sont également limitées et proviennent de microhabitats et hôtes fréquemment étudiés, mais elles pourraient avoir des rôles différents dans des hôtes moins étudiés, comme les bryophytes. La métagénomique, la métatranscriptomique et la protéomique sont des alternatives pour disposer à la fois d'informations taxonomiques et fonctionnelles afin de mieux comprendre les fonctions écologiques des microorganismes étudiés (Starke et al. 2021). Cependant, ces approches sont assez coûteuses et demandent beaucoup de temps. Bien que nos résultats mettent en évidence les différences dans la composition des communautés microbiennes entre les microhabitats et les types de forêts, d'autres études seront nécessaires pour relier les taxons microbiens à leurs fonctions écologiques. Même si le séquençage à haut débit a encore plusieurs limites, il fournit des données de grande valeur qui peuvent répondre à des questions biologiques importantes et nous aider à améliorer notre connaissance des communautés microbiennes qu'autrement les techniques de culture *in vitro* manqueraient la majorité de la diversité microbienne.

Le lien entre la phyllosphère des mousses et les fonctions écologiques a été évalué en particulier pour la fixation d'azote par les cyanobactéries, car c'est l'une des principales fonctions écologiques qui a été étudiée dans la forêt boréale, notamment par des groupes de recherche en Europe du Nord (DeLuca et al. 2002 ; Lindo et al. 2013 ; Rousk et al. 2013). Contrairement à nos attentes, nos résultats montrent une plus grande abondance relative de cyanobactéries dans les forêts de peupliers faux-trembles que dans les forêts d'épinettes noires. Nous n'avons pas mesuré directement l'activité de fixation d'azote par les cyanobactéries associées aux mousses en raison de contraintes de temps et de logistique ; car ce n'était pas l'objet de nos objectifs de recherche et car cela avait été étudié précédemment (Lindo et al. 2013 ; Liu et Rousk 2022). Ainsi, d'autres études ont déjà observé une corrélation entre la présence de cyanobactéries et

la fixation d'azote (DeLuca et al., 2007 ; Rousk et al., 2013) et les *Nostocaceae* sont un taxon confirmé de fixation d'azote associé aux mousses (Holland-Moritz et al., 2021). Par conséquent, nos résultats se sont concentrés sur les différences dans les communautés bactériennes (y compris les bactéries diazotrophes) entre les types de forêts et nous ne pouvons que spéculer sur la base d'études précédentes sur la corrélation entre l'abondance des cyanobactéries et les fonctions écologiques dans les forêts feuillus. Les études futures pourraient également intégrer la communauté endophyte des feuilles et des mousses, afin de la comparer à la communauté épiphyte évaluée dans notre étude. En outre, un lien entre les communautés microbiennes, les fonctions écologiques et les différences de caractéristiques des feuilles entre les types de forêts pourrait être analysé pour différentes espèces de mousses, comme cela a été récemment évalué (Liu et Rousk 2022). Bien que nos résultats sur la composition de la phyllosphère des mousses proviennent de forêts naturelles dominées par l'épinette noire ou le peuplier faux-tremble, nous pourrions explorer des possibles différences en forêts mixtes et voir si la composition de la phyllosphere de mousses et ses fonctions écologiques sont aussi différentes.

Notre projet a permis de mieux comprendre deux types de forêts fréquemment dominantes dans le biome boréale, en comparant les changements dans les forêts coniférines d'épinettes noires par rapport aux forêts feuillus de peuplier faux-tremble et les différents facteurs associés qui influencent la composition du sous-bois et des communautés microbiennes associées. Nous soulignons que la diversité biologique ne doit pas être étudiée et comprise comme des composantes indépendantes, mais qu'elle doit plutôt être considérée comme un tout, en tenant compte des symbioses et des interactions intrinsèques entre les organismes qui dépendent les uns des autres pour survivre, se reproduire dans le temps et répondre de manière synergique aux changements dans leur habitat.

ANNEXE A

SUPPLEMENTARY INFORMATION CHAPTER 1

Figure S 3.1: continued

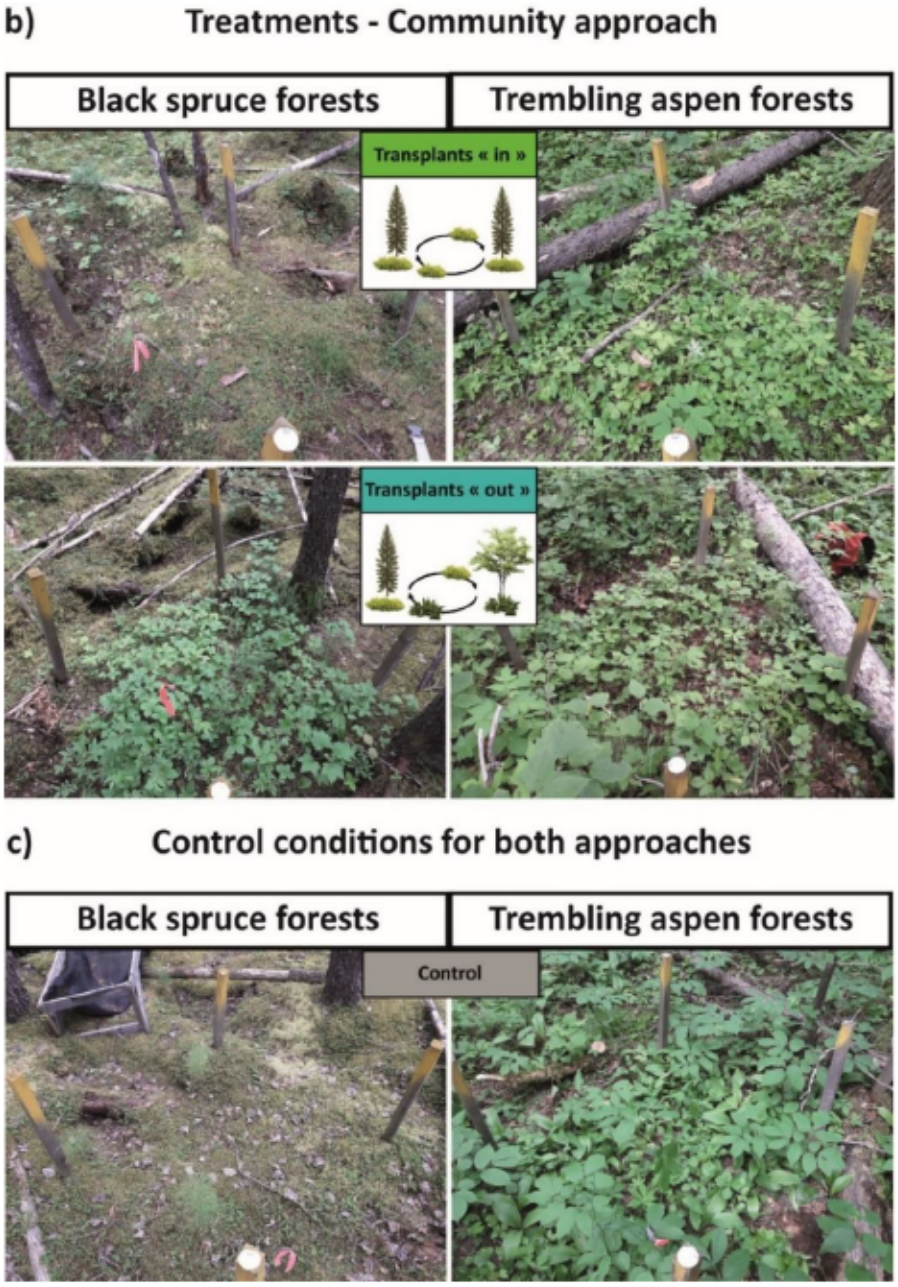

Figure S 3.1 Visual examples of treatment plots in forests dominated by black spruce and by trembling aspen forests, for each treatment of a) the ecosystem approach, corresponding to Light (Li, in yellow), Nutrients (Nu, in purple), Single-litter (1F, in orange) and Double-litter (2F, in red); b) the community approach, corresponding to Transplants-out (To, in blue), Transplants-in (Ti, in green), and c) Control conditions (C, in grey) for both approaches.

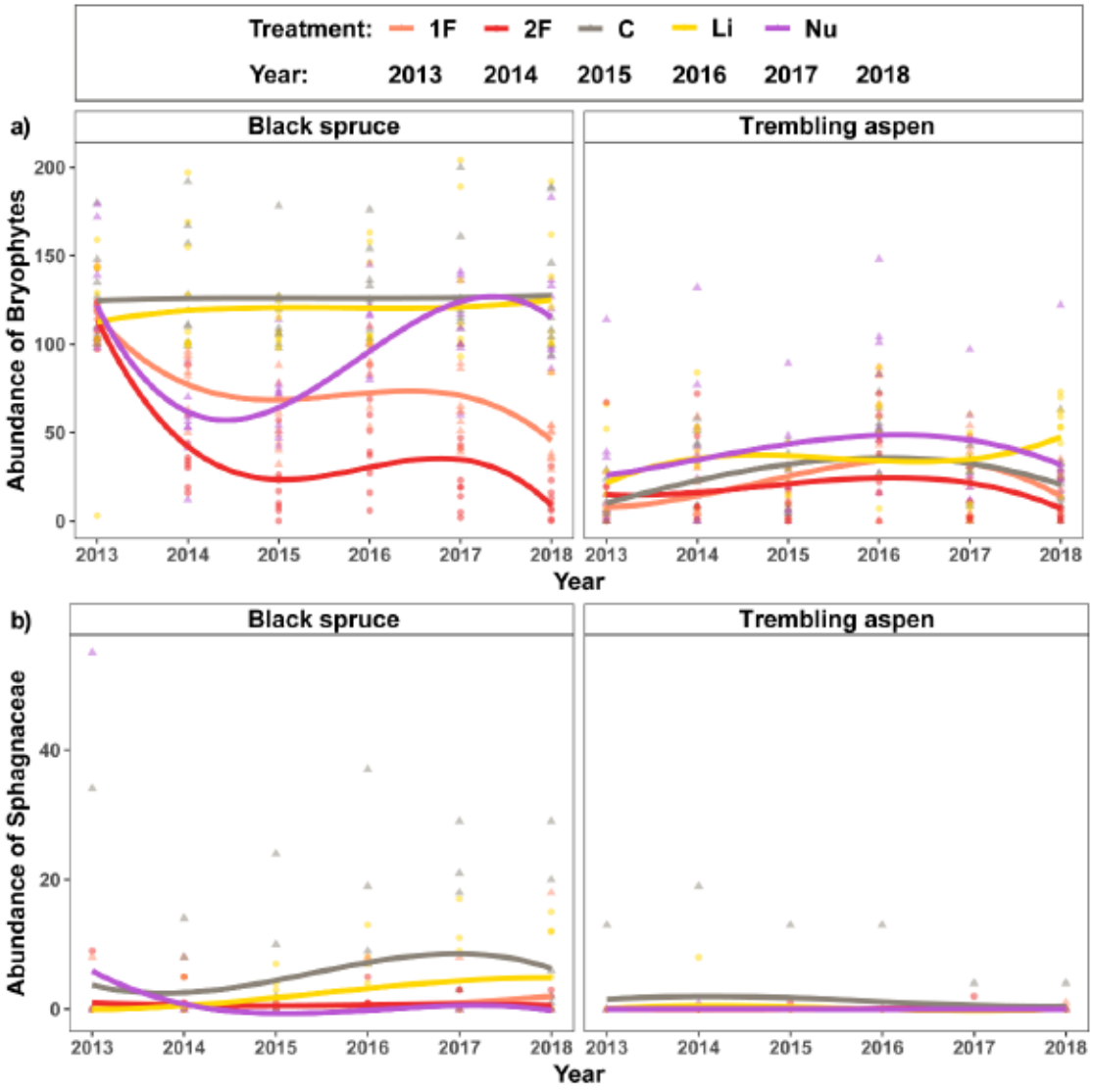

Figure S 3.2: continued

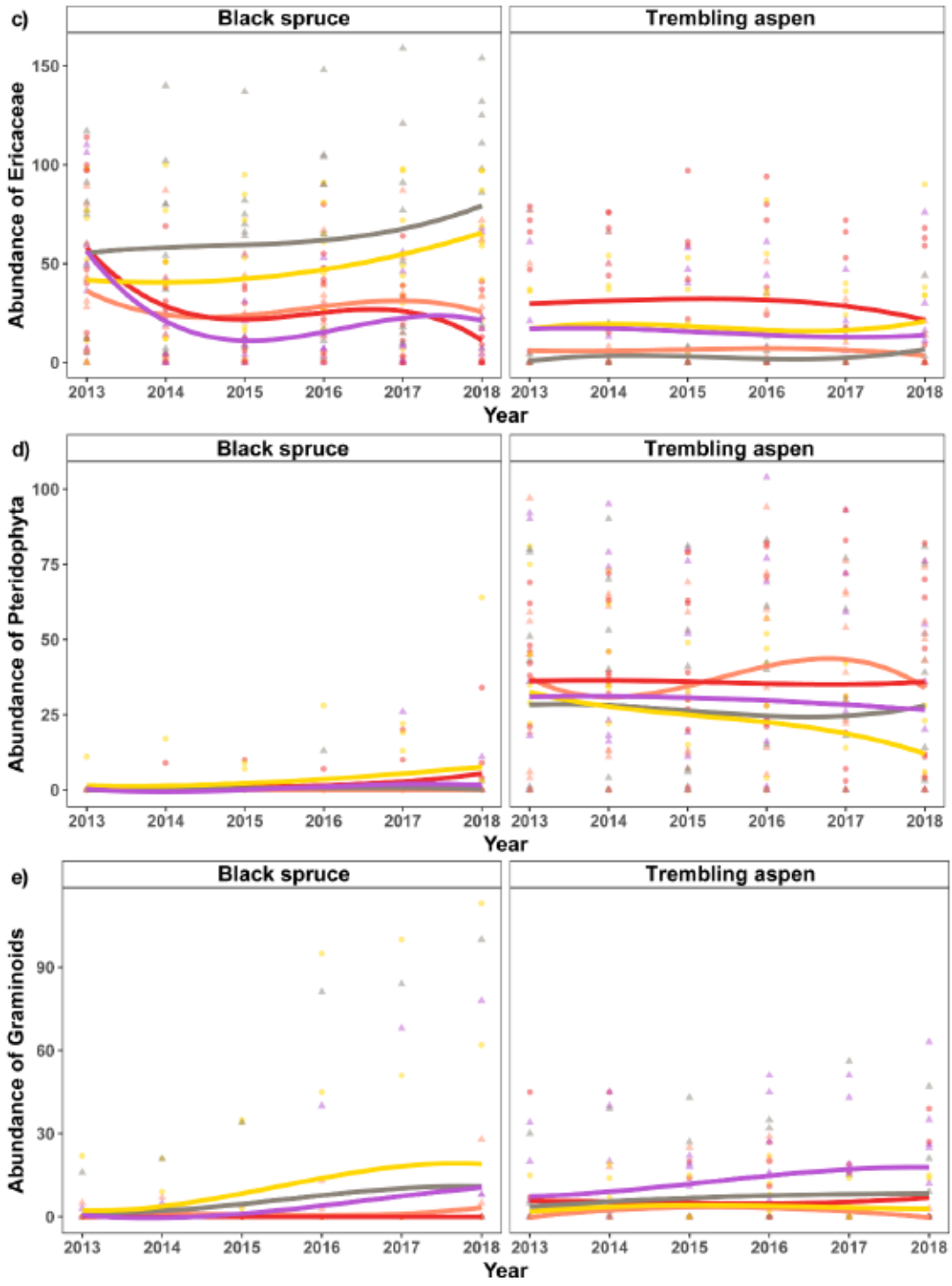

Figure S 3.2: continued

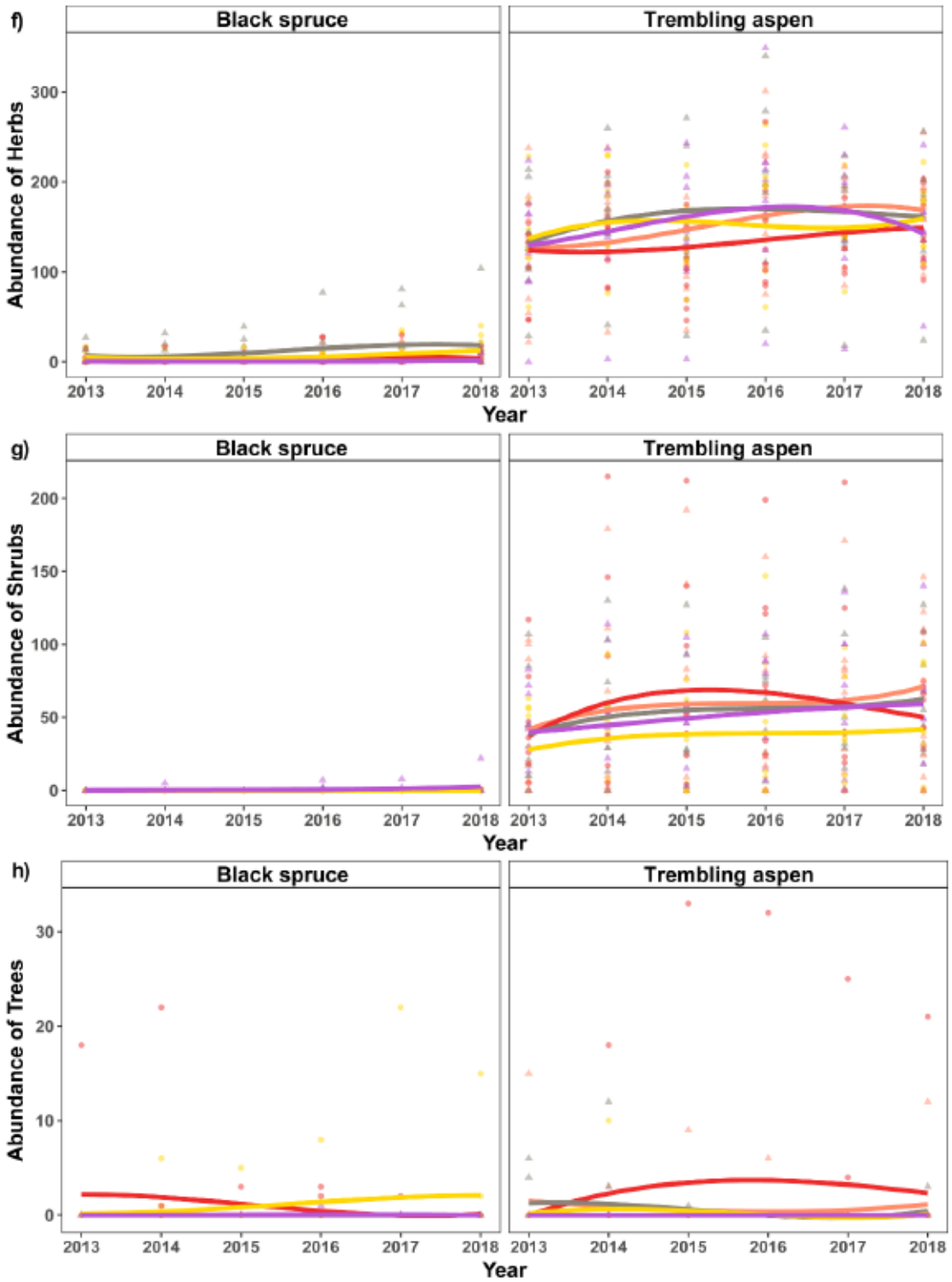

Figure S 3.2 Variation in abundance of functional groups over time (from 2013 to 2018) for black spruce (left panels) and trembling aspen stands (right panels). Functional

groups: (a) Bryophytes, (b) Sphagnaceae, (c) Ericaceae, (d) Peridophyta, (e) Herbs, (f) Shrubs, and (g) Trees. Treatments correspond to ecosystem approach: Light (Li, in yellow), Nutrients (Nu, in purple), Single-litter (1F, in orange) and Double-litter (2F, in red) and Control conditions (C, in grey). Smooth lines are based on the linear model of understory species abundances, each point in different colors (treatments) from data each year of the study.

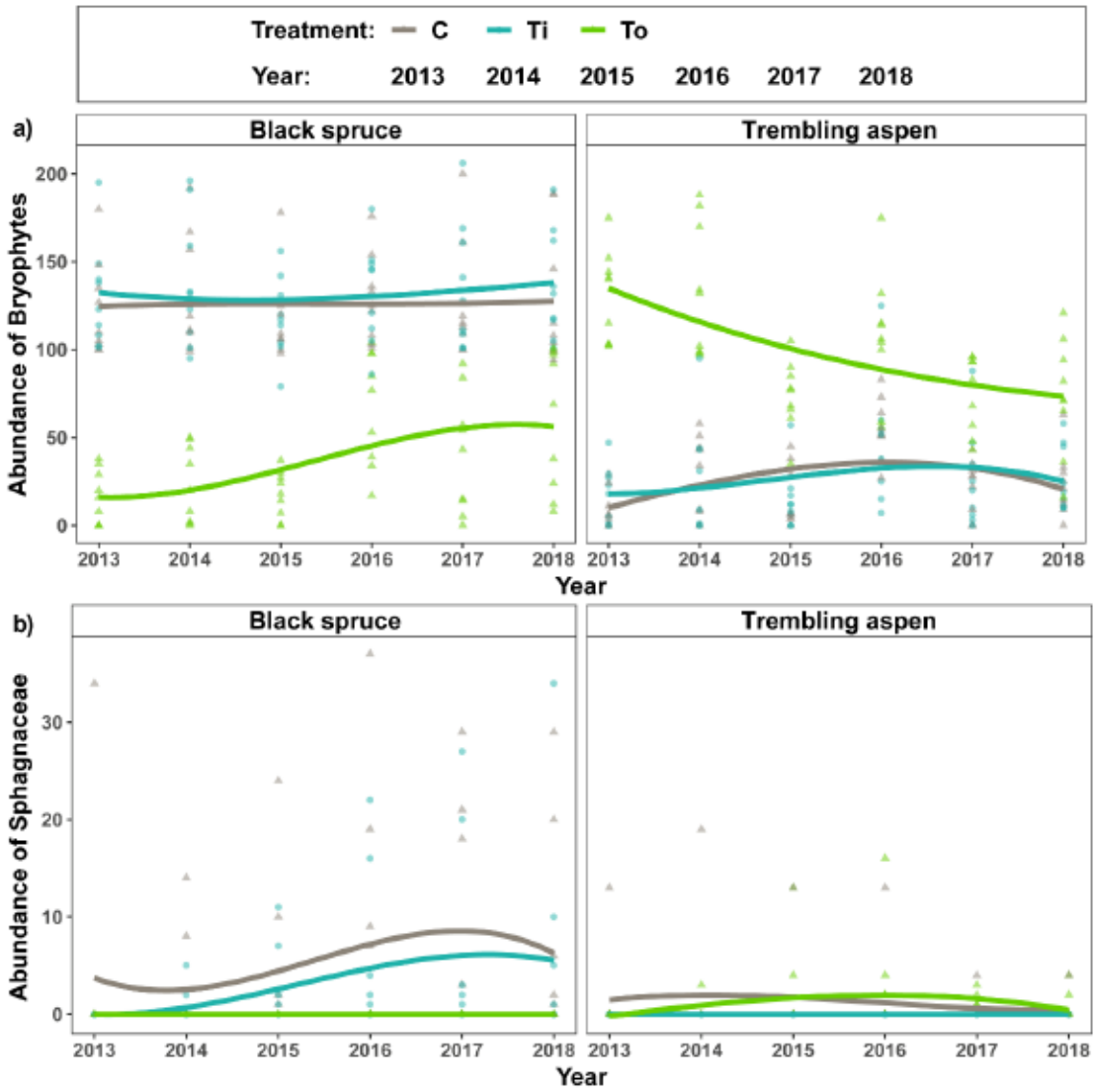

Figure S 3.3: continued

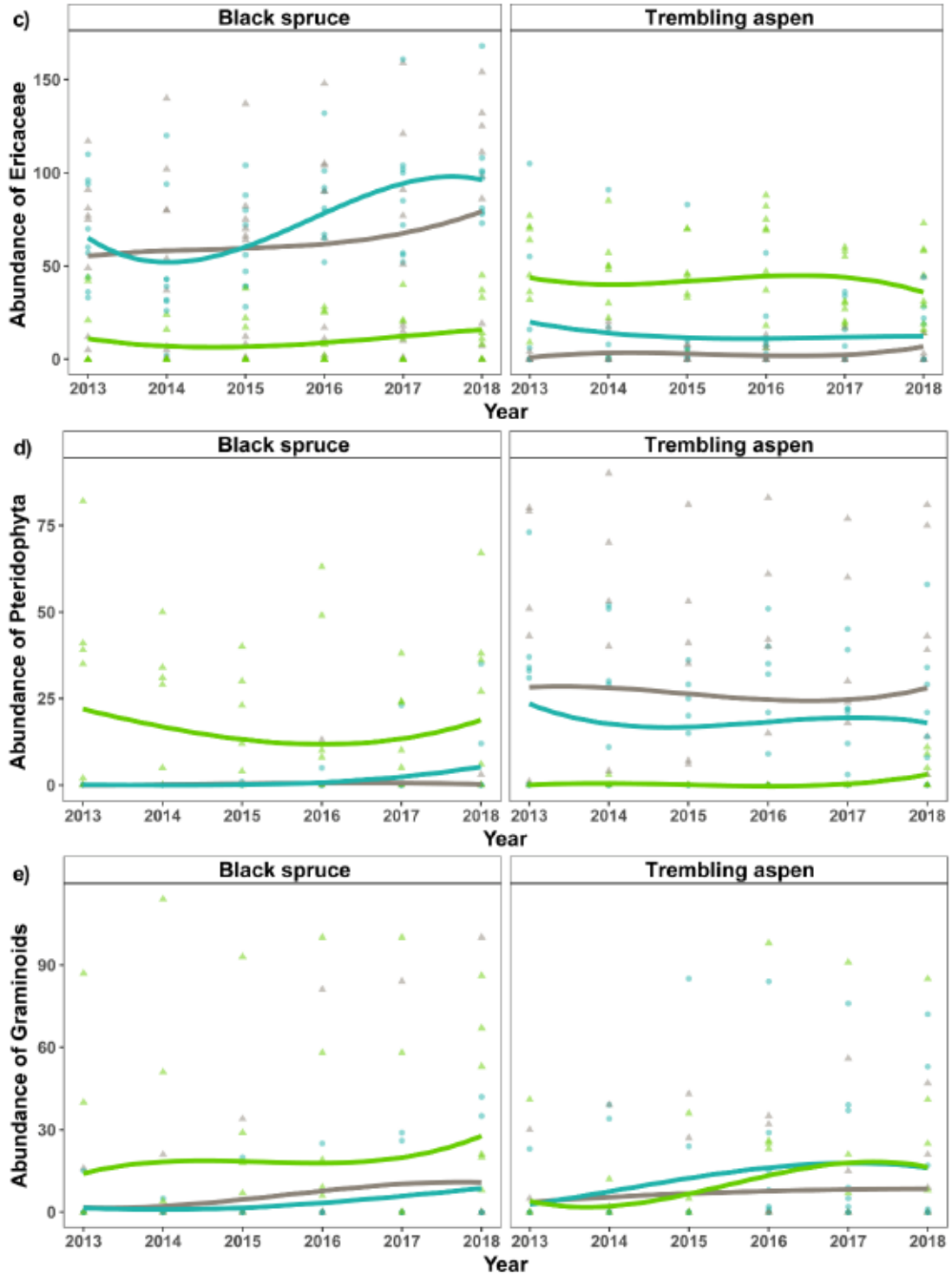

Figure S 3.3: continued

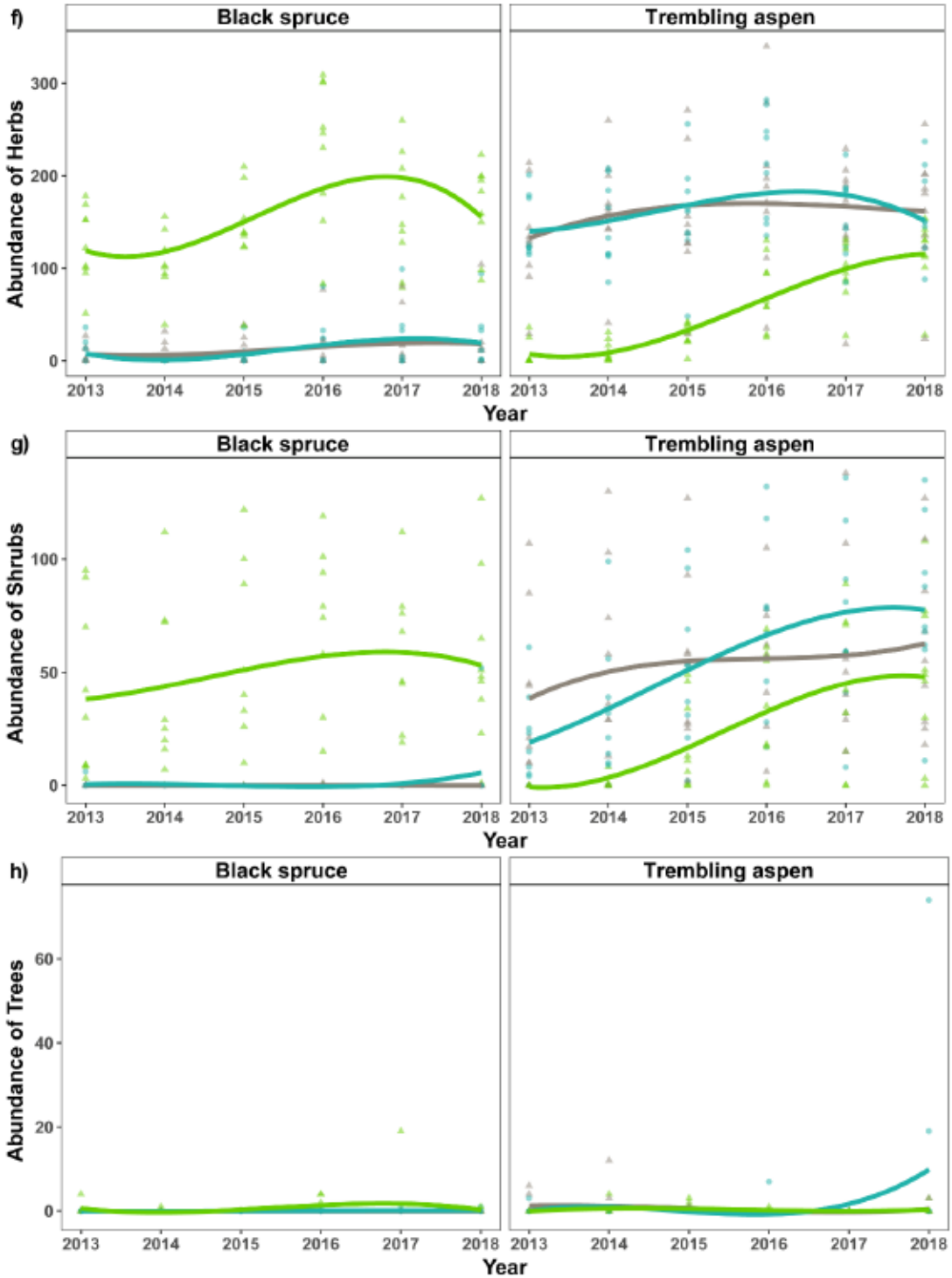

Figure S 3.3 Variation in abundance of functional groups over time (from 2013 to 2018) for black spruce (left panels) and trembling aspen stands (right panels). Functional groups: (a) Bryophytes, (b) *Sphagnaceae*, (c) *Ericaceae*, (d) *Peridophyta*, (e) Herbs,

(f) Shrubs, and (g) Trees. Treatments correspond to community approach: *Transplants-out* (To, in blue), *Transplants-in* (Ti, in green) and *Control* conditions (C, in grey). Smooth lines are based on the linear model of understory species abundances, each point in different colors (treatments) from data each year of the study.

Table S 3.1 Average abundance of understory plants of the cumulative effect of treatments in 2018 in black spruce and trembling aspen forests, for the Control (C, in grey) and the different treatments of the ecosystem approach (Objective 1): Light (Li, in yellow), Nutrients (Nu, in purple), Single-litter (1F, in orange) and Double-litter (2F, in red), and of the community approach (Objective 2): Transplants-out (To, in blue), Transplants-in (Ti, in green). Species, with their corresponding abbreviations, were classified in functional groups: Bryophytes, Sphagnaceae, Ericaceae, Pteridophyta, Graminoids, Herbs, and Shrubs, and Trees. Data correspond to the average and standard deviation (in grey numbers) of blocks and sites for each species among treatments and forest types. Values for each functional group (in colors) correspond to the average abundance per functional group for each treatment.

FUNCTIONAL GROUP		BLACK SPRUCE							TREMBLING ASPEN						
	Treatments of each approach	Control	Obj.1				Obj.2		Control	Obj.1				Obj.2	
Abbreviation	*Species*	C	1F	2F	Li	Nu	Ti	To	C	1F	2F	Li	Nu	Ti	To
BRYOPHYTES		**61.4**	**70.6**	**54.9**	**63.3**	**76.1**	**59.2**	**13.0**	**9.7**	**7.7**	**6.2**	**13.1**	**12.8**	**9.1**	**43.1**
DIP	*Dicranum polysetum*	2.2	3.0	4.2	2.5	0.7	4.8	9.2	2.8	4.3	3.3	1.9	3.5	2.8	4,8
HYS	*Hylocomium splendens*	0.5	0.0	0.1	0.0	0.0	0.0	6.1	0.3	16.2	4.3	14.0	0.0	1.1	0,0
PLG	*Plagiomnium sp.*	0.0	0.0	0.0	0.0	0.2	0.0	10.9	12.1	4.0	5.8	17.4	10.8	19.3	0,2
PLS	*Pleurozium schreberi*	59.0	69.6	71.9	66.7	75.8	64.0	40.5	27.9	49.3	42.8	30.9	33.3	22.7	64,9
POC	*Polytrichum commune*	0.0	0.0	0.3	1.3	2.1	1.5	2.6	12.4	1.9	3.3	8.6	24.7	28.0	1,7
PTC	*Ptilium crista-castrensis*	33.9	25.8	21.2	26.1	18.7	26.1	14.1	19.0	7.6	20.4	14.1	12.5	8.1	22,9
PTI	*Ptilidium ciliare*	0.1	0.1	0.5	1.4	0.0	1.1	0.0	0.1	0.3	0.0	0.0	0.4	0.1	1,3
RYT	*Rhytidiadelphus triquetrus*	0.0	0.2	0.3	0.0	1.5	0.0	16.6	20.9	16.2	19.8	12.7	14.7	18.0	3,0
SPS	*Sphagnum sp.*	4.1	1.2	1.6	2.0	1.0	2.4	0.0	4.5	0.1	0.3	0.4	0.0	0.0	1,1
PTERIDOPHYTA		**0.1**	**0.0**	**2.4**	**1.8**	**0.5**	**0.6**	**5.5**	**9.6**	**13.3**	**12.7**	**8.6**	**9.9**	**6.6**	**0.2**
DRD	*Gymnocarpium disjunctum*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	1.4	1.7	3.3	0.0	0,0
DRS	*Dryopteris carthusiana*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	2.6	3.4	0.0	0.0	0,0
EQP	*Equisetum pratense*	100.0	0.0	100.0	100.0	100.0	100.0	0.7	0.1	0.8	0.2	0.0	22.9	0.4	45,2
LYA	*Spinulum annotinum*	0.0	0.0	0.0	0.0	0.0	0.0	93.2	95.4	90.5	93.9	75.0	68.4	97.5	54,8
LYC	*Lycopodium clavatum*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	2.0	0.2	18.6	1.6	0.0	0,0

Table S 3.1 : continued

	LYO	*Dendrolycopodium obscurum*	0.0	0.0	0.0	0.0	0.0	0.0	6.1	4.2	2.8	1.0	1.3	3.8	2.2	0,0
	LYS	*Lycopodium sp.*	0.0		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0,0
ERICACEAE			**29.7**	**26.2**	**36.4**	**25.2**	**19.1**	**32.6**	**3.5**	**1.1**	**2.1**	**10.4**	**6.7**	**5.0**	**4.6**	**18.0**
	GAH	*Gaultheria hispidula*	83.4	97.3	85.1	91.0	62.1	91.2	34.7	49.4	76.3	12.8	23.2	34.9	24.4	94,4
	KAA	*Kalmia angustifolia*	0.7	0.3	0.0	0.4	0.2	4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0,0
	LEG	*Rhododendron groenlandicum*	3.8	0.2	8.3	0.2	14.3	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0,3
	VAA	*Vaccinium angustifolium*	4.6	0.3	3.9	4.8	21.9	2.3	0.0	42.6	10.3	36.7	33.7	8.9	0.0	2,2
	VAM	*Vaccinium myrtilloides*	7.5	1.8	2.7	3.7	1.6	1.1	65.3	8.0	13.4	50.5	43.1	56.3	75.4	3,0
HERBS			**8.8**	**3.2**	**5.0**	**9.1**	**3.6**	**7.1**	**60.3**	**60.1**	**55.6**	**49.5**	**57.6**	**55.4**	**60.3**	**28.1**
	ARN	*Aralia nudicaulis*	0.0	0.0	0.0	0.0	0.0	0.0	2.6	5.7	4.9	3.0	7.3	4.7	1.1	4,2
	ASA	*Oclemena acuminata*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	1.5	0.0	0.2	1.5	0.1	0,0
	ASM	*Eurybia macrophylla*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.2	0.4	2.8	0.2	0.1	0,0
	CAX	*Carex sp.*	25.2	33.9	0.0	31.6	67.5	19.7	3.3	0.0	0.0	0.3	0.4	0.3	1.0	8,3
	CHL	*Leucanthemum vulgare*	1.1	0.0	0.0	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.7	0.0	0.2	0,0
	CIA	*Circaea alpina*	0.0	10.8	0.0	1.4	0.0	0.0	5.1	0.1	2.9	1.3	0.7	0.7	1.8	1,9
	CLB	*Clintonia borealis*	0.0	1.6	0.0	1.3	0.0	0.0	5.0	9.6	5.8	5.9	5.6	8.9	5.5	7,7
	COL	*Geocaulon lividum*	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.2	0.0	0,0
	CON	*Cornus canadensis*	44.5	47.8	84.2	30.3	9.1	62.2	22.3	32.9	39.7	38.1	27.3	31.1	27.4	37,2
	COT	*Coptis trifolia*	0.4	1.6	0.0	0.0	0.0	0.0	3.0	1.0	1.8	2.3	3.6	2.3	3.9	0,8
	EPA	*Chamaenerion angustifolium subsp. angustifolium*	0.0	0.0	0.0	1.3	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0,0
	FRA	*Fragaria sp.*	0.0	0.0	0.0	0.0	2.0	0.0	1.1	1.3	1.4	0.3	3.5	1.0	0.7	1,5
	GAA	*Galium asprellum*	0.0	0.0	0.0	0.0	0.0	0.0	4.6	3.3	2.2	1.7	4.9	2.7	5.7	1,5
	GOR	*Goodyera repens*	6.2	0.0	7.2	0.2	5.2	0.8	0.0	0.1	0.2	0.2	0.3	0.1	0.1	0,0
	HIA	*Pilosella aurantiaca*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0,0
	LIB	*Linnaea borealis*	14.6	0.0	0.0	0.0	0.0	12.0	11.0	11.3	4.2	8.9	7.3	8.2	8.9	7,7
	MAC	*Maianthemum canadense*	0.0	0.0	1.0	0.8	0.0	0.0	7.8	10.4	10.4	12.3	10.7	9.4	9.0	2,1
	MIN	*Mitella nuda*	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.5	0.0	2.4	0.6	1.4	0.0	0,0
	OXM	*Oxalis montana*	0.0	0.5	0.0	0.0	0.0	0.1	4.3	3.2	6.5	4.8	6.5	3.1	4.8	0,3
	PES	*Petasites frigidus var. palmatus*	0.0	0.0	0.0	0.0	0.0	0.0	1.2	4.5	4.1	1.7	3.8	2.8	7.1	8,8
	POA	*Poa sp.*	8.0	1.6	0.0	30.5	15.9	2.9	7.8	4.0	1.2	3.7	1.8	7.5	6.0	7,2
	PYE	*Pyrola elliptica*	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.2	0.1	0.0	0.0	0,0
	PYR	*Orthilia secunda*	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.3	0.3	0.1	0.0	0,2

Table S 3.1 : continued

	SMT	*Maianthemum trifolium*	0.0	2.2	7.7	2.6	0.4	1.5	0.1	0.0	0.0	0.0	0.1	0.0	0.1	0,0
	SOM	*Solidago macrophylla*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.9	0.3	0.6	0.2	0,0
	STA	*Streptopus amplexifolius*	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.4	0.6	0.2	0.0	0.0	1,2
	TRB	*Trientalis borealis*	0.0	0.0	0.0	0.0	0.0	0.8	3.2	2.3	3.1	1.2	1.4	0.5	2.4	4,0
	VEJ	*Vicia cracca*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.5	0.0	0.0	0,0
	VIS	*Viola sp.*	0.0	0.0	0.0	0.0	0.0	0.0	15.3	6.8	7.6	9.6	9.2	11.0	13.8	5,5
SHRUBS			**0.0**	**0.0**	**1.3**	**0.6**	**0.7**	**0.5**	**17.7**	**19.5**	**21.3**	**21.2**	**13.9**	**16.9**	**19.4**	**10.5**
	AME	*Amelanchier sp.*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.1	0.0	0.0	0.0	0,0
	AUC	*Alnus alnobetula subsp. Crispa*	0.0	0.0	0.0	0.0	4.2	0.0	0.2	0.0	0.0	0.0	1.0	0.0	0.0	0,0
	AUR	*Alnus incana subsp. Rugosa*	100.0	0.0	0.0	0.0	10.4	0.0	0.0	0.3	9.2	0.0	2.9	2.3	0.2	1,7
	BOP	*Betula papyrifera*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0,0
	DIE	*Diervilla lonicera*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.5	0.0	0.0	0.0	0.0	0,0
	EPN	*Picea mariana*	0.0	0.0	98.1	100.0	2.1	4.9	0.4	0.1	0.8	0.0	0.0	0.0	0.2	0,1
	LON	*Lonicera canadensis*	0.0	0.0	0.0	0.0	0.0	85.2	0.7	0.0	1.5	0.0	1.5	0.4	0.4	0,0
	MEP	*Mertensia paniculata*	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.8	0.2	1.5	0.1	0.0	0.0	0,0
	PET	*Populus tremuloides*	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.9	0.2	0.0	0.5	0.0	3.2	1,0
	RIG	*Ribes glandulosum*	0.0	0.0	0.0	0.0	0.0	0.0	13.3	10.0	8.4	8.9	10.4	7.3	15.2	6,0
	RIT	*Ribes triste*	0.0	0.0	0.0	0.0	0.0	9.8	3.0	1.6	0.4	0.0	1.8	3.3	4.2	0,5
	ROA	*Rosa acicularis*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.5	0.1	1.2	2.0	0.4	2.0	1,3
	RUI	*Rubus idaeus*	0.0	0.0	0.0	0.0	31.3	0.0	0.5	5.1	4.2	1.2	3.2	6.3	6.9	14,7
	RUP	*Rubus pubescens*	0.0	0.0	1.9	0.0	52.1	0.0	73.7	61.2	42.8	48.7	67.8	69.7	61.6	70,6
	VIE	*Viburnum edule*	0.0	0.0	0.0	0.0	0.0	0.0	6.7	14.5	22.3	34.3	8.7	10.2	5.9	4,1
TOTAL			**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table S 3.2 Differences in α-diversity (Shannon-Weiner index) based on the ANOVA of the linear mixed effect model of understory species abundance to evaluate the factors of Year, Forest type, Treatment and all interactions, using Sites and Blocks as nested random factors.

	Sum of Squares	**Mean of Squares**	**Df**	**Df_{den}**	***F***	***P***
Year	9,45	1,89	5	664	21,410	<.0001***
Forest type	120,36	120	1	664	1364,028	<.0001***
Treatment	14,15	2,36	6	664	26,722	<.0001***
Year:Forest type	0,99	0,2	5	664	2,241	0.04875*
Year:Treatment	4,53	0,15	30	664	1,712	0.01097*
Forest type:Treatment	57,46	9,58	6	664	108,522	<.0001***
Year:Forest type:Treatment	**4,14**	**0,14**	**30**	**664**	**1,565**	**0.02909***

Table S 3.3 *Post-hoc emmeans* contrasts of the lineral mixed effect model (Table S 3.2) of the α-diversity (Shannon index) of plant understory species in each forest type (Black spruce – BS, Trembling aspen - TA), comparing each treatment to the control. Significant differences correspond to P < 0.0001 ***, <0.001 **, < 0.05 *.

Forest type	**Year**	**Contrasts (Treatment Vs. Control)**					
		Treatment	**Estimate**	**SE**	**df**	**t.ratio**	***P***
BS	**2013**	Li	-0.09	0.14	664	-0.66	0.9242
		Nu	-0.09	0.14	664	-0.623	0.9357
		1F	-0.21	0.14	664	-1.485	0.4707
		2F	-0.07	0.14	664	-0.528	0.9596
		To	**0.89**	**0.14**	**664**	**6.384**	**<.0001*** **
		Ti	0.10	0.14	664	0.693	0.9134
	2014	Li	-0.08	0.14	664	-0.586	0.9458
		Nu	-0.23	0.14	664	-1.663	0.363
		1F	-0.31	0.14	664	-2.175	0.1372
		2F	-0.03	0.14	664	-0.227	0.9966
		To	**0.98**	**0.14**	**664**	**6.973**	**<.0001*** **
		Ti	-0.05	0.14	664	-0.335	0.9891
	2015	Li	-0.05	0.14	664	-0.385	0.9837

Table S 3.3 : continued

		Nu	-0.33	0.14	664	-2.337	0.0945
		1F	**-0.39**	**0.14**	**664**	**-2.75**	**0.0319***
		2F	-0.18	0.14	664	-1.291	0.5965
		To	**0.96**	**0.14**	**664**	**6.852**	**<.0001*****
		Ti	0.08	0.14	664	0.591	0.9443
	2016	Li	-0.04	0.14	664	-0.265	0.9946
		Nu	-0.35	0.14	664	-2.471	0.0678
		1F	-0.24	0.14	664	-1.741	0.3195
		2F	-0.01	0.14	664	-0.051	1
		To	**1.11**	**0.14**	**664**	**7.951**	**<.0001*****
		Ti	0.15	0.14	664	1.056	0.7429
	2017	Li	0.01	0.14	664	0.085	0.9998
		Nu	**-0.39**	**0.14**	**664**	**-2.815**	**0.0264***
		1F	-0.35	0.14	664	-2.52	0.0597
		2F	-0.14	0.14	664	-0.962	0.7953
		To	**0.86**	**0.14**	**664**	**6.156**	**<.0001*****
		Ti	0.21	0.14	664	1.53	0.4424
	2018	Li	0.11	0.14	664	0.777	0.882
		Nu	-0.34	0.14	664	-2.45	0.0715
		1F	-0.35	0.14	664	-2.462	0.0693
		2F	-0.24	0.14	664	-1.684	0.3513
		To	**0.96**	**0.14**	**664**	**6.838**	**<.0001*****
		Ti	0.19	0.14	664	1.378	0.5397
TA	**2013**	Li	0.18	0.14	664	1.307	0.5856
		Nu	0.09	0.14	664	0.629	0.9338
		1F	-0.08	0.14	664	-0.579	0.9474
		2F	0.06	0.14	664	0.456	0.9734
		To	**-0.82**	**0.14**	**664**	**-5.867**	**<.0001*****
		Ti	0.06	0.14	664	0.443	0.9755
	2014	Li	0.16	0.14	664	1.121	0.7037
		Nu	-0.01	0.14	664	-0.057	0.9999
		1F	-0.05	0.14	664	-0.383	0.9839
		2F	-0.15	0.14	664	-1.04	0.7516
		To	**-1.00**	**0.14**	**664**	**-7.14**	**<.0001*****
		Ti	0.00	0.14	664	-0.008	1
	2015	Li	0.00	0.14	664	0.032	1

Table S 3.3 : continued

	Nu	-0.09	0.14	664	-0.629	0.9338
	1F	-0.15	0.14	664	-1.064	0.7377
	2F	-0.17	0.14	664	-1.19	0.6612
	To	**-0.61**	**0.14**	**664**	**-4.357**	**0.0001*****
	Ti	-0.04	0.14	664	-0.252	0.9953
2016	Li	0.09	0.14	664	0.62	0.9363
	Nu	0.04	0.14	664	0.314	0.991
	1F	-0.03	0.14	664	-0.192	0.9979
	2F	-0.07	0.14	664	-0.48	0.9692
	To	-0.32	0.14	664	-2.263	0.1126
	Ti	0.07	0.14	664	0.467	0.9715
2017	Li	0.06	0.14	664	0.453	0.9738
	Nu	0.00	0.14	664	-0.014	1
	1F	-0.01	0.14	664	-0.077	0.9999
	2F	-0.04	0.14	664	-0.253	0.9953
	To	-0.14	0.14	664	-1.01	0.7687
	Ti	0.15	0.14	664	1.046	0.7483
2018	Li	0.01	0.14	664	0.037	1
	Nu	0.00	0.14	664	0.001	1
	1F	-0.13	0.14	664	-0.894	0.8298
	2F	-0.11	0.14	664	-0.806	0.8702
	To	0.02	0.14	664	0.15	0.999
	Ti	0.12	0.14	664	0.821	0.8635

Table S 3.4 PERMANOVA based on the Hellinger-transformed data (Bray-Curtis distance) of understory vegetation abundance for the ecosystem and community approaches to test the variables of Year, Forest type, Treatment and all possible interactions, using Site and Blocks as nested random factors.

Objective	Variable	Df	Df_{total}	Sum of squares	R^2	F	Pr(>F)
Ecosystem approach	Year	1	179	0.464	0.00414	1.0918	0.2523
	Forest type	1	179	37.170	0.33192	87.5023	0.0001
	Treatment	4	179	1.816	0.01622	1.0687	0.2460
	Year:Forest type	**1**	**179**	**0.966**	**0.00863**	**2.2741**	**0.0360**
	Year:Treatment	4	179	1.075	0.00960	0.6327	0.8757
	Forest type:Treatment	4	179	1.714	0.01530	1.0085	0.3206
	Year:Forest type:Treatment	4	179	0.815	0.00728	0.4798	0.9895
Community approach	Year	1	107	0.696	0.01112	1.8002	0.0808
	Forest type	1	107	5.315	0.08485	13.7424	0.0001
	Treatment	2	107	0.882	0.01407	1.1396	0.2307
	Year:Forest type	1	107	0.477	0.00761	1.2325	0.1946
	Year:Treatment	2	107	0.545	0.00870	0.7044	0.6405
	Forest type:Treatment	2	107	15.459	0.24679	19.9846	0.0001
	Year:Forest type:Treatment	**2**	**107**	**2.137**	**0.03411**	**2.7625**	**0.0037**

ANNEXE B

SUPPLEMENTARY INFORMATION CHAPTER 2

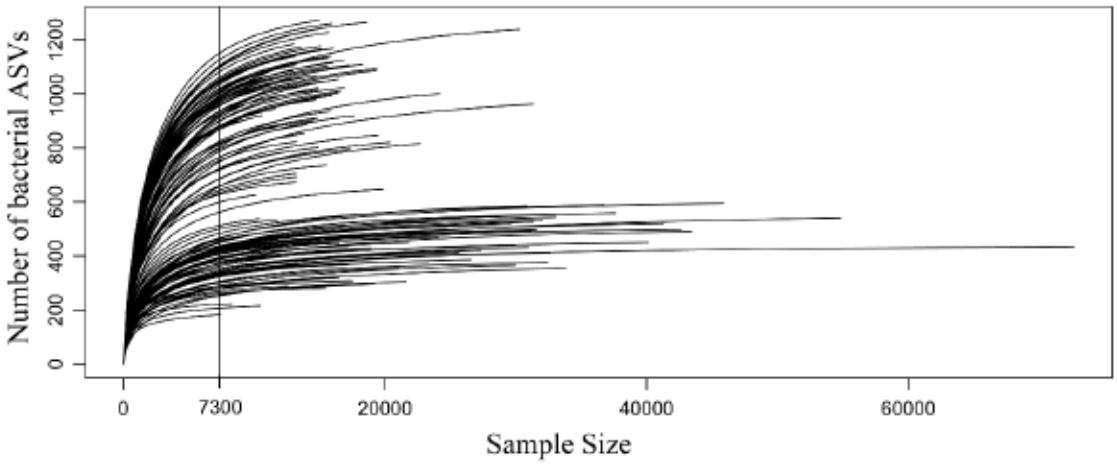

Figure S 3.4 Rarefaction curve of sample size (number of sequences) versus number of bacterial ASVs in samples from soil microbiome and tree phyllosphere. A threshold of 7300 was chosen to randomly rarefy the data and to capture the vast majority of ASVs in samples conserving all samples.

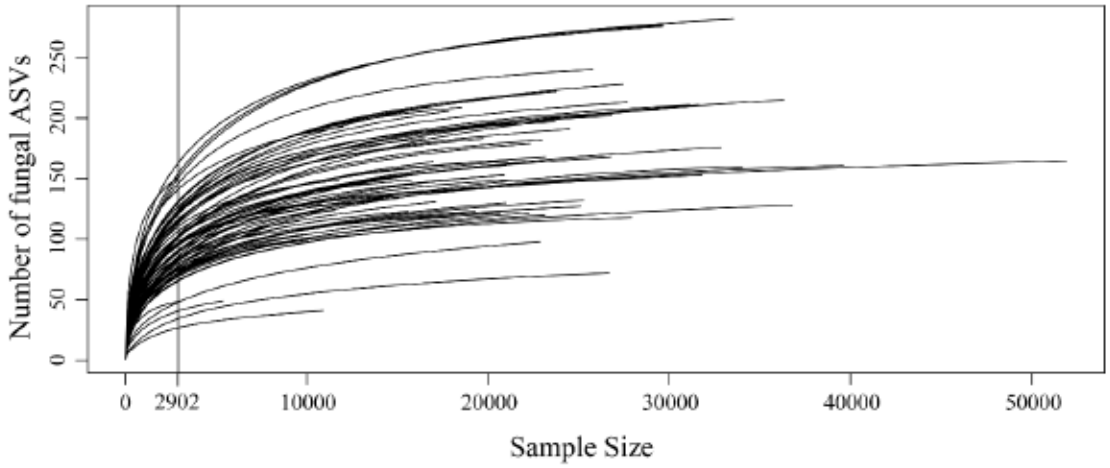

Figure S 3.5 Rarefaction curve of sample size (number of sequences) versus number of fungal ASVs in soil microbiome samples. A threshold of 2902 was chosen to randomly rarefy the data and to capture the vast majority of ASVs in samples conserving all samples.

Table S 3.5 Bacterial Shannon diversity between microhabitats (phyllosphere and soil microbiome) and forest types (black spruce and trembling aspen), based on mixed effect model with interaction of both factors and using Sites and Blocks as random factors.

	Sum of Squares	**Mean of Squares**	DF	DF_{den}	***F***	***P***	**Sig.**
Canopy	0.285	0.285	1	121	2.5045	0.1161	
Habitat	59.282	59.282	1	121	521.2927	<.0001	***
Canopy:Habitat	**5.202**	**5.202**	**1**	**121**	**45.7438**	**<.0001**	***

Table S 3.6 Differences in bacterial Shannon diversity between microhabitats (phyllosphere and soil microbiome) and forest types (black spruce and trembling aspen). Post hoc pairwise Tukey contrasts based on the mixed effect model with interaction of both factors and using Sites and Blocks as random factors.

Contrasts			**Estimate**	**SE**	**Df**	**t.ratio**	***P***	**Sig.**
BS_Phyllosphere	-	TA_Phyllosphere	0.509	0.0927	113	5.494	<.0001	***
BS_Phyllosphere	-	BS_Soil	-0.981	0.0859	113	-11.424	<.0001	***
BS_Phyllosphere	-	TA_Soil	-1.297	0.0859	113	-15.108	<.0001	***
TA_Phyllosphere	-	BS_Soil	-1.490	0.0869	113	-17.158	<.0001	***
TA_Phyllosphere	-	TA_Soil	-1.807	0.0869	113	-20.799	<.0001	***
BS_Soil	-	TA_Soil	-0.316	0.0795	113	-3.979	0.0007	**

Table S 3.7 Differences in relative abundance of bacterial ASVs among microhabitats (tree phyllosphere and soil microbiome) and forest types (black spruce and trembling aspen) and their interaction, including Site and Block as random factors. Significant differences of the interaction among microhabitat and forest type (PERMANOVA, $P < 0.05$).

Variable	Df	Df_{total}	Sum of Squares	R^2	*F*	*P*	Sig.
Canopy	1	124	9.626	0.1076	30.624	0.0001	***
Habitat	1	124	30.645	0.3425	97.491	0.0001	***
Canopy:Habitat	**1**	**124**	**11.182**	**0.1250**	**35.573**	**0.0001**	***

Table S 3.8 Differences in relative abundance of ASVs assigned to phyla from leaves bacteria between forest types and from soil bacteria and fungal phyla between each forest types, treatments and their interaction. Significant differences between forest types for some Phyla (ANOVA, all Benjamini–Hochberg-adjusted $P < 0.0001$***, $P < 0.001$**, $P < 0.05$*).

LEAF BACTERIAL PHYLA	Variable	Df	Df_{den}	Sum of Squares	Mean of Squares	*F*	*P*	P_{adj}	
Acidobacteria				**27.591**	**27.591**	**402.470**	**0.0000**	**0.0000**	***
Actinobacteria				**12.227**	**12.227**	**231.430**	**0.0000**	**0.0000**	***
Armatimonadetes				0.348	0.348	3.593	0.0637	0.0693	
Bacteroidetes				**30.497**	**30.497**	**535.870**	**0.0000**	**0.0000**	***
Chlamydiae				**17.941**	**17.941**	**109.870**	**0.0000**	**0.0000**	***
Chloroflexi				**31.464**	**31.464**	**65.279**	**0.0000**	**0.0000**	***
Deinococcus-Thermus	**FT**	1	51	**42.111**	**42.111**	**559.410**	**0.0000**	**0.0000**	***
Firmicutes				**2.559**	**2.559**	**17.402**	**0.0001**	**0.0002**	**
Fusobacteria				0.007	0.007	2.165	0.1473	0.1473	
Gemmatimonadetes				**0.107**	**0.107**	**5.275**	**0.0258**	**0.0328**	*
Patescibacteria				**20.146**	**20.146**	**88.716**	**0.0000**	**0.0000**	***
Planctomycetes				**0.222**	**0.222**	**6.357**	**0.0149**	**0.0208**	*
Proteobacteria				**2.505**	**2.505**	**262.610**	**0.0000**	**0.0000**	***
Verrucomicrobia				0.047	0.047	3.574	0.064	0.069	

Table 3.8 : continued

SOIL BACTERIAL PHYLA	Variable	Df	Df den	Sum of Squares	Mean of Squares	F	P	P_{adj}	
	FT	**1**		**0.458**	**0.458**	**16.734**	**0.0001**	**0.0006**	**
Acidobacteria	T	3	64	0.025	0.008	0.304	0.8227	0.9228	
	FT x T	3		0.016	0.005	0.192	0.9015	0.9516	
	FT	**1**		**0.079**	**0.079**	**12.728**	**0.0007**	**0.0022**	*
Actinobacteria	T	3	64	0.028	0.009	1.507	0.2212	0.9228	
	FT x T	3		0.002	0.001	0.102	0.9586	0.9586	
	FT	1		0.199	0.199	2.001	0.1621	0.2053	
Armatimonadetes	T	3	64	0.069	0.023	0.231	0.8742	0.9228	
	FT x T	3		0.454	0.151	1.520	0.2177	0.8275	
	FT	**1**		**0.481**	**0.481**	**15.765**	**0.0002**	**0.0007**	**
Bacteroidetes	T	3	64	0.054	0.018	0.594	0.6212	0.9228	
	FT x T	3		0.043	0.014	0.471	0.7036	0.8355	
	FT	**1**		**0.690**	**0.690**	**10.293**	**0.0021**	**0.0044**	*
Chlamydiae	T	3	64	0.063	0.021	0.312	0.8167	0.9228	
	FT x T	3		0.144	0.048	0.715	0.5466	0.8355	
	FT	**1**		**3.801**	**3.801**	**10.060**	**0.0023**	**0.0044**	*
Chloroflexi	T	3	64	0.541	0.180	0.477	0.6994	0.9228	
	FT x T	3		0.698	0.233	0.615	0.6076	0.8355	
	FT	1		0.001	0.001	1.000	0.3211	0.3211	
Deinococcus-Thermus	T	3	64	0.004	0.001	1.000	0.3987	0.9228	
	FT x T	3		0.004	0.001	1.000	0.3987	0.8355	
	FT	**1**		**0.897**	**0.897**	**7.391**	**0.0084**	**0.0146**	*
Dependentiae	T	3	64	0.282	0.094	0.776	0.5120	0.9228	
	FT x T	3		0.748	0.249	2.054	0.1151	0.8275	
	FT	1		0.333	0.333	3.504	0.0658	0.0893	
Elusimicrobia	T	3	64	0.198	0.066	0.695	0.5585	0.9228	
	FT x T	3		0.491	0.164	1.725	0.1708	0.8275	
	FT	**1**		**2.201**	**2.201**	**11.332**	**0.0013**	**0.0035**	*
Fibrobacteres	T	3	64	0.153	0.051	0.263	0.8519	0.9228	
	FT x T	3		0.386	0.129	0.663	0.5778	0.8355	
	FT	**1**		**5.908**	**5.908**	**22.051**	**0.0000**	**0.0001**	***
Firmicutes	T	3	64	0.012	0.004	0.014	0.9976	0.9976	
	FT x T	3		0.574	0.191	0.714	0.5472	0.8355	

Table 3.8 : continued

	FT	1		0.003	0.003	1.000	0.3211	0.3211	
Fusobacteria	T	3	64	0.009	0.003	1.000	0.3987	0.9228	
	FT x T	3		0.009	0.003	1.000	0.3987	0.8355	
	FT	**1**		**3.363**	**3.363**	**40.130**	**0.0000**	**0.0000**	***
Gemmatimonadetes	T	3	64	0.129	0.043	0.514	0.6742	0.9228	
	FT x T	3		0.087	0.029	0.346	0.7923	0.8855	
	FT	**1**		**1.382**	**1.382**	**10.721**	**0.0017**	**0.0041**	*
Nitrospirae	T	3	64	0.115	0.038	0.299	0.8263	0.9228	
	FT x T	3		0.617	0.206	1.597	0.1988	0.8275	
	FT	**1**		**0.970**	**0.970**	**19.418**	**0.0000**	**0.0003**	**
Patescibacteria	T	3	64	0.364	0.121	2.428	0.0735	0.9228	
	FT x T	3		0.276	0.092	1.839	0.1490	0.8275	
	FT	1		0.116	0.116	1.177	0.2821	0.3153	
Planctomycetes	T	3	64	0.282	0.094	0.950	0.4219	0.9228	
	FT x T	3		0.146	0.049	0.491	0.6897	0.8355	
	FT	1		0.006	0.006	3.587	0.0628	0.0893	
Proteobacteria	T	3	64	0.003	0.001	0.649	0.5863	0.9228	
	FT x T	3		0.006	0.002	1.123	0.3465	0.8355	
	FT	**1**		**0.320**	**0.320**	**6.575**	**0.0127**	**0.0201**	*
Spirochaetes	T	3	64	0.055	0.018	0.378	0.7690	0.9228	
	FT x T	3		0.071	0.024	0.486	0.6935	0.8355	
	FT	1		0.041	0.041	1.628	0.2066	0.2453	
Verrucomicrobia	T	3	64	0.035	0.012	0.455	0.7145	0.9228	
	FT x T	3		0.037	0.012	0.491	0.6900	0.8355	

SOIL FUNGAL PHYLA	**Variable**	**Df**		**Sum of Squares**	**Mean of Squares**	**F**	**P**	**P_{adj}**	
	FT	1		0.146	0.146	0.741	0.3927	0.4487	
Ascomycota	T	3	63	0.192	0.064	0.325	0.8072	0.8832	
	FT x T	3		0.293	0.098	0.496	0.6863	0.9999	
	FT	1		0.007	0.007	0.971	0.3283	0.4467	
Basidiobolomycota	T	3	63	0.020	0.007	0.989	0.4037	0.8832	
	FT x T	3		0.020	0.007	0.980	0.4082	0.9999	
	FT	1		0.002	0.002	0.341	0.5616	0.5616	
Basidiomycota	T	3	63	0.010	0.003	0.505	0.6801	0.8832	
	FT x T	3		0.000	0.000	0.002	0.9999	0.9999	

Table 3.8 : continued

	FT	1		0.003	0.003	1.043	0.3110	0.4467	
Chytridiomycota	T	3	63	0.010	0.003	1.105	0.3537	0.8832	
	FT x T	3		0.011	0.004	1.209	0.3138	0.9999	
	FT	**1**		**5.810**	**5.810**	**26.780**	**0.0000**	**0.0000**	***
Mortierellomycota	T	3	63	0.299	0.100	0.459	0.7118	0.8832	
	FT x T	3		0.075	0.025	0.116	0.9506	0.9999	
	FT	**1**		**2.150**	**2.150**	**21.262**	**0.0000**	**0.0001**	***
Mucoromycota	T	3	63	0.066	0.022	0.219	0.8832	0.8832	
	FT x T	3		0.113	0.037	0.371	0.7744	0.9999	
	FT	1		0.008	0.008	0.944	0.3350	0.4467	
Rozellomycota	T	3	63	0.079	0.026	3.082	0.0337	0.2692	
	FT x T	3		0.025	0.008	0.974	0.4107	0.9999	
	FT	1		0.012	0.012	1.848	0.1788	0.4467	
Zoopagomycota	T	3	63	0.013	0.004	0.697	0.5574	0.8832	
	FT x T	3		0.014	0.005	0.713	0.5478	0.9999	

Table S 3.9 Differences in the ASVs Shannon diversity of bacteria (a) and fungi (b) in soils from black spruce and trembling aspen forests. Comparisons among treatments: Control conditions (C, in grey), Litter addition (1F, in orange), Transplants-in (Ti, in green) and Transplants-out (To, in blue). Analysis of the interaction between forest type and treatments (ANOVA tests $P < 0.05$). Post hoc pairwise Tukey contrasts based on the mixed effect model with interaction of both factors and using Sites and Blocks as random factors.

	Factor	**Sum of Squares**	**Mean of Squares**	**DF_{num}**	**DF_{den}**	***F***	***P***	**Sig.**
Bacteria	**Forest type**	1.800	1.800	1	62	18.1387	**<.0001**	***
	Treatment	0.195	0.065	3	62	0.6548	0.5830	
	Forest type : Treatment	0.083	0.028	3	62	0.2796	0.8399	
Fungi	**Forest type**	0.700	0.700	1	63	1.6113	0.2090	
	Treatment	0.081	0.027	3	63	0.0620	0.9796	
	Forest type : Treatment	0.953	0.318	3	63	0.7310	0.5374	

Table S 3.10 Abiotic factors including local environmental conditions (Overstory density, Light inputs, Soil humidity and Soil temperature) and soil physicochemical properties for each forest type (BS – Black spruce and TA – Trembling aspen) for the different treatments in colors: Control conditions (C, in grey), Litter addition (1F, in orange), Transplants-in (Ti, in green) and Transplants-out (To, in blue). Data represent the average and standard deviation (in grey numbers) for sites and blocks. Variables in orange were significantly different between forest types. We found no significant differences among treatments or the interaction between Forest type and Treatment.

Treatment	Forest type	Overstory Density	Light	Soil Moisture	Soil Temperature	Soil pH		Soil chemical properties																
						pH-1	pH-2	N	C	K	Mg	Ca	Na	H	CEC	C:N	N:P	P:Al	P:Cs	P	Al	Mn	Fe	S
		(%)	(µmol m2s-1)	(VWC)	(°C)	(1:1)	(SMP)	(%)							(meq*100g-1)	(ratio)				(mg g-1)				
C	BS	94.2	51.5	15.3	10.9	4.1	4.9	0.8	26.2	2.1	10.2	19.2	0.5	0.7	38.3	34.2	331.1	0.0	0.1	0.0	1.8	0.0	0.5	0.0
		4.0	25.4	9.1	0.6	0.7	0.6	0.2	3.8	0.6	6.0	16.6	0.1	0.2	9.0	8.2	167.6	0.0	0.1	0.0	0.4	0.0	0.1	0.0
	TA	94.7	47.7	12.4	11.2	4.5	5.3	0.6	15.4	2.2	10.1	30.4	0.3	0.6	37.1	26.1	218.4	0.0	0.0	0.0	1.3	0.0	0.3	0.0
		2.8	16.1	3.1	0.8	0.4	0.3	0.2	3.3	0.5	4.8	11.7	0.1	0.2	8.0	5.9	163.5	0.0	0.1	0.0	0.2	0.0	0.1	0.0
1F	BS	94.7	46.5	13.0	10.9	4.0	5.0	0.7	24.2	2.3	9.9	18.2	0.4	0.7	38.0	35.3	267.6	0.0	0.1	0.0	1.7	0.0	0.4	0.0
		2.1	13.5	5.2	0.6	0.6	0.6	0.3	7.7	1.0	7.5	18.1	0.2	0.3	11.2	8.1	166.5	0.0	0.1	0.0	0.5	0.0	0.1	0.0
	TA	95.7	45.8	10.4	11.0	4.6	5.6	0.6	13.5	2.3	11.6	34.6	0.3	0.5	36.3	23.8	221.6	0.0	0.0	0.0	1.2	0.1	0.3	0.0
		2.4	22.1	2.2	0.6	0.3	0.3	0.2	6.0	0.4	5.0	10.3	0.1	0.2	9.9	6.4	142.8	0.0	0.0	0.0	0.2	0.0	0.1	0.0
Ti	BS	95.3	48.6	16.3	10.8	4.0	4.9	0.8	23.7	2.3	9.8	18.7	0.4	0.7	37.4	32.3	342.1	0.0	0.0	0.0	1.7	0.0	0.4	0.0
		2.0	17.7	10.8	0.7	0.4	0.4	0.2	4.7	0.8	4.5	13.0	0.2	0.2	9.1	9.3	244.7	0.0	0.1	0.0	0.4	0.0	0.1	0.0
	TA	96.2	47.6	14.6	10.9	4.6	5.4	0.5	12.2	2.4	10.0	30.2	0.3	0.6	35.6	22.5	213.4	0.0	0.0	0.0	1.4	0.1	0.3	0.0
		2.4	28.1	3.6	1.2	0.3	0.4	0.2	4.1	0.8	4.3	11.6	0.1	0.2	11.1	2.1	118.8	0.0	0.0	0.0	0.3	0.0	0.0	0.0
To	BS	95.3	48.6	15.9	11.3	4.0	5.0	0.8	25.4	2.4	10.8	18.8	0.4	0.7	37.4	33.1	287.7	0.0	0.0	0.0	1.7	0.0	0.4	0.0
		2.6	16.4	11.0	0.7	0.5	0.4	0.2	6.4	0.9	5.5	12.4	0.2	0.2	9.0	6.9	166.3	0.0	0.0	0.0	0.4	0.0	0.1	0.0
	TA	95.3	47.4	15.7	10.8	4.6	5.4	0.5	12.4	2.4	10.1	27.8	0.3	0.6	34.1	24.1	228.5	0.0	0.1	0.0	1.4	0.0	0.3	0.0
		3.8	19.2	5.2	1.1	0.3	0.4	0.2	4.2	0.6	4.0	10.2	0.0	0.1	11.1	2.9	145.7	0.0	0.1	0.0	0.1	0.0	0.0	0.0

Table S 3.11 Correlations of bacterial and fungal community composition with abiotic factors (local environmental conditions and soil physicochemical properties), understory vegetation and microbial phyla. Variables correlated with black spruce forests (in blue) and with trembling aspen forests (in green) and comparing physicochemical values of Table S4. Significant correlation with the microbial community composition based on the NMDS for bacteria and fungi (Envfit, $P < 0.0001$***, $P < 0.001$**, $P < 0.05$*).

	BACTERIA					FUNGI				
Variable	**NMDS 1**	**NMDS 2**	R^2	P	**Sig.**	**NMDS 1**	**NMDS 2**	R^2	P	**Sig.**
Local environmental conditions										
Soil temperature	0.80	0.60	0.0850	0.0360	*	0.28	0.96	0.0293	0.3790	
Soil moisture	0.20	0.98	0.1394	0.0060	**	-0.10	-0.99	0.1131	0.0180	*
Light	0.63	0.78	0.1999	0.0020	**	0.53	-0.85	0.0117	0.6560	
Overstory density	-0.30	-0.95	0.0756	0.0620	.	0.19	-0.98	0.0031	0.8980	
Soil physicochemical properties										
N (%)	0.11	0.99	0.4331	0.0010	***	-0.82	-0.57	0.0985	0.0280	*
C (%)	-0.16	0.99	0.4838	0.0010	***	-0.86	0.50	0.4248	0.0010	***
C : N	-0.77	0.63	0.4302	0.0010	***	-0.58	0.82	0.6500	0.0010	***
N : P	0.16	0.99	0.1954	0.0020	**	-0.50	-0.87	0.0316	0.3430	
P : Al	-0.04	-1.00	0.0524	0.1640		0.28	-0.96	0.0850	0.0460	*
P : Ca	-0.83	-0.55	0.2805	0.0010	***	-0.93	0.36	0.0752	0.0570	.
pH (H_2O)	0.99	-0.15	0.7942	0.0010	***	0.82	-0.57	0.5053	0.0010	***
pH (buf)	0.95	-0.32	0.2571	0.0010	***	0.69	-0.72	0.3324	0.0010	***
K (%)	-0.48	0.88	0.0115	0.6940		0.10	0.99	0.0030	0.8990	
Mg (%)	0.57	0.82	0.6404	0.0010	***	0.72	-0.69	0.0769	0.0730	.
Ca (%)	0.99	0.12	0.7584	0.0010	***	0.87	-0.48	0.3534	0.0010	***
Na (%)	-0.07	1.00	0.4685	0.0010	***	-0.92	-0.40	0.2652	0.0010	***
H (%)	-0.91	-0.41	0.7273	0.0010	***	-0.86	0.52	0.2668	0.0010	***
CEC	0.59	0.80	0.4085	0.0010	***	0.74	0.68	0.0118	0.6870	
P (mg)	-0.25	-0.97	0.0777	0.0630	.	0.14	-0.99	0.0770	0.0630	.
Al (mg)	-0.62	0.79	0.4397	0.0010	***	-0.93	0.37	0.3391	0.0010	***
Mn (mg)	0.71	-0.70	0.4872	0.0010	***	1.00	0.02	0.3795	0.0010	***
Fe (mg)	-0.35	0.94	0.5966	0.0010	***	-0.98	-0.21	0.5585	0.0010	***
S (mg)	-0.31	0.95	0.2991	0.0010	***	-0.91	0.42	0.2066	0.0010	***
Plant understory vegetation										
ARN	0.53	-0.85	0.1474	0.0090	**	0.96	0.29	0.1529	0.0050	**
ASM	0.01	-1.00	0.0557	0.1010		0.38	0.92	0.0329	0.3180	

Table S 3.11: continued

AUR	0.93	-0.38	0.0444	0.2110		0.87	-0.49	0.0409	0.2390	
CAX	0.21	0.98	0.0652	0.1000	.	-0.34	-0.94	0.0446	0.2330	
CHL	0.21	0.98	0.0574	0.1140		-0.60	-0.80	0.0761	0.0580	.
CIA	-0.35	-0.94	0.0047	0.8820		0.72	0.69	0.0039	0.8870	
CLB	0.22	-0.97	0.0669	0.0920	.	0.78	-0.63	0.0831	0.0480	*
COL	0.39	0.92	0.1003	0.0100	**	-0.63	-0.77	0.0024	0.9870	
CON	0.29	-0.96	0.2637	0.0010	***	0.95	-0.31	0.2711	0.0010	***
COT	0.47	-0.89	0.0320	0.3200		0.64	-0.77	0.0475	0.1860	
DIE	-0.21	-0.98	0.0159	0.7880		0.12	0.99	0.0321	0.3350	
DIP	-0.72	0.70	0.1040	0.0230	*	-0.95	0.31	0.1532	0.0020	**
DRD	0.39	-0.92	0.0423	0.1700		0.98	-0.19	0.0119	0.7550	
EPN	0.09	-1.00	0.0088	0.7690		0.20	0.98	0.0364	0.2890	
EQP	0.05	1.00	0.0420	0.2390		-0.54	-0.84	0.0261	0.4060	
FRA	0.39	-0.92	0.0323	0.3400		0.95	-0.31	0.0589	0.1400	
GAA	0.38	-0.92	0.0732	0.0740	.	0.94	0.34	0.1647	0.0030	**
GAH	-0.41	0.91	0.2177	0.0010	***	-0.99	0.14	0.2945	0.0010	***
GOR	0.96	-0.27	0.0181	0.6170		1.00	-0.05	0.0351	0.3110	
HYS	-0.92	0.39	0.0004	0.9760		0.89	-0.45	0.0029	0.9170	
KAA	-0.96	0.28	0.0281	0.4090		-0.31	0.95	0.1714	0.0020	**
LEG	-0.91	0.42	0.0449	0.2110		-0.56	0.83	0.0730	0.0620	.
LIB	0.52	-0.86	0.0652	0.0990	.	0.96	0.28	0.0956	0.0280	*
LON	0.81	-0.58	0.0183	0.5460		0.19	-0.98	0.0428	0.2050	
LYA	0.28	-0.96	0.1130	0.0150	*	0.92	-0.40	0.1582	0.0040	**
LYC	0.39	-0.92	0.0247	0.5390		0.74	0.68	0.0233	0.5300	
LYO	-0.64	-0.77	0.0287	0.3780		-0.27	0.96	0.0194	0.5330	
MAC	0.28	-0.96	0.3275	0.0010	***	0.98	0.19	0.2529	0.0030	**
MIN	-0.96	-0.28	0.0256	0.5040		-0.13	0.99	0.0154	0.6930	
OXM	0.09	-1.00	0.0083	0.7730		0.53	-0.85	0.0344	0.3000	
PES	0.54	-0.84	0.3067	0.0010	***	0.95	0.32	0.3297	0.0010	***
PET	0.00	-1.00	0.0496	0.1650		0.39	-0.92	0.0751	0.0700	.
PLG	0.23	-0.97	0.0122	0.6720		0.71	0.71	0.0551	0.1530	
PLS	-0.44	0.90	0.3379	0.0010	***	-1.00	0.04	0.4273	0.0010	***
POA	0.73	-0.68	0.0261	0.3890		0.90	0.43	0.0887	0.0260	*
POC	0.09	1.00	0.0082	0.7800		-0.03	-1.00	0.0508	0.1780	
PTC	-0.43	0.90	0.1311	0.0110	*	-0.99	0.15	0.1618	0.0060	**
PTI	0.08	-1.00	0.0067	0.8330		0.18	0.98	0.0181	0.5360	
PYR	-0.97	0.24	0.0125	0.6650		-0.96	0.28	0.0393	0.2710	
RIG	0.35	-0.94	0.0224	0.4380		0.99	-0.14	0.0516	0.1600	

Table S 3.11: continued

RIT	0.14	-0.99	0.0111	0.7220		0.99	-0.16	0.0203	0.4790	
ROA	0.54	-0.84	0.0448	0.2030		0.84	0.54	0.0561	0.1370	
RUI	0.46	-0.89	0.1806	0.0010	***	0.99	0.12	0.1697	0.0030	**
RUP	0.46	-0.89	0.2514	0.0010	***	0.98	0.18	0.4088	0.0010	***
RYT	0.02	1.00	0.0160	0.7850		-0.72	0.69	0.0046	0.9310	
SMT	-0.19	0.98	0.0538	0.1450		-0.86	-0.52	0.0429	0.2220	
SOM	0.95	-0.32	0.0232	0.5490		1.00	-0.10	0.0152	0.6690	
SPS	-0.16	0.99	0.1232	0.0060	**	-0.68	-0.73	0.1048	0.0220	*
STA	-0.42	-0.91	0.0114	0.6990		0.22	0.97	0.0148	0.6360	
TRB	0.55	-0.83	0.1818	0.0010	***	1.00	0.04	0.1910	0.0010	***
VAA	-0.36	-0.93	0.0277	0.3610		-0.49	0.87	0.0082	0.7610	
VAM	0.32	0.95	0.0405	0.2320		0.63	-0.78	0.0041	0.8820	
VIE	0.35	-0.94	0.1116	0.0140	*	0.99	0.11	0.1105	0.0190	*
VIS	0.14	-0.99	0.1197	0.0120	*	1.00	0.01	0.1627	0.0010	***
Bacterial phyla										
Acidobacteria	-0.99	0.17	0.8094	0.0010	***					
Actinobacteria	0.67	-0.74	0.2605	0.0020	**					
Armatimonadetes	-0.30	0.95	0.0901	0.0420	*					
Bacteroidetes	0.99	-0.12	0.6495	0.0010	***					
Chlamydiae	-1.00	0.07	0.5295	0.0010	***					
Chloroflexi	0.85	0.52	0.8383	0.0010	***					
Deinococcus-Thermus	0.99	-0.11	0.0288	0.4010						
Dependentiae	0.21	-0.98	0.0588	0.1250						
Elusimicrobia	-0.27	0.96	0.1247	0.0130	*					
Fibrobacteres	0.01	1.00	0.3789	0.0010	***					
Firmicutes	0.53	-0.85	0.2324	0.0010	***					
Fusobacteria	-0.92	-0.39	0.0118	0.8310						
Gemmatimonadetes	0.84	-0.54	0.7225	0.0010	***					
Nitrospirae	0.94	0.34	0.2360	0.0010	***					
Patescibacteria	0.65	-0.76	0.2998	0.0010	***					
Planctomycetes	0.82	0.57	0.2437	0.0010	***					
Proteobacteria	1.00	-0.07	0.1589	0.0020	**					
Spirochaetes	0.17	0.99	0.3447	0.0010	***					
Verrucomicrobia	0.75	0.66	0.2212	0.0010	***					
Fungal phyla										
				Ascomycota		-0,30	-0,96	0,1560	0,0040	**
				Basidiobolomycota		0,98	-0,19	0,0119	0,7670	
				Basidiomycota		0,10	1,00	0,0683	0,1070	

Table S 3.11: continued

Chytridiomycota	0,26	0,96	0,0043	0,9430	
Mortierellomycota	0,98	-0,17	0,3256	0,0010	***
Mucoromycota	-0,85	0,53	0,2969	0,0010	***
Rozellomycota	1,00	0,00	0,0008	0,9700	
Zoopagomycota	-0,55	-0,83	0,0093	0,7440	

Table S 3.12 Differences in relative abundance of microbial ASVs between forest types, treatments, and the interaction, for bacterial and fungal soil microbiome. Significant differences between forest types (PERMANOVA, $P < 0.0001$***, $P < 0.001$**, $P < 0.05$*), but not among treatments or the interaction.

Soil microbiome	Variable	Df	Df_{total}	Sum Sq	R^2	F	P	
Bacteria	**Forest type**	**1**	**70**	**3.8492**	**0.13143**	**10.1952**	**0.0001**	***
	Treatment	3	70	0.6170	0.02107	0.5447	0.9974	
	Interaction	3	70	0.6572	0.02244	0.5802	0.9889	
Fungi	**Forest type**	**1**	**70**	**5.002**	**0.08179**	**6.1052**	**0.0001**	***
	Treatment	3	70	2.170	0.03547	0.8827	0.7315	
	Interaction	3	70	2.372	0.03878	0.9650	0.4304	

ANNEXE C

SUPPLEMENTARY INFORMATION CHAPTER 3

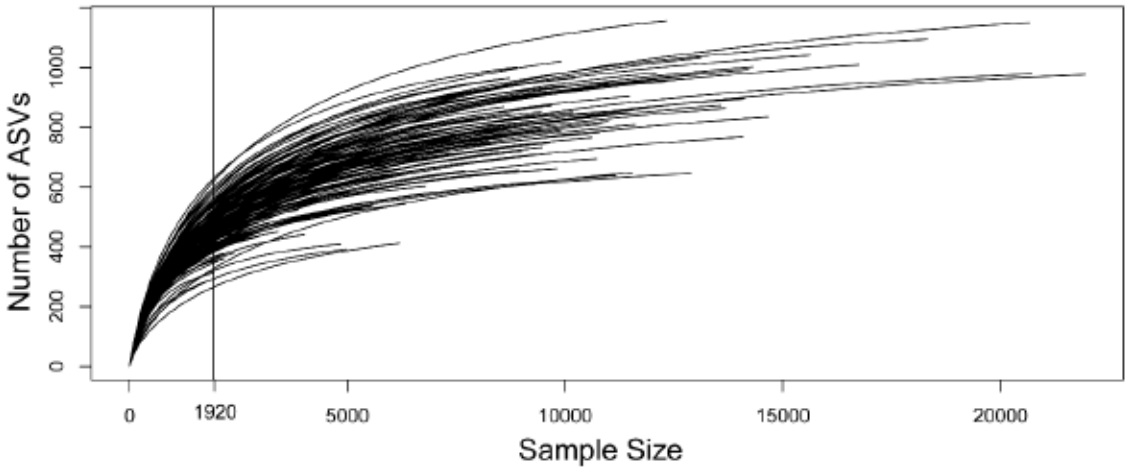

Figure S 3.6 Rarefaction curve of sample size (number of sequences) versus number of bacterial ASVs in feather-moss phyllosphere. The vertical line at 1920 was chosen as cut off in the rarefaction corresponding to the smallest number of sequences per sample with at least 1000 sequences.

Table S 3.13 Differences in bacterial phyla relative abundance between forest types, host species and their interaction, based on rarefied data and the generalized linear mixed model using Template Model Builder (glmmTMB) with a negative binomial family (quadratic parameterization). The phyla marked (*) indicate the use of the glmmTMB optimization with BFGS method for optimization for convergence of the model. Significant values $P < 0.0001$***, $P < 0.001$**, $P < 0.05$*.

Bacterial phyla	Factor	Estimate	Standard error	*z*	*P*	Sig.
*Acidobacteria**	(Intercept)	6.225	0.067	92.380	0.0000	***
	Forest-TA	**-0.634**	**0.096**	**-6.640**	**0.0000**	***
	Host-S	0.088	0.095	0.920	0.3550	
	Forest-TA : Host-S	-0.091	0.135	-0.680	0.4990	
Actinobacteria	(Intercept)	3.879	0.071	54.970	0.0000	***
	Forest-TA	-0.270	0.096	-2.820	0.0049	**
	Host-S	-0.195	0.096	-2.040	0.0413	*
	Forest-TA : Host-S	**0.556**	**0.136**	**4.100**	**0.0000**	***
Armatimonadetes	(Intercept)	3.008	0.110	27.423	0.0000	***
	Forest-TA	**0.416**	**0.111**	**3.737**	**0.0002**	***
	Host-S	**-0.569**	**0.118**	**-4.835**	**0.0000**	***
	Forest-TA : Host-S	0.289	0.162	1.790	0.0734	.
Bacteroidetes	(Intercept)	4.946	0.081	61.020	0.0000	***
	Forest-TA	**0.887**	**0.114**	**7.780**	**0.0000**	***
	Host-S	**-0.712**	**0.116**	**-6.170**	**0.0000**	***
	Forest-TA : Host-S	0.282	0.162	1.740	0.0820	.
*Chlamydiae**	(Intercept)	-12.846	102.641	-0.125	0.9000	
	Forest-TA	10.361	102.643	0.101	0.9200	
	Host-S	9.262	102.646	0.090	0.9280	
	Forest-TA : Host-S	-9.262	102.650	-0.090	0.9280	
Chloroflexi	(Intercept)	-22.132	10543.091	-0.002	0.9980	
	Forest-TA	22.956	10543.091	0.002	0.9980	
	Host-S	-0.708	18350.608	0.000	1.0000	
	Forest-TA : Host-S	0.890	18350.608	0.000	1.0000	
Cyanobacteria	(Intercept)	2.617	0.225	11.634	0.0000	***
	Forest-TA	**2.815**	**0.259**	**10.854**	**0.0000**	***
	Host-S	**-0.894**	**0.266**	**-3.354**	**0.0008**	***
	Forest-TA : Host-S	0.631	0.368	1.715	0.0863	.

Table S 3.13 : continued

Deinococcus-Thermus	(Intercept)	-24.407	33254.654	-0.001	0.9990	
	Forest-TA	22.769	33254.654	0.001	0.9990	
	Host-S	-6.912	890285.444	0.000	1.0000	
	Forest-TA : Host-S	6.065	890285.444	0.000	1.0000	
Dependentiae	(Intercept)	-0.406	0.456	-0.888	0.3740	
	Forest-TA	-0.470	0.665	-0.707	0.4790	
	Host-S	0.406	0.635	0.639	0.5230	
	Forest-TA : Host-S	-0.169	0.925	-0.183	0.8550	
Firmicutes	(Intercept)	-0.492	0.455	-1.083	0.2790	
	Forest-TA	-0.606	0.672	-0.902	0.3670	
	Host-S	-0.318	0.656	-0.485	0.6270	
	Forest-TA : Host-S	-0.087	0.980	-0.089	0.9290	
*Gemmatimonadetes**	(Intercept)	-15.721	432.097	-0.036	0.9710	
	Forest-TA	15.395	432.097	0.036	0.9720	
	Host-S	-3.076	2057.786	-0.001	0.9990	
	Forest-TA : Host-S	1.898	2057.786	0.001	0.9990	
Patescibacteria	(Intercept)	-0.488	0.360	-1.354	0.1760	
	Forest-TA	-0.711	0.536	-1.328	0.1840	
	Host-S	-0.677	0.565	-1.199	0.2310	
	Forest-TA : Host-S	0.916	0.783	1.169	0.2420	
Planctomycetes	(Intercept)	3.901	0.105	37.150	0.0000	***
	Forest-TA	**-0.362**	**0.107**	**-3.390**	**0.0007**	***
	Host-S	**0.255**	**0.105**	**2.430**	**0.0152**	*
	Forest-TA : Host-S	0.288	0.149	1.920	0.0543	.
Proteobacteria	(Intercept)	7.019	0.021	326.800	0.0000	***
	Forest-TA	-0.163	0.030	-5.500	0.0000	***
	Host-S	0.029	0.030	1.000	0.3262	
	Forest-TA : Host-S	**0.102**	**0.042**	**2.400**	**0.0148**	*
Tenericutes	(Intercept)	-28.641	273891.550	0.000	1.0000	
	Forest-TA	3.011	279747.265	0.000	1.0000	
	Host-S	3.011	280779.027	0.000	1.0000	
	Forest-TA : Host-S	20.422	286441.694	0.000	1.0000	
Verrucomicrobia	(Intercept)	2.996	0.117	25.527	0.0000	***
	Forest-TA	**0.290**	**0.131**	**2.220**	**0.0264**	*
	Host-S	0.213	0.131	1.632	0.1026	
	Forest-TA : Host-S	-0.188	0.183	-1.022	0.3066	

Table S 3.14 Comparisons of the relative abundance of bacterial phyla between the interaction of forest type (black spruce and trembling aspen) and host species (*P. schreberi* and *P. crista-castrensis*). Results of emmeans (pairwise Tukey test $P < 0.0001$***, $P < 0.001$**, $P < 0.05$*, significant values in bold), based on the generalized linear mixed model of each bacterial phyla, using Template Model Builder (glmmTMB) with a negative binomial family (quadratic parameterization). The phyla marked (*) indicate the use of the glmmTMB optimization with BFGS method for optimization for convergence of the model.

Bacterial phyla	Contrasts	Estimate	Standard error	DF	t-ratio	*P*	Sig.
Acidobacteria*	**BS-C - TA-C**	**0.634**	**0.096**	**137**	**6.6360**	**<.0001**	***
	BS-C - BS-S	-0.088	0.095	137	-0.9240	0.7921	
	BS-C - TA-S	**0.637**	**0.096**	**137**	**6.6720**	**<.0001**	***
	TA-C - BS-S	**-0.722**	**0.096**	**137**	**-7.5600**	**<.0001**	***
	TA-C - TA-S	0.003	0.096	137	0.0360	1.0000	
	BS-S - TA-S	**0.725**	**0.096**	**137**	**7.5950**	**<.0001**	***
Actinobacteria	**BS-C - TA-C**	**0.270**	**0.096**	**137**	**2.8170**	**0.0282**	*
	BS-C - BS-S	0.196	0.096	137	2.0400	0.1785	
	BS-C - TA-S	-0.090	0.095	137	-0.9530	0.7761	
	TA-C - BS-S	-0.075	0.097	137	-0.7740	0.8661	
	TA-C - TA-S	**-0.361**	**0.096**	**137**	**-3.7660**	**0.0014**	*
	BS-S - TA-S	**-0.286**	**0.096**	**137**	**-2.9870**	**0.0174**	*
Armatimonadetes	**BS-C - TA-C**	**-0.416**	**0.111**	**137**	**-3.7370**	**0.0015**	*
	BS-C - BS-S	**0.569**	**0.118**	**137**	**4.8350**	**<.0001**	***
	BS-C - TA-S	-0.136	0.112	137	-1.2060	0.6240	
	TA-C - BS-S	**0.985**	**0.116**	**137**	**8.5010**	**<.0001**	***
	TA-C - TA-S	0.280	0.111	137	2.5310	0.0596	
	BS-S - TA-S	**-0.705**	**0.117**	**137**	**-6.0200**	**<.0001**	***
Bacteroidetes	**BS-C - TA-C**	**-0.887**	**0.114**	**137**	**-7.7750**	**<.0001**	***
	BS-C - BS-S	**0.712**	**0.116**	**137**	**6.1660**	**<.0001**	***
	BS-C - TA-S	**-0.457**	**0.114**	**137**	**-3.9980**	**0.0006**	**
	TA-C - BS-S	**1.600**	**0.115**	**137**	**13.9080**	**<.0001**	***
	TA-C - TA-S	**0.430**	**0.114**	**137**	**3.7810**	**0.0013**	*
	BS-S - TA-S	**-1.169**	**0.115**	**137**	**-10.1500**	**<.0001**	**

Table S 3.14: continued

Chlamydiae*	BS-C - TA-C	-10.360	102.643	137	-0.1010	0.9996	
	BS-C - BS-S	-9.260	102.646	137	-0.0900	0.9997	
	BS-C - TA-S	-10.360	102.643	137	-0.1010	0.9996	
	TA-C - BS-S	1.100	1.236	137	0.8890	0.8105	
	TA-C - TA-S	0.000	0.927	137	0.0000	1.0000	
	BS-S - TA-S	-1.100	1.236	137	-0.8890	0.8105	
Chloroflexi	BS-C - TA-C	-22.956	10543.090	137	-0.0020	1.0000	
	BS-C - BS-S	0.708	18350.610	137	0.0000	1.0000	
	BS-C - TA-S	-23.138	10543.090	137	-0.0020	1.0000	
	TA-C - BS-S	23.664	15019.730	137	0.0020	1.0000	
	TA-C - TA-S	-0.182	0.380	137	-0.4790	0.9636	
	BS-S - TA-S	-23.846	15019.730	137	-0.0020	1.0000	
Cyanobacteria	**BS-C - TA-C**	**-2.815**	**0.259**	**137**	**-10.8540**	**<.0001**	***
	BS-C - BS-S	**0.894**	**0.266**	**137**	**3.3540**	**0.0056**	*
	BS-C - TA-S	**-2.552**	**0.255**	**137**	**-10.0290**	**<.0001**	***
	TA-C - BS-S	**3.708**	**0.250**	**137**	**14.8050**	**<.0001**	***
	TA-C - TA-S	0.262	0.245	137	1.0720	0.7071	
	BS-S - TA-S	**-3.446**	**0.255**	**137**	**-13.5230**	**<.0001**	***
Deinococcus-Thermus	BS-C - TA-C	-22.769	33255.000	137	-0.0010	1.0000	
	BS-C - BS-S	6.912	890285.000	137	0.0000	1.0000	
	BS-C - TA-S	-21.922	33255.000	137	-0.0010	1.0000	
	TA-C - BS-S	29.681	889978.000	137	0.0000	1.0000	
	TA-C - TA-S	0.847	1.000	137	0.8660	0.8222	
	BS-S - TA-S	-28.834	889978.000	137	0.0000	1.0000	
Dependentiae	BS-C - TA-C	0.470	0.665	137	0.7070	0.8941	
	BS-C - BS-S	-0.405	0.635	137	-0.6390	0.9192	
	BS-C - TA-S	0.234	0.654	137	0.3570	0.9843	
	TA-C - BS-S	-0.875	0.654	137	-1.3390	0.5401	
	TA-C - TA-S	-0.236	0.673	137	-0.3510	0.9851	
	BS-S - TA-S	0.639	0.643	137	0.9940	0.7533	
Firmicutes	BS-C - TA-C	0.606	0.672	137	0.9020	0.8036	
	BS-C - BS-S	0.318	0.656	137	0.4850	0.9622	
	BS-C - TA-S	1.012	0.702	137	1.4410	0.4762	
	TA-C - BS-S	-0.288	0.684	137	-0.4200	0.9749	
	TA-C - TA-S	0.405	0.729	137	0.5570	0.9446	
	BS-S - TA-S	0.693	0.714	137	0.9710	0.7664	

Table S 3.14: continued

Gemmatimonadetes*	BS-C - TA-C	-15.400	432.097	137	-0.0360	1.0000	
	BS-C - BS-S	3.080	2057.786	137	0.0010	1.0000	
	BS-C - TA-S	-14.220	432.097	137	-0.0330	1.0000	
	TA-C - BS-S	18.470	2011.908	137	0.0090	1.0000	
	TA-C - TA-S	1.180	0.516	137	2.2850	0.1065	
	BS-S - TA-S	-17.290	2011.908	137	-0.0090	1.0000	
Patescibacteria	BS-C - TA-C	0.711	0.536	137	1.3280	0.5470	
	BS-C - BS-S	0.677	0.565	137	1.1990	0.6286	
	BS-C - TA-S	0.473	0.536	137	0.8820	0.8143	
	TA-C - BS-S	-0.035	0.584	137	-0.0590	0.9999	
	TA-C - TA-S	-0.239	0.560	137	-0.4270	0.9738	
	BS-S - TA-S	-0.204	0.568	137	-0.3600	0.9840	
Planctomycetes	**BS-C - TA-C**	**0.362**	**0.107**	**137**	**3.3920**	**0.0050**	*
	BS-C - BS-S	-0.255	0.105	137	-2.4280	0.0766	
	BS-C - TA-S	-0.180	0.105	137	-1.7100	0.3225	
	TA-C - BS-S	**-0.617**	**0.106**	**137**	**-5.8090**	**<.0001**	***
	TA-C - TA-S	**-0.542**	**0.107**	**137**	**-5.0810**	**<.0001**	***
	BS-S - TA-S	0.075	0.105	137	0.7130	0.8918	
Proteobacteria	**BS-C - TA-C**	**0.163**	**0.030**	**137**	**5.4660**	**<.0001**	***
	BS-C - BS-S	-0.029	0.030	137	-0.9820	0.7600	
	BS-C - TA-S	0.031	0.030	137	1.0470	0.7218	
	TA-C - BS-S	**-0.192**	**0.030**	**137**	**-6.4480**	**<.0001**	***
	TA-C - TA-S	**-0.132**	**0.030**	**137**	**-4.4190**	**0.0001**	**
	BS-S - TA-S	0.060	0.030	137	2.0290	0.1823	
Tenericutes	BS-C - TA-C	-3.010	279747.000	137	0.0000	1.0000	
	BS-C - BS-S	-3.010	280779.000	137	0.0000	1.0000	
	BS-C - TA-S	-26.440	273892.000	137	0.0000	1.0000	
	TA-C - BS-S	0.000	86949.000	137	0.0000	1.0000	
	TA-C - TA-S	-23.430	61370.000	137	0.0000	1.0000	
	BS-S - TA-S	-23.430	61350.000	137	0.0000	1.0000	
Verrucomicrobia	BS-C - TA-C	-0.290	0.131	137	-2.2200	0.1230	
	BS-C - BS-S	-0.213	0.131	137	-1.6320	0.3640	
	BS-C - TA-S	-0.316	0.130	137	-2.4250	0.0771	
	TA-C - BS-S	0.077	0.129	137	0.5940	0.9339	
	TA-C - TA-S	-0.026	0.129	137	-0.2000	0.9971	
	BS-S - TA-S	-0.103	0.129	137	-0.7970	0.8558	

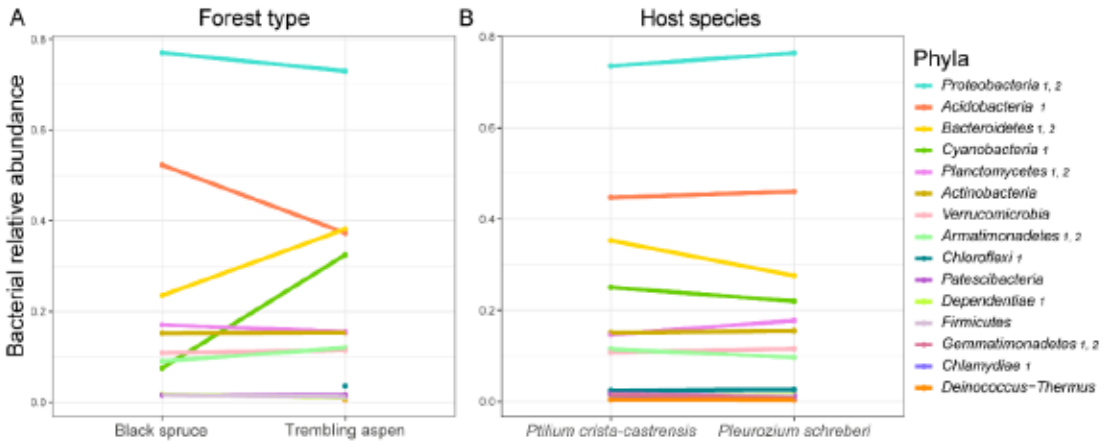

Figure S 3.7 Differences in relative abundance of bacterial phyla (in colors) a) between forest types (black spruce and trembling aspen) and b) between host species (*Pleurozium schreberi* and *Ptilium crista-castrensis*). Relative abundances were based on rarefied data. Significant differences between forest types (1 = ANOVA, all Benjamini–Hochberg-adjusted $P < 0.05$) and host species (2 = ANOVA, all Benjamini–Hochberg-adjusted $P < 0.05$) are indicated with labels next to phyla names in the legend.

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