Université du Québec
Institut National de la Recherche Scientifique
Centre Armand Frappier Santé Biotechnologie

SOYBEAN MICROBIOME STRUCTURE AND ASSEMBLY PROCESS

Par
Itumeleng Petros Moroenyane

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Président du jury et examinateur interne	Richard Villemur Armand-Frappier Santé Biotechnologie Institut National de la Recherche Scientifique 531 Boulevad des Prairies Laval H7V 1B7
Examinateur externe	Isabelle Laforest-Lapointe Départment de Biologie Univerité de Sherbrooke 2500 Boulevard de l'Université Sherbrooke J1K 2R1
Examinateur externe	Martin Filion Centre de Recherche et de Développment de Saing-Jean-sur-Richelie 430 Goin Boulevard Saint-Jean-sur-Richelieu J3b 3E6
Directeur de recherche	Étienne Yergeau Armand-Frappier Santé Biotechnologie Institut National de la Recherche Scientifique 531 Boulevad des Prairies Laval H7V 1B7

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ABSTRACT

The plant microbiome consists of the combined microbial communities that reside on and within the plant; these communities have an intrinsic relationship with their hosts, and some confer benefits to the plant host. The plant microbiome is recognised as an extension of the plant immune response and agents that abet stress. Although the role of stress in influencing the plant microbiome is well documented, a comprehensive understanding of successional patterns and prevailing assembly processes of soybean-associated microbes is still lacking. This thesis aimed to investigate different soybean microbial communities' spatial and temporal colonisation patterns and their overall ecological assembly processes. This thesis's overarching hypothesis was that there are spatial and temporal microbial niches spaces within the soybean microbiome, and these niche spaces are under strict plant-mediated selection. The second chapter focused on discerning bacterial and fungal successional patterns across all plant compartments and tested for niche differentiation along spatial and temporal axes. It highlighted that interactions between spatial and temporal dynamics influenced microbiome diversity patterns. Moreover, it emphasised the existence of a strong temporal dependence of communities. The third chapter focused on elucidating the prevailing assembly processes across spatial and temporal axes. Using complementary community assembly models, I highlighted that the plant compartment and developmental stage modulated the balance between niche-based and neutral processes. Also, it showed the importance of dispersal limitations in structuring plant microbiomes. The fourth chapter contrasted the different colonisation patterns of seed and soil microbiomes. Using a reductionist approach, I used near-axenic seedlings, which were inoculated with varying microbiome sources. It highlighted that the seed microbiome colonised the shoot compartment during early developmental stages, whilst the soil microbiome colonised the rhizosphere. The seed microbiome was capable of outcompeting members of the rhizosphere to colonise the endophytic space quickly. Different microbiome sources also influenced the abundance of N-cycling genes across all plant compartments, with an increased abundance of N-cycling genes in the soil treatment. Overall, this thesis shows that the soybean microbiome is temporally nested, and microbiome sources influenced colonisation patterns. Lastly, plant-mediated selection along with dispersal limitation played a role in their assembly. This adds to ongoing efforts to manipulate plant microbiomes for increased beneficial services and more sustainable agriculture.

RÉSUMÉ

Le microbiome de la plante est composé des communautés microbiennes qui résident sur et dans la plante et qui entrent en relation avec la plante hôte. Le microbiome de la plante est reconnu comme une prolongation du système immunitaire de la plante et contient des organismes pouvant réduire le stress éprouvé par la plante. Quoique l'influence du stress sur le microbiome de la plante est bien documenté, la compréhension détaillée de la succession et des processus d'assemblage des communautés microbiennes du soya est encore rudimentaire. L'hypothèse principale de cette thèse est qu'il existe des niches microbiennes spatiales et temporelles dans le soya, et que ces niches sont strictement sous le contrôle sélectif de la plante. Le deuxième chapitre compare les successions bactériennes et fongiques pour tous les compartiments de la plante et examine les preuves quant à l'existence de niches spatiales et temporelles. J'y démontre que la diversité microbienne est influencée par l'interaction entre les dynamiques temporelles et spatiales, en plus de la forte dépendance temporelle des communautés. Le troisième chapitre décortique les processus d'assemblage des communautés à travers le temps et l'espace. Je démontre, en utilisant des modèles d'assemblage des communautés complémentaires, que l'influence relative des processus neutres et de niches dépendent du compartiment et du stade de développement de la plante. De plus, l'importance des limitations de la dispersion sur la structure du microbiome y est démontré. Le chapitre 4 compare la colonisation microbienne de plantules de soya par le microbiome de la semence et du sol. Pour ce faire, j'ai utilisé une approche réductionniste où des semences pratiquement axéniques ont été inoculées avec des microbiome de différentes sources. Le microbiome des semences a colonisé les parties aériennes de la plante tandis que le microbiome du sol a colonisé la rhizosphère. Le microbiome des semences a été plus efficace pour coloniser l'intérieur des plantes que le microbiome du sol, même lorsque ces deux étaient mis en compétition. La source du microbiome a aussi influencé l'abondance des gènes du cycle de l'azote, ceux-ci ayant une plus forte abondance dans les plantules inoculées avec le microbiome du sol. Cette thèse démontre que le microbiome du soya est imbriqué temporellement, que la provenance du microbiome influence la colonisation de la plantule et que l'assemblage des communautés est influencé par la sélection de la plante et la limitation de la dispersion. Ces connaissances joueront un rôle critique dans les efforts en cours pour manipuler les microbiome des plantes afin d'augmenter les services bénéfiques des microbes pour une agriculture plus durable.

SYNOPSIS

Les écosystèmes agricoles sont des systèmes uniques où les cultures et les microbes sont intrinsèquement liés et sélectionnés pour promouvoir la santé des cultures et augmenter la productivité. Les progrès en sélection végétale ainsi qu'une meilleure compréhension de l'écologie des communautés microbiennes associées aux plantes ont permis d'améliorer les pratiques de gestion de ces écosystèmes dynamiques. Le microbiome végétal est constitué de l'ensemble des communautés microbiennes qui résident sur et dans la plante ; ces communautés entretiennent une relation innée avec leurs hôtes et leur confèrent des avantages. Ce microbiome est constitué d'espèces de mycorhizes bénéfiques, c'est-à-dire d'ectomycorhizes, comme la mycorhize arbutoïde, et d'endomycorhizes, comme la mycorhize arbusculaire, la mycorhize éricoïde, la mycorhize d'orchidée et la mycorhize monotropoïde, qui facilitent l'acquisition de nutriments en canalisant activement les nutriments du milieu environnant vers le cortex racinaire interne. Dans certains cas, ces communautés fongiques bénéfiques forment un réseau d'hyphes fongiques qui relie des plantes conspécifiques ou hétérospécifiques et transfère les nutriments dans ce réseau souterrain. De même, plusieurs bactéries favorisant la croissance des plantes influencent le développement et la croissance de leur hôte végétal en augmentant la production d'hormones spécifiques et en régulant les interactions avec les micro-organismes pathogènes. En outre, le microbiome végétal est reconnu comme une extension de la réponse immunitaire des plantes et contribue à atténuer le stress abiotique et biotique qu'elles subissent.

Dans les écosystèmes agricoles, le rôle et la fonction du microbiome végétal sont considérablement renforcés car ils influencent directement la performance et le rendement des plantes. Des efforts considérables ont été déployés pour identifier et documenter les microbes bénéfiques et pathogènes de plusieurs espèces de cultures. Plus précisément, les recherches se sont concentrées dans le passé sur les principales cultures mondiales, à savoir le riz, le maïs, le soja, le blé et la canne à sucre. Parmi celles-ci, le soja est la seule légumineuse et des efforts considérables ont été déployés pour comprendre et démêler la dynamique de l'interaction du soja avec ses rhizobactéries. Le soja est capable de former une relation symbiotique avec des souches spécifiques de rhizobia pour former des nodules racinaires capables de convertir l'azote atmosphérique en ammoniac qui est ensuite utilisé par la plante. Cette relation intrinsèque ne se limite pas à la fixation de l'azote. En fait, il a été démontré que les bactéries rhizobiennes augmentent la solubilisation des phosphates, la production de phytohormones et la régulation de

la production de métabolites secondaires qui améliorent l'immunité des plantes en amorçant leur résistance systémique.

Par conséquent, une grande importance a été accordée à la composition, à la dynamique et aux rôles de ces communautés microbiennes associées aux racines. Le microbiome de la rhizosphère du soja ne favorise pas seulement la santé des plantes, mais il est directement influencé par les métabolites végétaux sécrétés, par exemple les isoflavones et les saponines, et cette sécrétion est liée aux stades de développement des plantes et à leur état nutritionnel. Les interactions entre le soja et le microbiome associé aux racines sont fortement modulées par la qualité et la diversité des exsudats racinaires, et ces exsudats semblent varier selon les stades de développement de la plante, à savoir l'émergence, la croissance végétative, la floraison, le remplissage des gousses et la maturation. On sait peu de choses sur l'étendue de l'influence des métabolites sécrétés par le soja sur le microbiome de surface. Cependant, il a été démontré qu'en cas de dysbiose microbienne, c'est-à-dire en cas de perturbation de l'homéostasie du microbiote causée par un micro-organisme pathogène, la composition de la communauté du microbiome du soja en surface tend à changer radicalement. En outre, peu d'études ont mis en évidence le fait que le microbiome de la phyllosphère du soja est considérablement influencé par le développement ; dans les premiers stades, la composition et la structure ressemblent à celles du sol, mais deviennent progressivement plus divergentes au fur et à mesure que la plante se développe. Il semble en partie que le stress tant abiotique que biotique joue un rôle important dans la régulation du microbiome du soja dans les compartiments aériens et souterrains de la plante. Bien que le rôle du stress dans l'influence du microbiome de la plante soit bien documenté, il manque encore une compréhension globale des schémas de succession et des processus d'assemblage prédominants des microbes associés au soja. À ce jour, les études qui ont cherché à étudier et à démêler la succession se sont concentrées uniquement sur la rhizosphère ou la phyllosphère, et ces études sont limitées à quelques stades de développement, soit l'émergence et la floraison. De plus, ces études se concentrent sur la documentation de la composition et de la structure du microbiome sans explorer le rôle et l'influence des processus d'assemblage qui interviennent dans la dynamique de cette communauté.

Les communautés microbiennes sont intrinsèquement limitées par les mêmes processus écologiques qui modulent l'influence de toutes les communautés écologiques, c'est-à-dire les processus basés sur la niche ou les processus neutres. En bref, la théorie de la niche suppose que les espèces d'une communauté occupent des espaces de niche uniques, tandis que la théorie neutre suppose que les espèces d'une communauté ont des espaces de niche qui se

chevauchent. Les processus basés sur les niches délimitent l'espace de niche des espèces en régulant l'environnement, exerçant ainsi une pression sélective qui contraint les espèces dans leurs espaces de niche (sélection). D'autre part, les processus neutres précisent que l'espace de niche occupé est dû à la capacité de l'espèce à se disperser efficacement et avec succès dans un nouvel environnement (limitation de la dispersion), aux fluctuations aléatoires de l'abondance des espèces qui accroissent les différences entre des communautés par ailleurs équivalentes (dérive écologique) et à l'évolution de caractéristiques divergentes au sein d'une lignée (spéciation). Ces processus agissent sur les microbiomes végétaux pour créer des communautés divergentes dans l'espace (différents compartiments végétaux) et dans le temps (différents stades de développement). Dans les environnements du sol, il a été démontré que ces processus agissent sur les communautés en tandem: tandis que l'un d'entre eux prévaudra comme principal axe discriminant, l'autre aura un impact secondaire non négligeable. Chez les plantes, le rôle de la sélection médiée par la plante a été démontré comme étant le processus d'assemblage prédominant, cependant ces études se sont principalement concentrées sur les microbiomes rhizosphériques. L'environnement rhizosphérique est notoirement et infailliblement caractérisé par des modifications de l'environnement dues aux fluctuations de la diversité et de la qualité des exsudats racinaires. Cela dit, des preuves émergentes suggèrent que l'influence de la limitation de la dispersion contribue également aux différences observées dans la composition et la structure du microbiome de la rhizosphère du soja. La compréhension du rôle de ces processus est essentielle pour débloquer le réel potentiel de l'ingénierie du microbiome végétal.

L'hypothèse générale de cette thèse est qu'il existe des niches microbiennes spatiales et temporelles dans le microbiome des plantes, et que ces niches sont soumises à des sélections strictes qui sont médiées par les plantes. À cette fin, le soja (*Glycine max*) a été un modèle d'étude, et les communautés bactériennes et fongiques associées ont été étudiées dans tous les compartiments de la plante (rhizosphère, racine, tige, feuilles et gousses) et à tous les stades de développement (émergence, croissance, floraison et maturation). Les objectifs primordiaux de cette thèse sont : 1) de déterminer le schéma de succession des communautés microbiennes du soja, 2) d'élucider l'importance des processus neutres et basés sur la niche dans le microbiome du soja, et 3) de discerner l'importance relative des différentes sources microbiennes et leurs schémas de colonisation dans les premiers stades de développement du soja.

Le deuxième chapitre se concentre sur la vérification des preuves de différenciation de niche au sein du microbiome du soja le long des axes spatiaux et temporels. La composition et la structure du microbiome du soja sont influencées par le type de sol, les stades de développement et, dans

une moindre mesure, le génotype de la plante. Cependant, l'influence de la dynamique spatiale et temporelle dans la modulation de la composition, de la structure et de la succession du microbiome fait défaut. La preuve de la différenciation des niches dans la création d'un environnement spatialement hétérogène, puis la création d'une communauté diversifiée d'archées, de bactéries et d'eucaryotes est bien documentée à l'interface racine-sol du système de plantes modèles. Cependant, par rapport à l'interface racine-sol, la présence d'une différenciation de niche distincte d'autres organes végétaux et d'espèces végétales non modèles et leur impact sur l'héritage et la diversité de la flore microbienne sont mal compris. Des études récentes ont révélé l'existence d'une différenciation de niche au sein de l'endosphère des peupliers, et commencent à montrer comment les organes végétaux et la biogéographie influencent fortement la niche microbienne des cultures. Dans les systèmes agricoles, la saisonnalité et l'interaction au sein des communautés microbiennes et entre elles constituent un facteur majeur influençant la succession et la diversité microbienne des feuilles. Les niches créées par les changements du métabolisme des plantes ont un effet profond sur la diversité et la structure de la flore microbienne, et la dynamique temporelle semble être un axe important de différenciation sur lequel ces communautés sont distribuées. Bien que largement acceptée, la sélection de la flore microbienne par les plantes n'explique pas entièrement l'énorme diversité ni la composition observée pour ces communautés.

À cette fin, les plantes ont été cultivées dans une chambre environnementale avec une photopériode de 18 heures à 25°C suivie d'une période d'obscurité de 6 heures à 20°C jusqu'à la maturation des graines. Cinq plantes ont été échantillonnées de manière destructive à différents stades de développement, à savoir l'émergence (VE), la croissance (V3), la floraison (R1) et la maturation des graines (R6). Pour chaque plante, l'ADN génomique total a été extrait de la rhizosphère, du rhizoplane, de l'endosphère des racines, de l'espace endophytique et épiphytique de la tige (entre le premier et le deuxième entre-nœuds), et des feuilles pour l'espace endophytique et épiphytique (la plus jeune et la plus ancienne trifoliée). La composition et la structure de la communauté du microbiome ont été évaluées à l'aide du séquençage du gène de l'ARNr 16S bactérien/archaéen et de la région ITS fongique. Pour les deux communautés, en utilisant des analyses complémentaires d'autocorrélation temporelle, c'est-à-dire l'ADF (Augmented Dickey-Fuller) et le KPSS (Kwiatkowski-Phillips-Schmidt-Shin), les deux tests ont indiqué que la richesse microbienne (OTUs observées) et la diversité (indice de Shannon) étaient autocorrélées dans le temps. Les deux tests sont arrivés indépendamment à la même conclusion, c'est-à-dire qu'il existe une unité racine, soulignant la présence d'une forte tendance saisonnière

(p>0,05). Le stade de développement a influencé de manière significative l'abondance et la diversité de la communauté bactérienne/archéenne, alors que seule la diversité fongique a été notoirement influencée. De plus, les interactions entre le compartiment végétal et les stades de développement ont influencé de manière significative l'abondance et la diversité des communautés bactériennes/archéennes et fongiques.

Il y avait des différences entre les modèles de diversité alpha de la surface (feuille et tige) et du sous-sol (rhizosphère, rhizoplane et endosphère des racines). Par exemple, la rhizosphère présentait systématiquement une abondance et une diversité plus élevées pour les deux communautés à tous les stades du développement. De même, le renouvellement des communautés a été modulé par les interactions des dynamiques spatiales et temporelles. Le renouvellement de la communauté a été évalué en utilisant la dissimilarité de Bray-Curtis, et l'analyse des coordonnées principales (PCoA) a été utilisée pour discerner l'influence relative du compartiment végétal et du stade de développement. Pour les deux communautés, la dynamique des communautés a été modulée par l'interaction entre le compartiment végétal et le stade de développement. Il y avait une séparation claire des niches le long de l'axe spatial et temporel pour les deux communautés dans les compartiments aérien et souterrain. À l'interface racine-sol, l'influence de l'interaction entre le compartiment végétal et le développement était plus prononcée. En particulier, ces communautés, c'est-à-dire la rhizosphère, le rhizoplane et l'endosphère racinaire, étaient différentes les unes des autres; mais également au sein de chaque communauté, il y avait une distinction claire en fonction du stade de développement. Ce fort effet à l'interface racine-sol est probablement dû à une sélection médiée par les plantes à travers la rhizodéposition. Comme les demandes et les besoins métaboliques des plantes changent avec chaque stade de développement, la plante sécrète différents métabolites dans la rhizosphère, qui agissent comme un puissant filtre sélectif du microbiome. Bien que l'interaction entre le compartiment végétal et le stade de développement ait eu un effet significatif dans le compartiment aérien, l'importance de l'effet n'était pas aussi importante que dans le compartiment souterrain. Une grande partie de la variation de la structure de la communauté n'était expliquée ni par le compartiment végétal ni par le stade de développement. Cela s'explique en partie par la plus faible abondance et diversité des microbes dans ces compartiments végétaux. Les communautés écologiques dont l'abondance et la diversité des espèces sont plus faibles sont beaucoup plus influencées par des forces externes (variation de l'environnement, limitations de la dispersion) que par la dynamique interne de la communauté (compétition inter et intraspécifique, taux de natalité et de mortalité). La faible diversité dans le compartiment aérien

et la forte tendance saisonnière suggèrent que : 1) il y a une influence accrue de la dérive écologique et 2) cette influence accrue de la dérive, à son tour, est un filtre stabilisateur qui crée des espaces de niche temporels. Ici, en échantillonnant tous les compartiments de la plante dans la même expérience, il a été possible de détecter une tendance saisonnière dans les données qui a influencé l'abondance et la diversité des communautés microbiennes. L'abondance et la composition des exsudats végétaux libérés à chaque stade de développement influencent les modèles de succession microbienne au sein de la rhizosphère et du compartiment racinaire. Cette sélection imposée par la rhizodéposition était plus prononcée chez la communauté fongique que chez la communauté de bactéries et d'archées. Par conséquent, la composition des microbiomes végétaux s'emboîte à partir de la base: là où les communautés rhizosphériques démontrent leur plus grande diversité. Le stade de développement explique une proportion plus importante de la variation observée dans les compartiments souterrains que chez les compartiments aériens. Cependant, la grande quantité de variation résiduelle au sein des communautés aériennes apparaît souvent dans les communautés pauvres en diversité et implique une forte influence de la dérive écologique. Dans le cas présent, les communautés microbiennes de surface étaient moins abondantes et le stade de développement influençait plus fortement la diversité globale. Les variations temporelles de la taille des communautés écologiques dues à des facteurs extrinsèques, tels que le stade de développement des plantes, amplifient l'influence de la dérive écologique. En outre, ces variations temporelles de l'abondance et de la diversité des communautés agissent comme des filtres écologiques stabilisants, créant différentes niches temporelles occupées par différents taxons. Dans les communautés écologiques, la dérive provoque des fluctuations stochastiques des abondances, réduisant la diversité et conduisant à la divergence des structures communautaires. Dans ce cas, la variabilité observée dans la communauté du microbiome végétal est le résultat de la dérive écologique, et l'interaction entre le stade de développement et l'emplacement de l'échantillon peut créer une niche microbienne particulière. Par exemple, différents taxons fongiques peuvent présenter des niveaux similaires de colonisation de l'hôte, mais il existe de nettes différences dans l'étendue et les modèles de développement de l'infection médiée par les réponses métaboliques des plantes. Par conséquent, les communautés fongiques végétales ont tendance à présenter des topologies d'imbrication et des structures communautaires discordantes. Cependant, la composition et la diversité des communautés bactériennes sont principalement influencées par des réponses spécifiques localisées de l'hôte, telles que la production de métabolites spécifiques, de métabolites secondaires qui régulent les interactions biotiques, et la communication plante-microbe.

J’ai constaté une diminution de l'abondance relative de la plupart des phyla bactériens sur tous les sites, à l'exception des Firmicutes et des Bacteroidetes, qui ont montré une augmentation dans tous les échantillons rhizosphériques et épiphytiques. De même, la plupart des taxons fongiques ont montré une diminution significative de leur abondance relative, à l'exception des Ascomycota et des Zygomycota. Le premier n'a augmenté que dans la communauté épiphyte et endophyte, tandis que le second n'a augmenté que dans la rhizosphère. Dans tous les échantillons, il y avait un enrichissement détectable des taxons tout au long du développement de la plante. Le compartiment de la plante et le stade de développement ont tous deux influencé l'abondance relative des microbes bénéfiques connus pour les plantes ; toutefois, l'influence du compartiment de la plante était plus prononcée. Par exemple, à l'interface sol-racine, l'abondance relative de *Bradyrhizobium* et *Pseudomonas* était plus influencée par le compartiment végétal. En revanche, l'abondance relative de *Streptomyces* a été fortement influencée par le stade de développement. *Pseudomonas* et *Streptomyces* sont connus pour se coordonner avec *Bradyrhizobium* pour améliorer l'acquisition des nutriments et modifier l'architecture des racines. Les algorithmes de forêt aléatoires ont prédit un changement dans l'abondance de ces taxons dans différents compartiments. Par exemple, dans l'endosphère racinaire, l'abondance relative des *Streptomyces* était censée être la plus élevée pendant le stade de la floraison. Dans ce compartiment, l'abondance relative de *Bradyrhizobium* était la plus élevée à l'émergence.

Dans des espaces de niche spatiaux contraints, les taxons microbiens se diversifient rapidement sous l'effet d'une radiation adaptative visant à réduire la compétition inter-espèces innée. Cependant, en présence d'espaces de niches temporelles claires, les communautés microbiennes ont tendance à maintenir leurs niveaux élevés de biodiversité au fil du temps. De même, il y a eu une séparation claire des niches et une spécialisation des taxons fongiques à travers les compartiments et les stades de développement des plantes.

Ce chapitre a réussi à mettre en évidence que les niches microbiennes sont influencées par les dynamiques spatiales et temporelles. Par exemple, bien qu'il n'y ait pas de signes visibles de nodulation à chaque période de récolte, il y a eu une augmentation significative de l'abondance relative du partenaire bénéfique du soja, *Bradyrhizobium*, au stade de développement le plus précoce. *Bradyrhizobium* était le moins abondant des autres taxons influencés par le stade de développement. Les besoins métaboliques des plantes varient en fonction des stades de développement et, pour compenser, la plante investit de la biomasse dans la production de racines pour augmenter l'acquisition de nutriments. *Bradyrhizobium* et *Pseudomonas* sont des partenaires microbiens du soja qui coordonnent et modifient l'architecture des racines pour

augmenter l'acquisition des nutriments. De même, *Streptomyces* coopère avec *Bradyrhizobium* pour améliorer l'acquisition des nutriments. Cette étude a montré que l'abondance relative de *Bradyrhizobium* et de *Pseudomonas* est influencée par l'endroit où les microbes se localisent ; de plus, le stade de développement influence l'abondance relative de *Pseudomonas*. Il est alors possible de spéculer que, bien que la dynamique spatiale et temporelle influence l'abondance relative de *Bradyrhizobium*, de *Pseudomonas* et de *Streptomyces* à des degrés divers, ces taxons occupent des niches spatiales et temporelles complémentaires. Par exemple, l'abondance relative de *Pseudomonas* était prévue et atteignait son maximum au stade de la floraison dans l'endosphère des racines, dans le même compartiment où l'abondance relative de *Bradyrhizobium* était la plus élevée au stade de l'émergence. Les communautés de bactéries et d'archées contraintes à une seule niche spatiale ont tendance à se diversifier rapidement sous l'effet du rayonnement adaptatif, cependant, en présence de niches temporelles, les communautés sont plus susceptibles de contourner la dynamique de dépassement du rayonnement adaptatif et de maintenir des niveaux élevés de biodiversité dans le temps. Ainsi, nous suggérons que l'immense abondance et la diversité des taxons de bactéries et d'archées est maintenue grâce à ces cloisonnements de niche spatiaux et temporels. De plus, les taxons prédits par les algorithmes de forêt aléatoire se sont révélés, dans des études dépendantes de la culture, très bénéfiques pour la croissance et le développement du soja.

Enfin, les données ont mis en évidence que la dynamique spatiale et temporelle modulait l'abondance relative des genres de champignons dominants. Par exemple, les algorithmes de forêt aléatoire ont prédit que les OTUs de l'un de ces genres dominants étaient fortement associés au stade de la floraison. L'abondance relative du genre *Mortierella* a augmenté au stade de la floraison à l'interface racine-sol. Les membres de ce genre utilisant l'enzyme xylanase pour métaboliser les sucres d'origine végétale, sont chitinolytiques et antagonistes des champignons pathogènes des plantes tels que ceux du genre *Fusarium*. Nos données montrent que la dynamique spatiale et temporelle influence l'abondance relative de la plupart des genres de champignons, mais cela contraste avec les prédictions des algorithmes de forêt aléatoire. Dans l'ensemble, les OTUs du genre *Fusarium* ont été prédits spatialement pour dominer à l'interface racine-sol; cependant, les OTUs de *Mortierella* ont dominé au stade de la floraison. La forte augmentation de l'abondance relative de *Mortierella* et l'appauvrissement de *Fusarium* à l'interface racine-sol peuvent en partie être expliqués par la nature antagoniste de *Mortierella*. Ainsi, cette étude apporte la preuve que :

1. au sein des communautés fongiques végétales, les spécialisations de niche évoluent indépendamment le long d'axes spatiaux et temporels,
2. seuls des taxons adaptés peuvent habiter ces niches spécialisées, et
3. les compétitions de niche à l'interface racine-sol sont le moteur de l'assemblage et de la colonisation fongiques.

Dans l'ensemble, ce chapitre a trouvé des preuves pour soutenir que : 1) la spécialisation des niches au sein des communautés fongiques végétales évolue indépendamment selon des axes spatiaux et temporels, 2) des taxons adaptés occupent ces niches spécialisées, et 3) la compétition à l'interface racine-sol délimite l'assemblage et la colonisation des communautés fongiques. De plus, ce chapitre a mis en évidence l'importance relative de l'interaction entre les dynamiques spatiales et temporelles dans la modulation des modèles de succession du microbiome du soja et a fourni des preuves à l'existence de niches spatiales et temporelles au sein du microbiome du soja.

Le troisième chapitre visait à élucider l'importance relative des processus neutres et de niche dans la délimitation du microbiome végétal du soja. La sélection médiée par la plante module strictement la composition et la structure du microbiome végétal - le recrutement et l'assemblage actifs des microbes par le biais de facteurs stimulants (apport de nutriments, de facteurs nodaux) et de facteurs inhibiteurs (antimicrobiens, composés organiques volatils). Des preuves qui mettent en évidence l'influence des processus neutres dans la structuration des communautés microbiennes associées aux plantes émergent lentement. Il y a un intérêt investi pour démêler le mécanisme qui médiatise l'équilibre entre la sélection basée sur la niche et les processus neutres; pour cette raison, ce chapitre élucide l'importance relative des processus d'assemblage à travers les compartiments des plantes et leurs stades de développement. Les processus d'assemblage de la communauté ont été modélisés à l'aide de deux approches complémentaires : l'une qui associe des modèles nuls à des données phylogénétiques et des pools d'espèces pour estimer le renouvellement phylogénétique de la communauté entre les assemblages, tandis que les modèles nuls quantifient la déviation par rapport aux attentes nulles, ainsi que les modèles de distribution des espèces qui adaptent les modèles neutres ou basés sur la niche aux données d'abondance des espèces pour décrire l'abondance et la distribution des taxons en fonction de l'espace de niche disponible.

Les plantes ont été cultivées dans une chambre de croissance Conviron (Winnipeg, Canada), et ont été échantillonnées de manière destructive aux stades de développement suivants : V1

(émergence), V3 (croissance), R1 (floraison) et R3 (maturation). Le sol a été récolté à l'automne 2017 dans un champ expérimental qui n'avait aucun antécédent de pratique agricole, passé à travers un tamis de 40 mm, et homogénéisé avant la mise en pot. Les plantes ont été suppléées avec une solution nutritive pour plantes de Hoagland modifiée chaque semaine. Un total de cinq plantes a été échantillonné de manière destructive à chaque stade de développement, et l'extraction de l'ADN a été effectuée juste après l'échantillonnage. Les échantillons ont été prélevés dans la rhizosphère, les racines, les tiges et les feuilles. Jusqu'à présent, les études qui ont élucidé les processus d'assemblage des communautés au sein des microbiomes végétaux ont utilisé l'une ou l'autre de ces approches et se sont principalement concentrées sur un seul compartiment végétal ou stade de développement. Ici, j'ai voulu utiliser à la fois des modèles phylogénétiques nuls et des modèles de distribution des espèces pour quantifier les processus d'assemblage des microbiomes du soja à des échelles spatiales (compartiments de la plante) et temporelles (stades de développement). Je me suis concentré sur l'élucidation des processus d'assemblage dans les plantes de soja poussant en pots dans des conditions expérimentales de chambre de croissance contrôlée. En utilisant les régions phylogénétiquement conservées du gène marqueur de l'ARNr 16S, j'ai cherché à 1) élucider la dominance relative des processus neutres et de niche dans l'assemblage de la communauté bactérienne végétale le long des axes spatiaux et temporels, et 2) comparer différentes approches complémentaires pour modéliser les processus d'assemblage.

Dans l'ensemble, l'abondance microbienne (richesse d'OTU et phylogénétique observée) a varié de manière significative dans tous les compartiments des plantes et à tous les stades de développement. De même, la diversité bêta et l'abondance relative des taxons dominants ont varié le long des axes spatiaux et temporels. Les deux ensembles de modèles, ceux basés sur la phylogénétique et ceux basés sur la distribution des espèces, ont indiqué que ces variations dans les modèles de diversité sont modulées par le compartiment végétal et le stade de développement. Plus précisément, chaque compartiment végétal et chaque stade de développement modulent l'équilibre entre la dominance des processus basés sur la niche et celle des processus neutres. Cependant, la sélection médiée par les plantes et les limitations de dispersion étaient les processus d'assemblage prédominants dans tous les compartiments végétaux et stades de développement. Les deux ensembles de modèles indiquent la prédominance de la sélection basée sur la niche à tous les stades de développement du compartiment de la rhizosphère, la limitation de la dispersion jouant un rôle marginal. Au premier coup d'œil, les valeurs moyennes des modèles phylogénétiques nuls s'écartaient de manière

significative des attentes nulles, mais indiquaient entre autres la dominance des processus neutres. En démêlant l'influence relative des différents processus d'assemblage, la sélection homogène et la limitation de la dispersion étaient les processus d'assemblage prédominants pour tous les compartiments végétaux. La sélection variable jouait un rôle mineur dans tous les compartiments végétaux. Les indices de diversité phylogénétique bêta, tel le taxon le plus proche bêta, montrent une quantification probabiliste (la probabilité que des taxons étroitement apparentés cooccurrent moins souvent que prévu par le hasard) plutôt qu'absolue des cooccurrences. Cette propriété des modèles les rend idéaux pour détecter les influences du filtrage environnemental plutôt que les processus écologiques nuancés tels que la compétition interspécifique, par exemple. De même, tous les modèles de distribution des espèces ont indiqué que, pour l'abondance et la distribution des communautés, les modèles basés sur les niches étaient toujours les meilleurs modèles. À l'interface racine-sol, lorsque les nutriments sont limitants, l'influence des processus basés sur la niche sera plus importante. Dans les essais en champ de soja, lorsque les micronutriments deviennent limitants, on observe une augmentation des taux de dispersion sur les axes temporels. Les deux modèles ont permis d'élucider la dominance de la sélection basée sur la niche (homogène) et l'augmentation de la dispersion à l'interface racine-sol. Cette zone est un environnement très sélectif, la rhizodéposition conduisant à l'assemblage d'une communauté microbienne qui contraste fortement avec les communautés du sol en vrac. Il est également possible que le montage expérimental réductionniste (c'est-à-dire une chambre fermée) ait influencé de manière significative la distribution et l'abondance de la communauté bactérienne telle que détectée par les SDM et ait augmenté les taux de dispersion au sein des communautés épiphytes.

En revanche, le modèle de distribution des espèces (modèle d'assemblage neutre) avait le meilleur pouvoir explicatif pour l'assemblage des communautés microbiennes de certains échantillons de feuilles et de racines, ce qui suggère que la rigueur de la sélection végétale de ces environnements est relativement plus relâchée. La colonisation réussie de nouvelles niches bactériennes est principalement dominée par le tri des espèces (basé sur la niche) et la limitation de la dispersion (neutre). La surface accrue des feuilles et des racines augmente les possibilités de dispersion des microbes du sol, qu'ils soient aériens ou libres, pour occuper ces espaces de niche, et la limitation de la dispersion renforce les processus actuels qui se sont produits lors de la colonisation initiale. L'endosphère de la tige est un environnement relativement pauvre en nutriments, ou du moins déséquilibré, avec une teneur en azote de la sève affectant directement la diversité et l'abondance des microbes. En tant que tel, la sélection homogène a dominé

l'assemblage à des stades de développement ultérieurs tandis que la sélection variable a dominé à l'émergence. Je suggère que pendant le stade végétatif plus court, lorsque la pression sélective exercée par la plante change entre les stades de développement, cela produit une sélection variable, alors qu'aux stades reproductifs plus longs, la sélection homogène domine.

Ces résultats sont conformes aux enquêtes de terrain qui ont mis en évidence l'influence de la limitation de la dispersion dans l'assemblage de la rhizosphère du soja. En effet, au fur et à mesure que les besoins et les demandes métaboliques de la plante évoluent au cours du stade de développement, la composition des facteurs stimulants libérés change. Cette demande accrue conduit à ce que davantage de taxons occupent de nouveaux espaces de niche. Il est intéressant de noter qu'au stade de la floraison, les modèles prévoyaient une augmentation des taux de dispersion dans la rhizosphère. Les taux de dispersion ont augmenté au stade de la maturation pour la communauté épiphyte des racines, tandis que la communauté épiphyte des tiges et des feuilles a connu une dispersion accrue au stade de la croissance végétative. L'augmentation des taux de dispersion au cours de la croissance végétative s'explique en partie par le fait qu'au fur et à mesure que la plante se développe, le nouveau développement offre des opportunités de dispersion accrues pour les microbes aériens et libres qui occupent les nouvelles niches créées. Les preuves suggèrent que pendant les stades de développement les plus courts, c'est-à-dire l'émergence et la floraison, la pression sélective exercée par la plante change rapidement d'une période à l'autre. Au cours de ces courts stades de développement, différents taxons seront sélectionnés au cours de différentes périodes tandis que, pendant le stade de croissance plus prolongé, les mêmes taxons seront continuellement sélectionnés. Dans tous les compartiments végétaux, les modèles phylogénétiques nuls ont indiqué que ni le processus basé sur la niche ni le processus neutre ne dominaient le stade de maturation. Cet état non dominé résulte d'un changement dans la qualité et la quantité des métabolites libérés par la plante lorsqu'elle entre en sénescence. Cependant, les modèles de distribution des espèces ont prédit la dominance de la sélection basée sur la niche. Ceci est imputable aux différences inhérentes au cadre de travail des modèles ; les modèles phylogénétiques nuls montrent la quantification probabiliste plutôt qu'absolue de la cooccurrence, tandis que les modèles de distribution des espèces prédisent comment les taxons occupent des espaces de niche similaires et coexistent via le partitionnement de la niche. Cela suggère que la dominance observée de l'assemblage basé sur la niche au stade de la maturation par les modèles de distribution des espèces est une relique de sélections antérieures maintenues par les interactions microbes-microbes.

En conclusion, j'ai pu démontrer que des approches apparemment complémentaires de la quantification de l'assemblage révèlent la prédominance des processus de niche sur les axes spatiaux et temporels. Tout d'abord, les deux classes de modèles indiquent que le compartiment végétal et le stade de développement modulent l'équilibre entre les processus basés sur la niche et les processus neutres. Les limitations de dispersion ont eu une certaine influence à certains stades de croissance spécifiques ou dans des compartiments définis. Ces stades et ces compartiments pourraient se prêter plus facilement à l'inoculation ou à d'autres approches de manipulation du microbiome, car les communautés soumises à des processus d'assemblage stricts basés sur la niche sont probablement difficiles à déplacer. Ces connaissances pourraient orienter les efforts en cours pour manipuler les microbiomes des plantes afin d'accroître leurs services bénéfiques et de favoriser ainsi une agriculture plus durable.

L'objectif du quatrième chapitre était de discerner comment différentes sources microbiennes influençaient la colonisation et les modèles de succession dans différents compartiments de la plante. On pense que les communautés microbiennes associées aux plantes sont recrutées dans l'environnement immédiat de leurs hôtes. Fondamentalement, les plantes échangent et recrutent leurs partenaires microbiens par le biais de connexions physiques (c'est-à-dire le rhizome et les hyphes mycorhiziens), d'interactions racine-racine conspécifiques et d'une transmission verticale par les graines. Des preuves suggèrent que le microbiome des graines joue un rôle essentiel dans la préservation de la graine et la promotion de la germination. Cependant, on ignore encore quelle pourrait être l'influence des effets de priorité sur les modèles de colonisation du microbiome et comment différentes sources microbiennes modulent ces modèles. Ainsi, l'hypothèse était que le microbiome inné des graines serait le principal colonisateur des racines et des pousses de soja. En revanche, le microbiome de la rhizosphère serait colonisé principalement par les communautés microbiennes du sol.

Pour réduire les charges microbiennes innées et la teneur en matière organique (teneur en C), le sable a été trempé dans de l'hypochlorite de sodium à 10,8 % pendant une nuit. Le sable blanchi a été soigneusement rincé à l'eau d'osmose inverse pour éliminer toute trace de NaClO. Le sable blanchi étant dépourvu de tout composé organique et d'oligo-éléments, le sable a été préchargé en chlorure de fer (III) ($FeCl_3$). Le $FeCl_3$ est un élément immobile dans les plantes, et sa carence entraîne une diminution de la capacité photosynthétique de la production car elle affecte la production de chlorophylle. Pour ce sable, une solution nutritive modifiée pour plantes (sans azote) a été passée à travers le sable jusqu'à ce que le pH du lavage soit de 5,5. Des tests préliminaires ont indiqué que les plantes qui ont été cultivées dans le sable qui n'a pas été traité

avec du $FeCl_3$ présentent des symptômes de chlorose comme cela a été rapporté précédemment. Les graines de soja ont été imbibées d'eau stérile pendant 24 heures à 4°C avant d'être stérilisées en surface. Les graines stérilisées en surface ont ensuite été séchées à l'air dans une boîte de Pétri ouverte, et une fois modérément sèches, les boîtes de Pétri ont été scellées. Tous les travaux ont été réalisés dans des conditions stériles sous des hottes à flux laminaire. Une fois que les graines stériles en surface ont rompu leur dormance, elles ont été exposées à une faible dose d'irradiation aux rayons X à 40 Gy afin de réduire la charge microbienne des endophytes des graines. Comme les graines de soja sont plus sensibles aux mutations causées par l'exposition aux rayons X que les plantes matures ou les semis, seuls les semis (une fois que la racine primaire a émergé/progressé) ont été exposés aux rayons X, et non les graines, à une dose dont il avait été démontré qu'elle ne provoquait aucune anomalie phénotypique chez les plantes. Dans l'ensemble, aucune anomalie n'a été observée dans toutes les plantules utilisées pour tous les traitements. Ces graines de soja quasi-axéniques ont été inoculées avec des sources microbiennes provenant de la graine et du sol. Au total, il y a eu quatre traitements, à savoir : 1) positif (graines stériles et non irradiées en surface + inoculum du sol), 2) négatif (graines stériles et irradiées en surface), graines (graines stériles et irradiées en surface + inoculum endophytique des graines), et sol (graines stériles et irradiées en surface + inoculum du sol).

Tous les traitements ont été cultivés dans des boîtes de culture de plantes stériles sur du sable stérile et remplacés par une solution nutritive filtrée au début de l'expérience. Les plantes ont été cultivées pendant 14 jours dans une chambre environnementale avec une photopériode de 18 heures à 25°C suivie d'une période d'obscurité de 6 heures à 20°C jusqu'au stade de développement "V1". Des échantillons ont été prélevés dans les compartiments souterrains (rhizosphère et racine) et dans la biomasse aérienne, le poids frais total étant enregistré. La composition de la communauté bactérienne a été évaluée en utilisant le séquençage des amplicons du gène de l'ARNr 16S et les gènes fonctionnels clés impliqués dans le cycle de l'azote ont été quantifiés par PCR quantitatif en temps réel (qPCR). En bref, le gène *amoA* impliqué dans l'oxydation de l'ammoniac en hydroxylamine pendant l'étape initiale de la nitrification, le gène *nirK* qui est impliqué dans la réduction du nitrite en oxyde nitrique pendant la dénitrification, le gène *nifH* impliqué dans la fixation de l'azote atmosphérique en ammoniac, et le gène *nxR* qui oxyde le nitrate en nitrite pendant la deuxième étape de la nitrification.

Le traitement du sol avait la fraction de masse foliaire (LMF) la plus élevée et a montré peu de variation entre les réplicats dans la biomasse accumulée par rapport aux autres traitements,

tandis que les graines avaient des valeurs LMF faibles et ont démontré une variation modérée pour tous les réplicats. Les mesures de la diversité alpha (abondance et diversité) ont été significativement influencées par le traitement et les compartiments de la plante, avec des variations significatives uniquement observées pour les compartiments souterrains. Le traitement du sol présentait l'abondance et la diversité les plus élevées dans les compartiments de la rhizosphère et des racines. Le traitement des semences présentait la deuxième plus grande abondance et diversité dans ces mêmes compartiments, tout traitement confondu. Dans tous les traitements, on a observé des niveaux comparables d'abondance et de diversité microbiennes. Cependant, l'homogénéité et l'analyse de dispersion multivariée du roulement de la communauté ont indiqué que les différences observées entre les traitements résultaient de leur structure inhérente et non de différences dans la composition des espèces. La structure de la communauté a été significativement influencée davantage par le traitement et le compartiment végétal et moins par leur interaction. La visualisation a montré une séparation claire des échantillons le long du premier axe (compartiment végétal) et du second axe (traitement). Les communautés de la rhizosphère et des racines du traitement du sol étaient divergentes de toutes les autres. Une tendance similaire a été observée en examinant uniquement les 100 taxons les plus abondants. L'occurrence et la distribution des taxons microbiens dans le traitement du sol étaient principalement observables le long de l'axe rhizosphère-racine, tandis que le traitement des graines était le long de l'axe racine-pousse. Cela suggère que les taxons microbiens des graines occupent de préférence les compartiments des tissus végétaux plutôt que ceux de la rhizosphère. Il y avait des différences au niveau de l'abondance relative des phyla microbiens à travers tous les traitements et les compartiments de la plante. Le traitement du sol présentait la plus grande abondance de taxons rares dans la rhizosphère mais pas au niveau du compartiment racinaire. De même, il y avait une abondance accrue des gènes de recyclage de l'azote (*nifH*, *nirK*, et *nxR*) et leurs rapports d'abondance avec le gène de l'ARNr 16S étaient systématiquement plus élevés selon le traitement du sol. Cette abondance accrue implique que l'exposition des semis au microbiome du sol pourrait avoir des effets positifs sur l'acquisition des nutriments par les plantes.

Les micro-organismes impliqués dans le cycle de l'azote inorganique jouent un rôle clé dans la modification de la disponibilité de l'azote du sol pour l'absorption par les plantes, et nous avons donc choisi ce groupe comme groupe fonctionnel modèle pour tester les effets de nos traitements. Il est intéressant de noter que l'abondance des deux gènes bactériens impliqués dans la nitrification (*amoA* et *nxR* bactériens) ont été les seuls à être influencés de manière significative par les traitements, étant généralement plus abondants sous le traitement "sol". Ceci est

probablement lié à leur plus grande abondance dans l'inoculum du sol, comme le soulignent les analyses LDA pour les *Nitrosomonadaceae*, une famille bactérienne oxydant l'ammoniac. Cependant, nous avons pu détecter tous les gènes fonctionnels dans tous les traitements, même dans le traitement négatif, ce qui suggère que ces groupes fonctionnels peuvent être transmis par les semences. Comme la nitrification transforme l'ammoniac, plus favorable sur le plan énergétique, en nitrate, moins favorable sur le plan énergétique, et que la dénitrification entraîne une perte nette d'azote du sol, la présence de ces gènes fonctionnels pourrait avoir un impact négatif sur la nutrition azotée des plantes, ce qui rend leur transmission par les semences intrigante. Il est intéressant de noter que dans notre dispositif expérimental, de nombreux gènes étaient significativement plus abondants dans les échantillons de racines et de pousses, et moins abondants dans la rhizosphère. Nous avons constaté une augmentation de l'abondance des bactéries oxydant le nitrite dans la rhizosphère des plantes du traitement "sol", ce qui indique une augmentation potentielle de la production de nitrate, qui s'avère être la forme préférée d'azote pour les légumineuses, bien que l'absorption du nitrate soit un processus énergétiquement coûteux pour la plante. Cela suggère que l'exposition des graines au microbiome du sol pourrait avoir des effets positifs sur la nutrition des plantes. De même, l'abondance accrue de bactéries contenant des nitrites réductases (*nirK*) dans la rhizosphère du traitement "sol" était cohérente avec le LDA qui a révélé des taxons dénitrifiants connus (par exemple *Paucimonas*). étant associés au traitement sol. Cela suggère que ces groupes fonctionnels, bien que plus abondants dans l'inoculum du sol en vrac, ont une forte capacité à coloniser les plantes. Quant à leur rôle à l'intérieur des racines et des pousses, je ne peux que spéculer qu'ils pourraient profiter de l'azote inorganique dans la plante comme substrat. La présence potentielle de champignons dans l'inoculum du sol pourrait avoir augmenté l'abondance des dénitrificateurs dans le traitement "sol" comme cela a été précédemment observé dans les champs agricoles. Dans l'ensemble, les tendances observées dans les gènes fonctionnels étaient très similaires à celles observées en utilisant les gènes marqueurs taxonomiques, ce qui suggère que la primauté du microbiome de la graine a non seulement des conséquences sur la composition de la communauté microbienne de l'environnement du soja, mais aussi sur les fonctions qui y sont associées. Il est intéressant de noter que l'abondance relative de la plupart des genres, à l'exception de *Sphingomonas*, semble être " recrutée " de manière constitutive à partir de l'inoculum de graines et faire partie de la communauté transmise par les graines. Plus précisément, l'analyse de l'abondance différentielle a révélé que les abondances relatives de *Pseudomonadaceae*, *Pantoea*, *Methylobacterium* et *Chryseobacterium* étaient systématiquement plus faibles dans l'inoculum du sol et dans les traitements du sol. Ces taxons sont essentiels au maintien de l'homéostasie de

l'holobionte du soja ; par exemple, *Methylobacterium* est connu pour métaboliser l'allantoïne (un sous-produit de la fixation de l'azote) et il est démontré que *Chryseobacterium* améliore la croissance du soja dans les champs agricoles. Néanmoins, l'abondance de la plupart des genres était plus élevée dans l'inoculum du sol et le traitement du sol. Le sol possède une abondance incommensurable de taxons microbiens ; ainsi, l'abondance accrue de taxons et de gènes de recyclage de l'azote dans le traitement du sol n'est pas surprenante.

En résumé, ce chapitre a fourni la preuve que le microbiome des graines a la primauté dans la colonisation des espaces de niche de la plante, tandis que le microbiome du sol colonisera préférentiellement le compartiment de la rhizosphère. De telles expériences réductionnistes permettent de distinguer le *modus operandi* des différents taxons microbiens dans la colonisation des espaces de niche disponibles au sein de l'holobionte végétal.

L'objectif de ce chapitre était de contraster les voies de colonisation microbienne des plantes par les graines et le sol. J'avais émis l'hypothèse que les communautés microbiennes des graines seraient les premières colonisatrices de l'environnement végétal, avec un effet plus important dans les environnements des pousses et des racines par rapport à la rhizosphère. La plupart des résultats ont montré que les plantes de soja cultivées à partir de graines stérilisées en surface et irradiées avant d'être inoculées avec un extrait microbien du sol présentaient une diversité, une structure, une composition et des fonctions de communautés microbiennes différentes dans leurs racines et leur rhizosphère au stade de l'émergence. Ces différences ne se sont pas étendues aux communautés microbiennes des pousses. Les plantes de soja cultivées à partir de graines stérilisées en surface et irradiées qui n'ont pas été traitées avec un inoculum de sol ont développé des communautés microbiennes étonnamment similaires à celles hébergées par les plantes cultivées à partir de graines stérilisées en surface et irradiées qui ont été inoculées avec un extrait de graines, ou à celles hébergées par les plantes cultivées à partir de graines qui n'ont pas été irradiées et inoculées avec un extrait de sol. Cela suggère que

1. les communautés microbiennes des graines n'ont pas été complètement détruites par notre méthode,
2. les communautés microbiennes des graines gravement perturbées peuvent recoloniser la plante si elles ne sont pas en compétition avec les communautés microbiennes du sol,
3. lorsque les communautés microbiennes des graines ne sont pas perturbées, elles ont la priorité sur les communautés microbiennes du sol pendant la colonisation des racines et de la rhizosphère de la plante, et

4. même si les communautés microbiennes des graines sont perturbées et exposées aux microbes du sol, elles peuvent coloniser avec succès les compartiments des parties aériennes des plantes.

En tant que telle, l'hypothèse est confirmée, avec l'ajout que les communautés microbiennes portées par les graines semblent également être capables de coloniser la rhizosphère même lorsqu'en concurrence avec les communautés microbiennes du sol. Même si nos résultats sont issus d'une expérience hautement contrôlée, ils sont alignés avec les rapports précédents qui ont montré que les communautés microbiennes des graines sont la source primaire de micro-organismes dans l'environnement des plantes.

En conclusion, cette thèse met en évidence les modèles temporels et spatiaux qui modulent les modèles d'assemblage et de colonisation du microbiome du soja. Plus précisément, elle met en évidence la primauté et l'importance des effets de priorité et la façon dont les différents régimes de dispersion agissent sur la trajectoire de succession des microbiomes du soja. Le premier objectif a mis l'accent sur l'existence de niches spatiales et temporelles et sur la façon dont elles sont occupées par des taxons spécialisés, le second a accentué les processus d'assemblage des communautés qui délimitent ces niches spatiales et temporelles ; tandis que le troisième objectif a distingué le rôle des différentes sources microbiennes dans l'influence des modèles de colonisation et a mis en évidence l'impact nuancé de l'effet de priorité. Dans l'ensemble, cette thèse montre les processus écologiques et évolutifs agissant sur le microbiome du soja ; elle identifie le rôle des interactions spatiales et temporelles dans l'influence de la communauté globale et identifie les points d'intervention clés qui peuvent maximiser les efforts d'ingénierie du microbiome.

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ABA	Abscisic acid
ADF	Augmented Dickey-Fuller
AMF	Arbuscular mycorrhiza
C_{org}	Carbon content
DAMP	Damage-associated molecular patterns
DNA	Deoxyribonucleic acid.
ECM	Ectomycorrhiza
ETI	Effector triggered immunity
ESVS	Exact Sequence Variants
HR	Hypersensitive response
ISR	Induced systemic resistance
JA	Jasmonate
KPSS	Kwiatkowski-Phillips-Schmidt-Shin
MAMPs	Microbe-associated molecular patterns
mRNA	Messenger Ribonucleic acid
NRE	Non-rhizobial endophytes
NOD	Nodulation factors
OTU	Operational taxonomic unit
PAMPs	Pathogen-associated molecular patterns
PCoA	Principal Coordinate Analysis (PCoA)
PCR	Polymerase chain reaction
PEP	Peptide elicitors
uORFs	Upstream open reading frames
PGPR	Plant growth-promoting rhizobacteria
RNA	Ribonucleic acid
RET	Root extracellular trap
SA	Salicylic acid
SAR	Systemic acquired resistance
VE	Emergence stages
VC	Cotyledon stage
VOCS	Volatile organic compounds (VOCs)
qPCR	Quantitative Polymerase chain reaction

1 CHAPTER 1 : GENERAL INTRODUCTION

1.1 The Plant Holobiont

The plant microbiome consists of the combined microbial communities that reside on and within the plant; these communities have an intrinsic relationship with their hosts and confer benefits to the plant hosts (Zablotowicz et al., 1991; Berendsen et al., 2012; Chaparro et al., 2012; Berg et al., 2014). Plant-associated microbial communities are now recognised as an extension of the plant immune and agents that abet abiotic stress (Vandenkoornhuyse et al., 2015; Teixeira et al., 2019). In fact, it is around this fundamental understanding of how microbial communities influence their host that the hologenome theory developed. The hologenome theory posits that the combined microbiome and host genome are acted upon by selective processes (Zilber-Rosenberg and Rosenberg, 2021). The plant holobiont is an assemblage of plant-associated microbes that interact with **1**) each other and **2**) the host (Figure 1.1).

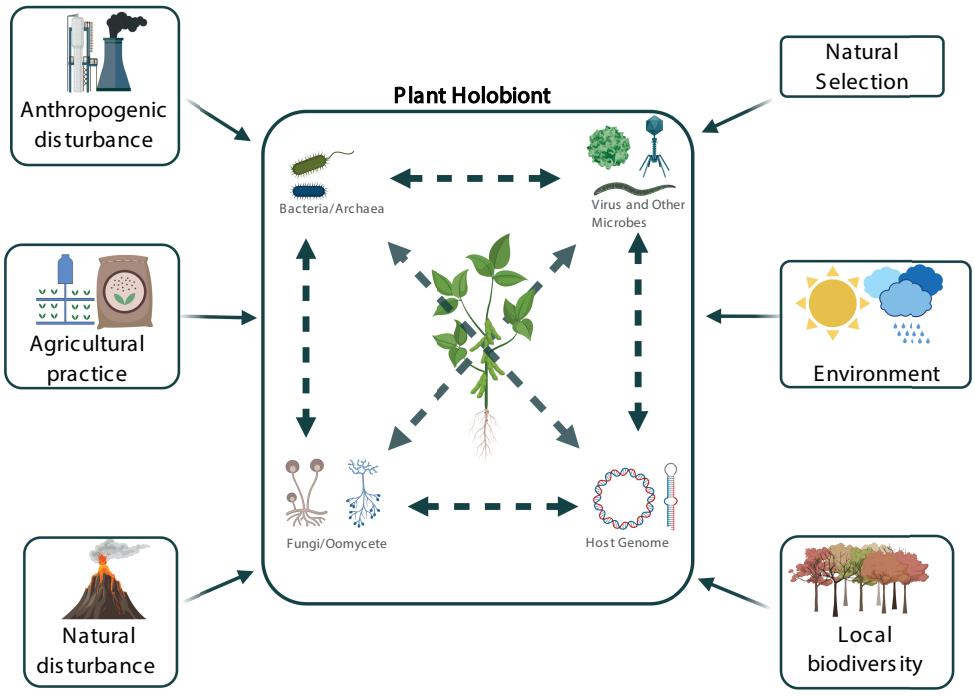

Figure 1-1 ***A schematic diagram of the plant holobiont and what influence it's fitness***

1.1.1 Assembly and structure of the plant holobiont

The plant microbiome is an assembly of archaea, bacteria, micro-eukaryotes, and virus communities that inhabit various plant organs with little overlap in taxonomic and functional composition (Turner et al., 2013b; Berg et al., 2014; Rout, 2014). The plant microbiome is defined by 1) their taxonomic composition, 2) functional capacity, and 3) dominance of the prevailing assembly processes. The plant host selects and regulates the composition and structure of the microbes that inhabit both the endophytic (space within the plant host where microbes reside as part of its life cycle *i.e*., intracellular space) and epiphytic (space outside the plant host where microbes reside as part of their life cycle *i.e.,* root or leaf surfaces) spaces.

The secretion of organic compounds into the intercellular and extracellular spaces by the host creates specialised ecological niches spaces that are occupied by microbes that have positive (mutualists), negative (pathogens, competitors), or neutral (commensalistic) interaction with the plant (Quiza et al., 2015; Hassani et al., 2018). These ecological relationship between host and microbes are often conserved across plant species and communities (Fierer and Lennon, 2011). The plant host, its associated microbial partner, and the interactions therein are acted upon by evolutionary processes to promote holobiont fitness.

The central dogma that competition alone explains the observed variation and patterns in ecological communities can no longer be accepted, especially for microbial communities. Coincidentally, there has been a paradigm shift in plant microbial community ecology from describing community patterns to understanding what processes delimiting community structure. Vellend (2010) and Nemergut et al. (2013) succinctly elucidated the processes that were important in assembling ecological communities: niche-based (selection) and neutral (ecological drift, speciation, and dispersal) (Figure 1.2).

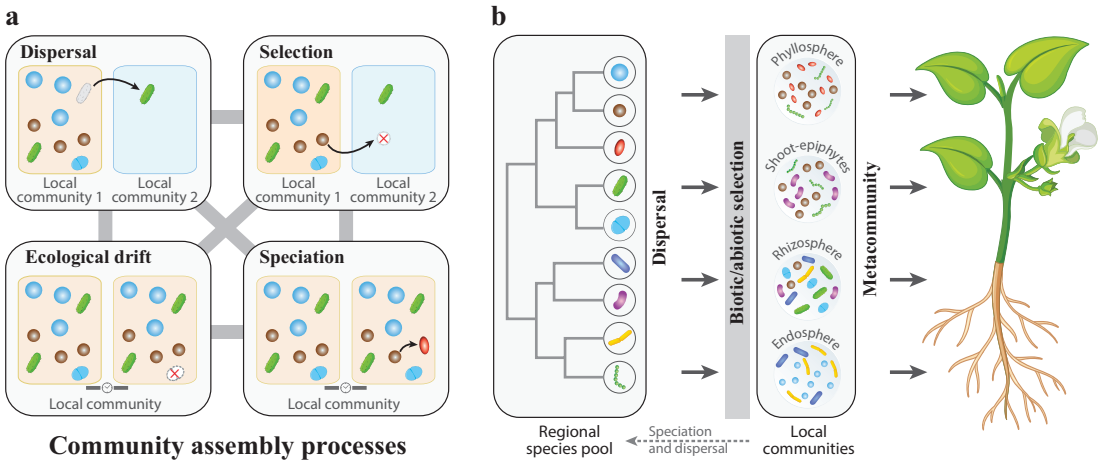

Figure 1-2 Ecological processes involved in plant microbiome assembly. (a) Details of how the four evolutionary processes structure community assembly and b) generic scheme of interactions between the regional species pool and metacommunity. Dispersal is defined as the movement of microorganisms between local communities and consequences of dispersal are highly dependent on the diversity in both communities . Selection is the consequences of interactions of abiotic and biotic selection that affect the establishment of microbes within a community. Ecological drift is changes in species abundance and diversity through time as a result of stochastic birth or death in community. Speciation is the process that generates genetic variation within a local community. The "X" indicates unsuccessful establishment or organismal removal. Adapted from Cordovez et al. (2019)

1.1.1.1 Niche-based selection

Niche theory stipulates that within any ecological ecosystem there exist a finite number of niches, each occupied by a single species. The realised niche describes 1) the environmental conditions that a species needs to survive and reproduce (the fundamental niche) and 2) considers the existence of the species in presence of biotic interactions (Vandermeer, 1972; Case and Gilpin, 1974). Thus, niche-based selection posits that ecological traits (e.g., life-history traits, nutritional requirements) within a community vary amongst species, which permits them to occupy their fundamental niches, but the fundamental niche of species is acted upon by biotic and abiotic factors, defining the realised niche (Leibold, 1995; Leibold and Chase, 2017). The influence of niche-based selection in the assembly of plant microbiomes is widely acknowledged. Specifically, the influence of plant-mediated selection involves the release of primary and secondary metabolites by both plant host and microbial partners (Figure 1.3) (Hartmann et al., 2009). The severity of niche-based selection and feedbacks varies across plant species and is highly dependent on nutrient strategies of the plant host. For example, leguminous plant (*e.g.*, soybean) have higher survival rates and stronger stimulatory feedbacks when grown in soils from non-conspecifics (Teste et al., 2017). This highlights that not only does the environment mediates plant holobiont fitness and assembly, but that also land-use history influences the strength of that selection.

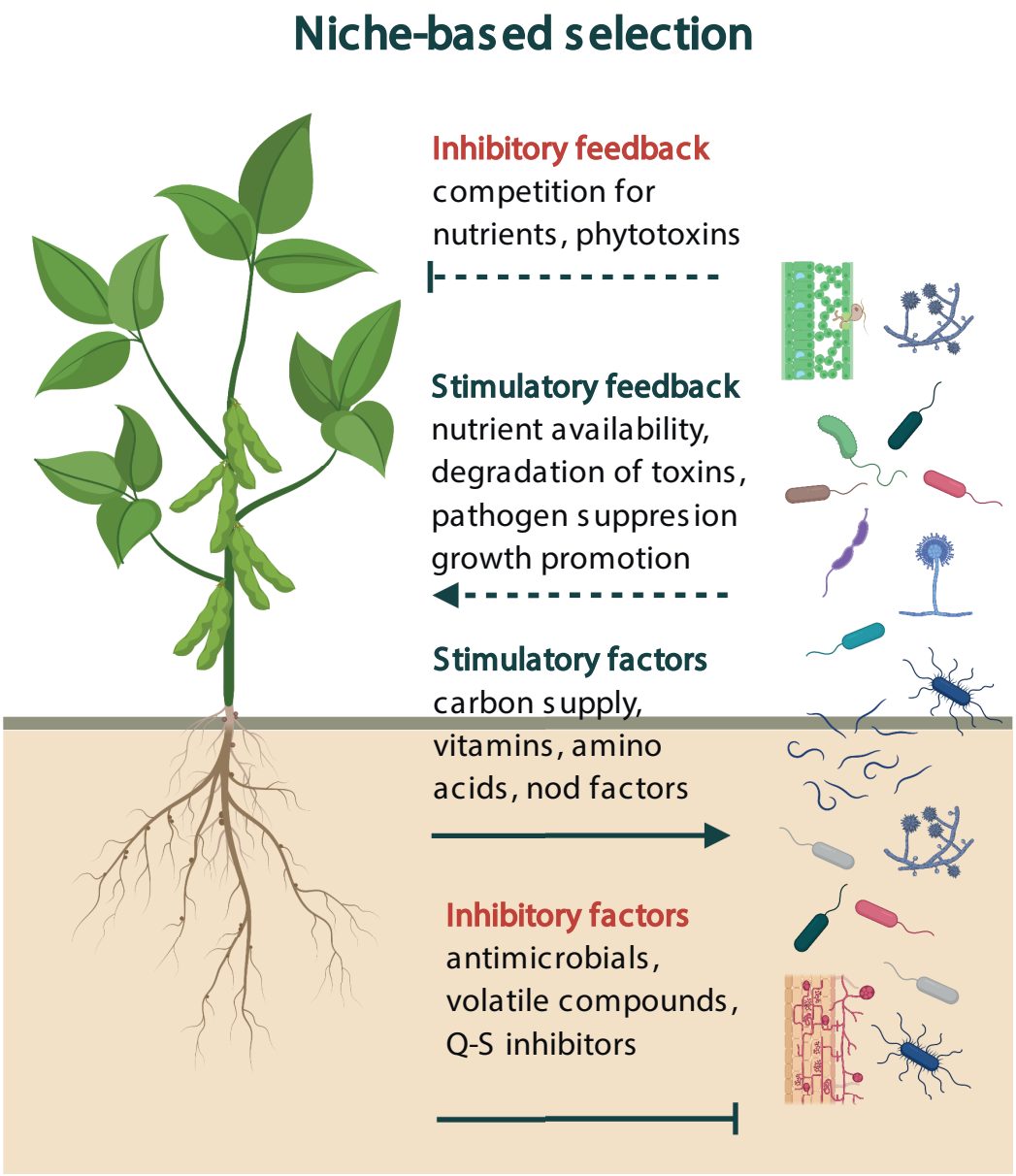

Figure 1-3 Niche-based selection highlighting interactions and possible feedback within the plant holobiont

This selection of microbial communities along the soil-plant continuum is highly modulated by both abiotic (*e.g.*, agricultural practices, anthropogenic disturbances) and biotic (*e.g.*, host genome, inter-species competition) dynamics (Figure 1.1). In agricultural ecosystems however, the assembly of the microbiome is mediated by plant compartment niches (Coleman-Derr et al., 2016) and host genome (Singer et al., 2019) more than agricultural practices, with plant-mediated selection increasing in severity along the soil-plant continuum (Xiong et al., 2020a). Furthermore, this selective pressure on the microbial communities is asserted at every plant developmental stage (Dini-Andreote and Raaijmakers, 2018; Cordovez et al., 2019). More importantly, recent works have highlighted the importance of historical contingencies (*i.e.*, timing and the effects of past events on community assembly), such as, anthropogenic/natural disturbances and priority effects (impact species have on community assembly and succession based on the order of their arrival) in facilitating plant-mediated selection within the holobiont (Fukami, 2015; Carlström et al., 2019). However, the significance of priority effects is more pronounced in actively growing plant tissues and compartment, as this the site of active niche creation. For instance, the emerging seedlings and expanding root systems, flowers, and emerging leaves are exposed to colonizers and priority effects along with plant-mediated selection will be more stringent (Dini-Andreote and Raaijmakers, 2018).

These fundamental ecological frameworks have emphasised the need to discern the relative influence of plant-mediated selection within the holobiont. Evolutionary adaptions of the plant holobiont to historical contingencies and biotic stresses occurs through the process of natural selection (Bordenstein and Theis, 2015; Rodriguez and Duran, 2020). Acquisition of microbes and microbial genes is a powerful mechanism for driving the evolution of complexity and improving holobiont fitness (Zilber-Rosenberg and Rosenberg, 2021). Selection of microbial partners proceeds both via cooperation and competition, working in parallel (Figure 1.3). However, this selective pressure acts across both temporal (across plant developmental stages) and spatial (across plant compartments). Seasonal environmental variations and natural disturbances at the spatial and temporal scales assert a strong selective pressure on plant holobiont fitness (Rodriguez and Duran, 2020). Previous studies have indicated that species-sorting (community assembly regulated by local niche processes) and neutral process (dispersal) interacted during microbial assembly and their relative importance was dependent on local species abundance (Langenheder and Szekely, 2011). Overall, plant-mediated selection enables the host to eliminate detrimental microbes/genes whilst increasing abundance and diversity of

beneficial microbial partners, in the absence of this plant-mediated selection, neutral processes that mediate microbial variation within the holobiont become inherently stochastic (Bordenstein and Theis, 2015).

1.1.1.2 Neutral theory and processes

Neutrality in the ecological context implies that species in similar trophic level (*i.e.,* bacteria in rhizosphere, and not species of different trophic position, like bacteria and gazelles) have similar birth rates, dispersal rates, and speciation rates, when compared to each other and the influence of niche does not affect species fitness and success (Hubbell, 2001; Hubbell, 2005). Neutral models predict the abundance and distribution of species as a direct consequence of random dispersal over time leading to ecological drift, whereas diversity in community is a result of unstable coexistence and balance between speciation and extinction (Hubbell, 2001; McGill et al., 2006). These implication on ecological community assembly are regarded as the null hypothesis to niche theory and led to a framework from which mechanisms of community assembly can be inferred (Webb et al., 2002). Null models that couple community composition and structure with randomisations have become the standard in describing community assembly processes (Graham and Fine, 2008; Cavender-Bares et al., 2009; Kembel, 2009; Stegen et al., 2013).

Under the neutral theory, diversity of microbial communities within the holobiont is mediated by the balance between extinction and speciation events, the influence of stochastic processes (*i.e*., death, dispersal limitation) (Figure 1.4) (Zhou and Ning, 2017). For example, each plant host (local community) is composed of an assemblage of species from the regional species pool, if some species colonise the host first, then the abundance and diversity of the local community is highly regulated by priority effects (Figure 1.4). When the influence of dispersal rates between local communities is high, then composition and diversity between the two communities will be similar (homogenising dispersal; Figure 1.4), however, when dispersal is weak the communities will vary (dispersal limitation; Figure 1.4) (Gilbert and Levine, 2017; Zhou and Ning, 2017). Though, within a community there can only be a finite number of species- this is known as the zero-sum assumption. The zero-sum assumption posits that when the community is full, then a new species can only be part of that community if a species goes extinct thus making space (Etienne et al., 2007). The zero-sum model subsequently infers that all species have an equal chance of occupying those niches spaces. New niche apportionment models, that is,

models that describe the break-up of the total niche space (spatial or temporal) and the relative abundance of species are best at describing patterns of colonisation for plant epiphytic communities (Tokeshi, 1990; Tokeshi and Schmid, 2002). Particularly, neutral apportionment models are best at describing communities in highly dynamics environments; it is this intrinsic aptitude of these models that makes them so invaluable in discerning assembly processes, recruitment, and colonisation patterns within the plant holobiont (Figure 1.4).

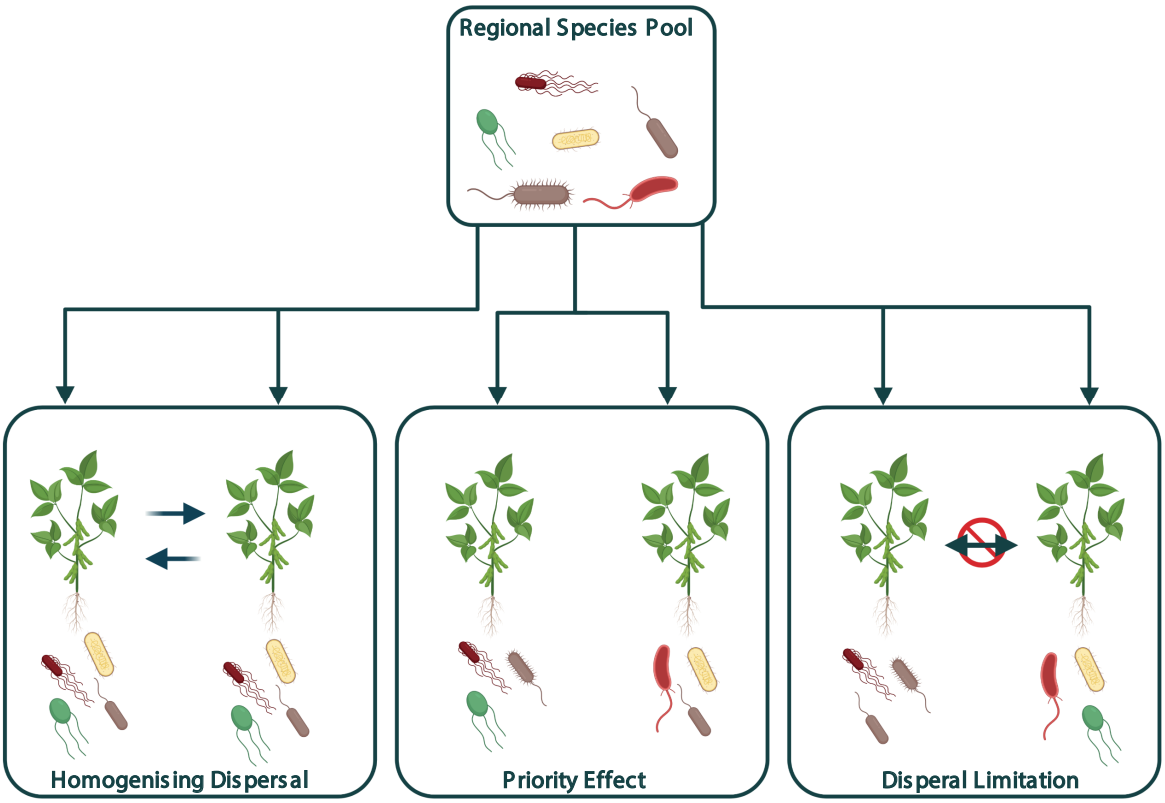

Figure 1-4 Importance and influence of neutral processes in plant holobiont

The importance and significance of neutral processes in modulating microbial community assembly within the plant holobiont has in the past been grossly overlooked. However, recent studies are highlighting the importance of dispersal limitation in discerning microbial communities in local communities (Marasco et al., 2018) and in moderating biogeographical patterns across landscapes (Moroenyane et al., 2019). The influence of neutral processes in creating and maintaining niche spaces across temporal scales has been highlighted in agricultural ecosystems. For instance, in highly degraded environments the influence of niche-based selection diminished whilst role of neutral processes is more pronounced. These drastic changes in abiotic parameters tapers the strong environmental filter *i.e.* the influence of niche-based selection and promotes the effects of neutral processes (Tripathi et al., 2018). In variable environmental conditions, the dominance of neutral processes on community assembly favours generalist taxa, whilst specialist taxa are recruited through niche-based processes (Liao et al., 2016). In practice, these shifts in dominance of either niche-based or neutral processes creates temporal niche spaces across plant microbiomes. Similarly, changes in agricultural practice enhance diversity of soil-borne microbes and creates a temporal niche space, that is, a period of time in which species differ in their competitive capacity given limited resources (Yu et al., 2015). Within the plant holobiont, this temporal niche is highly modulated by 1) host circadian rhythm and 2) developmental stages, and occupation of host niche space is density dependent. In the presence of a constant spatial and temporal niches, speciation events within the microbial communities are favoured and that promotes species coexistence and biodiversity (Tan et al., 2013). In like manner, when plant neighbourhoods are composed of conspecifics, the assembly and structure of the phyllosphere is modulated by neutral processes with increased dispersal rates between individuals (Meyer et al., 2022). It seems when there are stochastic fluctuations in environmental condition there is a dominance of neutral processes that assembles plant microbiomes, especially amongst conspecifics. These observations are corroborated also along spatial axes; biogeographical patterns and assembly of *Eucalyptus* phyllosphere bacterial community is dominated by neutral processes along regional scales (Yan et al., 2021). It is important to highlight that dispersal, speciation, drift, and stochasticity are not unique to the neutral theory alone and are shared with *e.g.*, the chaos theory. However, neutral models are best at describing community assembly processes as they incorporate dispersal and stochasticity as part of their working framework.

Overall, the occupations of niche spaces along spatial and temporal axes is strongly mediated by the balances between plant-mediated selection and neutral processes. Along temporal axis, as the plant develops from seed and new niche spaces are created (leaves, stem, roots, and flowers), neutral process and plant-mediated selection modulate assembly and colonisation of these niche spaces (Figure 1.5) (Shade et al., 2017; Amend et al., 2019). Dispersal effects (homogenising and limitation) coupled with plant-mediated selection have been implicated in creating distinct communities across spatial (transfer amongst organs and different hosts) and temporal (developmental stages) in agricultural systems (Figure 1.5) (Perez-Jaramillo et al., 2016; Goss-Souza et al., 2019). Plant holobionts exchange microbial symbiont through physical connection (*i.e.,* rhizome and mycorrhizal hyphae), intra and interspecies connections belowground, and through the dispersal of seed (Figure 1.5). These microbial symbionts are highly nested along spatial and temporal scales and their relationships are predictable by the hierarchical theory, that is, ecological processes are organised in scales of interactions (O'Neill et al., 1986; Vandenkoornhuyse et al., 2015).

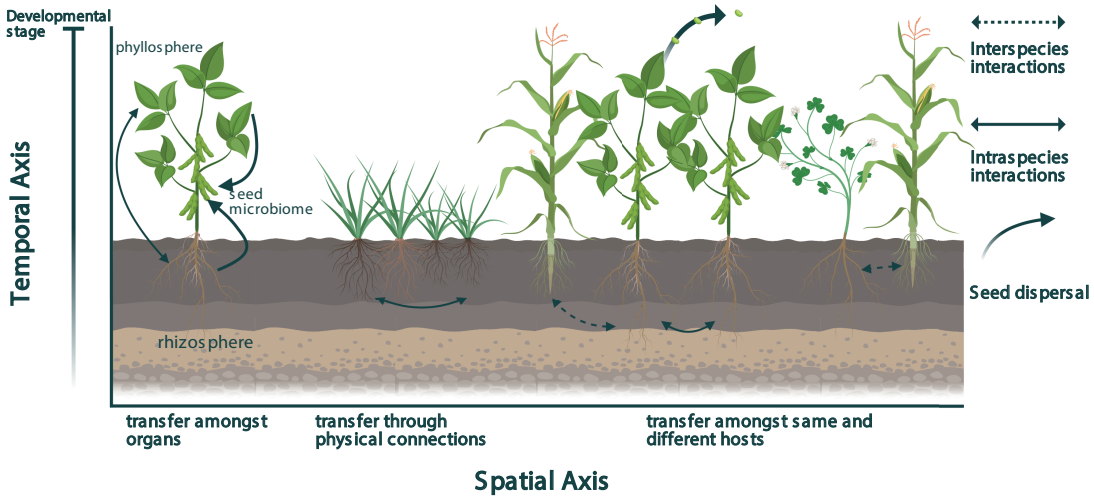

Figure 1-5 Spatial and temporal dynamics in plant holobionts

1.1.2 Compartmentalisation within the plant holobiont

The different plant compartments *i.e.* reproductive (flower and fruit), leaf, stem, root, and rhizosphere each have unique biotic and abiotic dynamics that influence the microbiome composition and structure (Morozov and Li, 2008; Hirsch and Mauchline, 2012). Microbial communities vary by their location on the plant, with the endosphere (within and between plant tissue), phyllosphere (the foliar surfaces of the plant), and the rhizosphere (Zablotowicz et al., 1991; Hirsch and Mauchline, 2012; Berg et al., 2014) harbouring distinct communities. The abundances and distribution of microbial symbionts are further influenced by plant genotype, composition of the plant-derived metabolites, and colonisations patterns (Dakora and Phillips, 2002; Lundberg et al., 2012). These studies emphasised the existence of spatial and temporal niches and how these are partitioned amongst and within communities. To date, a majority of studies have focused on the bacterial and fungal components of plant microbiome investigating mainly the rhizosphere communities (Malloch et al., 1980; Redman et al., 2002; Bulgarelli et al., 2012; Bulgarelli et al., 2015). However, archaeal communities have also been shown to confer benefits to the plants and are key players in nutrient cycling (Chen et al., 2008; Herrmann et al., 2008; Buee et al., 2009).

1.1.2.1 The phyllosphere microbiome

The phyllosphere is the most dynamic plant compartment with constant abiotic fluxes (temperature, wind, radiation, relative humidity, and plant surface topology) (Turner et al., 2013a; Copeland et al., 2015). Although the phyllosphere is generally considered to be nutrient poor compared to other rhizosphere, studies have indicated that microbial communities found there are extremely diverse and adapted to surviving in these 'harsh' conditions (Lv et al., 2013; Vorholt, 2014; Venkatachalam et al., 2016). The advent of high-throughput sequencing has enabled researchers to characterise these communities, but there has been an emphasis on studying plant pathogens and the phyllosphere of agricultural crops (Penuelas and Terradas, 2014). Successional patterns of the phyllosphere community are unique and vary across plant species and communities. A survey of phyllosphere community of Canadian agricultural crops: bean, soybean, and canola over a growing season revealed that initially the community was highly influenced by the soil microbial community and as the season progresses the community is not influenced by plant genotype as a shared leaf community emerges (Copeland et al., 2015). Furthermore, another study that used a synthetic plastic as a control to discern the successional

pattern of phyllosphere community between plastic and real plants, highlighted that temporal changes are important drivers in community structure and composition (Ottesen et al., 2016). Although it seems in part that the plant does not exert a strong selection force on the phyllosphere community, it has been shown that phyllosphere microbiome are transferred across generations (Maignien et al., 2014; Massoni et al., 2020). To date, there is a lack of a proven account of the discriminatory mechanistic processes involved in the selection and assembly of these 'inherited' microbiome. The niche optima (the most desirable a/biotic conditions for species to survive) and width (an ecological range of desirable abiotic conditions and biotic interactions needed for species to persists) for phyllosphere microbiome communities is influenced by several biotic and abiotic conditions (Figure 1.6). Microbial dysbiosis, that is, an imbalance in plant microbiome compositions and structure that results in perpetuation of disease-causing microbial partners is well documented for phyllosphere (Hooks and O'Malley, 2017; Liu et al., 2020a). Essentially, when plant holobiont dynamics are disrupted through introduction of pathogens via density-independent dispersal mechanism (*e.g.,* wind, insects, water), it leads to 1) shifts in balance of beneficial communities, 2) altered plant-mediated selection, and 3) ultimately commencement of the plant defence mechanism (Figure 1.6).

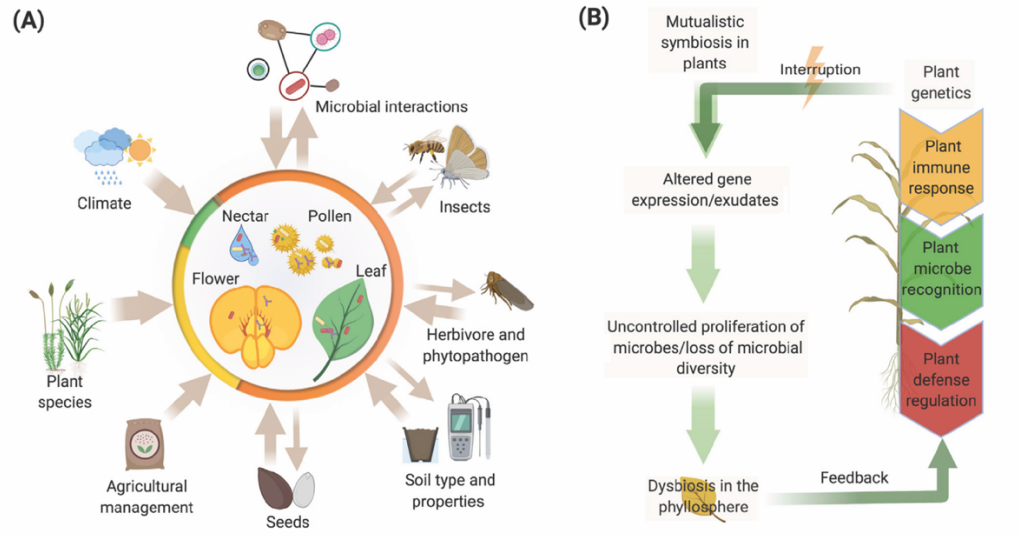

Figure 1-6 Assembly and dysbiosis of phyllosphere (Liu et al., 2020a)

1.1.2.2 The endosphere and seed microbiome

The endosphere microbial community has been well-characterised using microscopy and classical microbiological techniques (Turner et al., 2013a). This community is unique as, in healthy plants, it is largely composed of beneficial microbes and free from pathogens or those that exist tend to be latent; additionally, the endosphere community is critical in promoting plant growth and health (Gaiero et al., 2013; Kandel et al., 2017). Microbes that reside inside plant tissue (also known as endophytes) have had to adapt to a nutrient-rich environment that has relatively subdued levels of competition compared to the rhizosphere community. It is then not surprising to find the endophyte community to have a limited repertoire of secondary metabolites than its rhizosphere or phyllosphere counterparts (Gaiero et al., 2013; Brader et al., 2014). However, this view has been challenged, as most endophytic microbes are facultative symbiont and must compete with soil borne microbes at the root-soil interface prior to entry into the plant (Bulgarelli et al., 2012; Beckers et al., 2017). In particular, different seed endophytic microbes seem to amplify priority effects (Ridout et al., 2019), and these highly influenced the colonisation patterns of different plant compartment (Robinson et al., 2016). Transplantation studies emphasised the influence of seed endophytic communities in modulating overall microbiome colonisation and successional patterns while highlighting the substantial overlap in the occupied niche between seed endophytic and plant communities (Tannenbaum et al., 2020; Wang et al., 2020a). However, the occupation of rhizosphere niche spaces by seed endophytic microbes occurs in soils with decreased microbial loads (Luo et al., 2019). Taken together, this implies that successful colonisation of plant niche spaces by the endophytic microbiome is under strict plant-mediated selection and different ecological assembly processes.

1.1.2.3 The rhizosphere microbiome

The rhizosphere represents a zone where the plant roots interact with the surrounding soils to actively recruit and assemble the rhizosphere microbiome (de la Porte et al., 2020b; Agoussar and Yergeau, 2021). The secretion of photosynthesis-derived metabolites into the rhizosphere (rhizodeposition) is proposed to be the mechanism the plant uses to assemble and maintain this microbiome (Dakora and Phillips, 2002; Jones et al., 2009; Berendsen et al., 2012) (Figure 1.3). However, this view has been recently challenged through the suggestions that gaseous compounds and miRNA could also play a central roles in assembling the rhizosphere microbiome (Middleton et al., 2021). Also, subtle differences in host plant genotype play an important role in selecting which microbial taxa occur in the rhizosphere microbiome (Bulgarelli et al., 2012; Lundberg et al., 2012; Bulgarelli et al., 2015). Bulgarelli et al. (2015), in their study of root-inhabiting microbiota in domesticated and wild barley, revealed that there were marked shifts in the community moving from the root-soil interface towards inside the plant. Moreover, they highlighted that in both dicotyledons (*Arabidopsis thaliana*) and monocotyledons (barley, wheat, and maize), there exists lineage-specific molecular cues that are conserved and contributed to these shifts. In addition to the plant-mediated selection, microbe-microbe interaction in the surrounding soils are key to creating these unique microbial communities (de la Fuente Canto et al., 2020; de la Porte et al., 2020a). The immense abundance and diversity (taxonomic, functional, and size) of the rhizosphere microbial community creates an active zone of selection (Figure 1.7) (Kuzyakov and Razavi, 2019).

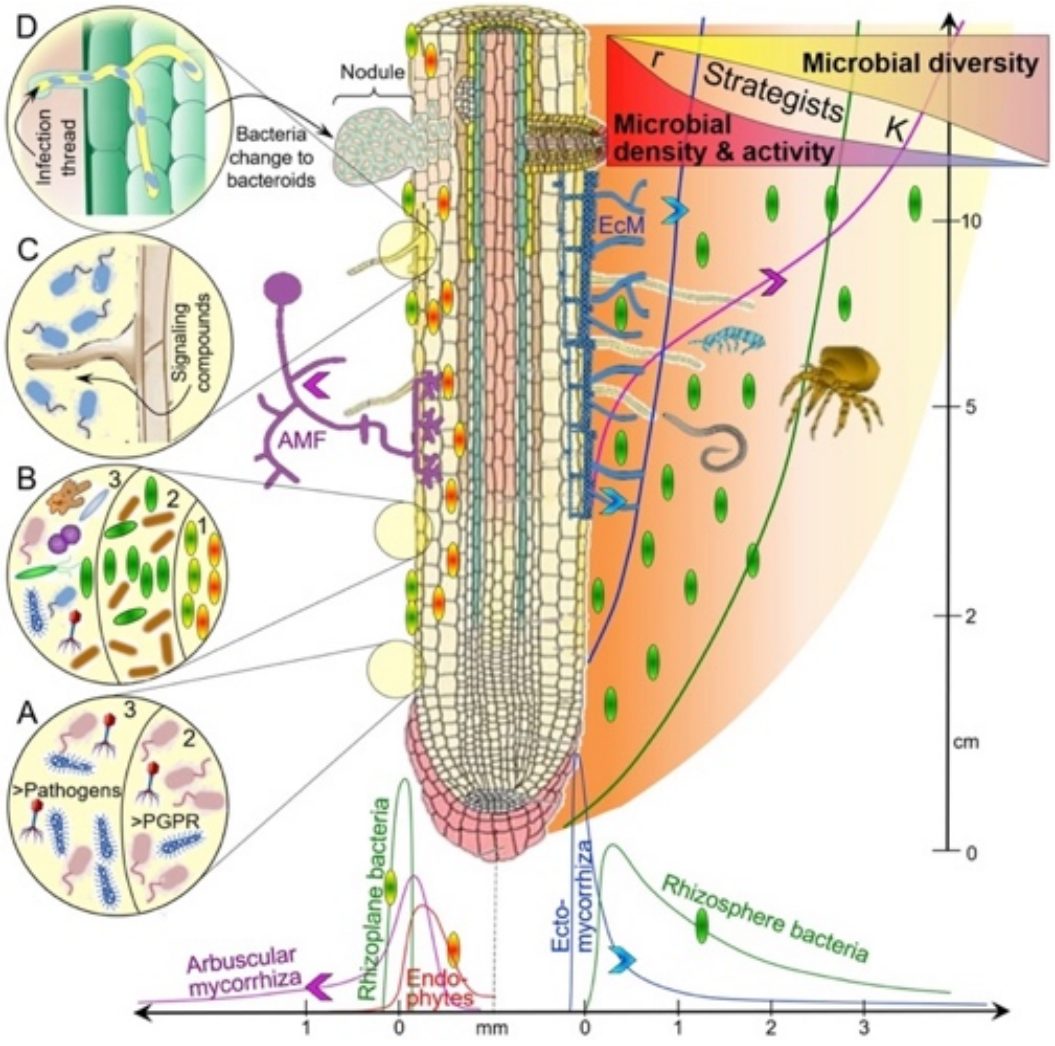

Figure 1-7 Plant rhizosphere size and dynamics (Kuzyakov and Razavi, 2019)

Advances in plant breeding and a broader understanding of the ecology of rhizosphere microbes agricultural ecosystems have led to better management practices in these dynamic ecosystems (Lemanceau et al., 2017; Toju et al., 2018b). These targeted approaches aim to increase overall fitness of the plant holobiont by mitigating the influence of external factors, especially in the rhizosphere. Rhizospheric microbes play multiple roles including but not limited to: increasing crop yield, acting as biological control agents of soil borne pathogens, promoting legume nodulation by nitrogen-fixing rhizobia, and increasing the emergence of seedlings (Malloch et al., 1980; Buee et al., 2009; Rout, 2014). In their comprehensive report Berg et al. (2016) highlighted the role the plant microbiome plays in increasing not only the host's metabolic repertoire, but also creating new nutritional and defence pathways. We owe much of our current understanding of plant microbiomes to discoveries made in the late 20th century- specifically, the discovery of plant growth-promoting rhizobacteria (PGPR) (Ross and Harper, 1970; Kloepper et al., 1989; Bashan, 1998; Burd et al., 1998). As such, the use of microbial inoculants and amendments to improve grain yield and disease suppression has become industry standard to mitigate the negative effects of artificial fertilizers on plant holobiont dynamics and ecosystems (Baez-Rogelio et al., 2017; Alori and Babalola, 2018). Other less conventional ways that plant holobiont fitness has been improved is by adopting an intercropping system, that is, cultivating multiple crops in the same field simultaneously (Ghosh et al., 2006). Healthy soils, that is, soils with sufficient drainage, good tilth, decreased pathogen and weed pressure, moderate levels of essential micro and macro nutrients, as well as increased diversity of microbes acts as species reservoir from which rhizospheric microbes are recruited (Moroenyane et al., 2017; Girsowicz et al., 2019; Liu et al., 2019b). Better agricultural practices improve soil health, organic matter content, and increase soil priming (when input of new carbon into soil system promotes the decomposition of old soil carbon) (Liu et al., 2020b). The plant holobiont can regulate this priming effect by modulating the amount and diversity of the nutrients released by the growing root (rhizodeposits) (Lloyd et al., 2016), the abundance and diversity of rhizospheric microbes (Kuzyakov, 2010), and moderating temperature at the root-soil interface (Hopkins et al., 2014). The significance of this priming effect is widely documented across landscapes (Chen et al., 2019a) and at a global scales (Bastida et al., 2019). This stringent regulation of abiotic conditions at the root-soil interface favours the creation of new spatial and temporal niche spaces whilst increasing the niche width of rhizospheric microbes. For example, the concentration of rhizodeposits and volatile organic compounds (VOCs) decreases with distance from the root, this has the unintended consequence of creating a higher density and activity of microbes at the root-soil interface (Figure 1.7). The release of root exudates into the rhizosphere directly influences

composition of rhizosphere microbiome upon plant defence response (Carvalhais et al., 2015). Activation of these immune responses ultimately induces systemic acquired resistance (SAR). Plants also have induced systemic resistance (ISR), which is activated by non-pathogenic microorganisms at the root surface (rhizosphere). The role and mechanisms of SAR in controlling the microbiome are widely documented across different plant taxa (Turner et al., 2013a). However, SAR is highly variable (e.g. translational control, intracellular signalling) and it is difficult to discern with absolute certainty how a plant will respond to microbial invasion (Zhang et al., 2020). This strongly suggests that radial communication of signal molecules between the root endodermis and rhizosphere plays a critical role in modulating plant immune response and thus rhizosphere composition. At the root-soil interface, there is increased abundance of PGPR (Figure 1.7a), root-endophytes (Figure 1.7b), arbuscular mycorrhiza (AMF), ectomycorrhiza (EcM) (Figure 1.7), and rhizobacteria (Figure 1.7d). These are highly specialised niches and typically occupied by plant mutualists, and the plant actively releases signalling compounds to attract these symbionts (Figure 1.7C).

Overall, plant-mediated selection of soil-borne microbes and their interactions become less complex with proximity to the root (Figure 1.7). Although widely accepted, plant-mediated selection of the rhizosphere microbiome does not fully expound on the diversity and composition of these communities (Buee et al., 2009). For instance, a network of diazotrohic microbes in wheat fields was less complex than bulk soil network structures and were highly modulated by soil pH (Fan et al., 2018). Similarly, in contaminated soils co-occurrence network structures of rhizosphere communities were less complex (Luo et al., 2021).

1.1.3 The plant holobiont response to colonisation

Plant holobiont interactions are immeasurably complex, but current research indicates that hosts play a significant role in selecting the composition of their microbiome and the plant immune response is key to this selection processes. The host's response to microbes depends on the nature of the microbe (pathogen or symbiont). Briefly, microbe-associated molecular patterns (MAMPs) are conserved features specific to each microbe- for instance, flagellum proteins or membrane protein structure and these initiate the plant immune system (Newman et al., 2013). Once MAMPs trigger an immune response, long or short-range signalling molecules are released and defence genes are activated; interplay between host-microbe begins with the release of effectors (which mitigate the plant's immune response) and this in turn triggers the effector triggered immunity (ETI) response by the plant (Bittel and Robatzek, 2007; Sundelin et al., 2012; Newman et al., 2013).

Both the MAMP and ETI are known as the systemic acquired response (SAR), and the role and mechanisms of SAR in controlling the microbiome are widely documented across different plant taxa (Turner et al., 2013a). The SAR response is highly variable, and it is difficult to discern with absolute certainty how plant will respond to microbial invasion. This varied immune response (between and within plant compartments) has made it difficult for researchers to determine what exactly delimits the plant microbiome, and it is unclear to what extent the microbial niches are divided within the plant microbiome and what environmental axis they divide along (Figure 1.8).

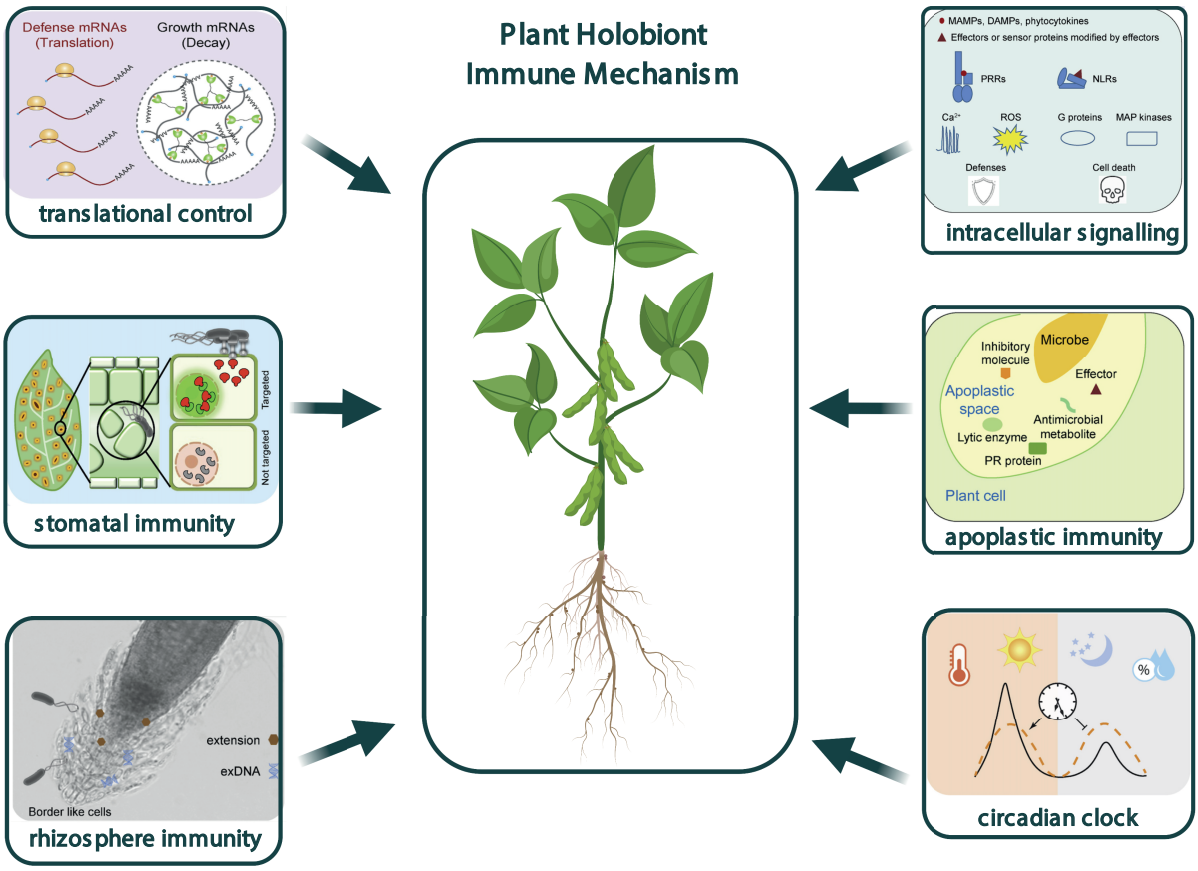

Figure 1-8 Plant holobiont immune mechanisms modified from (Zhang et al., 2020)

1.1.3.1 Translational control

Host-mediated translational control governs the utilisation efficiency of messenger ribonucleic acid (mRNA) by modulating positively or negatively the activity levels of rate-limiting protein factors in response to endogenous or exogenous signals *i.e.,* abiotic stress, biotic stress, hormones, or nutrient supply (Xu et al., 2017). At the molecular and cellular level, all biological activities within the plant holobiont are carried out by proteins. Plant regulate the synthesis of proteins by modulating the expression and release of mRNAs at the proper time and in specific tissue. Typically, these mRNA contain sequences that encode for R-motifs (resistance motifs) and uORFs (upstream open reading frames), and these ensure that right proteins are synthesised in the right amounts. Plant pathogens use the host's ribosomal machinery for protein synthesis, and this shift in plant cell biochemistry acts as a signal to actively translate pre-existing mRNA that encode for 1) defence proteins (Figure 1.9; fungal infection) and 2) or interfering RNA that silences the pathogenic mRNA (Figure 1.9; animal infection) (Yoo et al., 2020; Zhang et al., 2020). Similarly, in contaminated soils, the plant holobiont utilises translational control to mitigate the abiotic stress by upregulating the genes related to carbon and amino acid utilization in the rhizosphere (Figure 1.9; heatmap) (Yergeau et al., 2014). At the root-soil interface, plant-mediated translational control is implicated in the creation and maintenance of spatial and temporal niche spaces (Nuccio et al., 2020), and mediating the expression of genes related to nutrient cycling and mobilisation (Li et al., 2017).

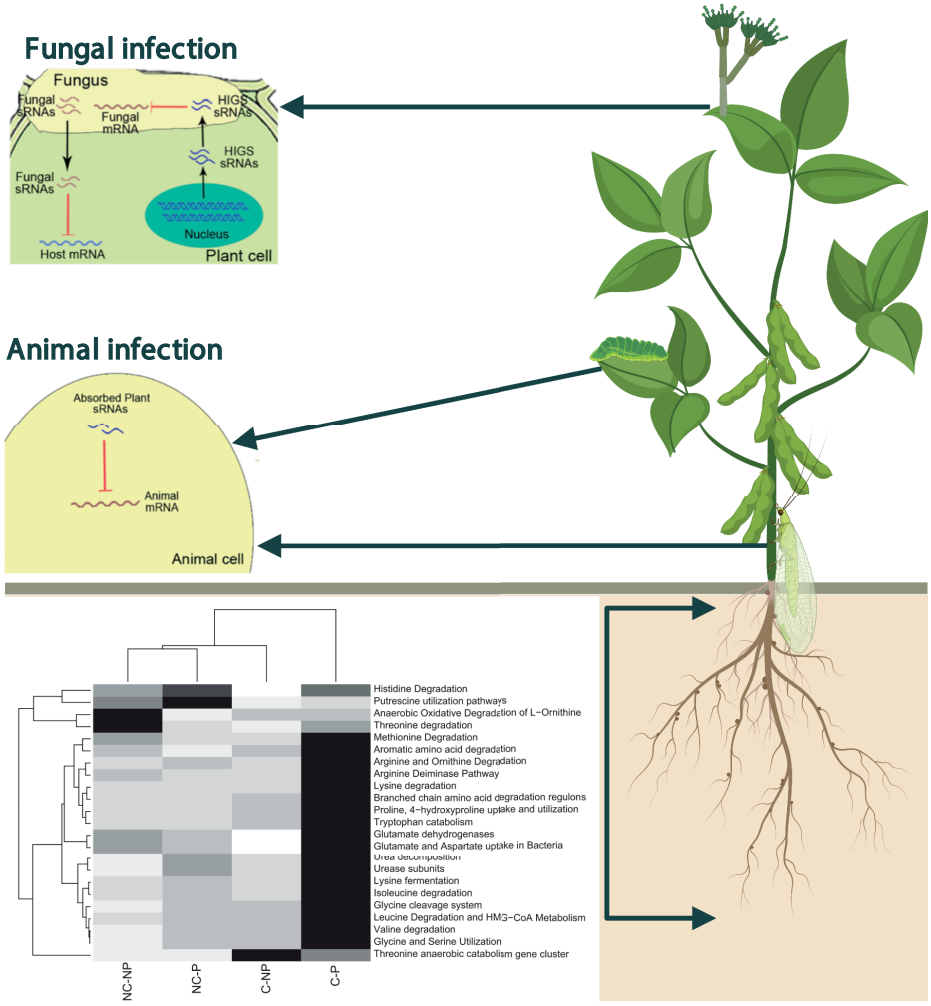

Figure 1-9 Translational control in response to plant microbiome adapted from (Yergeau et al., 2014; Zhang et al., 2020) where "C" is contaminated, "NC" non-contaminated, "P" rhizosphere, and "NP" is bulk soil

1.1.3.2 Intracellular signalling

Plant cell-to-cell communication is important in the co-ordination of key biochemical and physiological processes in response to abiotic stimuli (light, temperature, heat), biotic stimuli (microbe invasion), and cooperation of different organs to facilitate development. Intracellular signalling connects the cell surface to the nucleus, by activation of transmembrane receptors (*i.e.,* serine/threonine protein kinases) that form an integral part of the signalling cascade and targets intracellular organelles that leads to 1) changes in levels of gene expression, 2) protein conformational changes, and 3) stimulation/ inhibition of target substrate (Drobak, 1993; Yang et al., 1997). Recently, there has been interest to identify and understand the molecular mechanisms that can be engineered to abate biotic and abiotic stress in agricultural crops. Pattern recognition receptors (PRR) and MAMPs initiate the immune response that mediates the microbial loads, whereas their diversity and abundance influence the diversity of the innate microbial community (Figure 1.10) (Kayum et al., 2016; Hacquard et al., 2017). Thus, interactions 1) within the holobiont and 2) between the microbes and plant immune system are critical in modulating microbial symbiosis. In susceptible plants, the detection of ligands does not elicit a plant response due to translational control that silences the expression of plant defence genes whereas, in resistant plants the detection of these ligands leads to appropriate pathogenesis response *i.e.,* strengthen of the cell wall, production of ROS (Figure 1.10). The plant immune system and response are primed by beneficial microbes against competing invaders and pathogens (Westman et al., 2019), herbivory attacks (Yi et al., 2009), volatile organic compounds (VOCs) (de la Porte et al., 2020b), and airborne defence cues from conspecifics (Wenig et al., 2019).

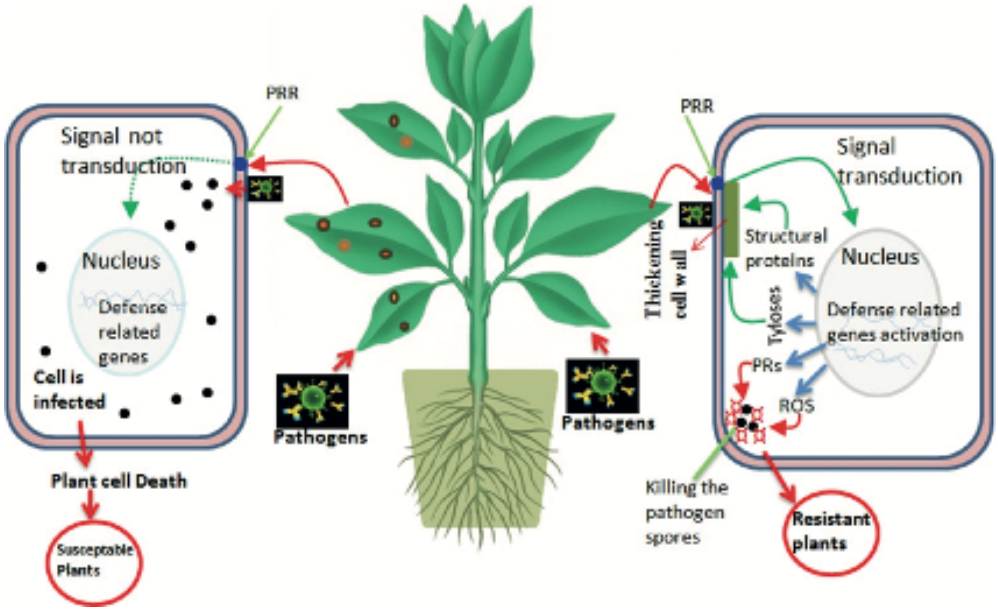

Figure 1-10 Plant intracellular signalling in susceptible and resistant plants (Kayum et al., 2016) where "PRR" pattern recognition receptors, , "PR" pathogenesis related, "ROS" reactive oxygen species.

1.1.3.3 Apoplastic immunity

The apoplast is formed by adjoined cell walls of adjacent plant cells and in the roots is interrupted by the Casprian strip, whilst in vascular tissue the extracellular space form part of the apoplast. The apoplast is a resource replete environment with adequate water and nutrient to support uninhibited microbial growth, however the apoplast is an active site where there increased immune response (Wang et al., 2020b; Godson and van der Hoorn, 2021). In fact, four major catalytic classes of proteases are constantly secreted *i.e.,* cysteine proteases, serine proteases, metallo proteases, and aspartic proteases and these strictly coordinate and control plant immune response by modulating 1) immune hydrolase activation, 2) damage-associated molecular patterns (DAMP) release, 3) direct antimicrobial activity, 4) hypersensitive response (HR), 5) effector perception, and 6) regulation of SAR and priming (Sattelmacher, 2001; Qi et al., 2017; Godson and van der Hoorn, 2021) (Figure 1.11). With that being said, symbiotic microbial partners have evolved strategies of abating plant immune response, for example, nitrogen-fixing *Sinorhizobium meliloti* evolved mechanism through divergence of MAMPs (Felix et al., 1999), while *Bradyrhizobium japonicum* secretes nodulation (Nod) factors to subdue the immune response and facilitate nodule formation in soybean (Liang et al., 2013; Im et al., 2014). Consequently, pathogenic microbes have also evolved mechanism of avoiding detection by the plant immune responses. Of these, *Pseudomonas syringe* is one the most common and well-studied and this pathogen uses effectors proteins along with toxin to incapacitate PAMP-incudes immune response within the apoplast (Sattelmacher, 2001; Xin et al., 2018). In all, successful invasion of the apoplast by either symbiont or pathogen involves avoidance of this initial step in plant immune response.

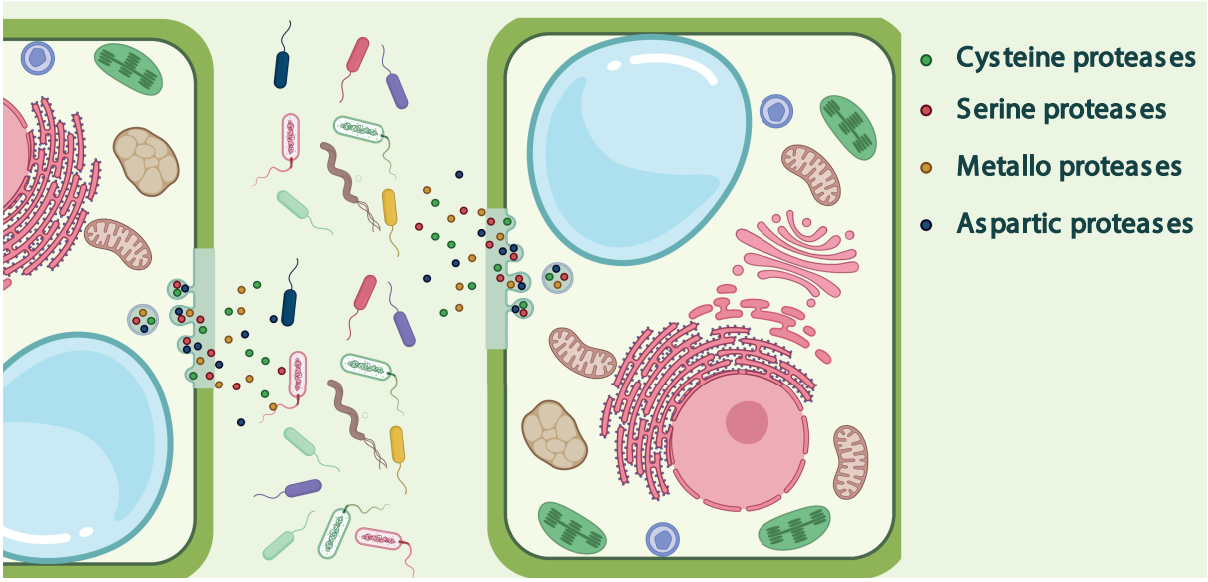

Figure 1-11 Apoplastic immune response adapted from Godson and van der Hoorn (2021) where adjoining plant cells are releasing a cocktail of apoplastic immune proteases into the extracellular space

1.1.3.4 Stomatal immunity

The stomata are opening formed by guard cells on the epidermis of the phyllosphere and are mainly involved in the exchange of water and gases between the endosphere and atmosphere, acting as the physical barrier to microbial invasion and initiating the plant immune response (Zeng et al., 2010). Stomatal immunity involves the perception of MAMPs or pathogen-associated molecular patterns (PAMPs) by PRR and begins a signalling cascade that initiates the immune response. This signalling cascades results in stomatal closure but beneficial microbe along with pathogens have evolved specific virulence factors and strategies to cause stomatal re-opening (Melotto et al., 2006; Melotto et al., 2017) (Figure 1.12). Plant hormones such as abscisic acid (ABA) which closes the stomata (Sirichandra et al., 2009), jasmonate (JA) open the stomata (Okada et al., 2009) and salicylic acid (SA) is implicated in regulating the stomatal immune response (Melotto et al., 2006; Zeng et al., 2010) are induced by perception of MAMPs. Invading foliar microbes for instance- *Pseudomonas syringae* have been shown to disrupt this hormonal crosstalk by producing phytotoxins (Sheard et al., 2010; Gimenez-Ibanez et al., 2017) and releasing effectors that interrupt perception by MAMPs (Ma et al., 2015; Melotto et al., 2017). Consequently, successful invasion of the phyllosphere through the stomata inextricably linked to abiotic conditions, increased humidity, rainfall, or frost (Melotto et al., 2006; Underwood et al., 2007). It seems that successful colonisation of the endosphere either by beneficial microbes or pathogen is to some extent modulated by environmental conditions.

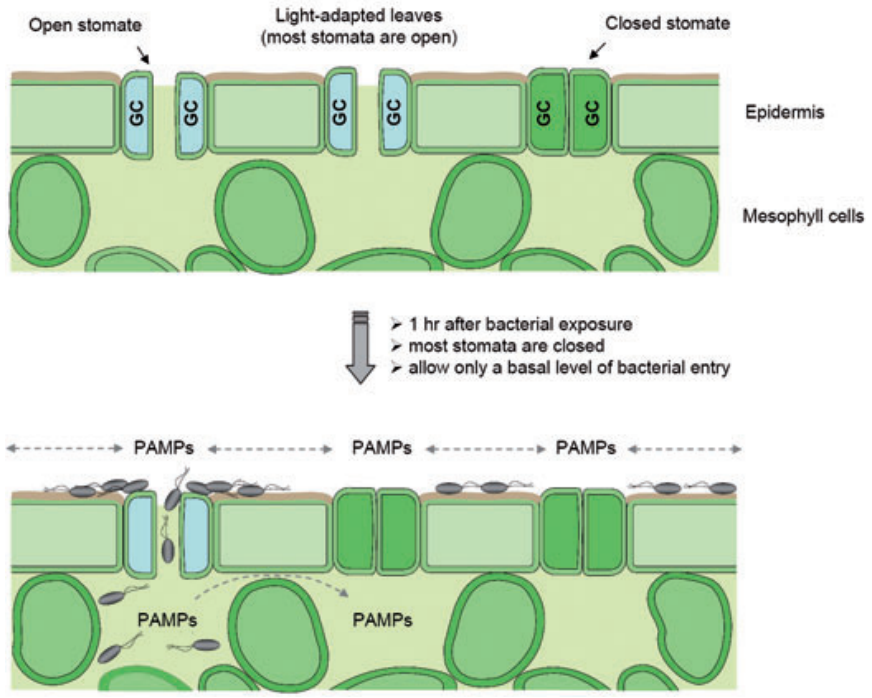

Figure 1-12 Stomatal closure and reopening in response to microbial invasion (Underwood et al., 2007)

1.1.3.5 Rhizosphere immunity

The rhizosphere microbiome plays a crucial role in promoting plant fitness and modulating plant physiology and soil abiotic conditions (Mendes et al., 2013; Turner et al., 2013b). Recently, the diversity of the rhizosphere microbiome has been shown to include taxa from different trophic levels and that these interact to promote plant health (Mendes et al., 2013; Xiong et al., 2020b). These microbes interact with the plant and prime the immune response by activation of induced systemic resistance (ISR). However, it is signalling within the rhizosphere that highly modulates the plant immune response, that is, 1) microbe-microbe signalling, 2) plant-microbe signalling, and 3) microbe-plant signalling (Figure 1.13) (Rasmann and Turlings, 2016; Venturi and Keel, 2016). The rhizosphere microbiome is considered to be an extension of the plant phenotype (de la Fuente Canto et al., 2020), in fact, parts of the plant immune response to invading pathogenic microbiome is delegated to beneficial microbes (Yu et al., 2019; Lee et al., 2021). Although interspecific (beneficial-pathogen) and intra-specific (pathogen-pathogen) microbe competition goes some way to account for plant defence, it is the release of plant exudates that accounts for a large proportion of rhizosphere immunity. Specifically, this role is played by the root extracellular trap (RET) which is composed of border cells, mucilage, polysaccharides, extracellular DNA, and antimicrobial compounds that are released at the growing tip of roots (Brinkmann et al., 2004; Driouich et al., 2013; Chuberre et al., 2018). Inter-species variations occur in the production and secretion of RET, however when plant defences are elicited there is marked increase in the production of RET (Curlango-Rivera et al., 2013a). In legumes, the distribution and retention of RET was improved when treated with compost extract which increased protection against fungal pathogens (Curlango-Rivera et al., 2013b). Recently, evidence is suggesting that virulence factors from pathogenic microbes evolved as counter-defence against RET (Hawes et al., 2016; Park et al., 2019), thus, highlighting the importance of signalling in rhizosphere as an extension of the plant immune response to microbial colonisation- whether friend or foe. In soybean, plant developmental stage and soil abiotic properties were shown to be important delimiting factors that structured the microbial community capable of producing DNase in the rhizosphere (Kamino and Gulden, 2021). In part, this suggests that interactions between microbes within the rhizosphere also shape the composition of RET- for instance, beneficial microbes have been shown to release microbial nuclease inhibitors that counter the onslaught from the invading pathogens (Hawes et al., 2016). Likewise, it seems that the presence of *Phytophthora* peptide elicitors (PEP-13) does not influence the abundance and composition of the released extracellular DNA in the RET of soybean (Chambard et al., 2021). Taken altogether, rhizosphere immunity is an interplay between plant RET, beneficial microbes, and soil abiotic factors working together to thwart prevalence of pathogens.

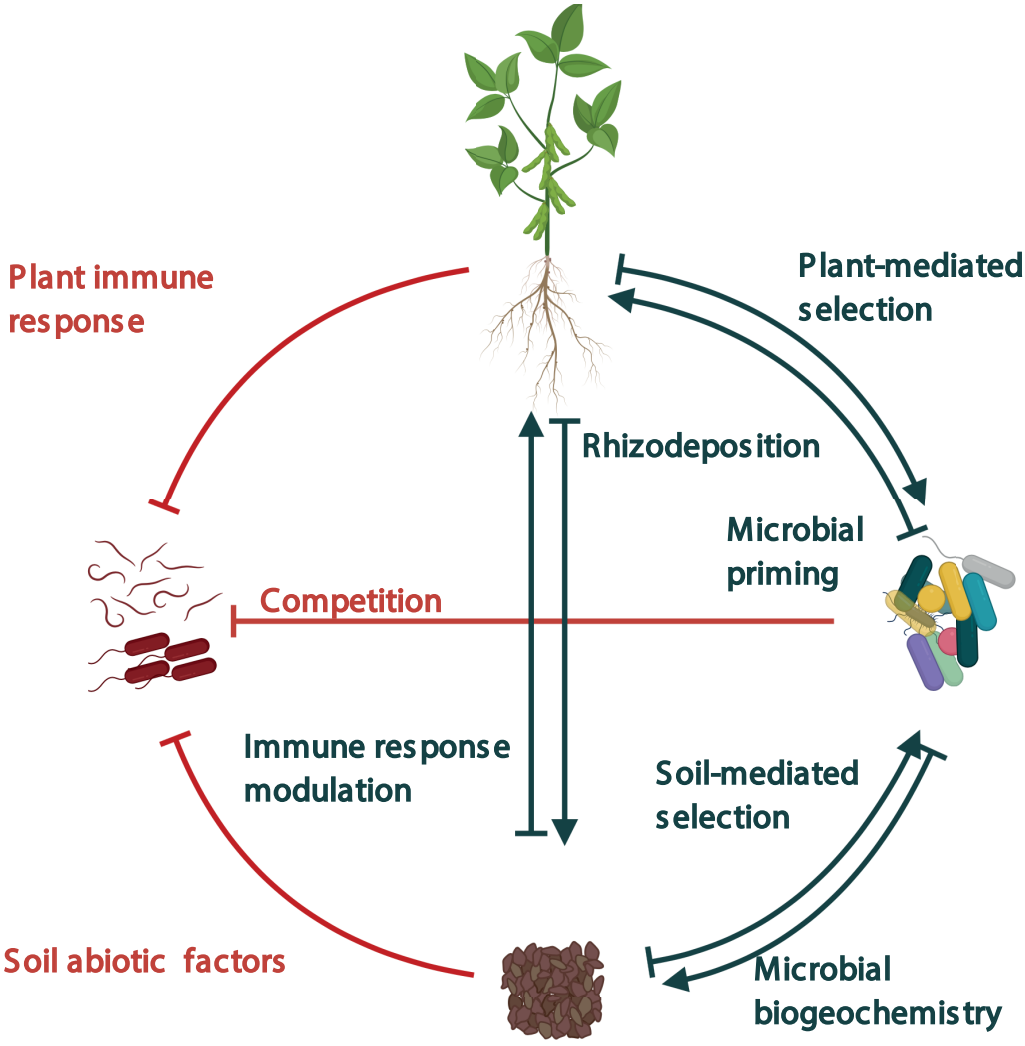

Figure 1-13 Rhizosphere interactions and immunity, with red lines indicating inhibition blue lines indicating stimulation.

1.1.3.6 Circadian clock

The plant circadian clock refers to a rhythm that mediates plant metabolic functioning and synchronises with light cycle of the surrounding environment, and influences aspects of plant physiology including but not limited to organ development, stomatal activity, signalling, and growth (Figure 1.14) (Webb, 2003). Fundamentally, the circadian clock consists of period (24 hours), phase, amplitude, and zeitgebers (internal and external cues that can reset the clock), microbial presence, light, temperature, and metabolic status (Lu et al., 2017). As these zeitgebers can influence aspects of the clock (phase and/or amplitude; red lines in Figure 1.14), it has the unintended consequences of manipulating signalling within the holobiont and thus influencing microbial colonisation patterns (Sharma and Bhatt, 2015). The circadian clock maintains a strict control over the expression and production of the major growth hormones (auxin, cytokinins, ethylene, gibberellic acids, abscisic acid, and brassinosteroids) and defence hormones (jasmonic acid and salicylic acid) (Atamian and Harmer, 2016). For instance, in *Arabidopsis thaliana* the circadian clock has been shown to be involved in priming, that is, the hormonal response to infection by *Pseudomonas syringae* varied by time of day (Bhardwaj et al., 2011; Lu et al., 2017). The molecular mechanism that underpin how the circadian clock regulates the expression of genes involved in the immune response such that the holobiont fitness is increased are widely documented (Srivastava et al., 2019), even in soybean (Wang et al., 2020c). Lastly, successful colonisation of plant depends on the tripartite interactions (the disease triangle), that is, 1) susceptible host, 2) virulent pathogen, and 3) a conducive environment (Francl, 2001). It is then poignant to highlight that any effort to try and engineer the holobiont depends on understanding the circadian clock molecular mechanism that regulate this disease triangle.

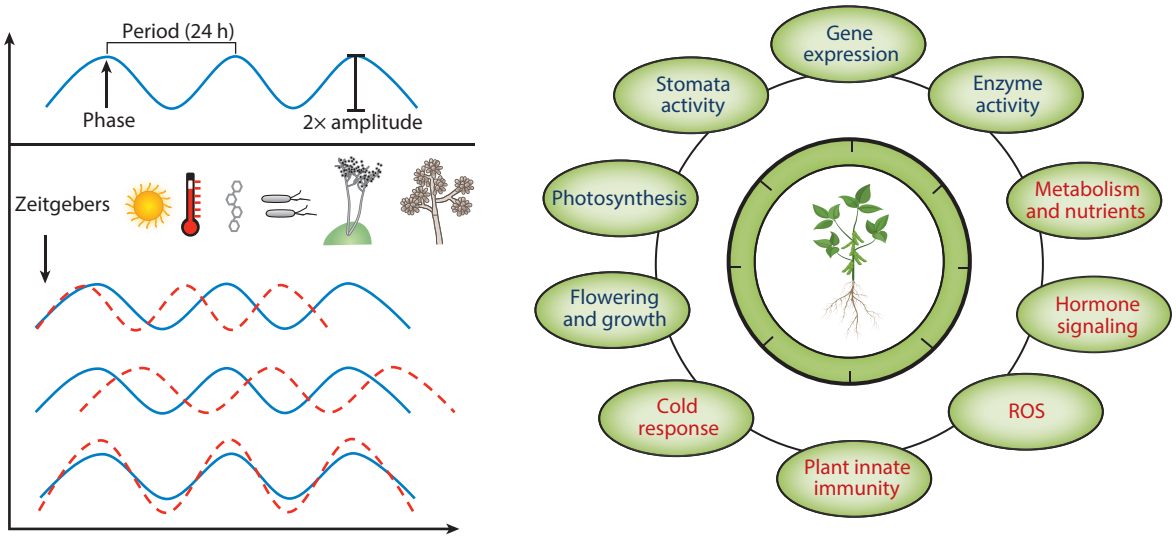

Figure 1-14 Plant circadian clock influence on plant metabolic function and immune response adapted from (Lu et al., 2017)

1.2 **The Study Model**

1.2.1 Soybean cultivar development

Drought and pathogen incidents are amongst the most significant threats to global agriculture, and will become more frequent and intense as climate change strengthens (Solomon et al., 2007). In Canada, this will particularly affect the eastern provinces that will see changes in annual precipitation. Soybean is economically the most important bean, that is a source of proteins for human and animals, and industrial products, including vegetable oil. Canada is the 7th biggest producer of soybean in the world, producing around 6.04 million metric tons of soybean in 2019 (FAOSTAT 2021 http://faostat.fao.org/). Simultaneously, the worldwide demand for soybean is increasing by roughly 1.3% annually, while current global production is insufficient to meet future demand (Ray et al., 2013). There is therefore a need for alternative approaches to improve soybean yields under sub-optimal conditions. Although there are numerous reports on the genetic, molecular and physiological bases of how plants respond to biotic stress (Langenbach et al., 2016), the nature of plant adaptation to high stress remains unresolved.

The soybean (*Glycine max*) cultivar used in these studies is AAC Edward was a cross between OT99-13 / OT02-18 / 2 / 90A01, conducted in 2003 at the Agriculture & Agri-Food Canada, Ottawa Research and Development Centre, Ottawa, Ontario. The cultivar is shorter with an average of height 40 ± 4.1 cm, the hairs on the main stem are tawny, violet flowers, the seeds are mustard yellow with a yellow hilum, and the plant mature earlier (AAFC 2021 https://inspection.canada.ca/english/plaveg/pbrpov/cropreport/soy/app00009627e.shtml). This cultivar was developed specifically for the Canadian climate and is well-adapted for short growing season and environmental conditions.

1.2.2 Developmental stages

The developmental stages of soybean are divided into the 1) vegetative stages and 2) reproductive stage with each stage subdivided numerically. The vegetative stage is further subdivided to denote the emergence stages (VE) and cotyledon stage (VC) (Figure 1.15). The vegetative stages are classified by the number of unrolled and fully developed uppermost node, that is, the edges of the leaflets are no longer touching and fully expanded. The reproductive stages are classified by the development of the reproductive organs, for instance, R1: beginning flowering, R3: beginning pod, R6 full seed, R7 beginning maturity, and R8 full maturity. It is worth noting that soybean development is highly influenced by environmental factors (irradiance received, climate, etc.), soil nutrient status, and to some extend soil management practices (Hodges and French, 1985; Pedersen and Lauer, 2004).

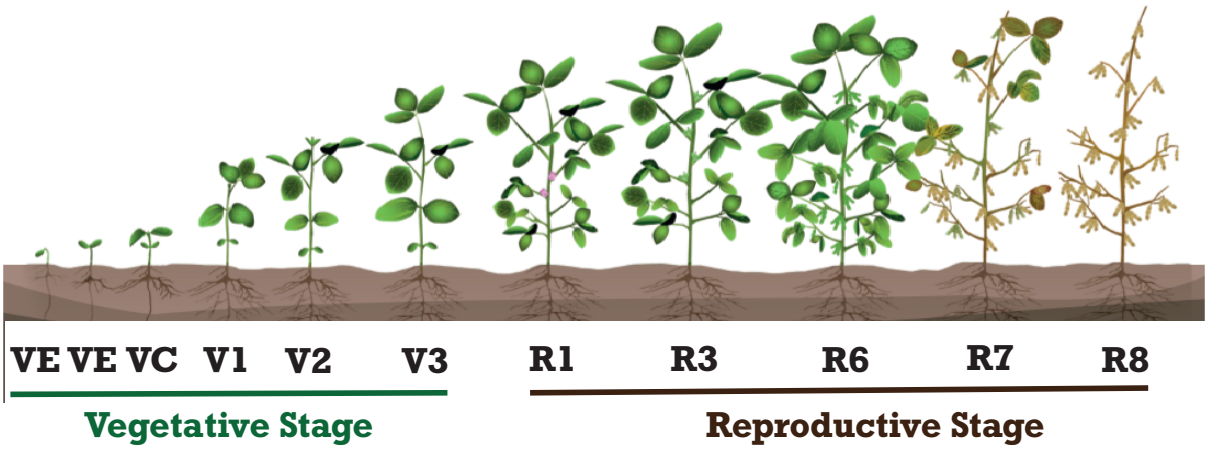

Figure 1-15 Developmental stages of soybean

1.2.3 Soybean rhizobia symbiosis and microbiome

One of the most valuable crops contributing to soil nitrogen is soybean, which develops a symbiosis with rhizobia. The bacterial endophytes of soybeans are known to play a critical role in the growth and resilience of the host. However, the exact roles and interactions of these organisms within the holobiont are not well-understood. Soybean contributes significantly to the soil nitrogen by developing a symbiotic relationship with certain rhizobia (Iannetta et al., 2016). The amount of N_2 that can be fixed depends on the bacterial symbionts living in the soil and the acidity and environmental conditions. Nodulation will only occur when the plant is in low N status (Albareda et al., 2009). Legumes prefer to take up soil nitrogen because it is metabolically cheaper to do so and N-fixation is restricted by the amount of fertilizer added to the soil. The host plant selection of rhizobia endophytes is influenced by various factors such as soil conditions and geographic locations. It is uncertain if the same rhizobia species are selected in all the root nodules of a plant (Olivares et al., 2013). Also, if the rhizobia are selected in individual nodules, the size or location of the nodule may vary. Although it can be observed that evolution tends to favour one species over another, this symbiosis can be maintained through natural selection (Young and Haukka, 1996; Chen et al., 2005; Tian et al., 2012). As part of the symbiosis, the bacteria secrete Nod factors, and when the plant is recognized as a participant in the symbiosis, it triggers various physiological responses. The plant recognition gene, known as the SYMRK, plays a crucial role in perceiving the various rhizobial Nod factors (Long, 1995; Stacey et al., 1995). The process of nodulation is a coordinated effort between the host plant and the Rhizobium bacteria. The host root contains a high concentration of flavonoids; these compounds are released by the host plant and interact with the bacterial nodD gene, and autoregulation of flavonoid secretion stimulates the production of the Nod factor (Stacey et al., 1995). This process is very rapid and involves the disruption of the cell wall, and the bacteria are then attached to the end of the root hair and stimulate the cell division in the root cortex. Additionally, non-rhizobial endophytes (NREs) and rhizobia endophytes are known to have a high diversity within the root nodules of soybeans (De Meyer et al., 2015; Zhang et al., 2018c). However, it is not known if the NREs are selected by the host plant or by random selection.

The soybean microbiome has in recent year been extensively studied across different soybean-producing regions and seasons. The soybean microbiome within the phyllosphere is highly variable and significantly influenced by temporal dynamics (seasonality and circadian rhythms) and less so by genotype (Copeland et al., 2015). This temporal variation in soybean

phyllosphere composition is influenced by differences in the environmental conditions, for instance, irradiance (Saber et al., 2014) and nitrogen fertilisation (Ikeda et al., 2010a; Ikeda et al., 2010b). Lastly, the belowground (root and rhizosphere) soybean microbiome is distinctly characterised by presence of PGPB (plant growth-promoting bacteria) and rhizobia. The microbial community of the rhizosphere is different from that found in bulk soil, and some of the taxa that are commonly recruited are also enriched in the soil (Zhang et al., 2018b). The comparison of the soybean's microbial community to the host genotype provides a comprehensive framework for studying the assembly process of the rhizosphere. It highlights the importance of incorporating plant genetic variability into the development of synthetic microbiomes (Liu et al., 2019a). The rhizosphere microbiome plays an integral role in the development and management of the rhizobia-soybean symbiosis and mitigation of stress (Han et al., 2020). Overall, understanding both temporal and spatial dynamics of the soybean microbiome will promote microbiome engineering efforts and promote plant health.

There are two approaches to plant microbiome engineering. A bottom-up approach that includes the isolation, evolution, and reintroduction of specific microorganisms, and a top-down approach that includes synthetic ecology using horizontal gene transfer to a wide range of plant host *in situ* and then characterisation of the microbiome (Ke et al., 2021). Modification and engineering this microbial assemblage to increase its functionality and confer added benefits to the host has been widely discussed (Mueller and Sachs, 2015; Quiza et al., 2015). There are three primary approaches that have been proposed to engineer the plant microbiome: synthetic microbiome (known consortia of microbes with specific functions are assembled into a community), microbiome manipulation (use of gnotobiotic system and changing specific parameters i.e. pH to elucidate the role of the microbiome in influencing host's performance), and host-mediated microbiome selection (beneficial microbial consortia are selected by the host i.e. only those that show to increase the host's performance are selected) (Mueller and Sachs, 2015; Agoussar and Yergeau, 2021). Of all these approaches, host-mediated microbiome selection is most efficient as it uses selection mechanisms that are evolutionary innate to the host (Mueller and Sachs, 2015); furthermore, any trait that is expressed by the host is tightly linked to the microbes associated with the host.

This thesis explores the interaction between plant and microbiome across spatial and temporal axis to highlight both dominant and nuanced processes that modulate the soybean microbiome, and identifies key intervention points for microbiome engineering effort. Thus, the

works presented in this thesis are timely and will lay the foundation for future research on soybean-microbe interactions and phytobiome ecology.

1.3 Hypotheses and Objectives

1.3.1 Hypotheses

The overall hypothesis of this thesis is that there exist spatial and temporal microbial niches spaces within the soybean microbiome, and these niches spaces are under strict plant-mediated selections.

The specific hypotheses of the thesis:

1. The soybean microbial communities will be strongly influenced by plant compartments and growth stage, and most importantly, by the interaction between these two factors.

2. As plants maintain a strict control of their internal environment, the assembled microbiome will be under constant niche-based selection across spatial (plant compartment) and temporal (developmental stages) axes with neutral processes (dispersal limitation) playing a minor role.

3. The innate seed microbiome will be the primary coloniser of the soybean roots and shoots whereas the rhizosphere will be colonised primarily by the soil microbial communities.

1.3.2 Objectives

The overall objective of the thesis is to investigate the spatio-temporal colonisation patterns of different soybean microbial communities and their prevailing ecological assembly processes.

The specific objectives of this project are:

1. To determine the successional pattern of plant microbiome communities in soybean and determine which spatial and temporal axis they divide along.

2. To elucidate the relative importance of neutral and niche-based processes in delimiting soybean plant microbiome.

3. To discern the source of plant microbial communities and understand microbiome transference in soybean.

2 CHAPTER 2 : PUBLICATION 1

2.1 **Temporal and Spatial Interactions Modulate Soybean Microbiome Succession and Diversity**

2.2 **Les interactions temporelles et spatiales modulent la succession et la diversité du microbiome du soja**

Moroenyane, I[a]., Tremblay, J[b] and Yergeau, É[a]*

a) Institut National Recherche Scientifique, Centre Armand-Frappier Santé, 531 Boulevard des Prairies, Laval, Québec, H7V1B7, Canada

b) Energy, Mining, and Environment, Natural Resource Council Canada, 6100 Avenue Royalmount, Montreal, Quebec, H4P 2R2, Canada

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Author contributions

I Moroenyane contributed to content and successful publication of the article. Itumeleng Moroenyane designed, performed the experiments and the statistical analyses, and wrote the manuscript. J Tremblay perfomed the bioinfomatics analysis and conributed to revising the manuscritp. É yergaeu obtained the research funding, supervised the project and participated in writing and revising the manuscript.

2.3 Abstract

Managed agricultural ecosystems are unique systems where crops and microbes are intrinsically linked. This study focuses on discerning microbiome successional patterns across all plant organs and tests for evidence of niche differentiation along temporal and spatial axes. Soybean plants were grown in an environmental chamber till seed maturation. Samples from various developmental stages (emergence, growth, flowering, and maturation) and compartments (leaf, stem, root, and rhizosphere) were collected. Community structure and composition were assessed with the 16S rRNA gene and ITS region amplicon sequencing. Overall, the interaction between spatial and temporal dynamics modulated alpha and beta diversity patterns.

Time lag analysis on measured diversity indices highlighted a strong temporal dependence of communities. Spatial and temporal interactions influenced the relative abundance of most abundant genera, whilst Random Forest predictions reinforced the observed localisation patterns of abundant genera. Overall our results show, spatial and temporal interactions tend to maintain high levels of biodiversity within the bacterial/archaeal community, whilst in fungal communities, OTUs within the same genus tend to have overlapping niches.

2.4 Introduction

The plant microbiome consists of the combined microbial communities that reside on and within the plant; these communities have an intrinsic relationship with their plant hosts (Berg et al., 2016). The plant microbiome is an assemblage of archaea, bacteria, micro-eukaryotes, and virus communities that inhabit various plant organs with little overlap in taxonomic and functional composition (Turner et al., 2013b; Berg et al., 2014; Rout, 2014). The distribution of these communities across space and time and how they are influenced by plant genotype (Lundberg et al., 2012), plant species (Copeland et al., 2015), climate change (Compant et al., 2010b), and plant nutrient status (Dakora and Phillips, 2002) was extensively studied. These studies highlighted the existence of spatial and temporal niches and how these are partitioned amongst and within communities.

Evidence of niche differentiation in creating a spatially heterogeneous environment and subsequently distinct archaea, bacteria and eukaryote communities is well documented in the root-soil interface of model plant systems (Bulgarelli et al., 2012; Duran et al., 2018). However, compared to the root-soil interface, the existence of clear niche differentiation in other plant organs and non-model plant species and their influence on microbiome succession and diversity is poorly understood. Recent advances have shown the existence of niche differentiation within the plant endosphere of poplar (Beckers et al., 2017), and begun highlighting how plant organs and biogeography strongly influence microbial niches in crops (Coleman-Derr et al., 2016).

In agricultural systems, seasonality and interactions within and between microbial communities are essential factors influencing foliar microbiome succession and diversity (Copeland et al., 2015; Ottesen et al., 2016). It seems in part that niches created by changes in plant metabolism significantly influence the diversity and structure of the microbiome, and temporal dynamics are a robust discriminatory axis on which these communities are distributed (Shade et al., 2013; Tkacz et al., 2015). Although widely accepted, plant-mediated selection of the microbiome does not fully expound on the observed immense diversity and composition of these communities.

In field experiments, soybean rhizosphere bacterial communities were shown to be sharply delimited by soil type and developmental stages (Xu et al., 2009), and spatial heterogeneity (rhizosphere-bulk soil) (Mendes et al., 2014). Contrariwise, fungal communities are

relatively stable over a growing season, but noticeable differences were observed between rhizosphere and bulk soil (Sugiyama et al., 2014a). Additionally, similar spatial and temporal dynamics have been highlighted within the leaf endophytic community of transgenic and conventional soybean cultivars (Montanari-Coelho et al., 2018). In the case of soybean, it seems that the influence of cultivar on microbiome diversity and succession is negligible (Xu et al., 2009; Copeland et al., 2015). These studies have shed light on microbial dynamics within the soybean rhizosphere. However, the role of spatial and temporal dynamics in influencing the entire soybean microbial community succession is lacking

Earlier efforts to manipulate the plant microbiome identified the molecular mechanisms that were influencing the plant-microbe interaction (Fray, 2002). However, even these earlier studies highlighted the role of interactions in plant microbiomes as important starting points for microbiome engineering. A recent review systematically outlines evidence to highlight the role of plant circadian rhythm, temperature, and nutrient needs (Cheng et al., 2019) on plant microbiomes. It seems then that, the key to improving attempts at engineering the plant microbiome ought to consider the following: 1) influence of spatial and temporal interactions between and within plant microbiomes on composition and structure, 2) influence of environmental conditions on the plant microbiome, and 3) influence of plan metabolic processes on the plant microbiome.

Here, using both bacterial and archaeal 16S rRNA gene and fungal ITS region sequence datasets, we hypothesized that the soybean microbial communities would be strongly influenced by plant compartments and growth stage, and most importantly, by the interaction between these two factors.

2.5 **Methods**

2.5.1 Plant growth conditions

Soybean plants (Pioneer: P19T01R) were grown with an 18-hour photoperiod at 25 °C followed by a 6-hour dark period at 20°C in a Conviron growth chamber (Winnipeg, Canada). Plants were supplied with a modified Hoagland's plant nutrient solution biweekly (Moscatiello et al., 2013). Plants were destructively sampled at each developmental stage V1 (emergence), V3 (growth), R1 (flowering), and R3 (maturation). Samples were collected from rhizosphere, root,

stem (between first and second internodes), and leaf (youngest and oldest trifoliate). A total of five plants were destructively sampled at each developmental stage, and DNA extraction was performed right after sampling.

Control seeds from cultivar were imbibed in sterile water for 24 hours at 4°C. To remove all traces of any contaminants attached to the seeds, the seeds were rinsed excessively with sterilised water. The seeds were then airdried in an open petri dish, and when moderately dry, the petri dishes were sealed. All works were performed under sterile conditions in laminar flow hoods. Seeds were crushed in liquid nitrogen using a sterile pestle and mortar. For each sample, 0.25 g of the crushed tissue was added to the bead tubes from the Qiagen Power Soil DNA kit (Hilden, Germany) and DNA was extracted following the manufacture's instructions except that DNA was eluted in 50 μl.

The control soil used was collected in summer of 2019 at the Institut National de la Recherche Scientifique (Laval, QC, Canada) from control unplanted plots of an experimental field that had been ploughed for the first time in 2016 with no history of agricultural practice for at least 20 years prior to that. Soils that have history of agricultural practice drastically regulate the composition of free-living microbial communities and change abundance of microbial N-cycling genes (Mendes et al., 2015; Merloti et al., 2019). For each sample, 0.25 g of the crushed tissue was added to the bead tubes from the Qiagen Power Soil DNA kit (Hilden, Germany) and DNA was extracted following the manufacture's instructions except that DNA was eluted in 50 μl. Soil edaphic properties were previously measured by AgroEnviro Lab (La Pocatiere, Quebec) and revealed an average pH of 7.2, P concentration of 193 (kg/ha), total N 0.15%, C/N of 13.1, density (g/cm^3) of 1.16, and porosity (%) of 55.14.

2.5.2 Microbiome sampling and sequencing

At each sampling period, the rhizosphere samples were considered as all the soil that was directly attached to the root surface. The epiphytic community (leaves, stem, and roots) was extracted using a modified protocol from Qvit-Raz et al. (2008). Briefly, the samples were placed in sterile 50 ml plastic Falcon test tubes (Corning, Tewksbury, MA, USA) and filled with sterile phosphate-buffered saline (PBS 0.1M, pH 7.4). The samples were then placed in sonication tub (Fisher FS20, Fisher Scientific, Waltham, USA) for 15 min and then vortexed for 10 s. The samples were then transferred into a new tube containing PBS and rinsed twice. The wash was pooled and spun down in a centrifuge at 2,000 g for 20 min, and the resulting pellet was

considered to be the epiphytic community (leaf, stem, and rhizoplane). The endophyte community was considered to be all the remaining microbes after the sonication and rinse treatment. Plant tissue was then pulverised in liquid nitrogen using a sterile pestle and mortar. For each sample, 0.25 g was added to the bead tubes from the Qiagen Power Soil DNA kit (Hilden, Germany) and DNA was extracted following the manufacturer's instructions. For all sample, the presence of DNA was visualised by electrophoresis in 1% agarose gels.

The bacterial/archaeal V4 hypervariable region of the 16S rRNA gene were amplified using 520F and 799R primer pairs, which were shown to exclude chloroplast sequences (Edwards et al., 2007) . Fungal ITS region was amplified using ITS1F and 58A2R (Martin and Rygiewicz, 2005). The average length of 16S amplicon sequences were of approximately 280 bp, and ITS sequences ranged between 250 and 493 bp. Briefly, extracted DNA was used to construct sequencing libraries according to Illumina's "16S Metagenomic Sequencing Library Preparation" guide (Part # 15044223 Rev. B), with the exception of using Qiagen HotStar MasterMix for the first PCR ("amplicon PCR") and halving reagent volumes for the second PCR ("index PCR"). The first PCR ("amplicon PCR") was carried out for 25 cycles with annealing temperatures of 55 °C. The resulting amplicons were pooled together and sequenced at the McGill University and Genome Québec Innovation Center (MUGQIC). Diluted pooled samples were loaded on an Illumina MiSeq and sequenced using a 500-cycle (paired-end sequencing configuration of 2x250 bp) MiSeq Reagent Kit v3. Bacterial and fungal sequences were processed bioinformatics using the AmpliconTagger pipeline (Tremblay et al., 2015; Tremblay and Yergeau, 2019). Briefly, raw reads were scanned for sequencing adapters, and PhiX spike-in sequences and remaining reads were merged using their common overlapping part with FLASH (Magoc and Salzberg, 2011). Primer sequences were removed from merged sequences, and remaining sequences were filtered for quality such that sequences having an average quality (Phred) score lower than 27 or one or more undefined base (N) or more than 10 bases lower than quality score 15 were discarded. Remaining sequences were clustered at 100% identity and then clustered/denoised at 99% identity (DNACLUST v3) (Ghodsi et al., 2011). Clusters having abundances lower than 3 were discarded. Remaining clusters were scanned for chimeras with VSEARCH's version of UCHIME denovo (Rognes et al., 2016), UCHIME reference (Edgar et al., 2011), and clustered at 97% (DNACLUST) to form the final clusters/OTUs. Bacterial and fungal OTUs were then assigned a taxonomic lineage with the RDP classifier (Wang et al., 2007), using the AmpliconTagger 16S and ITS training sets (Tremblay, 2019), respectively. The RDP classifier gives a score (0 to 1) to each taxonomic depth of each OTU. Each taxonomic depth having a score >= 0.5 was kept to

reconstruct the final lineage. Multiple sequence alignment was then obtained by aligning OTU 16S r RNA sequences on the SILVA R128 database (Quast et al., 2013) and ITS sequences were aligned on the ITS UNITE database (sh_taxonomy_qiime_ver7_dynamic_20.11.2016) (Kõljalg et al., 2005) using the PyNAST v1.2.2 aligner (Caporaso et al., 2010a). Alignments were filtered to keep only the hypervariable region of the alignment. Taxonomic lineages were combined with the cluster abundance matrix obtained above to generate a raw OTU table. For cross-sample comparisons, ten iterations were performed on a random subsample of 1,000 reads rarefactions, and the average number of reads of each OTU of each sample was then computed to obtain a consensus rarefied OTU table. Rarefaction curves of alpha diversity indices are provided in the supplemental material (Fig.S2-S4). Alpha (observed species) and taxonomic summaries were then computed using the QIIME v1.9.1 software suite using the consensus rarefied OTU (Caporaso et al., 2010b; Kuczynski et al., 2011). In general, final OTU tables were filtered to exclude eukaryotes from the 16S rRNA gene dataset and to keep only fungi in the ITS data. Reads that were identified as mitochondria or plastids were removed from the datasets. On average, 5 908 858 high quality ITS reads were assembled, and 4 851 927 16S rRNA gene reads were assembled.

2.5.3 Statistical analyses

The OTU abundance tables for both communities were normalised such that the summed relative abundance of all OTUs of each sample was equal to one. All statistical analyses were performed using R version 3.5.0 (R Core Team, 2020)

Alpha diversity patterns

OTU accumulation curves were constructed to determine if the sampling effort (number of samples) could recover most of the taxa, whilst the Preston log-normal curves were used to evaluate the estimated richness and occurrence of rare taxa across all samples (the presence of a normal distribution indicates a higher probability of rare taxa being represented). A three-way ANOVA was performed to evaluate the effects of developmental stage, plant organ (rhizosphere, root, stem, and leaves), and location (endophyte or epiphyte) on the number of observed OTUs (S_{obs}) and Shannon diversity indices for both communities. Pairwise comparisons were performed using Tukey's HSD tests, and where data was not normal, using distributed Kruskal-Wallis pairwise Wilcox test with Bonferroni correction applied. To determine the influence of temporal autocorrelation on diversity Augmented Dickey-Fuller (ADF) and Kwiatkowski-Phillips-Schmidt-Shin (KPSS) tests were performed using S_{obs} and Shannon diversity as input variables.

2.5.4 Microbial community structure

Principal Coordinate Analysis (PCoA) was used to visualise the community structure of the bacterial and fungal communities using the Bray-Curtis dissimilarity matrix. Multivariate dispersion and homogeneity across all samples were quantified and confirmed using ANOVA with 999 permutations. A Permutational Multivariate Analysis of Variance (PERMANOVA) test was performed with 999 permutations to assess the relative significances of temporal (developmental stage) and spatial dynamics (plant organ and location) on the community structure. Multivariate dispersion in the community data was evaluated and confirmed using ANOVA with 999 permutations.

2.5.5 Taxonomic profiles and Random Forest Models

The relative abundance of microbial taxa at the phylum and genus taxonomic levels were evaluated across developmental stages and plant organs. In both communities, random forest algorithms were used to predict the prevalence of taxa associated with each developmental stage at the genus and OTU taxonomic levels. Random forest prediction was made using the

randomForest algorithm (Liaw and Wiener, 2002) and implemented on the MicrobiomeAnalysis pipeline (Chong et al., 2020)

2.5.6 Data availability

The 16S and ITS rDNA raw reads from the microbiota analyses have been deposited at the NCBI BioProject repository under study accession number PRJNA601979.

2.6 Results

2.6.1 Alpha diversity patterns

In both cases, OTU accumulation curves indicated that a substantial amount of microbial OTUs was recovered and the sampling effort was enough to reach saturation or near saturation (Fig.S1). Furthermore, Preston log-normal curves for both taxa were shown to be a near-complete bell-shape indicating that samples were sequenced enough to detect rare and low-abundance taxa. (Fig.S1). Overall, these i*ndicat*e that the sampling effort was enough to capture not only the most abundant but also rare taxa and thus giving a comprehensive outlook of the community.

Overall, the interactions between these spatial and temporal components significantly influenced the abundance and diversity of fungal and bacterial/archaeal community (Fig.1; Fig.S6). Typically, the aboveground and belowground microbial communities are assembled and influenced by different ecological processes. As such, in addition to analysing the total community, the aboveground and belowground communities were analysed separately. At the root-soil interface, developmental stage significantly influenced the abundance and diversity of fungal (Observed OTUs χ^2=15.66***; Shannon diversity χ^2 =13.48*) and bacterial/archaeal (Observed OTUs χ^2=13.42**; Shannon diversity χ^2 =11.96*) communities (Fig.1; Fig.S6). Although there was a significant influence of the developmental stage on the abundance and diversity of both communities, pairwise Wilcox test with Bonferroni correction did not reveal any differences between the developmental stages. There were significant influences of sample site (rhizosphere, rhizoplane, and root endosphere) on the abundance of fungal (Observed OTUs χ^2=40.31***; Shannon diversity χ^2 =38.01***) and bacterial/archaeal (Observed OTUs χ^2=42.23***; Shannon diversity χ^2 =43.32***) communities (Fig.1; Fig.S6). In both communities, the rhizosphere consistently had the highest abundance and was significantly different to both rhizoplane and root endosphere (p<0.05), whilst only the bacterial/archaeal rhizoplane and root endosphere communities were marginally different from each other (p=0.08). Both the developmental stage and sample site influenced alpha diversity in both communities. In the aboveground compartment, developmental stage significantly influenced only diversity of the fungal community (Shannon diversity F value =3.77*) and influenced the abundance and diversity bacterial/archaeal (Observed OTUs χ^2=9.52*; Shannon diversity χ^2 =11.36**) communities (Fig.1; Fig.S6). Fungal alpha diversity significantly differed between the emerging and maturation stage (p<0.01; Tukey's HSD; Fig. S6). The bacterial/archaeal alpha diversity varied significantly between the emergence and flowering stage (p≤ 0.05; Wilcox test with Bonferroni correction; Fig.1; Fig.S6). Within the

aboveground communities there seem to be no influence of samples site (leaf endophyte, leaf epiphyte, stem endophyte, and stem epiphyte) on both fungal and bacterial/archaeal communities.

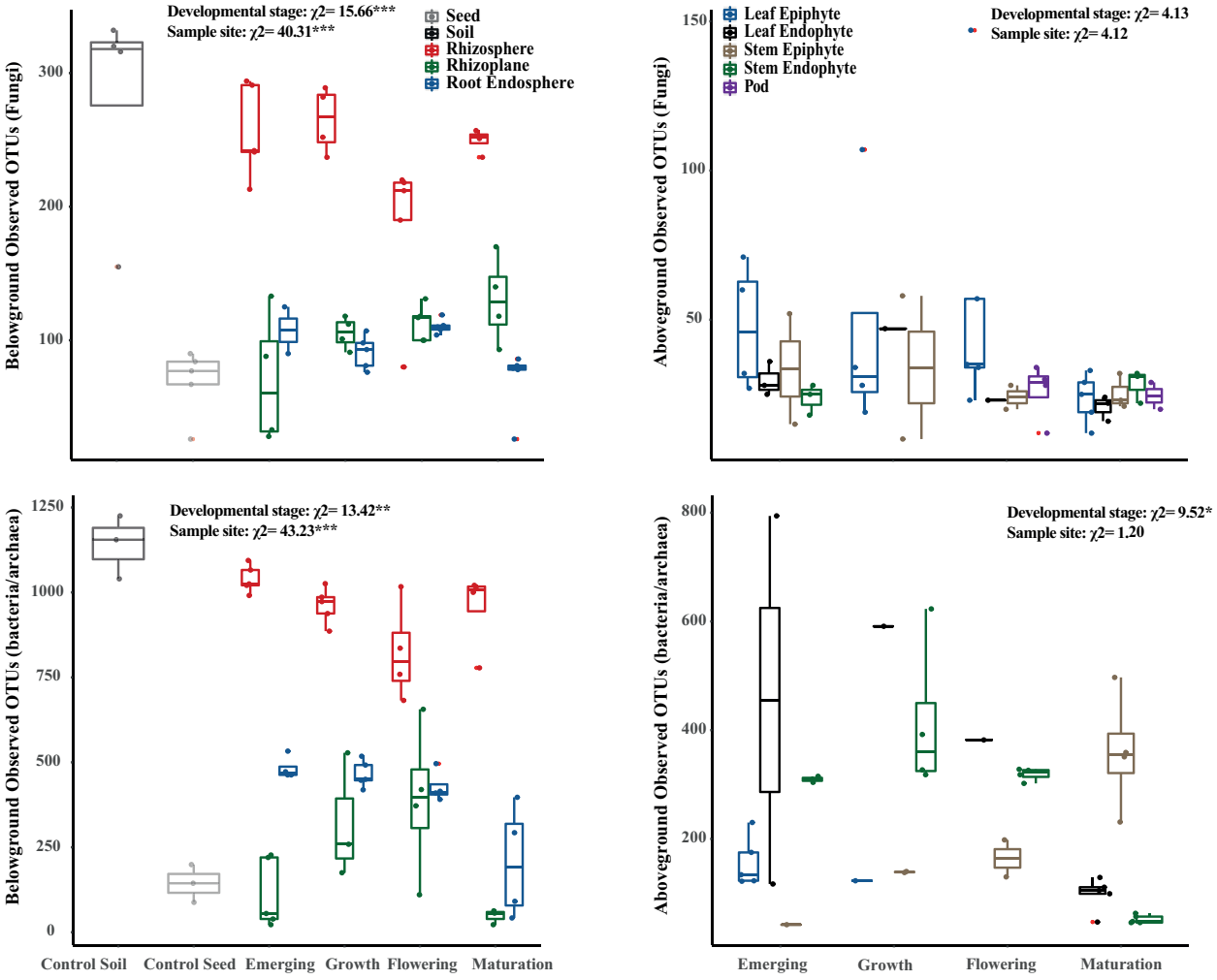

Fig.1 Belowground alpha diversity patterns. Top panel indicating fungal diversity and bottom panel indicating bacteria/archaeal diversity. Total OTU abundance influenced by developmental stage and samples site (Kruskal-Wallis chi-squared χ^2, *p*-value) and pairwise Wilcox test with Bonferroni correction (*p* value *<0.05, ** <0.01, *<0.001). Pairwise comparisons were not reported for control and pod samples. The lines inside the box indicate the mean of samples where n=3~5.**

Lastly, both analyses for temporal autocorrelation (ADF and KPSS) indicated that in both microbial communities Sobs and Shannon diversity index were temporally autocorrelated. The tests both rejected the null hypothesis that the datasets were stationary ($p>0.05$). Both tests independently arrived at the same conclusion that there is an existence of a root unit, highlighting the presence of a strong seasonal trend in microbial a-diversity patterns and indicating that these patterns are dependent upon one another.

2.6.2 Beta diversity patterns

For both communities, the Bray-Curtis dissimilarity matrix was used to infer community beta-diversity patterns and structure. Firstly, homogeneity and multivariate dispersion analysis were used to determine the relative influence of between community composition (between-sample variation) and within community composition (variation within replicates) on overall microbial community structure. In both communities, there was increased multivariate dispersion across plant organs and not developmental stages except the control (soil and seed) and pod samples (Fig.S7). When considering developmental stage, both communities displayed significant heterogeneous dispersion (bacteria: F value= 14.48, p<0.001; fungi: F value= 5.58, p<0.001; Fig.S7). Similarly, different plant organs highlighted that both communities displayed significant heterogeneous dispersion (bacteria: F value= 17.74, p<0.001; fungi: F value= 20.94, p<0.001; Fig.S7). Lastly, when considering location, i.e. endophyte or epiphyte, the multivariate dispersion analysis indicated that the endophytic and epiphytic communities had variable dispersion (bacteria: F value= 4.12, p<0.05; fungi: F value= 12.66, p<0.01).

Principle Coordinate Analysis (PCoA) was used to evaluate and visualise the microbial community structure of both communities with Bray-Curtis dissimilarity as input. For both communities, the interactions amongst variables had a significant influence on community structure (PERMANOVA, Table S1; Fig.S8). Multivariate analysis of variance of belowground and aboveground compartments recapitulated the influence of interactions in influencing community structure. Firstly, the belowground communities were significantly influenced by the interactions of developmental stage and sample site (Rhizosphere, Rhizoplane, and Root Endosphere) (Table 1). Similarly, the aboveground communities were also influenced by the interactions between developmental stage and sample site (Leaf Endophyte, Leaf Epiphyte, Stem Endophyte, Stem Epiphyte, and Pod) (Table 1). Additionally, PCoA highlighted a significant spatial and temporal niche separation amongst aboveground communities (Table 1; Fig.2; Fig.3). There was pronounced niche separation of communities along a spatial and temporal axis within the belowground compartments for both bacterial/archaeal and fungal communities (Table 1; Fig.2; Fig.3). Overall, the aboveground and belowground communities are modulated by the same spatial and temporal interactions. However, it is within the belowground compartment that the influence of these interactions is pronounced.

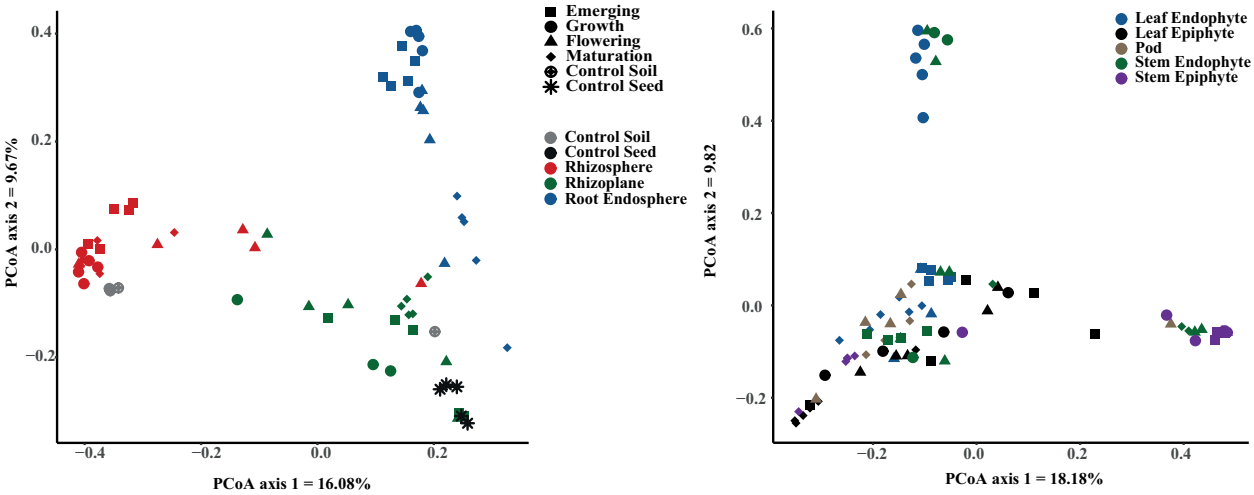

Fig.2 Principle Coordinate Analysis (PCoA) of bacterial/archaeal community based on Bray-Curtis dissimilarity across different plant compartment and developmental stages (Compartment= Control Soil, Control Seed, Rhizosphere, Root, Stem, Leaf, and Pod). Samples that are closer to each other have similar community composition, whereas samples that are further apart are distinct to each other in composition

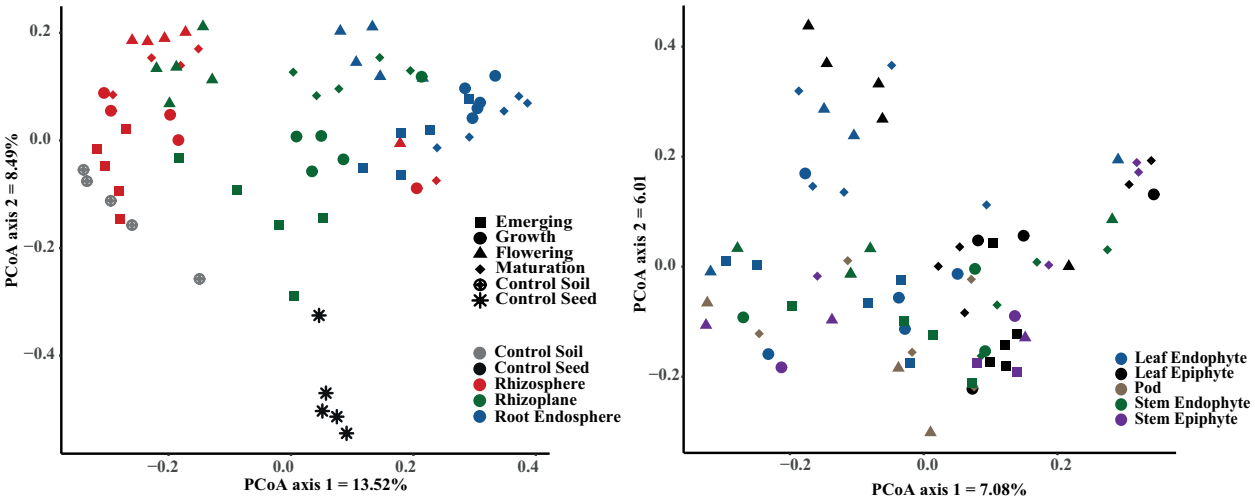

Fig.3 Principle Coordinate Analysis (PCoA) of fungal community based on Bray-Curtis dissimilarity across different plant compartment and developmental stages (Compartment= Control Soil, Control Seed, Rhizosphere, Root, Stem, Leaf, and Pods). Samples that are closer to each other have similar community composition, whereas samples that are further apart are distinct to each other in composition

Table 1 Permutation analysis of variance (PERMANOVA) results indicating the influence of temporal and spatial interaction on microbial community structure *(p* value *<0.05, ** <0.01, ***<0.001)

Bacterial/Archaeal Community	**Belowground**			**Aboveground**		
Source of variation	***MS***	***F value***	***R^2***	***MS***	***F value***	***R^2***
Developmental stage	1.00	5.91	**0.21*** **	0.90	4.52	**0.08*** **
Sample site	3.01	17.76	**0.25*** **	1.48	7.46	**0.19*** **
Developmental stage × Sample site	0.52	5.58	**0.13*** **	0.75	3.73	**0.24*** **
Residuals	0.16		0.38	0.19		0.46
Fungal Community						
Source of variation	***MS***	***F value***	***R^2***	***MS***	***F value***	***R^2***
Developmental stage	1.11	11.67	**0.30*** **	0.62	1.78	**0.06*** **
Sample site	1.23	76.48	**0.13*** **	0.71	2.06	**0.10*** **
Developmental stage × Sample site	0.23	7.30	**0.07** **	0.41	1.20	**0.14***
Residuals	0.15		0.47	0.34		0.62

Taxonomic profiles and Random Forest Models

Overall, there were variations in the relative abundance of dominant phyla across all samples for both microbial communities. For bacteria/archaea, *Proteobacteria* was the dominant phylum across all plant organs and developmental stages (Fig.S9). At the plant organ level, the relative abundance of dominant bacterial phyla was similar between the internal (endophyte) and external (epiphyte) with the noticeable increase of *Thaumarchaeota* in the root epiphyte, rhizosphere, and leaf endophyte. At the genus level, *Pseudomonas* had the highest the relative abundance in both the endophyte and epiphyte communities across all plant organs apart from the stem and pod organs (Fig.S9). There were similar taxonomic profiles at each developmental stage for the endophyte and epiphyte community at the phylum level. However, at the genus, there were divergences in the relative abundance of dominant taxa between the endophyte and epiphyte communities. Strikingly, although *Pseudomonas* was prevalent across all samples, there was a dominance of *Acinetobacter* at the growth developmental stage in the endophyte community (Fig.S9). The control seed taxonomic profile was divergent to both the immature and mature pods. However, there was a marked dominance of *Streptomyces* at the immature pods whilst the abundance of *Shigella* was higher in both mature seeds and control seed (Fig. S9). The relative abundance of the most abundant genera was comparable between above and below compartments (Fig.4; Fig.S10).

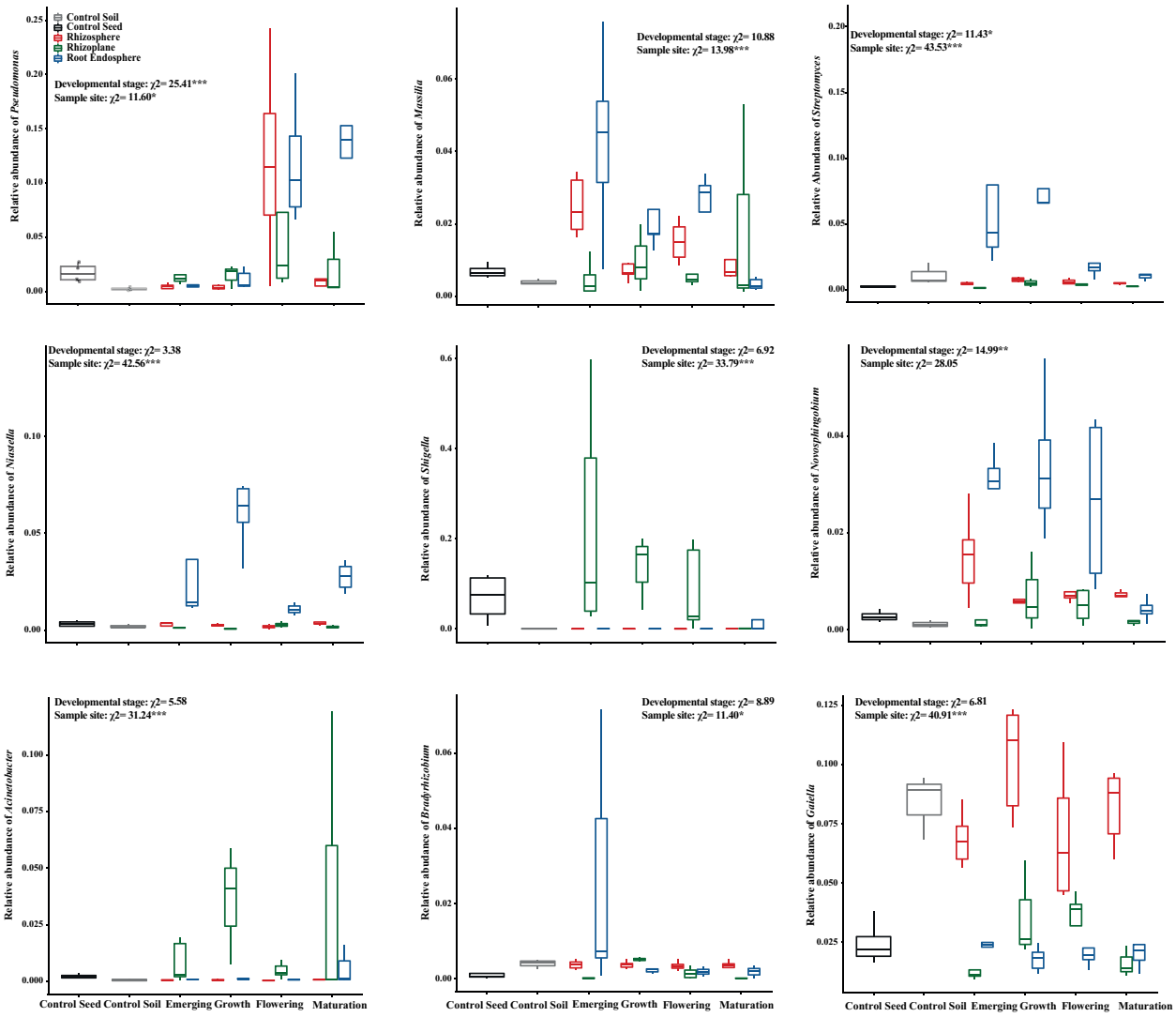

Fig.4 Belowground bacteria/archaeal relative abundance of most abundant genera that were significantly influenced by developmental stage and samples site (Kruskal-Wallis chi-squared χ^2, *p*-value) and pairwise Wilcox test with Bonferroni correction *(p* value *<0.05, ** <0.01, *<0.001). The lines inside the box indicate the mean of samples where n=3~5.**

In the belowground compartment, the relative abundance of *Novosphingobium* was exclusively modulated by developmental stage (Fig.4; χ^2=14.99**). For the rest, sample site significantly influenced their relative abundance: *Massilia* (Fig.4; χ^2=13.98***), *Niastella* (Fig.4; χ^2=42.56***), *Shigella* (Fig.4; χ^2=33.79***), *Gaiella* (Fig.4; χ^2=40.91***), *Bradyrhizobioum* (Fig.4; χ^2=11.40*), and *Acinetobacter* (Fig.4; χ^2=31.24***). *Pseudomonas* is one of two genera whose relative abundance was influenced by both developmental stage (Fig.4; χ^2=25.41***) and sample site (Fig.4; χ^2=11.60*). The other is *Streptomyces* whose relative abundance is influenced by developmental stage (Fig.4; χ^2=11.43*) and sample site (Fig.4; χ^2=43.53***). In the aboveground compartment, the relative abundance of *Pseudomonas, Streptomyces, Novosphingobium, Shigella,* and *Acinetobacter* were significantly influenced by both factors (Fig.S10).

The relative fungal abundance was mainly dominated by *Ascomycota, Zygomycota,* and *Basidiomycota* all showed variation across developmental stages and plant organs (Fig.S11). Across all plant organs and in both endophyte and epiphyte communities*, Ascomycota* was the most prevalent phyla except in the pods- *Basidiomycota* was more abundant (Fig.S11). Relative abundances of most dominant genera in the epiphyte and endophyte communities were similar across plant organs, apart from the root where there was an increase in abundance of *Dactylonectria* and *Nectriaceae* (known soybean growth-promoting genera) in the endophyte community (Fig.S11). Across developmental stages, there was a clear dominance of *Ascomycota* in both the epiphyte and endophyte community.

Noticeably, *Zygomycota* was the second most abundant phyla in the epiphyte community whilst *Basidiomycota* dominated the endophyte community (Fig.S12). *Basidiomycota* was the second most prevalent phylum in the pods (immature and mature) and control seed (Fig.S11). At the genus level, the abundance of *Aspergillu***s** was more ubiquitous across all developmental stages and in the endophyte and epiphyte communities, except for pods (immature and mature) and control soil where it was nearly absent (Fig. S11). Interestingly, although the taxonomic composition at the maturation stage was similar in both communities (endophyte and epiphyte), there was a marked difference in the relative abundance of taxa.Noticeably, the relative abundance of *Aspergillus, Dactylonectria***,** and *Monographella* were much higher in the endophyte communities, whereas *Mortierella* was nearly absent (Fig. S11).

In the belowground compartment, the relative abundance of fungal taxa were influenced by both developmental stage and sample site: *Penicillium* (Fig.5; developmental stage χ^2=30.35***; sample site χ^2=14.67**), *Fusarium* (Fig.5; developmental stage χ^2=20.53***; sample site χ^2=18.07*), *Mortierella* (Fig.5; developmental stage χ^2=25.83***; sample site χ^2=32.86***),

Dactylonectria (Fig.5; developmental stage χ^2=26.73***; sample site χ^2=37.26***), *Metarhizium* (Fig.5; developmental stage χ^2=13.96**; sample site χ^2=43.83***), *Monographella* (Fig.5; developmental stage χ^2=20.21***; sample site χ^2=14.36***). The relative abundances of *Aspergillus* (χ^2=13.89*), *Gibberella* (χ^2=24.50***), and *Malassezia* (χ^2=11.56*) were solely influenced by developmental stage (Fig.5). In the aboveground compartments, *Aspergillus* and *Malassezia* were the only taxa whose relative abundance was influenced by both developmental stage and sample site (Fig.S12).

Random forest algorithms using a small training set were used to predict the likelihood of association of OTUs across developmental stages and samples sites. There were specific taxa whose abundance and presence was significantly associated with the developmental stage and sample site. Of the predicted bacterial genera, four had the highest Mean Decrease Accuracy (taxa with a substantial value are more critical for the classification of the plant organ/ developmental stage). For instance, an OTU belonging to the genus *Azospirillum* were consistently shown to be associated with the root endosphere and stem communities (endophyte and epiphyte), whilst the other was highly associated mainly with root endosphere (Fig.6). Many of the OTUs associated belowground compartment were also associated with the stem compartment, except for a single OTU from the genus *Niastealla* was highly associated with leaf epiphyte and root endosphere (Fig.6). Across developmental stages, OTUs from the genus *Pseudomonas* and *Rhizobium* were highly associated with the flowering stage (Fig.6). Two OTUs from *Klebsiella* and *Paenibacillus* genera were associated with the maturation stage along with an OTU from family *Sphingomonadaceae* (Fig.6). The majority of OTUs were associated with the emergence stage.

Generally, random forest algorithms had increased accuracy when predicting the presence and abundance of fungal general across developmental stages and sample site. Firstly, four OTUs from the genus *Fusarium* were mainly highly associated with the belowground compartments and control soil (Fig.7). OTUs from *Dactylonectria* and *Tetracladium* were highly associated with the belowground compartments; also, an OTU from *Monographella* associated with the control seed. (Fig.7). The zoosporic *Olpidium* was only significantly associated with the emergence and growth developmental stages only (Fig.7). Five OTUs from genus *Mortierella* were highly associated with the flowering stage, whilst an OTU from genus *Gymnostellatospora* was positively associated with the emergence stage (Fig.7). Lastly, *Chaetomidium* was highly associated with the growth and to a lesser extend flowering developmental stage (Fig.7)

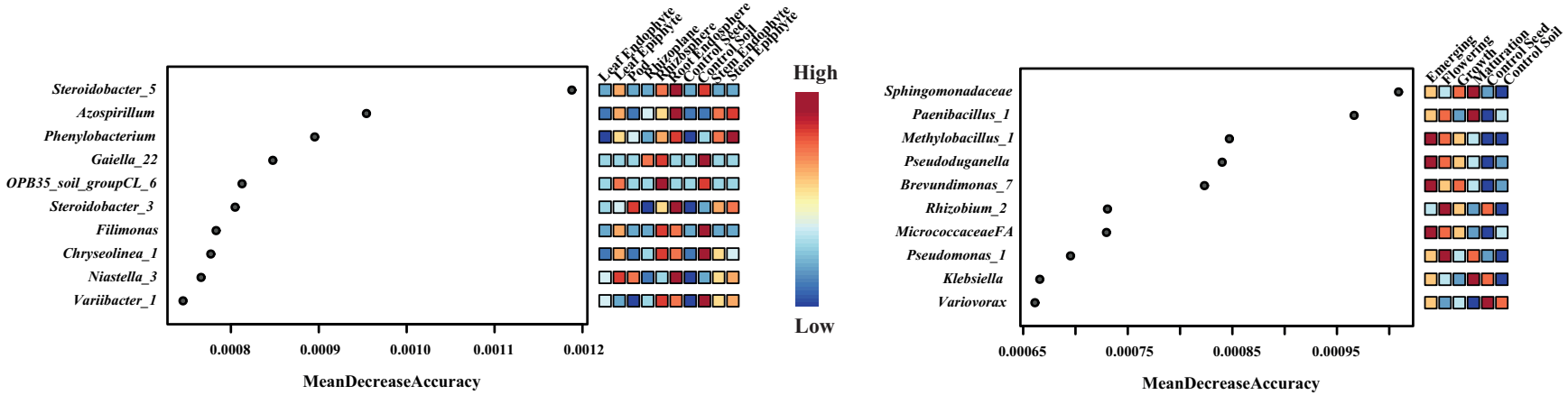

Fig.6 Random forest analysis based on the overall taxonomic profile and could distinguish amongst different developmental stages with increased predictive accuracy. The more the accuracy of the random forest decreases due to the exclusion (or permutation) of a single taxa, the more important that taxa is deemed, and therefore taxa with a large mean decrease in accuracy are more important for classification of the data. The figure shows taxa with the highest discriminatory power and their classification (panel: OTU identity) based on their importance for the identification each developmental stage and sample site

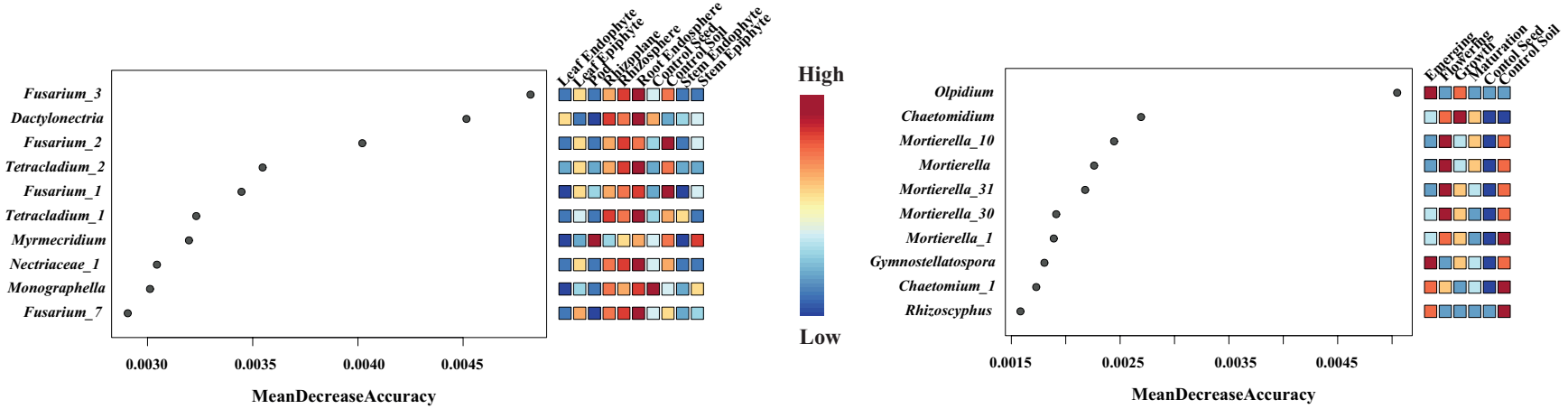

Fig.7 Random forest analysis based on the overall taxonomic profile and could distinguish amongst different developmental stages with increased predictive accuracy. The more the accuracy of the random forest decreases due to the exclusion (or permutation) of a single taxa, the more important that taxa is deemed, and therefore taxa with a large mean decrease in accuracy are more important for classification of the data. The figure shows taxa with the highest discriminatory power and their classification (panel: OTU identity) based on their importance for the identification each developmental stage and sample site

2.7 Discussion

To our knowledge, this is the first report that simultaneously provided evidence for the existence of microbial niches across spatial and temporal axes for a major crop. Here, our aim was to (1) highlight the influence of spatial and temporal dynamics on soybean microbiome and (2) testing for the existence of temporal niche spaces. Our results support our hypothesis that spatial and temporal dynamics both influence the plant microbiome, but also their interaction assert a strong selection filter on the microbiome structure.

2.7.1 Influence of spatial and temporal dynamics

Spatial and temporal dynamics are strong discriminatory axes for plants microbiomes as our data shows. However, the influence and importance of one of these factors cannot be overstated as their interactions modulate community structure and composition. Evidence of microbial niche differentiation along these spatial and temporal axes is emerging for model plant systems (Ofek-Lalzar et al., 2014; Niwa et al., 2018; Toju et al., 2018a), and recent interest in the role and the use of microbes in agriculture has spurred research into microbiomes associated with crops (Montanari-Coelho et al., 2018; Toju et al., 2018a; Merloti et al., 2019). Most importantly, these studies have indicated that the assembled microbiome of greenhouse plants are compositionally similar to their wild and field counterparts (Bai et al., 2015).

Developmental stage influenced the composition and structure of soybean phyllosphere (Copeland et al., 2015) and the rhizosphere communities (Xu et al., 2009; Sugiyama et al., 2014b). Here, by sampling all plant compartments in the same experiment, it was then possible to detect a seasonal trend in the data that influenced the abundance and diversity of microbial communities. The abundance and composition of the released plant exudates at each developmental stage influences microbial successional patterns within the rhizosphere and root compartment (White et al., 2015; Lian et al., 2017). This selection imposed by rhizodeposition was more pronounced for the fungal community than the bacterial/archaeal community. Consequently, plant microbiomes become compositionally nested from the ground up- where rhizosphere communities contain the highest diversity (Amend et al., 2019). Developmental stage explained a more substantial proportion of the observed variation within the belowground compartments than in the aboveground. However, the large amount of residual variation within

aboveground communities often arises in diversity deplete communities and implies a strong influence of ecological drift (Leibold and Chase, 2017). Here, the aboveground microbial communities had lower abundances, and developmental stage influenced overall diversity. Temporal variations in the size of ecological communities as a result of extrinsic factors, such as plant developmental stage amplifies the influenced of ecological drift (de Mazancourt et al., 2013; Gilbert and Levine, 2017). Further, these temporal fluctuations in community abundance and diversity, act as a stabilising ecological filter and creates different temporal niches that are then occupied by various taxa (Adler and Drake, 2008; Gilbert and Levine, 2017). In ecological communities, drift causes stochastic fluctuations in abundances and lowers diversity and this further results in divergence in community structure (Gilbert and Levine, 2017). It is then possible that observed variations in the aboveground communities are a result of ecological drift and the interactions between developmental stage and sample site create specialised microbial niches. For instance, various fungal taxa can exhibit similar levels of host colonisations, but there are clear distinctions in their degree of infection and developmental patterns that are mediated by plant metabolic response (Macia-Vicente et al., 2009). As a result, plant-fungal communities tend to display discordant nestedness topology and community structure (Toju et al., 2015). However, bacterial community composition and diversity are influenced primarily by specific localised host response, such as, production of organ-specific metabolites (Horton et al., 2014), secondary metabolites that regulate the biotic interactions (Cotton et al., 2019), and plant-microbe communication (Lareen et al., 2016).

In model systems, the successional pattern of leaf and root-associated microbiomes has tended to mirror one another with taxonomic and function overlap (Bai et al., 2015). However, this study found that spatial dynamics are durable discriminatory axes for belowground microbial communities. Plant organs and sample site strongly influence the abundance and distribution of available microbial niches in soybean (Miller and Roy, 1982). Plant samples site is a robust discriminatory axis for microbial diversity across different plant systems (Poudel et al., 2019; Singer et al., 2019). Microbial successional patterns tend to be strongly linked to sample site in part because only adapted taxa can inhabit these specialised niches within plant tissues (Dickie et al., 2002; Duran et al., 2018; Qian et al., 2019). In soybean, these successional patterns are highly variable between the primary root and secondary lateral roots (Sakamoto and Kaji, 2017), whilst fungal colonisation is a result of niche competition within the rhizosphere (Niwa et al., 2018). These findings indicate that in soybean root-soil interface niche differentiation is delimited along spatial and temporal axes, whereas it is their interaction that modulates communities. Equally, ecological processes that explain these patterns have been extensively reviewed (Turner et al.,

2013b). Our findings further support the notion that assembled microbiomes from greenhouse plants recapitulate not only similar community structure (Bai et al., 2015), but also internal community dynamics that are comparable to those of their field counterparts (Copeland et al., 2015). Dispersal rates vary across all plants sample sites, and these often influence microbial abundance diversity (Amend et al., 2019). It is then possible that in a closed dispersal-limited growth chamber, soil microbes that are not subsurface soil-bound could be airborne and colonise the aboveground organs. Altogether, these results highlight the nuanced ecological processes modulating plant microbiome succession.

2.7.2 Existence of temporal niche spaces

Ecological succession characterises the dominance and abundance of taxa and their influence on community dynamics over time. However, in the microbial ecosystem, the most abundant taxa may not be drivers of ecosystem change and functioning (Elshahed et al., 2008; Lynch and Neufeld, 2015). This study set out to detect temporal niche spaces occupied in soybean by 1) highlighting the influence of spatial and temporal interactions on composition and structure of the microbiome, and 2) detecting of specialised temporal niche spaces. In this attempt, the study succeeded in highlighting microbial niche spaces influenced by spatial and temporal dynamics. For instance, although there were no visible signs of nodulation at each harvest period, there was a significant increase in the relative abundance of the soybean beneficial partner *Bradyrhizobium* at the earliest developmental stage. *Bradyrhizobium* was the least abundant of other taxa that were influenced by developmental stage. Plant metabolic needs demands vary with developmental stages, and to compensate; the plant invests biomass in root production to increase nutrient acquisition (Hodge, 2004). *Bradyrhizobium* and *Pseudomonas* are soybean microbial partners that co-ordinate and modify root architecture to increase nutrient acquisition (Egamberdieva et al., 2017; Kumawat et al., 2019). Likewise, *Streptomyces* co-operates *Bradyrhizobium* to improve nutrient acquisition (Htwe et al., 2018). This study found that the relative abundance of *Bradyrhizobium* and *Pseudomonas* to be influenced by where the microbes localise; additionally, the developmental stage influenced the relative abundance of *Pseudomonas*. It is then possible to speculate that although spatial and temporal dynamics influence the relative abundance of *Bradyrhizobium* and both *Pseudomonas* and *Streptomyces* to varying extends, these taxa occupy complementing spatial and temporal niches. For instance, the relative abundance of *Pseudomonas* was predicted and peaked at the flowering stage in the

root endosphere, the same compartment the relative abundance of *Bradyrhizobium* was highest at the emergence stage. Bacterial/archaeal communities constrained to a single spatial niche tend to quickly diversify under adaptative radiation (Rocabert et al., 2017), however, in the presence of temporal niches, communities are more likely to circumvent the overshooting dynamics of adaptive radiant and maintain high levels of biodiversity over time (Tan et al., 2013). Thus, we suggest that the immense abundance and diversity of bacterial/archaeal taxa is maintained through these spatial and temporal niche partitioning. Moreover, taxa that were predicted by random-forest algorithms were shown in culture-dependent studies to be highly beneficial for soybean growth and development (Kuklinsky-Sobral et al., 2004).

Plant host interactions strongly modulate the realised niche of fungal communities, and niche specialisations are not delimited along abiotic and biotic (plant host age) axes (Chaloner et al., 2020). Our study highlighted both spatial and temporal dynamics modulated the relative abundance of dominant fungal genera. For instance, random-forest algorithms predicted that OTUs from one of these dominant genera to be highly associated with the flowering stage. The relative abundance of the genus *Mortierella* increased at the flowering stage at the root-soil interface. Members of the genus use the enzyme xylanase to metabolise plant-derived sugars and are chitinolytic (Brzezinska et al., 2014), and are antagonistic to plant fungal pathogens such as those in the *Fusarium* genus (Liu et al., 2019c). Our data show that both spatial and temporal dynamics influence the relative abundance of most fungal genera, but this contrasts with the predictions from random-forest algorithms. Overall, OTUs from genus *Fusarium* were spatially predicted to dominate at the root-soil interface; however, OTUs from *Mortierella* dominated at the flowering stage. The sharp increase in the relative abundance of *Mortierella* and depletion of *Fusarium* at the root-soil interface can in part be explicable by the antagonist nature of *Mortierella*. Thus, this study provides support that:

1. within plant-fungal communities niche specialisations evolve independently along spatial and temporal axes (Chaloner et al., 2020),
2. Only adapted taxa can inhabit these specialised niches (Qian et al., 2019), and
3. Niche competitions at the root-soil interface drive fungal assembly and colonisation (Toju et al., 2015).

Lastly, we argue that innate physiological (generation time, colonisation capacity, and cell size) characteristics contribute to the divergent response in bacterial and fungal communities.

2.8 Conclusion

In conclusion, this study confirms our hypothesis that the plant microbiome is influenced by spatial and temporal interactions, and further highlight the complexity of the soybean microbiome and call for more spatially- and temporally resolved studies to capture this complexity, with the view of harnessing the crop microbiome to optimise microbial services in agriculture. We highlight the influence of spatial and temporal dynamics on the occupied niche within soybean microbiome, by focusing on microbes that seem to be consistently influenced by the interaction between spatial and temporal dynamics as potential candidates for microbiome engineering efforts.

2.9 **Acknowledgements**

The authors would like to thank the Benjamin Mimee from Agriculture and Agri-Food Canada for providing the seeds used in the study. This work was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC) grant RGPIN 2014-05274 to EY. IM was supported by the Innovation and Scarce Skills scholarship from South African National Research Foundation (NRF), Fonds de Recherche du Québec (FRQNT), and partly by Foundation Armand-Frappier. We also wish to acknowledge Compute Canada for access to the University of Waterloo's High-Performance Computing (HPC) infrastructure (Graham system) through a resources allocation granted to EY.

2.10 Supplementary material

Table S1 Permutation analysis of variance (PERMANOVA) results indicating the influence of temporal and spatial interaction on microbial community structure *(p* value *<0.05, ** <0.01, ***<0.001)

Bacterial Community				
Source of variation	**df**	**MS**	**F Value**	**R^2**
Developmental stage	5	1.29	6.95	**0.11*** **
Sample site	7	2.08	11.19	**0.25*** **
Developmental stage × Sample site	19	0.64	3.44	**0.21*** **
Residuals	124	0.18		0.40
Total	155			
Fungal Community				
Source of variation	**df**	**MS**	**F value**	**R^2**
Developmental stage	5	0.99	3.95	**0.09*** **
Sample site	7	1.67	6.68	**0.22*** **
Developmental stage × Sample site	19	0.39	1.58	**0.14*** **
Residuals	111	0.25		0.53
Total	142			

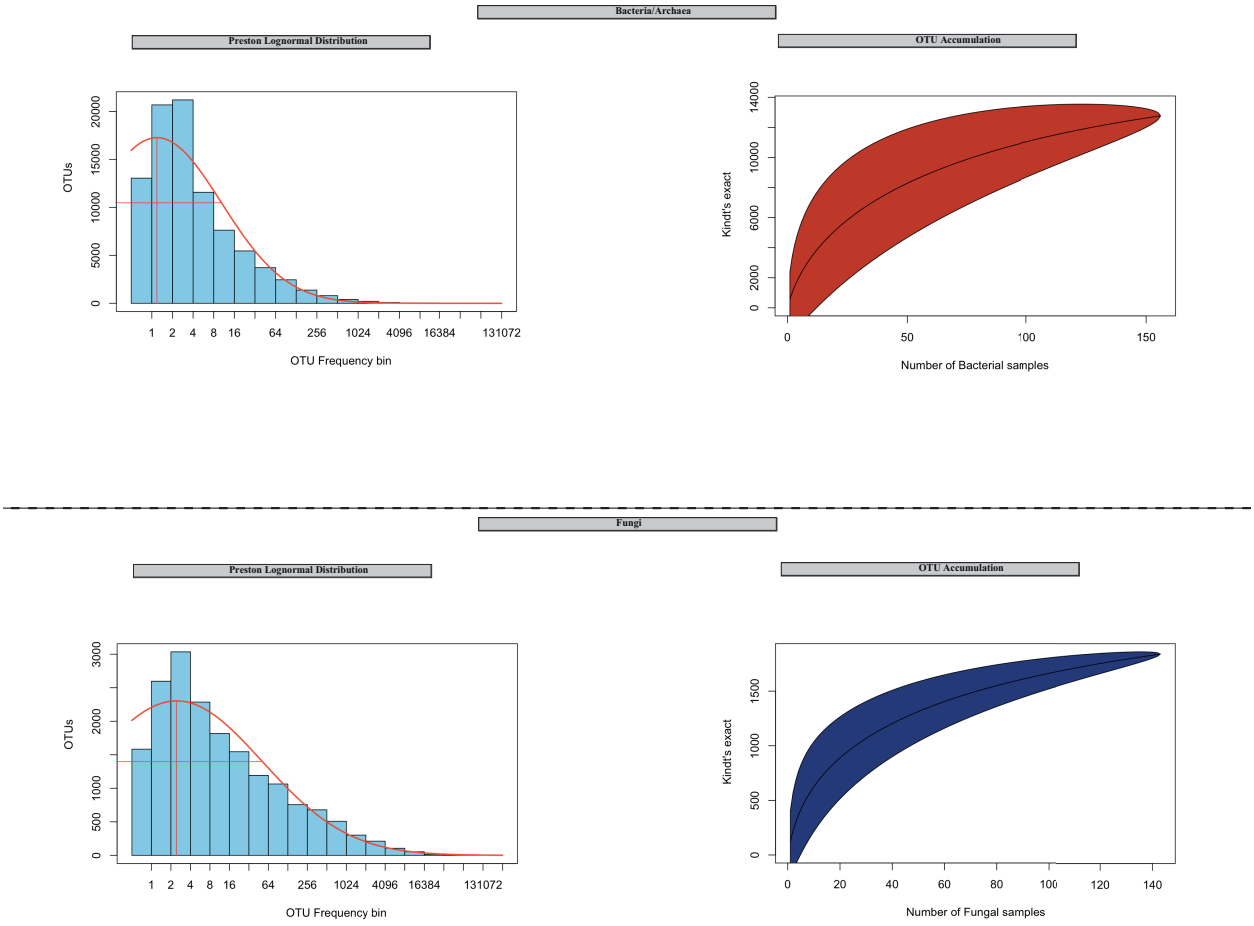

Fig.S1 OTU distribution and accumulations curves. Preston lognormal graphs indicating total number of recovered OTUs (area under the curve) and the proportion of rare OTUs indicating few rare OTUs (truncation point far below the mode) and OTU accumulation curve indicating the number of recovered OTUs as a function of sampling effort (number of samples) with 95% confidence interval for bacteria (TOP) and fungi (BOTTOM)

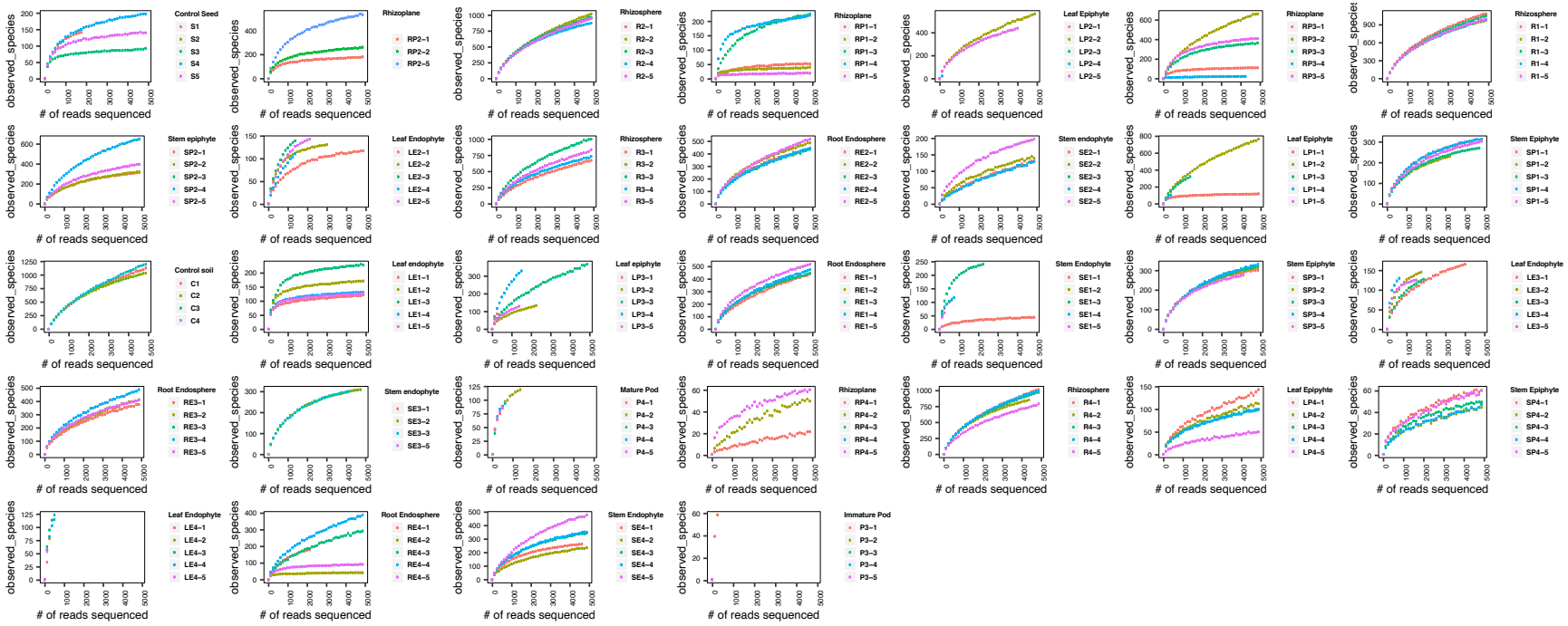

Fig.S2 Alpha diversity rarefication curves of observed species (bacterial /archaea), raw OTU tables were rarefied to 5000 reads ten times.

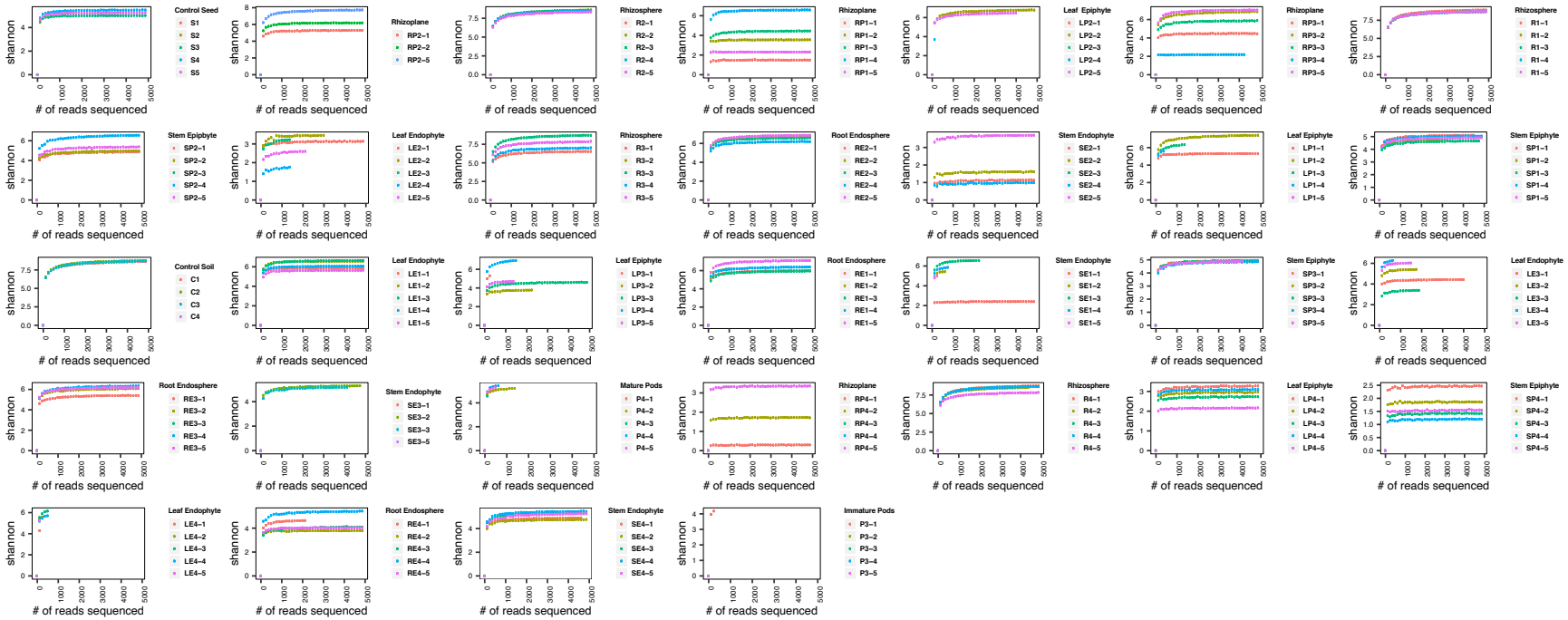

Fig.S3 Alpha diversity rarefication curves of Shannon diversity (bacterial /archaea), raw OTU tables were rarefied to 5000 reads ten times.

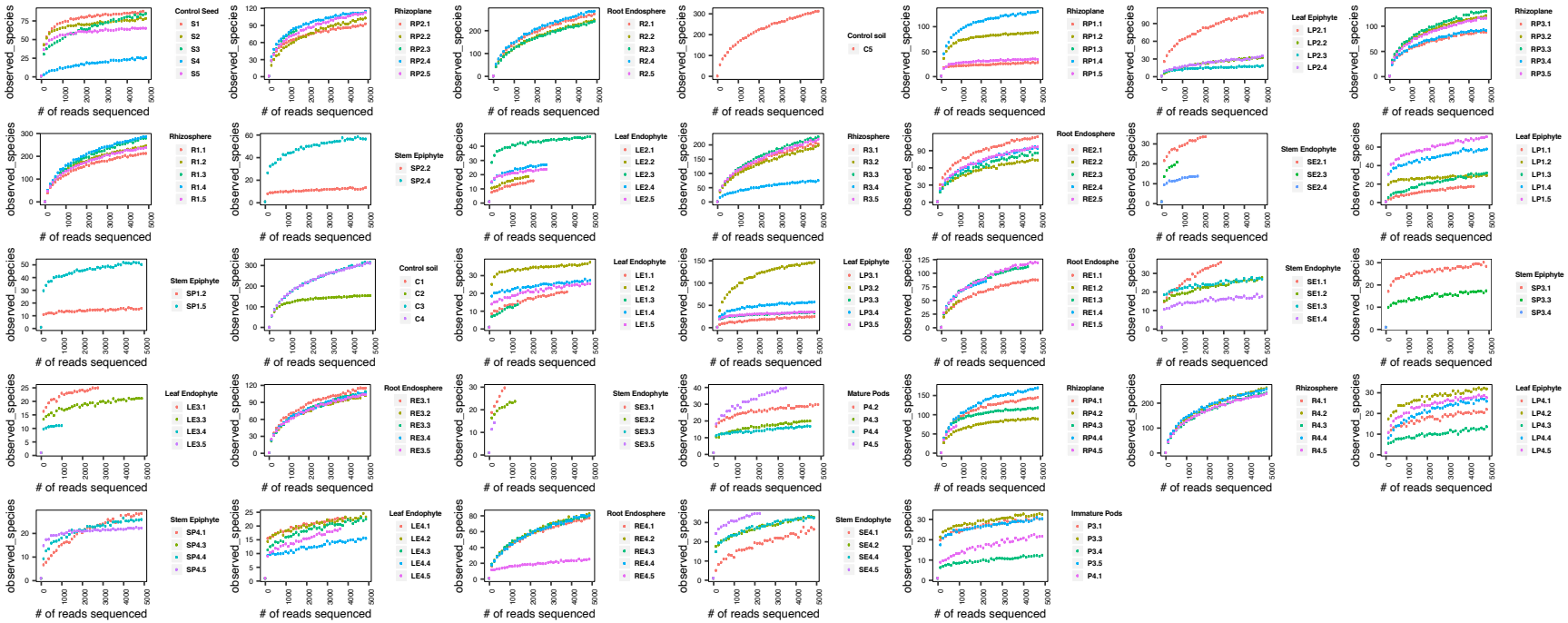

Fig.S4 Alpha diversity rarefication curves of observed species (fungi), raw OTU tables were rarefied to 5000 reads ten times.

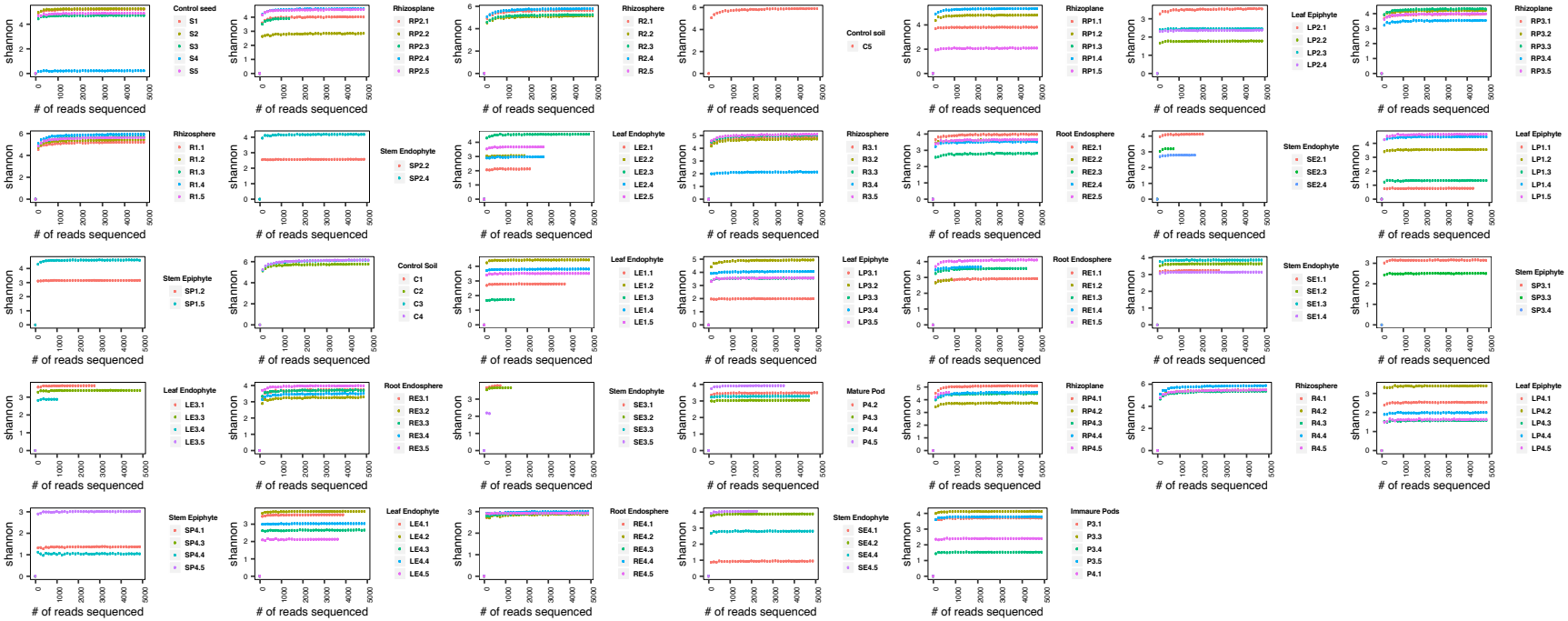

Fig.S5 Alpha diversity rarefication curves of Shannon diversity (fungi), raw OTU tables were rarefied to 5000 reads ten times.

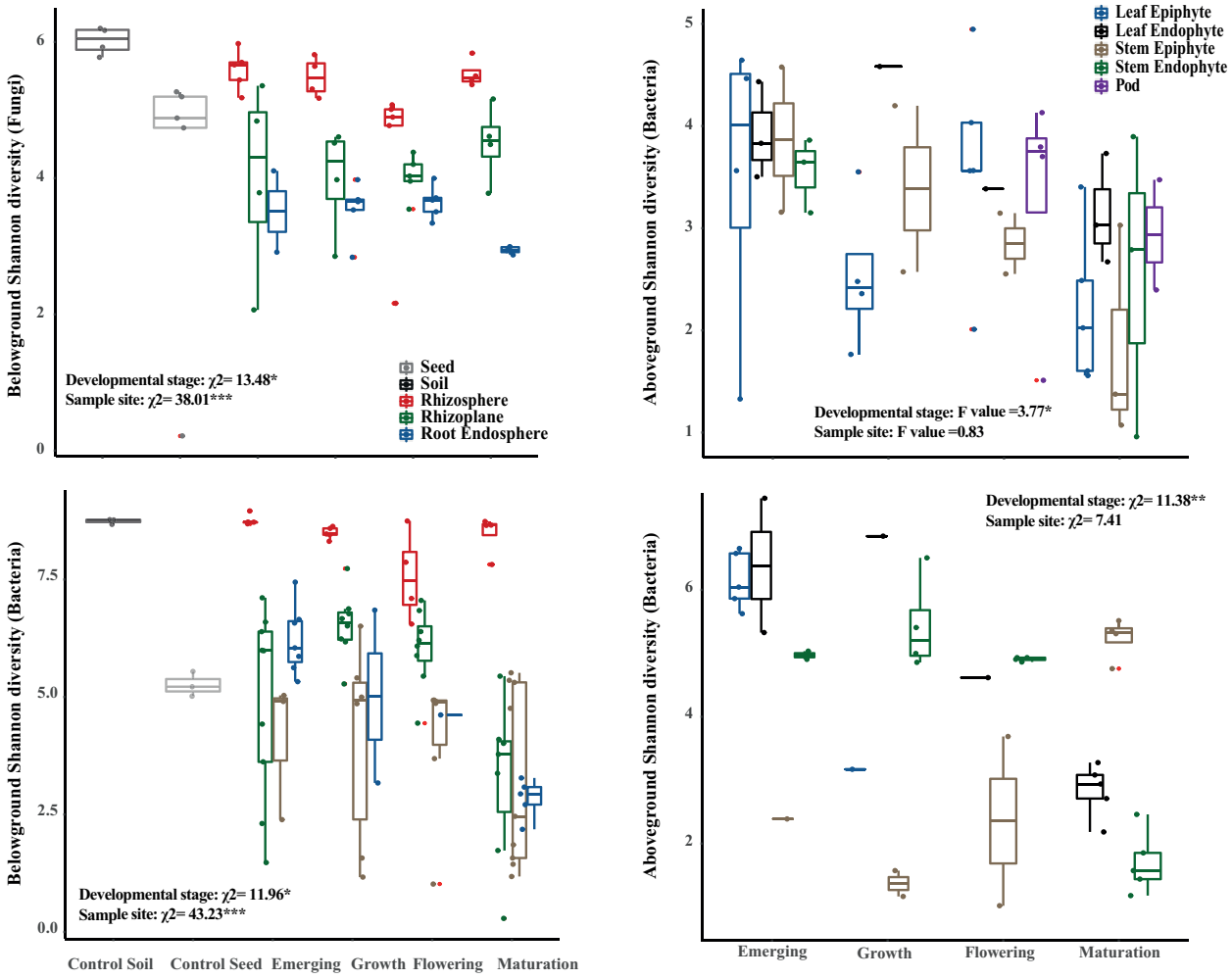

Fig.S6 Top panel indicating fungal diversity and bottom panel indicating bacteria/archaeal diversity. Total Shannon diversity influenced by developmental stage and samples site (Kruskal-Wallis chi-squared χ^2, *p*-value or ANONVA F value) and pairwise Wilcox test with Bonferroni correction (*p* value *<0.05, ** <0.01, *<0.001). Pairwise comparisons were not reported for control samples.**

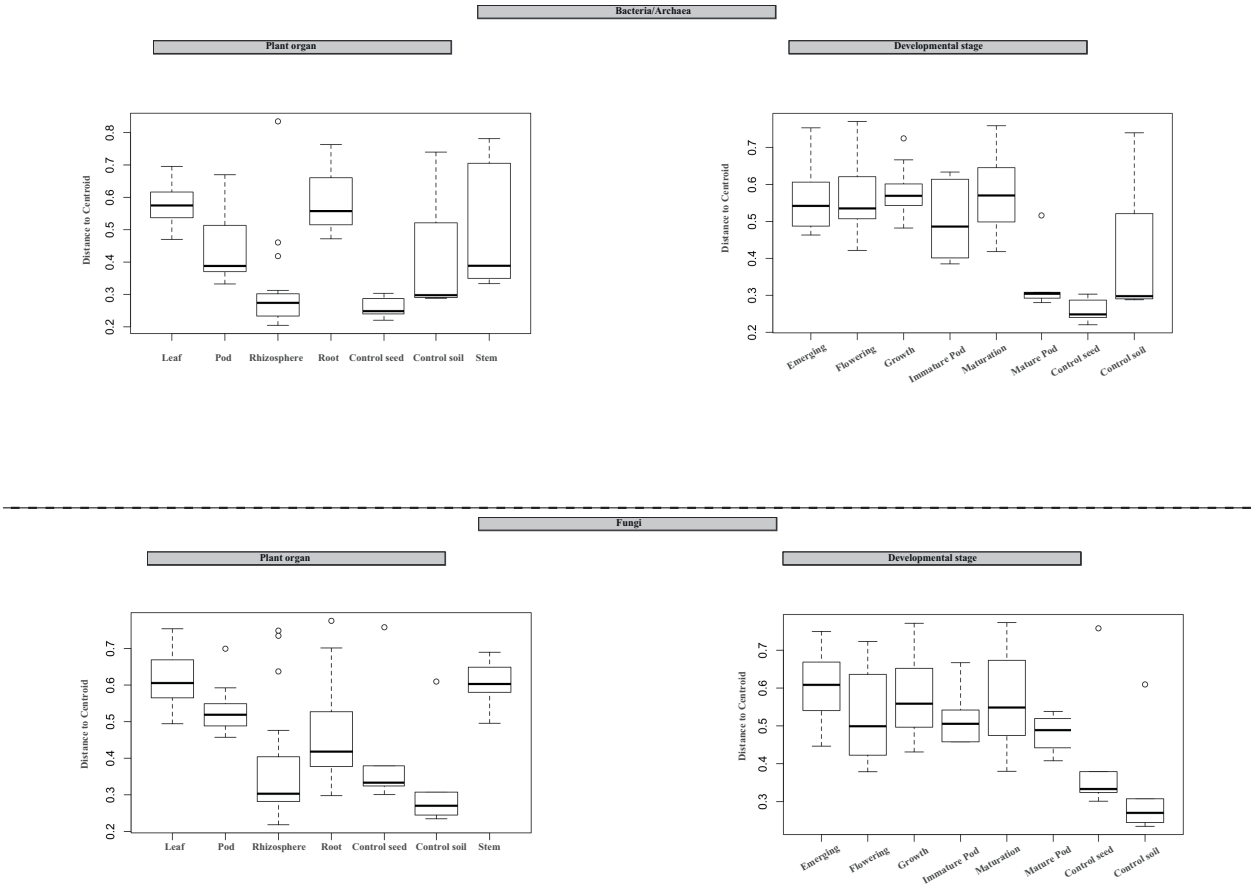

Fig.S7 Homogeneity of multivariate dispersion of microbial community based on Bray-Curtis dissimilarity across different plant organs and developmental stages.

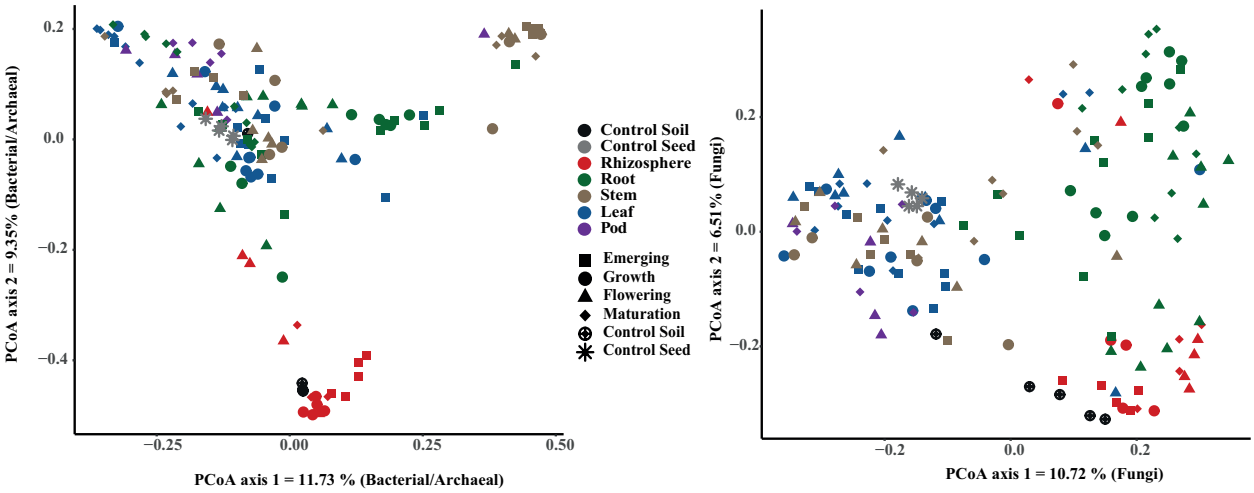

Fig.S8 Principle Coordinate Analysis (PCoA) of microbial community based on Bray-Curtis dissimilarity across different plant compartment and developmental stages (Compartment= Control Soil, Control Seed, Rhizosphere, Root, Stem, Leaf, Pods). Samples that are closer to each other have similar community composition, whereas samples that are further apart are distinct to each other in composition

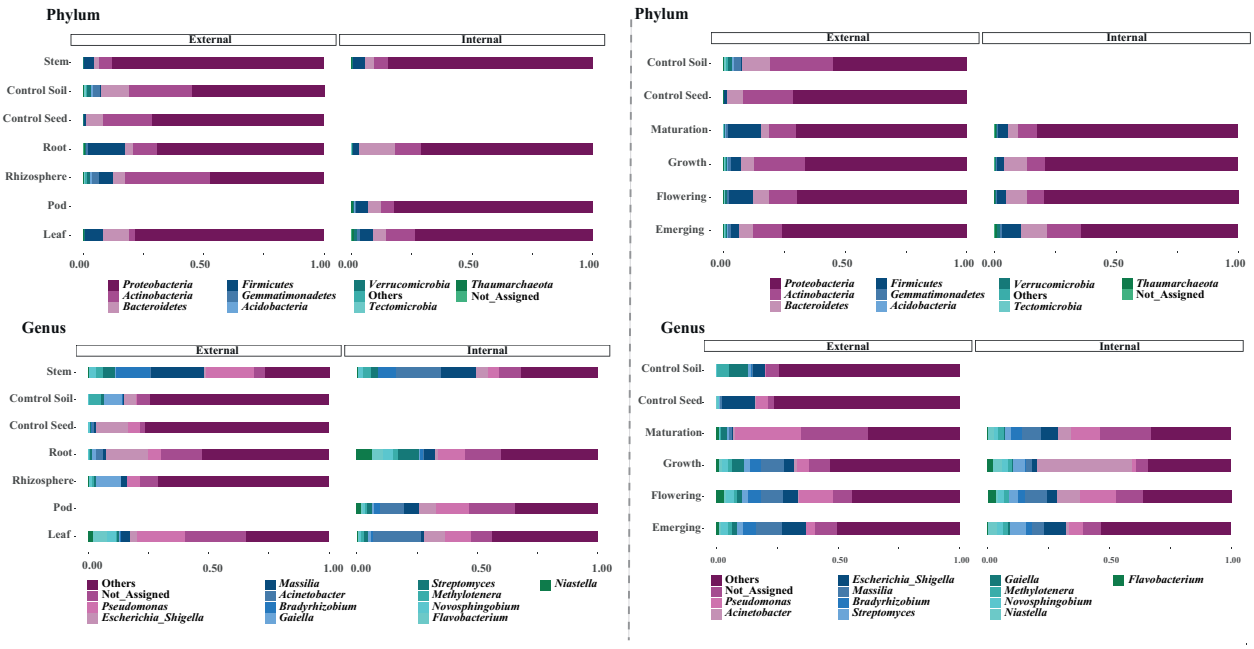

Fig.S9 Comparison of the relative abundance of dominant bacterial/archaeal taxa across plant compartment and developmental stages. Top panel: Relative abundance of the most dominant bacterial phyla. Bottom panel: Relative abundance of the most dominant bacterial genera

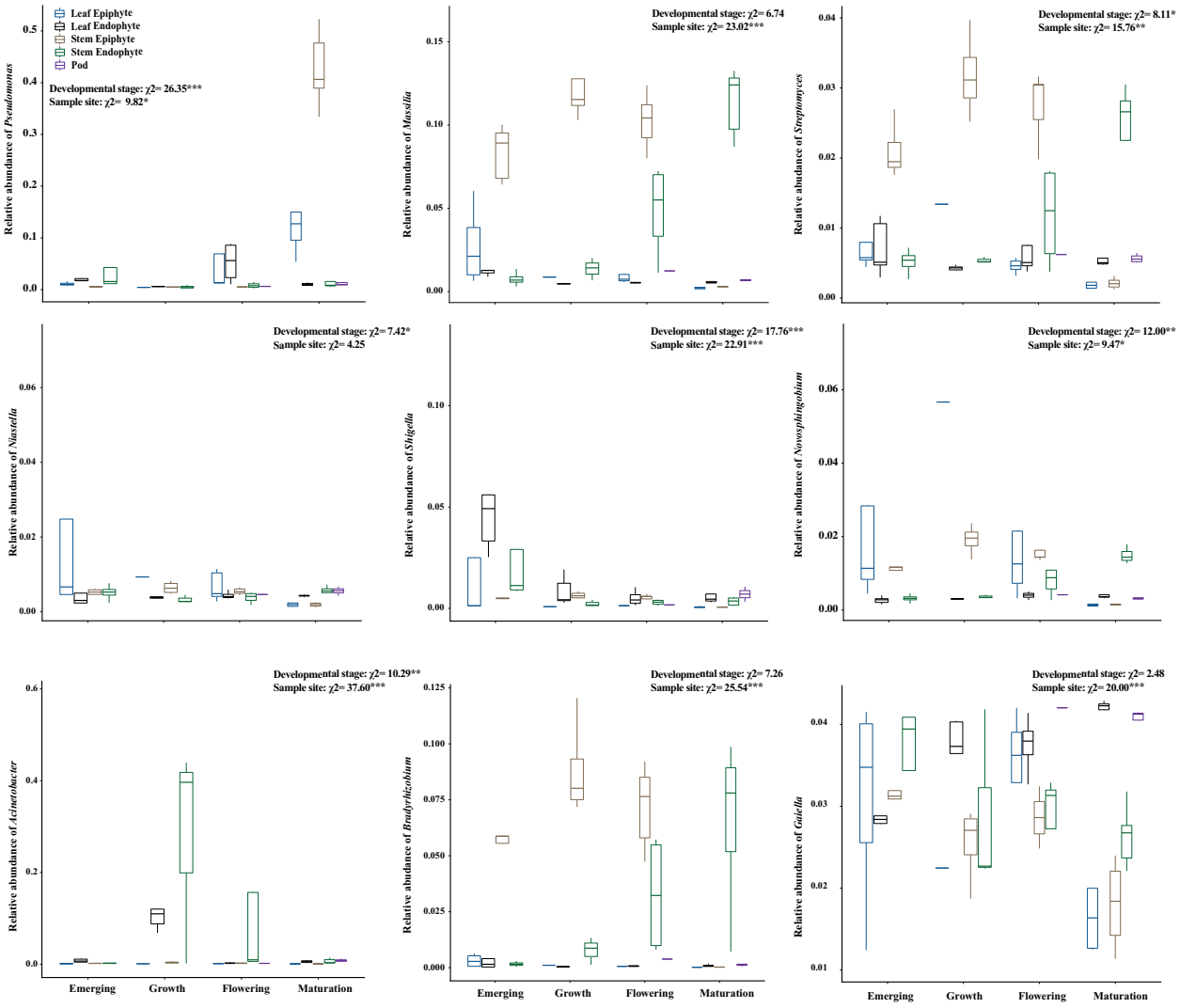

Fig.S10 Aboveground bacterial/archaeal relative abundance of most abundant genera that were significantly influenced by developmental stage and samples site (Kruskal-Wallis chi-squared χ^2, *p*-value) and pairwise Wilcox test with Bonferroni correction *(p* value *<0.05, ** <0.01, *<0.001**

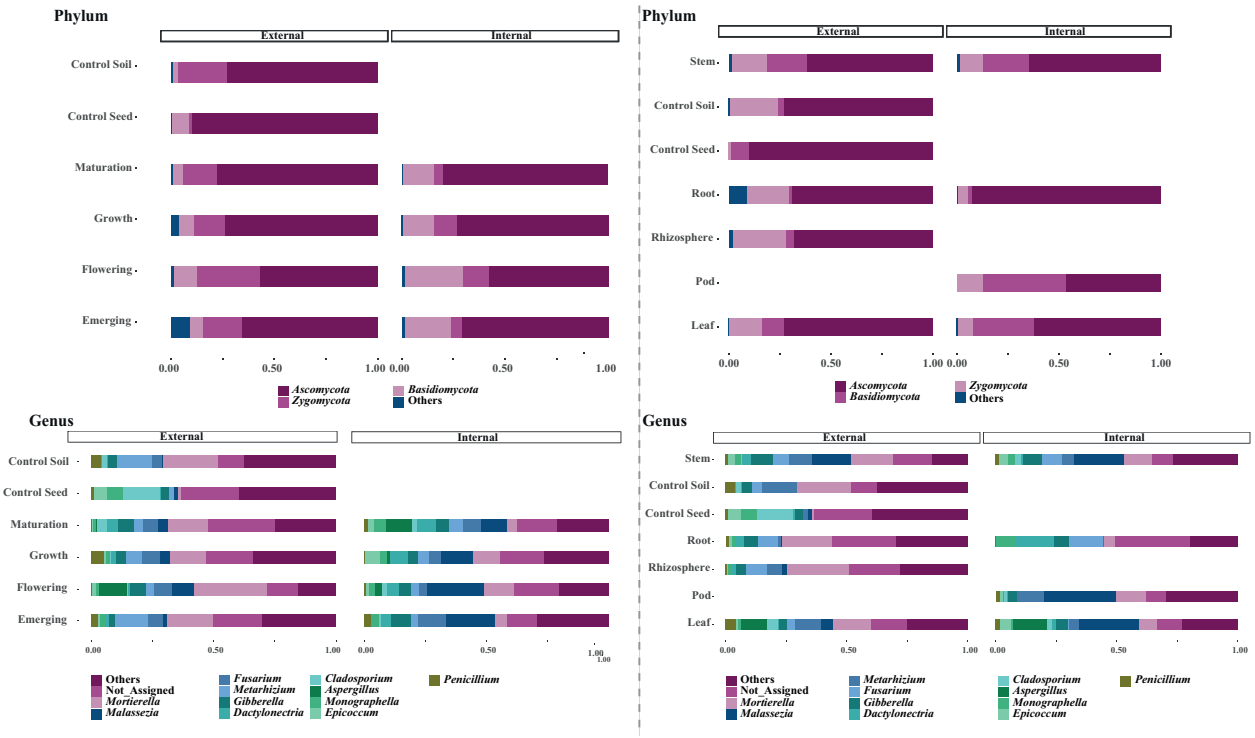

Fig.S11 Comparison of the relative abundance of dominant fungal taxa across plant compartment and developmental stages. Top panel: Relative abundance of the most dominant fungal phyla. Bottom panel: Relative abundance of the most dominant bacterial genera

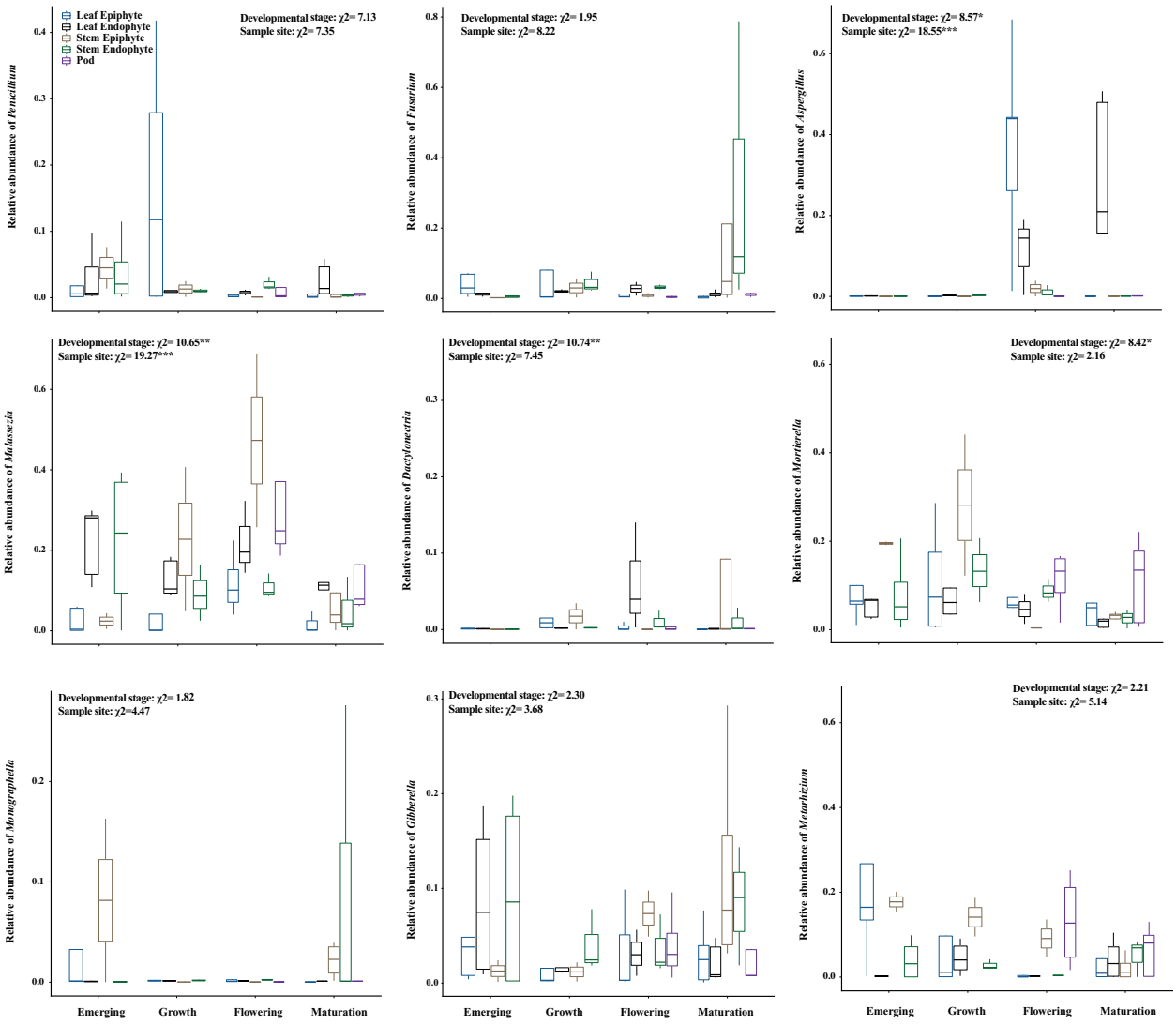

Fig.S12 Aboveground fungal relative abundance of most abundant genera that were significantly influenced by developmental stage and samples site (Kruskal-Wallis chi-squared χ^2, *p*-value) and pairwise Wilcox test with Bonferroni correction *(p* value *<0.05, ** <0.01, *<0.001)**

3 CHAPTER 3 : PUBLICATION 2

3.1 **Plant compartments and developmental stages modulate balance between niche-based and neutral processes in soybean microbiome**

3.2 **Les compartiments de la plante et ses stades de développement régulent les processus de niche et neutres du microbiomes du soja**

Moroenyane, I[a]., Tripathi, B[b]., Mendes, L[c]., Tremblay, J[d], and Yergeau, É[a]*

a) Institut National Recherche Scientifique, Centre Armand-Frappier Santé Biotechnologie, 531 Boulevard des Prairies, Laval, Québec, H7V1B7, Canada
b) Korea Polar Research Institute, Incheon, 406-840, Korea
c) Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP 13400-970, Brazil
d) Energy, Mining, and Environment, Natural Resource Council Canada, 6100 Avenue Royalmount, Montreal, Quebec, H4P 2R2, Canada

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Author contributions

I Moroenyane contributed to content and successful publication of the article. Itumeleng Moroenyane designed, performed the experiments and the statistical analyses, and wrote the manuscript. J Tremblay perfomed the bioinfomatics analysis and conributed to revising the manuscritp. B Tripathi and L Mendes were consulted and assisted with running null models and species distribution models. É yergaeu obtained the research funding, supervised the project and participated in writing and revising the manuscript.

Research links

The previous chapter and article focused on defining the successional patterns of the entire soybean microbiome across spatial and temporal niches. It highlighted that both spatial and temporal dynamics interacted to produce the observed diversity patterns; more importantly, these interactions were temporally nested. This article goes further by specifying the ecological processes that assemble and maintain the observed diversity patterns. Moreover, it spotlights the role of dispersal and niche-bassed selection as essential processes that create and maintain soybean microbial spatial and temporal niches.

3.3 Abstract

Understanding the dynamics of plant-associated microbial communities within agriculture is well documented. However, the ecological processes that assemble the plant microbiome are not well understood. This study elucidates the relative dominance of assembly processes across plant compartments (root, stem, and leaves) and developmental stages (emergence, growth, flowering, and maturation). Bacterial community composition and assembly processes were assessed using 16S rRNA gene amplicon sequencing. Null models that couple phylogenetic community composition and species distribution models were used to evaluate ecological assembly processes of bacterial communities. All models highlighted that the balance between the assembly process was modulated by compartments and developmental stages. Dispersal limitation dominated amongst the epiphytic communities and at the maturation stage. Homogeneous selection dominated assembly across plant compartments and developments stages. Overall, both sets of models were mostly in agreement in predicting the prevailing assembly processes. Our results show, for the first time, that even though niche-based processes dominate in the plant environment, the relative influence of dispersal limitation in community assembly is important.

3.4 Introduction

Microbial communities that colonise plant surface from the roots to the leaves and the inside of plant organs help overcome abiotic stress (Cordovez et al., 2019). The colonisation, diversity, and succession patterns of these microbial communities have become a research focus of interest for ecologists, including efforts to identify and include microbial communities in sustainable agricultural practices (Toju et al., 2018a; Bell et al., 2019). One of the prerequisites to such efforts is to understand the ecological processes that delimit microbiomes across plant compartments and growth stages, not only at the root-soil interface (Toju et al., 2018a; Jiao et al., 2020). Ecological communities are assembled simultaneously by both niche-based (environmental filtering) and neutral processes (dispersal limitations, ecological drift, and speciation events)(Vellend, 2010; Nemergut et al., 2013). However, the dominance of these processes across developmental stages and plant compartments within a single genotype remains unknown.

Fundamentally, plant microbial communities are defined by 1) their taxonomic compositions, 2) functional capacity, and 3) dominance of assembly processes. These inherent community characteristics are influenced by plant genotype(Wagner et al., 2016) , plant species (Fitzpatrick et al., 2018) , and plant nutrient status(Dakora and Phillips, 2002). These studies have highlighted that there is an interaction between the different components of the microbiomes. For instance, microbial taxa in the rhizosphere tend to influence community assembly processes by modulating the expression of crucial plant functional genes (Hartmann et al., 2009; Perez-Jaramillo et al., 2016), and assembly processes within rhizosphere microbiome vary across crops (Matthews et al., 2019).

Essentially, there are two classes of models from which community assembly can be inferred. Firstly, phylogenetic null models (PNM), where the integration of phylogenetic and species pool data has led to a framework from which mechanisms of community assembly can be inferred (Webb et al., 2002; Fine and Kembel, 2011). At their core, these approaches combine a phylogenetic community structure index such as beta mean nearest taxon distance (βMNTD) which estimates phylogenetic turnover between assemblages(Stegen et al., 2012; Stegen et al., 2013) and null models to quantify deviation from null expectations(Hardy, 2008; Kembel, 2009; Stegen et al., 2013). The null model randomly shuffles the taxa across tips of the phylogenetic

tree and βMNTD is recalculated, and this provides one null value for βMNTD(Stegen et al., 2013; Wang et al., 2013). After several rounds of iterations, the model provides a distribution of βMNTD values and deviations between the observed βMNTD value and null βMNTD distributions are quantified as beta nearest taxon index (βNTI) (Stegen et al., 2013; Dini-Andreote et al., 2015). Niche-based selection imposed by the environment are then quantified as 1) homogenous selection (βNTI less than 2) implies that selective pressure exerted by the environment is spatially homogenous and does not significantly change between periods, 2) heterogeneous selection (βNTI greater than 2) implies that the selective pressure changes between periods (Dini-Andreote et al., 2015). Under homogenous selection, taxa that are selected at a specific period will be continuously selected; whereas, under heterogeneous selection, different taxa will be selected across different periods. These models have been used to quantify the relative influence of different assembly processes (Jiao et al., 2020) to predict niche constraints of soil microbes (Tripathi et al., 2018) and to elucidate microbial biogeographical patterns(Moroenyane et al., 2016a; Moroenyane et al., 2016b). Secondly, species distribution models (SDM) use taxonomic composition and niche-based or neutral assembly models to predict the prevailing assembly processes. Typically, niche-based SDM models predict that changes in species abundance and distribution are interconnected to changes in environmental conditions (environmental filtering) (MacArthur, 1957; Dumbrell et al., 2010a). These models aim to describe the abundance distribution of taxa given the occupied niche space. Broadly, these models predict how taxa that occupy similar niche spaces can coexist by niche partitioning (Tokeshi, 1990, 1993; Chen, 2014). Under niche-based assembly, niche partitioning within communities can be modelled with several models: 1) broken stick, pre-emption, log-normal, and Zipf-Mandlebrot (MacArthur, 1957; Sugihara, 1980). Species distribution models use abundance and distribution of taxa to quantify niche partitioning. Conversely, neutral SDM models predict that the abundance and distribution of taxa is a direct consequence of dispersal limitation and species abundance (Hubbell, 2001; Etienne and Olff, 2005). The zero-sum model (ZSM) predicts that the abundance and distribution of taxa into niche spaces will be dominated by neutral processes (McGill, 2003; Etienne and Olff, 2005). Similar to PNM models, SDM models have been useful in predicting soil microbial biogeographical patterns (Moroenyane et al., 2019), soybean rhizosphere taxonomic and functional patterns (Mendes et al., 2014; Goss-Souza et al., 2019), and predict the composition of fungal leaf communities (Feinstein and Blackwood, 2012).

To date, studies that have elucidated community assembly processes within plant microbiomes have used either of these approaches and have focused mainly on a single plant compartment or developmental stage. Here, we were interested in using both PNMs and SDMs

to quantify assembly processes of soybean microbiomes across spatial (plant compartments) and temporal (developmental stages) scales. We focused on elucidating assembly processes in soybean plants growing in pots under controlled growth chamber experimental conditions. Using the phylogenetically conserved regions of the 16S rRNA marker gene, we aimed at 1) elucidating the relative dominance of neutral and niche-based processes in assembling the plant bacterial community along spatial and temporal axes, and 2) comparing different complementary approaches to model assembly processes.

3.5 Methods

3.5.1 Plant growth conditions and microbiome sampling

Plants were grown in a Conviron growth chamber (Winnipeg, Canada), and were destructively sampled at the following developmental stages: V1 (emergence), V3 (growth), R1 (flowering), and R3 (maturation). The soil was collected in autumn of 2017 from an experimental field that had no history of agricultural practice, passed through a 40 mm sieve, and homogenised prior to potting. Soil analyses were performed in October 2017 by AgroEnviro Lab (La Pocatiere, QC) and revealed an average pH of 7.2, P concentration of 193 (kg/ha), total N 0.15%, C/N of 13.1 and other soil properties reported in Table S1. Plants were supplemented with a modified Hoagland's plant nutrient solution weekly (Moscatiello et al., 2013). A total of five plants were destructively sampled at each developmental stage, and DNA extraction was performed right after sampling. Samples were collected from rhizosphere, root, stem, and leaves. At each sampling period, the rhizosphere samples were considered as all the soil that was directly attached to the root surface. The entire epiphytic community (leaves, stem, and roots) was extracted using a modified protocol from Qvit-Raz et al. (2008). Briefly, the samples were placed in sterile 50 ml plastic Falcon test tubes (Corning, Tewksbury, MA, USA) and filled with sterile phosphate-buffered saline (PBS 0.1M, pH 7.4). The samples were then placed in a sonication tub (Fisher FS20, Fisher Scientific, Waltham, USA) for 15 min and vortexed for 10 s. The samples were then transferred into a new tube containing PBS and rinsed twice. The wash was pooled and spun down in a centrifuge at 2,000 g for 20 min, and the resulting pellet was considered to be the epiphytic community. The endophyte community was considered to be all the remaining microbes after the sonication and rinse treatment. Plant tissue was then pulverised in liquid nitrogen using a sterile pestle and mortar. For each sample, 0.25 g was added to the bead tubes from the Qiagen Power Soil DNA kit (Hilden, Germany) and DNA was extracted following the manufacturer's instructions.

3.5.2 16S rRNA gene amplification and sequencing

The bacterial/archaeal V4 region of the 16S rRNA gene were amplified using 520F and 799R primer pairs, which were shown to exclude chloroplast sequences (Edwards et al., 2007). The average lengths of 16S amplicon sequences were of approximately 280 bp. Briefly, extracted DNA was used to construct sequencing libraries according to Illumina's "16S Metagenomic Sequencing Library Preparation" guide (Part # 15044223 Rev. B), with the exception of using

Qiagen HotStar MasterMix for the first PCR ("amplicon PCR") and halving reagent volumes for the second PCR ("index PCR"). The first PCR ("amplicon PCR") was carried out for 25 cycles with annealing temperatures of 55 °C. The resulting amplicons were pooled together and sequenced at the McGill University and Genome Québec Innovation Center (MUGQIC). Diluted pooled samples were loaded on an Illumina MiSeq and sequenced using a 500-cycle (paired-end sequencing configuration of 2x250 bp) MiSeq Reagent Kit v3. In total, 4,851,927 16S rRNA gene reads were received. Reads were processed using the AmpliconTagger pipeline (Tremblay et al., 2015; Tremblay and Yergeau, 2019). Briefly, raw reads were scanned for sequencing adapters, and PhiX spike-in sequences and remaining reads were merged using their common overlapping part with FLASH (Magoc and Salzberg, 2011). Primer sequences were removed from merged sequences, and remaining sequences were filtered for quality such that sequences having an average quality (Phred) score lower than 27 or one or more undefined base (N) or more than 10 bases lower than quality score 15 were discarded. Remaining sequences were clustered at 100% identity and then clustered/denoised at 99% identity (DNACLUST v3) (Ghodsi et al., 2011). Clusters having abundances lower than 3 were discarded. Remaining clusters were scanned for chimeras with VSEARCH's version of UCHIME denovo (Rognes et al., 2016), UCHIME reference (Edgar et al., 2011), and clustered at 97% (DNACLUST) to form the final clusters/OTUs. OTUs were then assigned a taxonomic lineage with the RDP classifier (Wang et al., 2007), using the AmpliconTagger 16S training sets (Tremblay, 2019), respectively. The RDP classifier gives a score (0 to 1) to each taxonomic depth of each OTU. Each taxonomic depth having a score >= 0.5 was kept to reconstruct the final lineage. Multiple sequence alignment was then obtained by aligning the 16S rRNA gene OTU sequences on the SILVA R128 database (Quast et al., 2013) using the PyNAST v1.2.2 aligner (Caporaso et al., 2010a). Alignments were filtered to keep only the hypervariable region of the alignment. For cross-sample comparisons of alpha diversity, ten iterations were performed on a random subsample of 1,000 reads rarefactions, and the average number of reads of each OTU of each sample was then computed to obtain a consensus rarefied OTU table (Fig.S1). Samples represented by less than 1,000 reads were removed from the analyses (2 samples were removed). Alpha (observed species) and taxonomic summaries were then computed using the QIIME v1.9.1 software suite using the consensus rarefied OTU table(Caporaso et al., 2010b; Kuczynski et al., 2011).

3.5.3 Statistical analyses

The OTU rank distribution for each sample was fit to niche-based models (null, pre-emption, log-normal, Zip f, and Mandelbrot) using the 'radfit' command in R (Oksanen et al., 2013), and neutral model (zero-sum model- ZSM) using TeTame v.2.1 (Jabot et al., 2008) using the same OTU table used to construct the phylogenetic tree. The Akaike Information Criterion (AIC) was used to assess the relative quality of each model, and the model that had the lowest AIC value was considered the best fit model for the data (Burnham and Anderson, 2003; Dumbrell et al., 2010b). The AIC values for each model were calculated using the equation AIC= -2×log-likelihood+ 2×npar, where npar is the number of parameters used in the model(Feinstein and Blackwood, 2012; Moroenyane et al., 2019). The statistical output is reported in Table 1. Dispersal rates were calculated by Etienne's formula, using TeTame Software (Jabot et al., 2008) (Table S3). Values of dispersal are between 0 and 1, where 0 means no tendency to migration and 1 means total tendency to migration in a specific community.

A maximum-likelihood tree was built from that all the aligned sequences of representative OTUs (a single representative sequence assigned to each OTU was used in subsequent analyses) with FastTree v2.1.10. using the GTR substitution model (Price et al., 2010). For cross-sample comparisons, the aligned fasta was subsampled to 1000 reads per samples, and samples with fewer than 1000s reads were discarded from all downstream phylogenetic analysis (Table S4; 26 samples were removed). Phylogenetic community turnover was evaluated using beta Nearest Taxon Index (βNTI) whose absolute magnitude reveals the relative influences of either niche-based or neutral processes. Briefly, using the mean nearest taxon index (MNTD), the standard effect size is calculated using the null mode 'taxa.labels' (999 randomisations in *Picante* (Kembel et al., 2010). The SES.MNTD index measures phylogenetic clustering in communities, with values >0 indicating phylogenetic overdispersion (distantly related taxa tend co-occur less than expected by chance) and values <0 indicating phylogenetic clustering (closely related taxa tend to co-occur more than expected by chance) (Webb et al., 2002). The phylogenetic turnover across all communities was calculated as the beta MNTD (βMNTD). The βNTI index is calculated as the difference between the observed βMNTD and mean of the normalised (standard deviation) null distribution of βMNTD. βNTI values that are <-2 indicating significantly less than expected phylogenetic turnover whilst values >+2 indicating significantly more than expected phylogenetic turnover (Stegen et al., 2012; Wang et al., 2013; Dini-Andreote et al., 2015). When βNTI values deviate from null expectation and value is between <-2 and >+2 it indicates the dominance of

neutral processes (Hardy, 2008), thus, observed differences in phylogenetic community compositions are the results of decreased dispersal rates (dispersal limitation), high dispersal rates (homogenising dispersal), or undominated by a specific process. The Bray-Curtis based Raup-Crick (RC_{bray}) was used to determine the prevailing processes on pairwise comparison with βNTI values that lie between <-2 and >+2 (Stegen et al., 2013; Dini-Andreote et al., 2015; Stegen et al., 2015). Briefly, the contributions dispersal limitation was calculated as the percentage of pairwise comparisons with $|\beta NTI| < +2$ and $RC_{bray} > +0.95$, homogenising dispersal $|\beta NTI| < +2$ and $RC_{bray} < -0.95$, and those that did not fall into those categories indicated undominated selections. This randomisation holds constant the observed taxa richness, occupancy and, turnover. Thus, this technique provides the expected level of βNTI given observed richness, occupancy, and turnover (Wang et al., 2013). A t-test was performed on the mean βNTI value to evaluate whether it significantly deviated from zero- which is expected under neutral assembly.

3.5.4 Sequence data deposition

The raw sequencing reads have been deposited in the NCBI SRA under Bioprect accession PRJNA601979: "Soybean microbiome - temporal and spatial development".

3.6 Results and discussion

To our knowledge, this is the first report that simultaneously provides evidence for the current assembly processes within bacterial niches across spatial and temporal axes in a controlled environment. Our aim to elucidate the overall processes within the plant microbiome highlighted that homogenous selection and dispersal limitations were the prevailing assembly processes across plant compartments and developmental stages. We were able to demonstrate that seemingly complementing approaches to quantifying assembly do reveal the dominance of similar processes across spatial and temporal axes, and these processes influence diversity patterns.

Overall, diversity patterns varied significantly across developmental stages and plant compartments. For instance, alpha diversity (OTU richness: developmental stage χ^2=12.37***; plant compartment χ^2=50.67***), beta diversity PERMANOVA (belowground: developmental stage R^2=0.21***, plant compartment R^2=0.25***; aboveground: developmental stage R^2=0.08***, plant compartment R^2=0.19***), and relative abundance of taxa at the phylum and order level varied significantly (Fig.S2). Recently, we demonstrated that these observed diversity patterns are modulated by interactions of spatial and temporal dynamics (Moroenyane et al., 2021c). At a glance, the mean ßNTI value of the community significantly deviated from null expectations but was between <-2 and >+2 indicating the dominance of neutral processes (Fig.1 one sample t-test p<0.05). When disentangling the relative influence of different assembly processes, homogenous selection and dispersal limitation were the prevailing assembly processes across all plant compartments with heterogenous selection playing a minor role across all plant compartments : Leaf (endophyte μ = -0.52***; epiphyte μ = -0.21***), Stem (endophyte μ = -0.64***; epiphyte μ = -1.01***), Root (endophyte μ = -0.82***; epiphyte μ = -0.76***), and Rhizosphere (μ = -0.14*) (Fig.1; Fig.S3). Phylogenetic beta diversity indices such as beta nearest taxon (ßNTI) show probabilistic (the likelihood of closely related taxa to co-occur less frequently than expected by chance) rather than absolute quantification of co-occurrences. This property of the models makes them ideal for detection of influences of environmental filtering rather than the nuanced ecological processes such as interspecific competition, for instance (Miller et al., 2017). Equally, all species distribution models (SDMs) indicated that, for the abundance and distribution of communities, niche-based models were always the best model with the lowest Akaike Information Criterion (AIC) (Table 1; Fig. 2).

Table 1. Bacterial Akaike Information Criterion (AIC) values of fitted rank abundance models. models with lowest Akaike Information Criterion (AIC) values were best fit. AIC values were calculated from the equation: $AIC = -2loglikelihood + 2 * npar$

			Akaike Information criterion (AIC)					
			Niche-based					Neutral
Organ	Developmental stage	ID	Null	Preemption	Lognormal	Zipf	Mandelbrot	ZSM
Leaf Endophyte	Emerging	LE1-1	1731.30	1636.61	**1063.21**	2057.79	1611.09	2151.64
		LE1-2	1259.84	1504.27	**1032.45**	1841.66	1453.29	2727.94
		LE1-3	4927.37	4868.88	**1573.91**	2518.82	2106.26	3785.24
		LE1-4	1675.18	1973.17	**1026.15**	1696.63	1544.46	2167.11
		LE1-5	6728.90	6915.60	**2123.10**	2694.90	2696.90	4424.70
	Growth	LE2-1	87015.80	39446.40	5198.60	2924.10	2926.10	**2257.24**
		LE2-2	7966.14	6809.24	2061.90	**1420.73**	1422.73	1964.87
		LE2-3	4508.65	4155.71	1519.77	**1049.60**	1051.60	1917.98
		LE2-4	6382.61	5047.45	1635.65	**1176.68**	1178.68	1559.45
		LE2-5	8214.80	7027.90	2245.40	**1572.40**	1574.40	2018.11
	Flowering	LE3-1	7277.60	3059.96	1126.86	1301.90	**767.68**	2633.86
		LE3-2	1671.15	1182.84	696.00	767.64	**550.13**	2261.44
		LE3-3	5405.77	4206.96	1192.67	**824.27**	826.27	1952.84
		LE3-4	537.73	597.40	466.02	**457.51**	459.18	1931.33
		LE3-5	673.46	719.96	**504.30**	608.39	542.89	2033.88
	Maturation	LE4-1	159.64	147.87	143.62	147.56	**132.80**	587.88
		LE4-2	280.67	290.54	252.62	243.32	**242.59**	1238.01
		LE4-3	445.21	474.87	385.28	370.36	**370.15**	1776.41
		LE4-4	515.30	535.25	382.43	347.64	**344.61**	1611.13
		LE4-5	239.03	235.14	222.83	221.16	**211.52**	1034.02

Leaf Epiphyte	Emerging	**LP1-1**	3453.59	3079.55	**1034.68**	1308.73	1310.73	3701.28
		LP1-2	169466.70	103572.90	15741.00	22986.80	NA	30959.80
		LP1-3	2468.73	2655.96	1352.99	**980.06**	982.06	3715.44
		LP1-4	501.13	512.65	381.30	338.84	**333.81**	1131.84
		LP1-5	51.54	47.80	44.24	**40.23**	42.23	186.31
	Growth	**LP2-1**	268.90	273.13	241.04	**222.59**	224.59	934.96
		LP2-2	23329.42	15662.00	3742.96	4127.47	**3187.77**	11376.68
		LP2-3	72.63	65.04	64.64	**62.46**	64.46	264.43
		LP2-4	132.36	120.38	111.37	116.40	**105.40**	419.49
		LP2-5	5917.21	4898.46	1668.12	1477.03	**1431.80**	5399.24
	Flowering	**LP3-1**	354.22	375.78	283.29	**237.85**	239.85	1086.25
		LP3-2	5337.65	3311.12	1059.60	**810.88**	812.88	1669.41
		LP3-3	224851.50	135978.60	10801.70	**5252.60**	5254.60	14874.92
		LP3-4	1739.63	1713.25	1129.09	1053.90	**949.51**	3577.40
		LP3-5	1973.75	1313.77	506.37	448.44	**412.82**	1666.46
	Maturation	**LP4-1**	116559.34	39012.06	3792.50	2946.54	**1960.75**	4721.30
		LP4-2	119928.30	36207.90	4763.70	3943.40	**3103.20**	4516.82
		LP4-3	49065.20	15185.40	3559.40	2884.50	**2191.40**	4789.04
		LP4-4	98097.00	23961.70	5364.30	5035.00	**2297.60**	6583.98
		LP4-5	45729.30	6258.10	5801.50	5962.80	3433.80	**2603.98**
Rhizosphere	Emerging	**R1-1**	20321.87	14809.94	7647.61	11695.92	**6704.83**	26104.20
		R1-2	9511.84	8118.59	4655.16	5932.30	**4314.59**	16121.94
		R1-3	47410.60	30584.00	10043.00	17440.50	NA	33673.20
		R1-4	46179.30	26828.40	10376.60	19500.10	NA	30958.80
		R1-5	16426.03	12405.64	6058.69	8738.74	**5853.93**	20814.50
	Growth	**R2-1**	41114.00	26828.60	8669.80	13987.40	NA	26697.80
		R2-2	56672.70	35468.50	11192.80	19230.20	NA	33378.60

		R2-3	12999.95	10448.33	5218.17	6893.44	**4773.15**	17314.98
		R2-4	4433.89	4253.87	2869.86	3178.24	**2653.35**	10115.56
		R2-5	49580.70	32668.20	9212.10	14600.40	NA	29040.20
	Flowering	**R3-1**	3160622.00	1724628.00	**67308.00**	92921.00	71980.00	73263.80
		R3-2	49.59	46.14	46.42	**44.67**	46.67	189.52
		R3-3	53947.70	30172.70	**12119.60**	23416.30	NA	33513.40
		R3-4	36796.10	31072.40	6960.60	**5443.90**	5445.90	13551.96
		R3-5	71502.90	45460.90	**8790.30**	13409.60	9315.70	23072.62
	Maturation	**R4-1**	87471.70	43841.50	17177.80	35477.10	NA	40397.20
		R4-2	3965.95	3930.50	2742.17	2951.24	**2547.70**	10118.84
		R4-3	13248.83	10139.84	5476.08	7514.48	**4981.22**	18473.70
		R4-4	17526.94	12942.67	6501.00	9229.46	**5658.92**	21676.72
		R4-5	23039.70	16685.50	5006.00	6359.60	**4743.60**	14187.52
Root Endophyte	Emerging	**RE1-1**	146354.40	76607.80	**7897.20**	11146.90	7947.10	19471.42
		RE1-2	1370.25	1340.06	758.77	**674.32**	675.92	2911.30
		RE1-3	20902.04	15263.92	3208.38	2501.23	**2343.76**	8547.12
		RE1-4	21411.70	13445.55	2967.40	3375.03	**2455.53**	9737.94
		RE1-5	13658.32	7898.62	3173.42	4789.58	**2403.20**	10172.64
	Growth	**RE2-1**	13370.81	7468.01	2613.56	3820.23	**2093.86**	8398.82
		RE2-2	22746.25	12710.13	3523.70	5213.65	**2688.32**	11384.34
		RE2-3	77678.60	31970.60	8343.10	15038.30	NA	15894.94
		RE2-4	37042.26	20805.52	3820.10	4827.30	**2869.46**	11608.16
		RE2-5	60672.40	28841.80	8044.70	13572.50	NA	17154.74
	Flowering	**RE3-1**	39375.78	25257.11	3278.44	**2444.07**	2446.07	8513.28
		RE3-2	73251.70	37916.20	**4272.60**	6508.50	5037.10	15300.64
		RE3-3	29.66	**27.86**	28.99	28.56	30.56	144.54
		RE3-4	35973.80	23017.40	3751.90	4135.70	**3675.20**	13186.74

		RE3-5	8248.63	5632.40	1801.59	1868.45	**1440.65**	6611.90
	Maturation	**RE4-1**	4195.19	3220.01	897.99	**593.40**	595.40	2861.04
		RE4-2	16427.30	4088.10	3376.10	5436.10	2255.20	**1876.60**
		RE4-3	147576.20	87397.90	14241.20	**9752.80**	9754.80	10994.52
		RE4-4	34640.62	22271.40	3890.23	2992.94	**2500.59**	9438.72
		RE4-5	17734.20	9474.50	2319.90	2203.70	**1829.90**	3447.56
Root Epiphyte	Emerging	**RP1-1**	104897.90	31644.80	3469.70	**1916.80**	1918.80	2225.02
		RP1-2	34518.20	10558.80	3661.40	6927.30	5677.50	**1710.09**
		RP1-3	121813.80	69599.20	8014.60	5366.00	**4859.80**	11663.26
		RP1-4	7467.70	6849.90	**3206.40**	5162.90	5164.90	4987.42
		RP1-5	75864.00	11715.40	7266.90	9161.00	6277.50	**1101.10**
	Growth	**RP2-1**	284881.00	138584.00	19381.00	30372.00	30374.00	**14825.64**
		RP2-2	105838.20	46109.50	**6829.80**	17734.50	15566.80	9980.66
		RP2-5	36322.40	17367.30	**5529.00**	15199.70	NA	16390.80
	Flowering	**RP3-1**	65812.60	31443.50	**3151.40**	4578.60	4169.20	5950.90
		RP3-2	20789.44	17338.29	4532.77	3505.16	**3207.61**	14351.18
		RP3-3	27353.80	25041.00	5857.20	**3600.90**	3602.90	7225.66
		RP3-4	5652.54	850.89	457.95	594.58	**366.96**	438.74
		RP3-5	11903.11	11242.08	**2901.61**	3080.43	3082.43	7934.16
	Maturation	**RP4-1**	39778.65	NA	578.41	**384.71**	386.71	862.00
		RP4-2	160817.00	26075.00	16549.00	14300.00	NA	**4041.64**
		RP4-3	**19.82**	21.42	22.72	21.39	23.39	85.16
		RP4-4	36.18	33.35	33.76	**32.92**	34.92	162.40
		RP4-5	53243.40	3953.90	6065.40	8679.20	NA	**3437.38**
Stem Endophyte	Emerging	**SE1-1**	22874.36	2627.93	2600.39	2920.11	**1123.27**	1695.79
		SE1-2	643.24	657.30	416.19	370.69	**365.87**	1499.59
		SE1-3	1742.77	1819.81	995.72	953.16	**884.26**	3527.46

		SE1-4	558.76	596.29	440.34	439.01	**421.40**	1612.81
		SE1-5	277.28	267.65	228.54	224.84	**209.64**	905.48
	Growth	**SE2-1**	68720.00	44720.40	5578.10	**4258.40**	4260.40	6061.14
		SE2-2	59392.30	32766.20	5150.70	**3839.90**	3841.90	6264.10
		SE2-3	49.68	47.12	42.08	**34.75**	36.75	146.82
		SE2-4	146357.20	123413.80	10741.40	**8709.60**	8711.60	8870.48
		SE2-5	14561.08	8078.75	1329.11	**773.92**	775.92	2801.10
	Flowering	**SE3-1**	13.15	**13.12**	14.00	14.00	16.00	72.16
		SE3-2	9750.87	8076.91	2265.21	**1569.14**	1571.14	4057.02
		SE3-3	8912.80	7555.40	2230.20	**1510.90**	1512.90	3947.50
		SE3-5	**10.22**	10.89	12.00	12.00	14.00	43.30
	Maturation	**SE4-1**	9931.74	7793.14	1872.30	**1196.93**	1198.93	3546.76
		SE4-2	24322.62	11803.20	1896.04	1893.60	**1133.83**	4774.00
		SE4-3	30694.20	22336.20	4075.80	**2807.70**	2809.70	6324.76
		SE4-4	19104.01	14162.74	3003.55	**2160.33**	2162.33	5867.80
		SE4-5	28519.10	23974.10	5248.40	**2944.50**	2946.50	8316.84
Stem Epiphyte	Emerging	**SP1-1**	9966.74	8050.04	2230.10	**1495.05**	1497.05	4244.22
		SP1-2	6853.10	5293.75	1514.06	**1053.47**	1055.47	3059.54
		SP1-3	11398.59	8603.62	1979.93	**1236.89**	1238.89	3814.10
		SP1-4	15320.50	12060.10	2788.30	**1780.60**	1782.60	4837.06
		SP1-5	11814.34	8919.97	2112.46	**1398.06**	1400.06	3938.26
	Growth	**SP2-1**	15898.19	12266.16	2765.02	**1712.02**	1714.02	4692.86
		SP2-2	21181.87	15766.97	3218.75	**2025.06**	2027.06	5385.90
		SP2-3	**12.70**	13.40	14.84	14.75	16.75	67.47
		SP2-4	29305.35	26887.04	6455.59	**3896.90**	3898.90	12699.78
		SP2-5	55594.30	41112.20	6428.70	**3804.00**	3806.00	8841.26
	Flowering	**SP3-1**	19746.85	15337.61	3314.05	**2022.32**	2024.32	5023.20

		SP3-2	12365.27	9677.30	2283.03	**1439.61**	1441.61	4442.32
		SP3-3	24216.64	17545.33	3432.94	**2177.00**	2179.00	5661.16
		SP3-4	17151.53	13248.13	3045.70	**1905.20**	1907.20	5151.12
		SP3-5	9455.30	7505.32	1863.38	**1160.44**	1162.44	3940.64
	Maturation	**SP4-1**	76934.07	11393.37	2497.32	2510.86	**901.79**	3230.02
		SP4-2	123765.90	12891.60	6943.20	6098.80	**2775.70**	2967.54
		SP4-3	34643.30	7384.83	1018.76	**611.51**	613.51	1965.01
		SP4-4	44753.33	8032.92	988.98	**592.04**	594.04	1952.83
		SP4-5	65893.30	18230.60	2018.50	**1646.60**	1648.60	2850.32

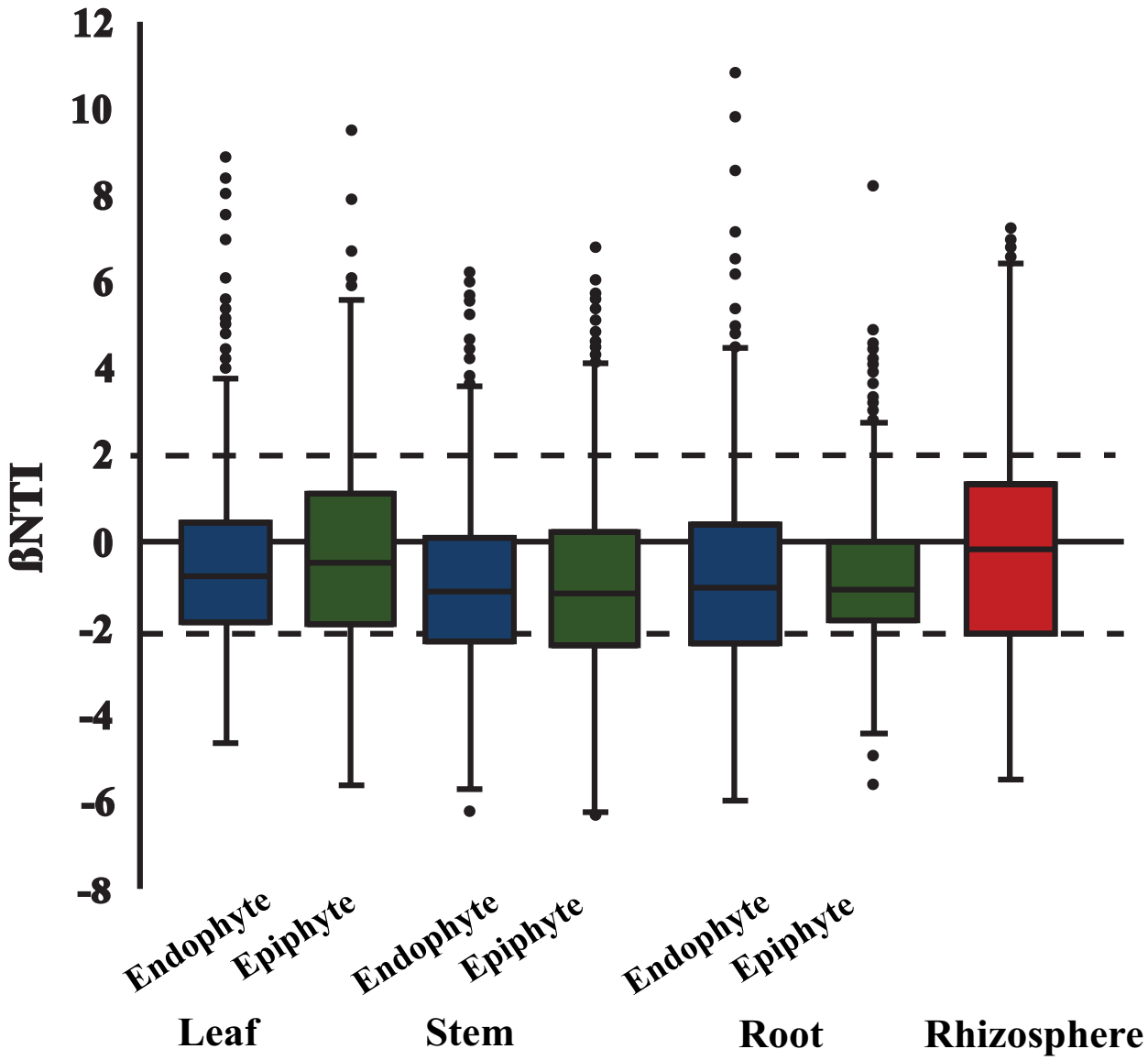

Fig.1 Boxplot of βNTI observations across plant compartments, where each observation is the number of null model standard deviations the observed value is from the mean of null distribution. The dashed blue lines indicate the significant upper and lower limits thresholds of βNTI at +2 and -2. A t-test was performed on the mean value of the βNTI to test if it significantly deviated from zero which is expected under neutral assembly: Leaf (Endophyte μ = -0.52*; Epiphyte μ = -0.21***), Stem (Endophyte μ = -0.64***; Epiphyte μ = -1.01***), Root (Endophyte μ = -0.82***; Epiphyte μ = -0.76***), and Rhizosphere (μ = -0.14*;)Where * indicates significance level (*<0.05; **<0.001, ***<0.0001)**

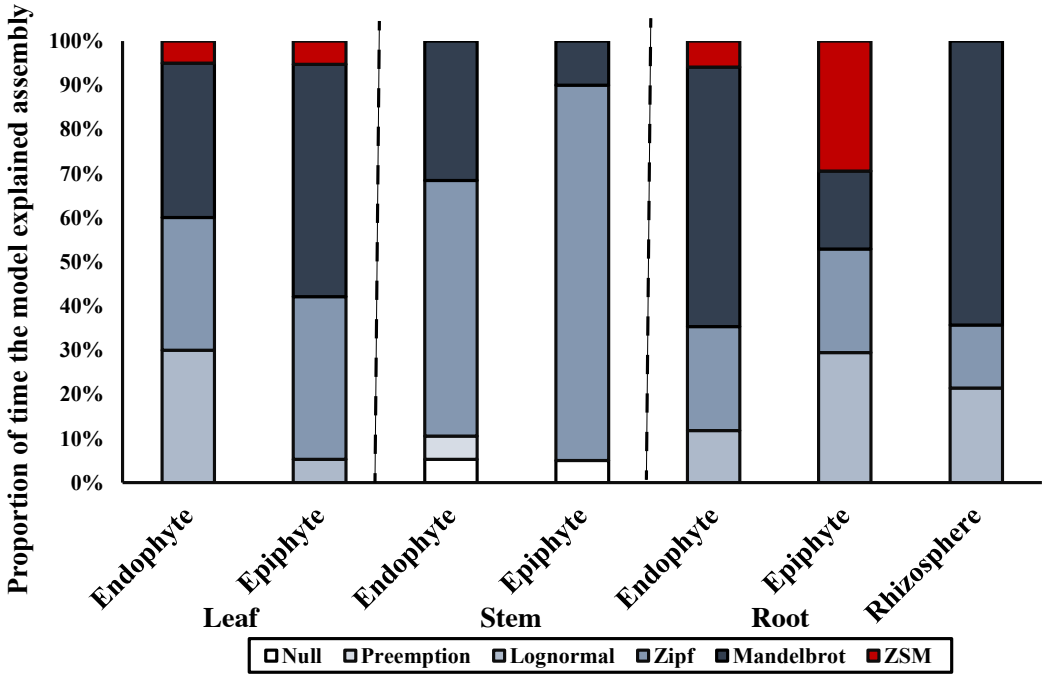

Fig.2 Bacterial community assembly processes (across plant organs) of fitted rank abundance models; models with lowest Akaike Information Criterion (AIC) values were best fit. AIC values were calculated from the equation: $AIC = -2loglikelihood + 2 * npar$

When nutrients are limiting, such as at the root-soil interface under certain conditions (Rengel and Marschner, 2005), there will be a more substantial influence of niche-based processes (Chase, 2010). In soybean field trials, when micronutrients become limiting, there are increased dispersal rates across temporal axes (Goss-Souza et al., 2019). Both PNMs and SDMs elucidated the dominance of niche-based selection (homogeneous) and increased dispersal at the root-soil interface (Fig.2; Fig.3; Fig.S3). This zone is a very selective environment (Smalla et al., 2001), with rhizodeposition leading to the assembly of a microbial community in sharp contrast with bulk soil communities (Hartmann et al., 2009; Mendes et al., 2014; Goss-Souza et al., 2019). Also, it is possible that the reductionist experimental setup (i.e. closed chamber) significantly influenced the distribution and abundance of the bacterial community as detected by SDMs and increased dispersal rates within the epiphytic communities.

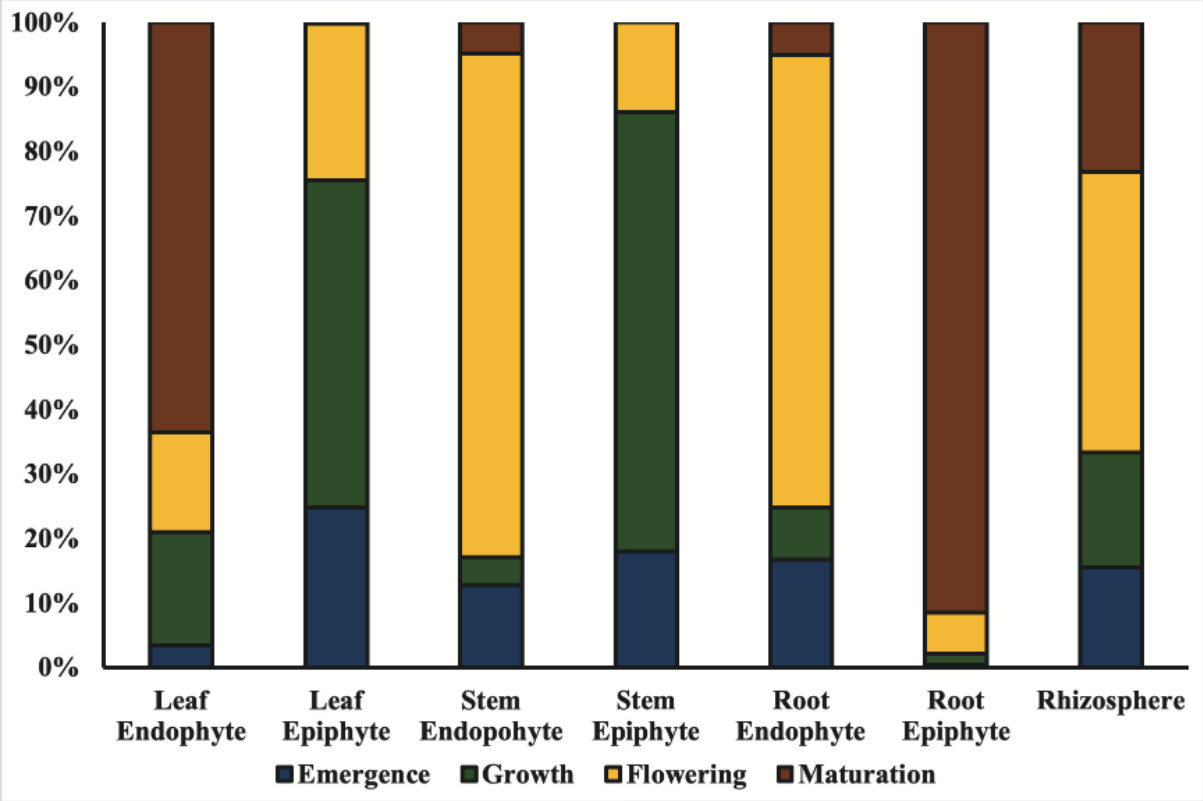

Fig.3 The percentage of dispersal in community assembly and dispersal rates were calculated using TeTame software with Etienne's formula, where *m* values are between 0 and 1. When *m*=1 indicates increased tendency to migrate and *m*=0 indicates no tendency to migrate across plant compartment.

Table 2. Dispersal rates across developmental stages and plant compartments of soybean-associated bacterial communities.

Organ	Developmental stage	Dispersal rate (*m*)
Leaf endophyte	Emerging	0.008
	Growth	0.041
	Flowering	0.036
	Maturation	**0.148**
Leaf epiphyte	Emerging	0.142
	Growth	**0.290**
	Flowering	0.139
	Maturation	0.001
Rhizosphere	Emerging	0.073
	Growth	0.084
	Flowering	**0.205**
	Maturation	0.109
Root endophyte	Emerging	0.033
	Growth	0.016
	Flowering	**0.139**
	Maturation	0.010
Root epiphyte	Emerging	0.001
	Growth	0.004
	Flowering	0.015
	Maturation	**0.215**
Stem endophyte	Emerging	0.087
	Growth	0.030
	Flowering	**0.531**
	Maturation	0.033
Stem epiphyte	Emerging	0.044
	Growth	**0.166**
	Flowering	0.034
	Maturation	6.17604E-07

Dispersal rates were calculated using TeTame software with Etienne's formula, where *m* values are between 0 and 1. when $m=1$ indicates no dispersal limitation and $m<1$ indicates dispersal limitation

In contrast, SDM neutral assembly model had the best explanatory power for the assembly of the microbial communities of some leaf and root samples, suggesting that the plant selection stringency of these environments is relatively more relaxed. Successful colonisation of new bacterial niche spaces is predominantly dominated by species-sorting (niche-based) and dispersal limitation (neutral) (Langenheder and Szekely, 2011). The increased surface area of leaves and roots provides increases dispersal opportunities for air-borne and free-living soil microbes to occupy these niche spaces, and dispersal limitation reinforces these current processes that occurred during initial colonisation (Maignien et al., 2014). The stem endosphere is a relatively nutrient-poor environment, or at least unbalanced, with a nitrogen content of sap directly affecting diversity and abundance of microbes (Subramanian et al., 2009; Ikeda et al., 2010b). As such, homogenous selection dominated assembly at later developmental stages whilst heterogenous selection dominated at emergence (Fig.S3). We suggest that during the shorter developmental stages (emergence/flowering) the selective pressure asserted by the plant produces heterogeneous selection; whereas, at the longer reproductive stages (vegetative growth and maturation) homogeneous selection dominates.

For the growth stages, again, the mean ßNTI value of the epiphytic community significantly deviated from null expectations but was between <-2 and >+2 indicating the dominance of neutral processes: Emergence (μ = -0.21***), Growth (μ = -0.19***), Flowering (μ = -0.23***), Maturation (μ = -0.07***), and Overall (μ = -0.70) (Fig.4). On average, homogenising dispersal and selection (homogenous and heterogenous) processes accounted for majority assembly processes *ca.*60% at each developmental stage (Fig.5). Similarly, SDMs highlighted that neutral processes play a minor role in community assembly across other developmental stages (Fig.6). Generally, niche-based processes (homogenous and heterogenous) dominated at the growth and flowering stage, and dispersal dominated at the growth and maturation stages. It is proposed that as the plant's metabolic demand for nutrient and carbon increases at this stage, there will be a stringent selection for microbial taxa that can help in the provision of those nutrients (Copeland et al., 2015; Hara et al., 2019). In the case of soybean, secondary metabolites (e.g. ethylamine and betaine) are produced during the flowering stage, and we suggest that the presence of these molecules act as a robust environmental filter (Hara et al., 2019). In fact, at the flowering stage, the abundance and distribution were best predicted solely by the niche-based model despite increased dispersal rates. It is then possible that within the communities, microbial taxa that were assembled by neutral processes (speciation or drift) are competitively excluded due to their inability to withstand strong environmental selection. These results presented here support observed successional patterns of field- and laboratory-grown soybean plants, as we found the

same specialist taxa (Fig. S2) that characteristically dominate at different developmental stages in soybean (Copeland et al., 2015; Zhang et al., 2018b; Liu et al., 2019a).

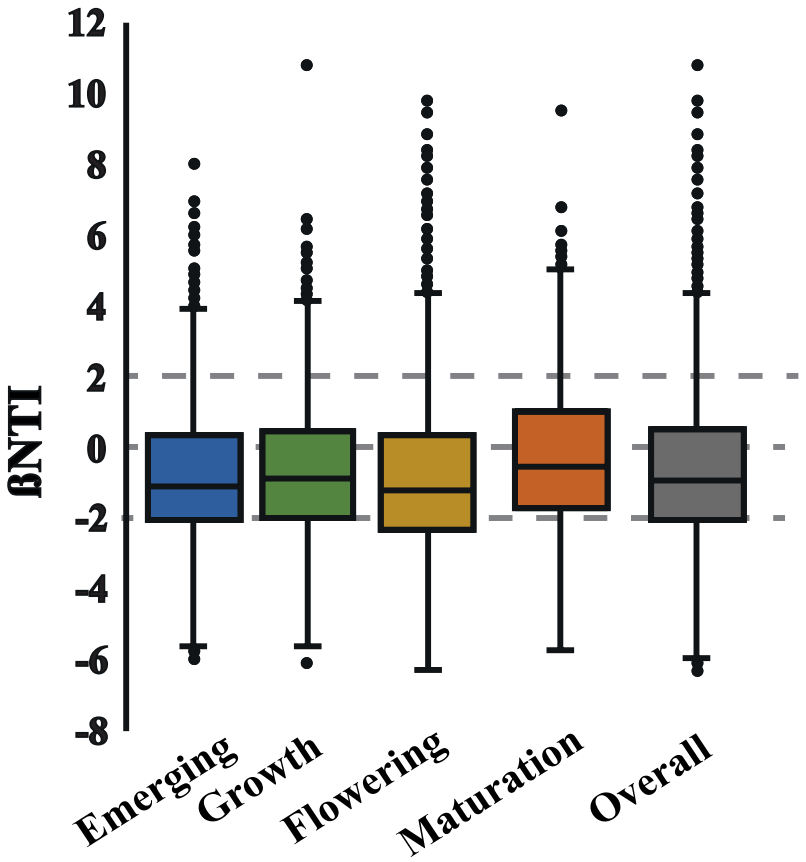

Fig.4 Boxplot of βNTI observations across developmental stages, where each observation is the number of null model standard deviations the observed value is from the mean of null distribution. The dashed blue lines indicate significant upper and lower limits thresholds of βNTI at +2 and -2. A t-test was performed on the mean value of the βNTI to test if it significantly deviated from zero which is expected under neutral assembly: Emerging (μ = -0.21*), Growth (μ = -0.19***), Flowering (μ = -0.23***), Maturation (μ = -0.07***), and Overall (μ = -0.70). Where * indicates significance level (*<0.05; **<0.001, ***<0.0001)**

Dispersal rates varied across the plant compartment and developmental stages (Fig.3; Fig.5; Fig.S3). The root and stem endophytic communities had a higher propensity for dispersal at the flowering stage, whilst the leaf and stem epiphytic was during the growth stage. The leaf endophyte and root epiphyte communities had increased dispersal rates at the maturation stage, whilst the rhizosphere community has little to intermediate dispersal rates across all developmental stages. For instance, SDMs neutral model had the best explanatory power for some communities at the emergence, growth, and maturation stages, indicating that both neutral and niche-based processes are essential in shaping the initial community, but also in explaining the temporal variation observed in the microbial communities associated to soybean (Hara et al., 2019)and other plants (Chaparro et al., 2014; Amend et al., 2019). Additionally, at the maturation stage, phylogenetic null models indicated that the community was neither dominated by niche-based nor by neutral processes. This shift in the community assembly processes suggests changes in plant metabolic quality, i.e. decrease in metabolites supplied to microbial symbiont as the plant enters senescence (Bell et al., 2015; Zhalnina et al., 2018). Here, we propose that the influence of niche-based processes on abundance and distribution of microbes at this stage, as shown by SDMs, may be a relic of previous environmental selection perpetuated by microbe-microbe interaction, as previously highlighted in the rhizosphere of desert plants (Marasco et al., 2018).

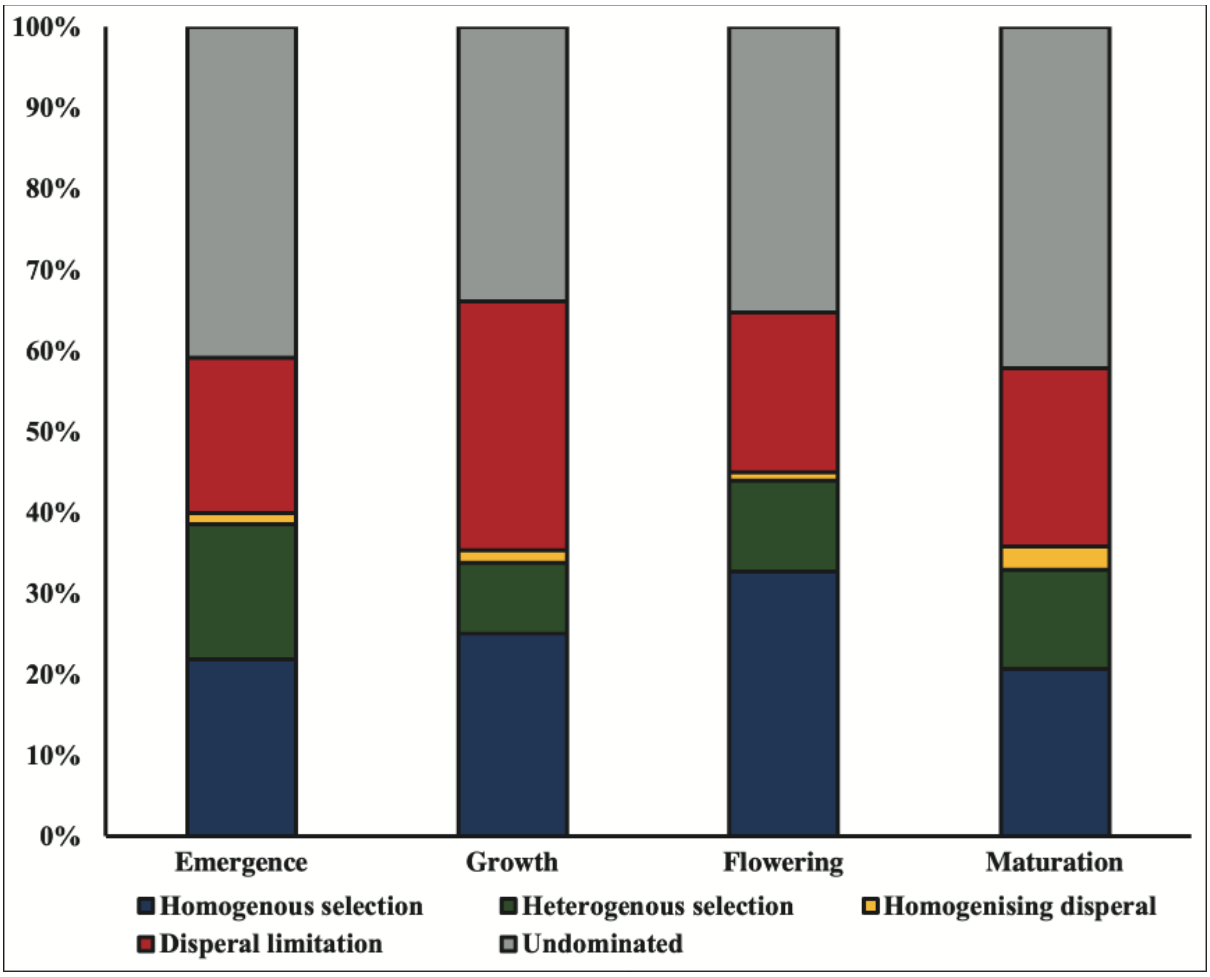

Fig. 5 The percentage of turnover in community assembly modulated by various niche-based (homogenous and heterogeneous selection), neutral processes (dispersal limitation and homogenising dispersal), and a fraction that was not dominated by any process across developmental stages.

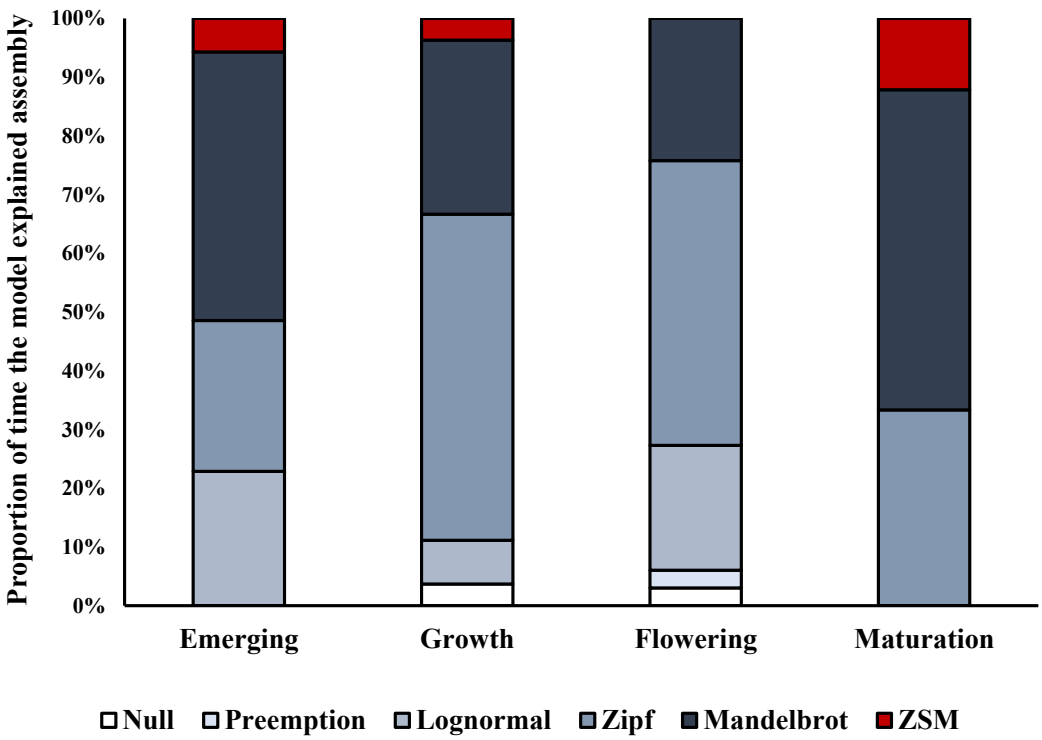

Fig.6 Bacterial community assembly processes (across developmental stages) of fitted rank abundance models; models with lowest Akaike Information Criterion (AIC) values were best fit. AIC values were calculated from the equation: $AIC = -2loglikelihood + 2 * npar$

Our study highlighted the difficulty in getting clear data on community assembly when considering niche space to be the same in different plant compartments, suggesting that modelling community assembly across space and time is far from trivial and would require some sort of normalization for volume and population size across compartments. With that cautionary note in mind, we were still able to demonstrate that seemingly complementing approaches to quantifying assembly do reveal the dominance of niche-based processes across spatial and temporal axes. Both classes of models indicated that the plant compartment and developmental stage modulate the balance between niche-based and neutral processes. Dispersal limitations did have some influence at some specific growth stages or in defined compartments. These stages and compartments might be more readily amenable to inoculation or other microbiome manipulation approaches, as communities under stringent niche-based assembly processes are probably challenging to displace. This knowledge could orient the ongoing efforts to manipulate plant microbiomes for increased beneficial services and more sustainable agriculture.

3.7 Acknowledgements

The authors would like to thank Benjamin Mimee from Agriculture and Agri-Food Canada for providing the seeds used in the study. This work was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC) grant RGPIN 2014-05274 to EY. IM was supported by the Innovation and Scarce Skills scholarship from South African National Research Foundation (NRF), Fonds de Recherche du Québec (FRQNT), and partly by Foundation Armand-Frappier. We also wish to acknowledge Compute Canada for access to the University of Waterloo's High-Performance Computing (HPC) infrastructure (Graham system) through a resources allocation granted to EY.

3.8 Conflict of interest

The authors declare no conflict of interest.

3.9 **Supplementary information**

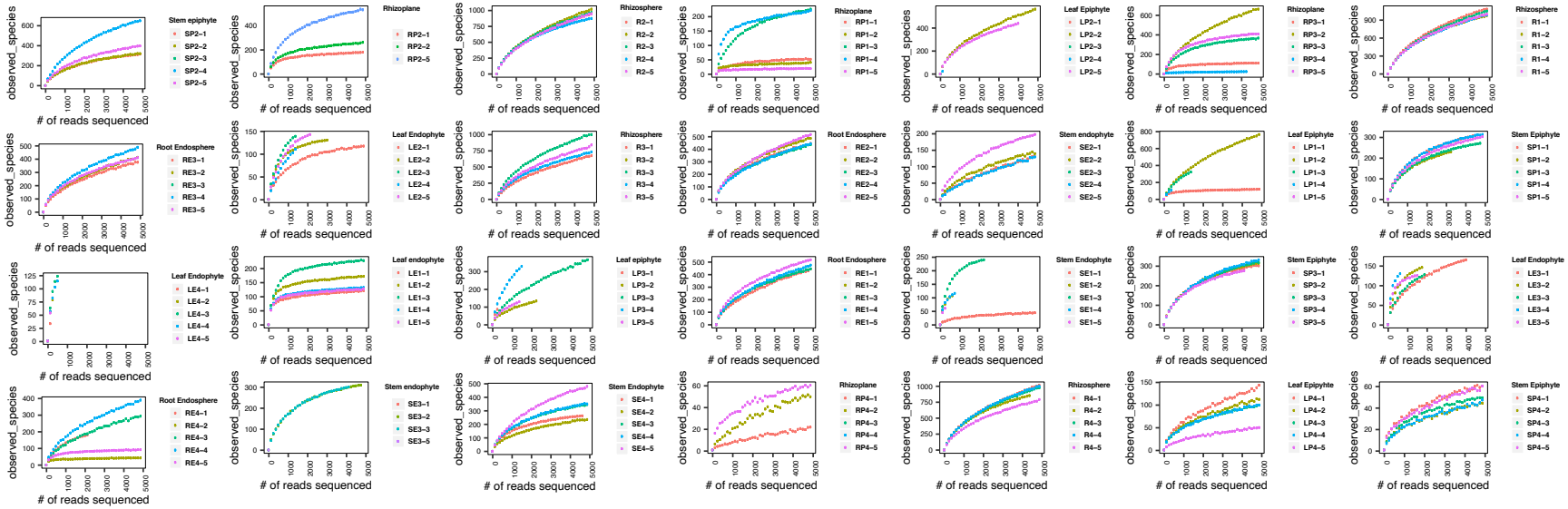

Fig. S1Alpha diversity rarefication curves of observed species (bacterial /archaea), raw OTU tables were rarefied

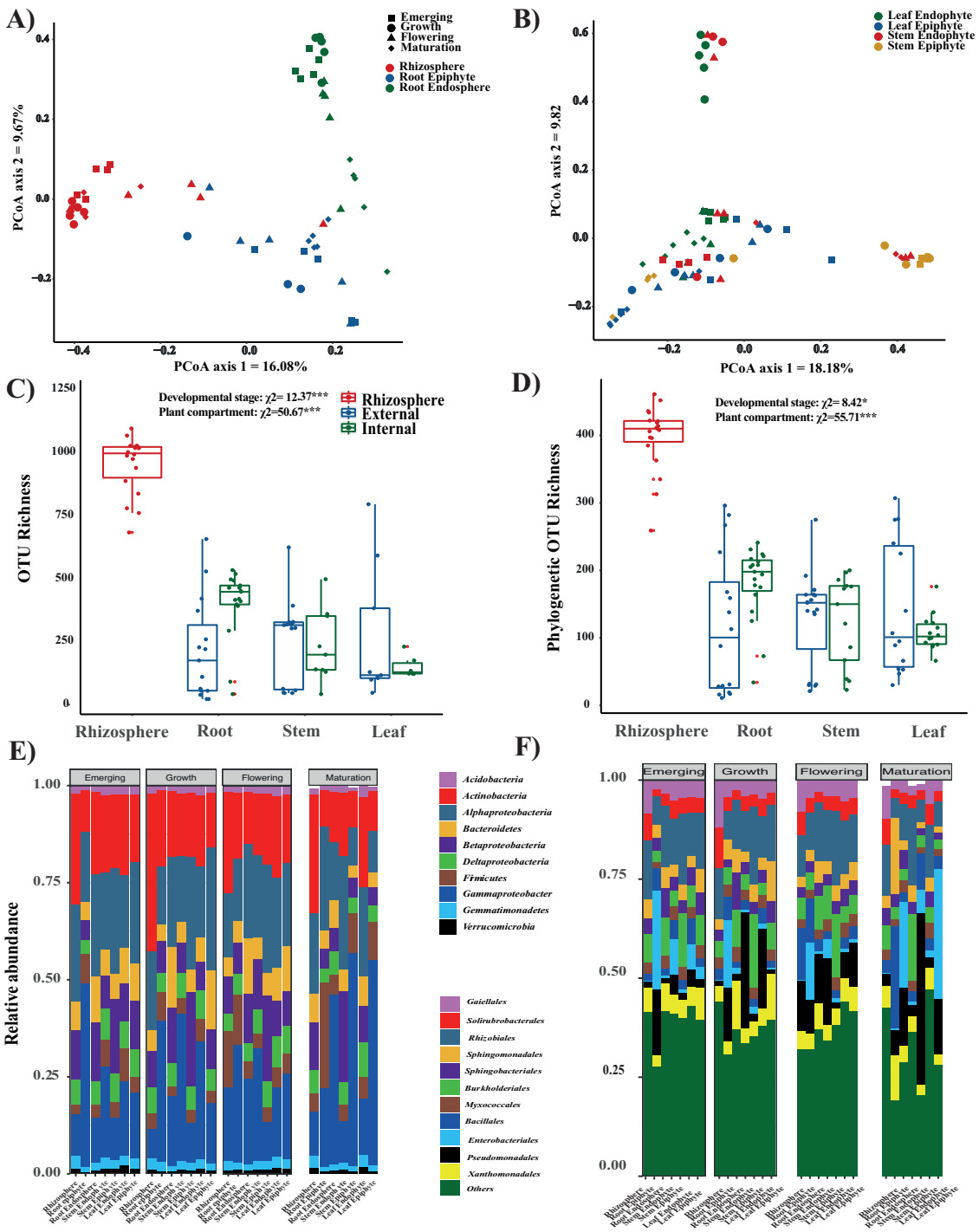

Fig.S2 Diversity across plant compartments and developmental stages. A) Bray-Curtis dissimilarity for below ground compartments, B) Bray-Curtis dissimilarity for above-ground compartments, C) OTU richness across plant compartment, D) OTU richness across developmental stages, E) Relative abundance at phylum level, F) Relative abundance at order level

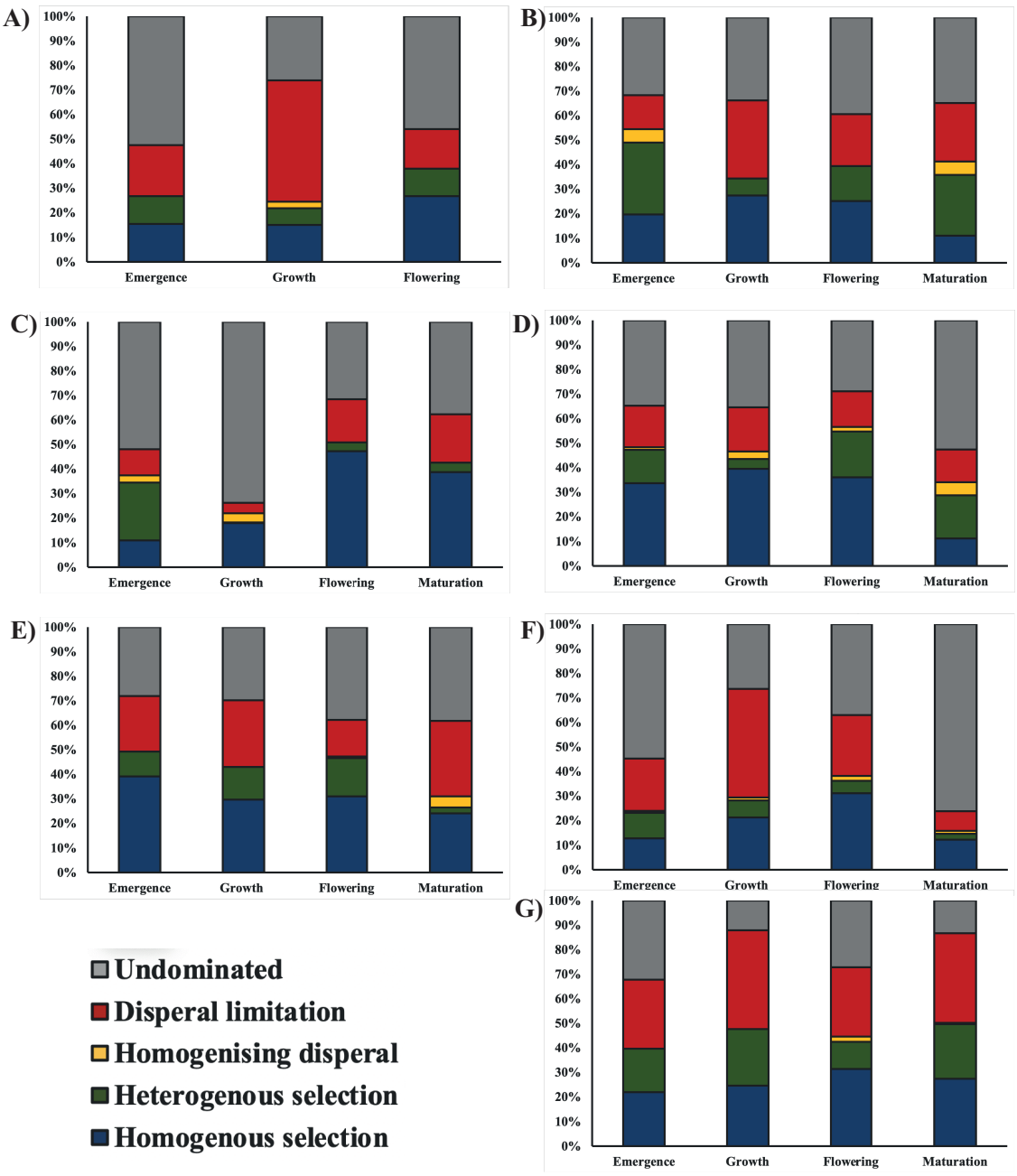

Fig. S3 The percentage of turnover in community assembly modulated by various niche-based (homogenous and heterogeneous selection), neutral processes (dispersal limitation and homogenising dispersal), and a fraction that was not dominated by any process across plant compartment; A) leaf endophyte, B) leaf epiphyte, C) stem endophyte, D) stem epiphyte, E) root endophyte, F) root epiphyte, and G) rhizosphere

Table S1 Measured soil physical properties and values represent a mean of five soil samples

Soil Properties	
pH	7.2
P (kg/ha)	193
K (kg/ha)	138
Ca (kg/ha)	7517
Mg (Kg/ha)	176
Al (ppm)	640
P/Al (ISP)	13.4
Mn (ppm)	25.7
Cu (ppm)	2.55
Zn (ppm)	4.53
B (ppm)	1.13
Fe (ppm)	188
N (total) %	0.15
C/N	13.1
NH_4^+ (ppm)	5.1
NO_3^- (ppm)	43.93
Conductivity (mS/cm)	0.33

Table S2 Total OTU richness across all plant compartments and developmental stages; LE= leaf endophyte, LP= Leaf Epiphyte, R= Rhizosphere, RE=Root Endophyte, RP= Root Epiphyte, SE= Stem Endophyte, and SP=Stem Epiphyte

Sample ID	OTU richness
LE1_1	126
LE1_2	177
LE1_3	260
LE1_4	140
LE1_5	145
LE2_1	152
LE2_2	134
LE2_3	145
LE2_4	114
LE2_5	148
LE3_1	167
LE3_2	148
LE3_3	131
LE3_4	138
LE3_5	128
LE4_1	42
LE4_2	83
LE4_3	124
LE4_4	117
LE4_5	76
LP1_1	121
LP1_2	2334
LP1_3	332
LP1_4	116
LP1_5	13
LP2_1	85
LP2_2	885
LP2_3	26
LP2_4	27
LP2_5	444
LP3_1	91
LP3_2	136
LP3_3	1151
LP3_4	338
LP3_5	132

LP4_1	303
LP4_2	257
LP4_3	153
LP4_4	197
LP4_5	86
R1_1	2008
R1_2	1351
R1_3	2510
R1_4	2347
R1_5	1685
R2_1	2086
R2_2	2612
R2_3	1470
R2_4	887
R2_5	2226
R3_1	5871
R3_2	18
R3_3	2518
R3_4	1248
R3_5	2108
R4_1	3050
R4_2	860
R4_3	1541
R4_4	1731
R4_5	1332
RE1_1	1267
RE1_2	226
RE1_3	591
RE1_4	691
RE1_5	715
RE2_1	578
RE2_2	769
RE2_3	1065
RE2_4	778
RE2_5	1174
RE3_1	615
RE3_2	916
RE3_3	11
RE3_4	825
RE3_5	428

RE4_1	183
RE4_2	58
RE4_3	662
RE4_4	593
RE4_5	108
RP1_1	71
RP1_2	54
RP1_3	343
RP1_4	268
RP1_5	37
RP2_1	411
RP2_2	546
RP2_5	931
RP3_1	175
RP3_2	936
RP3_3	441
RP3_4	25
RP3_5	503
RP4_1	33
RP4_2	136
RP4_3	7
RP4_4	13
RP4_5	104
SE1_1	55
SE1_2	113
SE1_3	242
SE1_4	119
SE1_5	70
SE2_1	201
SE2_2	211
SE2_3	10
SE2_4	294
SE2_5	203
SE3_1	5
SE3_2	315
SE3_3	303
SE3_5	4
SE4_1	265
SE4_2	312
SE4_3	444

SE4_4	419
SE4_5	623
SP1_1	311
SP1_2	232
SP1_3	272
SP1_4	345
SP1_5	309
SP2_1	341
SP2_2	373
SP2_3	5
SP2_4	899
SP2_5	589
SP3_1	348
SP3_2	322
SP3_3	391
SP3_4	365
SP3_5	279
SP4_1	105
SP4_2	93
SP4_3	63
SP4_4	64
SP4_5	92

Table S3. Dispersal rates across developmental stages and plant compartments of soybean-associated bacterial communities.

Organ	Developmental stage	Dispersal rate (*m*)
Leaf endophyte	Emerging	0.008
	Growth	0.041
	Flowering	0.036
	Maturation	**0.148**
Leaf epiphyte	Emerging	0.142
	Growth	**0.290**
	Flowering	0.139
	Maturation	0.001
Rhizosphere	Emerging	0.073
	Growth	0.084
	Flowering	**0.205**
	Maturation	0.109
Root endophyte	Emerging	0.033
	Growth	0.016
	Flowering	**0.139**
	Maturation	0.010
Root epiphyte	Emerging	0.001
	Growth	0.004
	Flowering	0.015
	Maturation	**0.215**
Stem endophyte	Emerging	0.087
	Growth	0.030
	Flowering	**0.531**
	Maturation	0.033
Stem epiphyte	Emerging	0.044
	Growth	**0.166**
	Flowering	0.034
	Maturation	6.17604E-07

Dispersal rates were calculated using TeTame software with Etienne's formula, where *m* values are between 0 and 1. When *m*=1 indicates increased tendency to migrate and *m*=0 indicates no tendency to migrate

Table S4 Total phylogenetic OTU richness across all plant compartments and developmental stages LE= leaf endophyte, LP= Leaf Epiphyte, R= Rhizosphere, RE=Root Endophyte, RP= Root Epitphyte, SE= Stem Endophyte, and SP=Stem Epiphyte

Sample ID	OTU richness
LE1.1	89
LE1.2	138
LE1.3	176
LE1.4	104
LE1.5	99
LE2.1	66
LE2.2	100
LE2.3	124
LE2.4	90
LE2.5	107
LE3.1	93
LE3.2	122
LE3.3	87
LE3.5	115
LP1.1	95
LP1.2	307
LP1.3	276
LP2.2	240
LP2.5	225
LP3.2	89
LP3.3	140
LP3.4	275
LP3.5	107
LP4.1	66
LP4.2	47
LP4.3	54
LP4.4	53
LP4.5	30
R1.1	461
R1.2	406
R1.3	452
R1.4	421
R1.5	422
R2.1	411
R2.2	408
R2.3	414

R2.4	396
R2.5	385
R3.1	259
R3.3	434
R3.4	313
R3.5	363
R4.1	436
R4.2	410
R4.3	419
R4.4	397
R4.5	335
RE1.1	187
RE1.2	198
RE1.3	213
RE1.4	194
RE1.5	241
RE2.1	221
RE2.2	217
RE2.3	208
RE2.4	208
RE2.5	224
RE3.1	175
RE3.2	178
RE3.4	231
RE3.5	205
RE4.1	139
RE4.2	34
RE4.3	125
RE4.4	164
RE4.5	73
RP1.1	28
RP1.2	29
RP1.3	113
RP1.4	159
RP1.5	17
RP2.1	138
RP2.2	168
RP2.5	296
RP3.1	88
RP3.2	282

RP3.3	227
RP3.4	16
RP3.5	267
RP4.1	11
RP4.2	19
RP4.5	31
SE1.1	23
SE1.3	197
SE2.1	39
SE2.2	67
SE2.4	36
SE2.5	86
SE3.2	177
SE3.3	176
SE4.1	150
SE4.2	121
SE4.3	186
SE4.4	173
SE4.5	200
SP1.1	164
SP1.2	138
SP1.3	140
SP1.4	163
SP1.5	153
SP2.1	135
SP2.2	164
SP2.4	275
SP2.5	192
SP3.1	156
SP3.2	152
SP3.3	171
SP3.4	171
SP3.5	142
SP4.1	32
SP4.2	30
SP4.3	31
SP4.4	21
SP4.5	29

4 CHAPTER 4 : PUBLICATION 3

4.1 Soybean microbiome recovery after disruption is modulated by the seed and not the soil microbiome

4.2 Le recouvrement du microbiome du soja après sa perturbation est influencé par le microbiome des graines et non pas le microbiome du sol

Itumeleng Moroenyane[1], Julien Tremblay[2], and Étienne Yergeau[1]*

a) Institut National de la Recherche Scientifique, Centre Armand-Frappier Santé Biotechnologie, 531 Boulevard des Prairies, Laval, Québec, H7V1B7, Canada
b) Energy, Mining, and Environment, Natural Research Council Canada, 6100 Avenue RoyalMount, Montreal, Quebec, H4P 2R2, Canada

***Corresponding author:** É.Yergeau Email: etienne.yergeau@inrs.ca
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Author contributions

I Moroenyane contributed to content and successful publication of the article. Itumeleng Moroenyane designed, performed the experiments and the statistical analyses, and wrote the manuscript. J Tremblay perfomed the bioinfomatics analysis and conributed to revising the manuscritp. É Yergaeu obtained the research funding, supervised the project and participated in writing and revising the manuscript.

Research links

The previous articles highlights focused on defining the successional patterns of the entire soybean microbiome across spatial and temporal niches. It highlighted that both spatial and temporal dynamics interacted to produce the observed diversity patterns; more importantly, these interactions were temporally nested. This article goes further by specifying the role of regional species pool in the colonisation of spatial niches. Moreover, it highlights the primacy of the seed species pool over the soil in colonisation of endosphere niche spaces.

4.3 **Abstract**

Endophytic microbiome of healthy seeds forms a symbiotic relationship with their host. Seeds and environment are sources of microbes that colonise the developing plant, however, the influence of each remains unclear. Here, using irradiation combined with surface sterilisation to generate near-axenic seeds with disrupted and reduced microbiome, we contrasted colonisation potential of seed and soil microbiome. We hypothesised that the seed microbiome would be the primary coloniser of the plant endophytic compartments. Our experimental design comprised four treatments, using soybean as a model plant: 1) nearly axenic seeds growing in a sterile environment , 2) non-axenic seeds inoculated with a microbial soil extract, 3) nearly axenic seeds inoculated with a microbial seed extract, and 4) nearly axenic seeds inoculated with a microbial soil extract. After 14 days of growth, plants were harvested, and DNA was extracted from the shoot, roots, rhizosphere, and subjected to 16S rRNA gene amplicon sequencing, qPCR of the total community and functional genes involved in the N-cycle. Community dynamics were similar for most treatments within their respective compartments, except for the soil treatment, where rhizosphere and root microbiome differed from other treatments, suggesting that the soil microbiome colonises the belowground compartment efficiently only when the seed microbiome is severely disrupted. For the shoot, all treatments resembled the seed microbiome treatment, suggesting that the seed-borne bacteria colonise the aboveground compartment preferentially. Our results highlight the primacy of the seed microbiome over the soils during early colonisation, putting seed microbes as potential candidates of microbiome engineering efforts.

4.4 Introduction

In agricultural ecosystems, plant-associated microbiomes were shown to improve crop yields (Chen et al., 2019b), nutrient uptake (Yang et al., 2009), and nutrient leaching reduction (Bender and van der Heijden, 2015). In recent year, there have been concerted efforts to manipulate the microbiome of diseased plants to recover and re-establish positive plant-microbe feedbacks. The identification of a beneficial microbiome community that encompasses microbial genes enhancing the holobiont fitness (Lemanceau et al., 2017; Toju et al., 2018a), and the critical role plant-associated bacterial communities play in mitigating plant pathogen colonisation (Duran et al., 2018; Hassani et al., 2018) have been enumerated. Untangling the patterns and mechanisms of colonisation within plant microbiome niches is the first step to unlocking holobiont fitness.

On the one hand, plant microbial communities are generally believed to be recruited from the soil and selected to occupy specific spatial and temporal niches spaces (Chi et al., 2005; Beckers et al., 2017; Kandel et al., 2017). Colonisation patterns of the endophytic niche space by soil-borne microorganisms have been extensively highlighted (Hardoim et al., 2015; Cordovez et al., 2019) along with the evolutionary processes governing assembly of microorganisms in agricultural soils (Jiao et al., 2020) and at the rhizosphere interface (Goss-Souza et al., 2019; Matthews et al., 2019). On the other hand, seeds are the dispersal unit of the plant, and the innate microbiome plays an integral role in the preservation and germination of the seeds. Seed-transmitted bacterial communities of domesticated wheat were shown to be less diverse than their wild counterparts (Özkurt et al., 2020). It was suggested that the seed-transmitted microbiome plays a crucial role in priming and niche partitioning (Truyens et al., 2015; Shade et al., 2017; Nelson et al., 2018). In wild acorns, there was clear niche differentiation of both bacterial and fungal seed-transmitted communities and effective differences in colonisation patterns of the phyllosphere and root compartments (Abdelfattah et al., 2020). Microbiome tracking experiments suggests that soil and seed-borne microbiome are major sources of phyllosphere communities with air-transmitted microbiome playing a minor role (Shu-Yi-Dan Zhou et al., 2020), whilst spatial distance from the air-soil interface was highlighted as a limiting factor in the successful colonisation of phyllosphere by soil-borne microbes (Zhang et al., 2021). Evidence of the role of the seed microbiome in characterising colonisation patterns of plant microbiome is slowly emerging, but its importance relative to the soil microbiome remains unknown.

A comprehensive seed survey of annual wild plants highlighted that photosynthetic

pathways are a strong discriminatory axis for the endophytic communities that are characterised by specialised taxa from *Firmicutes* (Girsowicz et al., 2019). In axenic wheat culture systems, competent seed endophytes were shown to colonise the root and shoot compartment whilst influencing overall microbiome colonisation patterns (Robinson et al., 2016). Wheat seeds inoculated with native endophytic bacteria demonstrate an amplified natural priority effect, but not when inoculated with their fungal endophyte partners (Ridout et al., 2019). Microbiome transplants studies emphasised the role of the seed endophytic community in modulating microbiome colonisation patterns and highlighted substantial overlap in the seed and shoot endophytic bacterial community composition (Tannenbaum et al., 2020; Wang et al., 2020a). These studies demonstrated the hereditability of the seed-borne microbial community and highlighted the significance of these transmitted taxa. Inoculation of flowers has been demonstrated as a viable method to incorporate beneficial microbes into seeds (Mitter et al., 2017); however, in another study only a subset of the community was shown to be transmitted (Rodríguez et al., 2020). In beans, there seems to high degree of phylogenetic niche conservatism of bacterial communities across different stages of seed maturations (Chesneau et al., 2020). Indeed, we had previously demonstrated the existence of bacterial temporal niche spaces across various developmental stages of soybean (Moroenyane et al., 2021c). Successful vertical transmission of the endophytic microbiome from the shoot into the rhizosphere appears to only occur in soil with a disrupted microbiome (Luo et al., 2019). This suggests that the establishment of the seed and endophytic microbiomes are not only under plant-mediated selection but influenced by other ecological factors. Thus, understanding how different microbiome sources influence community colonisation patterns is the first step in unlocking the promises of microbiome engineering.

Ecological assembly processes within the soybean rhizosphere are influenced by the microbiome composition (Goss-Souza et al., 2019; Liu et al., 2019b), and nutrient acquisition is highly regulated by rhizosphere composition and structure (Bender et al., 2015; Bender and van der Heijden, 2015). Nutrient acquisition in soybean is modulated by the plant metabolic needs (Copeland et al., 2015; Hara et al., 2019) and there are strong seasonal patterns of nutrient accumulation, with nitrogen being accumulated during the vegetative growth and pod-filling developmental stages (Bender et al., 2015). Soil nitrate levels strongly modulate the levels of root colonisation and yield is increased when soybean utilise both soil nitrate and symbiotic N_2 fixation (Harper, 1974; Gai et al., 2017). Therefore, for plant nutrition, not only is the taxonomic composition of the recruited microbes important, but their functional capacity. Virtually nothing is known about the functional differences of seed- and soil-transmitted microbiomes, and if this

follows that trends highlighted above for taxonomic composition.

Here, we were interested in disentangling the influence of different microbiome sources on the composition of the soybean microbiome and its functional consequences, using the N-cycle as an example. We focused on elucidating the role of different microbiome sources (seed and soil) on the plant microbial community diversity, structure, composition, N-cycle function, and abundance under controlled growth chamber experimental conditions. We hypothesised that the innate seed microbiome would be the primary coloniser of soybean roots and shoots whereas the rhizosphere microbiome would be colonised primarily by the soil microbial communities. We used an innovative approach to generate near-axenic seeds and set up a carefully controlled experiment to test our hypothesis.

4.5 **Methods**

4.5.1 Sand sterilisation

To reduce the innate microbial loads and organic matter content (C content), sand was soaked in 10.8 % sodium hypochlorite (NaClO) (Sany, Montreal, Canada) overnight. Bleached sand was thoroughly rinsed with reverse osmosis water to remove all traces of NaClO. As bleached sand is devoid of any organic compounds and trace elements, the sand was preloaded with iron (III) chloride ($FeCl_3$). $FeCl_3$ is an immobile element in plants, and its deficiency results in decreased production photosynthetic capacity as it affects chlorophyll production. Briefly, 200 μM of $FeCl_3$ (pH = 2) was passed through a 850 μm sieve containing rinsed sand until the wash-through was below pH = 4 (Henry et al., 2006). To this sand, a modified plant nutrient solution (with no nitrogen) was passed through the sand until the pH of wash-through was ≥ 5.5 (Henry et al., 2006). Preliminary tests indicated that plants that were grown in the sand that was not treated with ($FeCl_3$) exhibit symptom of chlorosis as previously reported in Henry et al. (2006). The sand was airdried overnight in a laminar flow hood prior to sterilisation in the autoclave at 121° C for 15 min at 101 KPa. After this procedure, no PCR-viable DNA could be extracted from the sand.

4.5.2 Seed surface sterilisation and irradiation

Soybean seeds from cultivar Pioneer: AAC Edwards were imbibed in sterile water for 24 hours at 4°C prior to surface sterilisation. Seeds were placed in 50 ml Falcon tubes with enough 70 % ethanol (EtOH) to cover the seeds and mixed by turning the tube for 2 min. Thereafter, the EtOH was poured out and 5% NaClO solution was added for 7 min whilst gently turning the tube. To remove all traces of NaClO solution, the seeds were rinsed excessively with sterilised water. The seeds were then airdried in an open petri dish, and when moderately dry, the petri dishes were sealed. All works were performed under sterile conditions in laminar flow hoods. Once surface sterile seeds broke dormancy, seeds were exposed to a low dosage of X-ray irradiation at 40 Gy in Rad Source RS 2000 (Buford, USA) in order to reduce the microbial load of seed endophytes. As bean seeds are more highly susceptible to mutation caused by exposure to X-ray irradiation than mature plants or seedlings (Genter and Brown, 1941), only seedlings (once primary root had emerged/growing) were exposed to X-ray irradiation treatment and not the seeds and at a dosage previously shown to not cause any phenotypic abnormalities to plants (Zappala

et al., 2013). In preliminary studies, it was determined that 40Gy was the most conservative upper limit we could expose the seedlings before abnormalities were observed (Fig. S1). Overall, there were no observed abnormalities in all the seedlings used in all treatments.

4.5.3 Experimental treatments

All plants were grown in sterile sand for 14 days and supplemented with 5 ml filter-sterile nutrient solution. As detailed above, seeds were surface sterilised and irradiated prior to the commencement of the experiment apart from the “positive” treatment. In total, there were four treatments: “positive” (surface sterile and non-irradiated seeds + soil inoculum), “negative” (surface sterile and irradiated seeds), “seeds” (surface sterile and irradiated seeds + endophytic seed inoculum), and “soil” (surface sterile and irradiated seeds + soil inoculum). We used soil extract as an inoculum instead of soil because seedlings emerging from surface-sterilized seeds that were in contact with real soil were rapidly killed by fungal pathogens. To make it more comparable, we also decided to use an endophytic seed inoculum. The endophytic seed inoculum was prepared by crushing five surface sterile seeds with a sterile pestle and mortar and suspending in sterile water. The soil inoculum was prepared by suspending 20 g of soil in 100 ml of sterile water. For both inocula, the suspension was vigorously vortexed, and 1 ml of the suspension was added to corresponding containers at the beginning of the experiment. The soil used was collected in summer of 2019 at the Institut National de la Recherche Scientifique (Laval, QC, Canada) from control unplanted plots of an experimental field that had been ploughed for the first time in 2016 with no history of agricultural practice for at least 20 years prior to that. Soils that have history of agricultural practice drastically regulate the composition of free-living microbial communities and change abundance of microbial N-cycling genes (Mendes et al., 2015; Merloti et al., 2019). Here, we wanted to discern if microorganisms from agricultural naïve soils had the potential to alter soybean colonisation patterns and abundance of N-cycling genes. Soil edaphic properties were previously measured by AgroEnviro Lab (La Pocatiere, Quebec) and revealed an average pH of 7.2, P concentration of 193 (kg/ha), total N 0.15%, C/N of 13.1, density (g/cm^3) of 1.16, and porosity (%) of 55.14 and other properties reported in Table S2.

4.5.4 Plant growth conditions and sampling

Seedlings were grown with an 18-hour photoperiod at 25 °C followed by a 6-hour dark period at 20°C in a Conviron growth chamber (Winnipeg, Canada). To ensure that plants were not nutrient stressed, containers were supplied with 5 ml modified Hoagland's plant nutrient solution at the beginning of the experiment as suggested in Moscatiello et al. (Moscatiello et al., 2013). The nutrient solution was filter-sterilized at the moment of application. Plants were grown in sterilised PLANTCON plant tissue culture containers (Fisher Scientific, Waltham, MA USA) until the vegetative growth developmental stage (V1). Samples were collected from rhizosphere (sand that directly attached to the root surface), root, and shoot (leaves and stem). Fresh weight of sampled tissue was recorded and placed in sterile 50 ml clear polyethylene Falcon test tubes (Tewksbury, USA) in a sterile laminar flow hood. Sampled plant tissue was crushed in liquid nitrogen using a sterile pestle and mortar. For each sample, 0.25 g of the crushed tissue or 0.25 g of rhizosphere soil was added to the bead tubes from the Qiagen Power Soil DNA kit (Hilden, Germany) and DNA was extracted following the manufacture's instructions except that DNA was eluted in 50 μl.

4.5.5 Quantitative real-time PCR

Key microbial functional genes involved in the nitrogen cycle were assessed using various primers pairs. *amoA*1-f∗ (5′-GGGGHTTYTACTGGTGGT-3′) and amoA2-r (5′ - CCCCTCKGSAAAGCCTTCTTC- 3′) (Levy-Booth et al., 2014), and crenamoA23-f (5′-ATGGTCTGGCTWAGA CG-3′) and crenamoA616-*r* (5′-GCCATCCATCTGTA-3′) (Tourna et al., 2008) targeting the gene encoding for, respectively, the bacterial and archaeal version of the ammonia monooxygenase subunit A gene (*amoA*), involved in the oxidation of ammonia to hydroxylamine during the first step of nitrification. nirK876-f∗ (5′-ATYGGCCAYGGCGA-3′) and nirK1040-r (5′ - GCCTCGATCAGRTTRTGGTT- 3′) (Henry et al., 2004) targeting the gene encoding the copper-containing nitrite reductase (*nirK*) involved in the reduction of nitrite to nitric oxide during denitrification. PoI-f∗ (5′-TGCGAYCCSAARGCBGACTC-3′) and PoI-r (5′ - ATSGCCATCATYTCRCCGGA- 3′) (Poly et al., 2001) targeting the gene encoding the component 2 of the nitrogenase (*nifH*) involved in the fixation of atmospheric nitrogen to ammonia. *F1norA*-f∗ (5′-CAGACCGACGTGTGCGAAAG-3′) and *R2norA* -r (5′ - TCCACAAGGAACGGAAGGTC- 3′) (Attard et al., 2010) targeting the gene encoding for the nitrite oxidoreductase (*nxR*) that oxidize

nitrate to nitrite during the second step of nitrification. Total bacterial and archaeal 16S rRNA gene abundance was estimated using primers 520F (5′- AGCAGCCGCGGTAAT-3) and 799R (5′-CAGGGTATCTAATCCTGTT3′) (Edwards et al., 2007). For all genes, standards and qPCR protocols were prepared and followed as previously described in Yergeau et al. (2020). Briefly, the standard curve was constructed from serially diluted linearised plasmids (10^8-10^2 copies/μl) and the iTaq universal SYBRGreen® kit was used following the manufacturer's protocol (Bio-Rad Laboratories Inc, Hercules, CA). All qPCR assays were performed on a Rotor-Gene 6000 and the data were analysed using the Rotor-Gene 7.1 software (Corbett Research Biosciences, Sydney, NSW, Australia).

4.5.6 16S rRNA gene amplicon sequencing

Extracted DNA was used to construct sequencing libraries according to Illumina's "16 S Metagenomic Sequencing Library Preparation" guide (Part # 15044223 Rev. B), with the exception of using Qiagen HotStar MasterMix for the first PCR ("amplicon PCR") and halving reagent volumes for the second PCR ("index PCR"). The first PCR ("amplicon PCR") was carried out for 25 cycles with annealing temperatures of 55°C using the primers 520F and 799R (Edwards et al., 2007), resulting in amplicons of an average length of 280 bp. Commercial microbial community standard was from ZymoBIOMICS® (Zymo Research Corporation, Irvine, CA, USA. Product D6300), negative PCR, and a blank from DNA extraction kit were used as internal standards. This primer pair targets the bacterial/archaeal V4 hypervariable regions of the 16S rRNA gene and were shown to exclude chloroplast sequences (Edwards et al., 2007). The resulting amplicons were pooled together and sequenced at the Centre d'expertise et de services Génome Québec (Montréal, Canada). Diluted pooled samples were loaded on an Illumina MiSeq and sequenced using a 500-cycle (paired-end sequencing configuration of 2 × 250 bp) MiSeq Reagent Kit v3. The total number of sequences per sample is reported in the supplementary (Table S1).

Sequencing data was analysed using AmpliconTagger (Tremblay and Yergeau, 2019) Briefly, raw reads were scanned for sequencing adapters and PhiX spike-in sequences. We removed single-end reads that failed to meet one of these conditions: having average quality Phred score lower than 25; having 30 bases of quality lower than Phred score 15; having 1 or more undefined bases (N). The remaining sequences were processed for generating Exact Sequence Variants (ESVs) (DADA2 v1.12.1) (Callahan et al., 2016). Since the quality filtering

step was performed in a separate upstream step, we used more lenient parameters for the dada2 workflow which is summarized as follows: filterAndTrim(maxEE = 2, truncQ = 0, maxN = 0, minQ = 0). Errors were learned using the learnErrors(nbases = 1e8) function for both forward and reverse filtered reads. Reads were then merged using the mergePairs(minOverlap = 12, maxMismatch = 0) function. Chimeras were removed with DADA2's internal removeBimeraDeNovo (method = 'consensus') method followed by UCHIME reference (Edgar et al., 2011). ESVs were assigned a taxonomic lineage with the RDP classifier (Wang et al., 2007) using the Silva release 128 database (Quast et al., 2013) supplemented with eukaryotic sequences from the Silva database and a customized set of mitochondria, plasmid and bacterial 16S sequences (see the AmpliconTagger databases DOI:10.5281/zenodo.3560150). The RDP classifier gave a score (0 to 1) to each taxonomic depth of each ESV. For each ESV, the taxonomic lineage was reconstructed by keeping only the taxa that had a score ≥ 0.5. Taxonomic lineages were combined with the cluster abundance matrix obtained above to generate a raw ESV table. From that raw ESV table, an ESV table containing only bacterial organisms was generated. Five hundred 1,000 reads rarefactions were then performed on this latter ESV table and the average number of reads of each ESV of each sample was then computed to obtain a consensus rarefied ESV table. ESVs pointing to non-bacterial taxa were removed. The ESVs detected in the negative and extraction kit controls were filtered out of the resultant ESV table, which was then used in all subsequent analyses.

4.5.7 Alpha diversity patterns

The ESV abundance table was normalised such that the summed relative abundance of all ESVs of each sample was equal to one. All statistical analyses were performed using R version 3.5.0 (R Core Team, 2020) unless otherwise stated. Taxon accumulation curves were constructed to determine if the sampling effort (number of ESV as a function of the number of samples) could recover most of the taxa, whilst the Preston log-normal curve was used to evaluate the estimated richness and occurrence of rare taxa across all samples (the presence of a normal distribution indicates a higher probability of rare taxa being represented) (Fig S2). Analysis of variance (ANOVA) or Kruskall-Wallis test when data were not normally distributed were performed to evaluate the effects of treatment (negative, positive, seed, and soil) and plant compartment (rhizosphere, root, and shoot) on the number of observed ESVs and Shannon diversity index.

4.5.8 Microbial community structure

Principal Coordinate Analysis (PCoA) was used to visualise the microbial community structure based on Bray-Curtis dissimilarity. Multivariate dispersion in the community data was evaluated and confirmed using ANOVA with 999 permutations using the “Vegan” pack in R (Oksanen, 2013). To assess the relative significances of treatment and plant compartment on the community structure, a Permutational Multivariate Analysis of Variance (PERMANOVA) test was performed with 999 permutations. Lastly, the 100 most abundant ESVs were used to determine their influence and were visualised using PCoA and heatmaps.

4.5.9 Taxonomic profiles, Random Forest Models, and Linear Discrimination Analysis

The relative abundance of microbial taxa at the phylum and genus taxonomic levels were evaluated across treatments and plant compartments. Ternary plots were generated to visualised enriched taxa across treatments. Random forest algorithms were used to predict the prevalence of taxa associated with each treatment at the genus and ESV taxonomic levels. Random forest prediction was made using the randomForest algorithm (Liaw and Wiener, 2002) and implemented on the MicrobiomeAnalysis pipeline (Chong et al., 2020). Differential abundance of genera was explored using the DESeq2 analysis pipeline (Love et al., 2014) and linear discrimination analysis was used to detect differentially abundant bacterial families and genera across all treatments (Chong et al., 2020)

4.5.10 Data availability

Raw sequencing data was submitted to NCBI SRA under BioProject accession PRJNA697966.

4.6 Results

4.6.1 Plant biomass

All seeds broke dormancy at the same time and all seedlings used in the experiment exhibited normal growth, irrespective if they were irradiated or not. Plant biomass accumulation varied across treatment and within replicates, although there were no statistical differences across treatments, there were interesting trends (Fig.1; Fig.S2). Firstly, the leaf mass fraction (LMF) highlights the fraction of the total biomass represented by leaves. The trend that emerged showed that the soil treatment had higher LMF values than other treatments, and the seed treatment had the lowest values. At the end, the positive control showed the highest phenotypic variation (fresh biomass) compared to all treatments (Fig.1; Fig.S2). The seed and negative treatments had similar levels of variation (fresh biomass), whilst the soil treatment showed less variation among replicates in accumulated biomass than other treatments (Fig.1; Fig.S2).

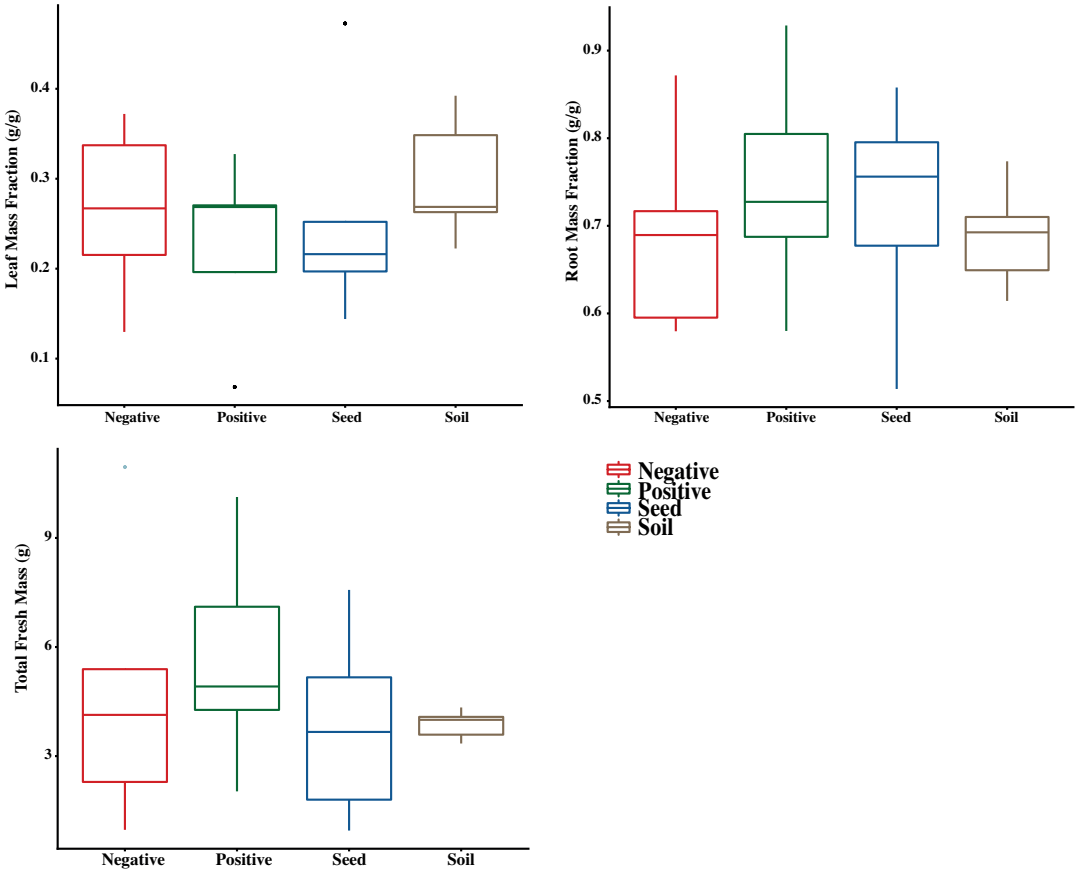

Fig.1 Plant biomass allocation across different treatments indicating Root mass fraction (RMF), leaf mass fraction (LMF) and total fresh biomass.

4.6.2 Alpha diversity

Exact sequence variants (ESVs) accumulation curve indicated that a substantial amount of microbial ESVs were recovered and the sampling effort was enough (number of recovered ESV as a function of the number of samples sequenced) (Fig.S3). Furthermore, the Preston log-normal curve highlighted that the sampling effort was enough to detect most of the rare and low-abundance taxa (Fig.S3). The number of observed ESVs in the mock community control corresponded exactly to the number of ESVs expected (n=10), indicating that the read processing resulted in an accurate picture of the microbial communities sequenced. The measured alpha diversity indices were all significantly influenced by treatment and plant compartments. Overall, there were significant differences between treatments only in the belowground compartments and not in the shoot. Total number of observed ESVs were significantly influenced by treatment (Chi-squared $\chi2= 19.01$, $p<0.001$; Fig.2A) and plant compartment (Chi-squared $\chi2= 34.27$, $p<0.001$; Fig.2A). The soil treatment had the highest abundance of ESVs in the rhizosphere and root compartments, whilst the seed treatment had the second-highest abundance of ESVs in the root compartment (Fig.2A). Shannon diversity index was significantly influenced by treatment (Chi-squared $\chi2= 19.97$, $p<0.001$; Fig.2B) and plant compartment (Chi-squared $\chi2= 40.25$, $p<0.001$; Fig.2B). At the rhizosphere, the soil and seed treatments had higher diversity levels, whilst only in the rhizosphere did the soil treatment significantly differ to all other treatments (Fig.2B).

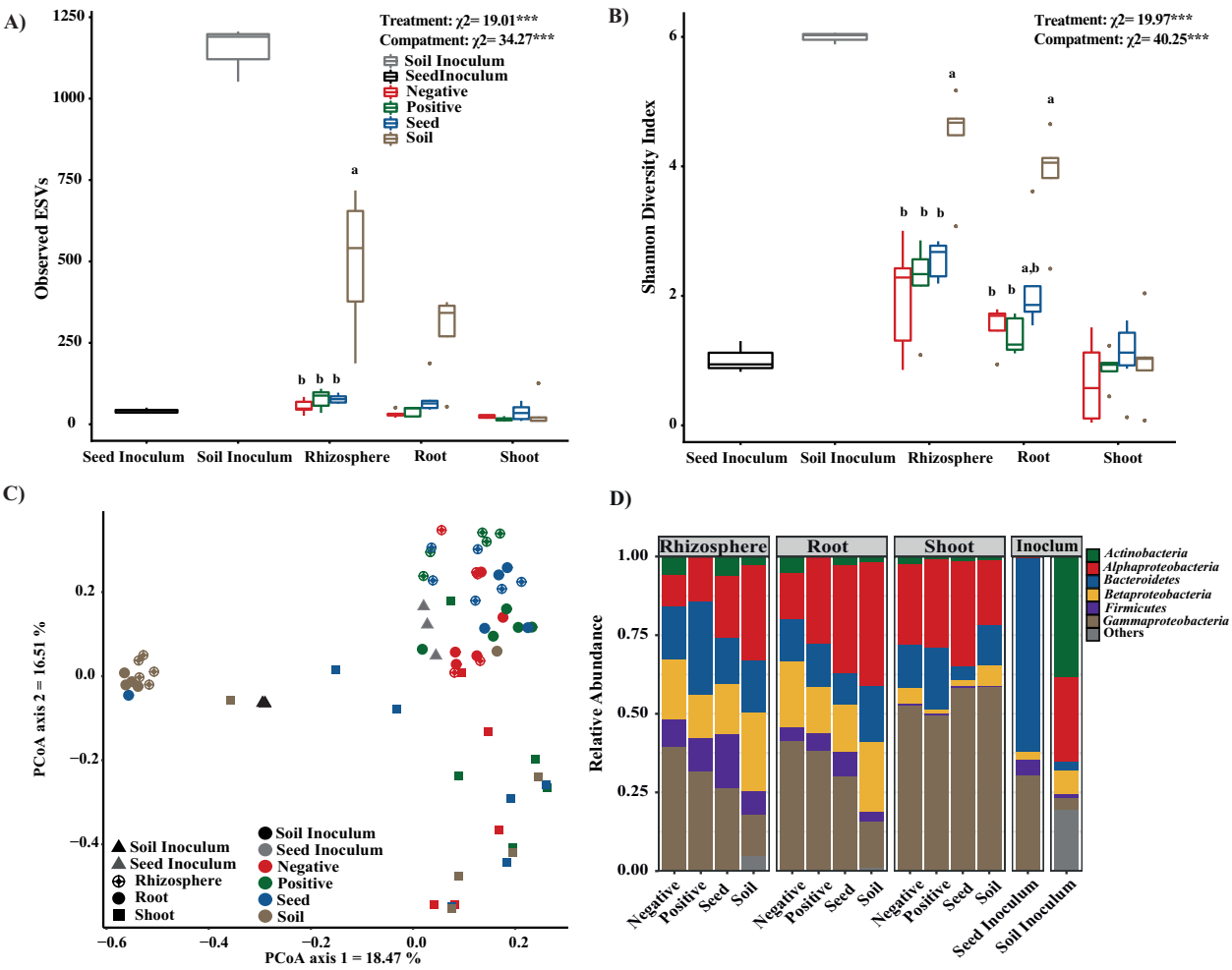

Fig.2 Microbial diversity patterns indicating bacteria/archaeal diversity influenced by treatment and compartment for A) Observed ESVs, B) Shannon diversity index, C) Principle Coordinate Analysis (PCoA) of bacterial/archaeal community based on Bray-Curtis dissimilarity, and D) Relative abundance of bacterial taxa across plant compartments and treatments; Taxa with relative abundance <1% were grouped as 'other'. *Kruskal-Wallis chi-squared χ^2, p-value; superscript letters represent pairwise Wilcox test comparison with Bonferroni correction. p value *<0.05, ** <0.01, *<0.001)***

4.6.3 Microbial community structure

Bray-Curtis dissimilarity was used to infer community beta-diversity patterns and structure. Firstly, homogeneity and multivariate dispersion analysis was used to determine the relative influence of between community composition (treatment variation) and within community composition (variation within replicates) on overall community structure. Community structure was influenced by differences in composition, with treatment and compartment having significant effects. When considering treatment, multivariate dispersion analysis indicated that there were detectable differences in multivariate dispersion across treatments (F value= 16.81, $p< 0.001$), and pairwise comparison indicated that the soil and seed inoculum were significantly different to all treatments ($p<0.05$, ANOVA; Tukey's HSD). However, there were no discernible difference across the four main treatment (negative, positive, seed, and soil) indication that difference in structure between this treatment was a result of community structure and not differences in composition. Principal Coordinate Analysis (PCoA) was used to evaluate and visualise the community structure. Treatment and plant compartments significantly influenced community structure (PERMANOVA, Table 1). There was a clear separation of samples by plant compartments along the second PCoA axis, and the first PCoA axis separated samples by treatment with the rhizosphere and root communities of the "soil" treatment being divergent from all others (Fig.2C).

Table 1 Permutation analysis of variance (PERMANOVA) results indicating the influence of treatment and compartment interaction on microbial community structure *(p* value *<0.05, ** <0.01, *<0.001)**

Bacterial/Archaeal Community	**Bray-Curtis Dissimilarity**		
Source of variation	***MS***	***F value***	R^2
Treatment	0.82	6.89	**0.26*** **
Compartment	1.23	10.36	**0.14*** **
Treatment × Compartment	0.24	2.04	**0.09** **
Residuals	0.11		0.49
Top 100 ESVs	**Bray-Curtis Dissimilarity**		
Source of variation	***MS***	***F value***	R^2
Treatment	0.61	6.94	**0.19*** **
Compartment	1.98	12.32	**0.22*** **
Treatment × Compartment	0.17	1.99	**0.11** **
Residuals	0.08		0.47

4.6.4 Taxonomic composition

Overall, there were variations in the relative abundance of dominant phyla across treatment and plant compartments (Fig.2D; Fig.S4). At the phylum level, *Proteobacteria* was the dominant phylum across all treatments and plant compartments. At the rhizosphere interface, the soil treatment had a higher relative abundance of rare taxa (relative abundance < 1%) in the rhizosphere but not in the root compartment (Fig.2D). The relative abundance of *Gammaproteobacteria* was consistently higher in the shoot compartment across all treatments (Fig.2D). Overall, ternary plots highlight the soil treatment had increased occurrence and abundance of ESVs across all treatments (Fig. 3). The soil treatment occurrence and distribution of ESVs were mainly along the root-rhizosphere axis across all treatments (Fig.3D), whilst the seed treatment had taxa that were enriched along the root-shoot axis (Fig.3C). Across all treatment ESVs belonging to *Gammaproteobacteria* were enriched in the shoot compartment (Fig.3). Across all treatments, ESVs belonging to *Firmicutes* and *Betaproteobacteria* were enriched along the root-rhizosphere axis, whilst in the seed treatment a proportion of those *Betaproteobacteria* were enriched along the root-shoot axis (Fig.3).

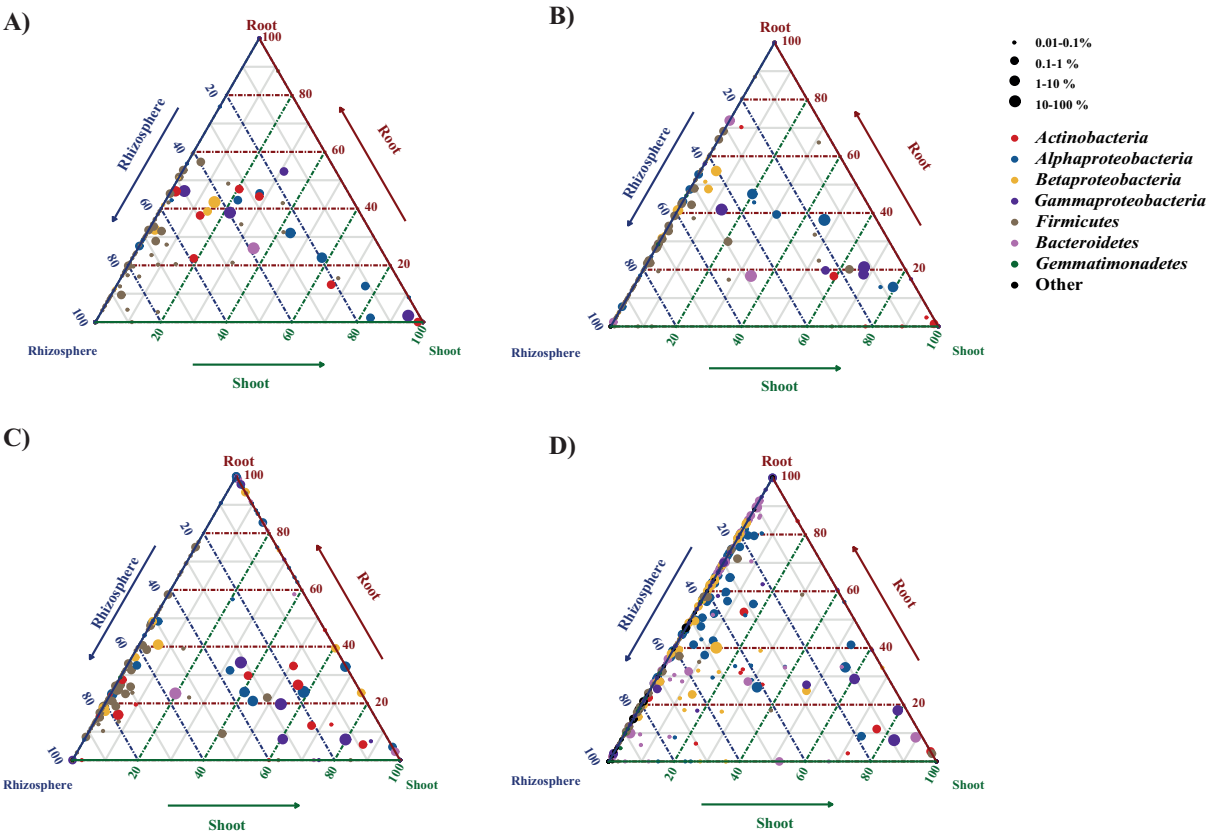

Fig.3 Ternary plots representing the relative occurrence of ESVs (circles) in A) Negative, B) Positive, C) Seed, and D) Soil treatments. Taxa in different plant compartments are coloured by their taxonomy at the phylum and class level for *Proteobacteria.* Size of circle is proportional to mean abundance in the community.

Overall, the relative abundance of the most abundant genera was influenced by treatment and plant compartment (Fig.S5). Apart from *Sphingomonas,* all other abundant genera appear to constitutively 'recruited' from the seed inoculum and/or part of the seed-borne community (Fig. 4; Fig.S6). The relative abundance of *Chryseobacterium* (Fig.4; χ^2=31.04***), *Methylobacterium* (Fig.4; χ^2=26.67**), *Pantoea* (Fig.4; χ^2=15.84**), and *Pseudomonadaceae* (Fig.4; χ^2=11.12*) was influenced by treatment. The relative abundance of these taxa was consistently lower for the soil treatment in the rhizosphere and root compartments. Differential abundance analysis of these taxa highlighted that these dominant taxa were significantly more prevalent across all treatment except for the soil inoculum (Fig.4; Fig.S5). For the rest, plant compartment significantly influenced the relative abundance of *Paenibacillus* (Fig.S4; χ^2=41.93***), *Sphingomonas* (Fig.S4; χ^2=19.07***; treatment χ^2=19.15***), *Stenotrophomonas* (Fig.S4; χ^2=11.04*; treatment χ^2=22.35***), and *Burkholderia.Paraburkholderia* (Fig.S4; χ^2=35.56***; treatment χ^2=13.16*). Of these, the relative abundance of *Paenibacillus* was lowest in the soil treatment and highest in the seed treatment within the rhizosphere (Fig.S5). Linear discrimination analysis indicated that majority of genera/families were significantly enriched in the soil inoculum and soil treatment (Fig.5; Fig.S7; Table S3). Lastly, the abundance of the nitrogen-fixing *Rhizobium* was significantly enriched in the soil and soil inoculum treatments (Fig.S8).

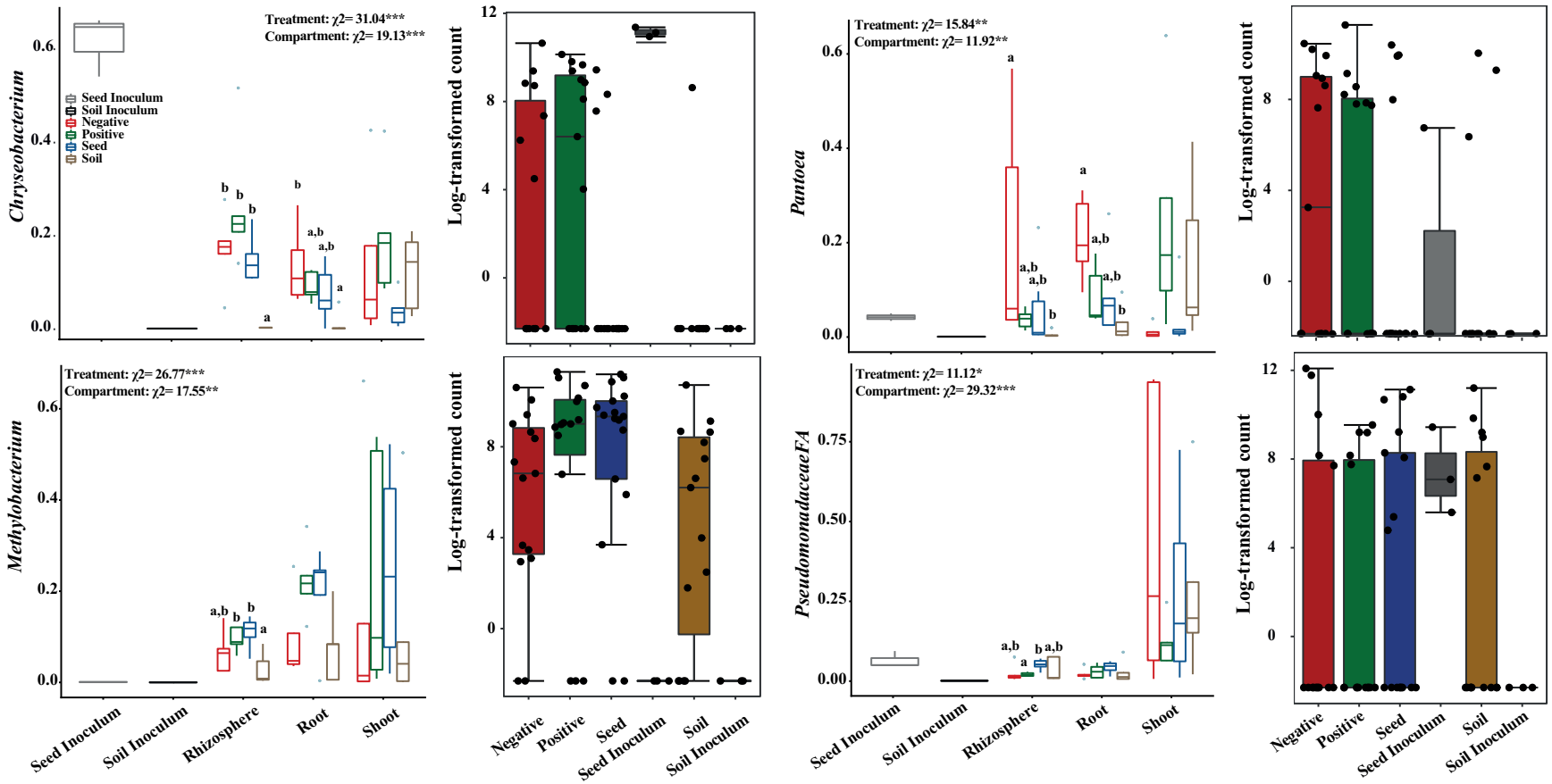

Fig.4 Left panel: Relative abundance of most abundant genera that were significantly influenced by treatment and compartment (Kruskal-Wallis chi-squared χ^2, *p*-value) and pairwise Wilcox test with Bonferroni correction (*p* value *<0.05, ** <0.01, *<0.001). Right panel: DESeq2 differential abundance analysis of most abundant genera (*p* value <0.05)**

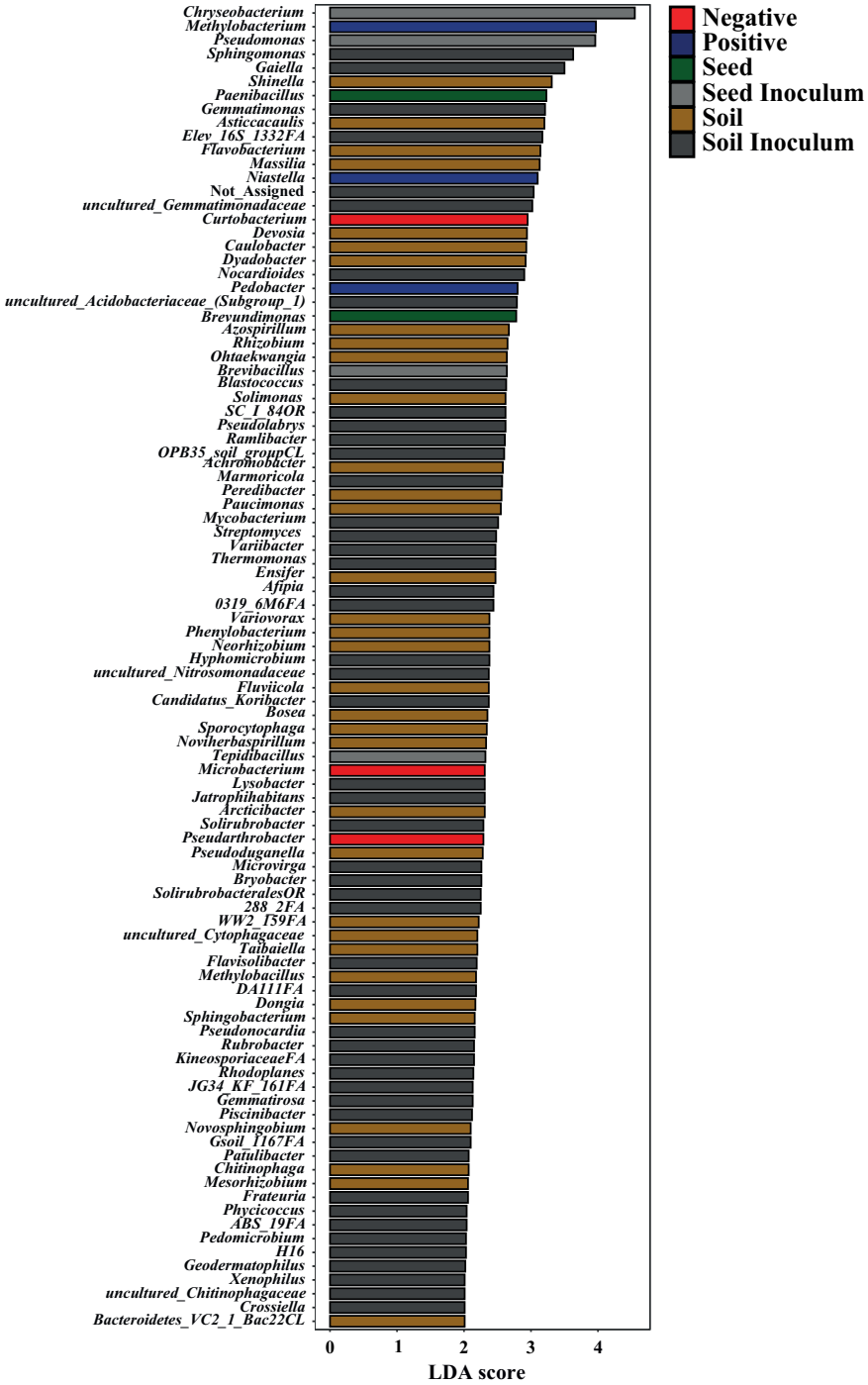

Fig.5 Linear Discriminatory Analysis (LDA) indicating differentially abundant genera across all treatments. LDA scores obtained from LEfSE analysis of microbiota across all treatments (LDA effect size > 2 was used as threshold of LEfSe analysis and p-value of 0.05)

4.6.5 Functional gene abundance

Treatment and plant compartment significantly influenced the abundance of all measured functional genes along with the 16S rRNA gene (Fig.7). Of all the measured microbial functional genes, the abundance of the bacterial ammonia monooxygenase subunit A gene (*amoA*) and the archaeal *amoA* significantly varied at different in the at the root compartment (Fig.7). Whilst, the abundance of nitrite oxidoreductase gene (*nxR*) significantly varied in both rhizosphere and shoot compartments (Fig. 7; Chi-squared χ^2=36.36***) The abundance of *nxR* was significantly higher in the soil treatment (Fig.7; Chi-squared χ^2=18.23**). The abundance of the gene coding for the component 2 of the nitrogenase (*nifH*) was not influenced by treatment, but there was an influence of compartment (Fig.7; Chi-squared χ^2=51.60***). The abundance of the Cu-containing nitrite reductase gene (*nirK*) was significantly higher in the root compartment across all treatments (Fig.7). The abundance of the 16S rRNA gene (as a proxy for bacterial and archaeal abundance) was marginally significantly affected by the treatment (Fig. 7; Chi-squared χ^2=11.15*). In the rhizosphere , the ratios in the abundance between N-cycling genes (*nifH, nirK,* and *nxR*) and the 16S rRNA gene were all significantly higher in soil treatment (Fig.8; $p<0.05$).

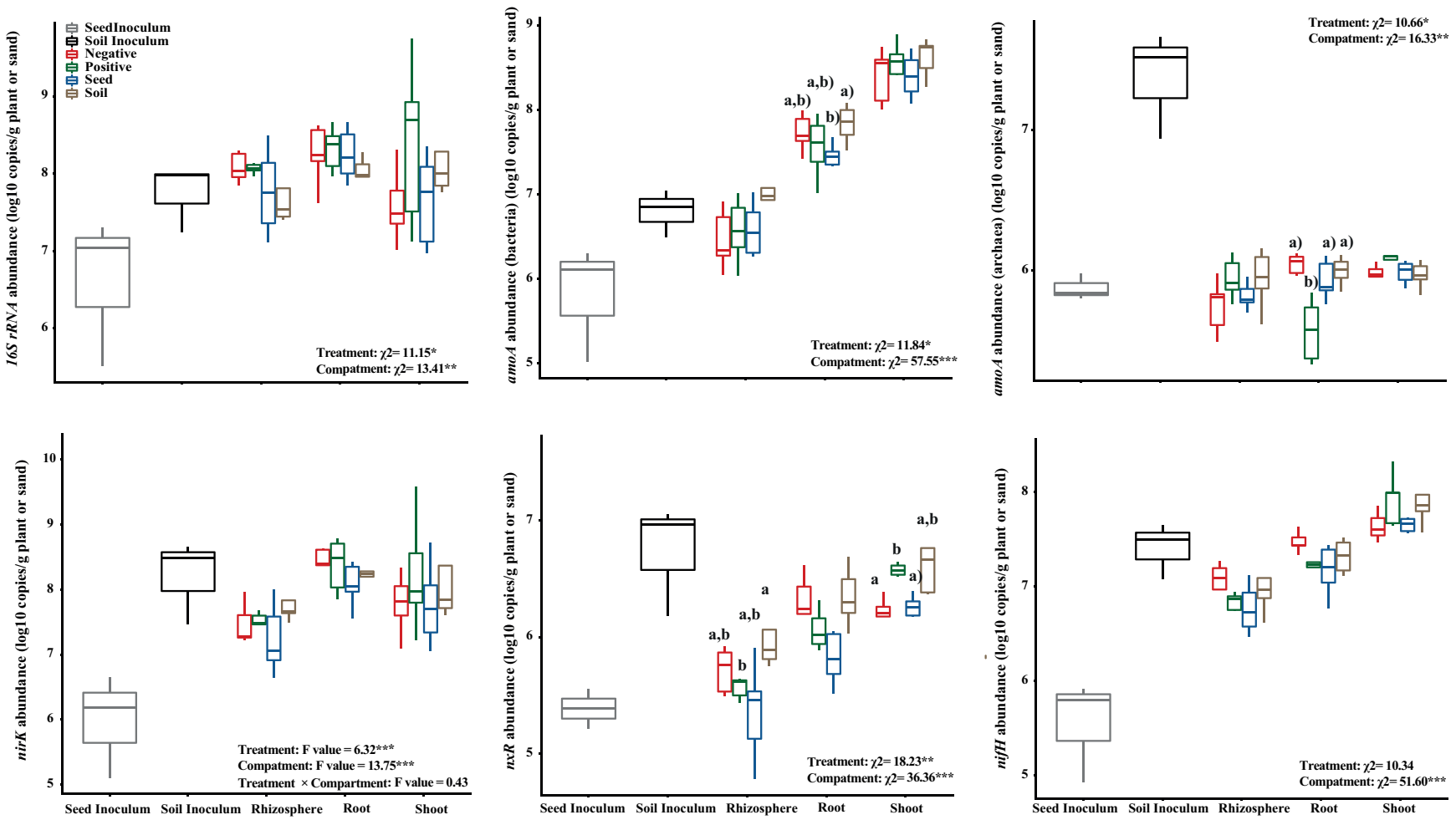

Fig.6 Abundance of microbial functions diversity influenced by treatment and compartment for A) 16S rRNA gene , B) *amoA* bacteria, C) *amoA* archaea ,D) *nirK*, E) *nxR* , and F) *nifH*. *Kruskal-Wallis chi-squared χ^2, p-value; ANOVA F value. p value *<0.05, ** <0.01, *<0.001).* Letters denote TukeyHSD pairwise or Wilcox test comparison with Bonferroni correction**

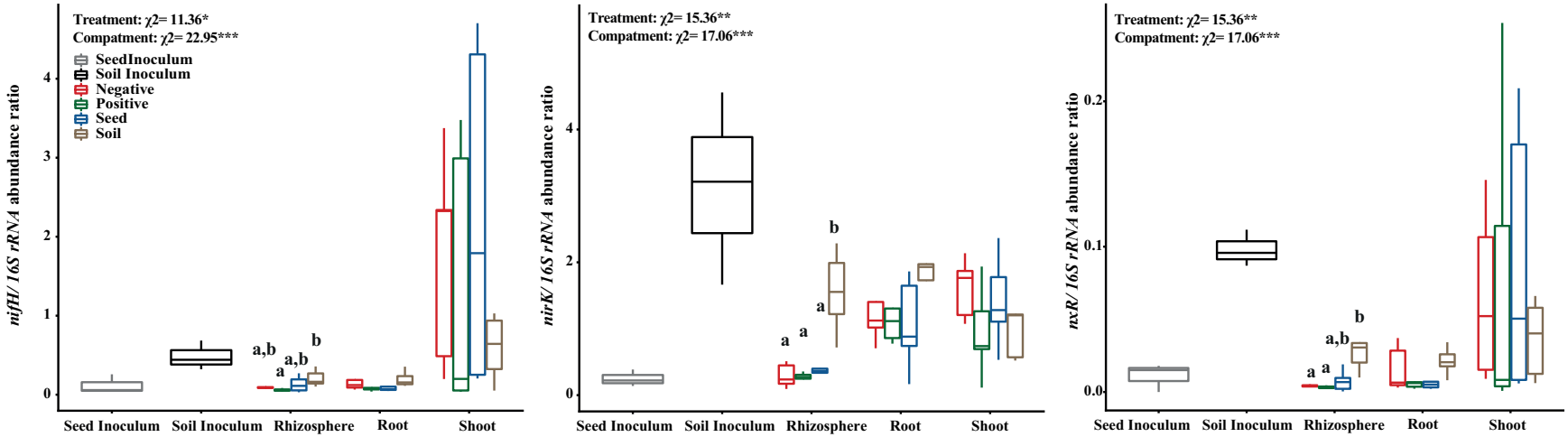

Fig.7 Abundance ratios of N-cycling genes and 16S rRNA genes by treatment and compartment for *nifH* /16S rRNA gene, *nirK*/16S rRNA gene, and *nxR*/16S rRNA gene. *Kruskal-Wallis chi-squared χ^2, p value *<0.05, ** <0.01, *<0.001).* Letters denote pairwise Wilcox test with Bonferroni correction**

4.6.6 Abundant microbial ESVs

The community structure and composition of the 100 most abundant ESVs across the four main treatments were visualised using PCoA of Bray-Curtis dissimilarity and Random Forest analyses were used to predict taxa that were associated with each treatment. Overall, the community structure of the 100 most abundant ESVs was similar to the overall community structure (Fig. S9). Treatment and plant compartments significantly influenced community structure (PERMANOVA, Table 1). A heatmap of the 100 most abundant ESVs (genus-level taxonomic affiliation) highlighted various clusters of taxa that were enriched in the soil and seed treatments (Fig. S10). Specifically, there were ESVs that were enriched in both the rhizosphere and root compartments, whilst another cluster highlighted ESVs were only enriched in the rhizosphere. Taxa that were predicted to be enriched in the seed treatment using Random Forest analyses, such as *Paenarthobacter* and *Paenibacillus* (Fig.S9; Fig.S10) also formed clusters of enriched ESVs in the heatmap (Fig.S10). Similarly, *Fluviicola*, *Dydobacter,* and *Peridibacter* were predicted to be associated with the soil treatment and were part of the cluster that was only enriched in the rhizosphere compartment (Fig. S9; Fig.S10).

4.7 Discussion

The goal of our study was to contrast the seed and soil routes for plant microbial colonisation. We had hypothesised that the seed microbial communities would be the primary colonisers of the plant environment, with a more substantial effect in the shoot and root environments as compared to the rhizosphere. Most of our results pointed out that the soybean plants grown from seeds that were surface-sterilised and irradiated before being inoculated with a soil microbial extract showed different microbial community diversity, structure, composition and functions in their roots and rhizosphere at the emergence stage. These differences did not extend to the shoot microbial communities. The soybean plants grown from surface sterilised and irradiated seeds that were not treated with a soil inoculum developed microbial communities strikingly similar to the ones harboured by the plant grown from surface-sterilised and irradiated seeds that were inoculated with a seed extract, or as the ones harboured by plants grown from seeds that were not irradiated and inoculated with a soil extract. This suggests that:

1. the seed microbial communities were not completely destroyed by our method,
2. severely disrupted seed microbial communities can recolonise the plant if not in competition with soil microbial communities,
3. when the seed microbial communities are not disrupted, they have priority over soil microbial communities during plant root and rhizosphere colonisation, and
4. even if the seed microbial communities are disrupted and exposed to soil microbes, they can successfully colonise the aboveground plant compartments.

As such, our hypothesis is confirmed, with the addition that the seed-borne microbial communities also appeared to be able to colonise the rhizosphere in the presence of competing soil microbial communities. Even though our results are stemming from a highly controlled experiment, they are aligned with previous reports that showed that seed microbial communities are the primary source of microorganisms in the plant environment (Chesneau et al., 2020; Rodríguez et al., 2020; Wang et al., 2020a).

In soybean, microbiome colonisation patterns are modulated by changes in the abiotic environment (Liu et al., 2019b; Sanz-Saez et al., 2019), a strong influence of priority effect (Hara et al., 2019), interactions of spatial (plant compartment) and temporal dynamics (developmental stage) (Miller and Roy, 1982; Moroenyane et al., 2021c), and plant-mediated selection with dispersal limitations influencing community assembly processes (Moroenyane et al., 2021a). Here, we sought to highlight colonisation patterns of different microbiome sources on near-axenic

plants grown in aseptic conditions. Surface sterilisation alone has been shown to be effective in the removal of microbial communities in legume seeds (Caetano-Anollés et al., 1990), and the use of irradiation to preserve and decontaminate agricultural crops, spices, and meat products is documented (Sommers, 2012). However, our fluorescence in-situ hybridization (FISH) investigation of the irradiated seeds indicated the presence of bacterial genomic material within the seed tissue (not shown). Ionising radiation interacts with DNA by causing irreversible degradation, and there is a positive correlation between dosage and DNA damage (Stuy, 1960; Moosekian et al., 2012; Borgognoni et al., 2017). Efficacy is influenced by dosage, the composition of the biological material (density, temperature, pH, and innate gases), and innate microbial composition; with gram-positive bacteria being more tolerant than gram-negative bacteria (Moosekian et al., 2012). X-ray irradiation decrease fruit-borne microbial communities by five-fold in tomatoes (Mahmoud, 2010) and tolerance is a strain-specific physiological trait (Beblo-Vranesevic et al., 2018). It is then possible that the low irradiation dosage administered in the experiment only reduced the microbial loads and not completely eradicated seed-borne microbes.

Surface sterilised soybean seeds harbour microorganisms that proliferate during germination and colonise the plant surface (Caetano-Anollés et al., 1990). Plant growth-promoting bacteria from seeds are considered to be the early colonisers of the spermosphere, in fact, endophytic bacteria from *Eucalyptus* seeds that were tagged with *gfp* gene have been recovered from the phyllosphere of seedlings (Ferreira et al., 2008). Similarly, known soybean seed growth-promoting bacteria tagged with the *gfp* gene were shown to colonise the inner compartment of roots and shoots (Batista et al., 2018). These studies highlighted that bacterial taxa from seed can easily colonise the shoot and root of host plant, but also the intercellular spaces. The intercellular space is enriched with carbohydrates, amino acids, and inorganic nutrients needed by the bacterial endosymbiont. Endophytic microbes have adapted to a nutrient replete environment that has relatively subdued levels of interspecific competition when compared to the rhizosphere or phyllosphere counterparts communities (Sturz et al., 2000; Bacon and Hinton, 2007). However, this view has been challenged, as most endophytic microbes are facultative symbiont and compete with soil-borne microbes at the rhizosphere interface prior to entry into the plant (Compant et al., 2010a). Here we propose that, at the rhizosphere interface, seed-borne microorganisms are capable of outcompeting members of the rhizosphere to quickly colonise the internal plant compartments, and even the rhizosphere itself. Furthermore, even when this innate endophytic seed microbiome is severely disrupted, it is still capable of colonising the rhizosphere and endosphere in the absence of competition from the rhizosphere microbiome.

Microorganisms involved in the inorganic N-cycle have a key role in changing the availability of soil N for plant uptake, and we therefore selected this group as a model functional group to test the effects of our treatments. Interestingly, the abundance of the two bacterial genes involved in nitrification (bacterial *amoA* and *nxR*) were the only ones significantly influenced by the treatments, being generally more abundant under the "soil" treatment. This was probably linked to their higher abundance in the soil inoculum, as highlighted in LDA analyses for the *Nitrosomonadaceae*, an ammonia-oxidizer bacterial family. However, we could detect all the functional genes in all the treatments even in the negative treatment, suggesting that these functional groups can be seed transmitted. As nitrification transforms the more energetically favorable ammonia in the less energetically favorable nitrate (Moreau et al., 2019), and that denitrification results in a net loss of nitrogen from the soil, the presence of these functional genes could negatively impact plant N nutrition, which makes their transmission through seeds intriguing. Interestingly, under our experimental set-up, many of the genes were significantly more abundant in root and shoot samples, and less abundant in the rhizosphere. We found that an increased abundance of nitrite-oxidising bacterial in the rhizosphere of plants of the "soil" treatment indicating potential increased production of nitrate (NO^{-3}), which is shown to be the preferred form of N for legumes (Cui et al., 2017; Liu et al., 2018) despite uptake of NO^{-3} being an energy exhaustive process for the plant (Moreau et al., 2019). This suggests that exposure of seeds to the soil microbiome might have some positive effects on plant nutrition. Equally, the increased abundance of nitrite reductases (*nirK*) containing bacteria in the rhizosphere of the "soil" treatment was coherent with the LDA that revealed known denitrifying taxa (e.g. *Paucimonas;* (Pichinoty et al., 1977). being associated with the soil treatment This suggests that these functional groups, though more abundant in the bulk soil inoculum, have a strong capacity to colonize plants. As for their role inside roots and shoots, we can only speculate that they might take advantage of the inorganic N *in planta* as a substrate. The potential presence of fungi in the soil inoculum could have further increased the abundance of denitrifiers in the "soil" treatment as previously observed in agricultural fields (Ma et al., 2017; Xu et al., 2019; Gui et al., 2021). Overall, the trends observed in the functional genes were highly similar to the ones observed using taxonomic marker genes, suggesting that the seed microbiome primacy not only has consequences on the microbial community composition of the soybean environment, but also on the associated functions.

Interspecific competition and priority effects are pronounced at the rhizosphere interface and influence microbial colonisation patterns (Compant et al., 2010a; Choi et al., 2020). Our data indicates that the intact seed microbiome has priority and outcompetes the invading soil

microbiome, and only when the innate seed microbiome is severely disrupted is there successful colonisation of the rhizosphere compartments by the soil microbiome. In axenic turfgrass culture, the extant soil and not the seed microbiome was shown to assert a more substantial influence on the microbiome structure (Doherty et al., 2020). Microbiome transplant studies tend to focus on measuring the performance of native plants grown in sterile soils that are inoculated with a foreign microbiome and reveal intricate plant-soil feedbacks where native plant performance is amplified when inoculated with native soil microbiome (Smith et al., 2018b). However, these studies tend to use autoclaved soil or peat to reveal these colonisation patterns of the belowground compartment and such experimental setups cannot highlight the role of the seed microbiome. Our closed chamber and reductionist experimental setup allow us to discern the nuanced influences of seed and soil microbiome on plant colonisation patterns and highlighted the primacy of the seed microbiome over the soil microbiome.

In the current study, the phyllosphere was significantly different from the other plant compartments, being not influenced by the soil microbiome even when the seed microbiome was severely disrupted. In culture-based experiments, intact seed endophytes influenced secondary colonisation of the rhizosphere (Ridout et al., 2019) and phyllosphere compartments (Carlström et al., 2019). Colonisation patterns within the phyllosphere microbiome were shown to be dominated by generalist bacterial taxa (Massoni et al., 2020). Our results highlighted that the community composition and structure of the phyllosphere were similar across all treatments, and all communities were dominated by ESVs from same genera. In field conditions, early colonisation patterns within the phyllosphere of agricultural crops are driven by recruitment from native soils and environment (de Souza et al., 2016; Grady et al., 2019), even within soybean (Copeland et al., 2015). Here, we showed that whether or not the recruitment from the environment is impaired, then innate seed microbiome will occupy the endophytic and epiphytic niche spaces of the phyllosphere, emphasising the nuanced influence of seed microbiome as potential priority effect agents. Overall, our experiments highlight that inoculation of the rhizosphere is insufficient to drive community change within the phyllosphere, and are in agreement with previous coalescence experiments that demonstrated that the strength of host-selection only increase with successive microbiome passages (Morella et al., 2020) and developmental stages (Copeland et al., 2015).

4.8 Conclusion

In conclusion, this study provides support for our hypothesis that only when the innate seed microbiome is severely disrupted does the soil microbiome will colonise the rhizosphere and influence the microbial community diversity, abundance and composition, both at the functional and taxonomical levels. In all other cases, the seed microbiome has priority over the soil microbiome. We also highlighted the significant differences in the influence of the soil microbiome between aboveground and belowground compartments. Our results are coherent with the hologenome theory of evolution, as a large part of the soybean microbiota appears to be seed-transmitted and to have the upper hand over microbes from the environment. Although these results would have to be confirmed in the field where conditions are more variable and environmental sources more abundant, our results are pointing toward the seed microbiome as the most promising candidate for plant microbiome engineering efforts.

4.9 Acknowledgements

The authors would like to thank the Department of Agriculture and Agri-Food Canada for providing the seeds used in the study. IM was supported by the Innovation and Scarce Skills scholarship from South African National Research Foundation (NRF), Fonds de Recherche du Québec (FRQNT), and partly by Foundation Armand-Frappier. This work was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council (NSERC) (grant RGPIN 2014-05274) to EY. We also wish to acknowledge Compute Canada for access to the University of Waterloo's High-Performance Computing (HPC) infrastructure (Graham system) through a resources allocation granted to EY.

4.10 **Supplementary Material**

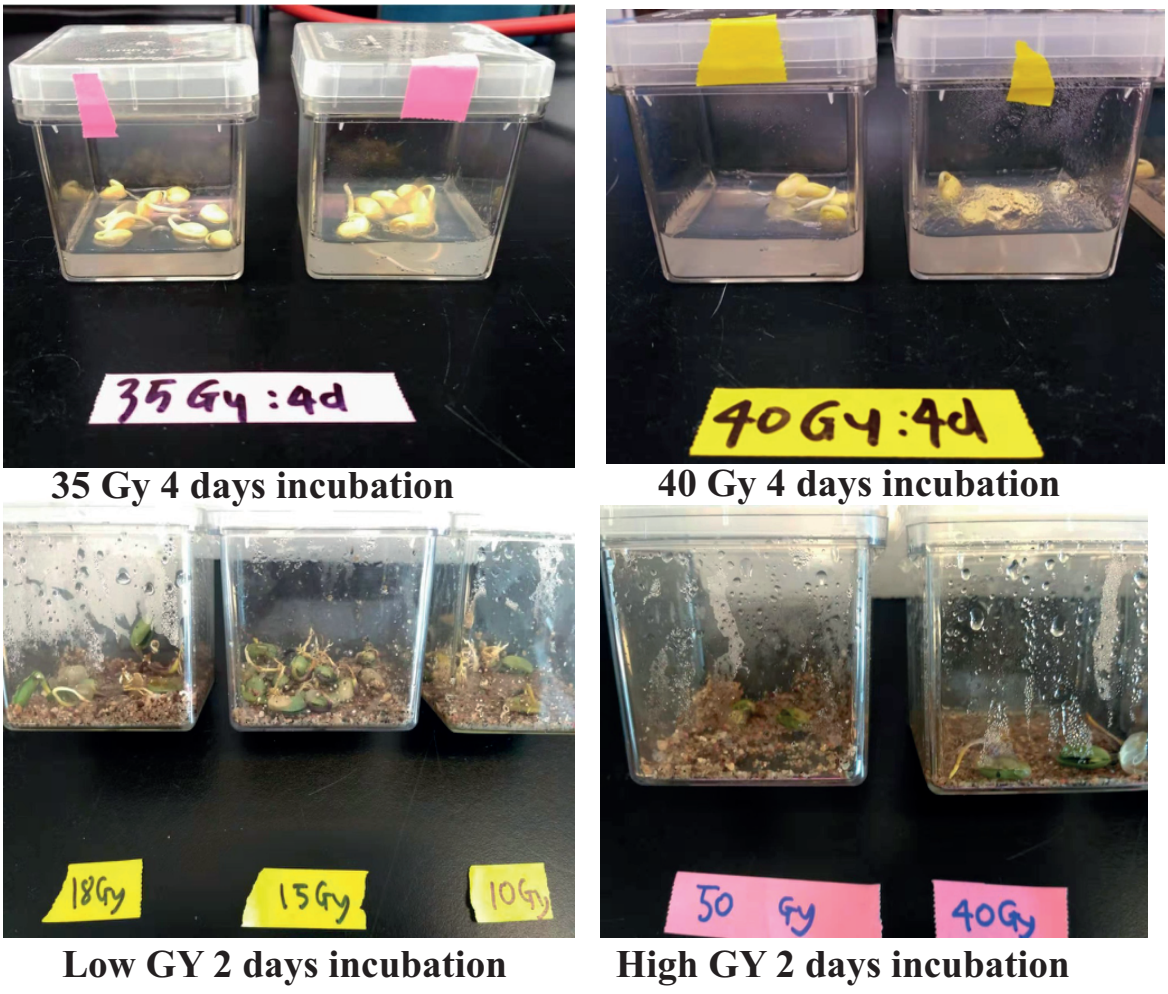

Fig.S1 Influence of irradiation on irradiated seedlings. Top: Irradiated seedling were able to grow on MS media with no microbial growth observed. Bottom: Irradiated seedlings growing on sterile sands, seedlings irradiated at 50Gy had abnormal growth

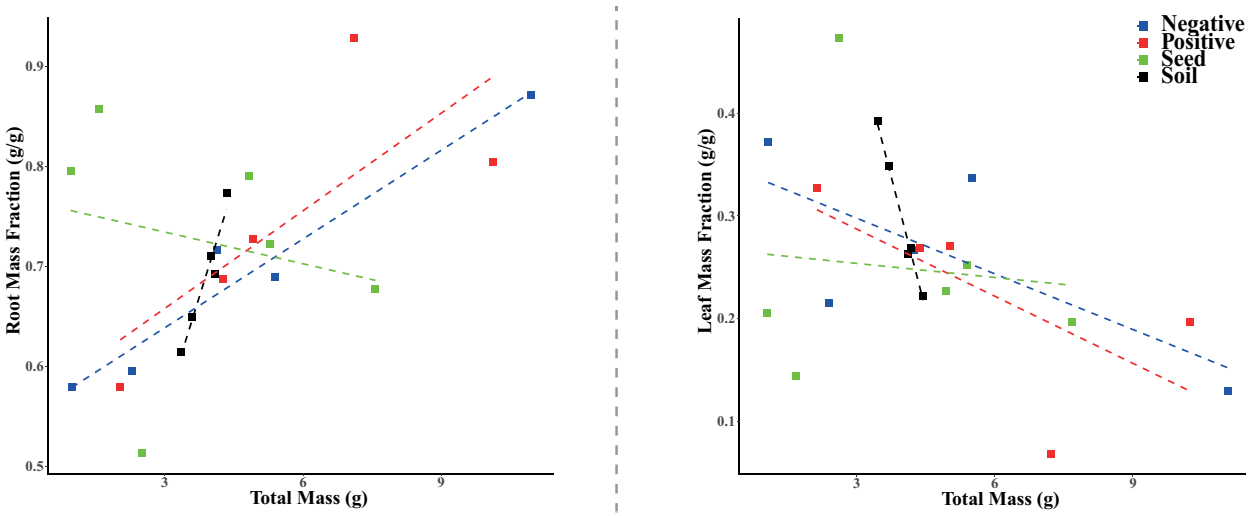

Fig.S2 Root mass fraction (RMF) and leaf mass fraction (LMF) plotted as function of total plant mass. The dashed lines indicate a generalised linear smooth (glm) for each treatment (blue=negative; red= positive; green= seed; soil= black)

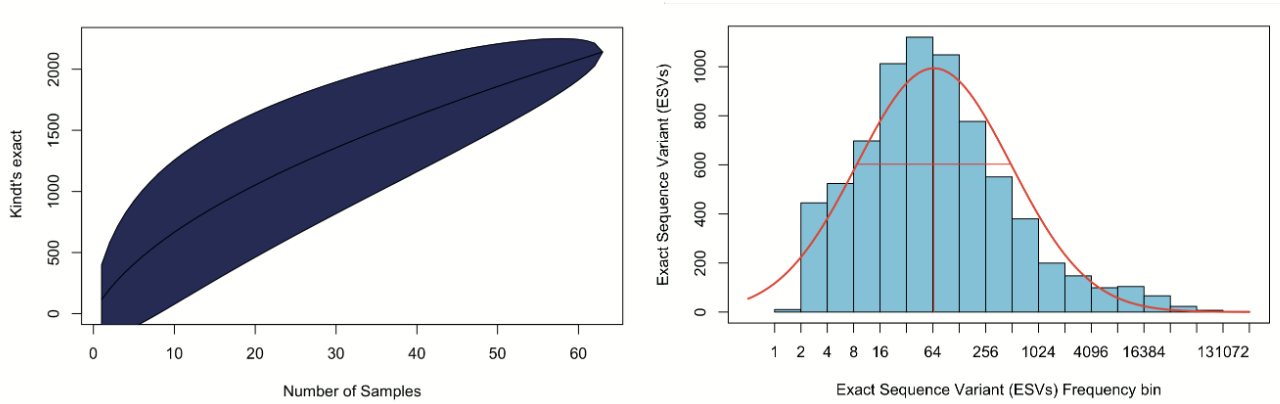

Fig.S3 ESV accumulation curve and Preston lognormal graph indicating total number of recovered ESVs (area under the curve) and the proportion of rare ESVs indicating few rare ESVs (bell shape).

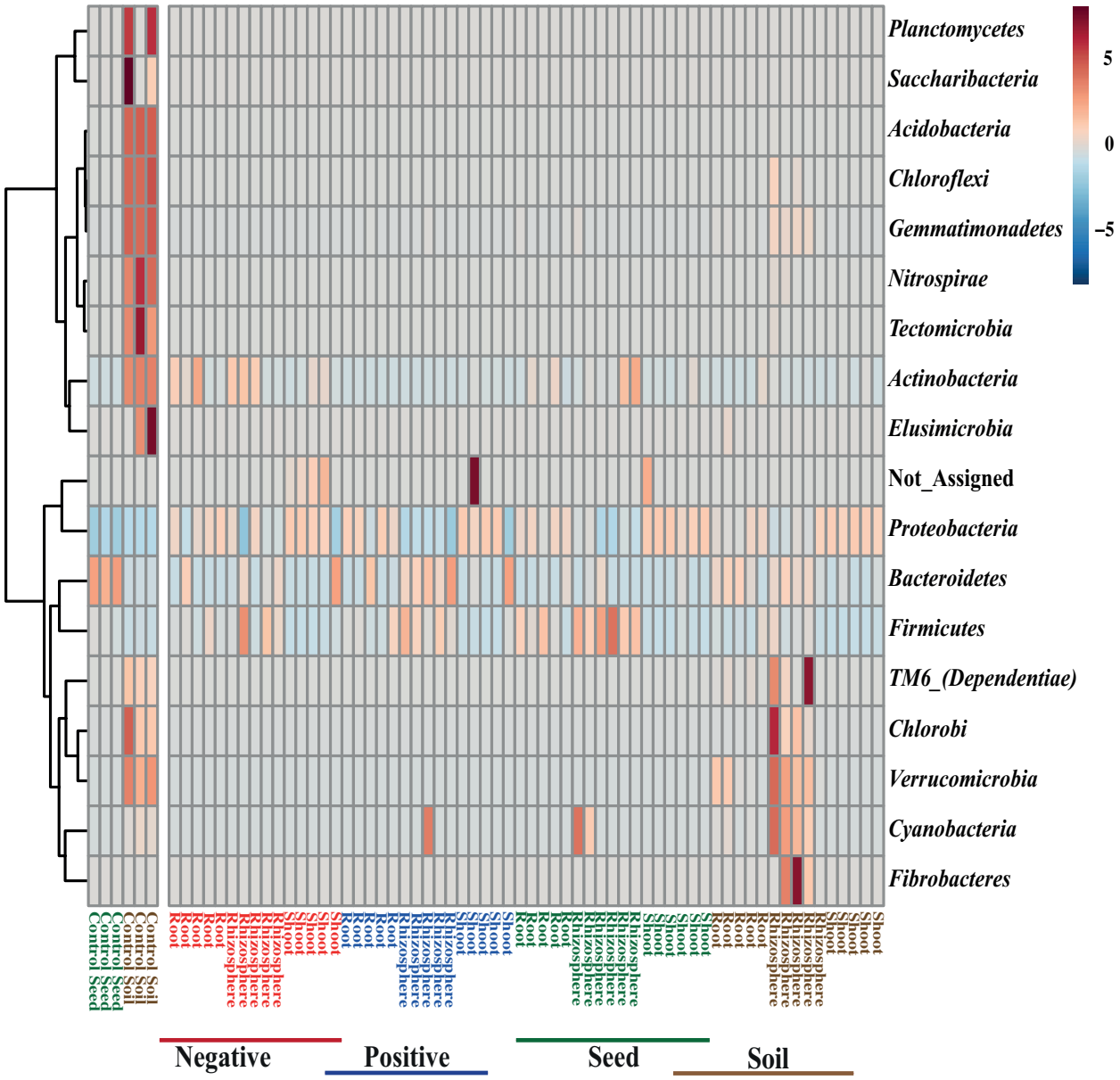

Fig.S4 Heatmap of relative abundance of the most abundant phyla using Ward clustering across plant compartments and treatment. Identity of treatment and compartment is indicted by columns annotation, whereas row annotation correspond to the phylum-level taxonomic affiliations of the ESV.

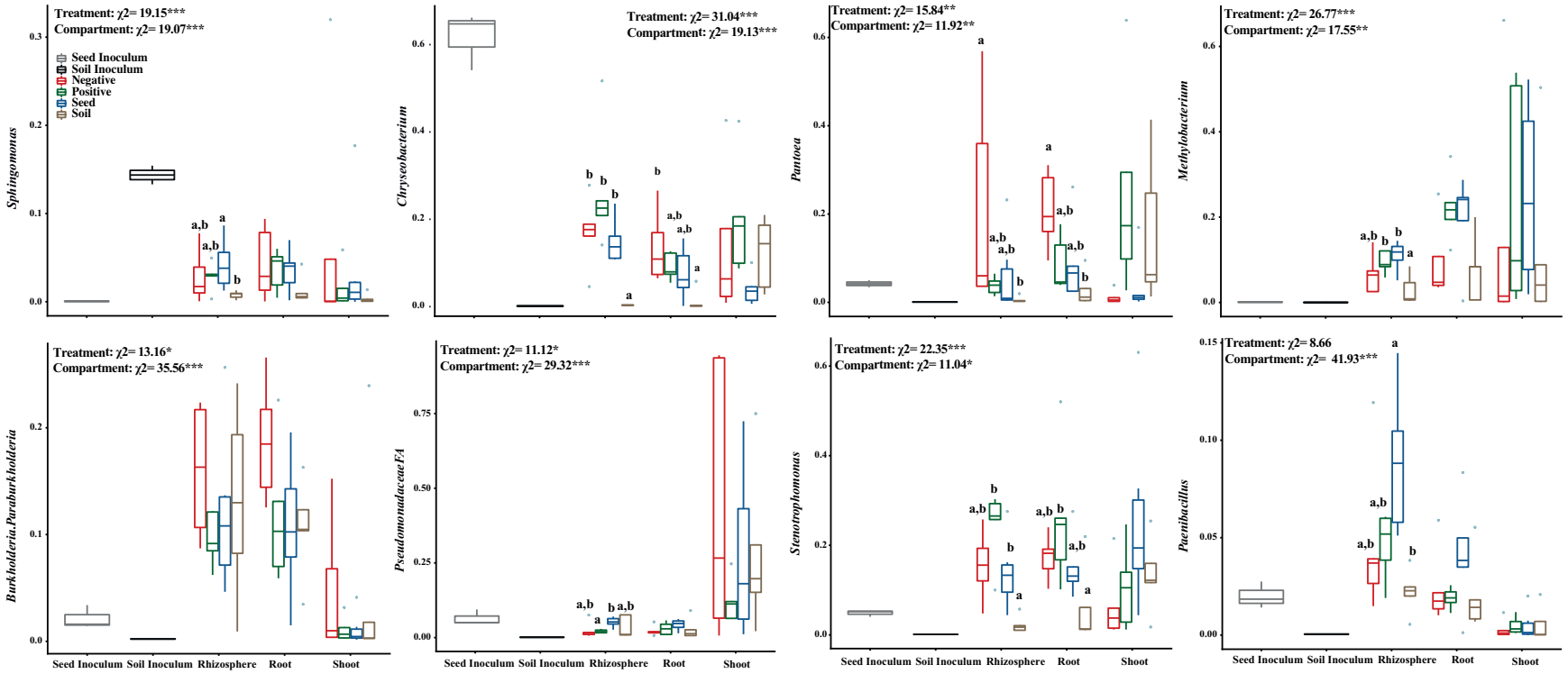

Fig. S5 Relative abundance of most abundant genera that were significantly influenced by treatment and compartment. (Kruskal-Wallis chi-squared χ^2, *p*-value) and pairwise Wilcox test with Bonferroni correction (*p* value *<0.05, ** <0.01, *<0.001)**

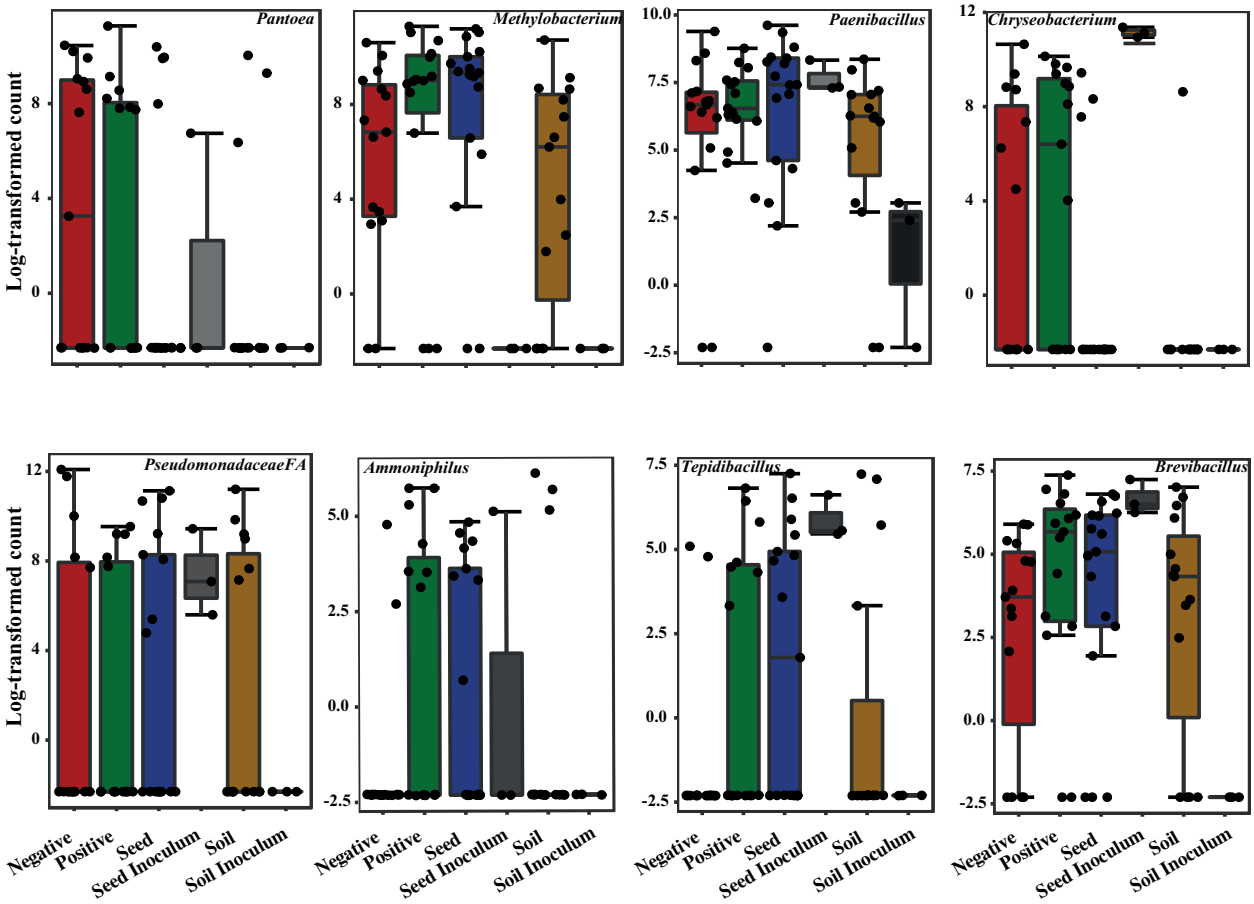

Fig.S6 Differentially abundant genera that was not present in the soil inoculum (p<0.05)

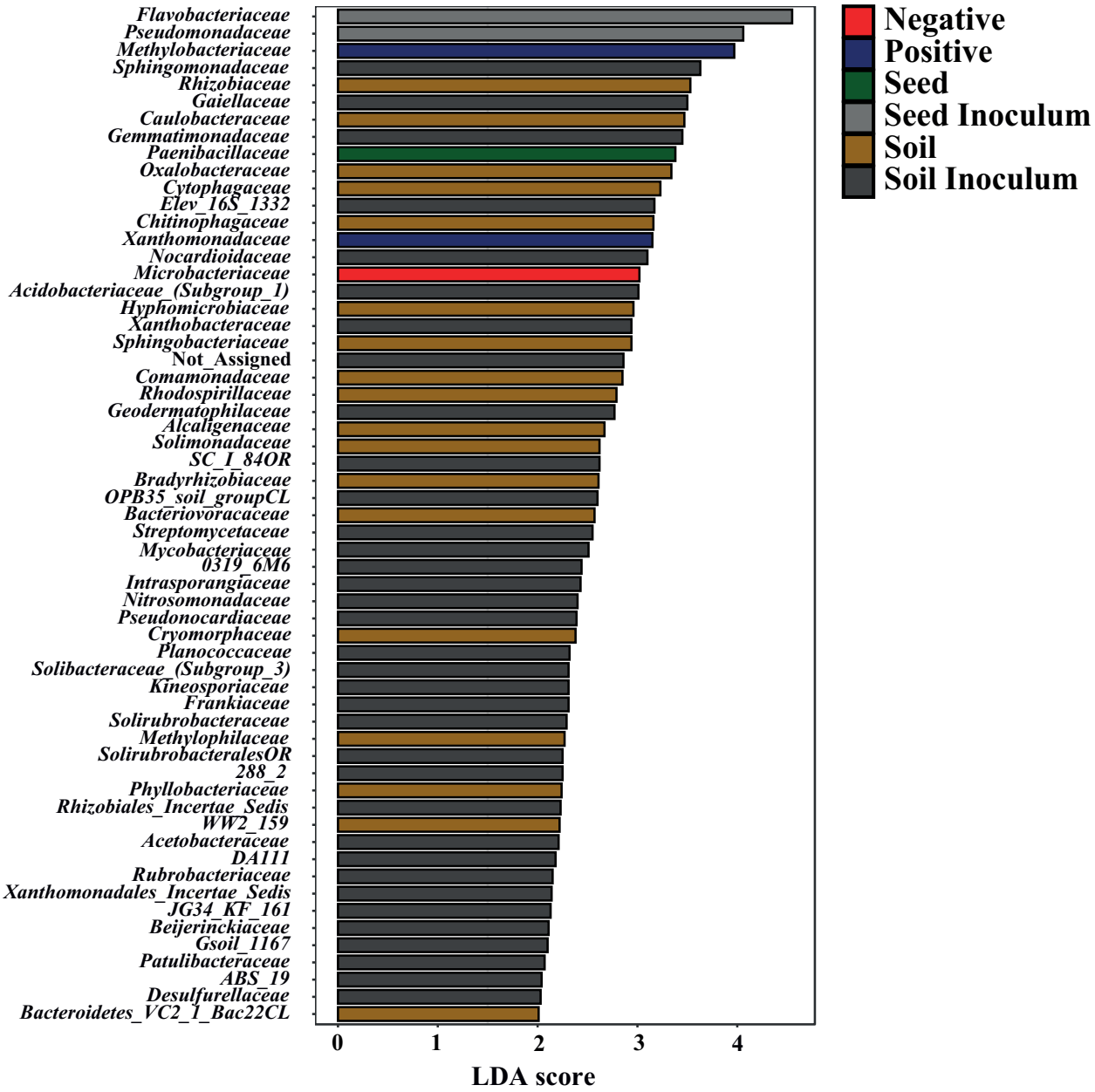

Fig.S7 Linear Discriminatory Analysis (LDA) indicating differentially abundant families across all treatments. LDA scores obtained from LEfSE analysis of microbiota across all treatments (LDA effect size > 2 was used as threshold of LEfSe analysis and p-value of 0.05

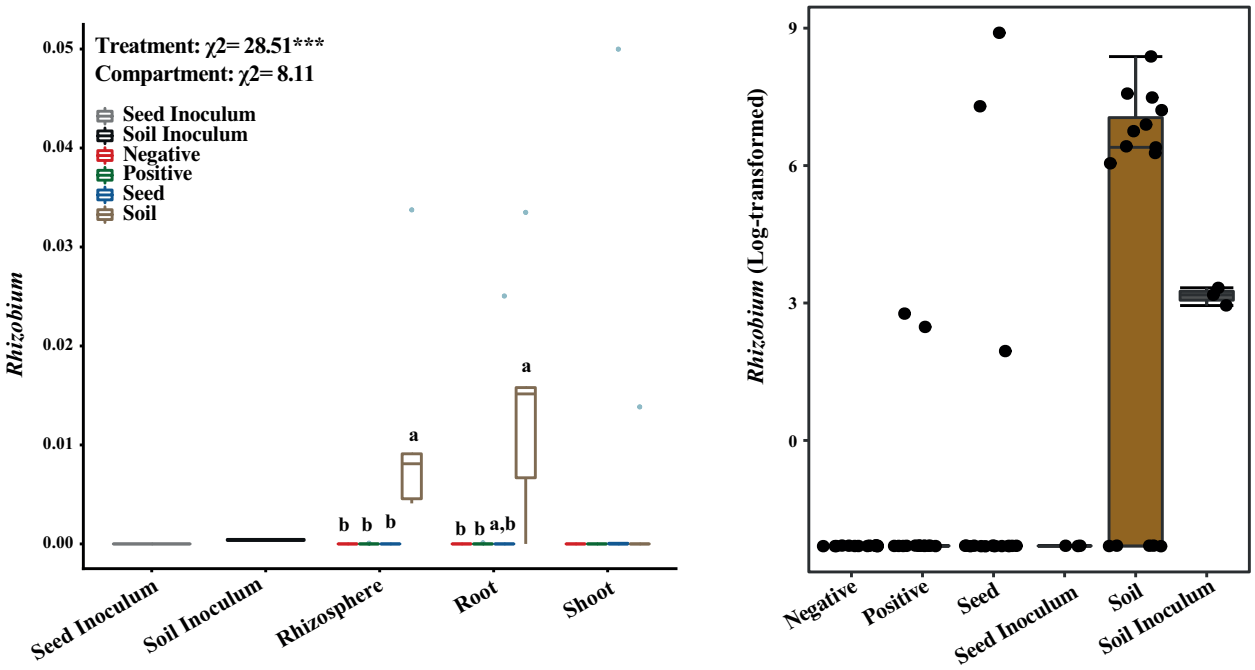

Fig.S8 Left panel: Relative abundance of *Rhizobium* that were significantly influenced by treatment and compartment (Kruskal-Wallis chi-squared χ^2, *p*-value) and pairwise Wilcox test with Bonferroni correction *(p* value *<0.05, ** <0.01, *<0.001). Right panel: DESeq2 differential abundance analysis of *Rhizobium* across all treatment *(p* value <0.05)**

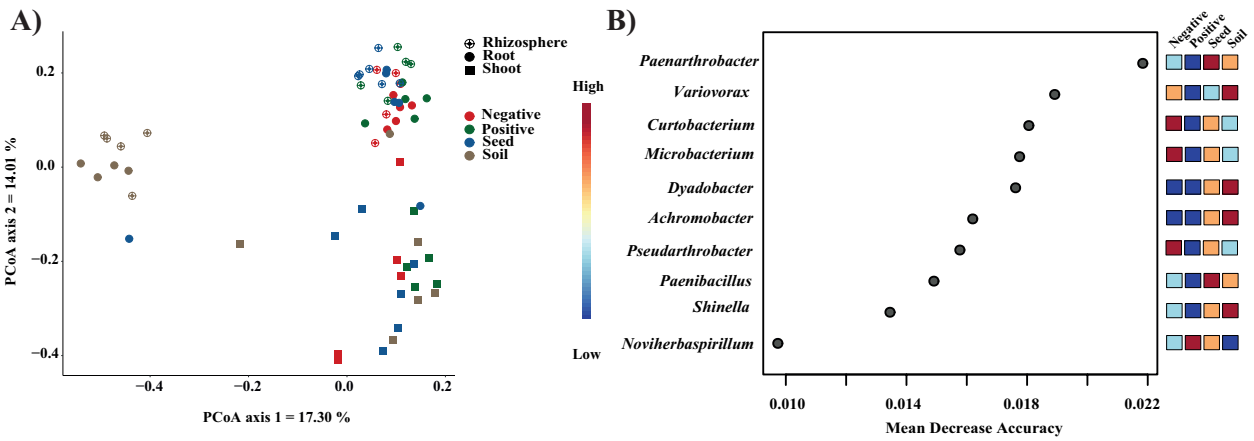

Fig.S9 Principle Coordinate Analysis (PCoA) of the 100 most dominant ESVs based on A) Bray-Curtis dissimilarity; samples that are closer to each other have similar community composition, whereas samples that are further apart are distinct to each other in composition. B) Random forest analysis based on the overall taxonomic profile could distinguish amongst different treatment with increased predictive accuracy. The more the accuracy of the random forest decreases due to the exclusion (or permutation) of a single taxon, the more important that taxa is deemed, and therefore taxa with a large mean decrease in accuracy are more important for classification of the data. The figure shows taxa with the highest discriminatory power and their classification. Blue colour indicates low probability of that genera being associated with that treatment, whilst red indicate high probability.

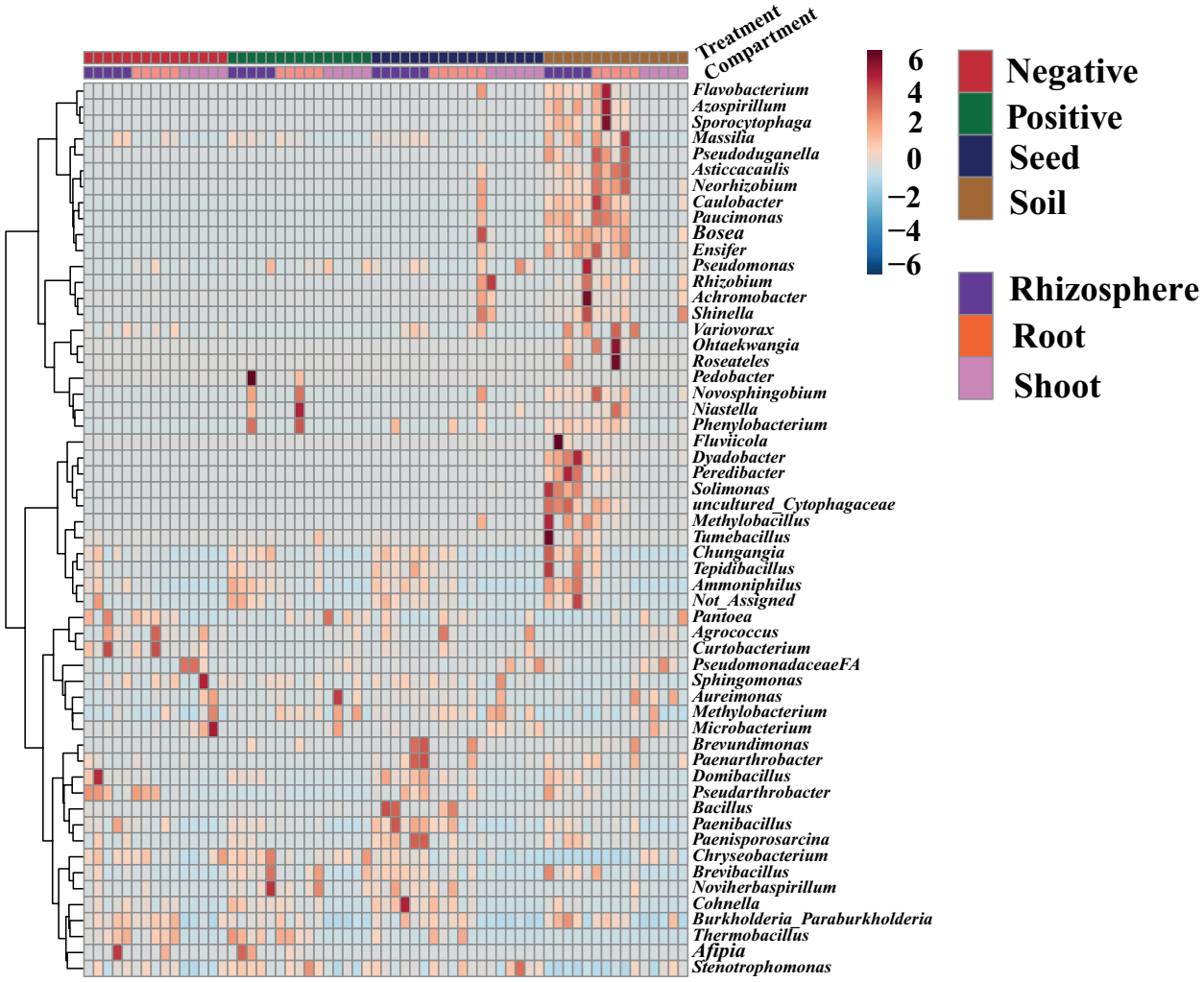

Fig.S10 Heatmap of relative abundance of the 100 most abundant ESVs using Ward clustering across plant compartments and treatment. Identity of treatment and compartment is indicted by columns annotation, whereas row annotation correspond to the genus-level taxonomic affiliations of the ESV.

Table S1. Total number of reads for each samples

Total_reads	**25,228,050**		
contaminants_reads	**86,368**		
phix_reads	**2,28**		
non_contam_non_phix_reads	**25,139,402**		
non_contam_non_phix_reads_1	**12,569,701**		
reads_1_QC_passed	**11,868,673**		
Cluster counts for reads1_clustered			
===BARCODES DISTRIBUTION===			
#name	**sequence**	**count**	**perc**
PCR2	AAAATAGGG	105,72	0.89%
NCR5	AAACGCTAG	143,369	1.21%
PCR3	AAACAATGA	191,293	1.61%
SoilR4	AAAAGGCCA	227,714	1.92%
ContSeed.1	AAACCGTTG	169,303	1.43%
SoilRZ4	AAACTAGCG	170,053	1.43%
SeedR2	AAACTGAAA	169,197	1.43%
SeedR4	AAACACCCG	675	0.01%
PCR1	AAACTCACA	208,436	1.76%
PCS1	AAAATTTTA	191,176	1.61%
SoilRZ1	AAACATATG	180,132	1.52%
SoilR1	AAACGACGA	173,125	1.46%
PCRZ3	AAACATGTA	252,944	2.13%
NCR4	AAAAGGTAG	166,608	1.40%
ContSoil.1	AAAATTAAA	100,083	0.84%
SeedR6	AAACCCGCA	128,563	1.08%
SoilR3	AAACCCACG	184,276	1.55%
SoilRZ2	AAACCTCTA	103,578	0.87%
SeedS2	AAAACGGCA	152,341	1.28%
SeedRZ5	AAAAGTCAA	211,689	1.78%
NCS2	AAAGAACCG	171,395	1.44%
NCR2	AAAAACTGA	145,728	1.23%
SeedS3	AAAATAATA	194,063	1.64%
SeedRZ2	AAACGCCCA	206,308	1.74%
SoilS4	AAACAGCAG	101,491	0.86%

SeedS6	AAACTCGAG	92,92	0.78%
PCR5	AAACCAAG G	197,594	1.66%
PCRZ2	AAACCAGG A	245,885	2.07%
SeedRZ3	AAACGTGG G	189,059	1.59%
PCS3	AAAAATCAG	96,251	0.81%
ContSeed.3	AAACTGCTG	150,405	1.27%
PCS5	AAAACTGAA	109,89	0.93%
NCRZ3	AAAATGGAG	127,306	1.07%
NCS4	AAACGGCA A	99,314	0.84%
ContSeed.2	AAAACTTTG	211,682	1.78%
PCR4	AAAACAATG	155,251	1.31%
Mock.Community	AAACAACG G	136,847	1.15%
PCRZ5	AAAACAGTA	93,857	0.79%
SoilS2	AAAGAATCA	133,078	1.12%
SoilR2	AAAAGCTCG	220,524	1.86%
PCS2	AAACGATCG	214,429	1.81%
SeedS4	AAACGTATA	119,093	1.00%
NCS5	AAAAATTAA	181,976	1.53%
NTC	AAACCGGA A	541,468	4.56%
KTC	AAAAGACTA	190,691	1.61%
SoilRZ5	AAAAAATTA	111,874	0.94%
SoilS3	AAAAGTGTG	139,606	1.18%
NCRZ1	AAACCGAA G	170,443	1.44%
SoilS1	AAACAGTAA	125,181	1.05%
NCS1	AAAATTCTG	216,495	1.82%
NCS3	AAAGACCA G	172,72	1.46%
PCRZ4	AAAAACCG G	139,338	1.17%
ContSoil.3	AAACTGTTA	129,992	1.10%
NCR3	AAAACCAG G	185,993	1.57%
SeedS5	AAAATCGCG	354,632	2.99%
SeedR1	AAAACTAAG	132,001	1.11%
SoilRZ3	AAACTTCGG	228,03	1.92%
SeedR5	AAAAAACTG	131,178	1.11%
PCRZ1	AAAACGAC G	165,471	1.39%

SeedRZ1	AAAAGCCGA	162,911	1.37%
NCRZ2	AAACACTCA	103,393	0.87%
NCRZ4	AAAATGACA	187,843	1.58%
SoilS5	AAACGGGTG	123,215	1.04%
SeedRZ6	AAACTTTGA	84,837	0.71%
SeedR3	AAAAAGTCA	175,23	1.48%
SoilR5	AAAAAAAAA	133,47	1.12%
NCR1	AAAAGATGG	107,193	0.90%
SeedRZ4	AAACCTTGG	115,143	0.97%
ContSoil.2	AAAAAGCCG	123,401	1.04%
SeedS1	AAACTAAGA	184,869	1.56%
NCRZ5	AAAACCGGA	224,79	1.89%
PCS4	AAAATCAGA	182,644	1.54%

Table S2 Measured soil physical properties and values represent a mean of five soil samples

Soil Properties	**Value**
pH	7.2
P (kg/ha)	193
K (kg/ha)	138
Ca (kg/ha)	7517
Mg (Kg/ha)	176
Al (ppm)	640
P/Al (ISP)	13.4
Mn (ppm)	25.7
Cu (ppm)	2.55
Zn (ppm)	4.53
B (ppm)	1.13
Fe (ppm)	188
N (total) %	0.15
C/N	13.1
NH_4^+ (ppm)	5.1
NO_3^- (ppm)	43.93
Conductivity (mS/cm)	0.33

Table S3 List of genera that are enriched in soil inoculum and soil treatment, based on Differential abundance analysis.

Taxon	Pvalues	FDR
Brevundimonas	0.00026468	0.0032583
Caulobacter	3.5294e-06	5.7273e-05
Duganella	0.00018113	0.0025866
Ensifer	4.4489e-07	8.8237e-06
Flavobacterium	4.9892e-11	2.2264e-09
Gemmatimonas	0.0020451	0.023552
Microvirga	1.2403e-06	2.1085e-05
ML635J_21CL	4.7173e-05	0.00073221
Novosphingobium	2.7208e-07	5.7137e-06
Oxalicibacterium	0.0031892	0.035579
Paenibacillus	6.5084e-12	4.4668e-10
Paucimonas	1.0542e-08	3.1364e-07
Pedobacter	0.00019906	0.0027332
PlanococcaceaeFA	0.00013735	0.0020431
Pseudarthrobacter	0.00025345	0.0032315
Pseudoduganella	0.00025217	0.0032315
Rhizobium	1.1558e-06	2.0631e-05
Steroidobacter	1.8449e-07	4.1164e-06
Xenophilus	1.1205e-06	2.0631e-05

5 GENERAL DISCUSSION

In recent years, the documented benefits conferred by the microbiome to the plant host has stimulated research on how the microbiome can be engineered. In principle, once the processes of assembling or maintaining the microbiome are understood, it is then possible to engineer them to increase the host's stress tolerance capacity, diseases resistance, and capacity of the host to colonise previously unavailable niches (Mueller and Sachs, 2015; Quiza et al., 2015; Agoussar and Yergeau, 2021). To this end, this thesis explored the colonisation patterns and prevailing processes that dominate the assembly of the soybean microbiome and further highlighted the nuanced and unexplored relevance of priority effects in microbiome succession. Phytobiome ecology has focused on the single snapshot of compositions change in time. However, abiotic and biotic changes occur at relative broad temporal scales, which requires a more detailed assessment of long-term community successional dynamics (Debray et al., 2021). The effects of local community composition on arriving species are primarily perceived during primary and secondary successions periods. These priority effects are caused by the 1) interaction between local communities and introduced species and 2) interaction between environment and arriving species (Fukami, 2015).

5.1 Priority effects modulate spatial and temporal microbial niche occupancy

The occupied microbial niche within the plant holobiont is acted upon by plant-mediated selections (niche-based processes) and neutral selection (dispersal, drift, and speciation) (Cordovez et al., 2019). The mechanistic processes that assemble and modulate the soybean microbiome have are broadly understood- genotype (Liu et al., 2019b), plant nutrient status (Bender et al., 2015), soil abiotic factors (Smith et al., 2016), environmental CO2 levels (Sanz-Saez et al., 2019; Christian et al., 2021), and climate (Compant et al., 2010b). However, the role of neutral processes (dispersal) in influencing plant microbiome is not well-understood. Ecological dispersal refers to individuals' migration from the regional species pool to local populations and promotes gene flow (Ronce, 2007; Fukami, 2015). Typically, microbial dispersal involves three distinct stages, that is, 1) departure from regional species pool, 2) transmission and 3) settlement into the local community/new environment. At the last settlement stage, the influence of priority

effects becomes more pronounced, and these effects bring to light the impact of the resident community on arriving species regardless of time (Debray et al., 2021). When exploring the successional patterns of soybean microbiome across the spatial and temporal axis, this thesis found evidence that strongly suggests that bacterial and fungal community composition were temporally autocorrelated (chapter 2, Moroenyane et al. (2021c)). The composition and structure of the community at the earlier developmental stages significantly influence the creation and colonisation of new microbial niches, thus community composition. Indeed, there was a detectable influence of plant compartment and developmental stages on microbial successional patterns for bacterial and fungal communities. The relative influence of the host (genotype, plant compartment, and development) and microbial arrival order was previously shown to be strong discriminatory axes that influence microbiome composition and functioning (Leopold and Busby, 2020). Intriguingly, the consequences of priority effects and historical contingency, in other words, the effect of order and timing of previous events on community assembly (Fukami, 2015), were shown to independently affect the host disease susceptibility (Leopold and Busby, 2020). Similarly, in field conditions, the order of arrival and subsequent microbial successional patterns significantly improved the survival of susceptible cultivars and promoted host defence priming (Plett et al., 2021).

Fundamentally, priority effects modulate community dynamics by acting on the realised species niche utilising two ecological processes: niche pre-emption and niche modification (Fukami, 2015). In niche pre-emption, primary colonisers (early arriving species) deplete or limit resources, such as nutrients (competitive exclusion) or space (competitive interference), available to late-arriving species (Fukami, 2015; Debray et al., 2021). More often than not, bacterial taxa have been shown to employ competitive exclusion of the colonisation on late-arriving species. For instance, transplant mesocosm experiments indicated that bacterial phylogenetic relatedness strongly predicted the strength of priority effects, with closely related taxa showing more substantial competitive exclusion (Tan et al., 2012; Venail and Vives, 2013). The genus *Pseudomonas* has been reported to play an integral role in maintaining plant holobiont homeostasis (Preston, 2004). However, there have been reports of competitive exclusion of early-arriving *Pseudomonas* species that colonise plant compartments (Lindow, 1987; Morella et al., 2020). Similarly, this thesis found evidence of the existence of temporal niches within the soybean microbiome, specifically, the increased species turnover across developmental stages and temporal niche displacement involving *Pseudomonas* and *Streptomyces* (Chapter 2; Moroenyane et al. (2021c)). There is a significant overlap in the metabolic and substrate

requirements of the inhabiting microbial species in plant systems. This increased metabolic niche overlap intensifies the influence of priority effects and competitive exclusion (Freilich et al., 2011). For example, facultative production of antibiotic by *Pseudomonas* and *Streptomyces* species was suggested as a mechanism that promoted competitive exclusion by limiting the risk of antibiotic resistance evolution and resource utilisation amongst competitors (Garbeva et al., 2011; Kinkel et al., 2014; Ghoul and Mitri, 2016).

Inversely, plant-associated fungal communities tend to have a far more positive interaction and exhibit reduced levels of competitive exclusion (Lee et al., 2019). In general, the fungal community tends to deploy competitive avoidance to facilitate the successful colonisation of dead trees to initiate decomposition. The influence of priority effects on fungal communities was shown to play a minimal role in the assembly of naturally occurring decomposing fungi, and these patterns were independent of tree species identity (van der Wal et al., 2016; Song et al., 2017). This thesis found evidence that supports contemporary discourse that states that competitive strength amongst species is the best predictor of fungal community assembly rather than priority effects (chapter 2; Moroenyane et al. (2021c)). For instance, the displacement of *Fusarium* taxa at the root-soil interface by *Mortierella* taxa was partly explicable by the antagonistic nature of *Mortierella*. This intense competition amongst taxa significantly influences the assembly and colonisation of fungal communities (Toju et al., 2015), and these occupied fungal niches tend to evolve independently across plant compartments and along the temporal axis (van der Wal et al., 2016; Song et al., 2017; Chaloner et al., 2020). Evidence suggests that the competitive strength of plant-associated fungi, directly and indirectly, modulates the occupied bacterial niche whilst strongly influencing the severity of priority effects (Johnston et al., 2019). However, *Lactobacillus* has been shown to use competitive exclusion as a mechanism that inhibits colonisation and assembly of fungal communities in animal products (Siedler et al., 2020). It is only in animal-microbe dynamics that shreds of evidence of competitive fungal exclusion are slowly emerging. For example, fungal entomopathogens were shown to deploy competitive exclusion within taxa (intraspecies) and between taxa (interspecies) to promote successful colonisation of host larvae (Li et al., 2021).

Although there is a lack of overwhelming evidence of competitive exclusion strategies within the plant-associated fungal community, it does not imply that there is no competition amongst fungal taxa. On the contrary, plant-associated fungal communities have been shown to use competitive interference strategies to dominate and direct successional patterns (Boddy,

2000; Drott et al., 2017). Equally, this thesis found evidence that fungal spatial niche occupation was primarily driven by taxa that were adapted to occupying those specialised niches, that is, leaf or root endosphere chapter 2; (Moroenyane et al., 2021c)). These findings were in direct support of previous reports that highlighted that these specialised spatial niches could only be occupied by adapted taxa (Qian et al., 2019), that fungal competitive interference reduced prevalence of pathogens (Hood et al., 2019), and ectomycorrhizal fungal community successional patterns were driven by competitive interference (Kennedy et al., 2011). Competitive interference and colonisation patterns of ectomycorrhizal fungi were recently shown to be important ecological drivers that can predict biogeographical patterns (Smith et al., 2018a). Overall, the difference in functional and life-history traits amongst different plant holobiont partners is a robust discriminatory axis that delimits the effect and severity of competition (exclusion and interference) and can be a strong predictor of the successional trajectory of the microbiome.

Early-arriving microbial taxa are capable of modifying their occupied niche space to either facilitate or inhibit further colonisation of the plant host (Fukami, 2015). At its core, niche modification occurs when the early-arriving taxa modify their niche by producing compounds that were not present in the environment, such as extracellular polymeric substances (EPS) (Poza-Carrion et al., 2013), break down large molecules into smaller molecules or cross-feeding (Ponomarova et al., 2017), modifying the plant tissue to elicit nutrient leakage (Monier and Lindow, 2005), or co-opting the plant biosynthetic pathways to produce novel molecules (Hooykaas and Schilperoort, 1992). However, microbe-mediated niche modification is double-edged, in that microbial taxa that are not dispersal-limited will be able to colonise unoccupied niches, whether plant commensal or pathogen. In both cases, successful colonisation of the new plant niche spaces involves suppressing the plant immune response (Hacquard et al., 2017). However, the severity of this suppression depends on the microbial taxa. For instance, microbial pathogens can suppress parts of the plant immune response that recognise them and other microbes (Halliday et al., 2020; Seybold et al., 2020). In plants with obligate and facultative mutualists, such as legumes, overall performance was directly influenced by the identity of the invading rhizobia taxa, and these facultative priority effects by rhizobia enabled the persistence of unbeneficial commensals within the microbiome (Boyle et al., 2021). Similarly, this thesis found evidence of early colonisation of soybean by *Bradyrhizobium* at the early developmental stages and the abundance of *Bradyrhizobium* was influenced by temporal dynamics (chapter 2; Moroenyane et al. (2021c)). Further, plant microbial community colonisation and coalescence

patterns are impelled by early arrival and niche modification by *Rhizobiaceae* taxa (Boyle et al., 2021; Ramoneda et al., 2021).

It seems that temporal fluctuations in soybean community composition and structure observed in this thesis are in part driven by the early arrival of specific microbial taxa (chapter 2; Moroenyane et al. (2021c)), and this, in turn, acts as a stabilising filter that modifies niche spaces that are later occupied by later-arriving taxa. But, hitherto overlooked were the relative influence of dispersal limitations and priority effects on soybean assembly. This thesis, utilising complementary community assembly approaches highlighted that in the case of soybean, dispersal limitations were significantly modulating community composition and structure (chapter 3; Moroenyane et al. (2021a)). Dispersal limitation combined with arrival order feedback to further intensify priority effects whilst directly influencing the composition and successional trajectory of the microbiome (Fukami et al., 2007). Intriguingly, intensive priority effects are the impelling force for increased adaptive radiation in microbial communities (Dieckmann and Doebeli, 1999; Rocabert et al., 2017). This in turn implies that although neutral processes (dispersal, ecological drift, and speciation rates) are important in the initial stages of community assembly, it is a niche-based process (selection) that seem to maintain the relatively high level of diversity in plant microbiomes. Here, it was found that dispersal rates along with stabilising homogenous selection were important ecological processes that assembled microbial communities and maintained the high levels of the observed phylogenetic and taxonomic diversity (chapter 2; Moroenyane et al. (2021c); chapter 3 Moroenyane et al. (2021a)). These findings are in direct support of empirical models that emphasise that the balance between the dominance of neutral and niche-based processes is mediated by dispersal rate and the existence of temporal niches (Cira et al., 2018)

5.2 **Priority effects and regional species pool synchronize microbial colonisation patterns**

Historical contingency provides novel insight on how regional species pool and priority effects influence community assembly, specifically, when taxa within the regional species pool can cause priority effects and the local environment is easily amendable by the arriving taxa (Fukami, 2015). Furthermore, diversity replete regional species pools contain microbial taxa that

may have the same functional capacity within the community and are also functionally redundant compared to diversity deplete communities. Thus, the impact of historical contingency and priority effects on community colonisation patterns and community structure is intrinsically linked to the diversity of the regional species pool (Wagg et al., 2019; Albright et al., 2020). The impacts of priority effects are heightened when the local environments are similar enough that dispersal from regional species pool favours early-arriving taxa (Urban and De Meester, 2009). This thesis explored this concept by employing a reductionist experimental approach. In chapters two and three, soybean plants were grown in environmental chambers, and the microbiome was systematically profiled along the spatial and temporal axis where the environments were similar. Moroenyane et al. (2021b) (chapter 4) highlights how regional species pool influences early colonisation patterns of near-axenic and diversity deplete soybean spatial niche spaces. In the resilient resident root microbial communities, priority effects and plant-mediated selection showed the preferential establishment of known commensal taxa (Wippel et al., 2021). Additionally, in sequential inoculation of axenic plants, commensal strains invade resilient and stable communities unaffected by late-coming strains (Carlström et al., 2019). The capability of plants to suppress disease prevalence and spread is highly dependent on the arrival order of the community and colonisation by the right commensal partner (Wei et al., 2019). In soybean, the rhizosphere microbiome was shown to be significantly influenced by genotype, indicating that in the case of soybean, the selected traits of each genotype can assemble a microbiome consisting of ideal commensals (Han et al., 2020). Additionally, sequential inoculation experiments added that commensals could colonise occupied niche space in the stable, resilient community across soybean genotypes (Zhong et al., 2019). This preferential colonisation of commensal was genotype-specific even when all commensals were from the same regional species pool. These findings imply that, in soybean, there is synchrony between regional species pool and priority effects, and these synchronous interactions assemble a unique microbiome that selects for commensals. Supplementary, this thesis found that disrupted microbiomes are capable of selecting the beneficial commensals across different sources of regional species pool (chapter 4; Moroenyane et al. (2021b)). Over and above, the unique community assembled from each regional species pool, different known soybean commensals were enriched. This new insight sheds light on our current understanding of how commensal recruitment in soybean occurs across genotypes and biogeographical scales (Zhang et al., 2018a), and suggests that when the microbiome is disrupted- secondary succession dynamics are still acted upon by priority effects and regional species synchronicity. That is, synchronised shifts in community structure and spatial

correlation fluctuations in population dynamics are key indicators of niche-based assembly processes (Griffin and Wells, 2017).

There is increased heterogeneity in the created spatial niche within plant hosts, and these niches also interact with temporal dynamics and change. This variation in spatial niches occurs when priority effects are modulated by metabolites (plant or microbe-derived) as these by-products are locally enriched; thus, niche modification occurs (Fukami, 2015; Jacoby and Kopriva, 2019; Chng et al., 2020). This thesis highlighted that across different spatial niche spaces (rhizosphere, root, stem, and leaves) within the soybean microbiome, temporal dynamics (developmental stages) interacted and led to community coalescence- an interchange of an entire microbiome (Rillig et al., 2015). Community coalescence is significantly influenced by the degree of priority effects and dispersal of taxa from the regional species pool (Vannette, 2020). In soybean, plant and microbe-derived metabolites facilitated community coalescence across developmental stages (Hara et al., 2019). This secretion of metabolites and increased dispersal from the species pool creates dynamic spatial and temporal niches within the soybean and, in turn, the perfect conditions for historical contingency to play a critical role, especially when multiple taxa will arrive together and occupy various niches (Fukami, 2015). In chapter three, the dominance of neutral and niche-based process and dispersal rates varied significantly across spatial and temporal niches. For instance, the epiphytic phyllosphere communities experienced increased dispersal rates during the vegetative developmental stages “emergence” and “growth”. At these stages when new spatial niches are constantly created, this thesis found evidence of spatial community coalescence across different regional species pools in all epiphytic niche spaces (chapter 4; Moroenyane et al. (2021b). Moreover, given the differences in diversity across regional species pools, microbial genes related to nutrient acquisition were significantly enriched in all treatments. These findings are in line with recent works that highlighted priority effects mediate the colonisation patterns of microbes that promote and increase microbial functional traits related to increased host performance (Chng et al., 2020; Cheong et al., 2021; Debray et al., 2021).

5.3 **Harnessing priority effects and regional species pool diversity to promote plant microbiomes engineering efforts.**

Here, I suggest that observed distinct successional patterns in plant microbiomes are, in fact, firmly restrained by the initial community, priority effects, and historical contingencies. Recently, a cohesive and comprehensive framework for engineering plant microbiome that incorporates robust ecological theory and synthetic microbial ecology was proposed (Agoussar and Yergeau, 2021). In their seminal work, Jones et al. (1994) first posited the idea that organisms acted as ecosystem engineers by sanctioning strong priority effects, such as niche modification. The promise of plant microbiome engineering depends on identifying plant compartments and developmental stages that are more amenable to synthetic communities. Recent approaches have focused on engineering the plant metabolic pathways to promote colonisation by the ideal commensals (Tatsis and O'Connor, 2016), and engineering the chemical interaction of plant microbiomes (Kenny and Balskus, 2018). In the past, such approaches have shown little reproductive success in field conditions; however, Hawkes and Connor (2017) argue that these failures can be circumvented by applying ecological theory to microbial communities and considering the dynamics of the whole community and not just focusing on a specific function or taxa. The works presented in this thesis, go a long way in responding to this “call to arms” by incorporating ecological theory and experimental design to identify plant compartments and developments that are dominated by strong priority effects and neutral community assembly processes. In microbiome reconstructions experiments where priority effects and community dynamics were not considered, microbiome dysbiosis often occurred (Suez et al., 2018). More importantly, this thesis highlighted that diversity and composition of the regional species pool drastically influences priority effect and microbiome colonisation patterns. Further signalling the importance of dispersal in modulating microbiome assembly and subsequent engineering. It also goes a long way to answer the question posited by Ronce (2007) “How Does It Feel to Be like a Rolling Stone? Ten Questions about Dispersal Evolution”. Specifically, it highlights that dispersal in plant microbiome is adapted to specific spatial and temporal niches spaces. These findings corroborate previous mesocosm dispersal studies that highlighted that dispersal facilitates the selection of taxa that will be able to adapt to the local community and habitat (Jacob et al., 2017). The diversity of regional species pools (dormant and active) and dispersal mechanisms of taxa has recently been shown to be critical factors to consider in achieving microbiome engineering success (Mestre and Höfer, 2020).

Thus, the culmination of the presented works in this thesis is timely and provides insight into how these fundamental ecological processes can be modelled and used to advance microbiome engineering efforts.

5.4 **Conclusion and perspectives**

In conclusion, this thesis sought to find evidence and support for its central hypothesis that spatial and temporal niches spaces exist. It highlighted the relative importance of priority effects and plant-mediated selection on these niches. In addition, highlighted the primacy of the regional species pool in affecting microbial colonisation patterns of the spatial and temporal soybean niche spaces. In this regard, this thesis successfully achieved its primary objective of investigating microbial colonisation patterns of different communities and the overall assembly processes.

The results presented in this thesis show that soybean microbial communities are temporally nested; that is, the composition and structure of the microbial community at any point along a temporal scale (*i.e.* developmental stage) significantly modulates the structure of a community further along the temporal scale. For instance, the abundance and composition of microbes at the vegetative stages will affect the structure of communities in the reproductive stages. This insight sheds new information on our current understanding of successional patterns of plant-associated microbial communities. These findings provide the first line of evidence for the primacy of priority effects in steering the successional patterns of microbial communities. It seems in part that temporal entanglement of plant-associated microbial communities is strongly mediated by the balance between priority effects (niche pre-emption and/or modification) and plant-mediated selection. For instance, there was evidence of temporal enrichment of specific microbial taxa across all developmental stages. These findings are in line with emerging research on the ecology of plant-associated microbial communities that prove that facultative priority effects by soybean symbiont- *Rhizobium* influenced overall plant performance and recruitment of commensals. In light of this, it is possible to surmise that the arrival order of obligate symbionts and plant metabolic demands are predictors of community composition and successional trajectory.

Spatial and temporal dynamics significantly influence the balance between neutral and niche-based processes that assemble plant-associated microbial communities. That is, not all spatial (*i.e.* endosphere, rhizosphere) and temporal niches (*i.e.* developmental stage) within the soybean microbiome are subjected to the same ecological processes at any given point. Furthermore, using complementary approaches, evidence suggested that dispersal was a vital process that delimited and facilitated microbial abundance and distribution. This strongly suggests that plant-associated microbial communities are transient and that their composition and coalescence patterns are driven by taxa that can occupy the niche early. These early-arriving taxa modify the occupied niche and act as a selection filter that determines which commensals are allowed to invade the already colonised niche.

This thesis provided evidence that emphasises the importance of the composition of the regional species pool and how it mediates spatial niche occupancy. It found that the source of regional species pools highly influenced the occupancy of spatial niches. Furthermore, facultative and obligate symbionts from the seed had primacy in the colonisation of specific niches. These results, combined with previous experiments, provide evidence of tripartite feedback amongst dispersal, arrival order, and regional species pools and how these influence the composition and successional trajectory of the plant microbiome. However, this is not to say that plant-mediated selection does not play a role; on the contrary, plant metabolic demands are a selection filter from which the beneficial symbiont can be recruited.

Taken altogether, these findings add to our current understanding of how soybean microbiomes are assembled and maintained. This knowledge brings the goal of microbiome engineering one step closer. A deep and fundamental understanding of the ecology of plant microbiomes will assist researchers in circumventing issues of early community coalescence and retention of beneficial microbes in synthetic and natural systems. The reductionist experimental approach utilised in this thesis highlighted the essential and nuanced effects of dispersal limitations and priority effects on soybean microbiome colonisation and successional pattern by stripping away excessive external noise. That said, the results from this thesis can be translated into field trials to ascertain the role of how environmental variability and field condition will mediate the balance between priority effect and niche-based selection.

5.5 Limitation and future direction

This dissertation has presented conceptual theory, empirical findings, and a roadmap for microbiome engineering. The conclusions reported in this dissertation are not without limitations due to the study question and method used. The reductionist approach of the experimental setup may have elucidated the mechanistic processes that assemble and influence soybean microbiome structure. However, these findings are yet to be corroborated by field observations at similar scales of sampling, analyses, and including other components of the plant microbiome. As a result, this thesis revealed more research avenues in plant-microbe interactions, which may aid in the advancement of research in this area. It generated a series of questions that can help guide future research efforts and increase interest in this field of study. These fields must be plant immune response and microbe-microbe interactions based on the thesis's content. Future study directions are thus dependent on the collection of restrictions that have been recognized and structured around these two fields. The logical next step based on these findings is to 1) identify and study plant systemic signalling molecules that affect plants and their associated microbiome as a unit, 2) identify how microbe-microbe interaction within the plant holobiont influence colonisation and succession, and 3) characterise the relative contribution of different communities (virus, bacteria, fungi, nematode…) to discover new, sustainable ways to protect crops and natural ecosystems.

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