Université de Montréal

Le microbiote rhizosphérique et racinaire du bleuetier sauvage

Par

Simon Morvan

Département des Sciences Biologiques

Faculté des Arts et des Sciences

Thèse présentée en vue de l'obtention du grade de *Philosophiae Doctor* (Ph.D.)

en Sciences Biologiques

Août 2022

Université de Montréal

Unité académique : Département de Sciences Biologiques, Faculté des Arts et des Sciences

---

Cette thèse intitulée

Le microbiote rhizosphérique et racinaire du bleuetier sauvage

Présenté par

Simon Morvan

A été évaluée par un jury composé des personnes suivantes

Frédéric Pitre
Président-rapporteur

Mohamed Hijri
Directeur de recherche

Maxime Charles Paré
Codirecteur

Geneviève Lajoie
Membre du jury

Philippe Constant
Examinateur externe

Résumé

Le bleuet sauvage (*Vaccinium angustifolium* Ait. et *V. myrtilloides* Michaux) représente un marché en plein essor au Canada, premier pays producteur et exportateur mondial de ce fruit. Pour faire face à la demande, les producteurs cherchent continuellement à adapter leurs pratiques de production dans le but d'améliorer leur rendement et l'état de santé de leurs bleuetiers. Or, les micro-organismes présents dans les racines et dans le sol jouent un rôle non négligeable en lien avec la santé des plantes. Ce microbiote est donc d'intérêt d'un point de vue agronomique, pourtant, contrairement à d'autres cultures, très peu d'études se sont penchées spécifiquement sur le microbiote du milieu racinaire du bleuetier sauvage. Ce doctorat s'inscrit donc dans l'optique d'accroître les connaissances sur les communautés bactériennes et fongiques présentes dans les bleuetières au Québec. Les objectifs de ce projet sont de détecter les taxons qui pourraient avoir un impact sur les variables agronomiques des bleuetiers telles que le rendement; d'identifier les variables physico-chimiques du sol influençant ces communautés; et d'étudier les impacts que peuvent avoir les différentes pratiques agricoles, telles que la fertilisation et la fauche thermique, sur ces micro-organismes.

Nous nous sommes appuyés sur le séquençage de nouvelle génération et le métacodage à barres de l'ADN environnemental de nos échantillons de racines et de sol afin d'obtenir une analyse des communautés bactériennes et fongiques de la rhizosphère et des racines des bleuetiers. Les analyses multivariées effectuées par la suite permettent de comparer ces communautés et de voir si certaines espèces sont spécifiques à une condition particulière.

Dans l'ensemble, cette thèse a donc permis de caractériser les communautés fongiques et bactériennes du milieu racinaire du bleuetier sauvage *in situ* dans plusieurs bleuetières du Québec. De nombreuses espèces de champignons mycorhiziens éricoïdes ont été systématiquement identifiées dans les trois études et leur prédominance suggère leur importance pour le bleuetier sauvage. Nous avons également trouvé que l'ordre bactérien des Rhizobiales, connu pour sa capacité à fixer l'azote atmosphérique, occupait une part importante de la communauté bactérienne. Les études sur la fertilisation et la fauche thermique ont

démontré que ces deux pratiques agricoles avaient peu d'impact significatif sur les communautés microbiennes étudiées. Enfin, cette thèse donne des pistes de réflexion sur la fixation d'azote par les communautés bactériennes et pose les premières bases pour des essais de bio-inoculation avec les espèces fongiques et bactériennes détectées ayant un potentiel impact bénéfique sur la culture des bleuets sauvages.

Mots-clés : Bleuet sauvage (*Vaccinium angustifolium* Ait.), Communautés microbiennes, Microbiote racinaire, Champignons mycorhiziens éricoïdes, Séquençage d'amplicons, ADN environnemental, Métacodage à barres, Rhizosphère, Fertilisation, Fauche thermique

Abstract

The wild blueberry (*Vaccinium angustifolium* Ait. and *V. myrtilloides* Michaux) market is booming in Canada, the world's leading producer and exporter of this fruit. In order to meet the demand, growers are constantly trying to adapt their production practices to improve their yields and the health of their blueberry fields. Micro-organisms present in the roots and in the soil play a significant role in the health of the plants. This microbiota is therefore of interest from an agronomic point of view, yet, contrary to other crops, very few studies have been conducted specifically on the microbiota of the root environment of wild blueberries. This doctoral project therefore aims at increasing our knowledge of the bacterial and fungal communities present in wild blueberry fields in Quebec. The objectives of this project are to detect taxa that could have an impact on agronomic variables of wild blueberry fields such as fruit yield; to identify soil physico-chemical variables influencing these communities; and to study the impacts that different agricultural practices, such as fertilization or thermal pruning, may have on these micro-organisms.

We relied on next generation sequencing and metabarcoding of environmental DNA from our root and soil samples to obtain an analysis of the bacterial and fungal communities in the rhizosphere and roots of blueberry shrubs. Subsequent multivariate analyses allow us to compare these communities and see if certain species are specific to a particular condition.

Overall, this thesis has characterized the fungal and bacterial communities in the root environment of wild blueberry in situ in several Quebec wild blueberry fields. Numerous species of ericoid mycorrhizal fungi were systematically identified in all three studies, and their predominance suggests their importance to wild blueberries. We also found that the bacterial order Rhizobiales, known for its ability to fix atmospheric nitrogen, occupied an important part of the bacterial community. Studies on fertilization and thermal mowing showed that these two agricultural practices have limited significant impacts on the microbial communities studied. Finally, this thesis provides insights into nitrogen fixation by bacterial communities and lays the

groundwork for bio-inoculation trials with the fungal and bacterial species detected to have a potential beneficial impact on wild blueberry cultivation.

Keywords: Wild blueberry (*Vaccinium angustifolium* Ait.), Microbial communities, Root microbiota, Ericoid mycorrhizal fungi, Amplicon sequencing, Environmental DNA, Metabarcoding, Rhizosphere, Fertilization, Thermal pruning

Table des matières

Liste des tableaux

Chapitre 2 – Plongée vers le microbiote rhizosphérique du bleuetier sauvage (*Vaccinium angustifolium*)

Chapitre 4 – Quels sont les effets de la fertilisation sur le microbiote du bleuetier sauvage ?

Liste des figures

Chapitre 1 – Introduction

Chapitre 2 – Plongée vers le microbiote rhizosphérique du bleuetier sauvage (*Vaccinium angustifolium*)

Chapitre 3 – Effet limité de la fauche thermique sur la culture de bleuets sauvages et de son microbiote associé à ses racines

Chapitre 4 – Quels sont les effets de la fertilisation sur le microbiote du bleuetier sauvage ?

Liste des sigles et abréviations

Al : *aluminum* |aluminium

ANOVA : *Analysis of variance* | analyse de variance

AMF : *Arbuscular mycorrhizal fungi* | Champignon mycorhizien arbusculaire

ASV : *Amplicon sequence variants* | Variants de séquences d'amplicons.

bp : *Base-pairs* | Paires de bases

Ca: *Calcium* | Calcium

CEC : *Cation exchange capacity* | Capacité d'échange cationique

CLR : *Centered log-ratio* | Log-ratio centré

DAP : *Diammonium phosphate* | Phosphate de diammonium

DNA : *Deoxyribonucleic acid* | Acide désoxyribonucléique

DSE : *Dark septate endophytes* | Endophytes racinaires sombres septés

ErM : *Ericoid mycorrhiza* | Mycorhize éricoïde

FAMES : *Free fatty acid methyl esters* |Ester méthylique d'acide gras

FDR : *False discovery rate* | Taux de fausses découvertes

Fe : *Iron* | Fer

GLMM : *Generalized linear mixed models* | Modèles linéaires mixtes généralisés

ITS : *Internal transcribed spacer* | Espaceur interne transcript

K: *Potassium* | Potassium

LMM : *Linear mixed models* | Modèles linéaires mixtes

LNC : *Leaf nitrogen content* | Concentration foliaire en azote

Mg : *Magnesium* | Magnésium

N: *Nitrogen* | Azote

NA : *Not assigned* | Non assigné

NGS : *Next generation sequencing* | Séquençage de nouvelle génération

NH_4^+ : *Ammonium* | Ammonium

NLFA : *Neutral lipid fatty acids* | Acide gras de lipide neutre

NMDS : *Non-metric multidimensional scaling* | Positionnement multidimensionnel non métrique

NO_3^-: *Nitrate* | Nitrate

NO_2^-: *Nitrite* | Nitrite

P: *Phosphorus* | Phosphore

PCA : *Principal component analysis* | Analyse en composantes principales

PCoA : *Principal coordinates analysis* | Analyse en coordonnées principales

PCR : *Polymerase chain reaction* | Réaction en chaine par polymérase

PERMANOVA : *Permutational multivariate analysis of variance* | Analyse permutationnelle multivariée de la variance

PGPR: *Plant growth promoting rhizobacteria* | Rhizobactéries favorisant la croissance des plantes

PhILR : *Phylogenetic Isometric Log-Ratio* | Log-ratio Isométrique Phylogénétique

PLFA : *Phospholipid fatty acid* |Acide gras phospholipidique

RA : *Relative abundance* | Abondance relative

RDA : *Redundancy analysis* | Analyse de redondance

Septoria LSD : *Septoria leaf spot disease* | Taches septoriennes

A mes grands-parents,

le fruit de ce travail provient d'un arbre ayant germé

quand, enfant,

je me promenais dans vos potagers.

Remerciements

Le doctorat est souvent comparé à un marathon, et bien que cette discipline se pratique de manière solitaire, elle ne serait pas possible sans une équipe qui nous accompagne tout au long du parcours.

Je remercie, en premier lieu, mon directeur Mohamed Hijri qui m'a donné l'opportunité d'effectuer ce doctorat au sein de son laboratoire ainsi que mon co-directeur Maxime Paré dont l'expertise dans la culture du bleuet sauvage a permis d'enrichir ce projet. Je remercie aussi les membres du jury qui ont permis d'améliorer cette thèse.

Je tiens également à remercier chaleureusement mes collègues qui étaient là pour m'épauler, et rendre l'épreuve du doctorat davantage plaisante : Andrew Blakney avec qui j'ai partagé l'intégralité du marathon et sur lequel je pouvais compter pour passer la ligne d'arrivée ; Alexis Carteron dont le parcours m'a inspiré et dont l'aide en bio-informatique et en statistiques a été primordiale dans mon cheminement; Julie Faure, Marion Leménager ainsi que tous les membres de l'AECBUM avec qui j'ai pu passer de bons moments ; Jacynthe Masse et Sébastien Renaud pour leur disponibilité et leur aide ; ainsi que Jocelyne Ayotte et Martin Lefrançois pour leur bienveillance. J'aimerais également remercier Stéphanie Shousha, Marie Barou Dagues et Marion Leménager pour le travail accompli durant l'année 2021 et qui a abouti à l'augmentation du salaire minimum garanti au doctorat au Département des Sciences Biologiques ainsi que l'indexation de ce montant à l'inflation. Malgré toutes les difficultés mises sur notre chemin, c'est un bel héritage que nous laissons aux futur·e·s étudiant·e·s des cycles supérieurs du Département. Je souhaite également remercier Marie-Christine Lafrenière et l'équipe des Lucioles pour leur engagement dans la vulgarisation et le rayonnement des sciences biologiques à l'Université de Montréal.

J'exprime ma reconnaissance au Centre de la Science de la Biodiversité du Québec qui m'a permis d'étendre mon réseau et d'élargir mes compétences grâce aux rôles que j'y ai pu jouer.

Comme on ne court pas un marathon sans s'être préalablement préparé, je souhaite remercier les entraineurs et entraineuses qui m'ont marqué et amené à entreprendre cette épreuve. Tout

d’abord, je remercie l’équipe de C’est Pas Sorcier qui a assurément initié mon intérêt envers les sciences. Je remercie également Dominique Mathieu, mon premier professeur de Science et Vie de la Terre au Lycée Français de Toronto, dont l’enseignement m’a tout de suite attiré vers la biologie. Un grand merci à l’équipe de Matis: Viggó Marteinson, Eyjólfur Reynisson, et tout particulièrement à Pauline Dechavanne qui a encadré mon premier stage en laboratoire et a confirmé mon attrait pour la recherche. Enfin, je souhaite remercier Claude-Olivier Sarde, responsable de la filière Conception et Innovation de Bioproduits à l’Université de Technologie de Compiègne pour ses enseignements en biotechnologie qui m’ont poussé à vouloir continuer la recherche dans cette voie.

Mon projet de recherche s’est déroulé dans des conditions particulières : pandémie, crise climatique, et contexte géopolitique international dégradé, rendant le doctorat, déjà éprouvant par nature, encore plus compliqué. J’aimerais rendre hommage aux différents artistes dont les œuvres m’ont permis de m’évader, notamment DJ Healer, Odezenne, Christian Löffler, Sofiane Pamart, Denis Villeneuve, Eiji Aonuma, Nicolas Jaar, Jérôme Niel, Saint DX, Hanna Reid, Flavien Berger…

Je tiens aussi à remercier mes amis ainsi que ma famille pour leur encouragement et leur soutien émotionnel durant ces 5 années. J’aimerais tout particulièrement remercier mes parents, Alain et Rozenn, car ce doctorat n‘aurait sûrement pas connu la même issue sans leur support psychologique, émotionnel et financier. Je n’aurais pas pu avoir de meilleur soutien.

Finalement, je souhaite remercier Romy dont les cavalcades dans le couloir et ses ronrons ont permis d’embellir les journées en télétravail. Enfin, Justine, je ne te remercierai jamais assez pour ton soutien indéfectible, ta compréhension et tes encouragements dans les moments difficiles; je ne suis pas sûr que je serai allé au bout de ce projet sans toi. J’ai beaucoup de chance de partager ma vie avec toi.

Chapitre 1 – Introduction

Le microbiote de l'environnement racinaire

Le terme « microbiote » désigne l'ensemble des micro-organismes dans un environnement donné. Depuis les premières observations au microscope au XVIIème siècle jusqu'à l'essor du séquençage à haut débit au milieu des années 2000, les scientifiques n'ont cessé de découvrir l'immense diversité, la complexité et les nombreux mécanismes et phénomènes impliquant les micro-organismes (Eisen, 2007; van Dijk et al., 2014). Présents dans une multitude de milieux (y compris les plus extrêmes (Merino et al., 2019)), les micro-organismes entretiennent une relation de longue date avec les végétaux terrestres puisque leurs interactions remonteraient à l'émergence des plantes sur la terre ferme, il y a 470 millions d'années (Martin et al., 2017). L'origine même de la première plante terrestre est censée résulter d'une symbiose entre une algue (famille des Charophycées) et un champignon ou un oomycète (Pirozynski and Malloch, 1975; Selosse and Le Tacon, 1998). Le fossile du Chert de Rhynie daté à 407 millions d'années est la preuve la plus ancienne d'une interaction directe entre des structures végétales et une structure s'apparentant à un champignon mycorhizien arbusculaire, nommée paramycorhize (Strullu-Derrien et al., 2015; Strullu-Derrien et al., 2018). Aujourd'hui, il est estimé que 72% des plantes vasculaires sont colonisées par des champignons mycorhiziens arbusculaires (Brundrett and Tedersoo, 2018). L'interaction entre les plantes et les bactéries remonte encore davantage dans le temps, puisque certains composants de leurs cellules tels que les mitochondries ou les chloroplastes, proviennent d'endosymbioses qui se seraient produites il y a un milliard d'années (McFadden, 1999; Archibald, 2015).

Les interactions entre une plante et ses micro-organismes sont si nombreuses et intrinsèques qu'il est plus adéquat de les considérer en tant que holobionte, un supra-organisme composé de la plante (hôte) et de son microbiote symbiotique (symbiotes) (Vandenkoornhuyse et al., 2015; Simon et al., 2019). Ce microbiote peut être composé d'organismes provenant de différents groupes taxonomiques tels que les virus, les champignons, les archées, les bactéries ou encore,

les nématodes. Dans cette thèse, nous nous sommes focalisés sur les champignons et les bactéries en raison de leur rôle prépondérant dans la nutrition et la santé des cultures végétales. De plus, bien que le microbiote des parties aériennes ne soit pas sans influence sur la productivité des végétaux (Laforest-Lapointe et al., 2017; Rosado et al., 2018; Chen et al., 2020), nous avons choisi de nous concentrer sur le microbiote du système racinaire. En effet, c'est grâce à leurs racines que les plantes puisent l'eau et les nutriments nécessaires à leur croissance. Or, les communautés bactériennes et fongiques peuvent, entre autres, avoir une influence sur ces apports nutritifs.

Le microbiote racinaire est une communauté complexe incluant des micro-organismes présents dans différentes niches écologiques. Certains peuvent pénétrer à l'intérieur des racines tandis que d'autres restent dans la rhizosphère, à la surface des racines et dans la fine zone de sol qu'elles influencent. Les micro-organismes de ces deux niches écologiques ont largement été étudiés, en raison des différents impacts que ceux-ci peuvent avoir sur les plantes (Parnell et al., 2016). En effet, à l'instar du microbiote intestinal humain, les micro-organismes du milieu racinaire remplissent de nombreuses fonctions. Ils peuvent être catégorisés selon le type d'interaction avec leur hôte, directe ou indirecte, et selon l'impact qu'ils ont sur la productivité de l'hôte (Bais et al., 2006; van der Heijden et al., 2008). La liste non exhaustive de micro-organismes et de leurs effets cités ci-après donne un aperçu des fonctions remplies par le microbiote racinaire. Les endophytes forment une catégorie de micro-organismes interagissant directement avec leur hôte en colonisant l'intérieur des racines. Parmi ceux qui ont des impacts globalement positifs, les micro-organismes symbiotiques ont fait l'objet d'une attention particulière en raison de leur contribution à l'apport en nutriments essentiels. Dans cette catégorie figurent notamment les champignons mycorhiziens (arbusculaires, ectomycorhiziens et ericoïdes, entre autres) (Smith and Read, 2008), les bactéries nodulatrices fixatrices d'azote chez les plantes appartenant à la famille des Fabaceae – communément appelées légumineuses – (Westhoek et al., 2017) ainsi que les actinobactéries nodulatrices du genre Frankia chez les plantes actinorhiziennes (Huss-Danell, 1997). Ainsi, chez certaines légumineuses (lentilles, soja, fèves), la proportion d'azote d'origine atmosphérique fixée grâce aux micro-organismes peut

dépasser les 70% (Franche et al., 2008; Peoples et al., 2009). Quant aux champignons mycorhiziens, leur contribution à l'apport en phosphore est avantageuse, ce nutriment étant peu disponible dans le sol et donc souvent limitant pour la croissance des plantes. Smith et ses collaborateurs ont comparé la quantité de phosphore intégrée par trois plantes (tomate, lin, luzerne) inoculées ou non par trois espèces de champignons mycorhiziens arbusculaires (*Gigaspora rosea*, *Glomus intraradices* et *Glomus caledonium*). Ces chercheurs ont ainsi montré que l'intégralité du phosphore d'une plante pouvait provenir de la symbiose avec des champignons mycorhiziens (Smith et al., 2003). D'autre part, les champignons ectomycorhiziens et éricoïdes sont connus pour produire des phosphatases, des enzymes qui clivent le phosphore de la molécule organique auquel il est lié, fournissant ainsi une source de phosphore autrement non accessible à la plante (Smith and Read, 2008). Les champignons mycorhiziens sont donc des alliés quasi incontournables pour l'apport en phosphore, surtout dans un contexte de crise des engrais phosphatés (Cordell et al., 2009; Roy-Bolduc and Hijri, 2011). Les avantages procurés par ces micro-organismes endophytes ne se limitent pas à la nutrition. Ils peuvent également protéger les racines des stress abiotiques, tels ceux occasionnés par les métaux lourds (Jourand et al., 2010; Hassan et al., 2013), mais aussi des stress biotiques dus aux micro-organismes phytopathogènes (Ismail et al., 2013). Ces micro-organismes phytopathogènes, tels que des oomycètes des genres *Phytophtora* (Tyler, 2007) ou *Pythium* (Li et al., 2019), des champignons du genre *Fusarium* (Punja and Rodriguez, 2018) ou encore des bactéries du genre *Ralstonia* (Peeters et al., 2013), infectent leur hôte via leurs racines et causent des dégâts considérables en agriculture (Singleton, 2002). De plus, malgré leur tendance mutualiste, les champignons mycorhiziens se situent en réalité sur un continuum mutualisme-parasitisme : l'impact du symbiote va varier en fonction de différents facteurs tels que la phénologie de la plante ou la concentration en nutriments du sol (Johnson et al., 1997). Dans une méta-analyse sur les champignons ectomycorhiziens, les résultats suggèrent que la position sur continuum mutualiste-parasite serait liée à des facteurs biotiques, soit l'identité de la plante hôte. De plus, cette étude n'identifie pas d'effet de la concentration en phosphore du sol sur la réponse de la plante hôte face à l'inoculation ectomycorhizienne (Karst et al., 2008). *A contrario*, pour les champignons mycorhiziens arbusculaires, leur colonisation serait influencée par la concentration en nutriments

avec une plus faible colonisation mycorhizienne dans des sols riches en nitrate et phosphate, ce qui implique une plus faible contribution en carbone aux champignons de la part de la plante (Jones and French, 2021). Les résultats de cette étude indique également que la colonisation mycorhizienne arbusculaire augmente en cas de faible concentration en nitrate ou en phosphate, suggérant ainsi le rôle de ces champignons dans le facilitation d'absorption de N et P par la plante hôte (Jones and French, 2021). Une autre étude testant l'effet de l'inoculation de 10 champignons mycorhiziens arbusculaires sur 64 espèces de plantes montre une grande variation en termes de croissance de la plante (biomasse aérienne et sous-terraine) variant de - 49% à + 46% dépendamment de l'association entre la plante et l'espèce de champignon (Klironomos, 2003). Ainsi, que leur influence soit positive ou négative, il y a un grand intérêt de connaître la composition du microbiote racinaire d'un hôte végétal.

Les plantes ne restent pas passives dans ce système d'holobionte et impactent, à leur tour, les communautés microbiennes à proximité de leurs racines. En effet, les plantes façonnent leur microbiote racinaire grâce aux rhizodépots (sécrétion d'exsudats, mucilage) (Bais et al., 2006). Ces molécules organiques relâchées dans le sol sont sous diverses formes, parmi lesquelles on trouve des sucres, des acides gras, des protéines, des phytohormones, des acides phénoliques, ou encore des métabolites secondaires (Bertin et al., 2003). Les plantes peuvent investir une proportion non négligeable de leur carbone dans ces rhizodépots, pouvant atteindre 40% du carbone fixé par photosynthèse (Bertin et al., 2003; Bais et al., 2006). Ces composés provoquent un profond changement au niveau du microbiote à proximité des racines, connu sous le nom d'effet rhizosphère (Bakker et al., 2013; Philippot et al., 2013; Fitzpatrick et al., 2018). L'effet rhizosphère est comparable à une pression de sélection qui a pour effet de recruter les micro-organismes favorables à la croissance des plantes, mais aussi de se défendre contre les micro-organismes pathogènes (Bertin et al., 2003; Bais et al., 2006). En raison de la sélection exercée par ces molécules d'origine végétale, la rhizosphère est moins riche en termes de diversité d'espèces microbiennes que le sol éloigné des racines (*bulk soil*), mais les micro-organismes présents y sont plus abondants et plus actifs (Bakker et al., 2013; Quiza et al., 2015; Rohrbacher and St-Arnaud, 2016). La pression de sélection est encore plus forte à l'intérieur des racines, les

micro-organismes devant être capables de déjouer les mécanismes de défense activés lors de leur intrusion. Cependant, les micro-organismes n'ont pas besoin d'être en contact direct avec la racine pour avoir une influence sur la plante. En effet, la rhizosphère abrite, entre autres, des bactéries et des champignons saprotrophes, décomposeurs de la matière organique du sol, qui rendent disponible une source additionnelle de nutriments à la plante (Barea et al., 2005; Vohník et al., 2012). De plus, diverses bactéries fixatrices d'azotes libres (non symbiotiques) sont également présentes dans la rhizosphère et contribuent à un apport en azote ammoniacal dans le milieu qu'elles occupent (Smercina et al., 2019). La fixation d'azote atmosphérique chez ses organismes est néanmoins beaucoup moins importante que chez les bactéries symbiotiques qui peuvent fixer entre 50 et 465 kg N ha^{-1} an^{-1} contre 1 à 80 kg N ha^{-1} an^{-1} pour les bactéries non-symbiotiques (Pankievicz et al., 2019). Les micro-organismes rhizosphériques sont également connus pour augmenter la résistance aux pathogènes, notamment grâce à la production d'antibiotiques ou en améliorant la résistance induite des plantes (Barea et al., 2005; Raaijmakers et al., 2008). L'impact des stress abiotiques auxquels la plante peut faire face peut également être atténué grâce aux micro-organismes rhizosphériques que ce soit un stress dû aux métaux lourds (Burd et al., 2000), aux hydrocarbures (Yergeau et al., 2014; Rohrbacher and St-Arnaud, 2016) ou encore à la sécheresse (Marasco et al., 2012). En résumé, il est important d'étudier à la fois les micro-organismes racinaires et rhizosphériques pour avoir une meilleure vue d'ensemble sur les communautés microbiennes pouvant influencer la santé et la productivité de la plante hôte.

Toutes ces fonctions attribuées aux micro-organismes vont non seulement impacter la productivité, mais également la diversité des plantes présentes dans un écosystème. En effet, ces micro-organismes n'agissent pas de manière équivalente en fonction de l'hôte. Ainsi, selon l'espèce hôte, la colonisation par une espèce de champignon mycorhizien arbusculaire ne va pas provoquer le même effet sur la croissance (van der Heijden et al., 1998; Klironomos, 2003). De plus, les végétaux dépendent plus ou moins de la colonisation par des champignons mycorhiziens pour assurer leur développement. De part et d'autre de ce spectre, citons les plantes non-mycorhiziennes telles que certains membres des Brassicacées qui produisent des composés antimicrobiens (Cosme et al., 2018) et les plantes myco-hétérotrophes non-photosynthétiques

dont les graines nécessitent une colonisation mycorhizienne afin de germer et de s'établir (Bruns and Read, 2000; Merckx et al., 2009). Le microbiote peut également procurer un avantage sélectif à sa plante hôte. Ainsi, la fixation d'azote atmosphérique via l'activité bactérienne favorise les légumineuses et les plantes actinorhiziennes lorsque l'azote est en concentration limitante dans le sol (Franche et al., 2008). A contrario, les micro-organismes phytopathogènes vont diminuer le succès reproducteur des végétaux qu'ils sont capables d'infecter (Morris and Moury, 2019). Ces divers exemples montrent à quel point les micro-organismes peuvent avoir un impact tangible sur la communauté végétale avec laquelle ils cohabitent et justifient donc l'intérêt de leur étude.

Un autre point à souligner est le dynamisme du microbiote de l'écosystème végétal, les micro-organismes étant impactés par de nombreux facteurs qu'ils soient biotiques ou abiotiques. Ainsi, une variation au niveau de l'acidité du sol, de l'hygrométrie, de la température, de la concentration en nutriments ou en matière organique va directement se répercuter sur la communauté microbienne (de Vries et al., 2012; Fierer, 2017). De plus, tout comme le microbiote racinaire est dynamique, la production d'exsudats racinaires par la plante l'est aussi. Chaparro et ses collaborateurs ont montré que la concentration d'exsudats racinaires produits *in vitro* par *Arabidopsis thaliana* varie en fonction de son développement (Chaparro et al., 2013). Leurs résultats montrent également une corrélation positive entre les concentrations d'exsudats racinaires et les gènes bactériens responsables de la métabolisation de ces exsudats. La concentration en composés phénoliques dans le sol augmente à mesure que la plante se développe, ce qui laisse croire à la mise en place d'une stratégie de défense plus stricte envers les micro-organismes (Chaparro et al., 2013). Cela va dans le sens de l'étude de De-la-Peña et collaborateurs, dont les résultats indiquent que des protéines de défense sont davantage excrétées au moment de la floraison d'*A. thaliana* (De-la-Peña et al., 2010). Toujours sur cette même plante modèle, une variation de la communauté bactérienne en fonction du stade de développement et des exsudats racinaires produits a été observée (Chaparro et al., 2014). Enfin, une étude a également montré que la production de métabolites secondaires (benzoxazinoids), produits par les racines de diverses céréales, provoque un changement au niveau de la composition des communautés fongiques et bactériennes des racines et de la rhizosphère (Hu et

al., 2018). Ces études tendent à montrer le rôle actif des plantes qui, via les exsudats racinaires, induisent une pression de sélection et influent sur les communautés microbiennes.

Du fait de ces nombreuses rétroactions entre la plante et les micro-organismes, mais aussi de celles des micro-organismes interagissant entre eux, l'écosystème est d'une grande complexité. Les techniques de séquençage génétique développées ces trente dernières années permettent d'avoir de plus en plus de résolution sur les taxons présents dans le microbiote racinaire et sur les gènes exprimés par ces micro-organismes. Ainsi le métacodage à barres de l'ADN environnemental permet de cibler certains règnes du vivant, tels que les champignons ou les bactéries, en séquençant des marqueurs génétiques. Ces marqueurs contiennent à la fois des parties conservées et des parties hypervariables permettant en même temps de cibler un règne (partie conservée du marqueur) et de distinguer les différents taxons de ce règne (partie hypervariable du marqueur). Contrairement aux techniques d'isolation classiques, le séquençage génétique permet d'avoir une idée beaucoup plus proche de la réelle communauté microbienne présente. En effet, seule une partie des micro-organismes présents dans le sol peuvent être isolés et cultivés en laboratoire, limitant ainsi notre compréhension de l'écosystème (Su et al., 2012). Ces techniques ont ainsi permis d'améliorer notre compréhension des communautés microbiennes et des facteurs biotiques et abiotiques qui les influencent (Dickie and St John, 2016; Abdelfattah et al., 2018). Malgré ces avancées, nos connaissances n'en demeurent pas moins parcellaires. Cependant, en dépit de nos lacunes, les impacts non négligeables des micro-organismes sur la productivité des plantes ont encouragé les chercheurs à trouver les moyens de tirer profit de leurs effets bénéfiques (Parnell et al., 2016). En agriculture, l'importance accordée aux micro-organismes est en plein essor, stimulée par le besoin de transformer l'agriculture traditionnelle, gourmande en intrants, en une agriculture durable, plus respectueuse de l'environnement (Adesemoye et al., 2009; Schlaeppi and Bulgarelli, 2015). Une des façons employées pour bénéficier des fonctions propres aux micro-organismes consiste à appliquer des bio-inoculants, produits contenant un ou plusieurs micro-organismes cultivables dont l'impact bénéfique a préalablement été constaté (Kaminsky et al., 2019). Ces inoculations peuvent avoir des résultats significatifs sur les rendements agricoles (Hijri, 2016; Schütz et al., 2018) et le marché

mondial de ces produits, chiffré à 425 millions de $USD en 2020, devrait atteindre les 750 millions $USD en 2025 (Market Data Forecast, 2020). Cependant, contrairement aux engrais chimiques dont l'application a des retombées relativement prévisibles sur le rendement, l'efficacité des bio-inoculants microbiens n'est pas garantie (van Veen et al., 1997; Kaminsky et al., 2019). En effet, le succès d'inoculation est incertain, car les micro-organismes inoculés font face à la communauté microbienne indigène déjà établie ainsi qu'à des conditions édaphiques qui peuvent être très différentes des conditions dans lesquels les inoculants ont été produit (Kaminsky et al., 2019). Le mode d'application de l'inoculant (application en champs ou enrobage de graine) peut également avoir un impact sur le succès d'inoculation (Kaminsky et al., 2019). De manière générale, des conditions biotiques et abiotiques défavorables à l'inoculum peuvent enrayer le succès de l'inoculation. Par conséquent, la compréhension des facteurs biotiques et abiotiques influençant les micro-organismes est donc une étape importante afin de pouvoir tirer parti de ces amendements de nouvelle génération. A ce jour, les recherches sur les micro-organismes en lien avec l'agriculture se sont essentiellement focalisées sur les grandes cultures (céréales, tomates, pomme de terre) étant donné l'importance que celles-ci ont dans notre alimentation. Pour les produits agricoles plus nichés, tels que le bleuet sauvage, les études sur leur microbiote sous-terrain sont cependant bien moins nombreuses.

La culture du bleuetier sauvage et ses particularités

La dénomination « bleuetier sauvage » désigne deux espèces d'arbustes pérennes, *Vaccinium angustifolium* Ait. et *V. myrtilloides* Michaux, produisant de petites baies comestibles, appartenant à la famille des Ericacées. Cette famille de plantes inclut notamment les canneberges (*V. macrocarpon*), les rhododendrons (*Rhododendron* spp.) ou la bruyère (*Calluna vulgaris*). Ces plantes sont connues pour leur habitat relativement hostile : sols acides (4<pH<6), riches en matière organique, pauvres en nutriments biodisponibles et concentrés en ions métalliques libres (Fe et Al notamment) (Cairney and Meharg, 2003; Leopold, 2016). La litière produite par ces végétaux contient de nombreux composés phénoliques ayant des effets allopathiques, limitant ainsi la colonisation du milieu par d'autres plantes (Jalal and Read, 1983; Cairney and Meharg,

2003). De plus, les Ericacées auraient un effet acidifiant sur le sol (Korcak, 1988), une étude ayant observé une corrélation hautement significative entre la taille de buissons de *Calluna vulgaris* et de *Erica cinerea* avec le pH du sol à une profondeur de 0-1cm au centre du buisson (Grubb et al., 1969).

Les bleuetiers sauvages sont essentiellement cultivés dans l'état du Maine aux États-Unis, ainsi qu'au Canada, dans les provinces maritimes et au Québec (Yarborough, 2012). Au Québec, en 2018, 478 exploitants de bleuetiers sauvages ont été recensés, pour un volume de production de 34 906 tonnes (MAPAQ, 2019). Le bleuet sauvage n'est pas l'unique type de bleuet produit en Amérique du Nord, il est concurrencé par le bleuetier en corymbe, *V. corymbosum*, dont la culture est plus conventionnelle. En effet, contrairement au bleuetier en corymbe, qui lui est cultivé à partir de cultivars plantés en rangées, la culture de bleuets sauvages a lieu dans des parcelles où les bleuetiers sont préexistants (Yarborough, 2012), essentiellement dans les forêts de pins gris. Cette particularité est principalement due au fait que les bleuetiers sauvages se propagent très lentement, essentiellement à partir de leur rhizome (Yarborough, 2012). La terminologie de « clone » est d'ailleurs utilisée pour désigner un individu, soit toutes les tiges et racines appartenant au même rhizome (Barker et al., 1964). Dans le cas des bleuetiers sauvages, il est donc compliqué d'identifier un clone visuellement, car les rhizomes de plusieurs individus peuvent s'entrecroiser, et chacun peut s'étendre sur plusieurs mètres (Pelletier et al., 2022).

Comme toutes cultures, le rendement en bleuet sauvage fluctue selon les aléas climatiques. La production est notamment négativement impactée par les gelées tardives, la sécheresse ainsi que la chaleur intense dans les bleuetières non irriguées (Strik, 2004). En hiver, la quantité de neige au sol est positivement corrélée au rendement en bleuets, car elle joue un rôle protecteur vis-à-vis du froid (Zapa, 2017). Indépendamment des conditions climatiques, le rendement peut également beaucoup fluctuer au sein même d'une bleuetière (Barker et al., 1964). Ainsi, une étude de 100 clones de bleuets sauvages prélevés dans un même champ a montré une variation de rendement allant de 400 à 17 000 $kg.ha^{-1}$ (moyenne de 7726 ± 3240 $kg.ha^{-1}$) (Hepler and Yarborough, 1991). Cette variabilité de rendement d'un clone à l'autre serait notamment expliquée par le bagage génétique de chaque individu (Strik, 2004). La suppression progressive

des clones les moins productifs afin de les remplacer par les plus fructueux permettrait donc d'augmenter le rendement (Hepler and Yarborough, 1991). Le rendement en bleuet est également très relié à la pollinisation croisée – le bleuetier sauvage étant peu autofertile (Bell et al., 2010) – ce qui incite les producteurs à installer des ruches afin d'assurer la meilleure pollinisation possible (MAPAQ, 2019). Parmi les pratiques culturales, la fauche est assurément la pratique qui fait la particularité de la culture de bleuet sauvage. Cette pratique s'inspirerait de l'expérience des peuples autochtones qui brûlaient des plants sauvages dans le but d'accroître leurs productions (Chapeskie, 2001; Theriault, 2006). Présentement, la fauche (thermique ou mécanique) a lieu après la récolte, à l'automne ou au printemps suivant. Les plants nécessitent alors un an de croissance végétative pendant laquelle les tiges repoussent à partir du rhizome et forment des bourgeons floraux et végétatifs. L'arbuste passe l'hiver sous la neige et reprend sa croissance au printemps pour produire des fruits vers la mi-août. La fauche thermique a l'inconvénient d'être plus dispendieuse et moins facile à mettre en œuvre que la fauche mécanique. L'utilisation de faucheuses mécaniques s'est donc généralisée étant donné que l'effet stimulant sur la productivité provient du fait de rabattre au sol les tiges des bleuetiers. Cependant, des études ont montré que la fauche thermique permet de contrôler des maladies fongiques telles que la pourriture sclérotique (*Monilinia vaccinii-corymbosi*), de réduire les populations de certains insectes indésirables (*Aroga trialbamaculella*, *Croesia curvalana*, *Altica sylvia*) et de réduire la germination de graines de mauvaises herbes (Lambert, 1990; Drummond et al., 2009; CRAAQ, 2013; White and Boyd, 2017). Dépendamment de l'intensité du brûlage appliqué, il peut y avoir également un effet sur la chimie du sol avec un apport en nutriments et une élévation du pH en raison du dépôt de cendre provoqué par le feu (Smith and Hilton, 1971). Ces avantages pourraient remettre la fauche thermique au goût du jour, notamment pour les parcelles sous régie biologique. En effet, ce mode de production doit faire face aux mêmes défis que l'agriculture conventionnelle, mais dispose de moins de produits phytosanitaires pour y faire face (Drummond et al., 2009). Ainsi, ni les pesticides, ni les engrais de synthèse, ni les herbicides tels que l'hexazinone, couramment utilisé dans les bleuetières (Kennedy et al., 2010; Li et al., 2017), ne sont permis. Par conséquent, l'effet phytosanitaire de la fauche thermique pourrait être une bonne alternative à ces produits.

Afin d'obtenir les meilleurs rendements possibles, l'application de fertilisants est également pratiquée dans les bleuetières. Au Québec, cet apport en nutriments est réalisé uniquement pendant l'année végétative (MAPAQ, 2016). Les amendements appliqués peuvent être sous forme minérale ou organique similairement à d'autres cultures. L'avantage d'une fertilisation organique réside dans une plus lente diffusion de nutriments, s'accordant avec les faibles besoins nutritionnels des bleuetiers sauvages (Mallory and Smagula, 2012). De plus, selon ces mêmes travaux, à dose d'azote équivalente (42 $kg.ha^{-1}$), deux types d'engrais organiques ont donné des rendements similaires à un engrais minéral (DAP), soit 70% plus élevés que le contrôle sans fertilisant (Mallory and Smagula, 2012). Au Québec, le seuil critique d'azote dans le sol au printemps a été estimé à 3,1 $mg.kg^{-1}$ (méthode d'extraction de l'ammonium à l'eau) (Lafond and Ziadi, 2013). Les bleuetiers sauvages ont donc des besoins assez faibles en azote (Marty et al., 2019). L'impact d'un apport en azote sur le rendement en bleuet n'est d'ailleurs pas bien défini, plusieurs études ayant des conclusions contradictoires sur le sujet (Eaton and Patriquin, 1990; Smagula and Dunham, 1995; Jeliazkova and Percival, 2003; Lafond and Ziadi, 2011). Il en ressort cependant que le bleuetier sauvage absorbe préférentiellement l'ammonium, davantage disponible que le nitrate dans un sol acide (Townsend, 1969). L'absorption sous cette forme est relativement rapide, une étude ayant mesuré qu'en 13 semaines les bleuetiers avaient absorbé entre 45 et 64% de l'azote appliqué au printemps sous forme de sulfate d'ammonium marqué au ^{15}N (Eaton and Patriquin, 1990). Cependant, un risque non négligeable associé à la fertilisation azotée minérale est la prolifération de mauvaises herbes (Penney and McRae, 2000), dont certaines (*Comptonia peregrina* et *Danthonia spicata*) sont plus efficaces à absorber l'azote minéral que les bleuetiers sauvages (Marty et al., 2019). En ce qui concerne la fertilisation en phosphore, son impact sur le rendement n'est pas évident à caractériser. En effet, certaines études indiquent une absence d'effet sur la croissance et sur le rendement (Eaton et al., 1996; Sanderson and Eaton, 2008; Lafond and Ziadi, 2011) tandis qu'une étude utilisant du diammonium phosphate (DAP) a trouvé une augmentation significative de la concentration foliaire en phosphore ainsi qu'une augmentation de croissance et de rendement (l'effet de l'azote et du phosphore étant cependant difficilement dissociable à cet égard) (Smagula and Dunham,

1995). Lafond et Ziadi suggèrent que les besoins en phosphore du bleuetier sauvage sont très faibles et que la quantité présente dans le sol pourrait être suffisante (Lafond and Ziadi, 2011). En effet, la couche de matière organique où se trouvent les racines des bleuetiers contient de l'azote et du phosphore en quantité suffisante, mais sous des formes récalcitrantes, non accessibles, par la plante (Adamczyk et al., 2016). La contribution en nutriments fournie grâce au microbiote racinaire et notamment aux champignons mycorhiziens éricoïdes est donc primordiale pour subvenir aux besoins nutritionnels des bleuetiers sauvages (Leopold, 2016). Ainsi, différentes études se sont penchées sur les effets du type et de la dose de fertilisants sur la colonisation mycorhizienne des bleuets.

Une expérience sur *V. corymbosum* a montré que cette colonisation mycorhizienne est plus élevée avec des engrais organiques qu'avec des engrais minéraux, et ce à dose égale de NPK (60-60-60 $kg.ha^{-1}$) (Montalba et al., 2010). De manière similaire, les résultats d'une autre équipe de recherche indiquent une plus grande colonisation mycorhizienne dans des exploitations de *V. corymbosum* en régie biologique, ce qui pourrait être attribué à la forme organique des engrais azotés appliqués (Sadowsky et al., 2012). La disponibilité des nutriments est plus importante pour les engrais minéraux, car les nutriments sont directement absorbables par les racines au moment de l'application. Ces concentrations élevées en nutriments absorbables pourraient être à l'origine de l'impact négatif sur les champignons mycorhiziens (Montalba et al., 2010). Cependant, ces effets négatifs de la fertilisation minérale sur le microbiote racinaire n'ont pas été systématiquement observés. Une expérience de fertilisation testant différentes doses de NPK (sous forme de sulfate d'ammonium, de superphosphate, et de chlorure de potassium) sur des bleuets sauvages n'a pas montré de changement sur la colonisation mycorhizienne (Jeliazkova and Percival, 2003). En revanche, cette même étude documente une augmentation significative de la colonisation mycorhizienne en août de l'année végétative en lien avec une fertilisation azotée en urée dosée à 35 $kg.ha^{-1}$ (Jeliazkova and Percival, 2003). D'autre part, les fertilisants organiques ont généralement pour effet d'augmenter progressivement le pH du sol, ce qui peut causer une diminution de la présence des mycorhizes chez les bleuetiers (Haynes and Swift, 1985; Caspersen et al., 2016). En effet, les champignons mycorhiziens éricoïdes (détaillés dans la

prochaine section) produisent des enzymes adaptées aux conditions acides (Leake and Read, 1990; Kerley and Read, 1995; Cairney and Burke, 1998).

Un dernier point digne d'être souligné est l'augmentation de la production des bleuets sauvages biologiques au Québec ces dernières années. La superficie dédiée à ce mode de production a été multipliée par environ 34 en 10 ans dans la région du Saguenay–Lac-Saint-Jean (Figure 1). Cet engouement s'explique en partie par la demande accrue des consommateurs qui, soucieux de leur alimentation, sont prêts à payer davantage pour les bleuets sauvages biologiques (Drummond et al., 2009; MAPAQ, 2016). De plus, le développement de la régie biologique est un des enjeux définis en 2016 par le Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ, 2016) et cela ne peut se faire sans une compréhension approfondie de l'écosystème des bleuetières (Drummond et al., 2009). Or, comme présenté en première partie, le microbiote racinaire est un des acteurs important de cet écosystème, et son étude semble donc tout à fait à propos. Pourtant, à ce jour, un faible nombre d'études concerne les communautés microbiennes du bleuetier sauvage.

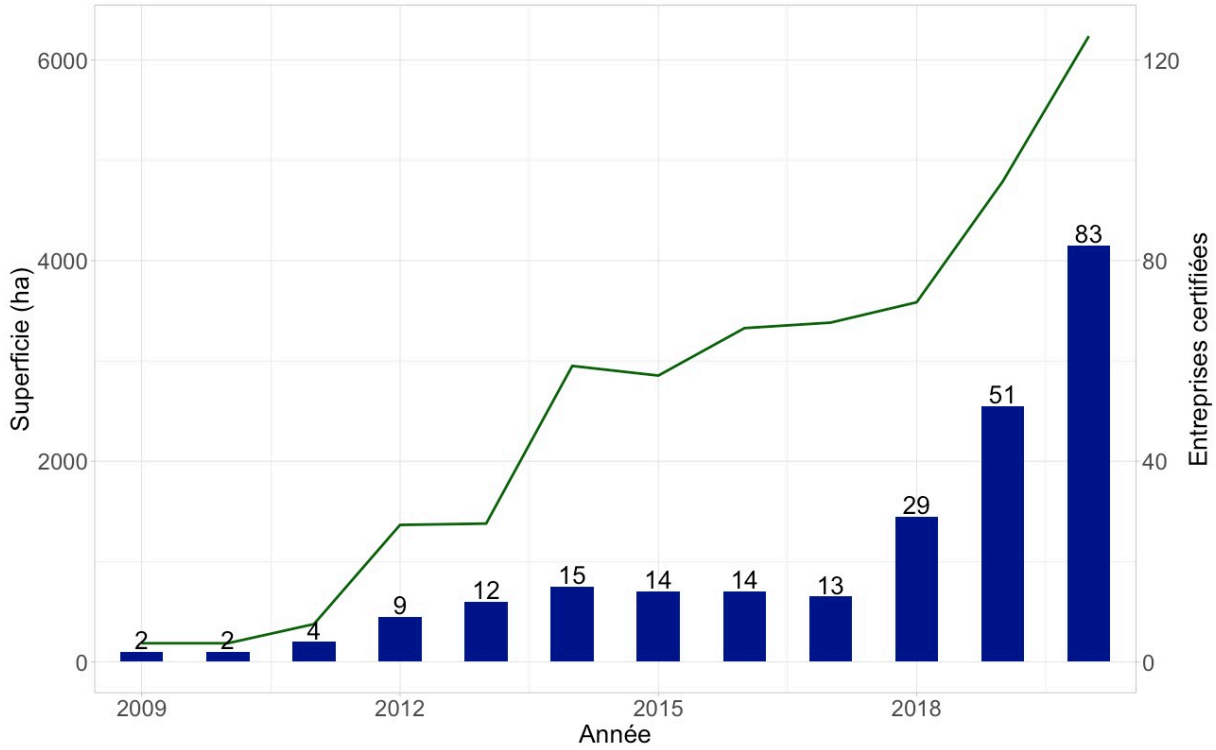


Figure 1. Évolution des superficies consacrées aux bleuets sauvages en régie biologique au Saguenay–Lac-Saint-Jean ainsi que le nombre d'entreprises certifiées. La courbe représente la superficie tandis que le diagramme en barres et les chiffre représentent les entreprises certifiées. Données obtenues de Pierre-Olivier Martel, agronome au Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec (MAPAQ).

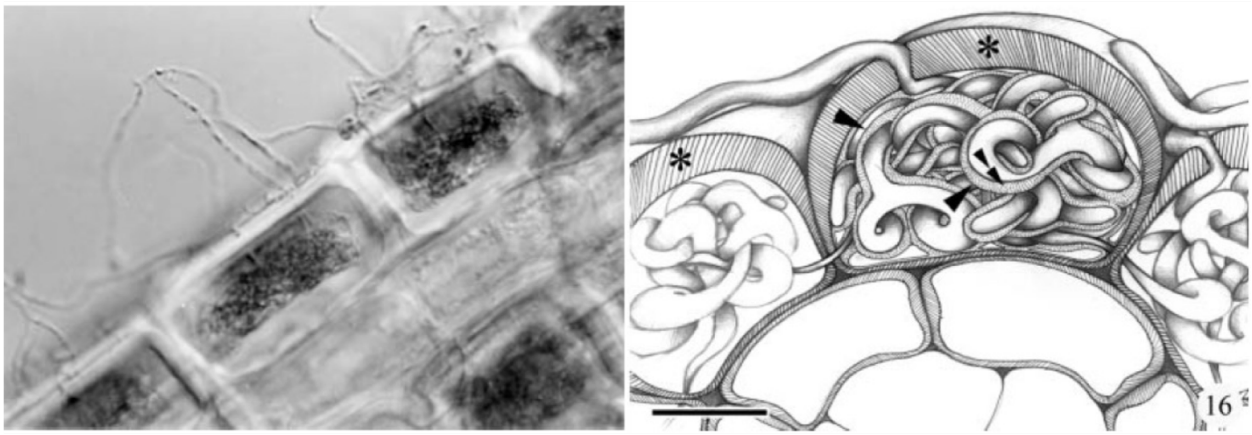
Le microbiote des Ericacées

Les études sur le microbiote de l'environnement racinaire du bleuetier sauvage ne sont apparues que récemment et demeurent peu abondantes. Yurgel et ses collaborateurs ont décrit les communautés d'eucaryotes et de bactéries au niveau du sol rhizosphérique et du sol brut de *Vaccinium angustifolium* ainsi que du sol sans végétation (Yurgel et al., 2017). Cette étude compare également des plants prélevés en forêt et en bleuetière et évalue les effets des propriétés chimiques édaphiques. Leurs principaux résultats indiquent une abondance relative des champignons (appartenant majoritairement aux Ascomycètes) négativement corrélée aux variables : azote total, matière organique et CEC. La structure des communautés bactériennes est principalement impactée par le pH et par les concentrations en aluminium et en magnésium. L'année suivante, les mêmes chercheurs se sont concentrés sur le lien entre le microbiote de *V. angustifolium* (au niveau du sol brut, du sol rhizosphérique et des racines) et le rendement en bleuet (Yurgel et al., 2018). Les chercheurs ont généré des réseaux de co-occurrence et ont notamment cherché à identifier les taxons pivots (hub taxa) en se basant sur les paramètres de centralité de degré, d'intermédiarité et de proximité. Ces taxons pivots ont donc davantage de connectivité que les autres, ce qui peut indiquer qu'ils jouent un rôle important dans la structuration du microbiote. Au niveau de la plante (échantillons racinaires et de sol rhizosphérique), les taxons pivots bactériens appartiennent au genre Bradyrhizobium et aux ordres Pedosphaerales, Actinomycetales et Solibacterales. Pour les eucaryotes, les taxons pivots appartiennent aux champignons de la classe Leotiomycetes (contenant l'ordre des Helotiales). Au niveau du sol (échantillons de sol rhizosphérique et sol brut) aucun champignon n'est identifié comme taxon pivot. Chez les bactéries, ce sont les taxons appartenant aux Acidobacteriaceae,

Caulobacteraceae, et aux Pedosphaerales qui ont été identifiés comme taxons pivots. En termes de rendement, le seul résultat obtenu est que le champignon parasite *Cryptomycotina sp.* est plus abondant dans les champs peu productifs. Enfin, le microbiote bactérien, fongique et eucaryotique (via séquençage des gènes 16S ARNr, ITS et 18S ARNr) de la rhizosphère de trois espèces du genre *Vaccinium* produisant des bleuets (*V. corymbosum*, *V. darrowii* et *V. virgatum*) ont été étudié plus récemment (Li et al., 2020). Dans cette étude, le *core* microbiome bactérien est caractérisé par une dominance des Alphaproteobacteria, notamment des Rhizobiales, et des Acidobacteria et Verrucomicrobia. Pour les champignons, les Leotiomycetes constituent la classe dominante et au niveau du *core* microbiome, les genres *Pezoloma* et *Hyaloscypha* (tous deux connus pour former des mycorhizes éricoïdes) sont les plus abondants (1ère et 3ème place respectivement). Malgré la présence de taxon en commun, la composition, la diversité alpha et l'abondance relative des communautés bactériennes et fongiques diffèrent significativement entre les trois espèces de *Vaccinium*, la rhizosphère de *V. darrowii* et *V. virgatum* étant notamment enrichis en taxons fongiques ayant déjà été identifiés comme pouvant être des champignons ErM (Li et al., 2020). Les chercheurs ont également trouvé une distribution différente de certains champignons ErM en fonction du génotype de l'espèce, ce qui indiquerait le rôle de l'hôte dans le recrutement de ses symbiotes (Li et al., 2020). Finalement, cette étude a également permis d'identifier des bactéries fixatrices d'azotes libres (Bradyrhizobiaceae, Methylocystaceae, Burkholderiaceae, Frankiaceae et Beijerinckiaceae) présentes dans la rhizosphère des trois espèces de *Vaccinium* étudiées (Li et al., 2020). A l'exception de ses trois études, la littérature parcourue investigue le microbiote d'autres espèces d'Ericacées ou s'intéressent spécifiquement à des espèces de champignons mycorhiziens éricoïdes (ErM).

Il y a quatre principaux types de champignons endophytes dans la famille des Ericacées : les champignons mycorhiziens arbutoïdes, monotropoïdes, éricoïdes et les Dark Septate Endophytes (DSE). Les mycorhizes arbutoïdes et monotropoïdes concernent des clades d'Ericacées ancestraux à celui contenant le genre *Vaccinium* (Kron et al., 2002; Smith and Read, 2008; Kariman et al., 2018) et n'ont donc pas été traitées dans ce projet. La symbiose mycorhizienne éricoïde serait la plus récente des types majeurs de mycorhizes, datée à 117 millions d'années (Leopold, 2016). Les

champignons impliqués forment un réseau d'hyphes peu dense autour de la racine et pénètrent la paroi cellulaire pour former des pelotes mycéliennes qui vont occuper la quasi-totalité du volume cellulaire (Smith and Read, 2008). La membrane plasmique des cellules hôtes s'invagine pour faire place aux pelotes mycéliennes et forme ainsi une matrice interfaciale (Figure 2). Les racines des Ericacées sont très fines (50-100-µm) et seule la couche épidermique est colonisée par les champignons ErM (Figure 2) (Smith and Read, 2008). Une racine peut être colonisée par plusieurs espèces, mais est souvent dominée par un unique taxon (Cairney and Meharg, 2003). La première espèce à avoir été isolée et identifiée est *Pezoloma ericae*, alors appelée *Pezillela ericae* (Pearson and Read, 1973a). La même année, le caractère mutualiste de la symbiose est démontré : le champignon transfère du phosphate et en échange reçoit des photosynthétats des pousses de deux Ericacées, *Calluna vulgaris* et *Vaccinium oxycoccos* (Pearson and Read, 1973b). Ce champignon est alors devenu le modèle de référence pour l'étude des champignons ErM. Depuis, d'autres espèces fongiques effectuant ce type de symbiose ont été identifiées. Nombreuses sont des Ascomycètes appartenant à l'ordre des Helotiales dont *Meliniomyces bicolor*, *Meliniomyces variabilis*, *Cadophora filandica*, et plusieurs espèces appartenant au genre *Oidiodendron* (Dalpé, 1991; Leopold, 2016; Kariman et al., 2018; Martino et al., 2018).



(a) (b)

Figure 2. Photographie et schéma de la colonisation mycorhizienne éricoïde. (a) Émergence d'hyphes de Rhizoscyphus ericacae de cellules racinaires épidermiques de Calluna vulgaris après lavage en série, macération et culture sur agar aqueux, photographie

de DJ Read tirée de (Smith and Read, 2008). (b) Diagramme d'un complexe d'hyphes intracellulaires dans les cellules épidermiques, typique d'une colonisation mycorhizienne éricoïde. La paroi tangentielle externe (*) des cellules épidermiques est épaissie. La membrane périfongique (pointes de flèche) et la matrice interfaciale (doubles pointes de flèche) séparent le champignon du cytoplasme de la cellule épidermique. Barre d'échelle = 25 µm. Diagramme tiré de (Peterson and Massicotte, 2004)

Comme mentionné dans la partie précédente, les Ericacées poussent dans des conditions difficiles et leur capacité d'adaptation serait étroitement liée à la présence des champignons ErM et à leur production d'enzymes (Cairney and Meharg, 2003; Mitchell and Gibson, 2006). À titre d'exemple, les enzymes phosphomonoestérases et phosphodiestérases produites par ces champignons permettent de libérer le phosphore emprisonné dans une forme organique, inexploitable telle quelle par la plante (Cairney and Burke, 1998). En termes d'azote, Kerley et Read ont prouvé la production de chitinase par *P. ericae* ainsi que sa capacité de transférer l'azote extrait de la chitine vers la plante hôte (*V. macrocarpon* et *C. vulgaris*) via la symbiose mycorhizienne (Kerley and Read, 1995). Les champignons ErM peuvent également protéger leurs plantes hôtes des métaux (Fe et Al notamment) qui sont davantage biodisponibles dans les sols acides (Perotto et al., 2002; Mitchell and Gibson, 2006). Les mécanismes en jeu ne sont pas encore tout à fait élucider mais appartiennent généralement à deux stratégies : la stratégie d'évitement (précipitation ou fixation des ions métalliques, suppression des transporteurs d'influx, efflux plus important des ions métalliques) ou bien la stratégie de compartimentation (translocation des ions métalliques dans des organes subcellulaires où ils seront moins toxiques) (Meharg, 2003). Une équipe de recherche de l'Université de Turin s'est servi d'*Oidiodendron maïus* comme champignon modèle pour étudier la résistance aux métaux lourds. Leurs études ont montré que ce champignon produisait d'avantage d'enzyme superoxide dismutase Cu/Zn lorsqu'en présence de concentrations élevées en zinc ou en cadmium. Cette enzyme permet d'endiguer l'accumulation des espèces réactives de l'oxygène dans les cellules et diminue donc le stress oxydatif (Daghino et al., 2016). Cette enzyme étant une métalloenzyme, elle pourrait également réduire la toxicité des métaux lourds en se fixant sur les ion métalliques. Cette équipe a également identifié des transporteurs

membranaires chez *O. maius* qui, une fois exprimés chez la levure (*S. cerevisiae*) non tolérante aux métaux lourds, permettaient de retrouver une croissance cellulaire. Un de ces transporteurs est présent sur la membrane du réticulum endoplasmique de la levure, impliquant une compartimentation des ions métallique, en l'occurrence du zinc, dans cet organelle. L'expression des gènes d'*O. maïus* en symbiose avec *V. myrtillus* en présence de cadmium a également été étudiée et les chercheurs ont identifié de nombreux gènes codant pour des transporteurs transmembranaires de type perméases, parmi les gènes surexprimés (Casarrubia et al., 2020). Les chercheurs ont également trouvé que les gènes codant pour de petites protéines sécrétées représentaient 11.76% des gènes surexprimés par *O. maïus* en présence de cadmium. Ces protéines pourraient donc être impliquées dans la réponse au stress induite par la présence de cadmium mais davantage de recherches sont nécessaires pour en comprendre le fonctionnement (Casarrubia et al., 2020).

Une étude de Martino et ses collaborateurs indique que les champignons ErM *O. maïus*, *M. variabilis*, *M. bicolor* et *P. ericae* possèdent tous des gènes codant pour des lipases, des protéases ainsi que pour des enzymes dégradant des polysaccharides (cellulose, lignine, chitine) (Martino et al., 2018). Une phylogénie basée sur ces gènes montre que ces champignons sont davantage apparentés à des champignons appartenant aux guildes saprotrophes et pathogènes qu'à la guilde mycorhizienne la plus proche (guilde ectomycorhizienne). Les champignons ErM auraient conservé leur arsenal d'enzymes saprotrophes n'ayant pas complètement évolué vers un mode de vie exclusivement mutualiste (Schlegel et al., 2016; Martino et al., 2018; Perotto et al., 2018). Ce mode de vie dual leur permet donc de s'adapter en fonction de la présence ou non d'une plante hôte.

Les taxons fongiques interagissant avec les Ericacées ne se limitent pas à l'ordre des Helotiales. En effet, certains Basidiomycètes sont également capables de s'associer aux racines d'Ericacées. L'inoculation de plants de *Vaccinium corymbosum* par *Mycena galopus* (jusqu'alors considéré comme étant un saprotrophe généraliste) colonise les racines en formant une structure en forme de doigts (peg-like structure), et provoque le même effet de croissance sur la plante induit par l'inoculation de *Pezoloma ericae* (Grelet et al., 2017). Un nouveau type d'association

mycorhizienne nommée mycorhize éricoïde gainée (sheathed-ericoïd mycorrhiza) entre un basidiomycète et des racines de *Vaccinium myrtillus* a amélioré la croissance de la plante in vitro (Vohnik et al., 2012). Certains champignons de l'ordre Sebacinales seraient également capables de mycorhizes ErM (Allen et al., 2003; Selosse et al., 2007). Enfin, certains champignons connus pour leur colonisation ectomycorhizienne sont également capables de symbiose éricoïde tels que *Cenococcum geophilum* qui colonise et améliore la croissance des plantules et des racines de *Rhododendron* sp. (Ericaceae) (Vohník et al., 2007). D'autres études ont également montré que certaines espèces de champignons (*Pezoloma ericae* et *Laccaria bicolor*) sont capables d'interagir à la fois avec des plantes ectomycorhiziennes et des Ericaceae (Villarreal-Ruiz et al., 2004; Villarreal-Ruiz et al., 2012) remettant en question la séparation entre deux guildes mycorhiziennes distinctes (Vrålstad, 2004). Cette liste non exhaustive montre la diversité de champignons supposément ou effectivement capables de symbiose mutualiste avec les racines des Ericaceae. En effet, il n'est toujours pas encore clair si certains de ces champignons sont bel et bien mycorhiziens ou si ce sont des colonisateurs opportunistes non mycorhiziens tels que des endophytes, des saprotrophes ou des nécrotrophes (Leopold, 2016). En effet, le troisième principe de Koch (preuve expérimentale du caractère mutualiste de la symbiose) n'est pas systématiquement vérifié, certaines études se contentant de la caractéristique morphologique pour établir que l'infection est mycorhizienne (Leake and Read, 1991). La question se pose notamment pour les "Dark Septate Endophytes" (DSE), avec l'espèce *Phialocephala fortinii* du complexe *Philocephala – Acephala*, qui colonise les racines à la manière des champignons ErM, mais dont la fonction demeure incertaine (Leopold, 2016; Hamim et al., 2017) . En effet, la présence de DSE dans les racines de plantes ne provoque pas toujours en une amélioration de la croissance et peut même être détrimentaire. Ils seraient néanmoins utiles pour la minéralisation de nutriment, notamment l'azote, dans les sols à forte teneur en matière organique (Newsham, 2011; Ruotsalainen, 2018).

En ce qui concerne la composition des communautés bactériennes des Ericacées, les études pertinentes sur le sujet sont plutôt rares. En 2017, des chercheurs ont étudié les communautés bactériennes au niveau des racines et de la mycosphère de trois Ericaceae : *Vaccinium myrtillus*,

Vaccinium vitis-idaea et *Calluna vulgaris* ainsi que celle de *Pinus silvetris* à titre de comparaison (Timonen et al., 2017). En termes d'abondance relative, la classe dominante est celle des Alphaproteobacteria, constituée de moitié par l'ordre des Rhizobiales, quel que soit le type d'échantillon (racines ou sol mycosphérique) ce qui suppose un fort potentiel de fixation d'azote (Timonen et al., 2017). Au sein des racines, seulement un tiers des OTUs est uniformément retrouvé au sein des quatre espèces indiquant une forte spécificité. La classe Acidobacteria avait une proportion relativement élevée dans les racines des Ericacées comparativement aux racines du pin. Enfin l'abondance de copies d'ARNr 16S bactérien est systématiquement plus élevée dans le sol mycosphérique des Ericacées que dans un sol contrôle sans plante. Ceci s'expliquerait par la présence des mycorhizes qui offrent une nouvelle niche écologique et produisent des exsudats (Timonen et al., 2017).

Cadre conceptuel et objectifs

Cette thèse s'intègre dans les Priorités de recherche au Québec définies par le Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec en 2016 dans sa Monographie de l'industrie du bleuet sauvage au Québec (MAPAQ, 2019). En effet, ce document évoque l'enjeu de la production et de l'amélioration des rendements en bleuet par différents moyens, dont l'étude de la fertilité des sols (et notamment des mycorhizes), de la fertilisation ou des effets de la fauche thermique sur les rendements. Comme décrit dans l'introduction de cette thèse, les micro-organismes du sol contribuent à sa fertilité, il est donc intéressant de connaître les communautés microbiennes de ce milieu et d'essayer de comprendre ce qui affecte leurs compositions dans le but d'optimiser leurs services écosystémiques, notamment en termes d'apports nutritifs pour les plantes. Cette thèse s'articule donc en trois chapitres ayant des objectifs distincts, mais qui permettent dans l'ensemble d'avoir une meilleure connaissance des communautés fongiques et bactériennes du milieu racinaire du bleuetier sauvage du Québec. Ces chapitres ont fait l'objet de publications dans des articles scientifiques revus par des pairs ou bien sont sous la forme de manuscrit visant à être soumis dans un futur proche. A la suite des trois chapitres, une conclusion générale synthétisant les résultats de ces trois études permettra également de montrer les limites de ce projet de doctorat et les futures expériences qui pourraient en découler.

Le **Chapitre 1** de cette thèse sert à introduire le contexte de ce projet et à expliquer les raisons qui ont poussé à étudier le microbiote racinaire et rhizosphérique du bleuetier dans différents contextes, testant l'effet de différentes pratiques agricoles sur cet écosystème.

La première étude présentée dans le **Chapitre 2** vise à caractériser les communautés bactériennes et fongiques du milieu racinaire des bleuetiers sauvages dans trois bleuetières commerciales et d'identifier des taxons ayant un potentiel bénéfique en termes de nutrition en azote pour le bleuetier sauvage. Les hypothèses qui sous-tendaient cette recherche étaient : 1. De retrouver une variété de taxons ayant été caractérisés comme étant des champignons mycorhiziens éricoïdes ; 2. D'identifier des taxons bactériens et/ou fongiques ayant potentiellement un rôle dans l'apport en azote des bleuetiers. Cette étude exploratoire dresse un

portrait jusqu'à lors encore méconnu de ces communautés microbiennes en bleuetière commerciale. En effet, comme précisé dans l'introduction, les études concernant le microbiote de l'environnement racinaire des bleuetiers sauvages *in situ* sont peu nombreuses. Deux études parues en 2017 et 2018 ont apporté de premiers éléments de réponse sur la composition des communautés bactériennes et fongiques de l'environnement racinaire de *V. angustifolium* (Yurgel et al., 2017; Yurgel et al., 2018). Cependant, le rang taxonomique utilisé pour caractériser ces communautés reste assez grossier (classe, ordre). De plus, les études ont utilisé le gène 18S ARNr qui cible l'intégralité des eucaryotes alors que nous souhaitons nous focaliser uniquement sur les champignons. Par conséquent, le séquençage de la région ITS est ici plus adéquat.

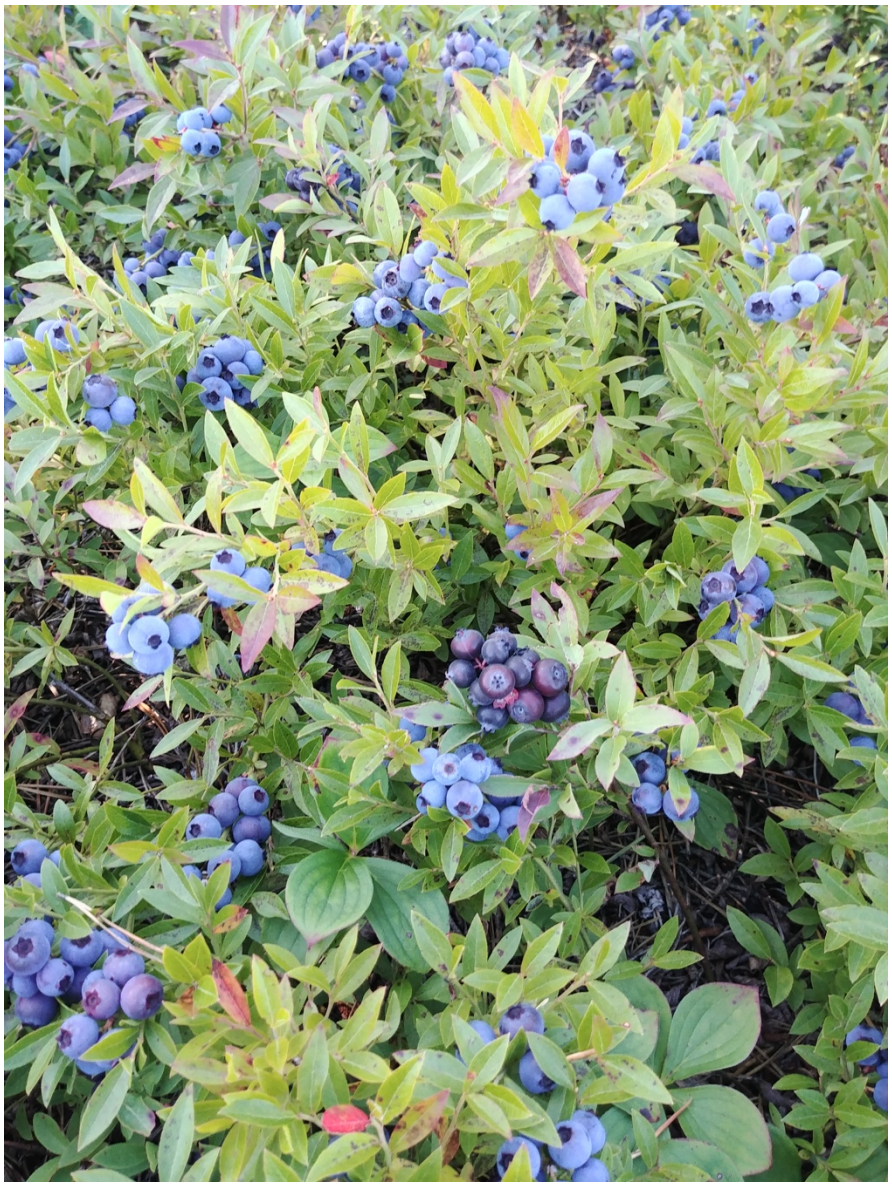
Toujours dans l'optique d'amélioration des rendements en fruits, les deuxième et troisième études de cette thèse ont porté sur l'impact de pratiques agricoles sur le bleuetier sauvage et son microbiote. Dans le **Chapitre 3**, nous avons voulu tester l'effet de la fauche thermique à différentes intensités sur les performances du bleuetier, sur son environnement et notamment son microbiote sous-terrain. Basées sur la littérature, nos hypothèses étaient que le brûlage allait diminuer l'acidité du sol, diminuer l'incidence de plantes adventices et de maladies fongiques et altérer la composition des communautés bactériennes et fongiques de la rhizosphère du bleuetier. Nous pensions également que plus l'intensité de brûlage était forte, plus les changements sur l'écosystème allaient être importants.

Le **Chapitre 4** a pour objectif de mesurer l'effet de la fertilisation, une autre pratique agricole mise en œuvre dans les bleuetières. Dans cette étude, l'effet d'une fertilisation minérale et organique sur le bleuetier sauvage et son microbiote a été comparé. Nous cherchions à vérifier que les doses de fertilisants utilisées ne soient pas détrimentaires aux taxons microbiens ayant un potentiel effet sur la nutrition du bleuetier sauvage (bactéries fixatrices d'azote et champignons mycorhiziens éricoïdes) et à statuer sur le type de fertilisant (minéral ou organique) à privilégier. D'après nos recherches, la fertilisation organique permet une libération progressive des nutriments, ce qui serait plus en phase avec les faibles besoins nutritifs des bleuetiers sauvages (Mallory and Smagula, 2012). En revanche, ces fertilisants ont tendance à augmenter le pH du sol, ce qui peut avoir un impact négatif sur les champignons mycorhiziens éricoïdes (Haynes and Swift, 1985; Caspersen et al., 2016). Notre hypothèse était donc que la fertilisation, qu'elle soit minérale

ou organique, allait diminuer la proportion de taxons ayant été caractérisés comme étant des champignons mycorhiziens éricoïdes, dans la communauté fongique racinaire et rhizosphérique par rapport à une parcelle non fertilisée.

Enfin, le **Chapitre 5** récapitule les principaux résultats de ce projet de recherche et présente également les limites de cette thèse et les pistes de recherches à réaliser dans le futur.

Chapitre 2 – Plongée vers le microbiote rhizosphérique du bleuetier sauvage (*Vaccinium angustifolium*)



Bleuets sauvages peu avant la récolte à la bleuetière Léon des Entreprises Gérard Doucet ltée (Bleuetière Saguenay), Saint-Honoré , Québec.

Into the wild blueberry (*Vaccinium angustifolium*) rhizosphere microbiota

Simon Morvan[1], Hacène Meglouli[1], Anissa Lounès-Hadj Sahraoui[2], Mohamed Hijri[1,3*]

[1] Institut de Recherche en Biologie Végétale, Département de sciences biologiques, Université de Montréal, 4101 Sherbrooke Est, H1X 2B2, Montréal, QC, Canada

[2] Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV), Université du Littoral Côte d'Opale, SFR Condorcet FR CNRS 3417, 50, Rue Ferdinand Buisson, 62228 Calais Cedex, France

[3] AgroBioSciences, Mohammed VI Polytechnic University, Lot 660, Hay Moulay Rachid, Ben Guerir 43150, Morocco

*** Correspondence:** mohamed.hijri@umontreal.ca

Keywords: Soil microbiota, rhizosphere, Ericaceae, Wild blueberry, *Vaccinium angustifolium*, Microbial community structures, Amplicon Sequencing, Phospholipid fatty acids, Ericoid mycorrhiza

Published in : *Environmental Microbiology*

DOI : https://doi.org/10.1111/1462-2920.15151

NOTE: Le texte de l'article a été modifié par rapport à la version publiée afin de corriger des fautes d'orthographe et de présenter la partie Méthodes avant la partie Résultats.

Abstract

The ability of wild blueberries to adapt to their harsh environment is believed to be closely related to their symbiosis with ericoid mycorrhizal fungi which produce enzymes capable of organic matter mineralization. Although some of these fungi have been identified and characterized, we still know little about the microbial ecology of wild blueberry. Our study aims to characterize the fungal and bacterial rhizosphere communities of Vaccinium angustifolium (the main species encountered in wild blueberry fields). Our results clearly show that the fungal order Helotiales was the most abundant taxon associated with V. angustifolium. Helotiales contains most of the known ericoid mycorrhizal fungi which are expected to dominate in such a biotope. Furthermore, we found the dominant bacterial order was the nitrogen-fixing Rhizobiales. The Bradyrhizobium genus whose members are known to form nodules with legumes, was among the ten most abundant genera in the bacterial communities. In addition, *Bradyrhizobium* and *Roseiarcus* sequences significantly correlated with higher leaf-nitrogen content. Overall our data documented fungal and bacterial community structure differences in three wild blueberry production fields.

Introduction

In the last decade, Next Generation Sequencing (NGS) technologies have revolutionized the biological sciences, allowing complete genome sequencing and metagenomes to be deciphered (van Dijk et al., 2014). Interactions between plants and micro-organisms have greatly benefited from the advances made by NGS technologies which have allowed the study of complex systems (Esposito et al., 2016). Analogous to humans and their gut microbes, a plant and its microbiota are so intrinsically linked that it is more appropriate to consider them as holobionts, defined as the interaction of a host (here the plant) and its micro-organisms (Vandenkoornhuyse et al., 2015; Vannier et al., 2018). Like in a human body, all plant organs harbor particular microbiota. The main niches that are studied are the phyllosphere (aerial surface of the plant), the endosphere (within plant tissue), and the rhizosphere (Turner et al., 2013). The micro-organisms that proliferate in these environments can have different life strategies ranging from pathogenicity to mutualistic symbiosis (Mendes et al., 2013; Saleem et al., 2017; Zhao et al., 2017).

In terms of benefiting agriculture, the more thoroughly studied micro-organisms are found in the soil, specifically, within the roots, and the surrounding rhizosphere (the portion of soil influenced by the root in terms of exudates, shredded cells and mucilage) (Turner et al., 2013). These exudates range from less than 10 up to 44 % of the plant-fixed carbon (Bais et al., 2006). In return, certain micro-organisms offer various beneficial functions to their host plants (Parnell et al., 2016; Fierer, 2017). Plant growth promoting rhizobacteria (PGPR) have been intensively investigated in this regard, as they could help to reduce the use of chemical inputs in agriculture (Adesemoye et al., 2009). The functions they provide to their host plants, such as nitrogen fixation, mineral mobilization, phytohormones production, pathogen protection, and abiotic stress tolerance, can have a significant effect on plant growth, health and yield increase (Berg, 2009; Mendes et al., 2013). Overall, bacteria have an important influence on the host plant's performance.

Fungi also play crucial roles in agriculture, they represent between 70 and 80% of crop diseases yielding significant economic losses (Moore et al., 2000). However, there are numerous groups of

beneficial fungi such as mycorrhizal fungi, which form symbiotic associations with plant roots. The most interaction involves arbuscular mycorrhizal fungi which colonize an estimated 72% of vascular plant species (Brundrett and Tedersoo, 2018). These fungi date back 460 million years and the oldest plant fossil found with arbuscular mycorrhizal fungi resembling structures (paramyccorhiza) is 407 million years old (Strullu-Derrien et al., 2018). Mycorrhizal fungi form an intraradical structure specialized for resource exchange between the plant host and the fungi (van der Heijden et al., 2015).

Wild or lowbush blueberries (*Vaccinium angustifolium* and *Vaccinium myrtilloides*) are species of shrubs belonging to the Ericaceae family, producing small edible blue berries. Within Canada, these shrubs are mainly cultivated in the Maritime Provinces and Québec, as well as the neighbouring state of Maine (USA) (Yarborough, 2012). In Québec alone, the total cultivated area was estimated at 35,579 hectares, with 29,000 hectares concentrated in the Saguenay-Lac-Saint-Jean region (MAPAQ, 2016). This makes Canada the world's biggest producer and exporter of wild blueberries with an export value of 238.8 million CAD $ (Agriculture and Agri-Food Canada, 2019). Among fruits, blueberries have one of the highest contents of anthocyanins which have a high antioxidant activity as well as beneficial effects on vascular, cognitive and glucoregulatory functions (Kalt et al., 2020).

Ericaceae plants, such as cranberries, rhododendrons and wild blueberries, grow in areas characterized by acidic soils, slow litter decomposition due to a high C:N ratio, and low nitrogen and phosphorus availability. In addition, the presence of toxic compounds such as trace metals and phenolic acids makes it even more difficult for these plants to grow in such harsh conditions (Cairney and Meharg, 2003; Mitchell and Gibson, 2006). The ability of Ericaceae plants to adapt to these hostile environments is believed to be aided by the presence of mycorrhizal fungi that have a high potential for releasing enzymes that mineralise organic matters (Cairney and Meharg, 2003; Mitchell and Gibson, 2006). Interestingly, the Ericaceae family has its own type of mycorrhization called ericoid mycorrhiza (ErM), and is thought to be the most recently evolved form of mycorrhizae, dating back only 117 million years (Leopold, 2016). Ericaceae roots are very

thin, with a diameter ranging between 50 and 100 µm. ErM fungi form a network of thin hyphae around the root and penetrate the cell wall of the epidermal layer to form mycelial coils that occupy most of the root cell's volume (Smith and Read, 2008). The first species that has been isolated and identified was *Pezoloma ericae*, (syn. *Pezillela ericae*, *Hymenoscyphus ericae*, *Rhizoscyphus ericae*) (Pearson and Read, 1973a). The same year, researchers illustrated the mutualistic nature of the ErM symbiosis; ErM fungi transfer phosphate and in exchange receive photosynthates from their host plants *Calluna vulgaris* and *Vaccinium oxycoccos* (Pearson and Read, 1973b).

The fungal species performing this type of ErM symbiosis are essentially Ascomycetes belonging to the order Helotiales, including *Meliniomyces bicolor*, *Meliniomyces variabilis*, *Cadophora filandica*, as well as several species belonging to the *Oidiodendron* genus that form the typical ericoid mycorrhizal structure in the root cells (Dalpé, 1991; Callahan et al., 2016b; Leopold, 2016; Kariman et al., 2018; Martino et al., 2018). However, Basidiomycetes are also able to interact with Ericaceae roots. Inoculation of the basidiomycete *Mycena galopus* (considered to be saprotrophic) on *Vaccinium corymbosum* caused the same growth effect on the plant as the one induced by the inoculation of the ascomycete *Pezoloma ericae* (Grelet et al., 2017).

A new type of mycorrhizal association called sheathed-ericoid mycorrhiza between a basidiomycete and *Vaccinium myrtillus* roots has improved the growth of the plant *in vitro* (Vohnik et al., 2012). Some fungi of the Sebacinales order (Basidiomycetes) were also capable of ErM mycorrhizae (Allen et al., 2003; Weiss et al., 2016). These examples show the diversity of fungi reported to be capable of symbiosis with Ericaceae roots. However, it is still not clear whether some of these fungi are actually mycorrhizal or whether they are non-mycorrhizal opportunistic colonizers such as endophytes, saprotrophs or necrotrophs (Leopold, 2016). Moreover, only a few studies have attempted to investigate the wild blueberry root microbial communities. Among these, Yurgel and collaborators studied the communities of eukaryotic microbes and bacteria in the rhizosphere and bulk soil of *Vaccinium angustifolium* and the effect of soil chemistry on these communities (Yurgel et al., 2017). Their results indicate that the

structure of bacterial communities is mainly affected by pH and aluminum and magnesium concentrations while the relative abundance of fungi (mainly Ascomycetes) is negatively correlated to total nitrogen, organic matter and cation-exchange capacity in the soil.

To our knowledge, no one has previously reported a comprehensive fungal community study using the ITS marker to investigate the wild blueberry rhizosphere. The objectives of our study were: i) to characterize the fungal and bacterial communities of *Vaccinium angustifolium* in the rhizosphere by identifying the most abundant taxa, a core microbiome and differences among alpha and beta diversity; ii) to complement the results obtained with sequencing by phospholipid fatty acid profiles; iii) to identify prospective taxa important for the blueberry leaf nitrogen content.

Experimental procedures

Study area

Sampling took place in the Saguenay-Lac-St-Jean region of Quebec, in three commercial wild blueberry fields owned by *Entreprises Gérard Doucet Ltée,* located around the town of Saint-Honoré (Fig. S8). All three blueberry fields were established from native stands by cutting down the spruce forest. The “Saguenay” field (48°31'21.5"N 71°00'51.4"W) is the oldest, established between 1984 to 1990, with an area of 121.1 ha. The “Shipshaw” field (48°30'38.2"N 71°12'57.3"W) was established in 1988 with an area of 64.6 ha. Finally, the du 40 field (48°33'51.4"N 71°12'16.2"W) was established between 2007 and 2016 and has an area of 180.7 ha. According to the pedological map of the Chicoutimi region by Raymond in 1971, the three plots were established on sandy and sandy loam soil (Raymond, 1971). The Saguenay and the Shipshaw fields begun transitioning to organic practices in 2015 and 2016, respectively. The du 40 field is still under a conventional agricultural model as it has been established more recently; there are more adventive plants that need to be eliminated. Conventional treatments include fertilizers and herbicides such as Spartan^{MD} and RoundUp.

Sampling

Field sampling occurred on August 16 2016, a few weeks before the blueberry harvest. In each of the three blueberry fields, 20 x 20 m plots were selected based on their estimated yields, on the advice of the producer. The goal was to have a yield heterogeneity within each blueberry field. However, yield was recorded on a larger scale than the sampling; consequently the yield data was not used in this study. Five plots were sampled in the Saguenay field, two in the Shipshaw field and two in the du 40 field. Within each plot, sampling consisted of extracting blueberry clusters including their shoots, roots, rhizomes and adhering soil, using a shovel. The surface extracted had an area of 20 x 20 cm and 15 cm deep. Five replicates, randomly located within each plot, were sampled, resulting in a total of 45 samples. Five samples were also taken following the same procedure in a spruce forest adjacent to the du 40 field. We separated the shoots from the rhizomes and its adhering soil and placed each compartment in separate Ziploc bags which were kept on ice until they could be processed and stored at -20°C. To recover the rhizospheric soil, the blocks of rhizomes and adhering soil were vigorously shaken in order to detach the soil which was then screened to remove roots. As the blueberry roots are very thin, it is difficult to be more precise than the method we used to recover the rhizospheric soil. Unfortunately, three of the five forest samples didn't have sufficient rhizospheric soil material to extract DNA, as the blueberry shrub sampled were growing on a layer of moss.

DNA extraction and amplification

To characterize the microbiota of wild blueberries, total environmental DNA was extracted from each sample and the 16S rRNA gene and ITS region were targeted for amplicon sequencing. DNA was isolated from 300 mg of rhizosphere using the NucleoSpin™ Soil kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. DNA quality was checked by gel electrophoresis (1%) then stored at -20°C until further use. Polymerase Chain Reaction (PCR) was done using HotStarTaq DNA Polymerase (1000U) kit (Qiagen, Hilden, Germany). The PCR mixture was made up of 1 unit of HotStarTaq DNA polymerase, 10X buffer, 1.5 mM MgCl2, 0.2 mM of each dNTPs, 0.5 mM of each primer and 1µL of DNA extract in a total volume of 25 µL. For bacteria, the primers

341(F): 5'- CCTACGGGNGGCWGCAG-3', and 805(R): 5'-GACTACHVGGGTATCTAATCC-3' were used to target the V3-V4 region of the 16S rRNA gene. The expected size of the amplicon was 464 nucleotides (Mizrahi-Man et al., 2013). For fungi, the primers ITS3_KYO2(F): 5'-GATGAAGAACGYAGYRAA-3', and ITS4_KYO3 (R): 5'-CTBTTVCCKCTTCACTCG-3', described in (Toju et al., 2012) were used. These primers target the ITS region, located between the 5.8S and LSU genes of the ribosomal RNA, and generate amplicons of around 430 nucleotides, though this region can vary in length. The ITS region has already been used successfully by Sietiö et al., (2018) to target ErM fungi (Sietiö et al., 2018). All primers used in PCR amplifications were coupled with CS1 (5'-ACACTGACGACATGGTTCTACA-3') and CS2 (5'-TACGGTAGCAGAGACTTGGTCT-3') at the 5' end, synthesized and purified using HPLC by Alpha DNA (Montréal, QC, Canada).

Thermal cycling conditions for both the 16S rRNA gene and ITS reactions were as follows: initial denaturation at 95°C for 3 min, 35 cycles of 94°C for 30 s, 53°C for 45 s and 72°C for 1 min and a final elongation at 72°C for 10 min. PCR reactions were done using an Eppendorf Mastercycler ProS thermocycler (Eppendorf, Hambourg, Germany). Amplification was confirmed by running each sample on a 1% agarose gel, and quantifying with a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to quantify the DNA concentration.

Sequencing data processing

Amplified PCR samples with CS1-CS2 adapters were sent to the Genome Quebec Innovation Center (Montréal, QC, Canada) for barcoding and sequencing using an Illumina MiSeq platform with a paired-end 2 x 300 base-pair method (Illumina, San Diego, CA, USA). The barcoding step adds an index (or barcode) to each sample as well as the sequence of Illumina adapters required for DNA binding to the flow cell (i5 5'-AATGATACGGCGACCACCGAGATCT-3' and i7 5'-CAAGCAGAAGACGGCATACGAGAT-3'). All MiSeq reads obtained were then processed using the DADA2 pipeline (Callahan et al., 2016a) on R (R Core Team, 2019). In this study, we didn't use Operational Taxonomic Unit (OTU) because they are typically determined by grouping sequences at 97% of sequence identity, depending on the data quality. This result in a low resolution usually insufficient to differentiate species within a genus. Singleton reads can lead to a large number of

false positives which results in over-inflation of OTUs. Instead, the DADA2 pipeline provides single-nucleotide resolution of amplicons which helps to identify chimeras and reduces the rate of false positives (Callahan et al., 2016a) .

Following the visualization of quality profiles, reads were filtered to eliminate poor quality sequences. For 16S rRNA gene reads, the filtering parameters used in the *filterAndTrim()* function were: trimLeft=c(17,21) to remove the primers, truncLen=c(280,255) and maxEE=c(3,3). For ITS reads, the parameters used were: trimLeft=c(18,18), truncLen=c(290,260), and maxEE=c(3,3). The other parameters of *filterAndTrim()* function were left at their default value. The error model was then computed on the filtered data using the *learnErrors()* function (Fig. S9 and S10). Filtered data were dereplicated using the *derepFastq()* function, before the amplicon sequence variants (ASVs) were inferred using the *dada()* function with the pool parameter set to "pseudo". Forward and reverse ASVs were then merged with an overlap of 12 base-pair (bp) using the *mergePairs()* function; sequences that didn't merge were eliminated. Chimeric sequences were eliminated by using the *removeBimeraDenovo()* function with the method parameter set to "pooled", which is required to adjust for using the "pseudo" pooling parameter. Taxonomy was then assigned using the implemented Naïve Bayesian Classifier RDP (Wang et al., 2007) and the *assignTaxonomy()* function. The default minimum bootstrap confidence cutoff value of 50% for the *assignTaxonomy()* function was kept. The reference taxonomic databases used were SILVA (Yilmaz et al., 2014 ; Callahan, 2018) for 16S rRNA gene sequences and UNITE for ITS sequences (Koljalg et al., 2013 ; UNITE Community, 2017). Sequences present only once (singletons) or twice (doubletons) in the whole data set were eliminated as they may be artifacts (Wen et al., 2017). Sequences which did not have a bacterial taxonomic assignment were eliminated (16 Archaea and 33 Eukaryota sequences) from the bacterial dataset. The same was done for the fungal dataset which eliminated 120 Chromista and 2 Rhizaria sequences. Samples that had fewer reads in the 16S rRNA gene data (sample 17, 19, 20, 24, 48 and 49) and the ITS data (sample 17, 19, 24, 48 and 49) were also eliminated from further analysis. Bacterial and fungal ASVs, with their assigned taxonomy and abundance per sample, are presented in Table S13 and S14 respectively.

Phospholipid fatty acids analyses

To estimate the biomass of the bacterial and fungal communities of wild blueberries, we extracted and measured the phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) markers. Rhizosphere samples were thawed, sieved (2 mm) and freeze-dried. Remaining plant debris was removed, then 3 g of freeze-dried soil were analyzed to measure the fatty acid content. Lipid extraction was performed according to (Frostegård et al., 1991). Extracted lipids were fractionated into neutral lipids, glycolipids and polar lipids on a SPE silica column (Solid Phase Extraction, Hypersep SILICA 500mg from Thermo Fisher Scientific, Waltham, MA, USA) by successive elution with chloroform, acetone and methanol respectively. Neutral lipids and phospholipids were then concentrated under a nitrogen stream, redissolved in toluene/methanol (1:1) and subjected to a trans-esterification using a base solution (KOH, (0.2 M) prepared in methanol) at 37°C for 15 min to release free fatty acid methyl esters (FAMES) from the PLFA and the NLFA. FAMES were analyzed by Fast GC-MS (Shimadzu 2010 Plus system, Kyoto, Japan). Samples were injected in split mode (ratio 100.0) at 280°C. The separation was performed on a Zebron ZB-1 MS capillary column (10 m length x 0.1 mm i.d., 0.1 µm film thickness, Phenomenex, Torrance, CA, USA). The system was operated at constant linear velocity (40 cm s-1) using helium as the carrier gas and the oven was programmed as follows: from 175°C to 275 °C at 25°C.min-1) and maintained for 0.5 min by means of a Shimadzu 2010 Plus system gas chromatograph (Shimadzu Corporation, Kyoto, Japan). This GC was equipped with a Shimadzu QP 2010 Ultra mass spectrometer detector (Shimadzu Corporation, Kyoto, Japan) and a Flam Ionization Detector (300°C) used alternatively. FAMES were compared to nanodecanoic acid methyl ester (C19:0-Me) as an internal standard by comparing their mass spectra with the standard mass (fatty acid methyl ester mixtures C4-C24:1, Sigma–Aldrich, Saint-Louis, MO, USA) in the NIST MS library. The amount of PLFA 16:1ω5 and NLFA 16:1ω5 in the soil of the different experimental plots were determined and used as indicators of the arbuscular mycorrhizal fungal biomass. The value of NLFA/PLFA C16:1ω5 ratio indicates the origin of the C16:1ω5 fatty acid. If the ratio is superior to 1, it indicates that the PLFA C16:1ω5 is originating from AMF and not from bacteria. PLFA 18:2ω6,9 was used as an indicator of fungal (except arbuscular mycorrhizal fungi) biomass (Frostegård et al., 2011). Gram-positive bacteria biomass was estimated by the quantification of the PLFA: i15:0, a15:0,

i16:0, i17:0, a17:0 amounts and Gram-negative bacteria biomass by the quantification of the PLFA: cy17:0, C18:1ω7 and cy19:0 amounts in the soil. The fungal:bacterial biomass ratio was measured by dividing the amount of the fungal PLFA 18:2ω6,9 by the sum of the bacterial PLFA (Bardgett and McAlister, 1999).

Elemental micro-analyses

To measure the impact of the bacterial and fungal communities of wild blueberries on the plant's performance, we measured the nitrogen content in the blueberry leaves. Shoot samples were oven-dried at 65°C for 72 hours, the blueberry leaves were clipped off the shoots and crushed in a cyclone mill grinder (2 mm screen). Leaf powder was then weighed at 7 mg (±0.2mg) in aluminum weighing boats on a Metler-Toledo XPR2 precision scale (Metler-Toledo, Columbus, OH, USA). 2mg (±0.2mg) of acetanilid was also weighed for calibration. The percentage of carbon and nitrogen of each sample were determined using an Elementar vario MICRO cube analyzer (Elementar, Langenselbold, Germany) (Table S15).

Visual and statistical analyses

All statistical analyses were performed in R 3.5.2 (R Core Team, 2019). Figures were generated using *ggplot2* (Wickham, 2016). In order to check the sequencing depth, rarefaction curves were obtained using the *rarecurve()* function from the *vegan* package (Oksanen et al., 2019).

Relative abundance profiles were obtained using the *phyloseq* package (McMurdie and Holmes, 2013).

For the core microbiota, we subset the samples by fields and then removed all the ASVs that were not present in all samples of these fields. We then compared the core microbiota of each field using the *intersect*() function of the *dplyr* package which allowed to pick out the common sequences belonging to two or more fields. For visualization, we used the *draw.quad.venn()* function of the VennDiagram package.

As the abundance of the sequences in a sample can impact alpha diversity measures, we rarefied samples to an even depth using the *rarefy_even_depth()* function of the *phyloseq* package (McMurdie and Holmes, 2013). The depth used was the minimum number of sequences found

across the samples. Two alpha diversity indices, Simpson and Shannon-Weaver, were computed using the *plot_richness()* function from the *phyloseq* package(McMurdie and Holmes, 2013).

In order to compare the bacterial and fungal communities of the samples, we used a non-metric multidimensional scaling ordination (NMDS). This type of ordination unlike Principle Component Analysis allows to constrain the variance into two dimensions which is more suitable for visualization. Complementary to this analysis, we used pairwise PERMANOVAs with 9999 permutations to test if the communities of the fields were statistically different. We decided to exclude the forest samples in the PERMANOVA as it only contained two samples and that clearly has an effect on the homogeneity of dispersion. The function used *calc_pairwise_permanovas()* of the mctoolsr package (https://github.com/leffj/mctoolsr/) calculates PERMANOVA results using the *adonis()* function of the vegan package for all factor-level pairs. It provides p_values with an FDR correction to take into account the multiple comparisons. The assumption of homogeneity of the dispersion among groups was checked using *betadisper()* and *permutest()* of the *vegan* package (Oksanen et al., 2019).

For the alpha diversity, PLFA analyses, and leaf nitrogen concentration analysis, we used one-way ANOVA tests to check for statistical differences using the *aov()* function of the vegan package. The assumptions of homogeneity of variance, normality of residuals, and homoscedascity of residuals were checked respectively with a Bartlett test using the *bartlett.test()* function, a Shapiro-Wilk test using the *shapiro.test()* and checking the distribution of the residuals. A post-hoc Tukey's Honest Significant Difference test was conducted if the ANOVA test was significant to identify which groups were statistically different. When the data did not meet the assumptions for the one-way ANOVA test, a Kruskall-Wallis test was used instead, using the *kruskal.test()* function. A post-hoc Wilcoxon-Mann-Whitney test with a Benjamini-Hochberg correction was computed when the Kruskall-Wallis test was significant using the *pairwise.wilcox.test()* function or *wilcox.test()* when there were only two groups to compare.

Given the statistical difference in leaf nitrogen content (LNC) between the Saguenay field and the du 40 and Shipshaw fields (Fig. 5), we looked into the bacterial and fungal communities, so as to search for taxon that are known for their impact on nitrogen fixation or solubilization that could therefore also have an impact on LNC. To that end, we used Spearman correlations between the

bacterial and fungal ASVs abundance and LNC. Correlation was computed using the *rcorr()* function of *Hmisc* package (Harrel, 2020). The two forest samples were not included in this analysis as no statistical difference was found between the forest and the fields (Fig. 5). The taxa were only considered for positive or negative Spearman correlation when their relationship to LNC was significant (linear regression, p_value < 0.05).

Additionally, we used an indicative species analysis which relies on predefined groups based on species abundance and fidelity. In this case, each field (Saguenay, du 40 and Shipshaw) was defined as a group, as well as the three combinations of two fields. Using the *multipatt()* function of the *indicspecies* package (de Cáceres and Legendre, 2009), an indicative species value (IndVal) was assigned to prospective indicative species based on their importance in the group they are found to be indicative of. We used 9999 permutations in order to compute the IndVal's p_values with the *p.adjust()* function of the *stats* package (R Core Team, 2019). To correct for multiple testing, we used a Benjamini-Hochberg, also known as False Discovery Rate (FDR), correction. Although this type of correction is less stringent than familywise error rate such as the Bonferroni or Holm correction, they are acceptable in exploratory studies (Noble, 2009) such as this. In order to refine the indicative taxa found to the ones that can potentially impact nitrogen uptake, we selected the indicative species that were significantly correlated to LNC (Spearman correlation, p_value<0.05). This analysis targets the ASVs that are more abundant or present in specific fields and can therefore pinpoint ASVs that would have gone unnoticed using the Spearman correlation alone.

The taxonomy assigned using DADA2 and SILVA and UNITE databases weren't always resolved to species levels. In order to have a finer taxonomy, we submitted the sequences of interest to NCBI's BLAST using the 16S ribosomal RNA sequences (Bacteria & Archeae), and the internal transcribed spacer region (ITS) from Fungi type and reference material databases. We retained the BLAST hits with the highest BLAST percentage of identification (percent ID) and lowest E value. We took into account all the species if our sequences matched to identical values. BLAST hits that did not exceed 97% in terms of percent ID were not presented in the results as it is hard to have confidence in these identifications (Tindall et al., 2010). For fungi, as UNITE was precise enough

to give species level identification for most of the sequences, we included them in the results. The fungal sequences that had no genera and species identification from UNITE and did not exceed 97% percent ID were not presented in the results.

The taxa that we present in both analyses are potential candidates to improve nitrogen uptake in blueberries. However, inoculation in controlled settings of these species is required to assess if these microbes do improve nitrogen uptake.

Accession numbers

All sequences have been deposited in the GenBank SRA database under the accession number PRJNA604263.

Results

9013 bacterial and 2964 fungal ASVs were inferred from high-quality Illumina sequencing.

Our first objective was to characterize the fungal and bacterial rhizospheric communities of the rhizosphere of *Vaccinium angustifolium.* To achieve this goal, we used a metabarcoding approach, where sequencing via Illumina MiSeq, resulted in a total of 7 334 284 reads (156 049 ± 58 938 reads per sample) for the bacterial 16S rRNA gene and 7 295 422 reads (155 222 ± 60 559 reads per sample) for the fungal ITS region. However, six samples (17, 19, 20, 24, 48 and 49) had approximately ten times less 16S rRNA gene reads than the other 42 samples (Fig. S1 A), which explains the large standard deviation. Five of these six samples (17, 19, 24, 48 and 49) also had approximately ten times less ITS reads (Fig. S1 B).

The quality profile plots show that some sequences had a poor quality especially the reverse sequences (mean quality score below 20 for the last 50-40 base-pair) (Fig. S2 and S3). This was

corrected with DADA2's filtering function, the mean quality score remains above 20 even for the reverse reads (Fig. S2 and S3).

After the final DADA2 step, a total of 2 103 026 reads were retained (51 293 ± 6 277 reads per sample) for the 16S rRNA gene data. From these reads, 9 013 amplicon sequence variants (ASVs) were inferred (1709 ± 210 ASVs per sample). For the ITS data, a total of 2 988 921 reads were retained (71 165 ± 25 348 reads per sample) and 2 964 ASVs were inferred (345 ± 113 ASVs per sample). Rarefaction curves obtained after quality filtering indicate a sufficient sequencing depth for both ITS and 16S rRNA gene data (Fig. S4).

The 16S rRNA gene sequences ranged from 397 to 461 base pairs with a median of 411 bp. According to the violin plot (Fig. S5 A), there seems to be two groups around the 1st quantile (402 bp) and the 3rd quantile (427 bp). For fungal sequences, the median size obtained is 358 bp with a maximum of 502 bp and a minimum of 272 bp. The higher dispersion for ITS reads illustrates the variability of length that characterizes the ITS region (Fig. S5 B).

The Rhizobiales and Helotiales were the most dominant bacterial and fungal orders, respectively.

From the bacterial ASVs, a total of 138 different orders were identified while 8.9% of the sequences did not receive a taxonomic assignment (Unknown). The abundance profile shows the top 10 orders in terms of relative abundance, which represented 68.1% of the sequences. The remaining 128 orders found were classified as "Other" on the plot (Fig. 1 A). The composition does not vary much within, or across, plots, or across blueberry fields. Remarkably, the structure of the 10 most abundant orders does not fluctuate much either. The most abundant orders were Rhizobiales (21.8%), Acidobacteriales (15.2%) and Acidobacteriia Subgroup2 (11.5%). More precisely, at the genus level, the most abundant genera were *Acidothermus* (5.2%), *Bryobacter*

(2.8%) and *Roseiarcus* (1.9%), belonging respectively to the Frankiales, Solibacterales and Rhizobiales orders (Table S1).

For fungal ASVs, a total of 80 different orders were identified but almost a quarter (24.2%) of the sequences were not assigned (Unknown). The top ten orders in terms of relative abundance represent 68.5% of the sequences and the remaining 70 orders were classified as "Other" (Fig. 1 B). Contrary to the bacterial dataset, the structure varies considerably even within replicates of a plot. The most abundant orders were Helotiales (16.9%), Valsariales (15.0%) and Chaetothyriales (13.5%) and Agaricales (11.5%). At the genus level, 54.3% of the reads were not taxonomically assigned. The most abundant genera are *Bambusaria* (15.0%), *Clavaria* (6.2%) and *Lactarius* (3.4%) belonging respectively to the Valsariales, Agaricales and Russulales orders (Table S2).

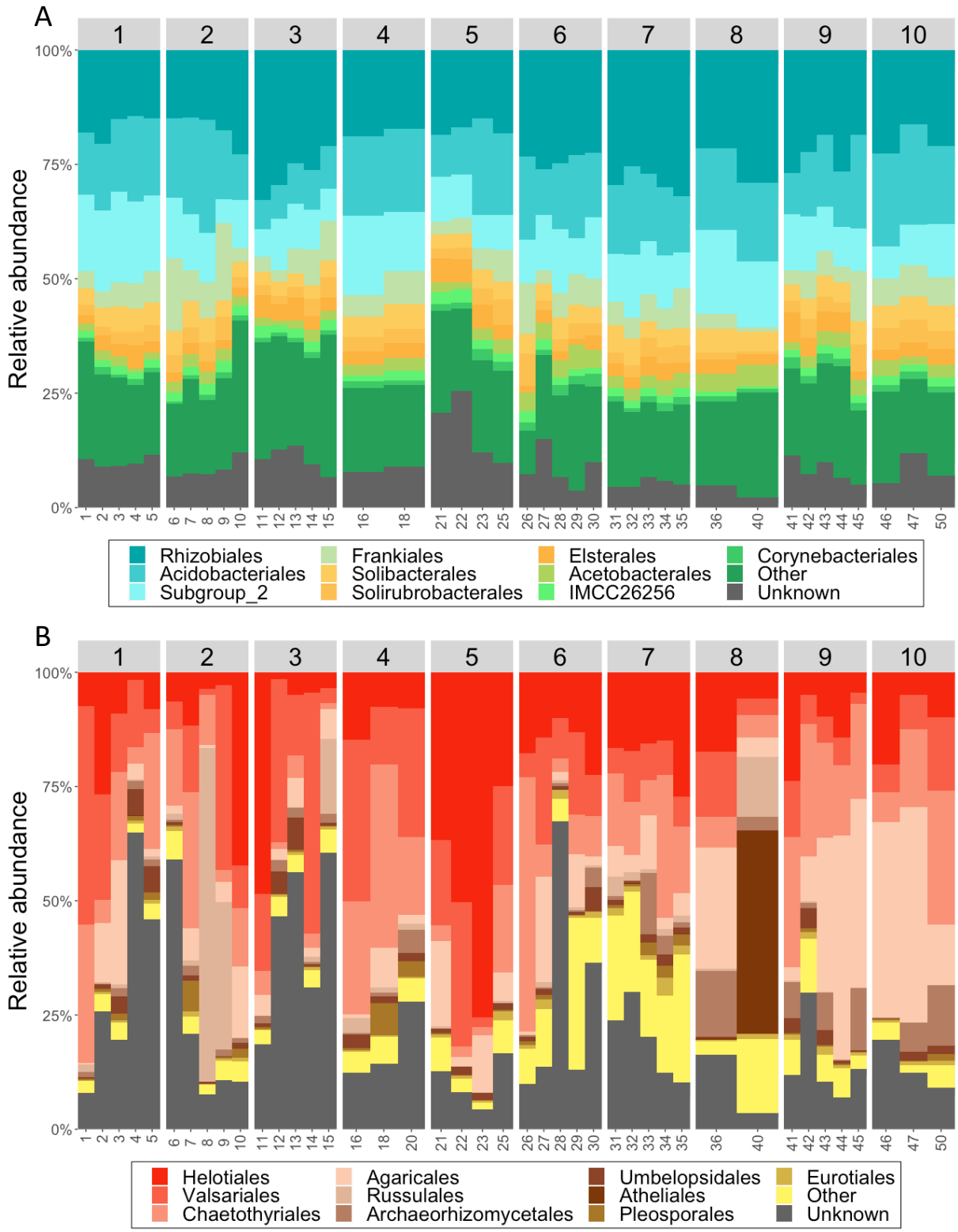

A
1
2
3
4
5
6
7
8
9
10
100%
75%
50%
25%
0%
Relative abundance
Rhizobiales
Acidobacteriales
Subgroup_2
Frankiales
Solibacterales
Solirubrobacterales
Elsterales
Acetobacterales
IMCC26256
Corynebacteriales
Other
Unknown
B
Relative abundance
Helotiales
Valsariales
Chaetothyriales
Agaricales
Russulales
Archaeorhizomycetales
Umbelopsidales
Atheliales
Pleosporales
Eurotiales
Other
Unknown

Figure 1. Abundance profile at the order level. A. Bacterial 16S rRNA gene amplicon data. B. Fungal ITS region amplicon data. The relative abundance was computed across the whole dataset. Different orders were ranked and only the ten most relatively abundant orders were plotted, the remaining orders (128 for 16S rRNA gene, 70 for ITS) were grouped under the "Other" category. The "Unknown" category corresponds to ASVs that did not obtain a taxonomic assignment at order level.

58 bacterial ASVs and one fungal ASV were identified as core across all plots.

To further characterize the fungal and bacterial communities of *Vaccinium angustifolium* in the rhizosphere, 58 bacterial ASVs inferred from the 16S rRNA gene data were present ubiquitously across the plots (Fig. S6 A). These ASVs belonged to 12 different orders, though three ASVs did not have an assigned identity at the order level (Table S3). The core ASVs that contributed the most in terms of relative abundance belong to the following orders: Rhizobiales (9.61%), Acidobacteriales (4.26%), Acidobacteriia Subgroup_2 (2.18%), Frankiales (2.00%) and Solibacterales (1.09%). One of the ASVs belonging to the Rhizobiales accounted on its own to 3.59% (75 583 reads) in terms of sequence abundance. It was identified as a *Bradyrhizobium sp.* using NCBI's BLAST tool on the nr/nt database. Nine additional ASVs were present exclusively in cultivated plots (forest plot excluded). Only one of those nine ASVs was identified up to species level (Table S3).

For the fungal ASVs, there was no overlap across all plots and only one ASV was found in all cultivated plots, after excluding the forest plots (Fig. S6 B) which was identified as *Solicoccozyma terricola* (synonym *Cryptococcus terricola*) an oleaginous basidiomycete yeast capable of turning starch to fatty acids (Close et al., 2016). Although it was found in all cultivated plots, its relative abundance is low, representing only 0.27% of the filtered sequences (Table S3). Finally, whether for the bacterial or fungal ASVs, the Shipshaw and du 40 fields share more common ASVs than with the Saguenay field (Fig. S6 AB).

Shannon-Weaver and Simpson alpha diversity indices both show a higher diversity for bacteria than fungi across all 10 plots.

Both 16S rRNA gene and ITS datasets were rarefied prior to alpha diversity computation. We randomly subsampled to 39 744, and 30 488, reads per sample for the 16S rRNA gene and ITS datasets, respectively. This resulted in the loss of 10 ASVs in the 16S rRNA gene dataset (0.1% loss) and 50 ASVs in the ITS dataset (1.7% loss).

For bacteria, the Shannon-Weaver diversity index (Fig. 2A) is quite homogenous across all cultivated plots. The forest plot has a lower diversity mean, however, this plot contains only two samples which could be biased. The Simpson diversity index (Fig. 2B) is close to its maximum for almost all plots with the exception of the forest, which has a sample with a lower diversity. Overall, the within field variation is larger for the Shannon-Weaver index than for the Simpson index. There is no significant difference between plots.

For fungal sequences, both Shannon-Weaver (Fig. 2C) and Simpson (Fig. 2D) diversity indices are globally low compared to bacteria and show a greater within-plot variation. Plot 7 has a higher diversity index than the other plots but this difference was not significant ($p\_value\_adj > 0.05$).

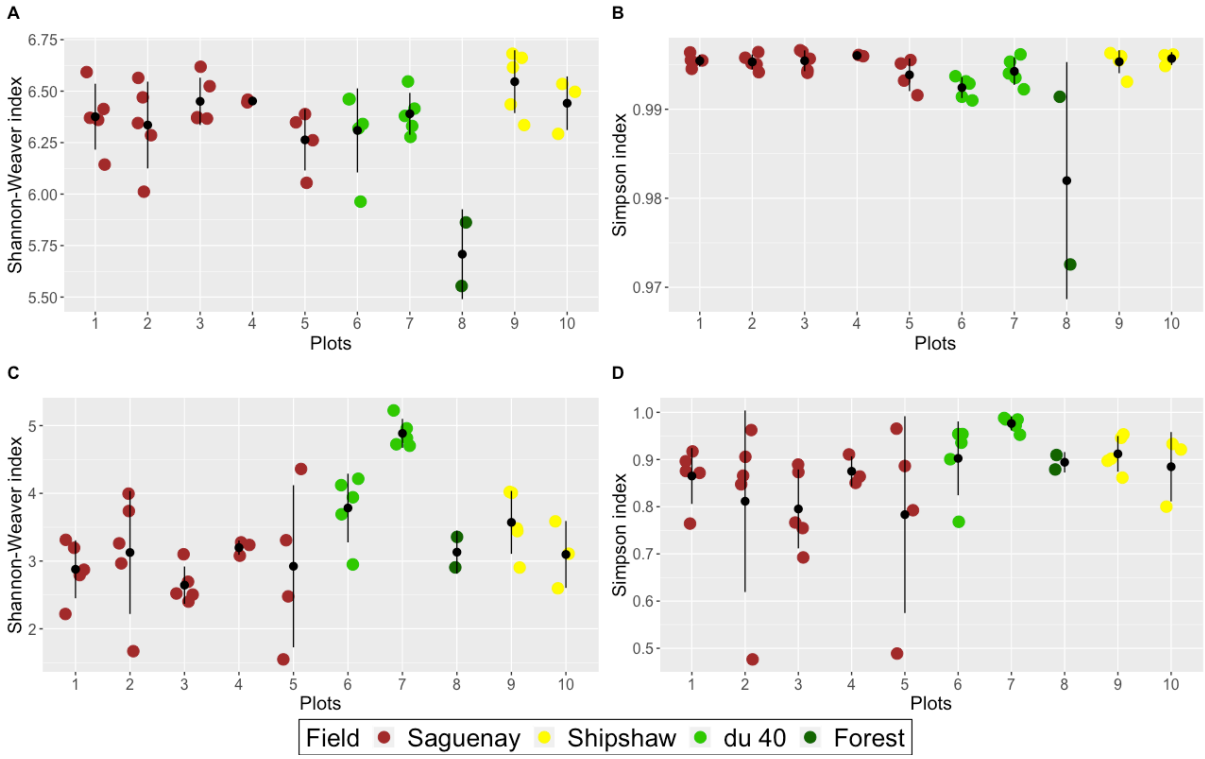


Figure 2. Alpha diversity. A. Shannon-Weaver diversity for 16S rRNA gene, B. Simpson diversity for 16S rRNA gene, C. Shannon-Weaver diversity for ITS, D. Simpson diversity for ITS.

Beta diversity indicates an age gradient, from the fields under management the longest to those more recently established.

Age seems to have a strong influence over fungal community structure as shown in the NMDS ordination plot (Fig. 3 B). Communities from the oldest managed field (Saguenay) are distinct from the communities belonging to the most recently managed field (du 40). The pattern is even more visible for bacterial community (Fig. 3 A). Bacterial and fungal communities in the forest soil adjacent to the du 40 field are relatively different from cultivated soil communities, as the two forest samples tend to be quite distant from the rest of the samples in the NMDS plots. Bacterial and fungal community composition differed significantly among the cultivated fields, according to the pairwise PERMANOVA tests ($p\_value\_FDR < 0.05$) with the exception of Saguenay and Shipshaw for the bacterial dataset (Table S6 and S7). Within group dispersion was homogeneous in the fungal data ($p\_value > 0.05$) but it was not the case for bacterial data ($p\_value < 0.05$).

For bacteria, the most abundant phyla (Proteobacteria, Acidobacteria and Actinobacteria) are located around the origin of the NMDS meaning that they are uniformly present in the different sites (Fig. 3 A, Table S4). However, Chloroflexi and Firmicutes (4th and 5th most abundant phyla, Table S4) seem to be more abundant in the Saguenay field which is the oldest field of wild blueberry production in our study. In terms of orders, Rhizobiales and Acidobacteriales (first and second most abundant orders) are located on the bottom half of the NMDS meaning that they are more characteristic of the forest and du 40 field (Fig. S7 A).

For fungi, Basidiomycota are more characteristic in the most recent plots (Fig. 3 B, Table S5). As for the orders, the three most abundant orders, Helotiales, Valsariales and Chaetothyriales are on the right-hand side of the NMDS suggesting that they are more prevalent in the du 40 field and the forest as the samples from these two groups are also on the right-hand side of the plot (Fig. S7 B), meaning that the PERMANOVA results must be taken with care. Nevertheless, the visual assessment of the NMDS plot does show a segregation among cultivated fields (Fig. 3 A).

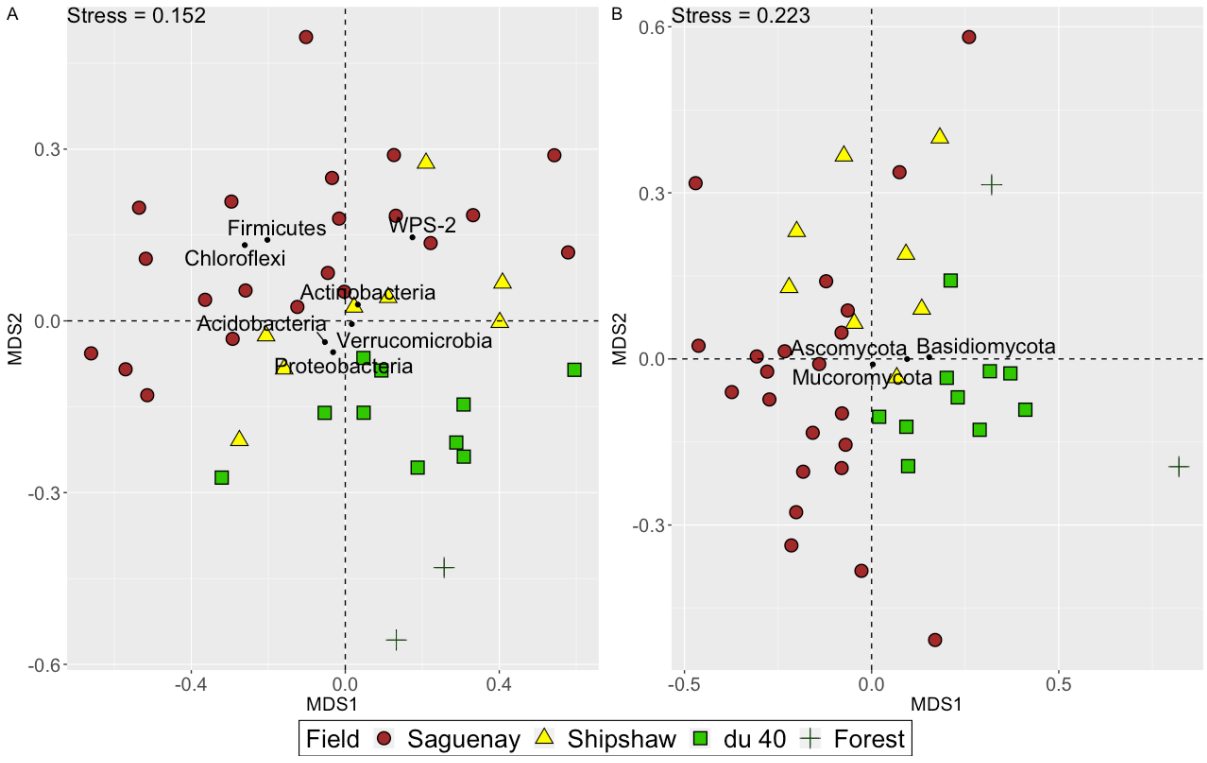


Figure 3. Beta diversity represented by a non-metric multidimensional scaling (NMDS) using Hellinger distance. A. 16S rRNA gene data, B. ITS data. Each coloured symbol represents a sample. ASVs were regrouped based on their phylum identity and their mean coordinates were computed and displayed on the graph using black dots and phylum name. For more clarity, only the phyla with an overall relative abundance above 1% were plotted (Table S4 and S5).

PLFA analyses indicate an absence of arbuscular mycorrhizal fungi.

The second objective of our study was to complement the results obtained through DNA sequencing with phospholipid fatty acid profiles. Gram-negative bacterial biomass was higher than Gram-positive bacteria biomass with a mean of 8.30 ± 2.08 µg/g of soil versus 5.87 ± 1.52 µg/g of soil (Fig. 4 A). The difference in PLFA content was found to be significant using the Wilcoxon-Mann-Whitney test (p-value = 2.495e-10). For Gram-positive bacteria PLFA, a significant difference was found between the Saguenay (5.38 ± 1.18 µg/g of soil) and the du 40 field (7.09 ± 1.63 µg/g of soil) using the pairwise Wilcoxon test with a Benjamini-Hochberg correction (p_value_adj = 0.013). However, for Gram-negative bacteria, no significant differences were found between plots or between fields.

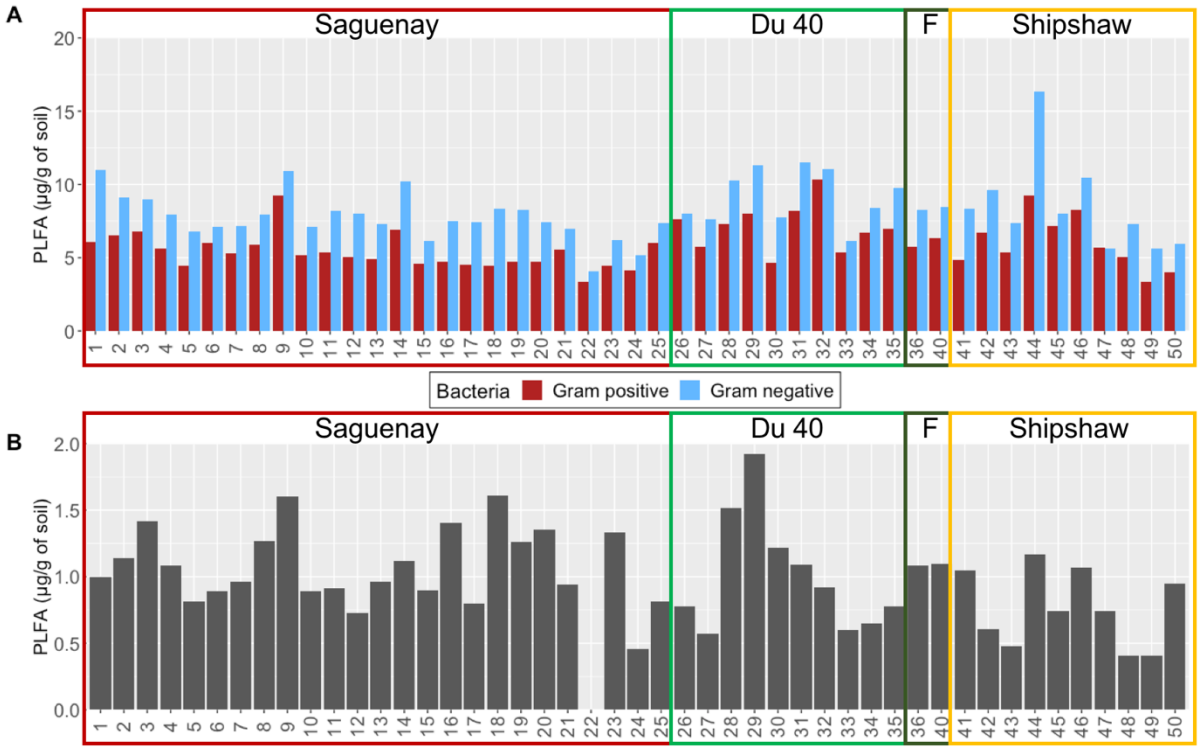


Figure 4. PLFA concentrations. A. Bacterial PLFA markers (PLFA markers i15:0, a15:0, i16:0, i17:0, a17:0 for Gram-positive bacteria. PLFA markers cy17:0, C18:1ω7 and cy19:0 for Gram-negative bacteria, B. Fungal (except AMF) PLFA marker (PLFA 18:2ω6,9). F is for Forest.

The ratio NLFA 16:1ω5 / PLFA 16:1ω5 was computed for every sample in order to account for the presence of arbuscular mycorrhizal fungi (AMF). The PLFA 16:1ω5 marker is present in AMF, but also in some bacteria, whereas the NLFA 16:1ω5 marker represents mainly the storage structures of the AMF (vesicles and spores). If the ratio is superior to 1, it indicates that the PLFA C16:1ω5 was originating from AMF and not from bacteria. However, this was not the case in any of our samples (Table S8).

The fungal PLFA 18:2ω6,9 had an average quantity of 0.97 ± 0.35 µg/g of soil (Fig. 4 B). Sample 22 had a null concentration of the PLFA 18:2ω6,9 marker which could be the result of an experimental error as the sample contains fungal DNA (Fig. S1 B). No significant difference was

found when comparing the mean fungal PLFA concentration per plot or per field using the Kruskall-Wallis test.

The fungal:bacterial biomass ratio had a mean value of 0.07 ± 0.02 (Table S8). A Kruskall-Wallis test comparing this ratio for the plots was significant (p=0.019), but the pairwise Wilcoxon test with a Benjamini-Hochberg correction, detected no significant difference between the plots. When comparing the fields, these same tests showed a significant difference between the Saguenay and Shipshaw field (p_value_adj = 0.031).

Significant difference in leaf nitrogen concentration between the Saguenay field and the du 40 and Shipshaw fields.

Our third objective was to identify prospective taxa important for blueberry leaf nitrogen content. First, the overall mean nitrogen concentration in dried leaves was 1.59 ± 0.28% (Fig. 5). The variation of the nitrogen concentration within plants from the same plot was high in plots 2 and 8. An ANOVA test followed by a post-hoc Tukey test, showed a significant difference of the dried leaf nitrogen concentration when comparing the Saguenay field to the du 40 and the Shipshaw field (Fig. 5). In the Saguenay field, the mean nitrogen concentration is 1.43 ± 0.24% whereas it reaches 1.82 ± 0.18% and 1.80 ± 0.18% for the du 40 and Shipshaw fields respectively (Fig. 5).

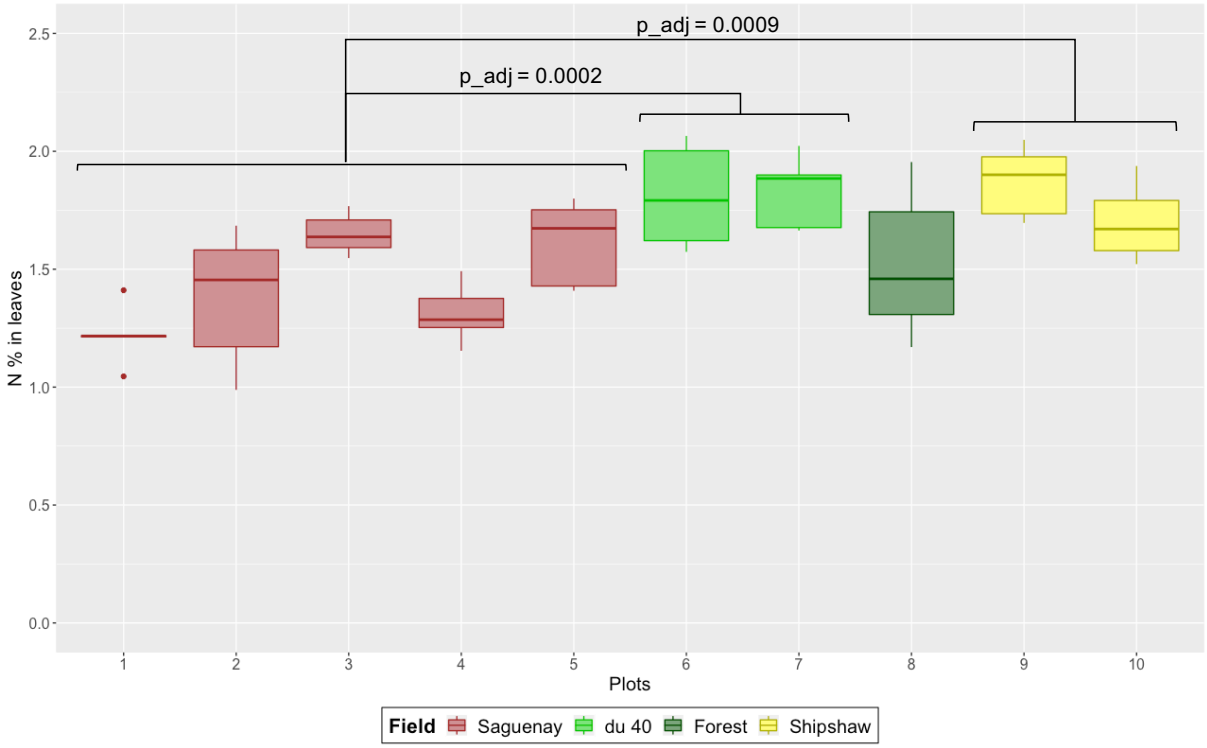


Figure 5. Dried leaf nitrogen concentration. Blueberry leaves were dried then crushed into a powder weighed at 7 mg (±0.2mg). The concentration of nitrogen was determined using an Elementar vario MICRO cube analyzer. P-values were obtained with a post-hoc Tukey test and were adjusted for multiple comparisons.

Bacterial ASVs matching to nitrogen fixing taxa are positively correlated to leaf nitrogen content.

After determining the leaf nitrogen content (LNC) among the fields, we studied the bacterial and fungal ASVs using Spearman correlation and analyzed the relationship they have with leaf nitrogen content (LNC) with the help of linear regression. Of the 8700 bacterial ASVs studied, we found 624 bacterial ASVs that have a significant relationship to LNC (linear regression, p_value < 0.05), with 411 that were positively correlated (Spearman correlation) (Table S9). Similar to our previous analysis, the Rhizobiales represented the most prevalent order identified within the 411 ASVs, with 68 ASVs, the majority of which (65) were positively correlated to LNC. Looking at the most abundant of those 624 bacterial ASVs, 19 had a relative abundance superior to 0.01%. Nine

of those ASVs have a positive correlation to LNC, with 5 belonging to the Rhizobiales. Three *Bradyrhizobium spp.* were identified, and all positively correlated to leaf nitrogen content; one of which, *Bradyrhizobium lupini / B. canariense*, was the most abundant ASV (3.26%). Furthermore, one of the Rhizobiales sequences was identified as *Roseirarcus fermentans* with a relative abundance of 0.80%.

Of the 8700 bacterial ASVs included in the analysis, 248 were detected as indicative species (p_value_FDR <0.05) and 124 of them were also statistically significant to LNC (linear regression, p_value < 0.05) (Table S10). Only 8 of these ASVs were negatively correlated to leaf nitrogen content, seven of which were indicative of the Saguenay field, which contains the least nitrogen (Fig. 5), while the remaining ASV is indicative of both the Saguenay and Shipshaw fields. For the indicative ASVs that were positively correlated to LNC, 79 were indicative of both du 40 and Shipshaw, while 33 and 4 were indicative of only du 40 and Shipshaw, respectively. Of the 124 ASVs that have a significant relationship to LNC (linear regression, p_value < 0.05), the Rhizobiales order represented the largest group with 20 ASVs, followed by the Acetobacterales and Acidobacterales orders both with 13 ASVs. As the Rhizobiales are known nitrogen fixers, we investigated further into these ASVs, all 20 of which were positively correlated to LNC. Four of those sequences had *Roseiarcus fermentans* as the best BLAST match. Additionally, 2 ASVs, identified as *Methylocella sp*. had an identification percentage superior to 99% (Table 1).

Table 1. Indicative Rhizobiales sequences correlated with LNC with a BLAST percent ID exceeding 97%.

Field	NCBI BLAST closest match	Percent ID	E value	Spearman correlation	Relative abundance
Du 40 and Shipshaw	*Roseiarcus fermentans*	99 %	0	0,41	0.013%
	Roseiarcus fermentans	98.51 %	0	0,53	0.042%
	Roseiarcus fermentans	97.26 %	0	0,60	0.010%
	Rhodoplanes roseus / piscinae	97 %	0	0,33	0.034%
	Methylovirgula ligni	99.5 %	0	0,43	0.036%
	Methylocella silvestris / tundrae / palustris	99 %	0	0,54	0.018%
	Devosia insulae	99.25 %	0	0,36	0.019%
	Blastochloris sulfoviridis / viridis	97.01 %	0	0,51	0.027%
Du 40	*Methylocella palustris*	99.5 %	0	0,52	0.022%
	Roseiarcus fermentans	98.01 %	0	0,49	0.019%

Fungal ASVs matching to ericoid mycorrhizal taxa are positively correlated to leaf nitrogen content.

From the 2817 fungal ASVs, we found 233 that have a significant relationship to LNC ((linear regression, p_value < 0.05), 185 of which were positively correlated to LNC (Table S11). The Helotiales represented the most prevalent order with 41 ASVs, the majority of which (35) were positively correlated to leaf nitrogen content, followed by Chaetothyriales and Valsariales both with 14 ASVs each (Table S11). Notably, UNITE wasn't able to assign orders to 61 of the 185 ASVs. Looking at the most abundant of the 233 ASVs, 26 had a relative abundance superior to 0.1%. Nineteen of which were positively correlated to LNC, with 4 ASVs belonging to the Helotiales order. The most abundant Helotiales ASV, representing 0.33% of the sequences included in this analysis, was identified as *Phialocephala fortinii* (Spearman correlation = 0.40). The three other Helotiales ASVs, like 13 of the 26 ASVs, did not have a BLAST percent ID superior to 97%. Four of these 13 ASVs were assigned to *Bambusaria bambusae* by UNITE but never gave a percent ID higher than 89% on BLAST. Regarding the ASVs in which we can be more confident concerning the identification, two sequences matched to *Exophiala abietophila*, yet their correlation to LNC is not congruent. Interestingly, the only fungal ASV in the core microbiome of the cultivated samples (forest samples excluded), identified as *Solicoccozyma terricola* (both by UNITE and BLAST), is among the 26 most abundant ASV and has a correlation of 0.54 to LNC.

Furthermore, we used an indicative species analysis to detect taxa that were preferentially abundant in certain fields or groups of fields. Of the 2817 fungal ASVs included in the analysis, 237 were detected as indicative species (p_value_FDR <0.05) and 97 of which have a significant relationship to LNC (linear regression, p_value < 0.05) (Table S12). Only 7 of these 97 ASVs were negatively correlated to leaf nitrogen content; five were indicative of the Saguenay field, while the two remaining are indicative of both the Saguenay and Shipshaw fields. For ASVs positively correlated to leaf nitrogen content, 71 are indicative of du 40, one of Shipshaw, and 18 of the combination of du 40 and Shipshaw fields. Of the 97 ASVs which have a significant relationship to LNC (linear regression, p_value < 0.05), the Helotiales order represented the largest group with 24 ASVs, followed by Chaetothyriales and Hypocreales orders with 10, and 9 ASVs, respectively (Table S12). Moreover, 16 ASVs did not have an order assigned by UNITE. We investigated more thoroughly the Helotiales order, as it contains most of the known ericoid mycorrhizal fungi that could therefore have an impact on nitrogen uptake. We found that all the 24 Helotiales ASVs were positively correlated with leaf nitrogen content. The identifications returned by UNITE and NCBI are not always consistent, nevertheless, known ericoid mycorrhizal fungi taxa were identified such as *Meliniomyces bicolor* and *Pezoloma ericae*, as well as *Oidiodendron* species (Table 2). Additionally, two ASVs were assigned to the dark-septate endophyte *Phialocephala fortinii* (Table 2).

Table 2. Indicative Helotiales sequences correlated to leaf nitrogen content. Percent ID and E value concern the NCBI BLAST closest match. The relative abundance was computed on the 2817 sequences included in the analysis.

Field	UNITE taxonomy	NCBI BLAST closest match	Percent ID	E value	Spearman correlation	Relative abundance
Du 40 and Shipshaw	*Lachnum virgineum*	*Chrysosporium filiforme*	92.28	3.00E-96	0.324	0.028%
	Mollisia NA	*Phialocephala scopiformis*	96.77	8.00E-147	0.583	0.108%
	NA	*Pezoloma ericae*	99.21	5.00E-129	0.383	0.032%
	Oidiodendron chlamydosporicum	*Oidiodendron chlamydosporicum*	99	6.00E-153	0.457	0.020%
	Phialocephala fortinii	*Phialocephala fortinii*	99.03	6.00E-158	0.447	0.093%

	Trimmatostroma NA	*Phialocephala scopiformis*	96.13	2.00E-143	0.673	0.060%
Du 40	*Cadophora orientoamericana*	*Molissia dextrinospora*	97.09	6.00E-148	0.440	0.027%
	Hyaloscypha NA	*Hyaloscypha bicolor*	93.36	6.00E-98	0.522	0.034%
	Lachnum pygmaeum	*Chrysosporium filiforme*	93.09	1.00E-99	0.352	0.003%
	Leptodontidium NA	*Hyphodiscus brevicollaris*	93.06	2.00E-143	0.350	0.003%
	Leptodontidium trabinellum	*Hyphodiscus brevicollaris*	93.06	2.00E-143	0.491	0.053%
	Leptodontidium trabinellum	*Hyphodiscus brachyconius*	93.06	6.00E-143	0.433	0.026%
	Meliniomyces bicolor	*Chloridium paucisporum*	97.64	3.00E-101	0.386	0.002%
	Meliniomyces bicolor	*Hyaloscypha bicolor*	96.12	2.00E-117	0.348	0.010%
	Meliniomyces bicolor	*Hyaloscypha bicolor*	96.51	5.00E-119	0.331	0.018%
	Meliniomyces NA	*Filosporella fistucella*	90.68	4.00E-120	0.364	0.006%
	Oidiodendron myxotrichoides	*Myxotrichum cancellatum*	96.84	2.00E-118	0.330	0.022%
	Oidiodendron NA	*Oidiodendron setiferum*	96.37	1.00E-140	0.323	0.007%
	Pezoloma ericae	*Chloridium paucisporum*	96.36	4.00E-100	0.334	0.038%

Discussion

Wild blueberry is a growing market and farmers are looking for production practices that can improve the yield and health status of their crop. The microbial communities associated to the wild blueberry root environment have not been thoroughly investigated although they could have an agronomical impact. We sampled wild blueberries from three different commercial production fields, and in order to i) characterize the bacterial and fungal communities of the wild blueberry rhizosphere; ii) to complement the results obtained by the phospholipid fatty acid profiles and sequencing; iii) to identify prospective taxa important for blueberry leaf nitrogen content. We sequenced portions of the bacterial 16SrRNA gene and fungal ITS region, and found Rhizobiales and Helotiales to be the most prevalent bacterial and fungal orders. Both Shannon-Weaver and Simpson alpha diversity indices were higher for bacteria than fungi and our beta diversity analysis

shows a community structure indicative of an age gradient. We complemented the sequencing results with a phospholipid fatty acid profile analysis which confirmed the results obtained. Finally, we investigated bacterial and fungal taxa that correlated to wild blueberry leaf nitrogen content and found positive correlations with both nitrogen-fixing bacteria and ericoid mycorrhizal fungi belonging to the Rhizobiales and Helotiales order, respectively.

Two nitrogen-fixing bacteria taxon among the top ten most abundant genera.

For our first objective, we sequenced the V3-V4 region of 16S rRNA gene in order to characterize the bacterial rhizosphere community. We found, *Acidobacteriales* and *Acidobacteriia Subgroup 2* (both belonging to the *Acidobacteria* phylum and *Acidobacteriia* class) to be among the most abundant orders representing 15.2% and 11.5% of the sequences. This is not surprising as blueberry plants grow on acidic soils with a typical pH range between 4.5 and 5.5 and these two orders contain acidophilic bacteria (Kielak et al., 2016). Surprisingly, the most abundant bacterial order is the *Rhizobiales* representing 21.8% of the sequences. In fact, two genera belonging to this order, *Roseiarcus* and *Bradyrhizobium*, are among the most abundant in the 16S rRNA gene dataset (Table S1) and are known to fix nitrogen (Kulichevskaya et al., 2014; Marcondes de Souza et al., 2014). Furthermore, one *Bradyrhizobium sp.* is part of the core microbiota, consequently, one could speculate that it could play an important role in nitrogen cycling with potential benefits for the wild blueberry plants.

The nitrogen requirements of wild blueberries are low, yet the addition of nitrogen (60 kg ha^{-1} of ammonium nitrate) during crop year has been shown to slightly increase the yields by 90 kg ha^{-1} (Penney and McRae, 2000). However, the plots they were working on were infested by weeds. When applying weed control, the nitrogen fertilization increased yields by 870 kg ha^{-1} when the fertilization was done during the crop year. This disadvantage in mineral nitrogen acquisition has also been investigated by Marty and collaborators who showed that due to its root system, wild blueberry is inefficient in acquiring inorganic nitrogen compared to poverty oat grass and sweet fern, two common weeds found in blueberry fields (Marty et al., 2019). Therefore, it may be

advantageous to promote and rely on an adequate microbiota for nitrogen acquisition rather than on mineral fertilization when in the presence of weeds.

In comparison to our results, Yurgel and collaborators found that the most abundant phylum for wild blueberry rhizosphere samples were *Acidobacteria*, from the DA052 and Acidobacteriia classes, and *Alphaproteobacteria* (which encompasses the *Rhizobiales* order) (Yurgel et al., 2017). On a broader scale, Timonen et al. (2017) focused on the bacterial diversity of the soil/mycospheres and roots of three Ericaceae and pine cultivated in microcosms made of Finnish boreal forest soil (Timonen et al., 2017). Their results indicate that across all their samples, *Alphaproteobacteria* sequences appeared to be most prevalent and that the order *Rhizobiales* comprised roughly half of those sequences, representing 17% of the sequences found overall. *Acidobacteria* sequences were also found in high proportions in all samples. These two studies show that there seems to be a similar trend in the prominent bacterial taxon encountered in soils adapted for Ericaceae.

Ericoid mycorrhizal fungi taxon among the top ten most abundant genera.

Similarly to what was done with bacteria, we sequence the ITS region to characterize the fungal community of the wild blueberry rhizosphere. The most abundant fungal order is Helotiales, which harbours several ericoid mycorrhizal fungal species. In fact, among the ten most abundant genera, two of them belong to this order: *Pezoloma* and *Oidiodendron*. At the species level, *Pezoloma* is only represented by *P. ericae* with a relative abundance of 1.773%. *Oidiodendron* is represented by eight species, *O. maius* being the most abundant (0.427 % in terms of relative abundance). Both of these are known to form ericoid mycorrhizal symbiosis (Leopold, 2016). Furthermore, the *Phialocephala* genus is also part of the ten most abundant genera. Both *P. fortinii* and *P. glacialis* are considered to be a dark septate endophyte yet both species colonized European blueberry (*Vaccinium myrtillus*) roots in a colonization pattern resembling ericoid mycorrhizal fungi (Lukešová *et al.*, 2015). These species had no significant effect on either root or shoot dry weight of *V. myrtillus*. However, it was shown that *P. fortinii* had the potential to

significantly increase the uptake of phosphorus for *Rhododendrons* cuttings (Vohník et al., 2005). Dark septate endophytes are not considered as mycorrhizal fungi as they do not have the intraradical structures specialized for exchange of nutrients with the host plants and have highly variable impact on the host plant (Ruotsalainen, 2018). Nevertheless, they may be useful for mobilizing nutrients in soils with high organic matter content, which is generally the case in blueberry fields (Ruotsalainen, 2018). When nutrients, like phosphorus and nitrogen, are trapped in organic compounds, they are unavailable to the plant. However, dark septate endophytes produce enzymes that can degrade these organic compounds, thus providing a new source of nutrients for the plant (Caldwell et al., 2000).

There is a striking difference in the structural variation when comparing the bacterial and fungal communities. The ten most abundant bacterial orders are roughly in the same proportions throughout the samples. This is not the case for the fungal dataset, as we observe large variations in the proportions of taxa even across replicates of a single plot. Bacterial communities are more sensitive than fungi to environmental changes such as drought (de Vries et al., 2018). The higher turnover in bacteria, versus fungi, could explain the observed difference in structural homogeneity, as the fungal community is not being replaced as quickly as the bacterial community. Sampling at multiple time points could help resolve this question.

No significant differences observed for alpha diversities but the beta diversity analysis shows an age gradient.

No significant difference was found between the plots for both the Simpson and Shannon-Weaver diversity indices both for the bacterial and the fungal dataset. The Shannon-Weaver and Simpson diversity indices are related to the Hill diversities of order 1 and 2, respectively. As the order increases, the Hill diversities are less sensitive to the rare species resulting in more robust estimation of the microbial diversity. Species richness was not included as it is very sensitive to rare species which makes it unreliable (Bent and Forney, 2008 ; Haegeman et al., 2013). Concerning the higher Shannon-Weaver index for fungal sequences in plot 7, it could be explained

by the presence of *Cornus canadensis*, an adventive plant which was an invasive weed in that plot. This plant may host additional fungal communities that are not generally found near other blueberry shrubs, explaining the increase in alpha diversity in plot 7. The difference between plot 7 and the rest of the plots is less obvious when looking at the Simpson index. As, the Shannon-Weaver index is more sensitive to rare taxa compared to the Simpson index, plot 7 must harbour rarer taxa than the rest of the plots. However, this explanation based on the presence of *C. canadensis* does not address the question of why we only observe a higher Shannon-Weaver index for the fungal dataset and not for the bacterial dataset.

The gradient of community structure that we observe (Fig. 3 AB) could be the result of an adaptation of the bacterial and fungal communities to the management practices. Unlike the highbush blueberry - *Vaccinium corymbosum* - which is planted, wild blueberry fields are established in areas where blueberries are pre-existing, usually spruce forests. Once the forest is cut down and the tree stumps removed, the blueberry-free soil portions are gradually filled by rhizome expansion from the blueberry shrubs and, to a lesser extent, seed germination (Jensen and Yarborough, 2004). Wild blueberry fields usually exceed 100 hectares and exhibit similar soil characteristics throughout, such as low pH and high organic matter. Therefore, we assume that differences in community structures would not be impacted by location in our study. Previous work has shown that domestication could alter the rhizospheric microbiota (Perez-Jaramillo et al., 2016). In the case of wild blueberries, as the name implies, domestication is not as severe as for other crops. There are no active selection of cultivars, and the plants remain in their native soil. The main differences are the agricultural practices (fertilization, treatments and pruning), the drastic decrease of plant diversity due to monoculture, and an increased access to sunlight. This explanation is only hypothetical, as the structural gradient observed between fields could also be due to chance. It would require to sample more blueberry fields belonging to different age groups to test this hypothesis. The fact that *Firmicutes* are more abundant in the oldest blueberry field could be a consequence of repeated nitrogen fertilization. Nitrogen addition to 28 soil samples collected across North America consistently increased the relative abundance of *Actinobacteria* and *Firmicutes* by an average of 11,8% and 2%, respectively, while reducing the relative

abundance of other phyla such as *Acidobacteria, Verrucomicrobia* or *Cyanobacteria* (Ramirez et al., 2012).

Phospholipid fatty acid profiles as a method to complete DNA sequencing.

After characterizing the bacterial and fungal communities in the Vaccinium rhizosphere, our second objective was to assess the results obtained by the phospholipid fatty acid profiles and sequencing. Phospholipid fatty acids profiles (PLFA) are less precise than DNA sequencing in terms of the diversity of distinguishable taxon but contrary to DNA sequencing, PLFA gives quantitative results which can serve to assess the abundance profiles obtained by sequencing. Furthermore, unlike DNA, which can be present in living or dead cells, phospholipids are only present in living soil microbes. Phospholipid fatty acids (PLFAs) are the main structural component of the phospholipid molecule and can serve as useful biomarkers to determine the living microbial types and abundance in the soil. The PLFA analyses showed a significantly higher biomass of Gram-negative bacteria than Gram positive. This is in accordance with the MiSeq sequencing data as the phyla *Proteobacteria* and *Acidobacteria* (both Gram-negative bacteria) represented 69% of the relative abundance. Interestingly, the samples that had a lower reads abundance (sample 17, 19 ,20 ,24, 48 and 49, Fig. S1 A), exhibited a PLFA concentration in the same magnitude compared to the rest of the samples that had a higher reads abundance. Therefore, the lower number of sequences for these samples could be due to a bias, perhaps during DNA extraction, as the same samples (except sample 20) have a low number of sequences in both 16S rRNA gene and ITS datasets. Furthermore, PLFA analyses also resulted in the non-detection of arbuscular mycorrhizal fungi. This is coherent with the fact that *Vaccinium* species are better known to form symbiosis with ericoid mycorrhizal fungi and not arbuscular mycorrhizal fungi (Smith and Read, 2008). This is also reflected in our ITS data, where only 180 sequences were assigned to Glomeromycota .

Beneficial bacterial and fungal species found to be positively correlated to leaf nitrogen content (LNC).

The final objective of our study was to identify prospective taxa important for blueberry leaf nitrogen content (LNC). The LNC measurements gave an average of 1.59 ± 0.28%, the Saguenay field had a significantly lower average (1.43 ± 0.24%) compared to the du 40 (1.82 ± 0.18%) and Shipshaw fields (1.80 ± 0.18%) (Fig. 5). Marty et al. (2019) used a similar procedure to measure the nitrogen concentration, but analyzed the integral aboveground biomass. The mean nitrogen concentration they found in the same species of wild blueberry was 1.15 ± 0.04% (Marty et al., 2019). Another study measured LNC in crop year which ranged from 1.42% to 1.75% depending on the treatment (fertilizer and/or pesticide) (Penney and McRae, 2000). The optimal LNC for the Saguenay-Lac-St-Jean region has been determined to be equal to 1.85% (Lafond, 2009). However, this concentration was estimated using leaves from vegetative plants, while we sampled plants that were in their crop year. An older study determined the satisfactory nitrogen content of leaf tissue to be 1.5 – 1.75% during crop year (Trevett, 1972).

As we observed significant differences in LNC between the Saguenay field and the two other fields, we searched for bacterial and fungal taxa that could be involved in the difference of LNC. Using Spearman correlations and indicative species analyses, we identified a subset of microbes and assessed their potential role in nitrogen uptake based on their taxonomy. We observed positive correlations (Spearman correlation) between the Rhizobiales bacterial order that had a significant relationship with LNC (linear regression, p_value>0.05). Among the 19 most abundant species (>0.01%) correlated to nitrogen, five of them belonged to the Rhizobiales, and all were positively correlated to LNC (Table S9). *Bradyrhizobium* species, especially *Bradyrhizobium lupini / B. canariense* are of particular interest: they are the most abundant and have a significant relationship to LNC (linear regression, p_value < 0.05) comprising 3.26% in terms of relative abundance, and both species are known nitrogen fixers (Hennecke et al., 1985; Chekireb et al., 2017).

Indicative species analysis also supports that Rhizobiales could positively impact blueberry leaf nitrogen content. All 20 of the indicative Rhizobiales species found in the indicative species analysis were positively correlated to LNC, four of which had a closest BLAST hit to *Roseiarcus fermentans* (Table 1). Interestingly, an ASV with a *Roseiarcus fermentans* BLAST hit was also among the 19 most abundant species with a relative abundance of 0.8%. The presence of this species in both the correlation and indicative species analyses could hint that it has an impact on leaf nitrogen content, which is plausible as this strain is a known nitrogen fixer (Kulichevskaya et al., 2014). Furthermore, among the indicative Rhizobiales, two ASVs had a BLAST match to *Methylocella* sp. (Table 1). The species mentioned in the results (*M. palustris, M. silvestris* and *M. tundrae*) are all methanotrophs isolated from acidic soils. Interestingly, this genus is able to fix nitrogen via an oxygen-sensitive nitrogenase (Dedysh et al., 2000; Dunfield et al., 2003; Dedysh et al., 2004). In addition, *Methylovirgula ligni*, also acidiphilic, contains fragments of the *nifH* gene and is capable of nitrogen fixation (Vorob'ev et al., 2009) and *Blastochloris* species have two sets of enzymes (Nif and Anf) for nitrogen fixation (Kyndt et al., 2020) (Table 1). We did not find information concerning the nitrogen-fixing capacity of *Rhodoplanes* sp. or *Devosia insulae.*

Among fungi, the Helotiales order is the most prevalent in both the LNC correlation and the indicative species analyses (Tables S11 and S12). Four of these Helotiales were found among the most abundant ASVs and were all positively correlated to LNC. One of these 4 Helotiales, identified with BLAST as *Phialocephala fortinii,* is a dark-septate endophyte which is often found in Ericaceae roots, but whose mycorrhizal potential remains uncertain, like previously discussed. Two ASVs assigned as *P. fortinii* are also present in the indicative species analysis and have a positive correlation to LNC (Table 2). These results suggest the positive impact this species of fungus has for *V. angustifolium* regarding nitrogen. Another ASV, identified as *Pseudogymnoascus roseus* has a *Geomyces* anamorph (explaining the dual identification from UNITE and NCBI) and was found to colonize *V. angustifolium* and *Gaultheria shallon* roots forming typical ericoid fungal structures in the root cell (Dalpé, 1989; Xiao and Berch, 1995) (Table 2). Additionally, *Pezoloma ericae, Meliniomyces bicolor* and *Oidiodendron* sp. were found to be indicative of the plots with higher LNC (Table 2). Both of *M.bicolor* and *P. ericae*, have been shown to translocate nitrogen to

their ericaceous host plant (Kerley and Read, 1995; Grelet et al., 2009) suggesting that the LNC variation observed could in part be due to these species. The *Oidiodendron* genus is known to have several species forming ericoid mycorrhizal structures, *O. chlamydosporicum* being one of them (Dalpé, 1991). However, no information was found regarding the mycorrhizal status of *O. myxotrichoides*.

Yurgel et al. (2018), focused on the link between the blueberry yield and the microbiota (bacteria and eukaryotes) of *V. angustifolium* (at the bulk soil, rhizosphere, and root level) (Yurgel et al., 2018). At the root and rhizosphere levels, they identified a bacterial hub taxon (microbial group significantly more connected within the network than other groups) belonging to the genus *Bradyrhizobium* and a fungal hub taxa composed of class Leotiomycetes which contains the Helotiales order. Despite not finding a relationship of these taxa with yield, the fact that these taxa were identified as hub taxa suggests their important role in the wild blueberry root and rhizosphere ecosystem. Our results tend to reinforce this as we find both *Bradyrhizobium* and Leotiomycetes to be abundant in our samples and they could be beneficial for their host regarding nitrogen uptake.

Overall, our results suggest that several bacteria belonging to the Rhizobiales order, (*Roseiarcus fermentans, Bradyrhizobium* sp., *Methylocella* sp. and *Blastochloris sp.)* as well as fungi species belonging to the Helotiales order (*Pezoloma ericae*, *Meliniomyces bicolor*, *Oidiodendron chlamydosporicum* and *Philocephala fortinii)* could potentially increase leaf nitrogen content in *Vaccinium angustifolium*. Although ericoid mycorrhizal fungi are already known to transfer nutrients to their host plants, contrasting results have been observed for the dark septate endophyte, *P. fortinii*. Our results suggest a beneficial outcome of the presence of this fungus for wild blueberry leaf nitrogen content. These results must be interpreted with caution as they are speculative. Further experimental work is required to test the possible biostimulant properties of these microbes by inoculating the isolated species on wild blueberry seedlings in controlled settings.

Author contribution

Simon Morvan: Data analyses, Writing of the manuscript.

Hacène Meglouli: PLFA extraction and analyses, Revision of the manuscript.

Anissa Lounès-Hadj Sahraoui: PLFA analyses, Revision of the manuscript.

Mohamed Hijri: Conceptualisation, Funding acquisition, Sampling, Supervision, Revision of the manuscript.

Acknowledgments

This study was supported by the Natural Sciences and Engineering Research Council (NSERC) Dicovery grant to MH (RGPIN-2018-04178).. We thank Gérard and Annick Doucet (Bleuetière Saguenay, formally Entreprises Gérard Doucet Ltée) for giving access to their blueberry fields and helping with the sampling. We thank Soon-Jae Lee and Mengxuan Kong for their assistance on sample preparation, DNA extraction and PCR amplification, Frédéric Laruelle for PLFA identification by GC-MS, Alexis Carteron, Xavier Guilbeault-Mayers, Nicholas Brereton, Stéphane Daigle and the QCBS for their help on bioinformatics and statistical analyses. We finally thank Andrew Blakney for English editing and commenting of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

Supplementary information

Supplementary Methods

Relative abundance plots

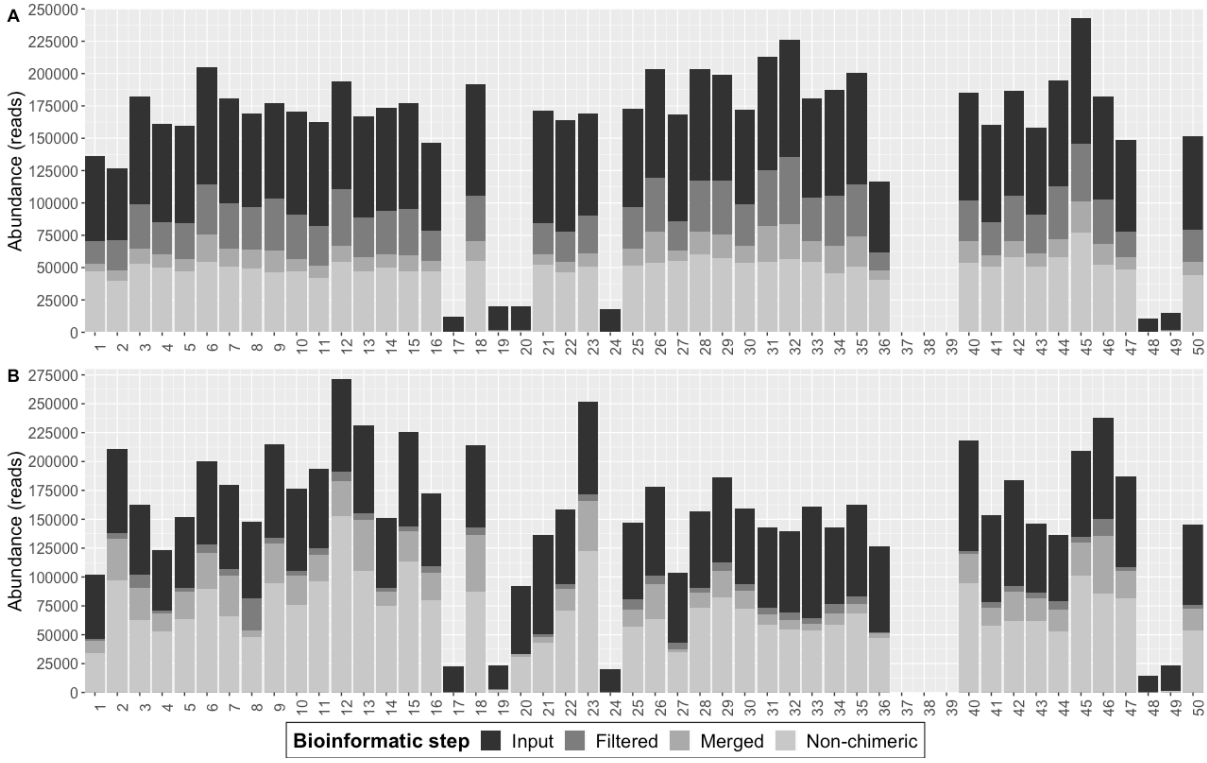
We decided to look at the taxonomic level of Order instead of the usually used Phylum to have for a more precise description of the communities. In order to have a visually intelligible graph, the different orders from both bacterial and fungal dataset were ranked in terms of relative

abundance, and only the ten most abundant orders were shown on the graph. The rest of the sequences not belonging to those ten orders were grouped under the appellation "Other". The sequences that had no taxonomic resolution at the Order level were classified as "Unknown".

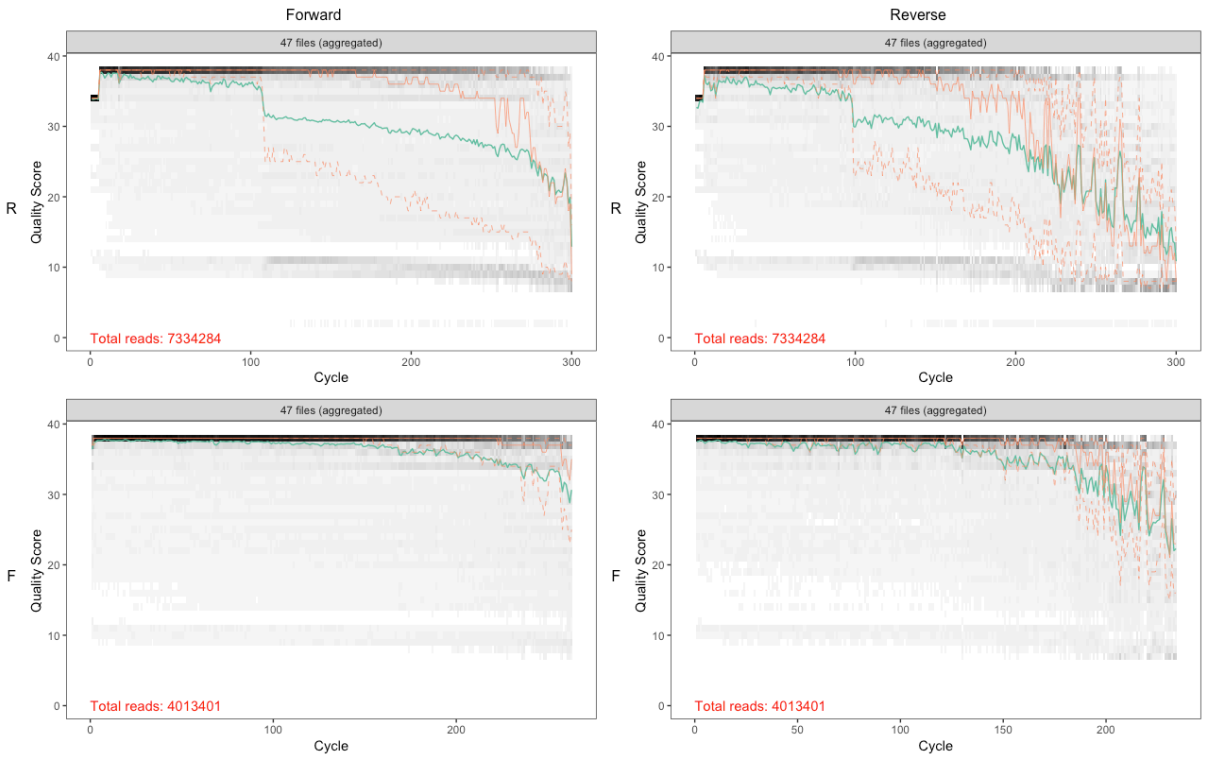
Beta diversity

First we performed a Hellinger transformation on the ASV abundance matrices using the *decostand()* function of the vegan package (Oksanen et al., 2019). A transformation is necessary as the Euclidean distance is inappropriate when using raw abundance datasets that contain absences which is the case for sequencing data (Legendre and Gallagher, 2001). It also corrects for the species abundance paradox (Legendre and Gallagher, 2001). We used the *metaMDS()* function with a Euclidean distance on the Hellinger transformed data to compute the NMDS coordinates. An ordination like an NMDS can plot two types of data, the objects and the variables, each having a set of coordinates. In our case, the objects are our samples and the variables are the ASVs. Instead of plotting thousands of ASVs on the graph, which would make it unreadable, we decided to regroup them by phyla. To do so, we replaced the ASV name by its corresponding phylum and computed the mean coordinates of all the ASVs within each phylum. This allows to see if the communities of groups of objects are particularly influenced by a phylum. For clarity, the phyla with a relative abundance inferior to 1% were not plotted.

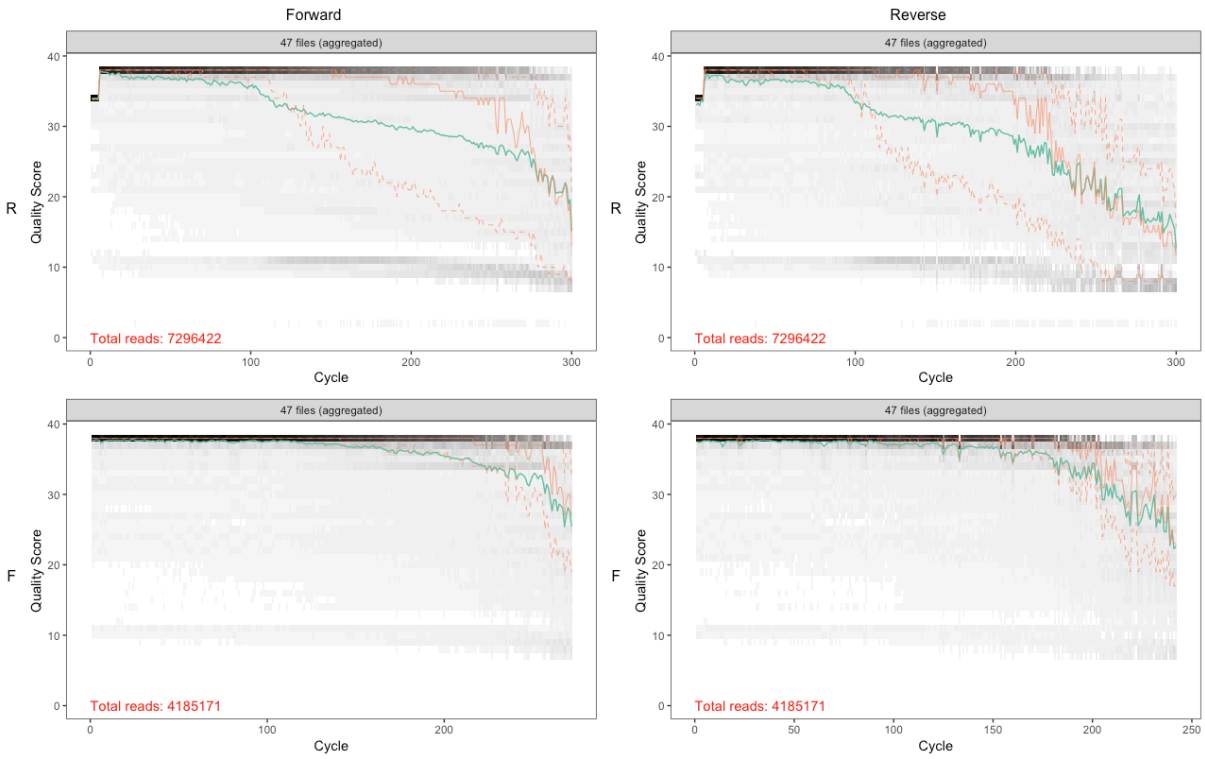
Supplementary Figures



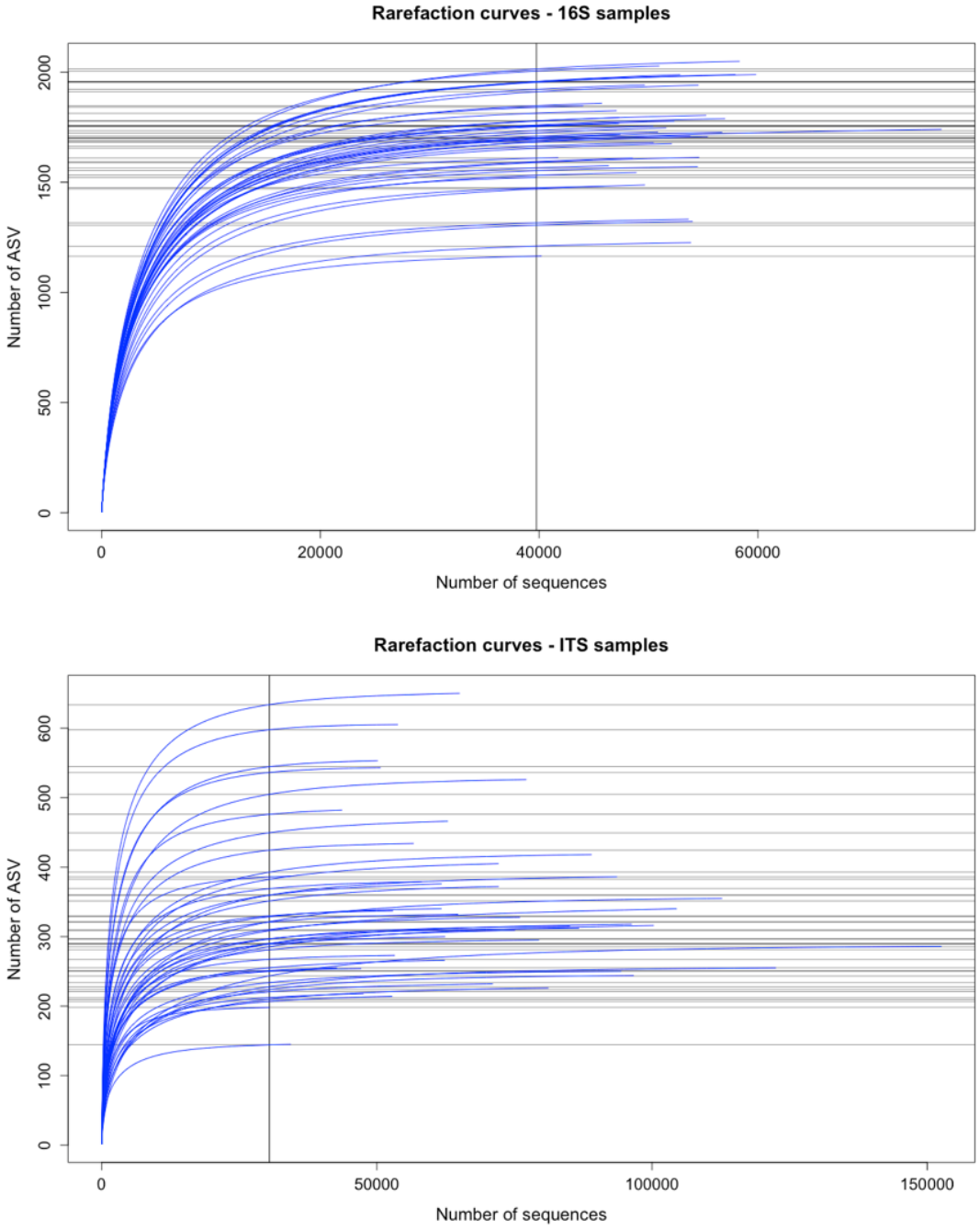
Supplementary Figure 1. Bioinformatic filtering steps. A. 16S rRNA gene amplicon data. B. ITS region amplicon data. Input corresponds to the initial number of reads per sample. Filtered corresponds to the number of reads retained after filtering (filterAndTrim step). Merged corresponds to the number of reads retained after merging (mergePairs step). Finally, non-chimeric corresponds to the number of reads retained after the removal of chimeras (removeBimeraDenovo step).



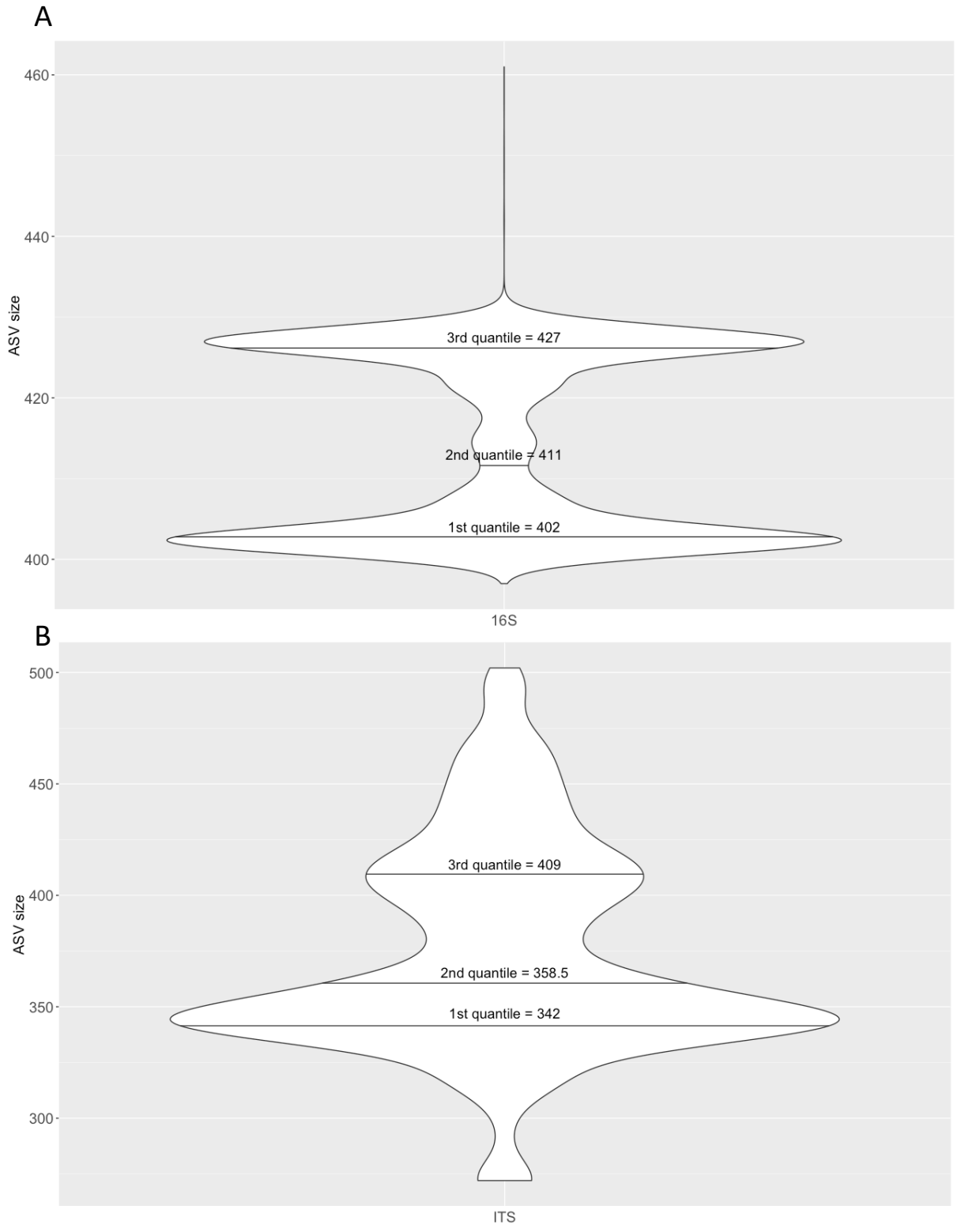
Supplementary Figure 2. Quality profiles for 16 rRNA sequences. R: raw sequences; F: filtered sequences. The green line represents the mean quality score, and the orange lines represent the quartiles. The grayscale is a heat map representing the frequency of each quality score at each base position.



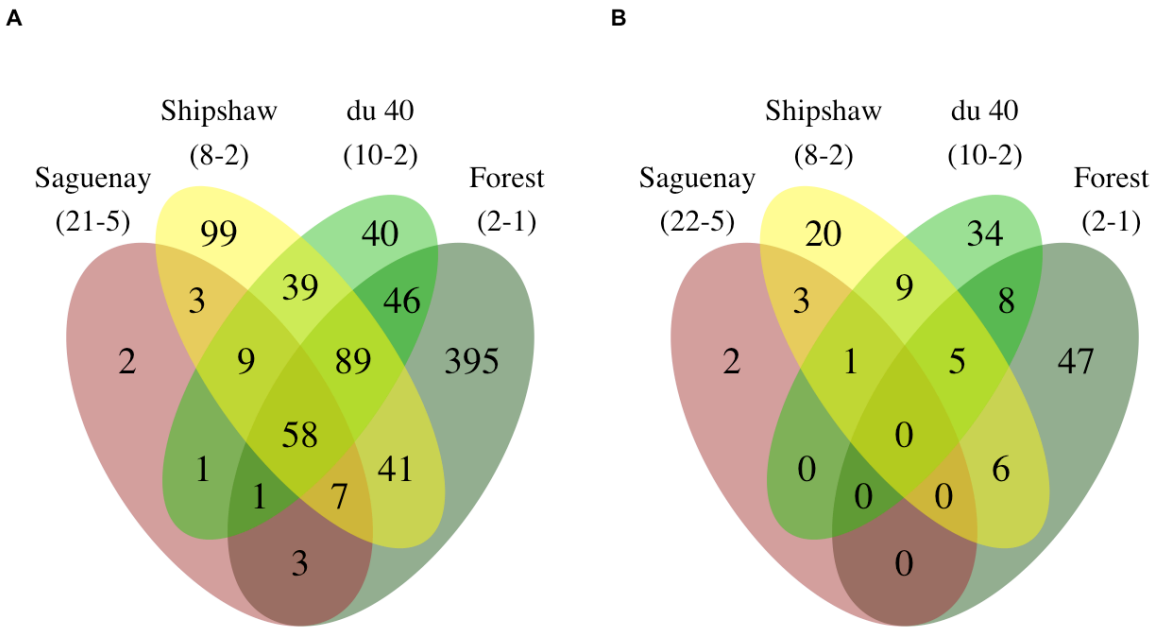
Supplementary Figure 3. Quality profiles for ITS region sequences. R: raw sequences; F: filtered sequences. The green line represents the mean quality score, and the orange lines represent the quartiles. The grayscale is a heat map representing the frequency of each quality score at each base position.



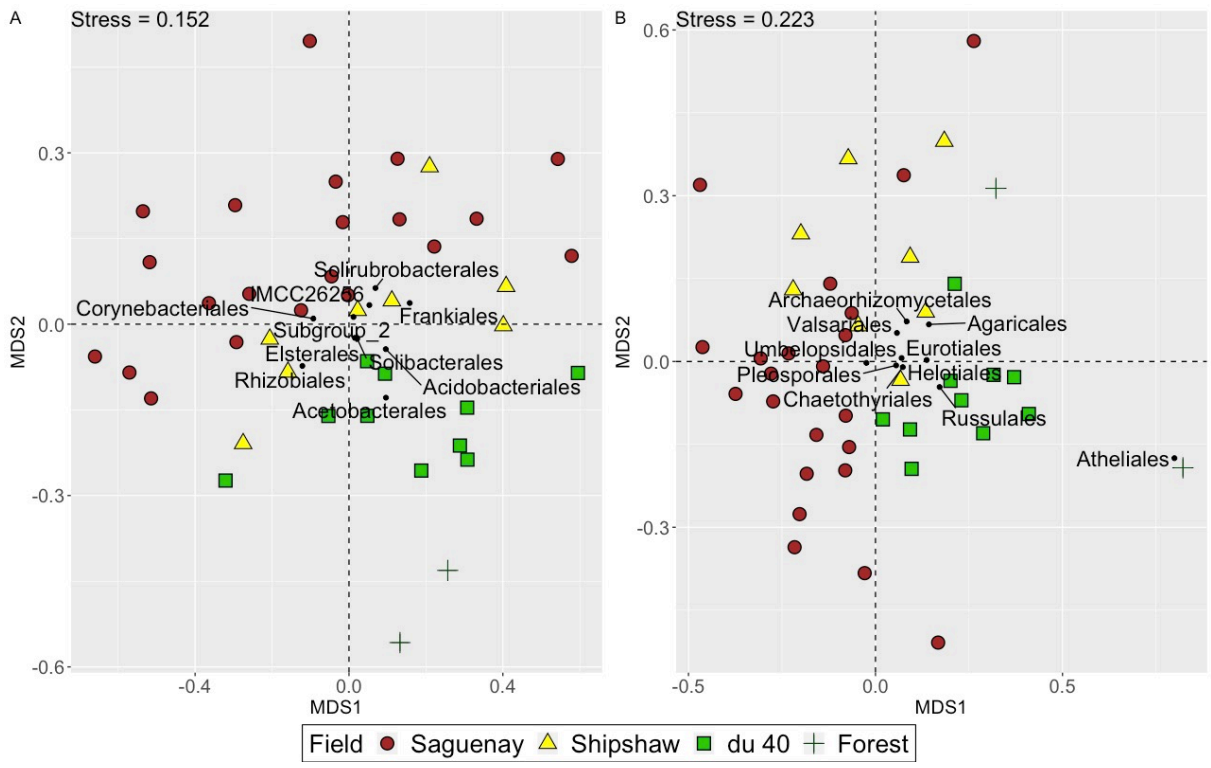
Supplementary Figure 4. Rarefaction curves for both the 16S rRNA gene and ITS datasets.


A
460
440
420
400
ASV size
3rd quantile = 427
2nd quantile = 411
1st quantile = 402
16S
B
500
450
400
350
300
ASV size
3rd quantile = 409
2nd quantile = 358.5
1st quantile = 342
ITS

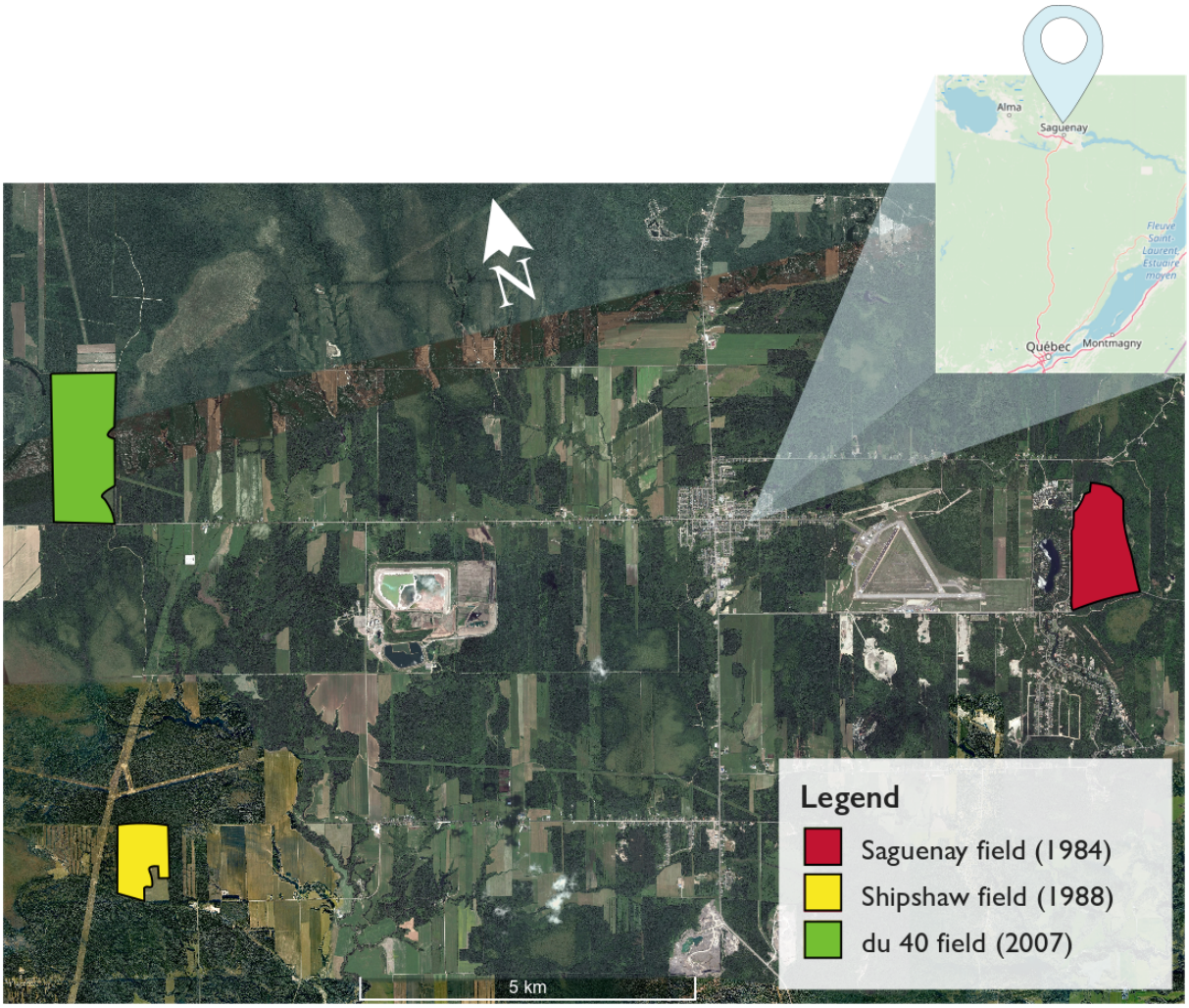
Supplementary Figure 5. ASV length. A. 16S rRNA gene amplicon data. B. ITS region amplicon data



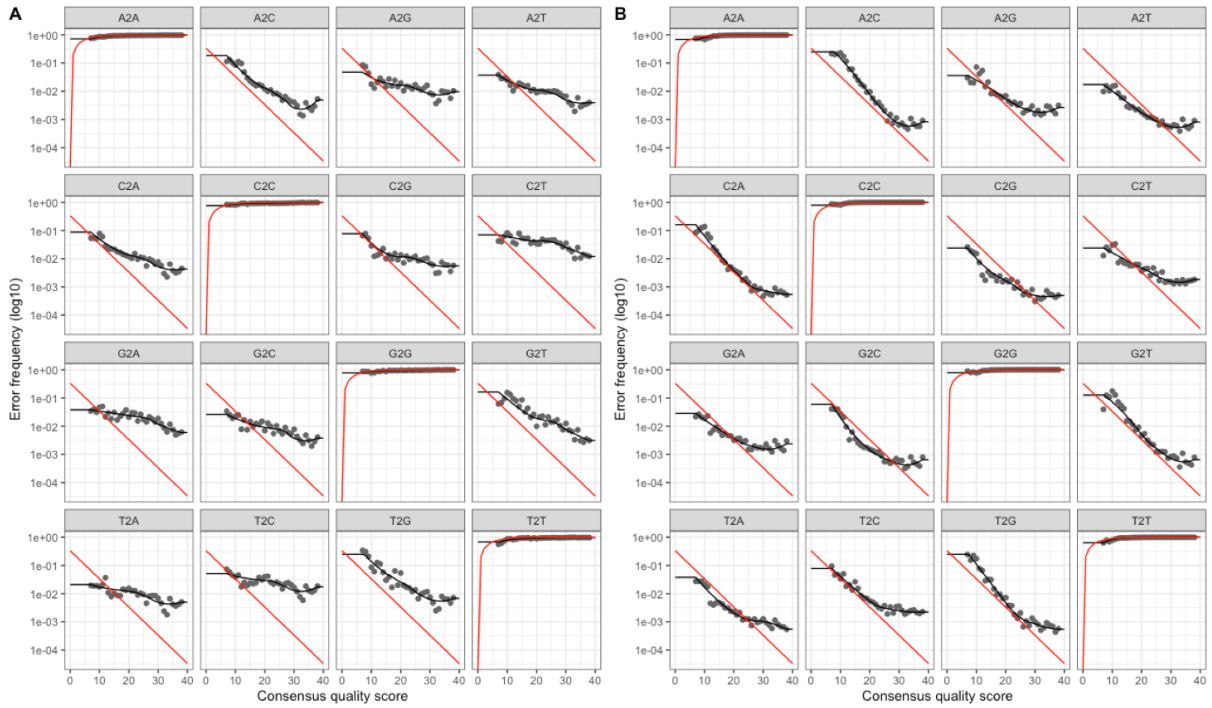
Supplementary Figure 6. Core microbiota Venn diagrams. A - 16S rRNA gene amplicon data, B - ITS region amplicon data. The first number under the field names represents the number of samples and the second represents the number of plots in these fields.



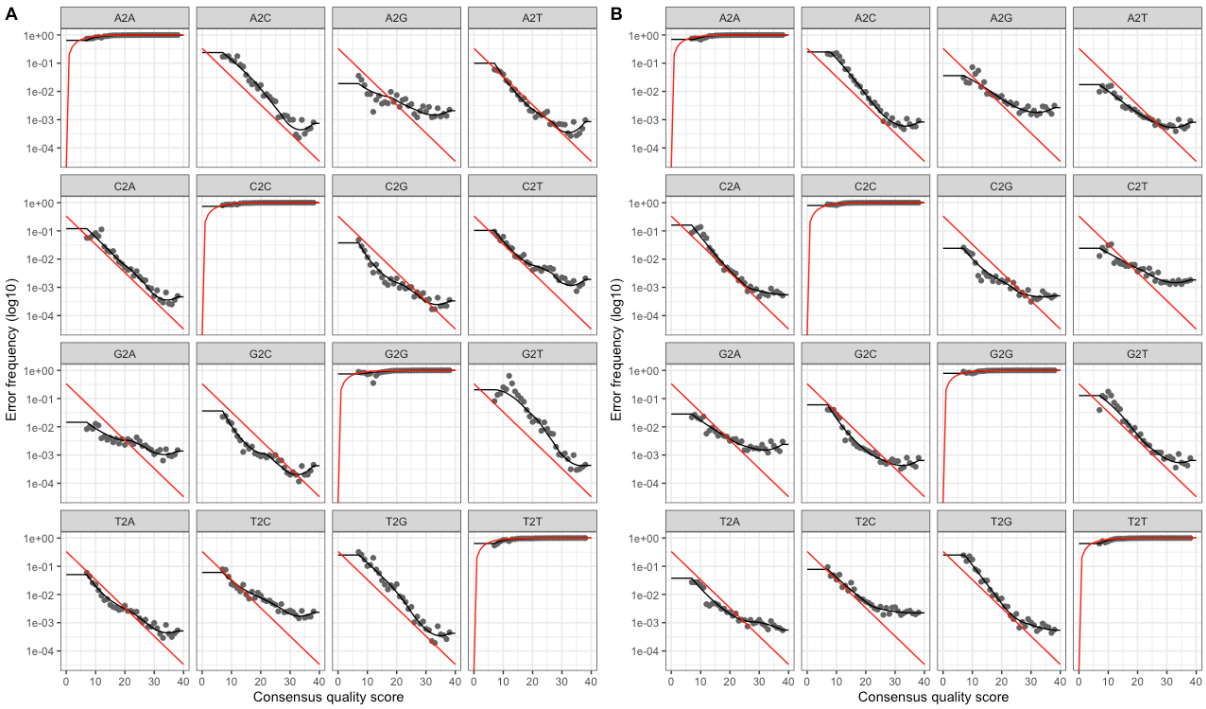
Supplementary Figure 7. Beta diversity at order level represented by a non-metric multidimensional scaling (NMDS) using Hellinger distance. A. 16S rRNA gene data, B. ITS data. Each coloured symbol represents a sample. ASVs were regrouped based on their order identity and their mean coordinates were computed and displayed on the graph using black dots and order name. For more clarity, only the ten most abundant order, reported on Fig. 1 were plotted.



Supplementary Figure 8. Map of the three blueberry fields and date of their establishment.



Supplementary Figure 9. Error model for the 16S rRNA gene amplicon data. A. Forward reads. B. Reverse reads



Supplementary Figure 10. Error model for the ITS region amplicon data. A. Forward reads. B. Reverse reads

Supplementary Tables

Par souci de clarté, les tableaux supplémentaires n'ont pas été inclus dans le document de thèse, mais sont accessibles sur la version en ligne de l'article.

Table S1 – 16S rRNA gene, 10 most abundant genera – species.	https://sfamjournals.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2F1462-2920.15151&file=emi15151-sup-0002-TablesS1-S7.xlsx
Table S2 – ITS region, 10 most abundant genera – species.	
Table S2 continued– ITS region, 10 most abundant genera – species.	
Table S3 – Core microbiota (bacterial and fungal).	
Table S4 – Relative abundance, total abundance and number of ASVs of the bacterial phyla identified using the SILVA database.	
Table S5 – Relative abundance, total abundance and number of ASVs of the fungal phyla identified using the UNITE database.	
Table S6 – Pairwise PERMANOVA results for the bacterial community on the Hellinger distances using 9999 permutations.	
Table S7 – Pairwise PERMANOVA results for the fungal community on the Hellinger distances using 9999 permutations.	
Table S8 – Phospholipid fatty acid data.	https://sfamjournals.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2F1462-2920.15151&file=emi15151-sup-0003-TableS8.xlsx
Table S9 – Bacteria – leaf nitrogen content Spearman correlations	https://sfamjournals.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2F1462-2920.15151&file=emi15151-sup-0004-TableS9.xlsx
Table S10 – Bacteria - Indicative species analysis	https://sfamjournals.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2F1462-2920.15151&file=emi15151-sup-0005-TableS10.xlsx
Table S11 – Fungi – leaf nitrogen content Spearman correlations	https://sfamjournals.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2F1462-2920.15151&file=emi15151-sup-0006-TableS11.xlsx
Table S12 – Fungi - Indicative species analysis	https://sfamjournals.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2F1462-2920.15151&file=emi15151-sup-0007-TableS12.xlsx
Table S13 – Bacterial ASV data.	https://sfamjournals.onlinelibrary.wiley.com/action/downloadSupplement?doi=10.1111%2F1462-2920.15151&file=emi15151-sup-0008-TablesS13-15.csv
Table S14 – Fungal ASV data.	
Table S15 – Elemental micro-analysis data.	

Chapitre 3 – Effet limité de la fauche thermique sur la culture de bleuets sauvages et de son microbiote associé à ses racines.

Brûleur au propane en action à la Bleuetière d'Enseignement et de Recherche, Normandin, Québec, © Maxime Paré

Limited effect of thermal pruning on wild blueberry crop and its root-associated microbiota

Simon Morvan[1*], Maxime C. Paré[2], Anne Schmitt[2], Jean Lafond[3], Mohamed Hijri[1,4*]

[1] Institut de Recherche en Biologie Végétale, Département de sciences biologiques, Université de Montréal, 4101 Sherbrooke Est, H1X 2B2, Montréal, QC, Canada

[2] Laboratoire sur les écosystèmes boréaux terrestres (EcoTer), Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Chicoutimi, QC, Canada

[3] Direction générale des sciences et de la technologie, Agriculture et Agroalimentaire Canada, Gouvernement du Canada, Normandin, QC, Canada,

[4] AgroBioSciences, Mohammed VI Polytechnic University, Lot 660, Hay Moulay Rachid, Ben Guerir 43150, Morocco

*** Correspondence:** simon.morvan@umontreal.ca **;** mohamed.hijri@umontreal.ca

Keywords: Thermal pruning, Wild blueberry, *Vaccinium angustifolium* Ait., Microbial community, Ericoid mycorrhiza, Amplicon Sequencing

Published in : *Frontiers in Plant Science*

DOI : https://doi.org/10.3389/fpls.2022.954935

NOTE: Le texte de l'article a été modifié par rapport à la version publiée afin de corriger des fautes d'orthographe.

Abstract

Thermal pruning was a common pruning method in the past but has progressively been replaced by mechanical pruning for economic reasons. Both practices are known to enhance and maintain high yields; however, thermal pruning was documented to have an additional sanitation effect by reducing weeds and fungal disease outbreaks. Nevertheless, there is no clear consensus on the optimal fire intensity required to observe these outcomes. Furthermore, fire is known to alter the soil microbiome as it impacts the soil organic layer and chemistry. Thus far, no study has investigated into the effect of thermal pruning intensity on the wild blueberry microbiome in agricultural settings. This project aimed to document the effects of four gradual thermal pruning intensities on the wild blueberry performance, weeds, diseases, as well as the rhizosphere fungal and bacterial communities. A field trial was conducted using a block design where agronomic variables were documented throughout the 2-year growing period. MiSeq amplicon sequencing was used to determine the diversity as well as the structure of the bacterial and fungal communities. Overall, yield, fruit ripeness, and several other agronomical variables were not significantly impacted by the burning treatments. Soil phosphorus was the only parameter with a significant albeit temporary change (1 month after thermal pruning) for soil chemistry. Our results also showed that bacterial and fungal communities did not significantly change between burning treatments. The fungal community was dominated by ericoid mycorrhizal fungi, while the bacterial community was mainly composed of Acidobacteriales, Isosphaerales, Frankiales, and Rhizobiales. However, burning at high intensities temporarily reduced Septoria leaf spot disease in the season following thermal pruning. According to our study, thermal pruning has a limited short-term influence on the wild blueberry ecosystem but may have a potential impact on pests (notably *Septoria* infection), which should be explored in future studies to determine the burning frequency necessary to control this disease.

Introduction

Blueberries are labelled as "functional food", a group of products that are supposed to have health benefits in addition to the nutrients they provide. In the case of blueberries, studies have shown that their consumption induces positive effects on cognitive, vascular and gluco-regulatory functions (Krikorian et al., 2010; Whyte et al., 2018; Kalt et al., 2020). Blueberries are among the fruits with the highest content of anthocyanins, pigments from the flavonoids family, which have antioxidant properties (Wu et al., 2006; Kalt et al., 2020). These health benefits may explain why the global demand for blueberries is increasing worldwide (Brazelton, 2013). To satisfy this increase in demand, producers are on constant lookout for agricultural practices that will increase their yield.

Wild blueberries (*Vaccinium angustifolium* Ait. and *V. myrtilloides* Michaux) are cultivated in the east of the United States (Maine) and Canada (Quebec and Maritimes provinces) (Yarborough, 2012), in fields where the plants are native, generally in boreal forests that are cut down or in abandoned farm fields (Yarborough, 2012). Another particularity of this crop is its production cycle as producers have found that pruning wild blueberry after fruit harvesting maintains a higher yield than if the plants were left unmanaged. Following harvest, wild blueberry stems are cut just above ground level. During the following growing season (vegetative stage) new stems emerge from the rhizome, and form flower buds, but the plant needs another growing season to produce its fruits. An alternative to mechanical pruning is the use of fire, termed thermal pruning. This method is thought to date back from early settlers who observed Native Americans enhancing blueberry growth by intentionally setting fire to patches of forests containing native shrubs (Chapeskie, 2001; Theriault, 2006). Thermal pruning was adopted by blueberry producers before being gradually replaced by mechanical pruning due to its lower cost (Lambert, 1990). Despite being more expensive, thermal pruning has the capacity of reducing diseases and damage caused by insects (Black, 1963; Blatt et al., 1989), fungal attacks (Blatt et al., 1989; Lambert, 1990; Hildebrand et al., 2016), as well as reducing some weed species (Penney et al., 2008; Smagula et al., 2008; White and Boyd, 2017). This sanitation effect is not observed when using mechanical pruning, which only has a stimulating effect on fruit production (Drummond et al., 2009). Fungal infections such as Monilinia blight (mummy berry), leaf rust, botrytis blight, or *Septoria* and

Valdensinia leaf spot diseases can cause severe production losses. Therefore, producers apply fungicides in the infected fields to control and prevent disease propagation (Hildebrand et al., 2016). Thermal pruning could be an alternative to fungicides as it significantly reduces some of these diseases. However their control can require intense and repeated thermal pruning (Hildebrand et al., 2016). Regarding weeds, prior knowledge of the species present in a field is required to decide whether burning will be effective, as they have varying degrees of resistance. The first category of wild blueberry weeds, which rely on their seed bank to propagate or that have shallow perennating organs, consist of a variety of grasses such as *Danthonia spicata, Agrostis* spp., *Festuca filiformis* or moss such as *Lycopodium* spp., *Poytricum* spp. or *Pleuzorium schreberi*. These weeds are more sensitive to thermal pruning and decrease over time when this practice is employed (Penney et al., 2008). The second, more problematic category consists of weeds that have deep rhizomatous and perennating organs such as *Comptonia peregrina*, *Kalmia angustifolia*, or *Cornus canadensis*. These weeds tend not to be negatively impacted by thermal pruning as it is not intense enough to damage their root system (Penney et al., 2008). Some even report an increase of abundance in weeds, such as *Cornus canadensis*, after thermal pruning (Hoefs and Shay, 1981; Penney et al., 2008). Furthermore, White and Boyd have shown that seed viability of three common blueberry weeds (*Tragopogon pratensis*, *Apocynum androsaemifolium* and *Panicum capillaire*) is impacted by the temperature and the duration of the treatment, having consistent reduction in germination with temperatures above 200°C (White and Boyd, 2017). Consequently, even when burning can have an positive effect, the intensity of the fire needs to reach a certain threshold in order to witness results.

A detailed investigation on thermal pruning practices demonstrates that there is no consensus on the method to use. First, burning may be done using different kinds of fuels, usually oil (Black, 1963; Smith and Hilton, 1971; Ismail et al., 1981; Ismail and Hanson, 1982; Warman, 1987), propane (Hoefs and Shay, 1981) and/or straw (Black, 1963; Penney et al., 1997). Different fuels have distinct burning temperatures: wheat straw can burn between 200°C and 480°C (Wang et al., 2009) when propane can theoretically reach 1976°C (adiabatic flame temperature of propane in air (Lide, 2005)). Second, fuels can be applied in different quantities (ex: 21t/ha of straw in (Penney et al., 1997) versus 4.5t/ha in (Smith and Hilton, 1971)). Third, the season of thermal

pruning can vary, usually occurring either in the late fall (Smith and Hilton, 1971; Ismail and Hanson, 1982; Smagula et al., 2008) or early spring (Black, 1963; Hoefs and Shay, 1981; Ismail et al., 1981; Warman, 1987; Penney et al., 1997). All of these parameters have a direct effect on burning intensity. Consequently, without a clear consensus on the method to use, conclusions drawn from the impact of this practice can be contradictory. From a production standpoint, the increase of organic blueberry demand entails a narrower range of synthetic phytosanitary treatments. Thermal pruning for pest management could, therefore, be of particular interests for organic growers. Nevertheless, empirical data are lacking on the fire intensity necessary to observe improvements in blueberry production and to determine if the additional cost implied is worth the investment. Furthermore, depending on its intensity, fire can give rise to significant changes in an ecosystem. Consequently our study also aimed to verify that thermal pruning did not induce any undesirable changes in the wild blueberry habitat.

Like other members of the *Ericaceae* plant family, wild blueberries grow naturally in acidic soil, with a pH ranging between 4 and 6. These soils are characterized by a high organic matter content, and a low nutrient availability as most of the nutrients required by the plant are bound to organic molecules and are, therefore, not in a readily available source. To prosper in such environments, wild blueberries rely on their root micro-organisms for their growth and development, especially on ericoid mycorrhizal fungi (Cairney and Meharg, 2003; Mitchell and Gibson, 2006). Some argue that blueberries are highly dependent on their ericoid symbiotic partnership and could not efficiently grow without it (Cairney and Burke, 1998). Ericoid mycorrhizal fungi produce a panoply of enzymes capable of degrading various organic compounds (Kerley and Read, 1995; Cairney and Burke, 1998; Martino et al., 2018), thereby releasing unavailable nutrients in the soil. These nutrients are then transferred to the plant root, which, in turn, provides photosynthates to the fungi (Pearson and Read, 1973b). Bacteria associated with wild blueberries, or *Ericaceae* in general, are much less studied than fungi but a recent study has shown that some taxa identified in proximity to wild blueberry roots are known nitrogen fixers, which could also be beneficial for blueberries (Morvan et al., 2020). As agricultural practices can modify the soil microbiome, thermal pruning could disturb the wild blueberry beneficial microbial communities. Throughout the literature, the effect of fire on the soil and its microbial communities remains unclear and

variable among studies, with most of the research focusing on the effect of forest or prairie fires. Three general effects are usually observed. First, heat superior to 90°C can kill most micro-organisms, and fire can, therefore, significantly decrease the biomass of bacteria and fungi (Neary et al., 1999; Hart et al., 2005; Mataix-Solera et al., 2009). Second, if the fire is intense enough to cause significant organic matter combustion, the resulting ash deposit induces an immediate but brief increase in pH and nutrient concentration in soil (Mataix-Solera et al., 2009). These changes will generally favor bacteria over fungi, and bacterial abundance can even increase compared to unburned soil (Mataix-Solera et al., 2009). Finally, intense fires can alter the aboveground plant community, and this turnover will, in turn, impact the microbiomes in relation with these plants, a phenomenon known as plant-soil feedback (Bever, 1994; Dangi et al., 2010; van der Putten et al., 2013). Symbiotic micro-organisms could be particularly vulnerable to the loss of their plant host as their fitness depends on the symbiosis. However, the range of changes on microbes caused by fire is highly correlated to the intensity of the fire in question (Neary et al., 1999; Hart et al., 2005; Whitman et al., 2019). Compared to forest or prairie fires, thermal pruning in wild blueberry fields is situated on the low intensity side of the spectrum. Therefore, findings drawn from the numerous previous studies on prescribed fires may not apply to the thermal pruning on wild blueberries. More precisely, the changes observed on soil chemistry and microbial communities should be mitigated by the reduced soil temperature and depth of burn reached during thermal pruning. Burning has been used in other agricultural practices that can be more comparable to wild blueberry thermal pruning, such as post-harvest stubble burning in cotton, sugarcane, rice (Hardison, 1976), as well as wheat and corn (Acree et al., 2020). However comprehensive studies on the effect of these prescribed fires on soil microbial communities are rare. Acree *et al.* (2020) found a significant decrease in abundance of soil total bacteria, saprotrophic and arbuscular mycorrhizae fungi 6h after wheat stubble burning but a recovery was observed 7 days after the treatment. However, they did not measure any changes in soil microbial abundance for corn stubble burning (Acree et al., 2020). Another study measured the effect of burning in a wheat-lupin-wheat crop sequence on soil biological activities (Alvear et al., 2005). They found a significant increase of urease and dehydrogenase activities in burned plots and a decrease in acid phosphomonoesterase, and glucosidase activities. Microbial carbon and nitrogen

were significantly lower in the burned plots in the 0-200 mm soil layer, 5 months after the burning had occurred (Alvear et al., 2005). These studies, therefore, show that burning in agricultural settings can have effects on the abundance of the microbial communities as well as on several biological activities. However, each agricultural system is unique, and it is, therefore, difficult to extrapolate on the results obtained in the cited studies.

The aim of our study was to assess the effects of three burning intensities on weed coverage, disease incidence, soil chemistry and blueberry performance. Furthermore, we characterized the bacterial and fungal communities of the rhizospheric soil to evaluate the repercussions of burning intensity on the microbial community. Our hypotheses were: (1) an increase in pH and nutrients would be correlated with the burning intensity; (2) a reduction of weed coverage and diseases; and (3) that the microbial community would be altered in diversity and/or composition due to the burning intensities. To address these hypotheses, we established an experiment in a random block design in a blueberry research field where three burning intensity treatments and an unburned control were applied at the beginning of spring 2018 during the vegetative stage. Agronomic measurements were taken throughout this year and during the subsequent harvesting year. Soil samples for microbial characterization were collected during the harvesting year, at the end of summer 2019.

Material and Methods

Experimental design

The experiment took place at the Research and Teaching Blueberry Field (Bleuetière d'Enseignement et de Recherche) located in Normandin, Quebec (48°49'40.2"N ; 72°39'36.9"W) in the North temperate zone (mean temperature: 0.9°C, annual precipitation: 871 mm (Government of Canada, 2021). This blueberry field was established in 2005, and has been attributed for research purposes since 2016. A weather station installed in the field allows to monitor several parameters such as rainfall and soil temperature. The field has a history of herbicide use; however no treatments (fertilization, herbicides, pesticides) were applied during the experiment (2018-2019).

The configuration of the experiment followed a randomized complete block design with 16 2.5-m-by-6-m plots organized into four blocks separated by 1 m wide strips. Quadrats of 1 m^2 were established at the centre of the plots resulting in a distance of 2.5 m between the quadrats (Fig. S1 A, B). Following a mechanical pruning with a blueberry mower (model TB-1072, JR Tardif, Rivière-du-Loup, Canada), the plots were thermally pruned using a high-pressure propane burner towed by a tractor on the 15th of May 2018 (Fig. S1 C). The fire prevention authorities (SOPFEU) have very strict policies and allow these fire treatments only during a specific period in order to protect the nearby forests. The home-made liquid propane burner included four individual propane burners that were placed 10 cm above soil surface and which allowed to burn a 2.5-m length.

Thermal pruning was carried out at three different intensities, with an additional unburned control in each block. The intensity of the burn corresponds to the speed at which the tractor moved over each plot. For the lowest intensity, the speed was set at 1.5 $km.h^{-1}$, while the speed was reduced to 0.5 $km.h^{-1}$ and 0.1 $km.h^{-1}$ for the medium and high intensity respectively. Considering net heating value of propane of 47 $MJ.kg^{-1}$ (Linstrom and Mallard, 2001), this fuel consumption represented about 6 580 $MJ.ha^{-1}$ of heat for the lowest intensity (consumption of about 140 kg of propane per hectare at pressure of 15 psi and tractor speed of 1.5 $km.hr^{-1}$). At medium and high intensity, this translates to around 19 740 $MJ.ha^{-1}$ and 98 700 $MJ.ha^{-1}$, respectively. Similarly to what was found by Vincent and collaborators who used a similar machinery, soil temperatures at 1 cm depth increased by <10°C (Vincent et al., 2018) (Fig. S1 D). One final note to be mentioned is that, in our study, the lowest intensity is already higher than the intensity used by some blueberry producers who drive at 4.8 $km.h^{-1}$ (3 $miles.h^{-1}$), which is more than three times as fast.

Data acquisition

Weeds, disease and blueberry coverage and blueberry biomass

After the thermal pruning was applied, 1 m^2 quadrats were set up randomly inside each of the 16 plots. These quadrats were used to implement the point interception sampling method: a quick, low observation bias and non-destructive method as described in (Lévesque et al., 2018)). This

method is based on the use of a 1 m^2 stand onto which a 1 m long aluminum bar with ten 10 cm spaced holes is secured. The operator vertically slides the rod into the holes and records the identity of the plants the rod touches, as well as the number of times the rod impacts a plant before the rod touches the ground. Once the 10 measures are recorded, the bar slides 10 cm farther along the stand and the processed is repeated over for a total of 100 measurements per quadrat.

This method was used to measure soil, weeds, blueberry coverage over the pruning year (4 measurements), and one measurement at the end of the production year. The coverage is computed using the ratio of the number of intercepts for soil or any given plant over the total number of intercepts. The blueberry biomass was estimated using the blueberry coverage based on the relation found in (Lévesque et al., 2018). Blueberry diseases were also measured using this method by recording the rod-intercepted blueberry leaf and stems state.

During the experiment, we identified symptoms corresponding to *Septoria* leaf spot disease and measured its prevalence by computing the ratio of impacted blueberry plant structures intercepts over the total number of blueberry plant structures intercepts.

The weed species identified in the experiment *Cornus canadensis*, *Maianthemum canadense* and *Gaultheria procumbens*. Specimens of *Kalmia angustifolia*, *Apocynum androsaemifolium* and *Carex sp.* were also observed but not presented in the results as there were too few occurrences for statistical analysis.

Soil chemical properties

The organic layer thickness, as well as the soil humidity content were measured before and after the thermal pruning had occurred. For the organic layer, 10 pseudo-replicates per plot were sampled using a soil corer to a depth of 15 cm, which allowed to measure the organic layer thickness. For soil humidity content, ProCheck moisture sensors were placed between 0 and 4 cm depth and measures were replicated five times for each plot. Furthermore, soil chemistry was analyzed multiple times during the experiment: 1 and 4 months after burning, as well as an additional sampling during the production year on the 24th of September 2019 to see the effect of burning at a longer scale. Soil samples were extracted using a soil corer at a rate of three

pseudo-replicates per plot. The three pseudo-replicates were pooled into one sample once the organic and mineral layers were separated in the lab. Both layers were dried and sieved through a 2 mm mesh. pH was measured using distilled water at a rate of 1:2 (Hendershot et al., 2007). Phosphorus, potassium, calcium and magnesium were extracted using Mehlich 3 solution (Ziadi and Tran, 2007). Phosphorus was quantified using colorimetry (Murphy and Riley, 1962), potassium with flame emission spectrophotometry, while an atomic absorption spectrophotometer was used for calcium and magnesium (Perkin Elmer AAnalyst 300, Überlingen, Germany). Finally, carbon and nitrogen concentrations were quantified by dry combustion on a LECO instrument with sieved soil <0.2 mm.

Fruit yields

On the 16th of August 2019, we quantified the blueberry yield by harvesting the fruits from within a 50 cm by 50 cm quadrat in each 16 experimental plots. The number of fruits in 125 mL volume, as well as their ripeness based on visual assessment of fruit colour, were also measured for each quadrat.

Microbial community analyses

Sampling

For the microbial community analysis, samples were collected during the harvest year on the 15th and 16th of August 2019. The sampling consisted of two, 10 cm wide, clumps of organic soil per plot, containing blueberry rhizomes. The two pseudo-replicates per plot were handled as separate samples and processed individually onwards to evaluate the microbial homogeneity of the plot. The samples were placed into Ziploc bags and kept on ice in a cooler until they were placed at -20°C. Frozen samples were thawed, and the rhizomes were then manually extracted from the clump of soil and placed in separate Ziploc bags. We placed soil aggregates containing roots were placed into 50 mL Falcon tubes with pierced cap in order to freeze-dry the samples. The freeze-dried soil samples were then sieved through 1 mm mesh to remove coarse organic matter and pebbles. The resulting soil material, henceforth named rhizospheric soil, contained thin root fragments, as well as organic matter. The samples were then ground to homogenize the material to a fine powder (Fig. S2).

DNA extraction and PCR amplification

We weighed 0.25 g of homogenized rhizospheric soil from each sample for the DNA extraction using the QIAGEN PowerSoil kit (Qiagen, Toronto, ON), following the manufacturer's protocol with the following modification: for cell lysis, samples were placed in TissueLyser II (Qiagen, Toronto, ON) instead of a vortex for 14 cycles of 45 s each at speed 4. In addition to the soil samples extractions, we added a negative control / blank sample by replacing the 0.25 g of soil by 250 µL of autoclaved and filtered water. Two mock communities, fungal and bacterial, were also included to be sequenced and act as positive controls. The fungal mock community was designed by Matthew G. Bakker and contains 19 fungal taxa (Bakker, 2018). In our experiment, we used the "even community" containing an equal amount of the 18S rRNA gene. The bacterial mock community contained 20 species (Table S1) with equimolar counts (10^6 copies/µL) of 16S rRNA genes (BEI Resources, USA). DNA extracts were stored at -20°C until they could be sent to Genome Québec Innovation Center (Montréal, QC, Canada) for PCR amplification and amplicon sequencing.

For the bacterial community, we targeted the V3-V4 region of 16S rDNA with the primers 341(F) and 805(R), resulting in an expected amplicon size of 464 base-pair long (Mizrahi-Man et al., 2013). Genome Quebec uses four versions of each primer (staggered primers) in order to increase base diversity in the Miseq flow cell. For the fungal community, we selected the ITS3KYO(F) and ITS4(R) primers to target the ITS region located between the 5.8S and LSU region of the ribosomal RNA gene (Toju et al., 2012). The mean amplicon length obtained with this pair of primers was 327.2 base-pair but this region is known to have varying length (Toju et al., 2012). Both sets of primers were coupled to CS1 (forward primers) and CS2 (reverse primers) tags that allow for barcoding. A second PCR was used to add a unique barcode per sample, as well as i5 and i7 Illumina adapters, that bind to the flow cell. Details on the PCR protocols, primers and adapters sequences, as well as the thermocycler parameters, can be found in Supplementary Methods. Sequencing was conducted on an Illumina MiSeq using a paired-end 2-x-300 base-pair method (Illumina, San Diego, CA, USA).

Sequencing data processing

The sequences from the 16S and ITS MiSeq data were downloaded from Genome Québec's platform and checked using the MD5 checksum protocol. We inferred sequence variants from the sequence data using the DADA2 pipeline (Callahan et al., 2016a) in R (R Core Team, 2021). Contrary to the commonly used 97% OTU clustering pipelines, DADA2 provides single-nucleotide resolution of amplicons, which helps to identify chimeras and reduces the rate of false positives (Callahan et al., 2016a). Furthermore, the amplicon sequence variants (ASVs) obtained with DADA2 are not clustered together to a given threshold of similarity and are, therefore, comparable between independent studies contrary to 97% OTUs as the clustering depends on the dataset analyzed. We processed the mock communities on their own in order to avoid any influence of our samples on the inferred mock ASVs.

For the fungal dataset, as the ITS region can vary drastically in length, we followed the DADA2 ITS Pipeline workflow. The first step of the pipeline consists in using cutadapt (Martin, 2011) in order to remove the primers and their reverse complements, by indicating the primers' nucleotide sequences. Once the primers removed with cutadapt, we proceeded with the filtering step using maxEE(2,2) and minLen(50) which removes sequences that are shorter than 50 nucleotides long. Regarding the bacterial dataset, as staggered primers were used, we followed a similar pipeline as the one used for the fungal dataset relying on cutadapt to remove the different versions of the primers. For the filtering step, we set truncLen to (270,240) and maxEE (2,2) based on a visual assessment of the quality profiles. After the different filtering step both bacterial and fungal datasets followed the same pipeline that used the default settings except those mentioned hereafter. In the learning error rates step, we used randomize = TRUE; in the sample inference step, we used "pseudo" as a pooling method and, accordingly, the "pooled" method for the bimera removal step. To add a taxonomy assignment to our inferred ASVs, we used the implemented naïve Bayesian RDP classifier with the *assignTaxonomy()* function. For the bacterial dataset, we used the SILVA reference database and two UNITE databases for the ITS sequences. Based on the taxonomy obtained, we removed non-bacterial ASVs as well as ASVs that were annotated as chloroplasts and mitochondria in the 16S dataset. The UNITE fungal reference database resulted in every ASVs to be labelled as Fungi but many sequences did not have an

assigned Phylum. We compared this taxonomic assignment to a second one obtained by using UNITE's eukaryotic reference database. Most of the unknown phyla fungi were labelled as non-fungal when using the eukaryotic reference database and were therefore removed in the ITS dataset. We then proceeded to further refine our datasets by removing singletons and doubletons (ASVs with a total abundance of 1 or 2) as they may be sequencing artefacts. Details on sequence data processing and mock community analyses can be found in the Supplementary information.

Statistical analyses

All analyses were performed in R version 4.1.1 (R Core Team, 2021), and figures were generated using the ggPlot2 R package (Wickham, 2016).

Soil and agricultural data

To test the effect of burning intensity on soil and agricultural data, we used either linear mixed models or generalized linear mixed-effect model. For data with a unique sampling date (blueberry performance apart from biomass), we computed linear mixed models (LMM), with blocks as random effects, followed by ANOVA tests to detect statistical differences between burning intensities. We used the lmerTest R package (Kuznetsova et al., 2017), which implements a Type III Analysis of Variance using Satterthwaite's method. For data with multiple sampling dates (weeds, disease, or soil chemistry), we either used LMM or generalized linear mixed-effect model (GLMM) using the *glmer()* function of lme4 R package (Bates et al., 2015). The choice of models depended on data distribution, and appropriate transformation (arcsin, log or square root for LMM) or regression (Poisson for GLMM) were employed. Burning intensity, date and the interaction between the two were set to be the fixed effects, and both experimental blocks and plots were set as random effects, with plots nested in experimental blocks. For the GLMM, we used the *anova()* function of the car R package (Fox and Weisberg, 2019) to perform a Type 3 Wald chi-square test. When the tests showed a moderate evidence of an effect ($P < 0.05$) of burning intensity or the interaction of burning intensity on the variable of interest, we reiterated the models for each individual date and removed date for the fixed factors and plot from the

random factors and checked for significant differences between treatments using *post-hoc* Tukey tests.

Microbial data

To check if our sequencing depth captured most of the bacterial and fungal communities, we generated rarefaction curves using the *rarecurve()* function of the vegan R package (Oksanen et al., 2020) (Fig. S3). We used phyloseq (McMurdie and Holmes, 2013) to facilitate data handling and to generate alpha diversity and beta diversity plots. We used Metacoder (Foster et al., 2017) for a more thorough analysis of the taxonomy of each dataset by plotting the ASV number and the relative abundance per taxa up to the genus level using the *heat_tree()* function.

Phylogenetic trees

To take into account the phylogenetic distance between the inferred ASVs and use Unifrac distances, we built phylogenetic trees following the method used in (Callahan et al., 2016b). First, a sequence alignment was generated using *AlignSeqs()* from DECIPHER (Wright, 2016). We then used the phangorn package (Schliep, 2011) to compute a distance matrix under a JC69 substitution model using *dist.ml().* A neighbour-joining tree was then assembled onto which we fitted a generalized time reversible with Gamma rate variation (GTR + G + I) model using *optim.pml().* The resulting trees were then added to their respective phyloseq objects.

Pseudo-replicate similarity and beta diversity

To compare the microbial communities in the pseudo-replicates (two samples originating from the same plot), we relied on a beta diversity analysis using four different dissimilarity metrics. First, to take into account the compositional aspect of sequencing data, we used the Aitchison distance (Quinn et al., 2018). Second, we used the Hellinger distance as its traditionally used in ecology (Legendre and Gallagher, 2001) and finally, we used the unweighted and weighted Unifrac distance to account for the phylogenetic resemblance of the communities. Ordinations allowed to visualize the pseudo-replicates community resemblance using principal components or principal coordinates analyses (PCA or PCoA). The pseudo-replicates were merged together after this analysis by summing their sequence abundance using *merge_samples()* from phyloseq. We then proceeded to the same analyses to estimate the similarity between the microbial

communities originating from different burning treatments. To test the significance of the differences observed, we used vegan's *adonis2()* function with 999 permutations, taking into account the experimental blocks. The homogeneity of dispersion assumption was checked using *betadisper()* and *permutest()* both in the vegan R package (Oksanen et al., 2020).

Alpha diversity

The Simpson and Shannon-Weaver alpha diversity indices were computed using the *plot_richness()* function of phyloseq. Both alpha diversity analysis were determined with and without rarefaction using *rarefy_even_depth()* as sequence abundance can impact alpha diversity measures. Statistical differences between different burning intensities were checked using one-way ANOVA's in a similar fashion than for soil and agricultural data (see above).

Core microbiota

In order to detect the shared and unique ASVs of each thermal treatment, we used *ps_venn()* from the MicEco R package (Russel, 2021). It allowed us to plot both the ASV number and the relative abundance that they represent in the datasets.

Representative taxa

Metacoder was used as a finer approach than the beta diversity analysis, to detect significant differences in each taxonomic rank between the burning intensities (Foster et al., 2017). The *compare_groups()* function was used after quantifying the per-taxon relative abundance using *calc_taxon_abund().* This function computes a nonparametric Wilcoxon Rank Sum test to detect differences taxon abundance in different treatments. In order to take into account the multiple comparisons, we corrected the *P*-values of the Wilcoxon Rank Sum test with false discovery rate (FDR) correction using the *p.adjust*() function of the stats R package (R Core Team, 2021). After setting a threshold of 0.05 for the *P* value, we used the *heat_tree_matrix()* to plot statistically different taxa based on their abundance using the log2 ratio of median proportions.

Additionally, we used DESeq2 to identify differentially abundant ASVs between two groups of samples (Love et al., 2014). In our case, we used the entire datasets for the analysis but chose to

only present the comparison of the negative control to the highest burning intensity as the difference in treatment between the two conditions is maximized and so is the potential effect of burning. We agglomerated the ASVs at species level prior to the analysis (see indicative species analysis for more details). As sequencing data are known to be sparse, some ASVs can be absent in one of the conditions. DESeq2 calculates a log 2-fold ratio by considering that the absence of the ASV is due to non-detection and spikes the data to allow the computation of the log-2-fold ratio. However, it is impossible to know with certainty if the absence is biologically supported (in which case the log-2-fold ratio has no meaning) or if it is due to a lack of detection. Therefore, in the case where an ASV was absent from one condition, the computed log-2-fold ratio value was changed to "– INF" or "+ INF", depending on which condition the ASV was absent from. To increase our confidence in the taxa found in this analysis, we present only the significant taxa that were present in at least three of the four replicates of a condition. We also proceeded with an indicative species analysis which is presented in supplementary information.

Accession numbers

Raw sequences have been deposited in the GenBank SRA database under the accession No. PRJNA803472.

Results

Effects of burning intensities on blueberry's agronomic variables

Blueberry performance

Burning delays the vegetative recover in spring with a significantly reduced blueberry coverage for the three burning treatments compared to the control (Fig. 1A). As blueberry biomass is derived from the blueberry coverage determined using the point intercept method, we also observed a similar significant difference in blueberry biomass between the burning treatments and the control (Fig. 1B). However, this difference subsided quickly in the next acquisition date, and both blueberry coverage and biomass of the four treatments remained homogenous throughout the rest of the experiment (Fig. 1).

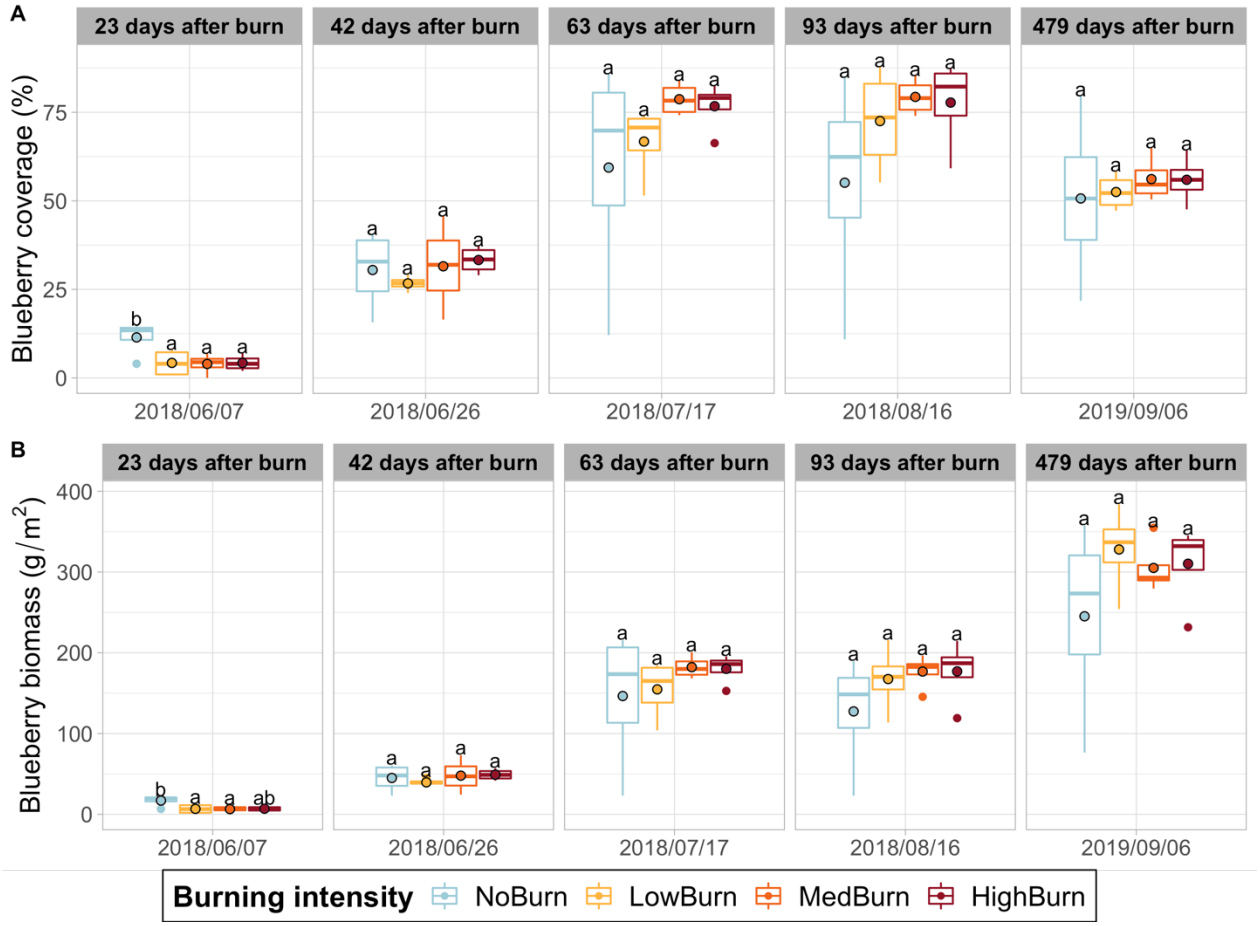


Figure 1. Blueberry coverage and biomass production over time. Mean value is indicated with a black circled dot. **(A)** Wild blueberry coverage. **(B)** Wild blueberry biomass production computed using the relation found by Levesque et al., 2017. Significant difference in each sampling date is indicated by letters according to post-hoc Tukey tests.

The high disparity between the replicates especially in the unburned control did not allow us to establish an effect of burning on shoot density (P = 0.2619), which varied between 322 per 1 m^2 for the control to 450 stems per 1 m^2 for the medium intensity treatment. Similarly, for shoot growth, a high variability in replicates of the unburned control prevented to find a significant effect of burning on shoot growth (P = 0.6412), although it tended to increase with burning with a mean length of 20.7 cm for unburned controls compared to 23.2, 23.5 and 23.7 cm at low-, medium-, and high-burning intensities, respectively.

The range of blueberry yields observed in a given treatment was too disparate to observe an effect of burning intensity (*P* = 0.9516). Blueberry yields were higher for the control than for the different burn treatments with 2.750 $T.ha^{-1}$ versus 2.100, 2.275, and 2.325 $T.ha^{-1}$ in order of increasing burning intensity.

Burning intensities did not increase the proportion of ripe fruits, as determined by the ANOVA test (*P* = 0.2112). Unburned control had 77.9% of the ripe fruits compared to 63.8% for the low-intensity burn treatment, while 55.5% and 55.9% of the fruits were ripe in the medium- and high-intensity treatments, respectively. Burning had no effect on the average weight of fresh ripe fruits (*P* = 0.1704) which was similar for the unburned control (0.283 g / 125 mL) and medium- and high-intensity treatments (0.288 g / 125 mL for both) but was lower for low-intensity burn (0.240 g / 125 mL).

Weeds and disease

Cornus canadensis was, by far, the most common and dominant weed species recorded, with a peak abundance in August 2018 (3 months after burning) when its coverage reached an average of 31.2% of the plots surface in the unburned controls. Although burning tends to decrease *C. canadensis* presence, the results were not statistically significant due to high variability (*P* = 0.3499). In low-intensity treatment, *C. canadensis* coverage was 15.2%, and 3.1% in medium, and 5.7% in high-intensity treatments. Date had a significant effect (*P* = 1.226e-08) but the interaction between burning and date was not significant (*P* = 0.5907) (Fig 2 A). Regarding the other two weed species found, burning did not have an effect on *Maianthemum canadense* (*P* = 0.8208) nor on *Gaultheria procumbens* (*P* = 0.9730) (Data not shown).

We did not test the effect of burning on the other weed species found as they were too sparse in our sampling. Instead, we summed up the coverage of all the weeds species recorded to test the effect of burning on total weed coverage. The ANOVA (Type 3 Wald Chisquare) test showed a strong evidence of an interaction between sampling date and burning intensity (*P* = 1.031e-06). The *post-hoc* Tukey tests identified statistical differences between the unburned control and the medium- and high-burning intensities for four out of the five sampling dates. The low-burning intensity also significantly reduced the total weed coverage compared to the unburned control in

the 3 last sampling dates but to a lesser extent than the more intense burning intensities (Fig 2 B).

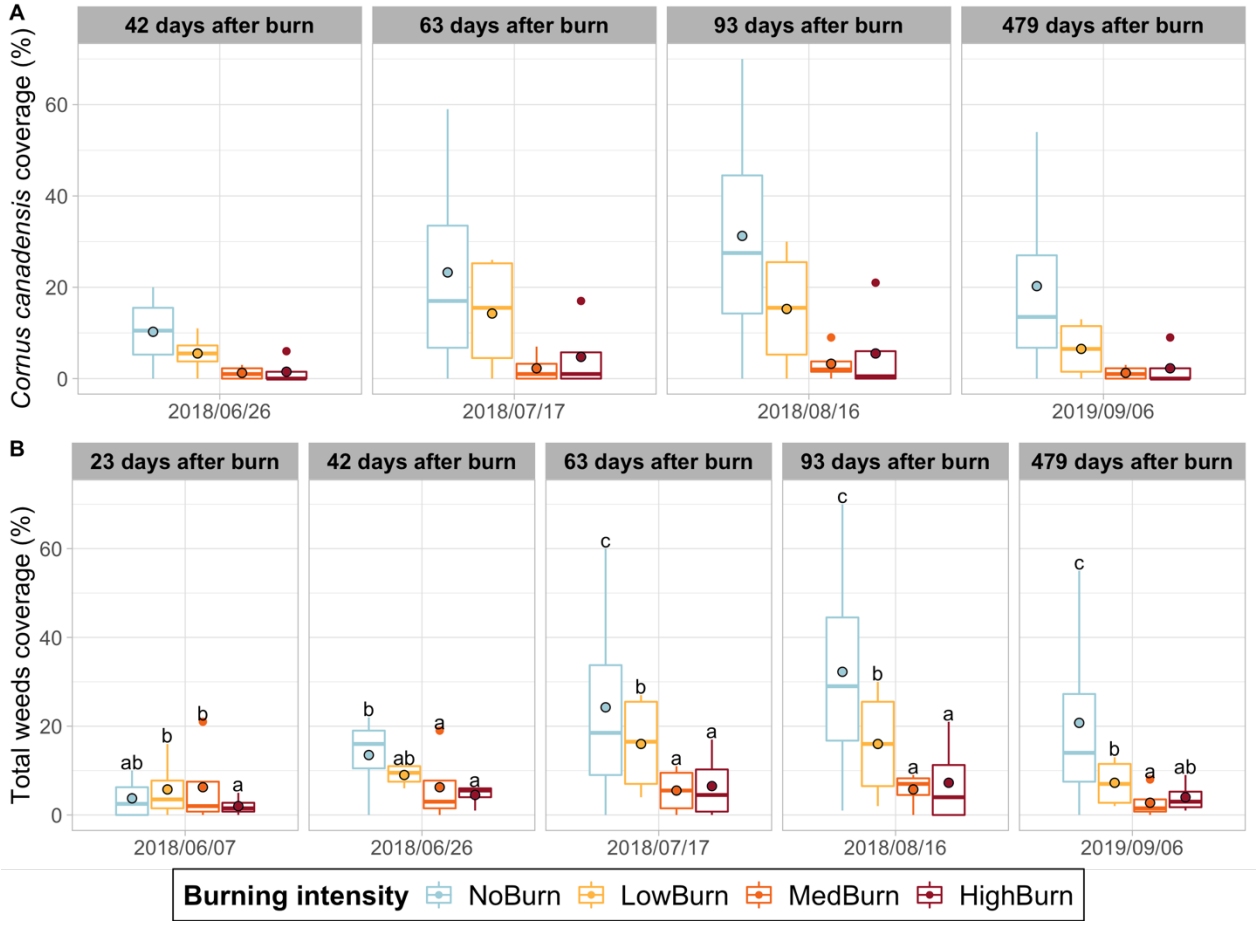


Figure 2. Weed coverage over time. Mean value is indicated with a black circled dot. (A) *Cornus canadensis* coverage. (B) Total weed coverage (pooled coverage of all the weed species observations). Significant difference in each sampling date is indicated by letters according to post-hoc Tukey tests.

The *Septoria* leaf spot was the only disease detected with recognized symptoms. We found a significant decrease (P = 0.0204) of around 23% of *Septoria* leaf spot disease between the unburned control (mean coverage of 81.4%) and the highest intensity burn treatment (mean coverage of 62.9%), 3 months after the burning treatment had occurred (Fig. 3). This difference did was not maintained as we did not find any significant difference between treatments at the subsequent date of sampling.

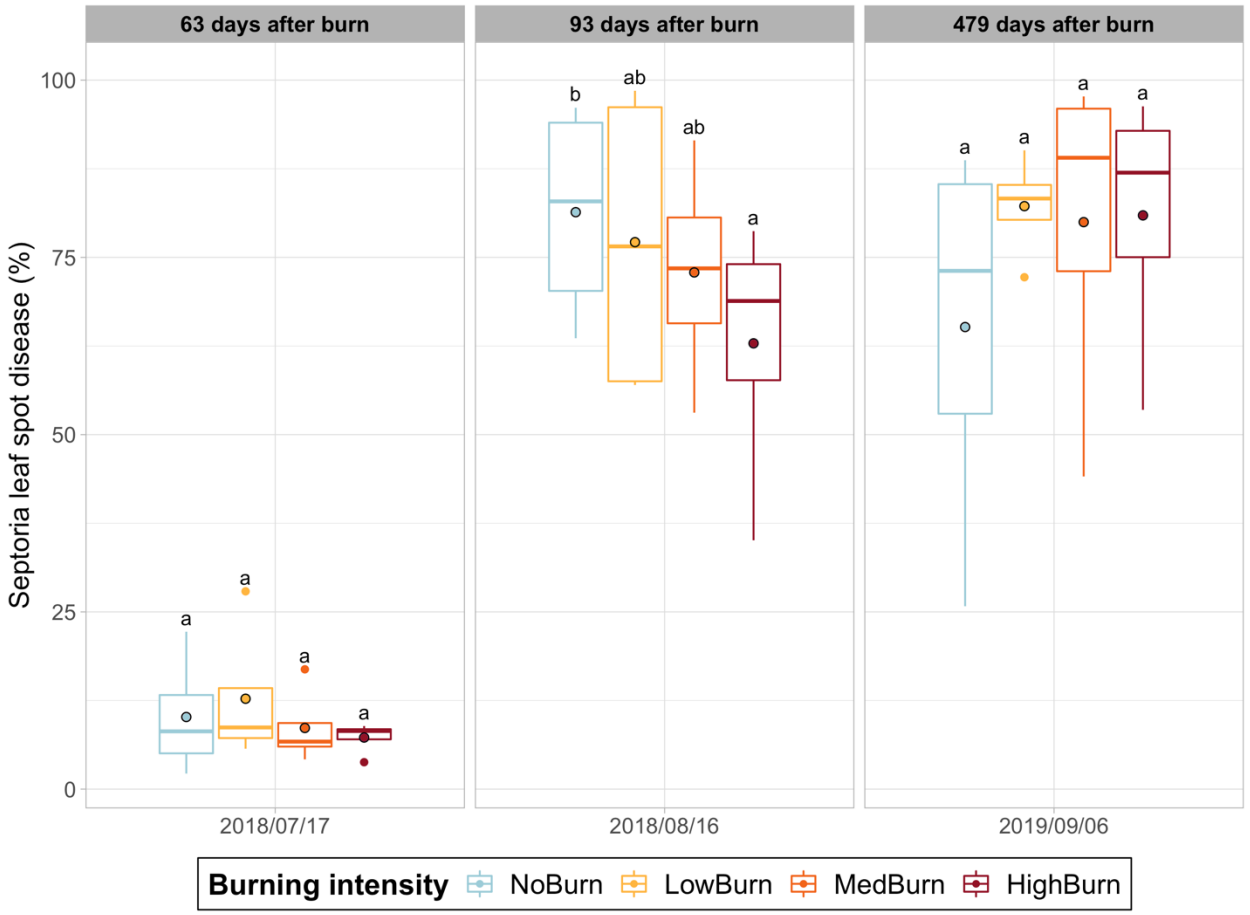


Figure 3. Septoria leaf spot disease incidence over time. Mean value is indicated with a black circled dot. Significant difference in each sampling date is indicated by letters according to post-hoc Tukey tests.

Soil chemistry

Burning had no significant effect on either organic layer thickness or its humidity content (Fig. S4). Similarly, we did not find any significant effect of burning on soil pH, which ranged from 3.9 to 4.8 in the organic layer and from 4.6 to 5.1 in the mineral layer, depending on the date of sampling (Fig. S5). The total carbon did not vary significantly due to burning either in the organic or in the mineral soil layer. As expected, the organic layer contained a higher carbon fraction ranging from 10% to 25% in the organic layer and from 0.85% to 1.1% in the mineral layer (Fig. S6). Regarding the total soil nitrogen, we did not observe any effect of burning for both soil layers. In the organic layer, the nitrogen content was highest in June 2018, and lowest in September 2018 (0.83% and 0.32%), while, in the mineral layer, it peaked at 0.048% in September 2018 and was the lowest in

September 2019, with an average of 0.02% (Fig. S7). For phosphorus (P) soil content in the organic layer (Fig. 4 A), we observed that burning significantly influenced P content between the highest burning intensity (59.9 mg.kg^{-1}) and the lowest intensity and unburned treatments (44.7 mg.kg^{-1} and 44.0 mg.kg^{-1} respectively) in June 2018 (P = 0.001785). A general trend can be seen as an increased intensity of burning tends to correlate with a higher P content in the organic layer. In the mineral layer, however, we did not observe any change in P content (ranging from 9.6 to 17.7 mg.kg^{-1}) due to burning intensity (Fig. 4 B).

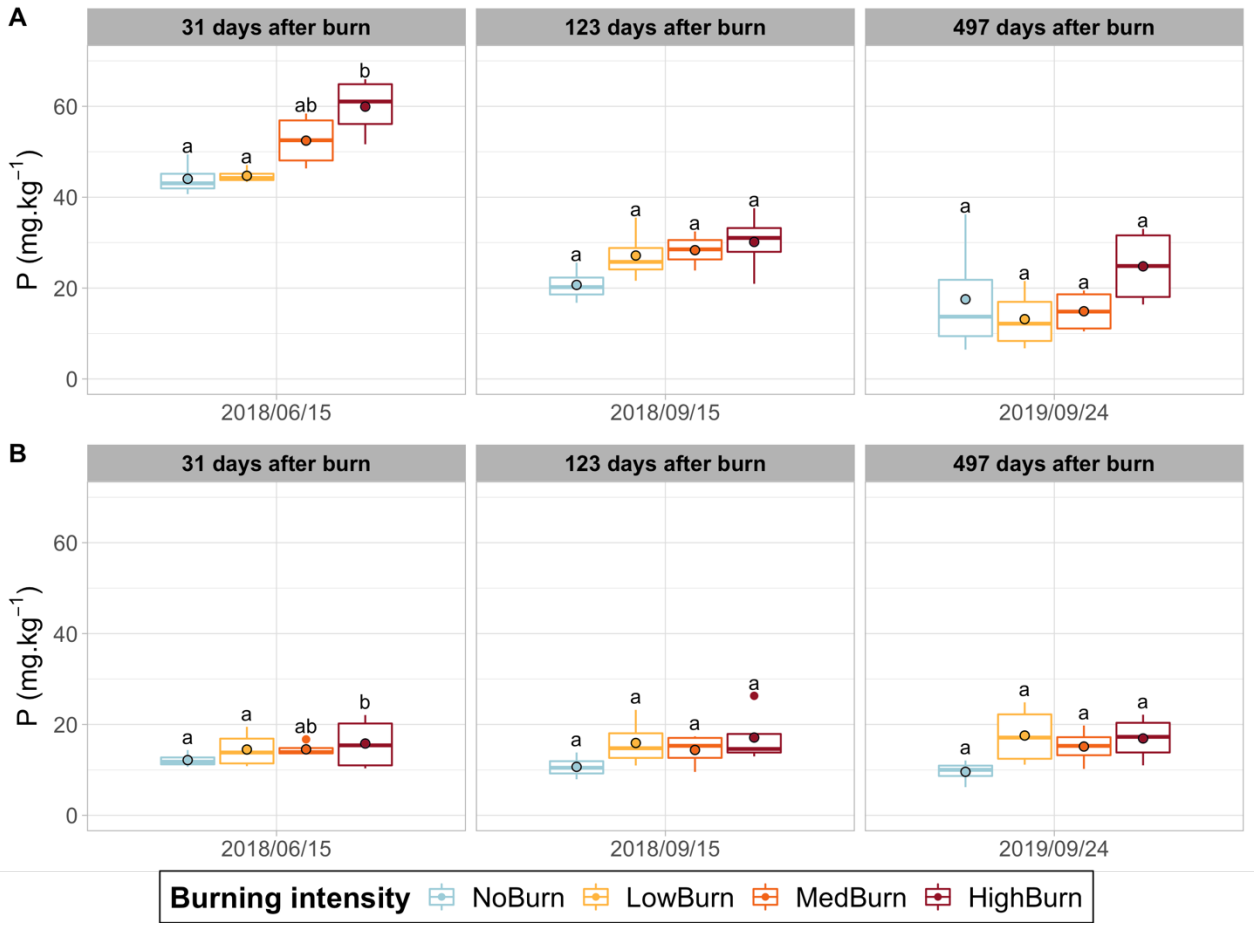


Figure 4. Phosphorus concentration in soil over time. Significant difference in each sampling date is indicated by letters according to post-hoc Tukey tests. Mean values are indicated with black circled dots. (A) Phosphorus concentration in the organic layer. (B) Phosphorus concentration in the mineral layer.

For potassium, magnesium and calcium, we obtain a significant difference between the low-intensity treatment and the control for the last sampling date (Fig. S8-S10). For magnesium and

calcium, we also obtain a significant difference in the mineral layer for this date (Fig. S9-10). However, we do not observe any significant differences for the two other dates nor for the more intense thermal intensities. In the organic layer, potassium ranges from 145 $mg.kg^{-1}$ to 394 $mg.kg^{-1}$, depending on the sampling date, while ranging from 4.8 $mg.kg^{-1}$ to 24.1 $mg.kg^{-1}$ in the mineral layer (Fig. S8). Magnesium concentrations were found to be lower in September 2019 and highest in June 2018 with 109.0 $mg.kg^{-1}$ and 319.5 $mg.kg^{-1}$ in the organic layer and 3.5 $mg.kg^{-1}$ and 7.7 $mg.kg^{-1}$ in the mineral layer (Fig. S9). Finally, calcium ranged from 820.7 $mg.kg^{-1}$ to 2525.3 $mg.kg^{-1}$ in the organic layer and from 10.1 $mg.kg^{-1}$ to 48.2 $mg.kg^{-1}$ in the mineral layer (Fig. S10).

Microbial communities

Taxonomic diversity of fungal and bacterial communities

From the ITS dataset, Fig. 5 shows a dominance of Ascomycota (P) – Helotiales (O) both in terms of relative abundance (46.8 %) and ASV numbers (244). Two Helotiales species particularly stand out: *Pezoloma ericae* and *Oidiodendron maius*, represented by 25 and 15 ASVs, respectively, and totalling 12.8% and 9.0% in terms of relative abundance (RA). Chaetothyriales is the second predominant order belonging to Ascomycota; most of its ASVs belonged to the Herpotrichiellaceae family (32 ASVs, 27% RA). In the Basidiomycota, the Agaricales order stands out with 61 ASVs but only 5.7% RA; *Clavaria sphagnicola* represented most of this relative abundance (3.8% RA dispatched into nine ASVs).

Fungal dataset

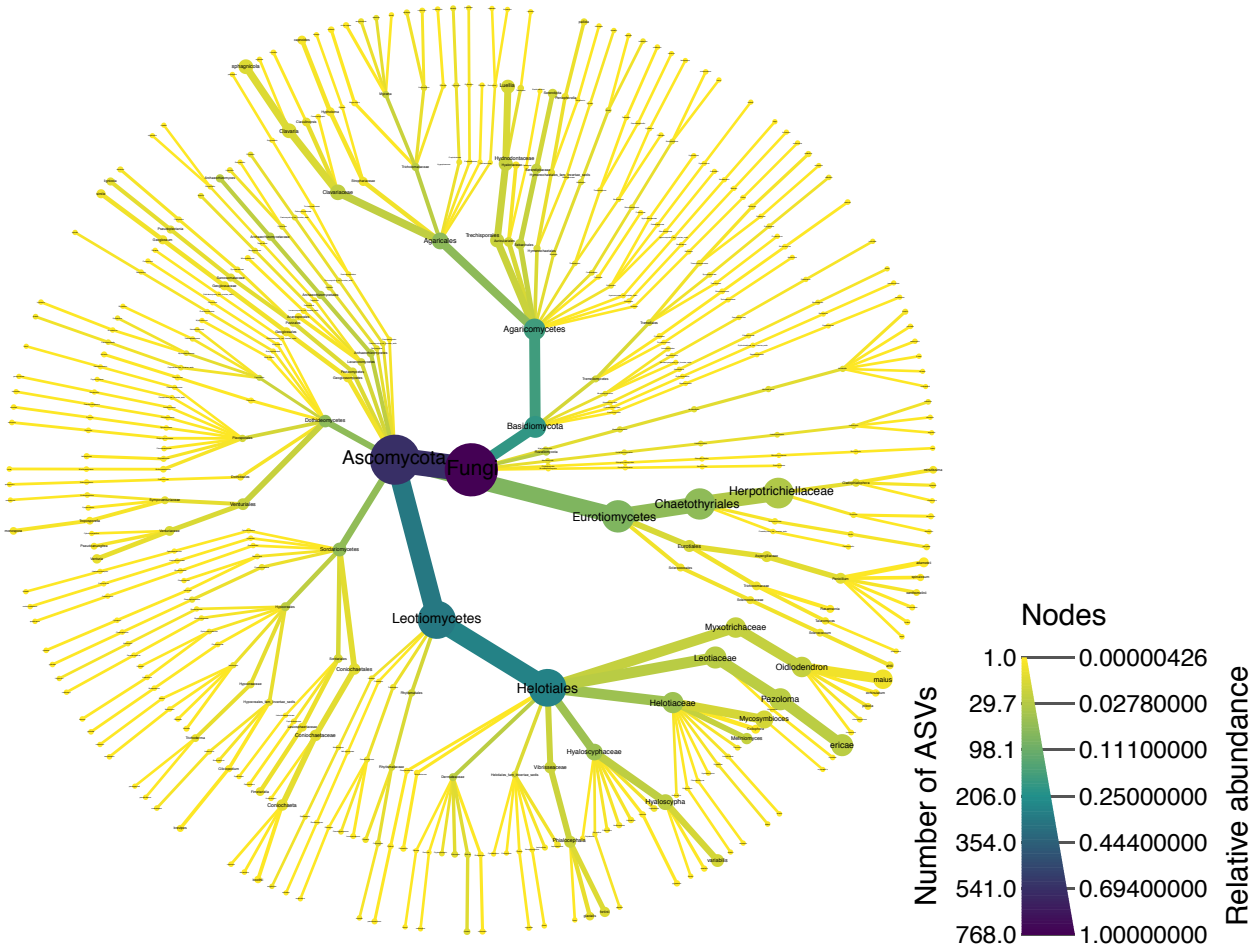


Figure 5. Taxonomy overview of the fungal community. This figure displays all of the fungal ASVs which were assigned to a taxonomic level (ASVs not assigned at a particular taxonomic level (NAs) are not shown). The colour of the edges and nodes indicates the number of ASVs found at a given taxonomic level, with darker colour indicating more ASVs and lighter colours indicating fewer ASVs. The size of the edges and nodes indicate the relative abundance of the ASVs assigned to a particular taxonomic level with wider edges/nodes indicating a high relative abundance and narrower edges/nodes indicating a low relative abundance.

For the bacterial dataset, the predominant taxa were Actinobacteriota (33.0% RA, 705 ASVs), Acidobacteriota (22.0% RA, 518 ASVs), Pseudomonadota (formally Proteobacteria) (18.9% RA, 768 ASVs) and Planctomycetota (18.4% RA, 798 ASVs) (Fig. 6). The Frankiales order was preponderant in the Actinobacteriota with 14.0% RA and 175 ASVs mainly assigned to the *Acidothermus* genus (12.9% RA, 134 ASVs). The Acidobacteriota was dominated by Acidobacteriales order (12.5% RA, 236 ASVs). In the Pseudomonadota phylum, the Rhizobiales order (10.4% RA, 150 ASVs) was preponderant which in turn was dominated by the *Roseiarcus* genus (2.5% RA, 31 ASVs). Finally, for Planctomycetota, the Isosphaerales order dominated by RA but not in terms of ASV richness (12.4% RA, 209 ASVs), as the Gemmatales order contained 304 ASVs but 2.7% in RA. The most abundant Isosphaerales belonged to the *Aquispharea* genus (10,1% RA, 81 ASVs).

Bacterial dataset

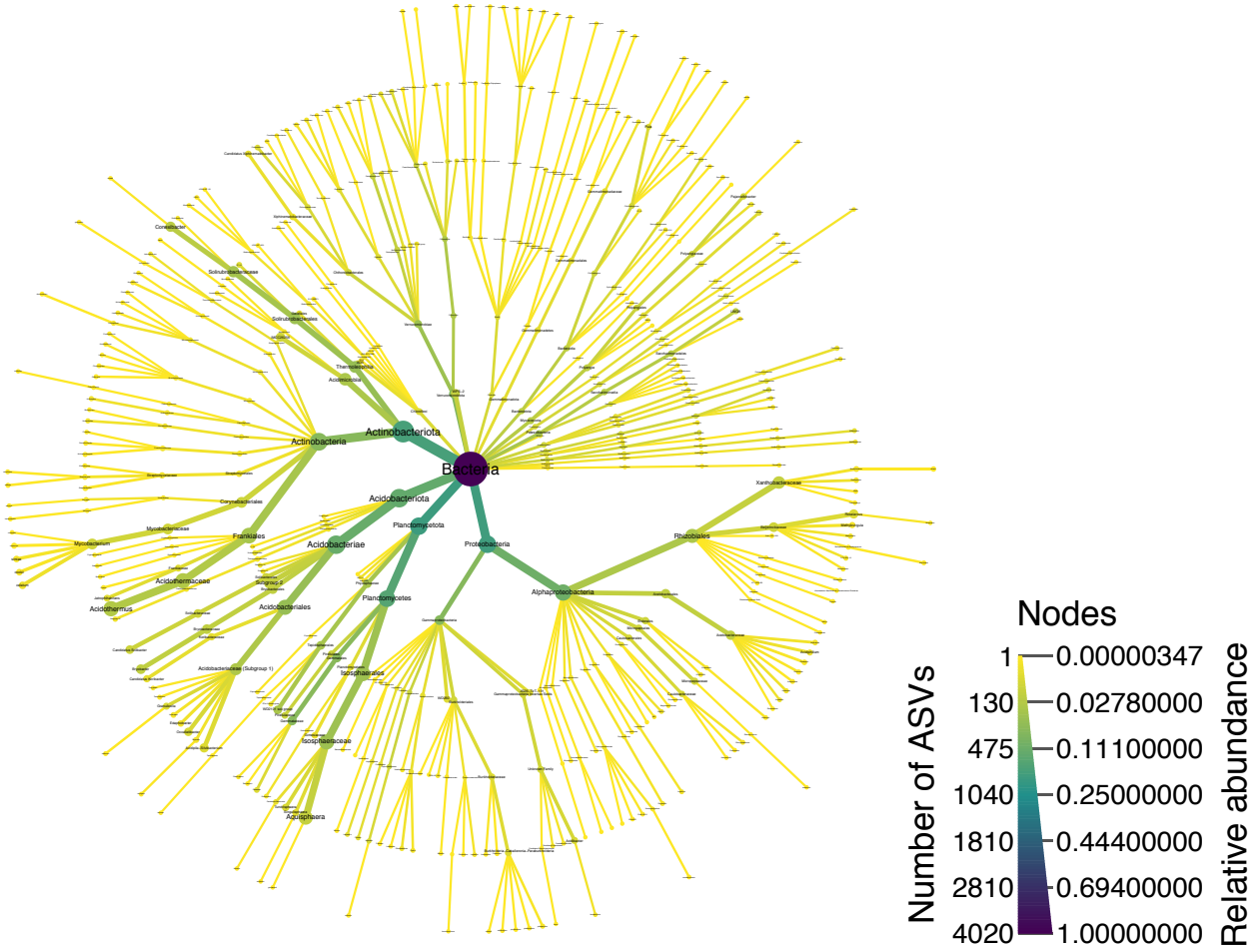


Figure 6. Taxonomy overview of the bacterial community. This figure displays all of the bacterial ASVs which were assigned to a taxonomic level (ASVs not assigned at a particular taxonomic level (NAs) are not shown). The colour of the edges and nodes indicates the number of ASVs found at a given taxonomic level, with darker colour indicating more ASVs and lighter colours indicating fewer ASVs. The size of the edges and nodes indicate the relative abundance of the ASVs assigned to a particular taxonomic level with wider edges/nodes indicating a high relative abundance and narrower edges/nodes indicating a low relative abundance.

Bacterial and fungal core microbiomes

Less than a third of the fungal ASVs (28.9%) are shared by all burning treatments but they represent most of the data with 81% RA. Each treatment had unique ASVs, ranging from 69 for the low-intensity burn to 144 for the unburned treatment; however, taken together, these unique ASVs represented a low RA in the fungal community (Fig S11 A). Among bacteria, 44.6% of the ASVs were found in all of the treatments and represented 95% of the RA. The unique ASVs in each treatment ranged from 294 to 522 but had a low abundance as their sum did not exceed 1% in terms of relative abundance (Fig. S11 B).

Pseudoreplicate similarity

The resulting ordinations based on different dissimilarity metric show a certain heterogeneity in both the fungal and bacterial communities, as pseudo-replicates originating from the same plot do not group close together in the ordination (Fig. S12 A B).

Effect of burning intensity on the microbial communities

Alpha diversity

There was no evidence that burning affected the fungal community (Shannon, $P = 0.473$, Simpson, $P = 0.698$). The assumptions were satisfied for all ANOVA's except for the heterogeneity of variances in the Simpson's measures. For both diversity metrics, the dispersion in each treatment was quite large, especially for the low-burning intensity treatment, where Plot 7 had a lower alpha diversity than the rest of the plots belonging to this treatment (Fig. S13 A). Similarly, no significant effect of burning intensity was found for the bacterial community diversity (Shannon, $P = 0.194$, Simpson, $P = 0.196$), with a tendency for a higher alpha diversity for the unburned control (Fig. S13 B). Overall, the fungal alpha diversity indices were lower than the bacterial indices. Furthermore, rarefying the sequence abundance to an even depth did not change the tendencies observed nor the outcome of the statistical tests (data not shown).

Beta diversity

We used four different metrics (Aitchison, Hellinger, unweighted and weighted Unifrac) to visualize and test the effect of burning intensities on microbial community compositions using

ordinations and PERMANOVAs. For the ITS dataset, the visualization of the Aitchison PCA and the unweighted Unifrac PCoA showed a separation of the unburned plots and the intensely burned plots (Fig. 7 A & B). However, regardless of the distance used, we found no evidence of the effect of burning intensity in shaping the fungal community composition when using PERMANOVA tests ($P > 0.05$). Finally, for each of the four distance metrics used, the variance in terms of community composition explained by burning intensity was around 20%. For the bacterial community, we also observed a separation of the unburned plots and the high-intensity burned plots in the Aitchison PCA, unweighted Unifrac PCoA (Fig. 7 C & D), as well as in the Hellinger PCA (data not shown). However, as for the fungal community, we did not find any statistical evidence of the effect of burning in shaping the bacterial community (*P* value > 0.05). The variance explained by burning intensity was also around 20% for the four metrics computed for the bacterial dataset.

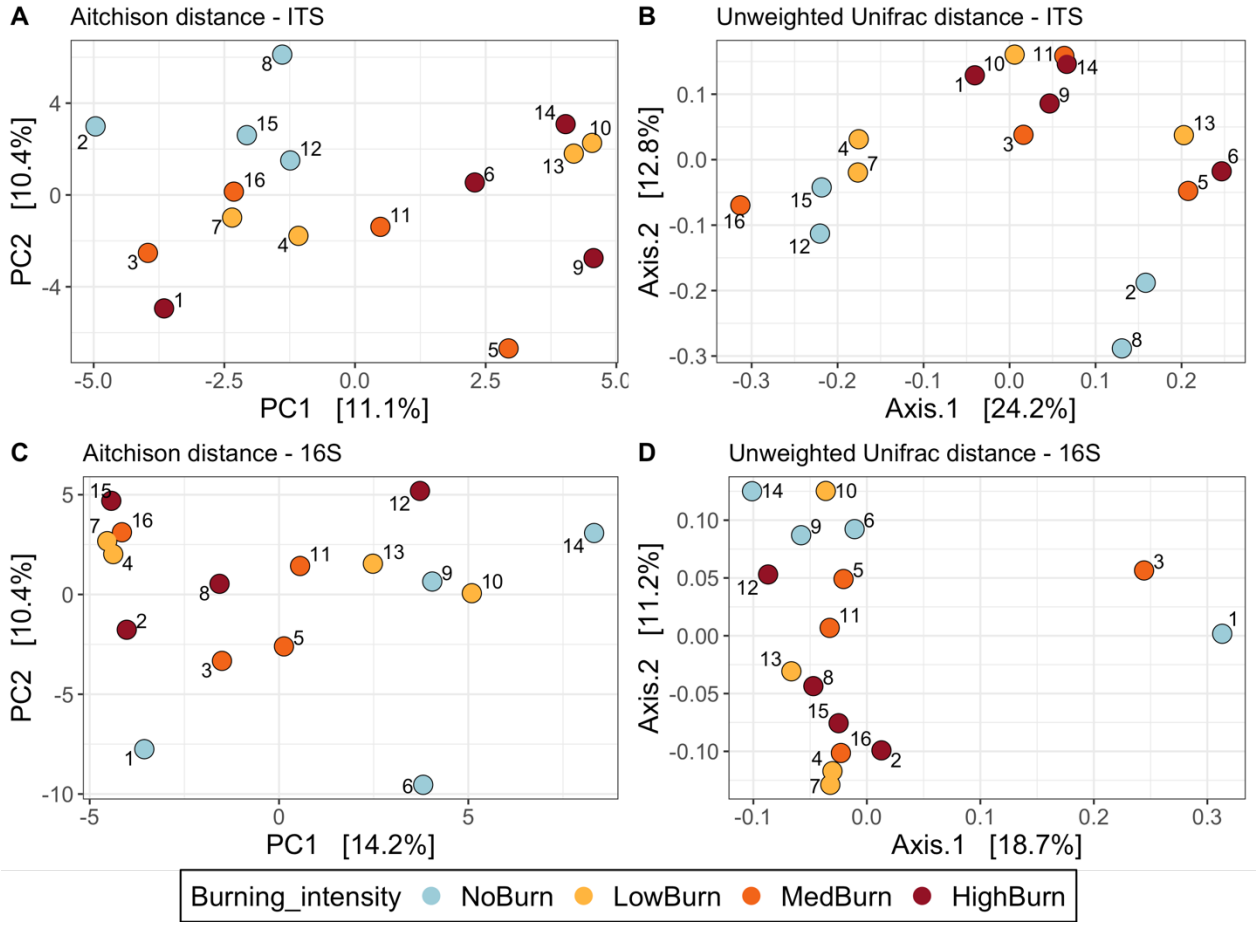


Figure 7. Beta diversity ordinations of the fungal and bacterial communities. Panels **(A)** and **(B)** represent principal component analysis (PCA) and principal coordinate

analysis (PCoA) ordinations of the fungal community using the Aitchison distance (Euclidean distance on centered-log ration transformed abundances and the unweighted Unifrac distance based on phylogeny dissimilarity, respectively. Panels **(C)** and **(D)** represent the same ordinations but of the bacterial community.

Representative ASVs

Although the beta diversity and PERMANOVAs indicated no significant differences in either the fungal or bacterial communities between each burning treatment, we looked at a finer level in order to detect ASVs that could be representative of a specific burn treatment. First, the differential heat tree plot showed no significant difference in the log 2-fold ratio of ASVs regrouped based on their taxonomy (FDR $P > 0.05$). The indicative species analysis using ASVs agglomerated at species level found no evidence for indicative ASVs (FDR $P > 0.05$) whether without or with relevant site combinations. Finally, the DESeq 2 analysis on species-level agglomerated ASVs (used to detect differential abundance between the highest burning intensity and the negative control) identified, with a moderate evidence, six fungal ASVs but no bacterial ASVs impacted by burning intensity (FDR $P < 0.05$). Half of these fungal ASVs were absent from the other condition, the other three being present in both treatments: two taxa belonging to the *Coniochaeta* genus were more abundant in the highest burning treatment and one *Pseudoanungitea sp.* was more abundant in the negative control treatment (Fig. S14).

Discussion

Thermal pruning had no significant impact on blueberry performance or agronomic variables, even at the highest intensities.

Thermal pruning had a negative effect on wild blueberry spring vegetative recovery with significantly lower coverage and biomass for the three burning intensities compared to the unburned control. However, the difference disappeared over time and all treatments had similar coverage and biomass during the rest of the experiment. Thermal pruning did not significantly impact stem density and growth, although we did see a slight increase in both variables, with

increasing burning intensity. Additionally, fruit yield and ripeness were not significantly different when comparing treatments. Finally, we did not observe any trend in the weight of ripe fruits. Overall these results indicate that wild blueberry recovers from thermal pruning very well. This was expected as wild blueberries populations are known to thrive after forest fires (Chapeskie, 2001; Wood, 2004). Furthermore, studies have shown that the use of thermal pruning in wild blueberry agricultural settings has promoted yields (Smith and Hilton, 1971; Warman, 1987; Penney et al., 1997). In our study, we do not measure any significant difference on blueberry performance during the harvesting year (yield, ripe fruit weigh, blueberry shrub biomass) when we compared the three treatments (thermal pruning after mechanical pruning) to the control, which was only mechanically pruned. Therefore, burning did not appear to contribute to a direct promotion of blueberry performance that mechanical pruning did not already provide. In fact, Smith and Hilton argued that the increased yield observed in their study, comparing thermal pruning to mechanical pruning, originated from the nutrients released from straw ash deposition (Smith and Hilton, 1971).

Thermal pruning has a temporary phytosanitation effect on *Septoria* leaf spot disease but this effect is unclear on weeds.

Due to the high discrepancy in the control replicates, which likely leads to a lack of statistical difference with the other burning treatments, the reduction of *Cornus canadensis* incidence observed must be taken with caution. The other two recorded weed species (*Maianthemum canadense* and *Gaultheria procumbens*) do not seem to have been affected by thermal pruning. When all weed species were pooled together, we did observe a significant difference between the unburned control and the burning treatments. However, the total weed coverage trend closely followed the *C. canadensis* trend as it was the predominant weed species in our plots. Although the experiment followed a random block design, there is a chance that the weed population density was disparate between the treatments, as we have no data on the pre-existing *C. canadensis* population in our plots. Additionally, *C. canadensis*, *M. canadense* and *G. procumbens*, like wild blueberries, are rhizomatous plants that primarily spread via their rhizome growth rather than by seed germination (Lee, 2004; Moola and Vasseur, 2009). As the highest

intensity burn only increased the temperature by 4°C at a depth of 1 cm (Fig. S1 D.), the majority of the rhizomes should have been left intact. Thus, we would expect these weed species to be largely resistant to our thermal pruning treatments depending on how deep their rhizomes are (Flinn and Wein, 1977; Penney et al., 2008). Finally, our results are in disagreement with previous studies, which report either no impact or a positive effect of thermal pruning on *C. canadensis* (Hoefs and Shay, 1981; Penney et al., 2008). Therefore, we suggest thermal pruning would not explain why we observed a decrease in weed coverage due to burning intensity. Accordingly, we cannot draw conclusions on the effect of burning on the weed species we observe in our experiment and this non-significant reduction in *C. canadensis* coverage should be investigated in-depth for validation. *C. canadensis* can be highly competitive in wild blueberry farms (Hall and Sibley, 1976; Yarborough and Bhowmik, 1993); therefore, if thermal pruning does reduce its population, this practice offers a great solution for organic producers who are not allowed to use herbicides.

Septoria leaf spot disease (*Septoria* LSD) causes a premature leaf drop, which can result in a loss of crop yield. We only observe a decrease of its incidence (negatively correlated with burning intensity) during the vegetative summer (3 months after the thermal pruning), with a significant difference between our negative control and the high-intensity burn. However this significant change in occurrence did not translate to reduced yield for our negative control. *Septoria* sp. propagates through spores produced on pycnidia that develop on pre-infected dead leaves from the soil litter (Hildebrand et al., 2016). We can hypothesize that burning diminished the number of viable spores, thus reducing the disease propagation (Hardison, 1976; Hildebrand et al., 2016). However, we still witness a high coverage of this disease with a mean incidence of 62.9% even for the high-intensity burn. Furthermore, there was no significant difference during the harvesting year where the burning treatments actually had higher *Septoria* LSD coverage than the negative control. This can be explained by the fact that plots were located close to each other and there were no means to prevent the spores arising from the more infested unburned control to contaminate the less infected high intensity burned plots. Interestingly, we did not capture any *Septoria sp.* in the fungal rhizosphere community even though Septoria LSD symptoms were

detected in our plots. As plant litter was excluded from our soil sampling for DNA sequencing, this could explain why we did not capture any *Septoria* sp. DNA sequence.

Thermal pruning increased soil phosphorus content but did not significantly influence other elements.

Overall, soil chemistry was not impacted by the burning treatments regardless of the burning intensity used. Apart from the phosphorus content which increased significantly with burning intensity in the organic layer 1 month after the treatment, there was no clear effect of thermal pruning on the rest of the elements measured (C, N, K, Mg and Ca) nor on soil pH. Previous studies on wild blueberry thermal pruning have shown that there was an increase in pH, phosphorus and potassium following the burning treatment when straw was added (Smith and Hilton, 1971). An increase in calcium and phosphorus in the organic soil layer was also observed in an experiment using a propane burner (Hoefs and Shay, 1981). However, these changes in nutrient content resulted from the combustion of organic matter, which was very limited in our plots. For instance, contrary to Smith and Hilton, we did not witness any ash deposit after the burning treatments suggesting that combustion in our experiment was low (Smith and Hilton, 1971).

Rhizosphere bacterial and fungal communities were homogenous throughout the thermal pruning treatments, more than a year after the burning treatment.

The alpha diversity analyses showed no significant difference between the burning intensities treatments either for bacterial or fungal communities. The alpha diversity indices were higher for bacteria than for fungi, with a mean range of 6.35 – 6.5 versus 3.1 – 3.7 for the Shannon-Weaver Index and 0.996 – 0.997 versus 0.89 – 0.95 for the Simpson reciprocal index. This difference is common, as alpha diversity indices rely on richness and evenness and that bacteria communities are commonly richer than fungi in soil, even though a low soil pH tends to generally decrease bacterial diversity (Fierer and Jackson, 2006; Rousk et al., 2010).

We obtained a similar outcome with our beta diversity analyses, regardless of the distance metric used. Furthermore, the DESeq2 analysis which aimed at detecting ASVs that were differentially

abundant between the negative control and the high intensity burning treatment only identified six fungal taxa, two of which were exclusive to the control (*Mycena sp.* and an Unknow genus) and one of which was exclusive to the high intensity burning treatment (*Lachnum* sp.*)*. The other three ASVs represented a very small portion of the relative abundance (RA), with 0.14% RA for *Pseudoanungitea* sp. , 0.25% RA for *Coniochaeta boothii*, and 1.35% RA for *Coniochaeta* sp. Therefore, their differential abundance between the negative and most extreme treatment does not induce a significant shift in community diversity. The two *Coniochaeta* sp. ASVs were more abundant in the high intensity burn which could be explained by the fact that this genus contains "fire-induced" species (Wicklow, 1975). The *Pseudoanungitea* genus (more abundant in the control) was described in 2018 and one species (*P. vacinii*) was isolated from the stem of *Vaccinium myrtillus,* a closely related plant from wild blueberry (Crous et al., 2018). Most of *Pseudoanungitea* are saprotrophic (Shen et al., 2020) but a sensitivity to fire has not been documented.

A shortfall of our study is that we sequenced the microbial communities more than a year after (~15 months) the burning treatments were performed, which precluded us from detecting an immediate shift in microbial communities. Wild blueberry generally grows in soil with low nutrient availability, and the plants must rely on their microbial communities, especially on ericoid mycorrhizal fungi, to uptake sufficient amounts of nutrients (Cairney and Meharg, 2003). Although wild blueberry rhizomes act as a nutrient source during growth, Grelet and collaborators showed that *Vaccinium* spp. do need exogenous nitrogen during the harvesting year, and cannot solely rely on its nutrient reserve (Grelet et al., 2001). Therefore, we chose to sequence the communities present at the end of the production cycle, at the time of harvest. Ideally, we would have sequenced before the burning treatment and throughout the two growing seasons to have a thorough analysis of the impact of thermal pruning on bacterial and fungal community. Our study cannot confirm that thermal pruning used at these intensities did not impact these fungal and bacterial communities, as there is a possibility that the communities are resilient and recovered from the initial disturbance caused by thermal pruning. Moreover, we believe that, if there was an initial disturbance it would have been very mild, such as observed for soil phosphorus content. Our results show that there was a very limited impact of burning on soil pH

and nutrient content. In addition, the highest fire intensity was not sufficient enough to cause a reduction in the organic layer depth or its humidity. Studies observing shifts in microbial communities after fires also see changes in soil chemistry, humidity; and temperature as well as a shift in the plant community (Hart et al., 2005; Dooley and Treseder, 2011; Dove and Hart, 2017; Whitman et al., 2019). However, in our study, thermal pruning is not inducing plant succession comparable to intense forest fires. Finally, the temperature at 1 cm depth increased only by 4°C with the highest intensity (Fig. S1 D.), while increasing as high as 80°C at the surface (data not shown). Although a good proportion of microbes at the surface may have been killed, the temperature in the soil did not rise high enough to cause a high mortality in the rhizosphere microbiome (Neary et al., 1999). Consequently, we can either hypothesize that thermal pruning in our study did not impact fungal and bacterial rhizosphere communities of wild blueberries or, if it did, we can affirm that both communities are resilient 15 months after burning.

Despite the fact that some plots were devoid of *C. canadensis*, while others were highly covered by the weed, we did not observe any significant change in the wild blueberry rhizosphere fungal and bacterial community. Although we took care to sample wild blueberry rhizosphere for DNA extraction, it is not possible to exclude all weed roots during the sample preparation. Nonetheless, we were surprised to see a lack of difference in the microbial communities since weeds act as additional hosts for microbes and can therefore alter the microbial community (Schlatter et al., 2015). Nevertheless, de Vries and collaborators have argued that plant traits and edaphic conditions also have strong impacts in shaping the microbial community (de Vries et al., 2012). Soil pH, in particular, exerts a high selection pressure on the microbial communities (Fierer and Jackson, 2006; Rousk et al., 2010; de Vries et al., 2012).

The pseudo-replicate similarity analysis is interesting as we could have expected that fungal and bacterial communities samples from the same plot, sampled a dozen centimetres apart, would be more similar than to the other samples. However, this is not what we have observed with our beta-diversity analysis ordinations, either relying solely on phylogenetic distance (unweighted Unifrac distance) or by sequence abundance (Aitchison distance). In both cases, the samples taken from the same plot do not group closer to each other than too other samples (Fig. S12). These results reinforce the fact that bacterial and fungal communities show a high diversity at

small scales (Bach et al., 2018; Smercina et al., 2021) and that scientists should consider composite sampling, when it is feasible, to try to capture a broader scope and a better representativeness of the microbial communities present in a given environment during the moment of sampling (Bullington et al., 2021).

The fungal community is dominated by known and putative ericoid mycorrhizal taxa.

Our analysis of the fungal community shows a predominance of the Helotiales order, totalling 244 ASVs and representing 46.8% of the relative abundance (RA). This fungal order contains known or putative ericoid mycorrhizae, most of which were identified in our dataset, including *Pezoloma ericae* (25 ASVs, 12.8% RA), *Oidiodendron maius* (15 ASVs, 9.0% RA), *O. chlamydosporicum* (2 AVS, 0.03% RA), *O. tenuissimum* (1 ASV, 0.02% RA), *Meliniomyces* sp. (19 ASVs, 1.7% RA), *Hyaloscypha variabilis* (12 ASVs, 2.6% RA) and *H. bicolor* (1 ASV, >0.01% RA), *Lachnum pygmaum* (1 ASV, 0.08% RA), and *Mycosymbioces* sp. (11 ASVs, 7.2% RA) (Vohník et al., 2005; Vohník et al., 2007; Grelet et al., 2009; Walker et al., 2011; Leopold, 2016; Fadaei, 2019). This order also contains the dark septate endophytes, *Phialocephala fortinii* (9 ASVs, 1.2% RA), and *P. glacialis* (5 ASVs, 0.5% RA) which may have possible beneficial outcomes on the plants they colonize (Newsham, 2011; Lukešová *et al.*, 2015). Still in the Ascomycota phylum, the Chaetothyriales order is the second most abundant with 59 ASVs and 28.6% RA, mainly composed of Herpotrichiellaceae (32 ASVs, 27.3% RA), a family often found in proximity with Ericaceae host plants (Midgley et al., 2004; Walker et al., 2011) and which contains the *Capronia* genus that was found to form hyphal coils in *Gaultheria shallon* roots (Allen et al., 2003). Switching to the less abundant Basidiomycota phylum, the three orders, which stand out: Agaricales, Trechisporales and Sebacinales, are also of interest. In our data, the Agaricales are dominated by *Clavaria sphagnicola* (9 ASVs, 3.8% RA), a genus known to form hyphal coils in Ericaceae roots (Peterson et al., 1980) and which is considered as a putative ericoid mycorrhizal fungal genus (Yang et al., 2018). *C. sphagnicola* is phylogenetically very close to *C. argillacea* which was found to make reciprocal exchanges of

nutrients with rhododendron roots (Englander and Hull, 1980). The Trechisporales order is mainly composed by *Luellia sp.* (11 ASVs, 3.4% RA), a genus containing saprotrophic fungi with some putative ectomycorrhizal fungi (Malysheva et al., 2018). Although no ErM fungi have been identified in this genus, research has shown that ectomycorrhizal fungi can also colonize Ericaceae roots (Villarreal-Ruiz et al., 2004; Vrålstad, 2004; Villarreal-Ruiz et al., 2012). Furthermore, saprotrophic fungi can help degrade plant debris and extract nitrogen and phosphorus from lignocellulose more efficiently than mycorrhizal fungi and can therefore be beneficial for plant growth (Vohník et al., 2012). Finally, the Sebacinales order mainly composed of *Serendipita* sp. (13 ASVs, 0.9% RA) was identified as having potential ericoid mycorrhizal fungi species (Vohník et al., 2016). Overall, our data shows a relatively high abundance of symbiotic and/or endophytic fungal taxa in the rhizosphere community, which may suggest their importance in the wild blueberry rhizosphere ecosystem. The confidence in our taxonomy annotation is reinforced with the mock community analysis which correctly assigned the correct genus to 17 on 19 taxa, and 10 to species level (Supplementary information). However, although we used an even mock community, where each taxon should be represented equally, we witnessed a higher abundance of Ascomycota taxa than Basidiomycota in our mock community results. While Ascomycota have been reported to dominate the wild blueberry rhizosphere (Yurgel et al., 2017; Morvan et al., 2020), the relative abundance obtained in our mock and experimental communities could be skewed as either the PCR amplification or sequencing could have introduced a bias toward Ascomycota over Basidiomycota sequences.

The bacterial community contains abundant taxa with carbon degrading capacity as well as dinitrogen fixation potential taxa.

The bacterial dataset was dominated by Frankiales (175 ASVs, 14.0% RA) mostly belonging to the *Acidothermus* genus (134 ASVs, 12.9% RA). To date, this genus has a sole known species : *A. cellulolyticus* isolated from acidic hot spring in Yellowstone National Park. As the name of the genus implies, the bacteria is acidophilic (optimal pH = 5) and thermophilic (optimal temperature

= 55°C) (Mohagheghi et al., 1986). Both the acidophilic and thermophilic nature of the bacteria are coherent in a wild blueberry context, as temperature records show that soil in the blueberry field (in which this experiment is part of) often reaches 40°C, with a max temperature of 53.07°C in the summer of 2021. Furthermore, *A. cellulolyticus* genome sequence contains numerous plant-biomass and fungal cell wall degrading enzymes (Barabote et al., 2009), a characteristic consistent in a wild blueberry soil which contains a high level of organic matter. Among the Acidobacteriales (236 ASVs, 12.5% RA), most belonged to the Acidobacteriaceae Subgroup 1 (148 ASVs, 6.2% RA) which was found to be abundant in a low pH soil and are able to degrade a variety of carbon sources (Kielak et al., 2016). The Isosphaerales order (209 ASVs , 12.4% RA) commonly found in acidic northern wetlands (Kulichevskaya et al., 2016) was dominated by the *Aquispharea* genus (81 ASVs, 10,1% RA), first identified from a freshwater aquarium (Bondoso et al., 2011). Although *Aquispharea* was not included in the study, Ivanova and collaborators have identified a common pool of carbohydrate-active enzymes in four other Isosphaeraceae species, suggesting a high potential of this bacterial family to use a variety of carbohydrates and glycoconjugates (Ivanova et al., 2017). The Rhizobiales order (150 ASVs, 10.4% RA), well known to harbor nitrogen-fixing bacteria, was dominated by the Xanthobacteraceae family (82 ASVs, 7.3% RA) and the *Roseiarcus* genus (31 ASVs, 2.5% RA). Xanthobacteraceae contain dinitrogen fixation potential (Sawada et al., 2003; Oren, 2014) among which *Rhodoplanes* (Buckley et al., 2007) and *Bradyrhizobium* (Ormeno-Orrillo and Martinez-Romero, 2019) have been previously reported to fix dinitrogen and are also present in our community. *Roseiarcus fermentans*, the only known species of this genus to date, was isolated from acidic Sphagnum peat and is capable of dinitrogen fixation. This genus was also found to be abundant in a previous study on wild blueberry rhizosphere (Morvan et al., 2020). Our bacterial mock community analysis supports our taxonomy assignment as 18 out of the 20 species present in the mock community were correctly assigned to species level and the two remaining had a correct genus identity. Regarding the abundance, we observed no bias towards a particular taxonomy level. Regrettably, there were no Acidobacteriota nor Planctomycetota taxa in the mock community that was sequenced while they represent a significant proportion of the community found in our experiment.

Conclusion

This study aimed to evaluate the impact of thermal pruning on agronomic variables and the microbiome of wild blueberry shrubs in an agricultural setting. Our results show limited, short-term benefits of burning. *Septoria* leaf spot disease was negatively impacted by burning at a high intensity in the short term. However, this temporary reduction had no effect on blueberry yield. We observed a tentative non-significant decrease of the predominant weed, *Cornus canadensis*, due to burning. Since *C. canadensis* is a common wild blueberry competitor, thermal pruning could be worthwhile but additional experiments are required to validate our results as they are in contradiction with the literature. Moreover, the burning intensities used in our experiment did not significantly disrupt the fungal and bacterial communities of the wild blueberry rhizosphere sampled during the subsequent harvesting year (15 months after the thermal pruning treatment). Otherwise, as we did not record any other significant beneficial outcome, thermal pruning may not be worth investing into. The use of alternative technics like vinegar as a herbicide (practice already used in cranberry fields) or products containing *Bacillus subtilis* as fungicides are more promising and ecofriendly than burning fossil fuels. This aspect should be taken into consideration especially during our times of climatic crisis.

Data Availability Statement

The agricultural datasets for this study can be found in Zenodo : https://doi.org/10.5281/zenodo.6544474. The microbial sequences have been deposited in the GenBank SRA database under the accession number PRJNA803472. The R scripts used to analyze the data and to generate figures are deposited on GitHub: https://github.com/SimonMorvan/thermal_BB_microbiome

Author contributions

MP and JL conceived and designed the agronomic part of the study while, MH and SM conceived and designed the microbial part of the study. AS and SM contributed to data acquisition. SM

performed the microbial community analysis experiments, analyzed the data and wrote the first draft of the manuscript. All authors reviewed and discussed the results, read and approved the submitted version of the article.

Funding

This study received funding from the following source: the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant to MH (Grant RGPIN-2018-04178); the Syndicat des Producteurs de Bleuets du Québec (SPBQ) and the Natural Sciences and Engineering Research Council of Canada (NSERC) (Grant RDCPJ-503182-16) to MP.

Acknowledgments

The authors would like to thank Geneviève Telmosse, Catherine Tremblay, and Stéphanie Cloutier for their help on data acquisition; Denis Bourgault for soil analysis; Stéphane Daigle for his help on mixed models, Andrew Blakney for critical reading and English editing. The authors also thank the Corporation d'Aménagement Forêt Normandin (CAFN) for providing access to their sites and infrastructure.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.954935/full#supplementary-material

Supplementary methods

16S PCR workflow

Projet Simon_1					517628	
2021-07-26						
PCR Amplification						

Comment
VAL12505178(1851) - 16S - A01-C09 + D01-H05

Master Mix

Master Mix Components	1X	96	Final Concentration		Volume to transfer in samples	Index volume to add
Q5 reaction Buffer 5X	5.00	480.0	1 X			
Roche_DMSO	1.25	120.0	5 %			
dNTP mix 10 mM_NEB	0.50	48.0	0.2 mM		24	
Q5 HiFi polymerase 2U-ul	0.25	24.0	0.02 U/ul			
H2O	16.70	1603.2			DNA Volume (ul)	Dilution / Conc. Used
341FP1-CS1; 341FP2-CS1; 341FP3-CS1; 341FP4-CS1	0.15	14.4	0.6 uM		1	nd
805RP1-CS2; 805RP2-CS2; 805RP3-CS2; 805RP4-CS2	0.15	14.4	0.6 uM			
Total	24.00	2304.0				

Thermocycler

Container	Thermocycler	Program		
VAL12505178_PCR_16S	Antonio	Q5		
		98C	0.5 min	
		98C	10 sec	
		60C	15 sec	25X
		72C	30 sec	
		72C	2 min	

Primers

Forward Primer	Localization	Sequence
341FP1-CS1	Oligos_Metagenomique_Boite4-C04	ACACTGACGACATGGTTCTACACCTACGGGNGGCWGCAG
341FP2-CS1	Oligos_Metagenomique_Boite4-C06	ACACTGACGACATGGTTCTACATCCTACGGGNGGCWGCAG
341FP3-CS1	Oligos_Metagenomique_Boite4-C07	ACACTGACGACATGGTTCTACAACCCTACGGGNGGCWGCAG
341FP4-CS1	Oligos_Metagenomique_Boite7-D03	ACACTGACGACATGGTTCTACACTACCTACGGGNGGCWGCAG
Reverse Primer	**Localization**	**Sequence**
805RP1-CS2	Oligos_Metagenomique_Boite5-B07	TACGGTAGCAGAGACTTGGTCTGACTACHVGGGTATCTAATCC
805RP2-CS2	Oligos_Metagenomique_Boite4-D01	TACGGTAGCAGAGACTTGGTCTTGACTACHVGGGTATCTAATCC
805RP3-CS2	Oligos_Metagenomique_Boite4-D02	TACGGTAGCAGAGACTTGGTCTACGACTACHVGGGTATCTAATCC
805RP4-CS2	Oligos_Metagenomique_Boite4-F07	TACGGTAGCAGAGACTTGGTCTCTAGACTACHVGGGTATCTAATCC

ITS PCR workflow

Projet Simon_1					517647	
2021-07-26						
PCR Amplification						

Comment
VAL12505179(1852) - ITS - A01-C09 + D01-H05

Master Mix

Master Mix Components	1X	96	Final Concentration		Volume to transfer in samples	Index volume to add
Qiagen 10X Buffer with15mM MgCl2	2.50	240.0	1 X			
Roche_DMSO	1.25	120.0	5 %			
dNTP mix 10 mM_NEB	0.50	48.0	0.2 mM		24	
Qiagen HotStarTaq 5U-ul	0.10	9.6	0.02 U/ul			
H2O	19.35	1857.6			DNA Volume (ul)	Dilution / Conc. Used
ITS3_KYO_02-CS1	0.15	14.4	0.6 uM			
ITS4R-CS2R	0.15	14.4	0.6 uM		1	1/30
Total	24.00	2304.0				

Thermocycler

Container	Thermocycler	Program		
VAL12505179_PCR_ITS	Antonio	Qiagen		
		96C	15 min	
		96C	30 sec	
		52C	30 sec	33X
		72C	60 sec	
		72C	10 min	

Primers

Forward Primer	Localization	Sequence
ITS3_KYO_02-CS1	Oligos_Metagenomique_Boite3-D10	ACACTGACGACATGGTTCTACAGATGAAGAACGYAGYRAA
Reverse Primer	**Localization**	**Sequence**
ITS4R-CS2R	Oligos_Metagenomique_Boite8-D08	TACGGTAGCAGAGACTTGGTCTTCCTCCGCTTATTGATATGC

Indexation PCR workflow

Projet Simon_1					**517803**	
2021-07-27						
PCR Indexation						

Comment
VAL12505178(1854) - 16S - **BC2** - A01-C09 + D01-H05

Master Mix

Master Mix Components	**1X**	**192**	**Final Concentration**		**Volume to transfer in samples**	**Volume of Index at 2uM to add**
Roche 10X Buffer without MgCl2	2.00	384.0	1 X			
Roche MgCl2 25mM	1.44	276.5	1.8 mM			
Roche_DMSO	1.00	192.0	5 %		17	2
dNTP mix 10 mM_NEB	0.40	76.8	0.2 mM			
Roche FastStart High Fi 5U-ul	0.10	19.2	0.025 U/ul		**DNA Volume (ul)**	**Dilution / Conc. Used**
H2O	12.06	2315.5				
					1	nd
Total	17.00	3264.0				

Thermocycler

Container	**Thermocycler**	**Program**		
VAL12505178_BC_16S	Antonio	Barcoding_Roche		
VAL12505179_BC_ITS	Enrique	**95C**	10 min	
		95C	15 sec	
		60C	30 sec	15X
		72C	60 sec	
		72C	3 min	

Supplementary information

Indicative species analysis

Complementary to the Metacoder analysis, we proceeded with an indicative species analysis. This method identifies specific species that are representative of predefined groups of samples based on the species abundance and fidelity. In our case, we used phyloseq's tax_glom function to agglomerate the ASVs at species level and set the predefined groups as the four burning intensities. We used the *multipatt()* function of the indicspecies R package (de Cáceres and Legendre, 2009). Each ASV is assigned an indicative species value (IndVal) based on its importance in the group it is found to be most indicative of. A second analysis allowed for relevant site combinations (example: highest with medium burning intensity but not highest and negative control) by setting option duleg=False and specifying which group combinations were relevant. For both analysis, with or without group combinations, 9999 permutations were used to compute the p-values of each IndVal which were corrected with an FDR method to account for multiple comparisons.

de Cáceres, M., and Legendre, P. (2009). Associations between species and groups of sites: indices and statistical inference. *Ecology* 90(12)**,** 3566-3574.

Data processing

After processing the sequences through the bio-informatic pipeline, we obtained 980 353 ITS sequences (30 636 ± 7 110 reads on average per sample) dispatched in 913 ASVs (159 ± 34 on average per sample) and 738 599 16S reads (23 081 ± 6114 reads on average per sample) dispatched in 4971 ASVs (1294 ± 132 on average per sample). Comparing the taxonomy assignment obtained using the UNITE fungal reference database and eukaryotic reference database allowed to remove 106 ASVs that were labelled with a non-fungal Kingdom, most of which (81) were labelled as Viridiplantae belonging to Ericaceae species**.** Singleton and doubleton removal eliminated an additional 45 ASVs. In the bacterial dataset, we removed 12 Archaea, 22 Chloroplasts and 11 Mitochondria ASVs as well as 261 singletons and 615 doubletons. Finally, to evaluate if we had any contamination, we searched for common ASVs between our samples and both the extraction and PCR blanks. Regarding the PCR blanks, none of the sequences originally present in the PCR blank made it through the fungal pipeline while one ASV with 52 reads was obtained in the bacterial dataset and was unique to the 16S PCR blank sample. For the extraction blanks, in the fungal dataset, we obtained 15 170 sequences dispatched in 4 ASVs. One of these ASVs was also present in 21 out of 32 samples totalling 437 reads. Out of precaution, we removed this ASV identified as *Troposporella monospora* from our sample dataset. For the bacterial dataset, the extraction blank resulted in 12438 reads dispatched in 18 ASVs. Seven of those 18 ASVs were also found in our sample dataset and were removed from the bacterial sample dataset as they could be potential contaminants. These extra refining steps resulted in a total of 768 fungal ASVs and 4016 bacterial ASVs in our samples. The rarefaction curves obtained for both bacterial and fungal datasets indicate a sufficient sequencing depth (Fig. S4).

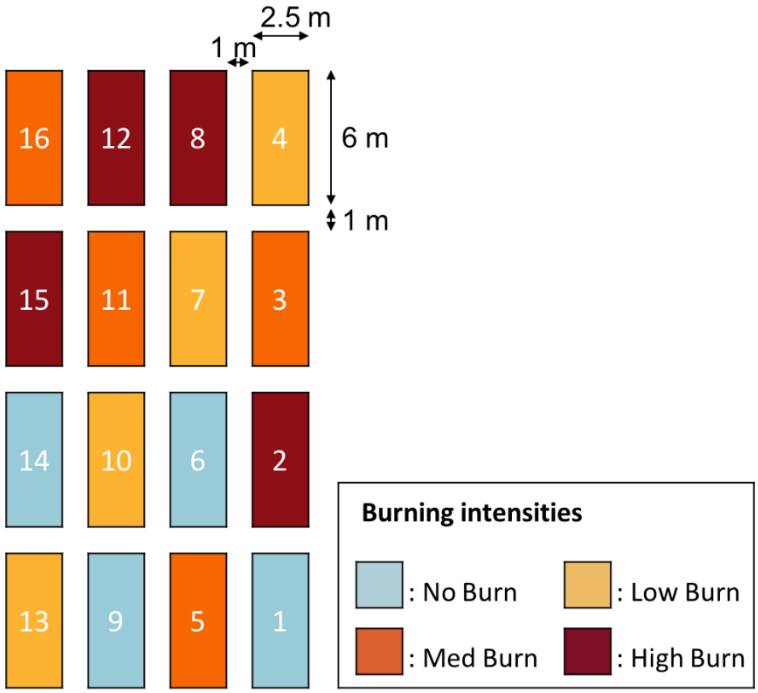
Mock community analysis

The fungal mock community contained 19 species belonging to the Ascomycota (12), Basidiomycota (3), Mucoromycota (2), Glomeromycota (1) and Chytridiomycota (1). Two species present in the mock community weren't found in our sequenced mock, *Rhizophagus irregularis* (Glomeromycota) and *Rhizomucor miehei* (Mucoromycota). The other 17 species were either correctly identified in our sequences mock (10) or had at least the same genus (7). One of the species, *Naganishia albida* (Basidiomycota) was separated into two ASVs in our sequenced mock. Although the mock community was conceived to contain even concentration of DNA material of each species, we observe a higher number of sequences for Ascomycota sequences than the rest of the fungal phyla which could indicate an amplification or sequencing bias (Table S2). The bacterial mock community contained 20 species with equimolar counts (10^6 copies/μL) of 16S rRNA genes. Out of the 20 species, 18 were identified to species level in the sequenced mock community. Our pipeline failed to assign a species to the *Actinomyces* sp. sequence, and *Bacillus cereus* was identified as *Bacillus anthracis*. Furthermore, there were additional taxa found in the sequencing data that are not listed in the mock community: *Staphylococcus caprae* (640 reads), *Lactobacillus paragasseri* (2 reads), Chloroplast (O, 12 reads), Mitochondria (F, 9 reads), and Xanthobacteraceae (F, 3 reads). The pipeline also found multiple ASVs assigned to a similar species with 6 *Bacteroides vulgatus* ASVs and 2 *Clostridium beijerinckii* ASVs (Table S3).

Supplementary figures

Supplementary Figure 1. Experimental design, visual aspect of the treatment and soil temperature profile measured during the burning treatment. A. and B. illustrate the experimental design. C. show the pruning treatment in action. D. displays the 1 cm depth temperature profile measured during the burning treatments.

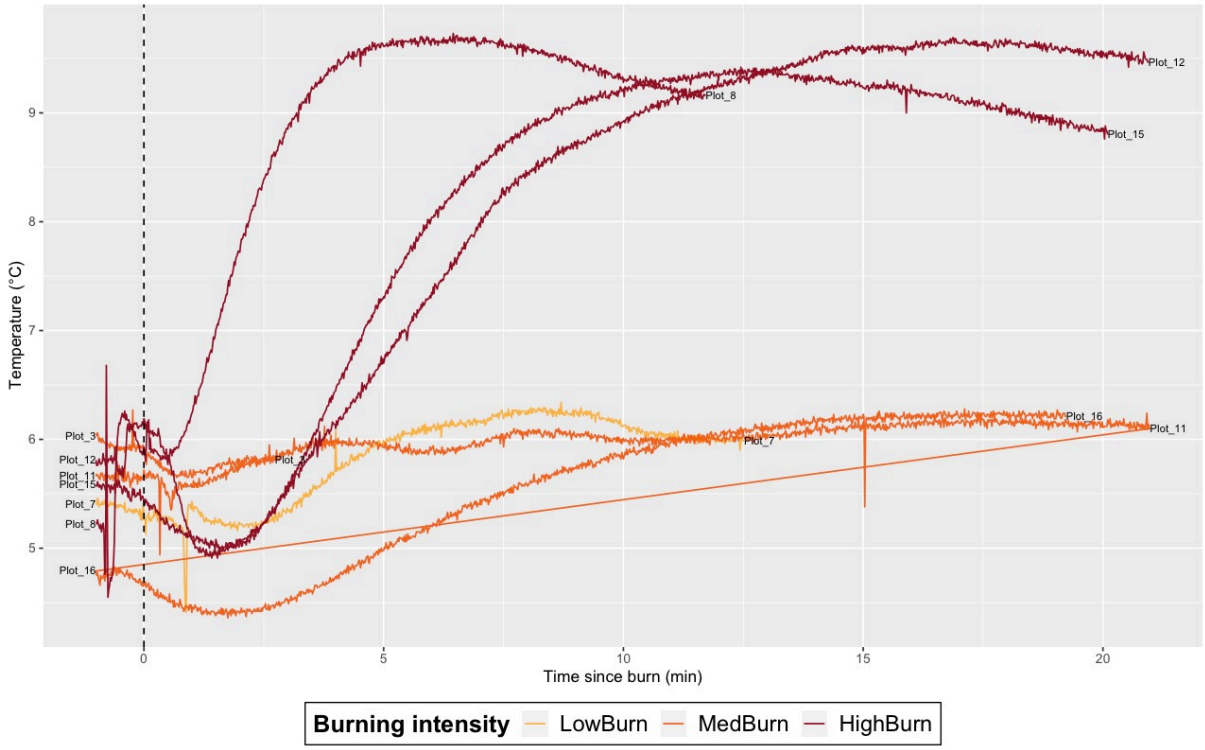
A.



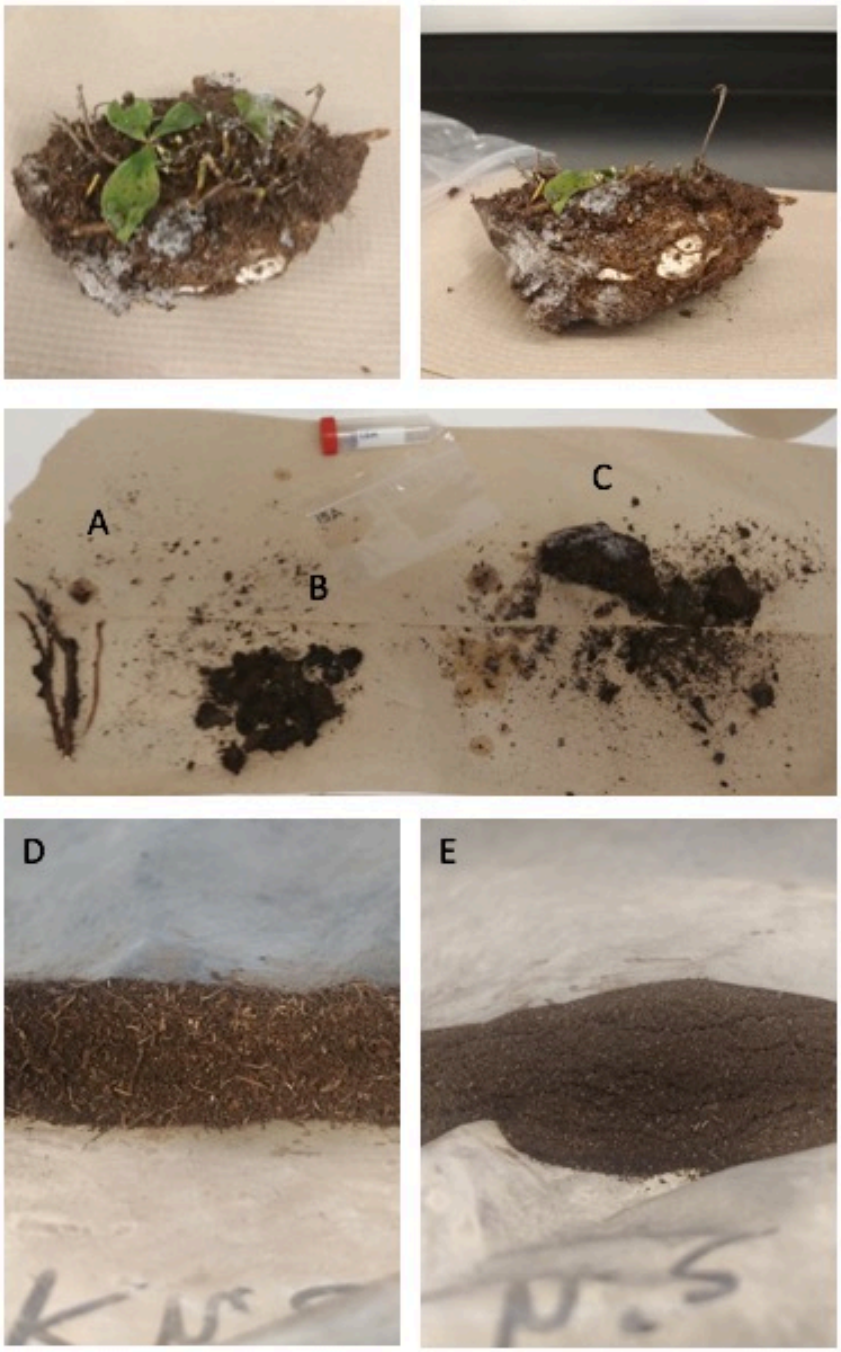
B.

C.

D.


Temperature (°C)
9
8
7
6
5
0
5
10
15
20
Time since burn (min)
Plot_12
Plot_8
Plot_15
Plot_16
Plot_11
Plot_7
Plot_3
Plot_2
Plot_12
Plot_11
Plot_15
Plot_7
Plot_8
Plot_16
Burning intensity
LowBurn
MedBurn
HighBurn

Supplementary Figure 2. Illustration of sample preparation for DNA extraction.



Initial sample

Processed sample

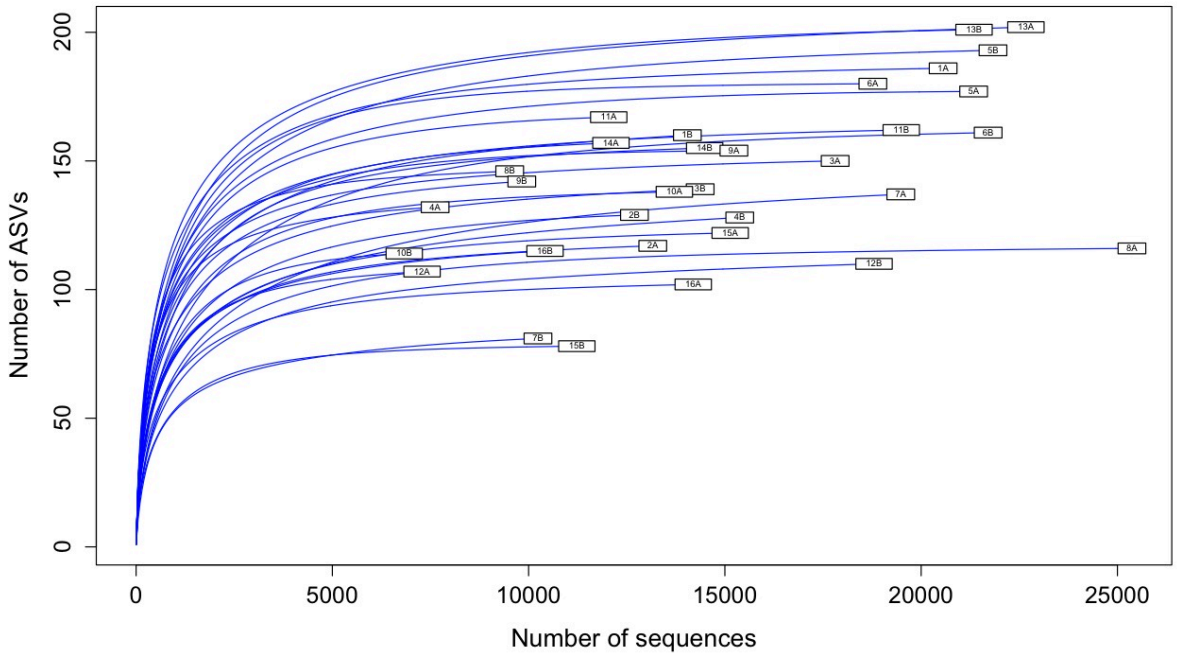
A. Rhizome
B. Rhizosphere aggregates
C. Rest of the soil clump soil

Dried and sieved samples

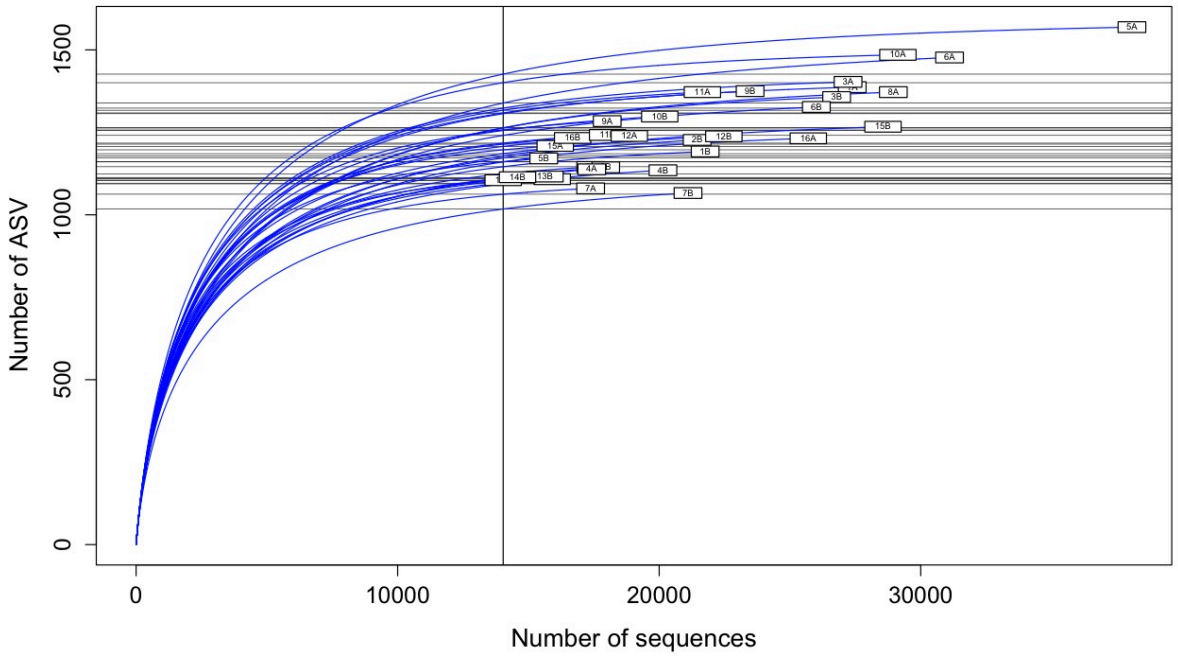
D. Before grinding
E. After grinding

Supplementary Figure 3. Rarefaction curves

A. Rarefaction curve for the fungal community.

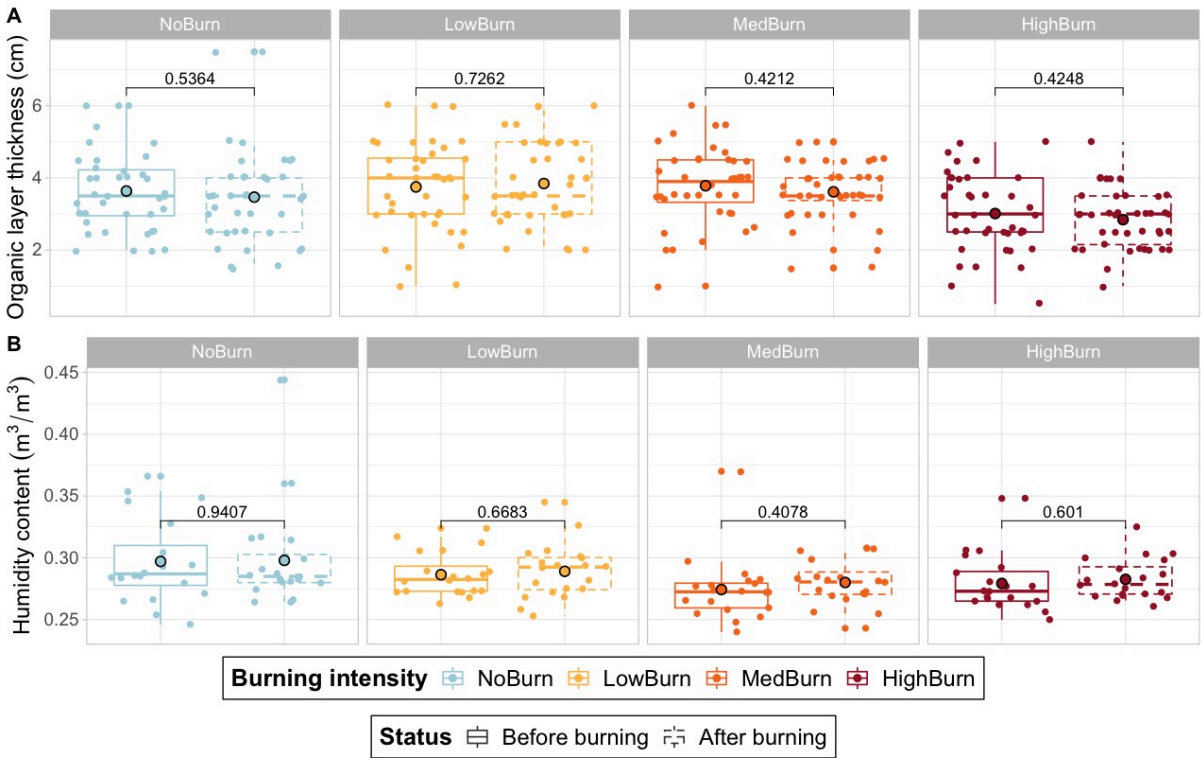


B. Rarefaction curve for the bacterial community.



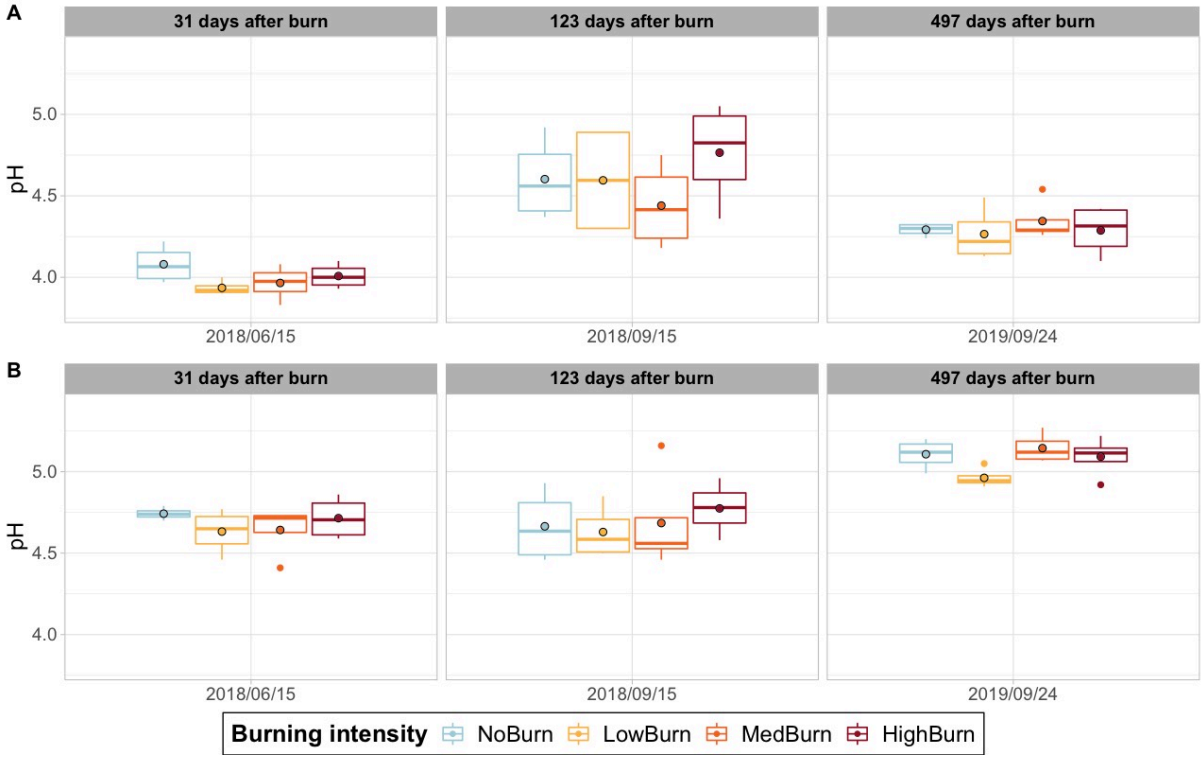
Supplementary Figure 4. Effect of burning on soil organic layer thickness (A) and humidity content (B).

Mean value is indicated with a black circled dot. P values computed by ANOVA are mentioned in each facet.



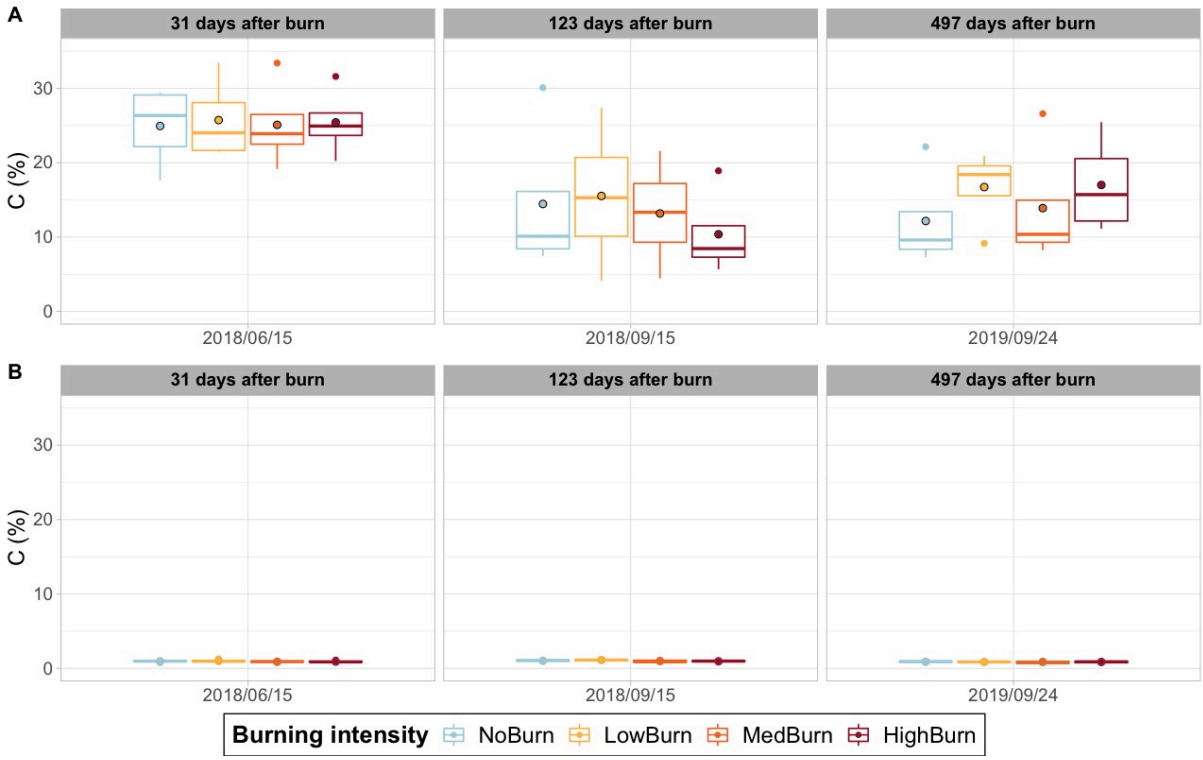
Supplementary Figure 5. Soil pH measurements over time.

No significant differences observed. Mean values are indicated with black circled dots. (A) Organic layer. (B) Mineral layer.



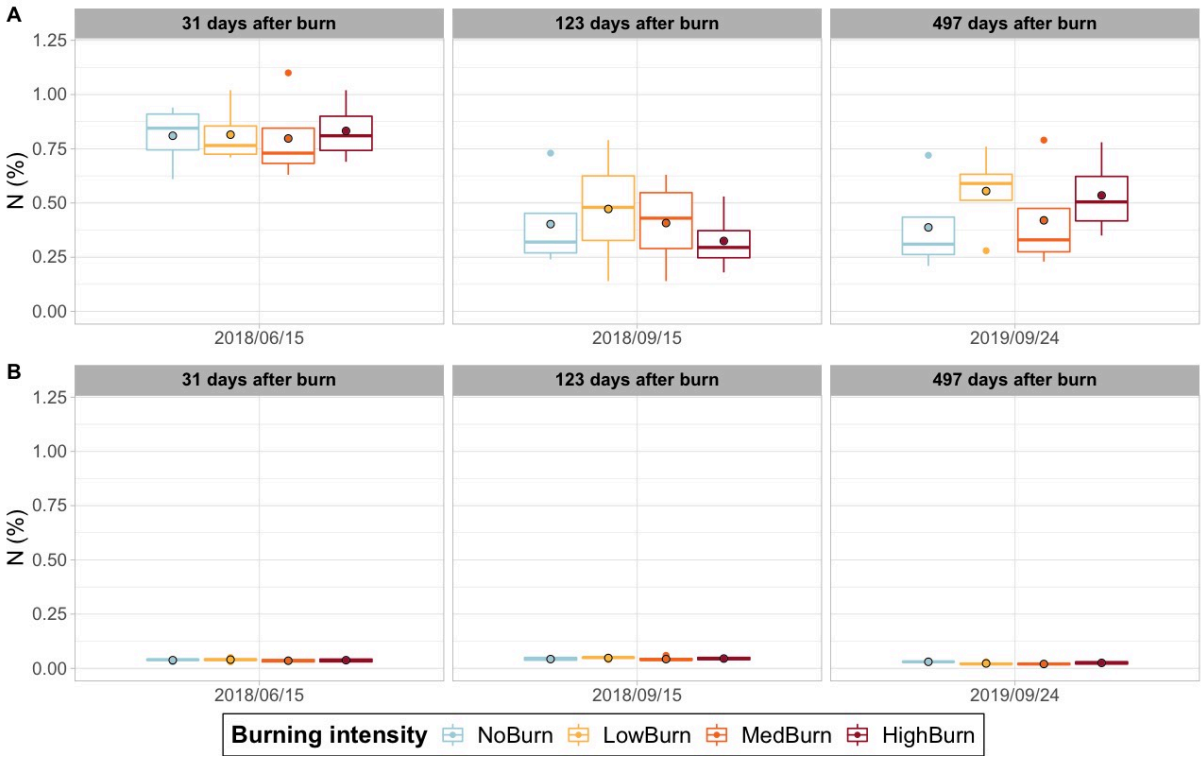
Supplementary Figure 6. Soil carbon concentration over time.

No significant differences observed. Mean values are indicated with black circled dots. (A) Organic layer. (B) Mineral layer.



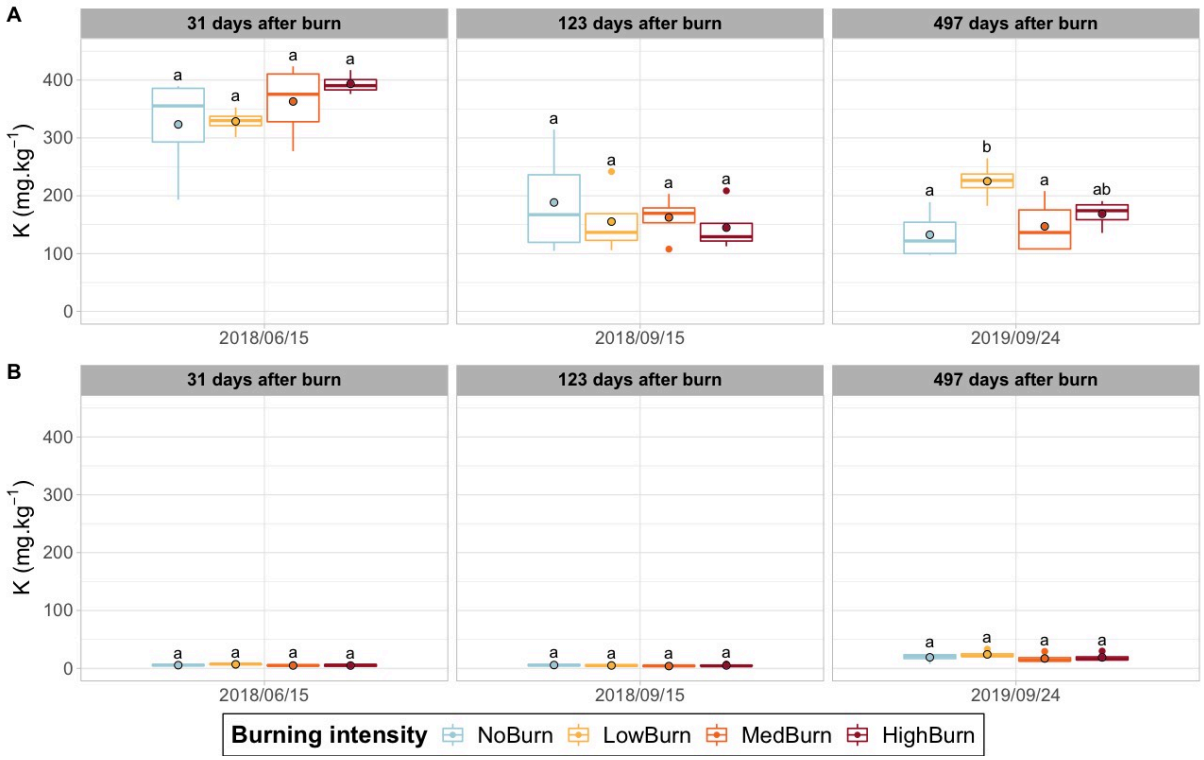
Supplementary Figure 7. Soil nitrogen concentration over time.

No significant differences observed. Mean values are indicated with black circled dots. (A) Organic layer. (B) Mineral layer.



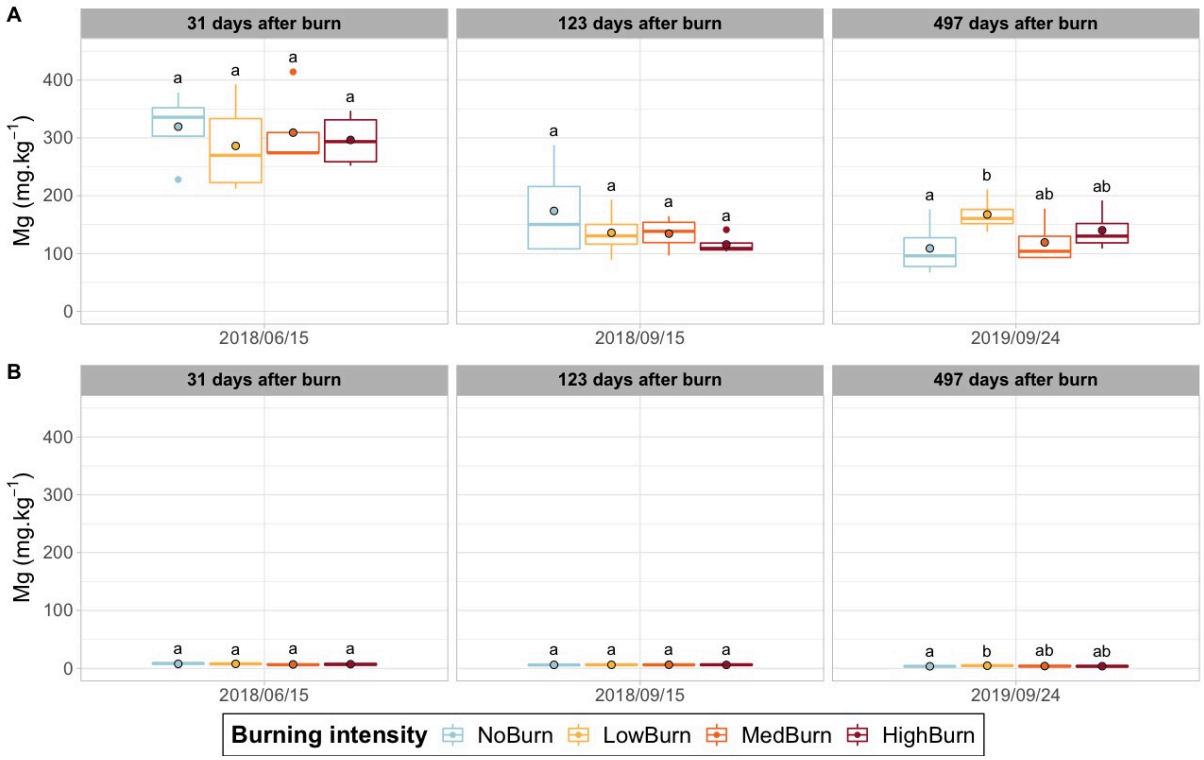
Supplementary Figure 8. Soil potassium concentration over time.

Significant difference in each sampling date is indicated by letters according to post-hoc Tukey tests. Mean values are indicated with black circled dots. (A) Organic layer. (B) Mineral layer.



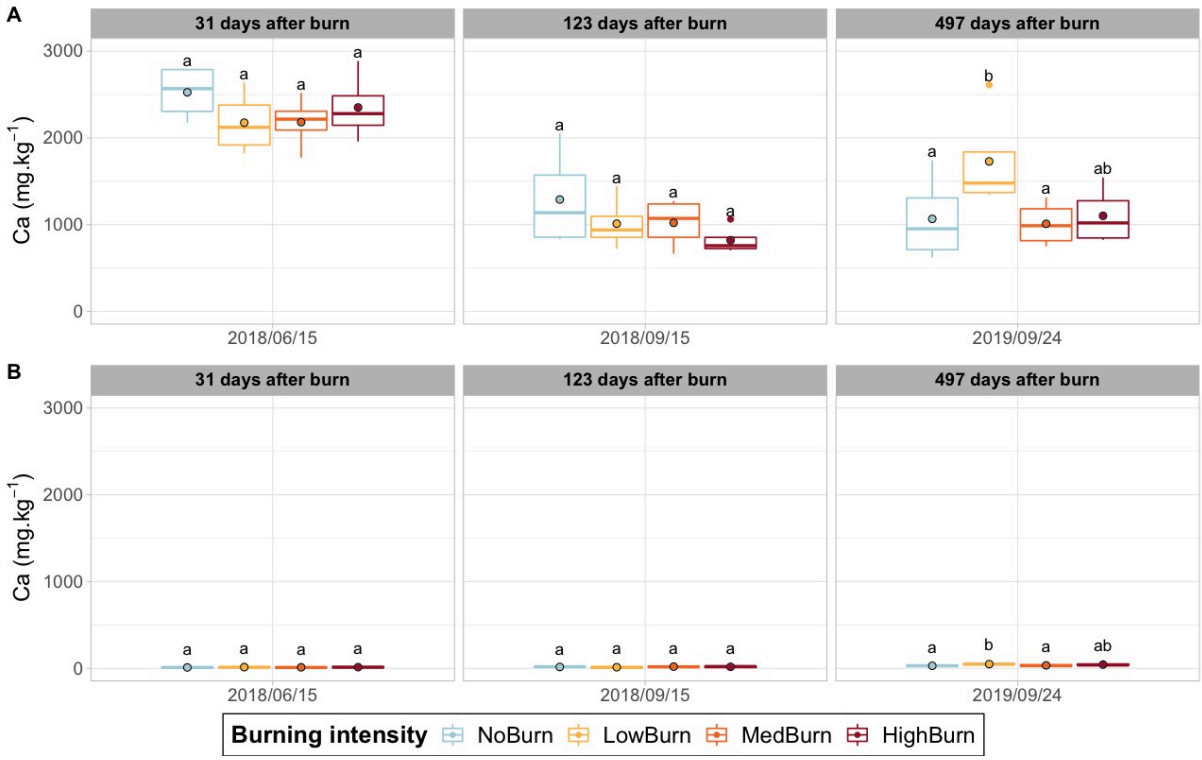
Supplementary Figure 9. Soil magnesium concentration over time.

Significant difference in each sampling date is indicated by letters according to post-hoc Tukey tests. Mean values are indicated with black circled dots. (A) Organic layer. (B) Mineral layer.



Supplementary Figure 10. Soil calcium concentration over time.

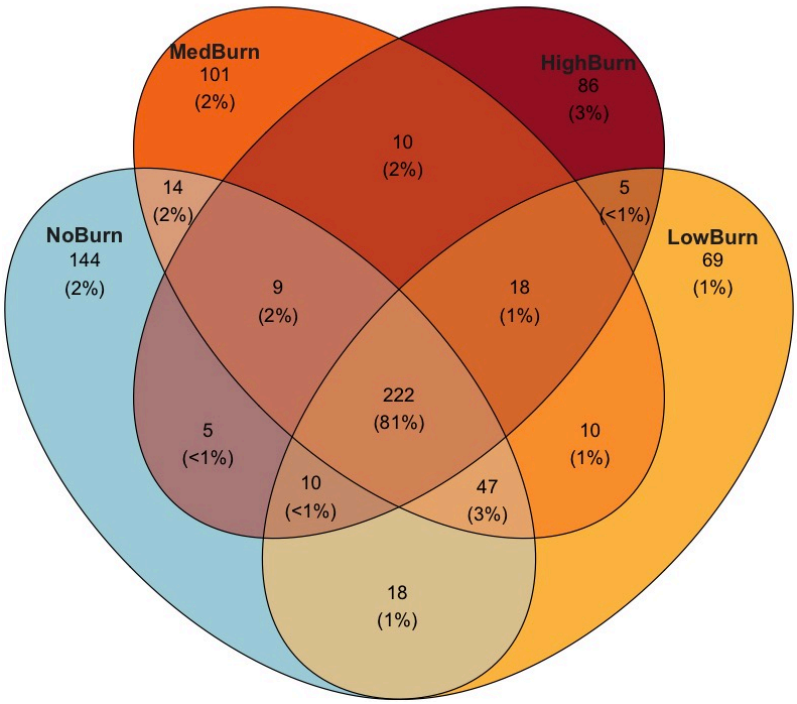
Significant difference in each sampling date is indicated by letters according to post-hoc Tukey tests. Mean values are indicated with black circled dots. (A) Organic layer. (B) Mineral layer.



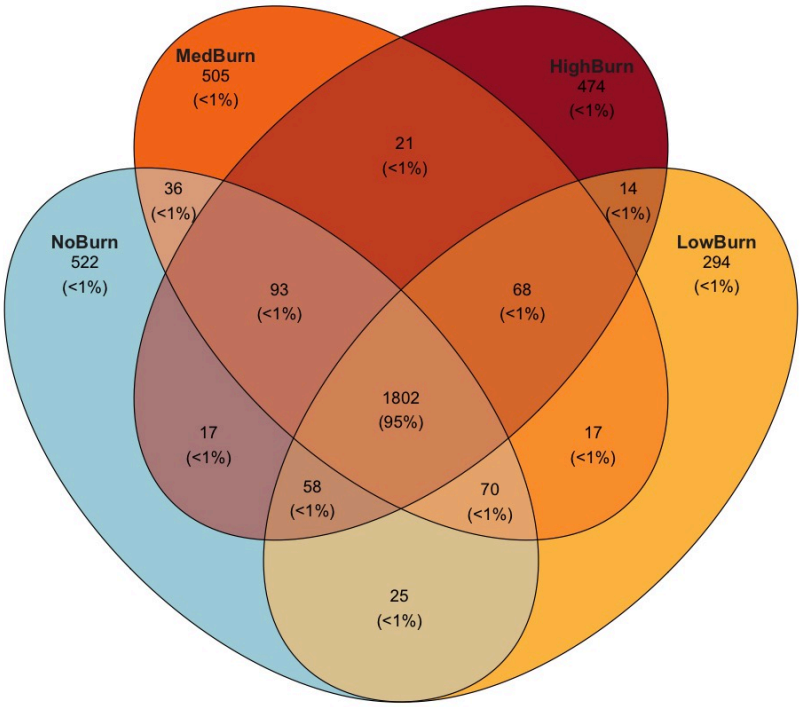
Supplementary Figure 11. Core microbiome of the fungal community (A) and the bacterial community (B).

Each section contains the number of ASV common to the overlap of the group as well as the relative abundance (in parentheses) these ASVs represent in the dataset.

A.

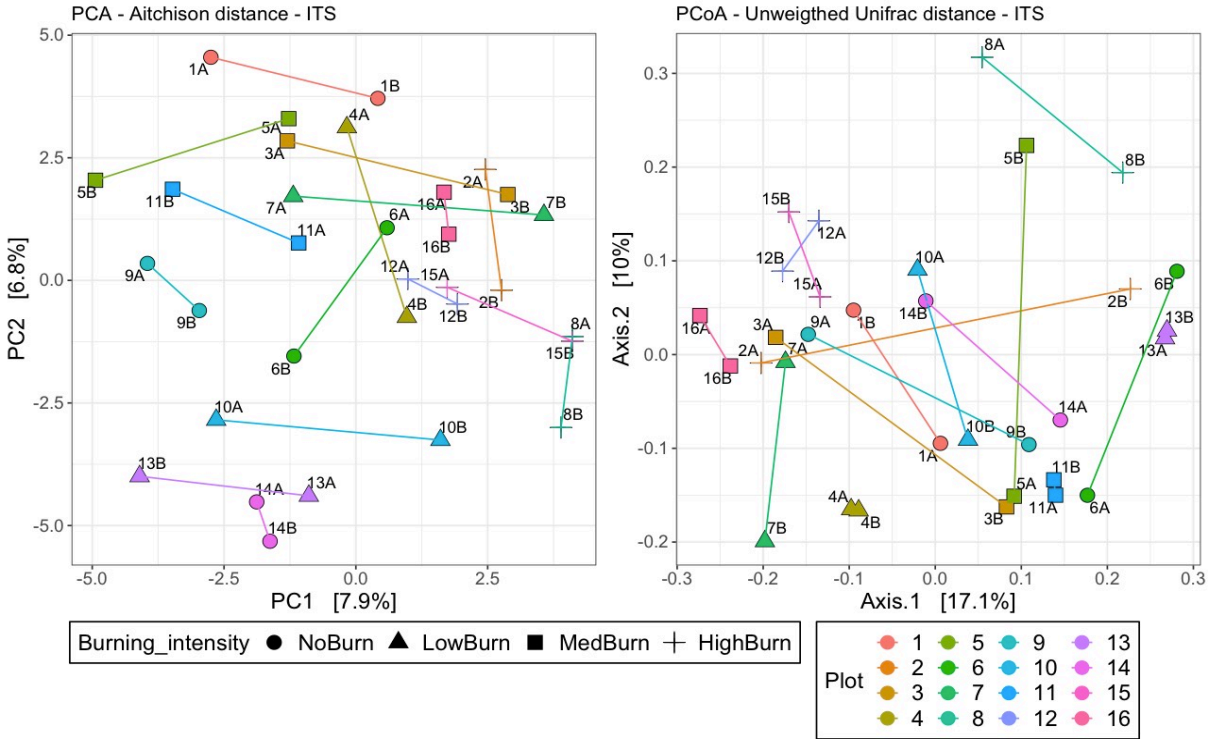


B.

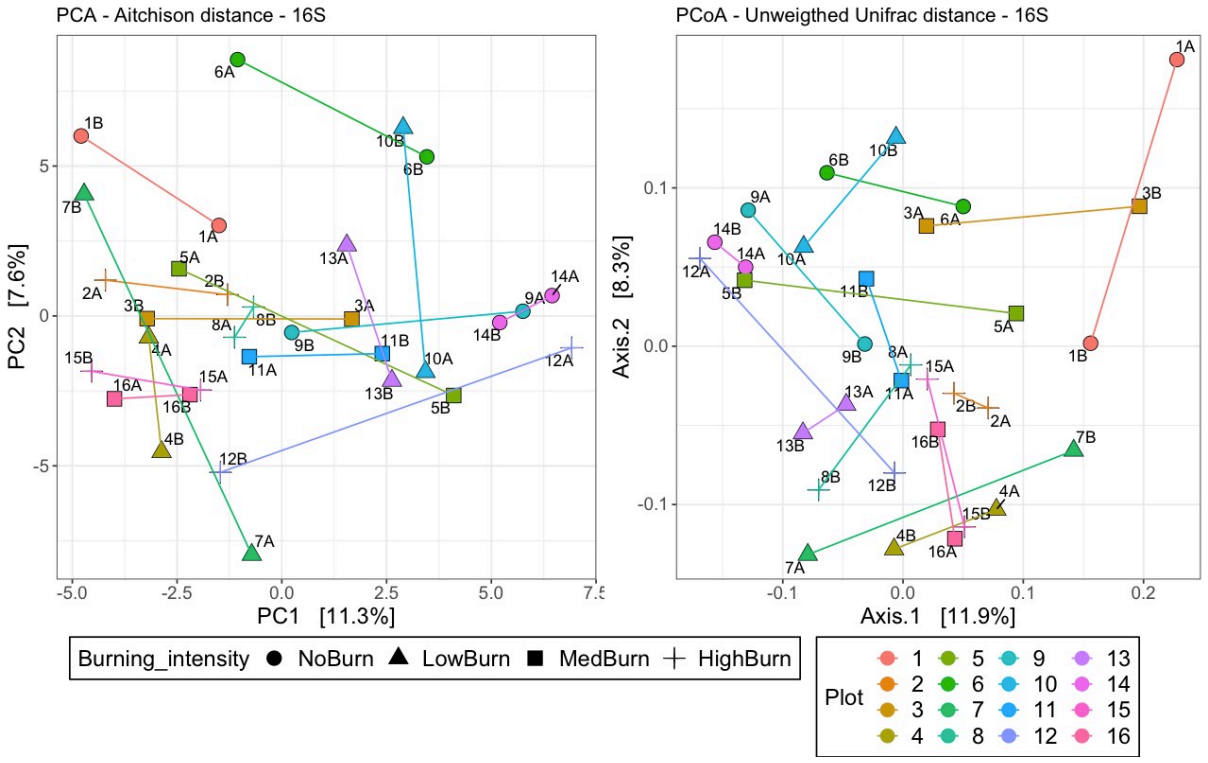


Supplementary Figure 12. Beta diversity of pseudo-replicate using the Aitchison and the unweighted Unifrac distances. A. Fungal community, B. Bacterial community.

A.



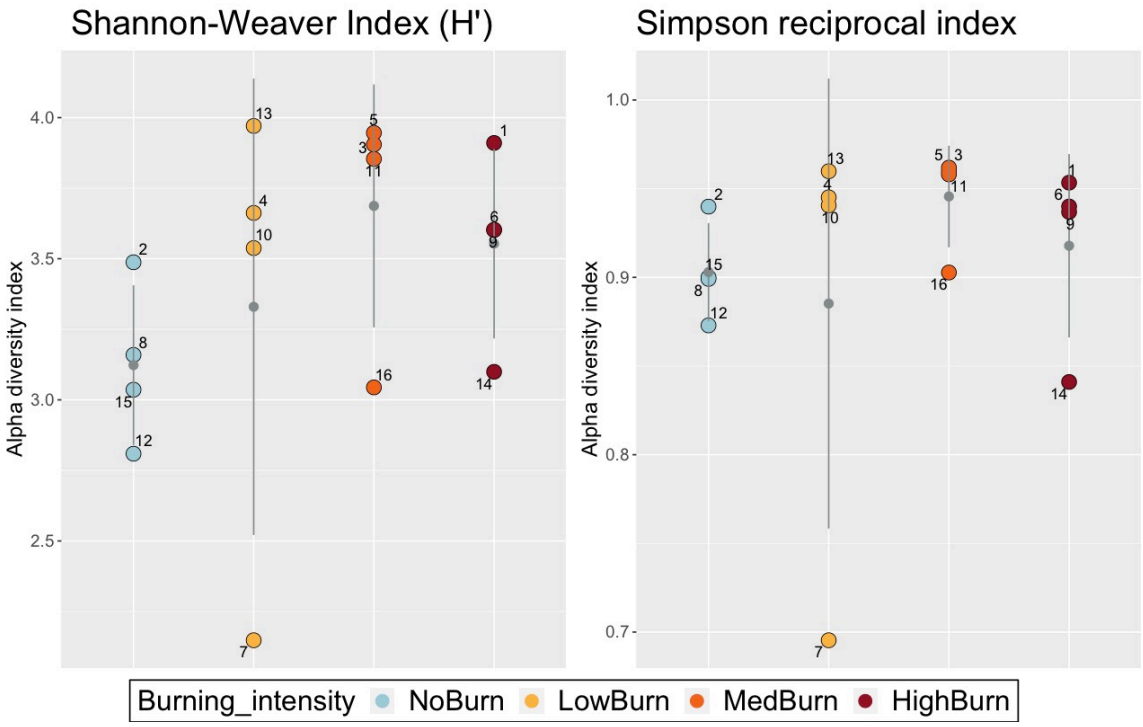
B.



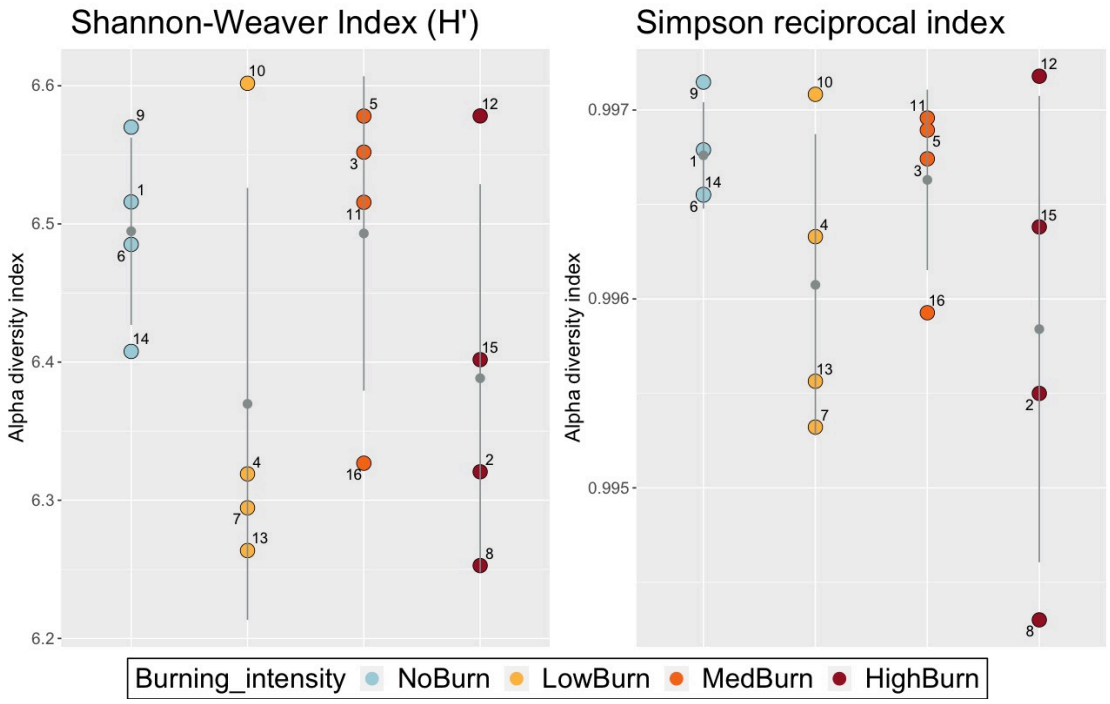
Supplementary Figure 13. Alpha diversity using Shannon-Weaver and Simpson reciprocal indices.

A. Fungal community, B. Bacterial community.

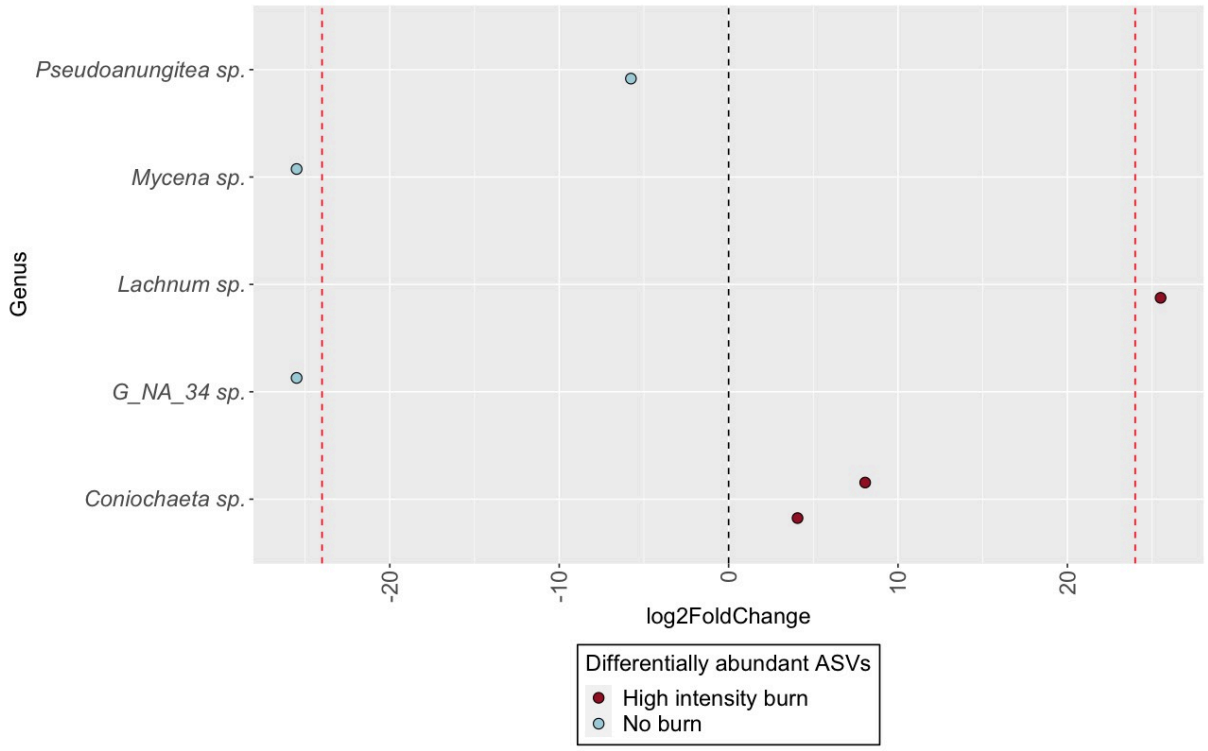
A.



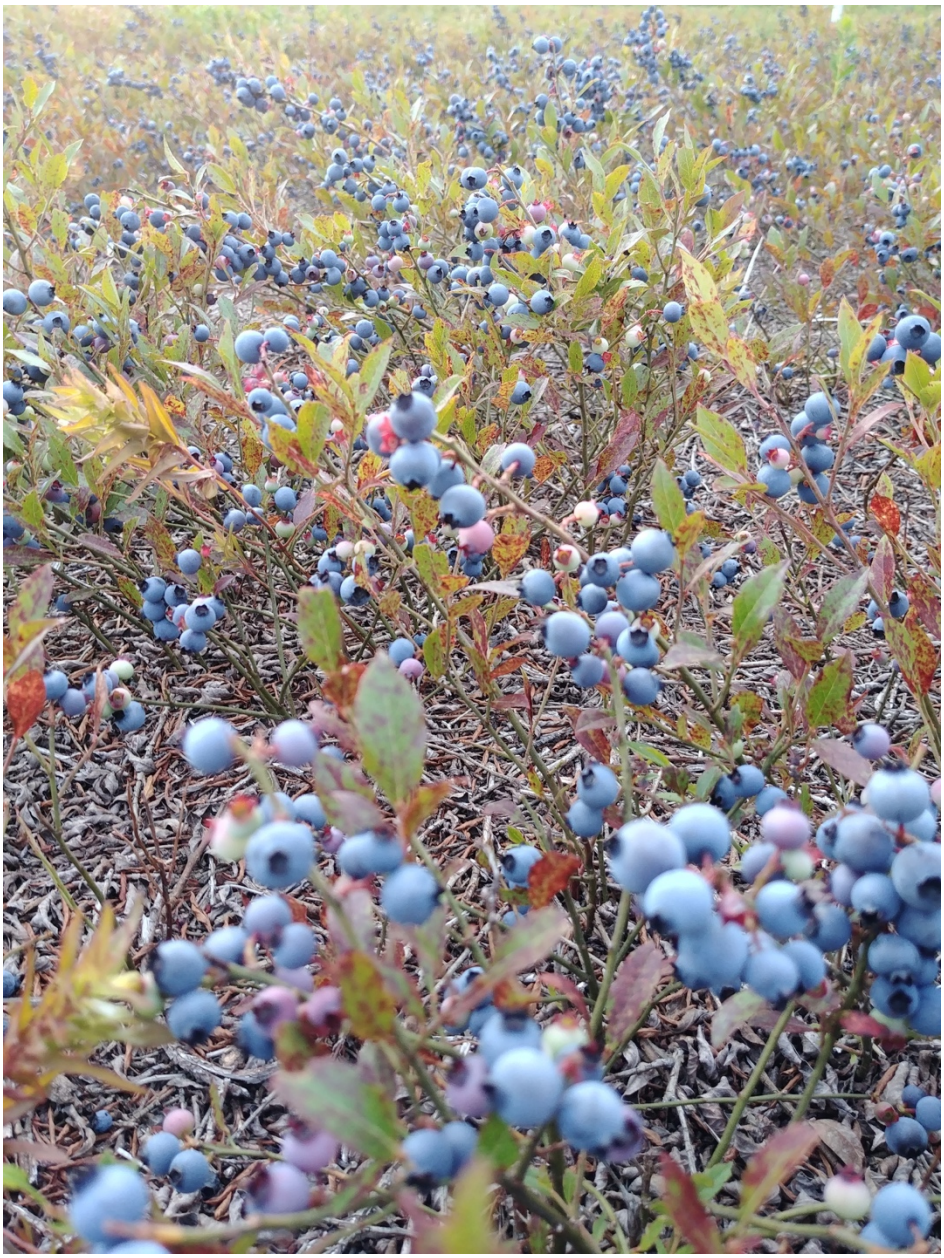
B.



Supplementary Figure 14. Differential abundance of fungal ASVs between the negative control and the highest burning intensity computed with DESeq2.



Chapitre 4 – Quels sont les effets de la fertilisation sur le microbiote du bleuetier sauvage ?



Bleuets sauvages prêts à être dégustés à la Bleuetière d'Enseignement et de Recherche, Normandin, Québec.

How does fertilization impact the wild blueberry microbiome ?

Simon Morvan[1*], Maxime C. Paré[2], Jean Lafond[3], Mohamed Hijri[1,4*]

[1] Institut de Recherche en Biologie Végétale, Département de sciences biologiques, Université de Montréal, 4101 Sherbrooke Est, H1X 2B2, Montréal, QC, Canada

[2] Laboratoire sur les écosystèmes boréaux terrestres (EcoTer), Département des Sciences Fondamentales, Université du Québec à Chicoutimi, Chicoutimi, QC, Canada

[3] Direction générale des sciences et de la technologie, Agriculture et Agroalimentaire Canada, Gouvernement du Canada, Normandin, QC, Canada,

[4] AgroBioSciences, Mohammed VI Polytechnic University, Lot 660, Hay Moulay Rachid, Ben Guerir 43150, Morocco

*** Correspondence:** simon.morvan@umontreal.ca / mohamed.hijri@umontreal.ca

Key words: Wild blueberry, *Vaccinium angustifolium* Ait., Microbial community, Amplicon Sequencing, Fertilization.

Draft in preparation for submission

Abstract

Wild blueberries (*Vaccinium angustifolium* Ait. and *V. myrtilloides* Michaux) production is regarded as less intensive than other agricultural systems, although several agricultural practices are commonly implemented to increase crop yields and to mitigate pets and pathogen attacks. Fertilization is generally applied during the vegetative year to increase the nutrient concentration in soils that are characterized by low nutrient availability. Whether organic or mineral, this nutrient amendment results in increased fruit yields. Blueberries are also known to rely on their microbiome to overcome the lack of nutrient availability in the soil. The symbiosis established between their roots and ericoid mycorrhizal fungi is thought to be crucial as these symbionts provide additional nutrients to their host plant. As fertilization can alter crop microbial communities, our study aimed to measure the impact of this practice in a wild blueberry setting, focusing on the bacterial and fungal communities found in the roots and rhizosphere of *Vaccinium angustifolium* Ait. We also looked at the effects of fertilization during the harvesting year (more than a year after fertilization) in order to see if there were any long-term impacts of this practice on the microbial community. Our study indicates that fertilization, whether mineral or organic, has a minimal effect on microbial communities, although it significantly increases yields (930 kg/ha for unfertilized plots compared to 1600 kg/ha and 1965 kg/ha for organic and mineral amendments, respectively) as well as plant nutrition. One year after application, fertilization does not seem to have a negative repercussion on the ericoid mycorrhizal (ErM) fungal community as no significant differences were observed in terms of relative abundance on known and putative ErM taxa between the control and the two fertilizing treatments. However, studies on the effect of repeated fertilization at a longer time scale are needed to ensure that it does not cause a shift in microbial communities that could be detrimental in the long run.

Introduction

Blueberry culture and agricultural practices

Wild blueberries, despite their name, are cultivated in Canada (Quebec and maritime provinces) and the United States of America (Maine). The vernacular name encompasses multiple species, mainly *Vaccinium angustifolium* Ait. and *Vaccinium myrtilloides* Michaux, that coexist in commercial blueberry fields (Yarborough, 2012). Contrary to the highbush blueberries culture (*V. corymbosum*) which resembles other fruit production with selected and planted cultivars, wild blueberries are grown from pre-existing stands that are usually found in abandoned farms or in the boreal forest understory. Once the land is cleared out of other plants and trees, wild blueberries propagate mainly through their rhizomes to progressively cover the entire field (Yarborough, 2012). This absence of sowing, transplanting or breeding explains the name of wild blueberries. However, similarly to other crops, producers manage their fields in order to maximize fruit yield. Among these agricultural practices are : pruning once every other year (Yarborough, 2004), pesticides applications to reduce problematic insects (Drummond and Groden, 2000; Collins and Drummond, 2004; Drummond et al., 2019), fungal diseases (Dedej et al., 2004; Esau et al., 2014; Hildebrand et al., 2016) and weeds (Jensen and Yarborough, 2004; Kennedy et al., 2010; Li et al., 2014) as well as fertilization (Eaton and Patriquin, 1988; Jeliazkova and Percival, 2003; Yarborough, 2004).

Fertilization effect on blueberry performance

Wild blueberries belong to the Ericaceae plant family along with cranberries (*Vaccinium macrocarpon*), rhododendrons (*Rhododendron* sp.) or heather (*Calluna vulgaris*). These plants are known to grow in acidic soils with low nutrient availability and high concentrations of trace metals. Essential nutrients are either contained in organic matter that cannot be directly absorbed by plants or bound to metals, mainly aluminum or iron in the case of phosphorus (Ohno and Severy, 2013)**.** Wild blueberries are adapted to this environment and have relatively low nutrient requirements (Hall, 1978; Lafond, 2009). Nevertheless, studies have found that fertilizers could increase fruit productivity (Penney and McRae, 2000; Gagnon et al., 2003; Lafond, 2004;

Starast et al., 2007; Maqbool et al., 2017). In Quebec, producers generally fertilize during the year following pruning using either mineral and/or organic amendments. Organic fertilizers have the advantage of slowly releasing nutrients, which could be more in phase with the wild blueberry nutrition requirements (Mallory and Smagula, 2012). However, organic fertilizers have the tendency to increase soil pH which can have a negative effect on ericoid mycorrhizal fungi, as they are adapted to acid soils (Haynes and Swift, 1985; Caspersen et al., 2016). Regarding their efficiency, Mallory and Smagula (2012) found no significant difference when they compared yields from fields fertilized with either mineral diammonium phosphate (DAP) or organic fertilizers (seafood waste compost and commercial organic fertilizers) (Mallory and Smagula, 2012). One of the downsides of mineral fertilization, with mineral nitrogen in particular, is the ability of common blueberry weeds to uptake this element more efficiently. Therefore mineral nitrogen fertilization can generate weed proliferation if nothing is done to hamper their development, resulting in the opposite intended effect on blueberry yield (Penney and McRae, 2000; Marty et al., 2019).

Wild blueberry microbial communities

The Ericaceae plant family forms a typical symbiosis within their roots with ericoid mycorrhizal fungi (Leopold, 2016). These fungi produce a myriad of extracellular enzymes, allowing them to efficiently degrade multiple organic molecules present in the soil matrix (Kerley and Read, 1995; Cairney and Burke, 1998; Read et al., 2004; Martino et al., 2018). Nutrients released through this process are absorbed by those fungi and transferred to the Ericaceae hosts in exchange of carbohydrates produced by photosynthesis (Pearson and Read, 1973b). Therefore, these fungal communities can play a major role in their host nutrition (Vohník et al., 2005) and studies have shown that inoculation with different ericoid mycorrhizal fungi increased plant growth and/or yield (Wei et al., 2016; Wazny et al., 2022). It has even been stated that the ability of wild blueberries to thrive in their harsh environment is due to this mycorrhizal symbiosis (Cairney and Meharg, 2003; Mitchell and Gibson, 2006). Furthermore, ericoid mycorrhizal fungi could also protect their host against trace metals toxicity through exclusion mechanisms (precipitation or fixation of metal ions outside of the plant cell, suppression of influx transporters, increased efflux of metal ions) or sequestration mechanisms (translocation of metal ions into subcellular organs

where they are less toxic)(Perotto et al., 2002; Meharg, 2003; Mitchell and Gibson, 2006). All of the mechanisms are not all well understood but undergoing research on *O. maïus* metal tolerance has shown an increased production of Cu/Zn superoxide dismutase when in presence of high concentrations of zinc or cadmium. This enzyme helps to control the accumulation of reactive oxygen species in cells and therefore reduces oxidative stress. As this enzyme is a metalloenzyme, it could also reduce the toxicity of heavy metals by binding to metal ions (Daghino et al., 2016). Gene expression of *O. maïus* in symbiosis with *V. myrtillus* in the presence of cadmium was also studied and the researchers identified many genes encoding permease-type transmembrane transporters among the overexpressed genes (Casarrubia et al., 2020). Finally, other authors have shown that they increase their host tolerance to abiotic stress such as drought (Mu et al., 2021). Hence, a better understanding of these communities is important to minimize any disturbance of these beneficial fungi, and to try to harness their ecological service. In addition to the fungal community, bacteria are also regularly explored when studying plants' rhizosphere and root microbiome (Adesemoye et al., 2009; Yang et al., 2009; Berendsen et al., 2012). To date, only a limited number of investigations have focused on the bacterial communities of wild blueberries or Ericaceae in general. However, bacteria could also be of importance, as studies have reported that Rhizobacteriales represented a large portion of wild blueberry rhizosphere bacterial communities. Furthermore, some of these Rhizobacteriales identified have previously been reported to be capable of nitrogen fixation, a number of which correlated to the wild blueberry leaf nitrogen content (Morvan et al., 2020; Morvan et al., 2022). Nevertheless further investigations are required to demonstrate nitrogen fixation by these bacteria in a wild blueberry soil setting. Additionally, the Rhizobacteriales order was also found to be abundant in multiple Ericaceae species (Timonen et al., 2017). Several other bacteria found in abundance in a previous study (Morvan et al., 2022) have previously been characterized and produce carbohydrate-active enzymes, enabling them to degrade multiple carbon sources, as is the case for several subdivisions of the Acidobacteriales order (Kielak et al., 2016), and Isosphaerales order (Kulichevskaya et al., 2016). Consequently, the bacterial community could also be of importance for the wild blueberry nutrition.

Fertilization effect on microbial communities

Fertilization is a common agricultural practices by which nutrients are added to the soil to mitigate its nutrient deficiency. This intervention provides additional nutrients to the crop but can also strongly influence the plant's microbiome (Weese et al., 2015 ; Li et al., 2020; Beltran-Garcia et al., 2021). Indeed, the input of nutrients can have different impacts depending on: the plants species and growth stage, cultivation condition (agricultural or unmanaged), the nature and composition of the nutrients applied (mineral or organic), the concentration, the application rate and the duration of fertilization (short or long term).

Focusing on nitrogen (N) which is one of the limiting nutrient, long-term mineral N fertilization trials have shown that microbial biomass increases by an average 15.1% in agricultural settings (if soil pH > 5) (Geisseler and Scow, 2014) while similar studies in unmanaged settings show the contrary with a decrease of 15% of the microbial biomass (Treseder, 2008). Mineral N fertilization boosts plant productivity which therefore tends to increase soil organic matter as it increases plant rhizodeposition. In turn, microbial biomass increases as it is positively correlated to soil organic matter (Geisseler and Scow, 2014). An explanation for the negative effect observed in unmanaged land is that N input under ammonium form causes a decrease in pH due to the nitrification process (Geisseler and Scow, 2014). This decrease in pH is generally countered in agricultural fields by adding lime whereas it is not the case in unmanaged lands. As pH is among the main important factors that influence soil microbiota composition (Fierer and Jackson, 2006; Rousk et al., 2010) and biomass (Rousk et al., 2009), this acidification may explain the biomass decrease. Additionally, N fertilization in a nitrate form has little effect on soil acidity (Malhi et al., 2000) and changes in soil bacterial biomass, composition and activity also seem alleviated when compared to ammonium fertilization (Enwall et al., 2007; Hallin et al., 2009). In a meta-analysis, a decrease in fungal biomass with N amendment (no matter the mineral form) was found to increase as total load and duration of fertilization increased (Treseder, 2008). However, bacteria biomass was not significantly affected by N fertilization. Phosphorus (P) fertilization, another limiting macronutrient in the soil, also causes shifts in microbial communities (Ducousso-Détrez et al., 2022). For instance, phosphorus sources (superphosphate or rock phosphate) explains

39.1% and 45.77% of the bacteria and fungi variability, respectively, in a sugarcane fertilization experiment (Gumiere et al., 2019). In a 40 year-long phosphorus fertilizer field trial in pastures (Wakelin et al., 2012) found that bacterial community composition did not vary overall, but certain taxa, such as Actinobacteria, had their structure impacted by phosphorus addition. Additionally, fungal composition also significantly varied when comparing control to fertilized soils. However, the difference in community composition between fertilized soils was small, even if they had different fertilizer rates, suggesting that the shift observed is not dose dependent. This variation was even stronger for the community of arbuscular mycorrhizal fungi, with different species either being more or less abundant in the fertilized or unfertilized plots (Wakelin et al., 2012).

Wild blueberries are rather uncommon as the soil in which they grow is already acidic and these plants require relatively low amounts of nutrients. For instance, the recommended dose of ammonium sulfate in Quebec for wild blueberry is 50 kg $N.ha^{-1}$ (Marty et al., 2019), whereas for spring wheat for example, the recommended N rate ranges from 90 to 120 kg $N.ha^{-1}$ depending on soil texture (Nyiraneza et al., 2012). Although the mineral nitrogen fertilizers recommended for wild blueberry are ammonium-based (Lafond, 2009), the lower concentrations applied compared to other commercial crops might mitigate soil acidification and therefore lower the impact on the soil micro-organisms. To date, we only found a single study that has looked into the impact of fertilization on the wild blueberry microbiota. Testing different rates of N-P-K fertilizers (from 0 to 60 $kg.ha^{-1}$), their results show that ericoid mycorrhizal colonization was not impacted by fertilization but found a significant increase of this colonization when applying 35 $kg.ha^{-1}$ of N in the form of urea (Jeliazkova and Percival, 2003). Looking at other *Vaccinium* species, mycorrhizal colonization was significantly higher in *V. corymbosum* roots with organic fertilizers compared to mineral fertilizers at equal doses N-P-K (60-60-60 $kg.ha^{-1}$) (Montalba et al., 2010). Higher mycorrhizal colonization in organic production could be higher due to the form of nitrogen applied (Sadowsky et al., 2012). Finally, in a phosphorus addition trial, Pantigoso *et al*. (2018) measured a shift in bacterial community composition in highbush blueberry rhizosphere when comparing low P (0 and 50 $kg.ha^{-1}$) to high P (101 and 192 $kg.ha^{-1}$) (Pantigoso et al., 2018).

On two other Vaccinium species (*V. myrtillus* and *V. vitis-idaea*) in natural settings (spruce and pine forests), N addition under ammonium nitrate form at the rate of 0, 12.5 or 50 kg.ha^{-1} during multiple years (12 or 4 years depending on the forest type) induced no significant change on the root-associated fungal community composition nor on the assumed ericoid mycorrhizal species (Ishida and Nordin, 2010).

The aim of our study was to document the effects of two types of fertilizers (organic and mineral forms) on soil chemistry, blueberry performance and on fungal and bacterial root and rhizosphere microbiota. Additionally, as fertilizers are only applied during the vegetative growth stage, we wanted to see if we could see a fertilizer effect on the fungal and bacterial communities, a year after its application. Our hypotheses were: (1) fertilization shifts fungal and bacterial community composition ; (2) this composition shift would be stronger in the pruning growth stage than in the harvesting growth stage. To address these hypotheses, we established an experiment in a random block design in a blueberry research field in Quebec, Canada where fertilizers were applied at the beginning of spring 2019 for the vegetative growth stage fields. The harvesting growth stage fields were fertilized in spring 2018. Blueberry agronomic variables were measured in all treatments during the 2019 season. Soil and root samples for microbial characterization were collected at the end of summer 2019 for both the pruning and harvest fields. In order to analyze the fungal and bacterial community of the roots and rhizosphere, we used amplicon sequencing, targeting the 16s rDNA region and ITS region, with the Illumina MiSeq technology.

Materials and Methods

Experimental design

The experiment took place at the Bleuetière d'Enseignement et de Recherche located in Normandin, Quebec (48°49'40.2"N 72°39'36.9"W) in the North temperate zone (mean annual temperature: 0.9°C, annual precipitation: 871 mm (Government of Canada, 2021). This field was converted to blueberry production in 2005, and has been attributed for research purposes since 2016. The experiment took place in four adjacent fields, half containing the harvesting growth

stage fields and the other the pruning growth stage fields. In each field, two blocks contained three 15x22m plot fertilization treatments (mineral, organic, none) resulting in four replicates for each fertilization and growth stage status for a total of 24 plots.

Fertilization was applied during the pruning growth stage for all treated plots, before plant emergence, in early June. Therefore, in our study the pruning growth stage plots were fertilized in June 2019, and the harvesting growth stage plots, in June 2018. The mineral fertilization consisted in applying ammonium sulfate, triple superphosphate, potassium sulfate and borate (Millibore) at a rate of 50 $kg.ha^{-1}$, 30 $kg.ha^{-1}$, 20 $kg.ha^{-1}$ and 1.24 $kg.ha^{-1}$ respectively. The organic fertilization provided the same concentration of N, P, K and B but using Actisol (5-3-2), a commercial natural hen manure fertilizer as well as the same dose of borate (Millibore).

Soil chemical properties

Soil samples were extracted on the 29th of September 2019 in both the pruning and harvesting plots, using a soil corer with a 2.54 cm (1 inch) diameter at a rate of three pseudo-replicates per plot. The three pseudo-replicates were pooled into one sample once the organic layer (0 cm to 5 cm depth) and mineral layer (5 cm to 20 cm depth) were separated in the lab. Both layers were dried and sieved through a 2 mm mesh. Soil pH was measured using distilled water at a rate of 1:2 (Hendershot et al., 2007). Phosphorus, potassium, calcium and magnesium were extracted using Mehlich 3 solution (Ziadi and Tran, 2007). Phosphorus was quantified using colorimetry (Murphy and Riley, 1962), potassium with flame emission spectrophotometry while an atomic absorption spectrophotometer was used for calcium and magnesium (Perkin Elmer AAnalyst 300, Überlingen, Germany). Finally, nitrate, nitrite and ammonium were also measured by proceeding with a 1 M KCl extraction followed by a colorimetric dosage on a Lachat colorimeter (Hach Company, USA) using Quikchem® Method 12-107-06-2-A for ammonium, and Quikchem® Method 12-107-04-1-B for nitrate and nitrite (Maynard et al., 2007).

Agricultural data

For the pruning year, leaves nutrient concentrations (N, P, K, Ca and Mg) were measured after a humid digestion of dried crushed leaves samples with a mixture of selenous acid and sulfuric acid - peroxyde (Isaac and Johnson, 1976; Lafond, 2009). For the harvesting year, these nutrients were

measured in fruits instead of leaves, using the same method. Yields were also measured during the harvesting year using a mechanized harvester with a 1.5 m wide comb (rake) mounted on a Kubota F90 series tractor. The fruits were weighed directly in the field, but a sub-sample of 1 kg of blueberries was cleaned in the lab to remove debris (leaves, stems). The proportion accounted by the weight of debris was measured to apply a correction on the total fruit yield harvested.

Microbial community analyses

Sampling

For the microbial community analysis, samples were collected between the 5th and 14th of August 2019. The sampling consisted in the collection of three, 10 cm wide and approximately 5 cm deep, clumps of organic soil per plot, containing blueberry rhizomes. Samples were placed in Ziploc bags, kept and transported into a cooler on ice until they could be placed at -20°C. Frozen samples were thawed to extract clumps of roots and soil from the original sample. The clumps obtained from the three pseudo-replicates were pooled into 50 mL Falcon tubes with pierced cap in order to freeze-dry the samples. The freeze-dried soil samples were then sieved through 1 mm mesh to remove coarse organic matter and pebbles. Roots and rhizomes were picked out of the sieve and placed in separate Ziploc bags. The resulting soil material, here forth named rhizospheric soil, contained thin root fragments that couldn't be removed as well as organic matter. The rhizospheric soil was ground to homogenize the material to a fine powder. For the roots, we selected roots fragments with a diameter <1 mm in order to focus on the youngest roots and made sure to manually eliminate any visible soil particles.

DNA extraction and PCR amplification

The DNA extraction, amplification and sequencing protocol was similar to the one described in a previous study (Morvan et al., 2022). For the rhizosphere, 150 mg of soil were weighed to carry out the DNA extraction using the DNeasy PowerSoil kit (Qiagen, Toronto, ON) following the manufacturer's protocol with the following modification: for cell lysis, samples were placed in TissueLyser II (Qiagen, Toronto, ON) instead of a vortex for 14 cycles of 45s each at speed 4.0. For

the roots, we used 20 mg of ground roots to extract DNA using the DNeasy Plant kit (Qiagen, Toronto, ON) following the manufacturer's instruction.

In addition to the DNA sample extraction, we included two negative controls consisting of DNA extraction of autoclaved and filtered water instead of either the roots or the rhizosphere soil. An additional PCR negative control was included by Genome Quebec. Two mock communities, fungal and bacterial, were also included in the sequencing procedure as positive controls. The fungal mock community was designed by Matthew G. Bakker and contains 19 fungal taxa (Bakker, 2018). In our experiment, we used the "even community" containing an equal amount of the 18S rRNA gene. The bacterial mock community contained 20 species (Table S1) with equimolar counts (10^6 copies/μL) of 16S rDNA genes (BEI Resources, USA). DNA extracts were stored at -20°C until they could be sent for amplification and sequencing by Genome Québec Innovation Center (Montréal, QC, Canada).

For the bacterial community, we targeted the V3-V4 region of 16S rDNA with the primers 341(F) and 805(R) resulting in an expected amplicon size of approximately 464 base-pair long (Mizrahi-Man et al., 2013). The sequencing platform (Centre d'expertise et de services Génome Québec, Montréal, QC) uses four versions of each primer (staggered primers) in order to increase base diversity in the Miseq flow cell. For the fungal community, we used the ITS3KYO(F) and ITS4(R) primers to target the ITS2 region located between the 5.8S and LSU region of the ribosomal RNA gene (Toju et al., 2012). The approximate amplicon length obtained with this pair of primers was 330 base-pair but this region is known to have varying length (Toju et al., 2012). Both sets of primers were coupled to CS1 (forward primers) and CS2 (reverse primers) tags that allow for barcoding. A second PCR was used to add a unique barcode per sample as well as i5 and i7 Illumina adapters that bind to the flow cell. Sequencing was conducted on an Illumina MiSeq using a paired-end 2 x 300 base-pair method (Illumina, San Diego, CA, USA).

Sequencing data processing

The sequencing data processing was identical to the one used in Morvan *et al.*, (2022) as the sequences originated from the same sequencing run (Morvan et al., 2022). Briefly, we used the DADA2 pipeline (Callahan et al., 2016a) in R (R Core Team, 2021) to process the raw fastq files. We processed the mock communities on their own in order to avoid any influence of our samples on the inferred mock ASVs. For both the fungal and bacterial datasets, we used cutadapt (Martin, 2011) in order to remove the primers, their complements, reverse and reverse complements by indicating the primers' nucleotide sequences. For the fungal dataset, we proceeded with the filtering step using maxEE(2,2) and minLen(50) which removes sequences that are shorter than 50 nucleotides long. Regarding the bacterial dataset, we set truncLen to (270,240) and maxEE (2,2) based on a visual assessment of the quality profiles. After the different filtering step both bacterial and fungal dataset followed the same pipeline that used the default settings except those mentioned hereafter. In the learning error rates step, we used randomize = TRUE, in the sample inference step, we used "pseudo" as a pooling method and accordingly the "pooled" method for the bimera removal step. In order to add a taxonomy assignment to our inferred ASVs, we used the implemented naïve Bayesian RDP classifier with the *assignTaxonomy()* function. For the bacterial dataset, we used the SILVA reference database and two UNITE databases for the ITS sequences. Based on the taxonomy obtained, we removed non-bacterial ASVs as well as ASVs that were annotated as chloroplasts and mitochondria in the 16S dataset. The UNITE fungal reference database resulted in every ASVs to be labelled as Fungi but many sequences did not have an assigned Phylum. We compared this taxonomic assignment to a second one obtained by using UNITE's eukaryotic reference database. Most of the unknown phyla fungi were labelled as non-fungal when using the eukaryotic reference database and were therefore removed in the ITS dataset. We then proceeded to further refine our datasets by removing singletons and doubletons (ASVs with a total abundance of 1 or 2) as they may be sequencing artefacts.

Statistical analyses

All analyses were performed in R version 4.1.1 (R Core Team, 2021) and figures were generated using the ggPlot2 R package (Wickham, 2016). We looked at each growth stage separately as the harvesting and pruning blocks were not grouped together and had a different fertilization legacy.

Soil and agronomic data

We computed linear mixed models (LMM), with fertilization and the variable of interest as fixed factors and blocks as random effects, followed by ANOVA tests to detect statistical differences between groups (compartment or fertilization). We used the lmerTest R package (Kuznetsova et al., 2017), which implements a Type III Analysis of Variance using Satterthwaite's method.

Microbial data

To check if our sequencing depth allowed to capture most of the bacterial and fungal communities, we generated rarefaction curves using the *rarecurve()* function of the vegan R package (Oksanen et al., 2020). We used the phyloseq R package (McMurdie and Holmes, 2013) to facilitate data handling and to generate alpha diversity and beta diversity plots. We used Metacoder (Foster et al., 2017) for a more thorough analysis of the taxonomy of each dataset by plotting the ASV number and the relative abundance per taxa up to the genus level using the *heat_tree()* function.

Phylogenetic trees

In order to take into account the phylogenetic distance between the inferred ASVs, we built a phylogenetic trees following the method used in (Callahan et al., 2016b). First, a sequence alignment was generated using *AlignSeqs()* from DECIPHER (Wright, 2016). We then used the phangorn package (Schliep, 2011) to compute a distance matrix under a JC69 substitution model using *dist.ml().* A neighbour-joining tree was then assembled onto which we fitted a generalized time-reversible with Gamma rate variation (GTR + G+I) model using *optim.pml().* The resulting trees were then added to their respective phyloseq objects.

Alpha and beta diversity

The Simpson and Shannon-Weaver alpha diversity indices were computed using the *plot_richness()* function of phyloseq. Statistical differences between the different fertilization treatments were checked using one-way ANOVA's in a similar fashion than for soil and agricultural data (see above).

To assess the similarity of microbial communities, we relied on a beta diversity analysis using two different dissimilarity metrics. We took into account the compositional nature of sequencing data by choosing appropriate dissimilarity metrics (Gloor et al., 2017). First, we chose the Aitchison distance which consists in transforming the sequence abundances using a centered log ratio transformation before computing the Euclidean distance between sites (Quinn et al., 2018). Second, to take into account the phylogenetic distance between the sequences, we used the phylogenetic isometric log-ratio transformation PhILR before computing the Euclidean distance between sites (Silverman et al., 2017). Principal component analysis (PCA) ordinations based on both of these dissimilarity allowed to visualize the samples resemblance based on their microbial communities. To test the significance of the differences observed, we used vegan's *adonis2()* function with 999 permutations and taking into account the experimental blocks. The homogeneity of dispersion assumption was checked using *betadisper()* and *permutest()* both in the vegan R package (Oksanen et al., 2020).

Representative taxa

Metacoder was used as a finer approach than the beta diversity analysis, to detect significant differences in each taxonomic rank between the burning intensities (Foster et al., 2017). The *compare_groups()* function was used after quantifying the per-taxon relative abundance using *calc_taxon_abund().* This function computes a nonparametric Wilcoxon Rank Sum test to detect differences taxon abundance in different treatments. In order to take into account the multiple comparisons, we corrected the p-values of the Wilcoxon Rank Sum test with false discovery rate (FDR) correction using the *p.adjust*() function of the stats R package (R Core Team, 2021). After setting a threshold of 0.05 for the p-value, we used the *heat_tree_matrix()* to plot statistically different taxa based on their abundance using the log2 ratio of median proportions. Additionally, we also proceeded with an indicative species analysis to identify specific species representative

of predefined groups of samples based on the species abundance and fidelity. Prior to this analysis, we agglomerated the ASVs at species level based on the obtained taxonomy. The significance of the results were obtained with permutation tests (9999 permutations) followed by an FDR correction to account for multiple testing. We used the *multipatt()* function of the indicspecies R package (de Cáceres and Legendre, 2009).

Redundancy analysis

We used constrained ordination in the form of distance-based redundancy analysis (db-RDA) to detect if the variation in the microbial datasets was linked to the variation of other variables such as fertilization, soil chemistry or agricultural variables. We used the distance matrices used for the beta diversity (Aitchison and PhILR distances) as the response variable and either the fertilization treatments, soil chemistry or agricultural variables as the explanatory variables. The fertilization treatments were converted to dummy variables (Legendre and Legendre, 2012). The soil chemistry and agricultural variables were standardized and we removed collinear variables prior to the db-RDA. The significance of the analysis was tested using permutation tests with the *anova.cca()* function of the vegan R package (Oksanen et al., 2020).

Accession numbers

Raw sequences will be deposited in the GenBank SRA database.

Results

Influence of fertilizers on soil chemistry

Fertilization had a very limited impact on soil chemistry in both of the growth stages sampled. In the pruning growth stage, only pH in the organic soil layer is different between the fertilization treatments with a significantly higher pH in the organic fertilized plots (pH = 4.58 ± 0.26) compared to the mineral fertilized plots (pH = 4.12 ± 0.35). In the harvesting growth stage, we found a significantly higher phosphorus concentration in the organic soil layer of the mineral fertilized plots (22.98 ± 4.67) compared to the control (17.00 ± 3.54) (Table 1). The fertilization treatments do not have significant effects on the mineral soil layer chemistry.

Table 1. Soil pH and chemistry in both growth stage according to fertilization treatment. “O” and “M” stand for organic soil layer and mineral soil layer respectively. ANOVAs were performed using Type III Analysis of Variance Table with Satterthwaite's method, appropriate transformations (log or square-root) were used when necessary to satisfy the normality assumption. The model used was a linear model with the variable of interest and fertilization as fixed factors and block as a random factor. Letters indicate a significant difference ($p < 0.05$, post-hoc Tukey test) in a given variable when comparing fertilization treatments. We did not proceed with statistical testing in certain cases for nitrate, nitrite and ammonium as the values obtained had a high variability (standard deviation superior to mean value) and that values were close to 0.

Pruning stage		ANOVA	Fertilization		
			Control	Organic	Mineral
pH	O	F = 3.31 ; p = 0.1073	**4.46 ± 0.21 ab**	**4.58 ± 0.26 b**	**4.12 ± 0.354 a**
	M	F = 2.28 ; p= 0.1836	4.97 ± 0.15 a	4.99 ± 0.18 a	4.77 ± 0.23 a
P ($mg.kg^{-1}$)	O	F = 1.19 ; p = 0.3672	23.92 ± 3.51 a	34.59 ± 16.69 a	26.15 ± 9.99 a
	M	F = 0.49 ; p = 0.6346	26.50 ± 17.18 a	17.92 ± 16.45 a	20.24 ± 4.73 a
K ($mg.kg^{-1}$)	O	F = 2.18 ; p = 0.1944	104.27 ± 30.79 a	237.61 ± 164.13 a	136.51 ± 57.36 a
	M	F = 0.77 : p = 0.4928	18.74 ± 5.43 a	21.97 ± 3.60 a	19.70 ± 0.87 a
Ca ($mg.kg^{-1}$)	O	F = 2.75 ; p = 0.1166	720.35 ± 296.47 a	1344.65 ± 478.86 a	800.33 ± 430.69 a
	M	F = 0.71; p = 0.5275	30.54 ± 14.88 a	41.42 ± 23.16 a	32.70 ± 18.40 a
Mg ($mg.kg^{-1}$)	O	F = 2.12 ; p = 0.2012	116.39 ± 47.55 a	219.57 ± 132.93 a	109.24 ± 41.69 a
	M	F = 0.93 ; p = 0.4454	5.67 ±1.68 a	7.81 ± 4.27 a	8.26 ± 3.65 a
NO_3^- ($mg.kg^{-1}$)	O	/	0.02 ± 0.05	2.40 ± 3.48	0.16 ± 0.21
	M	/	0	0.305 ± 0.44	0.27 ± 0.42
NO_2^- ($mg.kg^{-1}$)	O	/	0	0	0
	M	/	0	0	0
NH_4^+ ($mg.kg^{-1}$)	O	F = 1.76 ; p = 0.2271	3.26 ± 1.48 a	7.29 ± 5.57 a	4.77 ± 1.32 a
	M	/	0.21± 0.43	0.62 ± 0.87	0.07 ± 0.15

Table 1. Continued

Harvesting stage			Fertilization		
			Control	Organic	Mineral
pH	O	F = 0.57 ; p = 0.5822	4.31 ± 0.21 a	4.40 ± 0.08 a	4.32 ± 0.03 a
	M	F = 3.20 ; p = 0.1135	5.00 ± 0.15 a	5.00 ± 0.08 a	4.87 ± 0.07 a
P ($mg.kg^{-1}$)	O	F = 3.04 ; p = 0.1227	**17.00 ± 3.54 a**	**18.94 ± 3.81 ab**	**22.98 ± 4.67 b**
	M	F = 0.24 ; p = 0.7903	16.32 ± 5.39 a	15.29 ± 6.45 a	16.33 ± 8.22 a
K ($mg.kg^{-1}$)	O	F = 0.18 ; p = 0.8389	120.17 ± 55.63 a	119.52 ± 61.07 a	99.85 ± 19.59 a
	M	F = 0.96 ; p = 0.4181	7.26 ± 1.34 a	9.48 ± 2.24 a	7.74 ± 3.19 a
Ca ($mg.kg^{-1}$)	O	F = 1.77 ; p = 0.2490	797.99 ± 456.17 a	851.14 ± 519.45 a	537.97 ± 184.87 a
	M	F = 2.26 ; p = 0.1853	92.61 ± 50.81 a	94.51 ± 38.10 a	61.11 ± 23.42 a
Mg ($mg.kg^{-1}$)	O	F = 0.85 ; p = 0.4736	124.15 ± 66.05 a	128.57 ± 87.45 a	90.10 ± 36.16 a
	M	F = 1.51 ; p = 0.2951	13.25 ± 5.50 a	14.17 ± 5.10 a	11.16 ± 4.60 a
NO_3^- ($mg.kg^{-1}$)	O	/	0	0	0
	M	/	0	0	0
NO_2^- ($mg.kg^{-1}$)	O	/	0.018 ± 0.01	0.013 ± 0.005	0.015 ±0.01
	M	/	0	0	0
NH_4^+ ($mg.kg^{-1}$)	O	F = 0.23 ; p = 0.8015	4.29 ± 2.54 a	3.63 ± 2.76 a	3.50 ± 1.15 a
	M	/	0.36 ± 0.44	0.29 ± 0.37	0.18 ± 0.23

Influence of fertilizers on plant nutrient content

The chemistry analysis of the leaves sampled in the pruning resulted in significant differences between mineral fertilization and the control with increased nitrogen, potassium and calcium in the leaves of mineral fertilized plots. Nitrogen and calcium leaf concentrations were also significantly higher in mineral fertilized plots compared to organic fertilized plots. No significant differences were found between the control and the organic fertilized plots.

Table 2. Chemistry analysis of leaves and fruits as well as fruit yield according to fertilization treatment. The presented values are concentrations of each element ($mg.g^{-1}$)while the yield is measured in kg of fruits per hectare. ANOVAs were performed using Type III Analysis of Variance Table with Satterthwaite's method, log transformations were used when necessary to satisfy the normality assumption. The model used was a linear model with the variable of interest and fertilization as fixed factors and block as a random factor. Letters indicate a significant difference in a given variable when comparing fertilization treatments using a *post hoc* Tukey test.

Leaf (Pruning year)	ANOVA	Fertilization		
		Control	Organic	Mineral
N ($mg.g^{-1}$)	F = 8.23 ; p = 0.0191	**1.31 ± 0.14 a**	**1.44 ± 0.09 a**	**1.64 ±0.23 b**
P ($mg.g^{-1}$)	F = 1.68 ; p = 0.2629	0.11 ± 0.01 a	0.11 ± 0.01 a	0.11 ± 0.02 a
K ($mg.g^{-1}$)	F = 3.98 ; p = 0.0578	**0.55 ± 0.04 a**	**0.62 ± 0.06 ab**	**0.65 ± 0.04 b**
Ca ($mg.g^{-1}$)	F = 4.6 ; p = 0.0610	**0.38 ± 0.02 a**	**0.39 ± 0.04 a**	**0.33 ± 0.03 b**
Mg ($mg.g^{-1}$)	F = 0.28 ; p = 0.768	0.16 ± 0.02 a	0.16 ± 0.02 a	0.015 ± 0.03 a
Fruit (Harvesting year)	ANOVA	Fertilization		
		Control	Organic	Mineral
N ($mg.g^{-1}$)	F = 0.43; p = 0.6615	0.89 ± 0.002 a	0.89 ± 0.003 a	0.90 ± 0.003 a
P ($mg.g^{-1}$)	F = 6.58; p = 0.0307	**0.60 ± 0.02 a**	**0.64 ± 0.05 ab**	**0.73 ± 0.08 b**
K ($mg.g^{-1}$)	F = 16.41; p = 0.0010	**0.12 ± 0.004 a**	**0.12 ± 0.007 a**	**0.14 ± 0.005 b**
Ca ($mg.g^{-1}$)	F = 10.07; p = 0.0121	**0.54 ± 0.07 a**	**0.60 ± 0.09 b**	**0.62 ± 0.06 b**
Mg ($mg.g^{-1}$)	F = 2.54; p = 0.1589	0.15 ± 0.02 a	0.16 ± 0.01 a	0.16 ± 0.01 a
Yield (kg/ha)	F = 8.59; p = 0.0173	**929.65 ± 422.99 a**	**1606.83 ± 470.25 b**	**1965.60 ± 358.51 b**

Fertilization also had an effect on the fruit nutrient concentration at harvest. Fruits from control plots contained significantly lower phosphorus and potassium compared to fruits from mineral plots. Additionally, organic and mineral fertilized plots contained a significantly higher calcium concentration than control plots. Finally, yield was significantly higher in both organic and mineral fertilized plots compared to control. No significant difference in yield was found between the two types of fertilizers (Table 2).

Microbial community overview

Bacteria

For the bacterial dataset, after the cutadapt step, we had 87 698 ± 13 678 reads per rhizosphere samples and 86 958 ± 10 534 for the root samples. In the rhizosphere, the DADA2 pipeline filtered out almost 80% of the initial reads as we obtained 19 658 ± 6 329 reads per sample on average. For the root samples, more than half of these initial reads were kept with 47 913 ± 8646 reads per sample on average. However, the number of inferred ASVs was quite similar with 1 229 ± 155 ASVs on average for the rhizosphere samples and 1 103 ± 375 ASVs for the roots samples. In the end, we kept 1 716 653 reads grouped into 7 233 ASVs. Looking at the taxonomy, we eliminated 16 Archaeas, 69 Chloroplasts and 39 Mitochondrias. We also removed 1128 ASVs that were only present once or twice across the dataset. Taking into account these pruning steps as well as excluding the controls from our dataset, we were left with 5869 bacterial ASVs summing up to 1 008 592 reads, dispatched into 48 samples. Despite, the severe loss of reads during this filtering process, the rarefaction curves obtained for the bacterial community show clear inflexions of the curves sometimes even reaching a plateau indicating that we were able to capture most of the diversity even after the harsh loss of sequences during the filtering step (Figure S 1). Looking at the negative controls, we found 5 common ASVs in the rhizosphere extraction blank and in our dataset as well as 9 ASVs common in the root extraction blank and our dataset. Although these ASVs could originate from a contamination, we did not exclude them from our dataset and treated these ASVs with caution in downstream analysis. The sequenced bacterial mock community identified 18 out of the 20 species present in the mock, and the two remaining were

correctly assigned to the genus level. We did not observe any abundance bias towards a particular taxonomic lineage, though the mock lacked both Acidobacteriota and Planctomycetota representative taxa while these two phyla represent a significant proportion of the community found in our dataset.

The bacterial community was mainly composed of four phyla : Actinobacteriota (1015 ASVs, 36.7% of relative abundance (RA)), Acidobacteriota (720 ASVs, 20.2% RA), Proteobacteria (1199 ASVs, 19.4% RA), and Planctomycetota (1024, 13.3% RA) (Figure S 2). In these four phyla, one order always stands out with Rhizobiales for Proteobacteria (208 ASVs, 10.0% RA), Frankiales for Actinobacteriota (256 ASVs, 18.1 RA), Acidobacteriales for Acidobacteriota (333 ASVs, 12.9% RA), and Isosphaerales for Planctomycetota (272 ASVs, 9.2% RA). At a finer scale, we observe several taxa with a higher relative abundance than the rest of the community. For instance, the *Roseiarcus* genus (38 ASVs, 2.1% RA) is the most abundant of the Rhizobiales order, as is the case for the *Acidothermus* (210 ASVs, 17.4% RA) and *Mycobacterium* (72 ASVs, 4.8 % RA) genera belonging to Actinobacteriota. The Isosphaerales are dominated by the *Aquisphaera* genus (101 ASVs, 6.9 % RA) while the Acidobacteriales order is more evenly distributed with the *Occallatibacter* (70 ASVs, 2.6 % RA), *Acidipila−Silvibacterium* (37 ASVs, 2.6 % RA) and *Granulicella* (65 ASVs, 1.3 % RA) genera. Finally, the *Conexibacter* genus comprised of 142 ASVs, representing 3.1% of the relative abundance, dominates the Solirubrobacterales order while the *Bryobacter* and *Candidatus Solibacter* are two other dominant genera of the Acidobacteriota phylum with 110 AVS, 1.9% RA, and 94 ASVs, 1.6% RA respectively.

Fungi

For the fungal dataset, we started with 66 848 ± 10 508 reads in the rhizosphere samples and 67 764 ± 14 224 reads in the roots samples excluding sample 69Mro. This sample initially contained a far superior number of reads (483 297), which could be due to a volume error added to the ITS pool for this sample (error during quantification) according to Genome Quebec. After DADA2, we conserved 44.0% and 44.7% of the initial reads for the rhizosphere and root samples respectively. In the rhizosphere samples, we obtained 201 ± 52 inferred ASVs compared to 89 ± 30 in the root samples, with a total of 1352 ASVs including the controls and mock community. Finally, we removed 206 non-fungal ASVs as well as 55 singletons and doubletons resulting in 1065 ASVs and

560 165 reads for 48 samples in our fungal dataset (excluding controls and mock community). In the end, after the filtering process, the 69Mro sample contained 34 665 reads dispatched in 73 ASVs compared to 4739 ± 2215 reads and 68 ± 28 ASVs in the other root samples. Similarly to bacteria, the rarefaction curves obtained for the fungal community indicate that we were able to capture most of the diversity even though more than half of the initial sequences were lost during the filtering process (Figure S 3). In terms of negative control, we only found one ASV assigned to *Pezoloma ericae* both present in our dataset and in the rhizosphere extraction blank. This fungus is commonly found in the blueberry root environment. Although this ASV could have originated from a possible contamination from the control and could skew the abundance of this taxon in our dataset, we kept the ASV as it was commonly found across the dataset regardless of the fertilizer treatment. Our pipeline assigned the correct genus for 17 of the 19 species present in the fungal mock community, and 10 of which had a correct species assignment. Although we used the even mock community (equal 18S rRNA gene abundance among strains), Ascomycota taxa were more abundant than Basidiomycota taxa in our sequenced mock.

Overall, the fungal community was clearly dominated by the Ascomycota phylum comprised of 794 ASVs summing up to 92.9% of the relative abundance (RA) (Figure S 4). A high proportion of the Ascomycota belongs to the Helotiales order (327 ASVs, 52.8 % RA) followed by the Chaetothyriales order (116 ASVs, 25.5% RA). The Basidiomycota phylum (219 ASVs, 6.7% RA) was dominated by the Agaricales order (84 ASVs, 4.0% RA) while other phyla were represented by fewer ASVs and low relative abundances. At a finer level, a couple of taxa stand out in the Helotiales order : *Pezoloma ericae* (24 ASVs, 16.2% RA), *Oidiodendron maius* (4 ASVs, 9.2% RA), *Phialocephala fortinii* (7 ASVs, 2.8% RA), *Hyaloscypha variabilis* (17 ASVs, 2.8% RA) as well as the *Belonopsis* genus (11 ASVs, 5.0% RA). The Chaetothyriales order is dominated by the Herpotrichiellaceae but the taxonomic resolution is quite poor with 53 out of 71 ASVs with no assigned genus. The Agaricales order is dominated by *Clavaria sphagnicola* (13 ASVs, 2.5% RA).

Effect of the biotope

Bacteria

Looking at the bacterial alpha diversity in both roots and rhizosphere, we observed a significant difference for the Simpson index in both the pruning and harvesting growth stages (Kruskal-Wallis test, $p < 0.001$) with a higher index for the rhizosphere. The mean Shannon-Weavers index is also higher for rhizosphere than for roots but the difference is not significant (Kruskal-Wallis tests, $p = 0.069$ for the harvesting growth stage, and $p = 0.064$ for the pruning growth stage) (Figure 1).

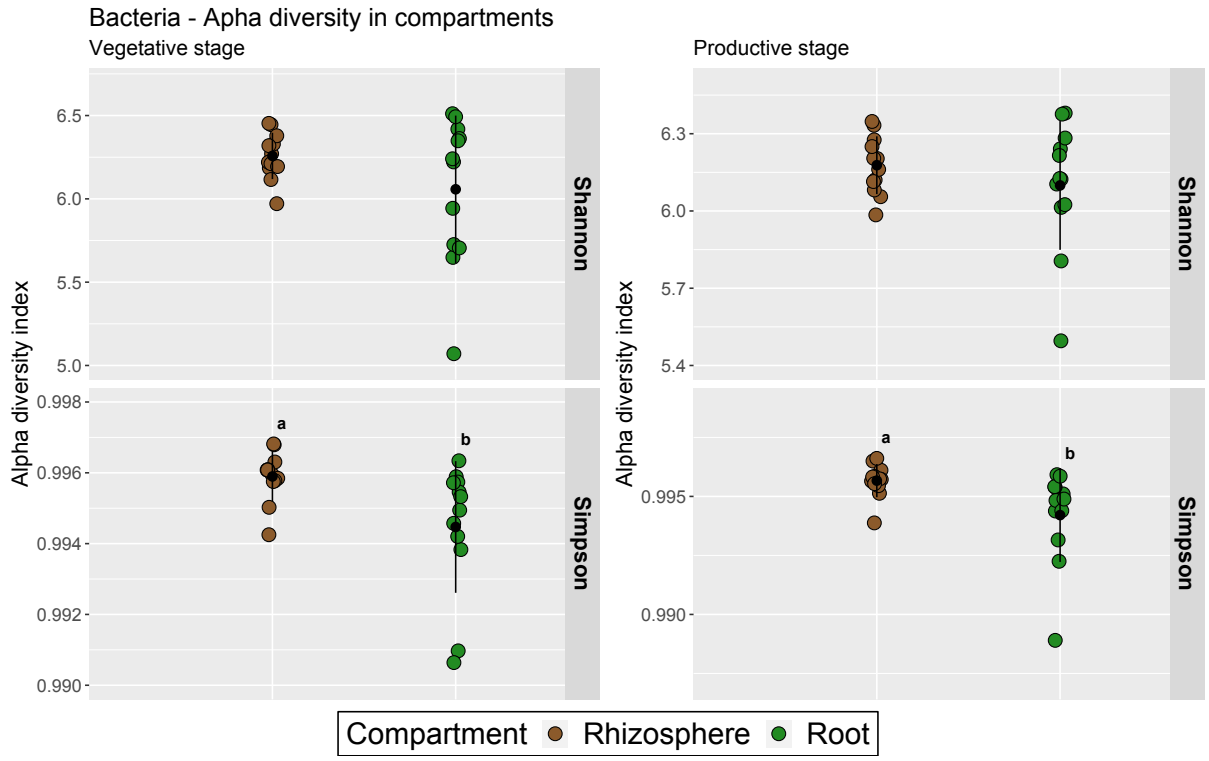


Figure 1. Alpha diversity indices for the bacterial community in each biotope (brown for rhizosphere, green for roots) for each growth stage (left column for the pruning year, right column for the harvesting year). Two indices are presented: the Shannon-Weaver index (top row) and the Simpson index (bottom row). The letters indicate a significant difference between the alpha diversity index found in each biotope.

The beta diversity analysis using Aitchison and PhILR distances on the pruning or harvesting subsets gave consistent significant effects of the biotope on the bacterial diversity (PERMANOVAs

p = 0.001). However, the homogeneity of dispersion assumption was systematically not validated (Betadisper, p <0.05) as shown in the PCA ordinations where the bacterial root community is more dispersed than the rhizosphere (especially when using the PhILR distance) (Figure 2). Consequently, the significance of the PERMANOVA tests must be handled with caution, though the visual assessment of the ordinations shows a clear separation between the two biotopes. Depending on the distance used and on the growth stage, biotope explains 10% to 28% of the variance observed.

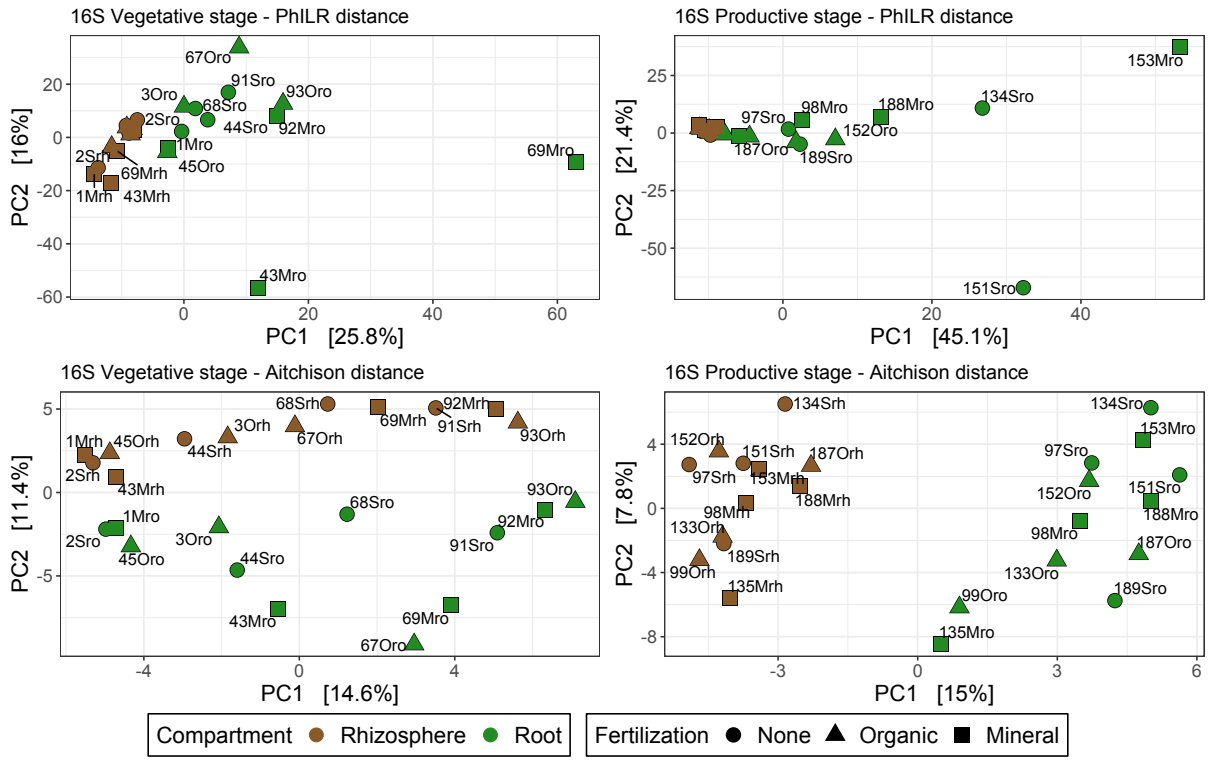


Figure 2. PCA ordinations of the bacterial community as a representation of beta diversity. Both growth stages are presented (left column : pruning year ; right column : harvesting year) as well as both distance metrics (top row: PhILR distance; bottom row: Aitchison distance). The samples are colour-coded according to their biotope and shaped according to the fertilization treatment they received.

The differential abundance metacoder plot allows for a finer approach by indicating which taxa are more abundant in a biotope. In the vegetative stage, across the 7 taxonomic levels, we found

56 taxa on the 660 observed, that have a significant differential abundance in either biotope (Figure 3). Overall, the roots are enriched in taxa ranging across multiple lineages including several Gammaproteobacteria while Planctomycetota, and more specifically the Isosphaerales order, are more abundant in the rhizosphere. In the Rhizobiales order, the only significant result concerns the *Methylovirgula* genus which is more abundant in the rhizosphere (log2fold ratio = 1.30, FDR p value = 0.03). In the Acidobacteriales order, the Acidobacteriaceae subgroup 1 is significantly more abundant in the roots (log2fold ratio = 0.85, FDR p value = 0.04). Finally, in the Actinobacteriota phylum, the dominant Frankiales order did not show any significant difference between biotopes.

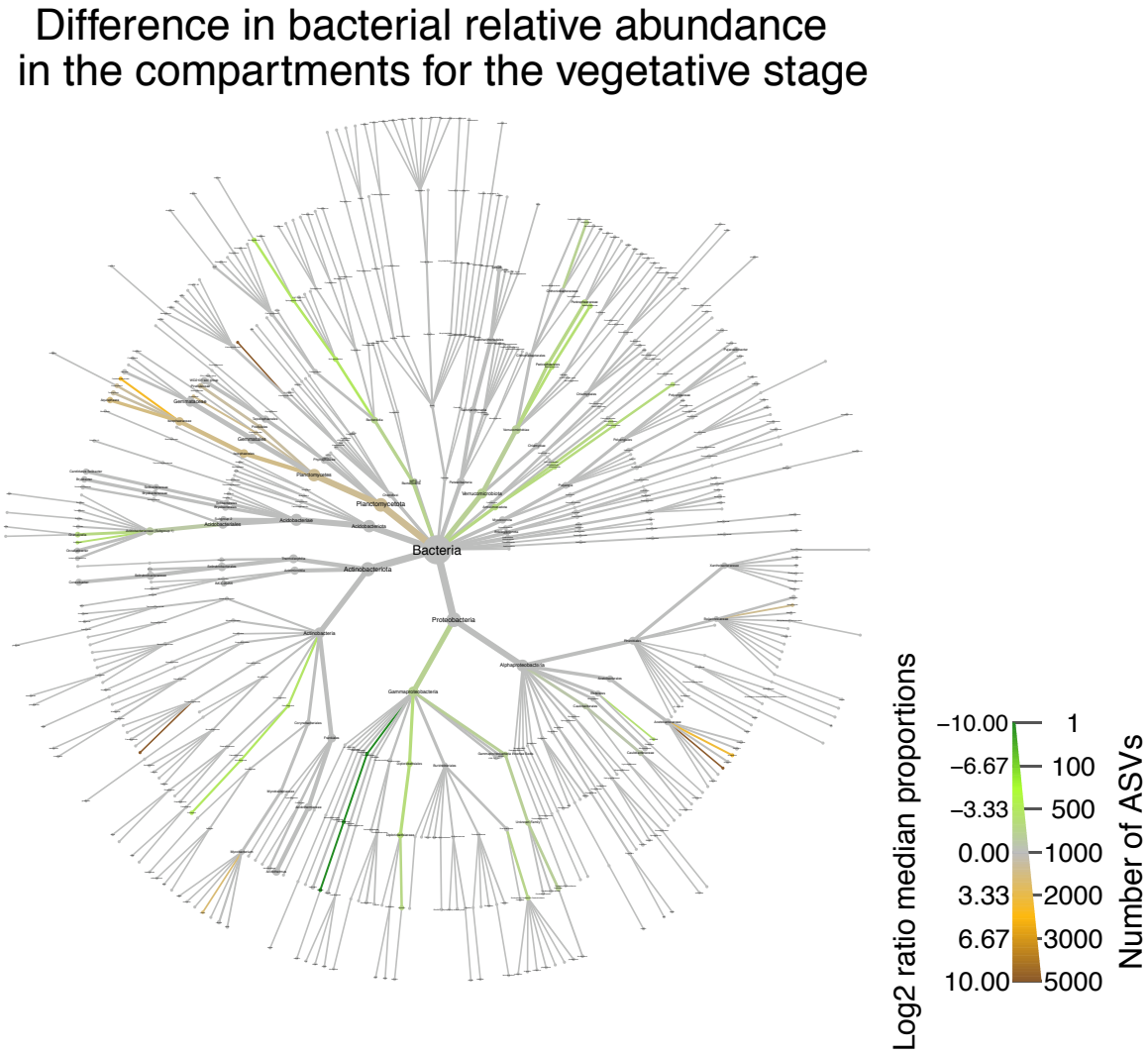


Figure 3. Differential abundance in the bacterial community for the pruning year comparing the root and rhizosphere. The coloured ASVs have a significant difference in relative abundance in either biotope (green for roots, brown for rhizosphere). A log2ratio of 10 (dark brown) or -10 (dark green) indicates that the taxon was exclusively present in the given biotope. The size of the edges and nodes indicate the number of ASVs assigned to a particular taxonomic level.

In the harvesting year, we found a higher proportion of taxa that have a significant differential abundance (107 taxa on 515 in total). The Proteobacteria phylum is significantly more abundant in the roots, with several taxa belonging to Gammaproteobacteria and Alphaproteobacteria

having an increased abundance in that biotope (Figure 4). In the Rhizobiales order, *Bradyrhizobium* spp. are more abundant in the roots (log2fold ratio = 1.72, FDR p value = 0.03) while the *Methylovirgula* genus is more abundant in the rhizosphere (log2fold ratio = 1.21, FDR p value = 0.01). Similarly to the vegetative stage, the Planctomycetota phylum and its Isosphaerales order are significantly more abundant in the rhizosphere.

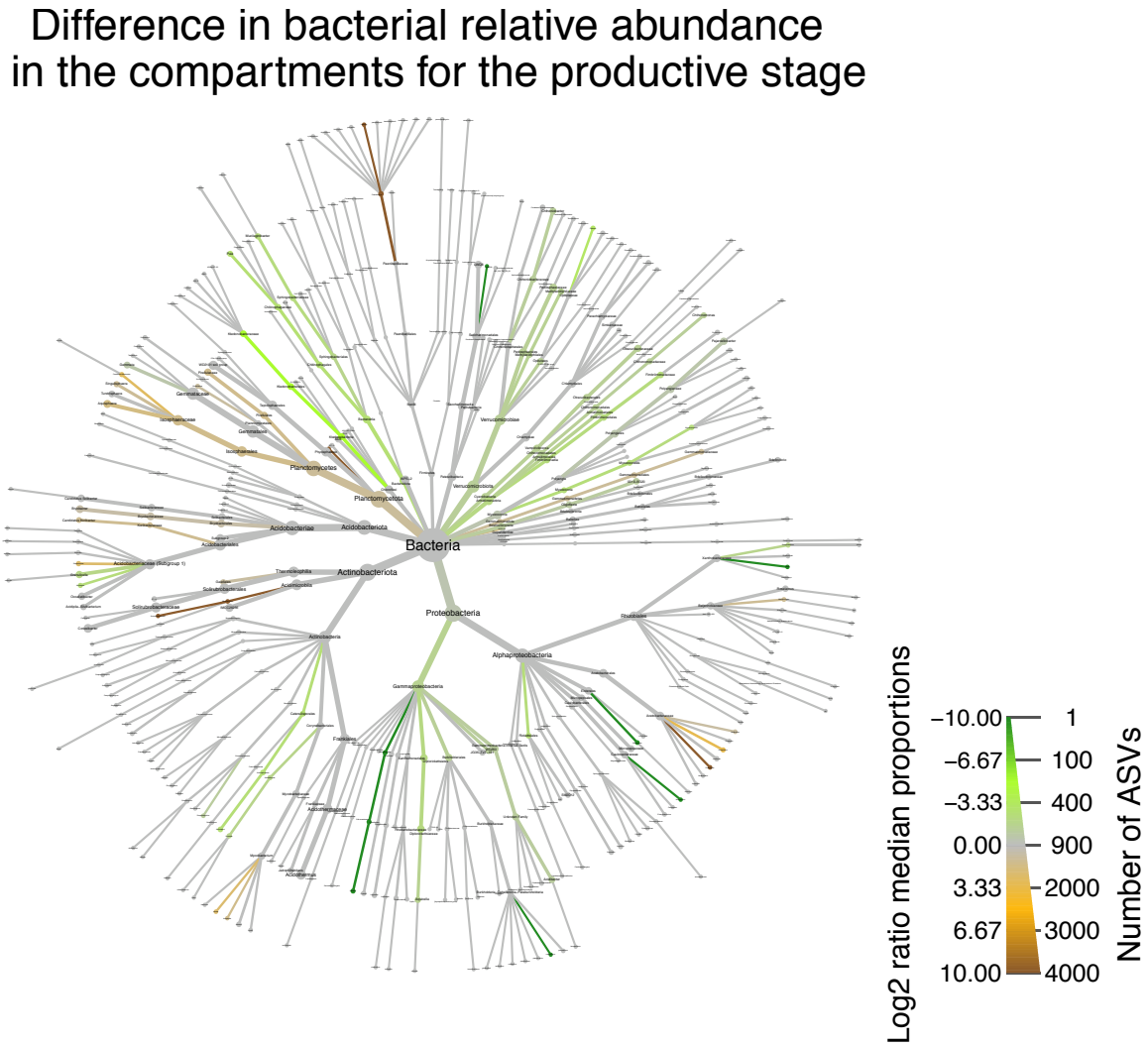


Figure 4. Differential abundance in the bacterial community for the harvesting year comparing the root and rhizosphere. The coloured ASVs have a significant difference in relative abundance in either biotope (green for roots, brown for rhizosphere). A log2ratio of 10 (dark brown) or -10 (dark green) indicates that the taxon was exclusively present in the

given biotope. The size of the edges and nodes indicate the number of ASVs assigned to a particular taxonomic level.

Fungi

Comparing the fungal alpha diversity in the two biotopes, the only significant difference was for the Shannon Weaver index in the vegetative growth stage, with a lower fungal alpha diversity in the roots compared to the rhizosphere ($p = 0.015$). There were no significant differences in fungal alpha diversity in the productive growth stage (Figure 5).

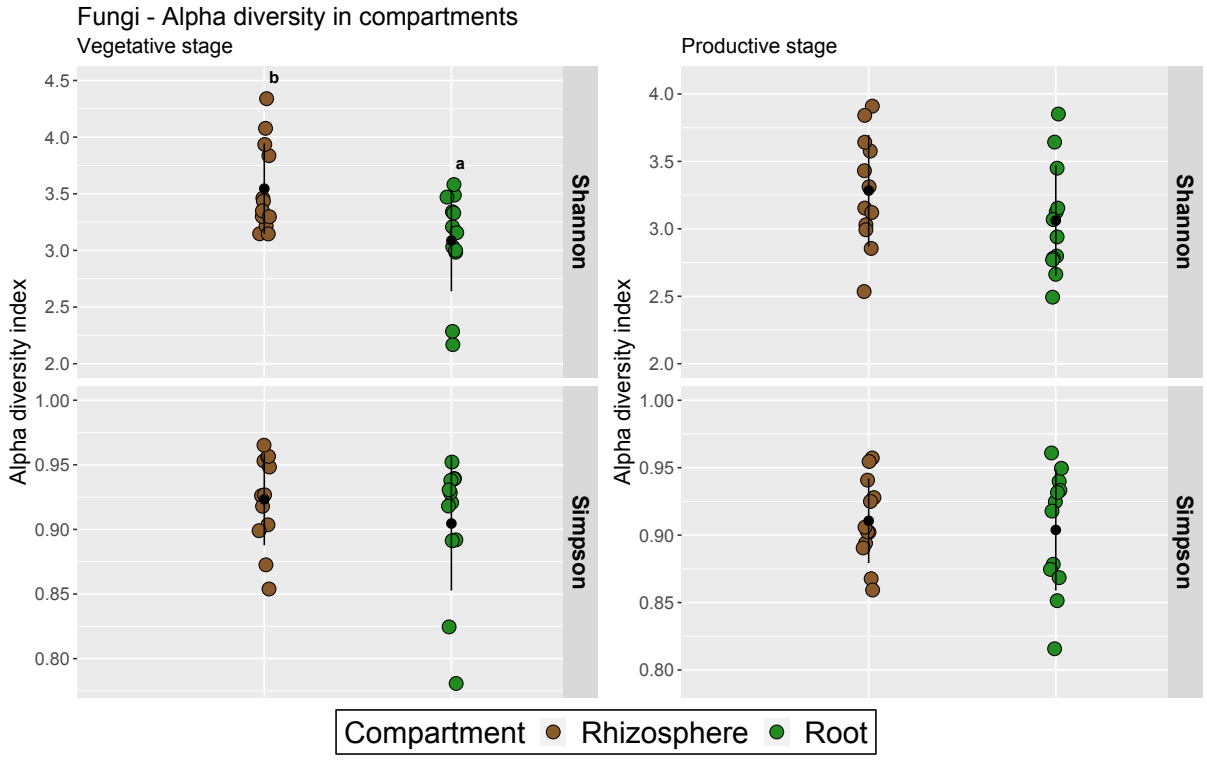


Figure 5. Alpha diversity indices for the fungal community in each biotope (brown for rhizosphere, green for roots) for each growth stage (left column for the pruning year, right column for the harvesting year). Two indices are presented: the Shannon-Weaver index (top row) and the Simpson index (bottom row). The letters indicate a significant difference between the alpha diversity index found in each biotope.

We obtained significant effects of compartments on the fungal beta diversity regardless of the growth stage or distance used (PERMANOVAs $p = 0.001$). For the PhILR ordinations, we observed

a higher dispersion of the rhizosphere fungal communities, which was significant for the vegetative year (Betadisper, p <0.05) (Figure 6). Compartment explains 12% to 18% of the variance depending on the growth stage and distance used. The variance explained by each axis of the PCAs are systematically higher in the distance incorporating phylogenetic signals (PhILR).

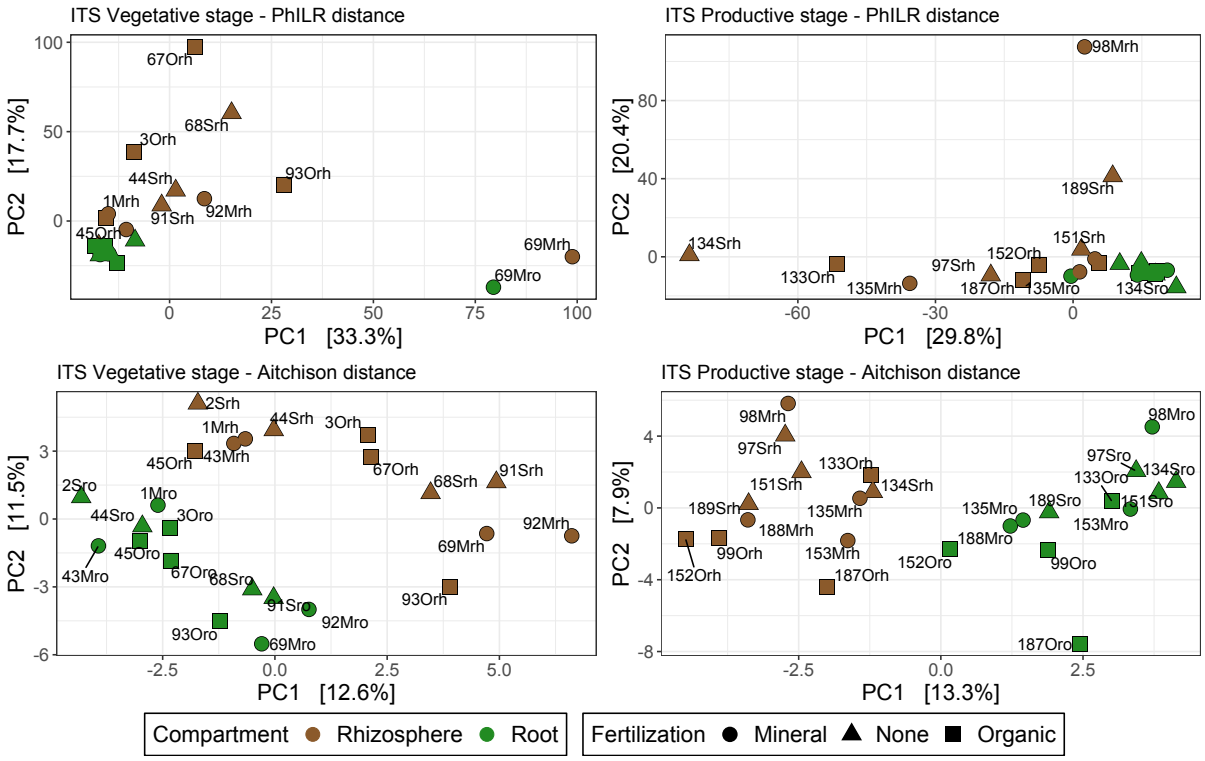


Figure 6. PCA ordinations of the fungal community as a representation of beta diversity. Both growth stages are presented (left column : pruning year ; right column : harvesting year) as well as both distance metrics (top row: PhILR distance; bottom row: Aitchison distance). The samples are colour-coded according to their compartment and shaped according to the fertilization treatment they received.

Looking more closely at this change in fungal community, in the pruning year, we find 80 taxa across the seven taxonomic levels (on a total of 484 taxa) that have a difference in relative abundance in either biotope. Overall, we find numerous taxa belonging to distinct lineages that are exclusive to the rhizosphere compartment (nodes in dark brown in Figure 7). In the root

compartment, *Belonopsis* sp., *Philocephala fortinii* and *Mycena sp.* are more abundant with log2fold ratios of 7.39, 4.25 and 2.69 respectively (FDR p value < 0.05).

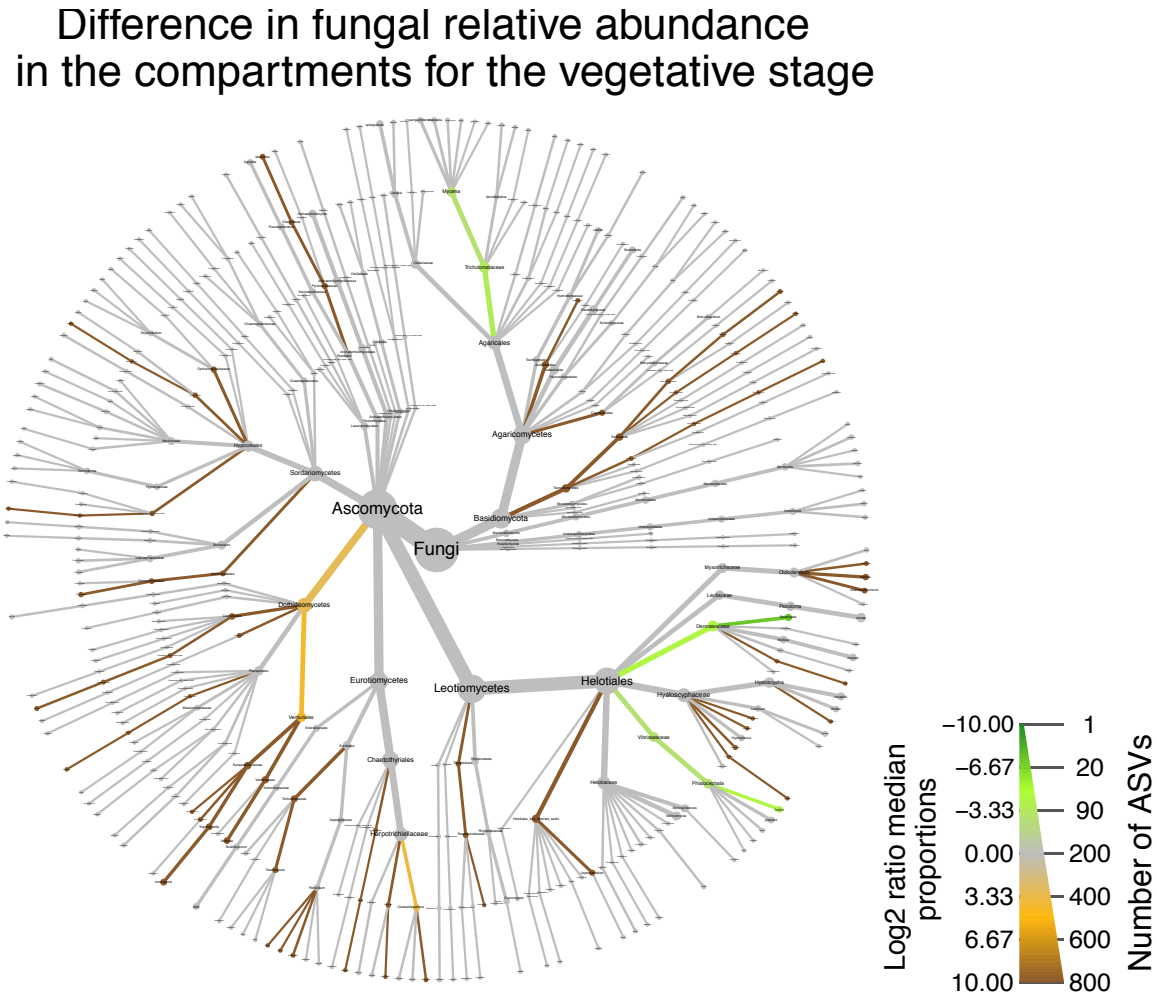


Figure 7. Differential abundance in the fungal community for the pruning year comparing the root and rhizosphere. The coloured ASVs have a significant difference in relative abundance in either biotope (green for roots, brown for rhizosphere). A log2ratio of 10 (dark brown) or -10 (dark green) indicates that the taxon was exclusively present in the given biotope. The size of the edges and nodes indicate the number of ASVs assigned to a particular taxonomic level.

In the harvesting year, we obtain 60 individual taxonomic levels with significant changes in relative abundance on a total of 405 individual taxonomic levels found. The analysis shows that the Helotiales order is significantly more abundant in the roots with a log2fold ratio of 0.66 (FDR p value = 0.023) (Figure 8). Similar to what was found in the pruning year, *Belonopsis* sp.,

Philocephala fortinii and the *Mycena* genus were more abundant in the roots with log2fold ratios of 6.74, 3.03 and 3.10 respectively (FDR p value < 0.05). Additionally, *Hyaloscypha variabilis* was also found to be significantly more abundant in the roots (log2fold ratio = 0.45, FDR p value = 0.047). Finally, numerous taxa were found to be exclusive to the rhizosphere, some of which, like *Exophiala xenobiotica* or *Troposporella monospora* were also exclusive to rhizosphere in the pruning year.

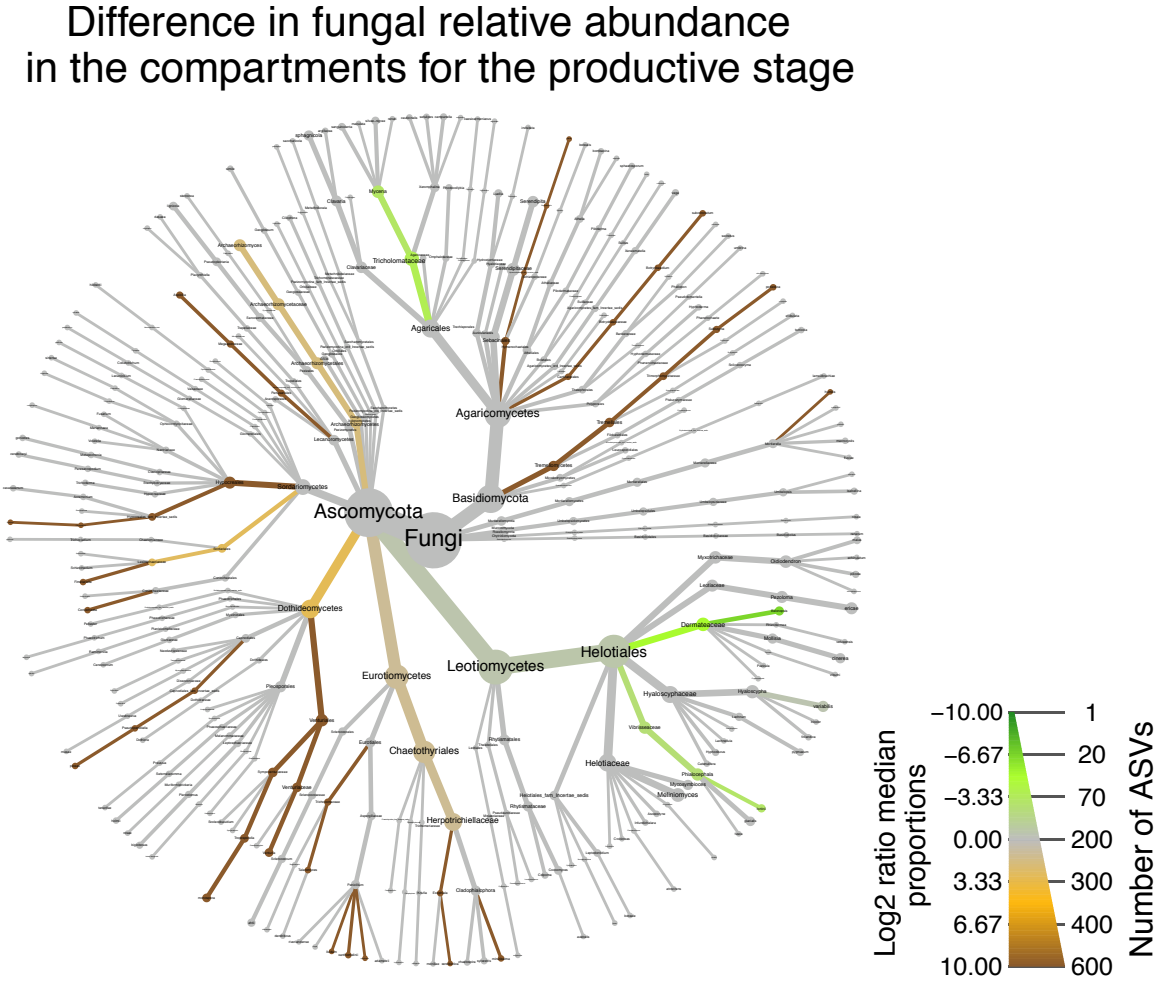


Figure 8. Differential abundance in the fungal community for the harvesting year comparing the root and rhizosphere. The coloured ASVs have a significant difference in relative abundance in either biotope (green for roots, brown for rhizosphere). A log2ratio of 10 (dark brown) or -10 (dark green) indicates that the taxon was exclusively present in the given biotope. The size of the edges and nodes indicate the number of ASVs assigned to a particular taxonomic level.

Fertilization effect

In the fungal dataset, the PERMANOVA tests indicated a significant effect of fertilization on the fungal communities (except when using the PhILR distance on the productive stage samples) and fertilization explained approximately 10% of the variance in the datasets. Similarly, fertilization was significant for the bacterial datasets regardless of the distance used or the growth stage and it explained 10 to 15% of the variance. For both fungi and bacteria, we found no interactions between biotope and fertilization. As biotopes had a stronger and more visible effect on the microbial communities than fertilization, we decided to look at each biotope individually in order to have a clearer interpretation of the fertilization effect. Consequently, there were four combinations between the growth stages and the biotopes. We used abbreviations VG (vegetative growth stage) for the pruning year and PD (productive growth stage) for the harvesting year as well as RO for roots and RH for the rhizosphere.

Fertilization on alpha diversity

In the pruning year, for bacteria, we found a significant difference between the mineral treatment and the control for the Shannon-Weaver index in both compartments, with a higher alpha diversity index for the control (Figure 9). We observe the similar trend with the Simpson diversity index but the difference between treatments are not significant.

For fungi, the Shannon-Weaver index of the organic treatment was significantly lower (2.66 ± 0.5) than the control (3.42 ± 0.185) and mineral treatment (3.18 ± 0.185) in the VGxRO combination. No significant differences were observed for the rhizosphere samples (Figure 10). Finally, in the harvesting year, no significant difference was observed on both alpha diversity indices for fungi or bacteria regardless of the growth stage and compartment combinations analyzed.

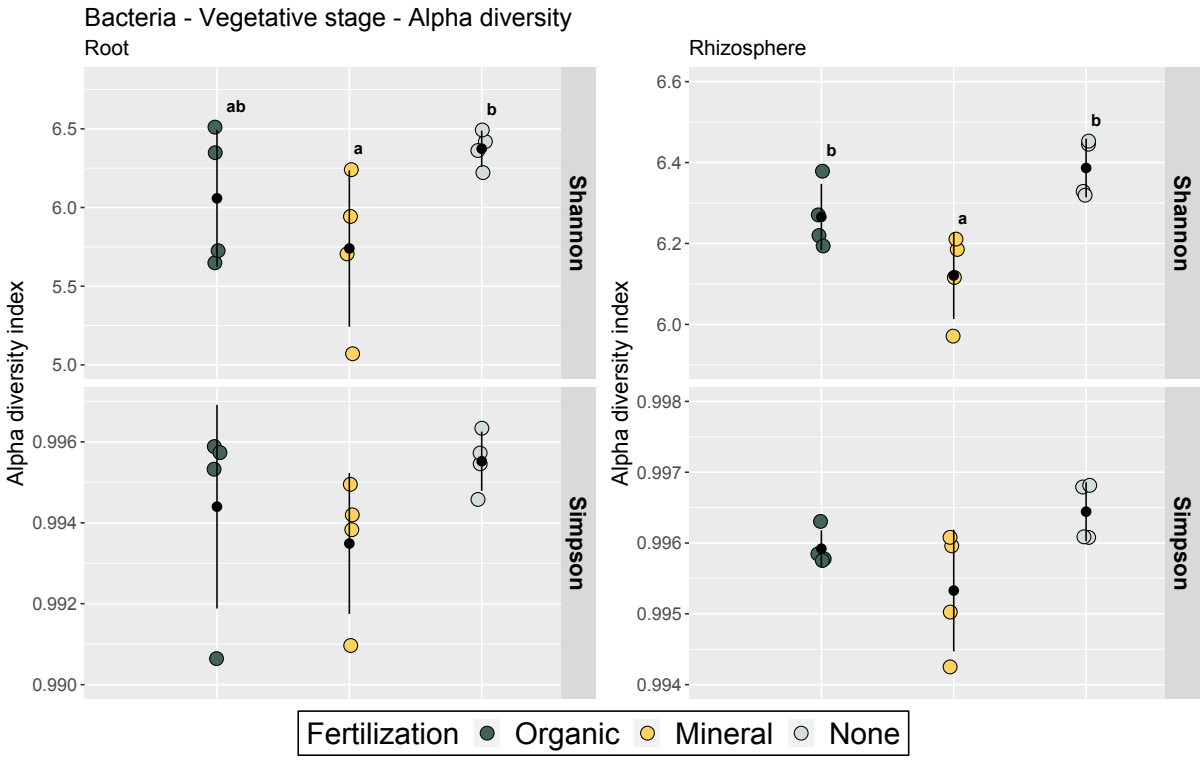


Figure 9. Alpha diversity indices for the bacterial community in the pruning year for each fertilization treatment (dark green for organic treatment, yellow for mineral treatment and grey for negative control). Both biotopes were analyzed with roots in the left column and rhizosphere in the right column. Two indices are presented: the Shannon-Weaver index (top row) and the Simpson index (bottom row). The letters indicate a significant difference between the alpha diversity index found in each biotope.

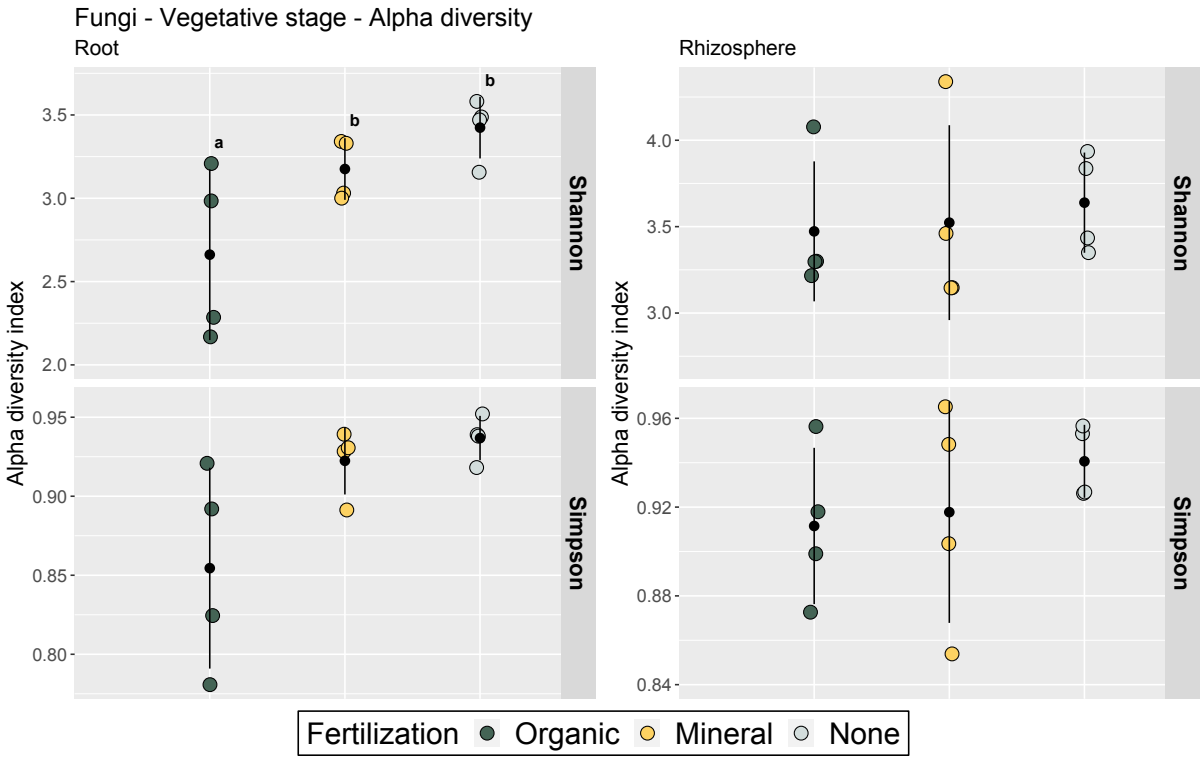


Figure 10. Alpha diversity indices for the fungal community in the pruning year for each fertilization treatment (dark green for organic treatment, yellow for mineral treatment and grey for negative control). Both biotopes were analyzed with roots in the left column and rhizosphere in the right column. Two indices are presented: the Shannon-Weaver index (top row) and the Simpson index (bottom row). The letters indicate a significant difference between the alpha diversity index found in each biotopes.

Fertilization on beta diversity and db-RDA

For the bacterial community, the PERMANOVA test indicated a significant effect of fertilization in both biotopes in pruning year (VGxRO p = 0.032 for Aitchison, p = 0.025 for PhILR ; VGxRH bac p = 0.006 for Aitchison, p = 0.014 for PhILR). However, the post-hoc tests did not give any significant difference on community composition in the three fertilization treatments. Additionally, the ordinations did not show a clear separation of the sample based on their fertilization treatment (Figures 11 and S4). In the productive growth stage, we found no significant effect of fertilization whether in the root or rhizosphere.

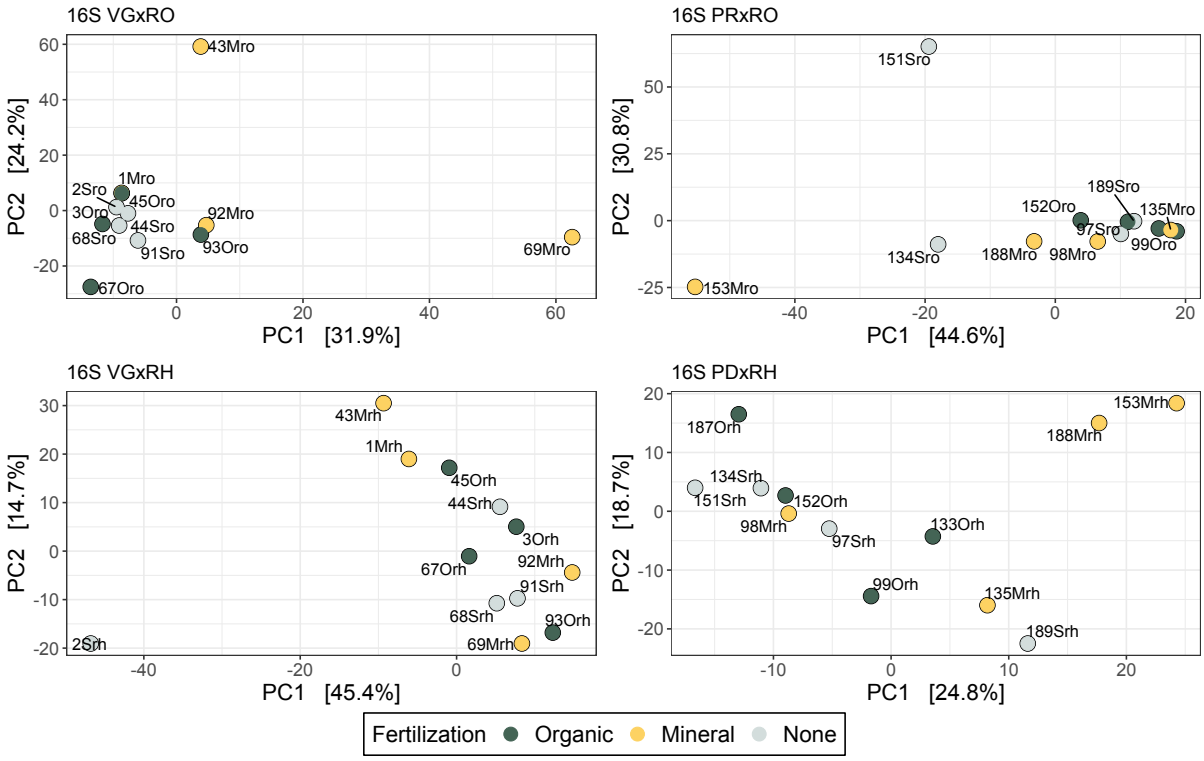


Figure 11. PCA ordinations of the bacterial community based on the PhILR distance as a representation of beta diversity (PhILR transformation and Euclidean distance). The rows are biotopes (roots : top row ; rhizosphere : bottom row) and the columns are the growth stage (pruning year: left row ; harvesting year: right row). The samples are colour-coded according to their fertilizer treatment (dark green for organic treatment, yellow for mineral treatment and grey for negative control).

Alternatively, the db-RDA constrained by fertilization indicated a poor explanation of the variance in the composition of bacterial communities with very low adjusted R-squared and nonsignificant tests for the productive growth stage. We had significant models for the Aitchison distance on both compartment for the pruning year ($p < 0.05$) but the explained variance was low with only 0.4% and 2.3% for the root and rhizosphere bacterial communities respectively.

In the fungal community, we did not find any significant difference in beta diversity, in any of the combinations of biotope and growth stage. Similarly to the bacterial community, the samples from each fertilizer treatment overlap and no clear partition is observed (Figure 12 and S6).

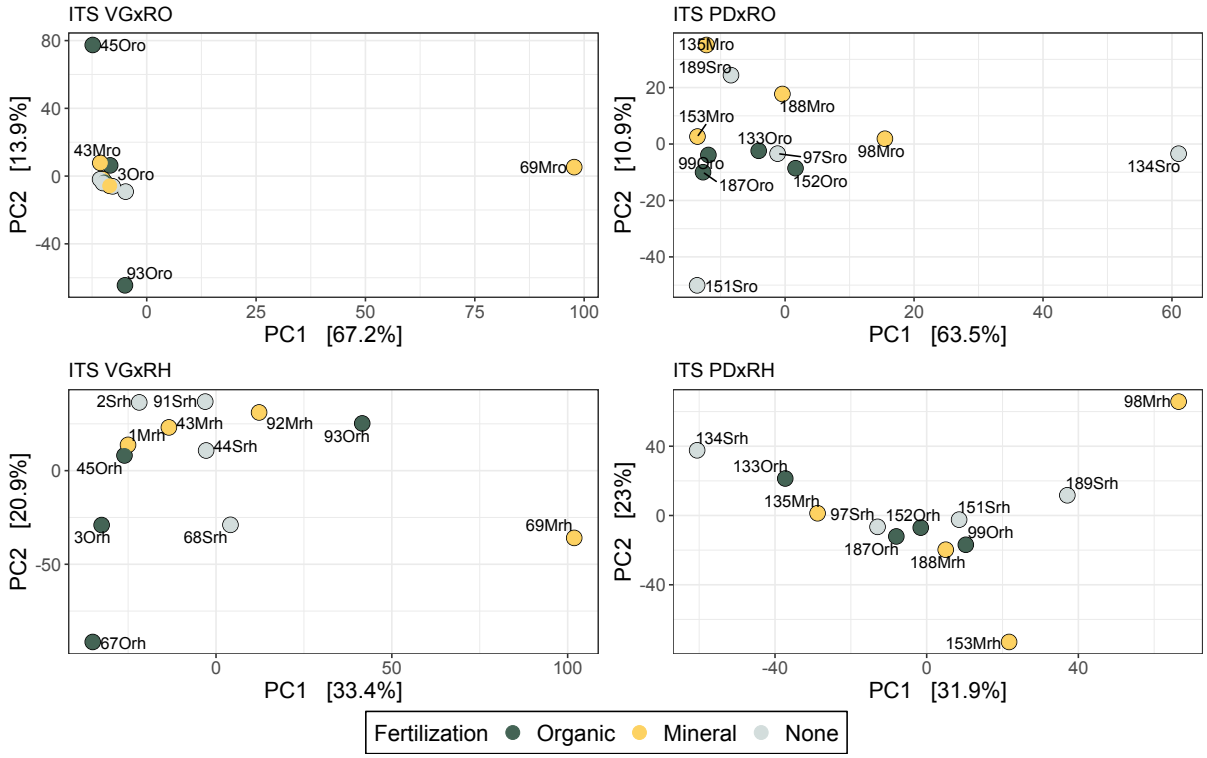


Figure 12. PCA ordinations of the fungal community based on the PhILR distance as a representation of beta diversity (PhILR transformation and Euclidean distance). The rows are biotopes (roots : top row ; rhizosphere : bottom row) and the columns are the growth stage (pruning year : left row ; harvesting year: right row). The samples are colour-coded according to their fertilizer treatment (dark green for organic treatment, yellow for mineral treatment and grey for negative control).

Additionally, similar to what was found for bacteria, the db-RDA analysis showed that the variation in fungal community composition was not redundant with the variance explained by fertilization. The only significant model was found with the Aitchison distance on the pruning year root dataset ($p = 0.03$) but the adjusted R-squared is extremely low ($_{adj}R^2 = 0.005$).

We also used the organic soil chemistry data as constraining variables in db-RDAs. For the fungal datasets, none of the variables were significant when using the Aitchison distance on the four combinations of growth stages and biotopes. When using the PhILR distance, we only found a significant db-RDA for the VGxRH with phosphorus, pH and nitrate as constraining variables ($p = 0.0049$, $_{ajd}R^2 = 0.31$). In the bacterial dataset, using the Aitchison distance we obtained a significant db-RDA ($p = 0.006993$) with phosphorus and nitrate as constraining variables for the VGxRO combination though the adjusted R-squared is low ($_{adj}R^2 = 0.065$). Using the PhILR distance, we find that phosphorus, pH, calcium and nitrate are all significant in the db-RDA and that these variables explain 43.5% of the bacterial community composition ($_{adj}R^2 = 0.435$, $p = 0.001$). Additionally, a significant db-RDA was also obtained for the VGxRH combination with phosphorus as the sole constraining variable ($p = 0.03197$, $_{adj}R^2 = 0.052$) but only for the Aitchison distance.

Fertilization on representative taxa

In order to have a finer approach than the beta diversity analysis which looks at the whole community, we searched for representative taxa in each of the three fertilization treatments in the four combinations of growth stages and biotopes using two approaches : indicator species and metacoder differential abundance analyses. In the bacterial community, neither analysis indicated any representative taxa of a fertilizer treatment. Similarly, we did not find any fungal representative taxa in any of the fertilization treatments.

Link between agronomic variables and microbiota

As a final analysis, we computed db-RDAs using agronomic variables from both growth stages as constraining variables to test whether the observed variance explained by these variables was redundant with the variance explained by microbial community composition. For fungi, we found no significant results for the harvesting year, and found a weak but significant result for the VGxRO combination using the Aitchison distance ($p = 0.03935$, $_{adj}R^2 = 0.07$). Similarly, for bacteria, the only significant result was for the VGxRO combination using the Aitchison distance ($p = 0.049$, $_{adj}R^2 = 0.087$).

Discussion

Fertilization has a minimal impact on microbial communities regardless of the growth stage and the compartment.

Overall, our results concerning the impact of fertilization on the fungal and bacterial root and rhizosphere communities tend to show a minimal influence in shaping these microbial communities. The only significant differences observed concerned the pruning year samples as expected, because fertilization was applied at the beginning of the pruning year.

For bacteria, we found that the mineral treatment reduces the bacterial Shannon alpha-diversity index in both compartments compared to the control in the vegetative stage. As the Shannon index is more sensitive to rare taxa than the Simpson index, and that there is no significant change for the Simpson index with fertilization, this suggests that mineral fertilization reduces the occurrence of bacterial rare taxa compared to unfertilized plots. However, we did not find any indicative bacterial taxa nor difference in relative abundance for the three fertilization treatments in any of the growth stage and biotope combinations. Therefore, we were not able to identify which bacterial rare taxa were depleted due to mineral nutrient addition. We obtained a significant effect of fertilization in both biotopes for the bacterial beta diversity in the pruning year. However the post-hoc tests did not result in a significant difference of the bacterial communities of the three fertilization treatments.

Regarding the fungal community, we observed a significant reduction of the Shannon index for the organic fertilizer in the roots of blueberries during the pruning year. This result was unexpected considering the fact that the mineral fertilization Shannon index is not significantly different from the control. In addition, there is a higher standard deviation for the organic treatment, as two samples had an index value around 3 (more similar to the mineral treatment and control Shannon index values), while the two other samples have a Shannon index value close to 2.25. The PERMANOVAs indicated no significant difference in beta diversity, in any of the

combinations of biotope and growth stage and we did not find any fungal indicative taxa nor change in relative abundance in any of the three fertilizer treatments.

Our results are consistent with a previous study, which did not observe any significant change on *Vaccinium* root-associated fungal community composition nor on the assumed ericoid mycorrhizal species under long-term (4 to 12 years) N fertilization (12.5 to 50 $kg.ha^{-1}$) in boreal forests (Ishida and Nordin, 2010). Additionally, testing different rates of mineral N-P-K (from 0 to 60 $kg.ha^{-1}$) in different combinations, Jeliazkova and Percival (2003) found that ericoid mycorrhizal colonization was not impacted by fertilization (Jeliazkova and Percival, 2003). Another study, measuring the effect of variable atmospheric nitrogen deposition rates, ranging from 5.27 to 29.65 N $kg.ha^{-1}.year^{-1}$ in ombrotrophic bogs containing *Vaccinium oxycoccos*, found that the root fungal diversity (Shannon index) was not impacted by nitrogen deposition rates (Boeraeve et al., 2022). The fungal community composition significantly varied with nitrogen deposition rates but this variable did not significantly impact putative ericoid mycorrhizal fungi diversity or composition. In our study, we also obtained sequences matching to know, ericoid mycorrhizal species such as *Pezoloma ericae*, *Oidiodendron maius*, *O. chlamydosporicum, Hyaloscypha variablis*, *H. bicolor* and *H. filandica* but did not find any variation in their relative abundance. In the same study, the authors also find that increasing nitrogen deposition rates did not significantly change the root bacterial community composition (Boeraeve et al., 2022), a pattern similar to what we observe in our experiment. Focusing on the link between fertilization and ericoid mycorrhiza, Kiheri *et al.*, (2020) found no significant increase in colonization in *Calluna vulgaris* and *Erica tetralix* (both Ericaceae) in both long-term N deposition and N-P-K fertilization trials (Kiheri et al., 2020).

However contrasting results were found in other studies. In a long-term fertilization experiment (23 to 30 years), Leopold *et al.*, (2021) measured a decrease in fungal richness and diversity in the *Vaccinium calycinum* rhizosphere when the soil was supplied with the limiting nutrient (N or P) (Leopold et al., 2021). However the nutrients were supplied at a higher dose of 100 $kg.ha^{-1}$ per year which could explain the difference. Additionally, Van Geel *et al.*, (2020) showed that soil

phosphorus as well as nitrogen deposition significantly reduced ericoid mycorrhizal richness in several Ericaceae species found in European bogs (Van Geel et al., 2020).

The fact that we did not see a strong impact of fertilization on microbial communities could be explained by the limited impact of fertilization on soil chemistry. Indeed, in the pruning year, pH was the only variable that significantly varied between fertilizer treatments (pH for mineral fertilization samples is significantly lower than pH for organic fertilization samples) while phosphorus significantly varied in the harvesting year. The higher phosphorus concentration in the mineral fertilization treatment compared to the control is difficult to interpret as we did not have any difference in the pruning year and that plots were not fertilized in the harvesting year. Finding a trend of higher pH in the organic treatment and a lower pH in the mineral treatment was to be expected as ammonia tends to acidify the soil (Geisseler and Scow, 2014), while organic fertilizers have already been reported to increase pH (Haynes and Swift, 1985; Caspersen et al., 2016). However, the changes in soil chemistry were perhaps too minimal to cause any significant shifts in the fungal and bacterial communities.

Another important point to consider is that, in the pruning year, fertilization occurred in early spring, while soil samples for DNA sequencing were collected approximately 2.5 months after the treatments had occurred, and soil chemistry was measured even later, at the end of September. This lapse of time between fertilization and sampling could be another factor that could explain why we did not find any major changes in the soil ecosystem; the possible initial perturbation of the microbial community caused by fertilization faded with time as the available nutrients were depleted from the organic soil layer. In the harvesting year, the time between fertilization and sampling are even more temporarily apart. A time-series experiment with regular sampling during the summer could show the dynamic of soil chemistry, and microbial community changes caused by fertilization. This type of study would allow to show if the impact of fertilization on the microbial communities is short-lived (<2.5 months) or if the microbial community is resistant to this change of conditions.

Bacterial and fungal diversity and community composition change with compartments

Regarding bacteria, we found a significant difference in the Simpson diversity index between rhizosphere and roots in the samples from both growth stages. However, no significant difference was found for the Shannon diversity index. These results are in line with numerous studies which find a higher diversity in the rhizosphere compared to the roots as the plant selection pressure is increased in its organs (Reinhold-Hurek et al., 2015; van der Heijden et al., 2015).

We also obtained significant community shifts between rhizosphere and roots for both the Aitchison and PhILR distances and a clear separation of both communities in the ordinations using these distances. The bacterial root samples tend to contain more phylogenetically distant taxa than the rhizosphere as samples are more dispersed in the ordinations plots based on the PhILR distance. Finally, looking at the most abundant phyla, the Metacoder plots indicate a higher relative abundance of Planctomycetota (P) – Isosphaerales (O) in the rhizosphere while several Proteobacteria are enriched in the roots. Species belonging to the Isosphaerales order contain numerous carbohydrate-active enzymes suggesting their ability to use a wide range of natural carbohydrates (Ivanova et al., 2017). Several of these bacteria including the *Aquispharea* genus were also found in abundance in the wild blueberry rhizosphere in a previous study (Morvan et al., 2022). As wild blueberry roots are mainly found in the soil organic layer, finding an increased abundance in hydrolytic bacteria in the rhizosphere is coherent. Concerning the higher presence of *Bradyrhizobium* spp. in the blueberry roots in the productive year, this genus is widely known for its nitrogen fixation capacity in legume nodules. Nevertheless, this genus is also found as an endophyte in non-legumes roots and may contribute to N_2 fixation (Yoneyama et al., 2017; Rosenblueth et al., 2018; Hara et al., 2019) or not (Schneijderberg et al., 2018). Hence an in-depth characterization of the *Bradyrhizobium* spp. present in the blueberry roots is required to elucidate their nitrogen fixation ability. As for the *Methylovirgula* genus being more abundant in the blueberry rhizosphere, this genus was isolated in 2009 from beechwood blocks at the surface of an acidic (pH 3.3–3.6) forest soil was characterized as acidophilic and the two isolates were also able to fix dinitrogen (Vorob'ev et al., 2009).

For the fungal community, alpha diversity was only significantly higher in the rhizosphere of the vegetative stage samples with the Shannon index, suggesting that it was enriched in low abundance taxa. Fungal community composition was also significantly different when comparing biotopes as it can be seen on the ordinations. Contrary to bacteria, the fungal root samples tend to have communities that are more phylogenetically close to one another when the rhizosphere communities are more phylogenetically different. In both growth stages, many fungal taxa are exclusive to the rhizosphere including known ericoid mycorrhizal fungi such as *Oidiodendron chlamydosporicum* and *Hyaloscypha finlandica*. However the dark septate endophyte *Phialocephala fortinii* as well as the *Mycena* genus are consistently more abundant in the roots. *P. fortinii* commonly colonizes Ericaceae roots and could have a beneficial effect on their fitness (Vohník et al., 2005; Newsham, 2011; Lukešová *et al.*, 2015). As for the *Mycena* genus , Grelet *et al.*, (2017) have proposed that this genus, previously characterized as a generalist saprotroph, could be transitioning between a full saprotrophic and a symbiosis lifestyle (Grelet et al., 2017). The authors obtained a comparable increase of growth in seedlings of *Vaccinium corymbosum* inoculated with either *Mycena galopus* or *Pezoloma ericae*, and observed peg-like structure in the root cells inoculated with *M. galopus*. However, as we did not look at colonization, we cannot confirm if we had a similar structure in the *Vaccinum angustifolium* roots that served to extract DNA.

Conclusion

The results of our study indicate a minimal impact of fertilization on both the fungal and bacterial communities of the rhizosphere and roots of *Vaccinium angustifolium*. Fertilization is clearly worthwhile for producers as yields more than doubled with the mineral fertilizer and were also significantly increased using the organic fertilizer. Our experiment did not measure drastic changes in soil chemistry in the organic layer, as pH was the only variable which significantly varied in the pruning year when comparing the two fertilizer treatment (significantly lower pH with the mineral fertilizer compared to the organic fertilizer). A long-term impact of fertilization would be interesting to measure as the plots sampled were relatively recent in terms of

agricultural management. Repeated nutrient addition could slowly modify soil chemistry which in turn could impact the microbial communities in the root environment of cultivated wild blueberries.

Author contribution

MP and JL conceived and designed the agronomic part of the study while MH and SM conceived and designed the microbial part of the study. SM contributed to data acquisition, performed the microbial community analysis experiments, analyzed the data and wrote the draft of the manuscript. All authors reviewed and discussed the results. All authors read and approved the submitted version of the article.

Funding

This study received funding from the following source: the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grant (Grant RGPIN-2018-04178) to MH ; the Syndicat des Producteurs de Bleuets du Québec (SPBQ) and the Natural Sciences and Engineering Research Council of Canada (NSERC) (Grant RDCPJ-503182-16) to MP.

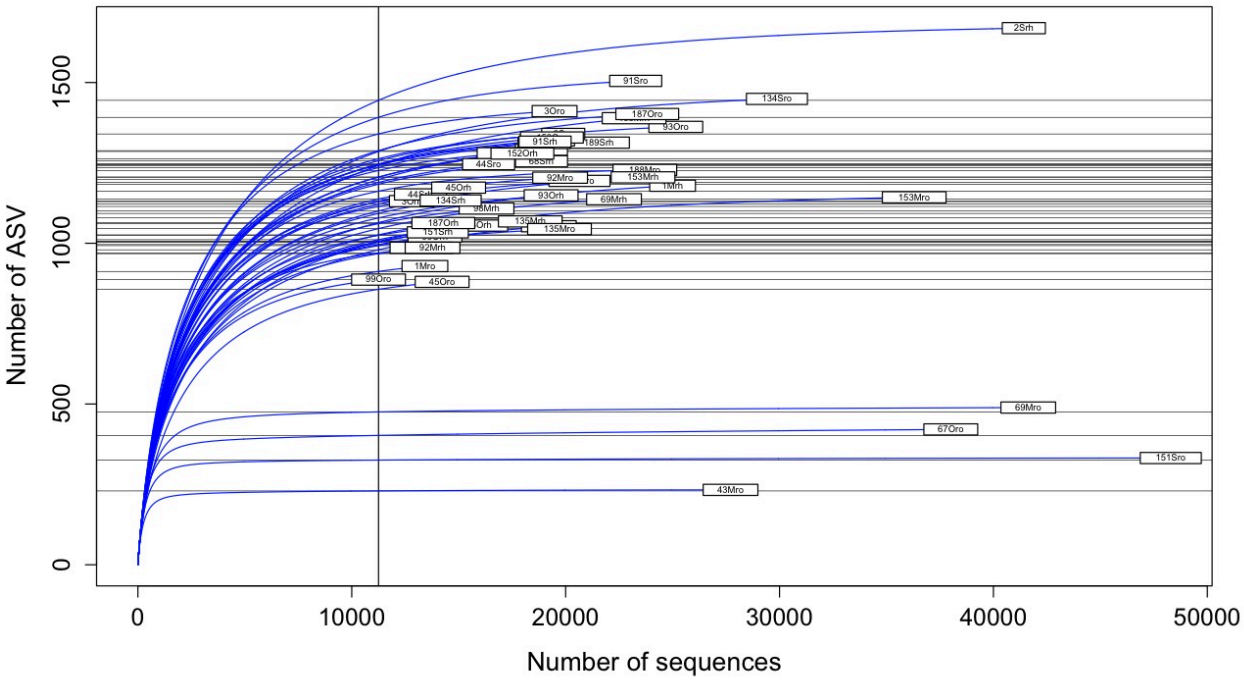
Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

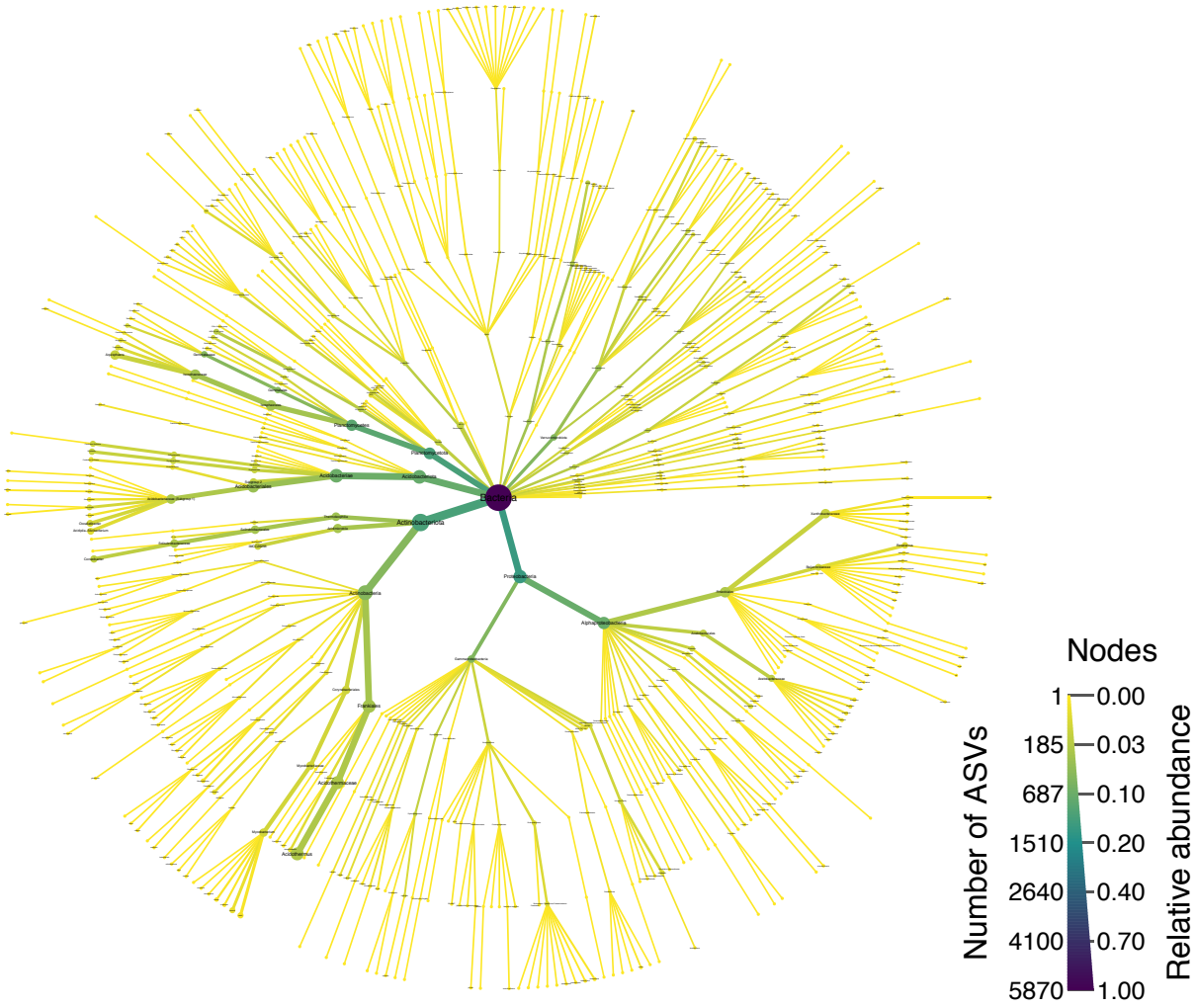
Acknowledgments

The authors would like to thank Geneviève Telmosse for her help on data acquisition; Denis Bourgault for soil analysis; Stéphane Daigle for his help on mixed models. The authors also thank the Corporation d'Aménagement Forêt Normandin (CAFN) for providing access to their sites and infrastructure.

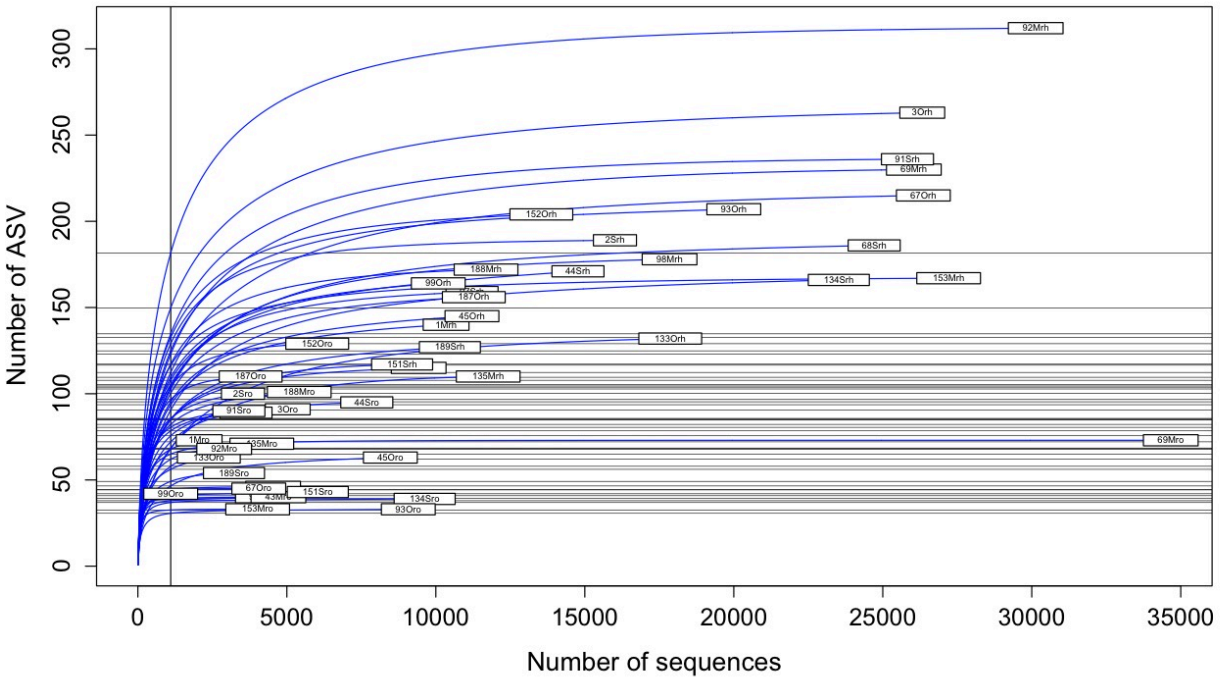
Supplementary Information



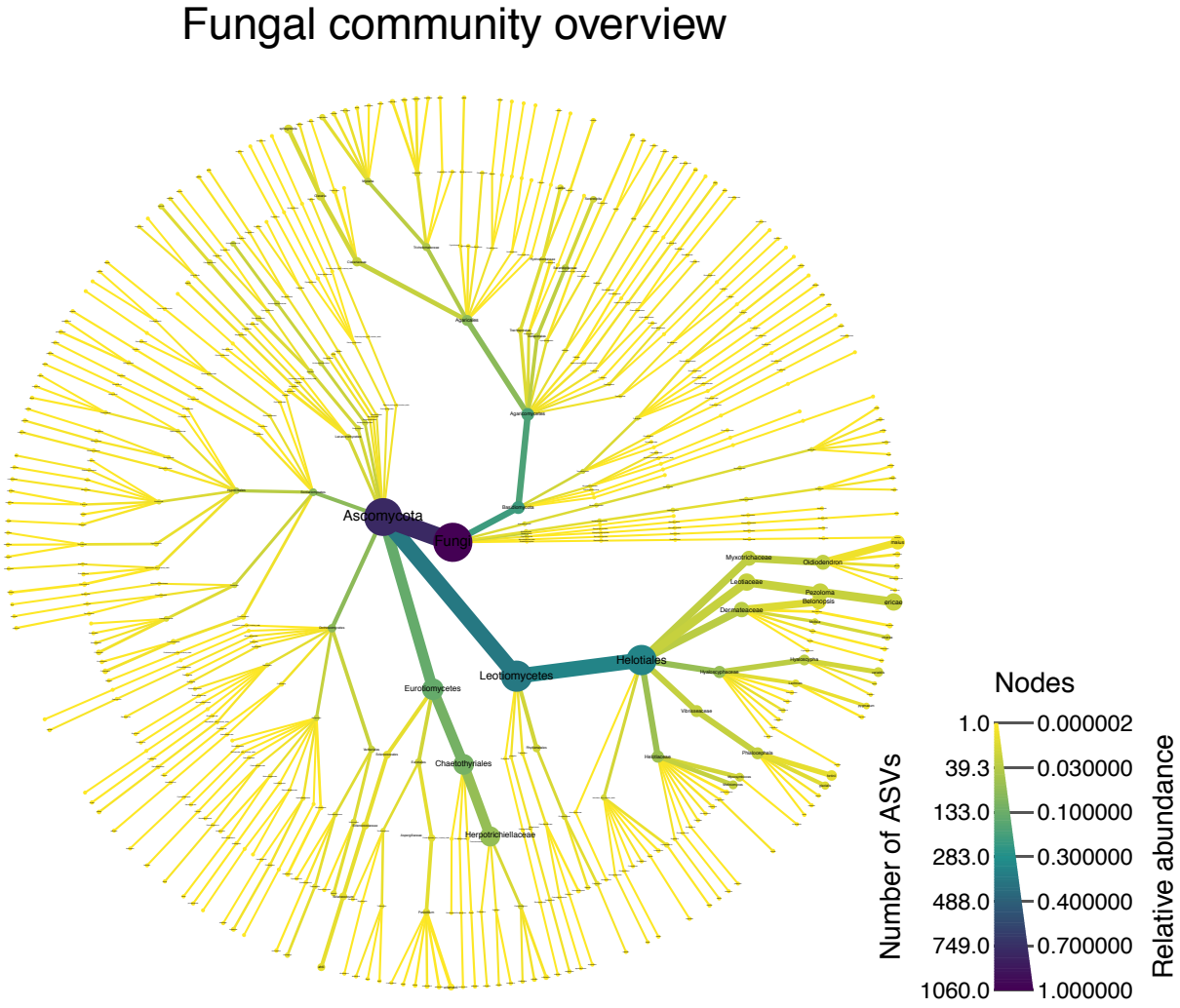
Supplementary Figure 1. Rarefaction curves of the bacterial community after the filtering pipeline.



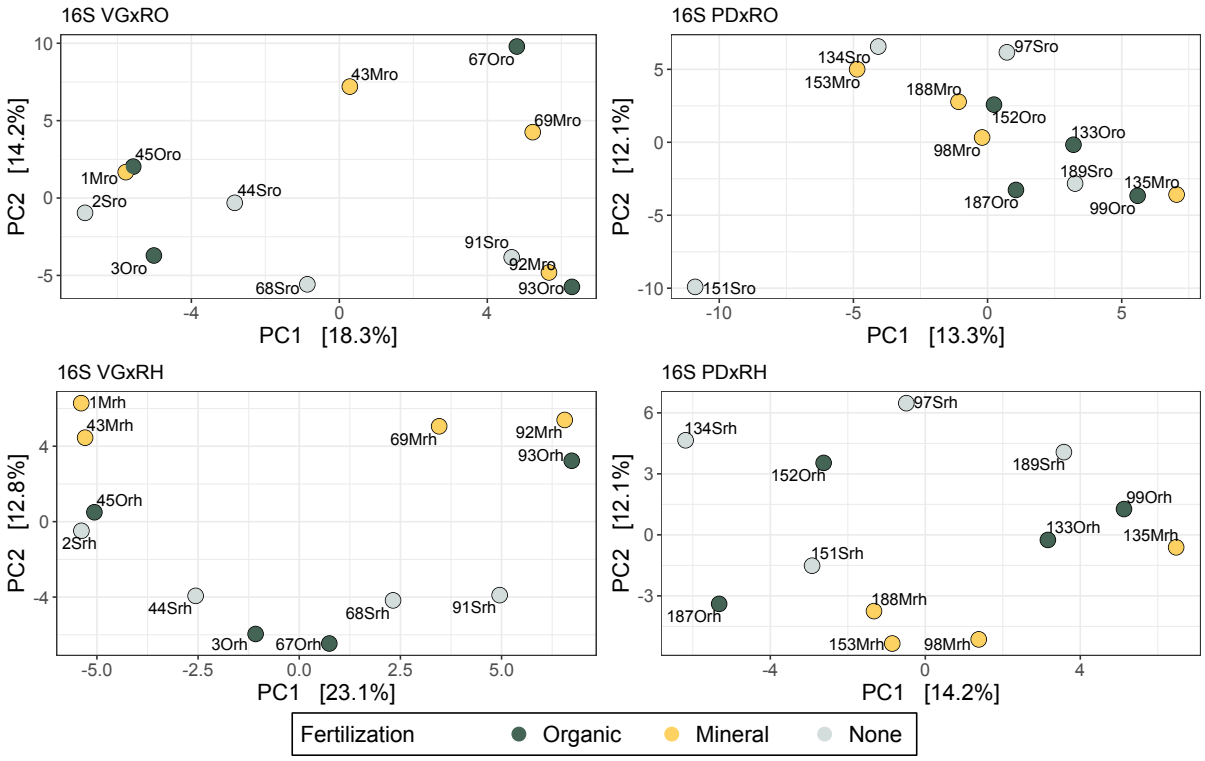
Supplementary Figure 2. Taxonomic overview of the bacterial community. The figure plots ASVs which were assigned to a taxonomic level (ASVs not assigned at a particular taxonomic level (NAs) are not shown). The colour of the edges and nodes indicates the number of ASVs found at a given taxonomic level, with darker colour indicating more ASVs and lighter colours indicating fewer ASVs. The size of the edges and nodes indicate the relative abundance of the ASVs assigned to a particular taxonomic level with wider edges/nodes indicating a high relative abundance and narrower edges/nodes indicating a low relative abundance.



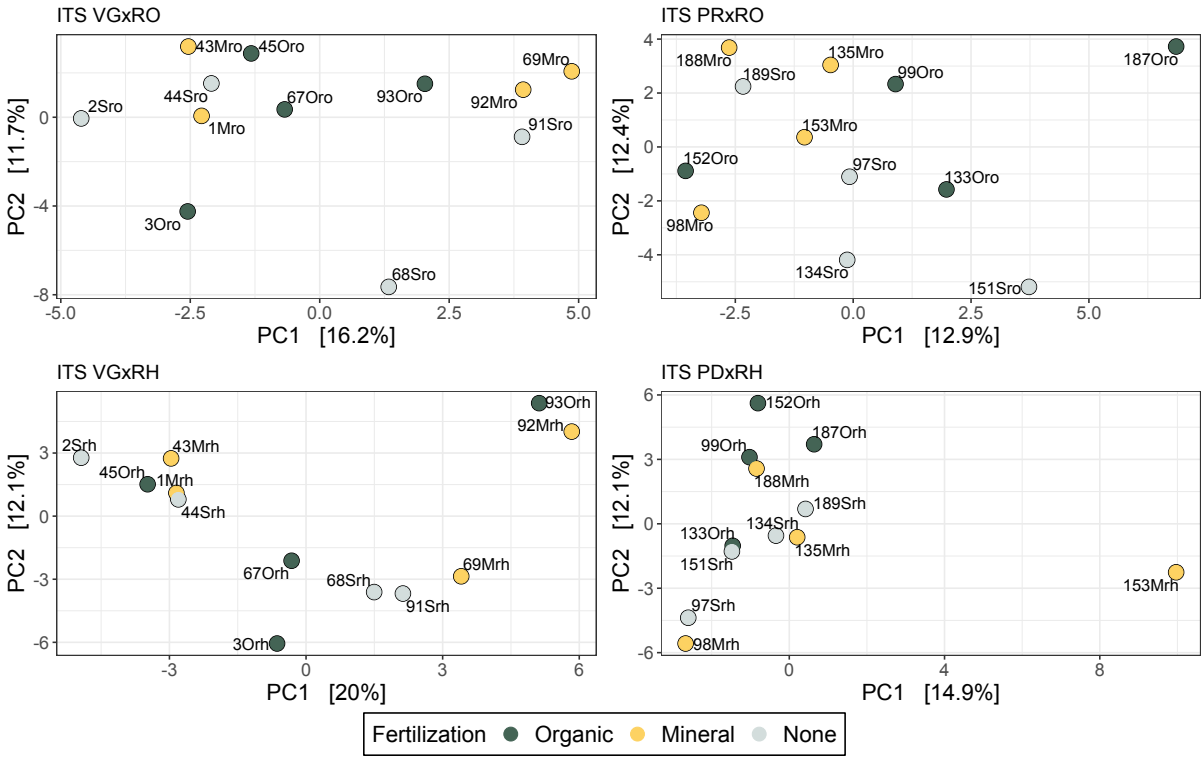
Supplementary Figure 3. Rarefaction curves of the fungal community after the filtering pipeline.



Supplementary Figure 4. Taxonomic overview of the fungal community. The figure plots ASVs which were assigned to a taxonomic level (ASVs not assigned at a particular taxonomic level (NAs) are not shown). The colour of the edges and nodes indicates the number of ASVs found at a given taxonomic level, with darker colour indicating more ASVs and lighter colours indicating fewer ASVs. The size of the edges and nodes indicate the relative abundance of the ASVs assigned to a particular taxonomic level with wider edges/nodes indicating a high relative abundance and narrower edges/nodes indicating a low relative abundance.



Supplementary Figure 5. PCA ordinations of the bacterial community based on the Aitchison distance (CLR transformation and Euclidean distance) as a representation of beta diversity (CLR transformation and Euclidean distance). The rows are compartments (roots : top row ; rhizosphere : bottom row) and the columns are the growth stage (pruning year : left row ; harvsting year : right row). The samples are colour-coded according to their fertilizer treatment (dark green for organic treatment, yellow for mineral treatment and grey for negative control).



Supplementary Figure 6. PCA ordinations of the fungal community based on the Aitchison distance (CLR transformation and Euclidean distance) as a representation of beta diversity (CLR transformation and Euclidean distance). The rows are compartments (roots : top row ; rhizosphere : bottom row) and the columns are the growth stage (pruning year : left row ; harvesting year : right row). The samples are colour-coded according to their fertilizer treatment (dark green for organic treatment, yellow for mineral treatment and grey for negative control).

Chapitre 5 – Synthèse

Rappels des objectifs et principaux résultats

Chapitre 2

Dans la première étude de cette thèse, nous souhaitions décrire les communautés bactériennes et fongiques, encore méconnues, du milieu racinaire des bleuetiers sauvages. En effet, au moment de l'échantillonnage, aucune étude ne s'était penchée sur la caractérisation des communautés bactériennes et fongiques de l'environnement racinaire du bleuetier sauvage. Or une meilleure connaissance du microbiote sous-terrain peut servir à adapter les pratiques agricoles pour mieux tirer profit de service écosystémiques rendus par ces micro-organismes. Le deuxième objectif était d'identifier des taxons ayant un potentiel bénéfique en termes de nutrition en azote pour le bleuetier sauvage. L'analyse des communautés fongiques obtenues dans les trois bleuetières commerciales échantillonnées indique une dominance de l'ordre des Helotiales contenant les principales espèces de champignons mycorhiziens éricoïdes connues. L'ordre des Rhizobiales, connu pour sa capacité à fixer l'azote atmosphérique, était prédominant pour les communautés de bactéries. Le fait de retrouver de nombreuses espèces de champignons mycorhiziens éricoïdes valide ainsi notre première hypothèse. De plus, nous avons également trouvé des corrélations positives significatives entre la concentration d'azote foliaire et l'abondance de taxons bactériens dont la fixation d'azote atmosphérique a préalablement été observée. De même, l'abondance de plusieurs espèces de champignons mycorhiziens éricoïdes était positivement corrélée à l'azote foliaire. La deuxième hypothèse de ce chapitre est donc également validée puisque nous avons identifié plusieurs taxons qui pourraient jouer un rôle dans la nutrition azotée du bleuetier sauvage. Cependant, des expériences additionnelles sont requises pour confirmer ces résultats.

Chapitre 3

La deuxième étude de cette thèse se penchait sur l'impact agronomique de la fauche thermique ainsi que son effet sur les communautés bactériennes et fongiques du bleuetier sauvage. L'objectif était de déterminer si cette pratique pourrait constituer une alternative à l'utilisation de produits phytosanitaires dans la lutte contre les mauvaises herbes et les maladies fongiques. Nous souhaitions déterminer les conséquences de la fauche thermique sur le microbiote sous-terrain du bleuetier sauvage afin de nous assurer que la perturbation occasionnée par cette pratique n'altère pas négativement le microbiote du milieu racinaire. L'effet du feu sur les communautés microbiennes du sol a été amplement étudié dans le cadre de feux de forêt mais notre contexte diffère en raison notamment de l'intensité du feu. Nos résultats ont indiqué un effet très limité de cette pratique sur les variables agronomiques et aucun effet sur le microbiote, et ce, même à forte intensité de fauche. Ainsi à l'exception d'une hausse temporaire de la concentration en phosphore dans le sol et d'une baisse temporaire des symptômes de la tâche septorienne, aucun effet sur le rendement en bleuet n'a été trouvé. Par conséquent, nos hypothèses n'ont soit pas été validées (acidité du sol et incidence des plantes adventices inchangées ; composition du microbiote inaltérée), soit été partiellement validées (baisse temporaire des symptômes de la tâche septorienne). Enfin, à l'instar de la première étude, dans l'ensemble du jeu de donnée, nous avons trouvé une prédominance des Helotiales et de Rhizobiales et identifié des espèces connues de champignons mycorhiziens éricoïdes.

Chapitre 4

La dernière étude de ce projet de doctorat visait à documenter l'effet de la fertilisation et du type d'engrais utilisé (minéral ou organique) sur le microbiote racinaire et rhizosphérique du bleuetier sauvage. A notre connaissance, aucune étude ne s'était concentrée sur l'effet d'une fertilisation contenant plusieurs nutriments sur le microbiote bactérien et fongique des racines et de la rhizosphère du bleuetier sauvage. L'objectif, en plus de mesurer l'impact de cette pratique au niveau agronomique, était de voir si l'ajout de nutriments dans le sol n'allait pas perturber les communautés de micro-organismes. Au niveau des variables agronomiques, la fertilisation augmente le rendement en fruit. L'effet de la fertilisation sur les communautés microbiennes a

été mesuré à court et moyen terme en séquençant l'ADN du sol et de racines prélevées lors de l'année végétative et productive. Les résultats indiquent globalement une absence d'effet de la fertilisation sur le microbiote racinaire et du sol rhizosphérique. L'échantillonnage pour le séquençage d'ADN à l'année végétative ayant lieu environ deux mois après la fertilisation, l'effet qu'ont pu avoir les nutriments sur les micro-organismes s'est peut-être estompé rapidement. Nous n'avons pas observé de différence en termes d'abondance relative pour les espèces de champignons mycorhiziens éricoïdes connus en comparant le contrôle négatif et les deux fertilisants. Notre hypothèse est donc infirmée, nous n'avons pas constaté une diminution de la proportion de taxons ayant été caractérisés comme étant des champignons mycorhiziens éricoïdes liée à la fertilisation.

Limites et perspectives

Les défis de l'analyse du microbiote

L'analyse du microbiote, ou comment tenter de comprendre ce que l'on ne voit pas ? Pour ma part, je pense qu'une des difficultés dans l'écologie microbienne réside dans l'invisibilité des sujets étudiés. Cette particularité implique d'avoir une certaine confiance dans les outils et les analyses utilisés afin de déchiffrer ces communautés. Or, malgré les avancées fulgurantes qu'ont permis le séquençage de l'ADN environnemental par métacodage à barres dans le domaine de la microbiologie, il demeure que cette technique et les analyses qui en découlent ne sont pas sans failles. Le premier biais réside dans l'échantillonnage et la différence d'échelle entre le monde microbien et notre monde. Pour une bactérie de 10 µm de diamètre, 1 m représente 100 000 fois sa taille. Si on rapporte ce nombre à notre échelle, cela représente la distance entre Montréal et Ottawa (pour un humain d'1m70). Ainsi, la communauté microbienne peut potentiellement beaucoup varier entre deux échantillons situés à 1 m l'un de l'autre. De plus, le sol n'est pas une matrice homogène, il est constitué de microagrégats qui ont des propriétés physico-chimiques variées influençant directement les micro-organismes qui peuplent ces milieux (Vos et al., 2013; Upton et al., 2019). Pour avoir une idée la plus représentative des communautés microbiennes dans un champ, les chercheurs sont encouragés à prendre de multiples échantillons qui seront regroupés par la suite, formant ainsi un échantillon composite. Cependant, cette méthode

demande un investissement supplémentaire en termes de temps d'échantillonnage et de préparation des échantillons, limitant le nombre de prélèvements composant l'échantillon composite. Par exemple, dans le chapitre 4, les échantillons composites étaient composés de trois prélèvements d'environ 10 cm de diamètre sur une parcelle de 15 m sur 22 m.

Le deuxième biais dans l'analyse du microbiote réside dans le processus d'obtention des séquences d'ADN analysées. Premièrement, l'extraction de l'ADN environnemental peut plus ou moins bien fonctionner sur les micro-organismes créant une première source de différence avec la communauté microbienne présente dans l'échantillon (Hermans et al., 2018; Saenz et al., 2019). De plus, la réaction en chaine par polymérase (PCR), permettant d'amplifier la région d'ADN ciblée et vouée à être séquencée, peut également introduire un biais. Bien que les amorces utilisées soient qualifiées d'universelles, il peut y avoir des problèmes d'appariement avec la séquence cible, réduisant l'amplification de certains organismes. Il peut y avoir également l'introduction de séquences chimériques ou des erreurs dans l'incorporation des nucléotides (Esposito et al., 2016; Fouhy et al., 2016; Eisenstein, 2018). D'autre part, les techniques de séquençage peuvent aussi introduire des erreurs, ne reproduisant pas fidèlement les séquences introduites dans les séquenceurs (Divoll et al., 2018 ; Tedersoo et al., 2018). L'utilisation de contrôles négatifs ainsi que de communautés connues (*mock communities*) comme contrôle positif, peut permettre de prendre conscience de ces biais, ce que j'ai tâché de faire pour les expériences du chapitre 3 et 4.

Un dernier biais réside dans l'analyse bio-informatique, où différentes méthodes donnent des résultats discordants qui peuvent mener à des interprétations contradictoires (Pauvert et al., 2019; Joos et al., 2020; Tedersoo et al., 2022). Dans cette thèse, j'ai utilisé un des plus récents pipeline bio-informatique (DADA2) qui, *a contrario* des anciens pipelines formant des unités taxonomiques opérationnelles (OTU) dont les séquences sont similaires à 97%, ne regroupe que les séquences identiques à 100% nommés variants de séquences amplifiées (ASV) (Callahan et al., 2016a). Cela permet une analyse plus fine du microbiote, et cela permet surtout de comparer entre eux, des ASVs provenant de différentes études ce qui n'est pas faisable avec la méthode des OTUs similaires à 97%. Les étapes de filtrage de ce pipeline bio-informatique permettent également de ne garder que les séquences de bonne qualité.

Tous ces biais sont donc à considérer lors de l'analyse des résultats qui représentent une version altérée de la communauté réellement présente. Cependant, comme nos analyses comparent des échantillons ayant reçu différents traitements, mais ayant été analysés de la même manière, on a tendance à estimer que les biais se sont appliqués de manière uniforme sur l'ensemble du jeu de données, permettant la comparaison des communautés présentent dans nos échantillons (Eisenstein, 2018).

Finalement, le séquençage de l'ADN par métacodage à barres n'indique que la présence d'un organisme à un instant donné. On peut comparer cela à une sorte d'arrêt sur image dans un film. De la même manière qu'il est compliqué de comprendre l'histoire d'un film avec une seule image, il en de même avec les communautés microbiennes qui sont dynamiques, fluctuantes dans le temps et l'espace, et dont nous prélevons un instantané pour établir un référentiel. De plus, l'ADN n'indique ni l'activité de l'organisme ni sa viabilité (Carini et al., 2016; Lennon et al., 2018), rendant davantage flou le référentiel établi. Plusieurs méthodes peuvent être employées pour faire face à ces problèmes. Ainsi un échantillonnage temporel permet de suivre l'évolution du microbiote et de prendre en compte son aspect dynamique (Lauber et al., 2013; Chaparro et al., 2014; Upton et al., 2019; Bullington et al., 2021). Du point de vue de l'activité et de la viabilité, le séquençage de l'ARN à la place de l'ADN peut être une alternative. L'ARN se dégrade plus rapidement que l'ADN et correspond donc plus fidèlement à la communauté microbienne présente au moment de l'échantillonnage (Cottier et al., 2018; Adamo et al., 2020). Toutefois, ces deux alternatives rajoutent des contraintes : l'échantillonnage temporel augmente nécessairement le nombre d'échantillons à analyser, et l'ARN étant plus instable que l'ADN, des précautions additionnelles doivent être mises en œuvre lors de l'échantillonnage pour préserver son intégrité.

Enfin, le métacodage à barres ne nous donne que l'aspect taxonomique de la communauté, mais ne renseigne par sur l'aspect fonctionnel de ces micro-organismes. Ces fonctions peuvent être insinuées par la taxonomie, en se basant sur des études ayant démontré que telle fonctionnalité est présente chez tel taxon. C'est ce qui a été fait dans cette thèse avec par exemple la possible importance de plusieurs espèces de Rhizobiales (*Roseiarcus fermentans* , *Rhodoplanes sp.*, *Bradyrhizobium sp.*, *Methylocella sp., Methylovirgula ligni, Blastochloris sp.*) dont la capacité à

fixer l'azote atmosphérique a été décrite dans de précédentes recherches. Malgré le fait que certaines Rhizobiales, telles que le genre *Azotobacter,* ne peuvent pas supporter des pH trop bas (<5 ou 6) (Aasfar et al., 2021), d'autres études ont montré une activité de nitrogénase de Rhizobiales à des pH acides (Barbosa et al., 2002; Avelar Ferreira et al., 2012). Toutefois, ces insinuations et suppositions doivent être vérifiées afin d'être validées, car les données de métacodage à barres ne permettent pas d'affirmer une fonctionnalité avec certitude. Les fonctions dignes d'intérêt pour de futures études seraient essentiellement nutritives, étant donné le faible pool d'azote et de phosphore directement accessible au bleuetier. Les champignons mycorhiziens éricoïdes, de par leur capacité mycorhizienne et leur activité enzymatique qui leur permet d'accéder aux pools de nutriments sous forme organique, ont donc attiré l'attention dans ce projet de recherche. Les enzymes dégradant la matière organique sont également présentes chez la guilde des champignons saprotrophes (Martino et al., 2018). Il y a donc une certaine redondance fonctionnelle, cependant la capacité de la plante de récupérer les nutriments libérés par les saprotrophes est sûrement moindre comparé à l'association mycorhizienne éricoïde. Idem, certaines bactéries que nous avons identifiées comme ayant une abondance relative élevée (plus de 5% de la communauté) ont été caractérisées comme ayant un potentiel de dégradation de la matière organique ce qui pourrait, *in fine,* bénéficier à la plante à proximité de ces bactéries.

Avec tous ces biais, on peut légitimement se demander ce que valent les résultats obtenus dans cette thèse. Plusieurs aspects peuvent, néanmoins, nous conforter dans une certaine cohérence de nos analyses. Premièrement, trouver des espèces de champignons mycorhiziens éricoïdes avec une abondance relative importante (ex : 12.8% et 16,2 % de la communauté fongique pour *Pezoloma ericae* dans le chapitre 3 et 4 respectivement) fait sens, et s'inscrit dans une lignée d'études indiquant l'importance que ces champignons jouent pour les Éricacées (Cairney and Meharg, 2003; Read et al., 2004). Pour les bactéries, moins d'études s'étant penchées sur ces communautés en lien avec les Ericacées, il est plus compliqué d'évaluer l'exactitude de nos résultats. Cependant, le fait de retrouver une forte abondance relative des Rhizobiales est en accord avec une autre étude ayant obtenu les mêmes résultats sur d'autres espèces de *Vaccinium* (Timonen et al., 2017; Li et al., 2020).

Cela nous amène à un deuxième aspect qu'est le caractère reproductible des résultats, un des principes de la science. Trois années et environ 150 km séparent l'échantillonnage du chapitre 2 et ceux des chapitres 3 et 4. De plus, les kits d'extractions d'ADN utilisés sont différents. Pourtant, on retrouve les mêmes tendances avec les Helotiales et les Rhizobiales qui dominent les communautés fongiques et bactériennes. Comme mentionné dans l'introduction, peu d'études se sont intéressées spécifiquement aux communautés microbiennes de *Vaccinium angustifolium, in situ* : les études de Yurgel *et al.* (Yurgel et al., 2017; Yurgel et al., 2018; Yurgel et al., 2019) (toutes basées sur le même échantillonnage), et de Lloyd *et al.* (Lloyd et al., 2021). Cela limite les comparaisons possibles, d'autant que ces publications proviennent toutes de la même équipe de recherche. Dans les analyses de Yurgel et collaborateurs, le niveau taxonomique retenu pour l'analyse du microbiote est le phylum ne permettant pas une analyse fine de la communauté microbienne. On retrouve néanmoins des similitudes avec une dominance des Proteobacteria, Acidobacteria et Actinobacteria chez les bactéries et d'Ascomycota chez les champignons. En revanche, les Planctomycetota ne sont pas mentionnés. Dans l'étude de Lloyd *et al*., (2021), les chercheurs obtiennent des résultats très différents en ce qui concerne la communauté fongique puisque c'est le phylum Basidiomycota qui domine la communauté (88% de l'abondance relative), principalement composé de la famille des Clavariaceae (60% de l'abondance relative) (Lloyd et al., 2021). Cette publication ne mentionne pas d'Helotiales dans leur communauté ni ne discute de leur absence alors que cet ordre contient la majeure partie des espèces de champignons mycorhiziens éricoïdes connus. En ce qui concerne les bactéries, on retrouve une forte abondance des Proteobacteria, Actinobacteriota et Acidobacteriota, mais les Planctomycetota sont moins abondantes que dans nos échantillons des chapitres 3 et 4.

En dépit de son imperfection, la popularité du séquençage d'ADN par métacodage à barres demeure, car cette technique est relativement peu coûteuse par rapport à de la métagénomique ou de la métatranscriptomique, et facile à mettre en place. Cette méthode est un bon moyen d'explorer le microbiote d'un milieu et peut permettre de poser des hypothèses à vérifier avec des expériences subséquentes. Enfin, comme tous pans de la science, la confiance à accorder aux résultats obtenus est liée à leur reproductibilité. Ainsi davantage d'études sur le microbiote du

bleuetier sauvage pourront confirmer ou infirmer les résultats obtenus dans ce projet et faire avancer nos connaissances sur ces communautés microbiennes.

Les champignons mycorhiziens éricoïdes

En raison de l'appartenance du bleuetier sauvage à la famille des Éricacées, ce projet de recherche porte un intérêt particulier à la symbiose mycorhizienne spécifique à cette famille. Les différents chapitres de cette thèse ont indiqué la prédominance de certaines espèces de champignons mycorhiziens éricoïdes connues, tels que *Pezoloma ericae* ou *Oidiodendron maius,* qui occupaient une part importante de la communauté rhizosphérique ou racinaire en termes d'abondance relative. Cependant, comme indiqué précédemment, le séquençage par métacodage à barres n'indique pas l'activité des micro-organismes, d'autant que pour valider l'aspect mycorhizien du champignon, il faut satisfaire les postulats de Koch soit l'isolation en culture pure, l'inoculation en condition contrôlée ainsi que le transfert réciproque de ressources (Leopold, 2016). Smith et Jones remettent d'ailleurs en question le caractère mutualiste d'une mycorhize stipulant que cette caractéristique est très compliquée à démontrer et, par conséquent, qu'elle ne devrait pas être obligatoirement requise dans les prérequis (Jones and Smith, 2004).

Ainsi, la présence en abondance de ces champignons ne permet pas de statuer sur l'effet qu'ils auraient sur le bleuetier, d'autant que nous n'avons pas trouvé de liens entre leur abondance et les variables agronomiques dans les chapitres 3 et 4. La corrélation entre l'azote foliaire et certains champignons mycorhiziens éricoïdes (dans le chapitre 2) suggère une relation, mais nécessite des études approfondies pour le vérifier. De plus, ces champignons ont la particularité de ne pas être symbiotrophes obligatoires, à l'inverse des champignons mycorhiziens arbusculaires, signifiant qu'ils peuvent se passer de l'association symbiotique, bien qu'elle leur offre un avantage compétitif (Martino et al., 2018 ; Ward et al., 2022). Les recherches sur les champignons mycorhiziens éricoïdes sont bien moins nombreuses que celles concernant les champignons mycorhiziens arbusculaires et ectomycorhiziens (Vohnik, 2020) et des recherches sont encore nécessaires pour décrire et comprendre cette symbiose. A l'instar des champignons mycorhiziens arbusculaires, le potentiel biostimulant des champignons ErM a déjà été exploité, notamment dans des pépinières, afin d'accélérer la croissance de jeunes plants de bleuets en

corymbe (Scagel et al., 2005), de canneberges cultivées en hydroponie (Kosola et al., 2007) ou de rhododendrons (Wei et al., 2016). Cependant, à ma connaissance, il n'existe pas encore de produits bio-inoculants à base de champignons ErM qui pourraient être dispersés dans les bleuetières afin d'augmenter leur abondance. Une des raisons réside peut-être dans le fait que les champignons ErM sont difficiles à cultiver artificiellement en raison de leur croissance très lente, et qu'une grande quantité serait nécessaire pour couvrir les superficies des bleuetières. De plus, nos études tendent à montrer que plusieurs espèces de champignons ErM sont déjà présentes naturellement dans la rhizosphère et les racines des bleuetiers sauvages. Ainsi, des produits biostimulants ne seraient pas forcément un investissement rentable pour les producteurs, d'autant que plusieurs limites sont associées avec ces produits (Kaminsky et al., 2019; Sessitsch et al., 2019). Cependant, une meilleure compréhension des conditions favorisant la symbiose mycorhizienne pourrait permettre de tirer davantage profit des espèces déjà en place.

Conclusion

Ce projet de recherche a permis de mettre en lumière la prédominance des Helotiales et des Rhizobiales dans les communautés fongiques et bactériennes de la rhizosphère et des racines du bleuetier sauvage. Ces deux groupes ont un intérêt nutritif pour le bleuetier, l'un contenant des champignons mycorhiziens éricoïdes, l'autre des bactéries fixatrices d'azote. Nous avons également trouvé un faible effet de la fauche thermique et de la fertilisation sur ces communautés microbiennes. De futures recherches sont nécessaires afin de tester le potentiel biostimulant des taxons d'intérêt identifiés dans cette thèse notamment les champignons mycorhiziens éricoïdes identifiés connus (*Pezoloma ericae, Oidiodendron maius, Oidiodendron chlamydosporicum, Hyaloscypha variabilis, Hyaloscypha bicolor, Mycosymbioces sp*.) et putatif (*Clavaria sphagnicola* , *Luellia sp., Serendipita sp.)*. Le dark septate endophyte *Phialocephala fortinii* serait également un bon candidat étant donné les résultats de la littérature à son sujet. Au niveau des bactéries, *Roseiarcus fermentans* , *Rhodoplanes sp.*, *Bradyrhizobium sp.*, *Methylocella sp., Methylovirgula ligni, Blastochloris sp.* seraient de bons candidats pour leur potentiel de fixation d'azote atmosphérique ainsi que des bactéries provenant des genres *Acidothermus sp*. et *Aquisphaera sp.* pour leur potentiel de dégradation de la matière organique.

Les fonctions à examiner seraient la présence de gènes codant pour des nitrogénases dans le cadre des bactéries susceptibles de fixer l'azote, la présence de gènes codant pour des enzymes de type phosphatase, chitinase et de « carbohydrate-active enzymes » (CAZymes) chez les champignons ainsi que la colonisation mycorhizienne des racines. D'autres fonctions connues comme ayant des impacts bénéfiques pour la plante, tels que la production de phytohormones tel que l'acide indole-3-acétique pourraient également être explorées. Enfin, une meilleure compréhension des conditions influençant ces taxons d'intérêt pourrait permettre d'envisager des ajustements des pratiques agronomiques dans les bleuetières afin d'optimiser les variables pour favoriser ces micro-organismes.

Références bibliographiques

Aasfar, A., Bargaz, A., Yaakoubi, K., Hilali, A., Bennis, I., Zeroual, Y. and Meftah Kadmiri, I. (2021). "Nitrogen fixing Azotobacter species as potential soil biological enhancers for crop nutrition and yield stability." Frontiers in microbiology **12**: 628379.

Abdelfattah, A., Malacrinò, A., Wisniewski, M., Cacciola, S. O. and Schena, L. (2018). "Metabarcoding: A powerful tool to investigate microbial communities and shape future plant protection strategies." Biological Control **120**: 1-10.

Acree, A., Fultz, L. M., Lofton, J. and Haggard, B. (2020). "Soil biochemical and microbial response to wheat and corn stubble residue management in Louisiana." Agrosystems, Geosciences & Environment **3**(1).

Adamczyk, B., Ahvenainen, A., Sietiö, O.-M., Kanerva, S., Kieloaho, A.-J., Smolander, A., Kitunen, V., Saranpää, P., Laakso, T., Straková, P. and Heinonsalo, J. (2016). "The contribution of ericoid plants to soil nitrogen chemistry and organic matter decomposition in boreal forest soil." Soil Biology and Biochemistry **103**: 394-404.

Adamo, M., Voyron, S., Chialva, M., Marmeisse, R. and Girlanda, M. (2020). "Metabarcoding on both environmental DNA and RNA highlights differences between fungal communities sampled in different habitats." PLoS One **15**(12): e0244682.

Adesemoye, A. O., Torbert, H. A. and Kloepper, J. W. (2009). "Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers." Microb Ecol **58**(4): 921-929.

Agriculture and Agri-Food Canada. (2019, 2019-07-09). "Little Berry, Big Impact." Retrieved 29th of july, 2019, from http://www.agr.gc.ca/eng/about-us/publications/discover-agriculture/little-berry-big-impact/?id=1412014431201.

Allen, T. R., Millar, T., Berch, S. M. and Berbee, M. L. (2003). "Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots." New Phytologist **160**(1): 255-272.

Alvear, M., Rosas, A., Rouanet, J. L. and Borie, F. (2005). "Effects of three soil tillage systems on some biological activities in an Ultisol from southern Chile." Soil and Tillage Research **82**(2): 195-202.

Archibald, J. M. (2015). "Endosymbiosis and eukaryotic cell evolution." Current Biology **25**(19): R911-R921.

Avelar Ferreira, P. A., Bomfeti, C. A., Lima Soares, B. and de Souza Moreira, F. M. (2012). "Efficient nitrogen-fixing Rhizobium strains isolated from amazonian soils are highly tolerant to acidity and aluminium." World Journal of Microbiology and Biotechnology **28**(5): 1947-1959.

Bach, E. M., Williams, R. J., Hargreaves, S. K., Yang, F. and Hofmockel, K. S. (2018). "Greatest soil microbial diversity found in micro-habitats." Soil Biology and Biochemistry **118**: 217-226.

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S. and Vivanco, J. M. (2006). "The role of root exudates in rhizosphere interactions with plants and other organisms." Annu Rev Plant Biol **57**: 233-266.

Bakker, M. G. (2018). "A fungal mock community control for amplicon sequencing experiments." Molecular Ecology Resources **18**(3): 541-556.

Bakker, P. A., Berendsen, R. L., Doornbos, R. F., Wintermans, P. C. and Pieterse, C. M. (2013). "The rhizosphere revisited: root microbiomics." Front Plant Sci **4**: 165.

Barabote, R. D., Xie, G., Leu, D. H., Normand, P., Necsulea, A., Daubin, V., Medigue, C., Adney, W. S., Xu, X. C., Lapidus, A., Parales, R. E., Detter, C., Pujic, P., Bruce, D., Lavire, C., Challacombe, J. F., Brettin, T. S. and Berry, A. M. (2009). "Complete genome of the cellulolytic thermophile Acidothermus cellulolyticus 11B provides insights into its ecophysiological and evolutionary adaptations." Genome Research **19**(6): 1033-1043.

Barbosa, H. R., Moretti, M. A., Thuler, D. S. and Augusto, E. F. (2002). "Nitrogenase activity of Beijerinckia derxii is preserved under adverse conditions for its growth." Brazilian Journal of Microbiology **33**: 223-229.

Bardgett, R. D. and McAlister, E. (1999). "The measurement of soil fungal : bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands." Biol Fert Soils **29**(3): 282-290.

Barea, J. M., Pozo, M. J., Azcon, R. and Azcon-Aguilar, C. (2005). "Microbial co-operation in the rhizosphere." J Exp Bot **56**(417): 1761-1778.

Barker, W. G., Hall, I. V., Aalders, L. E. and Wood, G. W. (1964). "The lowbush blueberry industry in eastern canada." Economic Botany: 357-365.

Bates, D. M., Maechler, M., Bolker, B. B. and S., W. (2015). "Fitting Linear Mixed-Effects Models Using lme4." Journal of Statistical Software **67**(1): 1-48.

Bell, D. J., Rowland, L. J., Stommel, J. and Drummond, F. A. (2010). "Yield variation among clones of lowbush blueberry as a function of genetic similarity and self-compatibility." Journal of the American Society for Horticultural Science **135**(3): 259-270.

Beltran-Garcia, M. J., Martínez-Rodríguez, A., Olmos-Arriaga, I., Valdes-Salas, B., Di Mascio, P. and White, J. F. (2021). "Nitrogen fertilization and stress factors drive shifts in microbial diversity in soils and plants." Symbiosis **84**(3): 379-390.

Bent, S. J. and Forney, L. J. (2008). "The tragedy of the uncommon: understanding limitations in the analysis of microbial diversity." ISME J **2**(7): 689-695.

Berendsen, R. L., Pieterse, C. M. and Bakker, P. A. (2012). "The rhizosphere microbiome and plant health." Trends in plant science **17**(8): 478-486.

Berg, G. (2009). "Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture." Appl Microbiol Biotechnol **84**(1): 11-18.

Bertin, C., Yang, X. and Weston, L. A. (2003). "The role of root exudates and allelochemicals in the rhizosphere." Plant and soil **256**(1): 67-83.

Bever, J. D. (1994). "Feedback between Plants and Their Soil Communities in an Old Field Community." Ecology **75**(7): 1965-1977.

Black, W. N. (1963). "The effect of frequency of rotational burning on blueberry production." Canadian Journal of Plant Science **43(2)**(2): 161-165.

Blatt, C. R., Hall, I. V., Jensen, K. I. N., Neilson, W. T. A., Hildebrand, P. D., Nickerson, N. L., Prange, R. K., Lidster, P., D., C., L. and Sibley, J. D. (1989). Lowbush blueberry production. Ottawa, ON., Agriculture Canada.

Boeraeve, M., Kohout, P., Ceulemans, T., Cajthaml, T., Tedersoo, L. and Jacquemyn, H. (2022). "Changes in the root microbiome of four plant species with different mycorrhizal types across a nitrogen deposition gradient in ombrotrophic bogs." Soil Biology and Biochemistry **169**.

Bondoso, J., Albuquerque, L., Nobre, M. F., Lobo-da-Cunha, A., da Costa, M. S. and Lage, O. M. (2011). "Aquisphaera giovannonii gen. nov., sp. nov., a planctomycete isolated from a freshwater aquarium." International Journal of Systematic Evolutionary Microbiology **61**(Pt 12): 2844-2850.

Brazelton, C. (2013). "World blueberry acreage & production." Folsom: USHBC.

Brundrett, M. C. and Tedersoo, L. (2018). "Evolutionary history of mycorrhizal symbioses and global host plant diversity." New Phytol **220**(4): 1108-1115.

Bruns, T. D. and Read, D. J. (2000). "*In vitro* germination of nonphotosynthetic, myco-heterotrophic plants stimulated by fungi isolated from the adult plants." New Phytol **148**(2): 335-342.

Buckley, D. H., Huangyutitham, V., Hsu, S. F. and Nelson, T. A. (2007). "Stable isotope probing with 15N2 reveals novel noncultivated diazotrophs in soil." Applied and Environmental Microbiology **73**(10): 3196-3204.

Bullington, L. S., Lekberg, Y. and Larkin, B. G. (2021). "Insufficient sampling constrains our characterization of plant microbiomes." Scientific Reports **11**(1): 3645.

Burd, G. I., Dixon, D. G. and Glick, B. R. (2000). "Plant growth-promoting bacteria that decrease heavy metal toxicity in plants." Canadian journal of microbiology **46**(3): 237-245.

Cairney, J. W. G. and Burke, R. M. (1998). "Extracellular enzyme activities of the ericoid mycorrhizal endophyte *Hymenoscyphus ericae* (Read) Korf & Kernan: their likely roles in decomposition of dead plant tissue in soil." Plant and Soil **205**(2): 181-192.

Cairney, J. W. G. and Meharg, A. A. (2003). "Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions." European Journal of Soil Science **54**(4): 735-740.

Caldwell, B. A., Jumpponen, A. and Trappe, J. M. (2000). "Utilization of Major Detrital Substrates by Dark-Septate, Root Endophytes." Mycologia **92**(2): 230-232.

Callahan, B. J. (2018). Silva for dada2: Silva taxonomic training data formatted for dada2 (silva version 132). Zenodo.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. and Holmes, S. P. (2016a). "DADA2: High-resolution sample inference from Illumina amplicon data." Nature Methods **13**(7): 581-583.

Callahan, B. J., Sankaran, K., Fukuyama, J. A., McMurdie, P. J. and Holmes, S. P. (2016b). "Bioconductor Workflow for Microbiome Data Analysis: from raw reads to community analyses [version 2; peer review: 3 approved]." F1000Research **5**(1492).

Carini, P., Marsden, P. J., Leff, J. W., Morgan, E. E., Strickland, M. S. and Fierer, N. (2016). "Relic DNA is abundant in soil and obscures estimates of soil microbial diversity." Nat Microbiol **2**: 16242.

Casarrubia, S., Martino, E., Daghino, S., Kohler, A., Morin, E., Khouja, H. R., Murat, C., Barry, K. W., Lindquist, E. A., Martin, F. M. and Perotto, S. (2020). "Modulation of Plant and Fungal Gene Expression Upon Cd Exposure and Symbiosis in Ericoid Mycorrhizal Vaccinium myrtillus." Frontiers in Microbiology **11**: 341.

Caspersen, S., Svensson, B., Håkansson, T., Winter, C., Khalil, S. and Asp, H. (2016). "Blueberry—Soil interactions from an organic perspective." Scientia Horticulturae **208**: 78-91.

Chaparro, J. M., Badri, D. V., Bakker, M. G., Sugiyama, A., Manter, D. K. and Vivanco, J. M. (2013). "Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions." PLoS One **8**(2): e55731.

Chaparro, J. M., Badri, D. V. and Vivanco, J. M. (2014). "Rhizosphere microbiome assemblage is affected by plant development." ISME J **8**(4): 790-803.

Chapeskie, A. J. (2001). Northern homelands, northern frontier: linking culture and economic security in contemporary livelihoods in boreal and cold temperate forest communities in northern Canada. Forest communities in the third millennium: linking research, business, and policy toward a sustainable non-timber forest product sector.**:** 31-44.

Chekireb, D., Crovadore, J., Brachmann, A., Chablais, R., Cochard, B. and Lefort, F. (2017). "Whole-Genome Sequences of 14 Strains of *Bradyrhizobium canariense* and 1 Strain of

Bradyrhizobium japonicum Isolated from *Lupinus spp.* in Algeria." Genome Announc **5**: e00676-00617.

Chen, T., Nomura, K., Wang, X., Sohrabi, R., Xu, J., Yao, L., Paasch, B. C., Ma, L., Kremer, J. and Cheng, Y. (2020). "A plant genetic network for preventing dysbiosis in the phyllosphere." Nature **580**(7805): 653-657.

Close, D., Ojumu, J. and Zhang, G. (2016). "Draft Genome Sequence of Cryptococcus terricola JCM 24523, an Oleaginous Yeast Capable of Expressing Exogenous DNA." Genome Announc **4**(6).

Collins, J. A. and Drummond, F. A. (2004). "Field-edge based management tactics for blueberry maggot in lowbush blueberry." Small Fruits Review **3**(3-4): 285-293.

Cordell, D., Drangert, J.-O. and White, S. (2009). "The story of phosphorus: Global food security and food for thought." Global Environmental Change **19**(2): 292-305.

Cosme, M., Fernandez, I., Van der Heijden, M. G. A. and Pieterse, C. M. J. (2018). "Non-Mycorrhizal Plants: The Exceptions that Prove the Rule." Trends Plant Sci **23**(7): 577-587.

Cottier, F., Srinivasan, K. G., Yurieva, M., Liao, W., Poidinger, M., Zolezzi, F. and Pavelka, N. (2018). "Advantages of meta-total RNA sequencing (MeTRS) over shotgun metagenomics and amplicon-based sequencing in the profiling of complex microbial communities." NPJ Biofilms Microbiomes **4**: 2.

CRAAQ, C. d. r. e. a. e. a. d. Q. (2013). Guide d'identification - Alliés et ennemis du bleuet nain. Insectes, maladies et végétaux.

Crous, P. W., Schumacher, R. K., Wingfield, M. J., Akulov, A., Denman, S., Roux, J., Braun, U., Burgess, T. I., Carnegie, A. J., Vaczy, K. Z., Guatimosim, E., Schwartsburd, P. B., Barreto, R. W., Hernandez-Restrepo, M., Lombard, L. and Groenewald, J. Z. (2018). "New and Interesting Fungi. 1." Fungal Systematics and Evolution **1**: 169-216.

Daghino, S., Martino, E. and Perotto, S. (2016). "Model systems to unravel the molecular mechanisms of heavy metal tolerance in the ericoid mycorrhizal symbiosis." Mycorrhiza **26**(4): 263-274.

Dalpé, Y. (1989). "Ericoid mycorrhizal fungi in the Myxotrichaceae and Gymnoascaceae." New Phytol **113**: 523-527.

Dalpé, Y. (1991). "Statut endomycorhizien du genre Oidiodendron." Canadian journal of botany **69**(8): 1712-1714.

Dangi, S. R., Stahl, P. D., Pendall, E., Cleary, M. B. and Buyer, J. S. (2010). "Recovery of soil microbial community structure after fire in a sagebrush-grassland ecosystem." Land Degradation & Development **21**(5): 423-432.

de Cáceres, M. and Legendre, P. (2009). "Associations between species and groups of sites: indices and statistical inference." Ecology **90**(12): 3566-3574.

de Vries, F. T., Griffiths, R. I., Bailey, M., Craig, H., Girlanda, M., Gweon, H. S., Hallin, S., Kaisermann, A., Keith, A. M., Kretzschmar, M., Lemanceau, P., Lumini, E., Mason, K. E., Oliver, A., Ostle, N., Prosser, J. I., Thion, C., Thomson, B. and Bardgett, R. D. (2018). "Soil bacterial networks are less stable under drought than fungal networks." Nat Commun **9**(1): 3033.

de Vries, F. T., Manning, P., Tallowin, J. R. B., Mortimer, S. R., Pilgrim, E. S., Harrison, K. A., Hobbs, P. J., Quirk, H., Shipley, B., Cornelissen, J. H. C., Kattge, J. and Bardgett, R. D. (2012). "Abiotic drivers and plant traits explain landscape-scale patterns in soil microbial communities." Ecology Letters **15**(11): 1230-1239.

De-la-Peña, C., Badri, D. V., Lei, Z., Watson, B. S., Brandao, M. M., Silva-Filho, M. C., Sumner, L. W. and Vivanco, J. M. (2010). "Root secretion of defense-related proteins is development-dependent and correlated with flowering time." J Biol Chem **285**(40): 30654-30665.

Dedej, S., Delaplane, K. S. and Scherm, H. (2004). "Effectiveness of honey bees in delivering the biocontrol agent Bacillus subtilis to blueberry flowers to suppress mummy berry disease." Biological Control **31**(3): 422-427.

Dedysh, S. N., Berestovskaya, Y. Y., Vasylieva, L. V., Belova, S. E., Khmelenina, V. N., Suzina, N. E., Trotsenko, Y. A., Liesack, W. and Zavarzin, G. A. (2004). "Methylocella tundrae sp. nov., a novel methanotrophic bacterium from acidic tundra peatlands." Int J Syst Evol Microbiol **54**(Pt 1): 151-156.

Dedysh, S. N., Liesack, W., Khmelenina, V. N., Suzina, N. E., Trotsenko, Y. A., Semrau, J. D., Bares, A. M., Panikov, N. S. and Tiedje, J. M. (2000). "Methylocella palustris gen. nov., sp. nov., a new methane-oxidizing acidophilic bacterium from peat bogs, representing a novel subtype of serine-pathway methanotrophs." Int J Syst Evol Microbiol **50 Pt 3**: 955-969.

Dickie, I. A. and St John, M. G. (2016). Second-generation molecular understanding of mycorrhizas in soil ecosystems. Molecular mycorrhizal symbiosis. J. W. S. Inc. Hoboken, NJ, USA**:** 473-491.

Divoll, T. J., Brown, V. A., Kinne, J., McCracken, G. F. and O'Keefe, J. M. (2018). "Disparities in second-generation DNA metabarcoding results exposed with accessible and repeatable workflows." Mol Ecol Resour **18**(3): 590-601.

Dooley, S. R. and Treseder, K. K. (2011). "The effect of fire on microbial biomass: a meta-analysis of field studies." Biogeochemistry **109**(1-3): 49-61.

Dove, N. C. and Hart, S. C. (2017). "Fire Reduces Fungal Species Richness and In Situ Mycorrhizal Colonization: A Meta-Analysis." Fire Ecology **13**(2): 37-65.

Drummond, F., Ballman, E. and Collins, J. (2019). "Population dynamics of spotted wing Drosophila (*Drosophila suzukii* (Matsumura)) in Maine wild blueberry (*Vaccinium angustifolium* Aiton)." Insects **10**(7): 205.

Drummond, F., Smagula, J., Annis, S. and Yarborough, D. (2009). "Organic wild blueberry production." MAFES Bull **852**: 43.

Drummond, F. A. and Groden, E. (2000). Evaluation of entomopathogens for biological control of insect pests of lowbush (wild) blueberry, Department of Biological Sciences, Deering Hall, University of Maine.

Ducousso-Détrez, A., Fontaine, J., Lounès-Hadj Sahraoui, A. and Hijri, M. (2022). "Diversity of Phosphate Chemical Forms in Soils and Their Contributions on Soil Microbial Community Structure Changes." Microorganisms **10**(3): 609.

Dunfield, P. F., Khmelenina, V. N., Suzina, N. E., Trotsenko, Y. A. and Dedysh, S. N. (2003). "Methylocella silvestris sp. nov., a novel methanotroph isolated from an acidic forest cambisol." Int J Syst Evol Microbiol **53**(Pt 5): 1231-1239.

Eaton, L. J. and Patriquin, D. G. (1988). "Inorganic nitrogen levels and nitrification potential in lowbush blueberry soils." Canadian journal of soil science **68**(1): 63-75.

Eaton, L. J. and Patriquin, D. G. (1990). "Fate of labelled fertilizer nitrogen in commercial lowbush blueberry stands." Canadian journal of soil science **70**(4): 727-730.

Eaton, L. J., Stratto, G. W. and Sanderson, K. R. (1996). Fertilizer phosphorus in lowbush blueberries: effects and fate. VI International Symposium on Vaccinium Culture 446.

Eisen, J. A. (2007). "Environmental shotgun sequencing: its potential and challenges for studying the hidden world of microbes." PLoS Biol **5**(3): e82.

Eisenstein, M. (2018). "Microbiology: making the best of PCR bias." Nat Methods **15**(5): 317-320.

Englander, L. and Hull, R. J. (1980). "Reciprocal transfer of nutrients between ericaceous plants and a *Clavaria* sp." New Phytologist **84**(4): 661-667.

Enwall, K., Nyberg, K., Bertilsson, S., Cederlund, H., Stenström, J. and Hallin, S. (2007). "Long-term impact of fertilization on activity and composition of bacterial communities and metabolic guilds in agricultural soil." Soil Biology and Biochemistry **39**(1): 106-115.

Esau, T. J., Zaman, Q. U., Chang, Y. K., Schumann, A. W., Percival, D. C. and Farooque, A. A. (2014). "Spot-application of fungicide for wild blueberry using an automated prototype variable rate sprayer." Precision agriculture **15**(2): 147-161.

Esposito, A., Colantuono, C., Ruggieri, V. and Chiusano, M. L. (2016). "Bioinformatics for agriculture in the Next-Generation sequencing era." Chemical and Biological Technologies in Agriculture **3**(1).

Fadaei, S. (2019). Effects of Ericoid Mycorrhizal Fungi on Growth and Salt Tolerance of Blueberry (Vaccinium myrtilloides), Lingonberry (Vaccinium vitis-idaea), and Labrador tea (Rhododendron groenlandicum): Implications for Oil Sands Reclamation. Master of Science, University of Alberta.

Fierer, N. (2017). "Embracing the unknown: disentangling the complexities of the soil microbiome." Nat Rev Microbiol **15**(10): 579-590.

Fierer, N. and Jackson, R. B. (2006). "The diversity and biogeography of soil bacterial communities." Proceedings of the National Acadameic of Sciences **103**(3): 626-631.

Fitzpatrick, C. R., Copeland, J., Wang, P. W., Guttman, D. S., Kotanen, P. M. and Johnson, M. T. J. (2018). "Assembly and ecological function of the root microbiome across angiosperm plant species." Proc Natl Acad Sci U S A **115**(6): E1157-E1165.

Flinn, M. A. and Wein, R. W. (1977). "Depth of underground plant organs and theoretical survival during fire." Canadian Journal of Botany **55**(19): 2550-2554.

Foster, Z. S., Sharpton, T. J. and Grunwald, N. J. (2017). "Metacoder: An R package for visualization and manipulation of community taxonomic diversity data." PLoS Computational Biology **13**(2): e1005404.

Fouhy, F., Clooney, A. G., Stanton, C., Claesson, M. J. and Cotter, P. D. (2016). "16S rRNA gene sequencing of mock microbial populations- impact of DNA extraction method, primer choice and sequencing platform." BMC Microbiol **16**(1): 123.

Fox, J. and Weisberg, S. (2019). An R Companion to Applied Regression. Thousand Oaks CA, Sage Publications.

Franche, C., Lindström, K. and Elmerich, C. (2008). "Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants." Plant and Soil **321**(1-2): 35-59.

Frostegård, Å., Tunlid, A. and Bååth, E. (1991). "Microbial Biomass Measured as Total Lipid Phosphate in Soils of Different Organic Content." J Microbiol Meth **14**(3): 151-163.

Frostegård, Å., Tunlid, A. and Bååth, E. (2011). "Use and misuse of PLFA measurements in soils." Soil Biology and Biochemistry **43**(8): 1621-1625.

Gagnon, B., Simard, R., Lalande, R. and Lafond, J. (2003). "Improvement of soil properties and fruit yield of native lowbush blueberry by papermill sludge addition." Canadian Journal of Soil Science **83**(1): 1-9.

Geisseler, D. and Scow, K. M. (2014). "Long-term effects of mineral fertilizers on soil microorganisms–A review." Soil Biology and Biochemistry **75**: 54-63.

Gloor, G. B., Macklaim, J. M., Pawlowsky-Glahn, V. and Egozcue, J. J. (2017). "Microbiome Datasets Are Compositional: And This Is Not Optional." Frontiers in Microbiology **8**: 2224.

Government of Canada. (2021). "Données des stations pour le calcul des normales climatiques au Canada de 1971 à 2000." Retrieved 29/11/2021, 2021, from

https://climat.meteo.gc.ca/climate_normals/results_f.html?searchType=stnProv&lstProvince=QC&txtCentralLatMin=0&txtCentralLatSec=0&txtCentralLongMin=0&txtCentralLongSec=0&stnID=5926&dispBack=0.

Grelet, G. A., Alexander, I. J., Proe, M. F., Frossard, J. S. and Millard, P. (2001). "Leaf habit influences nitrogen remobilization in Vaccinium species." Journal of Experimental Botany **52**(358): 993-1002.

Grelet, G. A., Ba, R., Goeke, D. F., Houliston, G. J., Taylor, A. F. S. and Durall, D. M. (2017). "A plant growth-promoting symbiosis between *Mycena galopus* and *Vaccinium corymbosum* seedlings." Mycorrhiza **27**(8): 831-839.

Grelet, G. A., Johnson, D., Paterson, E., Anderson, I. C. and Alexander, I. J. (2009). "Reciprocal carbon and nitrogen transfer between an ericaceous dwarf shrub and fungi isolated from Piceirhiza bicolorata ectomycorrhizas." New Phytologist **182**(2): 359-366.

Grubb, P. J., Green, H. T. and Merrifield, R. C. J. (1969). "The Ecology of Chalk Heath: Its Relevance to the Calcicole--Calcifuge and Soil Acidification Problems. ." The Journal of Ecology **57**(1): 175-212.

Gumiere, T., Rousseau, A. N., da Costa, D. P., Cassetari, A., Cotta, S. R., Andreote, F. D., Gumiere, S. J. and Pavinato, P. S. (2019). "Phosphorus source driving the soil microbial interactions and improving sugarcane development." Scientific reports **9**(1): 1-9.

Haegeman, B., Hamelin, J., Moriarty, J., Neal, P., Dushoff, J. and Weitz, J. S. (2013). "Robust estimation of microbial diversity in theory and in practice." ISME J **7**(6): 1092-1101.

Hall, I. (1978). Vaccinium species of horticultural importance in Canada. Horticultural Abstracts.

Hall, I. V. and Sibley, J. D. (1976). "THE BIOLOGY OF CANADIAN WEEDS: 20. *Cornus canadensis* L." Canadian Journal of Plant Science **56**(4): 885-892.

Hallin, S., Jones, C. M., Schloter, M. and Philippot, L. (2009). "Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment." The ISME journal **3**(5): 597-605.

Hamim, A., Miche, L., Douaik, A., Mrabet, R., Ouhammou, A., Duponnois, R. and Hafidi, M. (2017). "Diversity of fungal assemblages in roots of Ericaceae in two Mediterranean contrasting ecosystems." Comptes rendus biologies **340**(4): 226-237.

Hara, S., Morikawa, T., Wasai, S., Kasahara, Y., Koshiba, T., Yamazaki, K., Fujiwara, T., Tokunaga, T. and Minamisawa, K. (2019). "Identification of nitrogen-fixing Bradyrhizobium associated with roots of field-grown sorghum by metagenome and proteome analyses." Frontiers in microbiology **10**: 407.

Hardison, J. R. (1976). "Fire and flame for plant disease control." Annual Review of Phytopathology **14**(1): 355-379.

Harrel, F. E. J. (2020). Hmisc: Harrel Miscellaneous.

Hart, S. C., DeLuca, T. H., Newman, G. S., MacKenzie, M. D. and Boyle, S. I. (2005). "Post-fire vegetative dynamics as drivers of microbial community structure and function in forest soils." Forest Ecology and Management **220**(1-3): 166-184.

Hassan, S. E., Hijri, M. and St-Arnaud, M. (2013). "Effect of arbuscular mycorrhizal fungi on trace metal uptake by sunflower plants grown on cadmium contaminated soil." N Biotechnol **30**(6): 780-787.

Haynes, R. J. and Swift, R. S. (1985). "Growth and nutrient uptake by highbush blueberry plants in a peat medium as influenced by pH, applied micronutrients and mycorrhizal inoculation." Scientia horticulturae **27**(3-4): 285-294.

Hendershot, W. H., Lalande, H. and Duquette, M. (2007). Soil reaction and exchangeable acidity. Soil sampling and methods of analysis. Second edition. Canadian Society of Soil Science. M. R. Carter and E. G. Gregorich. Boca Raton, FL., Lewis Publishers**:** 171-178.

Hennecke, H., Kaluza, K., Thöny, B., Fuhrmann, M., Ludwig, W. and Stackebrandt, E. (1985). "Concurrent evolution of nitrogenase genes and 16S rRNA in *Rhizobium* species and other nitrogen fixing bacteria." Archives of Microbiology **142**: 342-348.

Hepler, P. R. and Yarborough, D. E. (1991). "Natural variability in yield of lowbush blueberries." HortScience **26**(3): 245-246.

Hermans, S. M., Buckley, H. L. and Lear, G. (2018). "Optimal extraction methods for the simultaneous analysis of DNA from diverse organisms and sample types." Mol Ecol Resour **18**(3): 557-569.

Hijri, M. (2016). "Analysis of a large dataset of mycorrhiza inoculation field trials on potato shows highly significant increases in yield." Mycorrhiza **26**(3): 209-214.

Hildebrand, P. D., Renderos, W. E. and Delbridge, R. W. (2016). "Diseases of lowbush blueberry and their identification." Agriculture and Agri-Food Canada.

Hoefs, M. E. G. and Shay, J. M. (1981). "The effects of shade on shoot growth of *Vaccinium angustifolium* Ait. after fire pruning in southeastern Manitoba." Canadian Journal of Botany **59**(2): 166-174.

Hu, L., Robert, C. A. M., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., van der Heijden, M. G. A., Schlaeppi, K. and Erb, M. (2018). "Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota." Nat Commun **9**(1): 2738.

Huss-Danell, K. (1997). "Tansley Review No. 93. Actinorhizal symbioses and their N2 fixation." New Phytol **136**(3): 375-405.

Isaac, R. A. and Johnson, W. C. (1976). "Determination of total nitrogen in plant tissue, using a block digestor." Journal of the Association of Official Analytical Chemists **59**(1): 98-100.

Ishida, T. A. and Nordin, A. (2010). "No evidence that nitrogen enrichment affect fungal communities of *Vaccinium* roots in two contrasting boreal forest types." Soil Biology and Biochemistry **42**(2): 234-243.

Ismail, A. A. and Hanson, E. J. (1982). "Interaction of method and date of pruning on growth and productivity of the lowbush blueberry." Canadian Journal of Plant Science **62**(3): 677-682.

Ismail, A. A., Smagula, J. M. and Yarborough, D. E. (1981). "Influence of pruning method, fertilizer and terbacil on the growth and yield of the lowbush blueberry." Canadian Journal of Plant Science **61**(1): 61-71.

Ismail, Y., McCormick, S. and Hijri, M. (2013). "The arbuscular mycorrhizal fungus, Glomus irregulare, controls the mycotoxin production of Fusarium sambucinum in the pathogenesis of potato." FEMS Microbiol Lett **348**(1): 46-51.

Ivanova, A. A., Naumoff, D. G., Miroshnikov, K. K., Liesack, W. and Dedysh, S. N. (2017). "Comparative Genomics of Four Isosphaeraceae Planctomycetes: A Common Pool of Plasmids and Glycoside Hydrolase Genes Shared by *Paludisphaera borealis* PX4(T), *Isosphaera pallida* IS1B(T), *Singulisphaera acidiphila* DSM 18658(T), and Strain SH-PL62." Frontiers in Microbiology **8**: 412.

Jalal, M. A. F. and Read, D. J. (1983). "The organic acid compostion of Calluna vulgaris heathland with special reference to phyto- and fungitoxicity." Plant and Soil **70**: 257-272.

Jeliazkova, E. A. and Percival, D. C. (2003). "N and P fertilizers, some growth variables, and mycorrhizae in wild blueberry (*Vaccinium angustifolium*)." Acta horticulturae: 297-304.

Jensen, K. I. N. and Yarborough, D. E. (2004). "An Overview of Weed Management in the Wild Lowbush Blueberry—Past and Present." Small Fruits Review **3**(3-4): 229-255.

Johnson, N. C., Graham, J. H. and Smith, F. A. (1997). "Functioning of mycorrhizal associations along the mutualism–parasitism continuum." New Phytol **135**(4): 575-585.

Jones, M. D. and Smith, S. E. (2004). "Exploring functional definitions of mycorrhizas: Are mycorrhizas always mutualisms?" Canadian Journal of Botany **82**(8): 1089-1109.

Jones, S. L. and French, K. (2021). "Soil nutrients differentially influence root colonisation patterns of AMF and DSE in Australian plant species." Symbiosis **83**(2): 209-223.

Joos, L., Beirinckx, S., Haegeman, A., Debode, J., Vandecasteele, B., Baeyen, S., Goormachtig, S., Clement, L. and De Tender, C. (2020). "Daring to be differential: metabarcoding analysis of soil and plant-related microbial communities using amplicon sequence variants and operational taxonomical units." BMC Genomics **21**(1): 733.

Jourand, P., Ducousso, M., Reid, R., Majorel, C., Richert, C., Riss, J. and Lebrun, M. (2010). "Nickel-tolerant ectomycorrhizal Pisolithus albus ultramafic ecotype isolated from nickel mines in New Caledonia strongly enhance growth of the host plant Eucalyptus globulus at toxic nickel concentrations." Tree Physiol **30**(10): 1311-1319.

Kalt, W., Cassidy, A., Howard, L. R., Krikorian, R., Stull, A. J., Tremblay, F. and Zamora-Ros, R. (2020). "Recent Research on the Health Benefits of Blueberries and Their Anthocyanins." Advances in Nutrition **11**(2): 224-236.

Kaminsky, L. M., Trexler, R. V., Malik, R. J., Hockett, K. L. and Bell, T. H. (2019). "The Inherent Conflicts in Developing Soil Microbial Inoculants." Trends Biotechnol **37**(2): 140-151.

Kariman, K., Barker, S. J. and Tibbett, M. (2018). "Structural plasticity in root-fungal symbioses: diverse interactions lead to improved plant fitness." PeerJ **6**: e6030.

Karst, J., Marczak, L., Jones, M. D. and Turkington, R. (2008). "The mutualism-parasitism continuum in ectomycorrhizas: a quantitative assessment using meta-analysis." Ecology **89**(4): 1032-1042.

Kennedy, K. J., Boyd, N. S. and Nams, V. O. (2010). "Hexazinone and Fertilizer Impacts on Sheep Sorrel (Rumex acetosella) in Wild Blueberry." Weed Science **58**(3): 317-322.

Kerley, S. J. and Read, D. J. (1995). "The biology of mycorrhiza in the Ericaceae: XVIII. Chitin degration by Hymenoscyphus ericae and transfer of chitin-nitrogen to the host plant." New Phytologist **131**(3): 369-375.

Kielak, A. M., Barreto, C. C., Kowalchuk, G. A., van Veen, J. A. and Kuramae, E. E. (2016). "The Ecology of Acidobacteria: Moving beyond Genes and Genomes." Frontiers in Microbiology **7**: 744.

Kiheri, H., Velmala, S., Pennanen, T., Timonen, S., Sietiö, O.-M., Fritze, H., Heinonsalo, J., van Dijk, N., Dise, N. and Larmola, T. (2020). "Fungal colonization patterns and enzymatic activities of peatland ericaceous plants following long-term nutrient addition." Soil Biology and Biochemistry **147**.

Klironomos, J. N. (2003). "Variation in Plant Response to Native and Exotic Arbuscular Mycorrhizal Fungi." Ecology **84**(9): 2292-2301.

Koljalg, U., Nilsson, R. H., Abarenkov, K., Tedersoo, L., Taylor, A. F., Bahram, M., Bates, S. T., Bruns, T. D., Bengtsson-Palme, J., Callaghan, T. M., Douglas, B., Drenkhan, T., Eberhardt, U., Duenas, M., Grebenc, T., Griffith, G. W., Hartmann, M., Kirk, P. M., Kohout, P., Larsson, E., Lindahl, B. D., Lucking, R., Martin, M. P., Matheny, P. B., Nguyen, N. H., Niskanen, T., Oja, J., Peay, K. G., Peintner, U., Peterson, M., Poldmaa, K., Saag, L., Saar, I., Schussler, A., Scott, J. A., Senes, C., Smith, M. E., Suija, A., Taylor, D. L., Telleria, M. T., Weiss, M. and Larsson, K. H. (2013). "Towards a unified paradigm for sequence-based identification of fungi." Mol Ecol **22**(21): 5271-5277.

Korcak, R. F. (1988). "Nutrition of blueberry and other calcifuges."

Kosola, K. R., Workmaster, B. A. A. and Spada, P. A. (2007). "Inoculation of cranberry (Vaccinium macrocarpon) with the ericoid mycorrhizal fungus Rhizoscyphus ericae increases nitrate influx." New Phytol **176**(1): 184-196.

Krikorian, R., Shidler, M. D., Nash, T. A., Kalt, W., Vinqvist-Tymchuk, M. R., Shukitt-Hale, B. and Joseph, J. A. (2010). "Blueberry supplementation improves memory in older adults." Journal of Agricultural and Food Chemistry **58**(7): 3996-4000.

Kron, K. A., Judd, W. S., Stevens, P. F., Crayn, D. M., Anderberg, A. A., Gadek, P. A., J., Q. C. and Luteyn, J. L. (2002). "Phylogenetic classification of Ericaceae: molecular and morphological evidence." The Botanical Review **68**(3): 335-423.

Kulichevskaya, I. S., Danilova, O. V., Tereshina, V. M., Kevbrin, V. V. and Dedysh, S. N. (2014). "Descriptions of Roseiarcus fermentans gen. nov., sp. nov., a bacteriochlorophyll a-containing fermentative bacterium related phylogenetically to alphaproteobacterial methanotrophs, and of the family Roseiarcaceae fam. nov." Int J Syst Evol Microbiol **64**(Pt 8): 2558-2565.

Kulichevskaya, I. S., Ivanova, A. A., Suzina, N. E., Rijpstra, W. I. C., Sinninghe Damste, J. S. and Dedysh, S. N. (2016). "*Paludisphaera borealis* gen. nov., sp. nov., a hydrolytic planctomycete from northern wetlands, and proposal of Isosphaeraceae fam. nov." International Journal of Systematic and Evolutionary Microbiology **66**(2): 837-844.

Kuznetsova, A., Brockhoff, P. B. and Christensen, R. H. B. (2017). "lmerTest Package: Tests in Linear Mixed Effects Models." Journal of Statistical Software **82**(13).

Kyndt, J. A., Montano Salama, D. and Meyer, T. E. (2020). "Genome Sequence of the Alphaproteobacterium Blastochloris sulfoviridis DSM 729, Which Requires Reduced Sulfur

as a Growth Supplement and Contains Bacteriochlorophyll b." Microbiol Resour Announc **9**(18).

Lafond, J. (2004). "Application of paper mill biosolids, wood ash and ground bark on wild lowbush blueberry production." Small Fruits Review **3**(1-2): 3-10.

Lafond, J. (2009). "Optimum leaf nutrient concentrations of wild lowbush blueberry in Quebec." Canadian Journal of Plant Science **89**(2): 341-347.

Lafond, J. and Ziadi, N. (2011). "Fertilisation azotée et phosphatée dans la production du bleuet nain sauvage au Québec." Canadian Journal of Plant Science **91**(3): 535-544.

Lafond, J. and Ziadi, N. (2013). "Biodisponibilité de l'azote et du phosphore dans les sols de bleuetières du Québec." Canadian Journal of Soil Science **93**(1): 33-44.

Laforest-Lapointe, I., Paquette, A., Messier, C. and Kembel, S. W. (2017). "Leaf bacterial diversity mediates plant diversity and ecosystem function relationships." Nature **546**(7656): 145-147.

Lambert, D. H. (1990). "Effects of pruning method on the incidence of mummy berry and other lowbush blueberry diseases." Plant disease **74**(3): 199-201.

Lauber, C. L., Ramirez, K. S., Aanderud, Z., Lennon, J. and Fierer, N. (2013). "Temporal variability in soil microbial communities across land-use types." ISME J **7**(8): 1641-1650.

Leake, J. R. and Read, D. J. (1990). "Proteinase activity in mycorrhizal fungi: I. The effect of extracellular pH on the production and activity of proteinase by ericoid endophytes from soils of contrasted pH." New Phytologist **115**(2): 243-250.

Leake, J. R. and Read, D. J. (1991). 20 Experiments with Ericoid Mycorrhiza. Methods in microbiology, Academic Press. **23:** 435-459.

Lee, P. (2004). "The impact of burn intensity from wildfires on seed and vegetative banks, and emergent understory in aspen-dominated boreal forests." Canadian Journal of Botany **82**(10): 1468-1480.

Legendre, P. and Gallagher, E. D. (2001). "Ecologically meaningful transformations for ordination of species data." Oecologia **129**(2): 271-280.

Legendre, P. and Legendre, L. F. (2012). Numerical ecology. Great Britain, Elsevier.

Lennon, J. T., Muscarella, M. E., Placella, S. A. and Lehmkuhl, B. K. (2018). "How, When, and Where Relic DNA Affects Microbial Diversity." Mbio **9**(3).

Leopold, D. R. (2016). "Ericoid fungal diversity: Challenges and opportunities for mycorrhizal research." Fungal Ecology **24**: 114-123.

Leopold, D. R., Peay, K. G., Vitousek, P. M. and Fukami, T. (2021). "Diversity of putative ericoid mycorrhizal fungi increases with soil age and progressive phosphorus limitation across a 4.1-million-year chronosequence." FEMS Microbiol Ecol **97**(3).

Lévesque, J.-A., Bradley, R. L., Bellemare, M., Lafond, J., Paré, M. C. and Willenborg, C. (2018). "Predicting weed and lowbush blueberry biomass using the point intercept method." Canadian Journal of Plant Science **98**(4): 967-970.

Li, J., Mavrodi, O. V., Hou, J., Blackmon, C., Babiker, E. M. and Mavrodi, D. V. (2020). "Comparative Analysis of Rhizosphere Microbiomes of Southern Highbush Blueberry (*Vaccinium corymbosum* L.), Darrow's Blueberry (*V. darrowii* Camp), and Rabbiteye Blueberry (*V. virgatum* Aiton)." Front Microbiol **11**: 370.

Li, N., Zhou, Q., Chang, K.-F., Yu, H., Hwang, S.-F., Conner, R. L., Strelkov, S. E., McLaren, D. L. and Turnbull, G. D. (2019). "Occurrence, pathogenicity and species identification of Pythium causing root rot of soybean in Alberta and Manitoba, Canada." Crop Protection **118**: 36-43.

Li, Z., Boyd, N., McLean, N. and Rutherford, K. (2014). "Hexazinone resistance in red sorrel (*Rumex acetosella*)." Weed science **62**(3): 532-537.

Li, Z., Boyd, N., McLean, N. and Rutherford, K. (2017). "Hexazinone Resistance in Red Sorrel (Rumex acetosella)." Weed Science **62**(3): 532-537.

Lide, D. R. (2005). Flame temperatures. CRC Handbook of Chemistry and Physics. D. R. Lide. Boca Raton, FL, USA, CRC Pres **85**.

Linstrom, P. J. and Mallard, W. G. (2001). "The NIST Chemistry WebBook: A chemical data resource on the internet." Journal of Chemical & Engineering Data **46**(5): 1059-1063.

Lloyd, A. W., Percival, D. and Yurgel, S. N. (2021). "Effect of Fungicide Application on Lowbush Blueberries Soil Microbiome." Microorganisms **9**(7).

Love, M. I., Huber, W. and Anders, S. (2014). "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2." Genome Biology **15**(12): 1-21.

Lukešová, T., Kohout, P., Větrovský, T. and Vohník, M. (2015). "The potential of Dark Septate Endophytes to form root symbioses with ectomycorrhizal and ericoid mycorrhizal middle European forest plants." PLoS One **10**(4): e0124752.

Malhi, S., Harapiak, J., Nyborg, M. and Gill, K. (2000). "Effects of long-term applications of various nitrogen sources on chemical soil properties and composition of bromegrass hay." Journal of Plant Nutrition **23**(7): 903-912.

Mallory, E. and Smagula, J. (2012). Effects of seafood-waste compost and mulch on soil health and soil nutrient dynamics in wild blueberry (*Vaccinium angustifolium* Ait.). X International Symposium on Vaccinium and Other Superfruits 1017.

Malysheva, E. F., Malysheva, V. F., Voronina, E. Y. and Kovalenko, A. E. (2018). "Diversity of fungal communities associated with mixotrophic pyroloids (*Pyrola rotundifolia*, *P. media* and *Orthilia secunda*) in their natural habitats." Botanica Pacifica **7**(2).

MAPAQ (2016). Monographie de l'industrie du bleuet sauvage au Québec.

MAPAQ. (2019). "Culture du bleuet - Bleuet du Québec en chiffre (2018)." Retrieved 26 Juin, 2020, from https://www.mapaq.gouv.qc.ca/fr/Productions/Production/Pages/Culture-du-bleuet.aspx.

Maqbool, R., Percival, D., Zaman, Q., Astatkie, T., Adl, S. and Buszard, D. (2017). "Leaf nutrients ranges and berry yield optimization in response to soil-applied nitrogen, phosphorus and potassium in wild blueberry (Vaccinium angustifolium Ait.)." European Journal of Horticultural Science **82**(4): 166-179.

Marasco, R., Rolli, E., Ettoumi, B., Vigani, G., Mapelli, F., Borin, S., Abou-Hadid, A. F., El-Behairy, U. A., Sorlini, C., Cherif, A., Zocchi, G. and Daffonchio, D. (2012). "A drought resistance-promoting microbiome is selected by root system under desert farming." PLoS One **7**(10): e48479.

Marcondes de Souza, J., Carrareto Alves, L., de Mello Varani, A. and E., d. M. L. (2014). The family *Bradyrhizobiaceae*. The Prokaryotes. E. Rosenberg, E. F. DeLong, S. Lory, E. Stackebrandt and F. Thompson. Berlin, Heidelberg, Springer 135 - 154.

Market Data Forecast. (2020). "Microbial Soil Inoculants Market." Retrieved 14 June 2020, 2020, from https://www.marketdataforecast.com/market-reports/microbial-soil-inoculants-market.

Martin, F. M., Uroz, S. and Barker, D. G. (2017). "Ancestral alliances: Plant mutualistic symbioses with fungi and bacteria." Science **356**(6340).

Martin, M. (2011). "Cutadapt removes adapter sequences from high-throughput sequencing reads." EMBnet. journal **17**(1): 10-12.

Martino, E., Morin, E., Grelet, G. A., Kuo, A., Kohler, A., Daghino, S., Barry, K. W., Cichocki, N., Clum, A., Dockter, R. B., Hainaut, M., Kuo, R. C., LaButti, K., Lindahl, B. D., Lindquist, E. A., Lipzen, A., Khouja, H. R., Magnuson, J., Murat, C., Ohm, R. A., Singer, S. W., Spatafora, J. W., Wang, M., Veneault-Fourrey, C., Henrissat, B., Grigoriev, I. V., Martin, F. M. and Perotto, S. (2018). "Comparative genomics and transcriptomics depict ericoid mycorrhizal fungi as versatile saprotrophs and plant mutualists." New Phytologist **217**(3): 1213-1229.

Marty, C., Levesque, J. A., Bradley, R. L., Lafond, J. and Pare, M. C. (2019). "Contrasting impacts of two weed species on lowbush blueberry fertilizer nitrogen uptake in a commercial field." PLoS One **14**(4): e0215253.

Mataix-Solera, J., Guerrero, C., García-Orenes, F., Bárcenas, G. M., Torres, M. P. and Barcenas, M. (2009). "Forest fire effects on soil microbiology." Fire effects on soils and restoration strategies **5**: 133-175.

Maynard, D. G., Kalra, Y. P. and Crumbaugh, J. A. (2007). Nitrate and Exchangeable Ammonium Nitrogen. Soil sampling and methods of analysis. M. R. Carter and E. G. Gregorich. Boca Raton, Florida, CRC press**:** 71-80.

McFadden, G. I. (1999). "Endosymbiosis and evolution of the plant cell." Current opinion in plant biology **2**(6): 513-519.

McMurdie, P. J. and Holmes, S. (2013). "phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data." PLoS One **8**(4): e61217.

Meharg, A. A. (2003). "The mechanistic basis of interactions between mycorrhizal associations and toxic metal cations." Mycological Research **107**(Pt 11): 1253-1265.

Mendes, R., Garbeva, P. and Raaijmakers, J. M. (2013). "The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms." FEMS Microbiol Rev **37**(5): 634-663.

Merckx, V., Bidartondo, M. I. and Hynson, N. A. (2009). "Myco-heterotrophy: when fungi host plants." Ann Bot **104**(7): 1255-1261.

Merino, N., Aronson, H. S., Bojanova, D. P., Feyhl-Buska, J., Wong, M. L., Zhang, S. and Giovannelli, D. (2019). "Living at the Extremes: Extremophiles and the Limits of Life in a Planetary Context." Front Microbiol **10**: 780.

Midgley, D. J., Chambers, S. M. and Cairney, J. W. G. (2004). "Distribution of ericoid mycorrhizal endophytes and root-associated fungi in neighbouring Ericaceae plants in the field." Plant and Soil **259**(1): 137-151.

Mitchell, D. T. and Gibson, B. R. (2006). "Ericoid mycorrhizal association: ability to adapt to a broad range of habitats." Mycologist **20**(1): 2-9.

Mizrahi-Man, O., Davenport, E. R. and Gilad, Y. (2013). "Taxonomic classification of bacterial 16S rRNA genes using short sequencing reads: evaluation of effective study designs." PLoS One **8**(1): e53608.

Mohagheghi, A., Grohmann, K. M. M. H., Himmel, M., Leighton, L. and Updegraff, D. M. (1986). "Isolation and characterization of *Acidothermus cellulolyticus* gen. nov., sp. nov., a new genus of thermophilic, acidophilic, cellulolytic bacteria. 36(3), ." International Journal of Systematic and Evolutionary Microbiology **36**(3): 435-443.

Montalba, R., Arriagada, C., Alvear, M. and Zúñiga, G. E. (2010). "Effects of conventional and organic nitrogen fertilizers on soil microbial activity, mycorrhizal colonization, leaf antioxidant content, and Fusarium wilt in highbush blueberry (Vaccinium corymbosum L.)." Scientia Horticulturae **125**(4): 775-778.

Moola, F. M. and Vasseur, L. (2009). The importance of clonal growth to the recovery of *Gaultheria procumbens* L. (Ericaceae) after forest disturbance. Forest Ecology: Recent Advances in Plant Ecology. A. G. Van der Valk. Dordrecht, Springer Netherlands**:** 319-337.

Moore, D., Robson, G. D. and Trinci, A. P. J. (2000). 21st Century Guidebook to Fungi, Cambridge University Press.

Morris, C. E. and Moury, B. (2019). "Revisiting the Concept of Host Range of Plant Pathogens." Annu Rev Phytopathol **57**: 63-90.

Morvan, S., Meglouli, H., Lounes-Hadj Sahraoui, A. and Hijri, M. (2020). "Into the wild blueberry (*Vaccinium angustifolium*) rhizosphere microbiota." Environmental Microbiology **22**(9): 3803-3822.

Morvan, S., Pare, M. C., Schmitt, A., Lafond, J. and Hijri, M. (2022). "Limited effect of thermal pruning on wild blueberry crop and its root-associated microbiota." Front Plant Sci **13**: 954935.

Mu, D., Du, N. and Zwiazek, J. J. (2021). "Inoculation with Ericoid Mycorrhizal Associations Alleviates Drought Stress in Lowland and Upland Velvetleaf Blueberry (*Vaccinium myrtilloides*) Seedlings." Plants **10**(12): 2786.

Murphy, J. and Riley, J. P. (1962). "A modified single solution method for the determination of phosphate in surface waters." Analytica chimica acta **27**: 31-36.

Neary, D. G., Klopatek, C. C., DeBano, L. F. and Ffolliott, P. F. (1999). "Fire effects on belowground sustainability: a review and synthesis." Forest Ecology and Management **122**(1-2): 51-71.

Newsham, K. K. (2011). "A meta-analysis of plant responses to dark septate root endophytes." New Phytologist **190**(3): 783-793.

Noble, W. S. (2009). "How does multiple testing correction work?" Nat Biotechnol **27**(12): 1135-1137.

Nyiraneza, J., Cambouris, A. N., Ziadi, N., Tremblay, N. and Nolin, M. C. (2012). "Spring wheat yield and quality related to soil texture and nitrogen fertilization." Agronomy Journal **104**(3): 589-599.

Ohno, T. and Severy, N. (2013). "Phosphorus and aluminium solubility relationships in acidic lowbush blueberry barren soils in M aine." Soil use and management **29**(4): 485-493.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. M., Szoecs, E. and Wagner, H. (2019). vegan: Community Ecology Package.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. M., Szoecs, E. and Wagner, H. (2020). vegan: Community Ecology Package.

Oren, A. (2014). "The Family Xanthobacteraceae." The Prokaryotes: 709-726.

Ormeno-Orrillo, E. and Martinez-Romero, E. (2019). "A Genomotaxonomy View of the *Bradyrhizobium* Genus." Frontiers in Microbiology **10**: 1334.

Pankievicz, V. C. S., Irving, T. B., Maia, L. G. S. and Ane, J. M. (2019). "Are we there yet? The long walk towards the development of efficient symbiotic associations between nitrogen-fixing bacteria and non-leguminous crops." BMC Biol **17**(1): 99.

Pantigoso, H. A., Manter, D. K. and Vivanco, J. M. (2018). "Phosphorus addition shifts the microbial community in the rhizosphere of blueberry (*Vaccinium corymbosum* L.)." Rhizosphere **7**: 1-7.

Parnell, J. J., Berka, R., Young, H. A., Sturino, J. M., Kang, Y., Barnhart, D. M. and DiLeo, M. V. (2016). "From the Lab to the Farm: An Industrial Perspective of Plant Beneficial Microorganisms." Front Plant Sci **7**: 1110.

Pauvert, C., Buée, M., Laval, V., Edel-Hermann, V., Fauchery, L., Gautier, A., Lesur, I., Vallance, J. and Vacher, C. (2019). "Bioinformatics matters: The accuracy of plant and soil fungal community data is highly dependent on the metabarcoding pipeline." Fungal Ecology **41**: 23-33.

Pearson, V. and Read, D. J. (1973a). "The biology of mycorrhiza in the Ericaceae: I. The isolation of the endophyte and synthesis of mycorrhizas in aseptic culture." New Phytologist **72**(2): 371-379.

Pearson, V. and Read, D. J. (1973b). "The biology of mycorrhiza in the Ericaceae. II. The transport of carbon and phosphorus by the endophyte and the mycorrhiza. New Phytologist, 1325-1331." New Phytologist **72**(é): 1325-1331.

Peeters, N., Guidot, A., Vailleau, F. and Valls, M. (2013). "Ralstonia solanacearum, a widespread bacterial plant pathogen in the post-genomic era." Mol Plant Pathol **14**(7): 651-662.

Pelletier, A. J., Lafond, J. and Paré, M. C. (2022). "Spatial dependency and independency of nitrogen in lowbush blueberry commercial fields." Canadian Journal of Soil Science.

Penney, B. G. and McRae, K. B. (2000). "Herbicidal weed control and crop-year NPK fertilization improves lowbush blueberry (Vaccinium angustifolium Ait.) production." Canadian Journal of Plant Science **80**(2): 351-361.

Penney, B. G., McRae, K. B. and Rayment, A. F. (1997). "Long-term effects of burn-pruning on lowbush blueberry (*Vaccinium angustifolium* Ait.) production." Canadian Journal of Plant Science **77**(3): 421-425.

Penney, B. G., McRae, K. B. and Rayment, A. F. (2008). "Effect of long-term burn-pruning on the flora in a lowbush blueberry (*Vaccinium angustifolium* Ait.) stand." Canadian Journal of Plant Science **88**(2): 351-362.

Peoples, M. B., Brockwell, J., Herridge, D. F., Rochester, I. J., Alves, B. J. R., Urquiaga, S., Bodley, R. M., Dakora, F. D., Bhattaria, S., Maskey, S. L., Sampet, C., Rerkasem, B., Khan, D. F., Hauggaard-Nielsen, H. and Jensen, E. S. (2009). "The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems." Symbiosis **48**(1-3): 1-17.

Perez-Jaramillo, J. E., Mendes, R. and Raaijmakers, J. M. (2016). "Impact of plant domestication on rhizosphere microbiome assembly and functions." Plant Mol Biol **90**(6): 635-644.

Perotto, S., Daghino, S. and Martino, E. (2018). "Ericoid mycorrhizal fungi and their genomes: another side to the mycorrhizal symbiosis?" New Phytol **220**(4): 1141-1147.

Perotto, S., Girlanda, M. and Martino, E. (2002). "Ericoid mycorrhizal fungi: some new perspectives on old acquaintances." Plant and Soil **244**: 41-53.

Peterson, R. L. and Massicotte, H. B. (2004). "Exploring structural definitions of mycorrhizas, with emphasis on nutrient-exchange interfaces." Canadian Journal of Botany **82**(8): 1074-1088.

Peterson, T. A., Mueller, W. C. and Englander, L. (1980). "Anatomy and ultrastructure of a Rhododendron root–fungus association." Canadian Journal of Botany **58**(23): 2421-2433.

Philippot, L., Raaijmakers, J. M., Lemanceau, P. and van der Putten, W. H. (2013). "Going back to the roots: the microbial ecology of the rhizosphere." Nat Rev Microbiol **11**(11): 789-799.

Pirozynski, K. A. and Malloch, D. W. (1975). "The origin of land plants: a matter of mycotrophism." Biosystems **6**(3): 153-164.

Punja, Z. K. and Rodriguez, G. (2018). "Fusarium and Pythium species infecting roots of hydroponically grown marijuana (Cannabis sativa L.) plants." Canadian Journal of Plant Pathology **40**(4): 498-513.

Quinn, T. P., Erb, I., Richardson, M. F. and Crowley, T. M. (2018). "Understanding sequencing data as compositions: an outlook and review." Bioinformatics **34**(16): 2870-2878.

Quiza, L., St-Arnaud, M. and Yergeau, E. (2015). "Harnessing phytomicrobiome signaling for rhizosphere microbiome engineering." Front Plant Sci **6**: 507.

R Core Team (2019). R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing.

R Core Team (2021). R: A language and environment for statistical computing. Vienna, Austria, R Foundation for Statistical Computing.

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C. and Moënne-Loccoz, Y. (2008). "The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms." Plant and Soil **321**(1-2): 341-361.

Ramirez, K. S., Craine, J. M. and Fierer, N. (2012). "Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes." Global Change Biology **18**(6): 1918-1927.

Raymond, R. (1971). Pédologie de la région de Chicoutimi. M. d. l. A. d. l. Colonisatio**:** 120.

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

Reinhold-Hurek, B., Bunger, W., Burbano, C. S., Sabale, M. and Hurek, T. (2015). "Roots shaping their microbiome: global hotspots for microbial activity." Annu Rev Phytopathol **53**: 403-424.

Rohrbacher, F. and St-Arnaud, M. (2016). "Root Exudation: The Ecological Driver of Hydrocarbon Rhizoremediation." Agronomy **6**(1): 19.

Rosado, B. H. P., Almeida, L. C., Alves, L. F., Lambais, M. R. and Oliveira, R. S. (2018). "The importance of phyllosphere on plant functional ecology: a phyllo trait manifesto." New Phytol **219**(4): 1145-1149.

Rosenblueth, M., Ormeño-Orrillo, E., López-López, A., Rogel, M. A., Reyes-Hernández, B. J., Martínez-Romero, J. C., Reddy, P. M. and Martínez-Romero, E. (2018). "Nitrogen fixation in cereals." Frontiers in Microbiology **9**: 1794.

Rousk, J., Baath, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R. and Fierer, N. (2010). "Soil bacterial and fungal communities across a pH gradient in an arable soil." The ISME Journal **4**(10): 1340-1351.

Rousk, J., Brookes, P. C. and Baath, E. (2009). "Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization." Applied and environmental microbiology **75**(6): 1589-1596.

Roy-Bolduc, A. and Hijri, M. (2011). "The Use of Mycorrhizae to Enhance Phosphorus Uptake: A Way Out the Phosphorus Crisis." Journal of Biofertilizers & Biopesticides **02**(1).

Ruotsalainen, A. L. (2018). Dark Septate Endophytes (DSE) in Boreal and Subarctic Forests. Endophytes of Forest Trees. A. M. Pirttilä and A. C. Frank, Springer International Publishing AG**:** 105-117.

Russel, J. (2021). MicEco: Various functions for microbial community data. R package.

Sadowsky, J. J., Hanson, E. J. and Schilder, A. M. C. (2012). "Root Colonization by Ericoid Mycorrhizae and Dark Septate Endophytes in Organic and Conventional Blueberry Fields in Michigan." International Journal of Fruit Science **12**(1-3): 169-187.

Saenz, J. S., Roldan, F., Junca, H. and Arbeli, Z. (2019). "Effect of the extraction and purification of soil DNA and pooling of PCR amplification products on the description of bacterial and archaeal communities." J Appl Microbiol **126**(5): 1454-1467.

Saleem, M., Meckes, N., Pervaiz, Z. H. and Traw, M. B. (2017). "Microbial Interactions in the Phyllosphere Increase Plant Performance under Herbivore Biotic Stress." Front Microbiol **8**: 41.

Sanderson, K. R. and Eaton, L. J. (2008). "Wild blueberry response to phosphorus applied to Prince Edward Island soils." Canadian journal of plant science **88**(2): 363-366.

Sawada, H., Kuykendall, L. D. and Young, J. M. (2003). "Changing concepts in the systematics of bacterial nitrogen-fixing legume symbionts." The Journal of general and applied microbiology **49**(3): 155-179.

Scagel, C. F., Wagner, A. and Winiarski, P. (2005). "Inoculation with Ericoid Mycorrhizal Fungi Alters Root Colonization and Growth in Nursery Production of Blueberry Plants from Tissue Culture and Cuttings." Small Fruits Review **4**(4): 113-135.

Schlaeppi, K. and Bulgarelli, D. (2015). "The plant microbiome at work." Mol Plant Microbe Interact **28**(3): 212-217.

Schlatter, D. C., Bakker, M. G., Bradeen, J. M. and Kinkel, L. L. (2015). "Plant community richness and microbial interactions structure bacterial communities in soil." Ecology **96**(1): 134-142.

Schlegel, M., Munsterkotter, M., Guldener, U., Bruggmann, R., Duo, A., Hainaut, M., Henrissat, B., Sieber, C. M., Hoffmeister, D. and Grunig, C. R. (2016). "Globally distributed root endophyte *Phialocephala subalpina* links pathogenic and saprophytic lifestyles." BMC Genomics **17**(1): 1015.

Schliep, K. P. (2011). "phangorn: phylogenetic analysis in R." Bioinformatics **27**(4): 592-593.

Schneijderberg, M., Schmitz, L., Cheng, X., Polman, S., Franken, C., Geurts, R. and Bisseling, T. (2018). "A genetically and functionally diverse group of non-diazotrophic *Bradyrhizobium* spp. colonizes the root endophytic compartment of *Arabidopsis thaliana*." BMC plant biology **18**(1): 1-9.

Schütz, L., Gattinger, A., Meier, M., Müller, A., Boller, T., Mäder, P. and Mathimaran, N. (2018). "Improving crop yield and nutrient use efficiency via biofertilization- a global meta-analysis." Front Plant Sci **8**: 2204.

Selosse, M.-A. and Le Tacon, F. (1998). "The land flora: a phototroph-fungus partneship?" Trends in Ecology & Evolution **13**(1): 15-20.

Selosse, M. A., Setaro, S., Glatard, F., Richard, F., Urcelay, C. and Weiss, M. (2007). "Sebacinales are common mycorrhizal associates of Ericaceae." New Phytol **174**(4): 864-878.

Sessitsch, A., Pfaffenbichler, N. and Mitter, B. (2019). "Microbiome Applications from Lab to Field: Facing Complexity." Trends Plant Sci **24**(3): 194-198.

Shen, M., Zhang, J. Q., Zhao, L. L., Groenewald, J. Z., Crous, P. W. and Zhang, Y. (2020). "Venturiales." Studies in Mycology **96**: 185-308.

Sietiö, O. M., Tuomivirta, T., Santalahti, M., Kiheri, H., Timonen, S., Sun, H., Fritze, H. and Heinonsalo, J. (2018). "Ericoid plant species and *Pinus sylvestris* shape fungal communities in their roots and surrounding soil." New Phytologist **218**(2): 738-751.

Silverman, J. D., Washburne, A. D., Mukherjee, S. and David, L. A. (2017). "A phylogenetic transform enhances analysis of compositional microbiota data." Elife **6**.

Simon, J.-C., Marchesi, J. R., Mougel, C. and Selosse, M.-A. (2019). "Host-microbiota interactions: from holobiont theory to analysis." Microbiome **7**(1): 1-5.

Singleton, L. L. (2002). Diseases of roots and crowns. Bread wheat: improvement and production. Rome, Italy, FAO. **Plant Production and Protection Series**.

Smagula, J. M. and Dunham, S. (1995). "Diammonium phosphate corrects phosphorus deficiency in lowbush blueberry." Journal of Small Fruit & Viticulture **3**(4): 183-191.

Smagula, J. M., Yarborough, D. E., Drummond, F. and Annis, S. (2008). Organic production of wild blueberries II. Fertility and weed management. IX International Vaccinium Symposium 810.

Smercina, D. N., Bailey, V. L. and Hofmockel, K. S. (2021). "Micro on a macroscale: relating microbial-scale soil processes to global ecosystem function." FEMS Microbiol Ecol **97**(7).

Smercina, D. N., Evans, S. E., Friesen, M. L. and Tiemann, L. K. (2019). "To Fix or Not To Fix: Controls on Free-Living Nitrogen Fixation in the Rhizosphere." Appl Environ Microbiol **85**(6): e02546-02518.

Smith, D. W. and Hilton, R. J. (1971). "The comparative effects of pruning by burning or clipping on lowbush blueberries in North-Eastern Ontario." Journal of Applied Ecology **8**(3): 781-789.

Smith, S. E. and Read, D. J. (2008). Mycorrhizal Symbiosis. Great Britain, Academic Press.

Smith, S. E., Smith, F. A. and Jakobsen, I. (2003). "Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses." Plant Physiol **133**(1): 16-20.

Starast, M., Karp, K. and Vool, E. (2007). "Effect of NPK fertilization and elemental sulphur on growth and yield of lowbush blueberry." Agricultural and food science **16**(1): 34-45.

Strik, B. (2004). Blueberry production and research trends in North America. VIII International Symposium on Vaccinium Culture**:** 173-184.

Strullu-Derrien, C., Selosse, M. A., Kenrick, P. and Martin, F. M. (2018). "The origin and evolution of mycorrhizal symbioses: from palaeomycology to phylogenomics." New Phytol **220**(4): 1012-1030.

Strullu-Derrien, C., Wawrzyniak, Z., Goral, T. and Kenrick, P. (2015). "Fungal colonization of the rooting system of the early land plant Asteroxylon mackiei from the 407-Myr-old Rhynie Chert (Scotland, UK)." Botanical Journal of the Linnean Society **179**: 201-213.

Su, C., Lei, L., Duan, Y., Zhang, K. Q. and Yang, J. (2012). "Culture-independent methods for studying environmental microorganisms: methods, application, and perspective." Appl Microbiol Biotechnol **93**(3): 993-1003.

Tedersoo, L., Bahram, M., Zinger, L., Nilsson, R. H., Kennedy, P. G., Yang, T., Anslan, S. and Mikryukov, V. (2022). "Best practices in metabarcoding of fungi: From experimental design to results." Mol Ecol **31**(10): 2769-2795.

Tedersoo, L., Tooming-Klunderud, A. and Anslan, S. (2018). "PacBio metabarcoding of Fungi and other eukaryotes: errors, biases and perspectives." New Phytol **217**(3): 1370-1385.

Theriault, M. K. (2006). Moose to moccasins: The story of ka kita wa pa no kwe. Dundurn, Natural Heritage.

Timonen, S., Sinkko, H., Sun, H., Sietio, O. M., Rinta-Kanto, J. M., Kiheri, H. and Heinonsalo, J. (2017). "Ericoid Roots and Mycospheres Govern Plant-Specific Bacterial Communities in Boreal Forest Humus." Microb Ecol **73**(4): 939-953.

Tindall, B. J., Rossello-Mora, R., Busse, H. J., Ludwig, W. and Kampfer, P. (2010). "Notes on the characterization of prokaryote strains for taxonomic purposes." Int J Syst Evol Microbiol **60**(Pt 1): 249-266.

Toju, H., Tanabe, A. S., Yamamoto, S. and Sato, H. (2012). "High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples." PLoS One **7**(7): e40863.

Townsend, L. R. (1969). "Influence of form of nitrogen and pH on growth and nutrient levels in the leaves and roots of the lowbush blueberry." Canadian Journal of Plant Science **49**(3): 333-338.

Treseder, K. K. (2008). "Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies." Ecology letters **11**(10): 1111-1120.

Trevett, M. F. (1972). "A second approximation of leaf analysis standards for lowbush blueberry. ." Res. Life Sci. **19**: 15-16.

Turner, T. R., James, E. K. and Poole, P. S. (2013). "The plant microbiome." Genome Biol **14**(6): 209.

Tyler, B. M. (2007). "Phytophthora sojae: root rot pathogen of soybean and model oomycete." Mol Plant Pathol **8**(1): 1-8.

UNITE Community (2017). UNITE general fasta release. Version 01.12.2017. UNITE Community.

Upton, R. N., Bach, E. M. and Hofmockel, K. S. (2019). "Spatio-temporal microbial community dynamics within soil aggregates." Soil Biology and Biochemistry **132**: 58-68.

van der Heijden, M. G., Bardgett, R. D. and van Straalen, N. M. (2008). "The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems." Ecol Lett **11**(3): 296-310.

van der Heijden, M. G., Martin, F. M., Selosse, M. A. and Sanders, I. R. (2015). "Mycorrhizal ecology and evolution: the past, the present, and the future." New Phytol **205**(4): 1406-1423.

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

van der Putten, W. H., Bardgett, R. D., Bever, J. D., Bezemer, T. M., Casper, B. B., Fukami, T., Kardol, P., Klironomos, J. N., Kulmatiski, A., Schweitzer, J. A., Suding, K. N., Van de Voorde, T. F. J., Wardle, D. A. and Hutchings, M. (2013). "Plant-soil feedbacks: the past, the present and future challenges." Journal of Ecology **101**(2): 265-276.

van Dijk, E. L., Auger, H., Jaszczyszyn, Y. and Thermes, C. (2014). "Ten years of next-generation sequencing technology." Trends Genet **30**(9): 418-426.

Van Geel, M., Jacquemyn, H., Peeters, G., van Acker, K., Honnay, O. and Ceulemans, T. (2020). "Diversity and community structure of ericoid mycorrhizal fungi in European bogs and heathlands across a gradient of nitrogen deposition." New Phytol **228**(5): 1640-1651.

van Veen, J. A., van Overbeek, L. S. and van Elsas, J. D. (1997). "Fate and activity of microorganisms introduced into soil." Microbiology and Molecular Biology Reviews **61**: 121-135.

Vandenkoornhuyse, P., Quaiser, A., Duhamel, M., Le Van, A. and Dufresne, A. (2015). "The importance of the microbiome of the plant holobiont." New Phytol **206**(4): 1196-1206.

Vannier, N., Mony, C., Bittebiere, A. K., Michon-Coudouel, S., Biget, M. and Vandenkoornhuyse, P. (2018). "A microorganisms' journey between plant generations." Microbiome **6**(1): 79.

Villarreal-Ruiz, L., Anderson, I. C. and Alexander, I. J. (2004). "Interaction between an isolate from the *Hymenoscyphus ericae* aggregate and roots of *Pinus* and *Vaccinium*." New Phytologist **164**(1): 183-192.

Villarreal-Ruiz, L., Neri-Luna, C., Anderson, I. C. and Alexander, I. J. (2012). "In vitro interactions between ectomycorrhizal fungi and ericaceous plants." Symbiosis **56**(2): 67-75.

Vincent, C., Lemoyne, P. and Lafond, J. (2018). "Management of blueberry maggot with high temperatures." Journal of economic entomology **111**(3): 1313-1317.

Vohnik, M. (2020). "Ericoid mycorrhizal symbiosis: theoretical background and methods for its comprehensive investigation." Mycorrhiza **30**(6): 671-695.

Vohník, M., Albrechtová, J. and Vosátka, M. (2005). "The inoculation with *Oidiodendron maius* and *Phialocephala fortinii* alters phosphorus and nitrogen uptake, foliar C: N ratio and root biomass distribution in Rhododendron cv. Azurro." Symbiosis **40**: 87-96.

Vohník, M., Fendrych, M., Albrechtová, J. and Vosátka, M. (2007). "Intracellular colonization of *Rhododendron* and *Vaccinium* roots by *Cenococcum geophilum*, *Geomyces pannorum* and *Meliniomyces variabilis*." Folia Microbiologica **52**(4): 407-414.

Vohník, M., Pánek, M., Fehrer, J. and Selosse, M.-A. (2016). "Experimental evidence of ericoid mycorrhizal potential within Serendipitaceae (Sebacinales)." Mycorrhiza **26**(8): 831-846.

Vohnik, M., Sadowsky, J. J., Kohout, P., Lhotakova, Z., Nestby, R. and Kolarik, M. (2012). "Novel root-fungus symbiosis in Ericaceae: sheathed ericoid mycorrhiza formed by a hitherto undescribed basidiomycete with affinities to Trechisporales." PLoS One **7**(6): e39524.

Vohník, M., Sadowsky, J. J., Lukešová, T., Albrechtová, J. and Vosátka, M. (2012). "Inoculation with a ligninolytic basidiomycete, but not root symbiotic ascomycetes, positively affects growth of highbush blueberry (Ericaceae) grown in a pine litter substrate." Plant and Soil **355**(1-2): 341-352.

Vorob'ev, A. V., de Boer, W., Folman, L. B., Bodelier, P. L., Doronina, N. V., Suzina, N. E., Trotsenko, Y. A. and Dedysh, S. N. (2009). "Methylovirgula ligni gen. nov., sp. nov., an obligately acidophilic, facultatively methylotrophic bacterium with a highly divergent mxaF gene." Int J Syst Evol Microbiol **59**(Pt 10): 2538-2545.

Vos, M., Wolf, A. B., Jennings, S. J. and Kowalchuk, G. A. (2013). "Micro-scale determinants of bacterial diversity in soil." FEMS Microbiol Rev **37**(6): 936-954.

Vrålstad, T. (2004). "Are ericoid and ectomycorrhizal fungi part of a common guild?" New Phytologist **164**(1): 7-10.

Wakelin, S., Mander, C., Gerard, E., Jansa, J., Erb, A., Young, S., Condron, L. and O'Callaghan, M. (2012). "Response of soil microbial communities to contrasted histories of phosphorus fertilisation in pastures." Applied soil ecology **61**: 40-48.

Walker, J. F., Aldrich-Wolfe, L., Riffel, A., Barbare, H., Simpson, N. B., Trowbridge, J. and Jumpponen, A. (2011). "Diverse Helotiales associated with the roots of three species of Arctic Ericaceae provide no evidence for host specificity." New Phytologist **191**(2): 515-527.

Wang, C., Wang, F., Yang, Q. and Liang, R. (2009). "Thermogravimetric studies of the behavior of wheat straw with added coal during combustion." Biomass and Bioenergy **33**(1): 50-56.

Wang, Q., Garrity, G. M., Tiedje, J. M. and Cole, J. R. (2007). "Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy." Appl Environ Microbiol **73**(16): 5261-5267.

Ward, E. B., Duguid, M. C., Kuebbing, S. E., Lendemer, J. C. and Bradford, M. A. (2022). "The functional role of ericoid mycorrhizal plants and fungi on carbon and nitrogen dynamics in forests." New Phytol **235**(5): 1701-1718.

Warman, P. (1987). "The effects of pruning, fertilizers, and organic amendments on lowbush blueberry production." Plant and Soil **101**(1): 67-72.

Wazny, R., Jedrzejczyk, R. J., Rozpadek, P., Domka, A. and Turnau, K. (2022). "Biotization of highbush blueberry with ericoid mycorrhizal and endophytic fungi improves plant growth and vitality." Appl Microbiol Biotechnol **106**(12): 4775-4786.

Weese, D. J., Heath, K. D., Dentinger, B. T. and Lau, J. A. (2015). "Long-term nitrogen addition causes the evolution of less-cooperative mutualists." Evolution **69**(3): 631-642.

Wei, X., Chen, J., Zhang, C. and Pan, D. (2016). "A New Oidiodendron maius Strain Isolated from Rhododendron fortunei and its Effects on Nitrogen Uptake and Plant Growth." Front Microbiol **7**: 1327.

Weiss, M., Waller, F., Zuccaro, A. and Selosse, M. A. (2016). "Sebacinales - one thousand and one interactions with land plants." New Phytol **211**(1): 20-40.

Wen, C., Wu, L., Qin, Y., Van Nostrand, J. D., Ning, D., Sun, B., Xue, K., Liu, F., Deng, Y., Liang, Y. and Zhou, J. (2017). "Evaluation of the reproducibility of amplicon sequencing with Illumina MiSeq platform." PLoS One **12**(4): e0176716.

Westhoek, A., Field, E., Rehling, F., Mulley, G., Webb, I., Poole, P. S. and Turnbull, L. A. (2017). "Policing the legume-Rhizobium symbiosis: a critical test of partner choice." Sci Rep **7**(1): 1419.

White, S. N. and Boyd, N. S. (2017). "Effect of Dry Heat, Direct Flame, and Straw Burning on Seed Germination of Weed Species Found in Lowbush Blueberry Fields." Weed Technology **30**(1): 263-270.

Whitman, T., Whitman, E., Woolet, J., Flannigan, M. D., Thompson, D. K. and Parisien, M.-A. (2019). "Soil bacterial and fungal response to wildfires in the Canadian boreal forest across a burn severity gradient." Soil Biology and Biochemistry **138**.

Whyte, A. R., Cheng, N., Fromentin, E. and Williams, C. M. (2018). "A Randomized, Double-Blinded, Placebo-Controlled Study to Compare the Safety and Efficacy of Low Dose Enhanced Wild Blueberry Powder and Wild Blueberry Extract (ThinkBlue) in Maintenance of Episodic and Working Memory in Older Adults." Nutrients **10**(6).

Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis. New York, Springer-Verlag.

Wicklow, D. T. (1975). "Fire as an environmental cue initiating ascomycete development in a tallgrass prairie." Mycologia **67**(4): 852-862.

Wood, G. W. (2004). "The Wild Blueberry Industry—Past." Small Fruits Review **3**(1-2): 11-18.

Wright, E. S. (2016). "Using DECIPHER v2. 0 to analyze big biological sequence data in R." R Journal **8**(1).

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E. and Prior, R. L. (2006). "Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption." Journal of Agricultural and Food Chemistry **54**(11): 4069-4075.

Xiao, G. and Berch, S. M. (1995). "The ability of known ericoid mycorrhizal fungi to form mycorrhizae with Gaultheria shallon." Mycologia **87**(4): 467-470.

Yang, H., Zhao, X., Liu, C., Bai, L., Zhao, M. and Li, L. (2018). "Diversity and characteristics of colonization of root-associated fungi of *Vaccinium uliginosum*." Scientific Reports **8**(1): 15283.

Yang, J., Kloepper, J. W. and Ryu, C.-M. (2009). "Rhizosphere bacteria help plants tolerate abiotic stress." Trends in plant science **14**(1): 1-4.

Yarborough, D. E. (2004). "Factors contributing to the increase in productivity in the wild blueberry industry." Small Fruits Review **3**(1-2): 33-43.

Yarborough, D. E. (2012). "Establishment and Management of the Cultivated Lowbush Blueberry (Vaccinium angustifolium)." International Journal of Fruit Science **12**(1-3): 14-22.

Yarborough, D. E. and Bhowmik, P. C. (1993). "Lowbush blueberry-bunchberry competition." Journal of the American Society for Horticultural Science **118**(1): 54-62.

Yergeau, E., Sanschagrin, S., Maynard, C., St-Arnaud, M. and Greer, C. W. (2014). "Microbial expression profiles in the rhizosphere of willows depend on soil contamination." ISME J **8**(2): 344-358.

Yilmaz, P., Parfrey, L. W., Yarza, P., Gerken, J., Pruesse, E., Quast, C., Schweer, T., Peplies, J., Ludwig, W. and Glockner, F. O. (2014). "The SILVA and "All-species Living Tree Project (LTP)" taxonomic frameworks." Nucleic Acids Res **42**(Database issue): D643-648.

Yoneyama, T., Terakado-Tonooka, J. and Minamisawa, K. (2017). "Exploration of bacterial N2-fixation systems in association with soil-grown sugarcane, sweet potato, and paddy rice: a review and synthesis." Soil Science and Plant Nutrition **63**(6): 578-590.

Yurgel, S. N., Douglas, G. M., Comeau, A. M., Mammoliti, M., Dusault, A., Percival, D. and Langille, M. G. I. (2017). "Variation in Bacterial and Eukaryotic Communities Associated with Natural and Managed Wild Blueberry Habitats." Phytobiomes Journal **1**(2): 102-113.

Yurgel, S. N., Douglas, G. M., Dusault, A., Percival, D. and Langille, M. G. I. (2018). "Dissecting Community Structure in Wild Blueberry Root and Soil Microbiome." Front Microbiol **9**: 1187.

Yurgel, S. N., Nearing, J. T., Douglas, G. M. and Langille, M. G. I. (2019). "Metagenomic Functional Shifts to Plant Induced Environmental Changes." Front Microbiol **10**: 1682.

Zapa, D. D. (2017). Développement d'un modèle prédictif de la productivité spatio-temporelle des plants de bleuets sauvages. MsC, Université de Sherbrooke.

Zhao, Y., Gao, Z., Tian, B., Bi, K., Chen, T., Liu, H., Xie, J., Cheng, J., Fu, Y. and Jiang, D. (2017). "Endosphere microbiome comparison between symptomatic and asymptomatic roots of Brassica napus infected with Plasmodiophora brassicae." PLoS One **12**(10): e0185907.

Ziadi, N. and Tran, T. S. (2007). Mehlich III-Extractable elements. Soil sampling and methods of analysis. Second edition. Canadian Society of Soil Science. M. R. Carter and E. G. Gregorich. Boca Raton, FL., Lewis Publishers**:** 81-88.

Annexes

Annexe 1 – The hidden side of wild blueberry allies – Microbiologist. Vol. 21 No 4.

Annexe 2 – Le bleuetier sauvage, un arbuste distingué – En collaboration avec Jean-François Laliberté et Sacha Lefebvre – Concours Illustre Recherche 2022 organisé par la FAECUM

Annexe 3 – Le microbiote à la rescousse du bleuetier sauvage – DIRE, Automne 2021, Vol. 30, no 3

The hidden side of wild blueberry allies

Simon Morvan [1] and **Mohamed Hijri** [1, 2]
[1] *Université de Montréal, Canada*
[2] *Université de Montréal, Canada / University Mohammed VI Polytechnic, Morocco*

Wild blueberries, or lowbush blueberries, are small shrubs that grow in generally hostile conditions for plants, as the soil is acidic and nutrient deprived. They can be mainly found in Quebec and the Atlantic coast of Canada and the USA. Contrary to their highbush blueberry relatives, wild blueberries are not planted in fields like a conventional crop. Instead, farmers locate a field where they are pre-existing, usually in the boreal forest, or abandoned farmland. The vegetation is removed to allow the wild blueberry rhizomes to spread. Another particularity of this crop is its two-year cycle. In the first year, blueberry shoots emerge from the rhizome and by the end of the growing season, they will have formed leaf and flower buds. After spending the winter under a thick layer of snow, the leaf and flower buds burst open during spring to be pollinated by bumblebees, and fruits are harvested at the end of the summer. This two-year process leads farmers to manage their fields in a way to have both growth stages occurring in the same season, allowing for annual fruit production. Once the fruits are harvested, the shoots are pruned either mechanically or thermally. Pruning allows enhanced fruit production, which would otherwise drop gradually.

One can wonder how a crop derived from such harsh conditions can lead to successful agricultural businesses. Blueberries belong to the Ericaceae plant family alongside cranberries, rhododendrons and heather. This plant family is known to form a unique, specialised symbiosis with ericoid mycorrhizal fungi. Estimated to date back 117 million years, this type of mycorrhizal symbiosis is the most recent one to have evolved, compared with the two more common mycorrhizal symbioses: arbuscular mycorrhizae and ectomycorrhizae. The hyphae of the ericoid fungi involved penetrate the cell wall of the epidermal layer of the thin (50–100 µm) Ericaceae roots. Mycelial coils, which occupy most of the cell's volume, are formed in order for the mutualistic exchange to take place.

Ericoid mycorrhizal fungi can be beneficial for their plant host in several ways. They contribute to heavy metal tolerance and they also produce a variety of enzymes able to degrade organic matter. In that sense, a recent study has shown that a few known species of ericoid mycorrhizal fungi shared more genes with saprotrophic fungi than with other kinds of mycorrhizal fungi (ectomycorrhizal or orchid mycorrhizal fungi). This capacity to degrade organic compounds is a blessing for the Ericaceae host plants. Indeed, most of the nutrients present in the soil in which they grow are trapped in organic forms, which are not readily absorbable by plants. By decomposing these compounds, ericoid mycorrhizal fungi provide an essential source of nutrients to their Ericaceae hosts. In return, they receive photosynthates in the form of sugars. It is believed that without this mutualism, Ericaceae would be unable to thrive in such harsh conditions.

This symbiosis has been known for almost 50 years, as the first ericoid mycorrhizal fungus was isolated and the nature of the symbiosis proven in 1973 by Pearson and Read. Nevertheless, it has been poorly studied compared with other forms of mycorrhizae. Furthermore, research on the wild blueberry root and rhizosphere microbial communities is only beginning, whereas it has been studied for decades for other crops such as wheat, corn and pulses. Therefore, in 2020, we intended to fill this gap by studying the fungal and bacterial communities found in the root and rhizosphere environment of wild blueberries in Quebec. In order to do so, we have relied on DNA extraction and sequencing of both the 16S rRNA gene and the internal transcribed spacer (ITS) gene allowing us to investigate both the bacterial and fungal communities, respectively. Our first study, focusing on describing these communities in wild blueberry rhizospheric soil, has confirmed the prevalence of the Helotiales fungal order, which contains most of the known ericoid mycorrhizal fungi. As for bacteria, the Rhizobiales order, which contains several nitrogen fixers, was the most abundant. By looking at the correlation between the leaf nitrogen content and the abundance of both bacterial and fungal taxa, we have found a positive correlation with several ericoid mycorrhizal fungi as well as nitrogen-fixing bacteria. Though correlation does not imply causation, these taxa are potential candidates for an improved nutrient intake for wild blueberries. Further studies are needed to test this hypothesis but if the results are conclusive, bio-inoculants adapted to wild blueberries containing a mixture of these microorganisms could be engineered in the near future.

FURTHER READING

Pearson V, Read DJ. The biology of mycorrhiza in the Ericaceae: I. The isolation of the endophyte and synthesis of mycorrhizas in aseptic culture. *New Phytologist* 1973; 72(2), 371–379

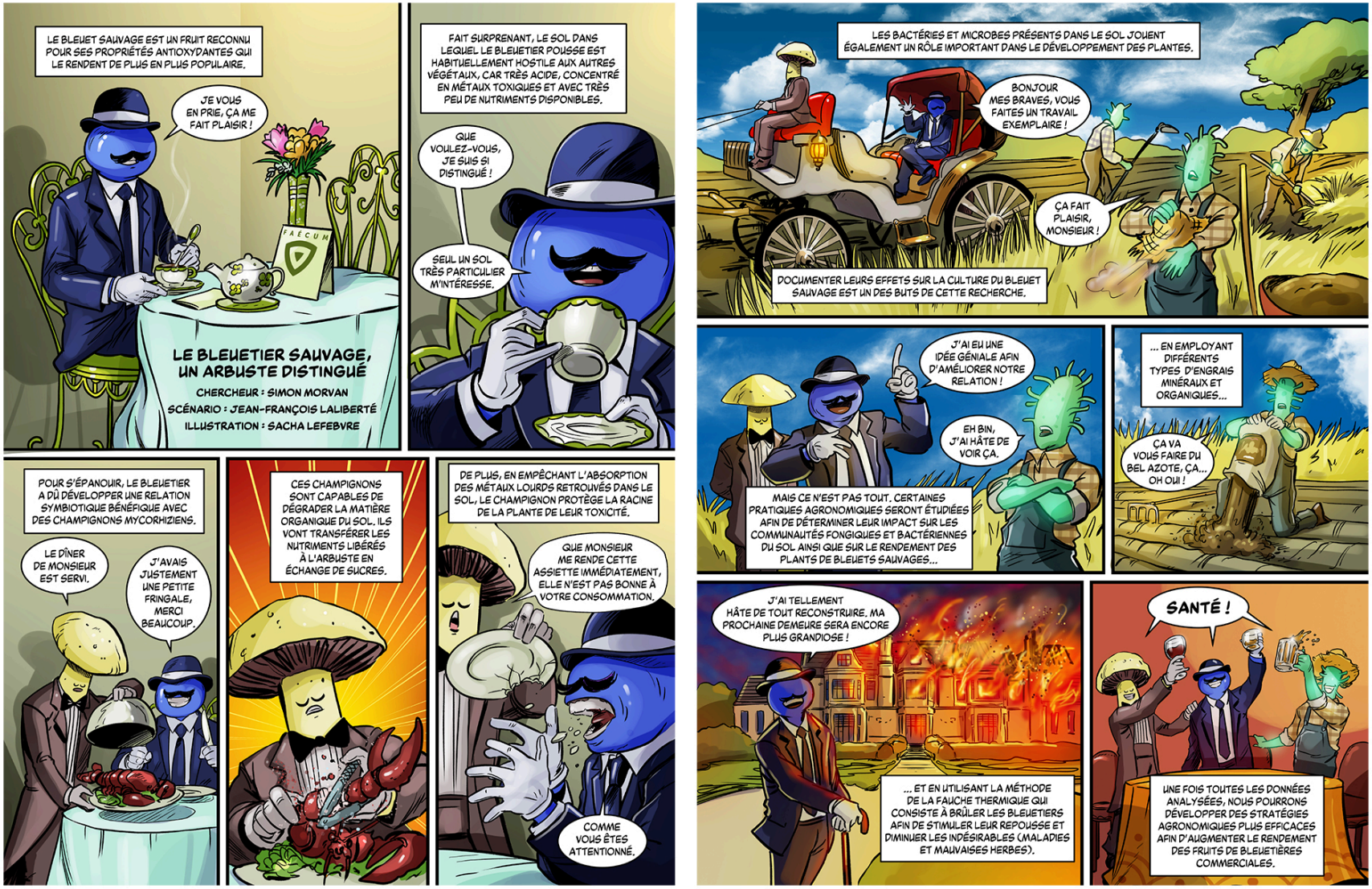
Cairney JW, Meharg AA. Ericoid mycorrhiza: a partnership that exploits harsh edaphic conditions. *European Journal of Soil Science* 2003; 54(4), 735–740

Leopold DR. Ericoid fungal diversity: challenges and opportunities for mycorrhizal research. *Fungal Ecology* 2016; 24, 114–123

Martino E, Morin E, Grelet GA, Kuo A, Kohler A, Daghino S *et al*. Comparative genomics and transcriptomics depict ericoid mycorrhizal fungi as versatile saprotrophs and plant mutualists. *New Phytologist* 2018; 217(3), 1213–1229

Morvan S, Meglouli H, Lounès Hadj Sahraoui A, Hijri M. Into the wild blueberry (*Vaccinium angustifolium*) rhizosphere microbiota. *Environmental Microbiology* 2020; 22, 3803–3822

The hidden side of wild blueberry allies – Microbiologist



Le bleuetier sauvage, un arbuste distingué – Concours Illustre Recherche de la FAECUM

Programme
Doctorat en sciences biologiques

Sujet de recherche
Le microbiote du milieu racinaire du bleuet sauvage

SIMON MORVAN
simon.morvan@umontreal.ca

Le microbiote à la rescousse du bleuetier sauvage

Le bleuet sauvage représente un marché en plein essor au Canada, premier producteur et exportateur mondial de ce fruit. Pour faire face à la demande, l'industrie cherche à adapter ses pratiques afin d'améliorer l'état de santé des bleuetiers et d'accroître leur rendement. Les micro-organismes du sol pourraient lui venir en aide. En effet, une équipe de recherche de l'Institut de recherche en biologie végétale du Département de sciences biologiques de l'Université de Montréal s'est penchée sur le sujet. Ses résultats ont montré que plusieurs espèces de bactéries et de champignons pourraient améliorer la performance et donc la productivité des bleuetiers sauvages grâce à une meilleure absorption en azote.

Le bleuet est un petit fruit qui existe depuis plus de 10 000 ans et trouve ses origines en Amérique du Nord. Les populations autochtones lui attribuaient une origine divine, leurs légendes racontant que le « Grand Esprit » envoyait cette baie dont l'extrémité affiche une forme d'étoile afin de subvenir aux besoins des enfants en période de famine. Également utilisé en médecine traditionnelle pour des thés relaxants faits à partir de ses racines, ce fruit était offert aux premières personnes venues d'Europe pour établir des colonies en Amérique du Nord, lesquelles ont vite été séduites par cette nouveauté dans leur alimentation. Les historiennes et les historiens estiment d'ailleurs que les chances sont élevées que le bleuet ait trouvé sa place sur la table de la première Action de grâce célébrée sur le territoire nord-américain.

Une baie emblématique

Le nom de cette petite baie bleue sert notamment à surnommer les personnes habitant la région du Saguenay–Lac-Saint-Jean, haut lieu de production du bleuet dans la province. Qu'il soit mangé frais ou incorporé dans des crêpes ou des muffins, le bleuet est apprécié pour ses qualités gustatives. De plus, sa concentration en anthocyanes, un antioxydant qui lui donne entre autres sa couleur bleutée, est l'une des plus élevées parmi les fruits offerts sur les étalages[1]. Des recherches ont permis d'établir que la consommation de bleuets a de nombreux effets bénéfiques sur les fonctions cognitives et cardiovasculaires[2]. Pour ces raisons, la demande en bleuets augmente dans le monde entier[3] et l'industrie est constamment à la recherche de pratiques agricoles pouvant améliorer le rendement des plants.

Comme c'est le cas pour le nom d'autres végétaux, le terme générique *bleuet* renvoie en fait à différentes espèces de cette baie. Les bleuetiers sauvages, ou bleuetiers nains, sont de petits arbustes cultivés dans l'est de l'Amérique du Nord, essentiellement au Québec, dans les provinces maritimes ainsi que dans l'État du Maine, aux États-Unis. La culture des bleuetiers en corymbe, une autre espèce de bleuetier, est plus répandue que celle des bleuetiers sauvages et se fait entre autres au Brésil, au Chili et au Mexique. Ces deux espèces se différencient notamment par la taille de leurs fruits, les bleuets sauvages étant plus petits. Les bleuets trouvés sur les étalages des épiceries sont généralement des bleuets en corymbe. Toutefois, s'ils proviennent du Saguenay–Lac-Saint-Jean, les chances sont plus grandes qu'ils soient des bleuets sauvages. De plus, la culture du bleuetier en corymbe s'apparente davantage à une culture en verger, avec des arbustes implantés en champs, tandis que les bleuetières produisant des bleuets sauvages sont établies sur des terres où des plants sont préexistants.

Au Québec, les plants réellement sauvages se trouvent dans la forêt boréale ou dans d'anciennes fermes que les bleuetiers ont colonisées[4]. En effet, le terme *sauvage* pour désigner cette espèce de bleuetier est plus ou moins bien choisi, puisque ces fruits sont en fait cultivés (à l'exception de ceux qui sont ramassés en forêt).

« Les plantes exercent une pression de sélection sur ces micro-organismes et peuvent, dans une certaine mesure, mobiliser les micro-organismes dont les fonctions leur seront bénéfiques. »

« Aussi, les bleuetiers sauvages s'établissent dans un sol ayant une forte teneur en matière organique et où les nutriments essentiels ne sont pas directement absorbables par la plante. »

Les bleuets sauvages n'ont de sauvage que leur origine : ce ne sont pas les plants qui sont implantés dans des champs, mais plutôt les champs qui se créent autour de plants natifs. Aussi, les bleuetiers sauvages s'établissent dans un sol ayant une forte teneur en matière organique et où les nutriments essentiels ne sont pas directement absorbables par la plante. De plus, ce sol est relativement acide, ce qui augmente la **biodisponibilité*** en métaux lourds, qui sont toxiques pour les végétaux. Dans ces conditions, comment fait le bleuetier sauvage pour prospérer et être autant en santé ?

FIGURE 1
Une bleuetière peu avant la récolte

Source : Simon Morvan

L'appui du microbiote

Le buisson du bleuetier n'est que la pointe de l'iceberg, car sous sa partie émergée, le sol entourant les racines est un milieu qui grouille de vie. En effet, le **microbiote*** racinaire est une communauté complexe incluant des micro-organismes présents dans différentes niches écologiques : certains peuvent pénétrer à l'intérieur des racines tandis que d'autres restent dans la **rhizosphère*,** à la surface des racines et dans la fine zone de sol qu'elles influencent. Les plantes exercent une pression de sélection sur ces micro-organismes et peuvent, dans une certaine mesure, mobiliser les micro-organismes dont les fonctions leur seront bénéfiques. Parmi les différents micro-organismes, les bactéries et les champignons sont les deux règnes couramment étudiés en raison des effets qu'ils peuvent avoir sur le végétal avec lequel ils interagissent.

* **Biodisponibilité :** capacité d'assimilation d'un élément par une plante, un micro-organisme ou par la faune présente dans le sol.

* **Microbiote :** ensemble de micro-organismes (bactéries, champignons, virus, etc.) vivant dans un milieu.

* **Rhizosphère ou sol rhizosphérique :** zone du sol sous l'influence des racines.

« En effet, les champignons mycorhiziens possèdent un arsenal d'enzymes adaptées aux conditions acides qui dégradent la matière organique et qui rendent ainsi les nutriments accessibles au champignon, lequel peut ensuite les transmettre à la plante. »

Les bleuetiers appartiennent à la famille des Éricacées, qui inclut entre autres la canneberge, les rhododendrons et les bruyères. Cette famille a la particularité de s'associer à de microscopiques champignons mycorhiziens, qui sont des champignons qui établissent une **symbiose*** bénéfique avec leur plante hôte. Les filaments du champignon (ou *hyphes*) pénètrent la première couche de cellules des jeunes racines et y forment un enchevêtrement dense semblable à une pelote de laine[5]. À partir de cette pelote, les deux organismes établissent les échanges caractéristiques de la symbiose mycorhizienne. La plante fournit ainsi au champignon des sucres qu'elle produit grâce à la photosynthèse (processus transformant l'eau et le dioxyde de carbone en sucres grâce à l'énergie lumineuse du soleil). Ces sucres servent au champignon à produire de l'énergie et l'aident à prospérer. En échange, le champignon procure des nutriments essentiels à la plante. En effet, les champignons mycorhiziens possèdent un arsenal d'**enzymes*** adaptées aux conditions acides qui dégradent la matière organique et qui rendent ainsi les nutriments accessibles au champignon, lequel peut ensuite les transmettre à la plante[6]. Cette capacité des champignons mycorhiziens de libérer les nutriments des formes organiques dans lesquelles ils sont bloqués représente une réelle aubaine pour les bleuetiers sauvages, puisqu'ils poussent pour la plupart dans un sol appauvri en nutriments disponibles.

Par ailleurs, les champignons mycorhiziens jouent un rôle protecteur vis-à-vis des métaux lourds en prévenant leur absorption par les racines des plantes qu'ils colonisent[7]. Ainsi, la capacité des bleuetiers sauvages à se satisfaire de ces conditions dépend notamment de cette symbiose

* **Symbiose :** association biologique, durable et réciproquement profitable entre deux organismes vivants.

* **Enzyme :** protéine capable de catalyser des réactions chimiques.

« Les résultats ont montré la dominance d'un groupe de champignons connu pour contenir de nombreuses espèces de champignons mycorhiziens qui s'associent aux Éricacées. »

mycorhizienne salutaire[8]. Plusieurs études ont en effet montré que la présence de certaines espèces de ces champignons améliorait la croissance[9] ainsi que l'absorption en azote[10] et en phosphore[11] des Éricacées.

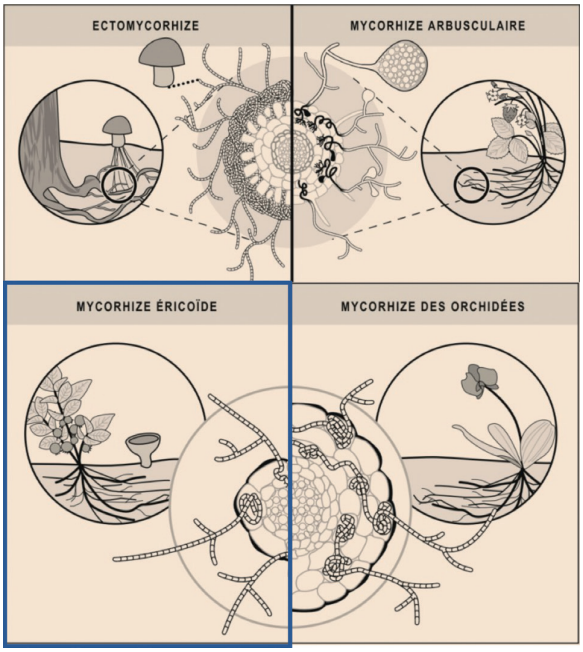


FIGURE 2
Les quatre principaux types de symbioses mycorhiziennes

Adaptée de Fortin, J. A., Plenchette, C. et Piché, Y. (2015). *Les mycorhizes : la nouvelle révolution verte.* MultiMondes, p. 17 et 22.

Des bactéries inattendues

En plus des champignons, un lot d'espèces de bactéries peut également influencer de manière positive la croissance d'une plante[12]. Parmi les fonctions bactériennes bénéfiques découvertes, les recherches ont révélé notamment la fixation de l'azote atmosphérique, la production d'hormones qui améliorent la croissance des plantes ou encore la protection contre des micro-organismes infectieux. Pourtant, l'effet des communautés bactériennes sur les bleuetiers a été peu étudié jusqu'à maintenant.

Bien que les connaissances sur les champignons mycorhiziens s'associant aux Éricacées datent de plus de vingt ans, les études se penchant sur le microbiote du milieu racinaire des bleuetiers sauvages en conditions réelles sont peu nombreuses. Le laboratoire du professeur Mohamed Hijri de l'Institut de recherche en biologie végétale de l'Université de Montréal s'est donc intéressé à ce sujet en récoltant des plants de bleuets et leur sol dans trois bleuetières de Saint-Honoré, dans la région du Saguenay–Lac-Saint-Jean. Grâce au séquençage à haut débit (une technique permettant d'identifier des espèces grâce à leur ADN), l'équipe de recherche a pu mettre au jour les communautés bactériennes et fongiques présentes dans le sol rhizosphérique des échantillons prélevés. Les résultats ont montré la dominance d'un groupe de champignons connu pour contenir de nombreuses espèces de champignons mycorhiziens qui s'associent aux Éricacées. Du côté des bactéries, le groupe qui domine la communauté inclut plusieurs espèces capables de fixer l'azote atmosphérique.

Par ailleurs, l'équipe du laboratoire a identifié plusieurs espèces de bactéries fixatrices d'azote et de champignons mycorhiziens d'intérêt. En effet, plus la quantité de ces espèces était élevée dans les échantillons, plus la concentration d'azote dans les feuilles des bleuetiers échantillonnés était haute. Ce résultat est doublement intéressant parce que l'azote est l'un des trois nutriments essentiels aux plantes (avec le phosphore et le potassium) et qu'il est difficilement accessible dans le sol où poussent les bleuetiers. Afin de valider l'importance de ces espèces bactériennes et fongiques, une prochaine étape serait de les **inoculer*** sur des bleuetiers pour voir si ceux-ci absorberont mieux l'azote que des bleuetiers non inoculés[13].

Les micro-organismes et l'agriculture

L'influence positive des micro-organismes sur la productivité des plants encourage les chercheurs et les chercheuses à trouver des moyens d'en tirer profit[14]. De plus, le nombre de bleuetières en agriculture biologique (qui fonctionne sans traitements ayant recours à des produits chimiques de synthèse) augmente fortement

***Inoculer :** introduire des cellules ou des organismes dans un milieu ou un autre organisme.

« Mieux comprendre les systèmes biologiques est donc essentiel pour avoir une agriculture à la fois productive et durable. »

au Québec. Ce mode de production doit faire face aux mêmes défis que l'agriculture conventionnelle, mais il dispose de moins de produits pour traiter les diverses maladies et les ravageurs[15]. Mieux comprendre les systèmes biologiques est donc essentiel pour avoir une agriculture à la fois productive et durable. C'est dans cette optique que deux autres études mesurant l'effet des pratiques agricoles sur le microbiote du bleuetier sauvage ont vu le jour au sein du laboratoire du professeur Hijri.

La première cherche à savoir si la fauche thermique, qui consiste à brûler les tiges après la récolte afin d'en stimuler la productivité, a une influence sur les communautés bactériennes et fongiques du milieu racinaire des bleuetiers sauvages. Les effets du feu sur les microbes ont déjà été bien étudiés dans le cadre des incendies forestiers, qui bouleversent la communauté microbienne, affectée par la chaleur. Cependant, cette pratique se distingue des feux de forêt par sa faible intensité et pourrait donc avoir des répercussions minimes sur le microbiote. La deuxième étude se penche sur les effets du type de fertilisation (minérale, organique ou absente) sur le microbiote racinaire. La fertilisation minérale permet d'accroître la disponibilité en nutriments directement assimilables par la plante. Cependant, elle pourrait avoir une influence négative sur la présence des champignons mycorhiziens, qui perdraient leur utilité en tant que fournisseurs de nutriments.

À terme, les résultats de ces différentes études pourraient aider à la confection de bio-inoculants. Ces produits composés de diverses espèces de bactéries et de champignons pourraient s'ajouter aux engrais chimiques appliqués sur les parcelles, voire les remplacer. Ces projets permettront d'en apprendre plus sur l'écosystème invisible, mais essentiel du microbiote racinaire. C'est en effet grâce à la compréhension des phénomènes naturels complexes que l'agriculture pourra progressivement se passer des produits chimiques néfastes à la biodiversité. ◎