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Le microbiome fongique de la rhizosphère du canola : Structure et variations.

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Résumé

Les champignons de la rhizosphère ont une grande influence sur le développement et la croissance des plantes. Certains de ces micro-organismes protègent les plantes contre les pathogènes, atténuent l'impact des stress abiotiques ou facilitent la nutrition des plantes. Ces organismes s'influencent mutuellement et forment des réseaux complexes d'interactions. Déterminer le fonctionnement du microbiome fongique de la rhizosphère des plantes cultivées est une étape nécessaire pour optimiser l'efficacité de la production végétale. Nous avons testé les hypothèses suivantes : (1) la diversification des systèmes de culture influe sur le microbiome fongique de la rhizosphère du canola; (2) le canola a un core microbiome, soit un ensemble de champignons toujours associés au canola quelles que soit les conditions du milieu; et (3) que certains de ces taxons ont une influence déterminante sur la structure des communautés (taxons nodaux) dans le core microbiome. Pour ce faire, en 2013 et 2016, nous avons échantillonné à la floraison, la phase de canola (*Brassica napus*) du système de culture, qui est l'un des deux types de canola (Roundup Ready® et Liberty Link®), utilisés dans le cadre d'une expérience de terrain à long terme (6 ans). Lacombe (Alberta), Lethbridge (Alberta) et Scott (Saskatchewan). En utilisant le séquençage d'amplicon par illumina, nous avons obtenus des résultats qui montrent que la diversification des cultures a un impact significatif sur la structure des communautés fongiques de la rhizosphère. Nous avons également découvert et décrit un core microbiome constitué de 47 OTU (Unité Taxonomique Opérationnelle) en 2013 et identifié *Preussia funiculata, Schizothecium sp., Mortierella sp., Nectria sp.* ainsi que deux taxons inconnus (OTU12 et OTU298) comme taxons nodaux parmi ce core microbiome. Cependant ce core microbiome s'est montré variable, et nous n'avons pu identifier qu'un OTU y appartenant en 2016 : *Olpidium Brassicae*. Nos résultats permettent de confirmer l'impact de la diversité culturale sur le microbiome fongique du canola et sont présentés comme une base pour le développement de stratégies d'ingénierie écologique pour l'amélioration de la production de canola.

Mots-clés : Communautés fongiques, écologie microbienne, agrosystèmes, rotations de cultures, *Brassica napus*

Abstract

The fungi in the rhizosphere have a large influence on plant development and growth. Some of these micro-organisms protect plants against pathogens, mitigate the impact of abiotic stress, or facilitate plant nutrition. These organisms influence each other and form complex webs of interactions. Deciphering the structure and function of the fungal microbiome of crop plant rhizosphere is a necessary step toward optimizing the efficiency of plant production. We tested the hypotheses that (1) the diversification of cropping systems influences the fungal microbiome of canola rhizosphere, (2) canola has a fungal core microbiome, i.e. a set of fungi that are always associated with canola, and (3) that some taxa have a determining influence on the structure of the communities (hub-taxa) within the core microbiome. In 2013 and 2016 we used the canola (*Brassica napus*) phase of five cropping system at blooming stage, from the less to the most diversified, that included one of two types of canola (Roundup Ready® and Liberty Link®), in an existing long-term (6 years) field experiment. The experiment has a randomized complete block design with four blocks, and is replicated at three locations: Lacombe (Alberta), Lethbridge (Alberta) and Scott (Saskatchewan). Our results show that crop diversification has significant impact on the structure of rhizosphere fungal communities. We also discover and described a canola core microbiome made of 47 OTUs in 2013 and identified *Preussia funiculata, Schizothecium sp., Mortierella sp., Nectria sp.* and two other unidentified taxa (OTU12 and OTU298) as the hub-taxa among this core. However this core microbiome was variable and could identify only one member in 2016 : *Olpidium brassicae*. Our results confirmed the effect of crop diversification upon the fungal microbiome of canola and are presented as a basis for the development of ecological engineering strategies for the improvement of canola production.

Keywords: Fungal communities, microbial egology, agrosystems, crop rotations, *Brassica napus*

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ADN : Acide Désoxyribonucléique

ANOVA : Analysis of Variance

ARN : Acide Ribonucléique

AMF: Arbusuclar Mycorhizal Fungi

DSE: Dark Septate Endophytes

IRBV : Institut de Recherche en Biologie Végétale

ITS: Internal Transcribed Spacer

MRPP: Multi Response Permutational Procedure

NGS: New Generation Sequencing

OTU: Operational Taxonomic Unit

PERMANOVA: Permutational Analysis of Variance

PCoA: Principal Coordinates Analysis

PCR: Polymerase Chain Reaction

F.A.O.: Food and Agriculture Organization

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Introduction

Les traces fossiles de symbiose entre les microbes et les plantes remontent au Dévonien il y a plus de 400 million d'années (Remy et al. 1994). Il s'agit d'une symbiose mycorhizienne arbusculaire (AMF). On pense que ce type de mutualisme a joué un rôle très important dans la conquête de l'environnement terrestre par les plantes et a contribué à leur diversification et leur évolution (Selosse and Le Tacon 1998). Cette proximité entre les plantes et les microbes les colonisant a été étudiée très tôt dans l'histoire de la botanique (B. Frank 1885). Mais ce n'est qu'à partir du moment où l'on a pu accéder à la technologie du séquençage de l'ADN que la mesure de la diversité des microbes présents dans les racines des plantes et interagissant avec elles a pu être dévoilée (Hannula et al. 2012), pointant un vide important dans la connaissance que nous avions du fonctionnement de la rhizosphère. Depuis quelques années, la recherche sur les communautés de micro-organismes racinaires s'est portée sur le microbiome des plantes (la diversité des micro-organismes associés aux plantes). Étudier le microbiome des plantes et plus particulièrement celui des racines implique de lier l'écologie microbienne au fonctionnement de la plante hôte. On considère alors les micro-organismes comme un pool de gènes et de fonctions profitables ou non à la plante. Prises une par une, les contributions des micro-organismes à la plante hôte peuvent paraitre, de prime abord, négligeables, notamment celles des micro-organismes commensaux, cependant les fonctions écologiques prises en charge par le microbiome racinaire d'une plante sont une contribution précieuse permettant à la plante d'augmenter considérablement l'exploitation de son milieu (Bulgarelli et al. 2013).

Comprendre le fonctionnement des différentes sortes d'interaction entre les microbes et les plantes au sein de leurs microbiomes est un sujet très important en écologie mais a aussi tout son sens en agronomie. Comme je l'ai précisé plus haut, l'utilisation de séquençage de nouvelle génération a permis d'ouvrir une nouvelle fenêtre de compréhension sur la diversité du microbiome racinaire des plantes. Cependant notre connaissance de l'importance de la fonction de ce microbiome et des interactions qui s'y déroulent reste limitée, même si les études s'y intéressant ont connu un épanouissement certain depuis le commencement de la dernière décennie (Ridout and Newcombe 2016; van der Heijden and Hartmann 2016). La compréhension du microbiome des plantes aura un impact certain sur plusieurs champs de recherche, notamment en agronomie.

Le microbiome, définition.

Le terme microbiome est apparu dans la littérature dans le cadre de l'étude de la diversité microbienne du système digestif humain (Shanahan 2002). Cependant, le concept de microbiome est applicable à tous les environnements hébergeant des communautés microbiennes. Le microbiome peut être décomposé en sous-unités. L'ensemble des micro-organismes que l'on trouve dans les racines des plantes forment le pan-microbiome racinaire, selon la définition de (Vandenkoornhuyse et al. 2015). Cet ensemble microbien inféodé au système racinaire présente des caractéristiques qui peuvent varier en fonction des conditions de l'environnement. Cependant, il est possible que des groupes de taxons soient présents peu importent les conditions abiotiques et biotiques du milieu. Ces derniers sont inféodés à la rhizosphère de la plante et vraisemblablement favorisés par la plante tout au long de son existence (Rout 2014), c'est le core microbiome. On peut aussi regarder uniquement la fraction du microbiome partagée entre deux milieux. On nomme cette fraction l'éco-microbiome. Enfin, le dernier groupe que l'on peut différentier est le microbiome non partagé qui regroupe les organismes qui ne sont pas systématiquement présents dans tous les échantillons ni systématiquement présents dans les échantillons qui partagent certaines conditions environnementales. Leur présence pourrait être due à des processus aléatoires et, selon la présente théorie, ils ne devraient pas être essentiels au fonctionnement de la plante ni à son adaptation aux conditions environnementales.

Les racines des plantes, un écosystème microbien

Les organismes microbiens vivant à proximité des racines des plantes, tels que les bactéries, les champignons, les oomycètes, et les archées sont attirés par les exsudats racinaires. Ces exsudats sont pour ces organismes une source de nutriments (van der Heijden and Hartmann 2016). Ils sont aussi un moyen pour la plante d'influencer la composition des communautés de microbes qui peuplent son système racinaire. De nombreuses études ont montré une forte relation entre la composition des communautés microbiennes des racines, et la composition des communautés végétales (Siciliano et al. 1998; Costa et al. 2006; Garbeva, van Elsas, and van

Veen 2007; Dias et al. 2012; Reinhold-Hurek et al. 2015). Pour exemple, DeAngelis et al. 2009 ont montré que sur 1917 taxons de bactéries de la rhizosphère de la Poaceae *Avena fatua*, 147 taxa étaient significativement plus abondants sur les racines de la plante que dans le sol. Parmi ces taxa, les plus nombreux appartenaient aux phyla *Firmicutes*, *Actinobacteria* ou encore à la classe des *Alphaproteobacteria*.

Les *Proteobacteria* comprennent des familles de bactéries très connues et étudiées comme les *Pseudomonadaceae* ou les *Burkholderiaceae.* Les *Proteobacteria* sont particulièrement abondantes dans le sol (Uroz et al. 2010). Cela s'expliquerait par le fort potentiel compétitif de ce groupe. Les *Proteobacteria* se divisent rapidement et peuvent utiliser un large spectre d'exsudats racinaires comme source de nutriments. Cependant, les niches écologiques des bactéries et des champignons se chevauchent dans l'écosystème racinaire, amenant ainsi à de la compétition. Par exemple, les champignons comme certains Ascomycètes ou encore les Gloméromycètes utilisent aussi les exsudats racinaires dans leur métabolisme (Hannula et al. 2012). En effet, les champignons peuvent émettent des composés nocifs, comme par exemple des antibiotiques empêchant la compétition des autres organismes comme les bactéries ou les autres champignons dans leur entourage proche (Lugtenberg, Rozen, and Kamilova 2017) ce qui rend la coexistence avec une grand nombre de bactérie saprophytes impossible.

Le microbiome de la rhizosphère est composé d'une multitude d'organismes, fongiques comme bactériens. Ceux-ci occupent un large spectre de niches écologiques allant de la surface des différentes parties des racines au milieu intracellulaire des racines. Les communautés microbiennes qui composent le microbiome rhizosphérique varient en fonction de différents paramètres comme l'humidité ou encore des éléments que l'azote ou le phosphore (Mosier et al. 1998; Dunfield and Germida 2003; Taktek et al. 2017). En effet, le microbiome est sujet à l'influence de facteurs biotiques et abiotiques. La principale influence qui s'exprime sur le microbiome est la nature du sol. En effet, de nombreuses études ont montré que les propriétés du sol ont un impact profond sur les communautés bactériennes et fongiques (de Ridder-Duine et al. 2005; Li, Voigt, and Kent 2016; Moll et al. 2016; van der Voort et al. 2016). Des études basées sur la métagénomique ont montré que le microbiome bactérien de différentes génotypes d'*Arabidopsis thaliana* est particulièrement influencé par le type de sol (Bulgarelli et al. 2012; K. Schlaeppi et al. 2014). C'est aussi le cas pour les bactéries diazotrophes associées à

Miscanthus x giganteus qui varient en fonction de leur localisation dans les racines et du type de sol (Li et al., 2016). Cependant, le type de sol n'est pas le seul facteur qui agit sur la variabilité des communautés microbiennes de la rhizosphère. En effet, la physiologie des plantes et plus particulièrement le processus d'excrétion des exsudats est influencé par leur appartenance phylogénétique.

La composition spécifique du couvert végétal influence fortement la composition spécifique des communautés de la rhizosphère. La plante, par la morphologie de ses racines et les particularités de ses exsudats racinaires, contribue à sélectionner les espèces qui peuplent son entourage racinaire (Bressan et al. 2009; Reinhold-Hurek et al. 2015). Certains composés relâchés dans la rhizosphère par les plantes provoquent des réactions spécifiques au sein du microbiome racinaire. Par exemple, les Bassicaceae (comme par exemple le canola : *Brassica napus, Brassica rapa, Brassica juncea*) sont connues pour relâcher des isocyanates dans le sol qui inhibent la croissance des populations de micro-organismes dans les racines (Rumberger and Marschner 2003). D'autres plantes émettent des flavonoïdes qui peuvent aussi stimuler la croissance des champignons mycorhiziens à arbuscules (AMF) et le développement des mycorhizes ainsi que la germination de leurs spores; ces mêmes classes de molécules sont aussi importantes dans la formation des nodulations racinaires de la famille des *Fabaceae* (Hassan and Mathesius 2012).

Si toutes les espèces de plantes ont leur profil d'exsudats racinaires spécifiques, les cultivars eux aussi peuvent avoir une influence distincte sur la structure des communautés de leur microbiome racinaire (Hwang et al. 2011; Dias et al. 2012; Nallanchakravarthula et al. 2014; Bazghaleh et al. 2015). C'est notamment le cas pour le canola, pour lequel le cultivar transgénique Quest diffère au niveau de ses communautés racinaires par rapport aux cultivars standard Excel et Parkland (Siciliano et al. 1998). Le cas des plantes cultivées est intéressant car durant les étapes de sélection des plantes, l'accent a été mis sur le développement du rendement et souvent au détriment du développement du système racinaire. Ainsi, l'importance du microbiome rhizosphérique de la plante dans sa santé et son développement a longtemps été négligé. Germida and Siciliano 2001, ont émis l'hypothèse que les techniques modernes de sélection pourraient avoir sélectionné des traits non-seulement essentiels à la défense des plantes cultivées contre les micro-organismes pathogènes du sol mais aussi qui interfèrent dans le développement de symbioses mutualistes. C'est notamment le cas pour le blé pour lequel les

auteurs ont comparé les communautés de bactéries endophytes des cultivars nouvellement électionnés contre celles d'anciennes variétés. Il en ressort que les nouveaux cultivars sont colonisés de manière agressive par des bactéries endophytes du genre *Pseudomonas* au détriment de bactéries plus bénéfiques (Germida and Siciliano 2001).

Le génotype des plantes détermine largement la composition des communautés du microbiome racinaire à l'intérieur de ce qui est permis par un environnement. C'est aussi le cas des interactions entre microbes. Il faut savoir que les micro-organismes interagissent dans le microbiome racinaire. Bien que de nombreuses études ont examiné ces interactions (Smith, Handelsman, and Goodman 1999; Hartmann et al. 2015; Edwards et al. 2015; Xue et al. 2015; Cha et al. 2016; Chapelle et al. 2016; Ridout and Newcombe 2016), on en sait remarquablement peu sur les interactions entre les différentes espèces de micro-organismes au sein du microbiome de la rhizosphère des plantes. On a pu observer des phénomènes d'antagonismes entre différents organismes. C'est notamment le cas chez le blé dont l'interaction avec un champignon de type *Penicillium* sp. a réduit l'impact du champignon pathogène *Fusarium culmorum* au niveau des racines et augmenté le rendement des plants infectés (Ridout and Newcombe 2016). Les interactions microbe-microbe sont relativement mal connues. On vient à peine de s'apercevoir que les micro-organismes comme les mycorhizes à arbuscules possèdent eux aussi leur microbiome (Naumann, Schüßler, and Bonfante 2010; Lecomte, St-Arnaud, and Hijri 2011; Iffis, St-Arnaud, and Hijri 2014, 2016; Salvioli et al. 2016; Iffis, St-Arnaud, and Hijri 2017). Ce dernier peut être intracellulaire et composé de biotrophes, notamment les bactéries gram-négatives similaires à *Glomerobacter gigasporarum* ou des bactéries coccoïdes relatives à la famille des endobactéries de type *Mollicutes* (Desiro et al. 2014). Il peut aussi être extracellulaire comme pour le cas du microbiome de l'hyphosphère des mycorhizes à arbuscules (Taktek et al. 2017, 2015) Il est difficile d'étudier ce genre d'interactions car bien souvent ni les hôtes ni les symbiontes ne sont cultivables. Souvent, les taxa qui y sont impliqués sont inconnus ou n'ont pas été répertoriés dans les bases de données. L'intérêt de l'étude de ces interactions réside dans le fait qu'elles ont des effets sur les communautés végétales. En effet, les symbioses entre microbes au sein du milieu racinaire peuvent augmenter la compétitivité des espèces de plantes qui leur sont liées au détriment des autres (Wardle et al. 2004).

Les micro-organismes du microbiome racinaire et les plantes vivent en équilibre dynamique. En effet, le microbiome rhizosphérique soumis aux changements

environnementaux. Les changements dans la structure des communautés de la rhizosphère au cours de la vie de la plante sont abondamment documentés (Dunfield and Germida 2003; Cavaglieri, Orlando, and Etcheverry 2009; Larsen, Gibbons, and Gilbert 2012; Hargreaves, Williams, and Hofmockel 2015). Par exemple, lorsque l'on s'est intéressé aux communautés de champignons et de bactéries de la rhizosphère à différents stades de développement chez *Medicago truncatula*, on a noté des différences en matière de composition d'espèces (Mougel et al. 2006). La succession des espèces microbiennes du sol associées à la plante commence dès les premiers stades de la germination (Nelson 2004). De plus, les micro-organismes de la rhizosphère ne sont pas répartis de manière homogène et sont influencés par les types de racines (primaires et secondaires) ainsi que par leur position (apicale ou basale) sur la racine en croissance. La comparaison entre des échantillons de rhizosphère de la base et de l'apex des racines a montré des différences nettes en matière de structure des communautés, notamment au niveau des proportions entre les microbes de stratégies r plus nombreux dans l'apex et K majoritaires dans la base (Folman, Postma, and Veen 2001). Ces variations au spatio-temporelles sont à prendre en compte lors de l'étude du microbiome de la rhizosphère.

Le microbiome de la rhizosphère et les techniques de séquençage.

La vaste majorité des micro-organismes du sol ne peuvent être cultivés à l'heure actuelle. Afin de pouvoir étudier de manière précise les interactions qui interviennent au sein du microbiome racinaire, il est alors nécessaire d'appliquer des méthodes d'analyse moléculaire direct de l'ADN ou de l'ARN environnemental. Des progrès formidables dans les technologies de séquençage et en informatique ont été accomplis au cours des dix dernières années et se poursuivent encore aujourd'hui. Ces progrès ont permis d'étudier la complexité du microbiome racinaire par l'étude de son information génétique. Ce peut être par le biais de l'ADN pour observer tout le génome microbien, ou de l'ARN pour étudier les fonctions exprimées par les micro-organismes dans la rhizosphère. On a alors besoin d'analyser une somme considérable d'information. La rhizosphère est un milieu extrêmement divers où la quantité de micro-organismes se compte milliards. Il est alors nécessaire d'avoir accès à des techniques de séquençage qui permettent l'acquisition d'une bonne résolution en ce qui concerne la diversité

microbienne des racines : on les nomme les techniques de séquençage de nouvelle génération (NGS).

Les différents systèmes NGS actuellement disponibles ont en commun qu'ils produisent une très grande quantité de données. Souvent les séquenceurs NGS sont classifiés comme étant de seconde ou de troisième génération de séquençage (Schadt, Turner, and Kasarskis 2010; Schatz, Delcher, and Salzberg 2010). Les technologies de seconde génération incluent les instruments 454 de Roche, les plateformes de type Illumina et les instruments de la firme Life Technologies comme le séquenceur par ligation d'oligonucléotides (SOLiD) et les séquenceurs Ion Torrent. La troisième génération de séquenceurs est définie par leur capacité à séquencer une molécule d'ADN sans étape de pré-amplification en conservant l'incorporation de nucléotides par cycle ou non. Les seules technologies de séquençage de troisième génération actuellement sur le marché sont la technologie PacBio développée par Pacific Biosciences et l'approche de Nanopore Sequencing.

Ces technologies permettent, dans le cas de l'étude du microbiome racinaire des plantes, de réaliser des études de métagénomique, c'est-à-dire étudier l'ensemble des génomes trouvés dans le sol ou la rhizosphère. La stratégie de séquençage optimale à suivre afin de mener un projet de métagénomique dépend beaucoup du but de l'étude que l'on veut mener. Une étude comparative entre la technologie illumina et le séquençage 454 a révélé que les données assemblées de l'une ou l'autre des méthodes reflétait de manière fidèle la composition génomique des échantillons qui étaient comparés ; illumina ne se montrant de meilleure qualité que sur les critères d'assemblage (Luo et al. 2012). Cependant, une différence majeure entre le 454 et Illumina (outre le fait que le 454 soit maintenant abandonné par Roche) est liée à la quantité de séquences (reads) produits par la réaction de séquençage : environ 500,000 pour le 454 contre plusieurs dizaines ou centaines de millions pour Illumina, tout dépendant de l'appareil et du protocole choisi. La longueur a aussi été un enjeu important avant l'abandon du 454 et le perfectionnement de l'Illumina. Si l'on veut étudier les gènes de la rhizosphère d'une plante, l'approche shotgun est recommandée et la plateforme de séquençage Illumina HiSeq est le meilleur choix au vu de son faible coût et qu'il permette donc un séquençage en profondeur, ce afin d'avoir une information solide même pour les organismes rares du système étudié (Knief 2014). Si l'on s'intéresse à l'identité des taxons présents dans le microbiome, utiliser le séquençage d'amplicons est alors plus approprié. Celui-ci est généralement fait avec un

séquenceur Miseq qui produit des reads plus longues mais moins nombreuses que le Hiseq qui est généralement réservé aux analyses métagénomiques ou metatranscriptomiques shotgun. Les études sur la diversité des micro-organismes utilisent usuellement l'information de marqueurs phylogénétiques comme le gène codant pour l'ARN 16S pour les bactéries ou l'ARN 18S et les séquences intergéniques ITS pour les champignons. La plateforme utilisée est alors Miseq de la firme Illumina (Claesson et al. 2010). Enfin, si l'on s'intéresse à l'abondance d'un organisme dans le microbiome, on peut utiliser la PCR quantitative. La technologie PacBio semble prometteuse afin de conduire des études de métagénomes, en raison de sa capacité à produire de grandes quantité de séquences de longue taille qui permettent de s'affranchir des problèmes reliés aux régions répétitives du génome (English et al. 2012). Cependant sa propension aux erreurs de séquençage, font que cette stratégie n'a pas encore été utilisée pour des études de métagénomique. font que cette stratégie est pour le moment difficile à appliquer en métagénomique (Castaño et al. 2016; Klaus Schlaeppi et al. 2016; Schloss et al. 2016).

Cooccurrence et analyse de réseau

Les outils de séquençage à haut débit permettent de décrire l'immense complexité du microbiome racinaire, mais encore faut-il avoir les outils statistiques pour les analyser les immenses fichiers de séquençage et leur donner du sens. En écologie, on peut utiliser les analyses de réseau afin de représenter les interactions biotiques particulières aux écosystèmes. Ces analyses permettent aux scientifiques d'accéder à une vue globale du système étudié et notamment de porter sa complexité à un niveau compréhensible (Johnson et al. 2009). Dans le cas des communautés de microbes, ces derniers interagissent de manière extrêmement fournie et diverses aussi bien avec leur hôte potentiel qu'avec les autres microbes de l'écosystème (van der Heijden and Hartmann 2016). On peut ainsi supposer que dans un système microbien donné, les OTUs partageant des corrélations positives soient dans la réalité liés par une relation bénéfique, et inversement pour les corrélations négatives. Cependant, s'appuyer sur des corrélations pour refléter la réalité d'un système est difficile. C'est en particulier le cas pour les systèmes d'interactions microbiens où les jeux de données peuvent avoir facilement plus de 5000 composantes, impliquant alors des dizaines de millier de corrélations. Ce faisant, le type de données générées par de telles techniques peut produire des résultats peu fiables, puisque les

données prennent la forme de fractions relatives de gènes où d'espèces plutôt que de leurs abondances absolues. Plusieurs méthodes ont été développé afin de pouvoir détecter le plus fidèlement possible les interactions entre microbes, parmi lesquelles CoNet (Faust and Raes 2016) et SparCC (Friedman and Alm 2012). CoNet part du principe qu'utiliser différentes techniques permet de compenser leurs forces et leurs faiblesses, ce afin de détecter de manière optimale différentes relations fonctionnelles. La méthode utilise alors une méthode d'ensemble avec la procédure ReBoot (Faust et al. 2012) pour le calcul de la valeur P pour chacune des méthodes statistiques utilisées. De son côté, la méthode SparCC est tout particulièrement pensée pour l'utilisation de données de compositions d'espèces et est basée sur l'analyse de log-ratio d'Aitchison (Aitchison 1982).

Certains taxa microbiens reliés à un grand nombre de taxa ont potentiellement un rôle important au sein du microbiome car ils peuvent peut-être agir sur une large partie du réseau. Certaines espèces « clef de voûte » ont un fort potentiel régulateur sur leur environnement et sur les autres espèces du microbiome (Benedek, Jordán, and Báldi 2007; Vaggi and Csikász-Nagy 2012; Berry and Widder 2014). A contrario, les taxons microbiens qui ne sont pas corrélés avec les autres sont plus à même de ne pas être affectés par les autres microbes du réseau et ont potentiellement moins de chance de développer des interactions microbes-microbes.

Agler et al. 2016 ont développé la notion de taxon nodal au sein d'un réseau microbien. Ce terme désigne les espèces particulièrement interconnectées aux autres. Leurs résultats ont montré que ces espèces étaient souvent extrêmement importantes pour les plantes et jouaient un rôle de médiateur entre la plante et son microbiome. En se focalisant sur les taxons nodaux on peut alors structurer l'information du réseau microbien. On regroupe les espèces du microbiome en cortèges gravitant autour de ces taxa nodaux. Cela permet par exemple de délimiter des ensembles d'organismes qui ont le potentiel pour en recruter d'autres bénéfiques à la plante. De telles espèces peuvent initier des processus de colonisation au sein des racines, organisant la défense de la plante contre les pathogènes. Cependant, les auteurs ont aussi mentionné qu'il était possible que quelques taxa nodaux soient pathogènes et influencent un nombre important d'autres taxa en menaçant les performances de la plante hôte.

Les réseaux d'interaction microbiens pouvant être très importants pour la fertilité des sols, ou pour la suppression des pathogènes, les nœuds microbiens ouvrent de nouvelles

perspectives en matière de compréhension et de gestion du microbiome dans le domaine agricole.

Le microbiome de la rhizosphère en agriculture

Les relations entre la plante et les micro-organismes sont diverses et les communautés microbiennes, complexes. Les microbes des racines sont connus pour leur importance en matière de nutrition des plantes, de leur protection contre les agressions pathogènes ainsi que contre les stress abiotiques mais surtout pour les infections qu'ils causent (Bakker et al. 2014; Fonseca-Garcia et al. 2016). Les relations entre les plantes et les micro-organismes peuvent être très spécifiques. Les micro-organismes fournissent une multitude de services à la plante hôte. Cependant, peu des interactions bénéfiques pour la plante sont réellement étudiées au regard de leur importance dans les systèmes agricoles. Parmi les symbioses mutualistes qui sont connues et qui font l'objet de travaux en agronomie, on inclut la formation des nodules fixateurs de l'azote chez la famille des *Fabaceae* réalisés par une symbiose avec des bactéries des genres *Bradyrhizobium, Mesorhizobium, Sinorhizobium et Azorhizobium* (Rhijn and Vanderleyden 1995; Galibert et al. 2001; Kaneko 2002; Wdowiak-Wrobel et al. 2017). Ces symbioses contribuent au maintien de la fertilité des sols en y injectant de l'azote (Barea et al. 2005; Mougel et al. 2006). De plus, près de 80% des plantes cultivées développent des symbioses racinaires avec les CMA (Hamel and Planchette 2007; Sessitsch and Mitter 2015; Smith and Read 2008). Ces CMA forment une interface intracellulaire et sont particulièrement importants pour la plante. En effet, le réseau d'hyphes des CMA est intégré aux racines dans lesquelles il pénètre et développe aussi une phase extra-racinaire qui peut être très abondante chez certains taxa. Ce système permet aux plantes d'accroitre de manière substantielle leur surface d'absorption ou encore de se protéger des maladies (Tuteja and Singh 2012). La relation entre les plantes de la famille des *Poaceae* avec le champignon endophyte *Epichloë* est aussi relativement bien connue pour sa production d'alcaloïdes repoussant les prédateurs (Clay 1988; Roberts and Ferraro 2015). Les symbioses faisant intervenir les champignons endophytes foncés à septums (Dark Septate Endophytes) ont aussi été relativement bien étudiées. Ces champignons de la famille des *Ascomycètes* colonisent les racines et leur milieu intracellulaire mais leurs associations ne

sont pas toujours bénéfiques pour la plante (A. R. I. Jumpponen and Trappe 1998; A. Jumpponen 2001).

Cependant, l'écrasante majorité des associations microbiennes potentiellement intéressantes pour l'agriculture reste inexplorée. Les pratiques culturales, qu'elles soient biologiques ou conventionnelles, ont une profonde influence sur les micro-organismes du sol (Baudron and Giller 2014). Par exemple, l'amendement du sol en matière organique, notamment par l'ajout de lisier de porc, permet une meilleure disponibilité des nutriments dans le sol pour les micro-organismes (Zhao et al. 2016). Cependant leur surdose conduit à une pollution des ressources limitrophes des agrosystèmes et notamment des nappes phréatiques et rivières entrainant des problèmes d'eutrophisation (Carpenter et al. 1998). La philosophie soutenant l'agriculture biologique, quant à elle, est concentrée sur l'utilisation de la biodiversité microbienne du sol (Thomas and Kevan 1993; Pershina et al. 2015). En agriculture biologique et conventionnelle, des *Fabaceae* sont utilisées de manière générale et importe de l'azote symbiotiquement fixé dans l'agrosystème (Gan et al. 2015). Le microbiome racinaire suscite beaucoup d'intérêt en agriculture. En effet, de nombreuses fermes se sont tournées vers l'utilisation d'inoculants afin de restreindre la quantité de produits chimiques épandus dans les champs. Cependant, les résultats prometteurs que les inoculants mycorhiziens ont montrés lors des essais en conditions contrôlées restent relativement imprévisible en champs mais peuvent malgré tout être positifs de façon générale (Sessitsch and Mitter 2015; Hijri 2016).

L'agriculture actuelle n'est généralement pas durable. La gestion du microbiome rhizosphérique peut contribuer à améliorer l'efficacité et la durabilité de sa production. En conséquence, il est urgent d'améliorer notre compréhension du microbiome racinaire et de ses interactions. En effet, la connaissance que nous avons des principaux mécanismes intervenant dans les relations plantes-microbes provient surtout de l'étude d'organismes modèles cultivés en conditions contrôlées (Smith, Handelsman, and Goodman 1999; Bulgarelli et al. 2012). Actuellement, la communauté scientifique a réalisé l'importance d'étudier les cultivars et leur influence sur les communautés microbiennes de la rhizosphère. Le but étant de découvrir des moyens de contrôler le microbiome des racines pour pouvoir sélectionner des communautés bénéfiques et agir sur la colonisation racinaire en augmentant l'efficacité de la production agricole (Siciliano et al. 1998; de Almeida Lopes et al. 2016; Perez-Jaramillo, Mendes, and Raaijmakers 2016). Cette utilisation des communautés microbiennes du sol, en complément de

l'amélioration des technologies agricole pourrait grandement changer la manière de faire de l'agriculture dans les prochaines décennies, afin que que les méthodes de productions soient compatibles avec une durabilité écologique des systèmes agricoles. Maintenir des fonctions microbiennes favorables aux plantes dans les champs est particulièrement important pour optimiser l'efficacité de production et augmenter la résistance des cultures en cette ère de changement climatique (sécheresse, humidité excessive ou infestation de pathogènes). De plus, agir sur le microbiome à des fins de production agricole n'est pas uniquement profitable aux agricultures mécanisées des pays développés. Le potentiel de la gestion du microbiome racinaire en agriculture vivrière pourrait amener vers une stabilisation de la production des pays en voie de développement avec un moindre coût pour les agriculteurs. La gestion des symbioses impliquées dans la nutrition des cultures est particulièrement importante pour la culture vivrière encore très répandue dans les pays en voie de développement.

Objectifs et Hypothèses du projet de recherche

Mon projet a pour objectif premier d'approfondir la connaissance des interactions entre plantes et champignons dans la rhizosphère dans l'optique de rendre la production agricole durable à long terme. La surface des sols agricoles représente près de 40% de la surface de la planète représentant ainsi la plus grande interface d'échange entre les activités humaines et l'atmosphère (F.A.O. 2015).

L'activité microbienne des sols agricoles est un facteur d'émission de gaz à effet de serre, notamment par le fait d'un apport substantiel en fertilisants pour améliorer la productivité des cultures, ce qui rend nécessaire la diminution de l'apport en fertilisants tout en maintenant des rendements similaires (Mosier et al. 1998). Il faut savoir que les champignons présents dans la rhizosphère d'une plante sont le pivot de sa croissance et de son développement (Hamel and Planchette 2007; Smith and Read 2008). Certains lui apportent une protection contre les stress et les pathogènes ou encore facilitent sa nutrition, augmentant son potentiel productif (Smith, Handelsman, and Goodman 1999; Ridout and Newcombe 2016). Ces organismes interagissent les uns avec les autres et tissent un réseau complexe d'interactions.

Au Canada, l'une des plus importantes productions agricoles du pays est celle de la culture du canola (*B. napus, B.juncea, B. rapa*). Mon deuxième objectif est de permettre d'ouvrir des pistes de recherches sur des organismes potentiellement bénéfiques au développement du canola. De plus, la compréhension des mécanismes régissant le microbiome fongique racinaire est importante pour l'amélioration de sa production en termes d'impacts environnementaux. La culture du canola au Canada se passe majoritairement dans les grandes plaines au Saskatchewan au Manitoba et en Alberta. Le canola y est cultivé en rotations de cultures afin d'éviter les pertes de rendement causées par l'accumulation des pathogènes dans le sol. Cependant si l'efficacité de ces rotations est avérée sur le maintien de la production agricole et sur la dilution de la population des pathogènes, peu de choses sont connues à propos de l'influence de ces rotations sur le microbiome fongique de la rhizosphère du canola (Guo, Fernando, and Entz 2005; Hilton et al. 2013; Harker et al. 2015). Nous avons alors émis l'hypothèse que la diversification de couverts végétaux successifs en champ influe sur le microbiome fongique racinaire du canola (en particulier sur sa diversité et sa structure) conduisant à une augmentation de la diversité microbienne liée à la diversité des rotations. Au sein du microbiome d'une plante, il existe en théorie un ensemble de taxa microbiens toujours présents en interaction avec la plante : le core microbiome (Vandenkoornhuyse et al. 2015). Afin d'aboutir à une meilleure compréhension de organismes clefs de la rhizosphère du canola des grandes prairies canadiennes, nous avons émis le postulat qu'il existe bien un core microbiome fongique et que ce dernier ne soit pas affecté par les diversifications de rotations. De plus au sein de cet ensemble de taxa nous avons émis l'hypothèse qu'il existe des taxa à fort effet structurant (nodaux) au sein de ce core microbiome.

Pour tester ces hypothèses, treize systèmes de rotations de cultures furent implantés en 2008 sur quatre fermes expérimentales d'Agriculture et Agroalimentaire Canada, i.e., Lacombe et Lethbridge en Alberta, et Scott en Saskatchewan. Nous avons échantillonné en 2013 et en 2016 la phase canola de six de ces rotations : les monocultures de génotypes de canola résistants à l'herbicide Liberty (LL) et à l'herbicide Roundup (RR), les rotations blé-LL et blé-RR, et les systèmes diversifiés pois-orge–LL et pois–orge–RR. Le sol de la rhizosphère fut récolté et son ADN extrait. Les amplicons de l'ITS des champignons du sol furent barcodés et séquencés par MiSeq, Illumina. Les séquences rapportées furent comptées et classifiées en unités taxonomiques opérationnelles (OTU) par le biais du pipeline USEARCH, puis ces dernières ont

été identifiées. Afin de savoir si les rotations de cultures influençaient la structure des communautés microbienne de la rhizosphère, nous avons utilisé une analyse de PERMANOVA sous R 3.3.3. Les relations entre les OTU du core microbiome ont été déterminées sur la base du calcul de réseaux de cooccurrence et par la méthode de groupement d'espèces développée par Legendre (2005).

Microbiome of Canola Root: Structure and Variations

INTRODUCTION

Understanding the microbiome of plants and more particularly the microbiome of their rhizosphere, involves linking microbial ecology to the functioning of the host plant. The roots are an interface of exchange between plants and microorganisms leading to the formation of symbiosis, particularly with fungi (Garbaye 1994; van der Heijden et al. 2006). Plant roots host symbiotic fungi facilitating nutrient uptake, preventing root infection by pathogens, mitigating the impact of abiotic stress, and modulating the levels of plant hormones (Mozafar et al. 2000; Guo, Fernando, and Entz 2005; Harker et al. 2015; Latz et al. 2016). Microorganisms are considered as a pool of genes and functions that are profitable or not, to the plant. Taken one by one, the contributions of microorganisms to the host plant may appear negligible at first sight. However, the ecological functions supported by the root microbiome of a plant are highly beneficial. They allow the plant to considerably increase the exploitation of its environment (Bulgarelli et al. 2013). Understanding how the different kinds of microbe-plant interactions behave within their microbiomes is a very important topic in ecology.

Little is known about the functioning of the plant root ecosystem. The use of next-generation sequencing has opened a new window of understanding about the ecology of the plant root microbiome. This microbiome is shaped with diverse microbial communities forming a complex web of interactions and can be divided into subunits (Ridout and Newcombe 2016; van der Heijden et al. 2016). All microorganisms found in in the rhizosphere are considered as

the pan-microbiome, as defined by Vandenkoornhuyse et al., (2015). This microbial set, which is subservient to the root system, has characteristics that can vary according to the conditions of the environment. However, it is possible that groups of taxa are present regardless of the abiotic and biotic conditions of the environment and are probably favored by the plant throughout its existence (Rout, 2014), it is the core microbiome.

In this group of microorganisms, some of them could play a key role in the functioning of the ecosystem and for the plant; interacting with a broad spectrum of partners and antagonists. To name that kind of microorganisms, Agler et al. 2016 developed the concept of hub taxa within a microbial ecosystem. This term refers to species that are particularly interconnected to others. Their results showed that these species are often extremely important for plants, and make the connection between the plant and its microbiome. By focusing on hub taxa, information from the microbial network can be structured. The microbiome species can be grouped into communities gravitating around these hub taxa. This allows, for example, to delimit sets of organisms that have the potential to recruit taxa beneficial to the plant (van der Heijden and Hartmann 2016). Such taxa can trigger plant's defense against pathogens. Some hub taxa can also be pathogenic and influence many other taxa by threatening the performance of the host plant (Agler et al. 2016). This study of hub taxa is a way to understand the ecology of the root ecosystem and lead to applications in agriculture. However, the fact that the rhizosphere is an open exchange interface subject to wide variations linked with the weather makes it difficult to observe the core microbiome. With the new generation of sequencing technologies available, it is now possible through amplicon sequencing to access this core microbiome and characterize it at different times to see its variations.

In the present research, we wanted to determine if there is a universal core microbiome in the rhizosphere of a given plant. To do this, we used *B. napus* because we had the opportunity to investigate the rhizosphere of this plant that represents a large part of Canada's agricultural production. Canola (*B. napus*) is one of the most important crop for the Canadian agroindustry so identifying the key microbes that likely impact its growth is crucial to optimize the management strategies for improving canola yield and health. Canola is also a simplified system. The *Brassicae* are known to release isocyanates in the soil that inhibits the growth of fungi leading to simpler microbial communities (Rumberger and Marschner 2003).

To reach this goal, we identified the core fungi of the rhizosphere microbiome in the Canadian prairies. Then, the effect of biotic or abiotic factors on the core microbiome structure in this environment were determined. Finally, we identified the hub taxa within the microbiome and their potential effect on the yield of canola. For this study, we used a long-term canola-based field experiment setup at three locations with contrasting edaphoclimatic conditions across western Canada (Scott, SK; Lacombe, AB; Lethbridge, AB) and different level of plant diversity. Canola rhizosphere soil was sampled in the summer of 2013 and 2016. To our knowledge, it is the first study of the core microbiome of *Brassica napus* using network analysis to define hub taxa. This study provides new insights into the effect of the environment on canola root associated fungal microbiome.

MATERIALS AND METHODS

Description of field experiments

The present study was conducted using a long-term field experiment testing the influence of canola (*Brassica napus* L.) frequency in crop rotation systems. The experiment was established in 2008 in three pedoclimatic zones of the canola-producing area of western Canada. Two sites were in Alberta at Lacombe (lat. 52.5°N, long. 113.7°W) and Lethbridge (lat. 49.7°N, long. 112.8°W) and the other at Scott, in Saskatchewan (lat. 52.4°N, long 108.8°W). At each site, 13 combinations of two factors, canola genotype and crop diversifications were randomized in four complete blocks. Information of site management and specificities were detailed in a previous paper (Harker et al. 2015). In 2013, we considered three levels crop diversification: (1) monoculture of canola, (2) wheat-canola and (3) pea-barley-canola. These rotation systems included either the canola genotypes Roundup Ready® (CRR) or Liberty Link® (CLL) for a total of six treatments considered (Table 1). In 2016, we used an additional highly diversified crop rotation system composed of lentils, wheat, canola LL, pea, barley, and CRR.

Sampling

Rhizosphere samples were collected during the fourth week of July in 2013 and 2016, corresponding to the end of canola bloom. Three to four plants from each of four randomly selected locations within each plot were uprooted with a shovel. The shoots were removed and roots were placed in plastic bags and brought to the laboratory in a cooler on ice at 4°C. About 5g of rhizosphere soil per plots were taken by gently brushing the roots. The samples were kept at 4°C before they were shipped to Swift Current, Saskatchewan, preserved at -80°C until DNA extraction.

DNA extraction and amplification

Total microbial DNA was extracted from rhizosphere soil using the PowerSoil DNA isolation kit (Mo Bio Laboratories, Calsbad, CA, USA) following the manufacturer's recommendations. DNA was eluted in 10 µL tris-HCL buffer and quantified using a Qbit 2.0 spectrophotometer (Invitrogen, Life Technologies, Carlsbad, CA, USA). We constructed amplicon libraries for fungal ITS sequences by using target-specific PCR primers attached to Illumina overhang sequences for Nextera preparation. The primer pairs were ACACTGACGACATGGTTCTACACTTG GTCATTTAGAGGAAGTAA (ITS1F-Illu) and TACGGTAGCAGAGACTTGGTCTCTG CGTTCTTCATCGAT (5.8A2R-Illu). Each 25 µL PCR reaction consisted of 0.10 µL of forward and reverse primers, respectively, with 19.6 µL H_2O, 2.5 µL 25 mM $MgCl_2$, 12.5 µL KAPA HiFi Hotstart ReadyMix (Kapa Biosystem, Cape Town, South Africa) and 1 µL of sample DNA. The reaction conditions were as follow: 95°C for 5 mins, 25 cycles of 94°C for 45 secs, 52°C for 60 secs, and 72°C for 30 secs with a final extension at 72°C for 7 min. PCR products were verified by electrophoresis on 1% agarose gels. Dual Nextera indices were then attached to PCR products based on the suggested protocol "16S Metagenomic Sequencing Library Preparation" provided by Illumina. The final purified product was quantified by Qubit Fluorometric Quantitation (Thermofisher Scientific). Libraries were pooled in equimolar ratios before sequencing with Miseq PE250.

Table 1. Nine of the 13 crop rotations treatments established in the pedoclimatic zones of the Canadian prairies. The phases of rotations used in this study appear in bold font.

Diversification level	2008	2009	2010	2011	2012	2013	2014	2015	2016
Monoculture	CLL †	CLL	CLL	CLL	CLL	**CLL**	CLL	CLL	**CLL**
	CRR	CRR	CRR	CRR	CRR	**CRR**	CRR	CRR	**CRR**
Low	W	CLL	W	CLL	W	**CLL**	W	CLL	W
	CLL	W	CLL	W	CLL	W	CLL	W	**CLL**
	W	CRR	W	CRR	W	**CRR**	W	CRR	W
	CRR	W	CRR	W	CRR	W	CRR	W	**CRR**
Medium	P	B	CLL	P	B	**CLL**	P	B	**CLL**
	B	CLL	P	B	CLL	P	B	CLL	P
	P	B	CRR	P	B	**CRR**	P	B	**CRR**
	B	CRR	P	B	CRR	P	B	CRR	P
High	Len	W	CLL	P	B	L	W	P	**CRR**

† CLL: Canola Liberty Link, CRR: Canola Roundup Ready, W: Wheat, P: Pea, B: Barley

OTU formation and bioinformatic pipeline

The preliminary processing of fungal ITS DNA gene libraries was performed using USEARCH v10 (Edgard, 2010) to join paired ends with the function "fastq_mergepairs". Cutadapt 1.13 was used to remove the primer part of the ITS sequences. Then we selected the sequences with more than 300 bp and less than 250 bp corresponding to the standard ITS length (Dorn-In et al. 2013). The sequences that did not reach the maxee quality filter (1.0) of USEARCH10 were removed with the command "fasq_filter". We removed the singletons using the command "fastx_uniques" of the USEARCH10 pipeline. To form the OTU we used the function "unoise3" of the pipeline and we choose a threshold of 1.0 for the sequence identity necessary to allocate a read to an OTU.

Data processing and statistical analyses

The α-diversity indices Chao1, Shannon and Simpson's, sere computed using the package vegan 2.4.6 of the software R 3.4.3. The effect of crop diversification on the indices was tested by analysis of variance (ANOVA) with Tukey's post-hoc analyses to group the treatments with the software JMP 13.2.1. The model was: block [random] + canola genotype [nested in block] + crop diversification [nested in block] for each year. The effect of crop diversification on fungal community structures was assessed at each site by permutational multivariate analysis of variance (PERMANOVA) using the "adonis" function of the "vegan" package in R. The model was tested site by site: block + canola genotype [block] + crop diversity [block]. The comparison of fungal communities was conducted with blocked multi-response permutation procedures (blocked MRPP) using the software PC-ORD6 and the p-values obtained were corrected using Šidák correction for two-way comparisons. The correction was calculated using $1 - (1 - \alpha)^{1/m}$, where α is 0.05, and m is the number of paired comparisons. To assess the clustering of samples in each group of comparisons, principal coordinates analysis (PCoA) plots were drawn using PC-ORD6.

To identify potentially important fungi in our communities, we used the concept of core microbiome as defined by Vandenkoornhuyse et al. (2015) to select core OTU. The OTUs that were present in every sample and every condition for each sampling year and site were considered as members of the core microbiome of canola rhizosphere.

To assess the biotic interactions of the fungi of the microbiome and create the network, the software Cytoscape 3.5.0 and its plugin CoNet was used (Faust and Raes 2016). The input data consisted of the matrix of the abundance of the OTU present in at least 50% of our samples to prevent false positive correlations. Spearman's and Pearson's correlations were included in the CoNet analysis with a threshold of $\alpha = 0.01$, with Bray-Curtiss and Kullback-Leibler dissimilarity. The bootstrapping was done with 1000 iterations with edgescore routine and we applied a filter to discard unstable edges with the Benjamin Hochberg multiple test correction with $\alpha = 0.05$. We then used the "organic" layout of Cytoscape to shape the network.

RESULTS

Taxonomic affiliation of the fungal microbiome of the canola rhizosphere

After merging the reads from the ITS data sets of summer 2013 and summer 2016, and filtering for quality, we retrieved from our 156 samples a total of 7,964,220 reads distributed within 2,156 OTUs. Read number per sample ranged from 8,805 to 112,115 reads. The dataset was composed mostly of four phyla: *Ascomycota* (57%), *Olpidiomycota* (21%), *Mortierellomycota* (14%) and *Basidiomycota* (6%) (Figure S1). Rarefaction curves indicated that read abundances were close to saturation for all the samples (Figure S2).

Effect of crop rotations on the alpha and beta diversity

The fungal diversity in 2016 was lower than the 2013 fungal diversity (Table S1). For both years, the level of crop diversification had no significant influence on the α-diversity of fungi (Table 2). The Tukey post-hoc tests showed no significant differences between the crop rotations.

The structure of the fungal communities was significantly influenced by the crop diversification (Table 3). PCoA projections (Figure 1) and the taxonomic profile of the fungal communities (Figure 2) reveal differences of due to the years and sites on the structure between the fungal community of canola rhizosphere. The most abundant families in the community across all sites were the *Olpidiaceae, Nectriaceae, Mortierellaceae,* and the *Chaetomiaceae*. The relative abundance of these families varied between 2013 and 2016. There was an increase in the abundance of the *Olpidiaceae* (near 70% of abundance in Lethbridge) from 2013 to 2016. Diversification of the rotation system influenced the fungal community of canola rhizosphere at all sites in both years, except at Scott in 2013 (Table 3). However, the genotype of canola did not appear to be determinant for the structure of microbial community in the rhizosphere. The genotype of canola was only significantly influent in interaction with the crop diversification on the microbial communities in 2013 at Lethbridge. Yet, this effect was not detected by blocked MRPP analysis (Table 4). If the effect of crop diversification is strong on the microbial communities, the differences between the levels of diversification in the rotation systems remains unclear: it changed between the years, sites and even between the canola cultivars (Table 4). It appears that the microbials communities of the monocultures in 2016 tends to be different to the communities of the other diversifications (Figure 1). The differences between the other levels of crop diversification remained variable depending the locality.

Some fungal OTU were consistently more abundant in certain cropping systems than in others, as per indicator species analysis (Table S3). These indicator species changes with time (Table S4).

Table 2. Probability (*P* value) of effects of canola type (canola), in 2013, and of cropping system diversification level (diversity), in 2013 and 2016, on the α-diversity of the fungal community residing in canola rhizosphere soil, as determined by ANOVA. Effects significant at α = 0.05 are indicated in bold.

A- Effect of the crop rotation on the fungal alpha diversity in 2013

Index	Genotype		Crop diversification		Genotype x Crop diversification	
	F	***P***	***F***	***P***	***F***	***P***
Chao	0.2634	0.9000	0.8852	0.5358	1.0425	0.4182
Richness	0.0301	0.9982	0.7140	0.6779	0.5908	0.7805
Simpson D	0.7237	0.5801	0.3579	0.9374	0.6337	0.7455
Shannon	0.4604	0.7643	0.3234	0.9531	0.5584	0.8062

B- Effect of the crop rotation on the fungal alpha diversity in 2016

Index	Genotype		Crop diversification		Genotype x Crop diversification	
	F	***P***	***F***	***P***	***F***	***P***
Chao1	0.0258	0.8728	2.2604	0.1368	0.0417	0.8386
Richness	0.4594	0.5000	2.0936	0.1520	0.9817	0.3249
Simpson D	0.0002	0.9881	3.7672	0.0559	0.4570	0.5010
Shannon	0.0517	0.8207	2.3493	0.1294	0.0945	0.7594

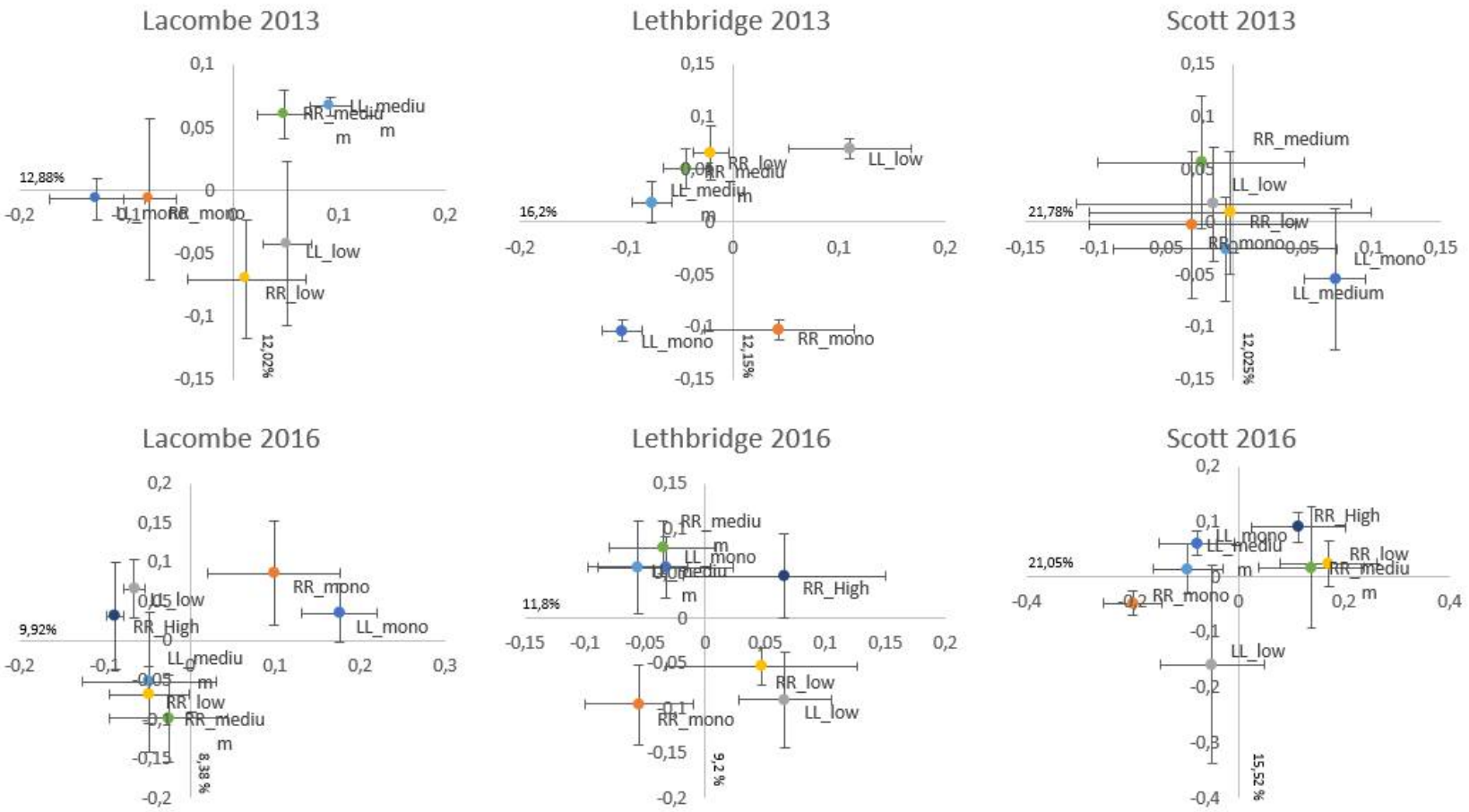

Figure 1. PCoA ordination of fungal communities found in the rhizosphere soil of canola grown at the three different experimental sites in 2013 and 2016. Monoculture: rotation with only canola, low: rotation wheat and canola, medium: rotation pea- barley-canola, high: rotation lentil-wheat-canola LL-pea-barley-canola RR.

Core microbiome and its variation between years, canola genotypes and crop rotations

Different fungal assemblages made the core of the microbiome of canola rhizosphere in 2013 and 2016. In 2013, the core microbiome of canola rhizosphere included 47 OTUs (Table 5). However, in 2016, the core microbiome was only composed of one OTU (OTU1, *Olpidium brassicae*). The relative abundance of the core OTU varied from 523 to 330460 reads per sample in 2013. Among the core in 2013, 22 OTU are taxa known as potential pathogens, 16 as potential saprophytes and 3 as potential endophytes, while the functional guild of the five remaining taxa is unknown. The diversification level of rotation systems affected the abundance of 3 core microbiome OTUs known as OTU27 (*Fusarium solani)*, OTU51 (*Dendryphion nanum),* and OTU63 (*Thielavia sp*.) (Table 5). It also appears that *F. solani* is significantly influenced by the genotype of canola. Yet, this effect was not detected by Tukey HSD. *D. nanum* is more abundant in the monoculture of canola than the other levels of crop diversification, and for *Thielavia sp*., no differences in its abundance was detected between the crop rotations.

Table 3. Effects of canola genotype, in 2013, and rotation crop diversification (crop diversification), in 2013 and 2016, on the structure of the fungal community in canola rhizospheric soil at each experimental site, according to PERMANOVA. Significant effects at $\alpha = 0.05$ are indicated in bold.

		2013		*2016*	
Site	**Source**	**DF**	***P***	**DF**	***P***
Lacombe	Canola genotype	1	0.821	1	0.628
	Crop diversification	2	**0.001**	3	**0.020**
	Canola genotype x Crop diversification	2	0.925	2	0.098
	Residuals	18	-	21	-
Lethbridge	Canola genotype	1	0.498	1	0.422
	Crop diversification	2	**0.005**	3	0.111
	Canola genotype x Crop diversification	2	**0.017**	2	0.777
	Residuals	18	-	21	-
Scott	Canola genotype	1	0.695	1	0.639
	Crop diversification	2	0.837	3	**0.001**
	Canola genotype x Crop diversification	2	0.716	2	0.240
	Residuals	18	-	21	-

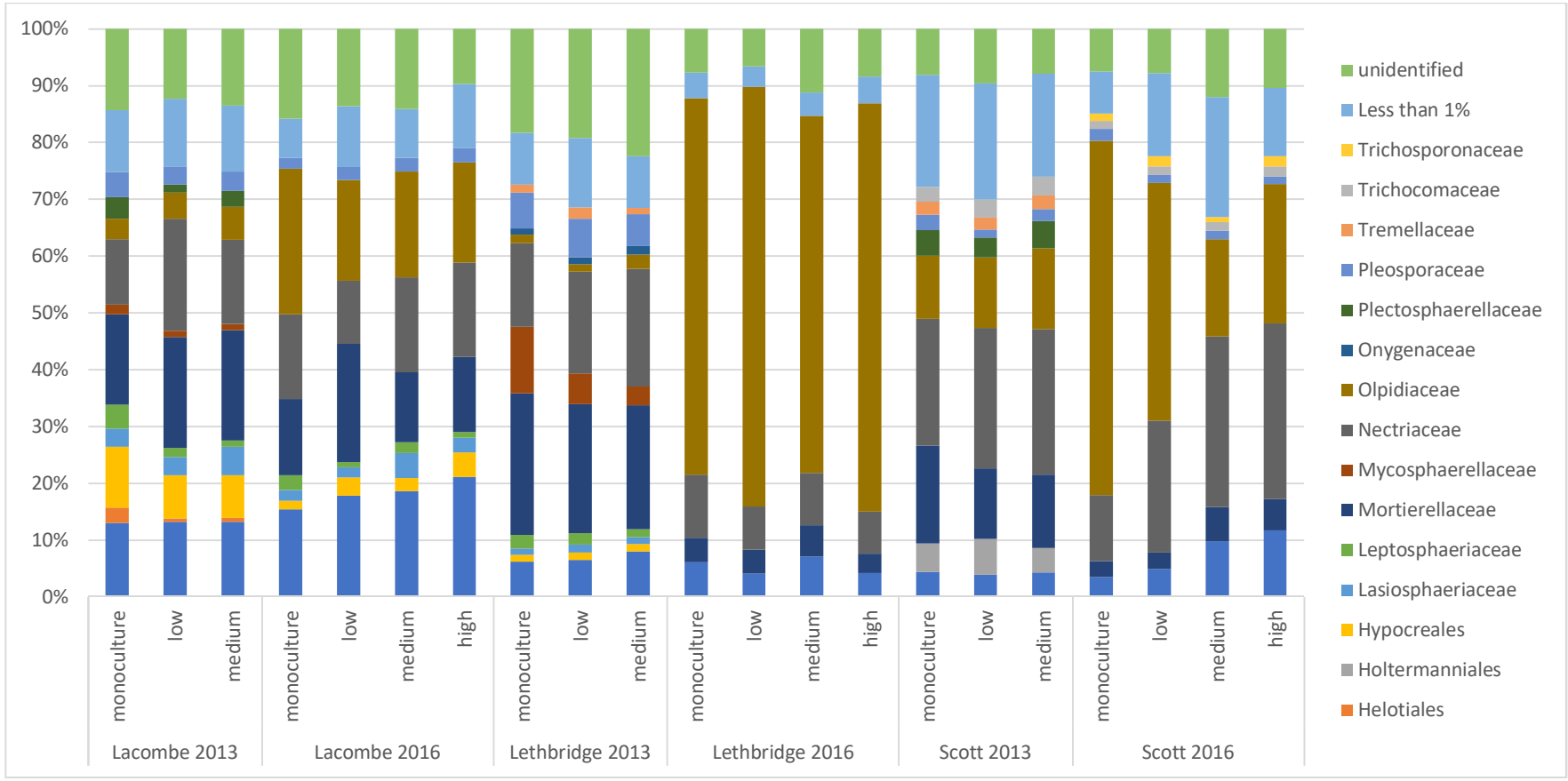

Figure 2. Taxonomic profile of the community of dominant fungal families found in the rhizosphere of canola as influenced by crop rotations, site and year (n = 4).

Table 4. Comparison of the compositions of the fungal communities of canola rhizosphere as influenced by canola type (canola) and cropping system diversification level (diversity), in 2013 and 2016 at each experimental site, as determined by Blocked Multi-Response Permutation Procedures (BMRPP) (n = 4, α = 0.035 as per Šidák correction for two-way comparisons).

Site	Diversification	Canola type	2013	2016
Lacombe	Monoculture ǂ	LL†	a §	a
	Monoculture	RR	a	a
	Low	LL	b	b
	Low	RR	b	b
	Medium	LL	c	b
	Medium	RR	c	b
	High	RR	- ¥	b
Lethbridge	Monoculture	LL	a	a
	Monoculture	RR	a	b
	Low	LL	c	b
	Low	RR	b	b
	Medium	LL	b	a
	Medium	RR	b	a
	High	RR	-	a
Scott	Monoculture	LL	a	a
	Monoculture	RR	a	a
	Low	LL	a	a
	Low	RR	a	ab
	Medium	LL	a	b
	Medium	RR	a	ab
	High	RR	-	b

† LL, canola liberty link; RR, canola Roundup Ready.

ǂ Monoculture, canola monoculture; Low, wheat-canola rotation; Medium, pea-barley-canola rotation; High, lentil-wheat-canola Liberty link-pea-barley-canola Roundup Ready.

§ Crop rotation associated with the same letter in a column and site are not different.

¥ Treatment absent in 2013.

Table 5. Significance levels of the effect of site, diversification of the crop rotation system and canola type on the core microbiome of canola in 2013, as determined by ANOVA. Significant effects greater than or equal to P<0.05 are indicated in bold.

OTU	Identity	Guild	Hub taxa	genotype	crop-rotation	genotype x crop-rotation	OTU	Identity	Guild	Hub taxa	genotype	crop-rotation	genotype x crop-rotation
OTU1	*Olpidium brassicae*	parasite	N	0,9886	0,9654	0,9079	OTU35	*Fusarium solani*	parasite	N	0,8426	0,88	0,9107
OTU10	*Plectosphaerella sp.*	parasite	N	0,5236	0,7643	0,9893	OTU387	*Candida parapsilosis*	saprophyte	N	0,8796	0,0947	0,2852
OTU102	*Trichoderma koningii*	endophyte	N	0,7485	0,8317	0,9779	OTU39	*Cylindrocarpon sp.*	parasite	N	0,9971	0,9079	0,9522
OTU11	*Fusarium sp.*	parasite	N	0,9571	0,2103	0,9855	OTU4	*Fusicolla sp.*	parasite	N	0,9999	0,9145	0,9967
OTU123	*Leptosphaeria sp.*	parasite	N	0,5964	0,1407	0,7165	OTU40	*Cylindrocarpon sp.*	parasite	N	0,9807	0,9892	0,9997
OTU13	*Fusarium avenaceum*	parasite	N	0,6406	0,0924	0,7228	OTU41	*Tetracladium sp.*	saprophyte	N	0,9532	0,7281	0,3717
OTU15	*Fusarium sp.*	parasite	N	0,9531	0,9855	0,9921	OTU42	*Alternaria infectoria*	parasite	N	0,9514	0,6336	0,9009
OTU154	*Mortierella sp.*	saprophyte	Y	0,9943	0,8241	0,9938	OTU49	*Clonostachys rosea*	endophyte	N	0,8791	0,732	0,686
OTU16	*Geomyces sp.*	saprophyte	N	0,4409	0,9466	0,9605	OTU5	*Nectria sp.*	parasite	Y	0,9934	0,9934	0,9999
OTU17	*Mortierella gamsii*	saprophyte	N	0,5589	0,8354	0,9342	OTU51	*Dendryphion nanum*	parasite	N	0,305	**0,004**	0,8249
OTU18	*Fusarium sp.*	parasite	N	0,9622	0,997	0,9996	OTU54	*Cladosporium tenuissimum*	saprophyte	N	0,9592	0,3295	0,416
OTU19	*Fusarium redolens*	parasite	N	0,7494	0,9021	0,9987	OTU56	*Exophiala sp.*	endophyte	N	0,9052	0,9765	1
OTU2	*Humicola grisea*	saprophyte	N	0,8602	0,9382	0,9343	OTU6	*Mortierella sp.*	saprophyte	Y	0,988	0,9932	0,9904
OTU21	*Fusarium sp.*	parasite	N	0,7295	0,9423	0,7214	OTU65	*Coniothyrium cereale*	undefined	N	0,9195	0,3052	0,4889
OTU22	*Mortierella sp.*	saprophyte	N	0,9759	0,9735	0,9965	OTU68	*Vishniacozyma victoriae*	undefined	N	0,9381	0,9725	0,9477
OTU229	*Aspergillus welwitschiae*	undefined	N	0,7349	0,8231	0,8132	OTU7	*Mortierella alpina*	saprophyte	N	0,9483	0,7525	0,9543
OTU24	*Alternaria alternata*	parasite	N	0,9943	0,7458	0,8596	OTU71	*Cladosporium sp.*	saprophyte	N	0,4908	0,3778	0,6439
OTU25	*Plectosphaerella sp.*	parasite	N	0,5265	0,0652	0,7133	OTU78	*unknown*	undefined	N	0,973	0,2826	0,9821
OTU26	*Solicoccozyma aeria*	sapophyte	N	0,9785	0,9581	0,9279	OTU8	*Phoma sclerotioides*	parasite	N	0,9972	0,9944	0,9997
OTU27	*Fusarium solani*	parasite	N	**0,0219**	**0,0067**	0,7657	OTU84	*Nectria sp.*	parasite	N	0,9641	0,9906	0,9941
OTU29	*Ulocladium dauci*	saprophyte	Y	0,8606	0,8468	0,9974	OTU864	*Trichocladium opacum*	saprophyte	N	0,9073	0,3466	0,2532
OTU3	*Mortierella sp.*	saprophyte	N	0,9005	0,7355	0,3246	OTU87	*Penicillium sp.*	saprophyte	N	0,502	0,6842	0,6647
OTU30	*Fusarium sp.*	parasite	N	0,971	0,0704	0,9993	OTU9	*Chaetomium globosum*	saprophyte	N	0,9245	0,9787	0,8676
-	-	-	-	-	-	-	OUT63	*Thielavia sp.*	undefined	N	0,5065	**0,0002**	0,0577

† $\alpha = 0.035$ as per Šidák correction for two-way comparisons

The details of the Relative abundancy differences between crop-rotation per core microbiome OTU are in Tables S5 S6 and S7

Interactions among the fungal microbiome and identification of hub taxa

In 2013, 119 correlations significant at $\alpha = 0.01$ with a $R \geq |0.5|$ were used to build a network of interactions between OTUs of the core microbiome (Figure 3). The network was composed of 97 copresences and 22 mutual exclusions between fungal taxa. It was composed of 48 OTUs, 15 of them were core microbiome OTUs: OTU15, OTU154, OTU2, OTU21, OTU22, OTU26, OTU29, OTU4, OTU40, OTU42, OTU5, OTU56, OTU6, OTU8 and OTU9 (for identifications, c.f. Table 5). The obtained network is modular: it is formed of one interaction group centered on OTU85 (*Preussia funiculata*), one other with lesser connections is centered on OTU298 (Unknown *Ascomycota*). The link between these two sub-networks is ensured by OTU5 (*Nectria sp.*) and OTU8 (*Nectria sp.*). Three other little sub-networks disconnected from the main structure are respectively centered on OTU165 (*Acremonium sp.*), OTU217 (*Penicillium janczewskii*) and the last one only composed of two OTU: OTU2 (*Trichocladium asperum*) and OTU40 (*Cylindrocarpon sp.*). Surprisingly, OTU1 (*Olpidium brassicae*) which ranked first for abundance in the microbiome had no connections with others OTU. Inside this correlative network, we identified six hub-taxa, i.e. six OTUs granted with more than 10 connections to the other members of the network. The most connected one was OTU85 (*Preussia funiculata*) with 17 connections. The second is OTU12 (Unknown *Ascomycota*) with 14 connections. Then, with the same number of connection than OTU12: OTU199 (Schizothecium sp.) and OTU6 (Mortierella sp.). The last ones were OTU298 (Unknown *Ascomycota*) and OTU5 (*Nectria sp.*) with 11 connections. Some OTU were particularly remarkable as they gathered high number of mutual exclusions: OTU56 (*Exophiala* sp*.,* 6 mutual exclusions), OTU5 (*Nectria sp.,* 4 mutual exclusions) and OTU8 (*Nectria sp.,* 4 mutual exclusions). OTU56 have a particularity: 7 over 8 of its links are mutual exclusions and 6 of these links are targeted on 4 different hub taxa: OTU85, OTU12, OTU6 and OTU199. No significant correlations matching our threshold were found in the fungal microbiome in 2016.

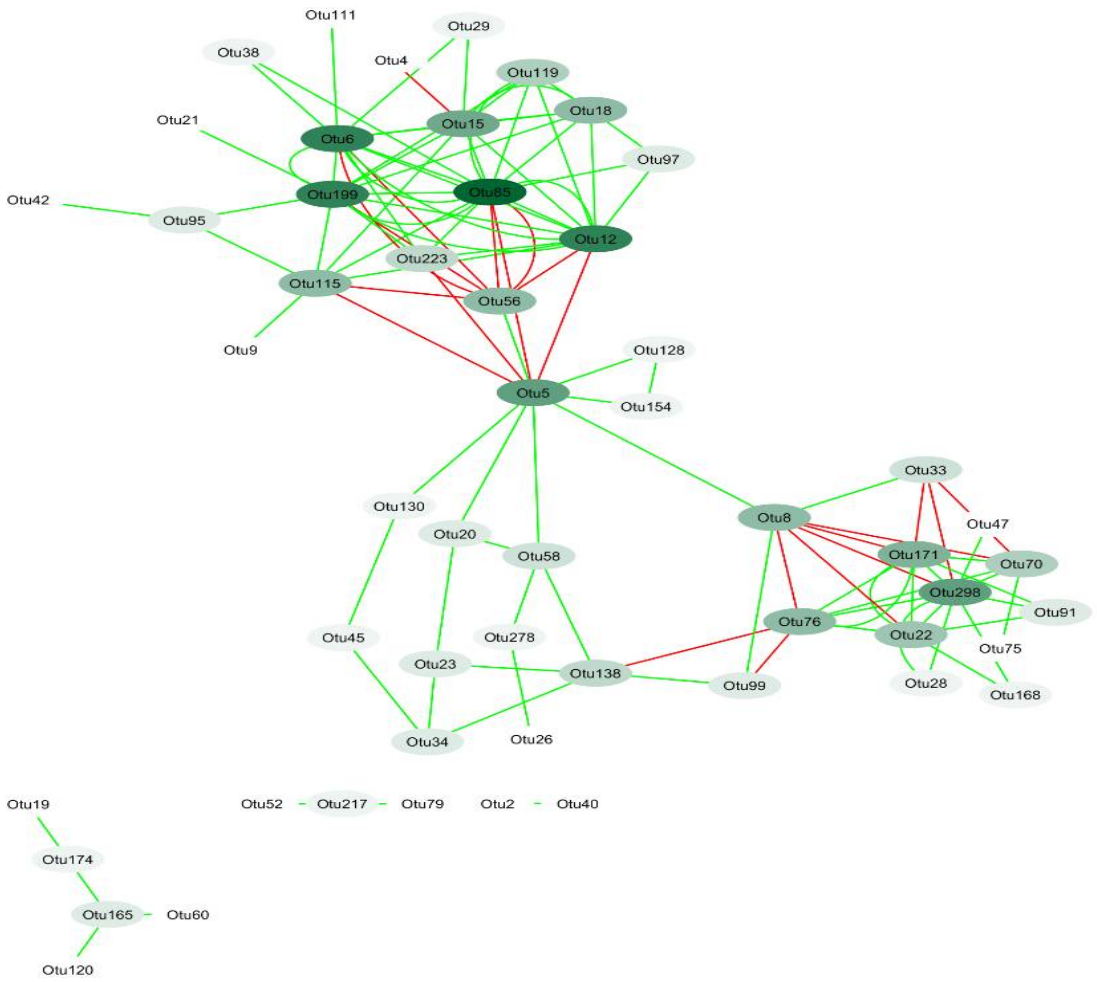

Figure 3. Network of interactions in the fungi forming the core microbiome of canola. Only the Spearman's correlations with $R > 0.50$ are shown. OTU size is proportional to the relative abundance of the taxa, and OTU shade indicates the frequency of correlations with other members of the network, darker OTU being more interrelated than light colored ones.

Correlation between Hub taxa, most abundant OTU and canola yield

We used Spearman's correlations to assess the relationships between the most abundant OTU (OTU1) of the fungal microbiome, the hub taxa and OTU56 on one hand, and canola yield in 2013 and 2016 on the other hand (Table 6). In 2013, Spearman's correlations have shown that the correlations between the yield and the hub-taxa could be variable in function of the locality. We could identify three taxa positively correlated with the yield: OTU56 (*Exophiala* sp.*)*, OTU5 (*Nectria sp.*) and OTU298 (Unknown *Ascomycota*). For the others hub taxa, the R values were more variables. The overall correlation values shown negative values but the intra-locality correlation shown positive and negatives values. In 2016, the core microbiome was only composed of one OTU: OTU1 (*Olpidium Brassicae*). This OTU was strongly negatively related with canola yield in Scott ($R = 0.69$) and overall ($R = 0.76$).

Table 6. Spearman's Correlations coefficients (*R*) of the correlation between canola yield and the relative abundancy the hub taxa plus OTU56 and OTU1, per site and year, $\alpha = 0.05$.

		OTU1	OTU56	OTU5 ǂ	OTU85 ǂ	OTU12 ǂ	OTU199 ǂ	OTU6 ǂ	OTU298 ǂ
2013	All sites	ns	0.390	0.450	-0.422†	-0.498	-0.414	-0.391	0.216
	Lacombe	ns	0.230	ns	0.336	0.390	0.531	ns	0.353
	Lethbridge	ns	ns	0.232	ns	ns	0.228	ns	ns
	Scott	ns	0.232	ns	ns	-0.307	ns	ns	ns
2016	All sites	-0.76	Na	Na	Na	Na	Na	Na	Na
	Lacombe	ns	Na	Na	Na	Na	Na	Na	Na
	Lethbridge	ns	Na	Na	Na	Na	Na	Na	Na
	Scott	-0.6936	Na	Na	Na	Na	Na	Na	Na

† Red font, significant negative correlations: green font, significant positive correlations: black font, non-significant correlations

ǂ Hub taxa

DISCUSSION

In this study, we used three sites at two different years to test de validity of the core microbiome concept for the canola. We could identify the canola core microbiome in the rhizosphere. This fungal core microbiome is composed of all the fungal taxa always found in the rhizosphere, independently of the environment where the plant is growing. The core microbiome was highly variable depending on time and location. The structure of the fungal communities was significantly affected by crop diversification. To identify the OTUs that could have a significant influence on the fungal microbiome, I constructed a network using the correlations between the fungi based on their abundances. Many OTUs sharing the same identity were strongly correlated to each other (e.g. *Nectria sp.*), so it is possible that the UNOISE3 pipeline which form OTUs based on a 100% sequence similarity, creates several OTUs corresponding to genetic variants of the same individual. Furthermore, we found many significant correlations between taxa forming the fungal microbiome in 2013, and identified six hub-taxa which might have a strong influence on the other members of the microbiome.

Variations in the canola fungal microbiome

Geographical and temporal variations of the fungal microbiome of plant rhizosphere have previously been reported in the literature (Gaiero et al. 2013; Edwards et al. 2015; Coleman-Derr et al. 2016). In our study, we expected the canola genotype and the level of crop diversification to be important factor of variation in the fungal microbiome of canola. The effect of canola genotype on microbial communities structure have been reported (Siciliano et al. 1998). However, in our study for both years, the canola types had no effect on fungal diversity and little impact on fungal community structure (Table S1 and Table 3). The genotype of canola appears to be, in our case, a very light source of variation in the fungal microbiome as its significant influence was only detected in Lethbridge in 2013. At the contrary, the levels of crop diversification had a lot more influence on the structure of the fungal communities in the canola rhizosphere. That could be explained by the fact that different crops are not recruiting similar

microbial communities, (Larkin 2003; Larkin and Honeycutt 2006). But if the crop diversification determined the community structure in most of our three localities in 2013 and 2016, it had no influence on the fungal diversity (Table 1). The diversity of crop rotation may be less important than the effect of the location of the fungal communities of the soil (Figure 1 and 2). Also, according to the indicator species analysis (Table S3 and S4) the levels of crop rotation diversity are not related to specific fungal taxa in a continuous way even if their influence on the fungal microbiome of the rhizosphere is significant.

Despite the fact that crop diversification was one of the main drivers in the structure of the fungal communities, our fungal microbiome of the canola rhizosphere was mainly composed of *Ascomycota*, which is consistent with the dominant fungal families reported in previous dedicated studies without crop diversification (Bennett et al. 2014; Tkacz et al. 2015; Gkarmiri et al. 2017). Interestingly, in these studies, *Olpidium*, has been reported to be the most abundant fungal genus in canola roots. In our case, *Olpidium brassicae* was one of the most abundant fungi in the canola rhizosphere microbiome in 2013 and 2016. But knowing that soil fungi are particularly sensitive to soil moisture levels (Zhang and Zhang 2016), it is possible that the weather conditions preceding the sampling might have caused an increase in the abundance of *Olpidium* in 2016 due to a drought in June and high level of moisture in July before sampling. Also, the condition of growth in the rhizosphere are not the same as in canola roots, so the ecology of *Olpidium* could change. The amount of rain was low in June 2016 (Figure 5), which may have favored the drought tolerant fungal species in the communities leading to a decrease of alpha diversity (Table S1) and an increase in *Olpidium*. Though, there is no information in the literature about how *Olpidium* behave in such situation. These annual variations are also perceptible in the core microbiome.

Canola fungal core microbiome

The fungal taxa forming the core microbiome of canola rhizosphere are likely to affect the health of the plant and the composition of microbial communities and should be in association with the plant at any location and time (Vandenkoornhuyse et al. 2015; van der Heijden et al. 2016). Interestingly, the fungal core microbiome of canola identified in 2013 in our study differs from that previously published (Lay et al. 2018) whereas the core microbiome

in 2016 was very similar. This study was conducted at similar locations in the Canadian Prairies, but in 2014 and 2016. Indeed, the one and only member of the core microbiome currently found in the canola rhizosphere in the Canadian prairies by Lay et al. (2018a) was *O. brassicae*. If *O. brassicae* was detected in the core microbiome of the canola rhizosphere of my study in 2013 (Table 6), it was not the only one. Here, 47 OTUs were allocated to the core microbiome, even using a more stringent threshold (100% presence in the plots against 75% in the previous study). This much higher number of OTUs attributed to the core microbiome is partly attributable to the deeper sequencing depth (7M reads versus 3M) in my study. Also, the core microbiome changed with time. That could be explained by the fact that the levels of moisture were not the same between the years and could highly influence the fungal species in the core microbiome. It is also possible that the core microbiome is affected by the plant phenology through the time (de Campos et al. 2013; Wagner et al. 2015). But in this study, we considered the same phenologic stage in 2013 and 2016 suggesting that abiotic factors (as moisture) are a major cause of variation in the core microbiome. This is coherent with the fact that sampling time and geographic location are known to greatly influence fungal communities (Barnes et al. 2016).

The core microbiome of 2013 had the particularity to include a large proportion of species from the genus *Fusarium* (Table 5). This is consistent with another study (Gkarmiri et al. 2017), where the genus *Fusarium* was dominant in canola rhizosphere. Colonization of the canola rhizosphere by *Fusarium* can lead to yield losses (Fernandez 2007; Fernandez et al. 2008), but not always (Hamel et al. 2005; Vujanovic et al. 2006; Yergeau et al. 2006). In our study most of the OTU related to the genus *Fusarium* were negatively correlated with the yield (Table S8), leading to a probable harmful influence of *Fusarium*. Non-pathogenic *Fusarium* species have also been detected in the roots of healthy plants and may be antagonistic to virulent *Fusarium* species. The antagonistic abilities of microbial species are particularly high in endophytes and are potentially beneficial to the plants, protecting them from parasitic intrusion (Clay 1988; Jumpponen and Trappe 1998; Xia et al. 2015). It could be the case of OTU35 which was sharing 100% identity with *Fusarium solani*, as it was positively related to yield in 2013. *Fusiarium solani* is a pathogenic specie, underlying the fact that short sequences are not well suited to identify correctly fungi, particularly species of the genus *Fusarium*.

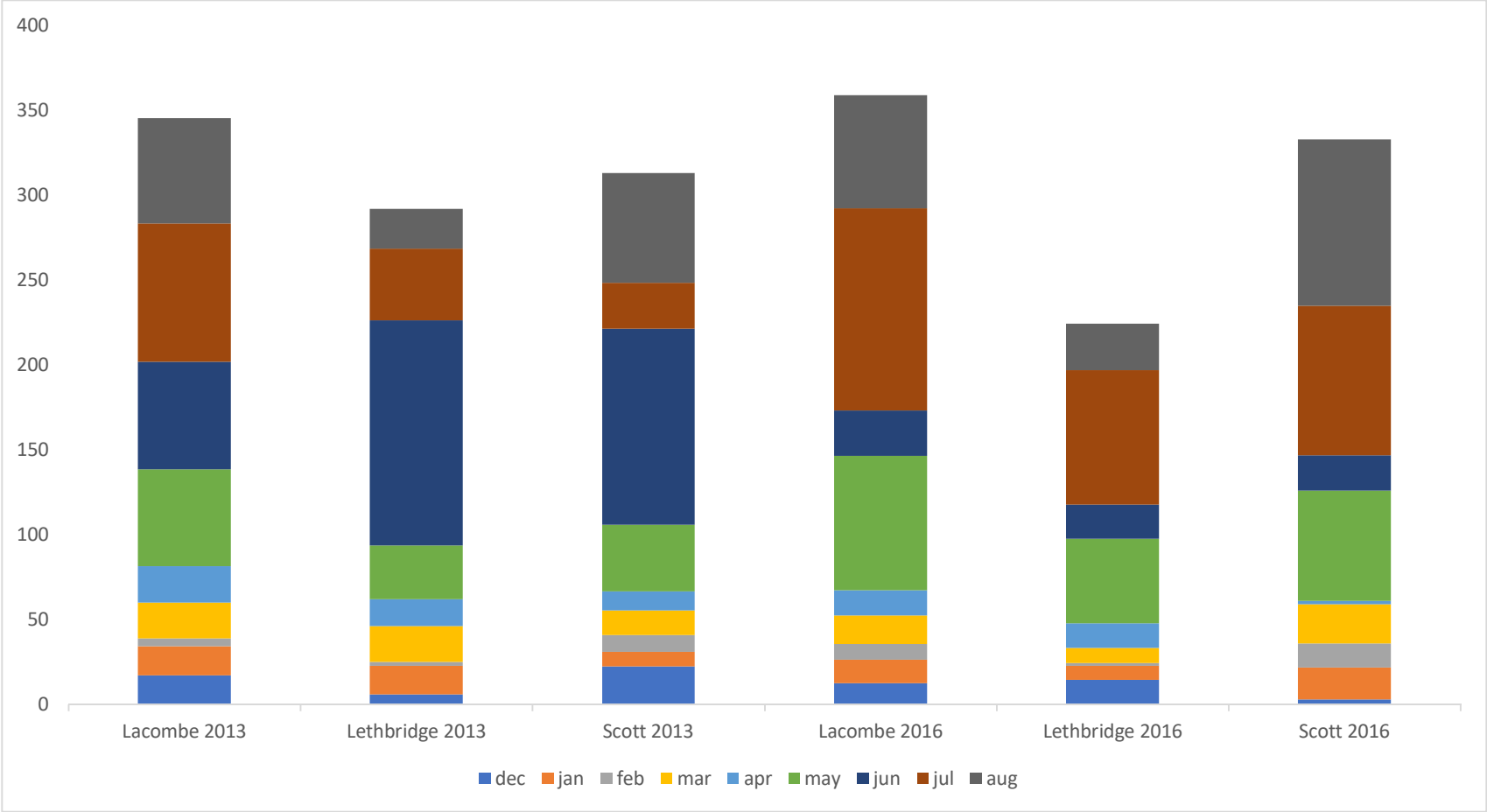

Figure 4. Cumulative precipitations for each site and year in mm.

The core microbiome of canola in 2013 included three potentially endophytic taxa: OTU102 (*Trichoderma koningii*), OTU49 (*Clonostachys rosea*) and OTU56 (*Exophiala* sp.). The genus *Trichoderma* is known for its antagonistic abilities and was recognized as a biocontrol agent. It was suppressive on *Sclerotinia* spp., a pathogen of canola (Hermosa et al. 2000; Hirpara et al. 2017; Jalali, Zafari, and Salari 2017). *Clonostachys rosea* was sometimes reported as a potential pathogen, but it was also found as a nematophagous fungus (Yang et al. 2007) that has potential for biocontrol (Vega et al. 2008). Finally, *Exophiala* is a dark septate endophyte (DSE). Certain DSE are known form symbiosis increasing the fitness of their host plant (Jumpponen and Trappe 1998; Jumpponen 2001). The genus *Exophialia* was also shown to increase drought resistance in sorghum (Zhang et al. 2017). This DSE is reported in canola rhizosphere for the first time. Further studies should test the influence of these taxa on canola.

Interactions in the microbiome and influence of hub taxa on canola yield

In the biotic interaction network of the canola rhizosphere, over 48 taxa, 12 were belonging to the core microbiome. Also, among the hub taxa, two over six were core microbiome OTUs: OTU5 (*Nectria sp*.) and OTU6 (*Mortierella sp*.). That is suggesting that a significant part of the core microbiome is not taking part of the microbe-microbe interactions inside the fungal microbiome of the canola rhizosphere. Six OTUs have been identified as hub taxa. Hub taxa are strongly interconnected to the other members of the core microbiome. These taxa have a very strong influence on the whole microbiome and on the health of the plant (Agler et al. 2016). The modular structure of the network suggests two different hotspots of interactions respectively centered on two hub taxa: one on OTU85 (*Preussia funiculata*) with 4 other hub taxa and the other on OTU298 (Unknown *Ascomycota*). Little is known about *Preussia funiculata,* this genus is described as coprophilous but a substantial part of its species are known to be endophytic with antimicrobial activities (Mapperson et al. 2014). Though, as OTU85 developed only two mutual exclusions with OTU56 (*Exophiala* sp.) and OTU5 (*Nectria sp.*), it is very unlikely endophytic. Its large number of positive co-occurrence with the other taxa of the microbiome, make it more suited of for a saprophytic role. OTU298 match with 95% identity to the sequence KX621287.1 of GenBank which belongs to the genus *Schizothecium*. As the

phylogeny of this genus remains questionable it is difficult to assess the ecological role of OTU298 (Cai, Jeewon, and Hyde 2005; Chang, Kao, and Wang 2010) but its poor but significant correlation with higher yield values could indicate an interaction with the canola. Another hub taxa remain unknown: OTU12 matches with 98% identity the sequence HM589219.1 identified as an unknown *Ascomycota* the next closest identification is with the sequence KX611037.1 known as *Preussia Africana* at 91%. As these two unknown hub taxa might be off ecological importance in the fungal microbiome of the canola rhizosphere further studies aiming at their identification are ongoing. OTU6 is a hub taxa known as *Mortierella sp.* The genus *Mortierella* is known in the literature to be saprophytic and highly represented in the Canadian prairies (Thormann, Currah, and Bayley 2004; Gkarmiri et al. 2017). We noted here that of the genus *Mortierella* were rather negatively correlated with canola yield (Table 7). As OTU6 is positively connected with OTU15 and OTU18 identified as fusariums, there could be an ecological association between *Mortierella* and canola pathogens. Plant root decay provoked by pathogens activity could benefit *Mortierella* by providing them with increased access to dead roots.

In contrast to these co-occurrence relationships, some taxa concentrate high number of mutual exclusions: *Nectria sp.* (OTU5 and OTU8) and *Exophiala sp.* (OTU56). OTU5 is a hub taxa but its co-presence with OTU8 deserves attention. These OTUs are sharing the same identification and their correlation is characterized by a very high *R* value (0.98). As we said earlier, the pipeline UNOISE creates OTUs based on a 100% sequence similarity, that could create several OTUs corresponding to genetic variants of the same individual. So, I decided to consider OTU5 and OTU8 as a single entity. Its high number of negative correlation suggest antimicrobial properties. That is coherent with the fact that many *Nectria* species are endophytic (Ikeda 2010; Cui et al. 2016; Ariefta et al. 2017). This dominance of suppressive behavior is even more present with OTU56. Its only positive link is with OTU5 which is also another endophyte. These two endophytic species are members of the core microbiome. It is interesting to note the positive relationship between high yield values and the OTU56 and OTU5. It would be interesting in the future to deepen the relationship between this fungus and canola. These correlations identify potentially profitable species for canola cultivation. These hub taxa must be isolated and tested in controlled experiment before we can conclude on how they influence canola growth and productivity.

Olpidium brassicae (OTU1)

OTU1 share 100% identity with the NCBI sequence AB205213 identified as *O. brassicae*. This fungal species was by far the most abundant in the core microbiome of canola rhizosphere in 2013 and was one of the most represented fungal species in 2016. It was also the only taxa that reached the criteria to be retained as member of the core microbiome in 2016. In previous studies, *O. brassicae* was also extremely abundant in the canola rhizosphere (Teakle 1960; Campbell and Sim 1994; Campbell, Sim, and Lecoq 1995; Sekimoto et al. 2011). The authors reported that *O. brassicae* is a plant root pathogen of the *Brassicaceae* family. However, Lay et al. (2018b) found no association between *O. brassicae* abundance and canola yield. In the present work, in 2013 when the abundance of this fungus was lower, it was not correlated with yield (Table 6). Hilton et al. (2013) similarly found canola yield losses in plots where *O. brassicae* was found in high amounts. That could suggest a switch in ecological behavior of *O. brassicae* when its population reach a certain threshold. In our study, however, the ecological role of *O. brassicae* remains questionable. Lay, et al. (2018b) shown that many taxonomic confusions were made between *O. brassicae* and *O. virulentus* in the literature, because of the genetic proximity of these two species. *O. brassicae* infects plants but does not carry viruses as is the case of *O. virulentus*. However, these correlations with yield would indicate that further studies on the abundance of *O. brassicae* would be required to determine the influence of this fungus on canola.

CONCLUSION

In this work, we described canola fungal core microbiome of the rhizosphere. We highlighted the potential beneficial role of two hub taxa: *Exophiala* sp. and *Humicola grisea* regarding their links to canola yield. We found that *O. brassicae* was the most abundant fungal species in the canola core microbiome of the rhizosphere. We also observed a large variation in the core microbiome composition between two years, ranging from 47 OTUs in 2013 to 1 OTU in 2016, suggesting a high effect of weather conditions on the fungal communities in the rhizosphere. The diversification of the crop rotation system did not appear to be a determining

factor in the variation of the fungal core microbiome of canola. This study also provides information about the fungal communities of canola rhizosphere that is very important for the future.

Conclusion et perspectives

Mon étude sur le microbiome fongique de la rhizosphère du canola apporte une précision nouvelle sur la structure de ce microbiome et des interactions entre microbes qui s'y développent. Par la caractérisation d'un core microbiome et le fait que ce dernier varie dans le temps mon étude a montré la nécessité de suivre l'évolution des communautés microbiennes afin d'identifier les acteurs les plus importants des dynamiques écologiques qui les composent. Dans le cas du canola, afin de poursuivre plus en avant la compréhension des mécanismes microbiens de sa rhizosphère un suivi des communautés dans le temps est envisagé pour la suite des recherches. La création d'un réseau écologique et l'identification de taxons nodaux au sein du microbiome, ainsi que la détermination de leur influence sur le rendement du canola, ont permis d'ouvrir des pistes de recherches futures centrées sur certains de ces taxons, à savoir Nectria sp. (OTU5) et Exophiala sp. (OTU56) qui par leur potentiel suppressif pourraient ouvrir la voie vers la mise en place d'agent de biocontrôle. La gestion du microbiome des plantes cultivées à des fins de protection contre les pathogènes des cultures ou encore pour l'augmentation des rendements semble de plus en plus accessible au fur et à mesure que les connaissances qui y ont trait s'accumulent. La production agricole dans les années à venir ne saurait s'affranchir de l'importance des communautés de microbes du sol et des racines.

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Supplementary Materials

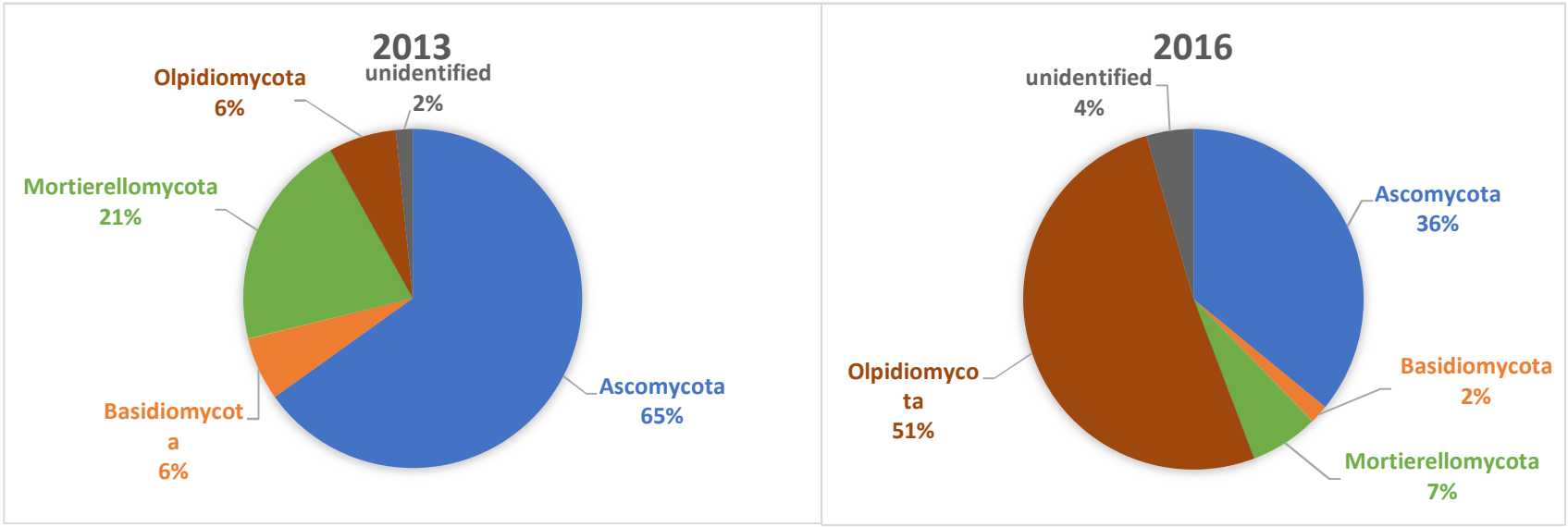

Figure S1: Phylums of the fungal communities of the canola rhizosphere (years 2013 + 2016)

a

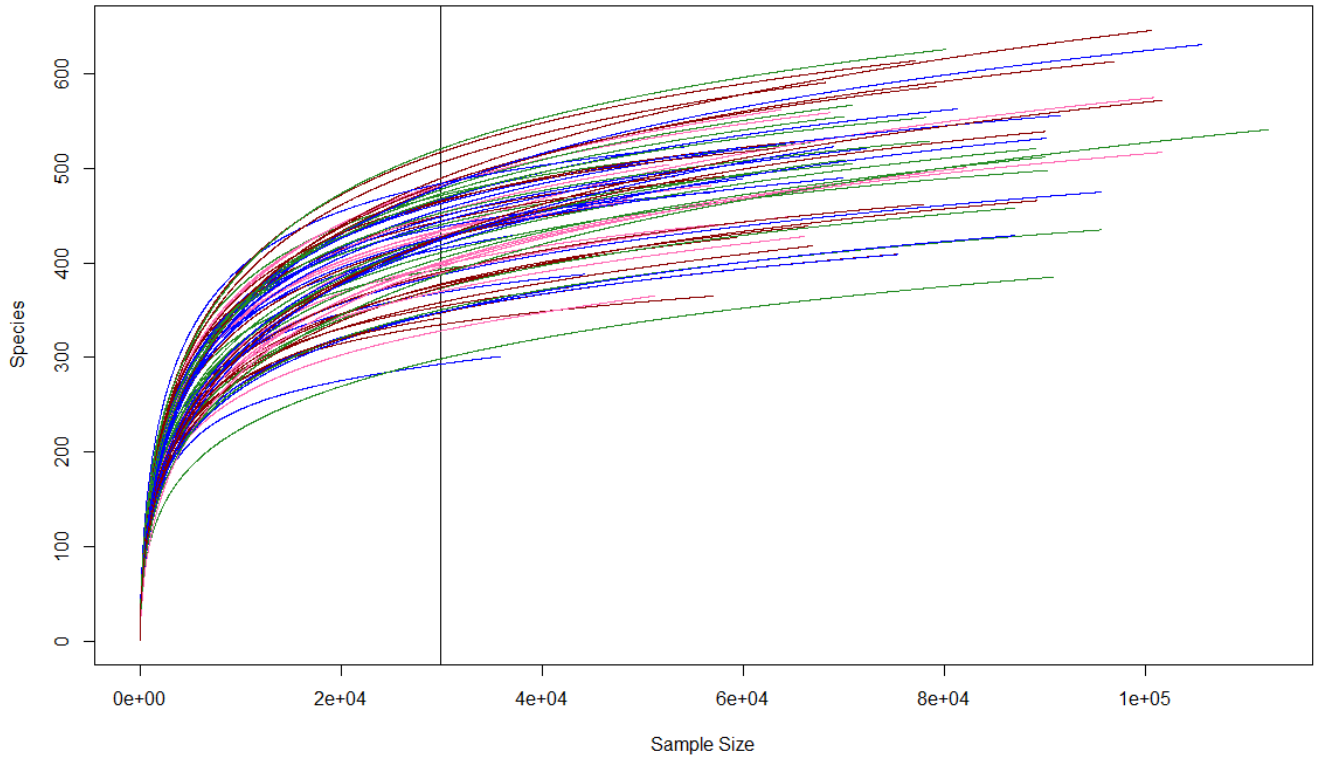

Figure S2: Rarefaction curves of our 70 samples (years 2013 + 2016)

b

Table S1: Mean values of α-diversity indexes in function of the genotypes of canola and the crop diversity in the plots in 2013 and 2016.

Diversity	Genotype	Richness	Chao1	Shannon	Simpson
2013					
Monoculture †	LL‡	190.917	285.641	3.963	0.958
	RR	183.917	269.237	3.767	0.932
Low	LL	202.273	290.738	3.911	0.947
	RR	199.000	303.038	3.949	0.949
Medium	LL	192.750	289.399	3.983	0.959
	RR	197.417	296.528	3.943	0.950
2016					
Monoculture	LL	58.667	68.928	2.166	0.679
	RR	50.667	66.635	2.071	0.662
Low	LL	62.083	53.220	2.235	0.676
	RR	56.000	70.927	2.449	0.756
Medium	LL	60.333	75.649	2.629	0.802
	RR	67.750	75.185	2.609	0.781
High	RR	67.000	81.164	2.485	0.731

†Crop diversity: Monoculture, canola monoculture; Low, wheat-canola rotation; Medium, pea-barley-canola rotation; High, lentil-wheat-canola Liberty link-pea-barley-canola Roundup Ready.

‡ LL : canola Liberty Link, RR : canola Roundup Ready

No significant differences between the values of each diversity indexes were found with Tukey HSD test.

c

Table S2: Relative abundancy of each OTU belonging to the core microbiome in 2013 in function of the crop diversity in field

OTU	Identity	Monoculture†	Low	Medium	OTU	Identity	Monoculture	Low	Medium
OTU1	*Olpidium brassicae*	0.0952‡	0.1136	0.1242	OTU35	*Fusarium solani*	0.0073	0.0077	0.0098
OTU10	*Plectosphaerella sp.*	0.0499	0.0363	0.0483	OTU387	*Candida parapsilosis*	0.0004	0.0004	0.0003
OTU102	*Trichoderma koningii*	0.0026	0.0014	0.0008	OTU39	*Cylindrocarpon sp.*	0.0056	0.0068	0.0063
OTU11	*Fusarium sp.*	0.0402	0.0281	0.0219	OTU4	*Fusicolla sp.*	0.0518	0.0600	0.0559
OTU123	*Leptosphaeria sp.*	0.0023	0.0018	0.0021	OTU40	*Cylindrocarpon sp.*	0.0065	0.0084	0.0058
OTU13	*Fusarium avenaceum*	0.0165	0.0254	0.0296	OTU41	*Tetracladium sp.*	0.0103	0.0124	0.0101
OTU15	*Fusarium sp.*	0.0202	0.0196	0.0289	OTU42	*Alternaria infectoria*	0.0055	0.0116	0.0115
OTU154	*Geomyces sp.*	0.0025	0.0038	0.0031	OTU49	*Clonostachys rosea*	0.0038	0.0073	0.0066
OTU16	*Mortierella sp.*	0.0289	0.0340	0.0285	OTU5	*Nectria sp.*	0.0470	0.0674	0.0589
OTU17	*Mortierella gamsii*	0.0293	0.0262	0.0236	OTU51	*Dendryphion nanum*	0.0168	0.0093	0.0077
OTU18	*Fusarium sp.*	0.0161	0.0165	0.0198	OTU54	*Cladosporium tenuissimum*	0.0088	0.0075	0.0054
OTU19	*Fusarium redolens*	0.0117	0.0122	0.0175	OTU56	*Exophiala sp.*	0.0058	0.0074	0.0078
OTU2	*Humicola grisea*	0.0525	0.0554	0.0585	OTU6	*Mortierella sp.*	0.0513	0.0541	0.0419
OTU21	*Fusarium sp.*	0.0153	0.0168	0.0182	OTU63	*Coniothyrium cereale*	0.0023	0.0089	0.0112
OTU22	*Mortierella sp.*	0.0096	0.0104	0.0155	OTU65	*Vishniacozyma victoriae*	0.0037	0.0092	0.0073
OTU229	*Aspergillus welwitschiae*	0.0013	0.0011	0.0013	OTU68	*Mortierella alpina*	0.0115	0.0109	0.0117
OTU24	*Alternaria alternata*	0.0224	0.0178	0.0145	OTU7	*Cladosporium sp.*	0.0789	0.0459	0.0336
OTU25	*Plectosphaerella sp.*	0.0143	0.0100	0.0205	OTU71	*unknown*	0.0040	0.0058	0.0026
OTU26	*Solicoccozyma aeria*	0.0172	0.0171	0.0157	OTU78	*Phoma sclerotioides*	0.0054	0.0054	0.0020
OTU27	*Fusarium solani*	0.0066	0.0072	0.0120	OTU8	*Nectria sp.*	0.0334	0.0431	0.0345
OTU29	*Ulocladium dauci*	0.0170	0.0151	0.0120	OTU84	*Trichocladium opacum*	0.0035	0.0032	0.0038
OTU3	*Mortierella sp.*	0.1033	0.0809	0.0735	OTU864	*Penicillium sp.*	0.0002	0.0002	0.0001
OTU30	*Fusarium sp.*	0.0028	0.0104	0.0199	OTU87	*Chaetomium globosum*	0.0027	0.0027	0.0060
-	-	-	-	-	OTU9	*Thielavia sp.*	0.0556	0.0431	0.0494

†Crop diversity: Monoculture, canola monoculture; Low, wheat-canola rotation; Medium, pea-barley-canola rotation; High, lentil-wheat-canola Liberty link-pea-barley-canola Roundup Ready.

‡ Each value of relative abundancy is a percentage of the total abundancy of fungi in the core microbiome

d

Table S3 ~ Operational taxonomic units (OTUs) of fungi residing in the rhizosphere of canola that were selected by canola type (canola) and cropping systems diversification level (diversity) in 2013, according to Indicator species analysis ($\alpha = 0.05$, $n = 4$), with their identity.

Canola †	Diversity ǂ	OTU	Identification	identity	*P* value
LL	Monoculture	OTU377	*Neurospora crassa*	99%	0.003 **
		OTU1365	*Myrothecium roridum*	99%	0.014 *
		OTU1806	*Zygopleurage zygospora*	95%	0.028 *
		OTU1367	*Humicola sp.*	93%	0.036 *
RR	Monoculture	-	-	-	-
LL	Low	OTU1449	unknown	81%	0.030 *
		OTU2014	*Kurtzmanomyces sp.*	95%	0.017 *
		OTU2109	*Myrmecridium hiemale*	99%	0.050 *
		OTU531	*Agrocybe pusiola*	99%	0.038 *
		OTU1324	unknown	82%	0.036 *
		OTU1602	*Schizothecium sp.*	94%	0.049 *
		OTU1454	*Schizothecium sp.*	93%	0.049 *
		OTU2195	*Pochonia suchlasporia*	92%	0.049 *
		OTU1495	*Praetumpfia obducens*	90%	0.046 *
		OTU1999	*Dinemasporium morbidum*	100%	0.049 *
RR	Low	OTU1011	*Dactylaria sp.*	90%	0.003 **
		OTU2067	unknown	85%	0.010 **
		OTU1889	*Cystobasidiomycetes sp.*	98%	0.024 *
		OTU1943	*Dioszegia butyracea*	100%	0.048 *
		OTU2143	unknown	88%	0.044 *
		OTU974	*Panaeolus retirugis*	99%	0.045 *
		OTU1168	*Oidiodendron truncatum*	99%	0.049 *
		OTU1786	*Pseudombrophila hepatica*	94%	0.050 *
		OTU2274	mycorrhizal fungus	93%	0.044 *
		OTU904	*Cephalotrichum sp.*	99%	0.044 *
		OTU1883	unknown	83%	0.039 *
		OTU1695	unknown	95%	0.042 *
LL	Medium	OTU1332	unknown	91%	0.010 **
		OTU1440	unknown	81%	0.007 **
		OTU2130	unknown	88%	0.048 *
RR	Medium	OTU473	*Pyrenophora tere*	100%	0.001 ***

† LL, canola liberty link; RR, canola Roundup Ready. ǂ Monoculture, canola monoculture; Low, wheat-canola rotation; Medium, pea-barley-canola rotation; High, lentil-wheat-canola Liberty link-pea-barley-canola Roundup Ready. *Level of significance, Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

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Table S4: Operational taxonomic units (OTUs) of rhizosphere fungi significantly selected by canola type and cropping system diversity level in 2016, according to Indicator species analysis ($\alpha = 0.05$, $n = 4$), with their identity.

Canola †	Diversity ǂ	OTU	Identification	identity	P-value
RR	Monoculture	OTU132	*unknown*	85%	0.032 *
LL	Low	OTU147	*Leptosphaeria sclerotioides*	100%	0.034 *
		OTU954	*unknown*	98%	0.012 *
RR	Medium	OTU168	*Kernia sp.*	98%	0.014 *
		OTU162	*Myrothecium roridum*	100%	0.038 *
RR	High	OTU109	*Hypocreales sp.*	100%	0.019 *

† LL, canola liberty link; RR, canola Roundup Ready.

ǂ Monoculture, canola monoculture; Low, wheat-canola rotation; Medium, pea-barley-canola rotation; High, lentil-wheat-canola Liberty link-pea-barley-canola Roundup Ready.

*Significant at $\alpha = 0.05$.

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