Microbial diversity and pathogen-microbiome interactions in crop residues : the case of Zymoseptoria tritici and Leptosphaeria maculans in a wheat-oilseed rape system

Lydie Kerdraon

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AgroParisTech
INSTITUT DES SCIENCES ET INDUSTRIES DU VIVANT ET DE L'ENVIRONNEMENT
PARIS INSTITUTE OF TECHNOLOGY FOR LIFE, FOOD AND ENVIRONMENTAL SCIENCES

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Microbial diversity and pathogen-microbiome interactions in crop residues: the case of *Zymoseptoria tritici* and *Leptosphaeria maculans* in a wheat-oilseed rape system

Thèse de doctorat de l'Université Paris-Saclay
préparée à AgroParisTech

École doctorale n°581 : agriculture, alimentation, biologie, environnement et santé (ABIES)
Spécialité de doctorat: Sciences agronomiques

Thèse présentée le 15 mai 2019 à Thiverval-Grignon par

Lydie Kerdraon

Composition du Jury :

Mme Jacqui Shykoff Directrice de recherche, CNRS (UMR ESE)	Présidente
Mme Patricia Luis Maître de conférences, Univ. Claude Bernard Lyon 1 (UMR LEM)	Rapporteur
M. Gaétan Le Floch Maître de conférences, Univ. Bretagne Occidentale (EA LUBEM)	Rapporteur
M. Lionel Ranjard Directeur de recherche, INRA Dijon (UMR AGROECOLOGIE)	Examinateur
Mme Marie-Hélène Balesdent Directrice de recherche, INRA Grignon (UMR BIOGER)	Co-encadrante
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M. Matthieu Barret Chargé de recherche, INRA Angers (UMR IRHS)	Co-encadrant invité

Thèse de doctorat

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Introduction Générale

Introduction générale

Le développement d'une agriculture durable et plus respectueuse de l'environnement fait partie des nouveaux défis auxquels sont confrontés tous les acteurs des filières agricoles en Europe. La propagation à grande échelle de maladies émergentes, le contournement de certaines résistances variétales, l'interdiction ou la baisse d'efficacité de certaines molécules fongicides due à l'apparition de résistances, font de cette problématique un défi majeur pour l'agriculture de demain.

Les programmes de recherche alliant les différentes composantes de la protection intégrée des cultures (prévention, surveillance, définition de seuils d'intervention, développement de méthodes non chimiques, déploiement de résistances variétales à différentes échelles spatio-temporelles, etc. [1]) se sont considérablement développés. Parmi ceux-ci, le projet EMPHASIS avait pour enjeu de maîtriser les menaces biotiques endémiques ou invasives pesant sur les agroécosystèmes européens, par des mesures de prédiction, de prévention, et d'éradication. Ce projet a été financé de 2015 à 2019 par la Commission Européenne dans le volet *SFS-03a-2014 Native and alien pests in agriculture and forestry* du programme H2020. Les objectifs d'EMPHASIS, à vocation appliquée, étaient aussi larges qu'ambitieux : les partenaires du projet, dont les unités INRA BIOGER (Biologie et Gestion des Risques en agriculture de Grignon) et IRHS (Institut de Recherche en Horticulture et Semences d'Angers), ont étudié les problèmes récurrents ou émergents causés par différents types de parasites et ravageurs des plantes cultivées (microorganismes phytopathogènes, insectes phytophages et plantes adventices) sur un large panel de systèmes agricoles et forestiers. Cette thèse s'est vue pleinement intégrée aux activités de recherche conduites par un groupe de travail s'intéressant au développement et la mise en œuvre de stratégies de biocontrôle.

Le biocontrôle est défini comme « l'ensemble des méthodes de protection des végétaux mettant en œuvre des microorganismes, des macroorganismes, des médiateurs chimiques ou des substances naturelles » [2]. L'utilisation d'agents de biocontrôle peut se faire de différentes façons : (1) la méthode classique, dont le but est d'introduire un nouvel agent dans le milieu et de favoriser son établissement afin qu'il puisse contrôler une espèce préjudiciable aux cultures ; cette méthode, qui implique le plus généralement l'utilisation d'espèces non-endémiques, est soumise à de nombreuses restrictions du fait des risques qu'elle fait courir à des organismes endémiques non-cibles. (2) La méthode inoculative, basée sur l'introduction d'un agent dans le milieu, en ayant pour objectif qu'il se reproduise, mais sans rechercher d'établissement à long terme ; la plupart des pratiques de biocontrôle en serre impliquant des insectes s'appuie sur cette stratégie. (3) La méthode inondative, pour laquelle l'agent est introduit dans de grandes quantités dans un milieu mais ne peut s'y reproduire et donc s'y établir, même provisoirement ; bien qu'efficace, cette méthode nécessite des applications régulières, et les coûts qui lui sont associés sont généralement élevés. Enfin, (4) la méthode de conservation consiste à induire des modifications du milieu dans le but de favoriser des organismes déjà présents et dont l'activité régule les populations de certains parasites ou ravageurs afin qu'elles passent sous le seuil de nuisibilité ; cette méthode est celle qui induit le moins de risques pour les organismes non-cibles [3].

De nombreuses études, réalisées en laboratoire, ont mis en évidence et caractérisé des interactions entre microorganismes (compétition, antagonisme, parasitisme, etc.). L'exploitation de ces interactions en conditions réelles (au champ) est toutefois délicate et reflète les difficultés de la mise en application du biocontrôle pour limiter de développement de maladies : les microorganismes vivent en associations complexes ; leurs interactions en conditions réelles sont difficiles à caractériser et encore mal connues. On considère, par exemple, que la population bactérienne est comprise entre 10^6 à 10^7 cellules par cm^2 de feuilles [4], et entre 10^8 à 10^{10} par gramme de sol [5]. L'utilisation d'un microorganisme comme agent de biocontrôle se heurte donc à la difficulté de savoir s'il pourra interagir efficacement et durablement avec l'agent pathogène cible, au sein d'un compartiment biotique complexe. Les techniques de séquençage haut débit utilisées depuis quelques années permettent aujourd'hui de décrire plus précisément les communautés de microorganismes, mais aussi leurs interactions. Elles offrent de nouvelles possibilités de biocontrôle : celles basées sur l'identification des taxons clés (genres, espèces) et de leurs interactions dans le milieu naturel, mais aussi celles d'étudier les facteurs qui influencent ces mêmes interactions dans une perspective appliquée. De fait, de nombreux travaux de recherche, qu'ils concernent l'humain, l'animal, le sol ou la plante, s'intéressent aux changements des communautés, qu'ils résultent de la prise d'un antibiotique, de l'application d'un traitement fongicide, de l'arrivée d'une nouvelle maladie ou d'un insecte ravageur. Ce type d'études a permis de reconsidérer le triangle épidémique, qui, auparavant, considérait l'hôte, l'agent pathogène et son environnement (et l'homme dans le cas des maladies des plantes). Le microbiome, et donc ses effets sur le développement des maladies, y a été ajouté. Les microorganismes qui interagissent avec l'agent pathogène font partie de son « pathobiome », c'est-à-dire l'environnement biotique capable modifier son développement et ses conséquences (pathogénèse, persistance, transmission, évolution) [6].

Les champignons phytopathogènes sont considérés comme l'un des risques majeurs pour les cultures, compte tenu des pertes de rendement qu'ils occasionnent [7]. Les épidémies provoquées par ces champignons peuvent être classées en deux catégories : les épidémies monocycliques, pour lesquelles l'inoculum à l'origine des infections n'est pas produit en cours de culture et provient d'une ou plusieurs sources fixes (sol, hôte alternant, plantes infectées présentes dans d'autres parcelles), et les épidémies polycycliques, pour lesquelles l'inoculum à l'origine de la majeure partie des infections est produit au cours de l'épidémie elle-même, sur des plantes infectées devenues à leur tour source d'inoculum [8]. Dans les deux cas, le début de l'épidémie est causé par l'inoculum primaire, et ce n'est qu'au cours du développement des plantes hôtes que l'importance relative de celui-ci par rapport à l'inoculum secondaire diminue (cas des épidémies polycycliques). Réduire la quantité d'inoculum primaire permettrait donc, théoriquement, de limiter le développement des épidémies, l'impact final dépendant de la nature « plus ou moins polycyclique » de celles-ci. Deux agents pathogènes, l'un monocyclique et l'autre polycyclique, ont fait l'objet de cette thèse.

Le premier agent pathogène étudié, *Zymoseptoria tritici,* est responsable de la septoriose du blé. Cet ascomycète hémibiotrophe peut provoquer des pertes de rendement allant jusqu'à 20 % sur des variétés de blé sensibles (*Triticum aestivum*) et de 5 à 10 % sur les variétés de blé sélectionnées pour

leur résistance et traitées avec un fongicide [9]. La protection contre cette maladie représente à elle seule 70% de l'usage annuel de fongicides sur blé en Europe [9,10]. Les ascospores de *Z. tritici*, issues de la reproduction sexuée, sont éjectées des débris contaminés de la culture de blé précédente [11] et dispersées sur de longues distances par le vent. Elles constituent la principale source d'inoculum primaire (Figure 1) [11]. La germination et la pénétration des spores dans les tissus de la plante se fait au niveau des stomates, initiant la première phase de l'épidémie [12]. Les lésions, au centre desquelles apparaissent les pycnides, fructifications asexuées, se développent sur les feuilles. Les pycnidiospores sont dispersées sur de courtes distances par la pluie [13]. Elles sont la principale source d'inoculum secondaire pendant la période de croissance du blé [11,14], mais ont longtemps été considérées comme étant les seules impliquées dans la dynamique épidémique printanière. Des quantifications dans l'air [15] et des travaux de modélisation [16] ont montré que les ascospores pouvaient aussi jouer un rôle significatif dans les phases épidémiques tardives. Les résidus de blé laissés sur le sol après la moisson sont le support de la reproduction sexuée et contribuent au maintien de la diversité génétique dans les populations pathogènes. En plus d'être une source quantitative d'inoculum primaire, ils influencent donc les dynamiques adaptatives de *Z. tritici*, notamment le contournement plus rapide des gènes de résistance [17] ou l'apparition de résistances aux fongicides [18–21].

Le deuxième champignon pathogène étudié, *Leptosphaeria maculans*, également un ascomycète, est, avec *Plenodomus biglobosus*, l'espèce qui domine le complexe fongique responsable du phoma du colza en Europe [22]. Cette maladie est, avec le sclérotinia, une des plus préjudiciables sur le colza. Le cycle de *L. maculans* est complexe (Figure 2, [23]). Les premières infections sont majoritairement provoquées par les ascospores éjectées des périthèces présents sur résidus de culture [24–26], bien que des pycnidiospores soient parfois produites sur résidus de culture et contribuent à accroître la quantité d'inoculum primaire [27,28]. Les résidus sont la principale source d'inoculum pour les épidémies de phoma, bien que les semences puissent également être infectées [29] et que la maladie se maintienne aussi sur des crucifères adventices. Sur résidus, la maturation des périthèces dépend de la température et de l'humidité [30,31] : la température affecte leur vitesse de maturation, mais pas leur quantité [31], tandis que l'humidité a un effet à la fois sur la dynamique de maturation et le nombre d'ascospores produites [32]. Les ascospores sont éjectées pendant toutes la durée de la culture suivante [33], permettant ainsi plusieurs cycles d'infection du colza à partir des résidus. Les spores déposées sur les feuilles germent et *L. maculans* entre par les stomates dès les premiers stades du colza [34]. Des macules (lésions) apparaissent alors sur les feuilles, sur lesquelles se développent des pycnides, qui libèrent des pycnidiospores ensuite disséminées par des éclaboussures d'eau de pluie sur de courtes distances. Si les pycnidiospores peuvent provoquer de nouvelles macules sur les feuilles adjacentes [35], l'importance des cycles d'infections secondaires sont de bien moindre importance pour le phoma du colza que pour la septoriose du blé. Le phoma est donc, de fait, considéré comme une maladie monocyclique. Après infection des feuilles, *L. maculans* colonise la tige par le xylème, puis la base de la tige. A la fin de l'hiver, le champignon provoque une nécrose au niveau du collet, ce qui entrave l'alimentation de la plante et peut provoquer la rupture des tiges, symptôme qui est le plus préjudiciable pour les cultures de colza.

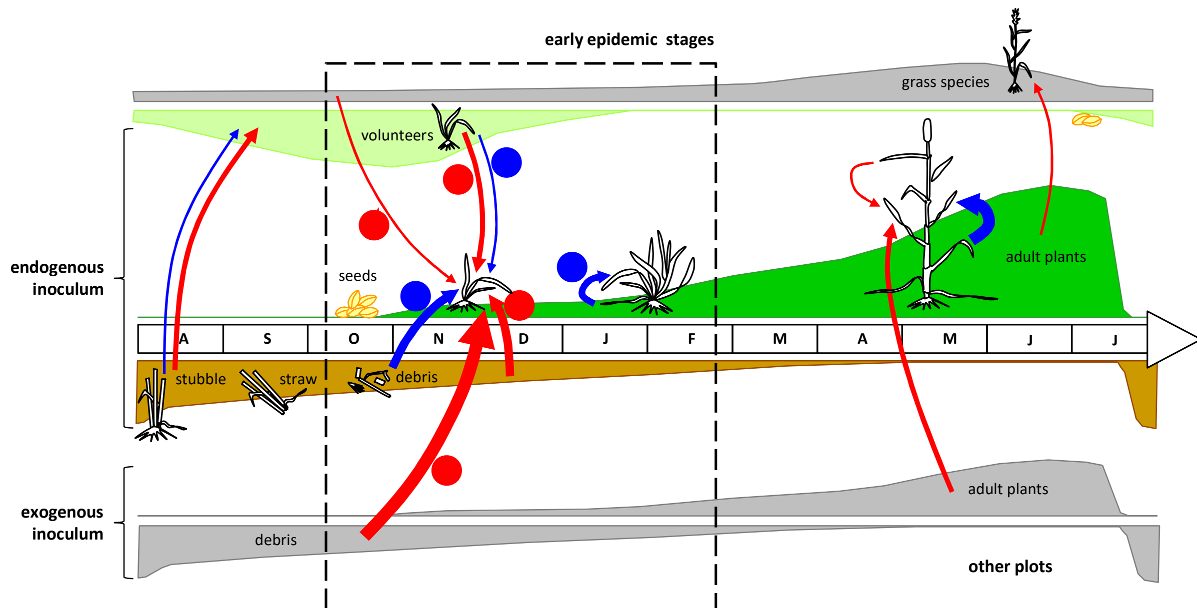

Figure 1. Représentation schématique de la dynamique des épidémies de septoriose (*Zymoseptoria tritici*), mettant l'accent sur les sources d'inoculum primaire [14]. Les flèches représentent les flux de spores (rouges : ascospores, bleues : pycnidiospores). La largeur des flèches est proportionnelle à l'importance du mécanisme représenté. Les premiers stades de l'épidémie dont il est question sont indiqués dans l'encadré en pointillés (1 : ascospores dispersées par le vent à partir de débris de blé infectés lointains ; 2 : ascospores dispersées par le vent à partir de débris de blé voisins ; 3 : ascospores dispersées par le vent à partir de repousses de blé ; 4 : ascospores dispersées par le vent à partir d'espèces de graminées ; 5 : pycnidiospores dispersées par les débris de blé voisins ; 6 : pycnidiospores dispersées par les repousses du blé ; 7 : pycnidiospores dispersées par les feuilles basales sénescentes).

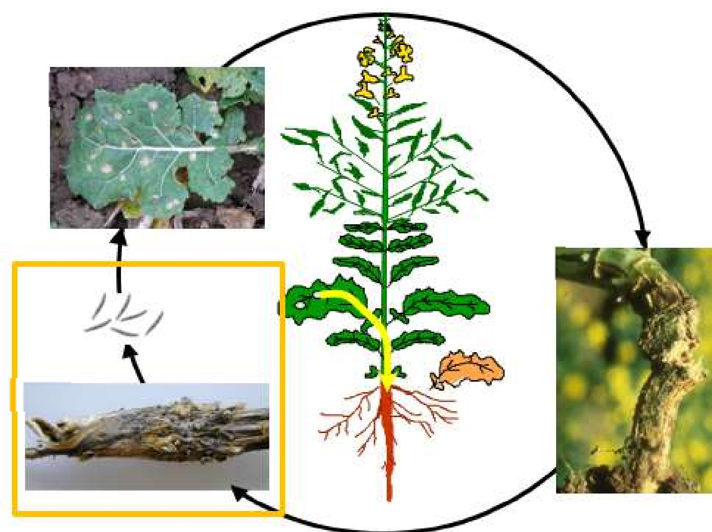

Figure 2. Cycle de vie de *L. maculans* [23], responsable du phoma du colza. (A) Périthèces sur résidus de colza. (B) Ascospores éjectées des résidus de colza. (C) Macules sur feuille de colza. (D) Colonisation systémique du colza. (E) Nécrose du collet.

L'importance des résidus de blé et de colza dans l'épidémiologie de la septoriose et du phoma est grande, parce qu'ils permettent le maintien de l'inoculum primaire, et donc la contamination précoce des cultures à plus ou moins grande distance, ainsi que le maintien de la variabilité génétique [17–21,36,37]. Alors que l'enfouissement des résidus est une méthode efficace pour réduire la quantité d'inoculum primaire, les techniques culturales simplifiées (sans labour) sont de plus en plus utilisées du fait de leurs effets bénéfiques, tels que la protection contre l'érosion des sols.

Le colza et le blé sont deux cultures d'importance majeure pour les usages domestiques et industriels en Europe du nord. Elles sont souvent associées dans les rotations. Les superficies consacrées au blé panifiable et au colza oléagineux en France étaient respectivement de $5{,}0 \times 10^6$ ha et $1{,}4 \times 10^6$ ha en 2017 [38]. Comme le colza oléagineux revient habituellement tous les trois ans dans la rotation et est utilisé presque systématiquement, soit directement avant ou directement après le blé, on peut estimer que cette rotation classique est utilisée sur près de $4{,}2 \times 10^6$ ha chaque année. Cela accroît considérablement l'intérêt porté aux microorganismes présents à la fois sur les débris de colza et de blé, notamment dans une perspective de biocontrôle : premièrement, parce que la collecte et l'identification de différentes espèces microbiennes peut être optimisée ; deuxièmement, parce que la probabilité d'identifier des espèces intéressantes pour chacune des deux cultures à partir de tests biologiques est accrue ; et troisièmement, parce que les méthodes développées pour gérer les maladies fongiques pourraient être efficaces pour l'une des cultures une année donnée et avoir des conséquences positives sur l'autre culture l'année suivante, illustrant l'intérêt de raisonner les stratégies de protection des grandes cultures à l'échelle de l'agrosystème (rotation blé-colza) (Figure 3).

La plupart des stratégies de protection des grandes cultures ciblent la période épidémique des maladies, dont l'impact sur le rendement est direct. L'objectif pour l'agriculteur est de limiter la sévérité des attaques au moment où la contribution de la plante au rendement est maximale (par exemple, remplissage des grains dans le cas du blé ou des siliques dans le cas du colza). Pourtant, la période inter-épidémique, moment où la culture est absente, joue un rôle important dans le développement des maladies. En effet, certaines pratiques sont susceptibles de contribuer davantage que d'autres au maintien de l'agent pathogène dans l'agrosystème et à la multiplication de son inoculum primaire.

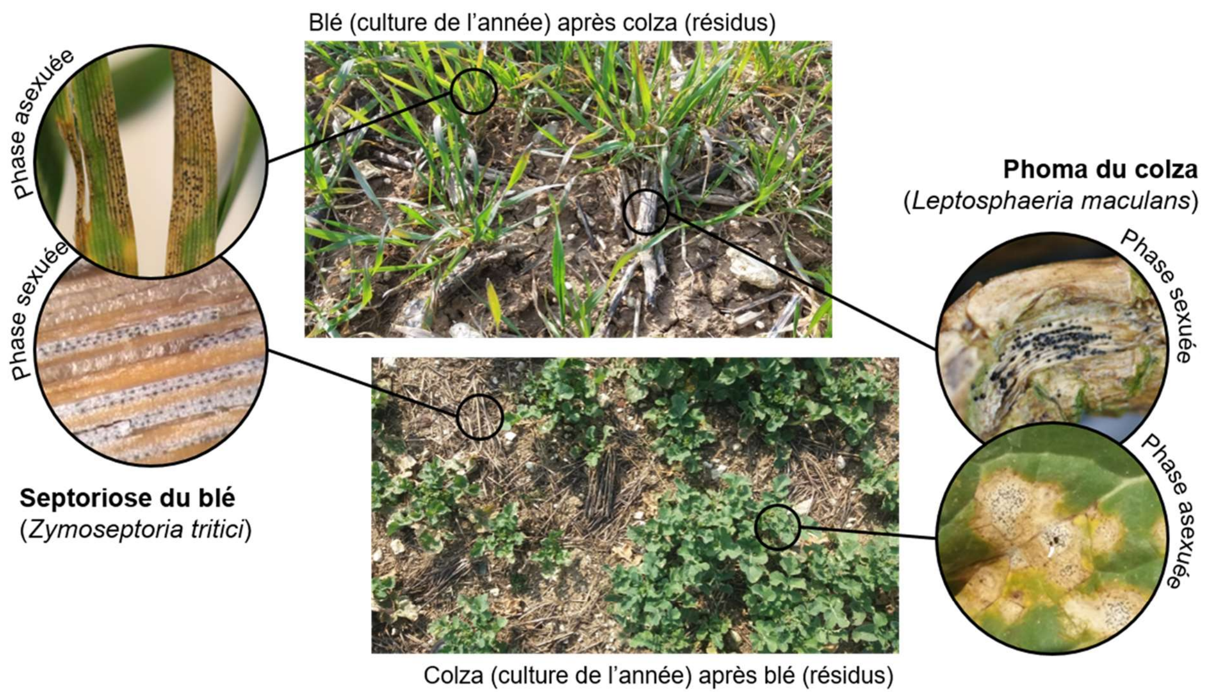

Figure 3 – Illustration de la présence de *Zymoseptoria tritici* en phase asexuée (lésions sur feuille de blé avec pycnides; photo F. Suffert) et en phase sexuée (résidus de blé avec périthèces; photo F. Suffert) et de *Leptosphaeria maculans* (macules sur feuille de colza avec pycnides; photo F. Suffert) et en phase sexuée (résidus de colza avec périthèces; photo M-H Balesdent) dans deux parcelles voisines de blé et de colza conduites en rotation avec un travail du sol superficiel (domaine expérimental de l'INRA de Grignon).

Dans cette thèse je me suis intéressée aux microorganismes cohabitant avec Z. tritici et L. maculans pendant la phase de reproduction sexuée, c'est-à-dire présents sur les résidus de blé et de colza. L'objectif était de caractériser la diversité microbienne de ce compartiment, jusqu'alors peu étudié, et de mettre en évidence les interactions entre ces agents pathogènes et les autres microorganismes.

Les microorganismes présents sur les résidus de cultures n'ont été que très peu étudiés dans une perspective d'application « phytosanitaire » : la plupart des travaux de recherche se sont focalisés sur ces résidus avec pour objectif d'identifier les taxons (genres, espèces, groupes fonctionnels) qui contribuent à leur dégradation, souvent dans une perspective agronomique (enrichissement du sol, amélioration de sa structure physique, minéralisation de composés organiques, etc.). La première partie de ma thèse a donc été une synthèse bibliographique réunissant les connaissances relatives à l'impact des résidus de culture dans les épidémies fongiques, et décrivant comment les microorganismes présents sur ces résidus pourraient être exploités pour développer des stratégies de protection raisonnées (Chapitre I). Partant de la vision classique des résidus comme source d'inoculum, ces derniers ont été définis comme un compartiment écologique dont les caractéristiques structurant les communautés microbiennes[1] ont été discutées. En associant les connaissances sur ces communautés microbiennes et l'analyse des interactions d'antagonisme décrites dans la littérature, j'ai proposé une réflexion sur la façon dont les microorganismes pourraient être utilisés pour réduire la place des agents pathogènes.

Pour mieux comprendre l'environnement biotique dans lequel évoluent *Z. tritici* et *L. maculans* pendant la période inter-épidémique, la deuxième partie de ma thèse (Chapitre II. 1) a consisté à décrire le microbiome associé aux résidus de culture de blé et de colza, dans un environnement agricole, en prenant en compte l'impact de la rotation culturale. Trois parcelles, l'une conduite en monoculture de blé, les deux autres en rotation blé-colza, ont été suivies. Afin de déterminer l'impact de la dégradation des résidus sur les microorganismes, des échantillons de résidus ont été collectés à différents pas de temps au cours de leur dégradation, et leur diversité microbienne a été analysée par metabarcoding. Cette approche a été renforcée par la mise en place des séries d'isolements fongiques et bactériens (Chapitre II. 2), dont le but était d'avoir à disposition une collection pouvant servir pour des études d'antagonisme, mais aussi de comparer les diversités obtenues par ces deux types de méthode, culture-dépendante et culture-indépendante.

[1] Le terme « communauté microbienne » est utilisé de manière ambigüe, voire de manière impropre à la place de « assemblage microbien ». Dans une revue qui fait référence, Boon et al. (2013) ont considéré qu'un « assemblage » est un ensemble de microorganismes (espèces, ou, plus généralement, taxons) dont on a pu établir, par des données morphologiques ou issues de séquençage, qu'ils occupent simultanément le même habitat ; une « communauté » correspond à un « assemblage » dont les taxons interagissent entre eux. Tant que l'existence d'interactions n'a pas été établie, il n'est donc pas souhaitable de parler de « communauté » *sensu stricto*. En revanche, dès lors que ces interactions ont été établies, on peut qualifier de « communauté » l'« assemblage » objet de l'étude. Ma thèse s'est attachée à mettre en évidence et caractériser les interactions au sein du microbiote des résidus de blé et de colza, en s'appuyant sur des études qui les avaient déjà établies dans les compartiments plante et sol. La perspective de devoir changer de terminologie, parfois même au sein d'un même chapitre, m'a conduit par souci de cohérence et de clarté à n'utiliser que le terme « communauté ».

Les deuxième et troisième parties de ma thèse (Chapitre III.1 et 2) ont consisté à estimer l'impact de la présence de chacun des deux agents pathogènes *Z. tritici* et *L. maculans*, sur les communautés microbiennes des résidus, et notamment à caractériser les interactions entre l'agent pathogène et les autres microorganismes. Du fait des différences de cycle biologique entre les deux champignons, deux stratégies expérimentales ont été utilisées. Pour le blé, l'étude a été faite en conditions semi-naturelles : des plants de blé ont été cultivés en serre ; seulement la moitié d'entre eux a été inoculée avec *Z. tritici*, avant d'être déposée en conditions naturelles. Pour le colza, l'étude a été conduite en conditions naturelles, en s'appuyant sur deux lignées isogéniques différant seulement par un gène conférant une résistance à *L. maculans*. Les communautés microbiennes présentes sur résidus de culture, pour le blé comme pour le colza, ont été caractérisées à plusieurs pas de temps au cours de la dégradation. L'évolution des interactions entre les microorganismes et chacun des deux agents pathogènes, en fonction de la présence ou l'absence de ces derniers, a été analysé par la comparaison de réseaux d'interaction.

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Chapitre I

Synthèse bibliographique

Caractériser les interactions microbiote - agents pathogènes dans les résidus de culture: une perspective pour gérer les maladies fongiques transmises par les résidus dans les systèmes de cultures céréalières ?

Disentangling microbiota-pathogen interactions in crop residues considered an ecotone: a promising way to manage residue-borne fungal diseases in cereal cropping systems?

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Abstract

The epidemiological contribution of crop residues as a source of inoculum for fungal diseases on plants, especially in cereal cropping systems, is well established. However, microbial ecologists have long reported positive effects of crop residues on the stability and productivity of several agrosystems. For this reason, no-till practices have become increasingly widespread, leading to the expression of trade-offs, or even incompatibilities, between different agro-ecological challenges. In this context, we propose a literature-based reflection on how to bridge the gap between "crop debris management" and "enhancing the action of microbes as direct or indirect biological control agents". Most studies focusing on the microbiota have suggested that microbial communities should be taken into account in plant disease management, but we still know much less about their ecological interaction with pathogens in the crop residue compartment than in the phyllosphere or rhizosphere. We provide here an overview of what is currently known about the impact of residues on plant disease epidemics and describe how microbial interactions on these residues could be exploited to develop innovative crop protection strategies. Starting from the classical view of residues as a primary source of inoculum, we considered possibilities for limiting their amount on the soil ground to reduce the pathogen pressure at the early epidemic stages. We describe residues as a transient half-plant/half-soil compartment constituting a key fully fledged microbial ecosystem: in other words, an ecotone which deserves special attention. We focus on microbial communities, the changes in these communities over time and the factors influencing them. Finally, we discuss how the interactions between the pathogens present on residues and these communities could be used: identification of keystone taxa and beneficial guilds of microorganisms naturally present, then their preservation by adapted agronomic practices, rather than the introduction of exogenous biocontrol agents designed as a “treatment product”.

Keywords: Agroecology, crop residues, fungal plant pathogen, microbiota, microbial communities, plant disease epidemiology, primary inoculum

Disentangling microbiota-pathogen interactions in crop residues considered an ecotone: a promising way to manage residue-borne fungal diseases in cereal cropping systems?

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Background

Crop residues (i.e. the parts of the crop plant not harvested), historically considered as waste, are now seen as a source of essential environmental services necessary for the perpetuation of productive agrosystems (Smil, 1999). No-tillage practices or conservation tillage (i.e. minimal soil disturbance) systems allow the formation of soil organic carbon, improve soil structure, prevent erosion, filter and retain water, and reduce evaporation (Derpsch et al., 2010; Govaerts et al., 2007). However, these practices, which are becoming increasingly common worldwide (Awada et al., 2014; de Freitas and Landers, 2014; Kertész and Madarász, 2014), increase the risk of "residue-borne" or "stubble-borne" disease epidemics, due to the presence of crop residues, which can act as a source of primary inoculum, on the soil surface (Bailey, 1996; Bailey and Lazarovits, 2003; Bockus and Shroyer, 1998). Indeed, several leaf-, stem-, and head-infecting microorganisms are known to survive on crop residues between cropping seasons (Bockus and Shroyer, 1998; Cook et al., 1978). Residue conservation tends to increase the risk of epidemics for many foliar diseases on cereals (Bailey, 1996; Bailey and Lazarovits, 2003; Bockus and Shroyer, 1998), particularly those initiated by ascospores produced by fruiting bodies resulting from sexual reproduction (perithecia). Wheat pathogens, such as *Pyrenophora tritici repentis* (Adee and Pfender, 1989), *Oculimacula yallundae* (Vero and Murray, 2016), and *Zymoseptoria tritici* (Suffert and Sache, 2011), have been shown to be more likely to infect the subsequent crop if wheat residues are present in the field.

Not only has there be a change in agricultural techniques, but epidemiological considerations have expanded to include new concepts, such as the “pathobiome”, defined as the pathogen and the cohort of microorganisms associated with it and likely to influence its persistence, transmission and evolution (Vayssier-Taussat et al., 2014). This concept encompasses the “microbiota” (defined as the assemblage of microorganisms present in a defined environment, whereas the “microbiome” is defined as the entire habitat, including the microorganisms, their genomes, and the surrounding environmental conditions; Marchesi and Ravel, 2015) as a distinct additional node, influenced by the three components of the classic epidemiological triangle (Figure 1; Paulitz and Matta, 1999; Foxman and Rosenthal, 2013; Hanson and Weinstock, 2016; Legrand et al., 2017). A particular feature of this concept is repositioning the “pathogen” into a broader community context: a microorganism responsible for disease as one of or many taxa interacting with other taxa present in the same ecological niche, which can affect each other positively or negatively. Disease expression seems to be the result of an imbalance between a potentially pathogenic species and the rest of the microbial community on host tissues, rather than simply a consequence of the presence of this pathogen species (Vayssier-Taussat et al., 2014).

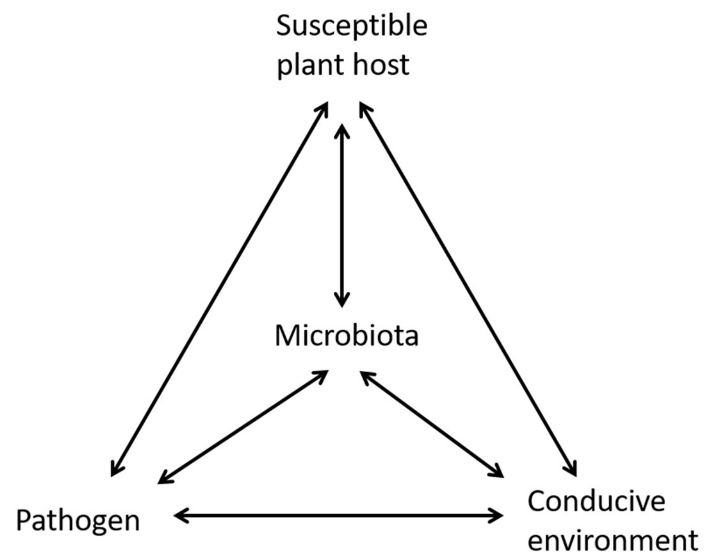

Figure 1. Disease triangle, serving as a conceptual model, presenting the factors that interact to cause a plant disease epidemic, completed by microbiota as a "fourth node" (adapted from Paulitz and Matta (1999) and Legrand *et al*. (2017)).

The situation is often rendered more complex by the presence of several microorganisms reported to act as crop pathogens or endophytes, which can develop without symptom development, or as saprophytes in the soil and plant residues. Microbial assemblage harbour “keystone taxa” (Banerjee et al., 2018), that exert individually or in a guild (group of species that exploit the same resources) a considerable influence on microbiome structure and functioning irrespective of their abundance across space and time. Thus, agronomic practices can theoretically cause a dramatic shift in microbiome structure and functioning by removing these keystone taxa, but can also reduce pathogen propagation and plant infection by beneficial microorganisms. The key questions are: which microorganisms should be considered as beneficial? Are they keystone taxa within the community? Which management practices could preserve them and enhance their positive activity against residue-borne diseases?

Despite the key role of residues in agrosystems, in terms of both soil conservation and disease risk, the communities present in this compartment have been little studied, while a large numbers of studies focused on plant microbiota, the detritusphere and bulk soil. Most studies have focused on the impact of residues on soil communities, rather than on the residue itself. In addition, the few studies focusing on crop residues conducted to date were performed in microcosms, with sterilized residues (Bastian *et al.*, 2009; Cookson *et al.*, 1998; Nicolardot *et al.*, 2007), greatly decreasing the complexity of this compartment, which is very rich in natural conditions, as it originates from the plant and is in close contact with soil.

This review, which is designed to be thought-provoking, gathers together knowledge about the impact of residues in plant disease epidemics and describes how interactions between the microorganisms present on these residues could be exploited to develop innovative crop protection strategies. To this end, we started from the classical view of residues as a source of inoculum, highlighting the limits of their quantitative management. We then move away from this view, by defining the residues as a “compartment” that has strong spatial and temporal relationships with other compartments of the agrosystem, firstly developing a typical static perception, and then focusing on more dynamic processes and interactions. We pass from the notions of substrate to pathobiome by focusing on the microbial communities present on residues, changes in these communities over time and the factors likely to influence them. Finally, we reflect on the possible uses of the communities present on the residues and possible interactions with pathogens, considering the possibility of identifying potential biocontrol agents from the cultivable part of these communities, through such integrative approaches.

I. Residue management in agrosystems: a brief history

Tillage, defined as the mechanical manipulation of soil and plant residues for seedbed preparation (Reicosky and Allmaras, 2003), has been associated with agriculture for several millennia. The "wooden plow" was developed in Mesopotamia at about 4000 to 6000 BC, and the "Roman plow" was developed at about 1 AD (Lal *et al.*, 2007). The emergence of "modern", "deep plow" tillage dates back to about 1000 AD, when a moldboard was added, making it possible to turn the soil over and, therefore, to bury residues (Lal *et al.*, 2007). Deep tillage systems has several benefits, including greater yields and root length densities for some species (Varsa *et al.*, 1997), but it also has negative effects on the soil. The retention of residues at the soil surface has repeatedly been show to have beneficial effects on soil preservation. The presence of residues prevents water erosion, by reducing the direct impact of raindrops (Chambers *et al.*, 2000; Hobbs, 2007), reducing runoff velocity and giving water longer to infiltrate (Pimentel *et al.*, 1995). It also prevents wind erosion by protecting the soil, and enhancing the soil's physical, chemical, and biological properties (Kassam *et al.*, 2015; Verhulst *et al.*, 2010). Conservation agriculture has another positive impact, in that it decreases the emissions from farming activities through the reduction of tillage operations (Govaerts *et al.*, 2007). Due to the negative consequences of tillage in terms of erosion (Borrelli *et al.*, 2017), conservation agriculture and no-tillage practices, and the use of permanent soil cover and rotations (Hobbs, 2007) have steadily increased (from 2.8 million ha in 1973/1974, to 110.8 million ha in 2007/2008; Derpsch *et al.*, 2010).

II. Impact of crop residues on the development of fungal disease epidemics in cereal cropping systems

Despite the benefits of conservation agriculture, the retention of residues at the soil surface can also have a negative effect: for instance, the reduction of soil temperatures in early spring, the stratification of nutrients with depth, and, mainly, the promotion of so-called “stubble-borne” diseases. These diseases, many of which are foliar, are caused by pathogens that can overwinter on residues (in different forms: mycelium, spores, sclerotia), or even carry out part of their life cycles on residues (Dyer *et al.*, 1996; Vero and Murray, 2016), including the production of primary inoculum (Leplat *et al.*, 2013; Shaw and Royle, 1989; Suffert and Sache, 2011; Vero and Murray, 2016). Unburied crop residues, lying on the soil surface and between crop plants in low-tillage systems, can be seen as a “brown bridge” of dead plant material that can harbor multiple pathogenic, saprophytic or endophytic species (Thompson *et al.*, 2015). This notion is comparable to the classical notion of a “green bridge” of crop volunteers or alternative weed hosts allowing biotrophic species, such as wheat leaf rust (*Puccinia triticina*), to survive locally between cropping seasons (Soubeyrand *et al.*, 2017).

Residues are the main, recurrent source of inoculum for several pathogens (“brown bridge”)

The survival of pathogens on residues seems to be inversely correlated with the degree of residue degradation (Gosende *et al.*, 2003; Hershman and Perkins, 1995; Leplat *et al.*, 2013; Marcroft *et al.*, 2003; Pereyra *et al.*, 2004). The rapid degradation of residues (Leplat *et al.*, 2013; Pereyra and Dill-Macky, 2008; Pereyra *et al.*, 2004; Summerell and Burgess, 1989), or their burial (Bockus and Claassen, 1992; Carignano *et al.*, 2008; Dill-Macky and Jones, 2000; Guo *et al.*, 2005; Jørgensen and Olsen, 2007), may therefore prevent ascospore release. Residue management is a particularly important issue as residues can have a major impact as a recurrent source of inoculum over long periods, often exceeding the interepidemic phase. For instance, ascospores of *Fusarium* species, a pathogen of cereals (wheat, maize), could be released for up to 3 years after harvest from maize residues (Pereyra *et al.*, 2004). However, it is difficult to generalize the quantitative impact of residues as an effective source of inoculum (e.g. Morais *et al.*, 2016), because the nature of the survival structures depends on the biology of the pathogen.

Equivocal relationship between the amount of residues and disease severity

Most disease management strategies target the epidemic phase of the disease, although interepidemic phases are also crucial for pathogen survival. Decreasing the presence of pathogens during this phase (i.e. reducing primary inoculum levels) could, theoretically, limit disease development in the next crop, and even over the next few years. Indeed, for some diseases, a correlation has been established between the amount of primary inoculum at the end of the growing season, and disease severity during the next season. Such a correlation has been demonstrated for *Leptosphaeria maculans*

(Lô-Pelzer *et al.*, 2009), *Pyrenophora tritici repentis* (Adee and Pfender, 1989; Bockus and Claassen, 1992), *Paragonospora nodorum* (Mehra *et al.*, 2015), and *Zymoseptoria tritici* (Suffert *et al.*, 2018).

A number of studies have demonstrated the value of managing the primary inoculum to limit disease severity during the year (Adee and Pfender, 1989; Filho *et al.*, 2016), but the inoculum generated on residues is not always the most important driver of epidemics when several cycles of the disease and environmental factors are considered. This is the case, for example, for polycyclic diseases, in which the secondary inoculum produced during the asexual phase of the pathogen's life cycle plays a crucial role. The amount of primary inoculum is not always a limiting factor for plant infection (Alabouvette *et al.*, 2006). Consequently, disease control by quantitative residue management alone would not be complete. The burial of residues at a field scale cannot therefore eliminate certain diseases.

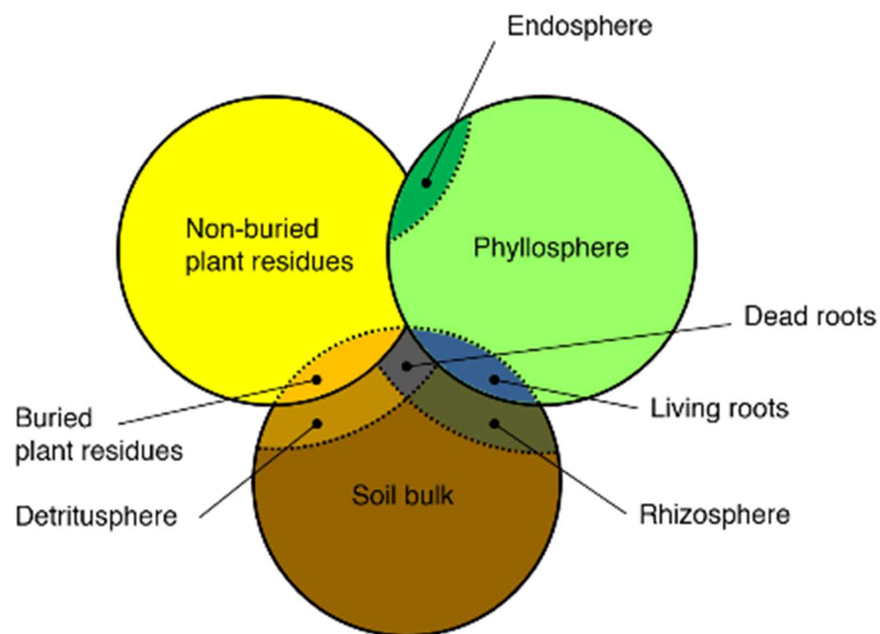

Figure 2. Positioning of the crop residues in relation to the other compartments constituting the different ecological niches of an agrosystem.

III. Crop residues, a key, shifting platform hosting microbial communities interacting with other compartments

The place of the residues "compartment", between plant and soil: definitions and concepts

Crop residues are the part of the crop plant that is not harvested (decaying plant material). Buried and non-buried plant residues can be distinguished (Figure 2) according to their location: above- and below-ground, respectively. This distinction makes perfect sense in terms of the epidemiology of plant diseases, as buried residues are no longer in the open air, they are no longer a source of inoculum for airborne diseases. It is tempting to adopt the term "residuesphere" to identify the microhabitat consisting of all crop residues, whether buried or non-buried. However, this term has occasionally been used as a synonym of "detritusphere" (Magid *et al.*, 2006; Sengeløv *et al.*, 2000), which is defined as the soil adjacent to plant residues (Marschner *et al.*, 2011; Pascault *et al.*, 2010a; Poll *et al.*, 2008). The detritusphere is considered to be the part of the soil immediately affected by residue decomposition, and is generally assumed to include the first 6 mm (Bastian *et al.*, 2009; Nicolardot *et al.*, 2007) or 10 mm (Magid *et al.*, 2006) of soil surrounding the residues at all times in stratified experiments. This top layer of the soil is very thin but has high levels of microbial activity (Kuzyakov and Blagodatskaya, 2015). The term "detritusphere" has been defined ambiguously by some authors as the layer of soil including the litter and the adjacent soil influenced by the litter (Gaillard *et al.*, 1999; Ingwersen *et al.*, 2008). An experimental comparison of the various soil zones (residues, detritusphere, and bulk soil) indicated that the bacterial and fungal communities are specific to a residue type in the detritusphere and to the location of residues (Nicolardot *et al.*, 2007). Residue degradation has been shown to induce a particular genetic structure of the microbial community with a gradient from residue to bulk soil. Based on these findings, it was concluded that the residues, detritusphere and bulk soil corresponded to different trophic and functional niches for microorganisms.

Residues should be considered as a distinct microbial substrate (Bastian *et al.*, 2009), characterized by the plant from which they originate, and by their "degradation" stage, implying chemical and physical changes dependent on their position relative to the ground surface. For this reason, as recently highlighted by Kerdraon *et al.* (Kerdraon *et al.*, 2019) in a study focusing on wheat-oilseed rape cropping systems, residues are not merely a specific "static" compartment and should be viewed as both a fully-fledged matrix and a transient compartment (Figure 3). They originate from the plant (temporal link; see part 3.2), are in close contact with the soil (spatial link; see part 3.3) and decay over the following cropping season, at rates dependent on plant species, cropping practices (Hadas *et al.*, 2004), and year (climate effect).

The microbial community of residues is inherited from the plant

The plant compartment hosting the "phytobiome" (plants, communities of organisms – micro- and macro-organisms – present in and on plants), including the phyllosphere, rhizosphere and endophytic

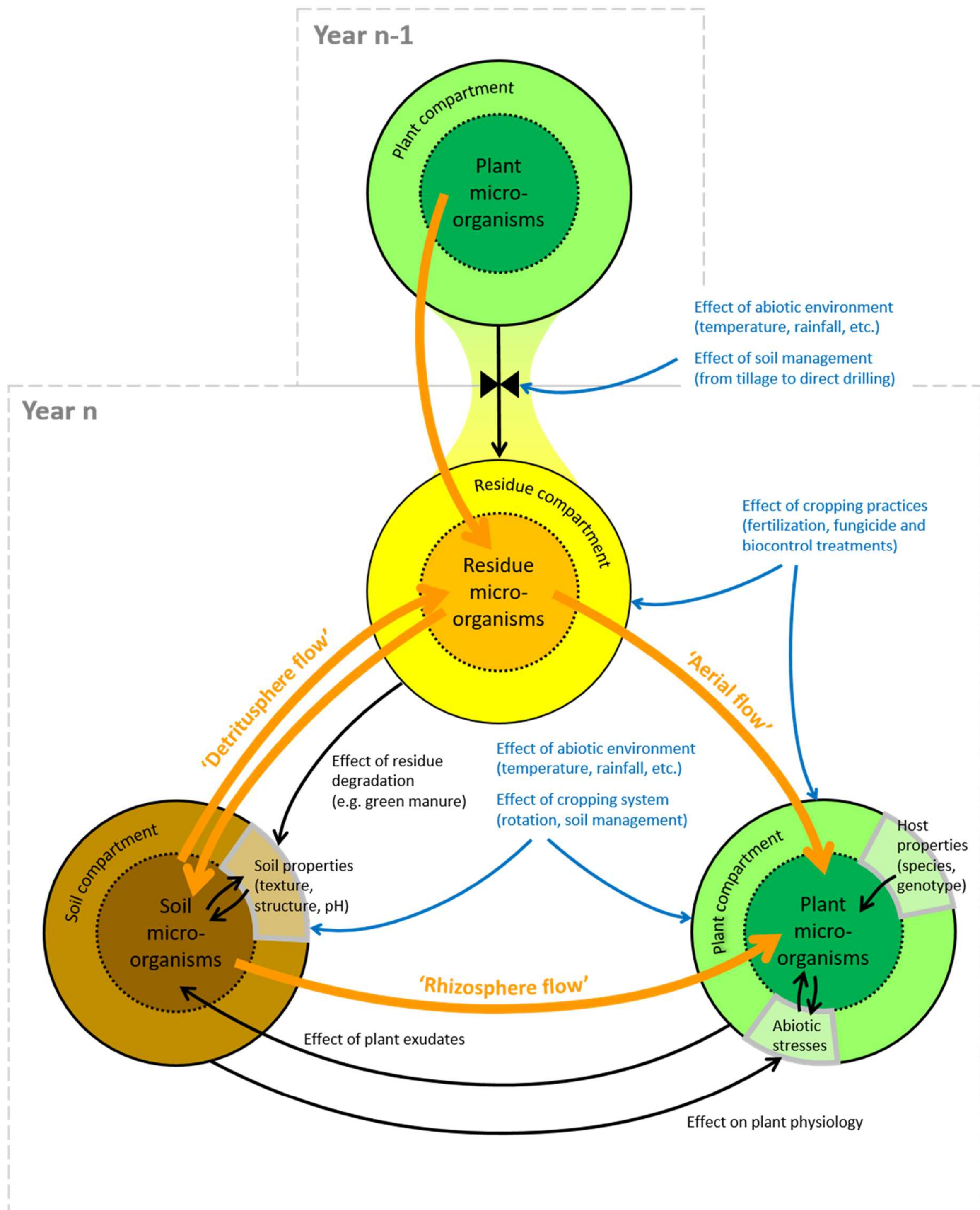

Figure 3. Representation of the most significant dynamic relationships – flow of microorganisms (orange arrows), biotic interactions (black arrows), abiotic or cropping effects (blue arrows) – between the crop residue, plant and soil compartments in an agrosystem.

compartments (Figure 2) makes a crucial contribution to the crop residues. Microorganisms are adapted to particular ecological niches and physiological conditions (Grudzinska-Sterno *et al.*, 2016; Larran *et al.*, 2007; Vorholt, 2012) and are driven by numerous biotic and abiotic factors (e.g. temperature, humidity, light; Carvalho and Castillo, 2018), including the plant itself, whether considered at species or genotype level (Bodenhausen *et al.*, 2014; Wagner *et al.*, 2016). The effect of the plant is therefore mainly due to two aspects: (i) it is already colonized by microorganisms that can remain on the residues (e.g. hemibiotrophic pathogens) and (ii) it has different biochemical compositions, which can affect the rate of degradation and the changes in the chemical and physical properties of the residues. These two aspects are closely linked, because the microorganisms present on plants are partly dependent on the biochemistry of the plant (species, genotype), and because these microorganisms can have a reciprocal impact on the plant properties, even before any degradation has occurred (changes in C/N ratio, production of defense compounds by plants in response to colonization by microorganisms, etc.). This aspect must be taken into account when trying to understand how new microorganisms colonize residues and how their microbiota changes over time in the agrosystem.

It may be theoretically possible to inoculate plants with endophytes such that residue is already colonized when it ceases to be plant tissue. Such biocontrol engineering practices are not yet largely used with exogenous fungal or bacterial strains during a cropping season, probably because of the complexity of the microbial interactions involved and their alteration by variations in agro-environmental conditions in field conditions (De Silva *et al.*, 2019). Nevertheless, it is an established fact that endophytic species naturally present in cultivated plants can limit the development of pathogens during the cropping season (Stone *et al.*, 2018). It is therefore likely that their action would continue on the residues during the intercropping season.

Pathogenic and non-pathogenic microorganisms present on plants can remain on the residues and influence the subsequent dynamics of colonization by other microorganisms

The structure of bacterial and fungal communities depends on plant species (Nicolardot *et al.*, 2007). Moreover, plant genotype also determines the structure of communities, particularly in cases of resistance to certain pathogens, but also for endophytes on wheat (Sapkota *et al.*, 2015). Comby *et al.* (2017) showed that the temporal variation of wheat microbial communities in natural conditions was driven by a succession of plant pathogens. The communities present on plants also depend on the organ considered. This is particularly true for aerial organs and roots, because of the differences between habitats in terms of nutrients and exposure (Comby *et al.*, 2016). However, several species, such as *Cladosporium* sp. and *Michrodochium nivale,* can colonize all parts of the plant (Gdanetz and Trail, 2017; Grudzinska-Sterno *et al.*, 2016). Moreover, the age of each organ has been shown to determine the communities present (Wagner *et al.*, 2016). In some cases, the proportion of pathogenic fungi increases with plant development (Grudzinska-Sterno *et al.*, 2016). Crop rotation does not appear to affect the communities associated with leaves, but does seem to affect root-associated communities, as demonstrated in a wheat-pea rotation (Granzow *et al.*, 2017). In summary, the microbial communities present on the residues at the beginning of their degradation depend on the plant (species, genotype,

organ) and on a pool of other organisms that differ according to the events in the plant's life and the environment in which it was grown (biotic and abiotic stresses).

The composition of the plant tissues and their changes over time also drive the colonization of residues by specific microorganisms

The biochemical composition of crop residues, which depends on the plant species from which they are derived, is one of the factors determining the structure and diversity of bacterial communities (Baumann *et al.*, 2009; Pascault *et al.*, 2010b). Even after the residues come into contact with the soil, the influence of the plant on residue colonization is evident. Residue decomposition has been shown to depend on the complex chemical composition of the residues, taking into account the C/N ratio, and the nitrogen and lignin contents of the plant (Kriaučiuniene *et al.*, 2012). The colonizing microbial communities differ between plant species, on identical soils. This has been established, for example, by a comparison between soybean, corn and wheat (Broder and Wagner, 1988), and by a comparison between wheat, rapeseed, and alfalfa, for which "easily degradable" residues underwent faster changes than "recalcitrant" residues, due to differences in the communities present, which consisted mostly of copiotrophic genera (developing preferentially in an environment rich in organic substrates) and oligotrophic genera (developing in an environment poor in organic substrates; Kerdraon *et al.*, 2019; Pascault *et al.*, 2010a). According to Boer (Boer *et al.*, 2005), fungi are the main decomposers of recalcitrant compounds, whereas bacteria break down simple substrates.

Biochemical transformations of wheat residues induce changes over time in the structure of microbial communities, and these microbial communities have a reciprocal effect, inducing biochemical transformations in the wheat residues hosting them. Bastian (Bastian *et al.*, 2009) described a change in the bacterial and fungal communities present on wheat residues between early stages of decomposition (14 and 56 days after the incorporation of residues into the soil) and later stages (56 to 168 days), which was interpreted as a change in the balance between copiotrophic and oligotrophic organisms. Residue communities are also influenced by cultivation techniques: residues are degraded differently in aerobic and anaerobic conditions (Cookson *et al.*, 1998). This results in different mobilized communities, including different bacterial and fungal cellulolytic species (Boer *et al.*, 2005), as degradation processes are not the same (Nicolardot *et al.*, 2007). The functional composition of the microbial communities (cellulolytic vs. lignolytic) depends on the physicochemical properties of the soil, such as pH.

Impact of the soil on changes in the microbial communities of residues during their degradation

Investigations of the impact of the soil compartment on changes in residue microbial communities during their degradation are subject to two difficulties. First, it is difficult to separate the "plant inheritance" effects (see part 3.2) from the "soil" effect when considering degradation processes, except

for species for which the origin can be determined with certainty because of specificities of their life cycle (phyllosphere for certain strictly biotrophic fungi, such as rust or mildew, or bulk soil for strictly telluric bacteria or arbuscular mycorrhizal fungi). This difficult issue has rarely been investigated. The second difficulty is that there are still significant gaps in our knowledge of the functional ecology and diversity of the soil microbial communities responsible for organic matter degradation from non-buried residues. Recent studies were performed under controlled laboratory conditions to characterize the impact of crop residues inputs on the diversity of soil microbial communities (e.g. Bastian *et al.*, 2009; Nicolardot *et al.*, 2007), but the converse is not true. These studies showed that the addition of crop residues to soil led to considerable heterogeneity in soil microbial community diversity, and identified three different zones in the soil: (i) the residues themselves, (ii) the detritusphere (the soil zone in close contact with the residues, see section 3.1), and (iii) the bulk soil. They reported the strongest microbial diversity dynamics on the residues, suggesting that the distinction between these compartments is not only “static” (Figure 2) but also “dynamic” (Figure 3).

Evidence for a major impact of soil compartment on the dynamics of microbial communities can only be indirect (comparison of communities) or partial (characterization of ecological functions). For instance, the importance of soil microorganisms in the mineralization of plant residues is well established, but the microbial colonization of residues left on the surface (for example in autumn, 3-4 months after harvest, when ascospore production peaks on residues of wheat infected with *Z. tritici*) is not documented.

Henriksen and Breland (Henriksen and Breland, 2002) showed that the degree of contact between crop residues and the soil matrix, which is determined by the method of residue incorporation, affects decomposition dynamics under both natural and experimental conditions. They showed that poor residue-soil contact reduces the decomposition of structural plant constituents by delaying colonization with microorganisms degrading cellulose and hemicellulose. Some studies have also focused on the location of residues (incorporated vs. left on the soil surface), to analyze the effect of different types of residue management on soil microbial communities during degradation, but very few have looked at the interactions between pathogens present on residues and the microbiota driving residue degradation. However, the richness of the residue compartment, at the interface between the plant and soil communities, suggests potentially interesting prospects for biocontrol.

IV. Characterization of the microbial communities in crop residues, for the sustainable management of residue-borne diseases: towards the identification of potential biological control agents to limit the impact of inoculum sources

This review shows that crop residues, a transient half-plant/half-soil compartment, constitute a key fully fledged microbial ecosystem; they might be considered an ecotone, as a boundary compartment between two biomes. The residue microbiota should be taken into account in the management of residue-borne diseases. It may be possible to identify guilds of beneficial microorganisms naturally present on residues, which could then be preserved, or even selected, characterized and used as biological control agents against the pathogens that complete their life cycle on residues.

Certain residue-borne fungal diseases can be managed by decreasing the amount of residues (Bailey and Lazarovits, 2003; Bockus, 1998; Bockus and Claassen, 1992; Guo *et al.*, 2005). For other plant diseases, the management of residues at the field scale may be less effective for decreasing final disease severity and yield losses over time in a sustained manner. Such a decrease could only be achieved by limiting the primary inoculum over a larger scale, considering the sources of local inoculum in a given plot to be sources of inoculum for more distant plots too. The best way to improve crop protection through a purely quantitative management of inoculum sources by cropping practices, therefore involves complex spatiotemporal management, based on more detailed knowledge of the rate at which concentrations of viable ascospores decline with distance from a source, for *Z. tritici,* for example. This review suggests that another, complementary approach could be considered.

There are currently no examples or studies supporting the strategy described above that have led to efficient and practical solutions applied in cereal cropping systems at a large scale. However, some promising results have been obtained. For instance, some microorganisms commonly present in the phyllosphere (*Paecylomyces lilacinus*, *Fusarium moniliforme* var. *anthophilum*, *Epicoccum nigrum*, *Bacillus* sp., *Cryptococcus* sp. and *Nigrospora sphaerica*) have been shown to affect the germination of *Z. tritici* spores (Perello *et al.*, 2002), and some members of the microbial community may have an impact on resistance to certain diseases (Ritpitakphong *et al.*, 2016). A number of effects can be targeted on residues, including increasing the rate of residue degradation, shortening the survival of certain organisms and promoting interactions affecting the saprotrophic development of pathogens, and the limitation of primary inoculum production. Some studies have reported beneficial effects of cropping practices, such as decreases in *F. graminearum* survival due to an increase in the population of microbial soil antagonists induced by the addition of green manure to the soil (Perez *et al.*, 2008). A recent study combining metabarcoding and co-occurrence network analysis allowed to profile microbial communities presents in maize residues and their potential interactions with the different pathogenic *Fusarium* species (Cobo-Díaz *et al.*, 2019). The author suggested that these communities present an important amount of taxa that may be of interest as part of biocontrol strategies against Fusarium Head Blight. Given the high diversity of microorganisms on residues, various modes of action could be used

to increase biocontrol efficiency (antibiosis, competition, antagonism; Alabouvette *et al*., 2006; Guetsky *et al*., 2001). Studies of this kind have been performed on chickpea residues infected with *Didymella rabiei,* for example (Dugan *et al*., 2005). Diverse modes of action were described for various organisms present on the residues. For example, *A. pullulans* can grow faster than *D. rabiei,* thereby limiting its propagation by competition, and *Clonostachys rosea*, which has mycoparasitic capacity, can decrease or even totally abolish the sexual and asexual reproduction of *D. rabiei*. *Microsphaeropsis* sp., which is also known to have mycoparasitic capacity (Benyagoub *et al*., 1998), has been shown to affect the production of *Fusarium graminearum* ascospores on wheat and maize residues (Bujold *et al*., 2001; Legrand *et al*., 2017). Bastian *et al.* (2009) highlighted the colonization of tailings (sterile tailings deposited on the soil) by bacteria such as *Pseudomonas fluorescens*, *Pseudomonas aurantiaca* and *Pseudomonas putida* and fungi such as *Chaetomium globosum*. All these species have been described as potential biocontrol agents (Clarkson and Lucas, 1993, Cordero *et al*., 2014; Flaishman *et al*., 1996; Kildea *et al*., 2008; Larran *et al*., 2016; Perello *et al*., 2002; Pfender *et al*., 1993; Ramarathnam and Dilantha Fernando, 2006).

Macroinvertebrates can also have an impact on the residue microbiome. This impact, expected to be more important when residues are buried, should be mentioned although it is not central in this review. Indeed, some empirical studies demonstrated that earthworms (e.g. *Lumbricus terrestris*; Wolfarth *et al*., 2011) can reduce the *Fusarium culmorum* biomass and deoxynivalenol concentration in wheat straw left on the soil ground. Amongst detritivorous species, collembolans (e.g. *Folsomia candida*) and nematodes (e.g. *Aphelenchoides saprophilus*) take also an important role in the control of phytopathogenic and toxinogenic fungi surviving on plant residues (Wolfarth *et al*., 2013; 2016): these organisms can be viewed as another potential driver to compensate negative consequences of conservation tillage.

Perspectives

Would it be possible for the same amount of residue to reduce the presence and activity of a pathogen in the microbial community of the residues, through interactions with other species: competition for resources, antagonism, or parasitism? If this is, indeed, possible, it would not be performed by classical biocontrol (introduction of an exogenous microorganism, which does not always give satisfactory results in field conditions), but would be through the exploitation of functional, agro-ecological relationships to identify the microorganisms that constitute the “beneficial fraction” of the community and then to promote them. The factors favoring microorganisms must first be identified, and this is an issue of growing importance for both academic and operational research working towards the development of biological control solutions. An accurate descriptive approach and the characterization of interactions within the residue microbiota are required. Next-generation sequencing, associated with ecological network analysis, is a promising technology for this approach (Toju *et al*., 2018). It provides access to the diversity of “non-culturable” microbes, facilitating the discovery of new species (Lagier *et al*., 2016) and more detailed community description. Some of the techniques available could be used to

characterize the diversity of the microbial communities associated with the pathogen throughout its life cycle, during both epidemic and interepidemic period, even if these periods are cryptic. Non-culturable microorganisms clearly cannot be used for biocontrol methods involving the introduction of exogeneous species, but we need to know more about their activity in natural conditions. It would then be possible to test, *in vitro* or *in planta,* the culturable species isolated from residues and identified as potential biocontrol agents, based on integrative strategies focusing on plants during their development (e.g. Gdanetz and Trail, 2017). Moreover, even if such species have an impact on the plant, it is important to be aware that the epidemic phase is probably not the only phase to study. The objective is not to find a species that can replace a fungicide applied during crop growth, but to understand how species, from single taxa to more complex microbial assemblage, can affect primary inoculum levels during the interepidemic period.

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Chapitre II

Les communautés associées aux résidus de culture

Chapitre II. 1

Les résidus de culture dans les systèmes blé – colza : une interface changeante, support des interactions microbiennes

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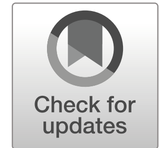

Crop Residues in Wheat-Oilseed Rape Rotation System: a Pivotal, Shifting Platform for Microbial Meetings

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Abstract
Crop residues are a crucial ecological niche with a major biological impact on agricultural ecosystems. In this study, we used a combined diachronic and synchronic field experiment based on wheat-oilseed rape rotations to test the hypothesis that plant is a structuring factor of microbial communities in crop residues, and that this effect decreases over time with their likely progressive degradation and colonisation by other microorganisms. We characterised an entire fungal and bacterial community associated with 150 wheat and oilseed rape residue samples at a plurennial scale by metabarcoding. The impact of plant species on the residue microbiota decreased over time and our data revealed turnover, with the replacement of oligotrophs, often plant-specific genera (such as pathogens) by copiotrophs, belonging to more generalist genera. Within a single cropping season, the plant-specific genera and species were gradually replaced by taxa that are likely to originate from the soil. These changes occurred more rapidly for bacteria than for fungi, known to degrade complex compounds. Overall, our findings suggest that crop residues constitute a key fully-fledged microbial ecosystem. Taking into account this ecosystem, that has been neglected for too long, is essential, not only to improve the quantitative management of residues, the presence of which can be detrimental to crop health, but also to identify groups of beneficial microorganisms. Our findings are of particular importance, because the wheat-oilseed rape rotation, in which no-till practices are frequent, is particularly widespread in the European arable cropping systems.

Keywords Community succession · Microbial diversity · Oilseed rape · Residue microbiota · Wheat

Background

Crop residues are an essential living element of agricultural soils. Smil [1] stressed that they 'should be seen not as wastes but as providers of essential environmental services, assuring the perpetuation of productive agrosystems'. When left in the field in the period between two successive crops, rather than being buried immediately, crop residues contribute to the formation of soil organic carbon, improve soil structure, prevent erosion, filter and retain water, reduce evaporation from the soil surface and increase the diversity and activity of microorganisms in the ground [2]. No-till practices are becoming increasingly widespread, as they take advantage of these attributes [3]. However, such practices are often considered likely to increase the risk of disease epidemics [4–6]. Indeed, several leaf-, stem-, head- and fruit-infecting microorganisms, classified as 'residue-borne' or 'stubble-borne' pathogens, are dependent on host residues for survival during the period between successive crops and for the production of inoculum for their next attack [7, 8]. The epidemiological contribution of residues as an effective source of inoculum is well established but difficult to quantify [e.g. 9] and generalise, because the nature of survival structures depends on the biology of the species. The situation is rendered even more complex by the presence of several species reported to act as crop pathogens in plants as endophytes, without symptom development in the plant, and in the soil and plant residues as saprophytes. Taking into account the inoculum from stubble-

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borne pathogens and possible competition with other microorganisms, it appears likely that the expression of a disease is the consequence of an imbalance between a potentially pathogenic species and the rest of the microbial community, rather than the consequence of the mere presence of this species [10].

Residues constitute a crucial ecological niche, not only for pathogenic species, but also for non-pathogenic and beneficial species. Residues can be viewed as both a fully-fledged matrix and a transient compartment, because they originate from the plant (temporal link), are in close contact with the soil (spatial link) and degrade over the following cropping season, at rates depending on the plant species, the cropping practices used [11], and the year (climate effect). It remains unknown whether the succession of microbial communities in residues is driven primarily by plant tissue degradation or edaphic factors [12]. Many studies have investigated the structure of the microbial communities present during the life cycle of the plant [e.g. 13–15], but few have investigated the microbiota associated with plant residues. Several ecological studies have investigated the impact of the residue compartment on the structure of soil microbial communities [2, 16–19], but not the impact of the soil compartment on structure of the residue communities. The detritusphere, defined as the part of the soil attached to residues [12, 20, 21], is the most extensive and broad hotspot of microbial life in the soil [22]. The residue compartment and the detritusphere are located in close physical proximity but are considered by microbiologists to be separate trophic and functional niches [23]. A description of the residue communities and the specific changes in these communities over time might, therefore, help agronomists to understand the impact of cropping practices on crop productivity. Fungi and bacteria play important roles in the degradation of plant tissues in debris (cellulose, hemicellulose, lignin), but the interactions between them within the microbial community remain unclear, due to the lack of information about their origins (air-borne, soil-borne or plant-borne), their individual functions and the drivers of community structure in residues.

Crop rotation induces changes in the composition of the soil microbial community and usually reduces pathogen pressure [e.g. 18]. For instance, wheat yields benefit from ‘break crops’ such as oilseed rape or other non-host crops to break the life cycle of wheat-specific pathogens [24]. We focused here on the wheat-oilseed rape rotation, one of the most widely used cropping systems in Europe. In 2017, the areas under bread wheat and oilseed rape in France were 5.0 million ha and 1.4 million ha [25], respectively. As oilseed rape usually recurs every 3 years in the rotation and is used almost systematically either directly before or directly after wheat, we estimate that this classical rotation is used on almost 4.2 million ha every year. Half the area occupied by these two crops is now grown without tillage, with at least some of the residues of the preceding crop left on the soil [26]. The issue addressed here is thus directly relevant to more than 2 million ha, or about one-tenth of the total arable area in France.

In this study, we deliberately focused on crop residues as a neglected, transient, but fully-fledged half-plant/half-soil compartment without describing the soil microbial communities, considering that it has been already performed in several studies [e.g. 27, 28]. We tested the specific hypothesis that plant is a structuring factor of bacterial and fungal communities in residues, and that this effect decreases over time, as contact with the soil induce progressive colonisation of residues by other microorganisms. Over the last few years, high-throughput metabarcoding has become an indispensable tool for studying the ecology of such complex microbial communities [29], partly due to the difficulties in isolating fungal and bacterial species and growing them in axenic conditions. We used this approach to describe and compare changes in the microbial community of wheat and oilseed rape residues left on the soil surface of three cultivated fields during two cropping seasons. We investigated whether the three main determinants of the diversity of fungal and bacterial communities—plant species, cropping season and cropping system (monoculture vs. rotation, focusing on wheat residues)—affected the microbiota of crop residues.

Methods

Experimental Design

Field Plots and Rotations

An extensive field experiment based on a wheat (W)-oilseed rape (O) rotation cropping system was carried out during the cropping seasons of 2015–2016 and 2016–2017 at the Grignon experimental station (Yvelines, France; 48° 51’ N, 1° 58’ E). This area is characterised by an oceanic climate (temperate, with no dry season and a warm summer). A combined diachronic and synchronic strategy [30] was used to investigate the dynamics of the residue microbial communities both over a 2-year period on the same plot and along a chronosequence substituting spatial differences (three plots) for time differences. A first monoculture plot (WWW) was sown with the winter wheat cultivar Soissons. This plot had been cropped with wheat since 2007 and was used in previous epidemiological studies focusing on the impact of wheat residues on the development of Septoria tritici blotch [e.g. 31–33]. Two other plots were cropped with oilseed rape cv. Alpaga and wheat cv. Soissons in rotation (OWO, adjacent to the WWW plot, and WOW, located 400 m away; Fig. 1). The size of the three plots was identical (20 m × 100 m). The OWO and WWW plots are characterised by a silty clay loam soil and plot WOW is characterised by a silty loam soil. Soil texture of the three

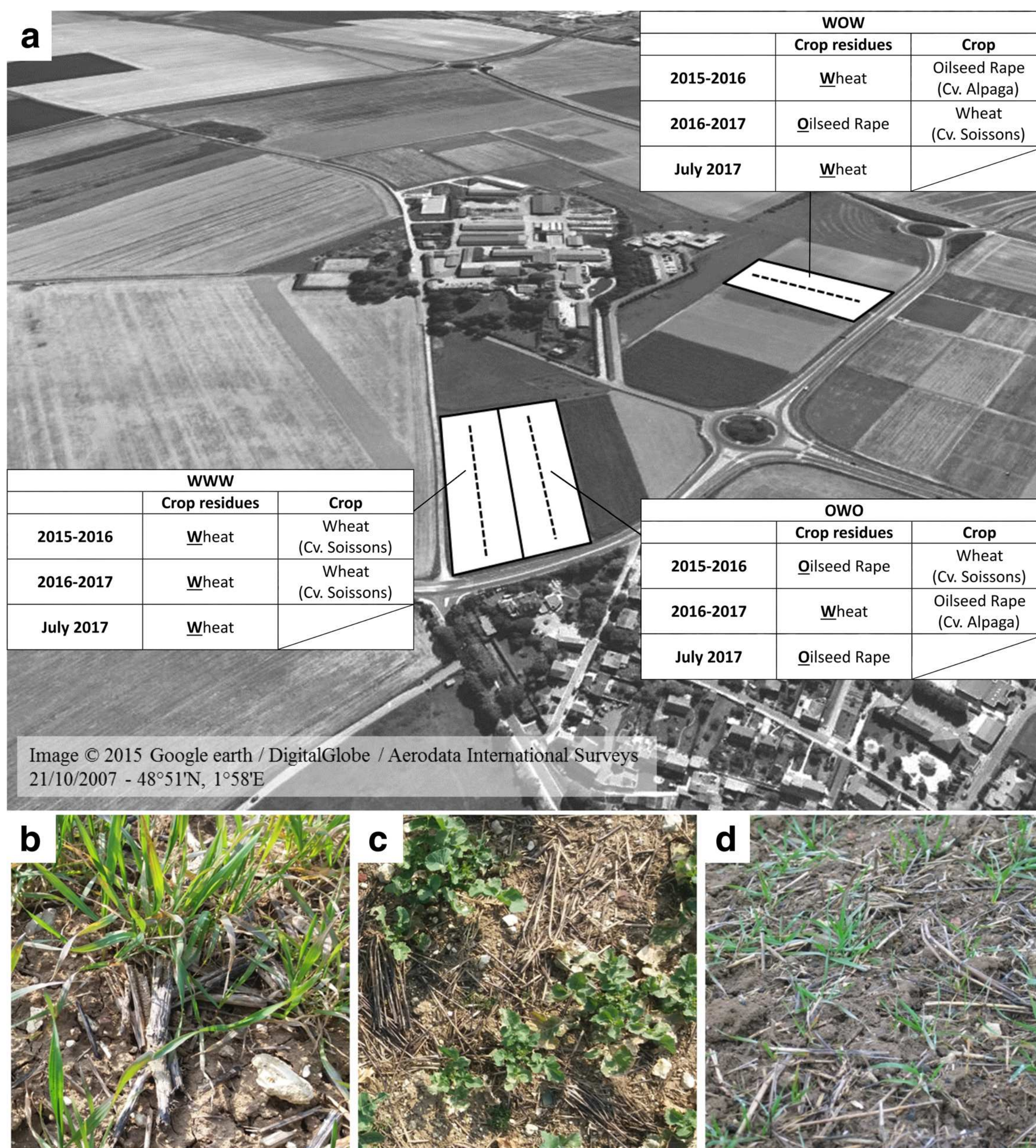

Fig. 1 Experimental layout of the experiment. **a** Plots (WWW, WOW and OWO) used during the two cropping seasons of the experiment at the INRA Grignon experimental station (Yvelines, France). WWW, plot cropped with winter wheat since 2007. WOW and OWO, plots cropped with a wheat-oilseed rape rotation since 2014. Wheat straw and oilseed rape residues were chopped at harvest and a large portion of these residues was then left on the surface. The dashed line indicates the sampling transect. **b** Oilseed rape residues in a plot cropped with wheat (OWO or WOW). **c** Wheat residues in a plot cropped with oilseed rape (WOW or OWO). **d** Wheat residues in the wheat monoculture crop (WWW)

plots is presented in Additional Table S1. The three plots were not tilled during the two cropping seasons. The wheat and oilseed rape residues were left on the soil surface after harvest. Soil was superficially disturbed to a depth of 10 cm with a disc harrow 6 weeks later (late September), leaving a large portion of residue on the surface. Crops were managed in a conventional way following local practices (nitrogen fertilisation, insecticide and herbicide treatments). No fungicide was sprayed on the leaves during the study.

Residue Sampling

Wheat and oilseed rape residues from the previous crop were collected over the two cropping seasons. The changes in the microbial communities during residue degradation were described on the basis of four sampling periods each year (October, December, February, and May). Sampling dates are presented in Additional Table S2. A supplementary sample was taken in July 2016, and a posteriori in July 2017, to

characterise the plant microbiota before the residues came into contact with the soil. For each sampling period, residues samples were collected at soil surface from five points in each plot, 20 m apart, along a linear transect (Fig. 1). Each sample was composed of twelve pieces of wheat residue or four pieces of oilseed rape residue. The five sampling points were located at the same place in the plots during the 2 years of the experiment.

DNA Extraction

Residues were cut to take off remaining roots, rinsed with water to remove the soil and air dried in laboratory conditions. They were then cut into small pieces, pooled in a 50-mL bowl and crushed with a Retsch™ Mixer Mill MM 400 for 60 s at 30 Hz in liquid nitrogen, in a zirconium oxide blender. The crushed powder was stored in 50-mL Falcon tubes at − 80 °C until DNA extraction. We transferred 40 mg of crushed residues to a 2.0-mL Eppendorf tube, which was stored to − 80 °C. Total environmental DNA (eDNA) was extracted according to the TriZol® Reagent protocol (Invitrogen, according to the manufacturer's instructions). Two independent extractions were performed per sample, giving a total of 300 eDNA samples. The two extractions were considered as technical replicates.

PCR and Illumina Sequencing

Fungal and bacterial community profiles were estimated by amplifying ITS1 and the v4 region of the 16S rRNA gene, respectively. Amplifications were performed with the ITS1F/ITS2 [34] and 515f/806r [35] primers. All PCRs were run in a reaction volume of 50 μL, with 1× Qiagen Type-it Multiplex PCR Master Mix (Type-it® Microsatellite PCR kit Cat No./ID 206243), 0.2 μM of each primer, 1× Q-solution® and 1 μL DNA (approximately 100 ng). The PCR mixture was heated at 95 °C for 5 min and then subjected to 35 cycles of amplification (95 °C (1 min), 60 °C (1 min 30 s), 72 °C (1 min)) and a final extension step at 72 °C (10 min). PCR products were purified with Agencourt® AMPure® XP (Agencourt Bioscience Corp., Beverly, MA). A second round of amplification was performed with 5 μL of purified amplicons and primers containing the Illumina adapters and indexes. PCR mixtures were heated at 94 °C for 1 min, and then subjected to 12 cycles of amplification (94 °C (1 min), 55 °C (1 min), 68 °C (1 min)) and a final extension step at 68 °C (10 min). PCR products were purified with Agencourt® AMPure® XP and quantified with Invitrogen QuantIT™ PicoGreen®. Purified amplicons were pooled in equimolar concentrations in five independent batches, and the final concentration of each batch was determined with the qPCR NGS library quantification kit (Agilent). The five independent batches were sequenced in five independent runs with MiSeq reagent kit v3 (300 bp PE).

Sequence Processing

Fastq files were processed with DADA2 v1.6.0 [36], using the parameters described in the workflow for 'Big Data: Paired-end' [37]. The only modification made relative to this protocol was a change in the truncLen argument according to the quality of the sequencing run. Each run was analysed separately. Taxonomic affiliations for amplicon sequence variants (ASV) generated with DADA2 were assigned with a naive Bayesian classifier on the RDP trainset 14 for bacteria [38] and the UNITE 7.1 database for fungi [39].

Only ASV detected in both technical replicates were conserved to ensure robustness [40] and were then added together. ASV classified as 'Cyanobacteria/chloroplast', or not classified at the phylum level, were discarded from the datasets. This resulted in suppression of 1.2% of reads for fungi (4.2% of unclassified ASV), and 1.5% of reads for bacteria (4.9% of unclassified ASV and 1.3% of ASV affiliated to Cyanobacteria/chloroplast). The remaining ASV were normalised according to the proportion of reads within each sample [41].

Microbial Community Analyses

Microbial community profiles were obtained for 100 wheat residue samples and 50 oilseed rape residue samples. The alpha-diversity was estimated for each sample by calculating the Shannon index [42], completed by Faith's phylogenetic diversity (PD) [43, 44] for bacterial communities. The compositional dissimilarity between samples (beta diversity) was estimated by the Bray-Curtis dissimilarity index.

Factors taken into account in the microbial community analyses were plant (wheat, oilseed rape), cropping system (monoculture, rotation), cropping season (2015–2016, 2016–2017), sampling period (July, October, December, February, May) and sampling plot (WWW, WOW, OWO, Fig. 1). We used a model to test these effects on each aforementioned index in a general way, and then conducted post hoc contrasts to characterise the differences. A complete model combining all the factors could not have been used because the experimental design did not include an oilseed rape monoculture field plot. Of note, oilseed rape monoculture is considered as an agronomic nonsense. Thus, a first model including the plant effect but not the cropping system effect (plant × cropping season × sampling period × sampling plot) was applied using the dataset from plots in rotation only (WOW and OWO). The effect of the cropping system (monoculture, rotation) was estimated separately using a second model (cropping system × cropping season × sampling period) applied on the dataset from wheat residues only; the sampling plot factor was not included in this second model as it would have been confounding with the cropping system factor.

The Shannon index was calculated for both bacterial and fungal communities with the ggpubr package in R [45], and Faith's phylogenetic diversity was calculated for bacterial communities with the picante package [46]. The effect of each factor on the Shannon index was assessed with two complementary ANOVA. A Kruskal-Wallis test also performed to assess significant differences in microbial diversity with time for each cropping season and sampling plot. Wilcoxon pairwise tests were also performed to compare the effects of sampling periods. Divergences were considered significant if $p < 0.05$.

The effect of each factor on the Bray-Curtis dissimilarity index was assessed with two complementary PERMANOVA using the adonis2 function of the vegan R package (version 2.4-4 [47] with 'margin' option, used to determine the effect of each term in the model, including all other variables, to avoid sequential effects. They were visualised by multidimensional scaling (MDS) on the Bray-Curtis dissimilarity index with the phyloseq package in R (version 1.22.3) [48] and completed for bacterial communities by incorporating phylogenetic distances using the UniFrac distance matrix. After the aggregation of ASV for each sampling condition 'sampling period/cropping year × crop within a rotation', the betapart R package [49] was used to determine whether temporal changes in community composition were due to turnover (i.e. replacement of ASV between two sampling periods) or nestedness (gain or loss of ASV between two sampling periods). The effect of the plant on the microbial communities associated with residues during degradation was also assessed with PERMANOVA on each sampling period, for each cropping season.

The genus composition of fungal and bacterial communities was analysed with a cladogram based on genus names. Only genera observed in three biological samples harvested on the same plot were incorporated into the cladogram. A cladogram representing the number of ASV for each genus, read percentage, occurrence and distribution for each sample, was constructed with the Interactive Tree Of Life (iTOL) [50] online tool for phylogenetic trees.

To illustrate taxonomic changes over time, especially between plant-derived communities and communities involved later in the colonisation of the residues, we focused on seasonal shifts (increase, decrease or stability) in the relative abundance of a selection of some fungal and bacterial genera and tested their statistical significance (Wilcoxon tests between sampling periods).

Results

The bacterial and fungal communities associated with wheat (W) and oilseed rape (O) crop residues were characterised on three plots: the wheat monoculture (WWW) and two oilseed rape wheat rotation plots (WOW and OWO) (Fig. 1). We assessed the composition of these microbial communities four times per year, during two consecutive cropping seasons (in October, December, February and May). An additional time point (in July) was also included for identification of the microorganisms present on the plant before contact with the soil. The analysis of raw sequence datasets for the 150 samples of wheat and oilseed rape residues collected over the two cropping seasons resulted in the grouping of 14,287,970 bacterial and 9,898,487 fungal reads into 2726 bacterial and 1189 fungal amplicon sequence variants (ASV). ASV not detected in both technical replicates (5.4% of bacterial reads and 1.5% of fungal reads) were removed from the datasets. A total number of reads remaining after ASV filtering are presented in Additional Table S3.

Alpha Diversity of Microbial Communities

Diversity dynamics, assessed by calculating the Shannon index, differed between the two cropping seasons and between fungi and bacteria. The diversity was significantly impacted by most of factors, including cropping system (monoculture, rotation) for wheat residues (Table 1; Fig. 2). Oilseed rape residues supported less fungal diversity and as much bacterial diversity than wheat residues in rotation. In addition, the diversity was significantly higher in wheat grown in monoculture than in wheat grown in rotation for both bacteria and fungi.

Fungal diversity increased over time in 2015–2016, whereas the differences between the samples in 2016–2017 did not reflect a gradual increase as the minimum was reached in December. Bacterial diversity followed a quite similar trend

Table 1 Results of the ANOVA performed to assess the effects of plant (wheat, oilseed rape), cropping season (2015–2016, 2016–2017), sampling period (July, October, December, February, May) and sampling plot on the Shannon index of the fungal and bacterial communities present in oilseed rape and wheat residues from the plots in rotation (OWO, WOW). The effect of the cropping system (monoculture, rotation) was estimated separately with a second ANOVA performed on the wheat residue samples dataset only (wheat in monoculture, i.e. in WWW; wheat in rotation, i.e. in WOW and in OWO)

		Fungi		Bacteria	
	Tested factors	*F* value	*p* value	*F* value	*p* value
Plots in rotation	Plant	22.42	< 0.001	0.33	0.567
	Cropping season	14.19	< 0.001	35.28	< 0.001
	Sampling period	14.25	< 0.001	37.73	< 0.001
	Sampling plot	31.06	< 0.001	61.57	< 0.001
Wheat residues	Cropping system	46.42	< 0.001	23.66	< 0.001
	Cropping season	51.43	< 0.001	33.34	< 0.001
	Sampling period	6.72	< 0.001	13.89	< 0.001

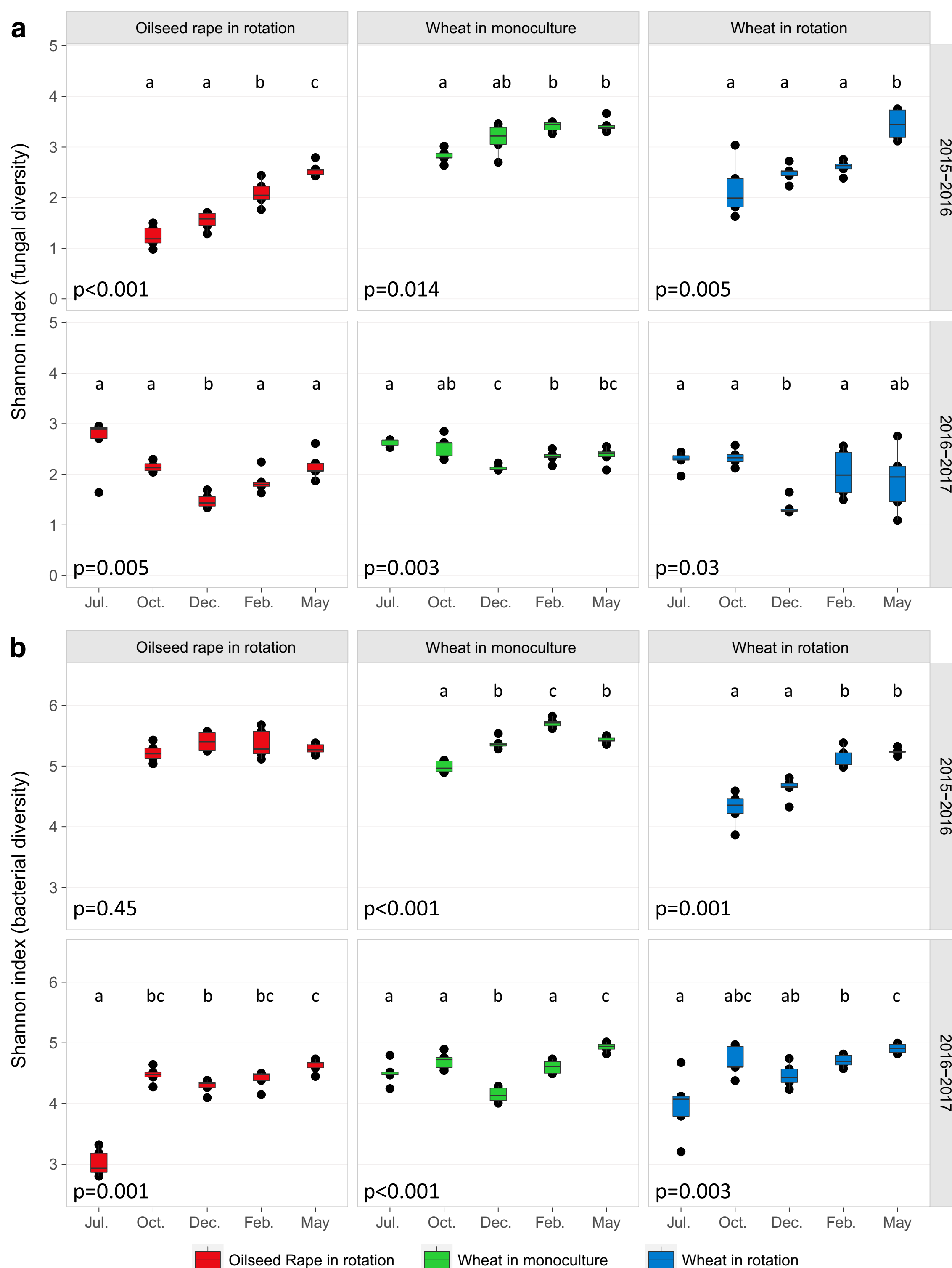

Fig. 2 Fungal (**a**) and bacterial (**b**) diversity in plants (July) and residues (October, December, February, May), as assessed with the Shannon index, according to sampling period, the crop within a rotation (oilseed rape in WOW and OWO, wheat in WWW, wheat in WOW and OWO) and the cropping season (2015–2016, 2016–2017). Each box represents the distribution of the Shannon index for five sampling points. Kruskal-Wallis tests were performed for each 'crop within a rotation × cropping season' combination (*p* values are given under each graph). Wilcoxon tests between sampling periods were performed when the Kruskal-Wallis test revealed significant differences. Samples not sharing letters are significantly different

with however a significant decreased from February to May, more pronounced for wheat residues than for oilseed rape residues during the first cropping season; this trend was also illustrated by the Faith's phylogenetic diversity (Additional Fig. S1). The climatic conditions during residue degradation (Additional Table S4) or differences in initial diversity on the plant before harvest may explain the less marked trends observed between the two cropping seasons.

Comparison of Microbial Communities Associated with Residues (Beta Diversity)

We analysed the effects of plant, cropping system, cropping season and sampling period on communities using the Bray-Curtis dissimilarity index and PERMANOVA. There was remarkably little heterogeneity between the five samples collected in the same sampling plots (Fig. 3) and the number of biological samples was, therefore, sufficient to assess differences due to the variables of interest (i.e. plant cropping season, sampling period and cropping system). This result was confirmed by the structure of bacterial communities visualised by incorporating phylogenetic distances using the UniFrac distance matrix (Additional Fig. S2).

The Structure of Bacterial and Fungal Communities Is Influenced by Plant Species and Cropping System

Oilseed rape and wheat residues presented different sets of ASV, for both bacterial and fungal communities (Fig. 3). Plant species was the main factor explaining differences between the communities, accounting for 38.4% for fungi and 26.6% of the variance for bacteria, as established with PERMANOVA (Table 2). For wheat, the cropping system (rotation, monoculture) accounted for 10.5% of the variance for fungal community composition and 6.6% of the variance for bacterial community composition.

The percentage of variance explained by the plant decreased over time for fungal community structure (e.g. from 75% in October 2016 to 40% in May), whilst for bacteria, the decrease of the percentage of variance explained by the plant was less pronounced (from 65 to 50%). The percentages of variance associated with the plant for each date are presented in Additional Table S5.

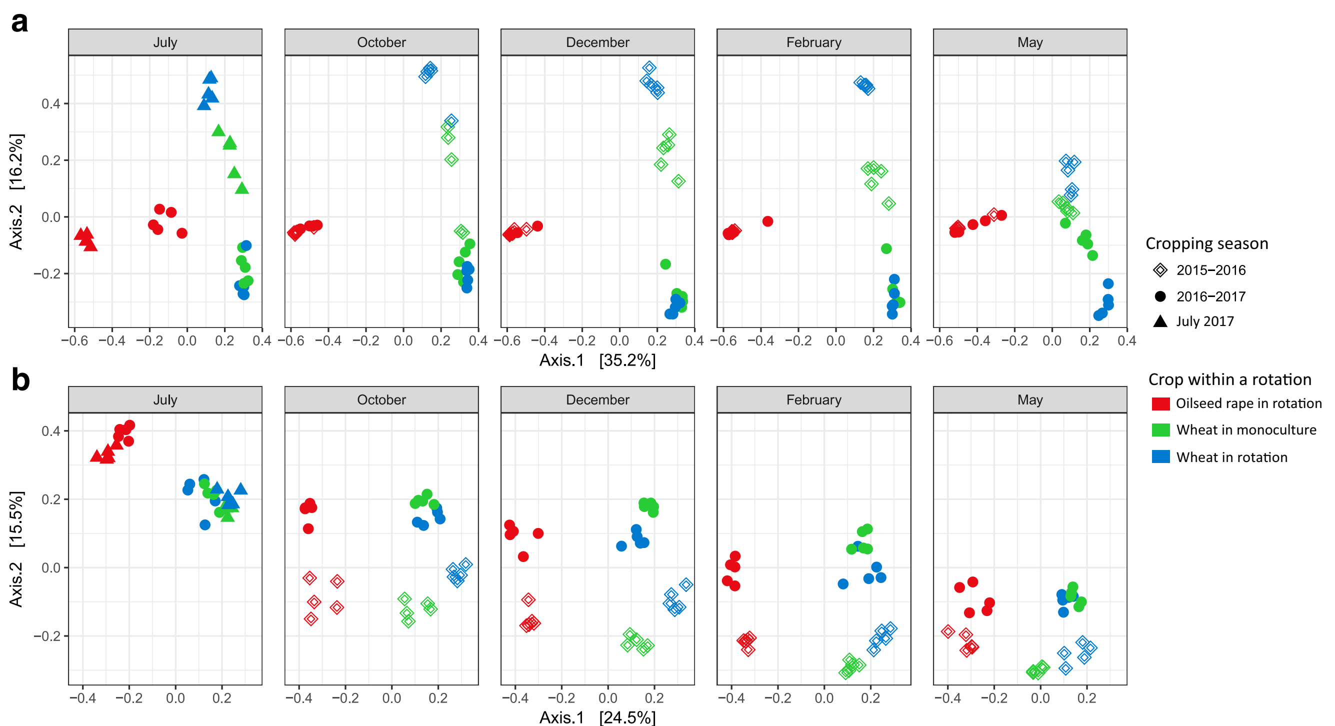

Fig. 3 Structure of the fungal (**a**) and bacterial (**b**) communities present in oilseed rape and wheat residues, according to compositional dissimilarity (Bray-Curtis dissimilarity index), after multidimensional scaling (MDS). The two MDS were performed on the overall dataset and faceted according to the sampling period. Each point represents one sample corresponding to a cropping season (shape 2015–2016, 2016–2017, July 2017) and crop within a rotation (colour, oilseed rape in rotation, i.e. in WOW and OWO; wheat monoculture, i.e. in WWW; wheat in rotation, i.e. in WOW and OWO)

Table 2 Results of the PERMANOVA performed to assess the effects of plant (wheat, oilseed rape), cropping season (2015–2016, 2016–2017), sampling period (July, October, December, February, May) and sampling plot on the Bray-Curtis dissimilarity index of the fungal and bacterial communities present in oilseed rape and wheat residues from the plots in rotation (OWO, WOW). The effect of the cropping system (monoculture, rotation) was estimated separately with a second PERMANOVA performed on the wheat residue samples dataset only (wheat in monoculture, i.e. in WWW; wheat in rotation, i.e. in WOW and in OWO). PERMANOVAs were performed using the adonis2 function with 'margin' option

		Fungi		Bacteria	
Dataset	Factors	R^2	*p* value	R^2	*p* value
Plots in rotation	Plant	0.384	< 0.001	0.266	< 0.001
	Cropping season	0.131	< 0.001	0.118	< 0.001
	Sampling period	0.068	< 0.001	0.149	< 0.001
	Sampling plot	0.100	< 0.001	0.053	< 0.001
Wheat residues	Cropping system	0.105	< 0.001	0.066	< 0.001
	Cropping season	0.292	< 0.001	0.248	< 0.001
	Sampling period	0.123	< 0.001	0.204	< 0.001

Community Structures Change Over Time

Cropping season was the main temporal factor underlying changes in community structure, accounting for 11.8% of the variance for bacteria when considering only the plots in rotation (or 24.8% when considering only the wheat residues without sampling plot effect), and 13.1% of the variance for fungi when considering only the plots in rotation (or 29.2% when considering the wheat residues without sampling plot effect) (Table 2). Sampling period had also a significant impact on community composition, accounting for 14.9% of the variance for bacteria when considering only the plots in rotation (or 20.4% when considering only the wheat residues without sampling plot effect) and 6.8% of the variance for fungi when considering only the plots in rotation (or 12.3% when considering the wheat residues without sampling plot effect). Theoretically, changes in ASV composition result from turnover (replacement of ASV between two sampling periods) and nestedness (gain or loss of ASV between two sampling periods [49]). We found that the dissimilarity between sampling periods was smaller for bacterial than for fungal ASV structure. By decomposing the dissimilarity between sampling periods, we found that, for fungi, 94% (± 5%) of dissimilarity was explained by turnover for oilseed rape, 89% (± 15%) for wheat in monoculture and 80% (± 13%) for wheat in rotation. For bacteria, 69% (± 17%) of dissimilarity was explained by turnover for oilseed rape, 61% (± 19%) for wheat in monoculture and 80% (± 16%) for wheat in rotation. Decomposition of dissimilarity between sampling periods is presented in Additional Table S6.

Changes in Communities, by Genus

We characterised potential taxonomic differences in communities over time by analysing wheat and oilseed rape residues separately. ASV were aggregated together at genus level, resulting in 84 fungal and 184 bacterial genera for wheat, and 63 fungal and 186 bacterial genera for oilseed rape. For the sake of clarity, the 60 most prevalent genera of fungi and bacteria were presented in Figs. 4 and 5, respectively. All detected genera and their evolution over time were presented in Additional Fig. S3, S4, S5 and S6. For both plant species, we identified genera that disappeared or displayed a significant decrease in relative abundance over time. The seasonal shifts of some genera and their significance are presented in Additional Fig. S6. Among these genera, some are known to be associated with plants, such as *Alternaria*, *Acremonium* [14, 51, 52], *Cryptococcus* [53], *Sarocladium* [54] and *Cladosporium* [13, 51–54].

Some of the fungal species detected on wheat, such as *Oculimacula yallundae* (all ASV of *Oculimacula* genera), *Zymoseptoria tritici* and *Pyrenophora tritici-repentis*, (Fig. 4; Additional Fig. S3) are known to be pathogenic. Some of the species detected on oilseed rape, such as *Verticillium* spp., *Leptosphaeria maculans* (= *Plenodomus maculans*) and *Leptosphaeria biglobosa* (= *Plenodomus biglobosa*), are also known to be pathogenic. Strikingly, *L. maculans* and *L. biglobosa* predominated over the other taxa. *Verticillium longisporum*, *V. dahlia* and *V. albo-atrum* were mostly detected during the second sampling year (Fig. 4; Additional Fig. S4). As samples were collected in two different fields, it was not possible to determine whether the occurrence of *Verticillium* spp., a soil-borne pathogen complex causing *Verticillium* wilt [55], was affected more by year or by the soil contamination. *Acremonium*, *Clonostachys* and *Alternaria* genera, which have also been described as associated with plants [56], were detected in the early sampling periods. Their relative abundances decreased over time (Additional Fig. S7). Most of the genera that were not present at early sampling points and with relative abundances increasing over time (e.g. *Coprinellus*, *Psathyrella*, *Torula*, *Tetracladium* and *Exophiala*) were common to wheat and oilseed rape residues (Fig. 4). These genera can thus be considered as probably derived primarily from the surrounding soil.

For bacteria, the difference in the genera detected between the two plants species was less marked than for fungi, as 146 genera were common to wheat and oilseed rape residues (Fig. 5). These 146 genera corresponded to the 98.7% most prevalent reads for wheat and 97.5% most prevalent genus reads for oilseed rape. *Proteobacteria* was the predominant phylum the first year. The most prevalent proteobacterial subgroup was *Alphaproteobacteria*, with a high prevalence of *Rhizobiales* and *Sphingomonadales*. *Rhizobium* and

Fig. 4 Distribution of the 60 most prevalent fungal genera detected in wheat residues in the five samples for each sampling date. Unclassified genera were removed from the visualisation

Neorhizobium, two major genera from *Rhizobiales*, decreased in abundance between October and May in both wheat and oilseed rape (Additional Fig. S7). *Sphingomonadales* genera were much more abundant on wheat than on oilseed rape, especially *Sphingomonas* (Fig. 5). *Bacteroidetes* genera, including *Pedobacter* in particular, were frequently detected and their prevalence tended to be stable for oilseed rape residues and to decrease for wheat residues (Additional Fig. S7). In parallel, an increase in *Actinobacteria*, particularly *Nocardioides*, was observed. Major differences between July and October were observed for oilseed rape, consistent with the beta-diversity analysis, in which the percentage dissimilarity between July and October was high, due to both species extinction and turnover. *Gammaproteobacteria* were highly abundant on oilseed rape in July. Their frequency then decreased rapidly from October to May, due largely to the decrease in *Pseudomonas* (Fig. 5; Additional Fig. S7). In parallel, we observed an increase in the levels of *Alphaproteobacteria*, especially *Rhizobium* and *Sphingomonas*, between July and October. A small decrease in levels of *Gammaproteobacteria* was observed between July and October for wheat in rotation, whereas the percentage of reads associated with this class increased between July and December for wheat in monoculture, due largely to the decrease in *Pantoea* and *Enterobacteria* (Fig. 5; Additional Fig. S7). The abundance of *Bacteroidetes*, especially *Pedobacter* and *Flavobacterium*, also increased between July and October.

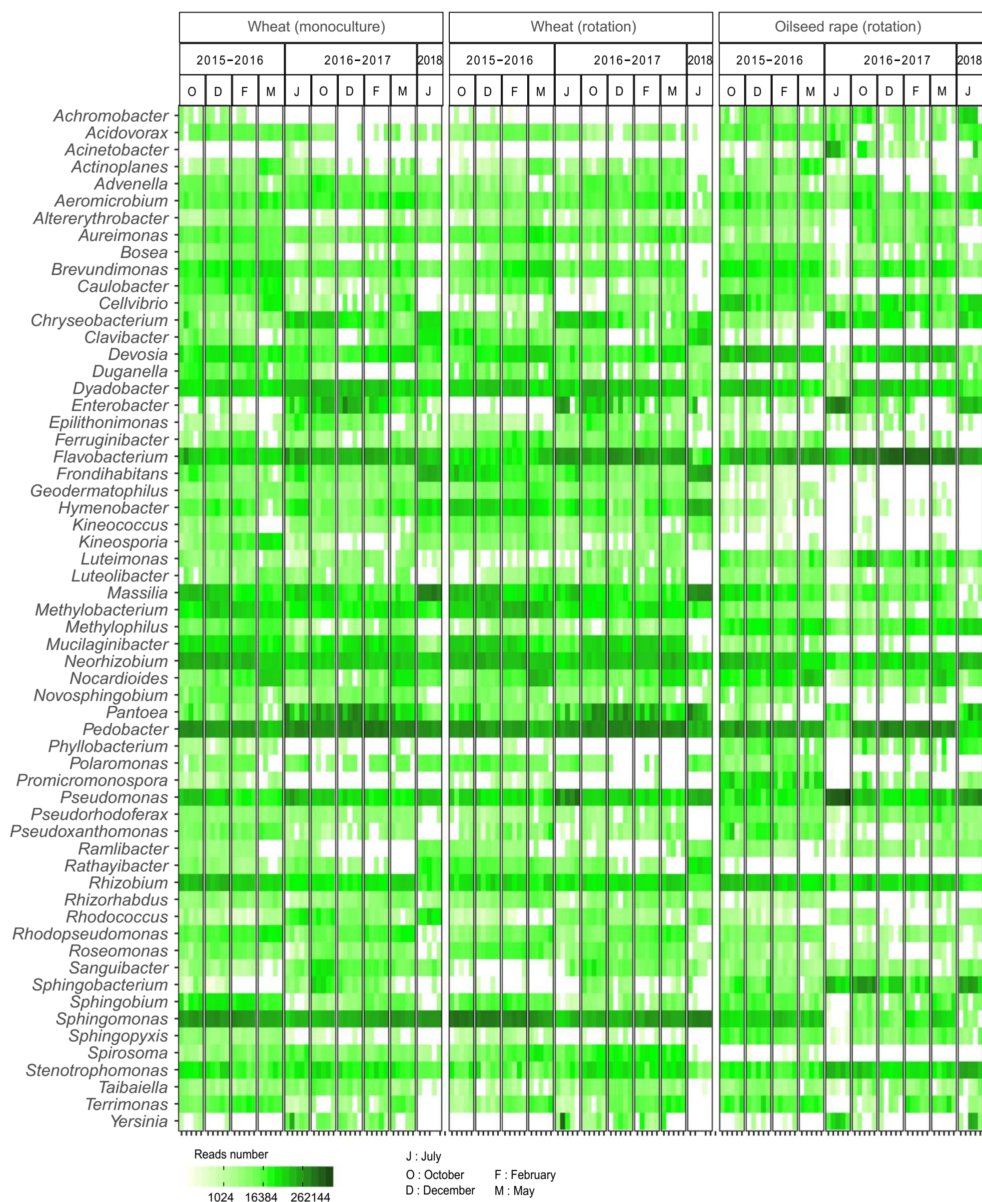

Fig. 5 Distribution of the 60 most prevalent bacterial genera detected in wheat residues in the five samples for each sampling date. Unclassified genera were removed from the visualisation

Discussion

Most studies on crop residues have focused on their impact on soil microbial communities [16], and the rare studies investigating the impact of soil on residue communities focused exclusively on bacteria [27, 28] or fungi [57]. Most of these studies were conducted on residues from a single year. Bastian et al. [12] established an extensive description of the species present in the soil, detritusphere and wheat residues, using sterilised residues and soil in a microcosm. In this study, we showed, under natural conditions, that three main factors (plant species, cropping season, rotation) simultaneously influence the composition of both fungal and bacterial communities present on residues. This study is the first to investigate the total fungal and bacterial communities associated with wheat and oilseed rape residues by a metabarcoding approach over two consecutive years. The very low variability of the communities for the five replicates is remarkable and shows that our strategy would be appropriate for comparing the effects of different treatments on microbial communities.

Crop Residues Should Be Viewed as a Shifting Platform for Microbial Meeting Strongly Affected by Plant Species

Oilseed rape and wheat residues contained different sets of microorganisms before soil contact and during the firsts sampling periods after harvest. Similar results were previously obtained for the bacterial communities of buried crop residues [28]. Consistent with the findings of this previous study, the

divergence between wheat and oilseed rape bacterial communities was probably due to differences in the chemical compounds present in the plants. The rapid change in the community observed at early stages of residue degradation for oilseed rape may be explained by the modification of simple compounds (sugars, starch, etc.), whereas wheat is composed of more complex compounds (lignin) and is, therefore, broken down less quickly, resulting in a slower change in the microbial community [28]. Overall, the change in bacterial community composition highlights turnover between copiotrophs and oligotrophs. Although copiotrophy and oligotrophy are physiological traits, several attempts have been made to classify microorganisms as oligotrophs and copiotrophs based on phylogeny [58]. According to this generalisation, bacterial and fungal taxa whose relative abundances are significantly decreased during succession belong mainly to copiotroph. These taxa include for instance *Alternaria*, *Cladosporium*, *Massilia* and *Pseudomonas* (Additional file Fig. S5). In contrast, the relative abundances of oligotrophic taxa such as *Coprinellus* or *Nocardioides* increased during residues degradation, which could be indicative of the superior abilities of these microorganisms to degrade complex polymers.

The initial fungal communities were structured mostly by the presence of species originating from the plant, several of which were highly specialised on the host plant. These species were gradually replaced by more generalist species, which colonised the residues of both plants. Most of these generalists, such as *Exophiala*, *Coprinellus* and *Torula*, are known to be soil-borne [59, 60], or involved in degradation, such as *Coprinopsis* [61]. The host-specific fungi identified in our study included a large number of ascomycetes known to be foliar pathogens (*O. yallundae*, *Fusarium* sp. and *Gibberella* sp., *Z. tritici*, *P. tritici-repentis*, *Parastagonospora nodorum*, *Monographella nivalis*, *L. biglobosa* and *L. maculans*). The lifestyles of some pathogens are well documented, as for *Z. tritici*, *P. tritici-repentis* and *L. maculans.* The decrease with time in levels of *Z. tritici* and other pathogens in wheat residues contrasts with the persistence of *L. maculans* and *L. biglobosa* in oilseed rape residues. These three pathogens are all known to reproduce sexually on the residues of their host plant [31, 62], but the life cycle of *L. maculans* is characterised by systemic host colonisation through intracellular growth in xylem vessels [63], whereas the development of *Z. tritici* is localised and exclusively extracellular [64]. Oilseed rape residues thus provide *L. maculans* with greater protection than is provided to *Z. tritici* by wheat residues. This likely explains differences in the persistence of the two pathogens and in the temporal dynamics of ascospore release: over up to 2 years for *L. maculans* [65, 66] but only a few months for *Z. tritici* [31, 67]. The predominance of *L. maculans* on oilseed rape residues was not surprising given that the oilseed rape cultivar Alpaga is known to be susceptible to *L. maculans*, but the high abundance of *L. biglobosa* was much more remarkable. One surprising finding of our study was the constant association of *L. maculans* with *L. biglobosa* on residues. Indeed, *L. biglobosa* is known to be more associated with upper stem lesions [68], and its presence in large amounts on residues has never before been reported.

Our findings are consistent with current epidemiological knowledge of emblematic wheat and oilseed rape diseases, but they highlight our lack of knowledge concerning the lifestyles of many other fungal pathogens present on residues. A key point to be taken into account is that the trophic status of many species known to be principally pathogenic or non-pathogenic is not definitive [69]. For instance, *Alternaria infectoria* is sometimes described as a pathogen of wheat [13, 70], sometimes as an endophyte [71], and has even been tested as a potential biocontrol agent against *Fusarium pseudograminearum* on wheat [72]. Crop residues, half-plant/half-soil, should be the focus of future studies aiming to disentangle the succession of microbial species with different lifestyles and to characterise their relative impacts on the development of currently minor, but potentially threatening diseases.

The Residue Microbiota Should Be Analysed in a Dynamic Manner, Both Within and Between Years

The results of our study highlight the importance of conducting multi-year studies focusing on ecological dynamics both within and between years in natural conditions. Year had a strong effect on both bacterial and fungal communities. Fluctuations of climatic conditions (temperature, rainfall, wind) have a major impact on pathogenesis (disease triangle concept [73]) and on the saprophytic survival of plant pathogens during interepidemic periods [74]. The 2 years of our study were marked by similar means of 10-day mean temperatures, but large differences in rainfall: mean 10-day cumulative rainfall in the first year was almost twice that in the second (Additional Table S5). The colonisation of residues by late colonisers may be affected by such climatic differences: in wheat, most prevalent degrading fungi (like *Coprinellus*, *Psathyrella*, *Coprinopsis*) were almost absent in the second year of the study. There was also considerable dissimilarity between the bacterial communities associated with each of the 2 years. For example, genus *Enterobacter*, which was highly abundant in the second year, was barely detectable in the first year.

Crop Rotation Has Little Impact on Residue Microbial Communities

Oilseed rape is never grown in monoculture, so the effect of crop rotation was assessed only for wheat. The effect of rotation on residue microbial communities was much smaller than the effect of year (cropping season). It was more marked for

fungi, for which diversity was greater in monoculture than in rotation. The use of a rotation may prevent the most strongly specialised species, in this case fungi, from becoming established, regardless of their pathogenicity. This finding is consistent with the greater development of some diseases in monoculture conditions, which promote the maintenance of pathogens through the local presence of primary inoculum. For instance, the presence of *P. tritici-repentis*, agent of tan spot disease, in the wheat monoculture plot and its absence from wheat-oilseed rape plots is consistent with epidemiological knowledge indicating that this disease can be controlled by leaving a sufficient interval between consecutive wheat crops in the same field [75].

Lesson to Be Learned from the Residue Microbial Communities for the Sustainable Management of Residue-Borne Diseases: a Delicate Balance Between Pathogenic and Beneficial Microorganisms

The maintenance of crop residues at the surface of the cultivated soil increases the microbial diversity of the soil and, in some ways, helps to maintain good functional homeostasis [76]. However, conservation practices tend to increase the risk of foliar diseases [4–6]. Most disease management strategies focus on epidemic periods, during which the pathogen and its host are in direct contact. Interepidemic periods are also crucial for pathogen development, although during these periods, the primary inoculum is not directly in contact with the new crop whilst not present in the field. Indeed, by carrying the sexual reproduction of several fungal pathogens, residues contribute to the generation and transmission of new virulent isolates potentially overcoming resistance genes, during monocyclic epidemics, as described for oilseed rape canker caused by *L. maculans* [77], but also polycyclic epidemics, as described for Septoria tritici blotch caused by *Z. tritici* [78].

However, the results of our study suggest that residues should not only be considered as a substrate for pathogens and a potential source of inoculum. Indeed, we detected several fungi identified as beneficial or even biocontrol agents in previous studies, such as *Clonostachys rosea*, *Aureobasidium pullulans*, *Chaetomium globosum* and *Cryptococcus* spp. *C. rosea*, which was detected in both oilseed rape and wheat residues, has been reported to limit the sexual and asexual reproduction of *Didymella rabiei* on chickpea residues by mycoparasitism [79]. It has also been reported to be effective against *Fusarium culmorum* on wheat plants, through antibiosis during the epidemic period [80], and on wheat residues, through antagonism during the interepidemic period [81]. *Cladosporium* sp., which was abundant in our study, has also been reported to inhibit the development of *P. tritici-repentis* on wheat plants [82] and of *Fusarium* sp. on wheat residues [81]. The presence of these fungal species on wheat and oilseed rape residues is of potential interest for future analyses of interactions. Due to the use of a low-resolution marker for bacterial characterisation, we were unable to identify similarly the bacteria potentially interacting with pathogenic fungi. For instance, the presence of *Pseudomonas* spp. suggests possible interactions both with other microbial species and with the host plant [83], but the nature of the potential interactions is indeterminate: species of the *Pseudomonas fluorescens* group are known to be beneficial to plants, whereas *Pseudomonas syringae* and *Pseudomonas aeruginosa* are known to be pathogens of plants and even humans.

Although our study reveals the presence of genera or species reported in the literature as biocontrol agents, it has not yet shown any interaction between them and the pathogens. This experimental study (sampling effort, residue treatments, etc.) was not designed to characterise such interactions. A strategy involving the inference of microbial interaction networks from metabarcoding datasets might help to identify the species beneficial against pathogens, through competition, antagonism or parasitism. This however requires a more analytical, comparative experimental approach that goes beyond the only description of shifts in natural communities composition, for example using different 'treatments' in a broad sense (e.g. artificial inoculation with a species or a group of species, change of biotic or abiotic environmental conditions, etc.) in order to modify interaction networks and so highlight the impact of some groups of microorganisms on the whole community or a given species.

Conclusion

This study shows that crop residues, which can be seen as half-plant/half-soil transient compartment, constitute a pivotal fully-fledged microbial ecosystem that has received much less attention than the phyllosphere and rhizosphere to date. This study therefore fills a gap in knowledge of the communities present on crop residues under natural conditions. It confirms that the microbiote of crop residues should be taken into account in the management of residue-borne diseases. Taking into account this ecosystem is essential, not only to improve the quantitative management of crop residues, but also to identify groups of beneficial microorganisms naturally present. The beneficial elements of the microbial community should be preserved, or even selected, characterised and used as biological control agents against the pathogens that complete their life cycle on the residues. These results are particularly important in that wheat-oilseed rape rotations are among the most widespread arable cropping systems in France and Europe.

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Authors' Contributions LK, FS, VL, MHB and MB conceived the study, participated in its design, and wrote the manuscript. LK conducted the experiments and analysed the data. FS and VL supervised the project. All authors read and approved the final manuscript.

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Data Availability The raw sequencing data is available from the European Nucleotide Archive (ENA) under the study accession PRJEB27255 (sample SAMEA4723701 to SAMEA4724326). We provide the command-line script for data analysis and all necessary input files as Additional File 2.

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Table S1 - Soil texture of the three plots (WWW, OWO and WOW).

	WWW, OWO	WOW
Clay (%)	27.4	18.2
Silt (%)	53.2	61.2
Sand (%)	18.8	20.4

Table S2 - Sampling dates of wheat and oilseed rape plants (July) and residues (October, December, February, and May) for each cropping season.

Cropping season	July	October	December	February	May
2015-2016	/	23.10.2015	04.12.2015	26.02.2016	19.05.2016
2016-2017	11.07.2016	17.10.2016	05.12.2016	06.02.2016	15.05.2017
2017-2018	07.07.2017	/	/	/	/

Table S3 - Total number of reads and percentage (in brackets) remaining after ASV filtering.

	After DADA2	After replicate suppression	After taxon suppression
Bacterial reads	14,287,970	13,509,461 (94.6%)	13,228,976 (92.6%)
Fungal reads	9,898,487	9,753,628 (98.5%)	9,628,995 (97.3%)
Bacterial haplotypes	19,235	2,905	2,726
Fungal haplotypes	3,587	1,241	1,189

Table S4 - Summary of meteorological data (temperature, rainfall) for the INRA Grignon experimental station (Yvelines, France), obtained from the CLIMATIK INRA database (https://intranet.inra.fr/climatik_v2/) from July 1st to May 31st of the following year, for the cropping seasons 2015-2016 and 2016-2017.

	10-day mean temperature (°C)		10-day cumulative rainfall (mm)	
	2015-2016	2016-2017	2015-2016	2016-2017
Mean	11.2	10.8	22.6	12.3
Minimum	2.0	0.9	0	0
Maximum	21.8	21.4	131	55

Table S5 - Plant effect (wheat vs. oilseed rape) on community dispersion. This effect was tested by applying the adonis function of the vegan R package to the Bray-Curtis dissimilarity index (PERMANOVA). *P*-values (not shown) were all < 0.02.

	Fungi				Bacteria			
	All	2015-2016	2016-2017	2017	All	2015-2016	2016-2017	2017
July	0.372	/	0.611	0.755	0.423	/	0.540	0.696
October	0.495	0.612	0.755	/	0.367	0.520	0.659	/
December	0.486	0.688	0.691	/	0.370	0.573	0.641	/
February	0.429	0.541	0.651	/	0.409	0.643	0.611	/
May	0.273	0.337	0.401	/	0.315	0.435	0.508	/

Table S6 - Decomposition of dissimilarity due to temporal changes in fungal (F) and bacterial (B) community composition. Total dissimilarity is broken down into turnover (replacement of ASV) and nestedness (gain or loss of ASV).

Crop within a rotation	Season	Sampling period compared	Total dissimilarity		Turnover		Nestedness	
			F	B	F	B	F	B
Oilseed rape	2015-2016	Oct. - Dec.	0.622	0.318	0.618	0.219	0.005	0.099
Oilseed rape	2015-2016	Dec. - Feb.	0.650	0.321	0.577	0.290	0.073	0.031
Oilseed rape	2015-2016	Feb. - May	0.591	0.390	0.565	0.202	0.027	0.188
Oilseed rape	2016-2017	Jul. - Oct.	0.652	0.554	0.648	0.250	0.004	0.304
Oilseed rape	2016-2017	Oct. - Dec.	0.620	0.353	0.549	0.217	0.071	0.136
Oilseed rape	2016-2017	Dec. - Feb.	0.585	0.353	0.516	0.276	0.068	0.077
Oilseed rape	2016-2017	Feb. - May	0.529	0.384	0.529	0.342	0.000	0.042
Wheat in monoculture	2015-2016	Oct. - Dec.	0.427	0.330	0.425	0.142	0.002	0.188
Wheat in monoculture	2015-2016	Dec. - Feb.	0.444	0.294	0.416	0.190	0.028	0.104
Wheat in monoculture	2015-2016	Feb. - May	0.444	0.458	0.424	0.255	0.020	0.203
Wheat in monoculture	2016-2017	Jul. - Oct.	0.438	0.346	0.424	0.300	0.014	0.046
Wheat in monoculture	2016-2017	Oct. - Dec.	0.463	0.330	0.257	0.113	0.207	0.217
Wheat in monoculture	2016-2017	Dec. - Feb.	0.386	0.248	0.311	0.200	0.075	0.048
Wheat in monoculture	2016-2017	Feb. - May	0.344	0.332	0.341	0.213	0.004	0.120
Wheat in rotation	2015-2016	Oct. - Dec.	0.425	0.317	0.409	0.157	0.016	0.160
Wheat in rotation	2015-2016	Dec. - Feb.	0.472	0.266	0.370	0.185	0.102	0.081
Wheat in rotation	2015-2016	Feb. - May	0.505	0.347	0.432	0.311	0.073	0.035
Wheat in rotation	2016-2017	Jul. - Oct.	0.498	0.313	0.427	0.272	0.071	0.041
Wheat in rotation	2016-2017	Oct. - Dec.	0.541	0.287	0.292	0.214	0.249	0.073
Wheat in rotation	2016-2017	Dec. - Feb.	0.350	0.284	0.292	0.284	0.059	0.000
Wheat in rotation	2016-2017	Feb. - May	0.424	0.334	0.329	0.287	0.095	0.047
Mean			0.496	0.341	0.436	0.234	0.060	0.107

Figure S1 - Bacterial diversity in plants (July) and residues (October, December, February, May), as assessed with the Faith's Phylogenetic Diversity index (PD), according to sampling period, the crop within a rotation (oilseed rape in OWO or WOW, wheat in WWW, wheat in WOW or OWO) and the cropping season (2015-2016, 2016-2017). Each box represents the distribution of PD for five sampling points. Kruskal-Wallis tests were performed for each "crop within a rotation * cropping season" combination (p-values are given under each graph). Wilcoxon tests between sampling periods were performed when the Kruskal-Wallis test revealed significant differences. Samples not sharing letters are significantly different.

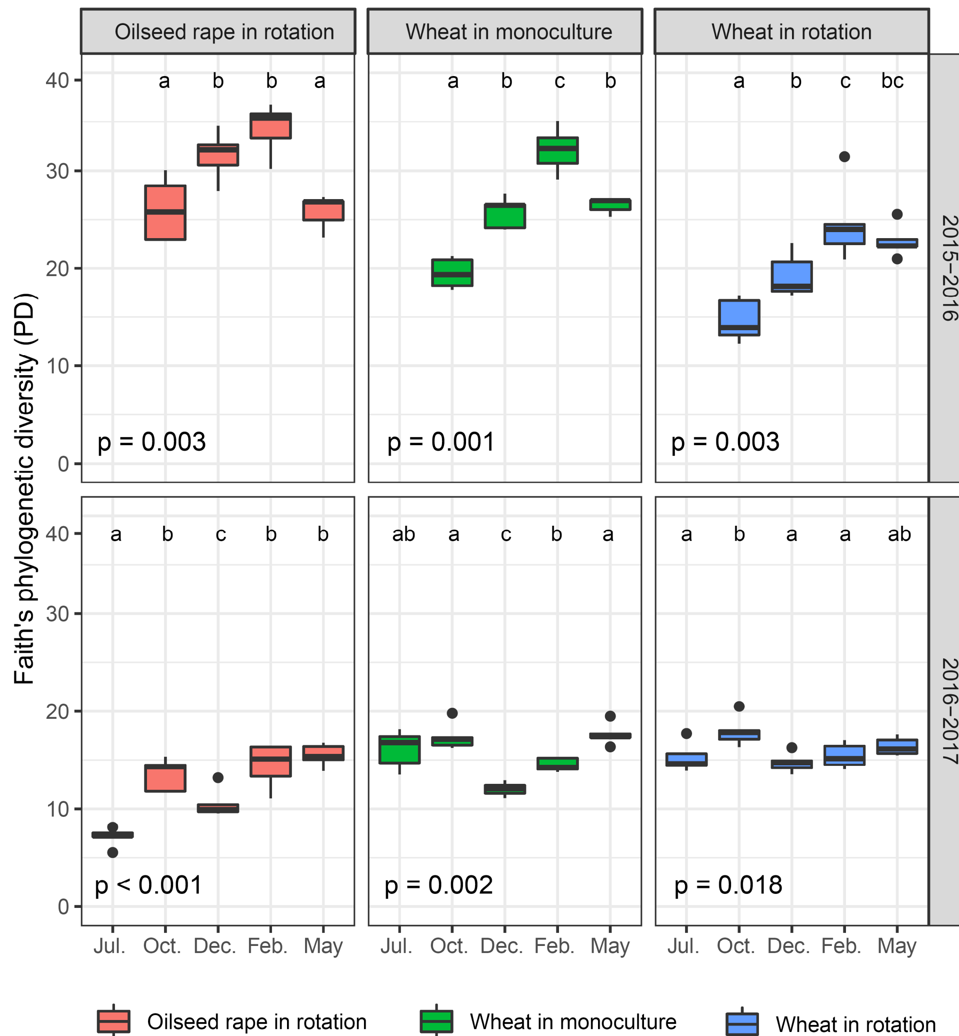


Figure S2 - Structure of the bacterial communities present in oilseed rape and wheat residues visualized by incorporating phylogenetic distances using the UniFrac distance matrix. MDS were performed on the overall dataset and faceted according to the sampling period. Each point represents one sample corresponding to a cropping season (shape: 2015-2016; 2016-2017; July 2017) and crop within a rotation (colour: oilseed rape in rotation, i.e. in WOW and OWO; wheat monoculture, i.e. in WWW; wheat in rotation, i.e. in WOW and OWO).

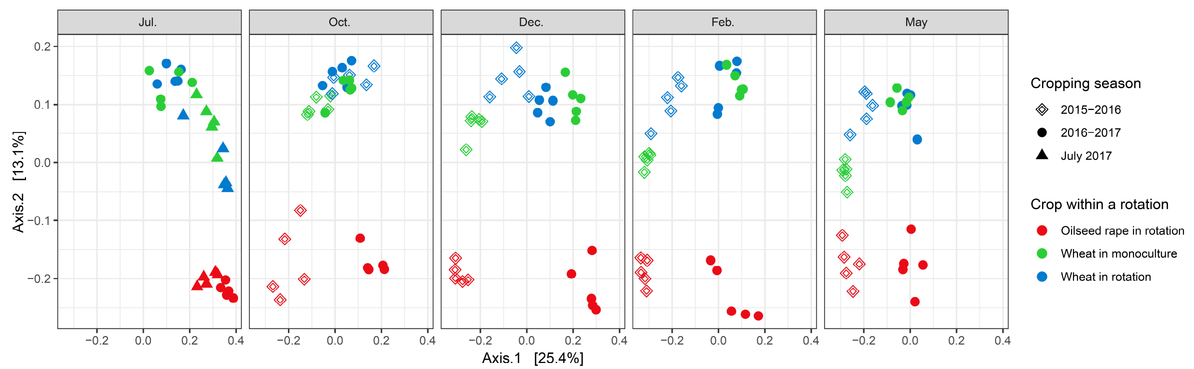


Figure S3 - Distribution of the most prevalent fungal genera detected in wheat residues. (**A**) Cladogram of the most prevalent genera. Genera were filtered according to their occurrence (at least three times in the five sampling points for each "crop within a rotation * cropping season * sampling period" combination). Unclassified genera were removed from the tree. (**B**) Number of ASV of each genus. (**C**) Occurrence of each ASV in the 100 samples of wheat residues. (**D**) Percentage of reads for each genus. (**E**) Distribution of each genus in the five samples per date (increasing number of reads shown on a scale running from yellow to red).

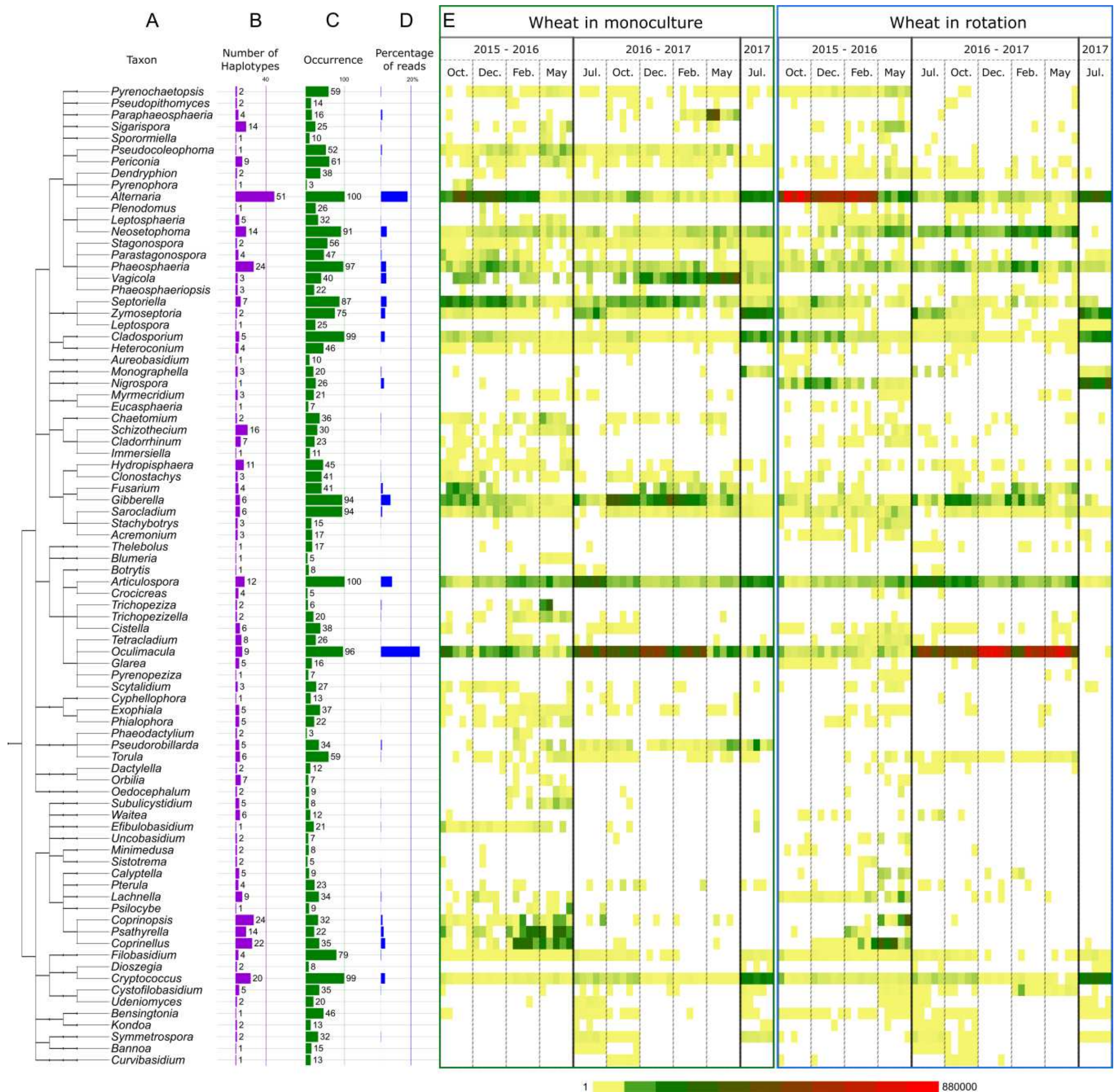


Figure S4 - Distribution of the most prevalent fungal genera detected in oilseed rape residues. (**A**) Cladogram of the most prevalent genera. Genera were filtered according to their occurrence (at least three times in the five sampling points for each "crop within a rotation * cropping season * sampling period") combination. Unclassified genera were removed from the tree. (**B**) Number of ASV for each genus. (**C**) Occurrence of each ASV in the 50 samples of oilseed rape residues. (**D**) Percentage of reads for each genus. (**E**) Distribution of each genus in the five samples per date (increasing number of reads shown on a scale from yellow to red).

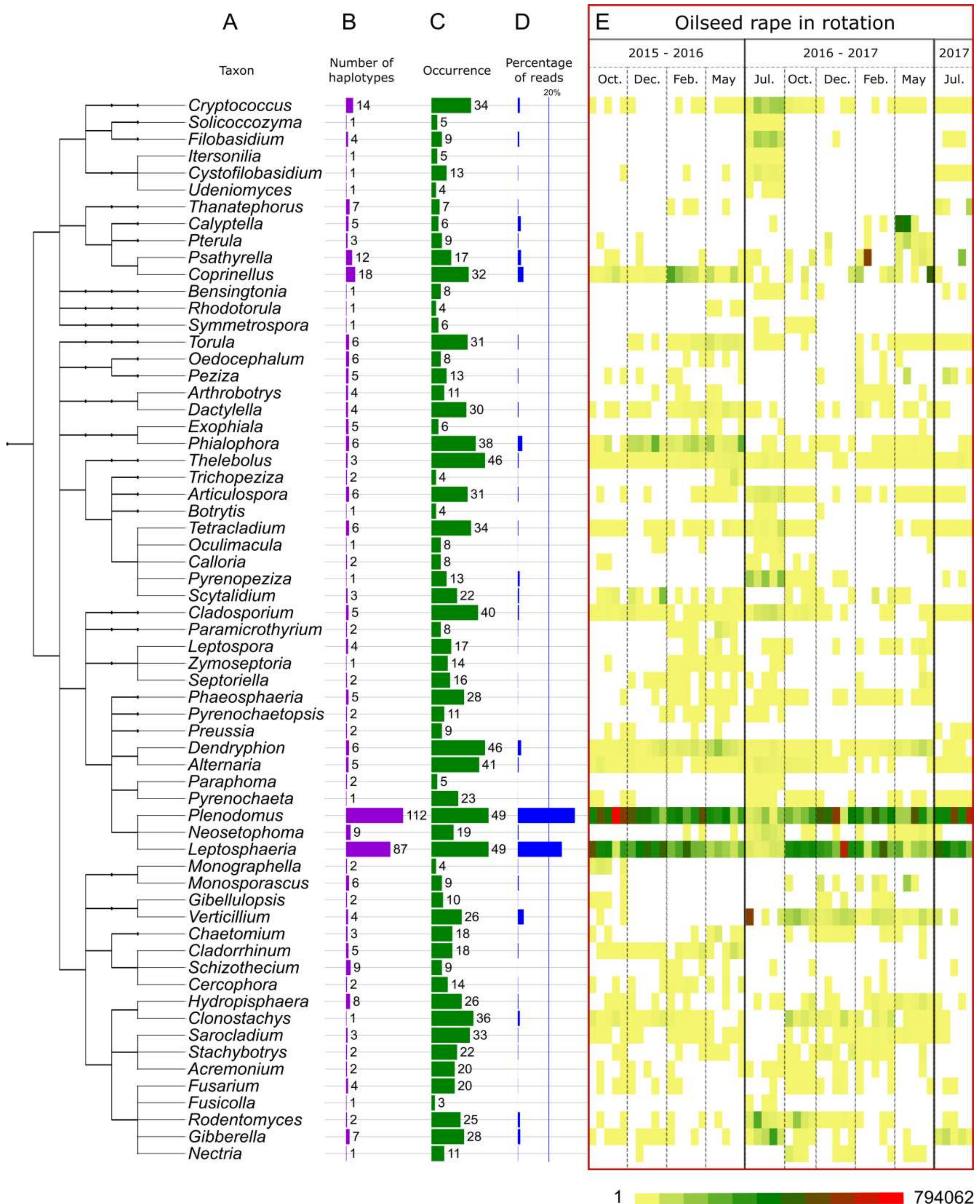
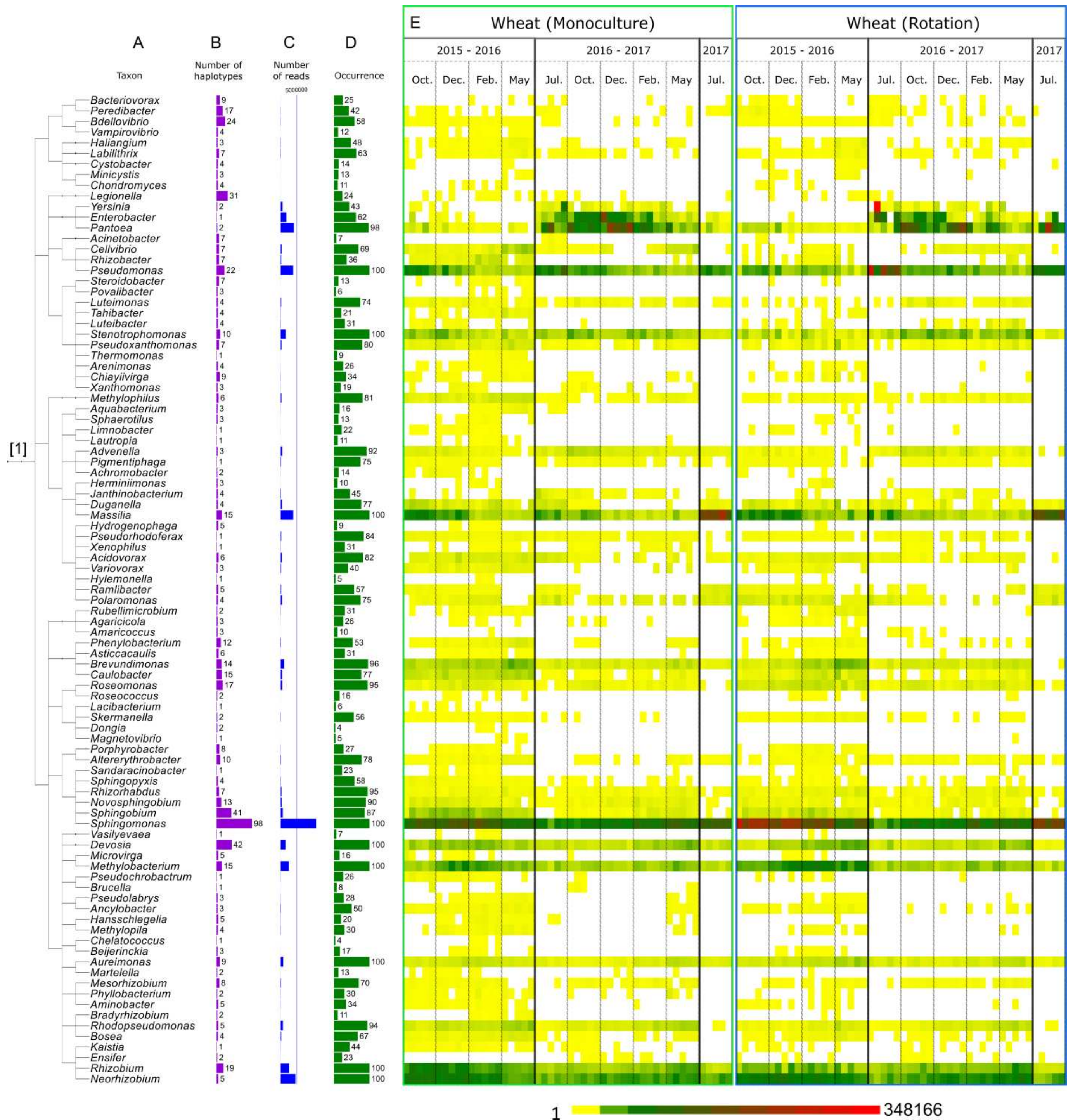
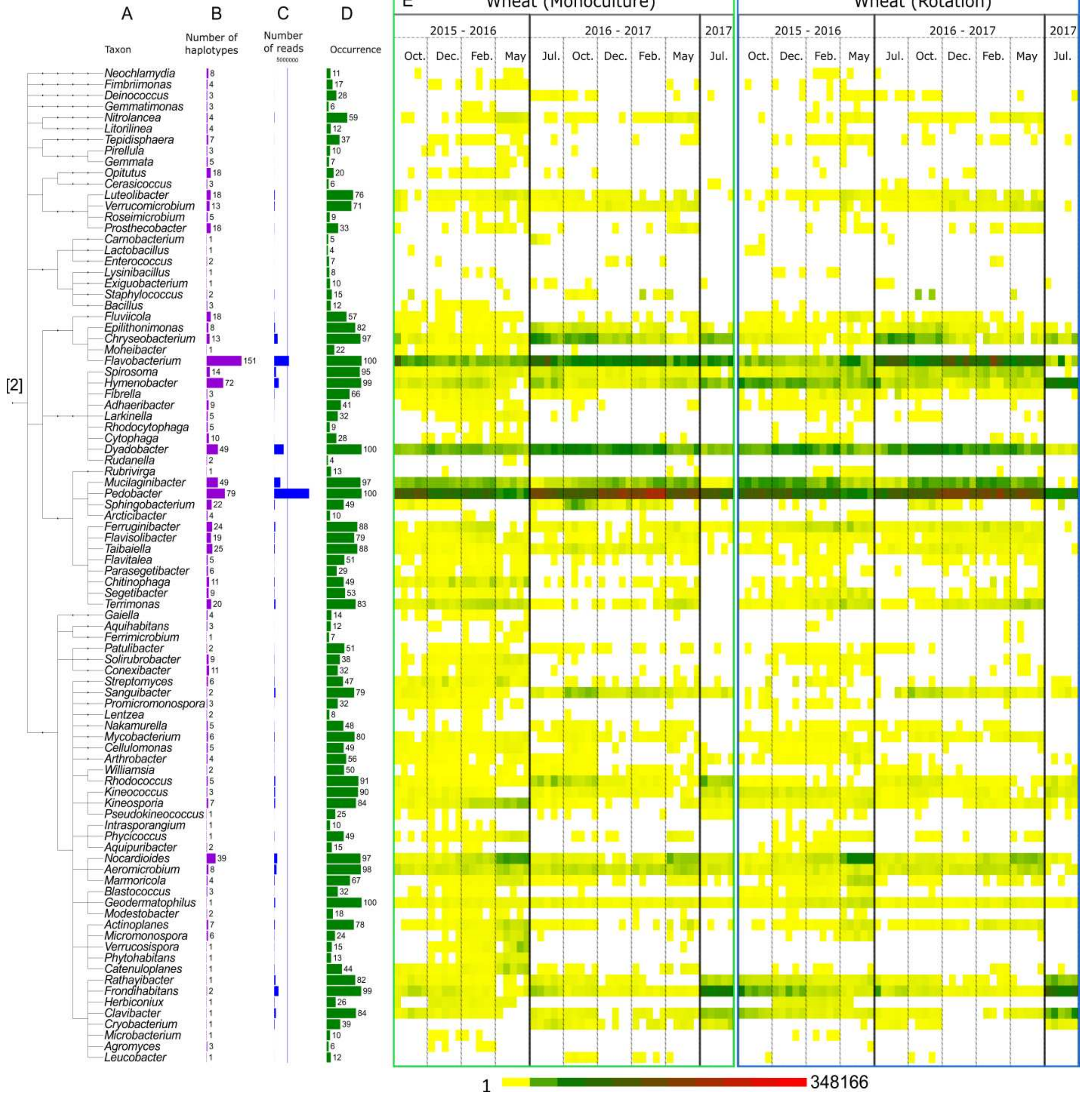


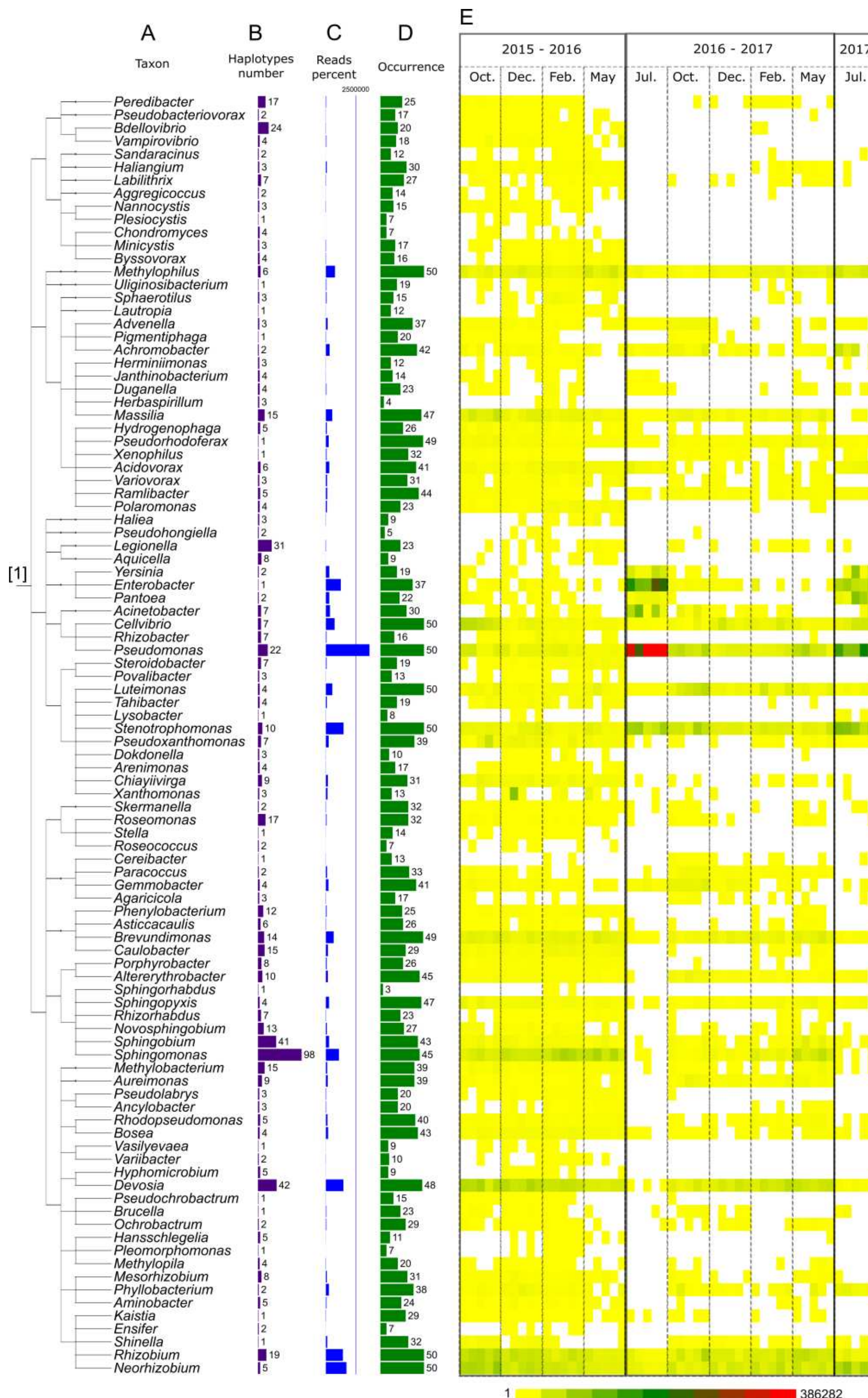
Figure S5 - Distribution of the most prevalent bacterial genera detected in wheat residues. **(A)** Cladogram of the most prevalent genera. Genera were filtered according to their occurrence (at least three times in five sampling points for each "crop within rotation * cropping season * sampling period" combination). Unclassified genera were removed from the tree. **(B)** Number of ASV for each genus. **(C)** Occurrence of each ASV in the 100 samples of wheat residues. **(D)** Number of reads for each genus. **(E)** Distribution of each genus in the five samples per date (increasing numbers of reads on a scale running from yellow to red). Due to the number of genera, the plot is separated in [1] proteo- and [2] other bacteria.

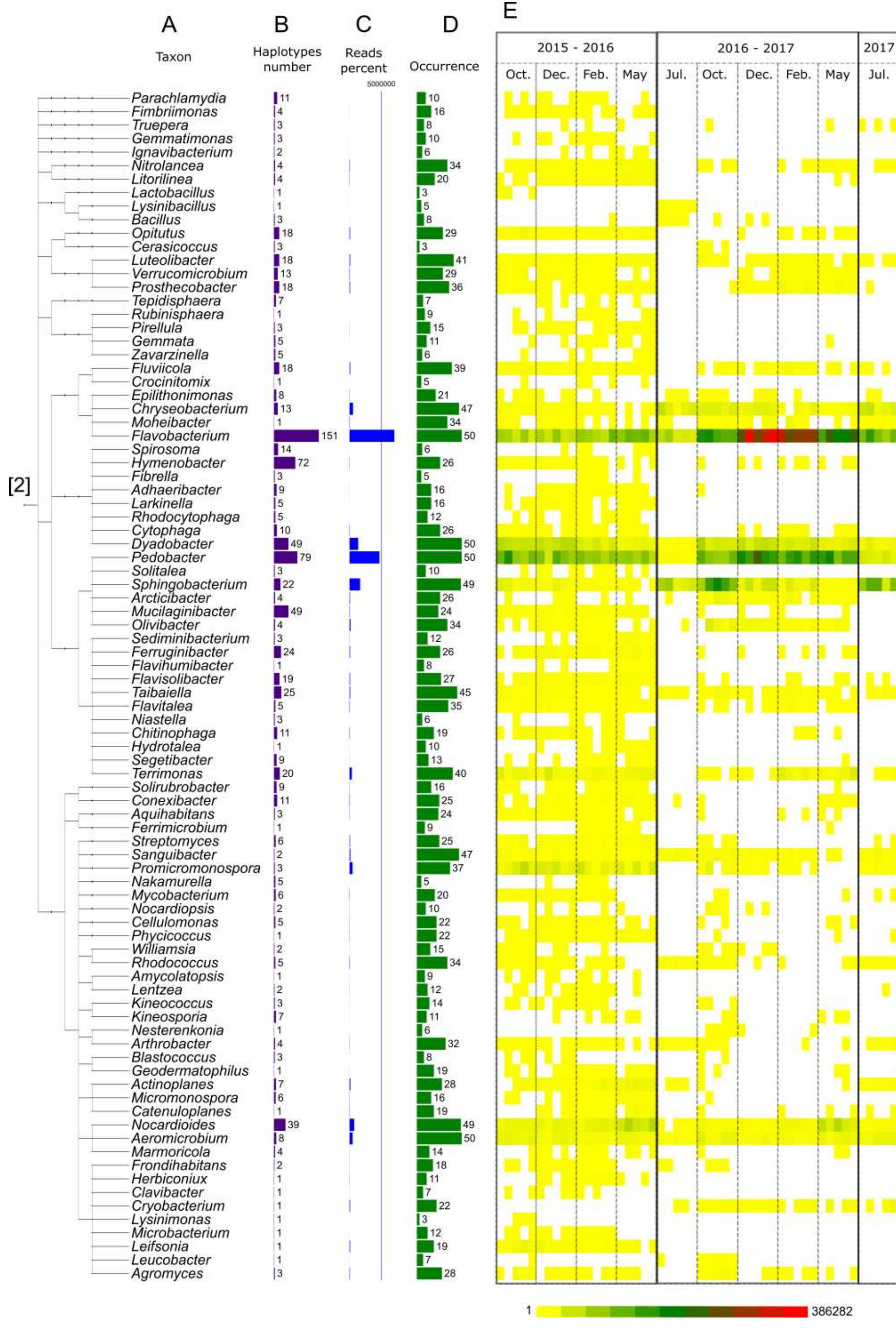




A
B
C
D
Taxon
Number of haplotypes
Number of reads
5000000
Occurrence
[2]
Neochlamydia 8 11
Fimbriimonas 4 17
Deinococcus 3 28
Gemmatimonas 3 6
Nitrolancea 4 59
Litorilinea 4 12
Tepidisphaera 7 37
Pirellula 3 10
Gemmata 5 7
Opitutus 18 20
Cerasicoccus 3 6
Luteolibacter 18 76
Verrucomicrobium 13 71
Roseimicrobium 5 9
Prosthecobacter 18 33
Carnobacterium 1 5
Lactobacillus 1 4
Enterococcus 2 7
Lysinibacillus 1 8
Exiguobacterium 1 10
Staphylococcus 2 15
Bacillus 3 12
Fluviicola 18 57
Epilithonimonas 8 82
Chryseobacterium 13 97
Moheibacter 1 22
Flavobacterium 151 100
Spirosoma 14 95
Hymenobacter 72 99
Fibrella 3 66
Adhaeribacter 9 41
Larkinella 5 32
Rhodocytophaga 5 9
Cytophaga 10 28
Dyadobacter 49 100
Rudanella 2 4
Rubrivirga 1 13
Mucilaginibacter 49 97
Pedobacter 79 100
Sphingobacterium 22 49
Arcticibacter 4 10
Ferruginibacter 24 88
Flavisolibacter 19 79
Taibaiella 25 88
Flavitalea 5 51
Parasegetibacter 6 29
Chitinophaga 11 49
Segetibacter 9 53
Terrimonas 20 83
Gaiella 4 14
Aquihabitans 3 12
Ferrimicrobium 1 7
Patulibacter 2 51
Solirubrobacter 9 38
Conexibacter 11 32
Streptomyces 6 47
Sanguibacter 2 79
Promicromonospora 3 32
Lentzea 2 8
Nakamurella 5 48
Mycobacterium 6 80
Cellulomonas 5 49
Arthrobacter 4 56
Williamsia 2 50
Rhodococcus 5 91
Kineococcus 3 90
Kineosporia 7 84
Pseudokineococcus 1 25
Intrasporangium 1 10
Phycicoccus 1 49
Aquipuribacter 2 15
Nocardioides 39 97
Aeromicrobium 8 98
Marmoricola 4 67
Blastococcus 3 32
Geodermatophilus 1 100
Modestobacter 2 18
Actinoplanes 7 78
Micromonospora 6 24
Verrucosispora 1 15
Phytohabitans 1 13
Catenuloplanes 1 44
Rathayibacter 1 82
Frondihabitans 2 99
Herbiconiux 1 26
Clavibacter 1 84
Cryobacterium 1 39
Microbacterium 1 10
Agromyces 3 6
Leucobacter 1 12
E
Wheat (Monoculture)
Wheat (Rotation)
2015 - 2016
2016 - 2017
2017
Oct.
Dec.
Feb.
May
Jul.
1
348166

Figure S6 - Distribution of the most prevalent bacterial genera detected in oilseed rape residues. **(A)** Cladogram of most prevalent genera. Genera were filtered according to their occurrence (at least three times in five sampling points for each "crop within rotation * cropping season * sampling period" combination). Unclassified genera were removed from the tree. **(B)** Number of ASV for each genus. **(C)** Occurrence of each ASV in the 49 samples of oilseed rape residues. **(D)** Number of reads for each genus. (E) Distribution of each genus in the five samples per date (increasing number of reads shown on a scale running from yellow to red). Due to the number of genera, the plot is separated in [1] proteo- and [2] other bacteria.

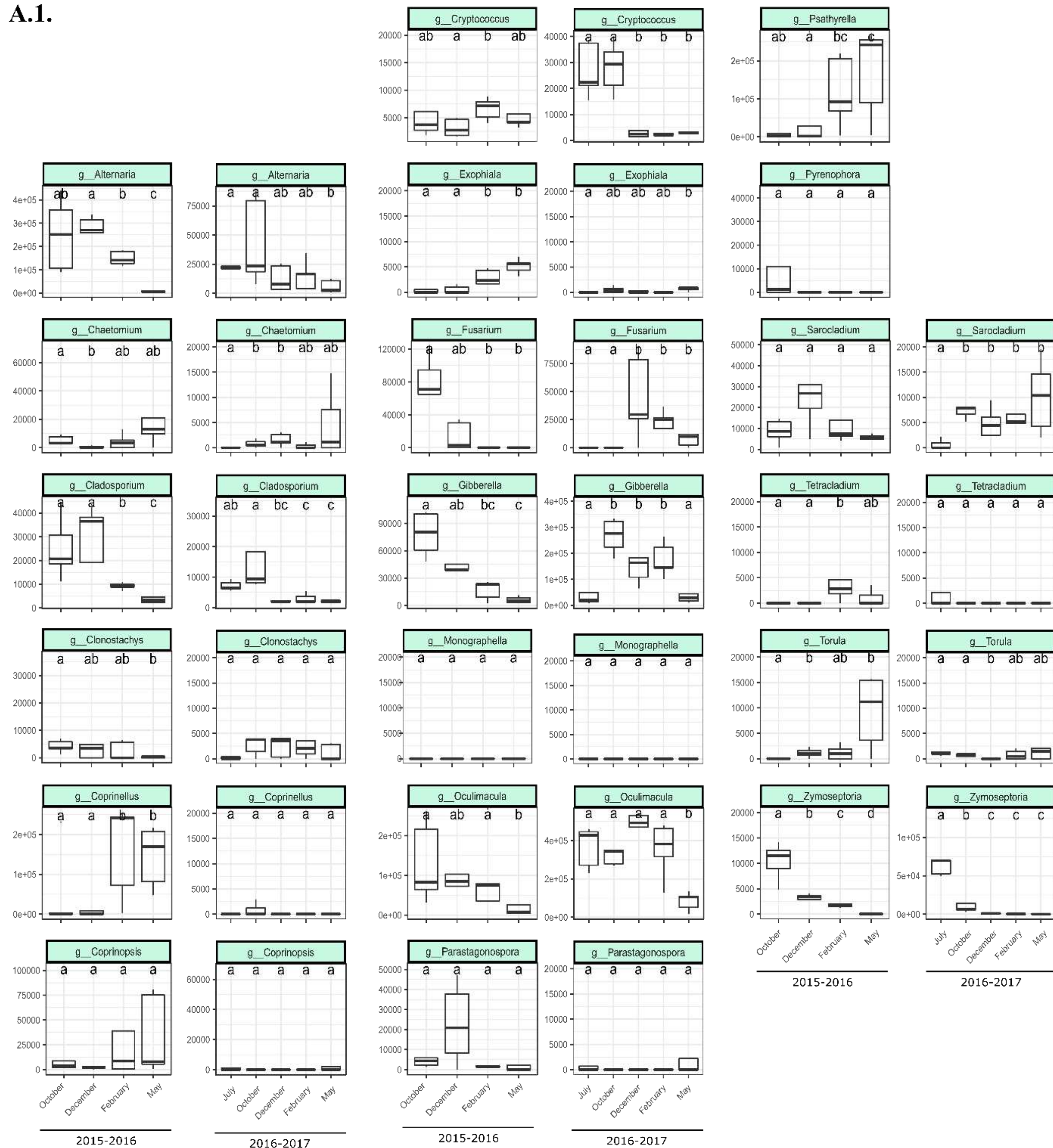




A
B
C
D
E
Taxon
Haplotypes number
Reads percent
Occurrence
5000000
2015 - 2016
2016 - 2017
2017
Oct.
Dec.
Feb.
May
Jul.
Oct.
Dec.
Feb.
May
Jul.
[2]
Parachlamydia
Fimbriimonas
Truepera
Gemmatimonas
Ignavibacterium
Nitrolancea
Litorilinea
Lactobacillus
Lysinibacillus
Bacillus
Opitutus
Cerasicoccus
Luteolibacter
Verrucomicrobium
Prosthecobacter
Tepidisphaera
Rubinisphaera
Pirellula
Gemmata
Zavarzinella
Fluviicola
Crocinitomix
Epilithonimonas
Chryseobacterium
Moheibacter
Flavobacterium
Spirosoma
Hymenobacter
Fibrella
Adhaeribacter
Larkinella
Rhodocytophaga
Cytophaga
Dyadobacter
Pedobacter
Solitalea
Sphingobacterium
Arcticibacter
Mucilaginibacter
Olivibacter
Sediminibacterium
Ferruginibacter
Flavihumibacter
Flavisolibacter
Taibaiella
Flavitalea
Niastella
Chitinophaga
Hydrotalea
Segetibacter
Terrimonas
Solirubrobacter
Conexibacter
Aquihabitans
Ferrimicrobium
Streptomyces
Sanguibacter
Promicromonospora
Nakamurella
Mycobacterium
Nocardiopsis
Cellulomonas
Phycicoccus
Williamsia
Rhodococcus
Amycolatopsis
Lentzea
Kineococcus
Kineosporia
Nesterenkonia
Arthrobacter
Blastococcus
Geodermatophilus
Actinoplanes
Micromonospora
Catenuloplanes
Nocardioides
Aeromicrobium
Marmoricola
Frondihabitans
Herbiconiux
Clavibacter
Cryobacterium
Lysinimonas
Microbacterium
Leifsonia
Leucobacter
Agromyces
1
386282

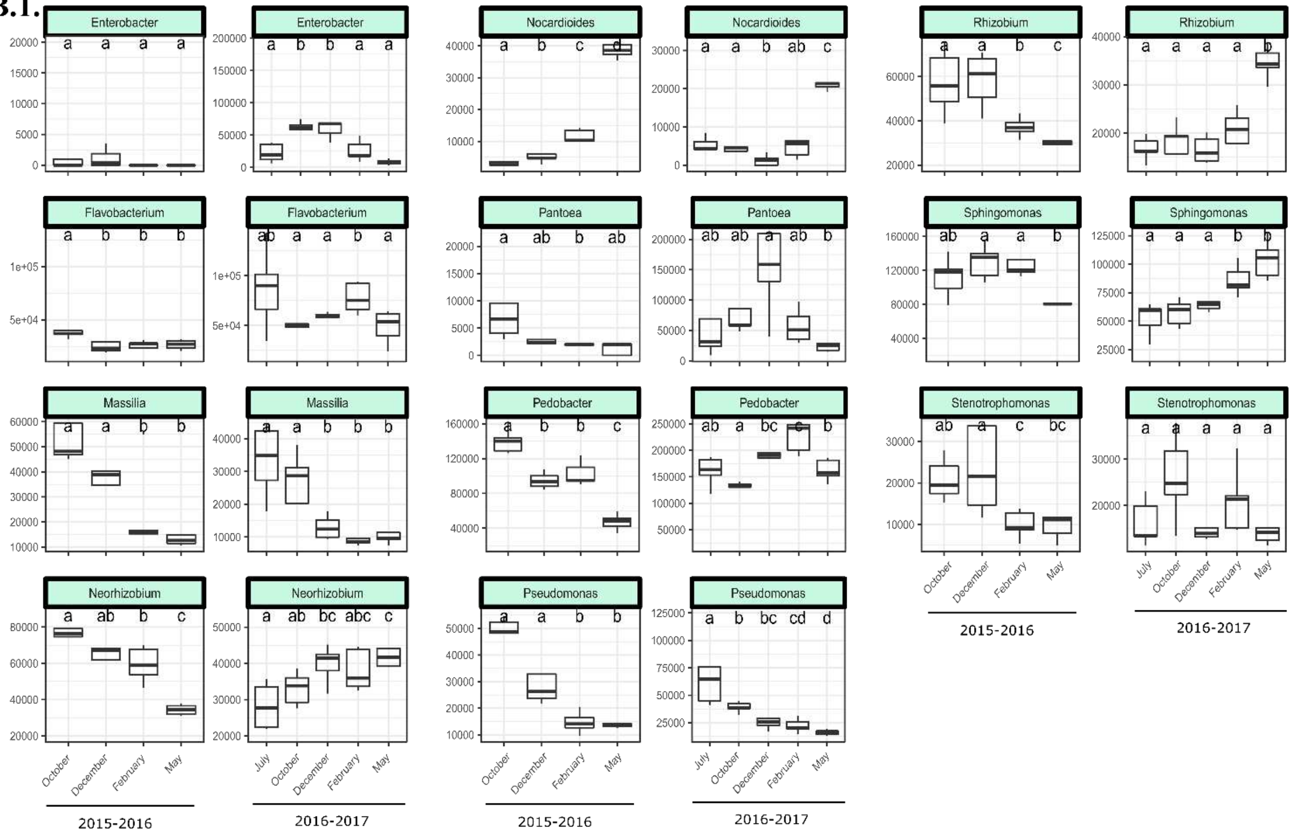
Figure S7 - Seasonal shift in the relative abundance of a selection of fungal **(A)** and bacterial **(B)** genera present on wheat and oilseed rape residues according to the system (wheat monoculture **[1]**, wheat in rotation **[2]**, oilseed rape in rotation **[3]**) and the cropping season (2015-2016, 2016-2017). Due to the plant impact (wheat and oilseed rape) in the fungal community, the fungal genera used here as examples are different for the two plants, unlike the case of the bacterial community. Each box represents the distribution of genera relative abundance for the five sampling points. Samples not sharing letters are significantly different (Wilcoxon tests between sampling periods).

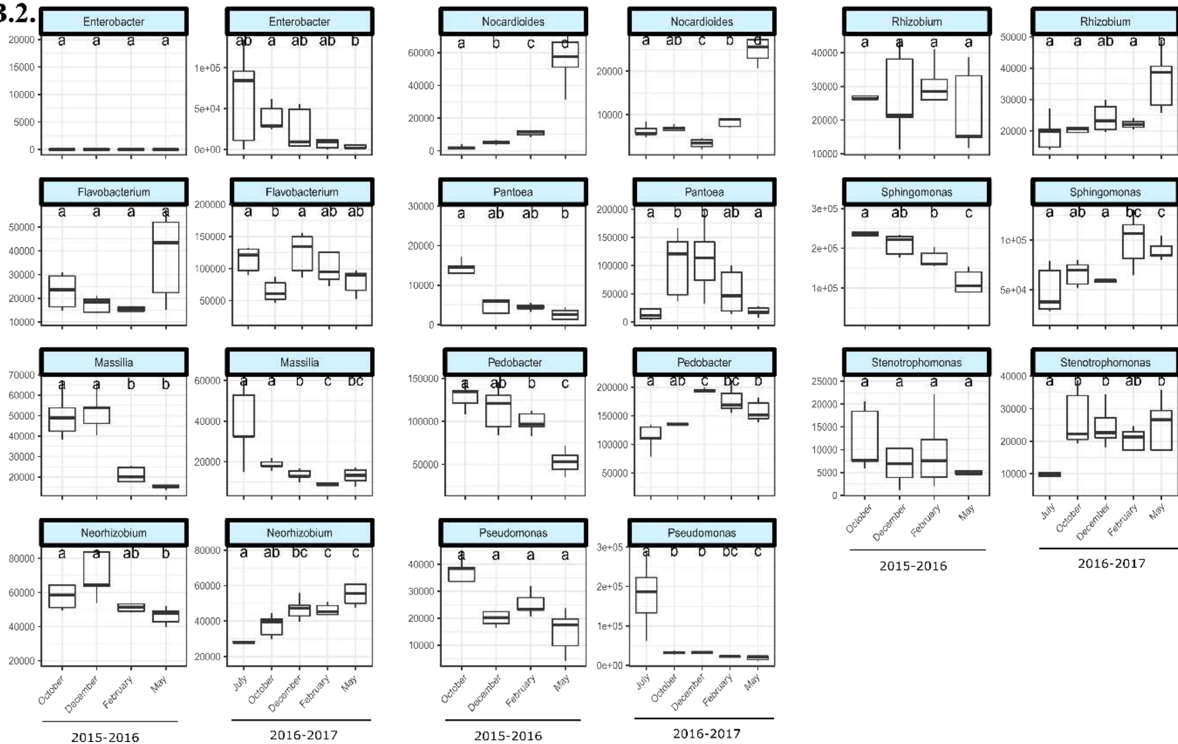
A.1.



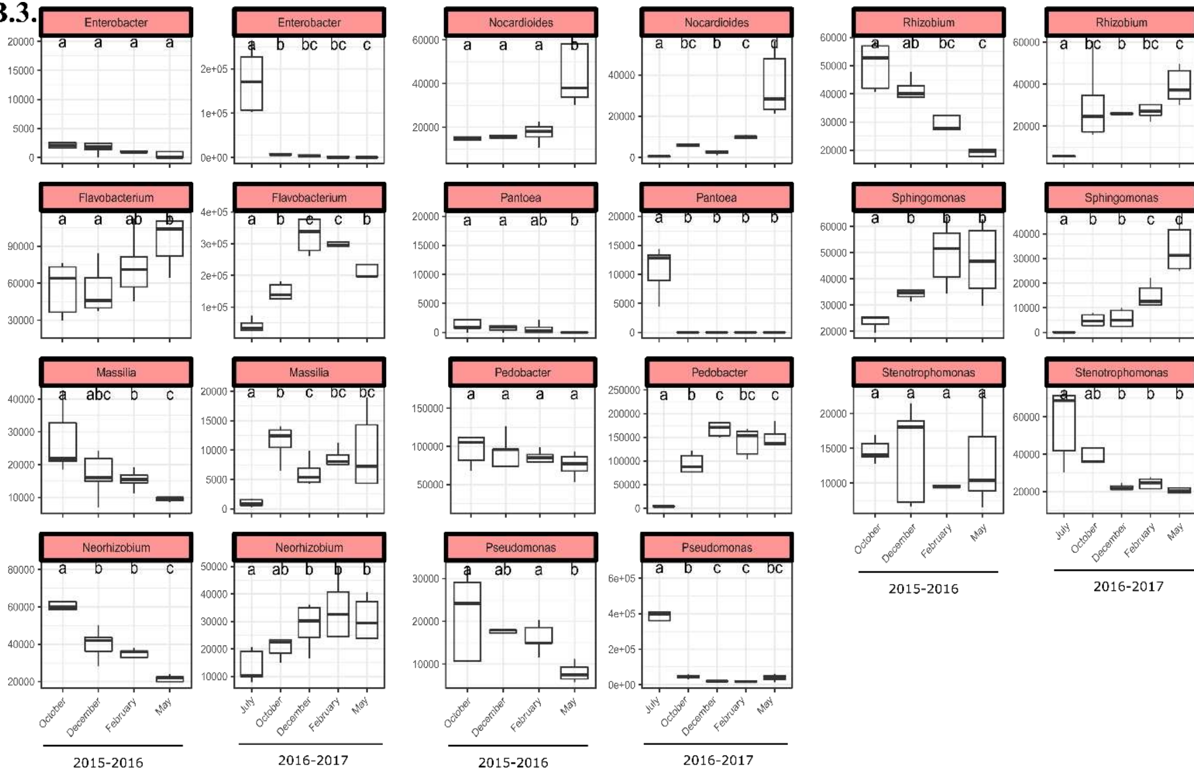
A.2.

A.3.
g__Acremonium
g__Cryptococcus
g__Pyrenopeziza
g__Alternaria
g__Exophiala
g__Sarocladium
g__Chaetomium
g__Fusarium
g__Tetracladium
g__Cladosporium
g__Leptosphaeria
g__Torula
g__Clonostachys
g__Plenodomus
g__Verticillium
g__Coprinellus
g__Psathyrella
July
October
December
February
May
2015-2016
2016-2017


B.1.
Enterobacter
Enterobacter
Nocardioides
Nocardioides
Rhizobium
Rhizobium
Flavobacterium
Flavobacterium
Pantoea
Pantoea
Sphingomonas
Sphingomonas
Massilia
Massilia
Pedobacter
Pedobacter
Stenotrophomonas
Stenotrophomonas
Neorhizobium
Neorhizobium
Pseudomonas
Pseudomonas
October
December
February
May
July
2015-2016
2016-2017


B.2.
Enterobacter
Enterobacter
Nocardioides
Nocardioides
Rhizobium
Rhizobium
Flavobacterium
Flavobacterium
Pantoea
Pantoea
Sphingomonas
Sphingomonas
Massilia
Massilia
Pedobacter
Pedobacter
Stenotrophomonas
Stenotrophomonas
Neorhizobium
Neorhizobium
Pseudomonas
Pseudomonas
October
December
February
May
July
2015-2016
2016-2017

B.3.



Chapitre II. 2

Comparaison entre deux méthodes culture-dépendante et culture-indépendante appliquées à l'analyse de la diversité bactérienne et fongique de résidus de blé et de colza

Comparaison entre deux méthodes culture-dépendante et culture-indépendante appliquées à l'analyse de la diversité bactérienne et fongique de résidus de blé et de colza

Lydie Kerdraon, Benjamin Boudier, Anne-Lise Liabot, Coralie Marais, Marion Fischer - Le Saux, Matthieu Barret, Mylène Balesdent, Valérie Laval, Frédéric Suffert[2]

Introduction

Au cours des dernières années, grâce à l'utilisation des techniques de séquençage haut débit, les connaissances sur la diversité des communautés microbiennes ont considérablement augmenté. L'utilisation de ces techniques élimine certains des biais qui existent avec celles basées sur la culture de microorganismes [1], et prennent en compte une plus grande complexité que celles qui sont exclusivement culture-dépendantes, par exemple pour les communautés de la phyllosphère [2] ou celle du sol [3]. Il a par exemple été estimé que moins de 1% des microorganismes du sol pouvaient être isolés et cultivés avec les techniques actuellement disponibles [4]. Une étude qui serait limitée, pour des raisons méthodologiques, à la fraction cultivable du sol sous-estimerait considérablement la diversité microbienne [5]. Cette prise de conscience a conduit à optimiser les techniques usuelles de microbiologie pour réduire la fraction considérée jusqu'alors comme « non-cultivable », par exemple par des approches dites de « culturomique ». La culturomique est définie comme une méthode haut débit faisant appel à de multiples conditions de culture et visant à détecter des espèces de microorganismes inconnues ou n'ayant encore jamais été isolées [6]. Une étude de culturomique ayant fait appel à 212 conditions différentes de culture a par exemple permis de récolter 32500 colonies représentatives de la microflore intestinale humaine [7] et de décrire 341 espèces bactériennes, dont 51 ont également été identifiées par une approche conjointe de metabarcoding. Parmi ces 341 espèces, 30 étaient de nouvelles espèces et 60 ont été isolées pour la première fois à partir de ce type d'échantillons. Ce type d'étude a permis de relancer les études basées sur des isolements [6,8], souvent réalisées en concomitance avec des études culture-indépendantes (DGGE [1,9], clonage et séquençage [10], metabarcoding [7]). Actuellement, les techniques culture-dépendantes font généralement appel à des approches moléculaires (PCR suivie d'un séquençage) pour identifier les isolats collectés. Les techniques culture-dépendante et culture-indépendante, en dépit de leurs différences fondamentales, font toutes les deux appel à l'identification des espèces par l'ADN [11]. Elles sont donc sujettes à certains

[2] *La partie expérimentale consacrée à l'analyse des communautés fongiques a été conduite dans sa majeure partie par LK, sous la supervision de VL et FS ; BB a réalisé la caractérisation de la collection de souches fongiques. La partie expérimentale consacrée à l'analyse des communautés bactériennes a été réalisée par ALL, CM, MLS et MB.*

biais communs : biais liés à la méthode d'extraction d'ADN employée, biais d'amplification par PCR ou manque de séquences de référence dans les bases de données, etc.

Si les méthodes culture-indépendantes permettent d'avoir une vision plus complète, voire exhaustive, du monde microbien, elles ne peuvent cependant pas s'affranchir totalement des méthodes culture-dépendantes : que ce soit pour la découverte de nouveaux produits naturels [4], la complémentation des bases de données nécessitant une approche mono-souche (i.e. de l'isolement à la caractérisation), ou pour tester les interactions entre microorganismes révélées par des analyses bio-informatiques à partir des données culture-indépendantes (ex. analyse de réseaux d'interaction). Il a également été démontré que les techniques culture-indépendantes, si complètes soient-elles, ne permettent pas d'avoir un recouvrement parfait de la communauté [12,13]. Un intérêt non négligeable des analyses culture-dépendantes est également la possibilité de décrire plus finement les microorganismes isolés. En effet, des techniques telles que le metabarcoding sont utilisées pour tenter de décrire une communauté dans sa globalité : les amorces utilisées pour les PCR donc des amorces généralistes permettant, certes, de décrire le maximum de la diversité (en utilisant des régions très conservées, comme, par exemple, celle de l'ADN codant pour l'ARN ribosomique 16S [ARNr 16S]), mais sont souvent peu résolutives. En isolant les microorganismes, on rend possible l'utilisation de différentes amorces, qui sont spécifiques des familles, genres, ou espèces isolés.

Les méthodes culture-dépendantes permettent d'identifier des microorganismes qui ne le sont pas toujours avec des approches culture-indépendantes [6,14]. Les deux approches sont donc complémentaires et permettent d'avoir une vision plus complète du monde microbien. Bien que souvent fastidieux, l'isolement des organismes est donc une étape encore indispensable, si, en plus de la description des communautés, on souhaite conserver les espèces qui la composent.

Un des objectifs de cette thèse était de caractériser les microorganismes susceptibles d'être associés aux champignons *Zymoseptoria tritici* et *Leptosphaeria maculans* sur résidus de culture de blé et de colza. Il semblait donc important de compléter les approches culture-indépendantes (issue de données de metabarcoding) du chapitre III.1 par une approche culture-dépendante dédiée (culture de champignons et de bactéries sur milieux, puis barcoding – i.e. séquençage individuel – d'une sélection d'isolats). L'intérêt des isolements était double : (i) avoir à disposition une collection d'isolats de référence pouvant être conservés et ensuite testés pour leur propriété antagoniste dans des études d'interactions microbiennes, à commencer par celles conduites dans le cadre du projet Européen H2020 EMPHASIS ; (ii) comparer la diversité des communautés obtenues par isolement à celles des communautés obtenues par metabarcoding.

La stratégie adoptée a consisté à isoler des souches fongiques et bactériennes à partir d'échantillons de résidus de blé et de colza collectés dans des parcelles agricoles suivant un dispositif expérimental pluriannuel et à les identifier individuellement par séquençage, en parallèle de l'analyse metabarcoding de la communauté réalisée directement sur la matrice complexe que sont les résidus (voir Chapitre III.1, [15]). Les deux stratégies utilisées pour l'obtention des collections bactériennes et fongiques, distinctes, sont détaillées ci-après.

II. Stratégie expérimentale différentielle bactérie vs champignon

Stratégie générale

La méthode d'analyse de la diversité microbienne par metabarcoding, dont les détails sont présentés dans le chapitre III.1 [15], a été appliquée conjointement aux populations bactériennes et fongiques en utilisant les mêmes échantillons de résidus de blé et de colza. En revanche, pour des raisons biologiques et techniques, les analyses de diversité (isolements microbiologiques suivis de barcoding) ont été menées de façon très différente entre les champignons et les bactéries. Par ailleurs, pour des raisons pratiques, les isolements ont été réalisés sous une seule condition de culture, tant pour les bactéries que les champignons.

Pour les bactéries, une stratégie de criblage haut débit a été mise en œuvre à partir de macérats de résidus. L'objectif était en effet d'isoler un grand nombre de bactéries provenant d'un nombre réduit d'échantillons afin de couvrir un maximum de la diversité présente dans quelques résidus (approche intensive basée sur un « échantillonnage réduit » et une « analyse de diversité haut débit »). Concrètement, trois échantillons ont été utilisés : deux échantillons de résidus de blé provenant des parcelles en monoculture (WWW) et en rotation WOW, et un échantillon de résidus de colza provenant de l'autre parcelle en rotation (OWO ; voir design expérimental présenté dans le chapitre III.1). Chacun de ces lots a été analysé avec une profondeur d'analyse différente : le nombre de bactéries isolées n'a pas été le même pour chacun des échantillons. Les bactéries ont été cultivées par étalement classique des macérats sur milieu de culture, puis sélectionnées aléatoirement. La caractérisation taxonomique des souches a été réalisée par séquençage du gène codant pour l'ARNr 16S. Chaque souche a été marquée par un barcode unique, et le séquençage a été réalisé en un seul run de séquençage MiSeq Illumina. La collection bactérienne a constitué un des produits de la thèse.

Pour les champignons, une stratégie d'isolement a été mise en œuvre à partir de fragments solides de résidus. L'objectif était d'avoir un aperçu global de la diversité microbienne sur un nombre élevé de fragments de résidus provenant de modalités d'échantillonnage différentes, sans pour autant que celle-ci soit estimée très finement pour chaque fragment (approche extensive basée sur un « échantillonnage large » et une « analyse de diversité bas débit »). Le protocole d'isolement a consisté à sélectionner et ne conserver qu'un seul morphotype par ensemble de fragments de résidus provenant d'un même échantillon (un point, une parcelle, une date ; voir ci-après), en ayant pour objectif d'atteindre 20-25 souches. Les souches ainsi mises en collection ont été caractérisées en effectuant un séquençage des espaceurs internes transcrits ITS1 et ITS2 et la petite sous unité ribosomale 5.8S par le biais des amorces ITS1F– ITS4.

Il est prévu que la collection, dont l'affiliation taxonomique a été améliorée avec l'ajout de la séquence du gène codant pour l'ARN polymérase II (RPB2) pour certaines souches, soit accessible sur R-Syst::database (https://www6.inra.fr/r-syst; https://github.com/r-syst/databases/tree/master/r-syst::fungi), constituant également un des produits de la thèse.

Figure 1. Prélèvement et traitement des résidus. (A, C) Prélevement des résidus au cours de la saison culturale 2015-2016. (B, D) Découpe des résidus de blé (B) et de colza (D) pour réaliser les étapes de caractérisation de la diversité microbienne par approches culture-indépendante (metabarcoding) et culture-dépendante (isolements et séquençage). Un fragment par résidu de blé a été prélevé pour les isolements fongiques. Trois fragments par résidu de colza ont été utilisés pour réaliser les isolements fongiques, au niveau du haut de la racine (1), du collet (2), et du haut de la tige (3). Le reste des demi-tronçons a été utilisé pour réaliser les macérats pour les isolements bactériens.

Echantillonnage

L'ensemble des isolements a été réalisé à partir des échantillons de résidus de blé et de colza collectés en 2015-2016 et 2016-2017 sur le dispositif expérimental pluriannuel décrit dans le chapitre III.1. Chaque année, 12 résidus de blé et 4 résidus de colza ont été prélevés en cinq points de collecte dans chacune des trois parcelles en rotation blé-colza (WOW et OWO) et monoculture de blé (WWW). Les prélèvements ont été réalisés à trois périodes de l'année (octobre, décembre et février). Chaque résidu de blé ou de colza, a été coupé en deux dans la longueur (Figure 1). Afin que la comparaison entre la méthode culture-indépendante (metabarcoding) et la méthode culture-dépendante (isolement et séquençage) soit la plus pertinente possible, les demi-tronçons de chaque résidu ont été utilisés respectivement pour chacune des deux approches. Les demi-tronçons de chaque résidu de blé et de colza utilisés pour le metabarcoding ont été broyés séparément (champignons et bactéries ; voir Chapitre III.1). Un fragment de 1 mm^2 de chacun des demi-tronçons de résidus de blé destinés aux isolements fongiques et des trois fragments de résidu de colza (au niveau du haut de la racine, du collet, et de la base de tige) ont été prélevés au scalpel (Figure 1) et déposés sur milieu en boîte de Pétri. Les isolements bactériens ont été réalisés à partir de trois échantillons de résidus prélevés pendant la saison 2016-2017 en un seul point de collecte par parcelle. Après avoir réalisé les isolements fongiques, les demi-tronçons ont été regroupés en vue de constituer un macérat par échantillon.

III. Analyse de la fraction bactérienne cultivable par une approche d'isolement basée sur un échantillonnage réduit couplé à une analyse de diversité haut débit

Matériels et méthodes

Isolement de la fraction bactérienne

Les macérats préparés en vue de l'isolement des bactéries ont été obtenus en immergeant les chacun des trois lots de résidus (issus de trois points de prélèvement en février 2017 pour les échantillons de colza (OR), et en octobre 2016 pour les deux échantillons de blé en rotation (WR) et en monoculture (WM)) dans 4 mL de PBS additionné de Tween20 (0,05 % v/v). L'ensemble a été homogénéisé avec un Stomacher (Lab-Blender 400) opérant à vitesse maximale pendant 2 minutes. Les suspensions ont été diluées en série et étalées sur milieu Tryptic Soy Agar (TSA) de 1/10 (17 $g.L^{-1}$ tryptone, 3 $g.L^{-1}$ peptone de soja, 2,5 $g.L^{-1}$ glucose, 5 $g.L^{-1}$ NaCl, 5 $g.L^{-1}$ K_2HPO_4 et 15 $g.L^{-1}$ agar) avec un complément en cycloheximide (50 $mg.L^{-1}$). Après 5 jours d'incubation à 18°C, 2231 colonies (1395 colonies pour le blé en monoculture, 744 colonies pour le blé en rotation et 93 colonies pour le colza) ont été identifiées, repiquées et stockées dans des plaques de 96 puits contenant 200 μL de bouillon de soja Tryptic. Un témoin négatif de culture (TSB sans colonie), un témoin d'eau et un témoin positif de PCR (*Agrobacterium tumefaciens* CFBP-2413) ont été utilisés dans toutes les plaques de culture. Après 5 jours d'incubation à 18°C sous agitation constante (70 $tr.min^{-1}$), 10 μL de chaque suspension bactérienne ont été transférés dans 90 μL d'eau stérile pour amplification PCR ultérieure. 190 μL d'eau glycérolée à 80% ont été ajoutés dans chaque puits contenant les suspensions bactériennes restantes et stockés à -80°C pour un usage ultérieur.

Caractérisation moléculaire basée sur le gène codant pour l'ARNr 16S

Le typage moléculaire de chaque isolat bactérien a été effectué avec la région v4 du gène codant pour l'ARNr 16S en utilisant la procédure décrite par Armani *et al.* [16]. Chaque ensemble d'amorces (515f et 806r [17]) a été marqué avec un tag unique par plaque. Les amplifications PCR ont été réalisées sur des mélanges réactionnels de 25 μL constitués de 2,5 μL de suspension bactérienne bouillie (99°C, 5 min), 5 μl de tampon Greenflexi (Promega), 2 μL de mélange dNTP (Promega U151B dilué à 2.5 mM), 1,5 μL de $MgCl_2$ (Promega 25mM), 0,25 μL de GoTaq flexi DNA Polymerase (Promega), 12,75 μL d'eau sans nucléase et 0,5 μL de chaque primaire (10 mM chacun). Les mélanges réactionnels ont été maintenus à 94°C pendant 5 minutes, suivis de 35 cycles d'amplification à 94°C (30 secondes), 50°C (45 secondes) et 72°C (2 minutes), avec une étape finale d'extension de 10 minutes à 72°C. L'efficacité de l'amplification a été évaluée par électrophorèse sur gel d'agarose à 1,5% et les produits d'amplification ont été purifiés avec des billes Sera-MagTM dans un rapport de 0,8 produit PCR/billes. Une deuxième amplification par PCR a été réalisée avec des amorces contenant un tag unique pour chaque puit. Les deuxièmes réactions PCR ont été effectuées dans des volumes réactionnels de 48 μL contenant 10 μL de tampon Greenflexi, 2 μL de mélange dNTP, 4 μL de $MgCl_2$, 0,2 μL de GoTaq

flexiDNA Polymérase, 26,8 µL d'eau sans nucléase, 2 µL de chaque primaire (2,5 mM chacun) et 5 µL du produit PCR1 purifié. Les mélanges réactionnels ont été maintenus à 94°C pendant 2 minutes, suivis de 12 cycles d'amplification à 94°C (1 min), 55°C (1 min) et 72°C (1 min), avec une étape finale d'extension de 10 minutes à 72°C. L'efficacité de l'amplification a été évaluée par électrophorèse sur gel d'agarose à 1,5% et les produits d'amplification ont été purifiés avec des billes Sera-MagTM dans un rapport de 0,8 PCR produit/billes. Les produits PCR ont été regroupés dans un seul tube et séquencés avec un kit de réactifs MiSeq v2 (500 cycles).

Filtrage des données

Les fichiers Fastq ont été traités avec DADA2 version 1.6.0 [18] en utilisant les paramètres suivants : filterAndTrim (fnFs, filtFs, fnRs, filtRs, filtRs, truncLen=c(170,130), maxN=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE, compress=TRUE, multithread=TRUE), filtFs et filtRs correspondants respectivement aux lectures 1 et 2. Les affiliations taxonomiques pour les variantes de séquences d'amplicons (ASV) générées avec DADA2 ont été assignées à un classificateur bayésien du Ribosomal Database Project [19].

Sur les 2231 colonies bactériennes analysées, 895 ASV ont été détectés. Les témoins positifs de PCR associés à chaque plaque ont mis en évidence un niveau de contamination des reads de 3,76±0.93 %. De fait, un filtre à 5% de la somme des reads de chaque échantillon a été utilisé sur l'ensemble des échantillons, réduisant le nombre d'ASV à 218 ASV. Après application de ce filtre, les puits contenant plusieurs ASV ont été considérés comme contaminés et retirés avant analyse, réduisant ainsi le nombre de séquences à 1933, et le nombre d'ASV à 108. Enfin, un filtre a été appliqué pour supprimer les échantillons dont le nombre de séquences était inférieur à 200, ce qui a finalement conduit à ne retenir que 1889 séquences, regroupées en 100 ASV. Ces 100 ASV ont été comparés aux ASV obtenus par analyse de metabarcoding de ces mêmes échantillons (voir Chapitre III.1).

Tableau 1 - Nombre de colonies bactériennes obtenues par la méthode culture-dépendante (isolements) par échantillon et par genre (WM : blé en monoculture, WR : blé en rotation, OR : colza en rotation). Les colonies non classifiées n'ont pas été comptabilisées dans le nombre total de genres identifiés.

	WM	WR	OR
Acidovorax	0	1	0
Advenella	22	13	0
Aeromicrobium	1	0	0
Agrococcus	2	0	0
Arthrobacter	7	2	0
Bacillus	1	0	0
Brevundimonas	0	0	9
Citricoccus	0	6	0
Clavibacter	75	20	0
Cryobacterium	1	0	0
Curtobacterium	265	126	0
Devosia	0	0	9
Dyadobacter	13	2	0
Enterobacter	0	1	0
Falsirhodobacter	0	0	1
Flavobacterium	0	0	12
Frigoribacterium	0	1	0
Frondihabitans	16	2	0
Herbiconiux	20	2	0
Labedella	11	5	0
Leucobacter	45	9	0
Luteimonas	0	0	6
Mesorhizobium	1	0	0
Methylobacterium	2	0	0
Microbacterium	82	31	10
Neorhizobium	18	8	0
Nocardioides	1	0	1
Paenibacillus	2	3	0
Pantoea	22	96	0
Pedobacter	1	4	3
Polaromonas	0	0	2
Pseudoxanthomonas	0	0	4
Rathayibacter	11	16	0
Rhizobacter	0	0	1
Rhizobium	6	6	1
Rhodococcus	5	1	0
Roseomonas	1	0	0
Sanguibacter	425	99	0
Sphaerotilus	0	0	3
Sphingomonas	15	6	3
Stenotrophomonas	3	5	1
Streptomyces	5	0	0
Xenophilus	1	1	0
Unclassified_Beutenbergiaceae	7	0	0
Unclassified_Microbacteriaceae	132	126	6
Unclassified_Micrococcaceae	0	5	0
Unclassified_Sphingomonadaceae	0	0	1
Nombre total de colonies	1219	597	73
Nombre de genres différents identifiés	30	25	15

Résultats

La stratégie utilisée a conduit à analyser 100 ASV correspondant à 43 genres bactériens différents (Tableau 1). Sur les mêmes échantillons de résidus l'analyse metabarcoding avait decelé 458 ASV correspondant à 303 genres différents. Ce résultat confirme la puissance de l'approche par metabarcoding, qui a permis de déceler un nombre d'ASV différents plus important. Entre 59% et 70% des ASV identifiés par la méthode culture-dépendante l'avaient également été par metabarcoding (Tableau 2), ce qui correspond à un nombre de colonies compris entre 63% et 85%. Réciproquement, la méthode culture-dépendante a permis d'identifier entre 10 et 16% de l'ensemble des ASV identifiés par metabarcoding. Sur les ASV correspondant aux isolats mis en collection, près des 2/3 ont donc été communs avec ceux la méthode culture-indépendante, et 1/3 ont été spécifiques de la méthode culture-dépendante. Si le metabarcoding donne un aperçu plus exhaustif de la diversité des ASV, il est important de noter que les isolements le complètent: au total ce sont 21 ASV, dont un ASV qui a représenté à lui seul 104 colonies, qui ont correspondu à une séquence qui n'a pas été identifiée par metabarcoding.

Des différences de proportion chez certains groupes taxonomiques sont à relever : en collection, les ASV correspondant aux *Actinobactéries* (*Microbacterium*, *Curtobacterium*, *Sanguibacter*) ont été beaucoup plus représentés que dans l'analyse metabarcoding (Figure 2) ; à l'inverse, les ASV correspondant aux *Dyadobacter*, *Pedobacter* et *Flavobactéries* ont été d'avantage représentés avec la méthode culture-dépendante. Malgré ces divergences, certaines différences de profils entre les échantillons de blé (WR et WM) et le colza (OR) mises en évidence par approche metabarcoding ont été retrouvées avec les isolements : les ASV correspondants aux genres *Flavobacterium* et *Devosia* ont par exemple été beaucoup moins représentés sur résidus de blé que sur résidus de colza, et les ASV correspondant au genre *Curtobacterium* ont été absents des résidus de colza.

Tableau 2 - Comparaison du nombre de séquences et d'ASV bactériens communs entre les méthodes culture-dépendante et culture-indépendante (WM : blé en monoculture, WR : blé en rotation, OR : colza en rotation).

Echantillon	WM	WR	OR	Total
Nombre de colonies prélevées	1395	744	93	2232
Nombre de séquences après filtrage	1219	597	73	1889
Nombre d'ASV issus de la collection	60	45	32	100
Nombre d'ASV issus de l'analyse metabarcoding	250	270	194	458
Nombre d'ASV issus de la collection et présents dans l'analyse metabarcoding, dans le même échantillon (nombre de colonies)	42 (1036)	3 (439)	19 (46)	-
Nombre d'ASV issus de la collection et présents dans l'analyse metabarcoding, mais dans un autre échantillon (nombre de colonies)	6 (80)	4 (58)	8 (15)	-
Nombre d'ASV présents dans la collection et absents dans l'analyse metabarcoding (nombre de colonies)	12 (103)	10 (100)	5 (12)	21 (215)

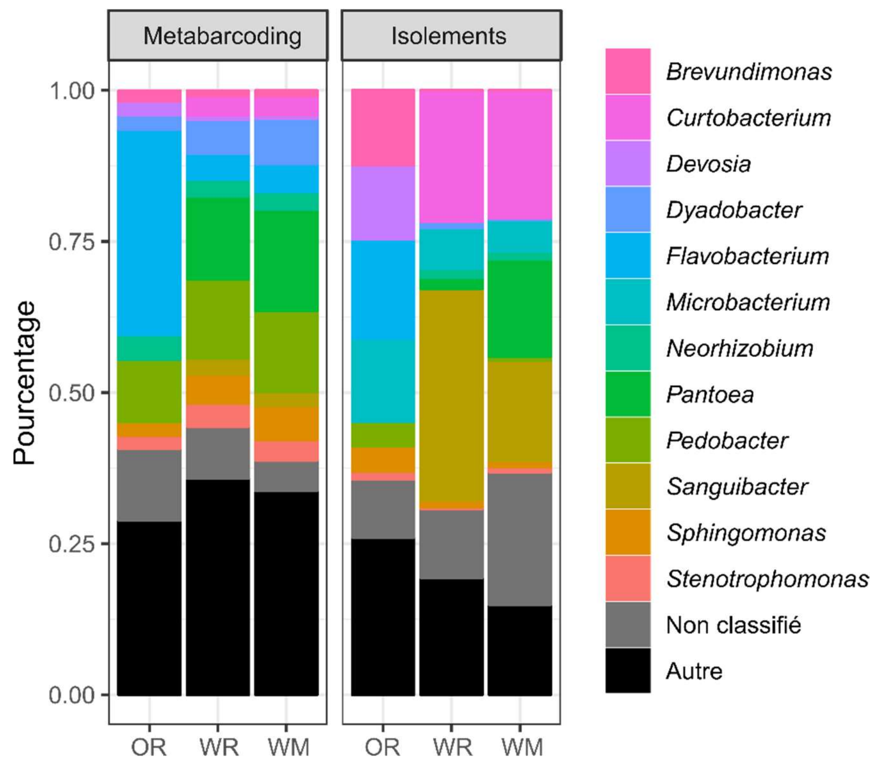


Figure 2 - Comparaison de la composition en genres bactériens des trois échantillons de résidus de blé et de colza (WM : blé en monoculture, WR : blé en rotation, OR : colza en rotation) obtenus par les méthodes culture-indépendante (metabarcoding) et culture-dépendante (isolements). Seuls les 12 genres les plus abondants sont représentés. Les genres moins abondants sont classifiés en « autre ».

IV. Analyse de la fraction fongique cultivable par une approche d'isolement basée sur un échantillonnage large couplé à une analyse de diversité bas-débit

Matériels et méthodes

Caractérisation moléculaire de la collection par séquençage Sanger

Les isolats fongiques collectés à partir des fragments de résidus de blé et de colza ont été purifiés par repiquages successifs sur milieu PDA et conservés dans des tubes d'eau glycérolée à 20% à -80°C.

Avant leur caractérisation, les isolats ont été remis en culture sur milieu PDA. Le mycélium de chaque isolat a été gratté et placé dans un puit de plaques de 96 puits. Les mycéliums ont ensuite été lyophilisés puis broyés avec une bille de tungstène dans 50 µL de tampon de lyse (AP-1) avec un broyeur Retsch-MM400 (Retsch France) opérant à une fréquence de 20 Hz pendant 30 secondes. L'ADN a été extrait à l'aide d'un tampon Qiagen DNeasy-Plant AP1, d'un réactif DX et de P3 (Qiagen, France), en suivant les instructions simplifiées du fabricant, les colonnes d'ADN n'ayant pas été utilisées. L'ADN de toutes les souches a été quantifié au spectrophotomètre Nanodrop (Thermo Scientific®), ajusté à 10 à 20 $ng.mL^{-1}$ et conservé à -20°C jusqu'à son utilisation. Pour l'identification moléculaire des séquences, les régions ITS (ITS1 et ITS2) et la petite sous unité 5.8S de l'ADNr ont été amplifiées à partir de chaque ADN avec les amorces ITS1F [20] et ITS4 [21]. La réaction PCR contenait 1x master mix pour le kit PCR Microsatellite Type-it (Qiagen, France), 3 μL de Q-Solution et 0,25 μM de chaque amorce dans un volume total de 30 μL. Les réactions PCR ont été réalisées à l'aide d'un thermocycleur Applied Biosystems 9700. Les paramètres d'amplification consistaient en : une étape initiale de dénaturation à 95°C pendant 5 min ; 35 cycles (95°C pendant 60 s, 60°C pendant 30 s, et 72°C pendant 60 s) ; une étape finale à 72°C pendant 10 minutes. La qualité des produits PCR a été contrôlée par électrophorèse sur des gels d'agarose à 2%. Les produits PCR ont ensuite été séquencés par la méthode Sanger (Eurofins, France). Les chromatogrammes ont été vérifiés en utilisant le logiciel Lasergene pour produire les séquences ensuite utilisées pour l'identification moléculaire. L'assignation taxonomique de chaque séquence a été réalisée avec un classifieur naïf de Bayes sur la base de données UNITE7.1, comme pour l'approche culture-indépendante (metabarcoding ; voir Chapitre III.1). Les séquences des isolats en collection, plus longues que les séquences metabarcoding (ITS1), ont été utilisées comme base de référence pour la comparaison de séquences.

Tableau 3 - Nombre d'isolats fongiques récoltés et séquencés pour chacun des échantillons de résidus de blé et de colza. Certains isolats ont été perdus lors de la décongélation, réduisant le nombre d'isolats séquencés pour trois échantillons sur 18 (*).

Période	Culture	Parcelle	Code parcelle	Nombre d'isolats
Oct. 2015	Blé	HDG3	WM	22
Oct. 2015	Blé	TTF7	WR	32
Oct. 2015	Colza	HDG2	OR	24
Dec.2015	Blé	HDG3	WM	19
Dec.2015	Blé	TTF7	WR	30
Dec.2015	Colza	HDG2	OR	24
Fév. 2016	Blé	HDG3	WM	2 *
Fév. 2016	Blé	TTF7	WR	0 *
Fév. 2016	Colza	HDG2	OR	5 *
Oct. 2016	Blé	HDG2	WR	24
Oct. 2016	Blé	HDG3	WM	31
Oct. 2016	Colza	TTF7	OR	33
Déc. 2016	Blé	HDG2	WR	31
Déc. 2016	Blé	HDG3	WM	23
Déc. 2016	Colza	TTF7	OR	45
Fév. 2017	Blé	HDG2	WR	33
Fév. 2017	Blé	HDG3	WM	28
Fév. 2017	Colza	TTF7	OR	18

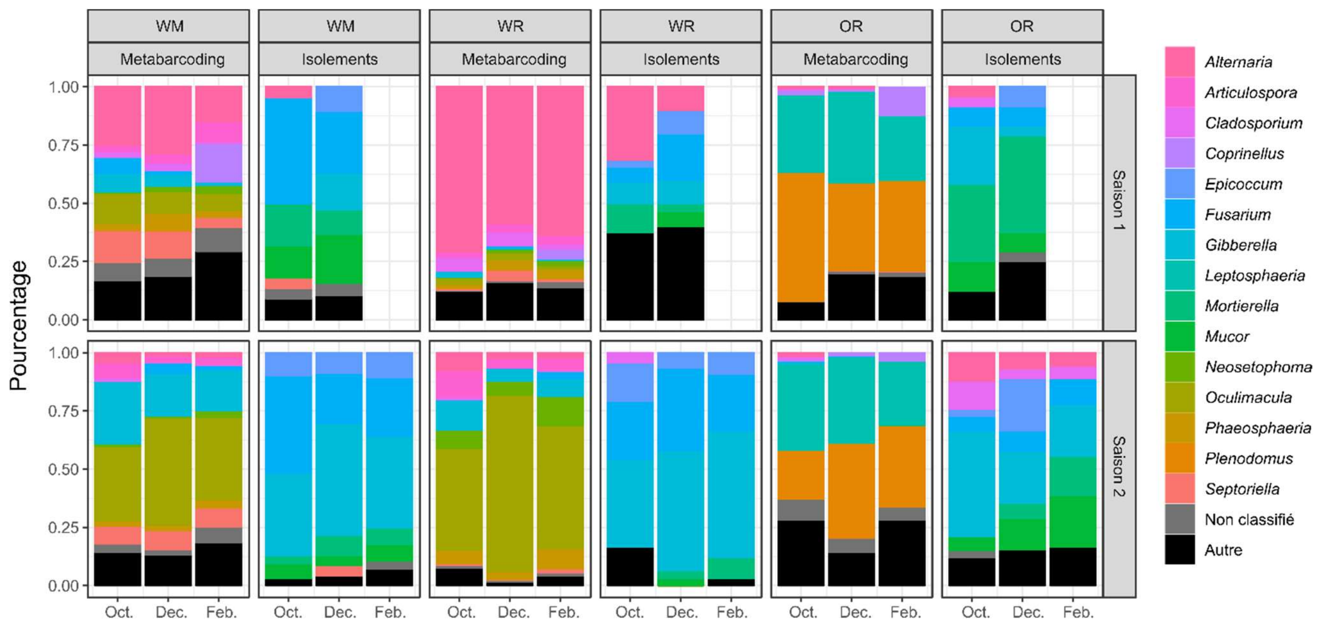


Figure 3 - Comparaison de la composition en genres fongiques des 18 échantillons de résidus de blé et de colza (WM : blé en monoculture, WR : blé en rotation, OR : Colza) obtenus par les méthodes culture-indépendante (metabarcoding) et culture-dépendante(isolements). Seuls les 12 genres les plus abondants sont représentés. Les genres moins abondants sont classifiés en « autre ». La composition en genres pour les trois échantillons dont le nombre d'isolats était faible n'est pas présentée (*)

Résultats

Au total 424 souches fongiques ont été isolées et mises en collection (Tableau 3 ; Tableau annexe 1[3]) et séquencées, correspondant à 279 ASV différents répartis en 22 genres, sur la base de 18 échantillons (Tableau 4). Sur les mêmes échantillons, l'approche culture-indépendante (metabarcoding ; voir Chapitre III.1) avait permis d'identifier 894 ASV correspondant à 148 genres différents.

De par les différences de longueur de séquences obtenues entre les approches culture-dépendante et culture-indépendante, certains ASV issus du metabarcoding ont eu 100% d'homologie avec plusieurs séquences issues du séquençage Sanger. 33 ASV (sur un total de 894) issus des échantillons analysés par metabarcoding ont eu 100% d'homologie avec 110 ASV de la collection identifiée par séquençage Sanger (sur un total de 279 ASV).

Globalement, les genres les plus présents dans la collection ont également été détectés en metabarcoding, à l'exception des ASV correspondant au genre *Mucor*, représentés par 24 isolats dans la collection. D'autres genres, moins représentés, ont également été absents de l'analyse metabarcoding (voir Chapitre III.1), comme les *Actinomucor*, *Boeremia*, *Botrytis, Neurospora* et *Stagonosporopsis*. Cette différence peut s'expliquer par une différence entre les amorces : celles utilisées pour caractériser les isolats de la collection ont permis d'affilier davantage d'ASV (ITS1 et ITS2) que par metabarcoding (ITS1). Pour cette raison, la comparaison des résultats obtenus par les deux méthodes s'avère délicate.

Il faut tout de même constater que les profils obtenus par les approches culture-dépendante et culture-indépendante ont été très différents (Figure 3). Les genres les plus détectés par isolement ont été très peu détectés par metabarcoding (ex. *Fusarium*, *Gibberella*), voire ont été absents (*Mucor*). Réciproquement, les genres les plus détectés par metabarcoding ont été très peu isolés (*Alternaria*), voire pas du tout (ex. *Oculimacula*, *Leptosphaeria* et *Plenodomus*).

[3] En annexe de la thèse

Tableau 4 - Répartition par genre des isolats fongiques obtenus par isolement en fonction de la nature des résidus (WM : blé en monoculture, WR : blé en rotation, OR : colza en rotation) et de la saison de culture (2015-2016, 2016-2017). Les trois dates de prélèvements (octobre décembre, février) ont été regroupées. Les isolats non classifiés n'ont pas été comptabilisés dans le nombre total de genres identifiés.

	2015-2016			2016-2017		
	WM	OR	WR	WM	WR	OR
Actinomucor	0	0	0	0	0	1
Alternaria	1	1	13	0	0	8
Boeremia	0	0	0	0	0	3
Botrytis	0	0	0	0	0	1
Chaetomium	0	3	0	0	0	4
Cladorrhinum	5	0	2	0	1	0
Cladosporium	1	0	0	1	0	7
Epicoccum	2	2	4	9	8	11
Fusarium	6	15	8	25	25	8
Gibberella	7	3	6	43	33	29
Khuskia	0	0	2	0	0	0
Laetisaria	0	0	1	0	0	0
Lophodermium	0	1	0	0	0	0
Monographella	0	0	2	3	1	0
Mortierella	20	6	5	4	5	6
Mucor	6	8	2	1	5	12
Neurospora	0	0	0	1	0	0
Nigrospora	0	0	8	0	0	0
Septoriella	0	1	0	0	1	0
Stagonosporopsis	0	0	0	0	0	1
Trichoderma	0	0	5	0	1	2
Waitea	0	0	1	0	0	0
Unclassified *Agaricomycetes*	0	0	1	0	0	0
Unclassified *Dothideomycetes*	0	3	0	0	1	0
Unclassified *Hysteriaceae*	4	0	2	1	1	2
Unclassified *Nectriaceae*	0	0	0	0	0	1
Unclassified *Pleosporales*	1	0	0	0	0	0
Nombre d'isolats	53	43	62	88	82	96
Nombre de genres différents identifiés	8	9	13	8	9	13

V. Discussion générale : comparaison des deux méthodes

Deux collections - l'une bactérienne et l'autre fongique - de souches issues de résidus de blé et de colza ont été obtenues et décrites avec des informations telles que le lieu de collecte, l'espèce hôte, la date, la séquence. Deux stratégies différentes ont été mises en œuvre pour les isolements bactériens et fongiques. Chacune de ces stratégies a conduit à l'identification d'ASV communs aux analyses metabarcoding, mais aussi d'ASV qui ne correspondaient pas aux ASV issus du metabarcoding.

L'approche intensive basée sur un « échantillonnage réduit » et une « analyse de diversité haut débit » pour caractériser la communauté bactérienne a révélé des profils relativement proches entre les méthodes culture-dépendante (isolements) et culture-indépendante (metabarcoding). Si les proportions de genres ne sont pas exactement les mêmes entre ces deux méthodes, ceux considérés comme les plus représentés en metabarcoding ont pu être isolés et identifiés par séquençage individuel de la région v4 du gène codant pour l'ARNr 16S, y compris dans l'échantillon de résidus de colza, avec seulement 73 bactéries isolées et séquencées (après filtre). Parmi les taxons cultivables, les colonies affiliées aux genres *Sanguibacter*, *Curtobacterium*, *Microbacterium*, et *Pantoea* ont été les plus abondantes. Dans chaque échantillon, au moins 60% des ASV isolés ont correspondu aux ASV identifiés par metabarcoding, ce qui est un ratio plutôt élevé comparé à d'autres études [14]. La différence importante de proportion de *Bacteroidetes* entre les deux méthodes (beaucoup plus représentés en isolement qu'en metabarcoding) a déjà été mise en évidence dans des études précédentes, où une comparaison entre des méthodes culture-dépendante et culture-indépendante a été faite et ou le milieu TSA avait également été utilisé [1]. Il ressort de ces résultats que l'utilisation d'un seul milieu de culture peut être à l'origine d'un biais d'analyse qu'il est ici difficile de quantifier. Certaines études de culturomique ont estimé un optimum à 70 conditions de culture (une trentaine de milieux, sélectifs ou non, en aérobie, anaérobie ou micro-aérophilie, à différentes températures et sur différents pas de temps) pour capter la totalité de la diversité bactérienne [6,7]. Bien que portant sur le microbiote humain, ces études illustrent la difficulté d'atteindre une exhaustivité descriptive des communautés bactériennes.

Parmi les taxons cultivés, certains ASV étaient également spécifiques de la méthode culture-dépendante (environ 40%). La moitié de ces ASV (13/22) appartiennent à la famille *Microbacteriaceae,* dont la moitié au genre *Microbacterium*.

L'approche extensive basée sur un « échantillonnage large » et une « analyse de diversité bas débit » utilisée pour caractériser la communauté fongique a révélé des profils totalement différents de méthode culture-indépendante. La plupart des ASV correspondant aux isolats de la collection n'a pas été révélée par metabarcoding. Cela peut s'expliquer, en partie, par un biais lié aux amorces utilisées. A l'exception d'*Alternaria*, pourtant minoritaire dans la collection, les genres les plus abondants dans l'approche metabarcoding ont été pour la plupart absents de la collection. De très nombreuses souches des genres *Fusarium* et *Gibberella* ont en effet été isolées bien qu'apparaissant en proportion faible dans l'analyse culture-indépendante. A l'inverse, les espèces *L. maculans* et *Oculimacula,* pourtant présentes en très grande proportion dans l'analyse metabarcoding, n'ont pas été isolés. Tout comme pour l'analyse bactérienne, l'utilisation d'un seul milieu peut expliquer ces différences. Les espèces fongiques associées aux genres *Fusarium* et *Gibberella* ont une croissance rapide sur milieu riche et

sont généralement surreprésentées, ce qui n'est pas le cas de *Leptosphaeria*. La technique d'isolement au regard de la biologie de l'espèce (présence en surface ou en profondeur des tissus de la plante hôte) est un autre biais possible : l'utilisation d'un fragment de résidu plutôt qu'un broyat et/ou d'un macérat peut conduire à sous-évaluer la diversité, car les isolements ne sont réalisés qu'à partir d'une infime partie du résidu. Enfin, la sélection des souches sur critère morphologique est également un des biais des isolements fongiques : la diversité d'espèces au sein de *Fusarium* est grande, impliquant la présence de nombreux morphotypes [22], et donc une probabilité plus importante pour ces souches d'être « choisies » et isolées. La comparaison de nos résultats à ceux de la littérature est assez délicate, car très peu d'études se sont intéressées à la culturomique sur champignons. Notons toutefois que l'une de ces études [23] fait état d'un faible recouvrement entre les séquences ITS1 et les séquences obtenues par culturomique.

Bien que basée sur un seul milieu, l'approche conduite pour les bactéries a permis d'isoler la plupart des taxons les plus abondants relevés en metabarcoding (Chapitre III.1). Pour ce qui concerne les champignons, l'approche metabarcoding a donné une vision probablement plus juste que les isolements, du fait des biais probables dont l'approche culture-dépendante ne permet de s'abstraire. En conclusion, si « chaque étape physique, chimique et biologique de l'analyse moléculaire d'un environnement est une source de biais qui conduit à une vision déformée du *monde réel* » [5], la multiplication des approches peut permettre de se rapprocher d'une vision la plus complète et la plus juste possible. Dunbar *et al.* [24] suggèrent que tout écart par rapport aux paramètres environnementaux initiaux pendant la culture, tel que l'utilisation de milieux enrichis, peut modifier la structure de la communauté en imposant de nouvelles conditions sélectives. Dans la plupart des cas il faut accepter de ne pouvoir accéder qu'à une fraction de la diversité microbienne d'un écosystème donné, et notamment des résidus de culture.

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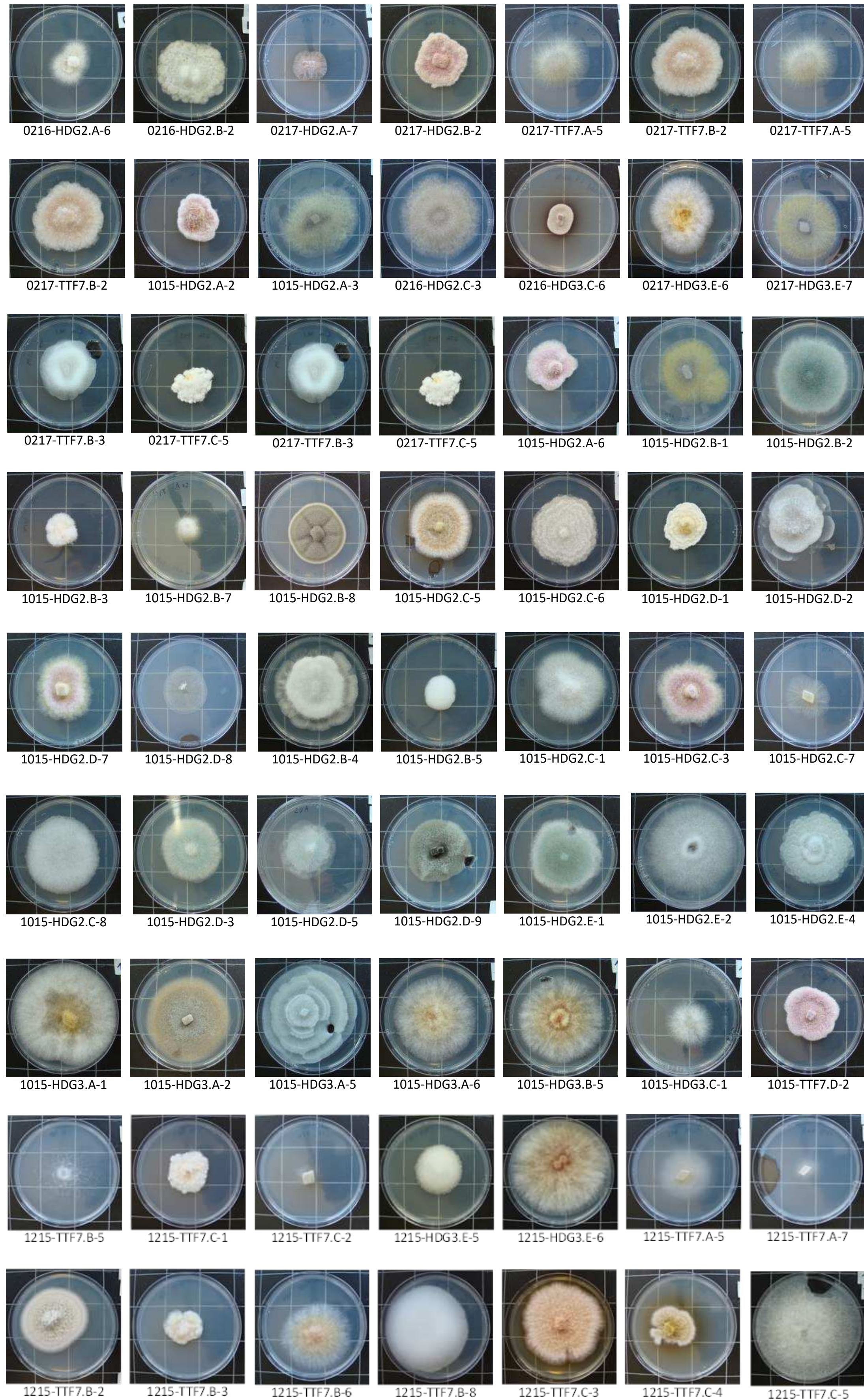
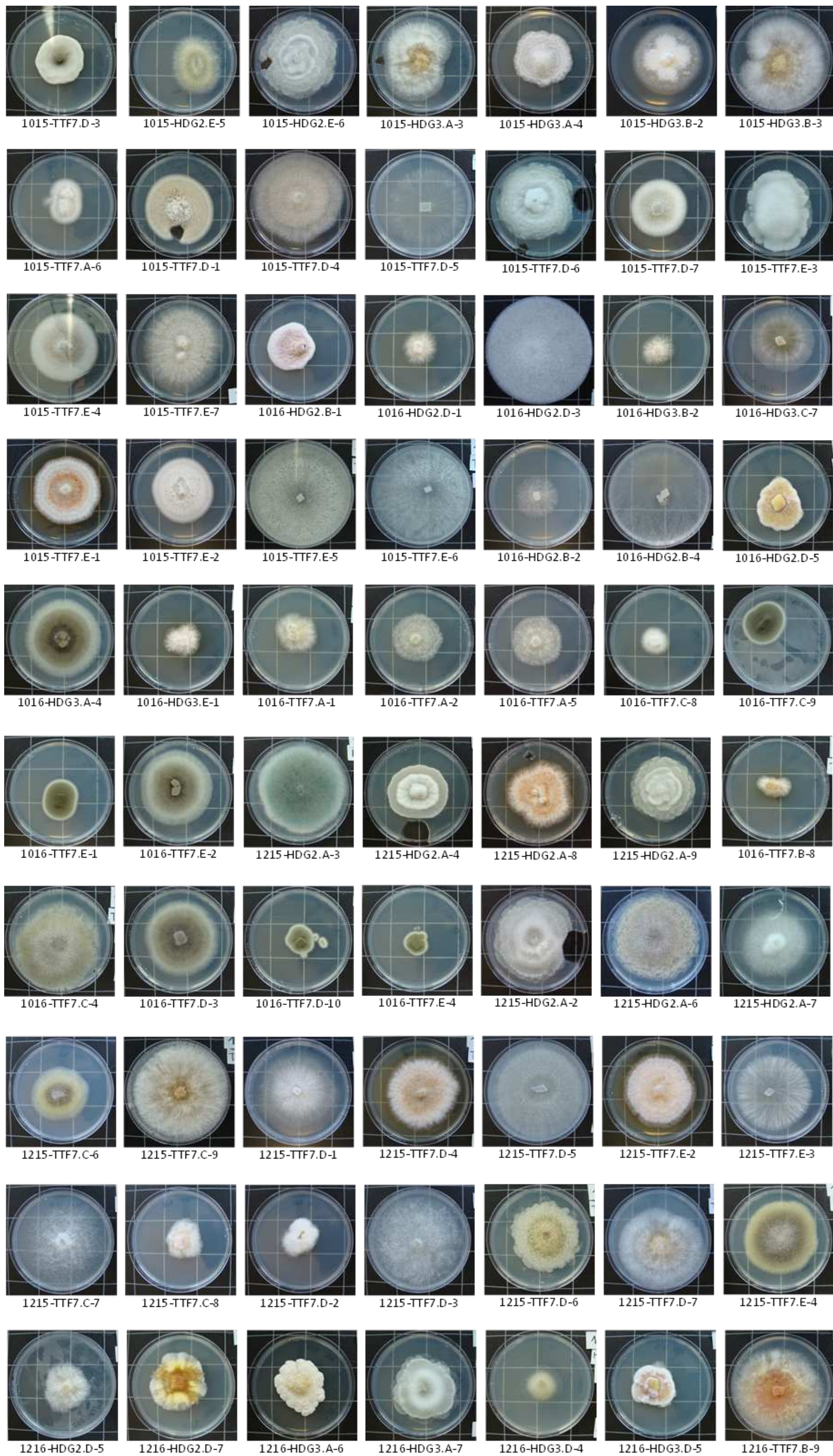
Figure supplémentaire 1 - Photographies de 194 souches fongiques représentatives de la collection des 424 souches isolées à partir de résidus de blé et de colza (R-Syst::database ; https://www6.inra.fr/r-syst; https://github.com/r-syst/databases/tree/master/r-syst::fungi). Les prises de vue ont été réalisées sur milieu PDA entre 3 et 12 jours de culture à 18°C (photos Benjamin Boudier, INRA BIOGER).

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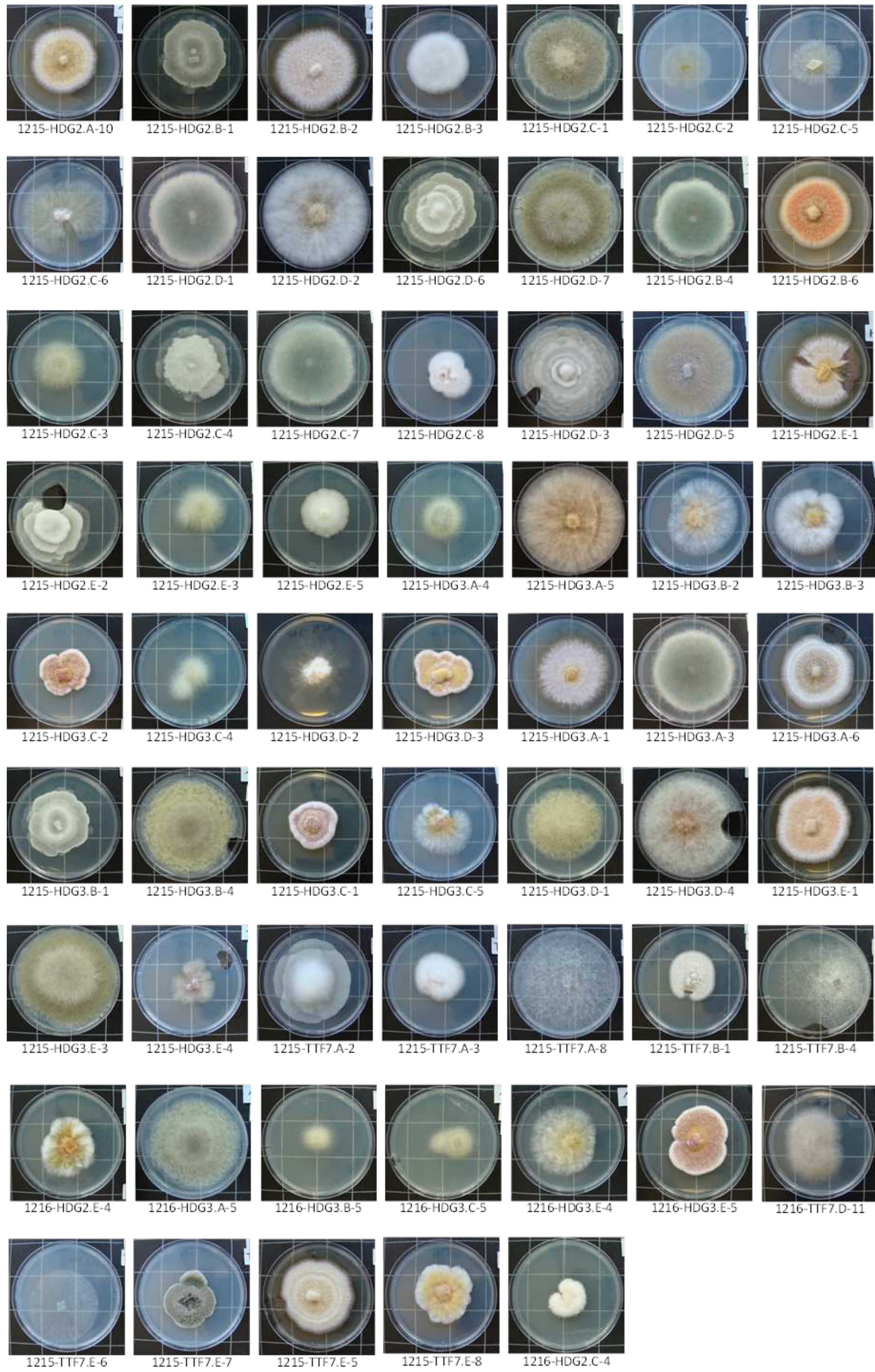
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Chapitre III

Interactions agents pathogènes - microbiome

Chapitre III.1

Identification des microorganismes qui interagissent avec les pathogènes par analyse de réseaux d’interactions: le cas de *Zymoseptoria tritici* dans le blé

Differential dynamics of microbial community networks help identify microorganisms interacting with residue-borne pathogens: the case of *Zymoseptoria tritici* in wheat

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Soumission prévue : Microbiome Journal

Abstract

Wheat residues are a crucial determinant of the epidemiology of Septoria tritici blotch, as they support the sexual reproduction of the causal agent *Zymoseptoria tritici*. We aimed to characterize the effect of infection with this fungal pathogen on the microbial communities present on wheat residues, and to identify microorganisms interacting with it. We used metabarcoding to compare the microbiome associated with wheat residues placed outdoors, with and without preliminary *Z. tritici* inoculation, comparing a first set of residues in contact with the soil and a second set without contact with the soil, on four sampling dates in two consecutive years.

The diversity of the tested conditions, leading to the establishment of different microbial communities according to the origins of the constitutive taxa (plant only, or plant and soil), highlighted the effect of *Z. tritici* on the wheat residue microbiome. Several microorganisms were affected by *Z. tritici* infection, even after the disappearance of the pathogen. Linear discriminant analyses and ecological network analyses were combined to describe the communities affected by infection. The number of fungi and bacteria promoted or inhibited by inoculation with *Z. tritici* decreased over time, and was smaller for residues in contact with the soil. The interactions between the pathogen and other microorganisms appeared to be mostly indirect, despite the strong position of the pathogen as a keystone taxon in networks. Direct interactions with other members of the communities mostly involved fungi, including other wheat pathogens.

Our results provide essential information about the alterations to the microbial community in wheat residues induced by the mere presence of a fungal pathogen, and vice versa. Species already described as beneficial or biocontrol agents were found to be affected by pathogen inoculation. The strategy developed here can be viewed as a proof-of-concept for crop residues serving as a particularly rich ecological compartment, with a high diversity of taxa originating from both the plant and soil compartments, and for *Z. tritici*-wheat as a model pathosystem. By revealing putative antagonistic interactions, we demonstrate that this strategy can be used to improve the biological control of residue-borne diseases.

Keywords: Ecological interaction networks, metabarcoding, microbial communities, microbiome, pathobiome, Septoria tritici blotch, wheat residues.

Differential dynamics of microbial community networks help identify microorganisms interacting with residue-borne pathogens: the case of *Zymoseptoria tritici* in wheat

Lydie Kerdraon, Matthieu Barret, Valérie Laval, Frédéric Suffert

Background

Septoria tritici blotch (STB) is one of the most important disease of wheat (*Triticum aestivum*), causing yield losses averaging 20% on susceptible wheat varieties and 5–10% on wheat varieties selected for disease resistance and sprayed with fungicide in Northwestern Europe [1]. It is caused by the hemibiotrophic, heterothallic, ascomycete fungus *Zymoseptoria tritici* [2], which initiates its sexual reproduction on senescent tissues [3]. STB is clonally propagated between wheat plants during the cropping season by pycnidiospores (asexual spores), which are splash-dispersed upwards over short distances. Wind-dispersed ascospores (sexual spores), mostly produced on wheat residues, initiate subsequent epidemics. Thus, wheat residues are a crucial, but often neglected determinant of the epidemiology of STB during the interepidemic period, as they support the sexual reproduction of the pathogen, maintaining diversity within populations and influencing adaptive dynamics in response to selection pressures [4], through the rapid evolution of fungicide resistance [5–8] or the breakdown of wheat resistance genes [9], for example.

The identification of microorganisms interacting with pathogens is an increasingly important issue for both academic and operational research on the development of biological control solutions [10,11]. In plant, animal and human epidemiology, increasing numbers of studies are trying to characterize variant microbial populations associated with specific disease stages, or temporal changes in the microbial populations during disease progression [12–14]. The pathogen and its cohort of associated microorganisms, which may influence its persistence, transmission and evolution, are together known as the “pathobiome” [15]. Pathobiome research has advanced significantly with the advent of high-throughput sequencing technologies, which have made it possible to describe and follow the diversity of the microbial communities associated with the pathogen during its life cycle, during both the epidemic and interepidemic periods.

The dynamics of microbial communities have been studied in detail during the vegetative and reproductive stages of the plant life cycle, but very few studies during and after plant senescence (e.g. [16,17]). The specific, central position of crop residues in agrosystems was long neglected, but these residues should be seen as both a fully-fledged matrix and a transient compartment: a compartment originating from the plant (temporal link), but in close contact with the soil (spatial link), with variable rates of degradation over the following cropping season, according to the plant species, the cropping practices used, and the climatic conditions in the year concerned [16,19–21]. In addition, the rare studies focusing on the evolution of microbial communities in crop residues performed to date were conducted

in microcosms, with sterilized residues (e.g. [22]), in which this compartment is much less complex than under natural conditions.

Several studies have investigated the potential beneficial effects of microorganisms for limiting the development of a plant pathogen during its saprophytic stage on natural crop residues (e.g. *Aureobasidium pullulans* and *Clonostachys rosea* inhibiting the sexual stage of *Didymella rabiei* on chickpea residues [23]; *Trichoderma harzianum* [24,25], *Microsphaerelopsis* sp. [26], *C. rosea* [27,28] and Streptomyces sp. [29] reducing *Fusarium graminearum* inoculum (perithecia, the sexual fruiting bodies) on wheat or maize residues, as exhaustively summarized in [30]). Other studies have focused on the general impact of cropping practices, such as the increase in microbial soil antagonists induced by the addition of green manure to the soil (e.g. [19,31]). Some phyllosphere microorganisms selected for their antifungal activity against *Z. tritici* (*Bacillus megaterium* [32]; *Pseudomonas fluorescens* [33]; *Cryptococcus* sp., *Rhodotorula rubra* and *Penicillium lilacinum* [34]; *T. harzianum* [35]; *Trichoderma koningii* [36]) have been tested *in planta* against the asexual, pathogenic stage of the pathogen (typically on wheat seedlings), but not against the pathogen during its sexual, saprophytic stage. Moreover, no microbial antagonists of *Z. tritici* have been isolated from wheat residues, despite the dense population of this habitat with a high diversity of microbial taxa [16].

The taxonomic structure of microbial communities associated with maize [17] and wheat [16] residues has recently been described under natural conditions. In addition to *Z. tritici*, the microbial communities associated with wheat include *Clonostachys* sp., *Aureobasidium* sp., *Chaetomium* sp. and *Cryptococcus* sp. [16], all of which are potential competitors. However, the presence of microorganisms in the same ecological niche, as highlighted in such descriptive approaches, does not necessarily mean that interactions actually occur between them. Many other non-interacting microorganisms (pathogens, endophytes) are also present on the residues. Moreover, microbial communities change during the physical degradation of the residues, probably modifying interactions between microorganisms over time [16]. Ecological network analysis has made it possible to detect putative interactions between microorganisms. For instance, Jakuschkin *et al.* [13] detected significant changes in foliar fungal and bacterial communities following the infection of pedunculate oak with *Ersysiphe alphitoides* (the causal agent of oak powdery mildew), and Cobo-Diaz *et al.* [17] identified candidate antagonists of toxigenic *Fusarium* spp. among the species present in maize residues. The use of co-occurrence networks in these two studies highlighted a set of bacteria and fungi that might be useful for managing plant pathogens.

In this study, our goal was to identify fungi and bacteria potentially interacting with *Z. tritici* during its sexual reproduction on wheat residues. To this end, we compared the structure of microbial communities associated with wheat residues with and without *Z. tritici* inoculation, by metabarcoding, combining linear discriminant analyses (LDA) and ecological network analyses. The response of microbial communities to *Z. tritici* infection was assessed during the interepidemic period between two successive crops, for two sets of wheat residues, one left outdoors in contact with the soil, and the other left outside but not in contact with the soil, at different sampling dates during two consecutive years. The diversity of experimental conditions was expected to lead to the establishment of different microbial

communities according to the origin of the constitutive taxa (plant or soil), thereby increasing the probability of detecting effects of *Z. tritici* on the residue microbiome, and of the residue microbiome on *Z. tritici*.

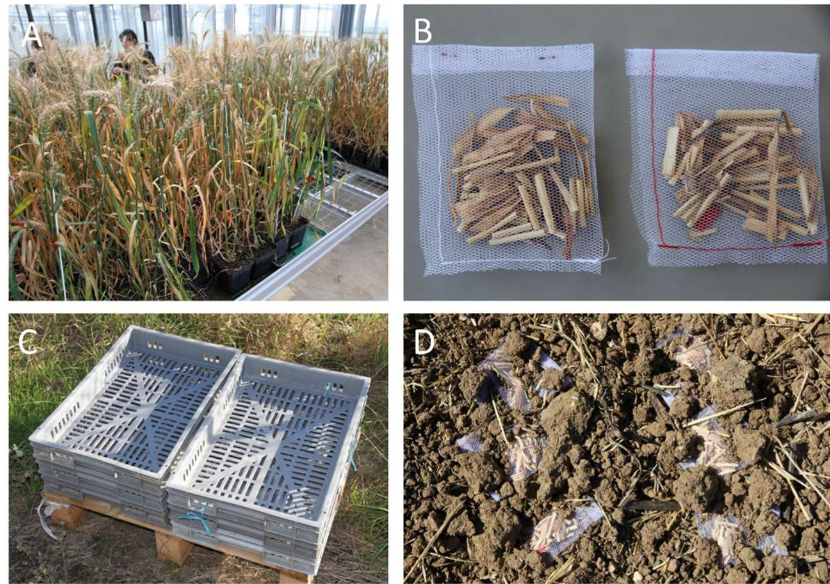

Figure 1. Preparation of wheat residues.
(A) Adult wheat plants were inoculated with *Zymoseptoria tritici* under greenhouse conditions. (B) Sealed nylon bags containing wheat residues, consisting of stem and leaf fragments of approximately 2 cm in length (red yarn for residues from wheat plants inoculated with *Z. tritici*; white yarn for those from non-inoculated plants). (C) "Soil contact" treatment: nylon bags were left on the ground of the field and partially covered with soil (one of the 15 sampling points). (D) "Above ground" treatment: plastic grids containing nylon bags placed outside the field.

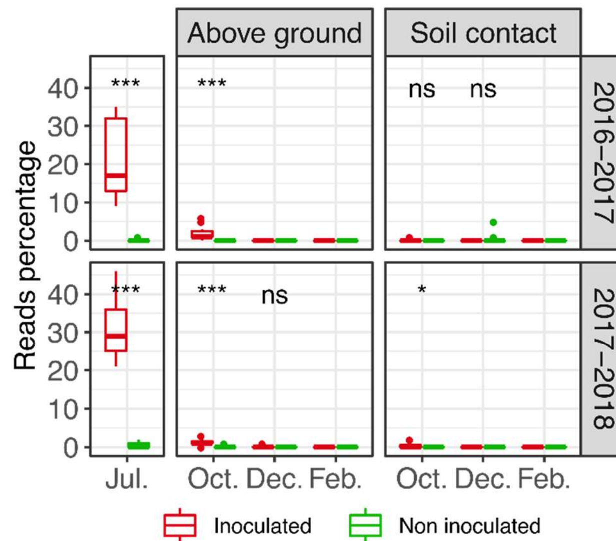

Figure 2. Relative abundance of *Zymoseptoria tritici*. Each box represents the distribution of the relative abundances of genera for the 15 sampling points. Wilcoxon tests were performed for inoculation condition (NS: not significant; * p-value<0.05; ** p-value <0.01; *** p-value <0.001).

Results

Overall diversity of the bacterial and fungal communities on residues

The response of the residue microbiome to *Z. tritici* inoculation was assessed by analyzing the composition of the fungal and bacterial communities of wheat residues, after inoculation with *Z. tritici* (*n*=240) or in the absence of inoculation (*n*=240). We also investigated the impact of cropping season (*n*=2), season (*n*=4), and soil contact (*n*=2) on the dynamics of these communities (see materials and methods for a detailed explanation of the experimental design; Figure 1).

We investigated the structure of the residue microbiome by analyzing the v4 region of the 16S rRNA gene and ITS1. Overall, 996 bacterial amplicon sequence variants (ASVs) and 520 fungal ASVs were obtained from 390 and 420 samples, respectively. Some samples (July 2016) were removed from the analysis due to the co-amplification of chloroplasts.

The high relative abundance (RA) of ASVs affiliated to *Z. tritici* in samples collected in July 2016 (21.5±9.8%) and 2017 (30.3±7.1%) highlights successful colonization of the wheat tissues by this pathogen following inoculation (Figure 2). However, the RA of *Z. tritici* rapidly decreased to 2±1.64% and 1.4±0.9% on residues not in contact with the soil ("above ground" residues) collected in October 2016 and 2017, respectively, and this species was below the limit of detection in December and February. For residues in contact with soil, this decrease occurred more rapidly, with *Z. tritici* already undetectable in samples collected in October.

Alpha diversity, estimated with the Shannon index, was low in July for both bacterial (2.70±0.75) and fungal communities (1.82±0.19; Suppl. Figure 1). A gradual increase was then observed during residue degradation. *Z. tritici* inoculation had no impact on bacterial alpha-diversity, but decreased fungal diversity (Kruskal-Wallis: $p = 0.008$). More specifically, bacterial diversity was higher in inoculated residue samples in July 2017 (2.92±0.80 for inoculated samples versus 2.47±0.6 for non-inoculated samples; Wilcoxon: $p = 0.022$), but no such difference was detected for the other sampling dates. Conversely, for fungal communities, inoculation had no effect in July, but led to a significant decrease in diversity in subsequent months during the second cropping season (October and December 2017, for the two soil contact conditions).

Beta diversity analysis (Bray-Curtis index) showed large dissimilarities between bacterial community composition in July and at the other sampling dates, as illustrated in the hierarchical clustering of the samples, justifying separate analyses and MDS representations (Figure 3). Inoculation with *Z. tritici* had a minor effect on bacterial communities, with only 5% of the variance explained for samples collected in July (PERMANOVA: $p = 0.004$). By contrast, in the same month, inoculation was the structuring factor for fungal communities, accounting for 33% of the variance (PERMANOVA: $p = 0.001$). For subsequent samplings (October, December and February), temporal conditions (seasonality and cropping season) were the main factors influencing fungal communities. Soil contact was the main structuring factor for bacterial communities, with a stronger effect than seasonality or cropping season (Table 1).

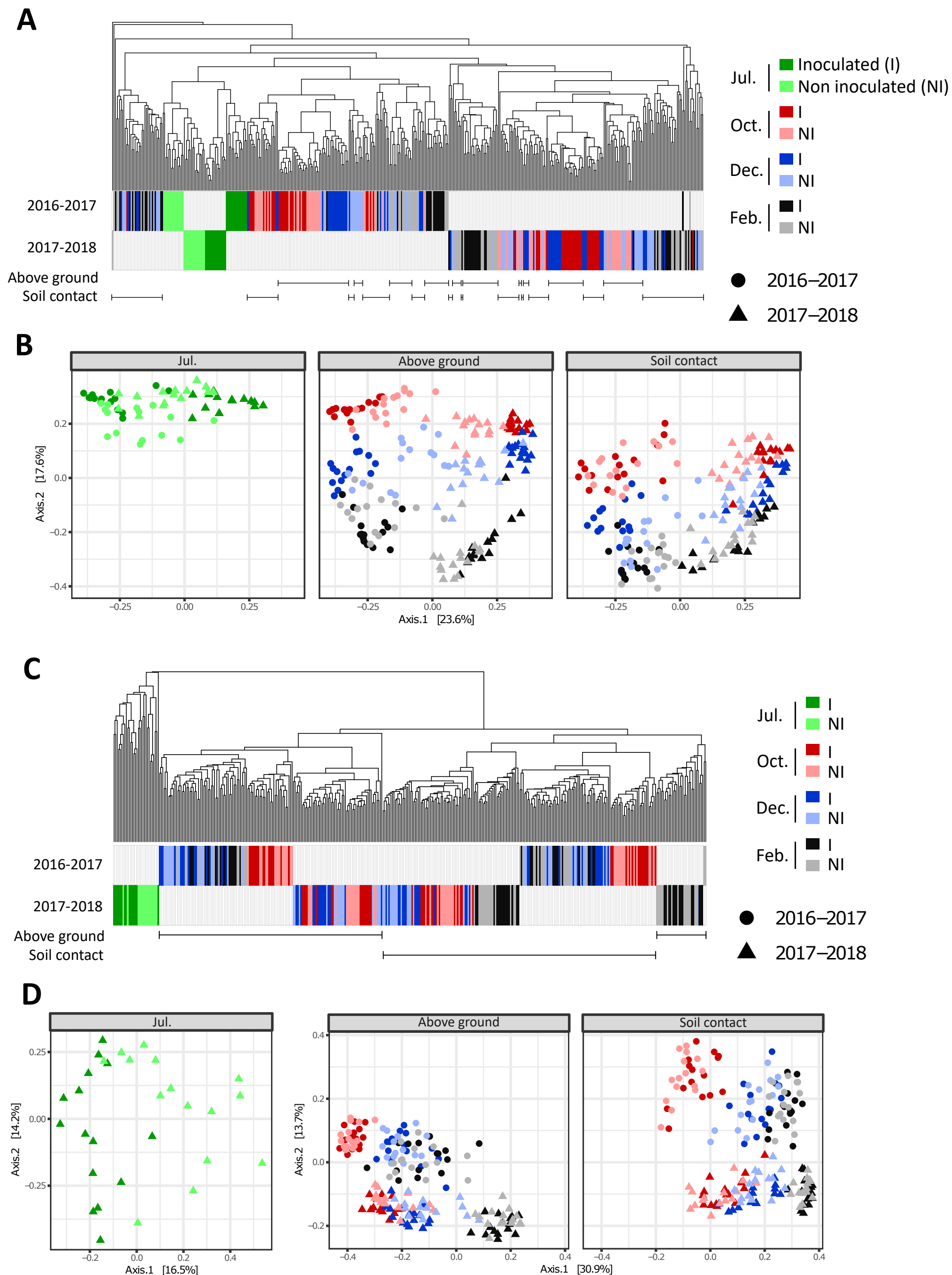

Figure 3. Dissimilarities between microbial communities. Beta diversity analyses for fungal (A, B) and bacterial (C, D) communities originating from 420 wheat residue samples. Hierarchical clustering (A, C) and multidimensional scaling (B, D) are based on the compositional distances between samples (Bray Curtis distance matrix). (A, C) Visualization of compositional distances between samples through hierarchical clustering with the average linkage method. The samples (15 sampling points per treatment) corresponding to the two cropping seasons (year) are represented by the two colored horizontal series (2016-2017, 2017-2018). Effects of seasonality are highlighted by different colours, corresponding to the different sampling dates (July: green; October: red; December: blue; February: gray). The intensity of the colors distinguishes between samples obtained from plants inoculated with *Z. tritici* (I, dark hues) and non-inoculated samples (NI, light hues). “Above ground” and “soil contact” treatments are represented by horizontal lines, with each sample considered separately. (B, D) Visualization of compositional distances between samples through multidimensional scaling (MDS). Each data point corresponds to one sample of wheat residues. The shape of the points (circles: 2016-2017; triangles: 2017-2018) corresponds to the cropping season (year effect); the colors, similar to those used in graphs A and C, correspond to the sampling dates (seasonality effect). For fungal communities, MDS analysis was performed on all samples together, whereas for bacterial communities, the analyses of the July samples and samples from all other sampling dates (October, December, and February) were separated, in accordance with the large differences between the communities of these samples shown in the clustering analysis (C). For a sake of clarity, the MDS are shown according to the soil contact condition.

Table 1 - Results of the PERMANOVAs analyzing the effects of cropping season, sampling date, contact with soil and inoculation on fungal and bacterial communities. Factors were tested with the adonis2 function of the vegan package. PERMANOVAs were performed with all tested factors together, with the “margin” option

		Factors tested	Proportion of the variance explained	*p*-value
Fungi	July	Season	0.197	0.001
		Inoculation	0.333	0.001
	Oct. - Dec. - Feb.	Season	0.217	0.001
		Sampling date	0.136	0.001
		Contact with soil	0.096	0.001
		Inoculation	0.012	0.001
Bacteria	July	Season	0.192	0.001
		Inoculation	0.051	0.004
	Oct. - Dec. - Feb.	Season	0.128	0.001
		Sampling date	0.168	0.001
		Contact with soil	0.195	0.001
		Inoculation	0.006	0.001

Impact of contact with the soil on microbial communities

The significant impact of soil contact on microbial communities highlighted differences in the process of wheat residue colonization. MDS analysis suggested that the communities of “above ground” residue samples collected in October were less different from those collected in July than from the communities of “soil contact” samples also collected in October (Figure 4). Contact with the soil, therefore, caused a greater change in communities, suggesting competition between plant-associated taxa and soil-borne taxa. Taxonomic differences between the communities present on residues in contact with the soil and those present in above ground residues were highlighted in linear discriminant analysis (LDA).

Some classes of taxa (e.g. *Bacilli*, *Sphingobacteria*, *Betaproteobacteria*, *Dothideomycetes*, *Pezizomycetes*) were particularly abundant only in above ground residues, suggesting that they were mostly derived from the plant. By contrast, other classes (e.g. *Alphaproteobacteria*, *Agaricomycetes*, *Cytophagia*, *Gammaproteobacteria*) were more prevalent in residues in contact with soil, suggesting that they originated from the soil (Suppl. Figure 2). The abundance of some classes varied with cropping season (e.g. *Flavobacteria*). Soil contact had a large impact for *Dothideomycetes* and *Bacilli*, which were highly abundant in July, but rapidly decreased in frequency when the residues were in contact with the soil. *Pezizomycetes*, absent in July, colonized only the above ground residues. Conversely, the percentage of reads associated with *Alphaproteobacteria*, which was quite high in July, and *Cytophagia*, which was low in July, increased over time, particularly in residues in contact with the soil. Similarly, *Agarycomycetes*, which was completely absent in July, colonized only residues in contact with the soil.

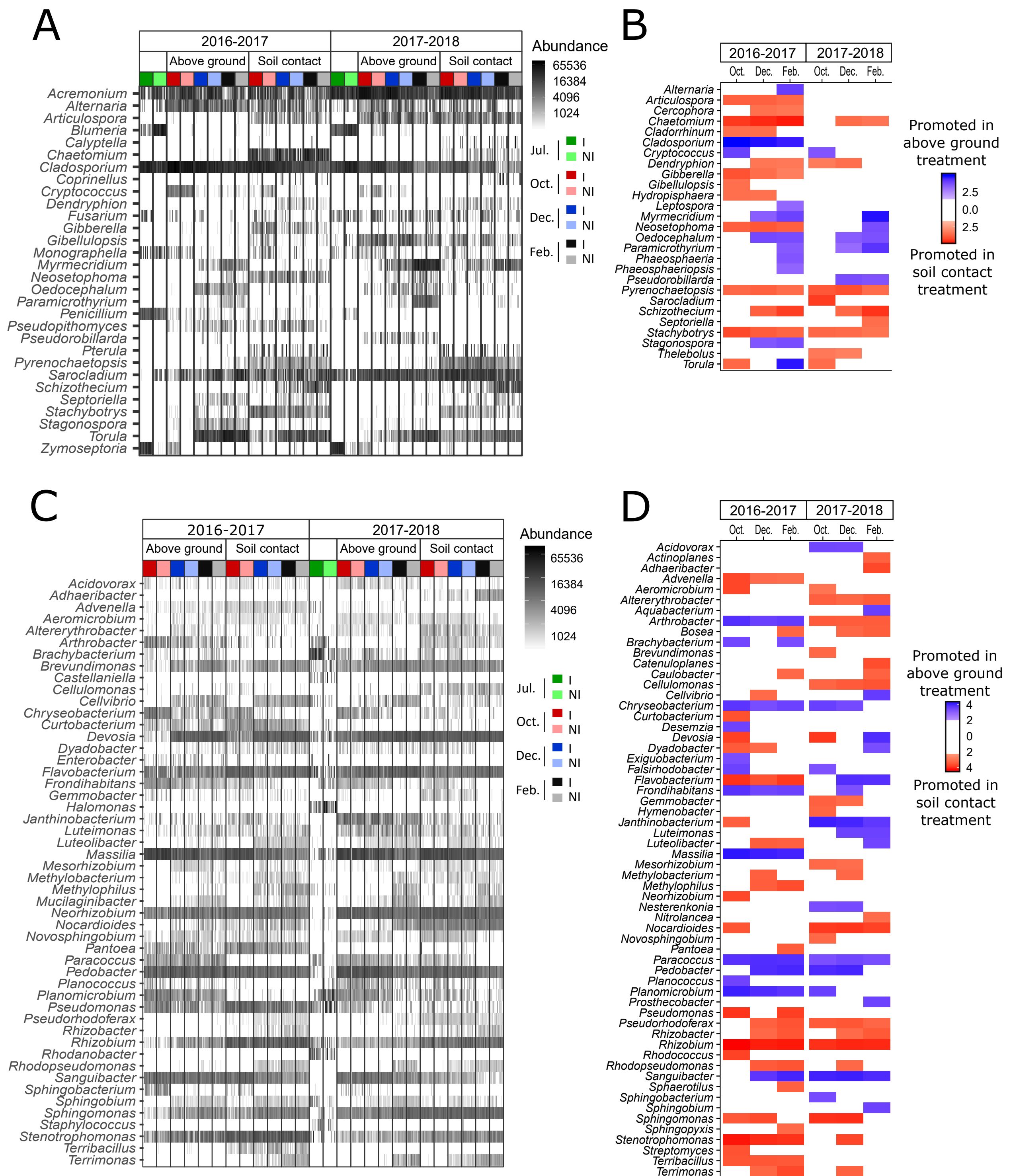

Figure 4. Changes in the relative abundance of microbial taxa over time.
(A, C) Diversity and dominance of the 30 most abundant (30/107) fungal genera (A) and the 50 most abundant (50/189) bacterial genera (B) distributed in all samples distinguishing between the different experimental conditions: i.e. cropping season (2016-2017; 2017-2018), contact with soil ("above ground" and "soil contact" treatments), seasonality (July: green; October: red; December: blue; February: gray), and inoculation with *Zymoseptoria tritici* (inoculated: dark hues; non-inoculated: light hues).
(B, D) Significant differences in relative abundance of fungal (B) and bacterial (D) genera between the samples in "soil contact" (red) and "above ground" (blue) samples in linear discriminant analysis (LDA). The Z. tritici inoculation condition was used as a subclass to avoid interference in the LDA. Only genera with a p-value < 0.05 for the Kruskal-Wallis test and an LDA score > 2 are displayed.

At the genus level, 87 (excluding "unclassified") of the 273 genera (60/190 for bacteria; 27/83 for fungi) identified displayed differences in abundance between above ground residues and residues in contact with the soil, for at least one date (Figure 4). For example, *Bosea*, *Rhizobium*, *Nocardioides*, *Pseudomonas*, and *Sphingomonas* were more abundant in residues in contact with the soil, whereas *Cladosporium*, *Massilia*, *Paracoccus*, *Stagonospora* and *Cryptococcus* were more abundant in above ground residues.

Impact of *Z. tritici* inoculation on microbial communities

The influence of *Z. tritici* on the RA of residue microbiome members was assessed, through LDA scores. In total, the RA of 115 ASVs (74 bacterial ASVs and 41 fungal ASVs) was significantly affected by *Z. tritici* inoculation, for at least one sampling date (listed in Suppl. Figure 3). The effect of inoculation on microbial communities persisted throughout the experiment, despite the absence of *Z. tritici* detection from December onwards (Figure 2). ASVs with significant differences in RA decreased over time for residues in contact with the soil (Suppl. Table 1). By contrast, for above ground residues, the number of differential ASVs increased until December, in both cropping seasons (20 ASVs in December 2016-2017; 31 ASVs in December 2017-2018).

Inoculation with *Z. tritici* decreased the number of fungal ASVs, including those affiliated to *Sarocladium*, *Gibellulopsis* and *Blumeria*, and increased the number of bacterial ASVs affiliated to *Curtobacterium* and *Brachybacterium* (listed in Suppl. Figure 3). The ASVs affected by inoculation differed between above ground residues and residues in contact with soil. The pattern of change (i.e. promoted or inhibited by inoculation) was always the same within a given year, regardless of soil contact conditions. For example, *Brachybacterium* and *Curtobacterium* were promoted by inoculation, in both soil contact conditions, whereas *Sarocladium* was inhibited by inoculation, in both soil contact conditions.

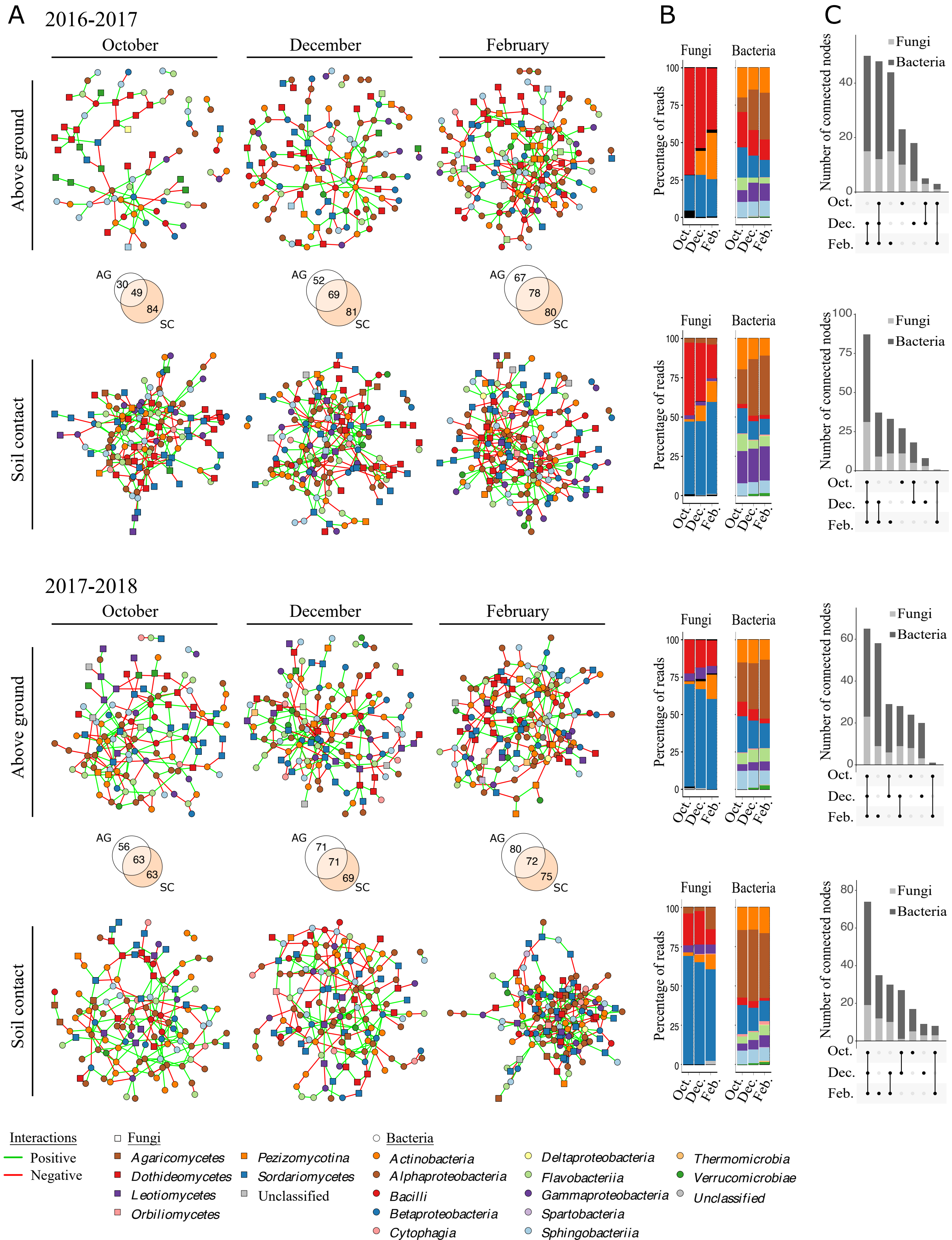

Figure 5. Temporal dynamics of co-occurrence networks.
(A) Networks based on bacterial and fungal ASVs combined. In all networks, circles and squares correspond to bacterial and fungal ASVs, respectively, with colors representing class. Isolated nodes are not shown. Edges represent positive (green) or negative (red) interactions. The Venn diagram highlights the number of non-isolated nodes common and specific to “above ground” (AG) and “soil contact” (SC) treatments for each sampling date (October, December, February).
(B) Percentage of reads associated with fungal and bacterial classes for each network. Isolated nodes are included. Colors are the same as in (A). (C) Upset plot of bacterial and fungal non-isolated nodes common and specific to sampling date for each treatment.

Impact of the actual presence of *Z. tritici* on microbial communities

Microbial analysis networks combining bacterial and fungal datasets were used to predict the potential interactions between *Z. tritici* and members of microbial communities associated with wheat residues.

Dynamics of ecological interaction networks – The dataset was split according to the effects previously described (cropping season, seasonality, soil contact conditions). Six ecological interaction networks were generated per experimental year, corresponding to residue samples in contact with the soil and above ground residues, collected in October, December, and February (Figure 5). The networks for July are presented in Suppl. Figure 4. The mean number of nodes in the network (205.3±47.5) increased over the season (Suppl. Table 1). Overall, networks were very sparse, with a mean node degree of 2.76□0.43. For each network, the positive/negative edge ratio decreased over time, reaching 1.0-1.5 in February. Most nodes were common to October, December and February. *Z. tritici* was one of the fungi with the largest number of degrees and greatest betweenness (measurement of centrality in a graph based on the shortest paths) for above ground samples in October. By contrast, for samples in contact with soil, it was absent the first year and had low betweenness and degree values for the second year (Figure 6).

***Subnetworks highlighting direct interactions between* Z. tritici *and other microorganisms* –** Ecological interaction networks were combined with LDA to investigate the interactions between *Z. tritici* and members of the microbial communities of residues (Figure 7). Only 13 of the 115 ASVs affected by inoculation (LDA) were in direct interaction with *Z. tritici*, indicating an indirect effect of *Z. tritici* on the community (no direct connection between the microorganisms). Microorganisms with the same differential pattern (i.e. "promoted by inoculation" or "promoted in the absence of inoculation") did not interact negatively with each other in networks. Conversely, microorganisms with opposite differential patterns systematically interacted negatively with each other. These results highlight the consistency of the LDA and ecological interaction network analysis approaches.

The subnetworks generated with microorganisms presenting differential relative abundances and their adjacent nodes were strongly connected: each subnetwork consisted of a principal component and, in some cases, smaller components of less than four nodes (Figure 7).

Only a few direct interactions between *Z. tritici* and other microorganisms were highlighted in ecological interaction networks. Some ASVs affiliated to the same genus had opposite interaction trends with *Z. tritici*, such as *Fusarium* ASVs in July 2017, or *Cladosporium* ASVs in October 2016, consistent with the findings of LDA analyses. In some cases, the same ASV had different interaction trends at different sampling dates or in different years. This was the case for *Acremonium* ASVs (negative interaction in October 2016, positive interaction in October 2017). Some genera, such as *Blumeria*, *Sarocladium*, and *Penicillium,* interacted only negatively with *Z. tritici*. *Symmetrospora*, *Brachybacterium*, and *Monographella* interacted only positively with *Z. tritici*.

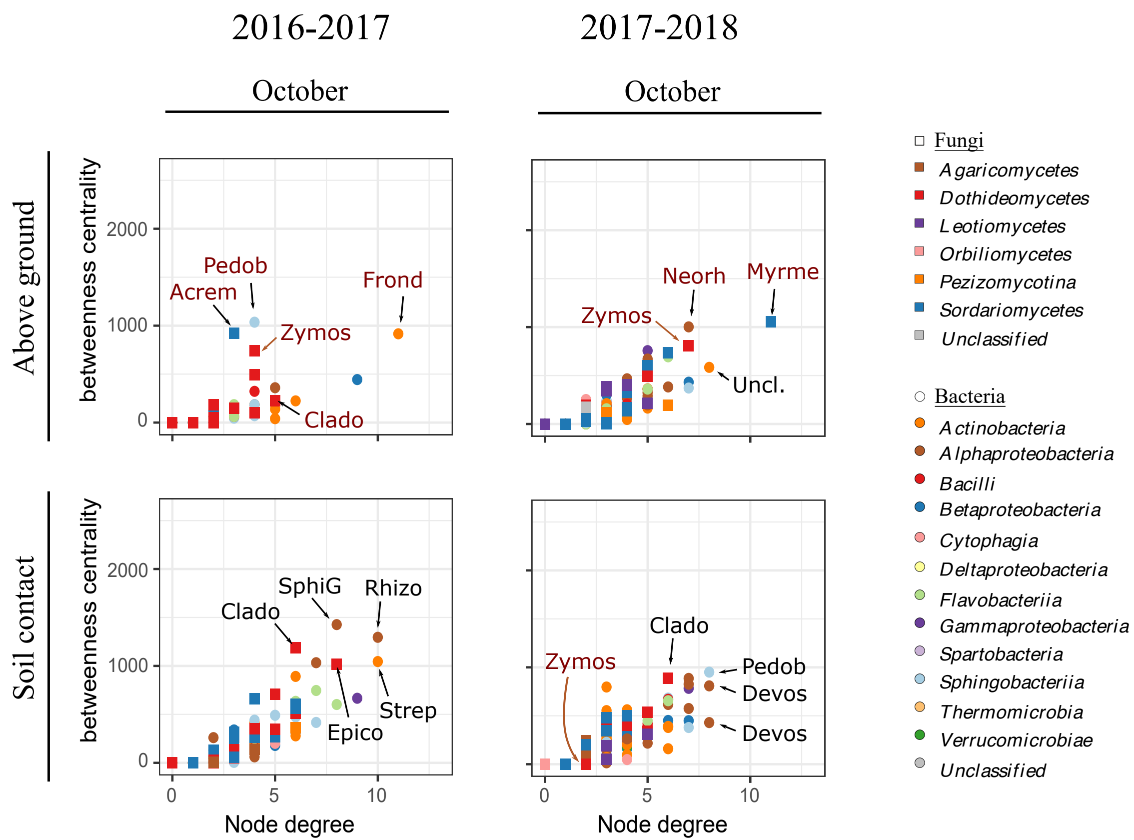

Figure 6. Betweenness, centrality and degree of each ASV in the October networks. Nodes with high betweenness, centrality and high degree values are considered to be keystone taxa in the networks. The genera of the fungal and bacterial ASVs with the highest degree and centrality are shown: *Acrem(onium); Clado(sporium); Devos(ia); Epico(ccum); Frond(ihabitans); Myrme(cridium); Neorh(izobium); Pedob(acter); Rhizo(bium); SphiG(=Sphingomonas); Strep(tomyces); Uncl.(assified); Zymos(eptoria)*. The betweenness, centrality and degree of each ASV in the networks for the other sampling dates (July, December, and February) are presented in Supplementary Figure 5.

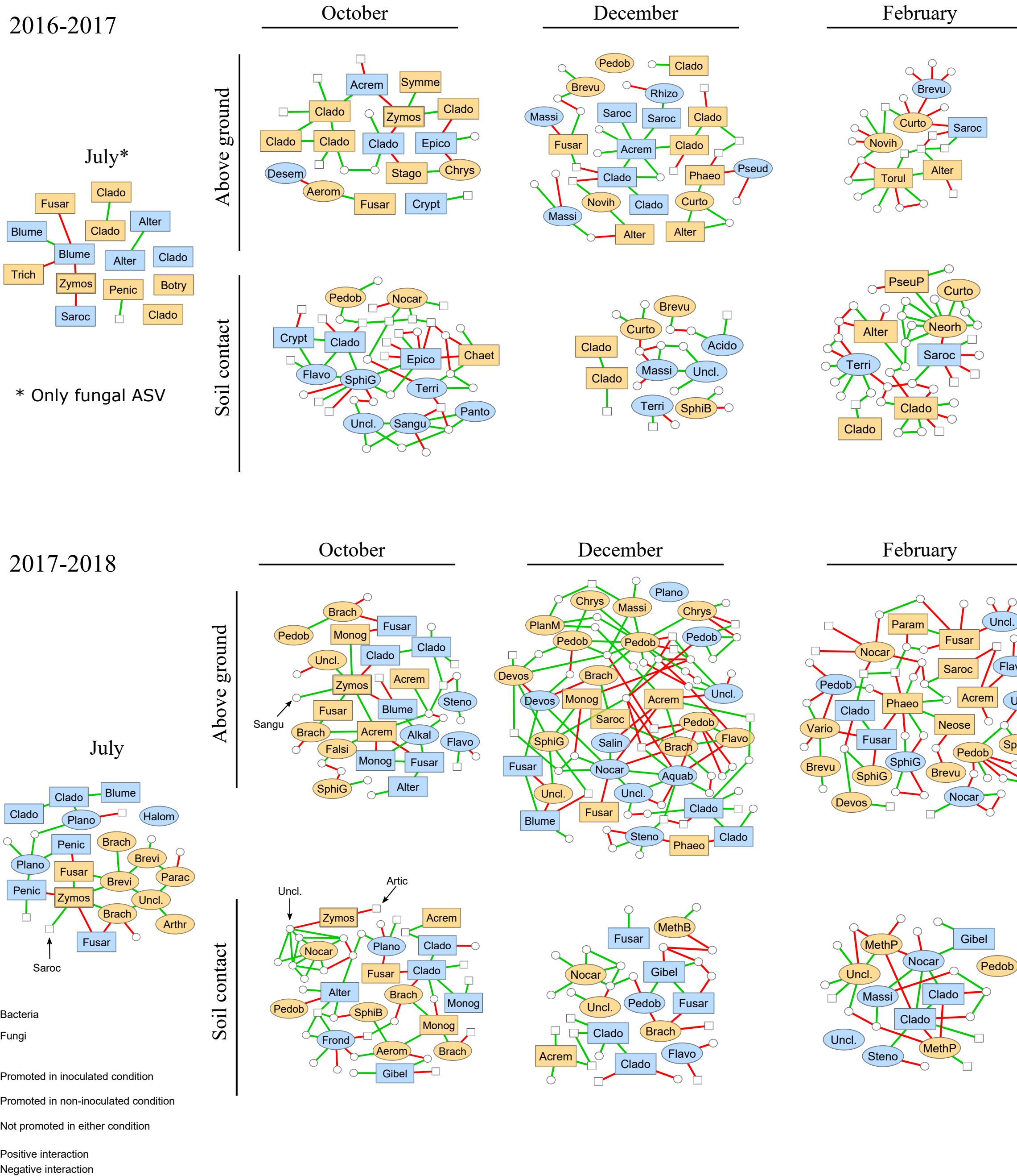

Figure 7. Subnetworks based on the data in Figure 4A and composed of differential bacterial and fungal ASVs identified in residue samples (originating from wheat plants inoculated and non-inoculated with *Zymoseptoria tritici*) and of the first adjacent nodes. Node color corresponds to the results of LefSe differential analysis between inoculated (orange) and non-inoculated (blue) treatments. Only genera with p-values < 0.01 for the Kruskal-Wallis tests and LDA scores > 2 were retained for the plot. The first adjacent nodes of each differential ASV are not named, except for ASVs interacting with *Z. tritici*. Edges represent positive (green) or negative (red) interactions. Differential ASVs are plotted with genus name abbreviations: *Acido(vorax); Acrem(onium); Aerom(icrobium); Alkal(ibacterium); Alter(naria); Aquab(acterium); Arthr(obacter); Blume(ria); Botry(osporium); Brach(ybacterium); Brevi(bacterium); Brevu(ndimonas); Chaet(omium); Chrys(eobacterium); Clado(sporium); Crypt(ococcus); Curto(bacterium); Desem(zia); Devos(ia); Epico(ccum); Falsi (rhodobacter); Flavo(bacterium); Frond(ihabitans); Fusar(ium); Gibel(lulopsis); Halom(onas); Massi(lia); MethB (=Methylobacterium); MethP(=Methylophilus); Monog(raphella); Neorh(izobium); Neose(tophoma); Nocar(dioides); Novih(erbaspirillum); Panto(ea); Parac(occus); Param(icrothyrium); Pedob(acter); Penic(illium); Phaeo(sphaeria); PhaeP(sphaeriopsis); Plano(coccus); PlanM(=Planomicrobium); Pseud(omonas); PseuP(=Pseudopithomyces); Rhizo (bium); Rhoda(nobacter); Salin(irepens); Sangu(ibacter); Saroc(ladium); SphiB(=Sphingobium); SphiG (=Sphingomonas); Stago(nospora); Steno(trophomonas); Symme(trospora); Terri(bacillus); Torul(a); Trich(oderma); Uncl.(assified); Vario(vorax); Zymos(eptoria).*

The subnetworks generated with microorganisms presenting differential relative abundances and their adjacent nodes were strongly connected: each subnetwork consisted of a principal component and, in some cases, smaller components of less than four nodes (Figure 7).

Only a few direct interactions between *Z. tritici* and other microorganisms were highlighted in ecological interaction networks. Some ASVs affiliated to the same genus had opposite interaction trends with *Z. tritici*, such as *Fusarium* ASVs in July 2017, or *Cladosporium* ASVs in October 2016, consistent with the findings of LDA analyses. In some cases, the same ASV had different interaction trends at different sampling dates or in different years. This was the case for *Acremonium* ASVs (negative interaction in October 2016, positive interaction in October 2017). Some genera, such as *Blumeria*, *Sarocladium*, and *Penicillium,* interacted only negatively with *Z. tritici*. *Symmetrospora*, *Brachybacterium*, and *Monographella* interacted only positively with *Z. tritici*.

Discussion

By sequencing the microbial communities of 420 samples of wheat residues, we obtained a total of 996 bacterial ASVs and 520 fungal ASVs. Using this large dataset, we estimated the potential interactions occurring between a plant pathogen (*Z. tritici*) and the members of microbial communities associated with crop residues in field conditions. By combining two approaches — LDA and network analysis — we were able to demonstrate an effect of pathogen infection, even after disappearance of the pathogen, on the structure and composition of the microbial communities during residue degradation.

Effect of soil contact on microbial communities

Our aim here was not to characterize the organisms colonizing wheat residues, but our findings nevertheless highlight major changes in the microbial community over time for residues in contact with soil. The taxa favored in above ground residues, such as *Cladosporium*, *Alternaria*, *Pedobacter* and *Massilia*, were already present on the plant. This is consistent with previous findings showing a decrease in the abundance of these plant-associated taxa during the degradation of residues in contact with soil and the colonization of these residues with soil-borne competitors, such as *Chaetomium*, *Torula*, and *Nocardioides* [16]. Some fungal genera not present in July were favored by above ground conditions (e.g. *Cryptococcus*, *Stagonospora*, and *Myrmecridium*). This finding is consistent with our knowledge of fungal dispersal processes, mostly involving aerial spores.

Disappearance of *Z. tritici* during residue degradation

Z. tritici rapidly disappeared between October and December. This finding is surprising in light of the quantitative epidemiological data acquired for the same plot, which suggested that *Z. tritici* ascospores may be ejected from residues until March [3,37]. The observed disappearance of *Z. tritici* may be due to lower levels of contamination of adult wheat plants in residues than would be achieved in the field after natural infection. Indeed, in field conditions, *Z. tritici* establishes itself on all parts of the plant (leaves, but also sheaths and stems) through multiple secondary infections, driven by the repeated splash dispersal of asexual spores, leading to an accumulation of contaminating raindrops at the points of insertion of the leaf sheaths. The single inoculation event in the greenhouse resulted in contamination principally of the leaves, the organs most exposed to spraying, with relatively little contamination of the stems and sheaths, the parts of the plant most resistant to degradation. Indeed, the results of a previous study [16] support this hypothesis: in the same field, during the same season, *Z. tritici* was detected in wheat residues originating from plants grown in natural conditions until February, and even May, with a similar metabarcoding approach.

Effect of *Z. tritici* on microbial communities

Endophytes and pathogens induce changes in plant tissues (e.g. necrosis), which may themselves modify the microbial communities inhabiting the plant (e.g. impact of secondary saprophytes or opportunistic pathogens [38]; selection of microorganisms by secondary metabolites produced by microorganisms or the plant; [39,40]). This general phenomenon may explain the impact of *Z. tritici* on the microbial communities observed in both LDA and network analysis. The impact of *Z. tritici* on residues, even after its disappearance between October and December, persisted until February, particularly for fungal communities. Within microbial networks, *Z. tritici* was one of the keystone taxa, despite its low abundance, in above ground residues in October (Suppl. Figure 5). The high levels of *Z. tritici* in July (between 10 and 40% of reads) account for its central position in the network. The number of microorganisms displaying changes in abundance due to *Z. tritici* inoculation decreased during residue degradation. This finding highlights the resilience of the community (i.e. its ability to return to its original composition after a disturbance, in this case, *Z. tritici* inoculation) [41].

Specific interactions with *Z. tritici*

Most of the predicted interactions with *Z. tritici* involved fungi, such as *Fusarium*, *Blumeria* or *Cladosporium*. *Z. tritici* infection has been shown to be associated with the accumulation of H_2O_2 [42]. This compound is known to inhibit biotroph fungal pathogens [43], such as *Blumeria graminis* [44,45]. This may explain the negative interaction between *Z. tritici* and *B. graminis* in July and October 2017-2018. In addition, *Z. tritici* infection induces leaf necrosis, potentially decreasing wheat susceptibility to *B. graminis,* due to a significant physiological interaction during the latent, endophytic period of *Z. tritici* development [45]. H_2O_2 is also known to promote necrotrophic agents, such as *Fusarium*. We detected both positive and negative interactions between *Z. tritici* and *Fusarium*, depending on the ASV considered. On adult wheat plants, such differential interactions have been demonstrated in log-linear analyses [46], with both species giving positive results on stem bases and negative results on the upper parts of stems. Positive interactions between *Z. tritici* and *Cladosporium* have also been demonstrated on adult plants [46], consistent with our findings for wheat residues. These results lend a biological meaning to the interactions detected, confirming the relevance of network analyses for highlighting ecological interactions within crop residue communities.

Trichoderma was more abundant in residues from wheat plants inoculated with *Z. tritici* (July 2016), as shown by LDA (Suppl. Figure 4). Conversely, *Epicoccum* and *Cryptococcus* were more abundant in residues from non-inoculated wheat plants (October 2016). The overabundance of those taxa, described as biocontrol agents in previous studies [34–36,47], was influenced by the presence of the pathogen. However, no direct interactions between *Z. tritici* and these species could be established. This exemplifies the difficulties highlighting beneficial species within complete microbial communities. These difficulties are not specific to the residue compartment and also apply to the spermosphere [48], phyllosphere [49] and rhizosphere compartments [14,50].

Other interactions

Other interactions between ASVs highlighted in the network analysis were examined in light of published results for fungal pathogens of cereals. For instance, it has already been shown that *B. graminis* growth on barley is inhibited by *Trichoderma harzianum* [51] and *Stagonospora norodum* [52], that *Stenetrophomas maltophila* attenuates the seedling blight of wheat caused by *F. graminearum* [53], that *Acremonium zeae* has antibiotic activity against *Fusarium verticillioides* [54], and that *Chaetomium sp.* produces compounds (e.g. chaetomin) active against *Alternaria triticimaculans* [34]. Conversely, certain non-pathogenic bacteria were shown to be associated with significantly more disease on wheat caused by *B. graminis* and *Z. tritici* and to "help" *Phaeosphaeria nodorum* to infect wheat tissues [55]. Newton *et al.* [38] has proposed the hypothesis of "induced susceptibility" to explain such an interaction between bacteria and biotroph fungal pathogens.

The analysis of ecological network also suggested that intra-kingdom interactions were favoured over inter-kingdom interactions in certain conditions (Suppl. Table 2). This may reflect differences in ecological niches and dynamics, as illustrated by the temporal changes in microbial communities over a season, with a densification of the networks during residue degradation. Further investigations are required to determine whether inter- or intra-kingdom interactions are more intense, and thus more promising for use in biocontrol engineering. Should we preferentially focus on fungal communities to improve the management of a fungal disease, and on bacterial communities to improve the management of a bacterial disease?

Identification of beneficial species, and potential biocontrol agents

Network models provide new opportunities for enhancing disease management and can be helpful for biocontrol. Our study, combining LDA and ecological network analyses based on a metabarcoding approach and differential conditions (plants inoculated with a pathogen or left non-inoculated; plant residues in contact with soil vs. residues not in contact with the soil), fits into the framework described by Poudel *et al.* [56], which considers several types of network analyses, including pathogen-focused analyses, taking into account diseased and healthy plant hosts, with a view to elucidating direct and indirect pathogen-focused interactions within the pathobiome.

Network analyses revealed no significant direct interactions between *Z. tritici* and microorganisms reported to be useful biocontrol agents. However, pathogen infection had a strong effect on the entire microbial community present in residues during the course of their degradation. Most of the interactions were difficult to interpret. Several interactions appeared to be transient, changing over time with residue degradation, and their presence or absence depended on whether the residues were in contact with the soil. This suggests that interactions between microorganisms are not very stable, and can be modified by changes in the environment, for example, or by the arrival of a new microorganism.

The neglect of complex interactions between biocontrol agents and their biotic environment (the plant, the soil and their microbiomes), the physical and chemical properties of which change over time, may account for lower levels of efficacy in field conditions than in laboratory conditions (concerning the

phyllosphere, e.g. [38], but also the residue compartment, e.g. [57]). Indeed, several studies have demonstrated the value of studying the effect of entire communities on biotic and abiotic stresses rather than the effects of single species. For example, resistance to *B. cinerea* in *Arabidopsis thaliana* was shown to be not due to a single species, but to the action of the microbiome as a whole [58]. By comparing the structure of microbial communities associated with *Brassica rapa* plants inoculated with the root pathogen *Plasmodiophora brassicae*, Lebreton *et al*. [14] showed significant shifts in the temporal dynamics of the root and rhizosphere microbiome communities during root infection. Moreover, the rhizospheres of plants infected with *P. brassicae* were significantly more frequently colonized with a *Chytridiomycota* fungus, suggesting interactions between these two microorganisms.

The most frequently studied cases of microbial community effects include "suppressive soils", which provide defense against soil-borne pathogens, rendering them unable to establish themselves or to persist in the soil or the plant [59]. The basis and dynamics of this disease suppression vary, and suppression may be general or specific, under the control of antibiotic-producing *Pseudomonas* or *Streptomyces* populations, for example [60]. Differences in the composition, structure and diversity of microbial communities on crop residues remain poorly understood, and further studies are required to determine the potential for use in biocontrol not of single agents, but of microbial communities, as for these suppressive soils. Despite this ecological reality, the current perception of biocontrol engineering is still too often limited to the action of a single species, even a single strain, with a direct, strong and durable effect against a plant pathogen.

Potential utility of the residue microbiome

Improving our understanding of the relationship between biodiversity and ecosystem functioning will require the development of methods integrating microorganisms into the framework of ecological networks. Exhaustive descriptions of microbial diversity combined with network analysis are particularly useful for identifying species within microbial communities of potential benefit for disease management [56]. By revealing antagonistic interactions between pathogen species (e.g. *Z. tritici*) and other microorganisms, our study suggests that this strategy could potentially improve the control of residue-borne diseases, as suggested by another recent study on *Fusarium* [17]. This strategy, which has been developed separately for the plant [61,62] and soil [14,50,63] compartments, would undoubtedly benefit from further development on crop residues. Indeed, decreasing the presence of pathogens on residues during the interepidemic period can decrease disease development on subsequent crops [21]. The strategy developed here can be viewed as a proof-of-concept for the use of crop residues as a particularly rich ecological compartment containing a high diversity of taxa originating from both the plant and soil compartments, and for the use of *Z. tritici*-wheat as a model pathosystem. Understanding the complex interactions between a pathogen, crop residues and other microbiome components in the shaping of a plant-protective microbiome is essential, to improve the efficacy of biocontrol agents and to preserve existing beneficial equilibria through the adoption of appropriate agricultural practices.

Methods

We investigated the effect of *Z. tritici* on the diversity of the wheat microbiome and the effect of the wheat microbiome on *Z. tritici*, by characterizing the composition of the microbial communities of 420 residue samples (210 per year) from plants with and without preliminary *Z. tritici* inoculation. The residues were placed outdoors, either directly in contact with the soil in a field plot or “above ground”, i.e. not in contact with the soil, to assess the effect of their colonization by microorganisms originating from the soil, the plant and the air on the saprophytic development of *Z. tritici*. We investigated the persistence of interactions between the pathogen and the whole microbial community, and changes in those interactions over time, by sampling the residues before exposure to outdoor conditions (in July), and every two months thereafter (in October, December, and February) (Figure 1).

Preparation of wheat residues

The 420 wheat residue samples were obtained from 60 winter wheat cv. Soissons plants grown in a greenhouse in each of the two years of the study, as described in [64]: two weeks after sowing, seedlings were vernalized for eight weeks in a growth chamber and then transplanted into pots. Three stems per plant were retained. Half the wheat plants were inoculated with a mixture of four *Z. tritici* isolates (two Mat1.1. isolates and two Mat1.2 isolates; [65]) to ensure that sexual reproduction occurred as in natural conditions. This equiproportional conidial suspension was prepared and adjusted to a concentration of 2×10^5 spores.mL^{-1}, as previously described [64]. Thirty plants were inoculated at the late heading stage in early May, by spraying with 10 mL of inoculum suspension. The other thirty plants were sprayed with water, as a control. Inoculated and non-inoculated plants were enclosed in transparent plastic bags for three days to ensure moist conditions favoring pathogen infection. Septoria tritici blotch lesions appeared three to four weeks after inoculation (Figure 1A). All plants were kept in the same greenhouse compartment until they reached complete maturity (mid-July).

For each “inoculated” and “non-inoculated” condition, stems and leaves were cut into 2 cm-long pieces and homogenized to generate the “wheat residues”, which were then distributed in 105 nylon bags (1.4 g per bag; **Figure 1B**) for each set of inoculation conditions, in each year.

Exposure of residues to natural conditions

Ninety nylon bags were deposited in contact with the soil in a field plot (the “soil contact” treatment) or without contact with the soil (“above ground” residue treatment). Thirty batches of residues (15 inoculated and 15 non-inoculated) were used to characterize the communities present in July before the exposure of the residues in the nylon bags to natural conditions. The field plot (“OWO” in [16]; Grignon experimental station, Yvelines, France; 48°51′N, 1°58′E) was the same in both cropping seasons. It was sown with wheat in 2015-2016, with oilseed rape in 2016-2017, and with wheat in 2017-2018. The 90 bags for the “soil contact” treatment were deposited in the OWO field plot (**Figure 1C**) in late July, at 15 sampling points 20 m apart (three “inoculated” and three “non-inoculated” bags at each

sampling point). The 90 bags of the "above ground" treatment were placed on plastic grids exposed to outdoor conditions and located about 300 m from the OWO field plot (Figure 1D).

We assessed the impact of seasonality on the fungal and bacterial communities on residues by collecting samples of each "inoculated" and "non-inoculated" treatment at three dates (October, December and February): 15 bags from plastic grids ("above ground" treatment) and one bag from each sampling point in the field ("soil contact" treatment) At each date, nylon bags were opened, the residues were rinsed with water and air-dried in laboratory conditions. Residues were then crushed with a Retsch™ Mixer Mill MM 400 for 60 seconds at 30Hz with liquid nitrogen in a Zirconium oxide blender.

Total DNA extraction

Total DNA was extracted with the DNeasy Plant Mini kit (Qiagen, France), with a slightly modified version of the protocol recommended by the manufacturer. Powdered residues (20 mg), 450 μL of Buffer AP1 preheated to 60°C, RNase A and Reagent DX (450: 1: 1) were mixed vigorously for 15 s in a 2 mL Eppendorf tube. Buffer P3 (130 μL) was added to each tube, which was then shaken manually for 15 s, incubated at -20°C, and centrifuged (1 min, 5000 g). The supernatant (450 μL) was transferred to a spin column and centrifuged (2 min, 20000 g). The filtrate (200 μL) was transferred to a new tube, to which sodium acetate (200 μL, 3 M, pH 5) and cold 2-propanol (600 μL) were added. DNA was precipitated by incubation at -20°C for 30 min and recovered by centrifugation (20 min, 13000 g). The pellet was washed with cold ethanol (70%), dried, and dissolved in 50 μL of AE buffer.

PCR and Illumina sequencing

Fungal and bacterial communities profiles were analyzed by amplifying ITS1 and the v4 region of the 16S rRNA gene, respectively. Amplifications were performed with ITS1F/ITS2 [66] and 515f/806r [67] primers. All PCRs were run in a total volume of 50 μL, with 1x Qiagen Type-it Multiplex PCR Master Mix (Type-it® Microsatellite PCR kit Cat No./ID: 206243), 0.2 μM of each primer, 1x Q-solution® and 1 µl DNA (approximately 100 ng). The PCR mixture was heated at 95°C for 5 minutes and then subjected to 35 cycles of amplification [95°C (1 min), 60°C (1 min 30 s), 72°C (1 min)] and a final extension step at 72°C (10 min). PCR products were purified with Agencourt® AMPure® XP (Agencourt Bioscience Corp., Beverly, MA). A second round of amplification was performed with 5 µl of purified amplicons and primers containing Illumina adapters and indices. PCR mixtures were heated at 94°C for 1 min, and then subjected to 12 cycles of amplification [94°C (1 min), 55°C (1 min), 68°C (1 min)] and a final extension step at 68°C (10 min). PCR products were purified and quantified with Invitrogen QuantIT™ PicoGreen®. Purified amplicons were pooled in equimolar concentrations, and the final concentration of the library was determined with the qPCR NGS library quantification kit (Agilent). Libraries were sequenced in four independent runs with MiSeq reagent kit v3 (600 cycles).

Sequence processing

Runs were analyzed separately. Primer sequences were first cut off in the fastq files with Cutadapt [68]. Files were then processed with DADA2 v.1.8.0 [69] according to the recommendations for the "DADA2 Pipeline Tutorial (1.8)" workflow [70], with quality trimming adapted for each run (Suppl. Table 3).

A mock sample consisting of equimolar amounts of DNA from known microorganisms was included in each run (see Suppl. Figure 6) to establish a detection threshold for spurious haplotypes. At a threshold of ≤ 0.3 ‰ of the size of the library, amplicon sequence variants (ASVs) were considered spurious and were removed from the sample. We used the naive Bayesian classifier on RDP trainset 14 [71] and the UNITE 7.1 database [72] to assign ASVs. ASVs as assigned to chloroplasts (for bacteria) or unclassified at the phylum level (for bacteria and fungi) were also removed from each sample. Due to large proportion of chloroplast sequences among the 16S rRNA gene products, all samples from July 2017 were removed from the analysis.

Differential community analysis

For microbial community analyses, the total library size of each sample was standardized by normalization by proportion. The experimental conditions taken into account were cropping season (2016-2017 and 2017-2018), seasonality (four sampling dates: July, October, December, and February), inoculation with *Z. tritici* (inoculated and non-inoculated), soil contact (soil contact and above ground treatments). The Shannon diversity index was used to assess the effect of each set of conditions on fungal and bacterial diversity. The divergence of microbial communities between samples was assessed by calculating the Bray-Curtis dissimilarity matrix with the phyloseq package (v 1.24.2 [73]), and then illustrated by MDS and clustering based on the average linkage method (ape package v 5.2. [74]). PERMANOVA was performed with the "margin" option, to test the effect of each factor on communities (adonis2 function, vegan package [75]).

A linear discriminant analysis (LDA) implemented in Galaxy [76] (LefSe, http://huttenhower.org/galaxy) was used to characterize the differential abundances of fungal and bacterial taxa between each soil contact condition and each *Z. tritici* inoculation condition. In this analysis, differences in the relative abundance of taxa between treatments were evaluated with a Kruskal-Wallis test; a Wilcoxon test was used to check, by pairwise comparisons, whether all subclasses agreed with the trend identified in the Kruskal-Wallis test. The results were used to construct an LDA model, to discriminate between taxa in the different conditions. For the comparison between "soil contact" and "above ground" treatments, inoculation condition was used as a subclass, with the Wilcoxon test alpha value set at 0.05, and the alpha value of the Kruskal-Wallis test set at 0.01. For the comparison between "inoculated" and "non-inoculated" treatments, the alpha value of the Kruskal Wallis test was set at 0.01 (no subclasses). For both analyses, the threshold for the LDA analysis score was set at 2.0.

Ecological interaction network analyses

For characterization of interactions within the different wheat residue microbial communities, we used ecological interactions networks calculated with SPIEC-EASI [77] for combined bacterial and fungal datasets [78]. The same parameters were used for all networks. The non-normalized abundance dataset was split on the basis of sampling date and soil contact condition. Each of the datasets included 30 samples (15 inoculated samples and 15 non-inoculated samples). Infrequent ASVs were filtered out by defining a threshold of a minimum of six occurrences. We used the neighborhood selection as graphical inference model (Meinshausen and Bühlmann MB method) with SPIEC-EASI, as this method has been shown to outperform most of the other available methods (e.g. CCREPE, SPARCC, SPIEC-EASI (glasso)) [77]. The StARS variability threshold was set at 0.05. Networks were then analysed with the igraph package (version 1.2.2. [79]). Scripts for network construction and analysis are available from GitHub (see Availability of data and materials).

Subnetworks for analysis of the *Z. tritici* pathobiome

We used a dual approach to characterize interactions between *Z. tritici* and the other taxa, based on: (i) the LDA scores obtained in differential analyses between *Z. tritici* inoculation conditions ("inoculated" and "non-inoculated" treatments); (ii) ecological network analysis. LDA identified taxa affected by inoculation conditions (definition of classes for samples) and network analysis identified interactions at the sample scale (without prior assumptions). Subnetworks of differential ASVs and their adjacent nodes were established by combining these two approaches. Subnetworks were visualized with Cytoscape V. 3.6.1 [80]

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Availability of data and materials

The raw sequencing data are available from the European Nucleotide Archive (ENA) under study accession number PRJEB31818. We provide the command-line script for data analysis and all necessary input files via GitHub (https://github.com/LydieKerdraon/MicrobialNetworkAnalysis-WheatResidues).

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Supplementary Table 1 - Sequence filtering for each run

Run	Primers	Sequence number (paired end)	Sequence quality trimming (F/R)	Selection by sequence length	Quality sequence number after DADA2 analysis
#1	ITS1F / ITS2	10 536 086 (×2)	220 / 210	-	7 164 826
#2	515f / 806r	8 368 872 (×2)	230 / 200	253 bp	5 562 335
#3	ITS1F / ITS2	10 216 508 (×2)	220 / 190	-	6 965 664
#4	515f / 806r	9 975 344 (×2)	220 / 170	253 bp	5 734 825

Supplementary Table 2 - Analysis of the proportion of intra-kingdom interactions (between two fungal ASVs and between two bacterial ASVs) and inter-kingdom interactions (between a fungal ASV and a bacterial ASV) in the ecological networks. The statistical significance of the under- or over-representation of inter-kingdom interactions (when F-B residuals < 1 or > 1, respectively) was established by a χ^2 test of independence performed on the contingency table ($\chi^2 < 0.001$).

Networks [1]	Number of species		Number of interactions			Theoretical maximum number of interactions			Residuals		
	F [2]	B [3]	F-F	B-B	F-B	F-F [4]	B-B [5]	F-B [6]	F-F	B-B	F-B
Oct. 2016-2017, above ground	32	73	17	57	17	496	2628	2336	1.856	0.545	-1.939
Oct. 2016-2017, contact with soil	52	90	32	121	69	1326	4005	4680	1.060	-0.736	0.358
Dec. 2016-2017, above ground	39	100	19	86	42	741	4950	3900	0.340	0.036	-0.266
Dec. 2016-2017, contact with soil	51	105	25	145	74	1275	5460	5355	-0.772	0.236	0.160
Feb. 2016-2017, above ground	48	107	30	110	65	1128	5671	5136	1.109	-0.866	0.508
Feb. 2016-2017, contact with soil	55	110	32	136	90	1485	5995	6050	0.208	-1.170	1.505
July 2017-2018	16	60	8	45	9	120	1770	960	0.216	1.476	-2.201
Oct. 2017-2018, above ground	39	93	28	93	62	741	4278	3627	1.309	-1.321	1.019
Oct. 2017-2018, contact with soil	31	109	17	140	43	465	5886	3379	-1.413	2.172	-2.144
Dec. 2017-2018, above ground	42	110	22	119	68	861	5995	4620	-0.598	-0.253	0.733
Dec. 2017-2018, contact with soil	35	116	19	149	50	595	6670	4060	-1.384	1.949	-1.849
Feb. 2017-2018, above ground	44	119	27	136	72	946	7021	5236	-0.207	-0.081	0.244
Feb. 2017-2018, contact with soil	46	114	26	135	91	1035	6441	5244	-0.752	-0.978	1.845

[1] according to sampling date, cropping season and contact with soil

[2] fungal species

[3] bacterial species

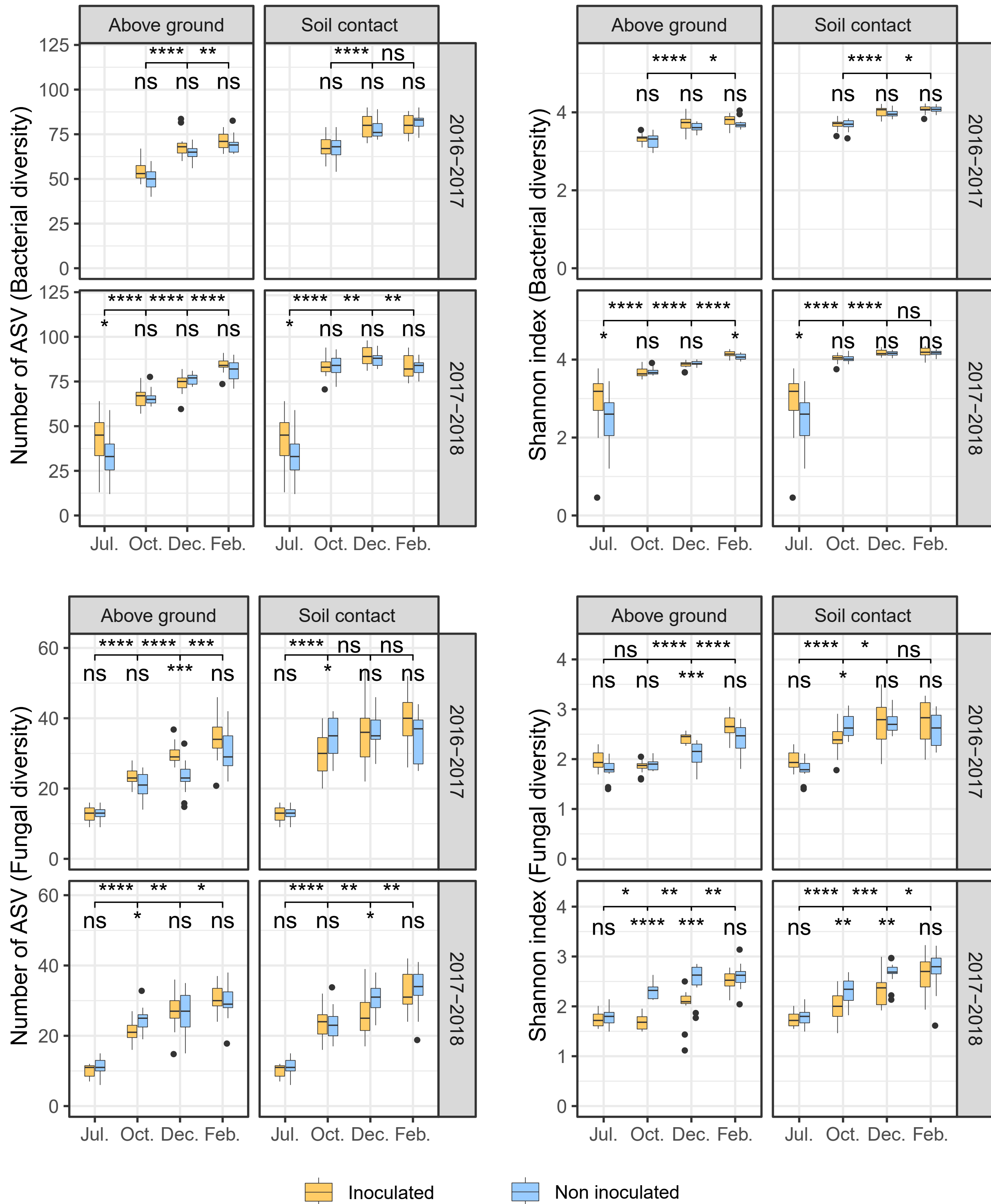
[4] estimated by $C_2^{n_F} = \frac{n_F!}{2 \times (n_F-2)!}$

[5] estimated by $C_2^{n_B}$

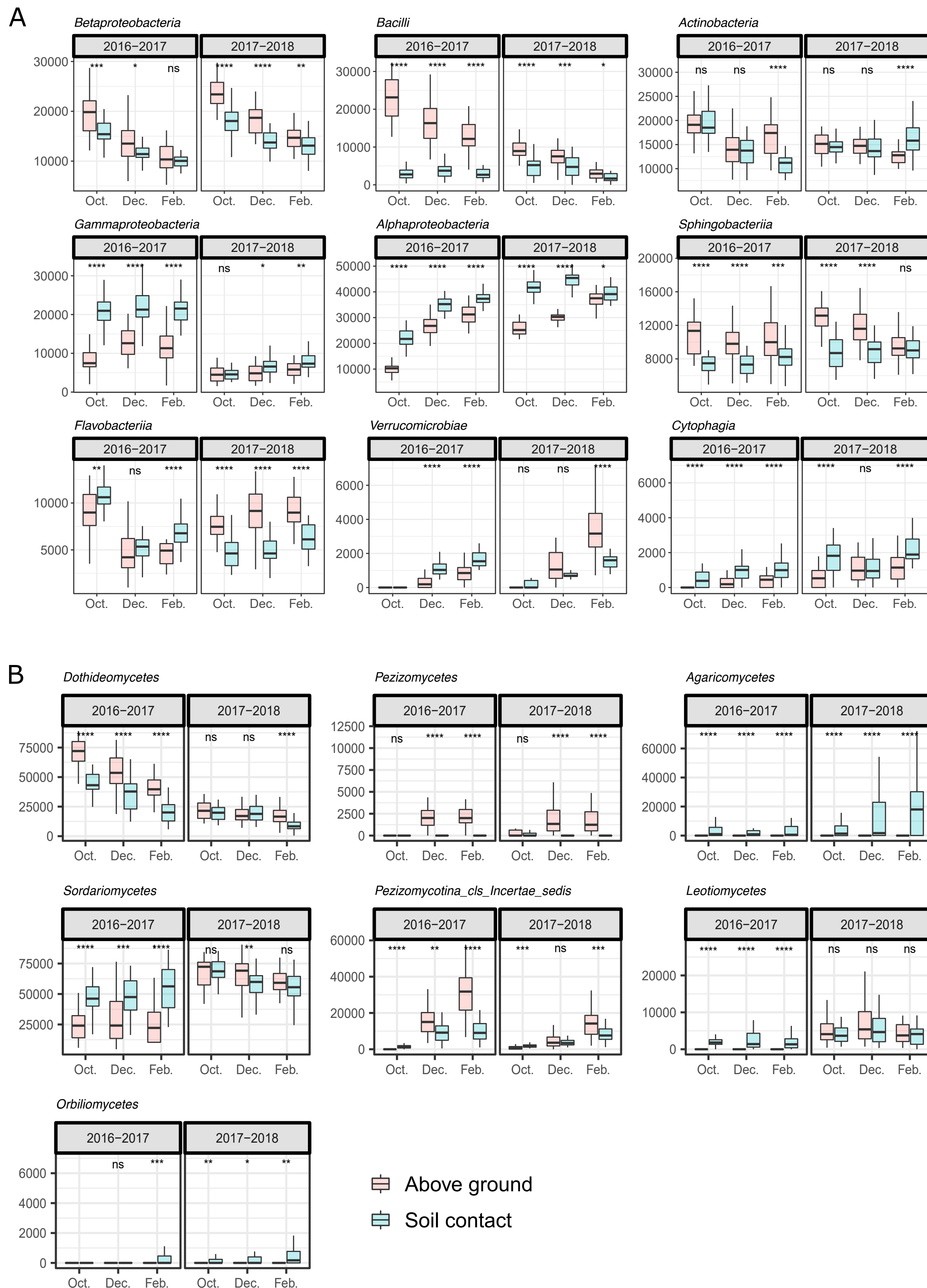
[6] estimated by $n_F \times n_B$

Supplementary Table 3 - Number of ASVs detected for each analysis performed on the dataset and properties of residue microbial ecological networks

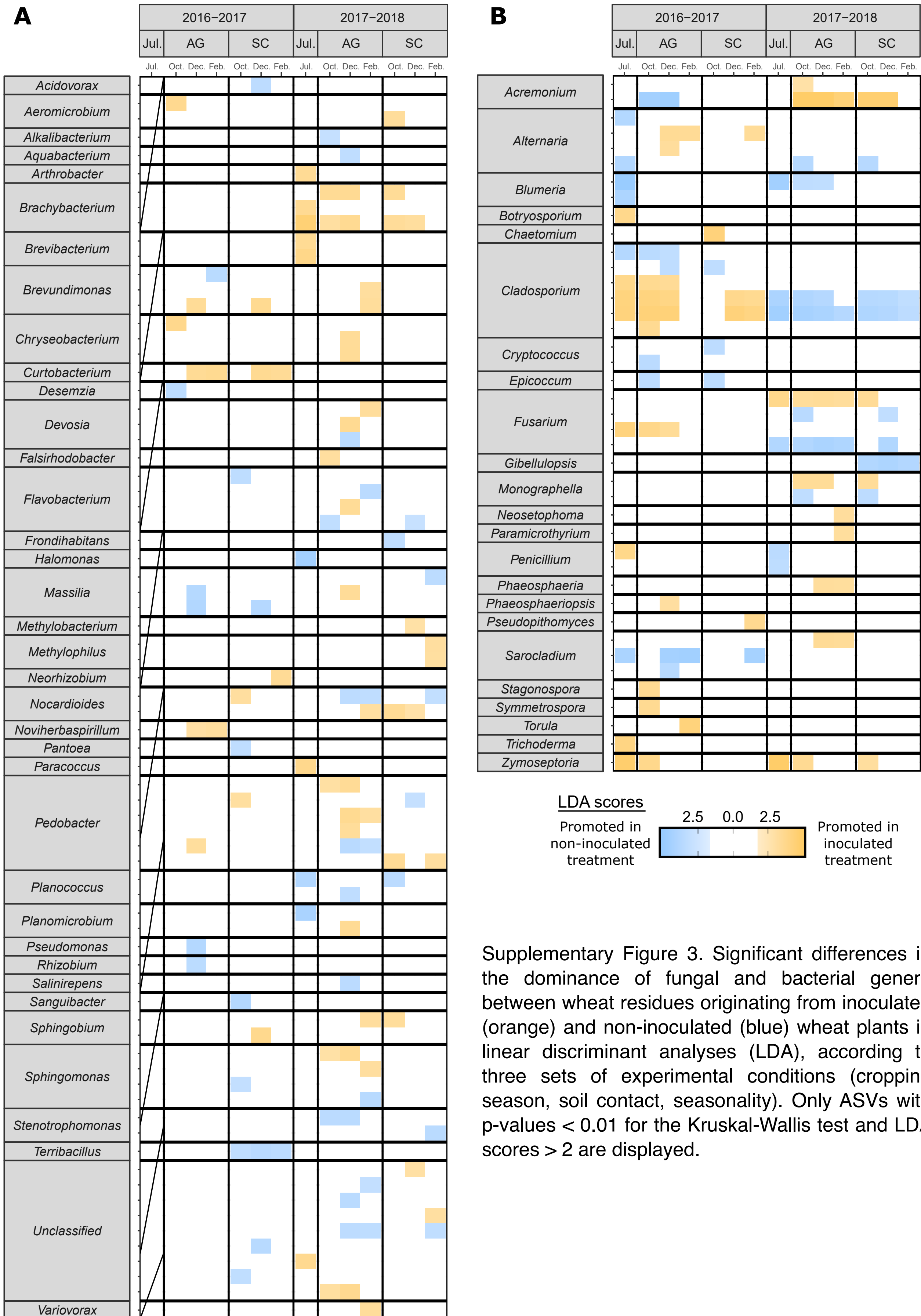
			All taxa		Number of taxa promoted in inoculated condition			Number of taxa promoted in non-inoculated condition			Number of taxa promoted in contact with the soil (SC)			Number of taxa promoted in above ground (AG)			Network analysis					
Year	Sampling date	Soil condition	F	B	F	B	Total	F	B	Total	F	B	Total	F	B	Total	F	B	Total	Interacting node	Isolated node	*Z. tritici* interaction
2016-2017	Jul.	-	36	/	8	/	8	6	/	6	-	-	-	-	-	-	20	/	20	12	8	2
2016-2017	Oct.	AG	61	182	8	2	10	4	1	5	13	31	44	9	18	27	32	73	105	79	26	4
2016-2017	Oct.	SC	106	167	1	2	3	3	6	9							52	90	142	133	9	nd
2016-2017	Dec.	AG	101	260	7	4	11	5	4	9	13	36	49	6	16	22	39	100	139	121	18	nd
2016-2017	Dec.	SC	164	227	2	3	5	0	4	4							51	105	156	150	6	nd
2016-2017	Feb.	AG	138	285	2	2	4	1	1	2	13	37	50	14	20	34	48	107	155	145	10	nd
2016-2017	Feb.	SC	179	238	4	2	6	1	1	2							55	110	165	158	7	nd
2017-2018	Jul.	-	36	286	2	7	9	6	3	9	-	-	-	-	-	-	16	60	76	60	16	6
2017-2018	Oct.	AG	87	197	5	6	11	7	3	10	8	36	44	5	20	25	39	93	132	119	13	7
2017-2018	Oct.	SC	117	216	4	6	10	5	2	7							31	109	140	126	14	2
2017-2018	Dec.	AG	104	252	5	13	18	4	9	13	7	43	50	6	22	28	42	110	152	142	10	nd
2017-2018	Dec.	SC	158	230	1	4	5	5	2	7							35	116	151	140	11	nd
2017-2018	Feb.	AG	135	340	6	8	14	2	6	8	8	33	41	10	21	31	44	119	163	152	11	nd
2017-2018	Feb.	SC	193	253	0	4	4	3	4	7							46	114	160	147	13	nd



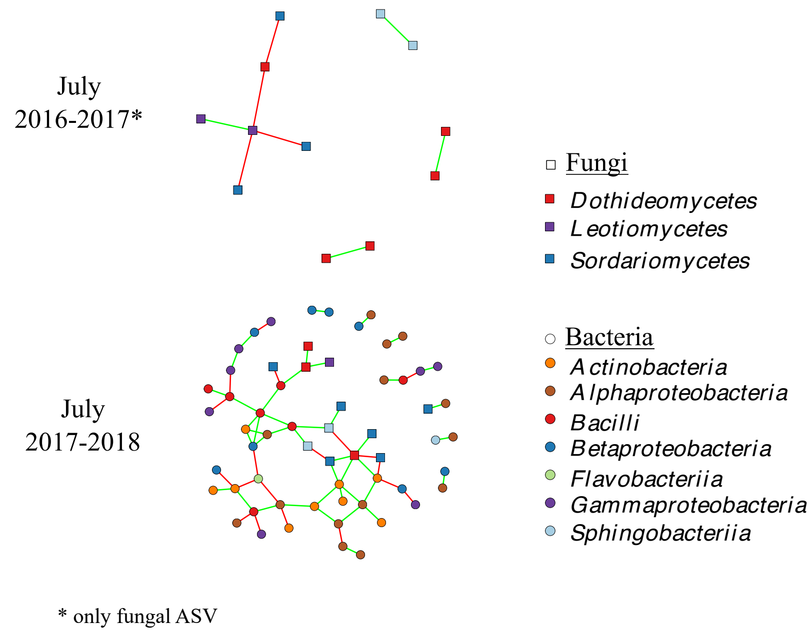
Supplementary Figure 1. Alpha diversity of microbial communities associated with residues. Observed richness (number of ASVs) and diversity (Shannon index), in four sets of experimental conditions (cropping season, contact with soil, seasonality, *Zymoseptoria tritici* inoculation). Each box represents the distribution of the number of ASVs and Shannon index for 15 sampling points per treatment. Wilcoxon tests were performed for inoculation condition (inoculated, non-inoculated) and sampling date (July, October, December, February; NS: not significant; * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001).



Supplementary Figure 2. Seasonal shift, from October to February, in the relative abundance of a selection of bacterial (A) and fungal (B) classes present on wheat residues (originating from wheat plants inoculated and not inoculated with *Zymoseptoria tritici)* according to cropping season (2016-2017, 2017-2018) and soil contact condition (in contact with the soil or above ground). Each box represents the distribution of class relative abundances for the 15 sampling points per treatment. Wilcoxon tests were performed for soil contact condition (NS: not significant; * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001).

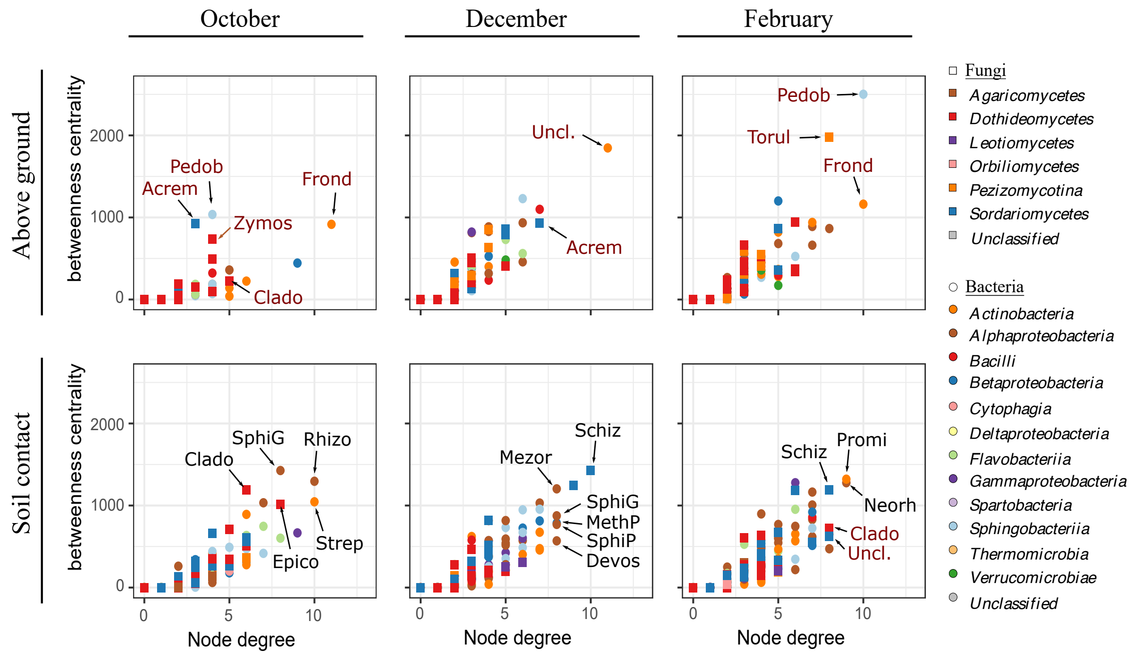


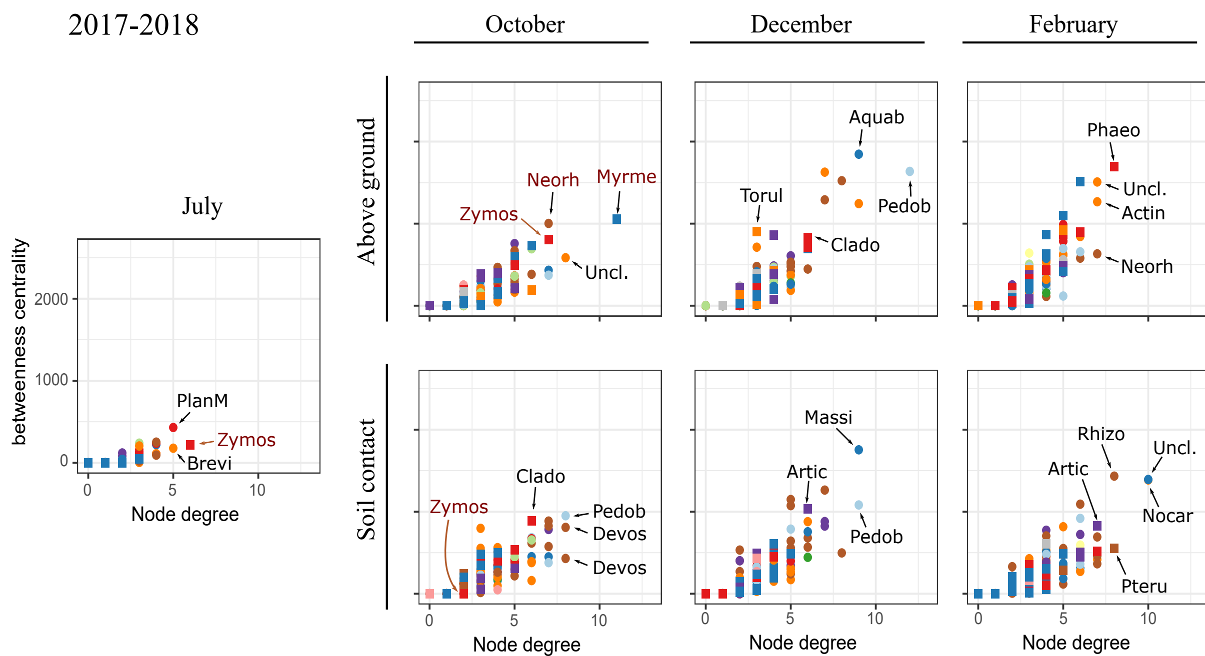
Supplementary Figure 3. Significant differences in the dominance of fungal and bacterial genera between wheat residues originating from inoculated (orange) and non-inoculated (blue) wheat plants in linear discriminant analyses (LDA), according to three sets of experimental conditions (cropping season, soil contact, seasonality). Only ASVs with p-values < 0.01 for the Kruskal-Wallis test and LDA scores > 2 are displayed.



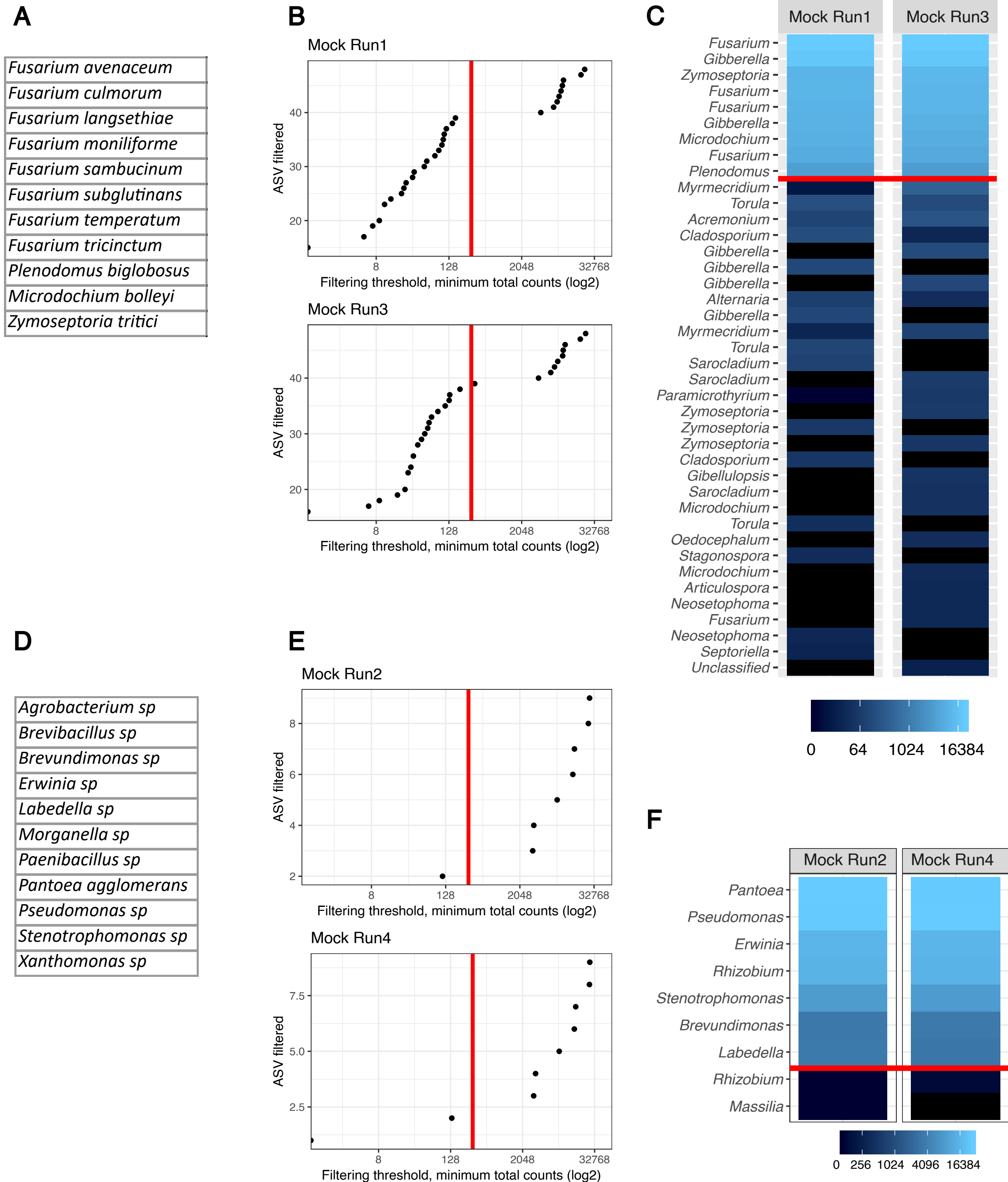
Supplementary Figure 4. Residue microbial ecological networks based on bacterial and fungal ASVs combined for July (no contact with soil) for each cropping season (2016-2017, 2017-2018). Circles and squares correspond to bacterial and fungal ASVs, respectively, with colors represent classes. Isolated nodes are not shown. Edges represent positive (green) or negative (red) interactions.

2016-2017





Supplementary Figure 5. Betweenness, centrality and degree of each ASV in the networks. Nodes with high betweenness, centrality and high degree values are considered to be keystone taxa in the networks. The genera of the fungal and bacterial ASVs with the highest degree and centrality are indicated: *Acrem(onium); Actin(oplanes); Aquab(acterium); Artic(ulospora); Brevi(bacterium); Clado(sporium); Devos(ia); Epico (ccum); Frond(ihabitans); Massi(lia); Mesor(hizobium); MethP(=Methylophilus); Myrme(cridium); Neorh (izobium); Nocar(dioides); Pedob(acter); Phaeo(sphaeria); PlanM(=Planomicrobium); Promi (cromonospora); Pteru(la); Rhizo(bium); Schiz(othecium); SphiG(=Sphingomonas); SphiP(=Sphingopyxis); Strep(tomyces); Torul(a); Uncl.(assified); Zymos(eptoria).*



Supplementary Figure 6. Mocks analysis for the two fungal sequencing runs (A, C) and the two bacterial sequencing runs (D, F). (A, D) Composition of the mocks. All microbial DNAs were pooled at equimolar concentrations. (B, E) Filter on the relative abundance of ASVs. The library size was normalized by proportion before analysis. The red line corresponds to a threshold at 3 ‰ of the size of the library. (C, F) ASVs detected in each mock. The 40 most abundant fungal ASVs are indicated (C), whereas all bacterial ASVs are indicated (F). The name of the ASVs corresponds to the taxonomic affiliation to the genus. All genera present in fungal mocks were detected (*Gibberella* and *Fusarium* are synonymous), while some bacterial genera were not detected in bacterial mocks, which differed only from one ASV. The red line corresponds to a threshold at 3 ‰ of the size of the library.

Chapitre III.2

Effets d'un gène de résistance à une maladie fongique sur le microbiome des résidus de culture : le cas *Leptosphaeria maculans*-*Brassica napus*

Effects of a resistance gene affecting fungal pathogen infection on the microbiome of crop residue: the case of the *Leptosphaeria maculans-Brassica napus* pathosystem

Lydie Kerdraon, Matthieu Barret, Marie-Hélène Balesdent, Frédéric Suffert, Valérie Laval

Soumission prévue : Molecular Plant Pathology

Abstract

Oilseed rape residues are a determinant of the epidemiology of stem canker, as they support the sexual reproduction of the causal agent *Leptosphaeria maculans*. Our goal was to characterize the effect of the presence of this fungal pathogen on the residue microbial communities, and to identify microorganisms interacting with it. To this end we used metabarcoding combining linear discriminant analysess (LDA) and ecological network analyses (ENA) to compare the microbiome associated with residues derived from a susceptible cultivar 'Darmor' and its derived isogenic line 'Darmor-*Rlm11*' obtained by the introgression of *Rlm11* from *Brassica rapa* into the French cultivar Darmor. The impact of the *Rlm11* gene was assessed by analyzing the composition of fungal and bacterial communities of 120 residues sampled collected at four dates (July, October, December, and February) in field conditions. In July, the fungal communities were significantly impacted by the presence of *Rlm11*, due for instance to a higher relative abundance of ASV affiliated to *Alternaria*, *Cladosporium*, *Filobasidium* and *Plenodomus* in Darmor-*Rlm11* residues, and a higher relative abundance of ASV affiliated to *Leptosphaeria* and *Pyrenopeziza* in Darmor residues. Bacteria colonizing residues were also impacted by the presence of *Rlm11*. The proportion of Darmor-*Rlm11* residue samples infected by *L. maculans* was smaller than for Darmor residue samples, although higher than expected likely because of some virulent strains in the French pathogen populations (estimated 5% in 2013). *L. maculans* appeared as having a weak role in networks, with few degrees (from 2 to 5 only), and ranked low in terms of betweenness centrality. In contrast, *Plenodomus biglobosus,* belonging to the same species complex than *L. maculans*, was considered keystone taxa in the July network. This approach might help identify and promote the development of beneficial microorganisms against residue-borne pathogens such as *L. maculans* and *P. biglobosus*. Whether the practical applications still seem far away, extensive results at the present time help to understand the relationship between biodiversity, pathogenicity and ecosystem functioning.

Effects of a resistance gene affecting fungal pathogen infection on the microbiome of crop residue: the case of the *Leptosphaeria maculans-Brassica napus* pathosystem

Lydie Kerdraon, Matthieu Barret, Marie-Hélène Balesdent, Frédéric Suffert, Valérie Laval

Background

Plants support a large number of microorganisms, whose assembly and structuring depends on many factors, such as the type of plant, the organ considered [1,2] and its age [3]. Many of these microorganisms are considered beneficial (e.g. plant growth-promoting bacteria) while others, pathogens, reduce the yield and quality of agricultural production. All these microorganisms are grouped into structured communities that can be defined by their composition and the interactions between them. The subset of the communities defined by a pathogen and the organisms that can influence and be influenced by this pathogen are defined as the pathobiome [4,5]. For instance it has been established that the foliar bacterial microbiota of *Arabidopsis thaliana* has an impact on infection by the necrotrophic fungus *Botrytis cinerea* [6]. The role of the microbiota in the plant's response to a disease or in the pathogenicity of a fungal pathogen is being studied on different pathosystems but is still poorly understood. With high throughput sequencing techniques, changes in microbial communities induced by the presence of plant pathogens, or temporal changes in assemblies over the progression of a disease, are increasingly being studied [4,7,8].

Stem canker is a widespread disease of oilseed rape (*Brassica napus*). The main causal agent of this disease is *Leptosphaeria maculans*, a heterothallic ascomycete fungus. This fungus has a complex life cycle. It enters the leaves through stomata [9], leading to the development of leaf spots and pycnidia producing rain-splashed pycnidiospores [10,11]. The fungus then progresses through the xylem from the leaf spots to the basis of the petiole and then into the stem [9,12]. This long stem and crown colonization phase remains symptom-free [12]. In late spring in Europe, *L. maculans* begins its necrotrophic phase, causing the "crown canker" (or "stem canker") at the stem base [13], which induces breakage of oilseed rape and yield losses. The fungus continues its life cycle as a saprophyte on infected stem bases left on the soil at harvest. Thus it survives during the intercropping season on stem residues [14], where it carries out its sexual reproduction, leading to the development of pseudothecia and the production of wind-dispersed ascospores [15,16], sometimes over several years [13,17]. Although the use of fungicides has been advised in some countries to control the disease [13], the main control strategy relies on the improvement of plant immunity by incorporating genetic resistance in oilseed rape cultivars, combining quantitative, polygenic resistance sources, and major resistance genes (*Rlm* genes) matching fungal effectors acting as avirulence (AvrLm) determinants [18].

In addition to *L. maculans,* another *Leptosphaeria* species, *Leptopshaeria biglobosa,* recently renamed *Plenodomus biglobosus*, shares the same life cycle as *L. maculans* and colonizes similarly oilseed rape plant tissues. Although less damaging in some countries [13], *P. biglobosus* was described

as the only causal agent of phoma stem canker in China [19]. *P. biglobosus* is present in all European countries but its agronomical impact is considered negligible compared to that of *L. maculans*.

Residues are an essential ecological niche for both *L. maculans* and *P. biglobosus* and the severity of stem canker disease is correlated with the amount of ascospores ejected by the residues of the previous crop [16,20]. Residues are also a compartment rich in microorganisms, where there is a shift between organisms from the plant and microorganisms from the soil [21], which can potentially interact with *L. maculans*, or be influenced by its presence, as previously established for *Fusarium* sp. in maize residues [22] and *Zymoseptoria tritici* in wheat residues [23]. Through culture-dependent approaches, it has been shown that some fungal species (e.g. *Alternaria* spp., *Trichoderma* spp., *Chaetomium* sp., *Gliocladium* sp.) were present jointly with *L. maculans* on buried oilseed rape residues [24]. Characterizing the interactions between pathogens and other microorganisms can open new opportunities for management of residues-borne diseases [25].

A first description of the microbiota associated to *L. maculans* during its saprophytic/reproduction stage on oilseed rape residues was performed by culture-independent approaches [26]. *L. maculans* and *P. biglobosus* were both found highly represented in the metabarcoding data set. However, other questions remain about the influence of these species on the fungal and bacterial communities and notably how the presence of *L. maculans* will influence the composition and evolution of the residue microbiome. To address this question, we took advantage of the existence of a pair of near-isogenic oilseed rape lines sharing the same genetic background but differing by the presence of the resistance gene *Rlm11:* 'Darmor' and 'Darmor-*Rlm11*'. The major gene *Rlm11* was introgressed from *Brassica rapa* into the French cultivar Darmor, characterized by a high level of quantitative, polygenic resistance, and population surveys showed that more than 95% of French *L. maculans* isolates were avirulent toward *Rlm11* [27]. This constituted the perfect frame to assessed by a metabarcoding approach and under field conditions, how the presence of one efficient major resistance gene, and hence of the lack or reduction of the presence of *L. maculans*, influenced the plant residue microbiome, including other plant pathogens of oilseed rape.

Materials and Methods

Oilseed rape field assay

The two cultivars Darmor (INRA-SERASEM, 1984) and its derived isogenic line Darmor-*Rlm11* [27] were sown in a 1.75 m × 8 m micro-plot each implanted on the Grignon experimental domain (INRA Grignon experimental station, Yvelines, France; 48°51′N, 1°58′E). Seeds were sown in September 2016 using an INOTEC single-seed speeder, at a density of 60 plants per m^2. Oilseed rape residues of a susceptible cultivar (cv. Alpaga) of the previous growing season (2015-2016) were added on the ground one month later (October 17, 2016) at a rate of 50 stubbles per plot, in order to re-inforce the inoculum pressure [28]. The presence of typical leaf lesions on the plants was observed three times between November 14 and December 2, 2016. First lesions appeared on leaves early December and the disease pressure was too low to allow a quantitative assessment of primary inoculum at the rosette stage. One month before full maturity (June), 60 plants of each cultivars were collected in each plot to establish the 'G2 score' [29], which characterizes the stem canker severity. Briefly, each plant was cut at the collar and the disease severity (proportion of necrosis caused by *L. maculans*) was estimated and converted in a score from 1 (no necrosis) to 6 (100% of the section necrosed). Then, the G2 score, ranging from 0 (no disease) to 9 (all plants lodged), was calculated for each plot as described by Aubertot *et al.* [9].

Oilseed rape residues preparation

After harvest (July), 60 plants of Darmor and 60 Darmor-*Rlm11* were collected in each plot. Plants were washed. Stem portions from 3 cm below and 6 cm above the crown were cut and thereafter called 'residues'. First, 15 Darmor and 15 Darmor-*Rlm11* residues were used to characterize microbial communities at harvest. Second, the remaining 45 residues of each cultivar were weighted and placed into a nylon bag (one residue par bag). The bags were deposited on the ground of a neighbouring plot in the same experimental area (wheat-oilseed rape rotation [26]) at 15 sampling points spaced 20 m apart (3 Darmor and 3 Darmor *Rlm11* bag-enclosed residues per sampling point) late July three months before subsequent wheat sowing.

Impact of seasonality on the fungal and bacterial communities of residues was assessed by collecting one Darmor and one Darmor-*Rlm11* bag-enclosed residue at three dates (October, December and February) in each sampling point (15 residue replicates per sampling date and per cultivar). The residues were rinsed with water, air dried, and weighted to assess degradation. Residues were then crushed individually with a Retsch™ Mixer Mill MM 400 for 60 seconds at 30 Hz with liquid nitrogen in a Zirconium oxide blender.

DNA extraction, PCR and Illumina sequencing

Total DNA was extracted with the DNeasy Plant Mini kit (Qiagen, France), according to the manufacturer's instructions with minor modification as described by Kerdraon *et al.* [23]. The Internal

Transcribed Spacer 1 (ITS1) genomic region and the v4 region of the 16S rRNA gene were amplified to estimate fungal and bacterial community profiles, respectively. Amplifications were performed with the ITS1F/ITS2 [30] and 515f/806r [31] primers. Amplifications were performed using Type-it® Microsatellite PCR kit, in accordance with Kerdraon *et al.* [23]. Second round of amplification was performed with primers containing Illumina adapters and indexes [23]. Libraries were sequenced in one run with MiSeq reagent kit v3 (600 cycles).

Sequence processing

Primer sequences of fastq files were first cut off using Cutadapt [32]. Files were then processed with DADA2 v.1.8.0 [33] according to the recommendations of the workflow DADA2 Pipeline Tutorial (1.8) [34]. A mock sample composed of DNA of known microorganisms was included in the run (Suppl. Figure 1) to establish a detection threshold for spurious haplotypes. At threshold of ≤ 3 ‰ of the library size, amplicon sequence variants (ASV) were considered spurious and were removed from each sample. Naive Bayesian classifier on RDP trainset 14 [35] and UNITE7.1 database [36] were used to assign ASV. ASV classified as chloroplasts (for bacteria) or unclassified at the phylum level have also been removed from each sample.

Analyses of microbial communities

Normalization by proportion was performed to standardize total library size of each sample. The effect of seasonality (sampling date) and *Rlm11* presence on alpha-diversity was assessed using Shannon index. The divergences in microbial communities composition, illustrated by MDS (ape package v 5.2. [37]), was assessed using a Bray-Curtis dissimilarity matrix calculated with the phyloseq package (v 1.24.2 [38]). To characterize the effect of seasonality and *Rlm11* presence on microbial communities structuration, PERMANOVAs were performed with 'margin' option (ADONIS2 function, 'vegan' package [39]). 'PairwiseAdonis' function were performed to characterize divergence between each condition, post-hoc tests [40].

Influence of the *Rlm11* gene and of the presence of *L. maculans* on the residue microbiome

In order to estimate the impact of the presence of *Rlm11* on the microbial communities, a linear differential analysis (LDA) was performed for each sampling date using LefSe [41] implemented in Galaxy (http://huttenhower.org/galaxy). LefSe uses the Kruskal-Wallis rank-sum test with a normalized relative abundance matrix to detect features with significantly different abundances between assigned taxa and performs LDA to estimate the effect-size of each feature. The alpha value of the Kruskal-Wallis test was set at 0.05 and the effect-size threshold was set at 2.0.

To identify interactions between *L. maculans* and other organisms, ecological interaction networks were constructed for each time point, whatever the cultivar (Darmor and Darmor-*Rlm11*

samples together). Networks were computed with SPIEC-EASI [42] on combined bacterial and fungal datasets [43] for each sampling date (30 samples per networks). To increase sensitivity of the network, rare ASV were removed [44] by defining a threshold of a minimum of six occurrences. For the four networks, Meinshausen and Bühlmann (MB) method was used as graphical inference model [42]. StARS variability threshold was defined as 0.05. Betweenness centrality of each node and node degree were estimated using igraph package (v. 1.2.2. [45]).

The two analyses - LDA and ecological interaction networks - were combined by constructing sub-networks focusing on the interactions between ASVs identified as differential in Darmor compared to Darmor-*Rlm11* by the LDA approach. The differential ASV highlighted by LDA and their adjacent nodes were used for analysing sub-networks constructed with Cytoscape (v. 3.6.1 [46]). Scripts for network construction and analysis are available from GitHub.

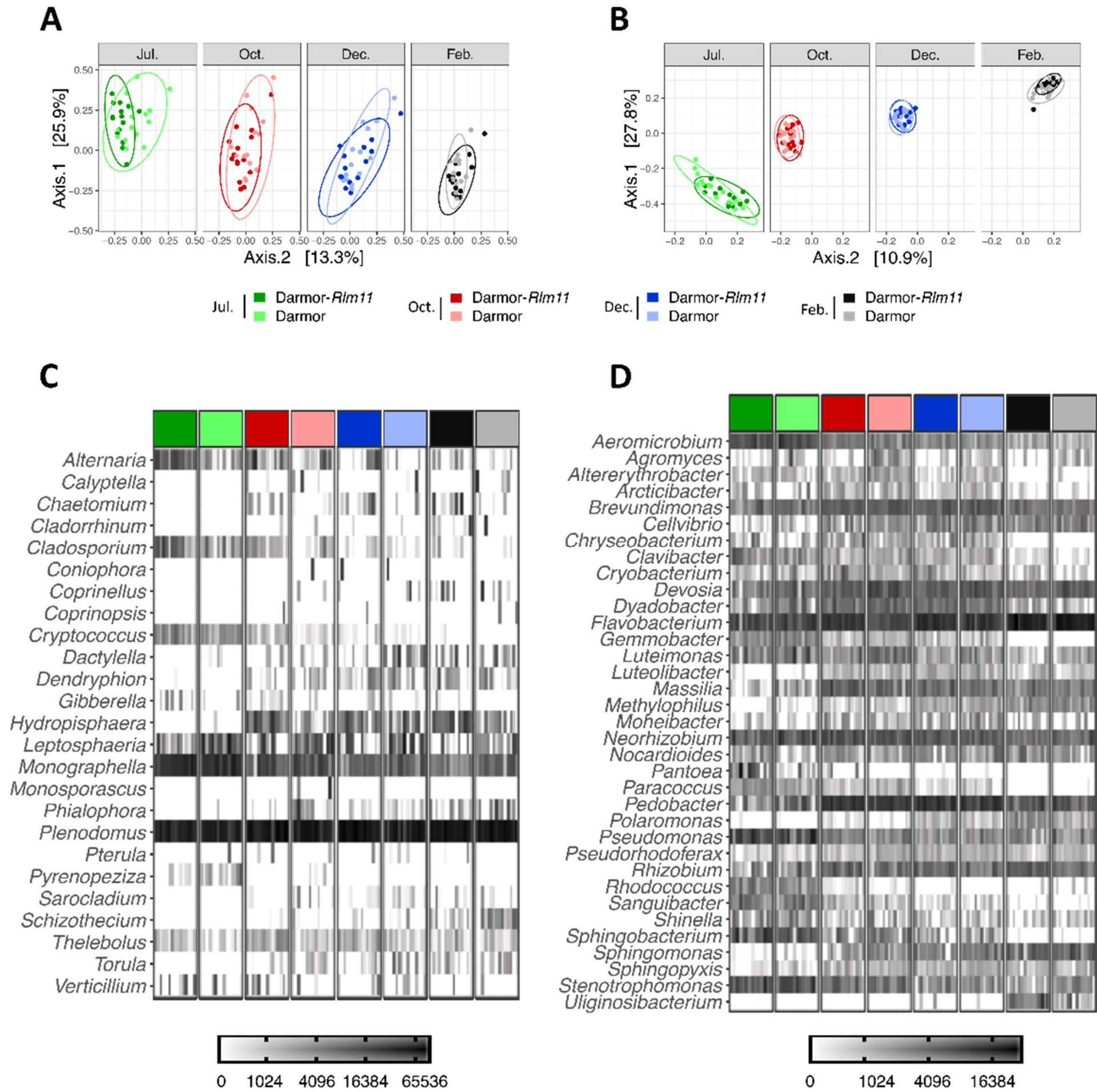


Figure 1. Effect of the presence of the *Rlm11* resistance gene and the sampling date (July, October, December and February) on fungal (A, C) and bacterial (B, D) communities originating from 120 samples of oilseed rape residues. (A, B) Visualisation of compositional distances between samples through multidimensional scaling (MDS) based on Bray-Curtis dissimilarity matrix. MDS analysis was performed on all samples together and was faceted according to the sampling date. Each data point corresponds to one sample of oilseed rape residues. The colours of the points distinguishes between sampling dates (July: green; October: red; December: blue; February: grey) and the cultivar (Darmor: light hues; Darmor-*Rlm11*: dark hues). (C, D) Diversity and predominance of the 25 more abundant (25/62 – excluding unclassified genera) fungal genera (A) and the 35 more abundant (35/134 – excluding unclassified genera) bacterial genera (B) distributed in all samples distinguishing between the different experimental conditions. The colors used to distinguish samples are similar to those used in (A) and (B).

Results

Overall diversity

The impact of the resistant gene *Rlm11* on oilseed rape residue microbiome was assessed by analyzing the composition of fungal and bacterial communities of 120 residues sampled at four dates (July, October, December, and February). Metabarcoding analysis resulted in 610 bacterial ASV and 335 fungal ASV. Fungal richness (Shannon index) was higher in Darmor-*Rlm11* residues compared to Darmor residues in July, but it was not significantly different and remained steady on the following sampling dates (Suppl. Figure 2). Bacterial richness increased from July to October for both cultivars and then decrease from December to February. Fungal richness remained steady during the whole experiment for both cultivars.

Structuration of microbial communities

Bray-Curtis index (beta diversity analysis) was used to characterize divergences in the structuration of communities according to presence of *Rlm11* and sampling dates. The presence of *Rlm11* induced significant changes, although minor, in the structuration of bacterial (PERMANOVA, R2 = 0.022, *p*-value = 0.001) and fungal communities (PERMANOVA, R2 = 0.042, *p*-value = 0.001). For fungi, the difference between Darmor and Darmor-*Rlm11* was significant only in July (Table 1, Figure 1). The beta diversity analysis highlighted an evolution of communities over time for fungi (PERMANOVA R^2 = 0.178, *p*-value = 0.001) as well as for bacteria (PERMANOVA, R^2 = 0.37, *p*-value = 0.001). The strong divergence observed between July and the other sampling dates for both bacterial and fungal communities (Table 1) is consistent with the rapid degradation of the residues after summer (almost 50% weight loss, Suppl. Figure 3). Some genera decreased or disappeared over time, such as *Cryptococcus*, *Alternaria*, *Sphingobacterium* or *Rhodococcus*, and others species appeared over time, such as *Torula*, *Schizotecium*, *Sphingomonas* or *Sphingopyxis* (Figure 1).

Table 1. Divergences of fungal (white corner) and bacterial (grey corner) communities structuration between the different oilseed rape residue status – Darmor-*Rlm11* (DR) and Darmor (D) – assessed with pairwise adonis. Significance of divergences was assessed with q-value (** means < 0.01).

	Jul. - DR	Oct. - DR	Dec. - DR	Feb. - DR	Jul. - D	Oct. - D	Dec. - D	Feb. - D
Jul. - DR	-	**0.335****	**0.385****	**0.459****	0.078	**0.345****	**0.364****	**0.457****
Oct. - DR	**0.201****	-	**0.104****	**0.329****	**0.286****	**0.105****	**0.132****	**0.333****
Dec. - DR	**0.277****	0.041	-	**0.212****	**0.346****	**0.137****	0.056	**0.217****
Feb. - DR	**0.376****	**0.096****	0.044	-	**0.446****	**0.339****	**0.217****	**0.069****
Jul. - D	**0.142****	**0.202****	**0.243****	**0.337****	-	**0.276****	**0.316****	**0.435****
Oct. - D	**0.223****	0.087	0.084	**0.155****	0.124	-	0.083	**0.324****
Dec. - D	**0.254****	0.083	0.048	0.089	**0.171****	0.041	-	**0.195****
Feb. - D	**0.352****	**0.113****	0.064	0.064	**0.283****	0.095	0.057	-

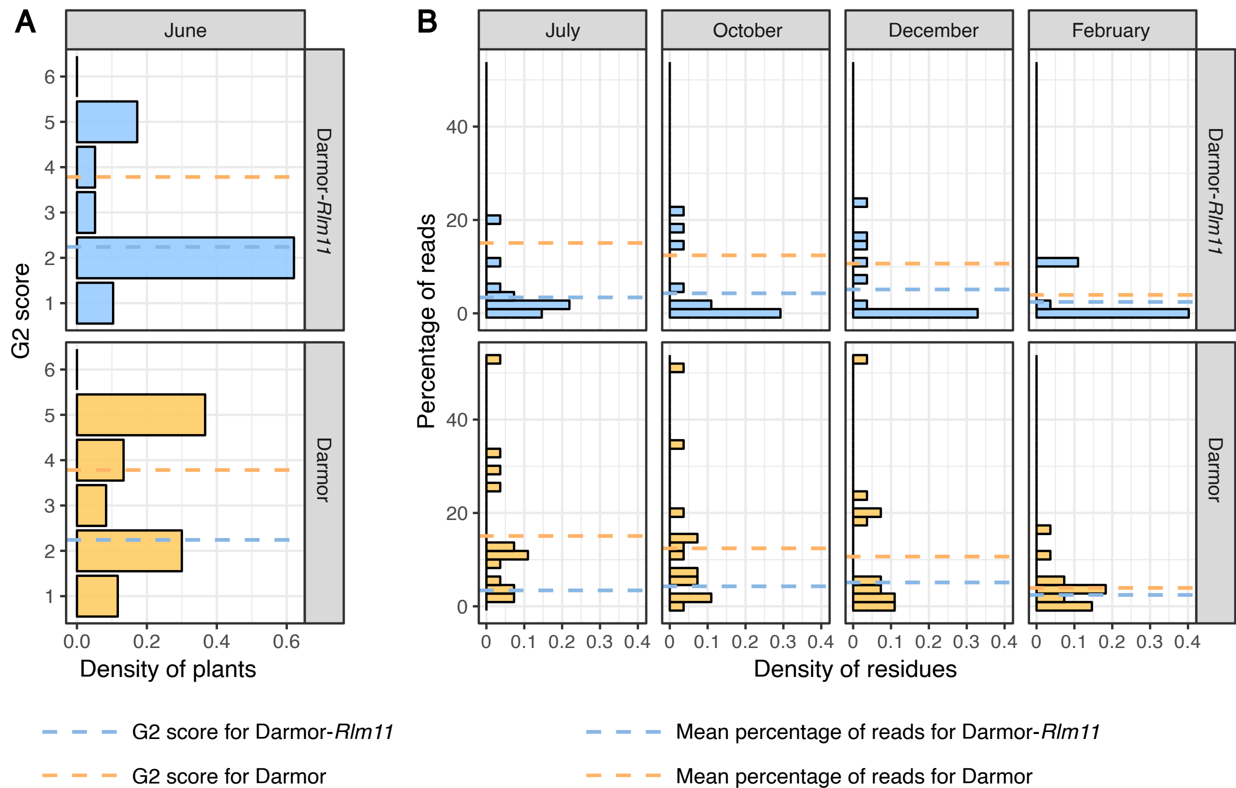


Figure 2. Influence of the presence of the *Rlm11* resistance gene and sampling date (July, October, December and February) on (A) the stem canker 'G2 score' (proxy of disease severity, estimated for 60 plants of Darmor and Darmor-*Rlm11*) and (B) the percentage of reads of *Leptosphaeria maculans*. (A) Percentage of plants affiliated to the six notes of the G2 score for both cultivars. The dashed line correspond to the G2 score, for each of the two cultivars (Darmor: yellow; Darmor-*Rlm11*: blue). (B) Percentage of reads affiliated with *L. maculans* in the 15 samples of each condition. The dashed line correspond to the mean of the reads percentage, for each of the two cultivars, at each sampling date (Darmor: yellow; Darmor-*Rlm11*: blue).

Disease assessment and presence of *L. maculans* and *Plenodomus* ASVs in residues

The G2 score assessed in June on the 60 oilseed rape residues exemplified the difference in susceptibility to *L. maculans* between Darmor (G2 = 3.78) and Darmor-*Rlm11* (G2 = 2.24). Although some Darmor-*Rlm11* plants displayed stem canker symptoms, the presence of *Rlm11* reduced stem necrosis due to *L. maculans* (Figure 2). Similarly, *L. maculans* was detected by metabarcoding less strongly and less frequently in Darmor-*Rlm11* residue samples than in Darmor. Moreover, the relative abundance (RA) of reads associated to *L. maculans* decreased during residue degradation for Darmor (from 15.18 ± 14.69 in July to 3.95 ± 4.63 in February) and was lower and steady for Darmor-*Rlm11* (3.85 ± 6.35). The RA of ASV affiliated to *Plenodomus* increased over time for Darmor (from 37.10 ± 11.67 to 53.18 ± 12.62) and Darmor-*Rlm11* (from 38.47 ± 11.80 to 55.32 ± 11.66).

Impact of the presence of *Rlm11* on the microbial community composition

Concerning fungi, RA of 29 ASV (represented by 16 genus, including unclassified) were impacted by the *Rlm11* presence/absence (Figure 3), as assessed by LDA. In July, the fungal communities were significantly different between Darmor and Darmor-*Rlm11* (Table 1), due to a higher RA of ASV affiliated to *Alternaria*, *Cladosporium*, *Filobasidium* and *Plenodomus* in Darmor-*Rlm11* residues, and a higher RA of ASV affiliated to *Leptosphaeria* and *Pyrenopeziza* in Darmor residues. Although the communities were not considered different in October, December and February (Table 1), some ASV displayed significantly different abundance between Darmor and Darmor-*Rlm11*. For example, one ASV affiliated to *Plenodomus* was promoted in Darmor-*Rlm11* throughout the degradation of the residues. The genus *Hydropisphaera*, that colonized residues after July (Figure 1), was promoted in Darmor-*Rlm11* residues compared to Darmor (Figure 3). Surprisingly, the genus *Pyrenopeziza*, among which *Pyrenopeziza brassicae* is the causal agent responsible for light leaf spot disease in oilseed rape and reproduces on oilseed rape residues [47], was promoted in Darmor in July.

Concerning bacteria, RA of 93 ASV (represented by 43 genus, including unclassified) were impacted by the *Rlm11* presence/absence (Figure 3). The structuration of bacterial communities was significantly different in October and February (Table 1). Bacteria colonizing residues were impacted by the presence of *Rlm11*. For example, ASVs affiliated to *Sphingomonas* preferentially colonized Darmor residues from October, and *Uliginosibacterium* preferentially colonized Darmor-*Rlm11* residues in February. Although there was no significant difference between microbial communities in July, 28 bacteria were differentially abundant between Darmor and Darmor-*Rlm11*, such as *Pantoea*, promoted in Darmor-*Rlm11* residue samples in July.

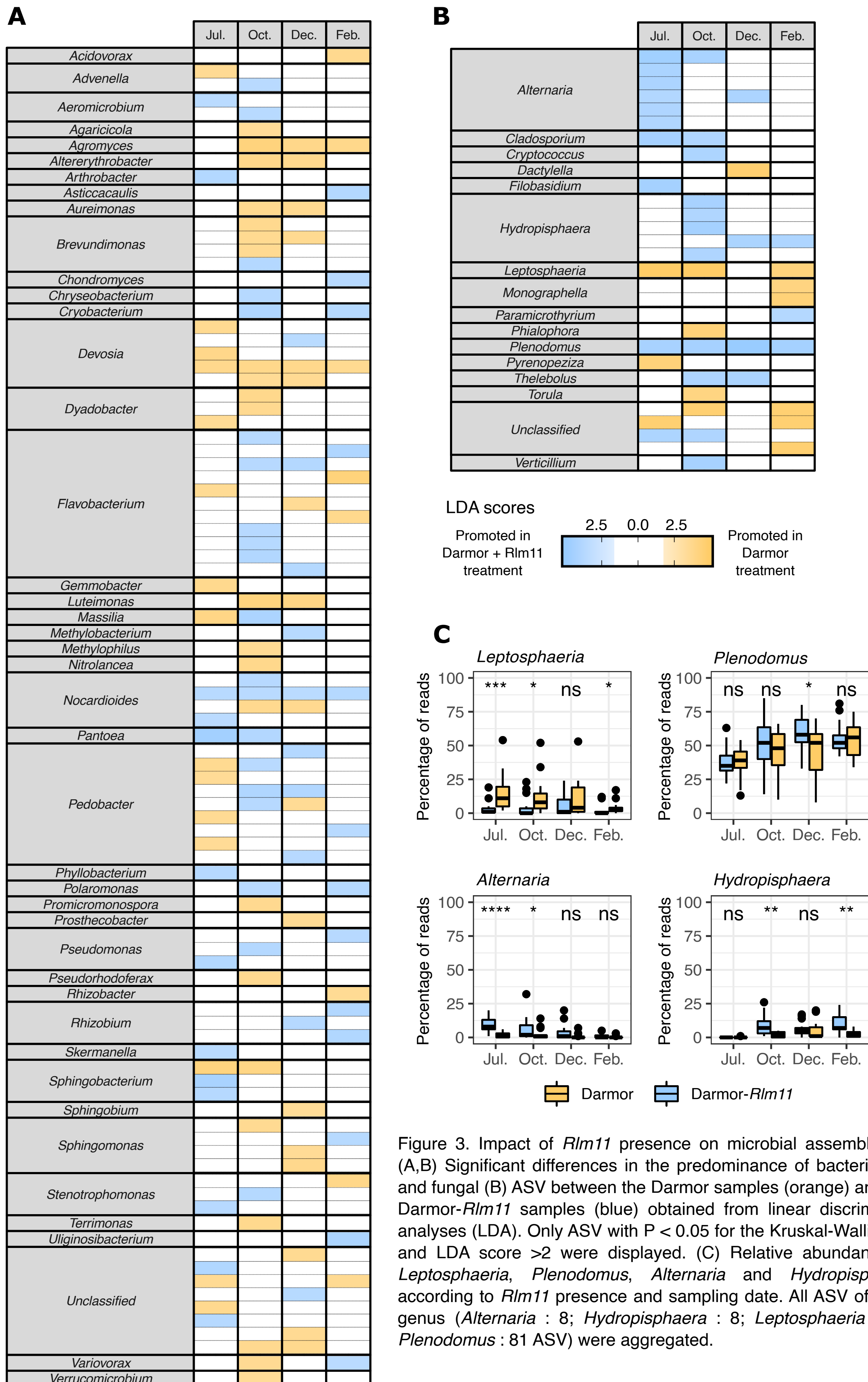


Figure 3. Impact of *Rlm11* presence on microbial assemblages. (A,B) Significant differences in the predominance of bacterial (A) and fungal (B) ASV between the Darmor samples (orange) and the Darmor-*Rlm11* samples (blue) obtained from linear discriminant analyses (LDA). Only ASV with P < 0.05 for the Kruskal-Wallis test and LDA score >2 were displayed. (C) Relative abundance of *Leptosphaeria*, *Plenodomus*, *Alternaria* and *Hydropisphaera* according to *Rlm11* presence and sampling date. All ASV of each genus (*Alternaria* : 8; *Hydropisphaera* : 8; *Leptosphaeria* : 13; *Plenodomus* : 81 ASV) were aggregated.

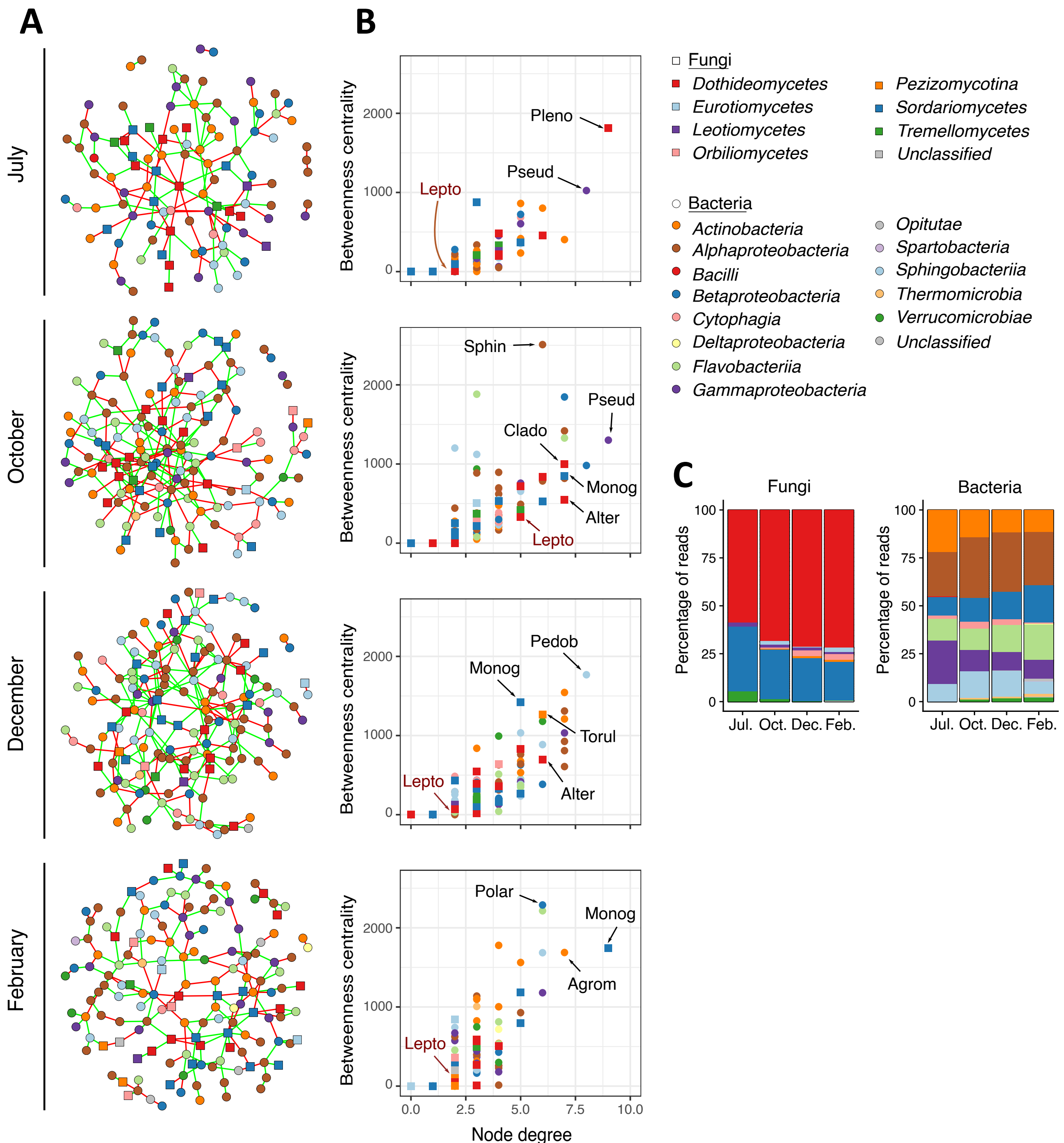


Figure 4. Temporal dynamics of co-occurrence networks. (A) Networks based on bacterial and fungal ASV combined. In all networks, circles and squares correspond to bacterial and fungal ASV, respectively, with color representing the class. Isolated nodes were not indicated. Edges represent positive (green) or negative (red) interactions. (B) Betweenness centrality and degree of each ASV in the networks. The place of *Leptosphaeria maculans* in the networks was indicated. Color and shape are the same as for (A). The genera of the fungal and bacterial ASVs with the highest degree and centrality were added: *Agrom(yces), Alter(naria), Clado(sporium), Lepto(sphaeria), Monog (raphella), Pedob(acter), Pleno(domus), Polar(omonas), Pseud(omonas), Sphin(gopyxis), Torul(a).* (C) Percentage of reads associated to fungal and bacterial classes for each network. Isolated nodes were indicated. Colors are the same as for (A).

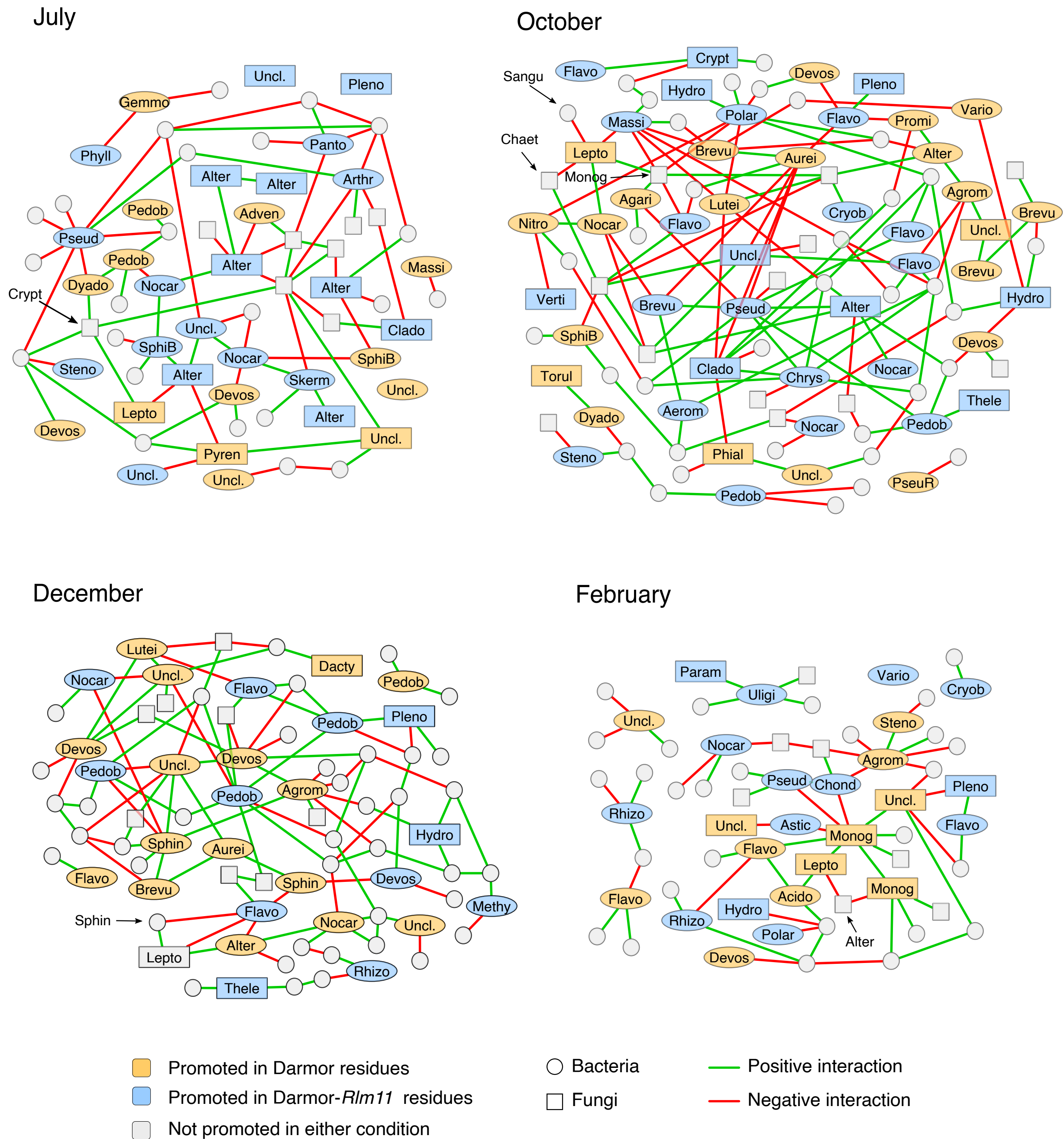


Figure 5. Subnetworks combining linear discriminant analyses (LDA; see Figure 3) and ecological interactions networks (see Figure 4), with focus on and fungal ASV identified as differential in LDA and of their first adjacent nodes. Node color corresponds to the results of LefSe differential analysis between Darmor (yellow) and Darmor-*Rlm11* (blue) treatments. Only genera with p-values < 0.05 for the Kruskal-Wallis test and LDA score > 2 were retained for the plot. Edges represent positive (green) or negative (red) interactions. Differential ASV were plotted with Genus name abbreviation: *Acido(vorax), Adven(ella), Aerom(icrobium), Agari(cicola), Agrom (yces), Alter(naria), Alter(erythrobacter), Arthr(obacter), Astic(cacaulis), Aurei(monas), Brevu(ndimonas), Chond (romyces), Chrys(eobacterium), Clado(sporium), Cryob(acterium), Crypt(ococcus), Dacty(lella), Devos(ia), Dyado(bacter), Flavo(bacterium), Gemmo(bacter), Hydro(pisphaera), Lepto(sphaeria), Lutei(monas), Massi (lia), Methy(lobacterium), Monog(raphella), Nitro(lancea), Nocar(dioides), Panto(ea), Param(icrothyrium), Pedob(acter), Phial(ophora), Phyll(obacterium), Pleno(domus), Polar(omonas), Promi(cromonospora), Pseud (omonas), PseuR(=pseudorhodoferax), Pyren(opeziza), Rhizo(bium), Skerm(anella), SphiB (=Sphingobacterium), Sphin(gomonas), Steno(trophomonas), Thele(bolus), Torul(a), Uligi(nosibacterium), Uncl. (assified), Vario(vorax), Verti(cillium).*

Direct impact of *L. maculans* on microorganisms

Dynamics of ecological interaction networks associated with oilseed rape residues - Ecological interaction networks combining bacterial and fungal datasets were built to characterize the interactions between L. maculans and the others members of bacterial and fungal communities, at each of the four sampling date (Figure 4). The average number of nodes in networks (138.8 ± 22.1) increased over the residue degradation. On average, 11.8 ± 5.5 nodes were isolated. Average node degree was 2.49 ± 0.34. L. maculans appeared as having a weak role in networks, with few degrees (from 2 to 5 only), and ranked low in terms of betweenness centrality (Figure 4). In contrast, one ASV affiliated to Plenodomus (P. biglobosus, the other oilseed rape pathogen associated to L. maculans), can be considered a keystone taxa in the July network.

Subnetworks highlighting direct interactions between* L. maculans *and other species - The combination of ecological interaction networks and differential analysis (LDA) highlighted the interactions between the ASV impacted by the *Rlm11* presence/absence. These ASV were mostly connected. *L. maculans* had as many interactions with ASVs promoted or inhibited by the presence of *Rlm11* as with ASV not impacted by the presence of this gene (Figure 5). ASVs interacting with *L. maculans* without being impacted by the presence of *Rlm11* were ASVs whose RA changed with the presence of *L. maculans*, regardless the cultivar. During residue degradation *L. maculans* interacted positively with ASVs affiliated to *Cryptococcus* (July), *Nocardioides* (October), *Monographella* (in October and February), *Altererythrobacter*, *Sphingomonas* (December) and *Acidovorax* (February*)*, and interacted negatively with *Alternaria* (July and February), *Massilia*, *Sanguibacter* and *Chaetomium* (October).

Discussion

The goal of this study was to characterize the effect of *L. maculans* on the microbiome of oilseed rape residues, using the two near-isogenic oilseed rape lines Darmor and Darmor-*Rlm11* carrying an efficient resistance gene against stem canker caused by *L. maculans*. This strategy allowed us obtaining a contrasted presence of *L. maculans* on the two types of residues to mimic both infected and healthy conditions. This efficient subterfuge was necessary because it would not have been possible to use inoculation of pathogen, as in Kerdraon *et al.* [23] with the wheat residue-borne pathogen *Z. tritici* or in Lebreton *et al.* [8] with the oilseed rape soil-borne pathogen *Plasmodiophora brassicae*. Indeed, infected oilseed rape residues hosting the sexual stage of *L. maculans* cannot be easily achieved under controlled conditions. In addition, there is usually a substantial difference between the microbial communities present on plants in field conditions and those obtained in greenhouses [6]. The use of fungicides, another plausible technical solution to obtain the healthy condition under field conditions would have had an impact on non-targeted endophytic or pathogenic fungal communities [48,49]. Furthermore, it has been shown that host genetics has a strong influence on the plant and root

microbiome [3,50,51], mainly because such cultivars differ by other traits than resistance to a plant pathogen [52]. Therefore, the use of true isogenic lines (differing only from a specific resistance gene) appeared the best technical solution. However one must be aware that this strategy did not allow distinguishing the effect of the presence/absence of *Rlm11* on the residue microbiome, from the presence/absence of *L. maculans*, which was itself strongly affected by *Rlm11*. To our knowledge, this study is the first to investigate the effect of a fungal plant pathogen on microbial communities, using near-isogenic lines [52]. In addition, very few studies have focused on *L. maculans*-associated microorganisms during the necrotrophic phase of this pathogen [24,26].

The proportion of Darmor-*Rlm11* residue samples infected by *L. maculans* was smaller than for Darmor residue samples, although higher than expected because of the presence of the efficient major resistance gene. The purity of the Darmor-*Rlm11* seeds has not been confirmed yet but a small contamination by plants devoid of *Rlm11* could explain the presence of *L. maculans* in only a few plants. Furthermore, an epidemiological survey revealed that 5% of *L. maculans* strains in the French pathogen populations were virulent against *Rlm11* in 2013 [27]. *L. maculans* populations collected from leaf spots on susceptible cultivars grown in the same experimental area from 2015 to 2017 will be pathotyped on oilseed rape seedling carrying *Rlm11* to estimate the frequency of the *avrLm11* virulence in the local population[4]. Another critical event to mention is that the *L. maculans* inoculum pressure was very low in Autumn 2016, likely due to hot and dry weather conditions in Summer and early Autumn, as illustrated by the lack of leaf spots observed until the beginning of December in the field trial. As a consequence, the stem canker severity was weak in June 2017 and the difference in the presence of *L. maculans* between Darmor and Darmor-*Rlm11* residues would have been probably greater in the case of a stronger stem canker epidemic. An important point revealed by the metabarcoding analysis is the high RA of *Plenodomus* genus compared to *L. maculans*, while similar data acquired in the same experimental area in 2016-2017 [26] highlighted a close RA of *L. maculans* and *P. biglobosus* on residues of cv. Alpaga, very susceptible to *L. maculans*. The low RA of *L. maculans* in cv. Darmor compared to cv. Alpaga can be attributed to the high level of quantitative resistance of cv. Darmor to *L. maculans*, which could explain the higher presence of *P. biglobosus* compared to *L. maculans* in Darmor.

Although the presence of *L. maculans* in Darmor-*Rlm11* residues was higher than expected in case of complete resistance, it was still lower than in Darmor. This difference allowed us to compare microbial community structures, but it was quite insufficient to compare effectively the communities in the presence and in the absence of *L. maculans*. In order to detect the effect of *L. maculans* on the whole residus microbiome, and on some given species, ecological interaction networks were performed aggregating the two cultivars datasets; by this way we were able to detect interactions between *L. maculans* and other fungi and bacteria simultaneously.

[4] La proportion de souches virulentes vis-à-vis de *Rlm11* et la pureté du lot de semences utilisé dans cette étude seront estimées avant la soumission de l'article.

L. maculans is known to survive for several years on residues [53]. Here we observed a reduction of the proportion of reads affiliated to *L. maculans* in Darmor from July (at harvest) to February, when less than 5% (in average) of the reads were affiliated to *L. maculans*. As previously established for wheat and oilseed rape together [26], bacterial and fungal communities changed as residues degrade during this period. Several microbial species colonizing the oilseed rape residues that were highlighted in both the previous and the current study have already been found associated with *L. maculans*. For example, *Chaetomium* detected on the residues between July and October were shown to colonize oilseed rape residues two months after deposition on the ground [24,26]. The genus *Monographella*, also present on the residues in our study, was isolated from the rhizosphere of senescent oilseed rape plants and was described as a *Verticillium* antagonist [54]. However, no significant direct interactions between *Verticillium* and *Monographella* were here detected by ecological interaction networks. Overall, all fungal genera and the majority of the most abundant bacterial genera identified here (Figure 1) were also found on oilseed rape residues in our previous studies [26]. The immediate proximity of the experimental plots of the two studies may explain these similarities.

The structuration of fungal communities was different between Darmor and Darmor-*Rlm11* in July, but not at the following dates. This suggests that the presence/absence of the *Rlm11* gene influences the microbiome of the stem basis when the plant is still alive, but does not induce a differential colonization of residues by fungi, partly from the rhizosphere, later on. The bacterial community structures were slightly impacted by the presence of *Rlm11*. The cultivar Darmor is known to be susceptible to the fungal pathogen *P. brassicae*, mainly prevalent in the western part of France and in the north of the UK [55]. Surprisingly, *P. brassicae* [47] but also other *Pyrenopeziza* species, were under-represented in Darmor-*Rlm11.* This unexpected difference between the two near-isogenic lines might be explained by one of the following hypotheses: (1) the presence of *L. maculans* facilitates the infection by *P. brassicae*; (2) the resistance of Darmor-*Rlm11* to *L. maculans* isolates carrying the avirulence gene *AvrLm11* gene indirectly prevents early infections of *B. napus* leaves by *P. brassicae,,* with significant impacts on the development of the polycyclic light leaf spot epidemics; (3) the introgression of *Rlm11* from *Brassica rapa* to *B. napus* was accompanied by the introgression of a resistance locus to *P. brassicae* or has replaced a susceptibility locus initially present in cultivar Darmor (Régine Delourme, INRA IGEPP, comm. pers.). These three hypothesis would need to be tested in further experiments.

Since stem canker caused by *L. maculans* is usually controlled by the deployment of resistant cultivars, few studies have focused on interactions between *L. maculans* and potential biocontrol agents. In this study, few direct interactions between *L. maculans* and other microorganisms in the residue microbial community were detected. These rare, but significant, interactions concerned both fungi (*Cryptococcus* in July, *Alternaria* in July and February; *Monographella* in October and February; *Chaetomium* in October), and bacteria (*Nocardioides*, *Sanguibacter* and *Massilia* in October, *Altererythrobacter* and *Sphingomonas* in December, and *Acidovorax* in February). Interestingly,

Chaetomium was previously shown to be a biocontrol agent against several pathogenic microorganisms, including *Sclerotinia sclerotiorum* in oilseed rape [56].

The interest of using near-isogenic lines differing by a single resistance gene to study the pathobiome was pointed out [52]. This strategy was used here to characterize the effect of the pathogen *L. maculans* on crop residues communities, but also the effect of given fungal and bacterial species members of these communities on this pathogen of major agronomic importance. If the level of contamination was not sufficient to obtain very clear-cut conditions, this study still have made it possible to complete our knowledge of the life cycle and species complex involved in stem canker and pointed out the central role of *P. biglobosus* on stem residues communities, a fungal species often neglected compared to *L. maculans. L. maculans* and *P. biglobosus* are two related species belonging to the same species complex, but little is known about their effective interaction. In Europe *P. biglobosus* is not considered as important as *L. maculans*. As the ecological niches of both pathogen species are suspected to be the same [57], a strong promotion of *P. biglobosus* might be hypothesised when *L. maculans* decreases. This hypothesis is however not supported by the results of the interaction network analyses as only the second most abundant ASV of *P. biglobosus* (4.5% of reads of this genus) was promoted in Darmor-*Rlm11* residues. For all other ASVs affiliated to *P. biglobosus*, a difference between Darmor and Darmor-*Rlm11* was only observed in December. At this sampling point, the proportion of *L. maculans* ASVs was not significantly different between the two cultivars. In addition, no direct interaction between *L. maculans* and *P. biglobosus* was detected in the interaction networks. However, the most abundant ASV of *P. biglobosus* (93.8% of this genus) had a central place in the networks of July, in terms of degree and betweenness centrality thus highlighting the importance of this species in the living plant. In contrast, the non-central position of *L. maculans* in networks suggests it has a low impact on the whole residue microbial community, and vice versa, despite its role as causal agent of the disease. All these observations may be linked to the high level of quantitative resistance to *L. maculans* in cv. Darmor, clearly illustrated by the low RA of *L. maculans* ASVs even in the absence of the *L. maculans* resistance gene *Rlm11*. *P. biglobosus* is considered as mainly responsible for producing upper stem lesions [57] and the *L. maculans* inoculum pressure was low in the 2016-2017 growing season. Nevertheless a rather high G2 score (3.78) was scored in the cv. Darmor plot here. G2 scores above 3 are considered as having a significant impact on yields [58]. All these data clearly suggests that the impact of *P. biglobosus* on the development of stem canker symptoms and yield losses may have been underestimated, at least in some European regions, and needs to be re-evaluated.

The dual approach used here is based on the LDA scores obtained in differential analyses between a resistant and a susceptible cultivar and ecological network analysis. This approach might be identify and used to promote the development of beneficial microorganisms against residue-borne pathogens such as *L. maculans* and *P. biglobosus*. Whether the practical applications still seem far enough away, extensive results at the present time help to understand the relationship between biodiversity, pathogenicity and ecosystem functioning, as it has already been done for *Fusarium* sp. in maize residues [22], and, in a companion study, for *Z. tritici* [23] in wheat residues.

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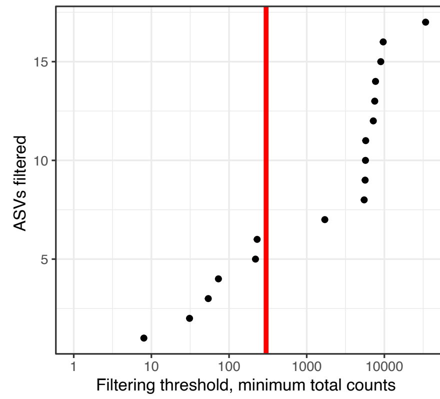
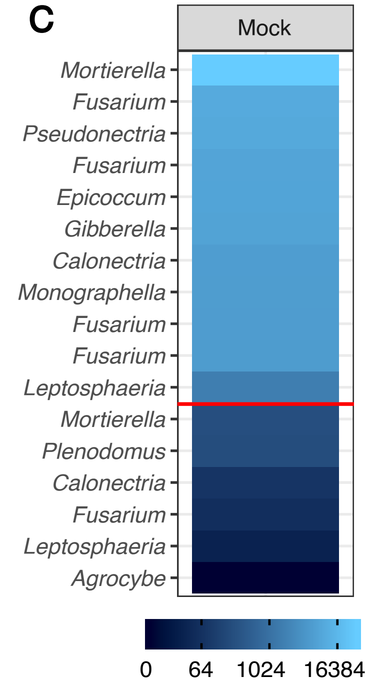
A

Microdochium majus
Fusarium sambucinum
Calonectria pseudonaviculata
Fusarium sporotrichioïdes
Epicoccum nigrum
Fusarium pseudonygamai
Mortierella elongata
Fusarium culmorum
Fusarium poae
Pseudonectria buxi
Leptosphaeria maculans

B



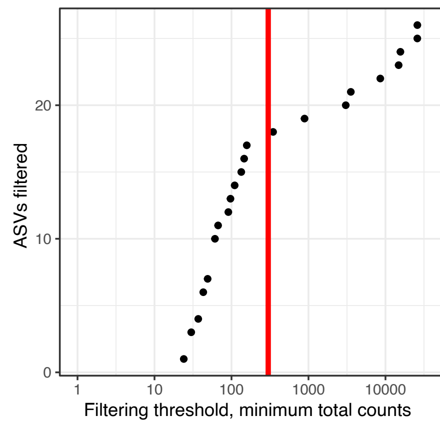
C



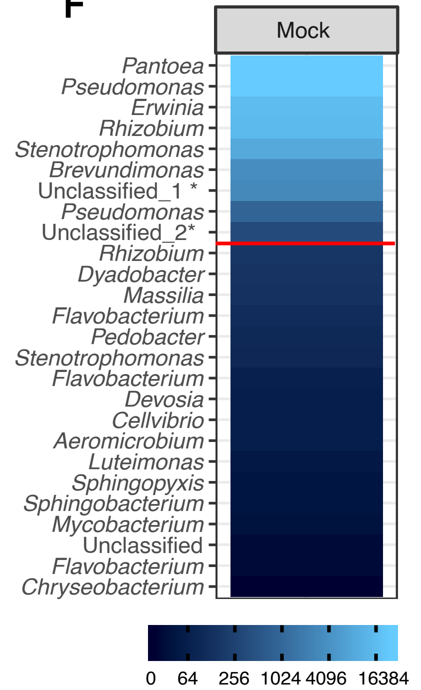
D

Agrobacterium sp
Brevibacillus sp
Brevundimonas sp
Erwinia sp
Labedella sp
Morganella sp
Paenibacillus sp
Pantoea agglomerans
Pseudomonas sp
Stenotrophomonas sp
Xanthomonas sp

E

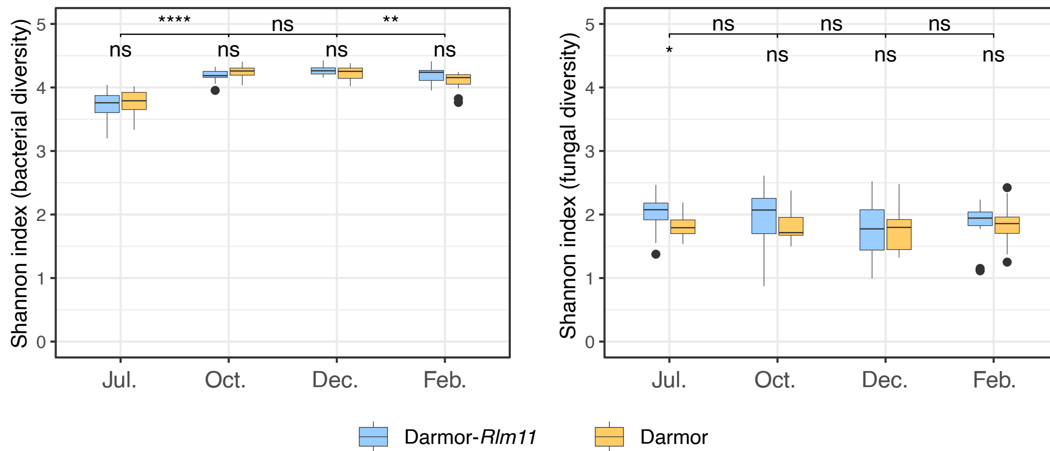


F

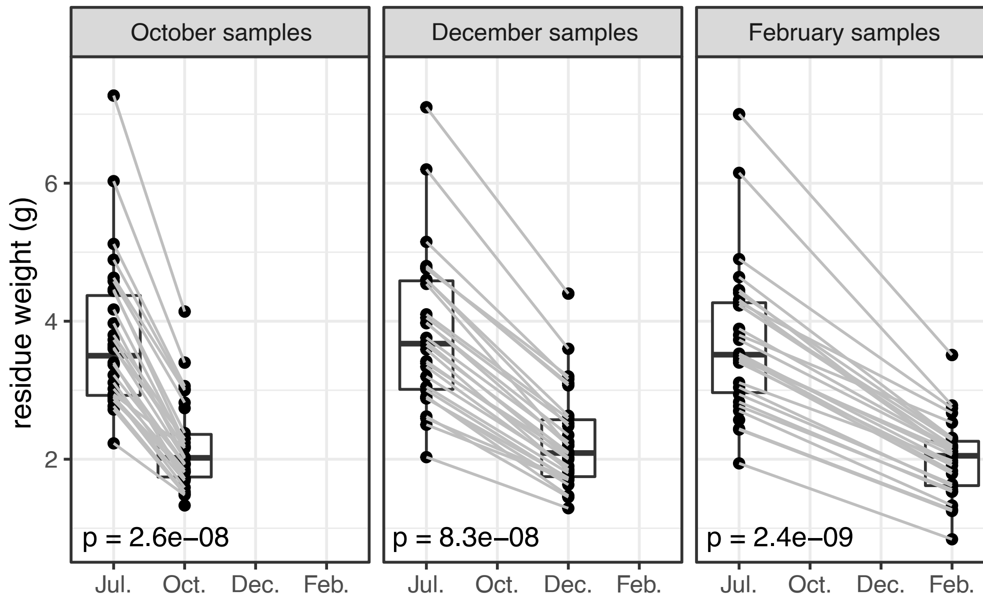


Unclassified_1 *: classified as Agrobacterium (ncbi Blast)
Unclassified_2 *: classified as Labedella (ncbi Blast)

Supplementary Figure 1. Fungal (A, B, C) and bacterial (D, E, F) mocks analyses. (A, D) Mocks composition. (B, E) ASVs filtering. Considering that all samples were sequenced in the same run, threshold (red line) was defined at 3 ‰ and was the same for both bacterial and fungal community analyses. (C, F) ASVs detected in each mocks. The name of the ASVs corresponds to the taxonomic affiliation to the genus.



Supplementary Figure 2. Alpha diversity of microbial assemblages associated with oilseed rape residues. Each box represents the distribution of Shannon index for 15 sampling points per treatment. Wilcoxon tests (NS: not significant; * p-value < 0.05; ** p-value < 0.01; *** p-value < 0.001) were performed for host cultivar (Darmor, Darmor-*Rlm11*) and sampling date (July, October, December, February).



Supplementary Figure 3. Weight loss of oilseed rape residues over time. Each set of residues was weighted in July before being placed in the nylon bags and at the different sampling dates. The grey lines indicate the decrease in the weight of set of residues from July to each sampling date. Wilcoxon tests were performed to test the significance of the weight decrease.

Conclusion générale

I. Eléments d'épidémiologie comparée de deux agents pathogènes majeurs des rotations blé-colza

Ce travail de thèse s'est concentré sur deux champignons pathogènes d'importance agronomique, *Zymoseptoria tritici*, agent causal de la septoriose du blé, et *Leptosphaeria maculans,* agent causal du phoma du colza. Ces deux microorganismes ont comme point commun de se maintenir sur les résidus de culture après la récolte, siège de leur reproduction sexuée. Les épidémies de septoriose du blé et de phoma du colza sont initiées par des ascospores issues de la reproduction sexuée et dispersées par le vent [1,2]. Les pics de production d'ascospores des deux espèces s'échelonnent de la fin de l'été au début du printemps [3,4]. Chez *Z. tritici*, la phase polycyclique de l'épidémie est assurée par les pycnidiospores, dispersés par les éclaboussures de pluie. A l'inverse, chez *L. maculans*, les infections en cours de culture proviennent en grande majorité des ascospores qui continuent à être libérées par les résidus [5] pendant toute la saison culturale, tandis que les pycnidiospores contaminant les feuilles adjacentes ne jouent qu'un rôle mineur [6]. Pendant l'hiver, *L. maculans* progresse dans la plante par les vaisseaux du xylème et descend jusqu'à la base de la tige sous forme mycélienne [7]. À la fin de l'hiver, le champignon provoque la nécrose du collet qui limite l'alimentation de la plante et peut provoquer la verse du colza. Si l'effet de ces deux agents pathogènes sur les rendements est plutôt dû aux infections secondaires en cours de culture, le rôle des résidus est assez similaire, en ce sens qu'ils permettent le maintien de l'inoculum primaire [8–11].

Les méthodes de protection des cultures contre ce type de maladies se concentrent principalement sur les phases épidémiques pendant lesquelles l'impact sur le rendement est majeur et souvent assez direct : positionnements ciblés de traitements fongicides et sélection de variétés dont la résistance s'exprime au stade adulte. Limiter le développement de l'agent pathogène pendant la phase inter-épidémique, c'est-à-dire l'inter-culture, fait appel à des méthodes considérées comme prophylactiques qui se raisonnent finalement à des échelles qui dépassent celle de l'exploitation agricole compte tenu de la distance de dispersion des ascospores, et peuvent sembler moins pertinentes à un agriculteur individuel. Le traitement « physique » des résidus, par enfouissement partiel (travail du sol simplifié) ou total (labour), permettrait théoriquement de réduire leur quantité. Les études précédentes menées à BIOGER sur *Z. tritici*, notamment dans le cadre de la thèse de David Morais [12], ont montré que la quantité d'inoculum n'était finalement jamais vraiment limitante [13], rendant ainsi illusoire ce moyen de gestion dès lors qu'il n'est pas conduit à l'échelle d'un paysage en tenant en compte de la façon dont les résistances variétales sont déployées. Un traitement « biologique » des résidus pourrait être envisagé comme moyen de gestion durable de l'inoculum primaire. Cela impliquerait de s'intéresser non plus aux aspects physique et quantitatif de l'inoculum et de ses sources, mais, comme nous l'avons fait dans cette thèse, à la diversité microbiologique des résidus, susceptible d'affecter le développement biologique de l'agent pathogène.

Nous avons en effet montré que les résidus de culture sont le support de communautés microbiennes et fongiques non pathogènes, évoluant au cours du temps en fonction de la vitesse de décomposition des tissus végétaux. Certains microorganismes jouent un rôle important dans la

dégradation de la matière qui constitue ces tissus (cellulose, hémicellulose, lignine), mais les fonctions et les interactions associées à ces communautés, en relation avec les maladies, avaient été jusqu'alors peu étudiées.

II. Retour sur la stratégie de recherche

L'objectif de cette thèse était de caractériser la diversité microbienne des résidus de blé et de colza et de mettre en évidence la façon dont *Z. tritici* et *L. maculans* interagissent avec les autres microorganismes. Plusieurs étapes ont été proposées pour caractériser les microorganismes associés à *Z. tritici* et *L. maculans* pendant la phase de reproduction sexuée.

La première étape, basée sur une synthèse bibliographique (Chapitre I), nous a conduit à montrer que le compartiment « résidus », en plus d'être un support des agents pathogènes, est également le support d'une communauté microbienne très active, et changeante. Cette recherche a permis d'identifier des microorganismes décrits sur les études de diversités des résidus, offrant des perspectives d'interactions avec les agents pathogènes, somme toute assez théoriques. Après avoir synthétisé et organisé les connaissances disponibles dans la littérature, en particulier celles relatives à des microorganismes déjà décrits comme présents dans le système cultural blé-colza, nous avons mis en pratique les résultats de nos réflexions dans une première étude menée en conditions naturelles.

Cette deuxième étape, menée conjointement et en appui au Chapitre I, nous a conduit à mieux caractériser l'environnement biotique des résidus en conditions naturelles, et démontrer l'effet de certains facteurs – la plante hôte, la saison, la rotation – sur la structuration des populations microbiennes de ces résidus. Pour cela, un dispositif biennal, composé d'une monoculture de blé et de deux parcelles en rotation blé-colza en non labour, a été utilisé. Dans une première sous-partie, les microorganismes présents sur les résidus – champignons et bactéries – issus d'une colonisation naturelle des parties végétales laissées sur le sol, ont été caractérisés par séquençage haut débit à cinq dates au cours de chaque saison culturale. En parallèle de cette caractérisation par metabarcoding, des isolements de champignons et de bactéries ont été réalisés dans une seconde sous-partie. La comparaison des deux méthodes, culture-indépendante et culture-dépendante, s'est avérée riche d'enseignement, démontrant notamment la complémentarité des deux approches. Cette seconde étape nous a effectivement permis de détecter, d'isoler et de mettre en collection plusieurs centaines de microorganismes – champignons (collection R-Syst BIOGER à Grignon) et bactéries (collection IRHS à Angers) – dont certains avaient été identifiés comme bénéfiques ou antagonistes potentiels dans le Chapitre I. A ce stade de l'étude, la présence de ces microorganismes dans le même environnement biotique que l'un des deux agents pathogènes *Z. tritici* ou *L. maculans* ne justifiait pour autant pas l'existence d'une interaction entre eux. Pour cette raison, la troisième étape de la thèse a permis de caractériser les interactions de manière approfondie par des stratégies expérimentales et des techniques d'analyse adaptées à la complexité de la problématique.

Cette troisième étape nous a conduit à caractériser (1) l'impact de la présence de chacun des agents pathogènes – *Z. tritici* et *L. maculans* – présents sous forme sexuée sur le microbiome des résidus de cultures et (2) leur interaction avec le reste de la communauté microbienne. Pour caractériser l'impact de la présence de l'agent pathogène sur les communautés, il était nécessaire de s'appuyer sur des cas contrastés de présence/absence, c'est-à-dire des conditions « d'infection » et des conditions « saine » lors de la culture de chacune des plantes hôtes. Compte tenu des différences de cycles biologiques de *Z. tritici* et *L. maculans*, deux stratégies ont été adoptées. Pour l'étude de *Z. tritici*, un dispositif semi-naturel a été mis en place : les plantes ont été élevés en serre, un lot d'entre-elles a été inoculé, et l'ensemble a été placé à l'extérieur après maturité pour suivre l'évolution des communautés microbiennes des résidus en conditions naturelles. Leur colonisation par les microorganismes du sol a été estimée en plaçant des lots de résidus en contact direct avec le sol, et d'autres sans contact. La complexité du cycle de *L. maculans* ne permettait pas d'envisager la même expérimentation avec le colza. La stratégie a donc été adaptée. Nous avons utilisé des lignées isogéniques différant par un gène de résistance à *L. maculans*. D'autres méthodes auraient théoriquement pu être adoptées pour générer ces deux conditions (parties basses de tiges de colza infectées vs. saines). L'utilisation d'un fongicide pour obtenir des conditions saines en conditions agricoles aurait toutefois eu un impact sur les communautés fongiques endophytes ou pathogènes non ciblés [14,15]. L'utilisation de variétés de colza non apparentées n'aurait pas non plus été satisfaisante étant donné qu'il a été démontré que la génétique de l'hôte a une influence forte sur le microbiome de la plante et de la racine [16–18], en particulier si les variétés diffèrent sur d'autres caractères que la résistance [19].

Avant de revenir sur les résultats en détail, il nous semble important d'analyser les méthodes utilisées dans cette thèse.

III. Méthodologie comparée

La puissance du metabarcoding ne fait pas pour autant tomber en désuétude les techniques d'isolements

La plupart des études réalisées au cours de cette thèse se sont appuyées sur du metabarcoding. Cette stratégie, bien que très efficace pour caractériser une communauté et mettre en évidence les changements dus à une perturbation (présence d'un agent pathogène, application de fongicides, introduction d'un gène de résistance, etc.), ne permet toutefois pas de caractériser les interactions en allant jusqu'au niveau de l'espèce : en effet, les amorces utilisées, généralistes, ne conduisent pas à une identification certaine des espèces présentes, et donc du rôle exact de chaque microorganisme dans la communauté. C'est le cas pour les bactéries, mais aussi pour un certain nombre de champignons, comme ceux rattachés au genre *Alternaria* sp. : certaines espèces sont parfois décrites comme agents de biocontrôle et d'autres comme agents pathogènes, tandis que les séquences ITS ne permettent pas de les distinguer. De fait, la réalisation de la collection au Chapitre II pourrait permettre de mieux caractériser ces communautés, en identifiant certaines souches avec les séquences d'autres gènes plus résolutifs (par exemple *gyrB* et *rpb2*). Par ailleurs, pour étudier précisément le rôle de chaque espèce de microorganisme de la communauté – seul ou sous-forme de complexe – il serait nécessaire d'isoler tout ou partie de cette communauté. Il pourrait ensuite être envisagé de réaliser des communautés synthétiques, à partir des microorganismes isolés, et de tester différentes combinaison de communautés afin de pouvoir décomposer les effets des organismes seuls ou en associations [20]. Une telle stratégie sous-évaluerait évidemment la diversité naturelle présente, mais pourrait permettre de mieux décrire les organismes présents et leur rôle dans la communauté : taxon clé ou sans incidence majeure ? Une autre stratégie pourrait consister à étudier la communauté par réduction de diversité : la communauté d'origine serait extraite et réimplantée sur une matrice stérile, après dilution en série. La dilution serait utilisée pour manipuler le nombre d'organismes différents présents, et donc le nombre de traits fonctionnels de la communauté, en partant du postulat que le nombre de traits fonctionnels augmente avec le nombre d'organismes présents [21]. Pour chacune de ces stratégies les techniques culture-dépendantes (isolements) sont essentielles : couplées aux analyses de metabarcoding, elles permettent d'avoir une vision non seulement plus précise, mais aussi plus fonctionnelle, du monde microbien.

Extraction et barcoding : des adaptations et des différences, à l'origine de biais potentiels

Les techniques d'analyse utilisées entre les Chapitres II et III ont présenté des différences. Le premier point à noter est le changement de technique d'extraction. Un test a été réalisé et a montré que ce changement n'avait pas eu d'incidence majeure sur la caractérisation des communautés (données non présentées). Le deuxième changement, et probablement le plus significatif, est la procédure de filtrage des données. En effet, dans la première expérimentation (Chapitre II) le filtrage a été réalisé sur la base d'une duplication d'extraction : les ASV présents dans une seule des deux extractions ont été supprimés. Pour les deux autres expérimentations (Chapitre III.1 et III.2), le filtrage a été fait sur la base

d'un pourcentage de reads, établi à l'aide de mélange d'ADN de composition connue. Malgré le nombre beaucoup plus important d'échantillons dans ces deux autres expérimentations, le nombre d'ASV est resté inférieur à celui de la première.

Inoculer une plante ou la rendre résistante : même combat méthodologique ?

Deux stratégies différentes ont été utilisées pour caractériser l'effet de la présence de l'agent pathogène sur les communautés.

Lors de l'étude réalisée au Chapitre II.1, *Z. tritici* a été détecté dans la grande majorité des résidus jusqu'au mois de février. *Z. tritici* n'a en revanche été détecté que jusqu'en octobre au Chapitre III.1. Cette disparition plus rapide peut s'expliquer par la stratégie expérimentale adoptée : en conditions naturelles *Z. tritici* s'établit sur toutes les parties de la plante par la réalisation de plusieurs cycles d'infections secondaires (estimé entre 6 et 7 au cours d'une saison de culture ; Frédéric Suffert, comm. pers.). Comme expliqué dans le Chapitre III.1, l'inoculation unique réalisée en serre a probablement été à l'origine de contaminations moins intenses des tissus plants de blé (un seul cycle infectieux) qu'en conditions naturelles, où les gouttelettes de pluies chargées de pycnidiospores ont pu s'amasser à la base du pétiole des feuilles à plusieurs reprises (à chaque épisode pluvieux). L'inoculation unique en serre a probablement eu pour conséquence l'infection des feuilles, parties qui se dégradent le plus rapidement, mais très peu des tiges, parties les plus résistantes qui constituent finalement la majeure partie des résidus à la fin de l'automne.

Pour le colza, la stratégie expérimentale a été différente : une colonisation naturelle par *L. maculans* a été privilégiée. Afin de maximiser les différences d'infection, pour pouvoir ensuite caractériser l'effet de la présence de *L. maculans* sur les communautés, deux cultivars ont été utilisés (Darmor et Darmor-*Rlm11*). Si cette stratégie ne nous a pas permis de générer des conditions aussi tranchées qu'avec une inoculation, elle nous a tout de même permis d'obtenir des échantillons ayant une différence d'abondance relative de *L. maculans* significative. Une inoculation en condition semi-naturelles est plus efficace pour obtenir des conditions contrastées mais ne permet pas d'obtenir des communautés aussi proches qu'en parcelles agricoles en conditions naturelles. De fait, les communautés des résidus de colza (et les interactions au sein de ces communautés) analysés dans le Chapitre III.2 ont été plus proches des communautés « réelles », naturelles, que celles analysées dans le Chapitre III.1 (Figure 1).

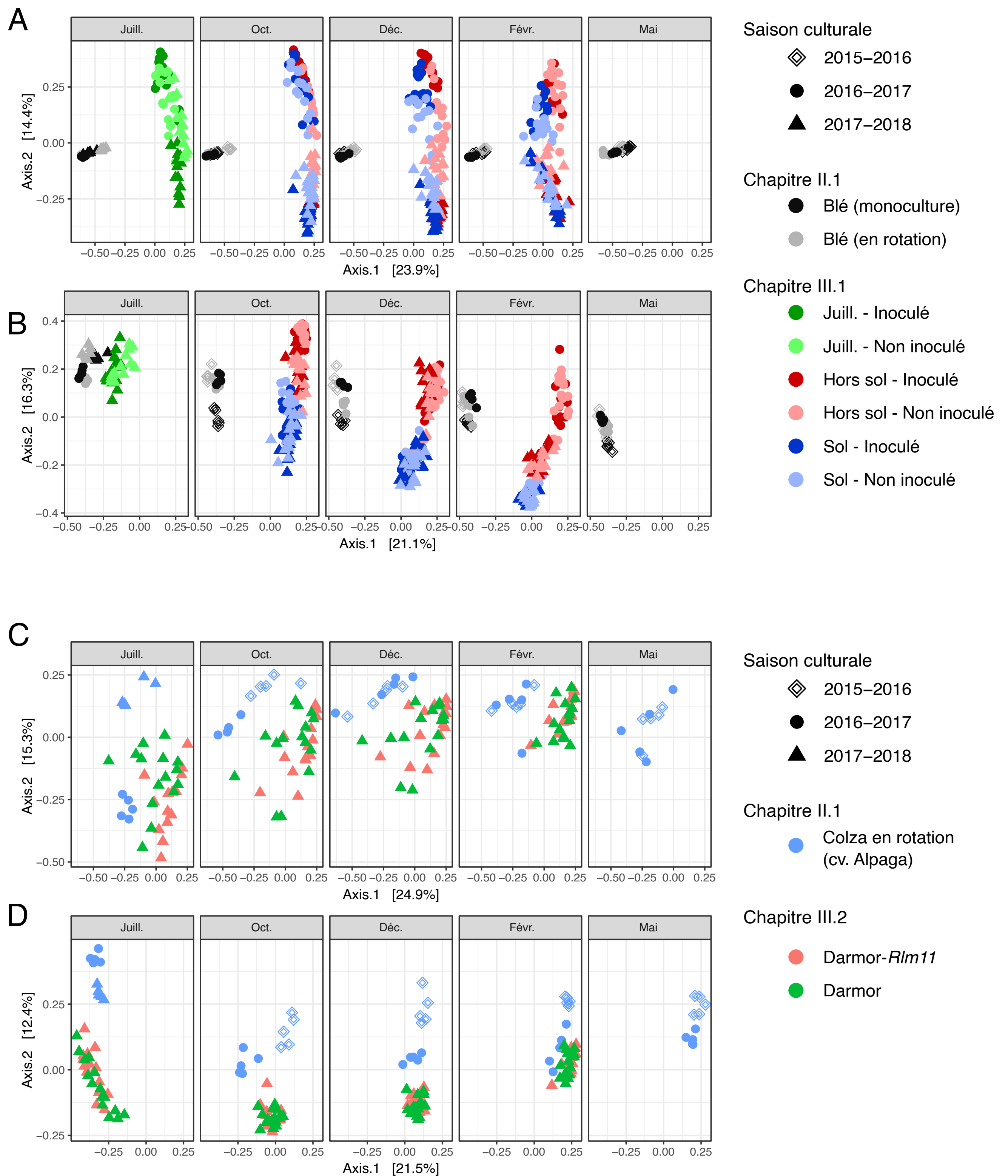


Figure1. Comparaison des communautés issues des résidus des Chapitre II.1 (blé et colza) , III.1 (blé) et III.2 (colza). Les résidus ont été échantillonnés au cours des saisons culturales 2015-2016 (oct., déc., févr., mai) , 2016-2017 (juill., oct., déc., févr., mai) et Juillet 2017 pour le Chapitre 2 ; au cours des saisons 2016-2017 et 2017-2018 (juill., oct., déc., févr.) pour le Chapitre III.1, et au cours de la saison 2017-2018 (juill., oct., déc., févr.) pour le Chapitre III.2.

Réseaux d'interactions

Dans les deux expérimentations du Chapitre III, la combinaison des deux approches – analyse différentielles avec a priori et analyse de réseaux d'interactions sans a priori – a permis de caractériser les impacts généraux de la présence de maladies (cas blé et colza) et les effets de l'introgression d'un gène de résistance (cas colza), mais aussi les interactions directes avec chacun des agents pathogènes. Le choix de la méthode de construction des réseaux ne nous permettait pas d'avoir des réseaux dirigés, c'est-à-dire indiquant lequel des deux organismes impliqués dans une interaction à un impact sur l'autre, ce qui a rendu l'interprétation des interactions assez difficile. Cette difficulté n'est pas spécifique de cette thèse et pointe les limites de l'analyse des réseaux d'interactions, à une époque où ils sont de plus en plus utilisés dans les analyses de communautés.

IV. Les résidus de culture : un compartiment à l'interface entre la plante et le sol, riche et évolutif

Tout au long de ce mémoire de thèse nous avons montré que les résidus devaient être perçus comme un écosystème microbien à part entière, à l'interface entre deux environnements : la plante et le sol. Nous avons montré dans le Chapitre I que ce compartiment est un support de l'inoculum primaire de nombreuses maladies, mais également un écosystème à part entière biologiquement très riche et évolutif, en terme de communautés microbiennes. Les communautés présentes sur résidus sont à la fois issues de la plante et du sol, ce qui en fait un compartiment dynamique source d'interactions uniques, ou les espèces biotrophes (en début de dégradation) et saprotrophes (en fin de dégradation) se rencontrent.

En conditions naturelles, les communautés fongiques des résidus sont initialement dominées par des agents pathogènes inféodés à la plante

En comparant les communautés microbiennes des résidus de blé et de colza, sur deux années et à plusieurs stades de dégradation, nous avons montré que la plante hôte était le facteur le plus structurant, à la fois pour les bactéries et pour les champignons. Pour les champignons, ce résultat peut être illustré par le nombre d'agents pathogènes détectés sur les résidus en conditions naturelles. En effet, parmi les champignons très présents au début, nous avons identifié, chez le blé, *Fusarium* et *Gibberella*, qui comptent des espèces responsables de la fusariose du blé, *Phaeosphaeria*, *Parastagonospora*, *Stagonospora*, qui comptent des espèces responsables de maladies foliaires, et *Occulimacula* sp., potentiel agent du piétin verse. Chez le colza, *L. maculans* et *Plenodomus biglobosa* ont été les deux champignons les plus abondants. *Verticillium* sp., potentiel agent de la Verticilliose, et *Pyrenopeziza* sp., potentiel agent de la Cylindrosporiose, ont également été présents au cours de la seconde année d'expérimentation. Une étude avait déjà montré que les variations temporelles des communautés fongiques du blé étaient provoquées, ou du moins associées, à des vagues successives de développement d'agents pathogènes [22].

Les différences de communautés entre le blé et le colza, notamment bactériennes, peuvent également être la conséquence de différences de composition chimique des résidus, comme cela a déjà été démontré dans des études précédentes [23]. Dans cette thèse, nous avons fait le choix d'éluder cette piste, d'une part parce qu'elle avait déjà fait l'objet d'études extrêmement poussées, et d'autre part en raison des contraintes techniques et du coût d'analyse physico-chimique des résidus, en particulier pour estimer les rapports C/N. Les études portant sur la phyllosphère avait également démontré que les communautés présentes sur plantes dépendaient de l'organe considéré, de l'âge de cet organe, de l'espèce et du génotype de la plante, en plus des facteurs environnementaux (radiation, pollution, fertilisation [24]). Par exemple, la comparaison entre les communautés bactériennes associées au trèfle, au soja, au riz et à *A. thaliana* a mis en évidence de forte différences de communautés, avec des proportion *d'Alphaproteobacteria*, *Actinobacteria* et *Bacteroidetes* qui varient en fonction de l'espèce végétale considérée [24].

Avec le temps l'effet de la plante sur la communauté microbienne décroît : les deux agents pathogènes s'éclipsent, plus ou moins rapidement, laissant la place à des taxons plus généralistes

Au cours de la dégradation, l'effet de la plante sur la structuration des communautés a diminué, et les résidus en contact avec le sol ont été rapidement colonisés par d'autres microorganismes, plus généralistes, tant chez le blé que le colza. Pour le blé, l'abondance relative des agents pathogènes originaires de la plante a diminué plus rapidement que pour le colza, chez lequel *L. maculans* et *P. biglobosus* sont restés les champignons les plus détectés au cours des premiers mois du processus de dégradation. Ce résultat peut être expliqué par les caractéristiques des cycles de vie de chaque agent pathogène : le développement de *Z. tritici*, par exemple, est extracellulaire, et le champignon ne progresse pas de façon systémique dans le blé, tandis que *L. maculans* croît de manière intracellulaire dans les vaisseaux du xylème. Les résidus de colza, par ailleurs plus trapus, offrent donc une meilleure protection à *L. maculans* que les résidus de blé à *Z. tritici*. Les microorganismes originaires de la plante sont remplacés par des microorganismes originaires du sol, à l'exemple de *Chaetomium* ou *Torula*. Nous avons montré que ces taxons ont colonisé les résidus dans toutes les expérimentations. D'autres taxons ont davantage colonisé les résidus de colza, comme *Dactylella*, *Dendryphon*, *Phialophora* (Chapitres II.1 et III.2), ou les résidus de blé, comme *Neosetophoma* ou *Pterula* (Chapitres II.1 et III.1). Il est également intéressant de noter que certains champignons appartenant aux genres *Cryptococcus*, *Stagonospora*, er *Myrmecridium* ont colonisé des résidus n'ayant jamais été en contact avec le sol : il est probable que des dépôts de spores par voie aérienne soient à l'origine de la contamination des résidus par ces taxons.

La saison culturale et la rotation ont un impact significatif sur le microbiote des résidus

La saison culturale a eu un impact fort sur la structuration des communautés microbiennes des résidus. Au tout début de leur dégradation, lorsque l'empreinte des communautés des résidus était encore proche de celle des communautés de la plante, cet effet a pu s'expliquer par la façon dont les conditions environnementales (conditions météorologiques, pratiques culturales) influencent le développement des agents pathogènes. En effet, les épidémies fongiques dépendent de différents facteurs climatiques, tels que la température, la pluviométrie ou l'humidité [25,26]. Les deux années d'expérimentation, contrastées en terme d'humidité et de pluviométrie, ont présenté des profils d'infection différents. Par exemple, les ASV correspondant à *Occulimacula yallundae* (piétin verse) ont été deux fois plus représentés dans le blé au cours de la saison 2016-2017 par rapport à la saison 2015-2016, et *Verticillium* et *Pyrenopeziza* ont été détectés dans les résidus de colza uniquement au cours de la saison 2016-2017. L'effet année a également été important sur les microorganismes originaires, selon toute vraisemblance, du sol : *Coprinellus*, *Psathyrella*, *Nigrospora* ou *Coprinopsis* ont par exemple été détecté en abondance en 2015-2016 (Chapitre II.1), comme colonisateurs tardif des résidus. Les saisons suivantes, ces organismes ont été détectés en abondance et occurrence beaucoup plus faible

que ce soit en 2016-2017 (Chapitre II.1 et Chapitre III.1) ou en 2017-2018 (Chapitre II.1). Le mois d'octobre de la saison culturale 2015-2016 a été marquée par une pluviométrie trois fois plus élevée que celle des autres années (INRA Climatik), ce qui pourrait avoir eu un impact sur ces microorganismes colonisateurs. Le printemps de cette même année culturale a également été plus pluvieux que celui des autres années d'étude, ce qui pourrait expliquer la prédominance de *Oculimacula yallundae* dans les résidus de blé pendant la saison 2016-2017 (issus des cultures de 2015-2016).

De manière analogue, l'influence du système cultural (rotation), bien que faible, s'est manifestée tout au long de l'évolution des résidus. L'impact a été plus marqué pour les champignons que pour les bactéries, ce qui peut encore une fois s'expliquer par la présence d'agents pathogènes de nature principalement fongique. La présence du champignon *Pyrenophora tritici-repentis* dans la monoculture est à noter, et n'est pas surprenante : l'helminthosporiose est caractéristique des zones de production où le blé revient fréquemment dans la rotation. Cet agent pathogène n'est pas le seul à être impacté par le système cultural : *Z. tritici* semple se conserver plus longtemps dans les résidus de blé en monoculture que les champignons issus d'une rotation blé-colza, de même que *Stagonospora* sp. et *Fusarium* sp., davantage présents dans les résidus issus de blé en monoculture que de blé en rotation. D'autres microorganismes colonisateurs ont également été plus abondants sur les résidus de blé de monoculture (*Trichopeziza*, *Hydropisphaera*).

V. La présence d'agents pathogènes structure les communautés microbiennes des résidus

Si plusieurs études avaient déjà montré l'impact de la plante, ou du sol, sur les communautés microbiennes des résidus, très peu d'entre elles se sont intéressées à l'effet des agents pathogènes sur ces communautés (et réciproquement). L'analyse des dynamiques de communautés en réponse à l'infection par un agent pathogène se sont multipliées ces dernières années, mais se sont plutôt concentrées sur la période où l'impact de l'agent pathogène est préjudiciable pour la culture, c'est-à-dire sur les organes aériens quelques semaines avant la fin du cycle végétatif. Jakuschkin *et al.* [13] ont par exemple mis en évidence des changements significatifs dans les communautés fongiques et bactériennes foliaires à la suite de l'infection du chêne pédonculé par *Ersysiphe alphitoides*. Pour ce qui concerne les parties racinaires, Lebreton *et al.* [27] ont mis en évidence des changements significatifs dans les communautés fongiques et bactériennes suite à l'inoculation des racines de chou chinois (*Brassica rapa*) avec *Plasmodiophora brassicae*.

Si les méthodes utilisées dans cette thèse n'ont pas permis de générer des conditions d'infections aussi tranchées entre le blé et le colza, les deux stratégies expérimentales adoptées ont clairement démontré l'impact de la présence de Z. *tritici*, et *L. maculans* sur les communautés microbiennes des résidus.

L'infection du blé par *Z. tritici* structure fortement le microbiome des résidus

L'infection des plants de blé par *Z. tritici* a eu un effet sur les communautés des résidus, même après la disparition (non détection) de l'agent pathogène. L'analyse discriminante a en effet mis en exergue un certains nombres de microorganismes dont l'abondance relative a été modifiée par l'infection par *Z. tritici*, qu'il soit encore présent ou non. L'effet de la présence passée de *Z. tritici* s'est toutefois estompé au cours du temps, illustrant ainsi une certaine résilience des communautés : la composition de celles de résidus issus de blé inoculé s'est progressivement rapprochée de la composition de celles de blé non inoculé. La place importante de *Z. tritici* dans les réseaux, en tant que taxon clé (degré élevé de centralité), est un indicateur du fort impact de sa présence sur les communautés.

Il est intéressant de noter que l'inoculation de *Z. tritici* a eu davantage d'effet sur la structuration des communautés fongiques que sur celle des communautés bactériennes. De plus, *Z. tritici* a eu davantage d'interactions directes avec des champignons qu'avec des bactéries. Malgré cela, peu d'espèces individuelles ont été identifiées comme étant en interaction directe avec *Z. tritici*. La plupart des champignons qui l'ont été sont des agents pathogènes (*Blumeria graminis, Fusarium* sp.), ce qui suggère que ces parasites du blé rentrent en compétition entre eux pendant la période épidémique. Plusieurs études avaient déjà mis en évidence de type d'interactions, à une échelle plus fine et fonctionnelle (par exemple, accumulation d'H_2O_2 dans les tissus colonisés par *Z. tritici*, préjudiciable aux champignons biotrophes mais profitables aux nécrotrophes).

L'introgression du gène de résistance à *L. maculans* a eu un effet significatif mais un peu plus limité sur le microbiome des résidus de colza.

Pour le colza, la différence entre la variété Darmor et Darmor-*Rlm11* n'a pas été aussi forte qu'entre le blé inoculé avec *Z. tritici* et le blé non inoculé. L'unique année de l'expérimentation a, de plus, été marquée par une pression parasitaire de *L. maculans* plus faible que *P. biglobosus*, par rapport aux années précédentes, ce qui peut également expliquer cette faible différence. On estime que 5% des souches locales de *L. maculans* sont malgré tout virulentes vis-à-vis du gène *Rlm11*, ce qui peut expliquer que Darmor-*Rlm11* ait été attaqué, même faiblement. De fait, les communautés microbiennes des résidus de colza ont été moins impactées par la différence entre Darmor et Darmor-*Rlm11* que ne l'ont été celles des résidus de blé inoculés et non inoculés par *Z. tritici*. Malgré des communautés proches, l'analyse discriminante a montré que d'autres microorganismes ont été impactés par l'introgression de *Rlm11*.

La très forte abondance de *P. biglobosus* et sa place centrale dans les réseaux a été mise en évidence de manière assez inattendue. Aucune interaction n'a toutefois été démontrée entre *P. biglobosus* et *L. maculans*, contrairement à ce que la proximité taxonomique et biologique des deux espèces aurait pu laisser supposer. Il est en effet connu que les deux espèces partage la même niche écologique [28]. Cependant, dans une étude culture-dépendante, West *et al*. [29] ont montré que *P.*

biglobosus est plutôt présent dans les tiges de colza, tandis que *L. maculans* colonise majoritairement le collet. Dans notre étude la proportion de *L. maculans* dans les résidus de colza a diminué avec le temps, ce n'a pas été le cas de *P. biglobosus*. Nous pouvons faire l'hypothèse d'une meilleure compétence saprophytique de *P. biglobosus* par rapport à *L. maculans* [29], sans toutefois avoir pu la tester. L'abondance relative de *L. maculans* a été plus faible que lors de l'expérimentation du Chapitre II.1, ce qui peut s'expliquer par la différence de sensibilité des cultivars utilisés : Alpaga est très sensible au phoma, alors que Darmor possède un niveau de résistance quantitative plus élevé.

Un dernier résultat intéressant à noter est la différence d'abondance relative du genre *Pyrenopeziza* entre les résidus Darmor et Darmor-*Rlm11*. *Pyrenopeziza brassicae* est le champignon responsable de la Cylindrosporiose, une maladie assez répandue dans l'ouest de la France et dans le nord du Royaume-Uni [30], et réalise également son cycle sexué sur les résidus de colza. Plusieurs hypothèses peuvent être formulées pour tenter d'expliquer cette différence : (1) la présence de *L. maculans* facilite l'infection par *P. brassicae* (2) ; la résistance à *L. maculans* conférée par *Rlm11* empêche la contamination précoce des feuilles de colza par *P. brassicae* ; (3) l'introgression de *Rlm11* de *Brassica rapa* à *B. napus* s'est accompagnée de l'introgression un locus de résistance à *P. brassicae* ou a remplacé un locus de sensibilité initialement présent chez Darmor (Régine Delourme, comm. pers.).

Au final ces résultats posent des questions disciplinaires – phytopathologiques – intéressantes qui mériteraient d'être traitées à BIOGER dans le cadre des travaux de recherche sur *L. maculans*, en tant que champignon pathogène modèle.

VI. Limiter l'inoculum primaire d'agents pathogènes en exploitant certains microorganismes inféodés aux résidus : une solution réaliste et opérationnelle ?

Les expérimentations réalisées dans le Chapitre II ont permis de montrer que, si les résidus sont un substrat important pour les agents pathogènes, ils hébergent également un certain nombre de microorganismes considérés comme des compétiteurs ou antagonistes potentiels. Parmi ceux-ci, on peut citer les champignons appartenant aux genres *Clonostachys*, *Aureobasidium*, *Chaetomium* et *Cryptococcus*.

L'étude des interactions entre les microorganismes offre des perspectives très intéressantes pour ce qui concerne la recherche d'agents « bénéfiques » dans le contexte de la protection des cultures. Plusieurs études partageant cette vision [31,32] ont déjà mis en évidence des modes d'action décrit sur résidus de culture. Par exemple, sur des résidus de pois chiche, *Aureobasidium pullulans* peut croître plus vite que l'agent pathogène *Didymella rabiei* responsable de l'Ascochytose et limiter ainsi sa propagation par compétition ; *Clonostachys rosea*, qui a une capacité mycoparasitaire, peut diminuer ou même supprimer totalement la reproduction sexuelle et asexuée de *D. rabiei* [32]. *Microsphaeropsis* sp., également connu pour sa capacité mycoparasitaire [33], peut réduire la capacité de production d'ascospores de *Fusarium graminearum* sur résidus de blé et de maïs [34,35]. Bastian *et al.* [36] ont montré que des résidus placés au contact du sol pouvaient être colonisés par des bactéries telles que *Pseudomonas fluorescens*, *Pseudomonas aurantiaca* et *Pseudomonas putida* et des champignons tels que *Chaetomium globosum*, espèces décrites comme des agents potentiels de biocontrôle [37–44].

Au cours de cette thèse, très peu d'organismes finalement décrits dans la littérature (Tableau 1) comme agents de biocontrôle ont eu des interactions directes avec les agents pathogènes étudiés (*Chaetomium* avec *L. maculans* ; *Cladosporium* avec *Z. tritici*), mais la présence de certains a été significativement impactée par celle d'un des deux agents pathogènes (*Chaetomium*, *Epicoccum* et *Trichoderma* sur blé ; *Cladosporium* et *Cryptococcus* sur colza). Les réseaux d'interactions ont mis en évidence des interactions significatives entre *Z. tritici* et des champignons appartenant au genre *Cladosporium*, dont certaines espèces ont déjà été décrites comme antagonistes de *Venturia inaequalis* sur pommier [45]. Une interaction directe entre *Chaetomium* et *L. maculans* a également été mise en évidence. La plupart des interactions décelées semblent toutefois n'être que temporaires et ont fait l'objet d'une certaine variabilité intra-parcellaire. En pratique, l'utilisation d'une espèce issue des résidus de culture et utilisée comme agent de biocontrôle contre *Z. tritici* ou *L. maculans* semble difficile à mettre en place sur la phase sexuée (résidus) ou asexuée (plantes en cours de croissance). En admettant que l'on arrive à identifier un agent de biocontrôle efficace contre ces agents pathogènes, comment estimer sa capacité à s'établir et à interagir avec un agent pathogène au sein d'une communauté, dont nous avons montré qu'elle était à la fois complexe par sa diversité taxonomique et sa résilience ?

Tableau 1. Liste non –exhaustive de microorganismes testés pour leurs effets antagonistes sur des agents pathogènes du blé, du colza, de l'orge, ou du pois chiche.

Antagoniste	Agent pathogène	Hôte	ref
Alternaria infectoria	*Fusarium pseudograminearum*	blé	[48]
Aspergillus niger	*Zymoseptoria tritici*	blé	[42]
Aureobasidium pullulans	*Phaeosphaeria nodorum*	blé	[49]
Aureobasidium pullulans	*Fusarium culmorum*	blé	[50]
Aureobasidium pullulans	*Didymella rabiei*	pois chiche	[32]
Bacillus megaterium	*Zymoseptoria tritici*	blé	[40]
Bacillus sp.	*Gaeumannomyces graminis var. tritici*	blé	[51]
Bacillus sp.	*Pyrenophora tritici-repentis*	blé	[41]
Bacillus cereus	*Leptosphaeria maculans*	colza	[44]
Bacillus sp.	*Zymoseptoria tritici*	blé	[42]
Chaetomium globosum	*Zymoseptoria tritici*	blé	[52]
Chaetomium globosum	*Pyrenophora tritici-repentis*	blé	[41]
Clonostachys rosea	*Fusarium spp*	blé	[53]
Clonostachys cladosporioides	*Fusarium spp*	blé	[53]
Clonostachys rosea	*Fusarium spp*	blé	[54]
Clonostachys rosea	*Fusarium graminearum*	blé	[55]
Clonostachys rosea	*Didymella rabiei*	pois chiche	[32]
Cryptococcus sp.	*Zymoseptoria tritici*	blé	[52]
Cryptococcus laurentii var. flavescens	*Phaeosphaeria nodorum*	blé	[49]
Cyathus striatus	*Leptosphaeria maculans*	colza	[56]
Drechslera teres	*Zymoseptoria tritici*	blé	[57]
Epicoccum nigrum	*Zymoseptoria tritici*	blé	[52]
Erwinia herbicola	*Leptosphaeria maculans*	colza	[58]
Fusarium equiseti	*Fusarium pseudograminearum*	blé	[48]
Fusarium nygamai	*Fusarium pseudograminearum*	blé	[48]
Fusarium moniliforme var. anthophylium	*Zymoseptoria tritici*	blé	[52]
Fusarium equiseti	*Fusarium spp.*	blé	[53]
Gliocladium catenulatum	*Leptosphaeria maculans*	colza	[59]
Laetisaria arvalis	*Pyrenophora tritici-repentis*	blé	[43]
Limonomyces roseipellis	*Pyrenophora tritici-repentis*	blé	[43,60,61]
Microsphaeropsis sp	*Fusarium graminearum*	blé	[34]
Microsphaeropsis ochracea	*Verticillium longisporum*	colza	[62]
Nigrospora sphaerica	*Zymoseptoria tritici*	blé	[52]
Paecilomyces lilacinus	*Pyrenophora tritici-repentis*	blé	[41]
Paenibacillus polymyxa	*Leptosphaeria maculans*	colza	[63]
Penicillium lilacinun	*Zymoseptoria tritici*	blé	[52]
Penicillium sp.	*Pyrenophora tritici-repentis*	blé	[41]
Pseudomonas fluorescens	*Zymoseptoria tritici*	blé	[40]
Pseudomonas putida	*Zymoseptoria tritici*	blé	[39]
Pseudomonas fluorescens	*Zymoseptoria tritici*	blé	[64]
Pseudomonas fluorescens	*Zymoseptoria tritici*	blé	[65]
Pseudomonas chlororaphis subsp. Aurantiaca	*Rhizoctonia cerealis*	blé	[66]
Pseudomonas fluorescens	*Pyrenophora tritici-repentis*	blé	[60]
Pseudomonas fluorescens	*Pseudocercosporella herpotrichoides*	blé	[37]
Pseudomonas fluorescens	*Gaeumannomyces graminis var. tritici*	blé	[67,68]
Pseudomonas fluorescens	*Fusarium proliferatum*	blé	[38]
Pseudomonas chlororaphis	*Leptosphaeria maculans*	colza	[44]
Pseudomonas aurantiaca	*Leptosphaeria maculans*	colza	[44]
Pythium sp.	*Fusarium culmorum*	blé	[69]
Rhodotorula rubra	*Zymoseptoria tritici*	blé	[52]
Serratia plymuthica	*Leptosphaeria maculans*	colza	[59]
Sporobolomyces roseus	*Phaeosphaeria nodorum*	blé	[49]
Sporobolomyces roseus	*Fusarium culmorum*	blé	[50]
Streptomyces griseoviridis	*Pseudocercosporella herpotrichoides*	blé	[37]
Streptomyces spp.	*Fusarium culmorum*	orge	[70]
Trichoderma harzianum	*Zymoseptoria tritici*	blé	[71]
Trichoderma koningii	*Zymoseptoria tritici*	blé	[71]
Trichoderma koningii	*Zymoseptoria tritici*	blé	[71]
Trichoderma aureoviride	*Zymoseptoria tritici*	blé	[71]
Trichoderma harzianum	*Fusarium pseudograminearum*	blé	[48]
Trichoderma harzianum	*Zymoseptoria tritici*	blé	[72]
Trichoderma koningii	*Zymoseptoria tritici*	blé	[72]
Trichoderma harzianum	*Zymoseptoria tritici*	blé	[73]
Trichoderma hamatum	*Pyrenophora tritici-repentis*	blé	[41]
Trichoderma koningii	*Pyrenophora tritici-repentis*	blé	[61]
Trichoderma spp.	*Gaeumannomyces graminis var. tritici*	blé	[74]
Trichoderma harzianum	*Fusarium graminearum*	blé	[75]
Trichoderma spp.	*Fusarium spp.*	blé	[53]

Dans la continuité directe des résultats acquis dans cette thèse, et pour tenter de répondre aux objectifs ambitieux du projet européen EMPHASIS, nous avons conçu et testé un protocole de traitement des résidus de blé qui puisse être utilisé pour estimer l'efficacité de microorganismes (espèce unique ou complexe d'espèce) pour limiter le développement de *Z. tritici* pendant sa phase de reproduction sexuée, en d'autre termes limiter le nombre d'ascospores produites. Les résultats de cette expérimentation préliminaire sont présentés dans l'Encadré 1. Des études de ce type mériteraient d'être reconduites, sur la base de protocoles améliorés, avec des microorganismes issus des collections fongiques et bactériennes (Chapitre II.2). Des « assemblages » constitués de plusieurs microorganismes issus de ces collections pourraient également être testés, afin d'étudier, par extrapolation, le comportement d'une « communauté » *sensu stricto*.

La plupart des études portant sur les méthodes de biocontrôle des maladies fongiques en systèmes de grandes cultures s'accordent sur le fait que les stratégies basées sur ces méthodes sont très complexes à mettre en œuvre. Compte tenu des résultats et des connaissances acquis pendant cette thèse nous pensons qu'il est difficile d'envisager qu'un agent de biocontrôle puisse être utilisé en tant que substitut à un « produit de traitement », par exemple un fongicide de synthèse dont l'efficacité est avérée. Nos résultats suggèrent en effet que, si certains microorganismes peuvent être identifiés comme bénéfiques, c'est probablement un complexe de microorganismes, voire une communauté microbienne dans son entièreté, qui pourrait avoir un effet, et pas forcément telle ou telle espèce considérée isolément. Dans une perspective appliquée, l'idéal ne serait-il pas finalement de favoriser des pratiques agricoles qui préservent (ou au moins évitent de dégrader) l'équilibre qui prévaut dans cette communauté ? Les perspectives d'utilisation d'agents de biocontrôle devraient aujourd'hui être révisées, pour ne pas dire relativisées, afin de travailler, non pas sur un microorganisme ciblant un agent pathogène, mais sur des communautés de microorganismes. Cela éviterait certaines désillusions. Plusieurs équipes de recherche étudient actuellement l'apport des communautés synthétiques. Certaines d'entre elles ont déjà montré que, si des microorganismes du sol peuvent modifier le phénotype de plantes (résistance au stress, aide à l'absorption de substances nutritives, etc.), leur maintien après leur introduction est loin d'être assuré, tandis que l'introduction de plusieurs organismes, dont on sait qu'ils interagissent entre eux, permet ce maintien [46,47]. Ce type d'interactions complexes est probablement impliqué dans le cas des « sols suppressifs », décrits il y maintenant plusieurs décennies. Ces sols, dont on admet qu'ils limitent l'établissement de certains agents pathogènes, sont toujours très étudiés, sans pour autant que la fonctionnalité des interactions entre les microorganismes qu'ils hébergent n'ait été complètement élucidée.

Encadré - Elaboration d'un protocole pour estimer l'effet de micro-organismes sur la phase sexuée de *Zymoseptoria tritici* sur résidus de blé

Objectifs et stratégie

L'objectif était de mettre au point puis de tester un protocole permettant d'estimer l'effet de micro-organismes sur la phase sexuée de *Z. tritici*. Un tel protocole pourrait être utilisé pour identifier des espèces ou des complexes d'espèces susceptibles de limiter la quantité d'inoculum primaire sur résidus de blé pendant la période d'inter-culture. Une expérimentation a été réalisée en 2017-2018 pour quantifier l'impact d'un traitement par des isolats fongiques sur la production d'ascospores sur des résidus de blé préalablement inoculés avec *Z. tritici*. Les isolats utilisés pour cette mise au point ont été choisis dans la collection EMPHASIS (Chapitre III.2) parmi ceux issus de résidus de blé collectés en parcelle agricole et dont l'affiliation taxonomique avait déjà été réalisée (Chapitre III.1 ; Kerdraon *et al.*, 2019). Ce choix n'a pas pu tenir compte des analyses de réseaux (Chapitre III.1), réalisées postérieurement, mais seulement de résultats d'études publiées ayant testé les propriétés antagonistes de certaines espèces fongiques vis-à-vis de certains agents pathogènes de céréales (Tableau 1).

Matériels et méthodes

Préparation des résidus contaminés par Z. tritici

Les résidus de blé ont été obtenus en contaminant 240 plants de blé adultes (cv. Soissons), élevés en serre, avec deux couples d'isolats parentaux de *Z. tritici* (10^5 spores.mL^{-1}). Le protocole suivi a été identique à celui décrit dans le Chapitre III.1, validé lors de précédentes études sur les déterminants de la reproduction sexuée [1,2]. Les plants de blé ont été maintenus en serre jusqu'à ce que l'ensemble des tissus soient secs, sous l'effet combiné de la septoriose et de la senescence naturelle. Ils ont ensuite été placés à l'extérieur (fin juillet) afin que la reproduction sexuée se produise (Figure 1.A). Mi-octobre, les tiges et les feuilles ont été découpées et ainsi transformées en « résidus de blé », ensuite répartis dans des sachets en voile de nylon, à raison de 20 g de résidus par sachet.

Inoculation des résidus avec les souches fongiques sélectionnées

Six souches de champignons issues de la collection EMPHASIS (*Trichoderma* sp. #1, 1015-HDG3.E-2; *Trichoderma* sp. #2, 1216-TTF7.E-4; *Trichoderma* sp. #3, 1216-TTF7.E-5; *Alternaria* sp., 1016-TTF7.B-5; *Cladosporium* sp., 1216-TTF7.C-5; mélange de trois souches *Epicoccum nigrum* ; voir Chapitre III.2) et une souche issue de la collection MNHN (*Trichoderma aureoviride*, LCP 3246) ont été cultivées sur milieu PDA pendant 10 jours à l'obscurité à 18°C. Les tissus fongiques ont été mis en solution après grattage des boîtes et filtrés afin de retirer les amas mycéliens les plus volumineux. Une suspension aqueuse de spores ajustée à 10^3 spores.mL^{-1} a été préparée dans 1 L d'eau stérile pour chaque souche, à l'exception du mélange *Epicoccum nigrum* dont la totalité de la fraction mycélienne a été utilisée (Figure 1.B). Cinq sachets de résidus ont été immergés pendant 20 minutes dans chacune des sept suspensions (Figure 1.C). Cinq sachets témoins ont été immergés dans de l'eau stérile. Les sachets ont ensuite été accrochés à un grillage à l'extérieur jusqu'à mi-janvier 2018 (Figure 1.D).

Estimation des effets des traitements sur l'intensité de la reproduction sexuée de Z. tritici

Mi-janvier, les résidus de chaque sachet ont été pesés et immergés dans de l'eau stérile pendant 20 minutes. Le protocole appliqué en routine au laboratoire [1–3] a été utilisé pour estimer l'intensité de la reproduction sexuée de *Z. tritici* sur les résidus de chaque sachet. Ils ont été répartis sur du papier filtre dans des boîtes rectangulaires (24 × 36 cm) laissées ouvertes pour qu'ils sèchent, le changement brutal d'hygrométrie entraînant l'éjection des ascospores. Huit boîtes de Pétri (90 mm de diamètre) contenant du milieu PDA ont été retournées et placées dans chaque boîte rectangulaire à 1 cm au-dessus des résidus pendant 18 h, avant d'être refermées et mises à incuber à 18°C à l'obscurité (Figure 1.E). Les colonies issues des ascospores éjectées ont été dénombrées entre 4 et 7 jours (Figure 1.F). L'effet des différents traitements sur le nombre d'ascospores éjectées par poids sec de résidus, proxy de l'intensité de la reproduction sexuée de *Z. tritici*, a été estimé comparativement au témoin.

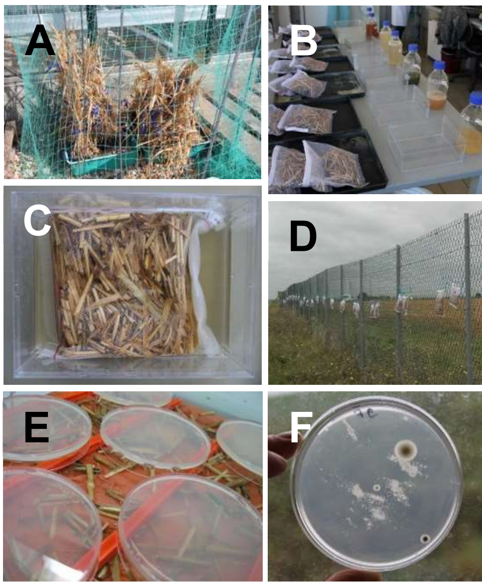


Figure 1 - Illustration des étapes clés du protocole expérimental.

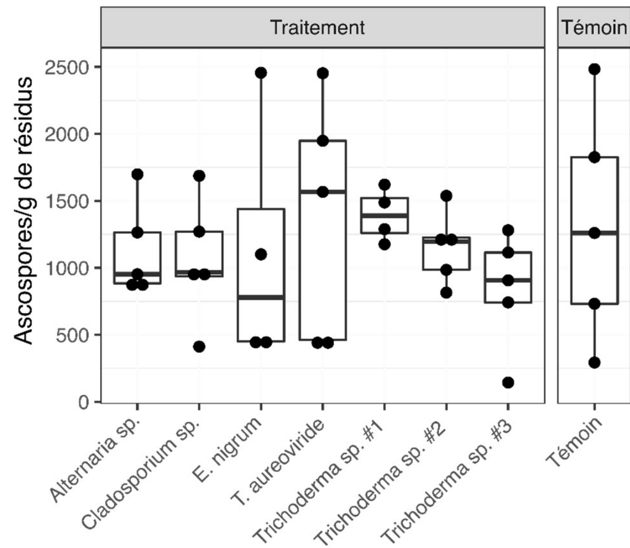


Figure 2 - Effet des traitements sur l'intensité de la reproduction sexuée de *Z. tritici*.

Résultats et conclusion

La production moyenne de l'ensemble des traitements a avoisiné 1200 ascospores par gramme de résidus, très proche de la moyenne du témoin (Figure 2). Malgré quelques différences visuelles, aucune modalité de traitement, c'est-à-dire aucune espèce fongique, n'a eu d'impact significatif sur la reproduction sexuée de *Z. tritici*. Pour autant, il n'est pas possible de démontrer l'absence d'effet : un impact faible est possible, mais les conditions expérimentales ne permettent pas de le détecter. Compte tenu de la variabilité non maîtrisée du traitement témoin, et de manière générale de l'ensemble des traitements, il est illusoire d'arriver à mettre en évidence de tels effets. On pourrait faire l'hypothèse qu'un traitement par un complexe d'espèces aurait davantage d'effet qu'un traitement mono-spécifique et aurait pu être détecté, sans pour autant pouvoir la tester en utilisant ce protocole qui souffre encore de nombreuses imperfections. Cette première expérience a toutefois permis de mettre en évidence son caractère « opérationnel ». Des améliorations sont indispensables pour obtenir des résultats desquels il soit possible de tirer des conclusions robustes.

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Annexe

Tableau annexe 1 - Liste des 424 souches fongiques isolées à partir de résidus de blé et de colza correspondant à la collection EMPHASIS (R-Syst::database ; https://www6.inra.fr/r-syst; https://github.com/r-syst/databases/tree/master/r-syst::fungi). Le code de chaque souche (ex. « 0217-TTF7.E-2 ») permet de connaître la période et l'année de prélèvement (« 02 » pour février et « 17 » pour 2017), la parcelle (« TTF7 ») et le point d'échantillonnage (« E »). Les photographies de certaines souches sont présentées à la Figure annexe 1. Les souches ont été classées par ordre alphabétique des espèces auxquelles elles ont été assignées. Les 424 souches ont été affiliées sur la base de l'ITS entier (amorces ITS1F et ITS4) en utilisant la base de données UNITE7.1. A noter que 148 de ces souches ont également été caractérisées par la séquence du gène *RPB2*. Le nom de la séquence ITS de chaque souche dans la base R-Syst peut être obtenu en ajoutant « _ITS4 » ou « _ITS1F » après le code de cette même souche (ex. « 0217-TTF7.E-2_ITS4 »).

Code souche	Plante	Photo	RPB2	Famille	Genre	Espèce
0217-TTF7.E-2	Colza	X		*Mucoraceae*	*Actinomucor*	*Actinomucor_sp*
1015-TTF7.D-5	Blé	X	X	*unclassified_Agaricomycetes*	*unclassified_Agaricomycetes*	*Agaricomycetes_sp*
1015-TTF7.A-4	Blé		X	*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1015-TTF7.D-1	Blé	X	X	*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1015-TTF7.B-9	Blé		X	*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1015-TTF7.D-7	Blé	X	X	*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1215-TTF7.B-2	Blé	X	X	*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1216-TTF7.C-11	Colza		X	*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1215-TTF7.B-1	Blé	X	X	*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1016-TTF7.C-8	Colza	X	X	*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1015-TTF7.C-1	Blé		X	*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1015-TTF7.A-6	Blé	X		*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1015-TTF7.A-8	Blé			*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1015-TTF7.B-5	Blé			*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1015-TTF7.E-4	Blé	X		*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1016-TTF7.E-3	Colza			*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1216-TTF7.D-10	Colza			*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1216-TTF7.D-9	Colza			*Pleosporaceae*	*Alternaria*	*Alternaria_infectoria*
1215-TTF7.E-7	Blé	X	X	*Pleosporaceae*	*Alternaria*	*Alternaria_tenuissima*
0217-TTF7.C-4	Colza		X	*Pleosporaceae*	*Alternaria*	*Alternaria_tenuissima*
1015-HDG3.B-1	Blé		X	*Pleosporaceae*	*Alternaria*	*Alternaria_tenuissima*
1015-TTF7.D-3	Blé	X	X	*Pleosporaceae*	*Alternaria*	*Alternaria_tenuissima*
1015-HDG2.D-9	Colza	X	X	*Pleosporaceae*	*Alternaria*	*Alternaria_tenuissima*
1016-TTF7.A-7	Colza			*Pleosporaceae*	*Alternaria*	*Alternaria_tenuissima*
1016-TTF7.B-5	Colza			*Pleosporaceae*	*Alternaria*	*Alternaria_tenuissima*
1216-TTF7.C-3	Colza		X	*Pleosporales_fam_IS**	*Boeremia*	*Boeremia_exigua*
1216-TTF7.B-4	Colza			*Pleosporales_fam_IS**	*Boeremia*	*Boeremia_exigua*
1216-TTF7.D-4	Colza			*Pleosporales_fam_IS**	*Boeremia*	*Boeremia_exigua*
1216-TTF7.B-6	Colza			*Sclerotiniaceae*	*Botrytis*	*Botrytis_cinerea*
1215-HDG3.D-2	Blé	X	X	*Chaetomiaceae*	*Chaetomium*	*Chaetomium_globosum*
1015-HDG3.D-1	Blé		X	*Chaetomiaceae*	*Chaetomium*	*Chaetomium_globosum*
1215-HDG3.E-4	Blé	X	X	*Chaetomiaceae*	*Chaetomium*	*Chaetomium_sp.*
0217-TTF7.D-4	Colza	X	X	*Chaetomiaceae*	*Chaetomium*	*Chaetomium_sp.*
1016-TTF7.A-2	Colza	X	X	*Chaetomiaceae*	*Chaetomium*	*Chaetomium_sp.*
1016-TTF7.A-5	Colza	X	X	*Chaetomiaceae*	*Chaetomium*	*Chaetomium_sp.*
1216-TTF7.C-1	Colza		X	*Chaetomiaceae*	*Chaetomium*	*Chaetomium_sp.*
1215-HDG2.C-2	Colza	X	X	*Lasiosphaeriaceae*	*Cladorrhinum*	*Cladorrhinum_flexuosum*
1215-HDG2.C-6	Colza	X	X	*Lasiosphaeriaceae*	*Cladorrhinum*	*Cladorrhinum_flexuosum*
1016-HDG3.C-7	Blé	X	X	*Lasiosphaeriaceae*	*Cladorrhinum*	*Cladorrhinum_flexuosum*
1015-HDG2.C-7	Colza	X	X	*Lasiosphaeriaceae*	*Cladorrhinum*	*Cladorrhinum_flexuosum*
1215-HDG2.C-5	Colza	X	X	*Lasiosphaeriaceae*	*Cladorrhinum*	*Cladorrhinum_flexuosum*
1015-HDG2.D-8	Colza	X	X	*Lasiosphaeriaceae*	*Cladorrhinum*	*Cladorrhinum_flexuosum*
1215-TTF7.A-5	Blé	X	X	*Lasiosphaeriaceae*	*Cladorrhinum*	*Cladorrhinum_foecundissimum*
1215-TTF7.A-7	Blé	X	X	*Lasiosphaeriaceae*	*Cladorrhinum*	*Cladorrhinum_foecundissimum*
1216-TTF7.D-8	Colza			*Davidiellaceae*	*Cladosporium*	*Cladosporium_funiculosum*
1016-TTF7.E-4	Colza	X	X	*Davidiellaceae*	*Cladosporium*	*Cladosporium_pseudocladosporioides*
1016-TTF7.D-10	Colza	X	X	*Davidiellaceae*	*Cladosporium*	*Cladosporium_pseudocladosporioides*
1015-HDG2.B-8	Colza	X	X	*Davidiellaceae*	*Cladosporium*	*Cladosporium_pseudocladosporioides*
1016-TTF7.C-9	Colza	X	X	*Davidiellaceae*	*Cladosporium*	*Cladosporium_pseudocladosporioides*
1016-TTF7.E-1	Colza	X	X	*Davidiellaceae*	*Cladosporium*	*Cladosporium_pseudocladosporioides*
0217-TTF7.A-5	Colza	X		*Davidiellaceae*	*Cladosporium*	*Cladosporium_pseudocladosporioides*

1216-TTF7.C-5	Colza			*Davidiellaceae*	*Cladosporium*	*Cladosporium_pseudocladosporioides*
1016-HDG2.B-2	Blé	X	X	*Davidiellaceae*	*Cladosporium*	*Cladosporium_sp.*
0217-HDG2.A-8	Blé		X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
0217-HDG3.C-5	Blé		X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
0217-HDG3.D-3	Blé		X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1016-HDG2.D-1	Blé	X	X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1215-HDG2.B-2	Colza	X	X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1215-HDG3.A-1	Blé	X	X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
0217-HDG2.A-5	Blé		X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
0217-HDG2.C-5	Blé		X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1016-HDG3.B-2	Blé	X	X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1016-HDG3.E-1	Blé	X	X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1215-HDG2.A-8	Colza	X	X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1215-HDG3.E-1	Blé	X	X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1215-TTF7.C-3	Blé	X	X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1215-TTF7.D-4	Blé	X	X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1215-TTF7.E-2	Blé	X	X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
0217-HDG3.C-2	Blé		X	*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1015-TTF7.E-1	Blé	X		*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1016-HDG2.A-7	Blé			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1016-HDG2.B-3	Blé			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1016-HDG3.C-10	Blé			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1016-TTF7.D-2	Colza			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-HDG2.B-6	Blé			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-HDG2.D-1	Blé			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-HDG3.A-1	Blé			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-HDG3.C-3	Blé			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-TTF7.A-1	Colza			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-TTF7.B-2	Colza			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-TTF7.B-3	Colza			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-TTF7.C-7	Colza			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-TTF7.C-8	Colza			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-TTF7.D-2	Colza			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-TTF7.D-3	Colza			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-TTF7.E-6	Colza			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-TTF7.E-7	Colza			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1216-TTF7.E-8	Colza			*Pleosporaceae*	*Epicoccum*	*Epicoccum_nigrum*
1016-HDG2.C-2	Blé			*Pleosporaceae*	*Epicoccum*	*Epicoccum_sp*
1016-HDG2.C-4	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_asiaticum*
1016-HDG2.E-5	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_asiaticum*
1016-HDG3.A-7	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_asiaticum*
1016-HDG3.C-6	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_asiaticum*
1216-HDG2.A-1	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_asiaticum*
1216-HDG2.A-3	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_asiaticum*
1216-HDG2.B-1	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_asiaticum*
1216-HDG2.D-6	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_asiaticum*
1216-HDG2.E-7	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_asiaticum*
1216-TTF7.B-10	Colza			*Nectriaceae*	*Fusarium*	*Fusarium_asiaticum*
0217-HDG3.B-4	Blé		X	*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0217-TTF7.D-5	Colza	X	X	*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0217-HDG2.A-9	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0217-HDG2.B-4	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0217-HDG2.B-6	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0217-HDG2.D-6	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0217-HDG2.D-8	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0217-HDG2.E-4	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0217-HDG3.A-1	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0217-HDG3.B-6	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0217-HDG3.C-4	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0217-HDG3.D-6	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0217-HDG3.E-6	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1015-HDG3.A-1	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1015-HDG3.B-5	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1015-HDG3.C-1	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1015-HDG3.D-7	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1015-HDG3.E-4	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1015-TTF7.A-3	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1015-TTF7.A-7	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1016-HDG2.A-4	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*

1016-HDG3.A-2	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1016-HDG3.A-3	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1016-HDG3.A-5	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1016-HDG3.A-8	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1016-HDG3.B-3	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1016-HDG3.B-5	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1016-HDG3.C-9	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1016-HDG3.D-2	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1016-HDG3.D-6	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1016-HDG3.E-3	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1016-TTF7.B-2	Colza			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1215-HDG2.D-2	Colza	X		*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1215-HDG3.B-2	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1215-TTF7.C-9	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1215-TTF7.D-7	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1216-HDG2.B-3	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1216-HDG3.A-4	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1216-HDG3.B-2	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1216-HDG3.C-4	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1216-HDG3.D-3	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1216-HDG3.E-4	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
1216-TTF7.B-1	Colza			*Nectriaceae*	*Fusarium*	*Fusarium_culmorum*
0216-HDG2.B-3	Colza			*Nectriaceae*	*Fusarium*	*Fusarium_oxysporum*
1216-TTF7.C-6	Colza			*Nectriaceae*	*Fusarium*	*Fusarium_oxysporum*
0216-HDG2.A-6	Colza	X		*Nectriaceae*	*Fusarium*	*Fusarium_redolens*
1015-HDG2.B-7	Colza	X		*Nectriaceae*	*Fusarium*	*Fusarium_redolens*
1015-HDG2.C-1	Colza	X		*Nectriaceae*	*Fusarium*	*Fusarium_redolens*
1016-TTF7.B-1	Colza			*Nectriaceae*	*Fusarium*	*Fusarium_solani*
0217-HDG2.B-7	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
0217-HDG2.C-6	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
0217-HDG3.A-3	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
0217-TTF7.A-1	Colza			*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1015-HDG3.A-3	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1015-HDG3.A-6	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1015-HDG3.B-3	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1015-HDG3.C-5	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1015-HDG3.D-6	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1016-HDG2.A-2	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1016-HDG2.A-3	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1016-HDG2.E-3	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1215-HDG2.B-4	Colza	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1215-HDG3.A-5	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1215-HDG3.B-3	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1215-HDG3.C-5	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1215-HDG3.D-4	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1215-TTF7.B-6	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1216-HDG2.D-5	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1216-HDG2.D-7	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1216-HDG2.E-4	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1216-TTF7.B-9	Colza	X		*Nectriaceae*	*Fusarium*	*Fusarium_sp.*
1016-HDG3.A-6	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_torulosum*
1216-HDG2.D-3	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_torulosum*
1215-TTF7.B-3	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_venenatum*
1215-TTF7.C-8	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_venenatum*
1215-TTF7.E-8	Blé	X		*Nectriaceae*	*Fusarium*	*Fusarium_venenatum*
1216-HDG2.C-5	Blé			*Nectriaceae*	*Fusarium*	*Fusarium_venenatum*
0217-HDG3.A-2	Blé			*Nectriaceae*	*Gibberella*	*Gibberella_acuminata*
0217-HDG3.E-3	Blé		X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.A-2	Blé			*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.A-3	Blé			*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.A-4	Blé			*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.A-6	Blé			*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.B-1	Blé			*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.B-2	Blé	X		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.B-5	Blé			*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.C-1	Blé			*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.C-3	Blé			*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.C-4	Blé			*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.D-1	Blé			*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*

0217-HDG2.D-2	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.D-4	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.E-1	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.E-3	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.E-6	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG2.E-7	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG3.A-4	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG3.A-5	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG3.B-2	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG3.B-3	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG3.B-5	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG3.C-3	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG3.D-4	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG3.D-5	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-HDG3.D-7	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
0217-TTF7.A-2	Colza		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1015-HDG2.A-2	Colza	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1015-HDG2.A-6	Colza	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1015-HDG2.C-3	Colza	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1015-HDG2.D-7	Colza	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1015-TTF7.B-1	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1015-TTF7.C-3	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1015-TTF7.D-2	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1016-HDG2.A-6	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1016-HDG2.B-1	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1016-HDG2.D-5	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1016-HDG2.E-1	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1016-HDG3.C-2	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1016-HDG3.C-5	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1215-HDG3.C-1	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1215-HDG3.C-2	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1215-HDG3.D-3	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-HDG2.A-2	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-HDG2.C-6	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-HDG2.E-2	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-HDG2.E-5	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-HDG3.A-2	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-HDG3.B-4	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-HDG3.C-6	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-HDG3.D-4	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-HDG3.D-5	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-HDG3.E-5	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-TTF7.C-10	Colza		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-TTF7.C-2	Colza		*Nectriaceae*	*Gibberella*	*Gibberella_avenacea*
1216-TTF7.B-7	Colza		*Nectriaceae*	*Gibberella*	*Gibberella_baccata*
0217-HDG2.A-1	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
0217-TTF7.C-5	Colza	X	*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1015-HDG2.B-3	Colza	X	*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1015-HDG2.D-1	Colza	X	*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1016-HDG2.D-2	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1016-HDG2.E-2	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1016-HDG3.C-1	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1016-TTF7.A-3	Colza		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1016-TTF7.B-6	Colza		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1016-TTF7.C-1	Colza		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1016-TTF7.C-5	Colza		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1215-HDG2.C-8	Colza	X	*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1215-TTF7.A-3	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1215-TTF7.C-1	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1215-TTF7.D-2	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1216-HDG2.B-5	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1216-HDG2.C-1	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1216-HDG2.C-3	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1216-HDG2.C-4	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1216-HDG2.D-2	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1216-HDG2.E-6	Blé		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1216-HDG3.A-6	Blé	X	*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1216-TTF7.B-8	Colza		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*
1216-TTF7.D-1	Colza		*Nectriaceae*	*Gibberella*	*Gibberella_intricans*

1216-TTF7.E-2	Colza			Nectriaceae	Gibberella	Gibberella_intricans
0217-TTF7.A-3	Colza			Nectriaceae	Gibberella	Gibberella_pulicaris
0217-TTF7.B-2	Colza	X		Nectriaceae	Gibberella	Gibberella_pulicaris
1016-TTF7.B-4	Colza			Nectriaceae	Gibberella	Gibberella_pulicaris
1016-TTF7.B-9	Colza			Nectriaceae	Gibberella	Gibberella_pulicaris
1016-HDG2.A-1	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1016-HDG2.C-1	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1016-HDG2.C-5	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1016-HDG3.A-1	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1016-HDG3.B-1	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1016-HDG3.C-3	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1016-HDG3.C-4	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1016-HDG3.D-3	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1016-HDG3.D-4	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1016-HDG3.D-5	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1016-HDG3.E-5	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1016-TTF7.A-4	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1016-TTF7.A-6	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1016-TTF7.B-7	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1016-TTF7.C-2	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1016-TTF7.C-6	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1016-TTF7.D-4	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1016-TTF7.D-5	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1016-TTF7.D-6	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1016-TTF7.D-8	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1216-HDG2.A-4	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1216-HDG2.B-2	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1216-HDG2.D-4	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1216-HDG2.D-8	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1216-HDG2.E-1	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1216-HDG2.E-3	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1216-HDG3.C-1	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1216-HDG3.C-2	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1216-HDG3.E-3	Blé			Nectriaceae	Gibberella	Gibberella_sp.
1216-TTF7.A-2	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1216-TTF7.C-12	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1216-TTF7.C-9	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1216-TTF7.D-5	Colza			Nectriaceae	Gibberella	Gibberella_sp.
1216-HDG3.E-1	Blé			Nectriaceae	Gibberella	Gibberella_tricincta
0217-HDG2.A-7	Blé	X	X	Hysteriaceae	unclassified_Hysteriaceae	Hysteriaceae_sp
0217-HDG3.E-1	Blé		X	Hysteriaceae	unclassified_Hysteriaceae	Hysteriaceae_sp
1015-HDG2.C-5	Colza	X	X	Hysteriaceae	unclassified_Hysteriaceae	Hysteriaceae_sp
1015-TTF7.C-4	Blé		X	Hysteriaceae	unclassified_Hysteriaceae	Hysteriaceae_sp
1016-TTF7.A-1	Colza	X	X	Hysteriaceae	unclassified_Hysteriaceae	Hysteriaceae_sp
1016-TTF7.B-8	Colza	X	X	Hysteriaceae	unclassified_Hysteriaceae	Hysteriaceae_sp
1215-HDG2.A-10	Colza	X	X	Hysteriaceae	unclassified_Hysteriaceae	Hysteriaceae_sp
1215-HDG2.B-6	Colza	X	X	Hysteriaceae	unclassified_Hysteriaceae	Hysteriaceae_sp
1215-TTF7.E-5	Blé	X	X	Hysteriaceae	unclassified_Hysteriaceae	Hysteriaceae_sp
1215-HDG2.E-3	Colza	X		Hysteriaceae	unclassified_Hysteriaceae	Hysteriaceae_sp
1215-TTF7.C-5	Blé	X	X	Trichosphaeriales_fam_IS*	Khuskia	Khuskia_oryzae
1215-TTF7.B-5	Blé	X		Trichosphaeriales_fam_IS*	Khuskia	Khuskia_oryzae
1215-TTF7.E-3	Blé	X	X	Corticiaceae	Laetisaria	Laetisaria_sp
1015-HDG3.B-2	Blé	X	X	Rhytismataceae	Lophodermium	Lophodermium_actinothyrium
1015-TTF7.E-7	Blé	X	X	Xylariales_fam_IS*	Monographella	Monographella_nivalis
1015-TTF7.B-4	Blé		X	Xylariales_fam_IS*	Monographella	Monographella_nivalis
1016-HDG2.C-3	Blé			Xylariales_fam_IS*	Monographella	Monographella_nivalis
1016-HDG2.C-6	Blé			Xylariales_fam_IS*	Monographella	Monographella_nivalis
1016-HDG2.E-4	Blé			Xylariales_fam_IS*	Monographella	Monographella_nivalis
1216-HDG3.A-3	Blé			Xylariales_fam_IS*	Monographella	Monographella_nivalis
0216-HDG2.B-2	Colza	X	X	Mortierellaceae	Mortierella	Mortierella_alpina
1215-HDG2.E-5	Colza	X	X	Mortierellaceae	Mortierella	Mortierella_alpina
1015-HDG2.C-6	Colza	X	X	Mortierellaceae	Mortierella	Mortierella_alpina
1015-HDG3.A-4	Blé	X	X	Mortierellaceae	Mortierella	Mortierella_alpina
1215-HDG3.E-5	Blé	X	X	Mortierellaceae	Mortierella	Mortierella_alpina
0217-HDG2.E-2	Blé		X	Mortierellaceae	Mortierella	Mortierella_alpina
0216-HDG2.C-2	Colza		X	Mortierellaceae	Mortierella	Mortierella_alpina
1015-HDG2.B-5	Colza	X	X	Mortierellaceae	Mortierella	Mortierella_alpina
0217-TTF7.D-2	Colza			Mortierellaceae	Mortierella	Mortierella_alpina
1015-HDG3.C-2	Blé			Mortierellaceae	Mortierella	Mortierella_alpina

1216-TTF7.D-7	Colza			*Mortierellaceae*	*Mortierella*	*Mortierella_alpina*
1215-HDG2.A-4	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1215-HDG2.D-6	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
0217-HDG2.C-2	Blé		X	*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1015-HDG2.E-4	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1015-HDG2.E-6	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1015-HDG3.A-5	Blé	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1015-TTF7.B-2	Blé		X	*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1015-TTF7.D-6	Blé	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1215-HDG2.A-9	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1215-HDG2.D-3	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1215-HDG2.E-2	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1016-HDG3.D-1	Blé			*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1216-HDG2.A-5	Blé			*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1216-HDG3.B-1	Blé			*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1216-TTF7.A-3	Colza			*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1216-TTF7.E-9	Colza			*Mortierellaceae*	*Mortierella*	*Mortierella_elongata*
1015-HDG2.D-2	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_exigua*
1215-HDG2.C-4	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_exigua*
1015-TTF7.E-3	Blé	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_exigua*
1015-HDG2.E-1	Colza	X		*Mortierellaceae*	*Mortierella*	*Mortierella_exigua*
0217-TTF7.B-3	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_gamsii*
1215-TTF7.A-2	Blé	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_gamsii*
0217-HDG3.D-1	Blé		X	*Mortierellaceae*	*Mortierella*	*Mortierella_gamsii*
0217-HDG3.C-1	Blé		X	*Mortierellaceae*	*Mortierella*	*Mortierella_gamsii*
1215-HDG2.A-2	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_gamsii*
1215-HDG2.B-1	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_gamsii*
0217-TTF7.B-1	Colza			*Mortierellaceae*	*Mortierella*	*Mortierella_gamsii*
1215-HDG3.B-1	Blé	X		*Mortierellaceae*	*Mortierella*	*Mortierella_gamsii*
1216-HDG3.A-7	Blé	X		*Mortierellaceae*	*Mortierella*	*Mortierella_gamsii*
1215-HDG2.B-3	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_minutissima*
0217-HDG2.E-5	Blé		X	*Mortierellaceae*	*Mortierella*	*Mortierella_minutissima*
1015-HDG2.B-4	Colza	X	X	*Mortierellaceae*	*Mortierella*	*Mortierella_minutissima*
1015-HDG3.B-4	Blé		X	*Mortierellaceae*	*Mortierella*	*Mortierella_minutissima*
1015-TTF7.C-2	Blé		X	*Mortierellaceae*	*Mortierella*	*Mortierella_minutissima*
1015-HDG2.C-8	Colza	X		*Mortierellaceae*	*Mortierella*	*Mortierella_minutissima*
0217-TTF7.D-3	Colza		X	*Mucoraceae*	*Mucor*	*Mucor_circinelloides*
1016-HDG3.C-8	Blé		X	*Mucoraceae*	*Mucor*	*Mucor_circinelloides*
1215-TTF7.C-6	Blé	X	X	*Mucoraceae*	*Mucor*	*Mucor_circinelloides*
0217-HDG3.E-7	Blé	X	X	*Mucoraceae*	*Mucor*	*Mucor_circinelloides*
1016-TTF7.D-3	Colza	X	X	*Mucoraceae*	*Mucor*	*Mucor_circinelloides*
1215-TTF7.E-4	Blé	X	X	*Mucoraceae*	*Mucor*	*Mucor_circinelloides*
1216-TTF7.D-6	Colza			*Mucoraceae*	*Mucor*	*Mucor_circinelloides*
1015-HDG2.B-1	Colza	X	X	*Mucoraceae*	*Mucor*	*Mucor_fragilis*
1015-HDG3.E-1	Blé		X	*Mucoraceae*	*Mucor*	*Mucor_fragilis*
1016-HDG3.B-4	Blé			*Mucoraceae*	*Mucor*	*Mucor_fragilis*
0216-HDG3.A-5	Blé		X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
0217-HDG3.E-8	Blé		X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
0217-TTF7.C-6	Colza	X	X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
0217-TTF7.D-6	Colza		X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
0217-TTF7.E-1	Colza		X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1015-HDG2.A-3	Colza	X	X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1015-HDG2.E-5	Colza	X	X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1015-HDG3.C-3	Blé		X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1015-HDG3.E-3	Blé		X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1215-HDG2.C-3	Colza	X	X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1215-HDG3.A-4	Blé	X	X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1215-HDG3.B-4	Blé	X	X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1215-HDG3.C-4	Blé	X	X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1215-HDG3.D-1	Blé	X	X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1216-HDG3.A-5	Blé	X	X	*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
0216-HDG2.C-3	Colza	X		*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1016-TTF7.C-4	Colza	X		*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1216-HDG2.C-2	Blé			*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1216-TTF7.A-4	Colza			*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1216-TTF7.B-11	Colza			*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1216-TTF7.C-4	Colza			*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1216-TTF7.D-11	Colza	X		*Mucoraceae*	*Mucor*	*Mucor_hiemalis*
1216-TTF7.E-3	Colza			*Mucoraceae*	*Mucor*	*Mucor_hiemalis*

1215-HDG2.C-1	Colza	X		*Mucoraceae*	*Mucor*	*Mucor_mucedo*
1016-HDG2.B-4	Blé	X	X	*Sordariaceae*	*Neurospora*	*Neurospora_tetrasperma*
1015-TTF7.A-5	Blé		X	*Trichosphaeriales_fam_IS**	*Nigrospora*	*Nigrospora_oryzae*
1015-TTF7.B-3	Blé		X	*Trichosphaeriales_fam_IS**	*Nigrospora*	*Nigrospora_oryzae*
1015-TTF7.E-2	Blé	X	X	*Trichosphaeriales_fam_IS**	*Nigrospora*	*Nigrospora_oryzae*
1215-TTF7.A-8	Blé	X	X	*Trichosphaeriales_fam_IS**	*Nigrospora*	*Nigrospora_oryzae*
1215-TTF7.B-4	Blé	X	X	*Trichosphaeriales_fam_IS**	*Nigrospora*	*Nigrospora_oryzae*
1215-TTF7.D-3	Blé	X	X	*Trichosphaeriales_fam_IS**	*Nigrospora*	*Nigrospora_oryzae*
1015-TTF7.B-7	Blé		X	*Trichosphaeriales_fam_IS**	*Nigrospora*	*Nigrospora_oryzae*
1215-TTF7.C-7	Blé	X		*Trichosphaeriales_fam_IS**	*Nigrospora*	*Nigrospora_oryzae*
1015-HDG3.A-2	Blé	X	X	*Dothideomycetes_fam_IS**	*Septoriella*	*Septoriella_hirta*
1216-HDG3.B-3	Blé			*Dothideomycetes_fam_IS**	*Septoriella*	*Septoriella_hirta*
0217-TTF7.C-3	Colza		X	*Pleosporales_fam_IS**	*Stagonosporopsis*	*Stagonosporopsis_sp.*
0217-HDG3.E-2	Blé		X	*Hypocreaceae*	*Trichoderma*	*Trichoderma_sp.*
1015-TTF7.E-6	Blé	X	X	*Hypocreaceae*	*Trichoderma*	*Trichoderma_tomentosum*
1015-TTF7.A-1	Blé		X	*Hypocreaceae*	*Trichoderma*	*Trichoderma_tomentosum*
1215-TTF7.D-1	Blé	X	X	*Hypocreaceae*	*Trichoderma*	*Trichoderma_tomentosum*
1015-TTF7.E-5	Blé	X	X	*Hypocreaceae*	*Trichoderma*	*Trichoderma_tomentosum*
1015-TTF7.B-6	Blé		X	*Hypocreaceae*	*Trichoderma*	*Trichoderma_velutinum*
1216-TTF7.E-4	Colza			*Hypocreaceae*	*Trichoderma*	*Trichoderma_viridescens*
1216-TTF7.E-5	Colza			*Hypocreaceae*	*Trichoderma*	*Trichoderma_viridescens*
1215-TTF7.E-6	Blé	X	X	*Corticiaceae*	*Waitea*	*Waitea_circinata_var*
1016-TTF7.C-3	Colza			*Nectriaceae*	*unclassified*	*unclassified*
0216-HDG3.C-6	Blé	X	X	*unclassified*	*unclassified*	*unclassified*
1215-HDG2.E-1	Colza	X	X	*unclassified*	*unclassified*	*unclassified*
1215-HDG3.A-6	Blé	X	X	*unclassified*	*unclassified*	*unclassified*
0217-HDG3.B-1	Blé		X	*unclassified*	*unclassified*	*unclassified*
1015-HDG3.C-4	Blé		X	*unclassified*	*unclassified*	*unclassified*

IS* = incertae sedis

Liste des productions

Productions scientifiques issues de la thèse (au 1er avril 2019)

Articles publiés

Kerdraon L, Balesdent M, Barret M, Laval V, Suffert F (2019) Crop residues in wheat-oilseed rape rotation system: a pivotal, shifting platform for microbial meetings. *Microbial Ecology*, sous presse (https://doi.org/10.1007/s00248-019-01340-8).

Articles en preprint

Kerdraon L, Laval V, Suffert F (2019) Characterization of residue-microbiome-pathogen interactions in field-crop systems applied to the management of epidemics caused by residue-borne fungal diseases. Déposé dans *arXiv* (https://arxiv.org/abs/1903.02246) et soumis à *Phytobiome*s *Journal.*

Kerdraon L, Barret M, Laval V, Suffert F (2019**)** Differential dynamics of microbial community networks help identify microorganisms interacting with residue-borne pathogens: the case of *Zymoseptoria tritici* in wheat. Déposé dans *bioRxiv* (https://www.biorxiv.org/content/10.1101/587667v1) et soumis à *Microbiome Journal.*

Articles en préparation

Kerdraon L, Barret M, Balesdent M, Suffert F, Laval V (2019) Effects of a resistance gene affecting fungal pathogen infection on the microbiome of crop residue: the case of the Leptosphaeria maculans-Brassica napus pathosystem. En préparation pour *Molecular Plant Pathology*.

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Kerdraon L (2019) Caractérisation de la diversité microbiologique des résidus de culture de blé et de colza dans une perspective de bio-contrôle de l'inoculum primaire de *Zymoseptoria tritici* et *Leptosphaeria maculans*. Colloque INRA-IRSTEA au Salon de l'Agriculture « *Développer les alternatives aux pesticides, l'affaire de tous* », 24 février 2019, Paris, France.

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Kerdraon L, Balesdent MH, Barret M, Laval V, Suffert F (2017) Microbiome diversity in crop debris and potential interactions with the fungal pathogens *Zymoseptoria tritici* and *Leptosphaeria maculans*. *12th EFPP-10th SFP Conference*, 29 mai-2 juin 2017, Malo-les-Bains, France.

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Kerdraon L, Balesdent M-H, Barret M, Suffert F, Laval V (2019) Dynamics of microbial community networks associated with the debris-borne wheat pathogen *Zymoseptoria tritici*. *10th International Symposium on Cereal Leaf Blights*, 22-24 mai 2019, Dublin, Irlande (résumé soumis).

Rapports scientifiques et livrables

Suffert F, Laval V, Balsesdent M-H, Vignolles N, Kerdraon L, Boudier B, Delestre G, Barret M, Le Saux M, Liabot A-L (2019) Screening of microbial BCAs candidate for *Zymoseptoria tritici* and *Leptosphaeria maculans* control on crop residues. Livrable D3.4 du projet Européen H2020 EMPHASIS, version 2, 23 janvier 2019, 197 pages.

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université PARIS-SACLAY

ÉCOLE DOCTORALE
Agriculture, alimentation, biologie, environnement, santé (ABIES)

Title: Microbial diversity and pathogen-microbiome interactions in crop residues: the case of *Zymoseptoria tritici* and *Leptosphaeria maculans* in a wheat-oilseed rape system

Keywords: crop residues, metabarcoding, microbiome, microbial community, pathobiome, phytopathology

Abstract: Crop residues, a transient half-plant/half-soil compartment, are a key fully-fledged ecological niche with major biological impact on agrosystems when maintained at the surface of the cultivated soil. They also contribute to the development of plant disease epidemics as main and recurrent source of primary inoculum. To deepen our understanding of this crop residue compartment and its importance regarding the management of residue-borne diseases, we investigated the interactions between the residue microbiome and two important residue-borne fungal pathogens in wheat-oilseed rape rotations systems, *Zymoseptoria tritici* and *Leptosphaeria maculans*. Firstly, we characterized the temporal dynamics of fungal and bacterial communities associated with a large set of residues sampled in three wheat-oilseed rape rotation plots over a two-year period. The communities were characterized by metabarcoding and complementary isolations. Beyond the constitution of fungal and bacterial reference collections, this allowed the comparison between the effectiveness of cultivation-independent and dependent methods. The impact of plant species, seasonality (cropping season and degradation), and rotation on the microbiome of crop residues was demonstrated for both fungal and bacterial communities. The impact of plant species on the residue microbiome decreased over time, with the replacement of plant-specific genera by more generalist taxa originating from the soil. The cultivation-dependent method used for bacteria enabled to isolate most of the abundant taxa identified by metabarcoding (unlike fungi, due to technical biases) demonstrating the complementarity of both methods. Secondly, the effect of the presence of *Z. tritici* and *L.maculans* on the wheat and oilseed rape residue microbiome, respectively, was assessed by combining linear discriminant analyses (LDA) and ecological network analyses (ENA). For wheat, we compared the bacterial and fungal communities associated with residues, with and without preliminary *Z. tritici* inoculation, in or without contact with the soil, on four sampling dates during two consecutive years. The number of microorganisms promoted or inhibited by *Z. tritici* infection decreased over time, and was smaller for residues in contact with the soil. Although many microorganisms were impacted by the infection with the pathogen, few interacted directly with *Z. tritici*, despite it was considered as a keystone taxa in ENA. In parallel, the effect of *L. maculans* on oilseed rape residue microbiome was assessed over one year using two isogenic oilseed rape lines 'Darmor' and 'Darmor-*Rlm11*' carrying a resistance gene against *L. maculans*. As for wheat, microbial communities changed as residues degraded. Despite close communities, LDA highlighted numerous microorganisms impacted by the *Rlm11* gene. However, *L. maculans* was not considered as keystone taxa in ENA. Finally, our results provide essential information on microbial community alterations in wheat and oilseed rape residues induced by fungal pathogens. In particular, species already described as pathogens (e.g. *Blumeria graminis*, *Fusarium*, *Cladosporium*, and *Alternaria*) or as biocontrol agents (e.g. *Trichoderma*, *Epicoccum*, *Cryptococcus*, *Chaetomium*) were affected by the presence of both pathogens. Metabarcoding, previously developed for plant and soil compartments, should now benefit from new applied developments to crop residues in order to identify beneficial microorganisms naturally present. The complexity and transience of interactions shows that the use of biological control agents against these diseases seems difficult to implement on residues. Further studies would be needed to use these interactions in a more applied perspective.

Titre : Diversité microbienne et interactions pathogène-microbiome dans les résidus de culture : le cas de *Zymoseptoria tritici* et *Leptosphaeria maculans* en système blé-colza

Mots clés : communautés microbiennes, metabarcoding, microbiome, pathobiome, phytopathologie, résidus de culture

Résumé : Les résidus de culture, compartiment à l'interface plante-sol et niche écologique à part entière, ont un impact biologique majeur sur les agrosystèmes lorsqu'ils sont maintenus en surface. Ils contribuent aussi, en tant que source récurrente d'inoculum, au développement d'épidémies végétales. Pour approfondir notre compréhension de ce compartiment et de son importance pour la gestion de maladies fongiques, nous avons étudié les interactions entre le microbiome des résidus et deux agent pathogènes importants dans les systèmes blé-colza, *Zymoseptoria tritici* et *Leptosphaeria maculans*. Dans un premier temps nous avons caractérisé la dynamique temporelle des communautés fongiques et bactériennes associées à des résidus échantillonnés dans trois parcelles en rotation blécolza pendant une période de deux ans. Les communautés ont été caractérisées par metabarcoding et des isolements complémentaires sur milieu. En plus de constituer deux collections de référence fongiques et bactériennes, cela nous a permis de comparer l'efficacité de méthodes culture-indépendante et culture-dépendante. L'impact de la plante, de la saisonnalité et de la rotation sur le microbiome des résidus de culture a été significatif pour les communautés fongiques et bactériennes. L'impact de la plante sur le microbiome des résidus a diminué au cours du temps, concomitamment au remplacement des taxons originaires de la plante par des taxons originaires du sol, considérés comme plus généralistes. La plupart des genres bactériens identifiés par metabarcoding ont également pu être isolés, contrairement aux champignons (à cause de biais techniques), démontrant ainsi la complémentarité des deux approches. Dans un second temps, les effets de la présence de *Z. tritici* et de *L. maculans* sur le microbiome des résidus de colza et de blé ont été évalués en combinant des analyses discriminantes linéaires (LDA) et des analyses de réseaux d'interaction (ENA). Nous avons comparé les communautés bactériennes et fongiques associées aux résidus de blé, avec et sans inoculation préliminaire de *Z. tritici*, en contact ou non avec le sol, à quatre dates d'échantillonnage pendant deux années. Le nombre de micro-organismes favorisés ou désavantagés par l'infection de *Z. tritici* a diminué au cours du temps et a été plus faible pour les résidus en contact avec le sol. Plusieurs micro-organismes ont été influencés par l'infection, mais peu d'entre eux ont été en interaction directe avec *Z. tritici*, pourtant un taxon clé. Parallèlement, l'effet de *L. maculans* sur le microbiome des résidus de colza a été évalué sur une période d'un an en utilisant deux lignées isogéniques 'Darmor' et 'Darmor-*Rlm11*' porteur d'un gène de résistance contre *L. maculans*. Les communautés ont évolué à mesure que les résidus se sont dégradés, comme pour le blé. Les LDA ont montré que plusieurs micro-organismes ont été impactés par la présence du gène *Rlm11*, sans que *L. maculans* ne soit considéré comme un taxon clé dans les ENA. Cette étude a finalement fourni des informations essentielles sur l'influence des champignons pathogènes sur les communautés microbiennes des résidus de blé et de colza. Leur présence a eu un impact sur des espèces déjà décrites comme pathogènes (ex. *Blumeria graminis*, *Fusarium*, *Cladosporium* et *Alternaria*) ou comme agents de biocontrôle (ex. *Trichoderma*, *Epicoccum*, *Cryptococcus*, *Chaetomium*). Le metabarcoding, déjà utilisé pour les décrire les communautés des compartiments plante et sol, gagnerait à être désormais appliqué aux résidus de cultures pour identifier les micro-organismes potentiellement bénéfiques qu'ils hébergent. La complexité et le caractère transitoire des interactions expliquent que l'utilisation d'agents de biocontrôle soit difficile à mettre en œuvre en ayant pour cible les résidus en tant que source d'inoculum. D'autres études seront nécessaires pour mieux exploiter ces interactions dans une perspective appliquée.

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