Université de Montréal

Caractérisation de la structure et de la composition des communautés microbiennes natives d'écosystèmes naturels hyperphosphatés et de leur contribution à l'élaboration d'inoculums solubilisateurs de phosphate de roche

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Cette thèse intitulée

Caractérisation de la structure et de la composition des communautés microbiennes natives d'écosystèmes naturels hyperphosphatés et de leur contribution à l'élaboration d'inoculums solubilisateurs de phosphate de roche

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RÉSUMÉ

Afin de réduire l'usage des fertilisants chimiques et leurs empreintes environnementales, la production de bioinoculants à base de bactéries rhizosphériques solubilisatrices de Phosphore (PSB) est une biotechnologie prometteuse. Lorsque celle-ci est associée à l'apport de phosphate de roche (RP), elle pourrait constituer une alternative écoresponsable d'amélioration de la fertilité des sols rhizosphériques, notamment à travers l'augmentation du phosphore (P) biodisponible. Pour répondre à cet enjeu environnemental majeur, il est indispensable d'étudier la biodiversité microbienne des sols en interaction avec le RP. Ainsi, ce travail de thèse a pour objectif de caractériser, d'un point de vue taxonomique, les communautés bactériennes et fongiques associées aux compartiments racinaires et rhizosphériques de plantes natives d'environnements singuliers, peu explorés, que sont d'anciens sites miniers riches en RP. Le séquençage Illumina MiSeq d'amplicons de gènes ribosomiques des différents groupes microbiens et l'assignation taxonomique des séquences regroupées en ASV, nous ont permis de mettre en évidence des valeurs de richesse et de diversité microbiennes comparables malgré la différence des teneurs en RP et en P des sols étudiés. De même, une grande similarité entre les profils des communautés a été identifiée à haut niveau taxonomique, avec une prévalence marquée des phyla Actinobacteriota et Ascomycota, et de l'ordre Glomerales pour les communautés de champignons mycorhiziens arbusculaires (CMA). À un niveau taxonomique plus bas, un core microbien de 26 ASV mycorhiziennes, persistantes à travers le gradient de RP et de P, a également été mis en évidence. Toutefois, l'analyse statistique des données environnementales nous a permis d'identifier des ASV indicatrices des habitats enrichis *versus* non enrichis en RP, pour chaque communauté microbienne étudiée. La synthèse de ces résultats originaux montre une influence mineure des teneurs en RP et en P sur le profil des communautés microbiennes des sites étudiés et questionne la signification écologique de la prévalence de certains taxa en lien avec l'histoire minière du site, sa restauration écologique et sa résilience, ainsi que les stratégies de vie et les attributs fonctionnels des taxa dominants. Par ailleurs, une collection de PSB a été réalisée à partir d'isolats racinaires issus des sites miniers étudiés, ou à partir d'isolats issus de spores et d'hyphes mycorhiziens piégés au contact d'apports en RP déposés dans la rhizosphère de plantes d'intérêt agronomique. Ces PSB racinaires ou hyphosphériques, testés par ailleurs pour plusieurs traits additionnels promoteurs de la croissance des plantes, ont été assignés à trois phyla : Proteobacteria, Firmicutes et, dans une moindre mesure, Actinobacteria ; *Bacillus* et *Pseudomonas* étaient les genres prédominants. Enfin, à partir de ces isolats, deux consortia bactériens avec différent traits promoteurs de la croissance des plantes (PGP), ont été constitués et testés seuls ou en combinaison avec l'apport de RP et/ou l'ajout d'un inoculum mycorhizien à base de *Rhizophagus irregularis*, sur la croissance de la tomate, espèce d'intérêt agronomique. Une augmentation du taux de germination, un accroissement de la hauteur des jeunes plantules et des modifications au niveau du système racinaire des plants adultes, en particulier des variations de la densité du tissu racinaire, ont été observés en réponse à l'inoculation simple par les consortia ou à leur interaction double ou tripartite avec le RP et/ou le CMA. Le profil taxonomique du microbiote natif avant et après apport des inoculants et du RP a été caractérisé. En conclusion, l'ensemble de nos travaux s'appuyant sur l'analyse des données environnementales, combinées aux données de laboratoire sur l'inoculation de plantes par des isolats bactériens, notamment d'origine minière, soulignent l'intérêt mais aussi la complexité de l'élaboration d'inoculants microbiens promoteurs de la croissance des plantes, solubilisateurs de P.

Mots clés : Phosphore, Rock Phosphate, Microbiome, Diversité Taxonomique, Metabarcoding, Bioinoculant, Consortium

ABSTRACT

In order to reduce the use of chemical fertilizers and their environmental footprint, the production of bioinoculants based on phosphorus solubilizing rhizospheric bacteria (PSB) is a promising biotechnology. When combined with rock phosphate (RP), it could be an eco-responsible alternative to improve rhizospheric soil fertility, especially by increasing bioavailable phosphorus (P). To address this major environmental issue, studying soil microbial biodiversity and its interactions with RP is required. Thus, the objective of this thesis is to characterize, from a taxonomic point of view, the bacterial and fungal communities associated with the root and rhizospheric compartments of native plants in singular, little explored environments, located in former mining sites rich in RP. Illumina MiSeq sequencing of ribosomal gene amplicons of the different microbial groups, then taxonomic assignment of the sequences grouped in ASV, allowed us to highlight comparable microbial richness and diversity indexes of soils despite their differences in RP and P contents. As well, a high degree of similarity between the community profiles was identified at a high taxonomic level, with a marked prevalence of the phyla Actinobacteriota and Ascomycota, and of the order Glomerales for the arbuscular mycorrhizal fungi (AMF) community. At a lower taxonomic level, a microbial core of 26 mycorrhizal ASV, persistent across the RP and P gradient, was also documented. However, statistical analysis of the environmental data allowed us to identify indicator ASV for PR-enriched *versus* non-rich habitats for each microbial community studied. All together, these original results contributed to rule on a minor influence of RP and P levels on the microbial community's profiles across the studied sites, and raised the questions of the ecological significance of the prevalence of certain taxa regarding the site's mining history, its ecological restoration, as well as the life strategies and functional attributes of the dominant taxa. In addition, a collection of PSB was made from mine site root isolates, or from isolates associated with spores and mycorrhizal hyphae trapped in contact with PR inputs deposited in the rhizosphere of agronomic plants. These root or hyphospheric PSB, tested elsewhere for several additional plant growth-promoting traits, were assigned to three phyla: Proteobacteria, Firmicutes, and, to a lesser extent, Actinobacteria; *Bacillus* and *Pseudomonas* were the predominant genera. Finally, from these isolates, two bacterial consortia with different plant growth promoting (PGP) traits were constituted and tested, alone or in combination with RP inputs and/or a mycorrhizal inoculum based on *Rhizophagus irregularis*, on the growth of tomato, a species with an agronomic interest. An increase in germination rate or height of young seedlings, and changes in the root system of adult plants, particularly changes in root tissue density, were observed in response to single inoculation by the consortia or to their dual or tripartite interaction with RP and/or AMF. The taxonomic profile of the native microbiota before and after inoculants and RP input was characterized. In conclusion, our work based on the analysis of environmental data, combined with laboratory data from plant inoculation experiments by bacterial isolates, especially of mining origin, underlines the interest but also the complexity of the development of microbial inoculants promoting plant growth, solubilizers of P.

Keys Words : Phosphorus, Rock Phosphate, Microbiome, Taxonomic diversity, Metabarcoding, Bioinoculant, Consortium

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Ce travail de thèse a été réalisé dans le cadre d'une cotutelle entre l'Université du Littoral Côte d'Opale (ULCO) et l'Université de Montréal, au sein de l'Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV) et du laboratoire de l'Institut de recherche en biologie végétale (IRBV). Il a été financé par la région des Hauts de France et l'Université de Montréal que je remercie vivement.

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AVANT-PROPOS

Cette thèse de doctorat est le fruit de trois années et demi de travail en cotutelle entre la France et le Canada, débutées dans l'Unité de Chimie Environnementale et Interactions sur le Vivant (UCEIV) de l'Université du Littoral Côte d'Opale (ULCO) pendant 15 mois puis poursuivie dans le laboratoire de Biologie Végétale (IRBV) de l'Université de Montréal pendant 23 mois, avant un retour en France pour la phase finale de rédaction d'articles et du mémoire.

Le contenu de cette thèse est bilingue. L'introduction générale, les introductions aux chapitres, la synthèse générale ainsi que la conclusion et les perspectives sont rédigées en français, langue imposée par l'Université de Montréal. Les chapitres présentés sous forme d'articles scientifiques acceptés ou soumis à des revues internationales sont rédigés en anglais.

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ARTICLE 1 : Amandine Ducousso-Détrez, Joël Fontaine, Anissa Lounès-Hadj Sahraoui, Mohamed Hijri (2022). Diversity of Phosphate Chemical Forms in Soils and Their Contributions on Soil Microbial Community Structure Changes. Microorganisms, 10, 609.https://doi.org/10.3390/ microorganisms10030609, (Publié)
J'ai réalisé l'ensemble du travail bibliographique sous la supervision de l'un de mes encadrants et rédigé la première version de la revue, et ainsi que les corrections apportées par l'ensemble des co-auteurs.

ARTICLE 2 : Amandine Ducousso-Détrez, Simon Morvan, Joël Fontaine, Mohamed Hijri, Anissa Lounès-Hadj Sahraoui, How high phosphate concentrations affect soil microbial communities after a century of ecosystem self-reclamation ? Land Degradation & Development (soumis)

ARTICLE 3 : Amandine Ducousso-Détrez, Robin Raveau, Joël Fontaine, Mohamed Hijri, Anissa Lounès-Hadj Sahraoui, Glomerales dominate arbuscular mycorrhizal fungal communities associated with spontaneous plants in phosphate-rich soils of former rock phosphate mining sites. Mircoorganisms, (Soumis)
Pour les articles 2 et 3 j'ai conçu le design expérimental et effectué l'échantillonnage de terrain après avoir réalisé les démarches administratives d'autorisation de prélèvement dans la zone d'étude. Puis j'ai mené l'ensemble des procédures d'isolement de l'ADN environnemental et de constitution des banques d'amplicons de gènes ribosomiques des communautés bactériennes et fongiques. J'ai également réalisé l'ensemble des traitements bio-informatique et statistiques des données de séquençage fournies par un prestataire, appuyé de Simon Morvan pour certaines analyses bio-informatiques, et de Robin Raveau pour la construction de l'arbre phylogénétique. Enfin, j'ai rédigé les premières versions des deux publications, auxquelles des corrections ont été apportées par les co-auteurs.

ARTICLE 4 : Amandine Ducousso-Détrez, Joël Fontaine, Anissa Lounès-Hadj Sahraoui, Mohamed Hijri, Culturable Phosphate Solubilizing bacteria isolated from rhizospheric and hyphospheric habitats enriched in rock phosphate. (En Préparation)
J'ai sélectionné 254 isolats bactériens à partir de différents prélèvements de sols, puis réalisé et entretenu une collection de 48 isolats d'intérêt. J'ai également assuré l'ensemble des expériences de caractérisation de ces différents isolats. Enfin j'ai rédigé la première version de la publication, à laquelle des corrections ont été apportées par les co-auteurs.

ARTICLE 5 : Amandine Ducousso-Détrez, Joël Fontaine, Anissa Lounès-Hadj Sahraoui, Mohamed Hijri. Inoculation of phosphate-solubilizing bacterial consortia affects germination rate and root traits of tomato plants in combinaison with amendment with an arbuscular mycorrhizal fungus and rock phosphate. Microbiological Research (En Préparation)
J'ai conçu l'expérience en serre sous la supervision de mes encadrants. J'ai assuré la surveillance des cultures en serre, de plus j'ai assuré la mise en culture des graines et la surveillance des plantes jusqu'au stade adulte (arrosage, veille sanitaire). J'ai réalisé le choix des isolats constitutif des inoculants bactériens et leur préparation. J'ai réalisé les inoculations et l'ensemble des mesures requises pour la caractérisation de leurs effets sur la croissance des plantes, y compris celles à partir du logiciel Winrhizo. J'ai également réalisé l'ensemble des traitements statistiques. Enfin, j'ai rédigé la première version de la publication qui a ultérieurement été retravaillée par et corrigée par l'ensemble des co-auteurs.

LISTE DES ABREVIATIONS

ADN : Acide Désoxyribonucléique

ARN : Acide Ribonucléique

ASV : Amplicon Sequence Variant – Variant de Séquence d'Amplicon

ATP : Adénosine Triphosphate

CMA : Champignon mycorrhizien à arbuscules

P : Phosphore

PCR : Polymerase Chain Reaction

Pi : Ion Phosphate

PSB : Bactérie solubilisatrice de P

PGP : promotrices de la croissance des plantes

PGPR : Plant growth promoting rhizobacteria (promotrices de la croissance des plantes)

PSM : microorganismes solubilisateurs de phosphore

RP : Rock Phosphate (Phosphate de roche)

INTRODUCTION GENERALE

INTRODUCTION GENERALE

A. La production agricole face à l'expansion de la population mondiale et à l'épuisement des ressources en phosphore (P) : un défi majeur pour l'avenir

Selon les prévisions et les statistiques démographiques des Nations Unies, la population mondiale devrait augmenter de 2 milliards d'individus au cours des trente prochaines années, passant de 7,7 milliards actuellement à 9,7 milliards en 2050. Face à cette expansion démographique, le développement de l'agriculture est l'un des leviers les plus importants à actionner pour lutter contre l'insécurité alimentaire et nourrir la population planétaire.
Selon l'organisation de coopération et de développement économiques (OCDE), la production agricole est en bonne voie pour suivre le rythme de croissance de la demande mondiale, et le secteur démontre une bonne capacité à faire face aux pics même inattendus de la demande. Cependant, en tant que premier utilisateur des ressources naturelles planétaires, la production agricole affiche une empreinte environnementale considérable. La banque mondiale et l'OCDE préconisent donc de repenser les pratiques du secteur agricole afin d'encourager des mesures plus respectueuses de l'environnement et des principes de durabilité, notamment dans le domaine agricole et les pratiques de fertilisation destinées à contrebalancer l'appauvrissement des sols surexploités.

Les engrais phosphatés apportés sous forme minérale permettent d'accroître dans les sols, le pool des formes phosphatées solubles et rapidement assimilables par les plantes. Ces engrais sont issus de l'exploitation de gisements naturels de phosphate de roche (désigné dans la suite du texte par RP pour rock phosphate en anglais), et de processus chimiques drastiques d'enrichissement en P du minerai. Mais leur utilisation excessive en agriculture intensive conduit à un gaspillage important et à des risques majeurs d'eutrophisation des écosystèmes aquatiques. En effet, par ruissellement et érosion, des pertes allant jusqu'à 80 % des apports sont relevées, contaminant partiellement les cours d'eau et les mers.
De manière concomitante, une exploitation massive des gisements géologiques phosphatés s'est également opérée face à la demande agricole de plus en plus croissante. Diverses études ont alors donné l'alerte quant à l'épuisement des réserves en phosphate. Le niveau exact des réserves mondiales demeurant mal connu, différents scénarii plus ou moins alarmistes ont été envisagés. Ainsi, l'étude de Cordell prévoit l'épuisement des réserves mondiales de phosphates au cours du XXI siècle (Cordell & White, 2015). Une autre estimation, celle de l'United States Geological Survey et de l'International Fertilizer Industry Association prédit la fin d'ici 100 à 125 ans. Un autre scénario plus optimiste, celui de l'International Fertilizer Development Center, repousse la disparition du P dans 300 ans. Mais il est évident que la qualité des roches phosphatées sur le marché mondial s'amoindrit peu à peu, processus accompagné d'une inaccessibilité grandissante aux gisements exploitables d'un point de vue économique. L'exploitation des gisements phosphatés risque donc de devenir plus coûteuse et plus polluante. Parallèlement, un manque de P engendrerait une baisse des rendements agricoles mondiaux et pourrait donc devenir une menace pour la sécurité alimentaire planétaire.
En outre, les gisements phosphatés sont inégalement répartis à travers la planète (Figure 1). Ainsi, environ 85% des gisements sédimentaires sont aux mains de quelques pays : le Maroc, la Chine, la Russie et les États-Unis. Ces derniers sont à l'origine de 72% de la production mondiale. A l'opposé, l'Europe et l'Inde par exemple, dépendent entièrement des importations. La gestion des réserves du phosphate est ainsi génératrice de tensions sociales et géopolitiques.

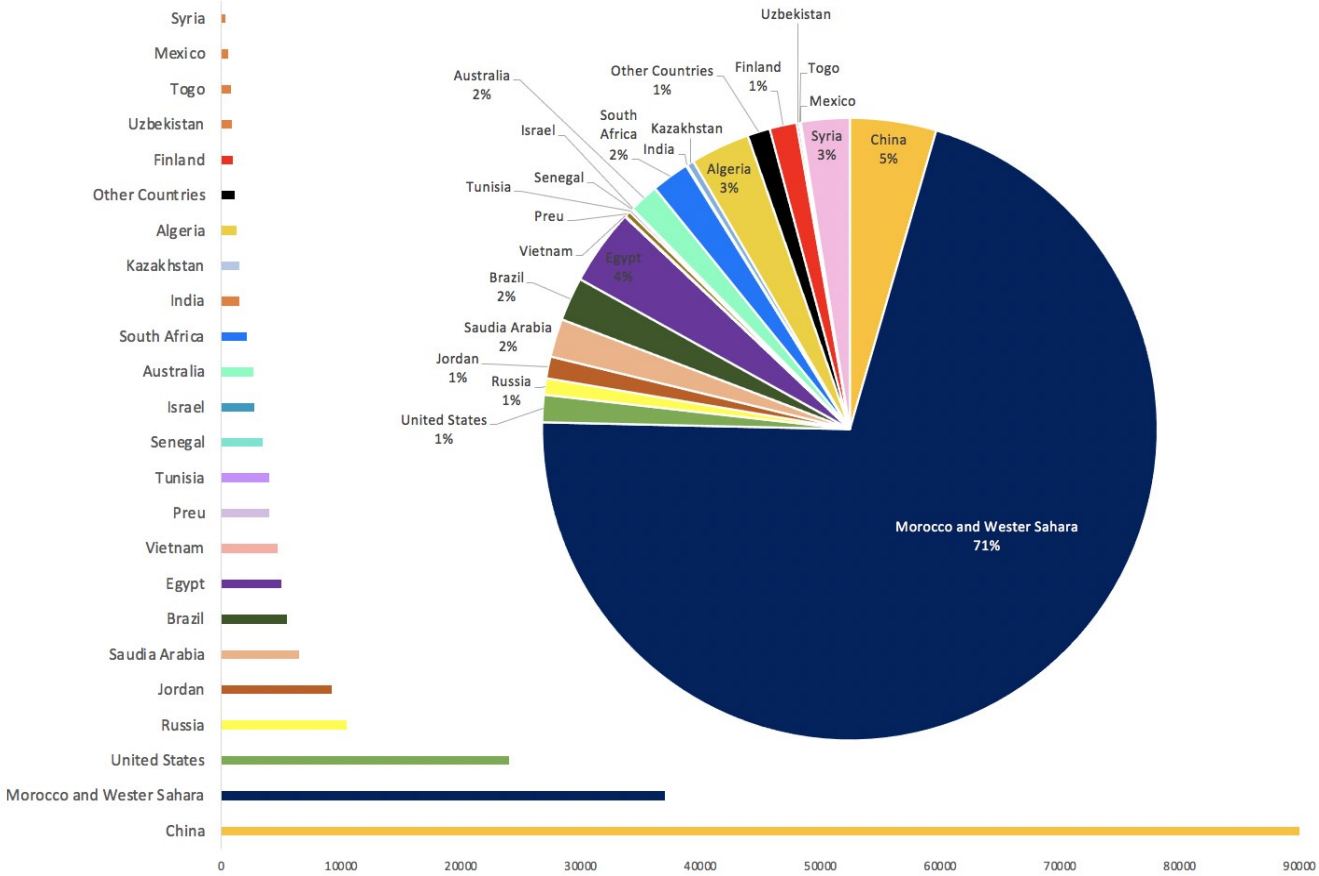

Figure 1 : Production mondiale de roches phosphates (barre plot) et réserves de roches phosphatées (secteur circulaire) d'après les chiffres fournis par U.S. Geological Survey pour l'année 2020.

B. La valorisation des ressources microbiennes du sol : une alternative pour une gestion plus durable du Phosphore

Face aux impacts environnementaux, économiques et politiques liées à l'usage intensif des engrais phosphatés, la recherche d'alternatives susceptibles de remédier la faible disponibilité du P dans les sols, et de limiter l'usage excessif des intrants phosphatés issus de l'industrie chimique, s'est opérée. Par exemple, l'utilisation d'espèce végétales ou variétés ayant une plus grande capacité à absorber et à utiliser le P malgré une faible disponibilité de P a été une stratégie efficace basée sur les plantes. Gao *et al.* (2016) ont notamment démontré que les plantes légumineuses telles que *Medicago sativa*, *Vicia faba* et *Phaseolus vulgaris* ont donné un meilleur rendement dans des conditions de carence en P et présentaient une augmentation de la fraction P disponible, de l'absorption de P et de l'activité des phosphatases acides (APase) du sol, en raison d'un système racinaire plus développé par rapport à d'autres cultures comme le *Sorghum bicolor*. L'application raisonnée de différentes sources de P, notamment organiques, est également développée.
De même, les efforts se sont notamment orientés vers l'utilisation directe du RP comme engrais phosphatée, une alternative moins coûteuse, et moins impactante que la production des engrais phosphatés chimiques pour les cycles biogéochimiques naturels.

La valorisation des compétences fonctionnelles des microorganismes du sol est ainsi recherchée. Il est en effet reconnu depuis longtemps que nombre de microorganismes associés aux plantes, notamment les champignons mycorhiziens à arbuscules (CMA) ou certaines bactéries de la rhizosphère, promotrices de la croissance des plantes (PGPR pour « Plant Growth Promoting Rhizobacteria »), jouent un rôle essentiel dans la libération de formes biodisponibles des nutriments à partir des formes initialement séquestrées par les composants du sol, et donc pour la nutrition minérale des plantes. En particulier, ces microorganismes sont susceptibles d'intervenir dans le cycle du P *via* la libération de composés permettant la solubilisation du P inorganique (en particulier des ions H^+, ou des acides organiques de faible poids moléculaire) ou d'enzymes (phosphatases) favorisant la minéralisation du P organique (Richardson *et al.*, 2005; Hinsinger, 2001). Ils constituent également une source (après reminéralisation liée à la libération de P microbien due à la mort microbienne) ou un puit (immobilisation liée à l'incorporation de P dans la biomasse microbienne vivante) de P biodisponible (Oehl *et al.*, 2004; Oberson & Joner, 2005). Par conséquent, l'introduction dans les sols de microorganismes solubilisateurs de P, promoteurs de la croissance des plantes, pourrait constituer une approche prometteuse pour améliorer la production agricole. Un grand nombre de microorganismes a ainsi été utilisé comme inoculant microbien sur de nombreuses espèces végétales d'importance agronomique (Bashan *et al.*, 2014), de même que divers inoculants commerciaux ont été développés (Calvo *et al.*, 2014; Singh *et al.*, 2016). Par exemple, en 2019, la Chine a enregistré plus de 800 brevets liés aux inoculants tandis que l'Inde a dépassé les 100 brevets (Santos *et al.*, 2019).
Cependant, la variabilité des résultats obtenus suite à l'application de ces inoculants en conditions naturelles ou agronomiques demeure problématique, et de nombreux travaux de recherche sont encore nécessaires afin de généraliser leur commercialisation et leur usage par les agriculteurs. Par exemple, des données plus précises sur les interactions plantes/microorganismes/sol sont nécessaires afin de réaliser un choix plus éclairé quant aux partenaires microbiens constitutifs d'un inoculant poly microbien favorable à la plante, aptes à remplir des fonctions ciblées et à s'implanter dans un environnement naturel ou agricole choisi en amont. En effet, le recrutement du microbiote racinaire (ou rhizobiote) par la plante est un processus en partie actif, résultant, au sein de la rhizosphère, des interactions entre les différents partenaires dans le continuum sol/plantes/microorganismes (Hartmann *et al.*, 2008). Il affecte la diversité, l'abondance et l'activité des taxons qui le composent. En particulier, les exsudats racinaires que la plante libère (Tkacz *et al.*, 2015), ou les interactions microorganismes/microorganismes sont autant de variables explicatives de la diversité microbienne de la rhizosphère (Peiffer *et al.*, 2013; Baker *et al.*, 2015). De même, les études de la dernière décennie ont révélé des assemblages microbiens très complexes, associés à différents paramètres édaphiques et d'importantes lacunes quant au rôle du RP en tant que moteur dans l'assemblage des communautés microbiennes du sol.

C'est pourquoi, des travaux de recherche portant sur l'étude des assemblages microbiens en fonction des conditions environnementales biotiques ou/et abiotiques, pourraient ainsi constituer un axe stratégique afin de réduire les intrants phosphatés chimiques.

C'est dans ce contexte général que s'inscrit mon projet de thèse qui combine à la fois de la recherche fondamentale et appliquée. Il a pour but d'apporter un éclairage sur les interactions entre le RP et les communautés microbiennes rhizosphériques, à la fois bactériennes et fongiques. Il vise également à identifier et isoler des souches bactériennes solubilisatrices de P, cultivables, adaptées à des environnements riches en RP et susceptibles d'être utilisées comme inoculants afin d'améliorer la nutrition phosphatée des plantes à partir de RP comme source de

P. Enfin, la finalité de ce projet est d'évaluer l'apport de ces inoculants sous forme d'un consortium poly-microbiens plurifonctionnels, sur une plante d'intérêt agronomique, la tomate.

Ce manuscrit de thèse sera structuré comme suit :

- A l'issue de cette **introduction** mettant en avant le contexte général du projet de thèse, la problématique ainsi que les objectifs globaux, **la première partie** présente une synthèse bibliographique qui s'articule autour de quatre points :

  - Le cycle du P et les différentes formes phosphatées du sol.

  - Les modalités de prélèvement du P par les plantes.

  - Les communautés microbiennes solubilisatrices de P et leur intérêt pour l'élaboration d'inoculants poly-microbiens plurifonctionnels.

  - Les interactions entre la diversité des formes phosphatées du sol et les communautés microbiennes du sol. Cette partie est présentée sous la forme d'une revue de synthèse **(Article 1, parue en 2022)** *: Ducousso-Détrez, A., Fontaine, J., Lounès-Hadj Sahraoui, A., & Hijri, M. (2022). Diversity of Phosphate Chemical Forms in Soils and Their Contributions on Soil Microbial Community Structure Changes. Microorganisms, 10(3), 609*

A l'issue de cette analyse bibliographique, les différentes hypothèses de travail seront définies et les principales questions scientifiques retenues dans le cadre de cette thèse seront présentées.

- Dans **une deuxième partie**, après un préambule qui présente les sites d'étude sur lesquels les recherches ont été conduites, une présentation des résultats obtenus est proposée sous forme d'article scientifiques ; elle est organisée en quatre chapitres.

  - **Le premier chapitre** décrit la diversité taxonomique des communautés bactériennes, fongiques **(Article 2)** et mycorhiziennes **(Article 3)** dans les divers sites échantillonnés, et analyse l'impact du P sur les profils microbiens détectés dans les sols rhizosphériques et dans les racines.

  - **Le deuxième chapitre** explore la diversité de quelques traits fonctionnels promoteurs de la croissance des plantes chez deux types d'isolats bactériens solubilisateurs de P, isolés à partir de sols échantillonnés riches en RP : les uns rhizosphériques, natifs de sols naturels, les autres issus de l'hyphosphère (volume du sol influencé par les hyphes des CMA), piégés à partir de sols agricoles **(Article 4).**

  - Enfin, le dernier chapitre propose de tester deux assemblages bactériens solubilisateurs de P, l'un rhizosphérique, l'autre hyphosphérique, comme inoculum microbien. Il présente également les éventuelles performances ou efficacités de ces inoculants bactériens solubilisateurs de phosphate sur la croissance et le développement d'une plante d'intérêt agronomique : la tomate **(Article 5).**

- **Une synthèse générale** met en lien l'ensemble des résultats obtenus suivie d'**une conclusion générale** qui vient clore ce manuscrit en exposant les principales **perspectives** sur lesquelles ouvrent ce travail de thèse.

Les références bibliographiques listées en fin de ce manuscrit correspondent exclusivement aux auteurs cités dans la synthèse bibliographique (hors celles citées dans l'article 1) ou dans la synthèse générale.

Les différentes valorisations et communications résultant de mes travaux seront présentées en annexe ; tout comme les modules de formation auxquels j'ai participé durant mes années de thèse dans le cadre des écoles doctorales SMRE, puis STS et de l'université de Montréal.

PARTIE 1

Synthèse bibliographique

SYNTHESE BIBLIOGRAPHIQUE

Le cycle du Phosphore

Dans les sols, le P provient, originellement, de l'altération oxydative des minéraux primaires constitutifs du matériau lithosphérique formé au cours des temps géologiques (Figure 2). De manière générale, le cycle global du P peut être envisagé comme la succession des étapes dominantes suivantes : (1) altération mécanique et chimique des roches P, qui permet la dissolution du P et qui constitue un processus lent, (2) réactions d'échanges entre les eaux interstitielles du sol et les particules du sol, (3) incorporation du P dans la biomasse terrestre et son retour dans le sol suite à la dégradation de ma matière organique, (4) transport du P vers les eaux de surface et les eaux souterraines et les lacs d'eau douce, (5) transport à travers les estuaires jusqu'aux océans où le P terrestre apporté par les eaux de ruissellement et par le processus de remontée des eaux de mer (up-welling) est partiellement assimilé par les êtres marins ou principalement retiré de la circulation pour devenir des sédiments sur les marges continentales et dans les eaux profondes, éventuellement. La phase atmosphérique joue généralement un rôle mineur dans le cycle global du P, et contrairement à de nombreux autres cycles, le cycle biogéochimique du P se déroule principalement à travers la lithosphère (roches de fond, sédiments), l'hydrosphère (rivière, lac et océan) et la biosphère.

Dans les écosystèmes naturels ou peu anthropisés, le cycle du P peut être considéré comme fermé. En effet, les apports par les pluies et/ou les poussières sont très faibles, de même que les apports par altération de la roche mère. Parallèlement, les sorties de P par lixiviation, lessivage ou ruissellement sont réduites. Dans ce contexte, la solution du sol peut être réapprovisionnée à partir d'autres formes phosphatées du sol (Darrah, 1993; Hinsinger, 2001) grâce à des processus physico-chimiques qui seront développés au paragraphe 3 de ce chapitre, dont les processus d'adsorption/désorption, précipitation/dissolution. L'activité microbiologique de la rhizosphère et l'activité racinaire vont également avoir un impact sur les processus de réapprovisionnement de la solution de sol comme nous l'avons décrit plus haut.

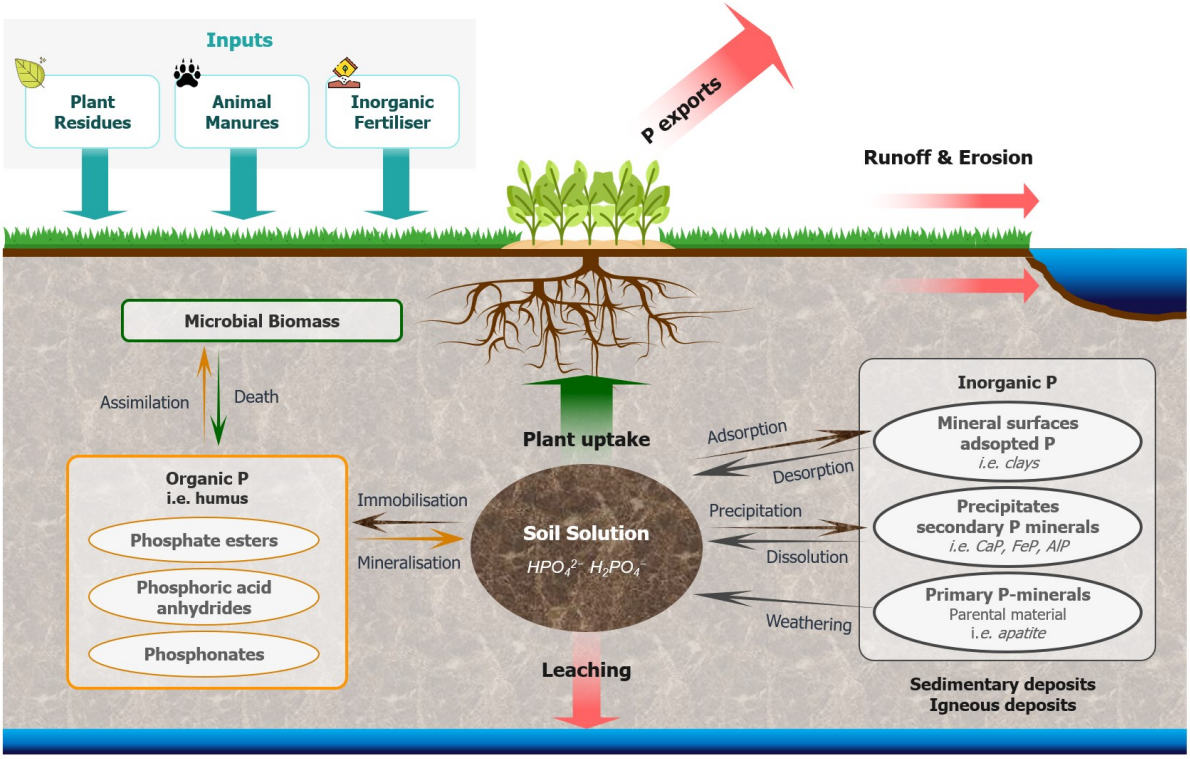

Figure 2 : Cycle du Phosphore dans l'écosystème terrestre : stocks géologiques, formes organiques et inorganiques, flux et réactions physicochimiques alimentant les formes ioniques solubles disponibles pour la nutrition phosphatée des plantes.

A l'opposé, le cycle du P est ouvert dans les écosystèmes fortement anthropisés et exploités à but commercial. En effet, les exportations de nutriments liées aux récoltes peuvent être importantes, et cette perte pour l'écosystème se traduit ensuite par une diminution de la fertilité des sols. En conséquence, en conditions agronomiques, le recours à des intrants fertilisants aptes à fournir des ions phosphates immédiatement disponibles pour supplémenter la nutrition des végétaux est largement développé. Les intrants P peuvent être d'origine chimique ou organique. Le fumier animal, les os d'animaux broyés, les excréments humains et d'oiseaux, les composts de déchets urbains et les cendres sont considérés comme des engrais organiques (Van Vuuren *et al.*, 2010). Les engrais P chimiques, quant à eux, font référence aux engrais produits à partir du RP (terme collectif désignant les roches sédimentaires ignées, métamorphiques, marines ou biogènes contenant des minéraux phosphatés) (Jones & Oburger, 2011). Les plus courants sont le SSP (superphosphate ordinaire ou superphosphate simple), le TSP (superphosphate triple), le MAP (phosphate mono-ammonique) et le DAP (phosphate di-ammonique) (Chien *et al.*, 2011) ; ils se distinguent notamment par des compositions et des teneurs en P variables, mesurée en oxyde de phosphore total (P_2O_5).

Le RP brut peut par ailleurs être utilisé comme intrant naturel et constitue une alternative aux engrais chimiques phosphatés. Il est en effet caractérisé par un coût industriel relativement plus faible et une meilleure acceptabilité en termes d'impacts environnementaux. Son efficacité est cependant variable selon la nature des sols où il est appliqué, et sa réactivité est plus faible que celle des engrais commerciaux lors d'une application directe sur le sol. Toutefois, la biodisponibilité du P des RP peut être accrue sous l'action de certains microorganismes, en particulier des bactéries, aptes à exprimer un potentiel solubilisateur du phosphore inorganique (Hanif *et al.*, 2015; Li *et al.*, 2015). Certaines stratégies fertilisantes reposent alors sur l'apport conjoint de RP et de biofertilisants microbiens promoteurs de la croissance des plantes, solubilisateurs de P.

Prélèvement du P biodisponible par les plantes

Le P est un élément chimique indispensable dans le monde vivant. Il est notamment constitutif des acides nucléiques et des phospholipides membranaires. Il joue un rôle essentiel dans la réserve et le transfert d'énergie, les régulations métaboliques et l'activation des protéines (cascades de phosphorylation). Il fait donc partie des éléments nutritifs majeurs nécessaires à la croissance des plantes.
Avec des teneurs estimées en P entre 0,05-0,8 % du poids sec des plantes (Schachtman *et al.*, 1998; Vance *et al.*, 2003; Sharma *et al.*, 2013), la nutrition phosphatée des plantes cultivées requière, avec des variations selon les espèces, environ 10 -100 kg P ha^{-1} (Kishore *et al.*, 2015). Elle s'opère essentiellement à partir des formes biodisponibles que sont les formes ioniques phosphatées (Pi) présentes en solution dans le sol (Barber, 1995) (Figure 3).

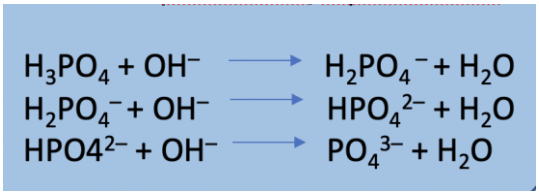

Figure 3 : Spéciation par protonisation/déprotonisation des formes ioniques phosphatées dans la solution du sol.

L'acquisition de P par la plante est alors dépendante d'une part de la mobilité dans les sols, des formes phosphatées biodisponibles, et d'autre part de l'interception de ces formes par la surface racinaire.
Une première modalité de transport des formes ioniques phosphatées correspond à un transfert par convection ou « mass flow »; il permet l'apport de P, du sol vers les racines avec le transport d'eau, lié à l'absorption d'eau par les racines. La quantité de P transporté par ce processus dépend de la concentration du nutriment en solution et de la quantité d'eau captée par la plante. Le « mass flow » fournit moins de 1 à 5% de la demande en P d'une plante (Lambers & Plaxton, 2015). Dans le cas du P, le transport par convection et l'interception racinaire ne sont pas suffisants pour approvisionner les racines. Mais le prélèvement par les racines crée alors une baisse de la concentration des ions P en solution à proximité des racines : une zone de déplétion apparaît rapidement après la capture du P par les racines, ce qui entraîne un gradient de concentration dans la rhizosphère (Schachtman *et al.*, 1998; Shen *et al.*, 2011). Ce gradient de concentration génère alors un transport des ions Pi par diffusion, depuis les zones les plus concentrées vers les zones les moins concentrées, à proximité des racines (Barber, 1995; Hinsinger, 2001). La diffusion est ainsi le principal processus de transport permettant l'acquisition des formes Pi par les racines (Barber, 1995).

Par ailleurs, un ensemble étendu de réponses adaptatives, à caractère morphologique, métabolique et moléculaire permet aux végétaux d'acquérir le P externe et/ou de mobiliser les ressources internes en P pour, d'une part s'adapter à la faible disponibilité du P dans les sols (Raghothama, 1999; Raghothama & Karthikeyan, 2005; Schachtman & Shin, 2007), et d'autre part maintenir l'homéostasie cellulaire (Figure 4). Ces réponses concernent différents domaines : i) l'expansion des capacités d'exploration du sol, ii) l'exploitation des ressources P du sol, iii) le prélèvement racinaire et le transport du P, et enfin, iv) la réaffectation et la remobilisation du P au sein de la plante. Elles sont en outre dépendantes de mécanismes moléculaires impliqués dans la signalisation locale (liées à la perception des teneurs locales en

P) et systémique (réponses à longue distance) (Nakamura, 2013; Lopez-Arredondo *et al.*, 2014; Baker *et al.*, 2015; Mitra, 2015).

Par exemple, étant donné la faible biodisponibilité et mobilité du P dans les sols, la capacité des systèmes racinaires à explorer efficacement le sol et à exploiter les ressources rhizosphériques en P est essentielle pour maximiser l'interception racinaire du P. Ainsi, en situation de carence en P, les réponses adaptatives locales correspondent principalement à des modifications morphologiques de l'architecture du système racinaire (c'est-à-dire la complexité des configurations spatiales du système racinaire). Notamment, la croissance de la racine primaire est stoppée, tandis que le développement des racines latérales et la prolifération des poils racinaires sont favorisés. La multiplicité des poils racinaires augmente alors la surface de contact entre la racine et le sol et induit une extension spatiale de la rhizosphère (Vance *et al.* (2003). Parallèlement, ces poils absorbants génèrent une expansion de la capacité d'exploration du sol en pénétrant des espaces poreux de diamètre inférieur à celui des racines.
Lors d'un déficit en P, les racines peuvent également présenter des changements dans l'angle de croissance et le diamètre de la racine latérale (Lopez-Arredondo *et al.*, 2014; Hinsinger *et al.*, 2015; Plassard *et al.*, 2015).

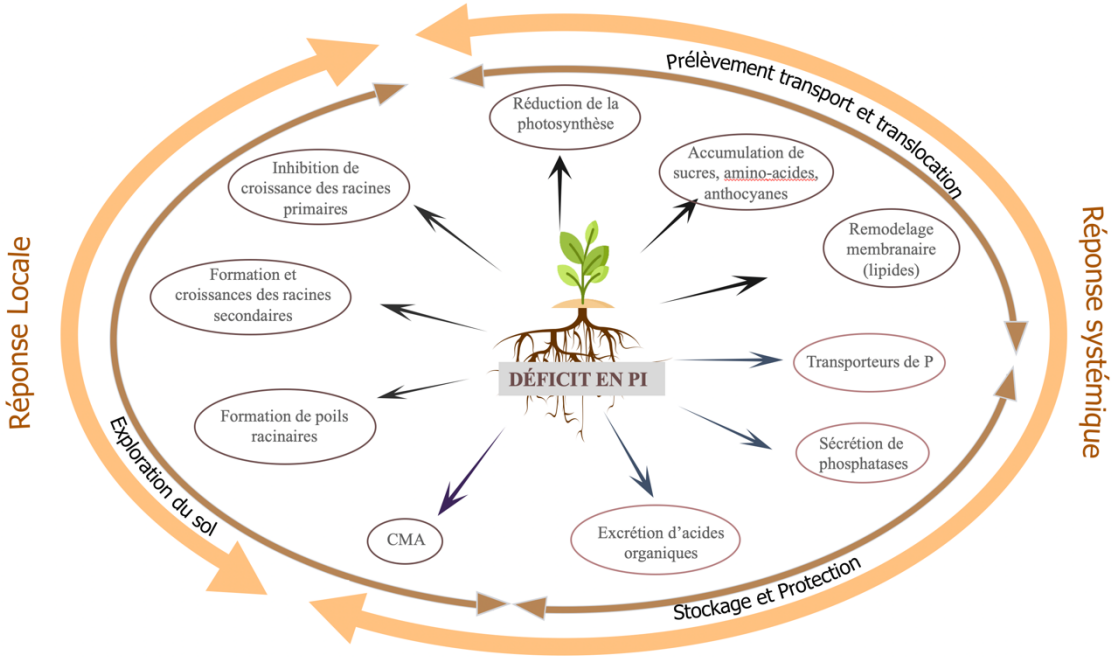

Figure 4 : Représentation schématique des réponses adaptatives des plantes face à un déficit en P d'après Baker *et al.* (2015).

Outre l'expansion des capacités exploratoires du système racinaire, la réponse adaptative des plantes à une carence en P peut également correspondre à une meilleure exploitation des ressources phosphatées du sol. Ainsi, des changements de pH induits par l'activité racinaire, en particulier l'acidification liée à la libération de H^+ par les racines, peut accroître la biodisponibilité du P inorganique et le prélèvement de P par les plantes. En effet, les racines prélèvent les éléments minéraux sous leurs formes ioniques. En conséquence, afin de maintenir l'électroneutralité dans leurs tissus, les racines doivent excréter d'autres composés chargés. Dans le cas d'un excès de cations prélevés, les racines excrètent des protons H^+. Dans le cas

d'un excès d'anions prélevés, elles excrètent les anions OH^- et HCO_3^-. L'excrétion d'anions organiques contribue également à l'acidification du sol (Darrah, 1993; Marschner *et al.*, 2003; Hinsinger, 2001). Les racines sont également responsables d'un changement du potentiel redox dans la rhizosphère et provoquer une acidification importante du sol (Hinsinger, 2001). Enfin, la respiration et la production de CO_2 par les racines (et les microorganismes rhizosphériques) peuvent également être une origine possible de l'acidification du sol (Hinsinger, 2001). Cette dernière va alors entrainer la dissolution des minéraux phosphatés et notamment ceux de la roche mère (Neumann & Römheld, 2002; Hinsinger, 2001) ou modifier la stabilisation abiotique des composés organiques phosphatés, les rendant plus ou moins accessible pour la dégradation enzymatique.
De même, dans des conditions de carence en P, la production et la sécrétion par la plante d'enzymes telles que les phosphatases contribuent au recyclage de formes phosphatées et à la libération d'ions P après hydrolyse enzymatique et minéralisation du P organique du sol ou re-minéralisation du P microbien (Raghothama, 1999; Vance *et al.*, 2003; Hinsinger *et al.*, 2011; Richardson & Simpson, 2011).

Par ailleurs, l'acquisition du P par la plante puis sa translocation sont optimisées par l'expression de divers transporteurs de P (PHT) qui permettent de répondre à une disponibilité médiocre et fluctuante du P.

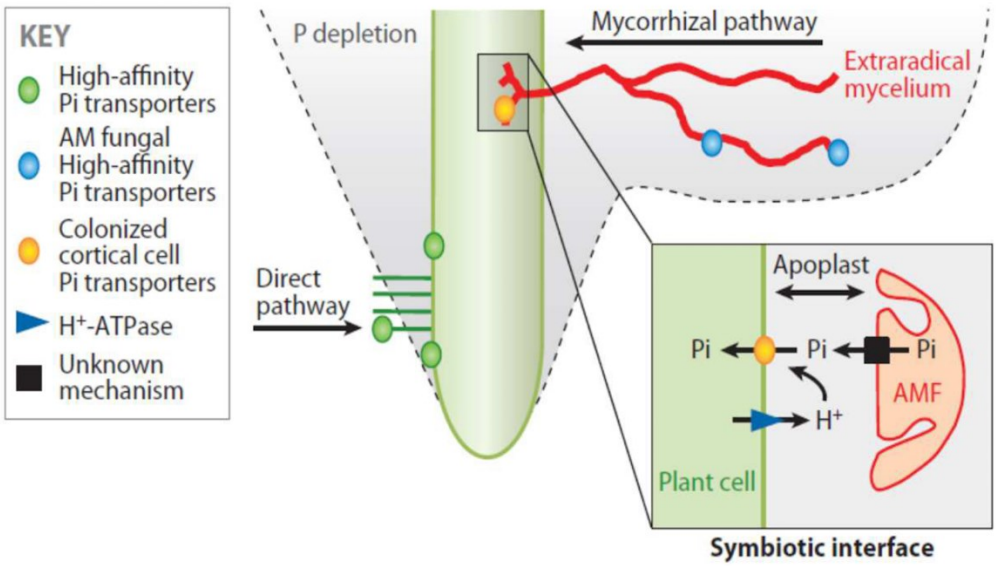

Figure 5 : Les différentes voies d'acquisition du Pi chez les plantes mycorhizées (Smith & Smith, 2011) :

- Voie directe d'acquisition du P (direct pathway) grâce à des transporteurs haute affinité situés sur les poils absorbants et dans la zone épidermique proche de l'apex. - Voie symbiotique faisant intervenir le mycélium extra-racinaire qui capte le Pi présent dans le sol grâce à des transporteurs haute affinité. Le Pi est ensuite transporté à travers les hyphes fongiques puis transféré aux racines au niveau des arbuscules grâce à des transporteurs de Pi végétaux spécifiquement exprimés au niveau de la membrane péri-arbusculaire.

En effet, la concentration de P dans les cellules de la racine pouvant être jusqu'à 1000 fois plus élevée que sa concentration dans la solution du sol, le transport du P à travers l'interface sol-racine a besoin d'un système de transport spécialisé dans le but d'acquérir le P contre ce gradient de concentration, d'ailleurs combiné à un gradient électrochimique (Bieleski, 1973; Schachtman *et al.*, 1998; Richardson *et al.*, 2009; Nussaume *et al.*, 2011; Wang *et al.*, 2017; Yang *et al.*, 2020). Chez les plantes, deux systèmes d'absorption de P ont été identifiés, un

système de haute affinité qui est régulé de manière transcriptionnelle et post-transcriptionnelle (Nussaume *et al.*, 2011; Baker *et al.*, 2015; Mitra, 2015; Poirier & Jung, 2015) en fonction des variations de concentrations en P, et un système de faible affinité qui est exprimé de façon constitutive. Il est admis que la majorité des transporteurs ioniques impliqués dans l'absorption du P au niveau de l'interface racine-sol sont à haute affinité tandis que ceux responsables de la mobilisation du Pi interne de la plante sont à basse affinité (Daram *et al.*, 1999).

Les PHTs végétales ont été communément classées en cinq familles, désignées PHT1-5 (López-Arredondo *et al.*, 2014; Liu *et al.*, 2016). Cependant, la plupart des études se concentrent essentiellement sur la famille PHT1 (Nussaume *et al.*, 2011). Celle-ci regroupe des transporteurs de haute affinité, habituellement présents dans la membrane plasmique (Pao *et al.*, 1998) et appartenant à la famille des PHS ($Pi:H^+$ symporter), eux-mêmes membres de la MFS (major facilitator superfamily) (Poirier & Bucher, 2002). Les membres de cette famille jouent un rôle important dans l'absorption et la remobilisation du Pi tout au long du développement de la plante (Karthikeyan *et al.*, 2002; Mudge *et al.*, 2002; Shin *et al.*, 2004; Ai *et al.*, 2009; Sun *et al.*, 2012; Chang *et al.*, 2018).
Les quatre autres familles de PHT sont principalement impliquées dans la redistribution de Pi au niveau tissulaire, cellulaire et subcellulaire. Par exemple, chez *Arabidopsis* AtPHT2;1 de la famille PHT2 est un transporteur chloroplastique de Pi à faible affinité (Daram *et al.*, 1999) ; les homologues de PHT3 agissent en tant que symporteurs Pi/H+ et antiport Pi/OH- pour jouer un rôle critique dans l'échange de Pi entre le cytoplasme et la matrice des mitochondries (Stappen & Krämer, 1994; Takabatake *et al.*, 1999) ; AtPHT4;6 dans la famille PHT4 transporte le Pi hors de l'espace luminal du Golgi pour être recyclé après la libération de la glycosylation (Rodríguez-Milla & Salinas, 2009) tandis que les membres de la famille PHT5 sont connus pour être des transporteurs de Pi vacuolaires (Liu *et al.*, 2016).

Outre la voie directe d'acquisition du P par le végétal à partir des réserves phosphatées du sol qui repose sur les transporteurs de haute affinité situés sur les poils racinaires et dans la zone épidermique proche de l'apex, les plantes engagées dans une symbiose mycorhizienne à arbuscules (dont nous exposerons les caractéristiques par la suite) présentent une voie supplémentaire d'acquisition du P : la « voie mycorhizienne » (Marschner, 1995; Richardson *et al.*, 2009; Baker *et al.*, 2015; Hinsinger, 2001) (Figure 5). Cette voie symbiotique fait intervenir le mycélium extra-racinaire qui capte le Pi présent dans le sol grâce à des transporteurs haute affinité.
Ainsi, dans les hyphes extra-racinaires, le Pi est absorbé par des symporteurs H+/Pi et Na+/Pi associés à des H+-ATPases et des Na+-ATPases, respectivement (Figure 6). Le Pi est ensuite incorporé dans l'ATP au sein des mitochondries, puis polymérisé en polyphosphates par le complexe de transporteur vacuolaire chaperon (VTC), et enfin accumulé dans les vacuoles tubulaires (Kikuchi *et al.*, 2014). Les polyphosphates peuvent alors être transloqués vers l'hôte, probablement par le flux d'eau qui est médié par une aquaporine fongique (AQP3) (Kikuchi *et al.*, 2016) à travers les hyphes non septés du champignon. Au cours de la translocation à longue distance, les polyphosphates pourront être dépolymérisés par les polyphosphatases, exportés vers le cytosol par l'intermédiaire de l'exportateur Pi vacuolaire (PHO91), puis incorporés dans l'ATP (Kikuchi *et al.*, 2014).

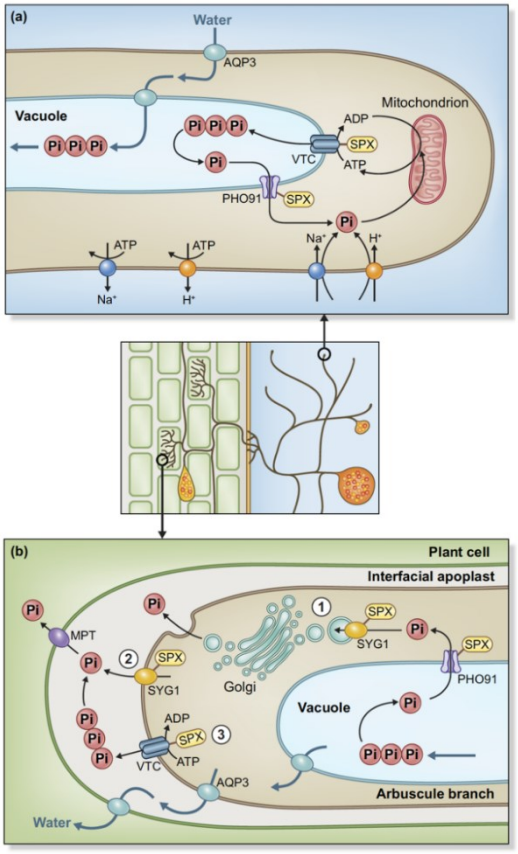

Figure 6 : La voie mycorhizienne de la nutrition phosphatée des plantes : Représentation schématique de l'absorption du P, sa translocation et son exportation dans les champignons mycorhiziens à arbuscules (Ezawa & Saito, 2018)

(a) Dans les hyphes extra-racinaires
(b) Dans les hyphes intra-racinaires

VTC : complexe de transporteur vacuolaire chaperon

AQP3 : aquaporine fongique

PHO91 : exportateur Pi vacuolaire (PHO91)

MPT : transporteurs de Pi inductibles mycorhiziens

Dans les hyphes intra-racinaires (Figure 6), les polyphosphates sont transloqués vers les arbuscules par le flux d'eau médié par l'aquaporine fongique AQP3, puis le P serait exporté par l'une des trois voies hypothétiques suivantes : (i) Le Pi est libéré par hydrolyse des polyphosphates, exporté vers le cytosol par l'intermédiaire de la PHO91, et chargé dans le réseau Golgi/trans-Golgi pour être exporté vers l'apoplaste; (ii) Le Pi libéré des vacuoles est directement exporté par le biais de SYG1 localisé sur la membrane plasmique du champignon ; (iii) Les polyphosphates sont directement exportés par le complexe VTC de la membrane plasmique vers l'apoplaste dans lequel la phosphatase végétale hydrolyse les polyphosphates. Le P est ensuite absorbé par les transporteurs de P inductibles mycorhiziens (MPT), localisés dans la membrane plasmique de la plante.

Par la suite, depuis les cellules épidermiques des racines, le P peut emprunter différentes voies : (i) il peut entrer dans le pool métabolique ; (ii) il peut être chargé et transporté dans les vaisseaux du xylème racinaire pour une translocation à longue distance, vers les parties aériennes, et différents organes de la plante. Dans ce contexte, la famille de gènes PHO1 chez *Arabidopsis* est par exemple une autre famille importante de transporteurs de P, jouant un rôle essentiel dans le transport de P à longue distance des racines vers les pousses, et dans la régulation de l'exportation de P des cellules épidermiques et corticales des racines vers les vaisseaux du xylème : (iii) il peut enfin être stocké dans les vacuoles pour le maintien de l'homéostasie du P.
Dans des conditions de carence en P, le P est également transloqué des parties aériennes à la racine *via* le phloème. Dans les mitochondries et les chloroplastes, l'homéostasie du P régule l'activité de l'ATP synthase pour maintenir des niveaux optimaux d'ATP.

A noter enfin, parmi les réponses adaptatives des plantes à un déficit en P qu'un recyclage du P interne par le catabolisme des phospholipides peut s'opérer. De même, une protection du métabolisme végétal contre les effets délétères de la privation de P peut s'engager, par exemple par stockage et l'accumulation de métabolites secondaires tels que les anthocyanes (Baker *et al.*, 2015).

Les communautés microbiennes solubilisatrices de P et leur intérêt pour l'élaboration d'inoculants poly-microbiens plurifonctionnels.

1. Divers groupes fonctionnels promoteurs de la croissance des plantes

1.1 Les bactéries PGPR solubilisatrices du P

Le sol est un environnement hétérogène composé d'une diversité de micro-habitats caractérisés par des propriétés physiques, chimiques et biologiques propres, et un réservoir de diversité microbienne (Vandenkoornhuyse *et al.*, 2015). Les propriétés du sol définissent également les propriétés de la rhizosphère, terme introduit par Hiltner en 1900 pour décrire la zone de sol située à proximité immédiate des racines et ainsi, directement influencée par les exsudats racinaires résultant de l'activité et la croissance racinaire. Par ailleurs, sous l'influence de l'exsudation d'une large diversité de nutriments organiques (acides organiques, phytosidérophores, sucres, vitamines, acides aminés, nucléosides, mucilages) et de signaux moléculaires (Bais *et al.*, 2006; Pothier *et al.*, 2007; Badri *et al.*, 2009; Shukla *et al.*, 2011; Drogue *et al.*, 2012), une sélection s'opère au sein du réservoir microbien du sol. Ainsi dans le micro-habitat rhizosphérique, la composition des communautés microbiennes associées aux racines des plantes, qualifiées de rhizomicrobiome (Chaparro *et al.*, 2014) est distincte de celle de la communauté microbienne du sol environnant, une conséquence directe de la compétition microbienne pour les nutriments libérés à proximité des racines des plantes (Raynaud *et al.*, 2008; Bulgarelli *et al.*, 2013; Chaparro *et al.*, 2014). La sélection des bactéries par les plantes est ainsi un domaine de recherche largement étudié et synthétisé dans plusieurs revues de littérature (Hartmann *et al.*, 2008; Doornbos *et al.*, 2012; Drogue *et al.*, 2012; Bulgarelli *et al.*, 2013).

Au sein du rhizomicrobiome, les communautés microbiennes jouent un rôle clé dans le maintien de multiples fonctions et services écosystémiques, incluant le cycle des nutriments, la production primaire, ou la décomposition de la litière (Van Der Heijden *et al.*, 2008; Wagg *et al.*, 2014). Par ailleurs, différents types d'interactions biotiques entre plantes et les microorganismes du sol s'y opèrent (Hartmann *et al.*, 2008; Doornbos *et al.*, 2012; Drogue *et al.*, 2012; Bulgarelli *et al.*, 2013), allant du commensalisme au mutualisme. Certaines d'entre elles sont des interactions symbiotiques mutualistes dans lesquelles les coûts et les bénéfices sont partagés par les deux partenaires. Parmi elles, deux catégories sont classiquement distinguées : i) les symbioses mutualistes qui sont des interactions intimes et le plus souvent obligatoires entre les microorganismes et une gamme restreinte de plantes hôtes, avec des structures dédiées spécifiquement à l'interaction (par exemple des nodules pour les genres *Rhizobiums* ou *Frankia*, ou des arbuscules pour les champignons mycorhiziens arbusculaires), ou ii) les symbioses associatives (coopératives), avec des bactéries libres (Drogue *et al.*, 2012). Ces dernières peuvent se développer dans, sur ou autour de la surface des racines et stimulant la croissance et la santé des plantes : elles sont qualifiées de bactéries PGPR (Kloepper, 1978). Comparée à la symbiose mutualiste, il est admis que les PGPR interagissent avec un plus large éventail d'espèces végétales et présentent une grande diversité taxonomique (Bashan *et al.*, 2014; Schütz *et al.*, 2018).

Les PGPR peuvent favoriser la croissance des plantes, les aider à résister aux stress abiotiques (Yadav *et al.*, 2017) ou les protéger en inhibant les agents phytopathogènes. Les mécanismes à la base de ces potentialités sont regroupés en deux types : des mécanismes dits « directs » et des mécanismes « indirects » (Couillerot *et al.*, 2009; Richardson *et al.*, 2009; Compant *et al.*, 2010; Vacheron *et al.*, 2013; Pérez-Montaño *et al.*, 2014) (Figure 7).

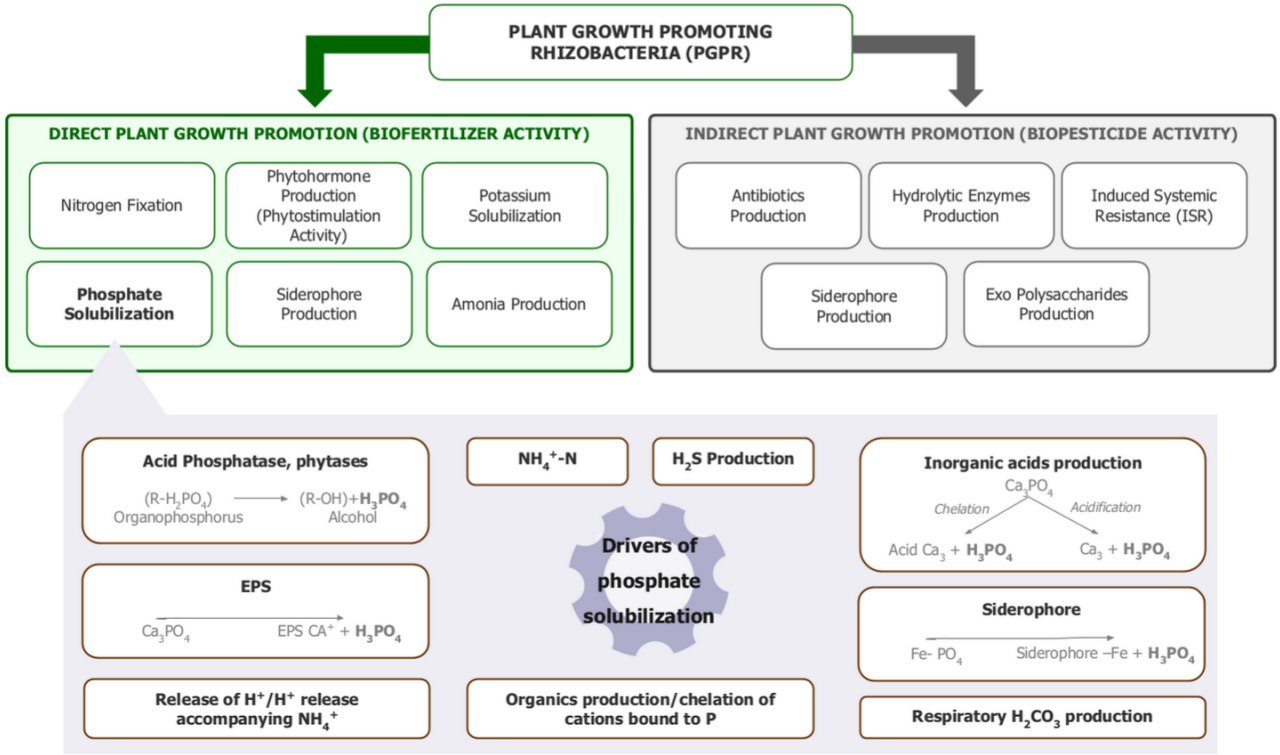

Figure 7 : Représentation schématique des mécanismes directs et indirects promoteurs de la croissance des plantes (d'après Prabhu *et al. (2019)* ; Figueiredo et al. (2016))

Les mécanismes dits « directs » sont principalement liés à la synthèse par les PGPR, de molécules qui vont favoriser la croissance des plantes, à des stades divers. Parmi ces substances, figurent des phytohormones telles que l'acide indole acétique (AIA), l'acide abscissique, des gibbérellines et cytokinines, ou des enzymes (1-aminocyclopropane-1-carboxylic acid (ACC) déaminase, Superoxyde dismutase …). Les mécanismes « indirects » sont associés à la protection vis-à-vis des stress biotiques et abiotiques, tels que la sécheresse (Fan *et al.*, 2015; Kaushal & Wani, 2016; Vurukonda *et al.*, 2016; Rubin *et al.*, 2017; Gouda *et al.*, 2018) ou la présence d'éléments traces (Vacheron *et al.*, 2013). La production de sidérophores ou l'acquisition de nutriments *via* la fixation d'azote atmosphérique ou la solubilisation des phosphates, sont également favorisées par les PGPR (Crowley, 2006). La formation de biofilm et la production d'exopolysaccharides jouent de même un rôle dans l'interaction des rhizobactéries avec la plante (Allison, 2003; Ferreira *et al.*, 2011).
Ces mécanismes ne sont pas exclusifs les uns des autres : concomitamment, une même espèce peut posséder plusieurs fonctions et donc appartenir à plusieurs communautés fonctionnelles. Par exemple, certaines bactéries ont montré des traits solubilisateurs de phosphate et un potentiel de biocontrôle contre des agents phytopathogènes (Rasul *et al.*, 2019). Il est également admis que les améliorations de croissance observées suite aux inoculations bactériennes peuvent être le résultat de plusieurs mécanismes. Par exemple, la modification du système racinaire par les PGPR est principalement due à la production de phytohormones telles que les auxines, l'acide abscissique, l'acide gibbérellique ou les cytokinines (Hayat *et al.*, 2010;

Vacheron *et al.*, 2013) ou la production de 2,4 diacétylphloroglucinol (Combes-Meynet *et al.*, 2011) ou d'oxyde nitrique (Molina-Favero *et al.*, 2008). Mais il peut également être associé à la réduction du niveau d'éthylène dans la plante par dégradation de son précurseur, le 1-aminocyclopropane-1- carboxylique acide (Glick, 2014) ; un niveau d'éthylène plus faible lève alors l'inhibition que cette phytohormone exerce sur l'élongation racinaire, entrainant un développement plus important des racines et donc une meilleure prospection du sol par le système racinaire (Vacheron *et al.*, 2013).

Parmi les PGPR, les bactéries solubilisatrices de P (nommées ci-après PSB pour « Phosphorus Solubilizing Bacteria ») se caractérisent par leur capacité à solubiliser le Pi du sol, la solubilisation du P étant définie comme la mobilisation du P inorganique par désorption du P adsorbé sur les constituants du sol, ou par dissolution des minéraux contenant du P (Richardson *et al.*, 2009; Hinsinger, 2001). Pour cette raison, les PSB ont été largement proposées comme un moyen d'augmenter, à partir de formes non solubles, la biodisponibilité des nutriments P dans le sol et l'approvisionnement de la plante hôte en P. Le paradigme sous-jacent étant que les PSB solubilisent le P du sol pour répondre suffisamment à leurs propres besoins, mais aussi au-delà, et donc répondre également aux besoins de la plante (Richardson, 2001; Jakobsen *et al.*, 2005). Dans ce contexte, un large éventail de PSB a été identifié *in vitro*. Leur diversité taxonomique est largement étendue (Ahemad & Kibret, 2014; Schütz *et al.*, 2018; Kour *et al.*, 2021).
Les mécanismes de solubilisation du P par les PSB sont relativement bien documentés notamment grâce à des expérimentations *in vitro* (Rodríguez & Fraga, 1999; Khan *et al.*, 2009; Hayat *et al.*, 2010; Oburger *et al.*, 2011). Ainsi, la solubilisation du Pi par les PSB peut avoir lieu principalement, par la sécrétion de protons (Hinsinger *et al.*, 2003; Jones & Oburger, 2011), ou par la production d'acides organiques de faible poids moléculaire (par exemple, citrate, oxalate, gluconate) (Illmer *et al.*, 1995; Delvasto *et al.*, 2006). Les protons sécrétés acidifient l'environnement autour de la PSB et entraînent la dissolution des minéraux inorganiques de P (par exemple, les minéraux de calcium (Ca-P). Pour leur part, les acides organiques de faible poids moléculaire peuvent soit abaisser le pH du sol, soit chélater les cations métalliques liés aux anions phosphates. Ils peuvent également entrer en compétition avec le phosphate pour les sites d'adsorption, et ainsi désorber l'orthophosphate P des minéraux du sol (par exemple, les argiles et les silicates) ou des oxydes d'aluminium et de fer, par échange de ligands (Jones & Edwards, 1998; Vance *et al.*, 2003; Rodríguez *et al.*, 2006; Khan *et al.*, 2009; Mander *et al.*, 2012; Sharma *et al.*, 2013).

1.2 Les champignons mycorhiziens arbusculaires

1.2.1. Origine de la symbiose mycorhizienne

La symbiose mycorhizienne peut être définie comme l'établissement d'une association intime, le plus souvent mutualiste, entre champignons mycorhiziens et les racines des plantes (Bonfante & Genre, 2010; Smith & Read, 2010). Parmi les différents groupes de champignons mycorhiziens, les CMA sont des biotrophes obligatoires qui requièrent un partenaire végétal pour l'accomplissement de leur cycle de développement. Ces champignons sont associés à plus de 80 % des espèces végétales terrestres (Brundrett & Tedersoo, 2018). La symbiose mycorhizienne arbusculaire serait apparue il y a environ 450 millions d'années, coïncidant avec l'émergence des premières plantes vasculaires (Martin *et al.*, 2017). Racines primitives et CMA auraient alors co-évolué (Brundrett, 2002), et l'établissement de la symbiose mycorhizienne

arbusculaire aurait joué un rôle clé dans la colonisation du milieu terrestre par les plantes, permettant à des végétaux aquatiques d'évoluer en plantes vasculaires (Bidartondo *et al.*, 2011; Field *et al.*, 2015).

1.2.2. Fonctions des CMA et nutrition minérale des plantes

L'établissement de la symbiose commence en particulier par l'échange de molécules de signalisation entre les deux symbiotes : les strigolactones d'origine végétale sont perçues par les CMA (Akiyama *et al.*, 2005; Harrison, 2005), qui, en retour, produisent un mélange de chito- et de lipo-oligosaccharides (Maillet *et al.*, 2011; Genre *et al.*, 2013), les « facteurs Myc ». Ceux-ci sont perçus par la plante, ce qui conduit l'activation de la voie de signalisation de la symbiose chez la plante et à l'expression de nombreux gènes impliqués dans la colonisation des racines et le fonctionnement de la symbiose (Delaux *et al.*, 2013). Le résultat est la colonisation fongique de la racine, un processus qui est caractérisé par la formation dans les cellules corticales de la racine, de structures différenciées hautement ramifiées et arborescentes appelées arbuscules, sièges d'échanges de nutriments entre les deux partenaires.
Dans les cellules à arbuscules, la membrane périarbusculaire (membrane dérivée de la plante et enveloppant l'arbuscule) est considérée comme le site principal des échanges et transfert de nutriments entre la plante et le champignon. Ainsi, dans cette symbiose, les CMA participent aux échanges souterrains de nutriments en s'associant aux racines des plantes, leur offrant un accès accru aux nutriments du sol, en échange du carbone issu de la photosynthèse (jusqu'à 20 %) (Bago *et al.*, 2000; Helber *et al.*, 2011; Berruti *et al.*, 2016; Keymer *et al.*, 2017; Luginbuehl *et al.*, 2017). La nutrition minérale de la plante est en outre facilitée par le fin réseau d'hyphes extra-radicaux fonctionnant comme un système d'absorption auxiliaire. Il est en effet estimé que le mycélium extra-racinaire est 10 à 40 fois plus étendu que les racines (Giovannetti *et al.*, 2001), et sa longueur pourrait varier de 10 à 22 mètres par plante en fonction de la plante hôte (Pepe *et al.*, 2017). Ainsi, tandis que l'absorption directe des nutriments par les racines entraîne la création de zones d'appauvrissement en minéraux, tel que le P dont la mobilité dans le sol est faible, le mycélium extra-racinaire des CMA se propage bien au-delà des zones d'appauvrissement (Smith & Read, 2008). Ainsi, le réseau mycélien représente une variable importante qui affecte la surface d'absorption et la capacité de prospection des champignons, et donc le taux d'absorption de P du sol par les plantes. Des expériences avec du P radioactif ont permis de quantifier le P entrant dans le système racinaire de la plante *via* les CMA (Pearson & Jakobsen, 1993; Smith *et al.*, 2003; Smith *et al.*, 2004). Si le principal bénéfice de la symbiose mycorhizienne arbusculaire est une amélioration du statut phosphaté de la plante, elle permet également le transfert à la plante d'autres nutriments tels que l'ammonium, le soufre, le potassium, le calcium, le magnésium, le cuivre, le fer, le zinc ou le potassium (Smith & Read, 2008; Smith & Smith, 2011; Lehmann *et al.*, 2014; Lehmann & Rillig, 2015; Ferrol *et al.*, 2016; Ferrol *et al.*, 2019). Ainsi, un effet direct de l'apport accru en nutriments et en eau est une augmentation de la croissance et du développement végétal (Smith & Read, 2008). D'un point de vue agricole, cela peut se traduire par une augmentation globale des rendements (Pellegrino *et al.*, 2015; Hijri, 2016; Ziane *et al.*, 2017).
En plus des contributions à l'absorption de macro et micronutriments qu'ils confèrent, les CMA sont susceptibles de seconder positivement la plantes dans divers domaines, notamment l'absorption d'eau, la stabilité des agrégats du sol grâce notamment à l'excrétion d'une glycoprotéine, la glomaline (Ortaş *et al.*, 2017; Makarov, 2019), l'atténuation du stress lié à la salinité et à la sécheresse, la détoxification des éléments traces métalliques et la protection contre les agents phytopathogènes (Smith & Read, 2008).

1.2.3. Taxonomie et phylogénie des CMA

Des analyses phylogénétiques basées sur la petite sous unité (SSU) du gène de l'ARNr ont permis de démontrer que les CMA formaient un groupe monophylétique bien distinct des autres embranchements de champignons, l'embranchement des Glomeromycotina (Schussler *et al.*, 2001; Spatafora *et al.*, 2016; Spatafora *et al.*, 2017), au sein duquel quatre ordres sont définis : les Glomérales, les Paraglomérales, les Archaeosporales et les Diversisporales (Figure 8).

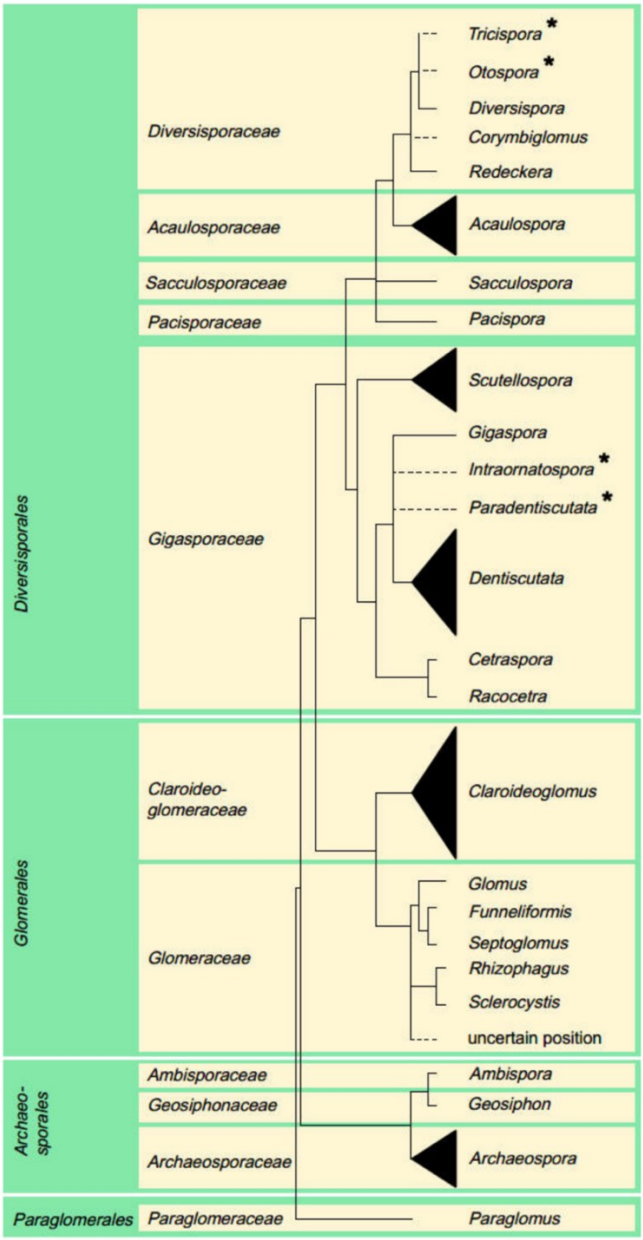

Figure 8 : Classification des Glomeromycota revue et formalisé par Redecker *et al*. (2013). La position phylogénétique des genres marqués d'un astérisque est encore questionnée par les auteurs (amf-phylogeny.com - November 2021)

L'ordre des Diversisporales compte cinq familles : les Gigasporaceae (dont les genres *Scutellospora, Gigaspora, Racocetra*), les Diversisporaceae (dont le genre *Diversispora*,), les

Acaulosporaceae, Sacculosporaceae et Pacisporaceae (un seul genre chacune : respectivement *Acaulospora, Sacculospora et Pacispora*). L'ordre des Glomérales compte deux familles : les Glomeraceae (sept genres : *Glomus*, *Funneliformis*, *Rhizophagus, Sclerocystis, Septoglomus, Dominikia*, *Kamienskia)* et les Claroideoglomeraceae (un seul genre : *Claroideoglomus).* L'ordre des Archaeosporales compte trois familles (les Ambisporaceae, les Geosiphonaceae et les Archaeosporaceae. L'ordre des Paraglomérales compte deux familles les Paraglomeraceae avec deux genres (*Paraglomus* et *Innospora*) et la famille des Pervetustaceae avec un seul genre (*Pervetustus*) (Błaszkowski *et al.*, 2017). Les Gigasporaceae, Glomeraceae, et Acaulosporaceae sont identifiées comme les familles les plus diversifiées au sein du phylum, regroupant ainsi 82% de l'ensemble des espèces (Stürmer *et al.*, 2018). La famille des Glomeraceae comprend les genres les plus abondants tels que *Glomus*, *Rhizophagus*, *Funneliformis* et *Septoglomus*, (Helgason *et al.*, 2007; Öpik *et al.*, 2010; Stürmer *et al.*, 2018)(Tableau 1).

Tableau 1 : Taxonomie des CMA : Liste des genres actuellement décrits de manière valide (amf-phylogeny.com - November 2021).

Paraglomerales	*Innospora* *Perveustus*	*Paraglomus*
Archaeosporales	*Ambiospora* *Geosiphon*	*Archaeospora* *Plonospora*
Diversisporales	*Acaulospora* *Cetraspora* *Dentiscutata* *Diversispora* *Intraornatospora* *Pacispora* *Racocetra* *Sacculospora* *Sieverdingia*	*Bulbospora* *Corymbiglomus* *Desetispora* *Gigaspora* *Otospora* *Paradentiscutata* *Redeckera* *Scutellospora* *Tricispora*
Glomerales	*Claroideoglomus* *Epigeocarpum* *Funneliglomus* *Halonatospora* *Microdominiki* *Nanoglomus* *Aehlia* *Sclerocarpum* *Sclerocystis*	*Domininkia* *Funneliformis* *Glomus* *Kamienskia* *Microkamienska* *Orientoglomus* *Rhizophagus* *Silvaspora* *Septoglomus*
Affiliation Inconnue	*Entrophospora*	

1.2.4. Génétique des CMA

Le nombre d'espèces de CMA décrites est faible comparé aux autres embranchements de champignons (Ohsowski *et al.*, 2014). Beaucoup de CMA sont connus uniquement grâce aux

séquences d'ADN environnementales et le nombre d'unités taxonomiques opérationnelles (moléculaire) différentes ou de taxons virtuels excède le nombre d'espèces de CMA décrites par les méthodes taxonomiques traditionnelles (sur critères morphologiques) (Öpik *et al.*, 2013; Öpik *et al.*, 2014). Actuellement, environ 341 espèces sont décrites et nommées (Redecker *et al.*, 2013), alors que les études de séquences d'ADN ribosomique environnemental suggèrent l'existence d'une diversité taxonomiques bien plus étendue (Öpik & Davison, 2016). Une diversité intraspécifique élevée a en outre été identifiée parmi les CMA, et a conduit à introduire la notion de pangénome, terme indiquant que le nombre total de gènes disponibles pour une espèce microbienne peut largement dépasser ceux codés par un seul individu (Mathieu *et al.*, 2018).

L'organisation génomique nucléaire des CMA est en effet complexe : les hyphes coenocytiques contiennent plusieurs milliers de noyaux circulants librement, les spores sont remplies de plusieurs centaines de noyaux et chacun d'entre eux présente des variabilités génétiques. Les CMA sont par ailleurs considérés comme asexués car aucun cycle sexuel n'a été observé au niveau cellulaire et morphologique. Cependant des publications récentes ont révélé que des gènes impliqués dans la méiose et la sexualité sont présents dans plusieurs CMA, suggérant donc l'existence d'un cycle sexuel cryptique (Riley *et al.*, 2014). De plus, une fréquence élevée d'éléments transposables au sein de séquences génomiques a été identifiée (Tisserant *et al.*, 2013; Lin *et al.*, 2014). Organisation nucléaire et variabilité intraspécifique complexifient ainsi les stratégies de séquençage et d'assemblages du génome et soulèvent des interrogations concernant la taxonomie des CMA. Plusieurs révisions de celle-ci ont d'ailleurs été nécessaires au fil des clarifications des relations phylogénétiques qui existent entre les espèces et isolats (Oehl *et al.*, 2008; Schussler & Walker, 2010; Redecker *et al.*, 2013; Baszkowski *et al.*, 2017; Bruns *et al.*, 2018).

1.2.5. Identification taxonomique des CMA

L'identification des CMA peut s'opérer à partir des spores. Elle est alors basée sur des critères morphologiques tels que l'apparence générale de la spore (taille, couleur), les structures pariétales (nombre, épaisseur, nature de chaque paroi) et sur des critères d'ontogenèse sporale. Cependant ce morphotypage est parfois insuffisant pour une identification au rang taxonomique d'espèce. Il nécessite par ailleurs un protocole d'isolement du CMA en culture pure. La culture sur plante hôte du symbionte obligatoire que sont les CMA est alors opérée *in vitro* ou *in vivo*. Généralement, pour une culture monospécifique *in vivo,* une inoculation est réalisée directement par dépôt des spores à la surface des racines de plantes hôtes pièges. Au total 4 à 8 mois sont nécessaires pour la multiplication du CMA. La culture *in vitro* est réalisée en milieu gélosé sur des racines génétiquement modifiées par *Agrobacterium rhizogenes* ou sur des explants racinaires non transformés. Une technique utilisant des boîtes de Pétri bi-compartimentées a été développée par St-Arnaud *et al.* (1996). Elle permet de cultiver les racines mycorhizées dans un compartiment de la boîte de Pétri, tandis que le deuxième compartiment de la boîte de Pétri est colonisé uniquement par le réseau mycélien du CMA ce qui permet d'obtenir une biomasse pure et importante du CMA. Cette méthode de culture est toutefois restreinte à quelques espèces du genre *Rhizophagus* (Figure 9).

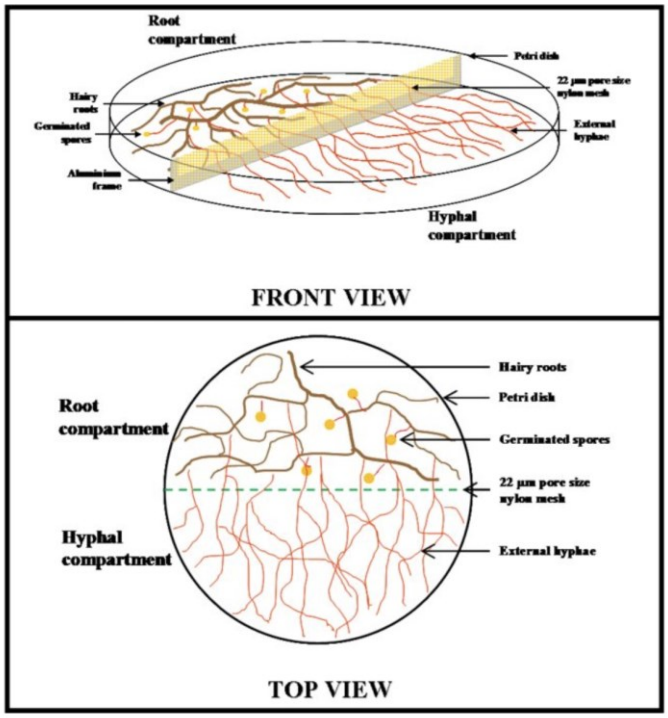

Figure 9 : Dispositif expérimental de culture en boîte bi compartimentés pour la culture axénique des CMA (Wang et al., 2017).

Le dispositif contraint le CMA à explorer les deux compartiments pour obtenir tous les nutriments essentiels à sa croissance : - le compartiment proximal racinaire contenant les racines transformées de carotte, cultivées sur milieu gélosé sans P et sur lequel est entretenue une culture pure de CMA, le compartiment distal hyphal contenant le milieu gélosé enrichi en P.

Dans le cas d'une étude sur le terrain ou en condition expérimentale, le dénombrement de spores dans une quantité de sol connue peut permettre une estimation de l'abondance en CMA dans le sol. Toutefois, le comptage des spores et leur identification ne reflètent pas suffisamment la structure des communautés réellement actives dans les racines. En effet, la production de spores n'est pas toujours corrélée avec la colonisation des racines car la sporulation de certains membres des Glomeromycota est assez discontinue voire absente (Schussler *et al.*, 2001). Il est également envisageable d'estimer le taux et la fréquence de mycorhization du système racinaire de la plante (Trouvelot, 1986; Vierheilig *et al.*, 1998) après coloration (bleu Trypan, noir de chlorazole, fuchsine, encre) des structures intra-racinaires fongiques (hyphes, vésicules et arbuscules). Toutefois, ces colorations ne permettent pas une estimation de l'abondance de différents taxa présents dans un système racinaire et ont une valeur taxonomique très limitée.

La taxonomie et la phylogénie moléculaire des champignons repose sur l'amplification par réaction de polymérase en chaîne (PCR) des régions de l'ADN nucléaire ribosomique (ADNr), à l'aide d'amorces nucléotidiques universelles et spécifiques des champignons. L'ADNr est répété en tandem et comprend les unités 5S, 5.8S, 18S et 28S, entre lesquelles se trouvent les séquences d'espaceurs transcrits internes (ITS pour « Internal Transcribed Spacer ») (Figure 10). Les séquences ITS ont ainsi été largement utilisées pour la taxonomie moléculaire fongique (Redecker, 2000; Renker *et al.*, 2006).

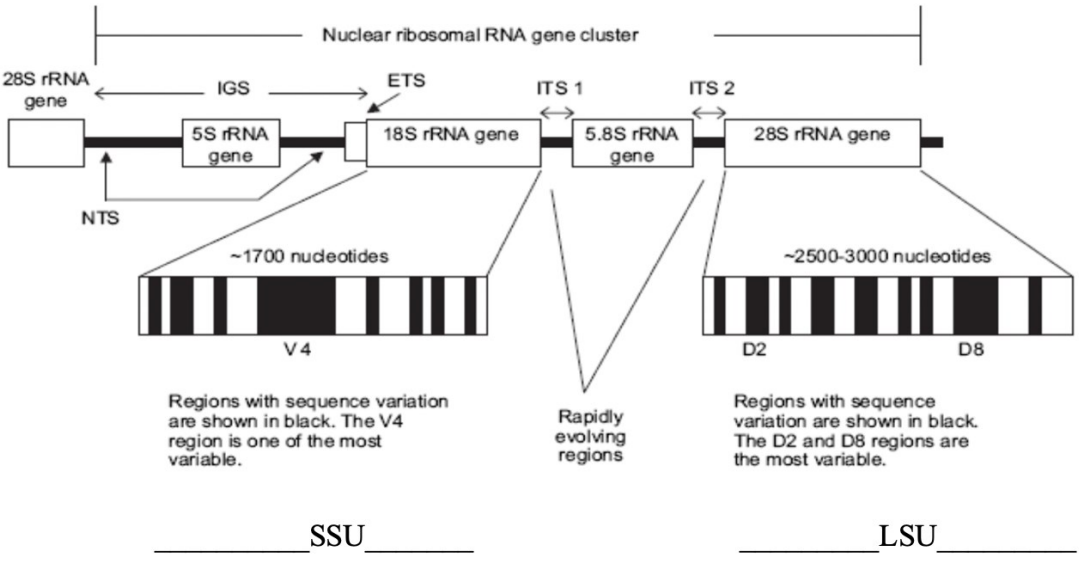

Figure 10 : Opéron du gène ribosomique des eucaryotes (Mitchell & Zuccaro, 2006).

Les ITS 1 et 2 sont les espaceurs internes transcrits (Internal Transcribed Spacers). L'IGS est l'espaceur intergénique (InterGenic Spacer) qui correspond au grand espaceur situé entre les unités codantes (géniques) 18S+5.8S+28S considérées ensemble. L'IGS comprend une portion transcrite (ETS, External Transcribed Spacer) et une portion non transcrite (NTS Non Transcribed Spacer). L'unité de transcription comprend ainsi l'ETS + les séquences codantes pour les trois ARN 18S, 5.8S et 28S + les ITS 1 et 2.

Cependant, les séquences ITS présentent un niveau élevé de variation au sein des espèces de CMA et même au sein de spores uniques (Sanders *et al.*, 1995; Lloyd-MacGilp *et al.*, 1996). C'est alors sur la base de la petite sous-unité de l'ARNr (ARNr SSU) que (Schussler *et al.*, 2001) ont démontré que les Glomeromycota constituaient un groupe monophylétique parmi les champignons. En 2008, Lee *et al.* (2008) ont développé des amorces AML1 et AML2, ciblant la SSU-ADNr, moins variable que l'ITS, mais qui permet une résolution discriminante suffisante jusqu'au niveau de l'espèce chez les CMA.

Un système universel de nomenclature a en outre été développé qui définit des groupes de similarité basés sur ce marqueur de séquence d'ARNr SSU (ARNr 18S) (base de données MaarjAM par Öpik *et al.* (2010) (Figure 11).

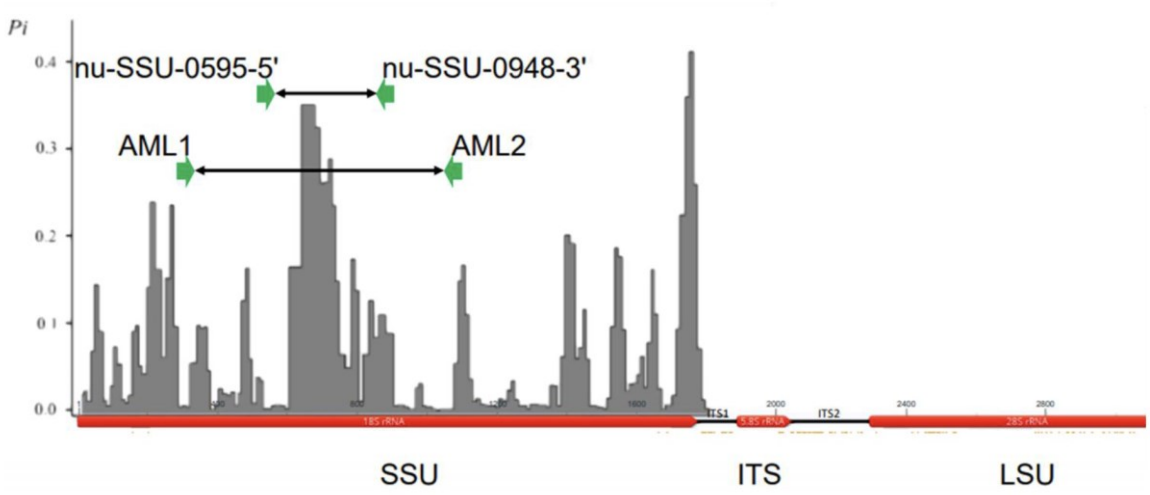

Figure 11 : Cartographie de la région amplifiée par PCR nichée (ADNr 18S) (Stefani *et al.,* 2020).

1.3 Bactéries mycosphériques et endophytes bactériens des champignons

Les CMA constituent des habitats particuliers pour des bactéries endophytes ou des communautés bactériennes vivant en étroite association avec les spores ou le mycélium extra-racinaire, dans la région définie comme mycosphère (Barea *et al.*, 2002; Bianciotto & Bonfante, 2002; Sbrana *et al.*, 2022).

Ainsi, plusieurs exemples d'endosymbiontes bactériens associés aux CMA ont été signalés. Notamment, dans une revue de synthèse portant sur l'interaction entre les CMA et les bactéries qui leur sont associées, Turrini *et al.* (2018) ont répertorié divers genres associés aux spores de CMA, tels que *Cellvibrio*, *Chondromyces*, *Flexibacter*, *Lysobacter*, *Pseudomonas*, *Burkholderia*, *Arthrobacter*, *Streptomyces*, *Bacillus*, *Paenibacillus*, *Pseudomonas*, *Herbaspirillum*, *Massilia*, *Rhizobium* et *Sinorhizobium*. De manière intéressante, les spores de *Gigaspora margarita* peuvent héberger à la fois des endobactéries liées aux Burkholderia et aux Mollicutes, ce qui soutient l'idée que certains champignons associés à la racine possède leur propre microbiome bactérien intracellulaire de faible diversité (Desirò *et al.*, 2014). La composition du microbiote associé aux CMA peut ainsi dépendre à la fois du taxon fongique et de l'espèce végétale hôte (Roesti *et al.*, 2005; Long *et al.*, 2008; Agnolucci *et al.*, 2015).

Nombre de ces bactéries, capables d'interagir avec les CMA dans des interactions tripartites plantes-bactéries-champignons mycorhiziens peuvent également exprimer des traits PGPR (Santoyo *et al.*, 2016). Par exemple, diverses souches bactériennes endophytes ont montré des caractéristiques bénéfiques, notamment la fixation de l'azote, la sécrétion de sidérophores ou la synthèse d'AIA (Crowley, 2006; Sharma *et al.*, 2013; Glick, 2014). Elles ont également montré la capacité à solubiliser le P minéral ou à minéraliser le P des phytates (Taktek *et al.*, 2015; Battini *et al.*, 2016; Giovannini *et al.*, 2020; Sharma *et al.*, 2020; Andrino *et al.*, 2021; Jiang *et al.*, 2021). Ainsi, les bactéries mobilisant le P pourraient augmenter la disponibilité du P pour les CMA, jouant ainsi un rôle clé dans l'acquisition de cet élément par les CMA et dans la facilitation de la nutrition en phosphatée des plantes.
En outre, certaines de ces bactéries exercent des effets bénéfiques sur les mycorhizes ; elles sont qualifiées par Garbay (1994) de bactéries auxiliaires de la mycorhization (MHB pour « Mycorhiza Helper Bacteria »). Il s'agit de bactéries associées aux CMA qui améliorent l'établissement de la symbiose mycorhizienne (Duponnois & Garbaye, 1991; Garbay, 1994).

Les bactéries détectées dans le cytoplasme des champignons, peuvent être activement acquises à partir de l'environnement et, dans la plupart des cas, sont héritées verticalement par les spores fongiques (Mitter *et al.*, 2017).

2. Des inoculants promoteurs de la croissance des plantes et solubilisateurs de P ?

2.1 Développement d'inoculums phytobénéfiques : démarches et contraintes

De manière générale, face à une prise de conscience écologique en agronomie, les potentialités et apports des PGPR et des CMA dans le domaine de la biostimulation et de la biofertilisation sont particulièrement recherchés et documentés (Hayat *et al.*, 2010; Ahemad & Kibret, 2014; Schütz *et al.*, 2018). Leur intérêt est de même reconnu pour favoriser la tolérance des plantes aux stress abiotiques et biotiques et la qualité des cultures (Vessey, 2003; Calvo *et al.*, 2014). Divers travaux font également état de leur utilisation pour la revégétalisation des sols afin notamment d'améliorer la survie des plants après leur transplantation sur le site (Bashan, 2018). Les propriétés développées par les PGPR et les CMA sont enfin largement utilisées dans les procédés de restauration écologique et de phytoremédiation des sols pollués (Glick, 2003; Khan, 2005; de-Bashan *et al.*, 2012; Ma *et al.*, 2016; Mishra *et al.*, 2017).

Le criblage d'isolats pour diverses caractéristiques favorisant la croissance des plantes est classiquement réalisé en laboratoire, à partir d'échantillons issus de différents compartiments tissulaires du végétal ou du sol comme indiqué dans la Figure 12. Il s'appuie de manière privilégie sur l'identification de fonctions microbiennes, telles que la solubilisation des phosphates, la fixation de l'azote ou la production d'antibiotiques, de sidérophores, d'hormones végétales et d'ACC désaminase. Grâce à cette approche, l'efficacité des PGPR a été mise en lumière par divers auteurs lors d'inoculation en pot ou en conditions de champs par divers auteurs (Wang *et al.*, 2015; Rasul *et al.*, 2019). Notamment, il a été démontré que l'inoculation par des PSB améliore accroit la biomasse, et/ou la teneur en P des pousses des plantes cultivées dans des sols non fertilisés avec une faible disponibilité en P (Wakelin *et al.*, 2007; Pande *et al.*, 2017). De même, un nombre conséquent d'inoculants à base de CMA sont commercialisés, variant en termes de formulations (poudre, granulés, liquide, types de propagules), de compositions d'espèces, de nombre de propagules, de déclarations de fonctions, de méthodes d'application et de recommandations. La plupart de ces inoculants sont introduits directement dans le sol ; certains d'entre eux sont appliqués par enrobage des semences (Bashan *et al.*, 2014).

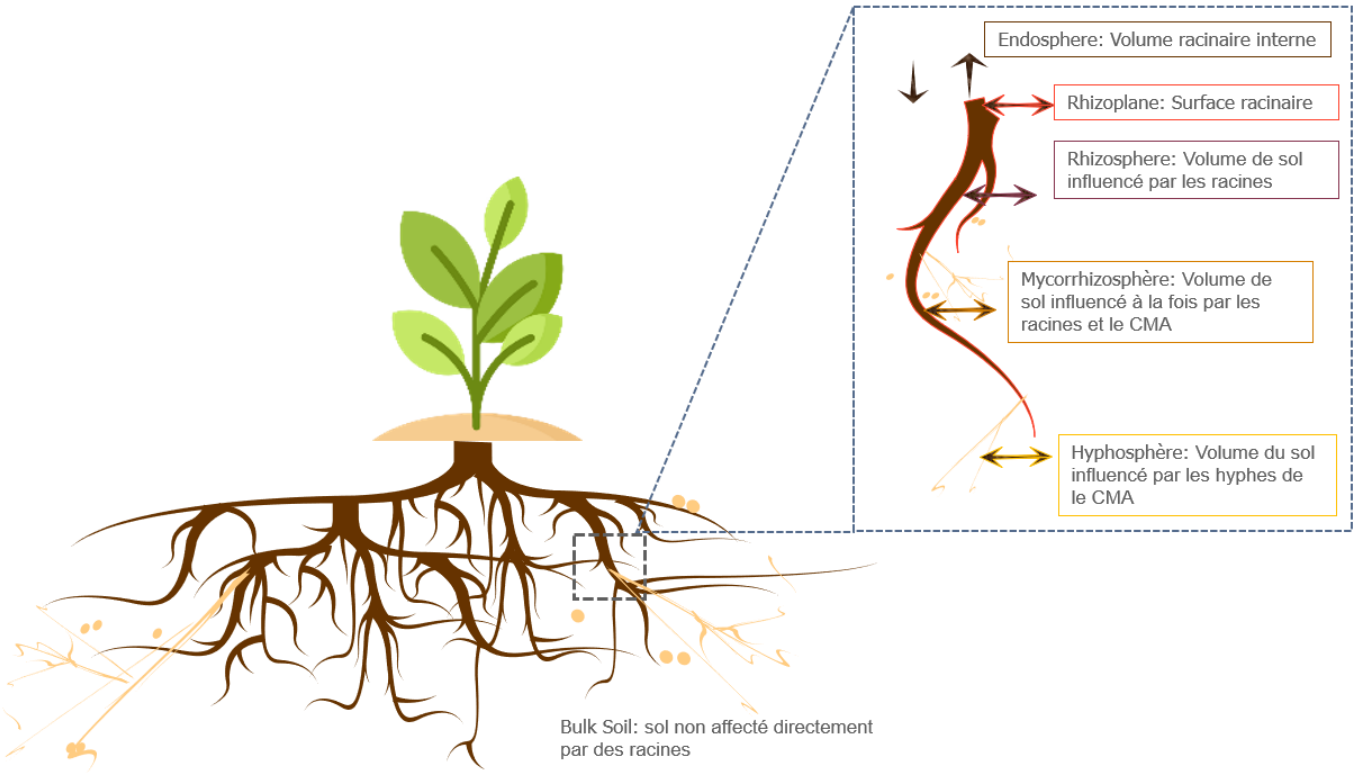

Figure 12 : Schéma conceptuel permettant de différencier les différents compartiments du sol étudiés par les données de la littérature (d'après Wang & Feng (2021)

Cependant, malgré un nombre élevé de brevets déposés et de produits commerciaux (Owen *et al.*, 2015; Koskey *et al.*, 2021), l'efficacité et les performances des inoculants ne fait pas consensus et des résultats contradictoires sont souvent observés. La formulation d'un inoculant (c'est-à-dire la combinaison par exemple d'une souche bactérienne à une matrice qui fournit des conditions micro-environnementales plus appropriées) est en effet un processus complexe comportant de multiples étapes et contraintes largement détaillées par plusieurs auteurs (Kaminsky *et al.*, 2019; Lawson *et al.*, 2019; Lobo *et al.*, 2019; Elhaissoufi *et al.*, 2021).
Ainsi la première étape du processus sélectionne des candidats microbiens promoteurs de la croissance des plantes, aptes à être cultivés *in vitro* (Tanaka *et al.*, 2014; Delgado-Baquerizo *et al.*, 2018). Elle constitue un premier biais expérimental qui limite l'exploration de la diversité microbienne et donc l'identification éventuelle de microorganismes pertinents en tant qu'inoculants.
Les souches les plus prometteuses sont ensuite testées en serre, puis parfois *in situ*. Or divers travaux de recherche soulignent un manque de reproductibilité des effets attendus lors du transfert au champ. Il est difficile d'établir des corrélations claires entre le gain de biomasse végétale et la mobilisation du P par les PSB (Whitelaw *et al.*, 1997; Krey *et al.*, 2013; Leggett *et al.*, 2015; Raymond *et al.*, 2019). Par exemple, alors que les souches d'*Azospirillum brasilense* utilisées par Fukami *et al.* (2016) ont permis une amélioration de la croissance du maïs et du blé dans des conditions contrôlées, aucun effet sur la croissance des plantes n'a pu être observé en plein champ. En effet, les technologies génomiques et moléculaires appliquées pour caractériser les communautés microbiennes dans des conditions naturelles ont révélé un haut niveau d'interactions sélectives entre l'inoculant microbien introduit, les communautés microbiennes autochtones, la plante et les propriétés physico-chimiques du sol récepteur. Ainsi, Raymond *et al.* (2019) ont suggéré que les mécanismes de sécrétion d'acides organiques et

d'acidification par les PSB, qui avaient été identifiés comme étant importants *in vitro* sont sensiblement moins efficaces dans le sol, en sus d'être spécifiques du génotype de la plante et des souches PGPR.
De même, à l'issue d'une étude portant sur 171 publications, Schütz *et al.* (2018) ont démontré que les microorganismes solubilisateurs de P utilisés comme inoculants étaient principalement efficaces dans les sols présentant des niveaux modérés de P disponible (25-35 kg de P), mais pas dans les sols à faible teneur en P ou à plus forte teneur.

En outre, les microorganismes autochtones du sol récepteur de l'inoculant sont hautement diversifiés et bien adaptés ; un microorganisme introduit est, quant à lui, confronté à des conditions compétitives qui peuvent réduire considérablement leurs effets bénéfiques, s'il n'est pas en mesure de concurrencer suffisamment la microflore résidente (Compant *et al.*, 2019). Or, la capacité concurrentielle d'une souche inoculée n'est généralement pas un critère de sélection au laboratoire. Par ailleurs, on ignore également si les PGPR introduits par inoculation vont exprimer un phénotype aux performances supérieures à celui des communautés autochtones, qui elles-mêmes contiennent potentiellement, pléthore de PGPR (Compant *et al.*, 2019). De même, des phénomènes d'antagonismes peuvent se mettre en place entres les souches introduites et natives, entrainant dans certains cas une absence d'effet phytobénéfique (Felici *et al.*, 2008; Trabelsi *et al.*, 2011).
Enfin l'inoculum à visée commerciale doit être apte à être fabriqué à grande échelle, facile à stocker et doit être formulé pour un usage aisé par les agriculteurs (Bashan *et al.*, 2014). Pour un usage en agriculture conventionnelle, les microorganismes doivent également être compatibles aux pratiques culturales telles que le labour, l'utilisation d'intrants chimiques et de produits phytosanitaires. Par conséquent, mettre en adéquation les traits fonctionnels d'un inoculant avec le contexte d'utilisation de cet inoculant constitue le principal verrou à lever afin d'optimiser l'utilisation et l'efficacité des inoculants (Martínez-Viveros *et al.*, 2010; Bashan *et al.*, 2014; de Souza *et al.*, 2016).

2.2 Formulation d'inoculants plurispécifiques, possédant diffèrent traits PGP en consortium

La construction de communautés artificielles, réunies en consortium, dont les propriétés se rapprocheraient partiellement de celles d'un microbiote naturel est une approche émergente pour l'élaboration de inoculants et l'amélioration de la production agricole (Parnell *et al.*, 2016; De Vrieze *et al.*, 2018; Compant *et al.*, 2019). Ainsi, il peut être envisagé de combiner au sein d'une même communauté, des microorganismes présentant des traits fonctionnels distincts et complémentaires. Par exemple, une complémentarité fonctionnelle pourrait être obtenue grâce à un inoculant poly-microbien associant des isolats améliorant la croissance des plantes et d'autres assurant le biocontrôle d'agents phytopathogènes.
La conception des inoculants en consortium peut également viser une redondance fonctionnelle et donc chercher à regrouper diverses souches présentant le même mode d'action. Ainsi, par exemple, plusieurs isolats présentant un même trait fonctionnel pourraient tolérer des conditions environnementales ou des génotypes de plantes différents, assurant alors une probabilité plus grande de persistance du trait ou de réponse aux particularités de l'environnement. Diverses données viennent étayer cette hypothèse. En effet, les fonctions biologiques du sol et les fonctions clefs pour le développement des plantes sont généralement portées par une importante diversité de taxons (Bashan, 1998; Van Loon, 2007; Glick, 2012; Vacheron *et al.*, 2013). Ainsi, la fixation de l'azote atmosphérique est retrouvée chez les Alpha, Beta, Gamma et Delta protéobactéries, les cyanobactéries et les archées. De même, le processus

de nitrification est retrouvé chez de nombreuses Beta et Gamma- protéobactéries, chez les archées (Leininger *et al.*, 2006) et chez certains champignons (Mothapo *et al.*, 2015). La structure du rhizomicrobiote naturel se caractérise donc par une forte redondance fonctionnelle où la perte de taxons n'est pas synonyme de perte de fonctions.
Expérimentalement, la complémentarité ou la redondance fonctionnelles au sein d'inoculant en consortia sont obtenues en augmentant le nombre de taxa, soit au niveau de l'espèce, du genre ou de la famille, faisant l'hypothèse d'une relation positive entre la diversité taxonomique et la complémentarité fonctionnelle (Van Der Heijden *et al.*, 2004; Maherali & Klironomos, 2007). En outre, il est apparu que des combinaisons de bactéries qui n'ont pas ou peu d'effets PGP en tant qu'inoculants individuels peuvent avoir des effets PGP dans un consortium (Berendsen *et al.*, 2018). Concernant la solubilisation du P, Braz & Nahas (2012) ont constaté que celle-ci et la production d'acide organique étaient plus importantes dans des cultures co-inoculées par *Aspergillus niger* et *Burkholderia cepacia* par comparaison à des inoculations individuelles. Explorant les effets de différents consortia assemblant un nombre variable de différentes souches de *Pseudomonas*, Hu *et al.* (2016) ont pour leur part montré qu'une plus grande diversité au sein des consortia de souches du genre *Pseudomonas* entraînait une plus grande production de biomasse végétale et une meilleure assimilation des nutriments dans les tissus végétaux. Concomitamment, il est apparu dans cette étude, que l'identité des souches était moins importante que le facteur « diversité », sur la production d'hormones végétales, de sidérophores et la solubilisation du P *in vitro*. De plus, la survie des consortia de *Pseudomonas* introduits augmentait avec la diversité. Dans une étude basée sur la cooccurrence de fonctions phytobénéfiques dans le groupe des *Pseudomonas fluorescencs* (Vacheron *et al.*, 2016) ont pour leur part montré que le maïs ne sélectionne pas préférentiellement des bactéries possédant beaucoup de fonctions mais plutôt celles comportant entre 1 et 5 fonctions, et favorisant une certaine redondance fonctionnelle. Dans une méta-analyse basée sur plus de 900 publications, (Yang *et al.*, 2017) ont démontré que les performances de la plante sont diversement influencées par la richesse taxonomique des communautés de CMA selon que l'on considère le rang taxonomique « famille » ou « espèce ». Ainsi, la croissance végétale serait plutôt améliorée par une richesse taxonomique au niveau la famille, plutôt que par la diversité spécifique au sein d'une famille.

Les inoculants en consortium ont montré leur pertinence pour la promotion de la croissance des plantes, à travers diverses études, par exemple sur la vigne (Rolli *et al.*, 2015), la pomme de terre (De Vrieze *et al.*, 2018), la tomate (Berg & Koskella, 2018), *Arabidopsis* (Berendsen *et al.*, 2018) et le maïs (Molina-Romero *et al.*, 2017). Cependant, certains travaux ont également montré des réponses contradictoires quant aux effets d'un inoculant en consortium. Il a ainsi été démontré qu'une double inoculation en consortium n'avait aucun effet, voire qu'elle réduisait l'effet PGP par rapport aux inoculants uniques (Felici *et al.*, 2008; Dodd & Ruiz-Lozano, 2012; De Vrieze *et al.*, 2018; Herrera Paredes *et al.*, 2018). Pour leur part, Raymond *et al.* (2019) soulignent que, même si des membres individuels des communautés microbiennes associées aux plantes peuvent posséder certains traits phytobénéfiques, la manifestation d'un trait dans la communauté est une propriété émergente qui ne peut être prédite à partir des membres individuels. Ces auteurs remettent ainsi en question le concept de PSB et la " finalité des PSB " en tant qu'inoculants au profit d'une vision de la fonction écologique des PSB pour le cycle du P en tant que composant de la communauté microbienne au sens large. Cette approche propose donc de se concentrer sur l'ensemble du microbiome rhizosphérique et de revenir sur la structuration des communautés microbiennes associées aux plantes.

2.3 Utilisation conjointe des acteurs microbiens impliqués dans le cycle du P et des engrais phosphatés

Les inoculants microbiens de type PSB peuvent être utilisés seuls ou en combinaison avec des engrais minéraux ou organiques pour améliorer l'accès des plantes aux intrants P tels que le super- et le triple-superphosphate, le RP, ou les composts et fumiers (Eisenlord *et al.*, 2012; Thonar *et al.*, 2017; Efthymiou *et al.*, 2018; Mpanga *et al.*, 2018). Par exemple, Nkebiwe *et al.* (2016); Nkebiwe *et al.* (2017) ont montré des effets synergiques en combinant des inoculants microbiens à l'apport d'engrais ammoniacaux. Comparant différents types d'engrais issus du recyclage de déchets organiques et inorganiques, Thonar *et al.* (2017) ont décrit les performances accrues d'un consortium *Bacillus/Pseudomonas/Trichoderma* en combinaison avec du fumier animal composté.

Les interactions entre la diversité des formes phosphatées du sol et les communautés microbiennes du sol.

1. Interaction entre P et les communautés microbiennes du sol

Les communautés microbiennes ayant une influence sur le cycle du P, on peut en retour, s'interroger sur l'influence de P sur le microbiome du sol. Ce questionnement a fait l'objet d'une revue de synthèse publiée dans la revue Microorganisms avec la citation suivante :

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

Cette publication présente, dans une première partie, les différentes formes de P en dynamique dans les sols sous l'influence de divers moteurs environnementaux. La deuxième partie est un état des lieux des dispositifs expérimentaux utilisés, et des connaissances acquises, dans l'analyse de l'influence de P sur les communautés fongiques et bactériennes. Il met en lumière la complexité des interactions en action dans les sols, et interpelle sur la nécessaire collaboration interdisciplinaire dans l'avenir afin de définir plus précisément les interactions entre les différentes formes phosphatées du sol et les communautés microbiennes et à terme, raisonner des formulations microbiennes promotrices de la nutrition phosphatée, utilisables en agriculture.

ARTICLE 1

microorganisms

MDPI

Review

Diversity of Phosphate Chemical Forms in Soils and Their Contributions on Soil Microbial Community Structure Changes

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Abstract: In many soils, the bioavailability of Phosphorus (P), an essential macronutrient is a limiting factor for crop production. Among the mechanisms developed to facilitate the absorption of phosphorus, the plant, as a holobiont, can rely on its rhizospheric microbial partners. Therefore, microbial P-solubilizing inoculants are proposed to improve soil P fertility in agriculture. However, a better understanding of the interactions of the soil-plant-microorganism continuum with the phosphorus cycle is needed to propose efficient inoculants. Before proposing further methods of research, we carried out a critical review of the literature in two parts. First, we focused on the diversity of P-chemical forms. After a review of P forms in soils, we describe multiple factors that shape these forms in soil and their turnover. Second, we provide an analysis of P as a driver of microbial community diversity in soil. Even if no rule enabling to explain the changes in the composition of microbial communities according to phosphorus has been shown, this element has been perfectly targeted as linked to the presence/absence and/or abundance of particular bacterial taxa. In conclusion, we point out the need to link soil phosphorus chemistry with soil microbiology in order to understand the variations in the composition of microbial communities as a function of P bioavailability. This knowledge will make it possible to propose advanced microbial-based inoculant engineering for the improvement of bioavailable P for plants in sustainable agriculture.

Keywords: phosphorus; chemical forms; inoculant engineering; microbial community; plant biostimulants

Citation: Ducousso-Détrez, A.; Fontaine, J.; Lounès-Hadj Sahraoui, A.; Hijri, M. Diversity of Phosphate Chemical Forms in Soils and Their Contributions on Soil Microbial Community Structure Changes. *Microorganisms* **2022**, *10*, 609. https://doi.org/10.3390/microorganisms10030609

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1. Introduction

Global demand for agricultural crops continues to increase and global food production is largely dependent on intensive agricultural management. In conventional agriculture, high-yielding crop varieties, irrigation, pesticides, and fertilizers are frequently used as common farming practices to attain higher crop yields. For instance, organic and/or inorganic fertilizers are used to provide available Phosphorus (P) compounds to plants by supplementing phosphate ions and to rapidly satisfy the nutrient requirements of crops. However, supporting plant growth and yield optimization has raised an overuse of P fertilizers, increased production costs and exerted negative impacts on agricultural soil quality and surrounding environments; in particular, surface water eutrophication [1]. In addition to these negative environmental impacts, an important proportion of P inputs is rapidly sequestered by P fixation, or precipitation by the soil matrix into insoluble complexes, inside which P is no longer directly available for biological assimilation by crops, further reducing fertilizer efficiencies.

Consequently, efforts have been made to develop alternate strategies to reach adequate levels of bioavailable P in soils and improve P uptake by plants. Among them,

crop-breeding programs incorporating the selection of beneficial plant-microorganism interactions to breed 'an optimized crop-holobiont' are promising alternatives. Agro-inputs exploiting soil microbiota ecosystem services inside microbial inoculants are also reported as a natural solution for promoting P cycling and P bioavailability [2]. Indeed, the soil microbial biomass is considered as a temporal sink and source of P compounds [3]. In addition, the soil microbial functions catalyze key processes in soil P turn-over and cycle, from various soil organic and inorganic pools [4]. In particular, some microorganisms, referred to as plant growth promoting microbes (PGPM), are identified as stimulating plant growth via direct and/or indirect mechanisms [5–7] and have been proposed as agriculturally beneficial microorganisms.

Among PGPM, phosphate solubilizing microbes (PSM) are identified as microbes exhibiting in vitro the ability to solubilize insoluble inorganic P-compounds producing available P-forms [8–13]. Thus, screening microbes for P-solubilizing taxa from field soils (P-deficient soils, or P-rich soils in mining zones) for use as microbial-based fertilizers in agronomic practices is recognized as an area of interest and has gained worldwide interest in past decades [2,14–18]. Some researchers even suggest the inoculation of phosphate-solubilizing bacteria (PSB) in soils can reduce the phosphate fertilizer application rate by 50% [19,20]. Different formulations have been proposed: engineered with a single strain or as a consortium of various microbial strains exhibiting diverse plant growth promoting properties (including P-solubilization). Nevertheless, while there is evidence of the efficacy of microbial formulations for soil fertility and plant productivity in different cropping systems, their performance as bio-fertilizers in field experiments remains debated by some authors who call for further evaluation of the PSM concept [21]. In addition, the impact of microbial inoculants on native communities in field applications is sometimes interrogated [22].

To progress in formulating widely efficient microbial-based inoculum, a deeper understanding of how P and the bacterial and fungal multi-kingdom community interact is required. In this context, we first review recent data concerning the drivers of P speciation in soils and of the dynamics of P-forms. Second, we report a growing set of recent studies investigating the contribution of P in shaping the responses of soil microbial communities, with a focus on fungal and bacterial microbiomes. Finally, we question how research in P and soil microbiome interactions is currently constrained from recent advances in our identification and understanding of soil P species diversity.

2. Diversity of Phosphorus-Forms and P-Dynamic in Soils

2.1. Back to Orthophosphate Ions and Distribution of P in Different Chemical Species

Phosphorus is not observed naturally as a free elemental form; it is highly reactive, combining rapidly with other elements. Notably, simply exposing it to air will stimulate a chemical reaction with oxygen. Therefore, P has many degrees of oxidation, from −III to +V [23]. In natural systems, P is almost exclusively present in the (+V) oxidation, i.e., as H_3PO_4, the tri-protic orthophosphoric acid, referred as phosphoric acid. It is a very polar molecule which makes it highly soluble in water. The dissociation successively produces dihydrogen phosphate anions ($H_2PO_4^-$), hydrogen phosphate anions (HPO_4^{2-}) and the tetrahedral oxy-anion orthophosphate (PO_4^{3-}) which are the fully dissociated orthophosphate anions of H_3PO_4 [23]. However, HPO_4^{2-} and $H_2PO_4^{3-}$ ions (referred to as iP) are the major mobile P forms in soil solution [24], in proportions relative to the soil pH, and linked to the dissociation constants of the successive protonization/deprotonization reactions of orthophosphoric acid [23,25]. For instance, when the soil pH is less than 7.0, $H_2PO_4^-$ is the predominant form in the soil. Moreover, the HPO_4^{2-} and $H_2PO_4^{3-}$ anions are the dominant bioavailable P forms, i.e., the fraction of total P in soil that is readily available for acquisition by microbes and plant roots [26]. However, iP is a highly reactive ionic form that interacts with numerous chemical compounds. Thus, much less than 1% of the total P will normally be found dissolved in the soil solution [10,24], and the soluble iP concentration rarely exceeds 10 µM in soil solutions [8,24,27]. The low availability

of phosphate, due to the poor solubility and mobility of soil P, frequently impairs plant growth and associated-metabolic pathways, and is therefore a major factor constraining plant performance in many natural and agricultural soils worldwide.

In addition, iP can be incorporated into a wide range of soil constituents, inducing a complex P speciation (i.e., P distribution in different chemical species), classically divided into two pools: inorganic P (inorgP), organic P (orgP) [28,29]. The inorganic pool usually accounts for 35% to 70% of total P in soil, mainly depending on soil age [28,30,31]. Apatite is the most common form of P, forming about 95% of the Earth's crust's P, but numerous other mineral P-forms are also observed, varying widely in their chemistry and structure. Indeed, primary apatite minerals exhibit a hexagonal crystal structure with long open channels. In its pure form, F^-, OH^- or Cl^- occupies sites along these channels to form fluorapatite, hydroxyapatite, or chlorapatite, respectively. In sedimentary rock, the most common form of natural apatite is francolite, a carbonate fluorapatite. However, the fact that the crystal lattice of apatite is "open" offers the possibility of many substitutions. Additionally, phosphorus can be abundant in many secondary phosphorus minerals. Phosphorus can also be present in trace amounts on the surface of particles such as clays, calcium carbonate, ferric oxyhydroxides or in the crystal lattices of other minerals. Ultimately, P occurs naturally in more than 300 different minerals [29].

Organic P forms (orgP) are characterized by the presence of carbon hydrogen bonds [32]. In natural soils, they are derived mainly from biological processes involving the assimilation of orthophosphate and subsequent release in soil. The orgP fraction classically constitutes 30–65% of the total P in most soils [33–35], although it may range from as low as 5% (in mineral soils) to as high as 95% in organic soils (>20–30% organic matter) [30,36–38]. OrgP consists of a large variety of compounds which are generally classified into four groups: monoester phosphates, diesters phosphates, phosphonates and organic polyphosphate [32,39]. Phosphate monoesters are the predominant form of orgP in soils under aerobic conditions, which occur mainly as inositol phosphates [40]. The abundance of inositol phosphates is highly variable, but frequently accounts for up to 50% to 80% of the total orgP [41], phytic acid being the most common stereoisomer [32,42]. Phosphate diesters include nucleic acids (i.e., DNA and RNA), phospholipids and teichoic acids [30,39,40]; nucleic acids and their derivatives account for 1% to 3% of total Po [30]. Phosphonates are characterized by a carbon-phosphorus bonds ($C\text{-}PO_3^{2-}$), a bond that gives them great chemical stability. Phosphoric acid anhydrides (i.e., organic polyphosphate) include compounds such as ADP and ATP.

Soil P content results also from immobilized P in biomass. Indeed, the microbial biomass can contain up to 26% (more typically c. 5–10%) of the total soil P [3,43]. Depending on seasonal conditions (especially soil moisture) and the turnover time of microbial biomass, the release of P from the microbial biomass can be significant; it therefore represents an important 'potential pool' of bioavailable P [44–46].

2.2. Geophysicochemical Processes Involved in P Cycling and Speciation

Weathering, sorption and precipitation/dissolution are essential geochemical processes that control P dynamics and speciation in soils [23,47]. Sorption does not refer to a specific mechanism but on the contrary, results from a set of abiotic reactions by which one chemical form becomes attached to another, through the pairing of ions with different soil compounds, with more or less strength, up to precipitation and formation of strong covalent bonds [48]. Sorption is the result of a mineral equilibrium, and two main physicochemical processes of sorption can be distinguished: dissolution/precipitation and adsorption/desorption. However, the mechanisms of sorption and subsequent transformation processes are only approximately known [48–50]. Moreover, it is sometimes difficult to distinguish accurately precipitation/dissolution and adsorption/desorption processes in soils because of the continuum between P adsorbed by more or less energetic bonds up to precipitated P crystallized.

Precipitation/dissolution are both very slow processes. The release of iP from apatite dissolution is the first key control on P availability in soil via weathering. The precipitation of negatively charged iP with a cation is observed when the concentrations of iP and cation are sufficiently high. It leads to a decrease in the concentration of iP in solution and the formation of a solid in which iP and the cation are present according to a stoichiometry characteristic of the mineral formed. Then, P-forms remain included in the constituents, reducing the possibility of transfer between soils compartments, towards living organisms or groundwater. On the contrary, adsorption is a fast and easily reversible process. It traps P-forms on the solid soil phase through mechanisms on surface sites and leads to the accumulation of ions at the interface between the solid phase and the liquid phase. Soil sorbents include many compounds that have surface charges variable and sorption occurs through physical or chemical processes such as mainly, anion exchange, precipitation of Ca phosphates, ligand exchange to Al and Fe oxides/hydroxides and edges of alumino-silicate minerals [50]. Compounds having hydroxyl groups, i.e., oxides and hydroxides of iron ($Fe(OH)_3$, aluminum ($Al(OH)_3$ and calcium ($CaCO_3$), that compose coatings on soil particles or are precipitated as interlayers in clays are the major factors for P sorption [51–53]. In alkaline soils, the clay content, Ca and Mg are the major factors for P sorption. In acid soils, iron and/or aluminum oxy-hydroxides and alumino-silicates dominate [9,37,54]. Po-compounds (more particularly phytic acid) are also frequently adsorbed by mineral clays and form complexes or precipitates with iron and/or aluminum oxy-hydroxides [52,55] in acidic soils. Organic matter such as fulvic acids in calcareous soils and humic acids in acidic soils can also sorb P compounds through various interactions: physical incorporation in organic matter by electrostatic connections of hydrophobic forces, chemical incorporation, direct or indirect adsorption through cationic bridge forming organic P-metal-organic matter complexes [56].

P compounds which differ significantly in their contributions to soil P availability due to their chemical and biological nature [57–59], are classified into different fractions: (i) soluble inorganic and organic P in the soil solution; (ii) labile inorgP and orgP, (iii) insoluble P. Labile P is adsorbed P-forms that are in permanent exchange with the dissolved forms through sorption process and move in and out of the soil solution according to pH, temperature, moisture and concentration. The balance between adsorption and desorption therefore governs the equilibrium with P in soil pore-water and the P-buffer capacity of the soil. Insoluble P includes Fe, Al and Mn oxyhydroxides forms, which constitute the main long term storage P-bearing phases in soils, as well as the insoluble organic P in undecomposed plant, animal, and microbial residues within the soil organic matter [24,26,60,61].

2.3. Multiple Drivers Shaped the P-Form Diversity in the Soil Matrix

Diverse measures allow partitioning of P-compounds into pools with different chemical properties that characterize "soil P diversity", which have been well detailed and accurately quantified [26,47,58,62–64]. Quantification of the microbial P pool is also sometimes proposed [39,58,65–70]. With various protocols and levels of P fractionation, the authors have collectively identified multiple factors acting as drivers of soil P dynamics and speciation: soil properties, climatic variables or soil management practices. Based on the literature, we propose, in Figure 1, a synthetic view of the multiple drivers that shape P form distribution in the soil-plant-microorganism continuum and that could be used as factors for P management.

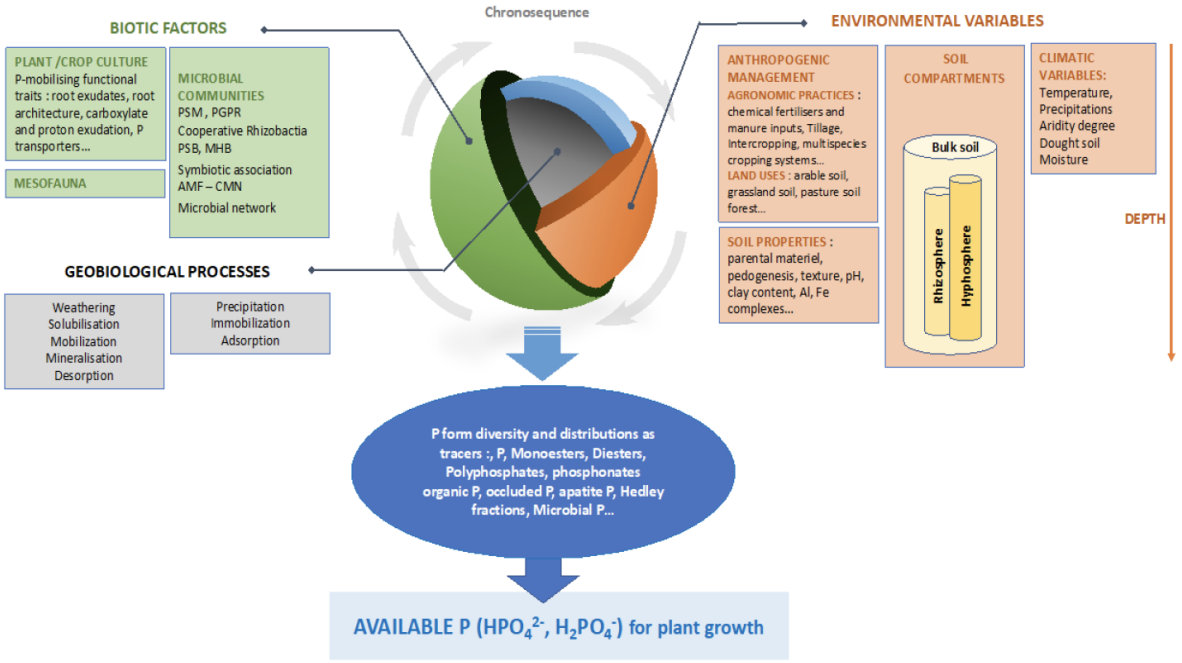

Figure 1. Conceptual representation of P dynamic drivers shaping P speciation. PSB: Phosphate solubilizing rhizobacteria: PSM: phosphate solubilizing microorganisms; MHB: mycorrhizal helper bacteria; CMN: common mycorrhizal network; PGPR: plant growth promoting rhizobacteria; AMF: arbuscular mycorrhizal fungi.

2.3.1. Soil Properties as Drivers of P-Speciation

The parent material (i.e., the primary geochemical reservoir of P chemical forms) and pedogenesis during geological timescale appear as essential factors to describe soil P dynamic and cycling [59]. This is notably supported in global maps generated by Yang et al. [65] who estimated the spatial distribution of different forms of P and the total P in soils based on global surficial lithology maps, literature reviews about rock P concentration in parent material, quantification of soil P transformation from conceptual models and depletion of total P during soil development. The general agreement between their estimation about total P and measured total P indicates the parent material the weathering of bedrock and P transport through leaching and erosion determines soil P cycle regulation in the long term. In agreement, from a dataset using ^{31}P nuclear magnetic resonance, Deiss et al. [71] highlighted how soil inorgP and orgP compounds responded to edaphic variables and soil weathering stages on a wide geographical scale. Ringeval et al. [68] combined several global datasets to analyze labile inorgP and total P of the soil. These authors thus established that one of the main drivers of the spatial variability of the P content of soils, with variations between total P and labile inorgP, originated from the biogeochemical background of the soil corresponding to the P of natural soils during the conversion to agriculture.

Besides soil geochemical characteristics, shifts in plant and microbial community during ecosystem development are also major drivers of pedogenesis. Notably, these drivers control the accumulation of organic matter and modifications in P sources with the occurrence of an increasing range of soil orgP during long term ecosystem development, relevant as a substrate for further P-cycling and turnover by microbes. In particular, as pedogenesis evolves due to both soil weathering stages and ecosystem development, changes in the complexity, as well as the absolute and relative abundances of orgP and inorgP compounds in soil, are observed [39,59,65]. For instance, chronosequence analyzes of natural soils around the world have shown that through long-term ecosystem development, cations and orthophosphate in young soils [72] are directly derived from bedrock weathering.

In contrast, older soils, therefore whose parent rock is more altered, can be profoundly modified with, in particular, a reduction or even a possible depletion of P, mainly in the form of apatite; consequently, during the evolution of soil, P can become a limiting element due to its accumulated losses, but also, due to its immobilization in secondary minerals or its complexation in organic compounds. Besides, it has been underlined that highly weathered soils generally have higher C/P ratios, a lower pH, and greater clay concentrations, which may affect the sorption capacity of the soil, and therefore, P speciation [71]. In addition, Hou et al. [73] concluded that pH and organic C concentration regulate competition between plants for P uptake and secondary minerals speciation. On the other hand, the development of organic matter also affects P-mobility by increasing the capacity of the soil to hold P via metal ligands such as Fe, Al and Ca Stutter et al. [58]. Furthermore, it has been shown that acid phosphomonoesterase activity and soil P fractions (such as labile inorgP, intermediately available inorgP, orgP, occluded P and apatite P) differ significantly during the different stages of forest succession: for instance, immediately available inorgP and orgP were prevalent in the late stages; occluded P was higher in the mid-successional forest and acid phosphomonoesterase activity was enhanced in mid- or late-successional forests [74]. Similarly, a marked increase has been observed in the DNA concentrations, long-chain polyphosphate and phospholipids in the organic horizon of old sites, whereas a peak of concentration in inositol hexakisphosphate was recorded in the intermediate stage sites [75]. In accordance, accumulations of pyrophosphate and polyphosphate have been reported as the result of the incorporation and stabilization of these biologically derived compounds in soil organic matter during ecosystem development [76]. In a more general manner, the soil chronosequences showed that the soil orgP speciation is dominated in aerobic conditions, by phosphate monoesters (mainly inositol phosphates) then by phosphate diesters (notably DNA) and phosphonates, irrespective of parent materials, plant cover and climatic conditions, and that most orgP forms increase with age to reach a peak, then decline with time [72].

In the same chronosequence, an analysis of the dynamics of P over the entire ecosystem taking into account the P of plant and microbial biomass and other pools of P in the soil made it possible to highlight the importance of the microbial pool which accounts for 68~78% of the total biomass P in mature soils where major changes in plant and microbial communities have been observed [72,74].

Soil pH is classically considered to be the major variable of soil chemistry due to its profound impact on countless chemical reactions. For instance, as previously described in this paper, pH affects the speciation of orthophosphate ions. Moreover, because the pH of the soil solution determines the number of charges developed on the surface of clays, organic matter and iron oxides, soil aluminum and the forms of orthophosphate ions, pH is a strong factor that affects P speciation, in particular through sorption [48–50,54].

As reported by Ziadi et al. [26], soil available P was also affected by soil physical properties such as texture. In particular, Suñer and Galantini [77] reviewed how soil texture affected P availability by affecting pore-size distribution and pore continuity, which in turn controls soil water availability, gas diffusion and the activity of soil biota. They notably concluded orgP contributed significantly to the total P in predominantly sandy soils, whereas in finer textured soils the inorgP pool was the highest.

2.3.2. Influence of Climatic Variables

Numerous studies have tried to assess how climate variables affect soil P cycle, speciation and availability. These have mainly focused on soil/air temperature and precipitation, as well as aridity, or drought [78]. Notably, Hou et al. [73] evaluated how climate patterns affected soil P cycle and availability in global terrestrial ecosystems using a global database of 760 soils, compiled from 96 published studies. They concluded soil P availability, indexed by Hedley labile inorganic P fractions, significantly decreased with increasing mean annual temperature and precipitation. They also suggested that temperature, precipitation and aridity all affected soil P availability, with the effects varying essentially with soil

particle size. Deiss et al. [71] established that climatic variables (mean annual precipitations and temperatures) diversely regulated soil inorgP and orgP pools, in combination with edaphic variables primarily determined by the parent material and soil forming factors. Hui et al. [79] noted that orgP concentration decreased with increasing temperatures.

Related to precipitation, the conclusions of Deiss et al. [71] underlined inorgP decreased and orgP should increase as precipitation increases; as the precipitation increased, proportions of orthophosphate decreased and pyrophosphate increased. Liebisch et al. [67] observed that seasonal patterns of fluctuations in available inorgP and microbial P, were mainly driven by soil moisture during a season. In a warm temperate forest, Zhang et al. [73] investigated how drought altered soil P dynamics and bioavailability. In a four-year field drought experiment, these authors found that drought significantly reduced soil calcium phosphate content and that this decrease was correlated with the formation of secondary minerals (Fe/Al oxides) accompanied by an increase in inorgP and orgP. In the analysis by Hou et al. [73], they suggested aridity, mainly determined by mean annual temperature, has a relatively minor effect on soil available P. Moreover, their analysis concluded that climate effects on soil available P contrasted between low-sand soils and high-sand soils, suggesting that temperature and precipitation interact with soil particle size to affect soil P availability. Ziadi et al. [26] highlighted that freeze–thaw cycles can stimulate soil mineralization and could therefore be one factor regulating soil available P concentration in early spring. They also emphasized that increased soil available P during the rewetting phase could result from release of organic P from lysed cells of microbial biomass.

2.3.3. Influence of Land Uses and Soil Management Practices

There is an increasing body of literature concerning how land use and soil management, through various agronomic practices, may drive P dynamics, including affecting diversity and abundance of P species. In this context, various environments are studied; arable lands, grassland, pasture soils, grazing sites, forest and agricultural management such as crop rotation or tillage [39,58,66,68,70,72,78,80]. Interestingly, Stutter et al. [58] estimated that aspects of land use were influential on P species and concentration, after examining thirty-two temperate soils and uses. They also noticed a complex interplay with key soil properties, controlling soil P accumulation and the P species occurring. Nevertheless, despite the large variation between soils, sites and management factors, they concluded that arable soils were dominated by inorganic orthophosphates, with P-monoesters to a lesser extent. Diesters, polyphosphates and microbial P were limited. Orthophosphates and monoesters dominated in the same proportions, in intensive grasslands, while in contrast, P is dominated by orgP, including monoesters, diesters and polyphosphates, as grazing becomes extensive and microbial P increases.

The impact of tillage, which involves plowing and harrowing to mix P imported in amendments and P recycled from crop residues throughout the plow layer, is also the subject of many studies [26,66,81,82]. For example, Xomphoutheb et al. [82] investigated the effects of different tillage systems on phosphorus and its fractions in the rhizosphere and non-rhizosphere. They observed that available P is increased in the non-rhizosphere area under different tillage methods, while the concentration of available P is reduced in the rhizosphere. They concluded that soil management minimizing soil disturbance increased the abundance of different P fractions and improved P availability. In agreement, Oberson et al. [83] reported in their review that total and available P concentrations are higher in the topsoil than in the lower soil horizons with no-tillage; as well, organic P and microbial P was higher in the topsoil of no-tillage soils than in plowed soils.

2.3.4. Interrelations, Coupling and Feedback between the Different Environmental Variables

While multiple drivers of P form distribution have been identified, the data on how these different drivers combine to explain P distribution and availability are still lacking, and research faces several difficulties. Indeed, the environmental drivers (soil properties, climatic variables, anthropogenic uses and agricultural practices) are interconnected vari-

ables with feedbacks between them [58,69,71,73,81,84,85]. In consequence, P compounds partitioning responds to different combinations of explicative variables and the relative contribution of a given driver taken individually is extremely difficult to decipher [86]. In addition, data analysis is made difficult because of the diversity of the analytical methods used to identify the P diversity and P fraction numbers. Moreover, the chemical fractionation procedures have been developed to evaluate the main P forms in soil, not the totality of the various P compounds. Moreover, these procedures were based on differences in the chemical resistance and biological susceptibility to degradation, giving rise to descriptive models, which in some cases, may exceed field validation possibilities. Together, such facts make data analysis about P speciation more complex.

3. Phosphorus as a Driver of Shifts in Soil Microbiomes

3.1. A Large Diversity of Experimental Designs Has Been Used to Track P as a Driver of Microbial Community Assemblages

From the literature, Table 1 compiles selected works that described P-dependent microbial community profiling, where analyses of how P impacts microbial communities have been addressed in different ways. For example, investigations studied different biotopes in distinct plant species using various experimental designs, including different agricultural practices, with or without repeated P inputs over a more or less long period. In addition, different analytical tools are used to describe microbial communities.

For instance, the impact of P inputs on soil microbial communities has been investigated in different global terrestrial ecosystems, including grasslands [87], arctic tundra [88], pastoral agricultural systems [89], forests [90], or estuarine areas [91], and in greenhouse experiments [92]. Authors have variously analyzed the impact of P within the bulk soil, and the different plant compartments (rhizosphere, roots, lateral versus axial roots), highlighting how P diversely affects microbial communities in these different ecological niches [92–95]. Across these studies, various plant species were included, and the evidence is well documented that the microbial response to P inputs is strongly influenced by the host plants. For example, it appears that non-mycorrhizal *Arabidopsis thaliana* and mycorrhizal *Petunia* sp. formed different microbial associations under the same low-P conditions [96]: notably, Burkholderiales (ex: *Candidatus Accumulibacter*), Rhodocyclales (ex: *Dechloromonas* sp.) and Bdellovibrionales are the most abundant *Arabidopsis* root bacteria while the *Petunia* root bacteria only included Burkholderiales (ex: *Candidatus Glomeribacter gigasporarum*) and Rhodocyclales as prevalent members. Similarly, addition of P significantly altered the microbial community composition in an old-growth tropical forest, but the same P treatment had no effect in a pine forest [90].

Changes in the microbial community composition have been examined with different types of P fertilizers: mineral fertilization with superphosphate (SP) or triple superphosphate (TSP) [89,92,97–99], manure fertilization [100,101], combinations of mineral and organic fertilizers, rock phosphate [89,98,102,103], or P-nutritive solution [90,96,104]. Notably, Gumiere et al. [103] have suggested that P sources can explain 39.1% and 45.77% of the variability in the bacteria and fungi community assemblages, respectively.

The impact of P levels on the microbial community has also been studied. High P levels (from the increase to excess) or low P levels (in nutrient limiting conditions, P-deficient soils or in depletion conditions) are both identified as inducing shifts in soil microbial communities [4,89,97,104,105]. Interestingly, taxa whose abundances varied substantially in response to P level or P fertility gradient were identified in both climate chambers with nutritive solutions [96], as well as in fields with contrasting histories of P fertilizer treatment [86].

In addition, there are experiments designed to target the impact of long-term P fertilization (i.e., multiple application patterns over a long period of time) under different P supplementation histories [92,95,98,100], or, instead, short-term fertilization experiments [93–95,102,103,105]. In both designs, shifts in soil bacterial and fungal communities were described. For example, as part of a proteogenomic comparison of soil microbial communities, Yao et al. [42]

reported the adaptation of soil communities to different P levels as a function of phosphorus fertilization. This adaptation was reflected in particular by changes in community structure, regulation of enzymatic abundances and the gain or loss of metabolic capacities.

Finally, determining the potential P effect on microbial communities was examined using various tools: for instance, some authors have used MiSeq or Hiseq amplicon sequencing from total DNA templates, RFLP analysis, DGGE fingerprints, or sequencing of targeted genes (i.e., alkaline phosphatase gene (*phoD*)) [70,91,105]. Others have assessed microbial communities by recording alkaline or phosphatase activities [80,106], by estimating microbial biomass [64,90] or quantifying phospholipid fatty acid (PLFAs) [80,90].

3.2. Occurrence of Microbial Communities with P-Dependent Structure and Composition

Phosphate inputs to soils are regularly reported to co-occur with changes in total microbial biomass, or functional profiles. For instance, Tang et al. [64] have shown significant increases in total microbial biomass-P inside the rhizosphere of intercrop plant species after P supplementation. By profiling bacterial and total PLFAs, Ali et al. [80] described changes in biochemical characteristics of microbial communities after P fertilization of cropping systems. Numerous studies also concluded that P can drive variation in microbial alpha and beta diversity indices across P fertilization treatment (RP, or TSP supplemented soils) in relation to the control (no P addition) [98] or according to the P level [94].

The identification of microbes with P-dependent abundances has also been reported. It is widely accepted that P is an important factor in the global biogeographic distribution of fungi [87,107,108]. Many studies have also concluded that P fertilization leads to changes in fungal communities, as developed for example by Wakelin et al. [86] in a long-term trial with P supplementation. Specifically, Yu et al. [31] observed that fungal community richness and species composition within roots varied with soil P contents. These authors also found that in axial roots β-diversity was similar at high or low P, whereas under low P conditions β-diversity was higher in lateral roots compared with high P. The species composition analysis they proposed, showed greater diversity for Basidiomycota at high-P and for Ascomycota at low-P in the lateral roots while under low-P conditions the diversity of Chytridiomycota was greater in the axial roots. In response to high or low soil P levels, changes in the composition of fungal communities have been observed in the bulk soil. Gomes et al. [94] also described significant differences in taxonomic composition linked to P level, mainly identified as variation in abundance of *Sordariomycetes*, *Dothideomycetes*, *Pleosporales* and *Phoma* across root or rhizosphere samples of maize.

Other studies also examined fertilizer induced changes on AMF, investigating mycorrhizal and non-mycorrhizal plant species [92,96]. Notably, high levels of mycorrhizal colonization under low P conditions and a lower hyphal and/or arbuscular mycorrhizal colonization rates of roots with high P level treatment have been repeatedly reported [105,109]. Accordingly, Gomes et al. [94] reported high abundance of *Glomeromycota* at low P content, with a prevalence of *Gigasporaceae*, *Scutellosporaceae* and *Racocetraceae*, in the root samples of maize. Similar to maize, Bodenhausen et al. [96] identified that the root microbiota of Petunia was enriched in *Glomeromycota* fungi in low-P conditions. However, in an apparent discrepancy, Silva et al. [98] reported that *Glomeromycota* were enriched in the RP-amended soils in comparison to other treatments (no P addition and TSP supplemented soil).

Wakelin et al. [89] concluded P-fertilizer application can either stimulate or depress AMF depending on the AMF species. They reported that the abundance of *Glomus intraradices* decreased as a component of the AMF community with P fertilization, while some taxa such as *G. mosseae*, *G. claroideum*, *Scutellospora*, and *Diversispora* respond positively to P fertilization and increased in abundance with the addition of fertilizer.

Similarly, Tang et al. [110] underlined the decrease of AMF colonization with P fertilization is not systematic in field-grown plant species, as P fertilization varied considerably with the plant species and indicator of root colonization by AMF. Thus, P-fertilization significantly reduced root colonization frequency and dramatically decreased arbuscular

intensity under P treatments relative to the unfertilized treatment in durum wheat, but not in faba beans [110]

Similar to fungi, some data highlighted that the occurrence/abundance of bacterial taxa may vary according to P-dependent patterns. For example, Gomes et al. [30] found significant differences in the proportion of proteobacteria between P-rich and P-poor soil samples when looking for changes in bacterial taxa in the roots and rhizosphere of maize grown in oxisoils after fertilization with TSP. These significant differences were supported by the prevalence of γ-proteobacteria when P levels are high. They also reported a decrease in the Proteobacteria/Acidobacteria ratio in low-P conditions (without TSP addition) due to a positive effect of low-P content on Acidobacteria. Further analysis at the lower family level, illustrated fast-growing and copiotrophic *Enterobacteriaceae* and *Pseudomonadaceae* were enriched in high-P soils (with TSP), while roots from low P soils contained abundant slow-growing bacterial taxa, such as *Burkholderiaceae*. Comparing the effects of long-term fertilization practices with RP versus TPS on the microbiomes of maize in tropical oxisol soils, [98] pointed out decreases in the abundance of Proteobacteria with the TSP or RP amendments compared to the control. In addition, they observed a predominance of *Oxalobacteraceae* in the RP fertilized soil compared to the control and TSP treatments.

Trabelsi et al. [38] showed that the different bacterial groups that make up the bean rhizosphere had differential responses depending on the type of phosphate fertilizer used. Thus, following TSP inputs, Proteobacteria increased while Actinobacteria and Firmicutes decreased in the rhizosphere, whereas in the uncultivated soil, a decrease in Proteobacteria and an increase in Actinobacteria were observed. In contrast, the same authors observed stimulation of, mainly, Proteobacteria, Actinobacteria and Firmicutes when applying RP at the same rate in the uncultivated soil. However, increasing the RP rate induced a significant decrease in species richness concerning mainly Proteobacteria and Firmicutes in the uncultivated soil, while in the rhizosphere, the change in taxonomic structure was mainly due to an increase in Proteobacteria and a decrease in Actinobacteria.

Across a study comparing how the root-associated bacterial communities of mycorrhizal Petunia and the non-mycorrhizal Arabidopsis responded to being supplemented with fertilizer solutions that mainly varied in the P concentration, Bodenhausen et al. [96] established that the two plant species formed different microbial associations under low-P conditions. Notably, they found marked changes in the root microbiota of Arabidopsis between low- and high-P fertilization, while the opposite was reported by Robbins [92]. Bodenhausen et al. [96] also described that the root microbiota of Petunia was enriched in slow-growing bacterial taxa such as *Burkholderiaceae* in low-P conditions, while *Enterobacteriaceae* and *Pseudomonadaceae* were enriched in high-P conditions, which is in accordance to Gomes et al. [94] in maize.

As reported across setups based on inorganic fertilization, variation in P content following organic fertilizer application also significantly influenced the soil microbial community structure and composition. In particular, manure applications were favorable for different taxa; a part of the changes could be explained by soil C and N contents and C/N ratio [100,101].

3.3. Importance of Interkingdom Interactions among Plant-Associated Microbial Communities

Numerous biotic interactions occur inside the soil microbiota, where microbe-microbe interactions are important selective forces sculpting and likely stabilizing the complex microbial assemblages associated with plants [75,76]. Among these interactions, are interkingdom microbial associations between root fungi and the endobacteria they host in their cells [74]. Thus, numerous examples of bacterial endosymbionts have been reported; they mostly belong to the families *Burkholderiaceae* or related *Bacillaceae*, and live in intimate association with plant-associated fungi such as *Rhizophagus*, *Gigaspora*, *Laccaria*, *Mortierella*, *Mucoromycota*, *Ustilago*, *Rhizopus* sp. [111]. Lee et al. [112] proposed that the inter-kingdom interaction within the plant holobiont is governed by an interrelated temporal organization

where arbuscular mycorrhizal symbiosis could be a determinant to coordinate circadian clocks in holobionts.

Moreover, the association between fungal hyphae and plant roots can lead to the establishment of a high interactive zone defined as the mycorrhizosphere that may stimulate other soil microorganisms, including PGPM. Hence, mycorrhizal plants, their colonizing fungi along with their mycorhizospheric bacteria and endobacteria, form a complex entity in the holobiont [113]. The evaluation of the effects of three sources of phosphorus in the presence or absence of AMF inoculation, on the microbial interactions of the soil led Gumiere et al. [39] to find that the percentage of P_2O_5 increased the number of bacteria-bacteria interactions while this percentage had the effect of reducing the number of fungi-fungi connections. They showed that AMF inoculation may drive a high percentage of variability inside the soil bacterial community, reaching 41% and they hypothesized that such a percentage may be associated with the recruitment of bacteria by AMF since the hypha-recruited bacteria may support the AMF for phosphorus acquisition.

In this sense, the analysis of microbial networks is now a privileged tool to assess microbial interactions in ecosystems. These analyses, pairwise comparisons between the abundance profiles of individual taxa, make it possible to identify positive, neutral or negative connections between the microbial partners of a plant. If such correlations do not necessarily predict causal relationships, they progressively increase the understanding of microbial communities. For example, co-occurrence network analysis has been used by Bodenhausen et al. [32] to characterize pairs or groups of microbes with similar abundance behavior along the P-fertilizer gradient. These authors did not find that P-rich conditions gave rise to groups of co-occurring OTUs (operational taxa units). On the other hand, they identified two major modules comprising groups of OTUs particularly abundant in P-poor conditions; the first module mainly includes Betaproteobacteria, Burkholderiales and Rhodocyclales [32]; the second module groups together a set of OTUs of the Glomeral order. An OTU assimilated to endobacteria closely associated with an AMF was revealed by their co-occurrence analysis. This OTU had an abundance consistent with the mycorrhizal OTUs identified along the P gradient [32]. The occurrence of these microbe-microbe interactions adds a further level of complexity to identifying and understanding how P influences soil microbial communities.

Table 1. Influence of P towards soil microbiome: Diversity of some selected experimental designs developed in microbial community profiling for assessing P-dependant shifts.

Experimental Design				Assessment of Shifts in Microbial Assemblages			References
Fertilization Practices P Sources	**P Levels in Amendment**	**Ecosystem Or Culture Conditions**	**Plant Species Culture Duration before Plant Sampling**	**Soil Compartments**	**Targeted Microbial Communities**	**Diversity Analysis**	
Long-Term Fertilization Practices—Repetitive Inputs During the Long Term							
~40 year fertilization trial Superphosphate Phosphate rock	188 SP; 250SP; 250RP; 376SP kg $ha^{-1}yr^{-1}$	pastoral agricultural system	grass/clover	soil	Actinobacteria Pseudomonas, AMF	Gene copie numbers (qPCR) PCR-DGGE profiles	[89]
~3 year fertilization trial Solutions of NaH2PO4	15 g m^{-2} $year^{-1}$ in 2 monthly portions	tropical forests	tree species of a mixed forest	soil	Bacteria Fungi AMF	Microbial biomass Community composition (PLFA)	[90]
2 or 4 year fertilization trial Triple-super phosphate	10 g of P per $m^2 \cdot yr^{-1}$	Broad range of natural sites	native plants Growing season	Soil	Bacteria Archaea Fungi	Sequencing of gene markers; Alpha diversity; Taxonomic structure Functional gene composition	[97]
43 year fertilization trial Triple-super phosphate	no inputs 11 kg P ha^{-1} yr^{-1} 33 kg P ha^{-1} yr^{-1}	field experiment	Intercropping *Faba bean*/ *Durum wheat* flowering stage	Rhizosphere Bulk soil	Actinobacteria, α-Proteobacteria Firmicutes	Microbial biomass Genes copies numbers (qPCR)	[110]
2 years trial $NaH_2PO_4.2H_2O$.	5 g P m^{-2} yr^{-1} 15 g P m^{-2} yr^{-1} 30 g P m^{-2} yr^{-1}	plantation	Subalpine spruce plantation	soil	Bacteria Fungi AMF	Microbial biomass Community composition (PLFA)	[104]
Fertilization since 1902 mineral fertilization (NPK) farmyard manure fertilization Combined farmyard manure and mineral fertilization	NPK = calcium ammonium nitrate+ superphosphate+ potassium chloride, 20t ha^{-1} of manure	field experiment	4-year crop rotation (*B. vulgaris*; *H. vulgare*; *S. tuberosum*; *T. aestivum*).	soil	Bacteria Fungi	microbial biomass Enzyme activities Sequencing of gene markers; Richness; Alpha diversity; Taxonomic structure	[100]
~30 years trial superphosphate Organic manure	40 kg P_2O_5 ha^{-1} $year^{-1}$	field experiment	*Triticum aestivum* L.	soil	Bacteria Archea	Sequencing of gene markers; Alpha/beta diversity	[101]

Table 1. *Cont.*

Experimental Design				Assessment of Shifts in Microbial Assemblages			References
Fertilization Practices P Sources	**P Levels in Amendment**	**Ecosystem Or Culture Conditions**	**Plant Species Culture Duration before Plant Sampling**	**Soil Compartments**	**Targeted Microbial Communities**	**Diversity Analysis**	
3 years fertilization trial Triple superphosphate Crude rock phosphate	P2O adjusted: 4 kg/ha 100 kg/ha	field experiment	Maize 60 days	Root Rhizosphere	Bacteria Fungi AMF	Sequencing of gene markers; Alpha diversity; Taxonomic structure T-RFLP	[114]
Since 1949 Superphosphate Basic slag Alkali sinter phosphate 6-year crop rotation	0; 5 kg P ha^{-1} $year^{-1}$	Greenhouse	*Arabidopsis thaliana* 7–8 weeks	Roots Rhizosphere Bulk soil	Bacteria Fungi	Sequencing of gene markers; Alpha/beta diversity; Taxonomic structure	[92]
long-term experiment	0; 150 kg ha^{-1}	field experiment	Maize 10 weeks	Axial roots Lateral roots Bulk soil	Fungi	Sequencing of gene markers; Alpha/beta diversity; Taxonomic structure transcriptome sequencing	[115]
Short term P fertilization/One-time phosphate fertilization/							
Triple-super phosphate Rock phosphate	50 kg P ha^{-1} 50 kg P ha^{-1} 250 kg P ha^{-1}	glasshouse Agricultural soil	*Phaseolus vulgaris* 10 weeks	Rhizosphere Bulk soil	Bacteria	PCR-TRFLP: Richness; Taxonomic structure DGGE fingerprintings	[102]
Potassium phosphate	0; 5; 10 and 20 kg P ha^{-1}	greenhouse Agronomic soil	*Lolium perenne* 14 weeks	soil	Bacteria Fungi AMF	Sequencing of gene markers;Alpha/beta diversity Gene abundance(phoD) Phosphatase activity	[105]

Table 1. *Cont.*

Experimental Design				Assessment of Shifts in Microbial Assemblages			References
Fertilization Practices P Sources	**P Levels in Amendment**	**Ecosystem Or Culture Conditions**	**Plant Species Culture Duration before Plant Sampling**	**Soil Compartments**	**Targeted Microbial Communities**	**Diversity Analysis**	
Soils from low/High P area (4.4 mg/dm^3 5.3 mg/dm^3) Additional superphosphate	90 kg/ha P_2O_5	field experiment	Maize 60 days	Roots Rhizosphere	Bacteria Fungi	Sequencing of gene markers; Alpha/beta diversity; Taxonomic structure	[94]
P-K or P-Na buffer	1; 20; 50 mM P	Phytochamber Agricultural soil	*Arabidopsis thaliana* 8 week	Roots Rhizosphere Bulk soil	Fungi	Sequencing of gene markers; Alpha diversity; Taxonomic structure Fungal Co-occurrence networks	[93]
Superphosphate Rock phosphate AMF inoculation (Rhizophagus clarus)	60 mg of P_2O_5 per kg	Greenhouse Agricultural soil	*Sugarcane* 120 days	Soil	Bacteria Fungi	DGGE analysis; Taxonomic structure Co-occurrence network	[103]
Nutritive solution KH2PO4	0; 0.03 mM; 1 mM; 5 mM	Climate chamber Agronomic soil+sand	*Petunia hybrida*/ *Arabidopsis thaliana* 10 weeks	Roots	AMF Fungal endobacteria	Sequencing of gene markers; Alpha diversity; Taxonomic structure Co-occurrence network	[96]

AMF: arbuscular mycorrhizal fungi. PLFA: phospholipid fatty acid analysis. TRFLP: Terminal-Restriction Fragments Length Polymorphisms. DGGE: Denaturing Gradient Gel Electrophoresis.

3.4. Identification of General Rules Explaining the Shifts in Microbiomes Following P Inputs Are Lacking

As detailed above, the literature documents numerous interactions between P and shifts in microbial community profiles. The data illustrate how P impacts microbial species compositions and diversity of bacterial and fungal communities in the bulk soil, the roots and in the rhizosphere, where different P-dependent taxa have been identified. Concomitantly, community responses have been captured at various taxonomic resolutions [92–94].

However, contradictory results have also been reported. For instance, Bodenhausen et al. [96] found marked root microbiota changes in *Arabidopsis* between low-P and high-P fertilized treatments, while Robbins et al. [92] did not. Silva et al. [98] obtained significantly higher Shannon and Simpson indices for bacteria in P added samples compared to control samples without P supplied. On the contrary, Silva and Nahas [114] and Toljander et al. [115] had originally described a higher bacterial diversity in the P unfertilized soil, while Huang et al. [104] indicated that soil microbes were insensitive to an elevated P availability in a subalpine spruce plantation.

Other research has looked more specifically at the possible impact of P on PSB. Ikoyi et al. [105] pointed out the relative abundance of bacterial genera, such as *Bacillus*, *Bradyrhizobium*, *Paenibacillus*, widely described elsewhere as genera harboring PSB strains, were significantly lower with P-fertilization compared to the control. In contrast, Gomes et al. [30] concluded that the relative abundance of taxa frequently associated with phosphate solubilization capabilities and more generally plant growth promotion did not necessarily reflect the effects of phosphate fertilization. Their findings were in line with those of Tang et al. [50] who concluded that the stimulation elicited by P fertilization was not limited to a specific group of PSB, but concerned the entire bacterial and fungal community. Similarly, at sites with extractable P levels ranging from 11.9 to 296.5 ppm, Fernandez et al. [59] did not observe any particular effect of P on PSB communities. Indeed, a review by Kour et al. [17] on bacterial taxa hosting PSB suggested that PSB are widely distributed in soils. Thus, these authors estimated that soil PSM accounts for 20–40% of the total population of the rhizosphere and about 10–15% of the total population of the bulk soil. They identified 551 P-solubilizers from the literature, and highlighted that PSM belongs to diverse phyla, most dominant being Proteobacteria (38%) and Firmicutes (22%), while only 1% of P solubilizers belonged to Bacteroidetes, and 6% to Actinobacteria. Among P-solubilizing fungi, 20% of P-solubilizing fungi were identified as Ascomycota; Basidiomycota represented 3%. Therefore, whether PSBs are impacted by P, strictly because of their PSM functionality, or rather, as components of an overall microbial community that is impacted as a whole, remains an open question. Further studies are needed to make a solid statement about the validity of the interaction.

Taken together, our review illustrates that large-scale conclusions are still hazardous and little consensus has emerged regarding possible key taxa with systematic dependence on P. Here, the diversity in experimental designs may explain the great complexity in analyzing the effect of P on the soil microbiome. Besides, studies of P impact on microbiomes typically focus on total or available P content data while changes in microbial community composition have been rarely analyzed and significantly correlated with the broad range of soil P compounds resulting from P cycling. Indeed, a soil matrix displays specific local environmental conditions with diverse biological, physical and chemical properties driving the dynamics of P compounds and the occurrence of a large diversity of P-compounds with different properties, in particular their bioavailability towards microbial nutrition and biomass. Consequently, we hypothesize the dynamics of P may have more significant effects on soil microbial communities than P supplementation *stricto sensu*. To support this hypothesis, in the next section, we revisit the literature on P speciation in soils, and some of the environmental or agronomic factors that influence the physicochemical equilibria of P compounds.

4. Assessing P-Impact on Microbial Communities to Identify Rules in P-Dependent Shifts, Require Appropriate Characterization of Amended P Forms and Their Fate

As reported above, a large diversity of P-compounds occurs in the soil. However, analysis of P impacts on soil microbiome generally focuses on total and available P, and there is a clear lack of in-depth analysis about the influence of the other P-forms linked to soil edaphic properties and P cycling. Yet, it would be relevant to consider that P dynamics may have more effects on soil microbial communities than P amendments stricto sensu. Accordingly, we must ask two questions. First, which range of the chemical P pool and species are precisely introduced as P-fertilizers in soils across the experimental designs? In unmanaged ecosystems, the P-content of soils can be predicted from the local soil properties resulting of transformations that occur on a geological time-scale and during soil development from the lithologic parent material [60,104]. Conversely, in agricultural systems, farming practices such as P fertilization, alter the P cycle by introducing different P compounds [63,116–120]. The mined RP is the raw material of manufactured chemical fertilizers. It can be profitably recovered from sedimentary inorgP deposits, igneous inorgP deposits and biogenic deposits. In addition, during the different industrial processes required to improve the purity of RP in P pentoxide (P_2O_5) [121], the tricalcium phosphate is converted diversely; consequently, fertilizers are available in over 100 different blends, notably with varying concentrations of nitrogen, phosphorus and potassium, sometimes also associated with varying amounts of heavy metals constituents as minor constituents in the ores, like arsenic, cadmium, chromium, lead, mercury, nickel, or vanadium [121]. As well, the P-forms in organic amendments [122–124] greatly vary depending, for instance, on animal species, age, diet and how manure has been stored [125,126]. In addition, organic sources used as P fertilizer usually result in higher soil organic matter content and in larger soil microbial biomass and activity, as well as higher soil organic C level, compared to soil receiving mineral fertilizers [39,79,117,127,128].

The second emerging question arising when studying impact of P inputs on microbial community, is: what is the fate of P-inputs shortly after application, according to soil properties? Indeed, a large diversity of P-compounds can be added to soils through P fertilization and many changes, more or less rapid and drastic, in the native and added P forms may be expected due to numerous drivers shaping the P-form diversity. For instance, a P fraction is immediately up taken by plant or immobilized by soil microbes. Furthermore, due to numerous physicochemical reactions that transformed the soluble to insoluble P pools in soils, up to 80% of P applied as fertilizer can be fixed into less or un-available fractions, shortly after application, mostly via sorption/precipitation with soil particles or microbial immobilization [123]. Due to such high P-fixing capacity of numerous soils, many agricultural soils have accumulated large amounts of P after past successive applications of chemical P fertilizer "in excess" of that required to support high yield of crop production. Consequently, to date, high legacy P stocks are available in some agricultural soils, but what could also be a supplementary P resource through full management of inherited soil P [129,130]. Furthermore, erosion or leaching can impact the introduced P-forms, leading consequently to modification of P-contents in sampled soils from different sites which are rarely analyzed when studying microbial community assemblages subsequently to fertilizer inputs. In addition, organic fertilizers can also help in mobilizing native P in soil through the action of organic acids and/or by chelation and sorption [101,131–133]. However, there is a lack of data to determine the extent to which P cycling may result in differences in soil microbial community profiles.

5. Perspectives

In this review, we discussed P soil status as a driver of microbial communities on one hand, and P-form diversity in soils on the other. We highlighted two constraints for the future in the field of plant microbiome research and for the formulation of microbial inoculants relevant for P plant nutrition [112]. Firstly, a deeper understanding of how multi-kingdom interactions inside the plant holobiont shape microbial communities is re-

quired. Here, meta-analysis to explore interactions, as well functional ecology approaches, and network analysis, may be relevant [76,130–132]. Second, we also suggest advancing concomitantly our knowledge about interactions between P and microbial communities in soils by taking into account the P dynamics and chemical form diversity and transformation. Here, we believe that in the future, multidisciplinary and integrated approaches should be preferred, in order to build quantitative models of P transformation in order to develop a dynamic approach to the phosphate fertility of soil. Thus, only joint advances in these different research areas developed in distinct laboratories will allow the design and deployment of effective microbial fertilizers efficient in contrasting conditions [84,133–136] with predictable behavior and robust results, in an efficient way at a large scale.

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PROBLEMATIQUE

ET

OBJECTIFS

QUESTIONNEMENTS SCIENTIFIQUES

La partie introductive a permis de mettre en évidence l'importance d'une meilleure gestion des ressources phosphatées. Celle-ci est devenue un enjeu mondial majeur compte tenu du contexte actuel de forte pression démographique, de besoins alimentaires croissants et de tensions socio-économiques et politiques.

Par ailleurs, l'analyse de la littérature scientifique a permis de mettre en lumière, d'une part, le potentiel des communautés et des interactions microbiennes dans la solubilisation des composés phosphatés séquestrés dans le sol et d'autre part, l'intérêt de faire appel à l'inoculation des plantes avec des agents microbiens phytobénéfiques, solubilisateurs des composés phosphatés. En effet, ces associations plantes/microorganismes bénéfiques pourraient augmenter l'approvisionnement de la plante en P, tout en réduisant les impacts environnements délétères des engrais minéraux industriels et les coûts globaux de production et d'exploitation agricoles.

En outre, certains travaux ont montré la pertinence de concevoir des consortia poly microbiens et poly fonctionnels, issus d'un assemblage d'isolats microbiens afin d'apporter à la plante un avantage en termes de croissance, de développement et de tolérance aux stress environnementaux. Nous avons par ailleurs souligné l'intérêt de l'utilisation des PSB en combinaison avec le RP afin d'accroître sa solubilisation et promouvoir son usage comme substituant aux engrais chimiques.

Cependant, nous avons souligné également la complexité des interactions entre les communautés microbiennes et les composés phosphatés (natifs ou introduits) du sol. Ce dernier point a conduit certains auteurs à faire l'hypothèse de la nécessité de raisonner le choix des isolats microbiens constitutifs d'un inoculant, en fonction des conditions finales de son utilisation agronomique. Le contexte écologique d'origine d'un isolat microbien, peut alors constituer un premier critère de choix raisonné.

Et en conséquence, si l'on se fixe comme objectif à long terme, une contribution à l'élaboration de consortia poly microbien utilisables seul, ou en présence de RP afin d'optimiser l'usage raisonné des engrais de synthèse à partir de ce minerai dont les réserves géologiques sont menacées, une meilleure connaissance des interactions entre RP et communautés microbiennes est indispensables. Ces interactions peuvent être abordées par l'analyse dans un environnement naturel ou avec une approche expérimentale in vitro ou en conditions contrôlées.

A partir de ces hypothèses, j'ai choisi de mener mes travaux avec le questionnement général suivant : **Quels apports des données environnementales sur les populations microbiennes et des données de culture *in vitro* pour la sélection de candidats PGPR peut-on combiner afin de contribuer à l'élaboration d'un inoculum synthétique, poly-bactériens, avec différents traits PGP, favorable à la croissance et au développement végétal, notamment par le trait solubilisateur de RP, et utilisable en présence de RP ?**

Le projet de thèse s'est alors articulé autour de deux problématiques majeures, déclinées en plusieurs questionnements :

1. **Quelles sont les caractéristiques des communautés microbiennes naturelles, natives de sols riches en RP**
   - ✓Quels environnements échantillonner ?
   - ✓Quelle est la réponse des communautés bactériennes ?
   - ✓Quelle est la réponse des communautés fongiques ?

2. **La sélection d'isolats microbiens issus d'environnements riches en RP peut-elle permettre de concevoir la composition d'un inoculum synthétique, poly-bactériens, avec différents traits PGP, favorable à la croissance et au développement végétal et utilisable en présence de RP ?**
   - ✓ Quels environnements singuliers échantillonner pour isoler des candidats bactériens adaptés à des environnements riches en RP ?
   - ✓ Sur quels critères sélectionner les isolats bactériens pour élaborer des inoculums poly microbiens et poly fonctionnels et promoteurs de la croissance des plantes en présence de RP ?
   - ✓ Quelle est l'influence des consortia bactériens élaborés, sur la croissance et le développement d'une espèce végétale d'intérêt agronomique telle que la tomate, cultivée en serre et amendée en RP
   - ✓ Quelle est l'interaction des consortia bactériens élaborés, avec un champignon mycorhizien arbusculaire, *Rhizophagus irregularis* ?
   - ✓ L'introduction des consortia bactériens influence-t-elle la structure des communautés microbiennes natives des sols ?

PARTIE 2

RESULTATS

ET

DISCUSSIONS

PREAMBULE

La partie « Résultats et Discussions » de ce manuscrit étant rédigée sous forme de publications scientifiques, acceptées ou soumises, l'ensemble des Matériels et Méthodes, utilisés dans ce travail de thèse est décrit dans chacun de ces articles. Afin d'éviter les redondances et faciliter la lecture du mémoire de thèse, seule la description des sites d'étude a été rappelée ci-après, en préambule de la présentation des articles.

Ainsi, mes travaux s'appuient sur deux dispositifs d'étude et /ou de prélèvement :
Le premier correspond à des sites naturels non agricoles, géologiquement riches en RP, situés dans la région du Quercy, en France. Ce dispositif a été retenu d'une part pour l'étude de la diversité taxonomiques des communautés microbiennes en réponse au RP, et d'autre part pour l'échantillonnage d'isolats bactériens rhizosphériques.
Le second dispositif correspond à une parcelle située dans le jardin botanique de Montréal, Canada, où diverses espèces agronomiques ont été cultivées sur des sols amendés expérimentalement en RP. Ce dispositif a été exclusivement utilisé pour les premières étapes d'isolement de bactéries hyphosphériques comme il sera détaillé ci-après.

Présentation des dispositifs expérimentaux

1. Les sites des phosphatières du Quercy : des écosystèmes riches en RP

Pour analyser l'impact du P sur la structure des communautés microbiennes, différents sites des « Phosphatières du Quercy » (**N** 44.351827, E 1.691021**)** dans le Sud-Ouest de la France, ont été retenus. Ils correspondent à d'anciennes exploitations minières de phosphates. A ce jour, ce sont des environnements naturels, non agricoles, caractérisés par un matériau parental géologique et minéralogique initialement identique, mais plus ou moins enrichi en P suite à l'exploitation minière passée.
En effet, au cœur des causses du Quercy, de nombreuses cavités karstiques se sont développées dans les carbonates du Jurassique moyen et supérieur. Par la suite, au cours du Cénozoïque, ces formations ont connu un remplissage progressif, enrichi en phosphate, à l'origine des Phosphatières du Quercy. Des dépôts lacustres sont ensuite venus colmater, sceller et préserver les remplissages de l'érosion. Puis, l'érosion des calcaires lacustres a permis par endroit, de reconnecter les Phosphatières à la surface, puis leur exploitation pour la fabrication d'engrais phosphatés.
L'exploitation industrielle des gisements de phosphorite pour la production d'engrais P s'est opérée en 1870. Puis, après 25 ans de « fièvre du phosphate », l'activité minière a délaissé les sites, permettant une revégétalisation spontanée et progressive des écosystèmes pendant un siècle, sans perturbation anthropique majeure jusqu'à aujourd'hui. Les phosphatières sont en effet inclues dans Réserve naturelle nationale d'intérêt géologique du Lot, au sein du Parc naturel régional des Causses du Quercy.
Singulièrement, au cours de l'exploitation, seules les fractions les plus enrichies ont été exportées et les sédiments les plus fins ont été laissés sur place, générant localement des dépôts. Cette pratique d'exploitation minière a ainsi engendré la présence contigüe, sur une même localisation minière, de deux types de sites adjacents, contrastés quant à leur enrichissement en Phosphate :

- Un site fortement perturbé, caractérisé par des déblais miniers de RP brut excavés et abandonnés à la surface du sol il y a un siècle
- Un site natif non perturbé par l'apport de RP excavé (Figure 13).

Les particularités de cette zone géologique permettent de disposer de sites contrastés quant à la teneur en RP et de cibler des environnements stables, à l'équilibre, c'est à dire non soumis à des perturbations récentes, notamment quant aux apports en RP. En effet, les phosphatières du Quercy se présentent comme des réseaux karstiques dont le remplissage naturel correspond à un mélange d'argiles (kaolinite, illite et des interstratifiés illite-smectite), de sables, de graviers, parfois de galets, d'ossements et de fragments du calcaire encaissant, avec la particularité d'être enrichies en phosphate (Cubaynes *et al.*, 1989). Chaque phosphatière propose un remplissage qui lui est propre où le minerai phosphaté, la phosphorite, peut contenir jusqu'à 38% de P_2O_5 (Bornuat, 2009). Le minéral de phosphore prédominant y est l'apatite (Slansky, 1975; Ruttenberg, 2003), sous forme de fluoro-apatite $Ca_5(PO_4)3F$, ou de carbonate fluoro-apatite $Ca_{10}(PO_4)_{6-x}$ $(CO3F)_x(F, OH)_2$ (Billaud, 1983).

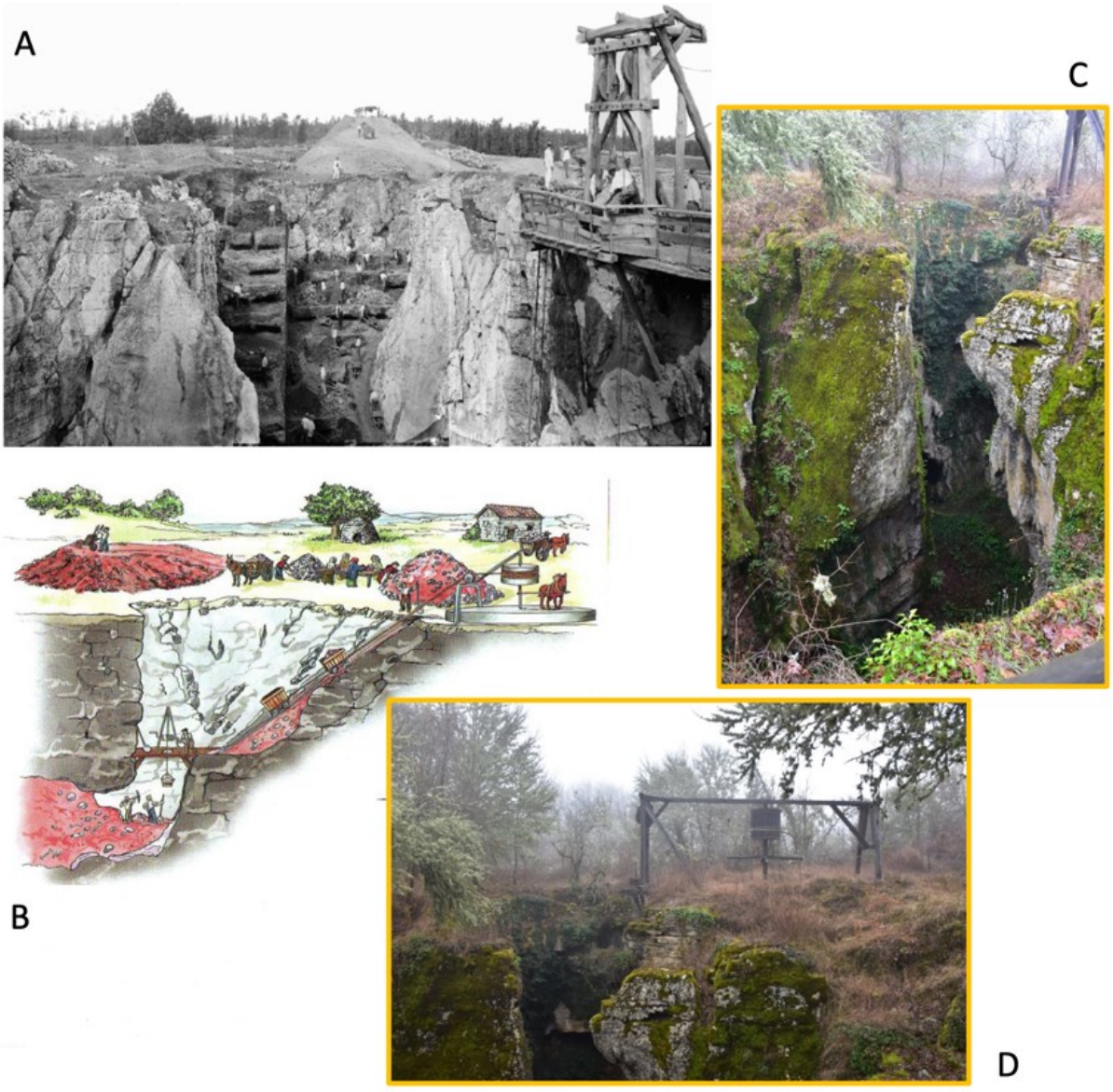
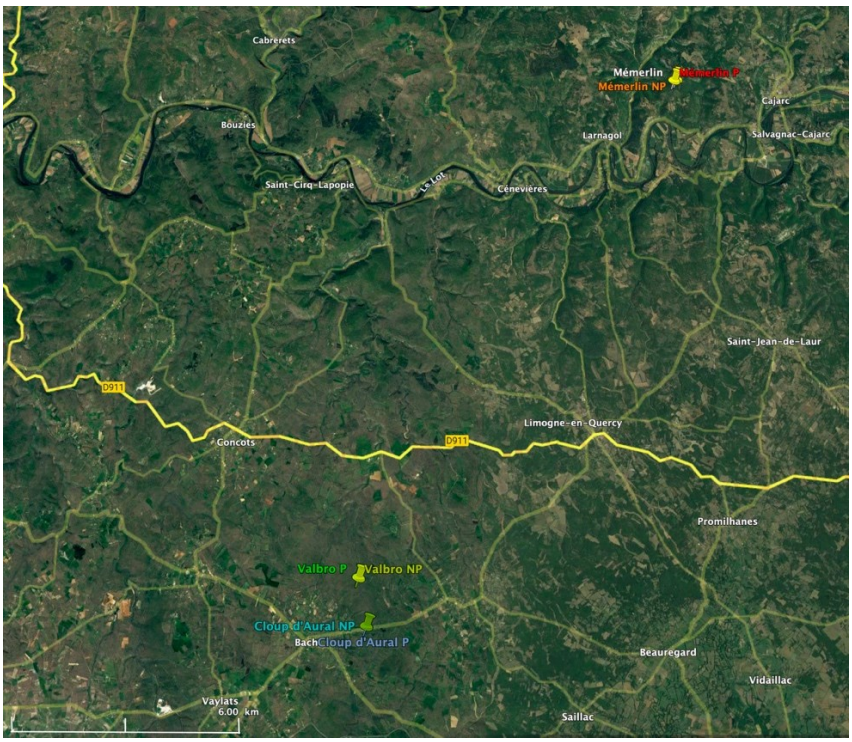


Figure 13 : Exploitation des phosphatières du Quercy
A : photographie d'époque ; B : schéma illustrant l'organisation de l'exploitation ; C : état des sites d'exploitation lors des échantillonnages en Janvier 2019 D : vestiges actuels de l'exploitation (extraits du projet de Réserve Naturelle Nationale d'intérêt géologique du département du Lot, 2013)

Dans ce contexte, trois localisations géographiques ont été retenues : Cloup d'Aural (L1), Valbro (L2) et Mémerlin (L3), chacune comportant deux sites d'échantillonnage, l'un enrichi en RP (i.e. site P), l'autre non enrichi en RP (site nP). Pour chacun des sites d'échantillonnage les données GPS ont été relevées (Figure 14).



MEMERLIN
Site P : 44°29'15,85''N ;
1°48'13,19''E
Site nP : 44°29'17,35''N ;
1°48'16,34''E

VALBRO
Site P : 44°21'44,65''N ;
1°41'16,02''E
Site nP : 44°21'44,24''N ;
1°41'16,43''E

CLOUP D'AURAL
Site P : 44°21'03,70''N ;
1°41'26,66''E ;
alt.322m

Site nP : 44°21'03,44''N ;
1°41'26,99''E ;

Figure 14 : Localisation géographique des Phosphatières du Quercy: Cloup d'Aural (L1), Valbro (L2) et Mémerlin (L3).

2. Plantes et sols échantillonnés

Sur chacun des six sites, quatre espèces végétales mycotrophes (Wang & Qiu, 2006), communes à tous les sites, ont été identifiées : *Ranunculus bulbosus, Bromus sterilis, Taraxacum officinale* et *Dactylis glomerata*. Trois plantes de chaque espèce ont été échantillonnées (Figure 15).
Pour chaque plante échantillonnée, le système racinaire a été prélevé. Les fractions « racines » (endosphère+rhizoplan) d'une part, et « sol rhizosphérique » d'autre part ont été soigneusement séparées (Figure 15).
Dans chacun des différents sites miniers étudiés, des isolements bactériens ont été réalisés afin d'identifier des PSB.

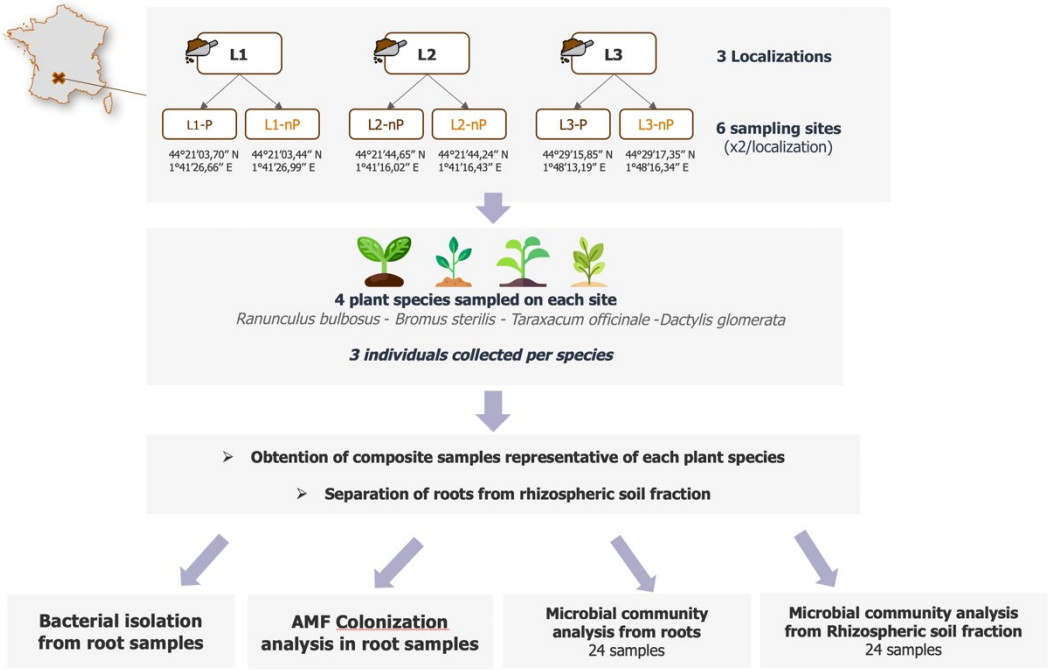


Figure 15 : Dispositif d'échantillonnage pour l'étude des communautés microbiennes des sols dans la région des Phosphatières du Quercy, France.

3. Isolement de bactéries hyphosphériques dans un écosystème agricole artificiellement enrichi en RP

Le dispositif d'isolement de bactéries hyphosphériques PSB a été réalisé en plusieurs étapes (Figure 16).

La première étape du dispositif avait un double objectif : 1. créer un environnement rhizosphérique riche en RP dans la rhizosphère d'une part, et 2. piéger des structures mycorhiziennes (hyphes et spores) au contact du RP d'autre part. Ainsi, différentes espèces végétales (pomme de terre, tomate, poireau, aubergine et maïs) ont été cultivées sur un sol de type Chernozem noir, dans une parcelle expérimentale du Jardin botanique de Montréal, réservée aux pratiques d'agriculture biologique (sans apport d'engrais chimiques notamment) (juin 2018). Puis des sachets (5 cm x 10 cm) faits d'une membrane de nylon (porosité 200 µm) contenant du RP ont été déposés à proximité des racines. Deux provenances d'échantillons de RP ont été utilisées : RP d'origine sédimentaire (Maroc) ou RP d'origine ignée (Canada). Après trois mois de culture sous ce dispositif, les sachets ont été retirés du sol et stockés (5°C). Leurs caractéristiques (provenance du RP et espèce végétale inoculée) ont été relevées.

La deuxième étape du dispositif d'isolement de bactéries hyphosphériques visait à intensifier la formation et la prolifération de spores mycorhiziennes dans les sachets. Ainsi, les sachets ont été réimplantés à la base de plantes cultivées en pots, sur un mélange stérilisé de vermiculite, sable et terreau (1 :1 :1). Après cinq mois de culture des plantes en conditions contrôlées (laboratoire de Calais ; température 23°C, lumière naturelle et artificielle pour une photopériode de 16h/8h), les sachets ont été de nouveau collectés. Des spores de CMA ont alors été isolées manuellement sous loupe binoculaire à partir de chaque sachet.

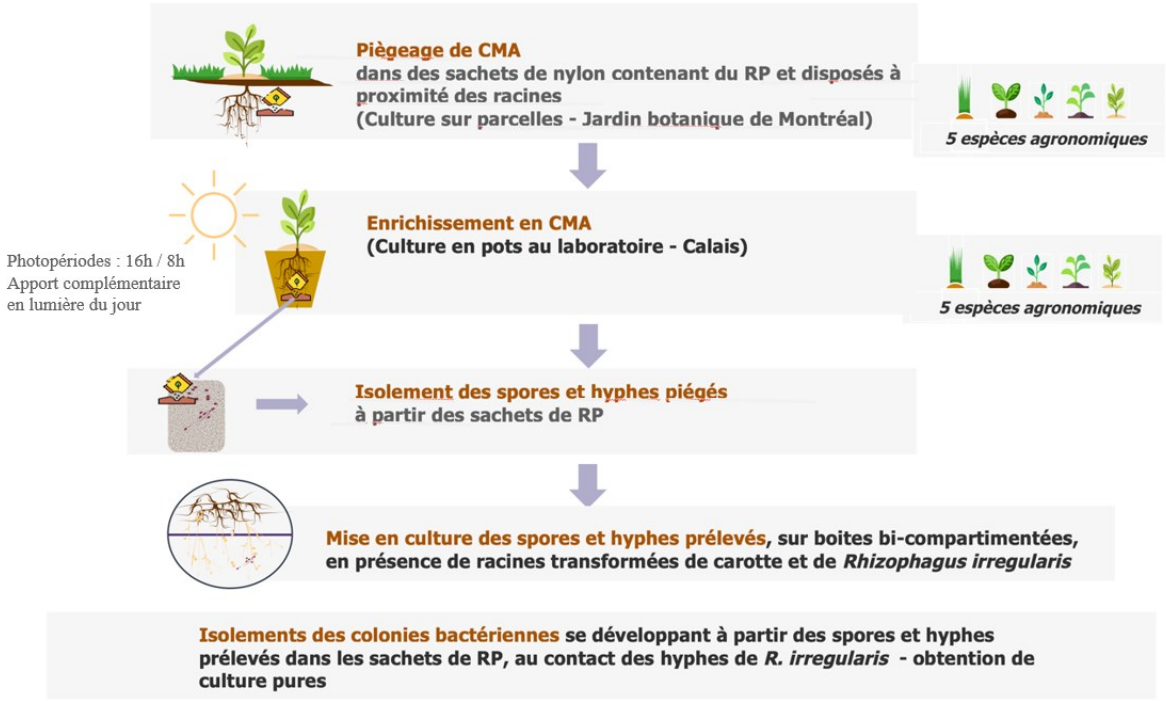


Figure 16 : Schéma du protocole d'isolement des souches bactériennes d'origine hyphosphériques.

Du piégeage in situ à l'aide de sachets contenant du RP sur différentes espèces végétales (*Solanum melongena L, Solanum lycopersicum L, Zea mays L., Solanum tuberosum L., Allium porrum L)*. Puis un piégeage en pots dans un substrat sableux, stérilisé pour l'enrichissement des sachets en CMA. Enfin la culture en boîte bi-compartimentée pour isolement bactérien sur les hyphes de *Rhizophagus irregularis.*

La troisième étape du processus visait l'isolement de bactéries hyphosphériques. Elle a été réalisée selon le dispositif expérimental décrit par St-Arnaud *et al.* (1995) puis repris par Taktek *et al.* (2017). Ce dispositif *in vitro* a été utilisé pour permettre l'isolement de bactéries ayant le potentiel de croître en utilisant les exsudats du mycélium extra-racinaire comme seule source d'énergie. Ainsi, *R. irregularis* a été cultivée sur des racines de carotte (*Daucus carota* L.), transformées par *Agrobacterium rhizogenes* (Riker *et al.*, 1930; Conn, 1942) dans des boîtes de Pétri à double compartiment : un compartiment contenait les racines de carotte transformées mycorhizées sur 20 mL d'un milieu M (Bécard & Fortin, 1988) avec 0,4 % (P/V) de Phytagel (Sigma). Le second compartiment contenait 20 mL de milieu M sans aucune source de carbone ni de vitamines. Il a été maintenu exempt de racines en les coupant régulièrement pour permettre uniquement la croissance des hyphes extra-racinaires du CMA dans le compartiment fongique. Les boîtes de Petri ont alors été incubées pendant 5 semaines à 25ºC dans l'obscurité jusqu'à ce que les hyphes colonisent le deuxième compartiment.
Les spores préalablement piégées à l'issue de la deuxième étape de culture en serre ont alors été déposées sur les hyphes pour permettre le développement des bactéries associées. Les boîtes de Pétri ont ensuite été incubées pendant 5 jours à 25ºC, et observées quotidiennement pour vérifier la viabilité des hyphes. Les colonies capables de se développer le long des hyphes de *R. irregularis*, sans aucun dommage visible pour ces derniers, ont été isolées et purifiées par ensemencements successives sur un milieu de gélose tryptique de soja à 10 % (TSA, Laboratoires QueLab, Canada) avant de poursuivre la caractérisation pour des traits PGP. Ces bactéries sont qualifiées d'« hyphosphériques » dans la suite du mémoire.

CHAPITRE 1

A. Réponse adaptative du microbiote de la rhizosphère et des racines à une contrainte édaphique rencontrée dans des sols miniers à fortes concentrations en RP, soumis à une restauration écologique spontanée : Diversité taxonomique du microbiote de sites riches en RP et analyse de l'impact du RP sur cette diversité

Synopsis

Les travaux décrits dans les sections A et B de ce manuscrit, pose pour hypothèse, sur la base des données de la littérature, qu'au sein du méta organisme plantes-bactéries-champignons-CMA, l'enrichissement d'un sol en RP peut générer des réponses adaptatives des communautés microbiennes, éventuellement différentes dans les compartiments rhizosphériques et racinaires.

Dans un premier temps, mon travail de thèse a nécessité la recherche et l'identification de sites naturellement riches en RP au Canada, au Maroc et en France. Notre choix s'est porté sur la région des Phosphatières du Quercy, en France, un site protégé car labellisé Parc Naturel Régional et Géoparc Mondial de l'UNESCO. Des autorisations d'échantillonnage auprès de la Préfecture du Lot étaient donc nécessaires pour effectuer des prélèvements.
J'ai ainsi mené l'échantillonnage de sols et racines sur divers sites où l'histoire minière permettait d'envisager des teneurs contrastées en RP, liées à de très anciens apports de RP sous forme de déblais de roches de phosphorites, résultant des modalités d'exploitation de sites miniers au cours du 19ème siècle.

Mon questionnement a été le suivant :

- ✓ Quelles sont les caractéristiques édaphiques des sols échantillonnés, notamment leurs teneurs en RP, P total et biodisponible ?
- ✓ Quelles sont la structure et la composition taxonomiques des communautés bactérienne, fongique et mycorhizienne des compartiments rhizosphériques et racinaires soumises à fortes concentrations en RP durant plus d'un siècle ?
- ✓ Des concentrations en RP et P contrastées conduisent-elles à des communautés microbiennes contrastées avec apparition de taxons dominants ou spécifiques des environnements échantillonnés ? Afin de répondre à ces questions, nous avons adopté une approche métagénomique, via le séquençage à haut débit d'amplicons et la technique Illumina MiSeq, suivi d'une analyse bio-informatique et statistique des données relatives aux différentes communautés, pour des analyses taxonomiques basées sur les Amplicon Sequence Variants (ASV, 100% d'homologie). Les résultats obtenus ont donné lieu à deux articles scientifiques présentées dans les sections A puis B.

Ces travaux sont présentés ci-après sous forme d'article scientifique :

Article 2 : Amandine Ducousso-Détrez, Simon Morvan, Joël Fontaine, Mohamed Hijri, Anissa Lounès-Hadj Sahraoui, How high phosphate concentrations affect soil microbial communities after a century of ecosystem self-reclamation ? Land Degradation & Development (soumis)

Article 3 : Amandine Ducousso-Détrez, Robin Raveau, Joël Fontaine, Mohamed Hijri, Anissa Lounès-Hadj Sahraoui, Glomerales dominate arbuscular mycorrhizal fungal communities associated with spontaneous plants in phosphate-rich soils of former rock phosphate mining sites. Mircoorganisms, (Soumis)

Résumé des principaux résultats obtenus :
L'analyse des diversités alpha et bêta des communautés bactériennes et fongiques mettent en évidence une différence significative entre les compartiments racinaire et celles du sol. Cependant, aucune différence significative n'a été détectée entre les différents sites d'échantillonnage, qu'ils soient enrichis ou non en RP, que l'on considère les communautés bactériennes, fongiques totales ou mycorhiziennes.

En outre, les données acquises montrent que les profils des communautés au niveau taxonomique le plus élevé (phylum) est persistant malgré les grandes variations environnementales des teneurs en RP et P. Ainsi, les communautés bactériennes sont dominées par les phyla des Actinobacteriota et Proteobacteria tandis que ceux des Ascomycota et Basidiomycota prédominent dans la communauté fongique, quels que soient les sites d'échantillonnage. De même dans l'ensemble des sites, les communautés de CMA sont principalement dominées par les Glomerales, et dans une moindre mesure par les Diversisporales. Un core microbien de 26 ASVs, persistantes à travers le gradient de RP et P, a également été mis en évidence. Des variations dans les abondances relatives des ASV mycorhiziennes du core microbien, ainsi qu'une partition des ASVs selon les sites enrichis ou non en RP ont toutefois été relevées. En particulier, une réduction ou même la disparition des ASVs assignées aux Archaeosporaceae et Paraglomeraceae dans les sols enrichis en P ont été mises en évidence.

Par ailleurs, la recherche d'espèces indicatrices a permis d'identifier pour chaque communauté, des ASV spécifiques soient des sites enrichis, soit des sites non-enrichis en PR, avec une affiliation taxonomique pouvant aller jusqu'au genre voire l'espèce.

L'influence du P comme moteur du profil des communautés microbienne est analysé. La signification écologique de la prévalence de certains taxa est discutée, notamment au regard d'une part de l'histoire minière du site et sa restauration écologique naturelle depuis un siècle et d'autre part en s'appuyant sur des données de la littérature concernant les stratégies et traits de vie des taxons microbiens.

ARTICLE 2 : Diversité taxonomique des communautés bactériennes et fongiques dans des sols riches en RP

Ce paragraphe est présenté sous forme d'un article scientifique, article soumis à la revue Land Degradation & Development.

Title: How high phosphate concentrations affect soil microbial communities after a century of ecosystem self-reclamation ? Land Degradation & Development

Authors: Ducousso-Détrez Amandine [1,2], Fontaine Joël[1], Hijri Mohamed[2,3], Lounès-Hadj Sahraoui Anissa[1]

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Abstract:
The use of rock phosphate (RP) instead of soluble phosphate fertilizers is preferred for the development of more sustainable agriculture. However, the impact of high concentrations in RP on bacterial and fungal communities remains poorly documented. Thus, next-generation sequencing was used to characterize bacterial and fungal communities in the soils and roots of four plant species *Ranunculus bulbosus* L.*, Bromus sterilis* L.*, Taraxacum officinale* F.H. Wigg. and *Dactylis glomerata* L. growing naturally in a self-restored ecosystem, on three former open-pit phosphate mines where past exploitation generated locally a substantial phosphate enrichment of the soil. Our results show that bacterial communities are dominated by Actinobacteria and Proteobacteria phyla, while the Ascomycota and Basidiomycota phyla predominate in the fungal community. The alpha and beta diversities of both bacterial and fungal communities differ significantly between the root and soil compartments but are not significantly affected by RP inputs. However, Amplicon Sequence Variants (ASVs) indicative of RP-enriched soils have been identified; among them ASV143-*Agromyces*, ASV285-*Actinophytocola*, ASV432-*Actinoplane* for bacteria and ASV17-*Epsilon*, ASV176-*Columnaris*, ASV439-*Glutinans* for fungi. These findings enable to develop and formulate microbial-based bioinoculants for sustainable agriculture applications based on more adapted microorganisms to high concentrations of RP.

Key Words: Rock phosphate, Phosphate Mining, Ecosystem restoration, Microbial diversity, Metagenomic

INTRODUCTION

Phosphate fertilization as soluble P (mainly as PO_4^{2-} ions), has been used in intensive agricultural systems to contribute significantly to current global food production and security. Thus, for decades, these agricultural soil amendments have contributed to increase the total phosphorus (P) stock of soils. Consequently, P amendments have significantly increased for replenishing the soil P pool in adequate levels of orthophosphate ions. However, most agricultural soils may contain more important total P stocks. Indeed, it is estimated that only a small fraction (0.1%) of the total P is immediately available for plants in a soluble P-form such as the orthophosphate ions, PO_4^{2-} (Rai *et al.*, 2013; Bünemann, 2015). Low P availability is a major productivity constraint in many natural or managed ecosystems. P-fertilization is mainly applied using chemical inputs derived from phosphate-rich rock deposits, mined for their high P_2O_5 grade, then industrially processed into marketable products. However, currently global reserves of high-quality RP are reaching alarmingly low levels (Cordell *et al.*, 2009; Cordell & White, 2011; Cordell & White, 2015; George *et al.*, 2016; Reitzel *et al.*, 2019). In addition, anthropogenic activities have altered the global P biogeochemical cycle and cause global environmental, social, economic and geopolitical challenges. In particular, environmental damages have emerged, such as water eutrophication or high accumulation of legacy P fixed to soil solid phase (Buczko & Kuchenbuch, 2007; Zhu *et al.*, 2018). Consequently, in order to avoid a potential global P crisis, a variety of strategies are invested. Among the potential strategic options, the valorization of soil resources and processes involved in soil P dynamics appears promising for improving P use efficiency in agronomic systems and for reducing the current waste of P fertilizers. Indeed, P-cycling and speciation into inorganic and organic compounds results from a set of interconnected physical, chemical and biological drivers (Chen *et al.*, 2021; Ducousso-Détrez *et al.*, 2022; Hu *et al.*, 2022). Increasing the capacity of plants to benefit from the total soil P stock, especially, its not directly available part, notably rock phosphate (RP) that has to be considered in combination with competent phosphate-solubilizing microorganisms (PSM) is a privileged research path for the development of sustainable agriculture. As biological activators of P cycling, numerous PSM, defined by their capacity to convert inorganic P and organic P into bioavailable forms facilitating uptake by plant roots, have been identified. This functional group is extensively and diversely studied (Rodríguez & Fraga, 1999; Sharma *et al.*, 2013; Kishore *et al.*, 2015; Alori *et al.*, 2017; Kafle *et al.*, 2019; Kalayu, 2019; Kour *et al.*, 2021; Raymond *et al.*, 2021; Tian *et al.*, 2021). It includes a wide range of organisms, among them being soil phosphate-solubilizing bacteria (PSB) (Gómez-Muñoz *et al.*, 2018; Mpanga *et al.*, 2018; Batool & Iqbal, 2019; Elhaissoufi *et al.*, 2020; Liu *et al.*, 2020) and phosphate-solubilizing fungi (PSF) (Leggett *et al.*, 2015; Elias *et al.*, 2016; Efthymiou *et al.*, 2018; Raymond *et al.*, 2018).

Concomitantly, the agronomic relevance of PSM for enhancing plant performance and agricultural yield has been proved. Current researches suggest their inoculation in soils to reduce the P fertilizer application rate by 50% without significant reduction of the crop yield (Jilani *et al.*, 2007; Rafi *et al.*, 2019). Thus, different types of microbial inoculum have been developed as P bio-fertilizer (Bashan *et al.*, 2014; Hijri, 2016; Lawson *et al.*, 2019). However, some uncertainties in the efficiency of inoculants in field applications are often underlined (Bargaz *et al.*, 2018).

For instance, is microbial P solubilization ability a trait linked to specific groups of soil taxa, rather than a general property of the overall soil microbial community? Do differences in the structural composition of a putative PSM community simply occur with changes in the overall microbial community with soil P levels, or is the selections pressure stronger for PSM taxa? A deeper understanding on how P shape the overall plant-associated microbiomes in the soil-plant-microbe continuum is required. Thanks to the progress of the next generation sequencing

(NGS) technologies several studies provide evidence that contrasting P fertilization regimes is a driver that shape microbial communities in interaction with plants (Silva *et al.*, 2017; Gomes *et al.*, 2018; Robbins *et al.*, 2018; Yu *et al.*, 2018; Fabianska *et al.*, 2019). To date work has focused on the influence of P sources (organic or inorganic) and concentrations in shaping microbial communities; the impact of RP has been notably analyzed (Pattanayak *et al.*, 2007; Silva *et al.*, 2017; Trabelsi *et al.*, 2017; Gumiere *et al.*, 2019). Indeed, the use of raw RP has been proposed in different agricultural systems as an alternative to reduce the use of industrial fertilizers (Zapata & Roy, 2004; Cordell *et al.*, 2009; Van Kauwenbergh, 2010; Richardson & Simpson, 2011). In addition, although RP has a lower reactivity than commercial fertilizers when applied directly in the field, P availability from these rocks can be increased through the action of the soil microbiota. Consequently, RP has been targeted as an effective agronomic product when applied directly to soils with microbial P-solubilizing inoculum to promote crop production by improving plant P acquisition (Manzoor *et al.*, 2016; Kaur & Sudhakara Reddy, 2017; Soltangheisi *et al.*, 2018).

Thus, the present study aims at investigating the patterns of native bacterial and fungal communities across natural sites inside a mining area, expected to be enriched in RP deposits at various grades, due to localized excavation of crude ores by past mining activities. We firstly proceeded to the characterization of soil geochemical properties to identify the major contrasted soil variables, notably the P levels. Using sequencing amplicons of the 16S rRNA gene and the ITS region and ASVs, we characterized the indigenous bacterial and fungal communities in the roots and rhizospheric soil of native plant species across soils “with” or “without” excavated RP ore inputs. Microbial diversity was evaluated in the different sampled soils using alpha and beta diversity indices. We then explored microbial community composition at different taxonomic levels. Indicator species analysis was also performed among communities of the different soils. The ecological significance of the microbial profiles observed is discussed in link to microbial response to RP-rich soils and ecosystem restoration after mining perturbations.

EXPERIMENTALS AND METHODS

Study area

The study was conducted in natural sites, located in the geographical area of the “Phosphatières du Quercy" (44° 22′ 22″ N, 1° 41′ 16″ E) near Bach, southwestern France. In this area, fillings of phosphorites, a phosphatic ore (containing up to 38% P_2O_5) (Bornuat, 2009) naturally stored in the clay of limestone plateaus of the Quercy paleokarst, were exploited from 1870 to 1907 for the production of P-fertilizers. Locally, due to mining practices, one-time ore inputs of excavated crude RP have been abandoned on the topsoil. Consequently, strips of strong ecologically disturbed soils (P-soils), enriched one century ago by excavated rocks including sedimentary phosphorite (e.g. deep soil layer brought in the surface as top soil), closely co-occurred side by side with native, non-disturbed soils (nP-soils). Following this single pulse of P nutrient via RP inputs, these mine lands were abandoned, re-colonized by spontaneous vegetation, and naturally revegetated during one century, without any notable anthropogenic disturbance until today.

Thus, in the Phosphatières area, we selected three old mines: Cloup d’Aural (L1), Valbro (L2) and Mémerlin (L3). In each localization, two site profiles have been investigated: an undisturbed native site “without” ancient mining inputs (hereafter referred as nP-soil), and a well-established adjacent disturbed site “with” mining inputs of excavated phosphorite rocks (hereafter referred as P-soil. Thus, six sites (two paired sites per location) were identified and geo-referenced (Figure 1).

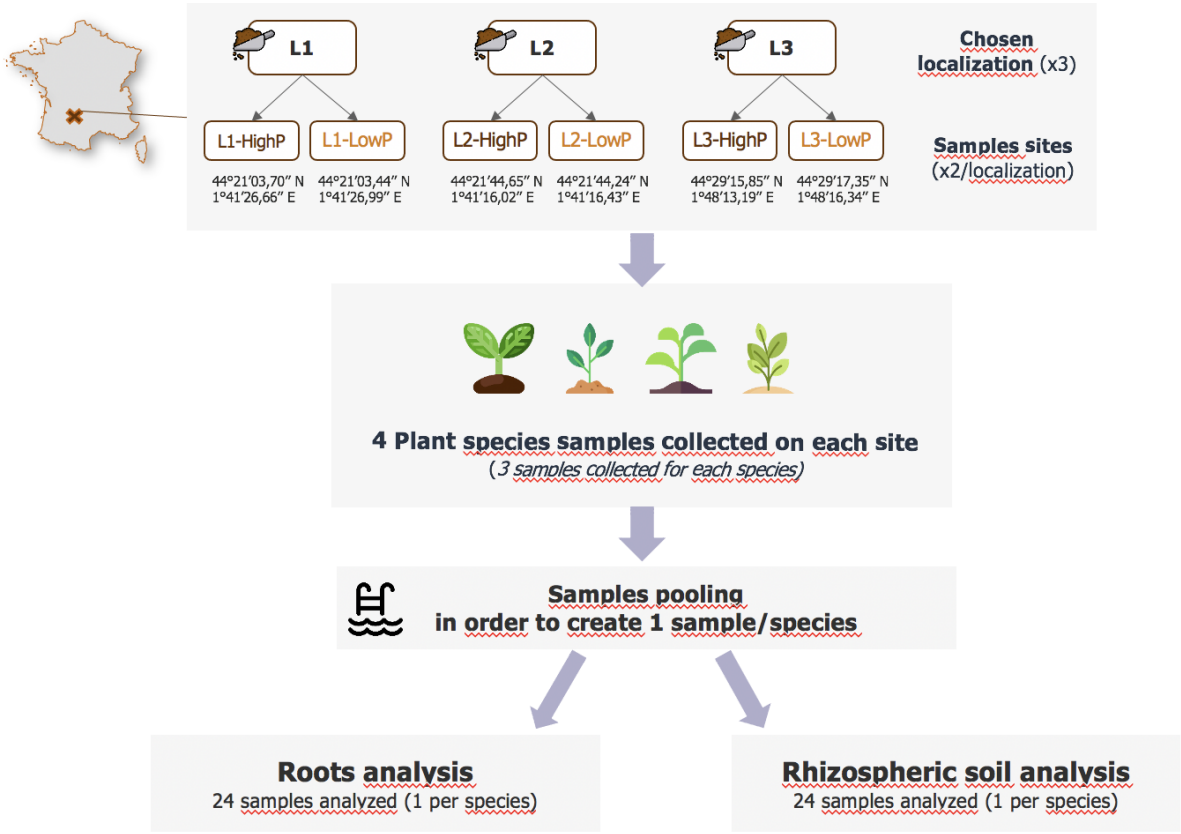

Figure 1: Study area and experimental setup

Harvesting of native plant species across six natural sites

At each of the six sites, the same set of native herbaceous species, were identified among the plant community present in each sites: *Ranunculus bulbosus* L., *Bromus sterilis* L., *Taraxacum officinale* F.H. Wigg. and *Dactylis glomerata* L. For each species, three individual plants were collected in each of the six studied sites. A total of 72 plants were harvested with their surrounding soil (5 - 20 cm deep) and transported quickly, in coolers, to the laboratory for further processing. The harvest took place on January 29, 2019.

Separation of root and rhizospheric soil samples

Two distinct fractions across the soil–plant continuum were harvested: (i) root samples, and (ii) rhizospheric soils closely associated with plant roots, referred as "soil" samples.

Each root sample was a composite of root fragments, collected from three plants per species and then pooled. The soil attached to the roots was removed by gentle agitation, and then the roots were washed in distilled water. Thus, four root samples per site were prepared, resulting in a total of 24 root samples for all six sites. For each root sample, one fraction was frozen at -20°C before subsequent molecular analysis.

In the same manner, each soil sample was a composite of soils collected from the three plants of the same plant species and subsequently pooled. The root fragments were removed from these samples. Consequently, we obtained 24 rhizospheric soil samples. These fresh samples were then immediately stored at -20°C until further DNA extraction.

Soil physico-chemical properties characterization

In each site, multiple soil fractions not adhering to plant roots were collected and then pooled to obtain a composite bulk soil sample; these were subsequently analyzed by the CIRAD-US

Analyze laboratory (Montpellier, France) for structural composition (granulometry: clays, silts and sands), pH and chemistry using notably, Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) measurements. To complement these data an X-ray fluorescence analysis (XRF Titan 800 S X-Ray Fluorescence) was carried on soil capsules pressed at 20T for 2 minutes, for the assessment of element concentrations from Mg to U.

DNA extraction from soils and roots

From the soil samples, total genomic DNA was extracted in triplicates (24 soil samples, 250 mg of soil per sample) using Nucleospin Soil R kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's instructions. Quality control of extracted DNA was performed on a 1% (w/v) agarose gels.

Genomic DNA from roots was extracted in triplicate using a method adapted from Abu-Romman (2011) and Aleksic *et al.* (2012). Briefly, roots were frozen in liquid nitrogen (–196°C) in a sterilized mortar and ground into fine powder. The ground roots (300 mg) were then subject to Cetyl trimethyl ammonium bromide (CTAB – 1.4 M NaCl, 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 2% CTAB), Polyvinylpyrrolidone (PVP 1% w/v), ß-Mercaptoethanol (5% v/v) and activated charcoal (0.5% w/v) extraction (30 min.; 55°C). After this incubation period, a centrifugation step was carried out (10 min.; 16,000 g) and the lysate extraction was then performed using two successive steps with chloroform: isoamylalcohol (24:1). DNA precipitation then occurred in the presence of isopropanol (1 h incubation; 25°C), followed by another centrifugation (10 min.; 700 g). The DNA pellet was then washed three times in a row by addition of ice-cold ethanol (70%) and centrifuged (10 min.; 900 g), before air drying at room temperature (approximately 90 min.; 20°C). Finally, the DNA pellet was dissolved in 50 mL of TE buffer (10 mM Tris-HCl, pH 8.0; 1.0 mM EDTA, pH 8.0).

For both types of samples (root or soil), the quality of the extracted DNA was verified using 1% (w/v) agarose gels. Quantification of extracted DNA was carried out on a Spectra Max R iD3 spectrophotometer (Molecular Devices LLC, Sunnyvale, CA, United States). The concentration of all samples was determined and DNA extracts were diluted to 25 $ng.mL^{-1}$ for further analyses. The extracted DNA was stored at –20°C until use.

PCR reactions, amplicon library generation and sequencing

In order to profile the bacterial communities, we used the primer pair 341(F): 5'-CCTACGGGNGGCWGCAG-3', and 805(R): 5'-GACTACHVGGGTATCTAATCC-3' targeting the V3–V4 hypervariable region of the bacterial 16S ribosomal RNA (rRNA) gene, with an expected size of the amplicon of around 400 base pairs (bp), in length (Herlemann *et al.*, 2011; Mizrahi-Man *et al.*, 2013). The PCR amplification were performed with reaction mixtures (25 µL) contained 5 µL of Q5 (5X) reaction buffer and 0.25 µL (2 U μL^{-1}) of Q5® High-Fidelity DNA Polymerase (New England Biolabs France, Évry, France), 0.8 µL of forward and reverse primers (0.4 µM), 1 µL of dNTPs (0.2 mM), 1 µL of DMSO, 1 µL of Bovine Serum Albumin (BSA; 100 $\mu g.mL^{-1}$) and 1 ng of environmental DNA template. The thermal profile used in order to obtain bacterial rDNA amplicons was as follows: preheating 95°C for 3 min. of initial denaturation, followed by 35 cycles at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 50 s and final extension step at 72°C for 5 min.

Regarding the fungal communities, the PCR amplification targeted the internal transcribed spacer (ITS) segments located between the 5.8S and LSU genes of the ribosomal RNA, using the primers ITS3_KYO2 (F): 5'-GATGAAGAACGYAGYRAA-3', and ITS4_KYO3 (R): 5'-BTTVCCKCTTCACTCG-3' (Toju *et al.*, 2012). Although this region can vary in length (Sietiö *et al.*, 2018), the primer pairs ITS3_KYO2/ITS4_KYO3 generate amplicons of around 430 bp (Toju *et al.*, 2012). The reaction mixture (25 µL) included 5 µL of Q5 (5X) reaction buffer and

0.25 µL of Q5® High-Fidelity DNA Polymerase (New England Biolabs France, Évry, France), 0.8 µL of forward and reverse primers (0.4 µM), 1 µL of dNTPs (0.2 mM), 1 µL of DMSO, 1 µL of BSA (100 $\mu g.mL^{-1}$) and 1 ng of environmental DNA template. The thermal cycling conditions for preparation of fungal ITS rDNA amplicon libraries were performed with an initial denaturation for 5 min. at 95°C, followed by 35 cycles of 94°C for 20 s, 47 °C for 30 s and 72°C for 20 s, and with a final extension step at 72°C for 7 min.
All primers used for PCR amplification were coupled with CS1 (5'-ACACTGACGACATGGTTCTACA-3') and CS2 (5'-TACGGTAGCAGAGACTTGGTCT-3') adapters at the 5' end. All the amplification reactions were conducted with PCR thermocycler (Agilent Surecycler 8800) and quality control for band size of the amplicons was operated on 1.5% agarose gel electrophoresis before sequencing of libraries.

The triplicate of each amplified PCR samples with adapters were pooled together and sent to the Genome Quebec Innovation Centre (Montreal, QC, Canada) for barcoding and sequencing using an Illumina MiSeq sequencer producing paired-end reads of 2 x 300 bp in length.

Bioinformatic processing for sequencing data analysis
Bioinformatic analyses were performed using the R 4.0.2 software (R Core Team, 2019). The DADA2 pipeline (v. 1.16) (Callahan *et al.*, 2016) was used to process the different sets of paired-end sequences of each gene data set and to infer ASVs. Following the visualization of quality profiles, a filtration step of reads was performed to eliminate poor quality sequences, non-merging sequences as well as chimeric sequences. Subsequently, the ASVs obtained from the DADA2 pipelines were filtered for very low-abundance ASVs before any further analysis: sequences present only once (singletons) or twice (doubletons) in the whole data set were eliminated across the microbial profiles (Wen *et al.*, 2017). Rarefaction curves, which show the observed ASVs numbers as a function of sequencing effort, were computed with the function 'rarecurve' from the Vegan R package to estimate if sequencing depth was sufficient to capture the whole diversity present. ASV count tables were generated using usearch_global from USEARCH package and the taxonomic assignment was carried out using the assignTaxonomy() function which implements the RDP naive Bayesian classifier method described in Wang *et al.* (2007). The Silva v132 database formatted for the DADA2 (Callahan *et al.*, 2018) was used to assign bacterial 16S rRNA gene sequences from kingdom to genus (minimum bootstrap 80). The UNITE web-based database was used to assign fungal taxa (Kõljalg *et al.*, 2013).

The gene sequences of the whole dataset have been deposited in NCBI Sequence Read Archive (SRA) database and can be found under the project accession number PRJNA784523.

Microbial community analysis
Statistical analyses (R 4.0.2 software, R Core Team, 2019) were run independently for bacterial and fungal communities. The impacts of compartmentalization (root *vs* soil) and P status (P-soil *vs* nP-soil) were tested; comparisons were performed either between samples of the same localization or by pooling localizations together and comparing soil P contents.

Diversity measures: alpha diversity and beta diversity statistics.
The local alpha diversity (Whittaker, 1972) was estimated in each sample by computing the Chao1, Shannon and Simpson statistics (Shannon, 1948; Simpson, 1949; Kim *et al.*, 2017) from the plot_richness() function from the phyloseq package (McMurdie & Holmes, 2013). No read depth normalizing or supplementary rarefaction were performed to more fully capture the

diversity and keep a maximum of ASVs. The sample data were graphed using ggplot2 to obtain boxplot (Wickham, 2016). Ranks of the alpha diversity indices were subjected to ANOVA test with the significance determined by a permutation test using the aov() function of the Vegan package (Oksanen *et al.*, 2018), followed, if required, by a post hoc Tukey's Honest Significant Difference (HSD) test ($p<0.05$) allowing pairwise comparisons to assess the effect of plant compartment and soil RP contents.

Beta diversity, which is the variations in microbial community structure among samples, was measured and visualized by principal coordinate analysis (PCoA) based on the Hellinger distances as dissimilarity measure. Permutational multivariate analysis of variance (PERMANOVA) was performed to statistically compare the clusters of samples and to test for significant differences among centroids, using adonis2 function in the Vegan package (significance tested by 9999 permutations). In addition, Permutest.betadisper function was used to consider the multivariate homogeneity of group dispersions (*i.e.* distance of group members to the group centroid) when interpreting the results of PERMANOVA. The statistical significance for all tests was set at P-values <0.05.

Variations in microbial taxonomic composition

The R package *metabarcoder* was used to parse, manipulate and visualize in a tree format using taxonomic classification, how samples vary in their taxonomic composition. Thus, to display differences in abundance for each taxon, at every taxonomic rank, between two sample groups, the compare groups() function in metacoder package was used after calculating the per-taxon abundance from the ASV read counts with the calc_taxon_abund() function. The Wilcox rank-sum test was computed to test for significance of difference between the median abundances of samples in each treatment followed by a Benjamini-Hochberg (FDR) correction for multiple comparisons. For visualization, the standard heat_tree() function was used to compute differential heat trees to quantitatively depict statistics associated with taxa and display, across pairwise comparisons, in which sample each taxon is more abundant in, using the color and the size of nodes and edges in a taxonomic tree colored with a diverging color scale (Foster *et al.*, 2017; Iburg *et al.*, 2021). The statistic used for plotting was the log2 of the ratio of median proportion of reads in the two samples groups compared.

Indicator species analysis

The indicator taxa are defined based on a combination of specificity (occurring in that environment more frequently than other environments) and fidelity (the majority of taxon members are found in that environment). The multipatt() function from indicspecies package was used on the ASV abundance data table (De Cáceres & Legendre, 2009). An indicative species value (IndVal's p_values with the p.adjust() function computed from 9999 permutations; significant at the $\alpha = 0.05$ level) was assigned to prospective indicative species based on their importance in the group they are found to be indicative of.

RESULTS

Soil characterization

In our 3 different mining areas each containing one site with mining inputs (P-soils) and one control site without mining inputs (nP-soils)(Figure 1), we did not find any significant differences across sites for pH which was close to near neutrality, ranging 6.9 and 7.3. When comparing sites from the same mining areas, significant differences were observed for P and Ca contents between P-soils and nP-soils. As phosphorus is mainly in the form of tricalcium

phosphate in these soils, it seems normal that variations in Ca concentrations follow to variations in P concentrations. The P soils had total P contents ranging from 2,880 to 13,927 $mg.kg^{-1}$ while nP-soils ranged from 1,057 to 1,496 $mg.kg^{-1}$. We also found clear differences in terms of available Olsen P levels were also noticed with higher concentrations in P-soils ranging from 46 to 339 $mg.kg^{-1}$ compared to nP-soils (ranging from 5 to 13 $mg.kg^{-1}$). Soil analysis highlighted presence of trace elements: chromium 176 to 227 ppm, nickel 38 to 52 ppm, arsenic 23 to 42 ppm and lead 35 to 50 ppm which are classically found in RP as minor constituents. Metal trace element contaminants such as cadmium, mercury or vanadium were below the limit of detection of our equipment (Table S1). However, no significant differences between P-soils and nP-soils were found for these trace elements.

Descriptive results of bioinformatic data.

To characterize the fungal and bacterial communities in the rhizospheric soil and root samples across the six sites, the metabarcoding approach *via* Illumina MiSeq sequencing resulted in a total of 4,890,709 reads for the bacterial 16rRNA gene and 3,642,907 reads for the fungal ITS region over the sample set (*i.e.* 24 soil samples and 24 root samples) (Table S2). Following the visualization and correction of the quality profile plots of reads with DADA2's filtering function, dereplication then merging of sequences, a total of 6,720 ASVs were inferred from the 16S rRNA gene data. The ITS data were clustered into 6,664 ASVs (Table S2). Information about sequencing depth for both 16s rRNA and ITS gene data are available from rarefaction curves for each sample (Figure S1-S2).

Alpha diversity in P-soils and nP-soils and in rhizospheric vs root compartment.

Computing the Chao1, Shannon and Simpson alpha-diversity metrics, we compared microbial diversity according to the plant-associated compartments (root *vs* rhizospheric soil and according to the RP status (nP-soils *vs* P-soils) across the six sampled sites (Table S3). Collectively, the results showed that fungal alpha diversity values were generally lower than the bacterial ones within each compartment, no matter the metrics (Table S3). Besides, the Shannon and Simpson indices of bacterial communities were not significantly impacted by RP status (Figure 2A). Similarly, fungal diversity indices did not differ significantly comparing P-soils and nP-soils (Figure 2B).

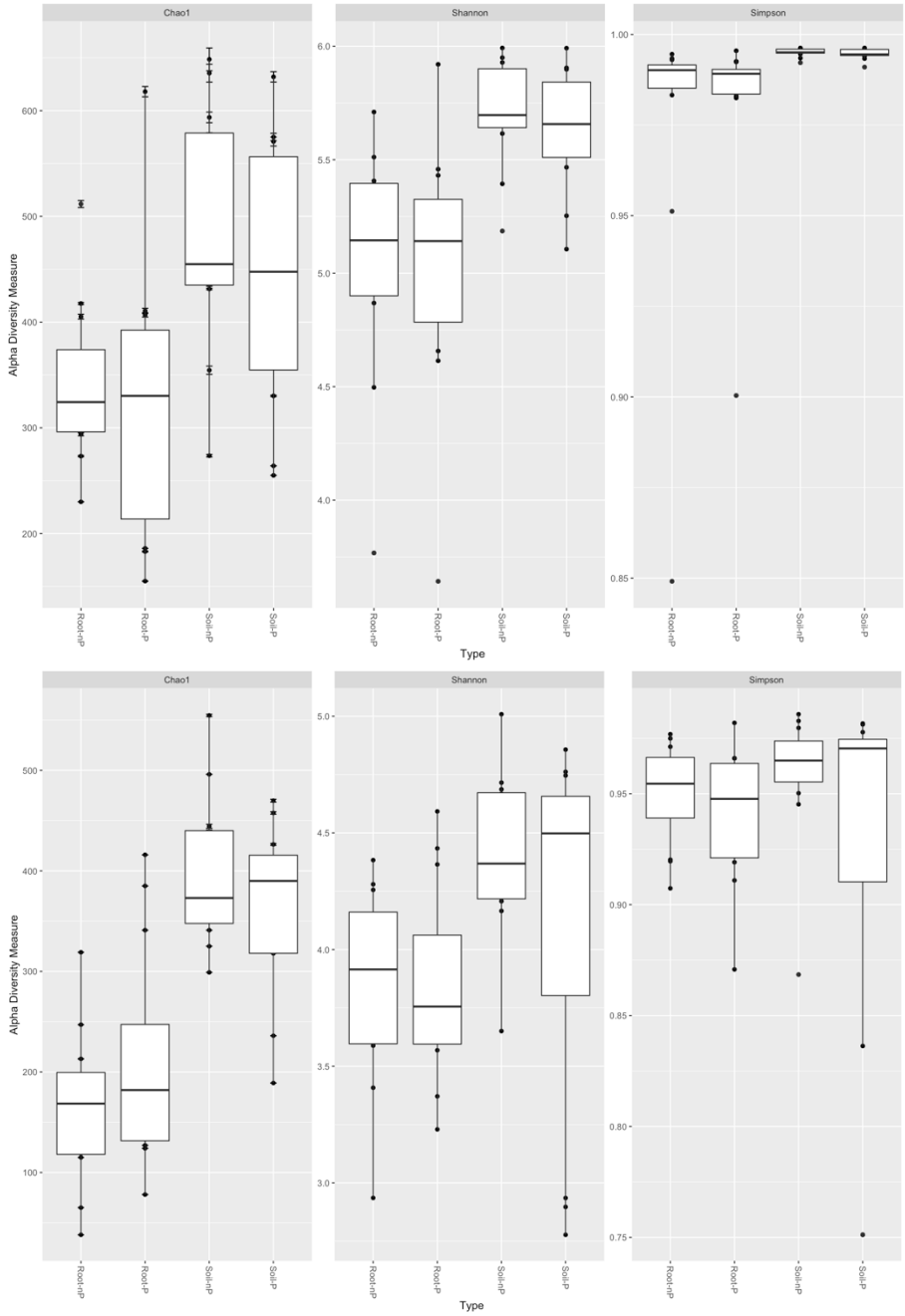

Figure 2: Alpha-diversitiy of the over-all 16S rRNA bacterial gene communities (A) and the over-all fungal ITS communities (B) communities, as measures by richness (number of ASVs, Chao1, richness Shannon (bacteria p.value = 7.126e-05; fungi p.value = 0.0899 and Simpson indices (bacteria p.value = 0.117; fungi p.value = 0.472), within rhizospheric soil and root compartment sampled in P and nP sites.
P: with mining RP ore deposit; nP: without mining RP ore deposit.

In contrast, significant variations in alpha diversity indexes were observed across the rhizospheric soil relative to the root-compartment. For the bacterial communities, higher Shannon and Simpson values were significantly noticed in rhizospheric soil, performing global analysis. However, Simpson metrics which are known to give less weight to rare ASVs, were not significantly affected by plant compartment effect. Moreover, analysis of the fungal dataset at local level showed the Shannon indices were significantly influenced by the nature of the compartment in L1 localization.

Beta diversity in P-soils and nP-soils and in rhizospheric *vs* root compartment.
Bacterial sample beta diversity were visualized using an ordination by Principal Coordinate analysis (PCoA) highlighted that the first axis, explaining 14.9% of the variance, identified two clusters on either side of the central vertical axis, corresponding to either soil or root samples (Figure 3A). Additionally, the PERMANOVA test indicated a significant effect of the compartment on the bacterial beta diversity.
A similar segregation pattern was observed for fungal beta diversity but the variation fraction attributable to compartment did not exceed 8.9% (Figure 3B).

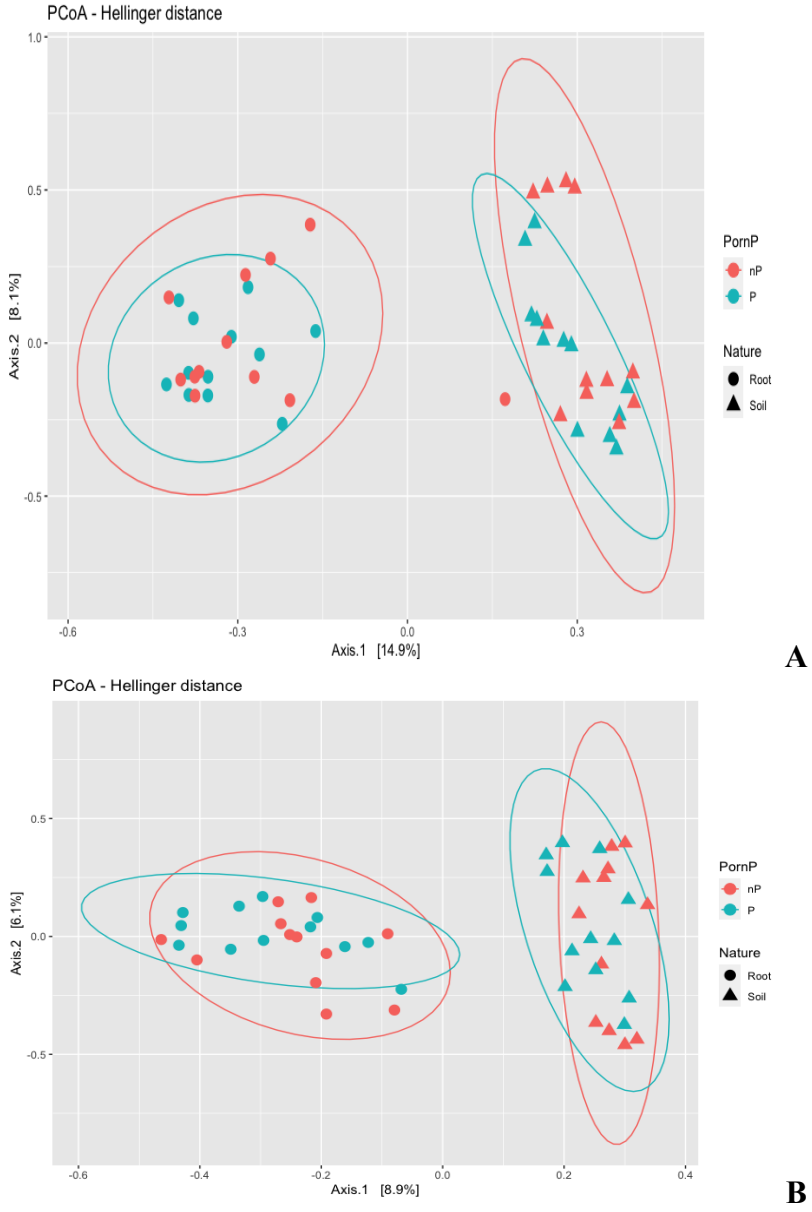

Figure 3: Beta-diversity analysis among the over-all data set: PCoA ordination, based on Hellinger distances, of bacterial 16S rRNA gene sequence data (A) with p value=0.024 and fungal ITS sequence data (B) with p value=0.0168.

Next, we investigated if a fraction of beta diversity was attributable to soil P-status. Indeed, a significant effect of P-status on bacterial and fungal community clustering was observed when assessing its effect from the over-all data set (respectively p value=0.024 and p value=0.0168). In contrast, when comparing matched soils from the same location, the assessment of its impact on fungal community diversity was significant in L1 and L2 (Figure 4), but not in L3. Futhermore, P-status did not have a significant effect on the bacterial communities ordination, when comparing matched soils, regardless of location.

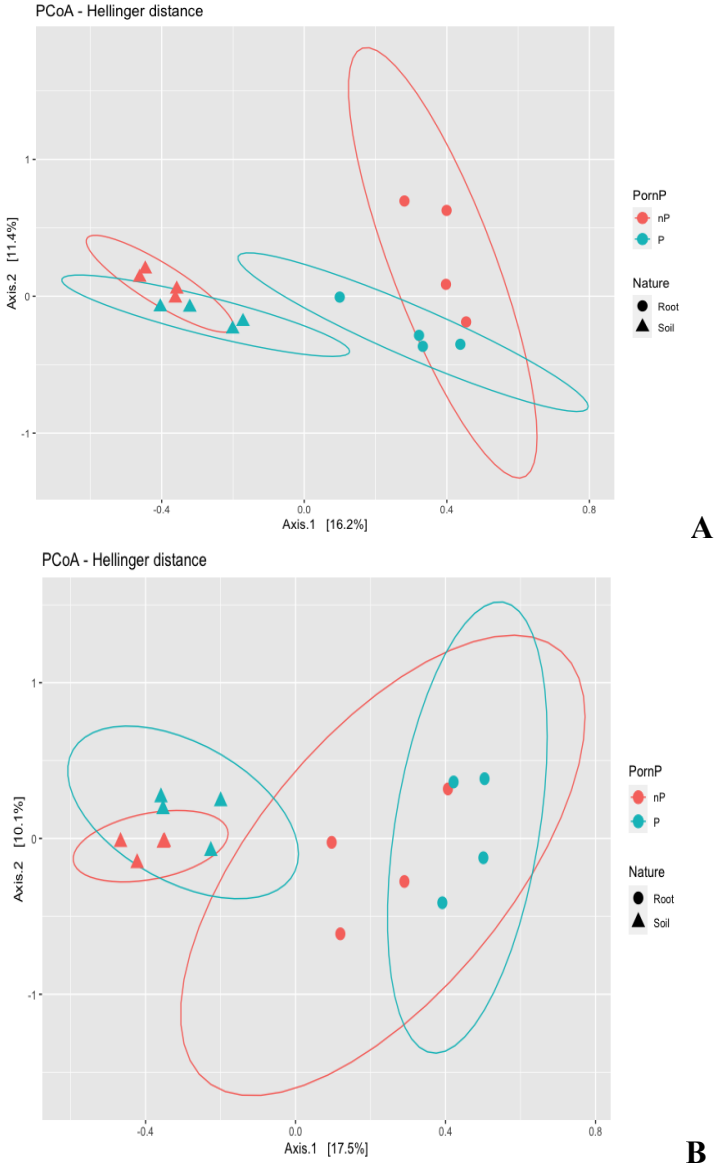

Figure 4: Beta-diversity analysis in pairwise soil comparisons: PCoA ordination, based on Hellinger distances, of fungal ITS sequence data when comparing (A) L1-P *vs* L1-nP soils within L1 localization (8 samples) (p value =0,0351), (B) comparison L2-P *vs* L2-nP soils within L2 localization (8 samples) (p value =0,0345)
P: with mining RP ore deposit; nP: without mining RP ore deposit

Global taxonomic composition of the overall bacterial and fungal communities.
From the overall bacterial ASVs obtained across the overall samples (48), 28 bacterial phyla were identified; 15% of the ASVs were non-assigned (NA) to a phylum. Based on the relative read counts, the prevalent phyla were Actinobacteriota (71%) and Proteobacteria (20%) (Figure

5A). Bacteroidota, Myxococota and Gemmatimonadota phyla were also identified, but are less abundant (less than 2.5%), as well as Acidobacteriota, Firmicutes, Verrucomicrobiota and Entotheonellaeota (less than 1% each). A total of 54 classes were recovered and among them, 6 are dominant: Actinobacteria is the major class (46%) while Thermoleophilia and Alphaproteobacteria reached around 15% and 13% respectively; Acidimicrobiia and Gammaproteobacteria accounted for 7% each (Figure 5C). The other classes reached less than 3% and the reads NA to a class represented about 1%. Among the 126 orders recognized, the most abundant are Micromonosporales (15.9%), Rhizobiales (8.5%), Solirubrobacteriales (8.3%), Propionibacteriales (7.9%), Gaiellales (5.7%) and Streptomycetales (5.2%). Non-assigned ASVs at the order level accounted for 4.75% of the reads.

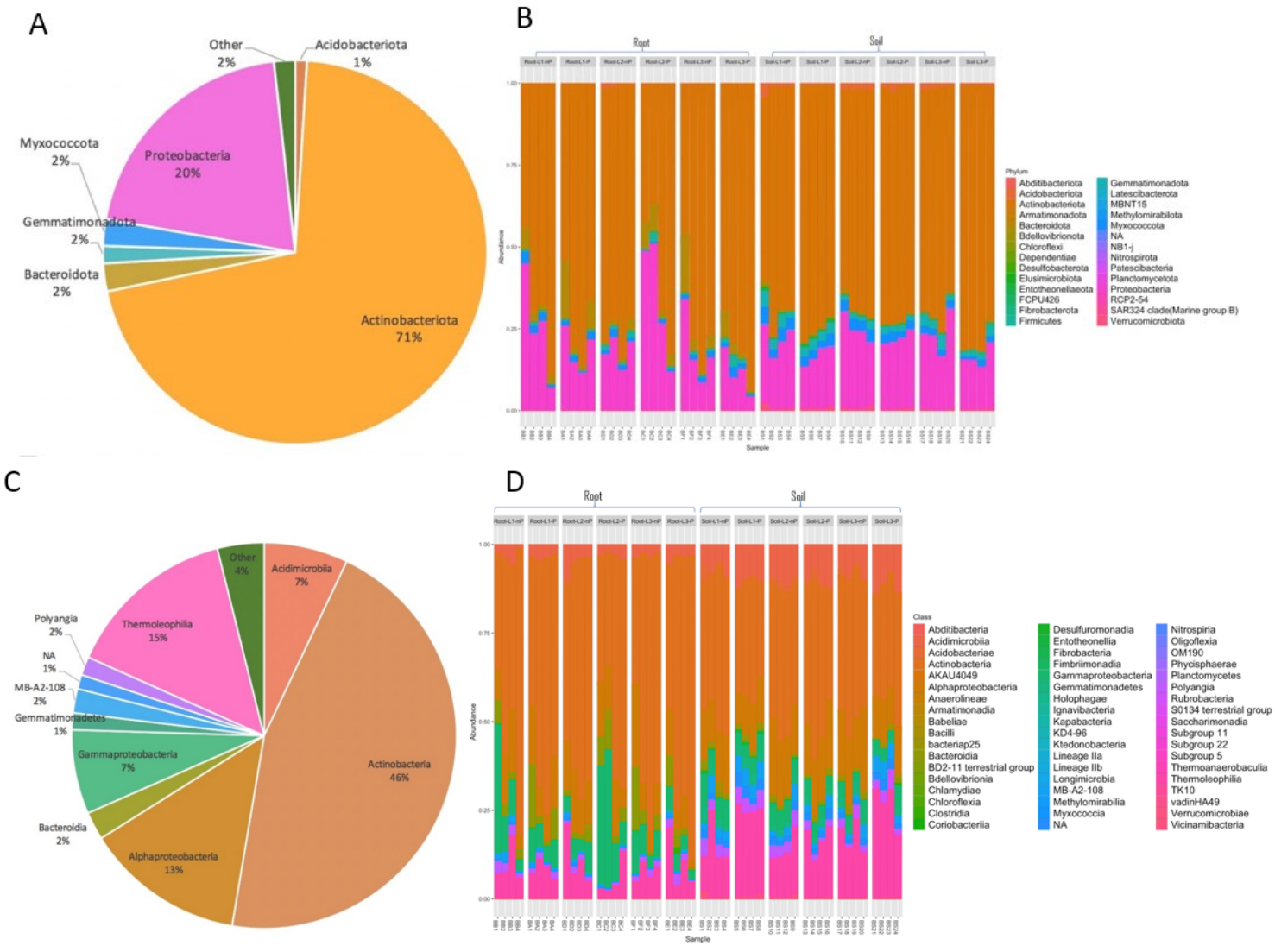

Figure 5: Taxonomic distribution patterns of the bacterial population at phylum (A, B) and class levels (C, D)
A and C: Distribution of the global bacterial community (overall populations from the 48 samples pooled) at phylum level (A) and across the major classes (*i.e.* more than 1% of total reads) (C).
B and D: Relative abundance profile (percentage of total 16S rRNA gene sequences) at the phylum level (B) and across the major classes (D) of bacterial 16S rRNA gene amplicon data, for each soil and root samples at each sampled site.
Relative abundance was computed across the overall bacterial data set. 'NA" category: ASVs that did not obtain taxonomic assignment at phylum or class level; "Other" category: mix of taxa with low abundance (less than 1%).

From the amplicons obtained with the primers targeting the fungal ITS region, NA ASVs to a phylum represented about 12% of the community in terms of relative abundance. Ascomycota was by far the most prevalent fungal phylum in all samples (63%) (Figure 6A). Basidiomycota represent 22% of the overall community profile. Agaricomycetes (23.3%), Sordariomycetes (21.9%), Eurotiomycetes (20%), Leotiomycetes (14.2%) and Dothideomycetes (8.1%) are the major classes observed, whereas NA at class level reached 5.1%. The major orders are Helotiales (13.6%), Chaetothyriales (9.9%), Hypocreales (9.1%), Agaricales (8.3%), Sebacinales (8.2%), Eurothiales (8%) and Pleosporales (6.3%) with 14.6% of NA (Figure 6C). Only about 2% of ASVs were affiliated to the phylum Glomeromycota, and only AMF-inferred to the Glomerales order, then to the Claroideoglomeraceae and Glomeraceae families, were identified using the ITS3_KYO2/ITS4_KYO3 PCR primers. Around 93% of Glomerales ASVs were NA at the genus level.
Overall, the dominance of bacterial phyla Actinobacteriota, Proteobacteria and fungal phyla Ascomycota and Basidiomycota persisted in all samples as well as across all sites (Figures 5B, D; and 6B, D).

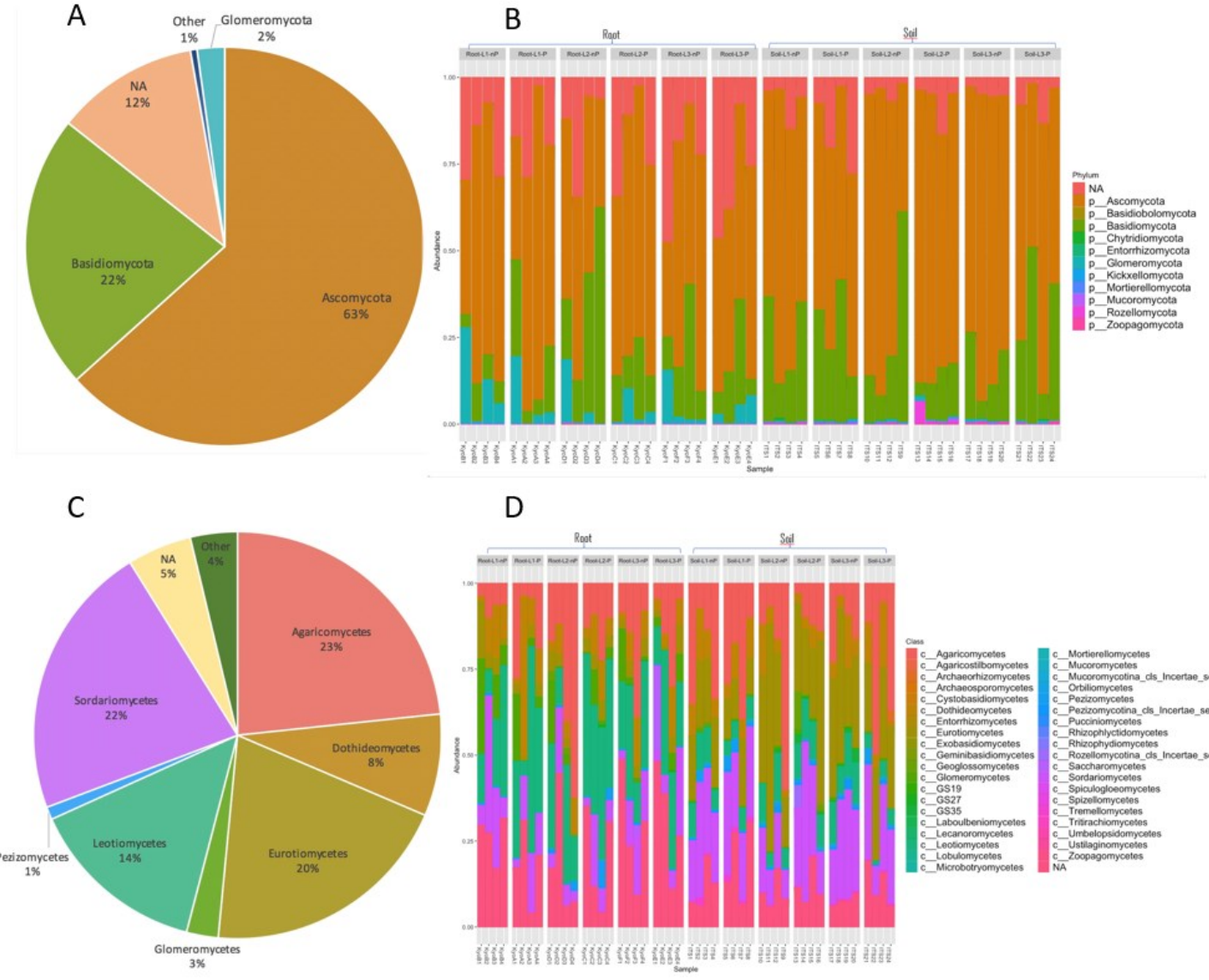

Figure 6: Taxonomic distribution patterns of the fungal population at phylum and order levels: Distribution of the global fungi community at phylum level (A) and across the major classes (C). Relative abundance profile (percentage of total ITS gene sequences) at the phylum level (B) and across the major classes (D) of ITS gene amplicon data, for each soil and root samples at each sampled site.
Relative abundance was computed from the number of fungal ITS region amplicons
'NA" category: ASVs that did not obtain taxonomic assignment at phylum or class level; "Other" category: mix of the classes of low abundance (less than 1%).

Taxonomic distribution between root and rhizospheric compartments

In root samples, we noted 26 bacterial phyla present, with Actinobacteria (70%), Proteobacteria (21%), Bacteroidota (4.7%) and Myxococcota (1.5%) having the highest relative abundances. Concomitantly, in soil samples, 24 different phyla were observed with Actinobacteria (70%), Proteobacteria (20%), Myxococcota (2.8%), Gemmatimonadota (2.3%) and Acidobacteriota (1.47%) being the most abundant.

Comparison of the bacterial composition across soil *vs* root samples indicated significant differences at different taxonomic levels (Metabarcoder differential abundance analysis with wilcox_p_value < 0.05) (Figure 5-A, S4). Thus, the relative abundance of Actinobacteriota, Acidobacteriota, Myxococcota, Gemmatimonadota, Verrucomicrobiota and alpha-Proteobacteria classes was significantly higher in the rhizosphere compared to the roots while the proportion of Bacteroidota and Chloroflexi were significantly increased in root compared to rhizosphere-compartment (all localizations pooled). At order level, bacteria identified as Streptomycetales, Cytophagales, Flavobacteriales and Pseudomonadales were enriched in roots.

In the fungal data set, 11 and 8 phyla were found respectively in soil *vs* root samples. Increased relative abundances of Ascomycota was observed in soils compared to roots, with a prevalence of Sordariomycetes, Dothideomycetes and Eurotiomycetes at class level. Similarly, preferential presence of Basidiomycota in the rhizosphere was observed. At class level, reads annotated as Agaricomycetes were significantly overrepresented in rhizosphere compartments. Interestingly, within the Glomeromycota phylum, the relative abundance of members of the Glomerales order was significantly higher in roots than in the rhizosphere.

Inputs of RP, effects in the higher microbial taxa ranks

Comparison of the bacterial composition across P-soils and nP-soils were performed at different taxonomic levels. No differential response was accurately established, neither at bacterial phylum, class or order taxonomic ranks, in any localization according to the Wilcoxon Rank Sum test (wilcox_p_value > 0.05). Likewise, no variations in abundance were measured in the fungal community, when analyzing the pairwise comparison of P-soils and nP-soils, whatever the location and the taxonomic ranks (from phylum to order) examined.

Microbial ASVs indicators of P-soils

Indicator bacterial ASVs (ibASVs) from P-soils account for 3.1% of the total bacterial ASVs. Among them, 50 were from the root compartment and 130 from the soil compartment.

Overall, the indicator fungal ASVs (ifASVs) from P-soils (Table S4) accounted for 1.4% of the total fungal community. Among them 13 ifASVs were from the root compartment, while 76 were from the soil compartment.

Looking at, the taxonomic assignment (Table S4) and mean relative abundances of these indicator taxa, we found that ibASVs and ifASVs. Taxonomic patterns of ibAVSs comprised at least 15 phyla, with the majority of community sequences belonging to the Actinobacteriota (74%) and Proteobacteria (19.4%). At the class level, most ibASVs were classified into Actinobacteria (46.7%), Alphaproteobacteria (14.7%), Thermoleophila (13.7%)

Acidimicrobiia (8.8%) and Gammaproteobacteria 4.7%. For fungi, ifASVs were dispatched in seven phyla, , with most of the sequences beloning to the Ascomycota (68.7%) and only 10.8% and 1.5% ifASVs were affiliated respectively to Basidiomycota and Glomeromycota phyla. Four other phyla have also been recorded, each for only one or three ifASVs: Chytridiomycota (3), Rozellomycota (3), Mucoromycota (1) and Zoopagomycota (1). Among the Ascomycota phylum, the Sordariomycetes class is dominant, and overall, the 99 ifASVs belonged to 20 differents orders, most of them being identified as Hypocreales or Helotiales.

DISCUSSION

An original experimental setup to characterize native microbial communities of P-rich soils

In this study, microbial and physico-chemical characterizations of soils and roots originating from sites formerly altered by mining inputs of excavated raw RP ore and non-altered sites (as reference plots) have been compared. As expected, concerning P contents, higher P contents were highlighted in P-soils compared to nP-soils. Classically, P is only present in minute quantities in the Earth's crust (0.09 wt%) (Filippelli, 2008) and the total P content in soils typically ranges from 50 to 3,000 $mg.kg^{-1}$ depending on parent material, vegetation cover and management history (Sanyal & DeDatta, 1991; Frossard *et al.*, 2004; Rai *et al.*, 2013). Consequently, in line with these values, all the sampled soils in our study can rather be qualified as P-rich soils.

Also, clear differences in terms of available Olsen P levels are observed in P-soils compared to nP-soils, ranging respectively from 46.1 to 339.5 $mg.kg^{-1}$ and from 5.04 to 12.82 $mg.kg^{-1}$. These values indicate that available-P fractions do not exceed 1% of total P in nP-soils (0.36 to 0.85%),

in accordance with the literature (Bieleski, 1973; Barber, 1995; Rodríguez & Fraga, 1999). In the P-soils available P is consistently higher, ranging from 1.61 to 2.43% of the total P content.

Plant species shape the bacterial and fungal communities in the soil – root continuum

Our results showed that bacterial and fungal communities were different across the plant-associated compartments in soils: root and rhizosphere, consistently with literature data about P supplemented soils (Gómez-Muñoz *et al.*, 2018; Robbins *et al.*, 2018; Fabianska *et al.*, 2019). Interestingly, higher bacterial indices in the rhizosphere compared to roots were computed no matter the alpha diversity indices and whether the analysis was global or performed separately within each localization (L1 or L2 or L3). Regarding the global fungal diversity, Shannon indices values were also significantly higher in soil compared to root compartment. Moreover, our data highlighted that compartments exert a significant effect on beta diversity for bacteria and fungi.

Soil and root microbial community response to P supply after ecosystem self-restoration

Considering the history of our experimental sampling sites, the RP input can be there described as a one-time pulse of nutrient P due to a drastic ecological disturbance related to RP ores inputs by mining exploitation, with subsequent, gradual whole-ecosystem restoration for over a century, and persistence of high P levels up to date. In such environmental context, no difference in alpha diversity of microbial communities was observed when comparing P-soils and nP-soils. However, some variation in microbial beta diversity indices were noticed according to RP status, depending on location and bacterial or fungal communities. At high taxonomic levels, comparable patterns in terms of prevailing taxa identity were displayed by all the microbial communities, whether or not the soils have been impacted by the past RP input event and whatever the soil P concentration. In contrast, at lower taxonomic level, occurrence

of indicator species of P-soils, including soils and plant roots were highlighted among bacteria and fungi.

Previous studies on the effects of P supplementation on soil microbial communities have been considered variously, including through a wide range of experimental setups, strongly different from ours. Thus, researchers investigated microbial communities across fields (Silva *et al.*, 2017; Gomes *et al.*, 2018), forests (Liu *et al.*, 2012), tree plantations (Huang *et al.*, 2016), pastoral systems (Wakelin *et al.*, 2012) or controlled environmental conditions (Robbins *et al.*, 2018; Bodenhausen *et al.*, 2019; Fabianska *et al.*, 2019). Concomitantly, various plant species and genotypes were also analyzed, as well as different soil compartments (*i.e.* bulk soil, root-associated compartment such as rhizosphere, rhizoplane and root endosphere) (Silva *et al.*, 2017; Gomes *et al.*, 2018; Robbins *et al.*, 2018; Yu *et al.*, 2018; Fabianska *et al.*, 2019). The role of P sources in shaping microbial communities was also investigated with various P-chemical forms (industrial fertilizers, RP, nutrient solutions, manure…) or according to different P levels ((Gomes *et al.*, 2018); Bodenhausen *et al.* (2019)). Furthermore, some authors have focused on the impacts of exogenous P amendments on microbial communities after a relatively short-term P fertilization regime (Trabelsi *et al.*, 2017; Gomes *et al.*, 2018; Ikoyi *et al.*, 2018; Bodenhausen *et al.*, 2019; Fabianska *et al.*, 2019; Gumiere *et al.*, 2019); while others studied soil biome responses after repetitive and consecutive pulses of P-availability across decades of fertilization processes (Liu *et al.*, 2012; Wakelin *et al.*, 2012; Leff *et al.*, 2015; Wang *et al.*, 2015; Francioli *et al.*, 2016; Huang *et al.*, 2016; Tang *et al.*, 2016; Silva *et al.*, 2017; Robbins *et al.*, 2018; Yu *et al.*, 2018). In most approaches, P is generally supplied at P levels close to, or slightly above, plant requirements, and the microbial response observed is a snapshot of the effect of a recent P-resource amendment even after a long-term fertilization process.

Literature also revealed microbial response to P supply are extensively diverse. For instance, studying long term fertilization impact of RP and triple superphosphate, Silva *et al.* (2017) underlined the bacterial Shannon indices showed significantly higher values for RP added samples in relation to the control. For their part, Robbins *et al.* (2018) concluded P had little effect on alpha and beta diversity, after long periods of exposure to long term contrasting fertilization regimes.

On the other hand, numerous authors identified, mainly in soil, but also in plant roots, shifts in microbial communities, but at different taxonomic levels. For example, minor shifts in soil bacterial and fungal communities were recorded in studies about long-term P effect (Wakelin *et al.*, 2012; Wang *et al.*, 2015). In contrast, the short-lived effects of P fertilization observed by Trabelsi *et al.* (2017) were significant: effects of RP fertilization on microbial composition at low P level (*i.e.* 50 kg P ha^{-1}) were marked by the increase of taxa including members of Betaproteobacteria and Actinobacteria while members of Gammaproteobacteria and Bacteroidetes were stimulated by high RP level (250 kg P ha^{-1}); Firmicutes decreased only at high RP level. These authors observed the stimulation of Actinobacteria both in the rhizosphere and in the uncultivated soil. Previously, Wakelin *et al.* (2012) have also hypothesized that soil Actinobacteria could be linked to P status. Comparing different P-fertilization treatments, Silva *et al.* (2017) noticed for their part, Proteobacteria was the predominant phylum in the microbial community of the maize rhizosphere whatever the P treatments. Additionally, they observed while Enterobacteriaceae taxa (Gammaproteobacteriacae) decreased with RP, Oxalobacteraceae (mainly *Massilia* and *Herbaspirillum*) and Burkholderiaceae (Betaproteobacteria) increased with RP addition. Bacillaceae (Firmicutes) also showed significantly higher abundance in RP soils. Finally, *Burkholderia* sp. and *Bacillus* sp. were enriched and *Klebsiella* was the second most abundant taxon in the RP-treated soil.

In summary, literature data showed inconsistency at the level of the belowground ecosystem, in the microbial community response to P and no clear consensus emerged from published data to identify, if they exist, key taxonomic groups responsive to P inputs. Part of explanation for this finding probably reside in diversity of experimental contexts above detailed briefly. However, beyond the experimental divergences, we hypothesize a better understanding of the distribution and abundance of soil microbial communities could emerge with more exhaustive data about the soil chemistry before P supply as well as the chemical P-forms added. In addition, a more comprehensive analysis of P chemistry could probably provide explanatory elements for variation in microbial patterns over studies; here however, more theoretical models for the estimation of P form turnover would be required (Yang *et al.*, 2013; Helfenstein *et al.*, 2018; Hou *et al.*, 2018; Oksanen *et al.*, 2018; Hou *et al.*, 2019).

Inference of ecological significance for the prevalent taxa observed among the plant-associated compartments from restored P-soils

Across this study, large similarities have been recorded across the sampled microbial assemblages in terms of prevailing taxa, namely Actinobacteriota and Ascomycota, as well as Proteobacteria and Basidiomycota to a lesser extent. These similarities are observed both in the root or soil compartments and in the plant species. Such similarities raise some questions; in particular, although it is generally difficult to infer the ecological function of a microbe solely based upon a taxonomic assignment, it is relevant to examine ecological significance of the dominance of specific microbial taxa. Notably, we note the prevalent phyla identified in soil and root host many taxa referred to as copiotrophic taxa according to the oligotrophic/copiotrophic theory (Fierer, 2017; Ho *et al.*, 2017). Thus, according to this theory, Betaproteobacteria, known to be more abundant in organic carbon-rich habitats, have been generally classified as copiotrophic microbes (Francioli *et al.*, 2016; Fierer, 2017; Ho *et al.*, 2017). The trophic categorization of Actinobacteria remained unclear thus far and mixed results about their response to nutrient status have been documented, with some results reporting a copiotrophic status with an increase in abundance with nutrient contents while others reported no change. Nevertheless, they are generally viewed as being ubiquitous and widely distributed across terrestrial ecosystems (Lewin *et al.*, 2016; Delgado-Baquerizo *et al.*, 2018; Sayed *et al.*, 2020; van Bergeijk *et al.*, 2020). They are also considered as important contributors to the process of plant biomass decomposition due to their cellulolytic enzymes. Moreover, although Actinobacteria are still generally viewed as free-living bacteria with a saprotrophic lifestyle, their common association with plants, including in the rhizosphere and as endophytes is well established (Chen *et al.*, 2019). If Actinobacteria and Proteobacteria are known as copiotrophic microbes which display more rapid growth rates under high C availability conditions, in contrast, Acidobacteria are typically referred to as oligotrophic microorganisms that rather exist in nutrient-deficient and strongly acidic environments (Yao *et al.*, 2017; Cui *et al.*, 2021).

The oligotrophic/copiotrophic theory is less frequently used to discuss the ecological roles of dominant fungal taxa. However, it is suggested that saprotrophic fungi may exhibit copiotrophic features (Yao *et al.*, 2017) and Ascomycota phylum which include important decomposers of organic substrates and many wood-decay saprotrophs, notably the Eurotiomycetes, Leotiomycetes and Dothideomycetes (Lundell *et al.*, 2014) are classified into the copiotrophic categories. Ascomycota has been found to be a prevalent fungal phylum in various environments due to their diversity in terms of metabolic capacities, owing to the wide variety of enzymes they produce. In contrast, many members of the fungal phylum Basidiomycota (for which a lower quantity of sequences was recorded in our samples) may predominantly behave as oligotrophs despite this phylum also hosts fungal decomposers of plant litter with high lignin content (Ma *et al.*, 2013) indicating copiotrophic traits.

All together, these data are in accordance with our sampling conditions. Firstly, we exclusively sampled root and rhizosphere compartments. Across such soil compartments, the dynamics of plant microbiome acquisition and profiling remain an ill-understood complex process (Peiffer *et al.*, 2013; Fitzpatrick *et al.*, 2018). Nevertheless, according to the classical models, the rhizosphere is described as a nutrient-rich compartment (Hartman *et al.*, 2018) attracting only a subset of microbes present in the bulk soil thanks to qualitative and quantitative patterns of root exudates (rhizodeposition) and thus, orchestrating the composition and function of microbial populations (Marschner *et al.*, 2004; Philippot *et al.*, 2013; Tkacz *et al.*, 2020). In turn, the rhizoplane and the endoderm are supposed to impose a selective filter that controls further microbial colonization inside the root compartment and depletion of rhizospheric taxa into the endosphere (Bulgarelli *et al.*, 2013; Hartman *et al.*, 2018; Orozco-Mosqueda *et al.*, 2018). Concomitantly, the root endodermis delimits a protective biotope, also with rich-nutritional characteristics. Consequently, being classically described as carbon-rich hotspots, root and rhizosphere compartments can be referred to as copiotrophic biotopes.
Besides, we can hypothesize an accumulation of organic matter and organic carbon during ecosystem restoration of the sample sites, due to the absence of anthropogenic disturbance or export of plant material for more than one century. Therefore, these ecosystems could also be compatible with the growth of copiotrophic populations in the bulk soil and upper soil layers, *i.e.* with a high abundance of Actinobacteria and Ascomycota among the most notable taxa observed in plant-associated compartments. However, presently, whether the response in microbial traits and functions is deeply rooted in taxonomy and phylogeny remains an open question and this hypothesis needs further substantiation (Ho *et al.*, 2017).
In addition, the homogeneity in microbial patterns observed in our study could be explained through the respective contributions and prevalence of the deterministic *vs* stochastic processes, two major ecological processes, proposed to explain the spatial patterns of soil microbial diversity (Caruso *et al.*, 2011; Vellend *et al.*, 2014; Zhang *et al.*, 2015; Deakin *et al.*, 2018). Thus, we hypothesize deterministic processes may have operated immediately after the drastic mining disturbance, inducing the environmental filtering of microbial species according to the ecological fitness of microbes for the environmental properties, generating microbial dissimilarity between P and nP soils. Then, after decades of soil restoration subsequently to mining inputs, both bacterial and fungal communities of disturbed-soils have reached patterns quite comparable to those of non-disturbed soils, due to the prevalence of stochastic dispersal processes known to determine the similarity of microbial communities, notably at small spatial scales, in neighboring sites (Deakin *et al.*, 2018). Additionally, it is nowadays well established that the overlying plant community with which the soil sample was associated is a critical factor contributing to the assembly of soil microbial communities (Bulgarelli *et al.*, 2013; Fitzpatrick *et al.*, 2018; Leff *et al.*, 2018; Thiergart *et al.*, 2020; Trivedi *et al.*, 2020; Klasek *et al.*, 2021). We can hypothesize the dominant microbial taxa observed across sampled soils may reflect influence of vegetation, especially since we exclusively sampled plant-associated compartments of plants (not bulk soil) across to the same botanical species set.

CONCLUSION and PERSPECTIVES

Using NGS, we examined the diversity and composition of fungal and bacterial communities of the roots and rhizospheric soil of four wild herbaceous species spontaneously growing in two types of soils with different P legacy. Our findings show that rhizospheric soils and roots are two different selective environments for microbial communities in accordance with numerous studies finding significantly different communities in these two compartments. In addition, despite contrasting P concentrations, microbial communities exhibit similar patterns in terms

of dominant taxa in P and nP-soils at a high taxonomic level (phylum). Actinobacteriota and Proteobactria dominated the bacterial communities while the fungal phyla Ascomycota and Basidiomycota were predominant overall. Nevertheless, indicator species analysis has led to identify specific taxa enriched or exclusive to P-soils. Indeed, bacterial ASVs assigned to different groups such as Rhizobiales, Sphingomonadales, Burkholderiales, Solirubrobacteriales, etc. which for many of them have the ability to solubilize tricalcium P were found specifically in the different compartments studied. Thus, our findings provide new insights into the microbial communities of ecosystem with significant RP inputs. The isolation and in-depth characterization of some culturable ASVs will be of interest for their assessment as potential candidate as inoculates adapted to RP supplemented soils.

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AUTHOR CONTRIBUTIONS

ADD conceived and designed the study, then operated the data acquisition and analysis. SM contributed to carrying out the data analysis. JF, MH, and AL-HS contributed to supervision, conception and design. ADD drafted the manuscript, and all authors were involved in revision of the final version. All authors read and approved the final manuscript.

AVAILABILITY OF DATA

The dataset supporting the conclusions of this article is available in the NCBI Sequence Read Archive (SRA) database and can be found under the project accession number PRJNA784523.

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SUPPLEMENTARY MATERIAL

Table S1: Physicochemical properties of soils at the sampled sites. Soil samples obtained, in each site, from multiple soil fractions not adhering to plant roots, collected and then pooled into a single composite bulk soil sample. Properties mainly measured by Inductively Coupled Plasma spectrometry-atomic emission spectrometry (ICP-AES).CEC: cation exchange capacity; CationsEch : Exchangeable cations; TS: Saturation rate ; OM: organic matter ; C_{org} : organic carbon.

	Localizations	**L1**		**L2**		**L3**	
	Sites	**P**	**nP**	**P**	**nP**	**P**	**nP**
pH(H_20)	pH(H_20)	7,058	6,956	7,552	6,868	7,324	7,267
Totals Carbonates	CT%	0.10	0.10	0.21	0.10	0.10	0.10
Organic matter by dry combustion (Dumas)	OM%	6.50	6.04	8.84	9.34	11.64	14.22
	C_{org}%	3.77	3.50	5.13	5.42	6.75	8.25
	N‰	3.04	3.12	3.65	4.49	5.63	6.99
	Corg/N	12.42	11.22	14.04	12.06	12.00	11.80
Granulometric fraction	Coarse sands %	2,9	2,5	14,1	4,5	12,9	7,0
Available P (Olsen)	P mg/kg	46,10	5,04	339,46	8,09	192,78	12,82
Phosphorus total	P mg/kg	2860,0	1380,2	13927,9	1067,1	10739,1	1496,3
Iron total	Fe mg/kg	50426,5	46905,7	66132,3	47679,1	50843,3	48395,1
Manganese total	Mn mg/kg	548,9	817,3	1382,3	1407,2	1328,9	1532,8
Aluminium total	Al mg/kg	127446,1	107072,7	108967,9	93340,1	101438,5	93827,2
Copper total	Cu mg/kg	43,9	35,6	71,9	24,0	65,2	35,8
Zinc total	Zn mg/kg	197,3	147,7	632,1	131,0	467,1	173,4
Calcium total	Ca mg/kg	11403,5	7896,5	45554,7	8666,6	33942,1	14260,3
Magnesium total	Mg mg/kg	3482,7	3286,4	1989,5	3754,3	3244,5	3551,1
Potassium total	K mg/kg	11590,6	11296,7	8917,8	12588,3	12326,4	12271,6
Sodium total	Na mg/kg	1139,9	1589,8	902,7	2039,3	2133,9	2168,8
Absorption complex (Co(NH3)6Cl3)	Ca me/100g	25,52	23,48	24,03	25,01	31,13	30,50
	Mg me/100g	1,05	1,14	0,72	1,26	1,15	1,55
	K me/100g	0,31	0,23	0,36	0,32	0,41	0,42
	Na me/100g	0,12	0,13	0,09	0,13	0,13	0,12
	CationsEch me/100g	27,01	25,00	25,21	26,76	32,83	32,60
	CEC me/100g	27,79	25,11	25,77	28,33	33,60	36,36
	TS%	0,97	1,00	0,98	0,94	0,98	0,90

Table S2: Data from the bioinformatic processing after Illumina MiSeq sequencing.

	BACTERIA	FUNGI
Nb Samples	48	48
Nb raw MiSeq reads	4.890.709 soil: 2.457.648 roots: 2.433.061	3.642.907 soil: 2.674.883 roots: 968.024
Nb reads filtered after DADA2 step	1 126 828	1 611 669
Nb reads per sample	from 9 660 to 45 661	from 825 to 127 566
Nb ASV inferred	6720	6 664
Nb singletons Nb doubletons	51 433	31 402
Nb ASV retained	5 671	6231

Table S3: Shannon and Simpson Alpha diversity of bacterial and fungal communities within the rhizospheric or root samples
48 samples harvest across 6 sites (L1-P, L1-nP, L2-P, L2-nP; L3-P, L3-nP), 2 plant-associated compartments (rhizospheric soil /root) and 4 plant species (one plant species sample being a composite sample mixed from 3 individuals)

Bacteria

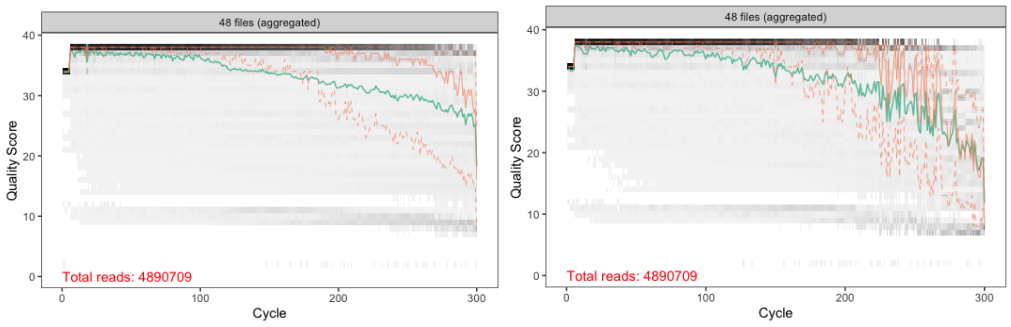
		Chao1	Shannon	Simpson			Chao1	Shannon	Simpson
Root L1-P	BA1	439,5	5,465	0,993	Soil L1-P	BS1	623375	5,959	0,996
	BA2	424125,0	5,484	0,993		BS2	381	5,562	0,995
	BA3	189,3	4,679	0,984		BS3	267	5,115	0,991
	BA4	381,0	5,316	0,990		BS4	450	5,605	0,995
Root L1-NP	BB1	311,2	4,496	0,951	Soil L1-NP	BS5	602	5,967	0,996
	BB2	316,0	5,223	0,991		BS6	688	6,045	0,996
	BB3	421,0	5,546	0,994		BS7	451	5,684	0,995
	BB4	322,0	3,807	0,851		BS8	606	5,956	0,996
Root L2-P	BC1	319125,0	5,143	0,989	Soil L2-P	BS9	436	5,609	0,994
	BC2	412,2	5,231	0,989		BS10	624	5,953	0,996
	BC3	239,0	4,839	0,983		BS11	546	5,781	0,995
	BC4	188,0	4,615	0,982		BS12	700	6,059	0,996
Root L2-NP	BD1	543,1	5,736	0,995	Soil L2-NP	BS13	640	5,944	0,996
	BD2	361,0	5,431	0,993		BS14	380	5,443	0,994
	BD3	449,0	5,409	0,990		BS15	288	5,205	0,992
	BD4	311,0	5,105	0,990		BS16	494	5,736	0,995
Root L3-P	BE1	659,1	5,975	0,996	Soil L3-P	BS21	500	5,865	0,996
	BE2	231,0	4,926	0,989		BS22	349	5,507	0,995
	BE3	383,3	5,162	0,987		BS23	280	5,278	0,993
	BE4	162,0	3,683	0,902		BS24	592	5,877	0,994
Root L3-NP	BF1	389,0	5,303	0,991	Soil L3-NP	BS17	668	6,009	0,996
	BF2	239,0	4,892	0,987		BS18	455	5,642	0,995
	BF3	386,1	5,099	0,986		BS19	470	5,745	0,995
	BF4	292,3	4,929	0,983		BS20	462	5,674	0,995

Fungi

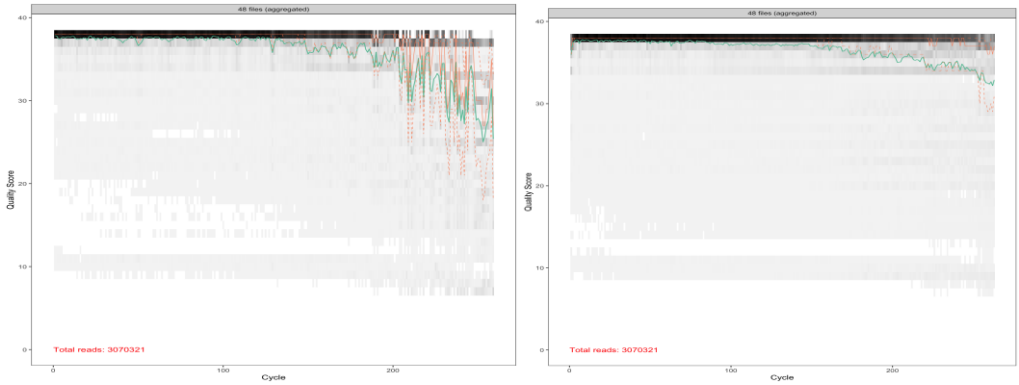
		Chao1	Shannon	Simpson			Chao1	Shannon	Simpson
Root L1-P	KyoA1	195	3,757	0,949	Soil L1-P	ITS1	406,0	4,598	0,974
	KyoA2	217	3,667	0,922		ITS2	424,3	4,748	0,981
	KyoA3	80	3,235	0,925		ITS3	330,1	4,446	0,971
	KyoA4	397	4,369	0,963		ITS4	432,1	4,557	0,970
Root L1-NP	KyoB1	118	3,411	0,920	Soil L1-NP	ITS5	459,7	4,428	0,972
	KyoB2	188	3,919	0,958		ITS6	453,0	4,514	0,972
	KyoB3	155	4,285	0,977		ITS7	354,1	4,168	0,945
	KyoB4	197	3,699	0,920		ITS8	567,2	4,670	0,957
Root L2-P	KyoC1	137	3,607	0,946	Soil L2-P	ITS9	417,7	2,779	0,751
	KyoC2	153	3,921	0,966		ITS10	397,6	4,632	0,973
	KyoC3	131	3,765	0,954		ITS11	241,0	2,900	0,751
	KyoC4	365	4,448	0,966		ITS12	487,0	4,765	0,978
Root L2-NP	KyoD1	325	4,385	0,971	Soil L2-NP	ITS13	331,0	4,240	0,965
	KyoD2	251	4,081	0,951		ITS14	379,0	3,653	0,869
	KyoD3	144	3,613	0,946		ITS15	305,0	4,689	0,983
	KyoD4	41	2,965	0,909		ITS16	359,0	4,210	0,957
Root L3-P	KyoE1	127	3,374	0,911	Soil L3-P	ITS21	471,2	4,861	0,982
	KyoE2	420	3,963	0,871		ITS22	193,0	2,937	0,836
	KyoE3	193	3,586	0,920		ITS23	336,0	4,428	0,968
	KyoE4	225	4,598	0,982		ITS24	368,2	4,097	0,935
Root L3-NP	KyoF1	216	4,130	0,955	Soil L3-NP	ITS17	388,0	4,223	0,950
	KyoF2	199	4,264	0,975		ITS18	385,1	4,319	0,965
	KyoF3	68	3,608	0,954		ITS19	410,2	4,720	0,980
	KyoF4	124	3,921	0,965		ITS20	512,2	5,012	0,986

Table S4: Bacteria and fungi indicator species analysis (excel format) https://docs.google.com/spreadsheets/d/1-JOCy_vcoA5xqWu3Na2ovrhR5oV2ESiE/edit?usp=sharing&ouid=102817051035010487074&rtpof=true&sd=true

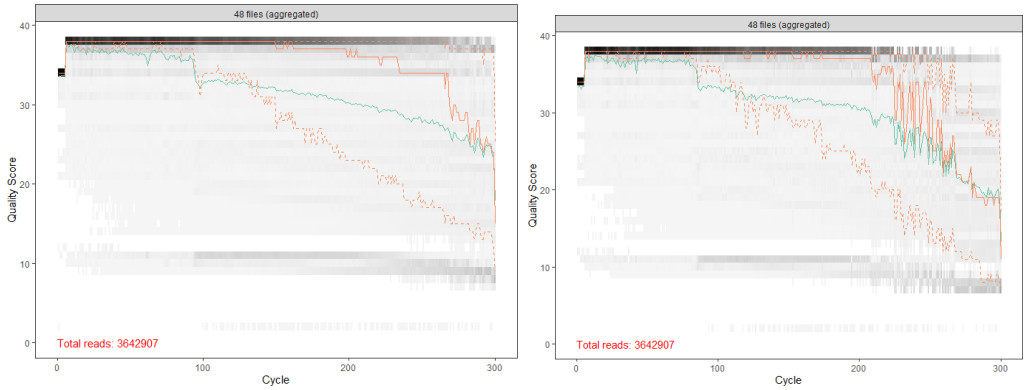
16 rRNA before filtration



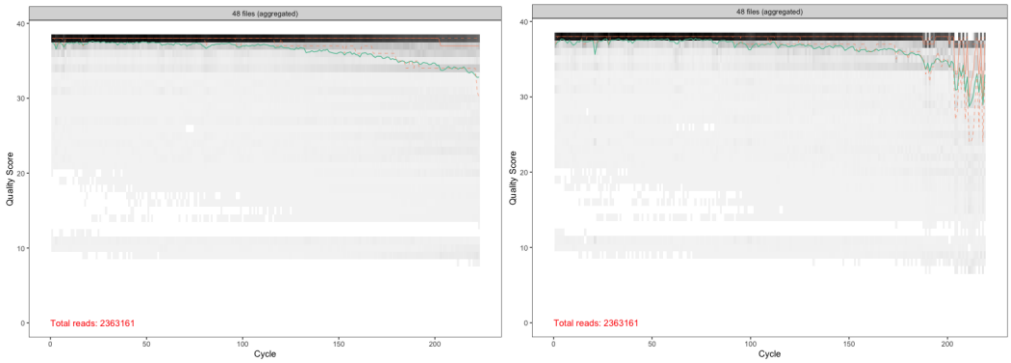
16 rRNA after filtration



ITS before filtration



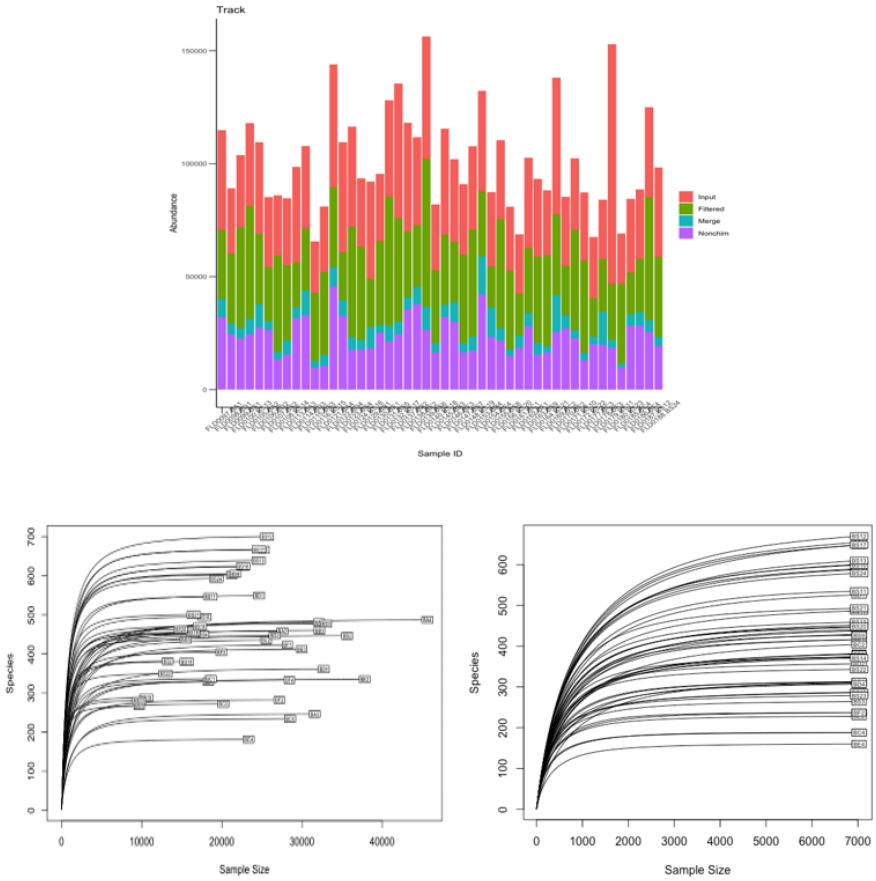
ITS after filtration



Forward sequences **R**everse sequences

Figure S1: Quality profiles for 16 rRNA and ITS sequences.

A



B

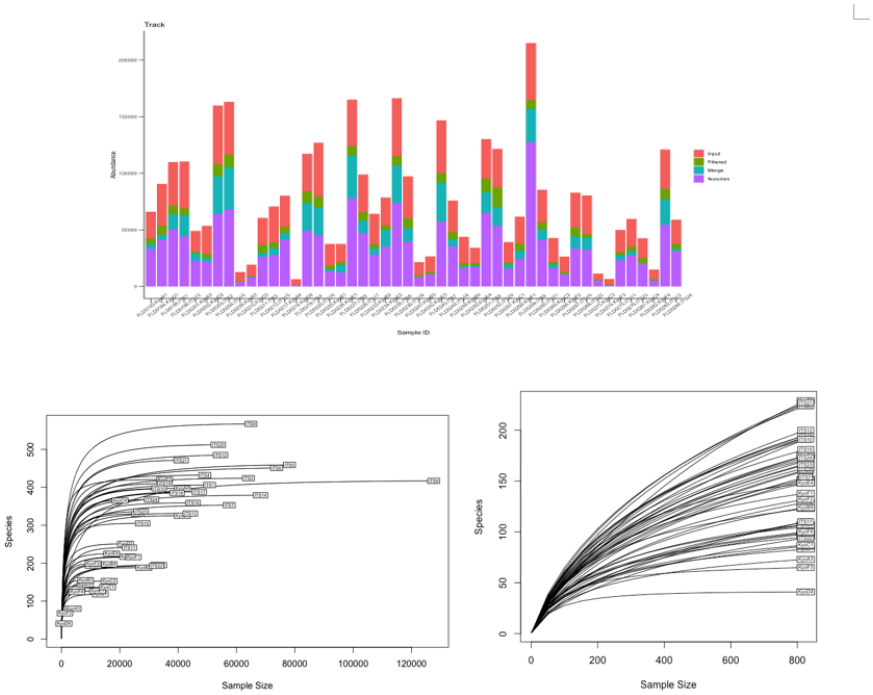


Figure S2: Rarefaction curves for 16S rRNA (A) and ITS (B) data sets.

ARTICLE 3 : Communautés mycorhiziennes : diversité dans des sols riches en RP

Ce paragraphe est présenté sous forme d'un article scientifique, article publié dans la revue Microorganisms.

Glomerales Dominate Arbuscular Mycorrhizal Fungal Communities Associated with Spontaneous Plants in Phosphate-Rich Soils of Former Rock Phosphate Mining Sites

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Abstract: Arbuscular mycorrhizal fungi (AMF) are key drivers of soil functioning. They interact with multiple soil parameters, notably, phosphorus (P). In this work, AMF communities of native plants grown spontaneously on former mining sites either enriched (P sites) or not enriched with P (nP sites) by mining cuttings of rock phosphate (RP) were studied. No significant differences were observed in the root mycorrhizal rates of the plants when comparing P and nP sites. The assessment of AMF diversity and community structure using Illumina MiSeq metabarcoding and targeting 18S rDNA in roots and rhizospheric soils showed a total of 318 Amplicon Sequence Variants (ASVs) of Glomeromycota phylum. No significant difference in the diversity was found between P and nP sites. Glomeraceae species were largely dominant, formed a fungal core of 26 ASVs, and were persistent and abundant in all sites. In the P soils, eight ASVs were identified by indicator species analysis. A trend towards an increase in Diversisporaceae and Claroideoglomeraceae and a reduction in Paraglomeraceae and Glomeraceae were noticed. These results provide new insights into AMF ecology in former RP mining sites; they document that P concentration is a driver of AMF community structures in soils enriched in RP long term but also suggest an influence of land disturbance, ecosystem self-restoration, and AMF life history strategies as drivers of AMF community profiles.

Keywords: arbuscular mycorrhizal fungi; phosphorus; rock phosphate; diversity; taxonomic composition

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1. Introduction

Many efforts have been deployed to sustain agricultural production systems while reducing their environmental footprint. Ecosystemic contributions of microbial resources to improve and maintain soil fertility are among the strategies that have been utilized. In particular, arbuscular mycorrhizal fungi (AMF) have gained a large interest as biological input in agroecosystems [1–3]. Indeed, AMF are ubiquitous fungi that form symbiotic associations with most plant species, including important crop plants, such as vegetables and cereals [4,5]. They are obligate biotrophs, receiving their carbon sources in the form of photosynthetic sugars and fatty acids from plant hosts [6,7]. In exchange, elements such as phosphorus (P), nitrogen, sulfur, or microelements, such as copper and zinc, may also be transferred to the host via arbuscules, favoring plant nutrient supply [1,8,9]. Mycorrhizal symbiosis can also contribute to plant protection against biotic or abiotic stresses [10–13]. In addition, evidence also indicate that AMF can be critical components to improve soil structure, water retention capacity, and soil fertility and reduce soil nutrient

leaching [1,14–17]. Concomitantly, the multiple benefits that AMF confer to their hosts and agroecosystems' functioning have raised opportunities for their application as commercial biofertilizers. In particular, AMF increase the uptake of relatively immobile ions (such as phosphate ions) for their host plant due to an extensive extraradical hyphal network that extends beyond the zone of direct uptake by roots. In this way, they contribute to greater soil prospection and enable mycorrhizal plants to access more soil P resources. Therefore, AMF have great importance to productivity and plant growth in most ecosystems [1,18]. However, despite the great potential of AMF and the evidence of their relevance for agriculture [19], a deeper understanding of AMF ecology is of primary importance to improving engineering and the use of AMF-based bioinoculants in ecosystems.

In such context, significant improvements have been made in the detailed analysis of AMF communities among complex habitats using AMF gene markers and high-throughput sequencing technology for taxonomic identification, phylogenetic reconstruction, or quantification of AMF [20–23]. Thus, due to such improvements, much compelling evidence has reported that different abiotic and biotic factors interfere with AMF communities at either a global or local scale [24–27]. In particular, the host plant can be considered a strong factor in shaping AMF communities [28–30], but these are also known to be sensitive to interactions with other soil organisms [31,32]. Edaphic properties [24,29,33] and climatic parameters [10,25,34,35] are also recognized as strong drivers of AMF communities. In addition, some studies have suggested a high diversity of AMF communities in natural habitats [36,37] while a lower diversity tends to be observed in agricultural ecosystems [38]. Notably, AMF communities' characteristics may vary with land use (usage intensity, cultivation duration, pasture, etc.) and with agronomic practices for soil management (chemical inputs, plowing, tillage, crop rotation, land conversion, etc.) [39,40]. In particular, the responses of AMF to soil P were largely investigated [41–43]. So, research efforts on the understanding of P as a key driver of AMF colonization, diversity, and community composition have gained interest, in particular, for identifying guides in the development of AMF inoculants and their use with a reduction in the use of chemical fertilizers [44,45]. In particular, how AMF communities interact with rock phosphate (RP) has attracted considerable attention because RP is a natural, low industrial-cost phosphate input that has more ecological and sustainable acceptability than chemical fertilizers. However, P as a key player in shaping AMF communities still remains a controversial subject in the literature. Thus, the impact of P concentrations on AMF colonization as well as on the diversity and composition of AMF communities is regularly discussed [28,39].

Thus, the current work aims at characterizing the diversity and composition of AMF communities across a former mining area of RP where two types of sites occurred: sites enriched or not enriched with phosphate deposits originating from the mining activity. The AMF community profiles in the soil and root samples were determined using MiSeq amplicon sequencing targeting 18S rDNA that generates Amplicon Sequence Variants (ASVs). The diversity and composition of AMF were analyzed at several levels of taxonomic resolution, and the potential role of the P gradient and soil restoration process in AMF community composition was discussed.

2. Materials and Methods

2.1. Study Area and Experimental Design

This work was carried out in the region of the "Phosphatières du Quercy" (southern France; N 44.351827, E 1.691021). This area is characterized by numerous paleokarst whose fillings are enriched in phosphorite (i.e., a phosphatic ore containing variable proportions of tricalcium phosphate). There, in the past, mining extraction was carried out in the form of open-pit mines (Figure S1a), and some excavated spoils were abandoned near the extraction points. Thus, each selected site corresponds to a former mining point (i.e., a site without enrichment with RP ores, referred hereafter as "nP site") associated with its spoil area (i.e., a site with ore deposits due to mining exploitation process, referred to as "P site"). Today,

in this former mining area, the dominant landscapes are dry grasslands that are punctually wooded (Figure S1).

The P concentrations are known to be more or less high in each karst phosphatic fill [46]. Three nP were therefore chosen, expecting significantly high P concentrations. The choice of locations was also constrained (i) by the possibility of locating potential P and nP sites in close proximity to each other from the ground surface and (ii) by the necessary presence of the same set of species constituting the plant sampling. Thus, four native herbaceous mycotrophic species were sampled: *Ranunculus bulbosus*, *Bromus sterilis*, *Taraxacum officinale*, and *Dactylis glomerata*, and three plants per species were collected. From these 3 plants, the soil closely attached to roots (i.e., rhizospheric soil) was manually removed by gentle agitation and pooled into a single soil fraction. Then, the roots were washed with sterile water to get rid of remaining soil particles and then pooled into one root fraction per species (Figure 1). Soil and roots were then, respectively, kept at 4 °C and −20 °C until further DNA extraction. This was repeated for each plant species in each site, leading to 24 root samples (6 sites × 4 plant species) and 24 rhizospheric soil samples. Detailed soil properties for each site are available in supplementary data (Table S1).

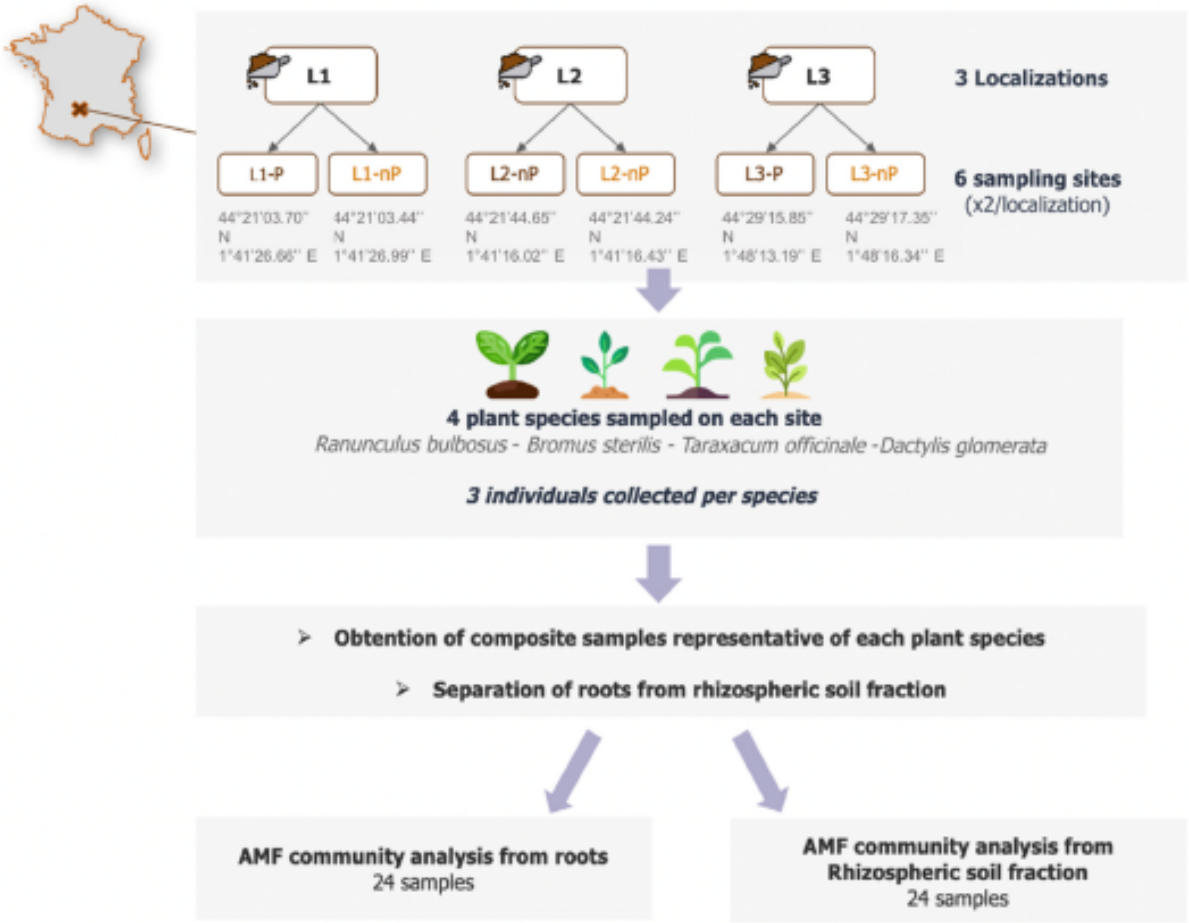

Figure 1. Sampling sites and experimental design.

2.2. Estimation of Arbuscular Mycorrhizal Root Colonization

Immediately after plant harvesting, from each plant species and site, fractions of fresh roots were stained using nonvital Trypan blue (0.5 $g \cdot L^{-1}$) according to the method described by [47]. From each stained root batches (24 batches), fragments of 1 cm were sampled, and 3 intersections per root fragment were examined through microscopic observations

(Nikon Eclipse E600, ×100 magnification). The rate of root colonization by AMF structures (vesicles and arbuscules) was quantified as described by [48].

2.3. Soil Physicochemical Properties

In each site, the soil physicochemical properties (pH, soil texture, chemical composition) were measured by the CIRAD-US Analyse laboratory (Montpellier, France) using inductively coupled plasma spectrometry, atomic emission spectrometry, and X-ray fluorometry. The 6 soils were characterized by near-neutral pH, ranging between 6.9 and 7.3. Regarding granulometry, all topsoil (0–30 cm) resulted in high levels of clay with additionally higher percentages of coarse sands in P soils. In nP soils, total P concentrations ranged from 1057.1 to 1496.3 $mg{\cdot}kg^{-1}$ while in P soils, they ranged from 2880.0 to 13,927.9 $mg{\cdot}kg^{-1}$. Likewise, available Olsen P concentrations in P soils were between 46.1 $mg{\cdot}kg^{-1}$ to 339.5 $mg{\cdot}kg^{-1}$ while nP soils were characterized by values ranging from 5.04 to 12.82 $mg{\cdot}kg^{-1}$. Thus, by comparing matched soils from the same localization, P and Olsen P ratios ranging from 2.07 to 13.05 and from 9.14 to 41.96 Pi, respectively, were obtained (Table S1), which allowed to qualify the P sites as high-P sites, and the nP sites as low-P sites.

2.4. DNA Extraction, PCR, and Sequencing

2.4.1. DNA Extraction

Genomic DNA extraction from the rhizospheric soil samples was directly performed from 250 mg of soil using a Nucleospin Soil® kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions.

Genomic DNA extraction from root samples was operated from roots initially frozen in liquid nitrogen and then reduced to fine powder. Then, the extraction from 250 mg of ground roots was performed using Cetyltrimethylammonium bromide (CTAB, 1.4 M $NaCl_2$ 100 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0; 2% CTAB), Polyvinylpyrrolidone (PVP 1% *w*/*v*), ß-Mercaptoethanol (5% *v*/*v*), and activated charcoal (0.5% *w*/*v*) extraction (30 min; 55 °C). A centrifugation step was then carried out (10 min; 16,000× *g*), and lysate extraction was performed with chloroform: isoamyl alcohol (24:1). DNA precipitation was obtained in isopropanol (1 h incubation; 25 °C) followed by centrifugation (10 min; 700× *g*). After three washings (with ice-cold ethanol (70%) followed by centrifugation—10 min at 900× *g*) and air-drying at room temperature (approx. 90 min; 20 °C), the DNA pellet was dissolved in 50 µL of TE buffer (10 mM Tris-HCl, pH 8.0; 1.0 mM EDTA, pH 8.0) [13,49,50].

All extractions were performed in triplicate. DNA quality of root and soil DNA extracts was assessed using 1% (*w*/*v*) agarose gel, and measures of the 260/280 nm and 260/230 nm ratios were performed with a SpectraMax® iD3 device (Molecular Devices LLC, Sunnyvale, CA, USA). After measures of DNA concentrations, root and soil DNA extracts were normalized to 25 $ng{\cdot}L^{-1}$ for further analyses. The extracted DNA was stored at −20 °C until further use.

2.4.2. PCR Targeting the 18S rRNA Gene of AMF

The DNA of AMF was specifically targeted using Nested-PCR (Surecycler 8800, Agilent Technologies, Les Ulis, France) with a set of two primer pairs as previously published by [50]. Thus, the first-round PCR was performed using the AMF-discriminating primer pair AML1 (3′-ATCAACTTTCGATGGTAGGATAGA-5′) and AML2 (3′-GAACCCAAACAC TTTGGTTTCC-5′) which generate amplicons of the small 18S subunit of the rRNA gene of about 800 bp in length [51,52]. The PCR conditions were as follows: initial denaturation at 94 °C for 3 min followed by 35 cycles at 94 °C for 1 min, 45 °C for 1 min, and 72 °C for 1 min, and a final elongation step at 72 °C for 5 min [13]. PCR reactions were performed in a reaction volume of 25 µL, and reagents were as follows: 5 µL of Q5 (5X) reaction buffer, 0.25 µL of Q5® High-Fidelity DNA Polymerase (New England Biolabs France, Evry, France), 0.8 µL of each primer (0.4 µM), 1 µL of dNTPs (0.2 mM), 1 µL of DMSO, 1 µL of BSA (100 $\mu g{\cdot}mL^{-1}$), and 1 ng of DNA template [13,51].

From the 800 bp length amplicons obtained after the first-round PCR, which are not compatible with Illumina sequencing technology, nested PCRs were performed using an in-house set of internal primers nu-SSU-0595-5-F (ACACTGACGACATGGTTCTACA CGGTAATTCCAGCTCCAATAG) and nu-SSU-0948-3-R (TACGGTAGCAGAGACTTGGTCT TTGATTAATGAAAACATCCTTGGC). This set of primers flanked the V4 region of 18S and hence downsized the length of the amplicons to about 400 bp, which is compatible with MiSeq 300 paired-end sequencing [53]. The two primers were complemented with CS1 and CS2 barcoded adapters [13,53]. The reaction mixture was the same as previously described, but 1 ng of DNA from the first-round PCR as template was used. The reaction conditions for the second PCR were as follows: initial denaturation at 94 °C for 3 min followed by thirty-five cycles at 94 °C (1 min), 58 °C (1 min), and 72 °C (1 min), and a final elongation step at 72 °C (5 min).

2.4.3. Sequencing

From genomic DNA of root and soil samples collected for each plant species harvested in each site, PCR reactions were performed in triplicates for each sample, and these PCR products were then pooled together. Sequencing was processed by Genome Quebec Innovation Centre (Montreal, QC, Canada) by using an Illumina MiSeq platform generating 2 × 300 bp paired-end reads.

2.4.4. Nucleotide Sequence Accession Number

The 18S rRNA gene sequences of the raw data set have been deposited in the NCBI Sequence Read Archive database under the project accession number PRJNA877825.

2.5. Bioinformatic and Statistical Analyses

Bioinformatic process and statistical analyses were operated in the R 4.0.2 software (R Core Team, 2019) environment. The DADA2 pipeline (v. 1.16) [54], an open-source program implemented in R package (https://benjjneb.github.io/dada2/tutorial.html accessed on 1 October 2022), was used to process the sequencing data. Briefly, sequence reads were filtered and trimmed using optimized parameter settings as recommended. Sequence reads were dereplicated, denoised, and merged using DADA2 default parameters. Then, sequences were aligned and categorized to infer amplicon sequence variants (ASVs) grouping amplicon sequencing data by using 100% of sequence identity [55]. Sequences presenting only once (singletons) in the whole data set were eliminated. Validity of sequencing depth was controlled from rarefaction curves, computed using the "rarecurve" function from the Vegan package in R.

The taxonomic assignment of ASVs was performed following a previously established two-step approach [13,53]. In the first step, the Silva v132 database formatted for DADA2 was used to assign ASVs from kingdom to genus (minimum bootstrap 80) with the assignTaxonomy () command and the blast parameters as constitutively formatted for DADA2 [56]. From the ASVs assigned as AMF with Silva database, affiliation of each ASV was assessed at the genus level by performing a BLAST analysis against the NCBI database and MARJAAM, a web-based database containing referenced Glomeromycota DNA sequence data. The ASVs identified as non-Glomeromycota at the phylum level at the end of these two stages of assignment were excluded from further analyses.

In a second step, aiming at a refined taxonomic identification of each ASV, a phylogenetic tree was subsequently constructed as proposed by Stefani et al., 2020 [53]: multiple alignment including ASVs identified as Glomeromycota and multiple consensus sequences was computed using the web portal Kalign [57] (http://msa.sbc.su.se/ accessed on 1 October 2022). Then, a maximum-likelihood tree was obtained using RAxML v8.2.10 [58], through the CIPRES web portal [59]. Visualization of the output was finally obtained with the FigTree v1.4.4 program.

With the aim to identify the influence of former RP inputs on AMF communities, we studied AMF communities across the six sampling sites as well as inside root or

rhizospheric soil using proxy richness, alpha diversity, distribution, and abundance of taxa. Then, data relative to P sites were compared to those of nP sites, inside each location, or after pooling data from the 3 P sites on the one hand and the 3 nP sites on the other hand. The Chao1 richness estimator and alpha diversity indices (Shannon and Simpson) [60–62] were computed from the plot_richness () function using the phyloseq R package.

The normality and homoscedasticity of the data were assessed from the Shapiro–Wilk and Bartlett tests, respectively. If both conditions were verified, ANOVA analysis complemented with a posthoc Dunn test was carried out. Otherwise, a Kruskal–Wallis nonparametric test ("kruskal.test" function in R) was used. The significance of the statistical analyses was considered for $\alpha = 0.05$.

Venn diagrams were constructed with the Vegan package to visualize the numbers of ASVs that are either specific to one site or shared between paired sites inside the mining area; significance of the difference in ASVs numbers was then conducted using chi2 test. Indicator species analysis was performed using the multipatt function from indicspecies package [63] to compute the indicator value index (IndVal), i.e., a measure of specificity (based on abundance values) and fidelity (computed from presence data) of each ASV to a targeted clustering group of samples related to a targeted ecological condition [60]. This index, therefore, led to identifying ASVs (hereafter referred to as "indicator ASVs") that can be considered closely related to the ecological condition of their group [64]. The significance of IndVal of each species was assessed by a random permutation procedure from 9999 permutations with a significance at the $\alpha = 0.05$ level (IndVal's *p*_values with the p.adjust () function).

3. Results

3.1. Mycorrhizal Colonization of Plant Roots

The AMF root colonization was estimated in each sampling site from 135 microscopic observations of different individuals per plant species. Root colonization rates ranged from 13 to 63% across the different sampling sites (L1-P vs. -nP, L2-P vs. -nP, and L3-P vs. -nP) and plant species (*Ranunculus bulbosus*, *Bromus sterilis*, *Taraxacum officinale*, or *Dactylis glomerata*). However, when considering all individuals of the four plant species as a pool (to reduce a putative effect only linked to plant species), no significant difference in mycorrhizal colonization between plants growing in P versus nP sites was observed, whether the estimate was made for the entire mining area or within each individual localization. Thus, considering the entire mining area, the mean percentages reached 42 and 43% in plant roots sampled in P versus nP sites, respectively (Table 1).

Table 1. Mycorrhizal rates of the four plant species (*Ranunculus bulbosus*, *Bromus sterilis*, *Taraxacum officinale*, and *Dactylis glomerata*) grown on the six sites (L1-P and -nP, L2-P and -nP, and L3-P and -nP). Data based on 135 root fragments from three plants per species in each sampling site.

		Mycorrhizal Rates (%)					
Sites	**Total P Concentration (mg kg^{-1})**	***Ranunculus bulbosus***	***Taraxacum officinale***	***Dactylis glomerata***	***Bromus sterilis***	**Average Per Site**	**Average P versus nP**
L1-P	2860	38	40	33	51	41	
L2-P	13,928	20	27	42	18	27	42
L3-P	10,739	53	53	62	63	58	
L1-nP	1380	53	58	40	56	52	
L2-nP	1067	47	13	5	16	32	43
L3-nP	1496	58	44	33	42.	44	

3.2. Analysis of AMF Diversity

After Illumina Miseq sequencing, the global dataset resulted in a total of 4,795,981 raw MiSeq reads (yielded across 24 soil samples and 24 root samples, Table S2). After quality

filtering and chimera removal, a total of 3,527,278 sequences were retained as the AMF 18S rDNA dataset (Table S3). The rarefaction curves obtained after quality filtering reached a plateau, indicating we had a good representation of the microbial community, as most of the abundant species were represented and the sequencing depth effort was adequate to progress to further AMF community analysis (Figure S2).

From the AMF 18S rDNA dataset and using the Silva v132 database formatted for DADA2, 392 ASVs were inferred and identified as Glomeromycota. Among them, 74 ASVs were not assigned at the phylum level after inferring the phylogenetic tree (Figure S3). These ASVs were therefore excluded from further analysis. Thus, a total of 318 ASVs were kept for further comparisons of the AMF communities within P and nP soils.

3.3. Richness and Alpha-Diversity

The diversity (Shannon and Simpson indexes) and richness (Chao1 estimator) were used to compare AMF communities across P versus nP sites as well as between root or rhizosphere biotopes. A significant difference was observed between the two habitats, with higher diversity in roots compared to rhizosphere soil biotopes. In contrast, no significant difference was recorded comparing the P and nP sites either for the entire mining area (Figure 2) or within each localization (data not shown). No impact of P versus nP status was noticed in either the root or rhizosphere biotopes (data not shown).

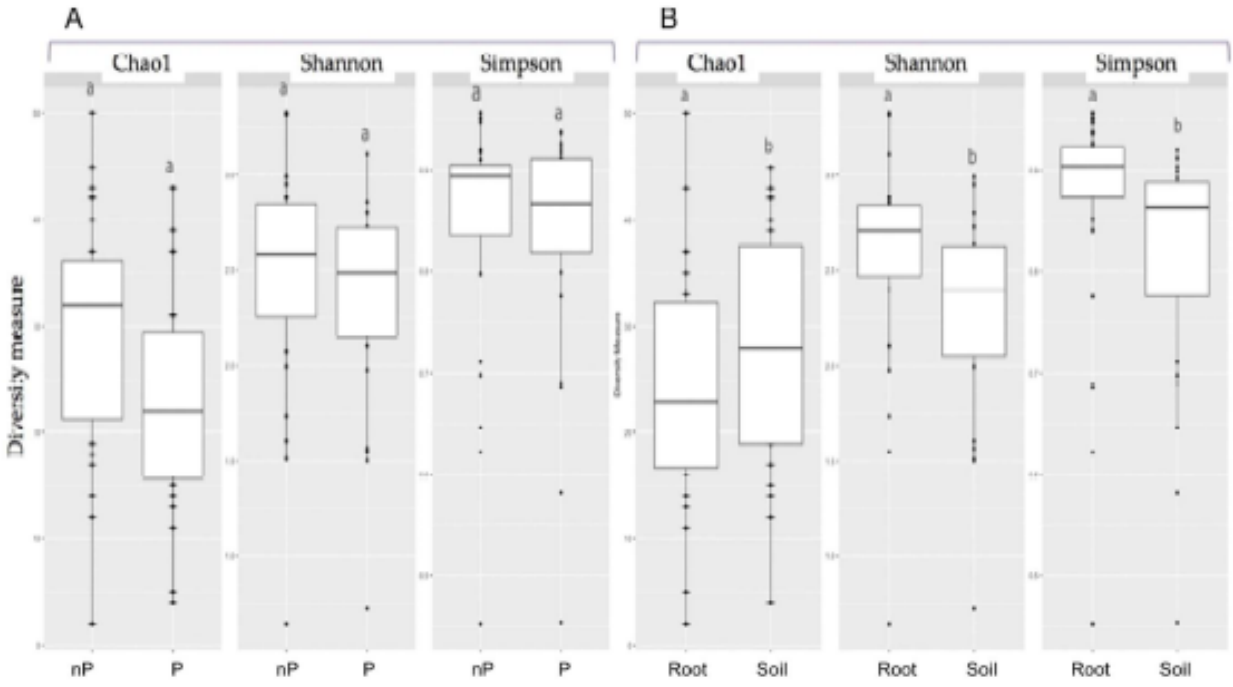

Figure 2. Richness (Chao 1 estimator) and diversity (Shannon and Simpson) indexes comparing AMF communities of (**A**) P versus nP soils, root and soil data being pooled; (**B**) root versus rhizospheric soil samples, P and nP site data being pooled. Within each graph, different letters denote significant differences using the Kruskal–Wallis nonparametric test ($p < 0.05$).

3.4. Taxonomic Assignment of ASVs

The 318 ASVs identified as Glomeromycota were assigned to Glomerales (81%), Diversisporales (12%), Paraglomerales (3%), and Archaeosporales (2%) (Figure 3A) while no taxonomic assignment at order level was obtained for 2% of them. Among them, five families were identified: Glomeraceae, Claroideoglomeraceae, Diversisporaceae, Paraglomeraceae, and Archeosporaceae. About 20.7% of the ASVs could be affiliated to a genus, mainly identified as *Rhizophagus*, *Funneliformis*, and *Glomus* (18, 15, and 14 ASVs,

respectively), but also, to a lesser extent, as *Archaeospora*, *Paraglomus*, *Septoglomus*, and *Claroideoglomus* (Table S4). With assignment up to the species rank, 39 ASVs (12.2%) could be named in line with referenced strains in the published databases. They were representative of eight species and six genera, namely *Archaeospora trappei*, *Claroideoglomus lamellosum*, *Funneliformis mosseae*, *Glomus indicum*, *G. iranicum*, *Rhizophagus irregularis*, *R. vesiculiferus*, and *Septoglomus africanum* (Figure 3B). Among them, the prevalent ones in terms of both read count and ASV numbers were *F. mosseae* (10 ASVs totaling together 12,591 reads, i.e., 2.7% of the readings), *G. indicum* (9 ASVs with 2698 reads, i.e., 0.6%), and *R. vesiculiferus* (6 ASVs with 8033 reads, i.e., 1.75%). Among ASVs assigned up to the species level, ASV 36, referred to as *F. mosseae*, was dominant in terms of relative abundance (2.6%) (Table S5).

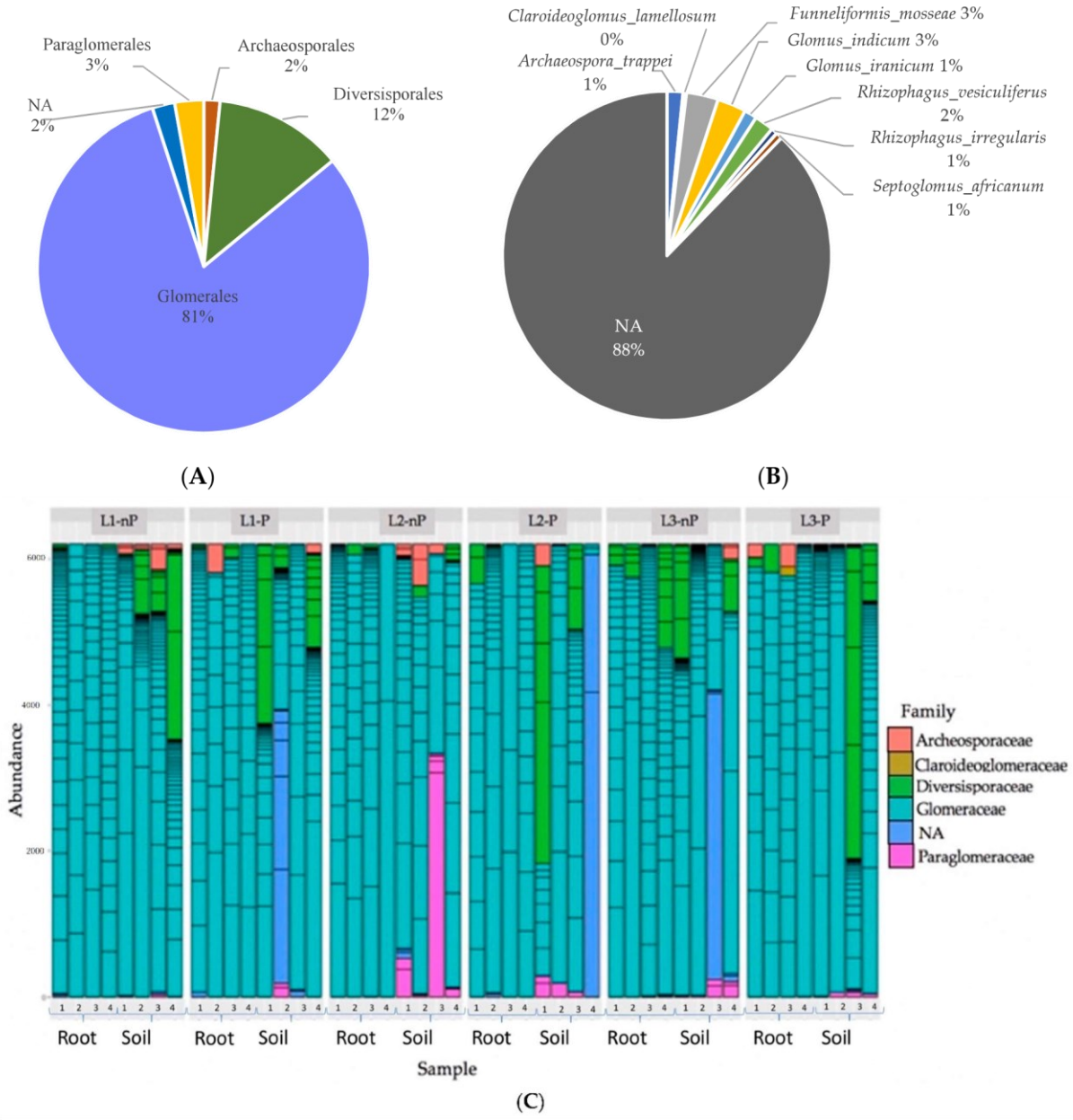

Figure 3. Taxonomic distribution of the 318 ASVs identified as Glomeromycota inside the entire mining area (**A**) at the order level, (**B**) at the species level, and (**C**) taxonomic distribution of the 318 ASVs at the family level inside each sample. "NA" category: ASV that did not obtain taxonomic assignment at the studied level.

Regarding variations across the samples (Figure 3C), Glomeraceae were largely dominant in all samples (i.e., across root and soil samples for each site) with Diversisporaceae and Paraglomeraceae mainly observed in soil.

3.5. ASVs Shared by the Six Mining Sites

When having a look at the different experimental conditions, a core of 26 ASVs (about 8.2% of the total ASV number) shared by the six sampling sites was identified (Table S6). These ASVs, ubiquitously present, correspond to about 64% of the total read number retained after the bioinformatic processing, taxonomic, and phylogenetic assignment. Among them, three were dominant (ASV61, ASV62, and ASV208), contributing to 57.1% of the read number of the 26 ASVs. With the exception of two ASVs assigned to Diversisporaceae, all core ASVs belonged to Glomeraceae. Two of them were identified up to the species level with a taxonomic identity inferred to *R. vesiculiferus*.

3.6. Dissimilarity in the AMF Community Composition and Identification of Indicator Species Comparing P and nP Sites

By comparing P versus nP sites, ASVs that were specific (i.e., occurring exclusively across the P versus nP sites) and ASVs that were shared (i.e., observed at both P and nP sites) were observed, as shown in Figure 4. Thus, considering the complete mining area, we identified 100 ASVs that are shared by the two profiles, representing 31.4% of the total ASV number but 86.5% of the total read number. In contrast, 138 ASVs (43.4%), accumulating 8.6% of reads, were identified as nP-site-specific (hereafter referred to as "nP-specific ASVs"). Concomitantly, 80 ASVs (25.2%) were P specific, displaying 4.85% of the reads (Figure 4).

Considering each localization, the same trend was observed, with P sites showing a lower number of specific ASVs compared to non-P sites. The most contrasting difference was observed in location two, where the number of nP-specific ASVs was almost twice the amount of P-specific ASVs (52 ASVs, or 41.6%, versus 28 ASVs, or 22.4%, respectively) (Figure 4). Variations in ASVs abundance among P vs. nP sites were also observed, as depicted in Figure 5, focusing on the 50 most abundant ASVs. In addition, it highlights the higher abundance of ASV 61, 62, and 208 in all sites.

Moreover, indicator species analysis was performed to search ASVs indicative of the following sample groups: the P versus nP group, the root versus soil group, as an individual group, or in combination (Table S7). Thus, in particular, seven nP-indicator ASVs and one P-indicator ASV were identified. All of them were assigned to Glomeraceae.

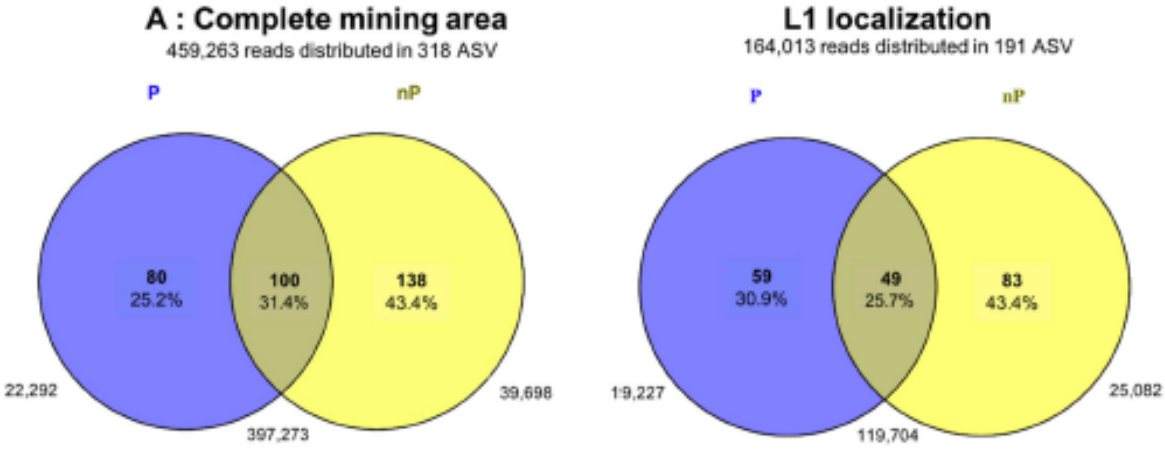

Figure 4. *Cont.*

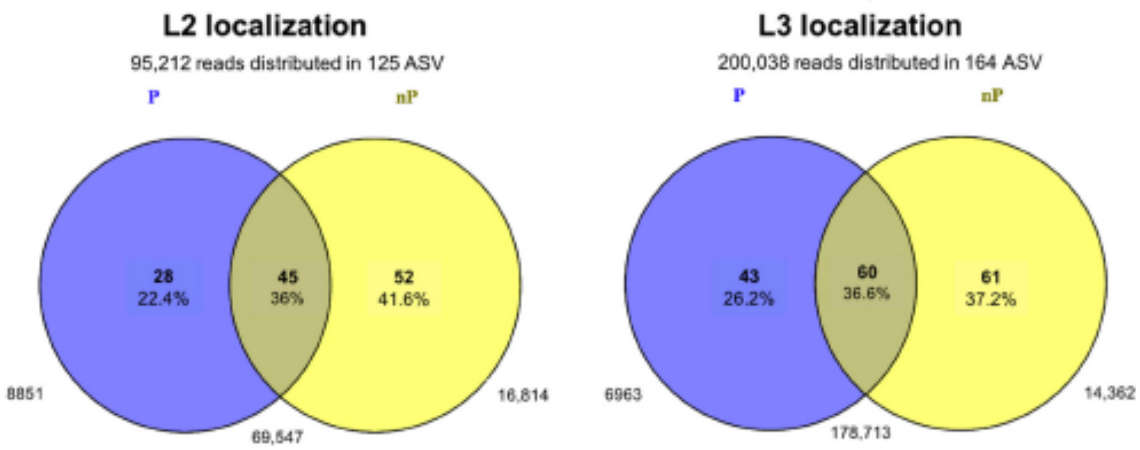

Figure 4. Venn diagrams showing the overlap of the AMF communities across P and nP sites, considering the entire mining area (A) or each localization individually (L1 localization, L2, and L3). Shared and specific ASV numbers and their relative abundance (in percent) are given inside the circles; the read numbers are given out of the circles.

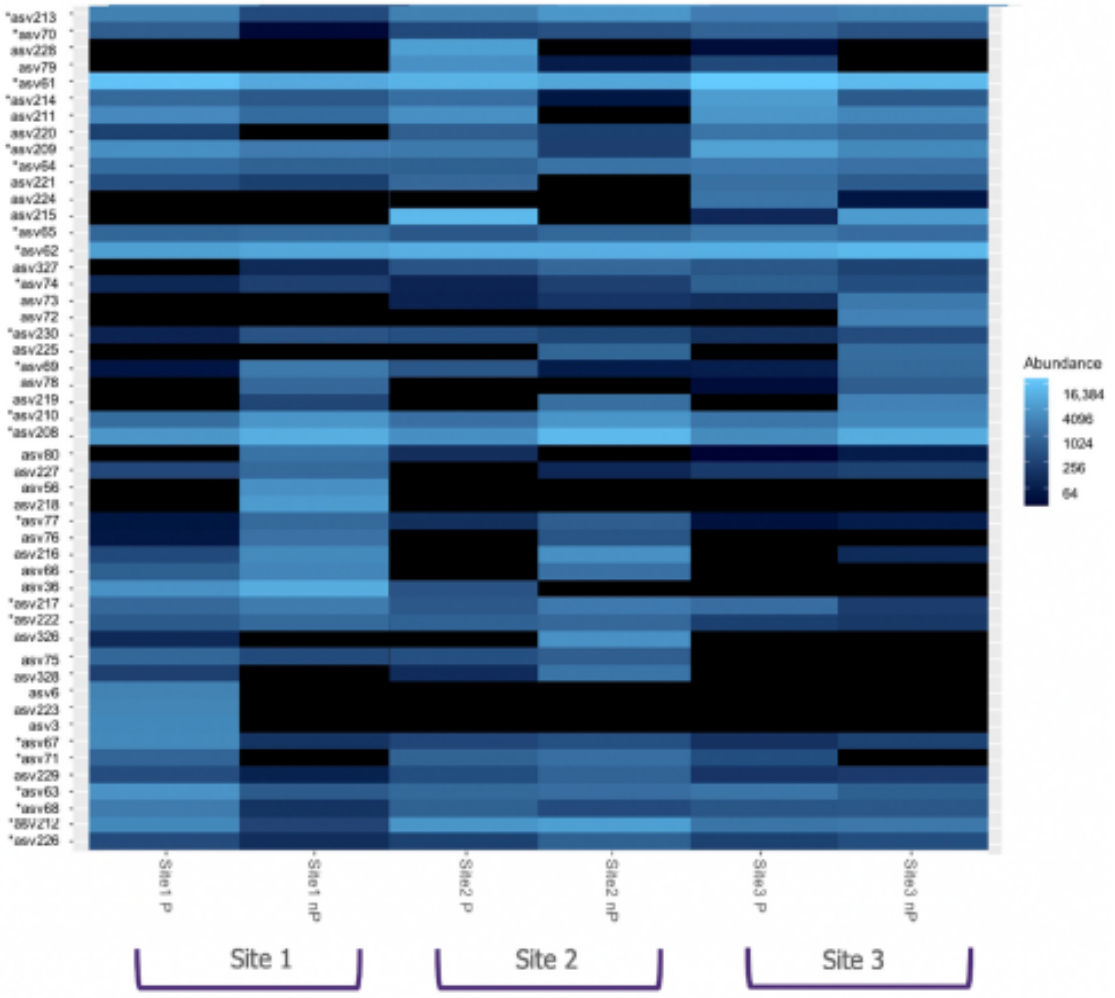

Figure 5. Heatmap representation showing the abundance of the 50 most abundant ASVs across all the sampling sites. The 50 most abundant ASVs include the 26 core ASVs shared by the six sampling sites *: Core ASVs shared by all sampling sites.

Regarding the taxonomic classification, no Archaeosporaceae member was observed in P soils. Paraglomeraceae and Glomeraceae tend to be reduced in P soils in terms of ASV percentages (Figure 6). In contrast, Diversisporaceae and Claroideoglomeraceae families tended to have increased with the P content. Lower percentages of assigned ASV were recorded at genus and species levels in P soils (Figure 6).

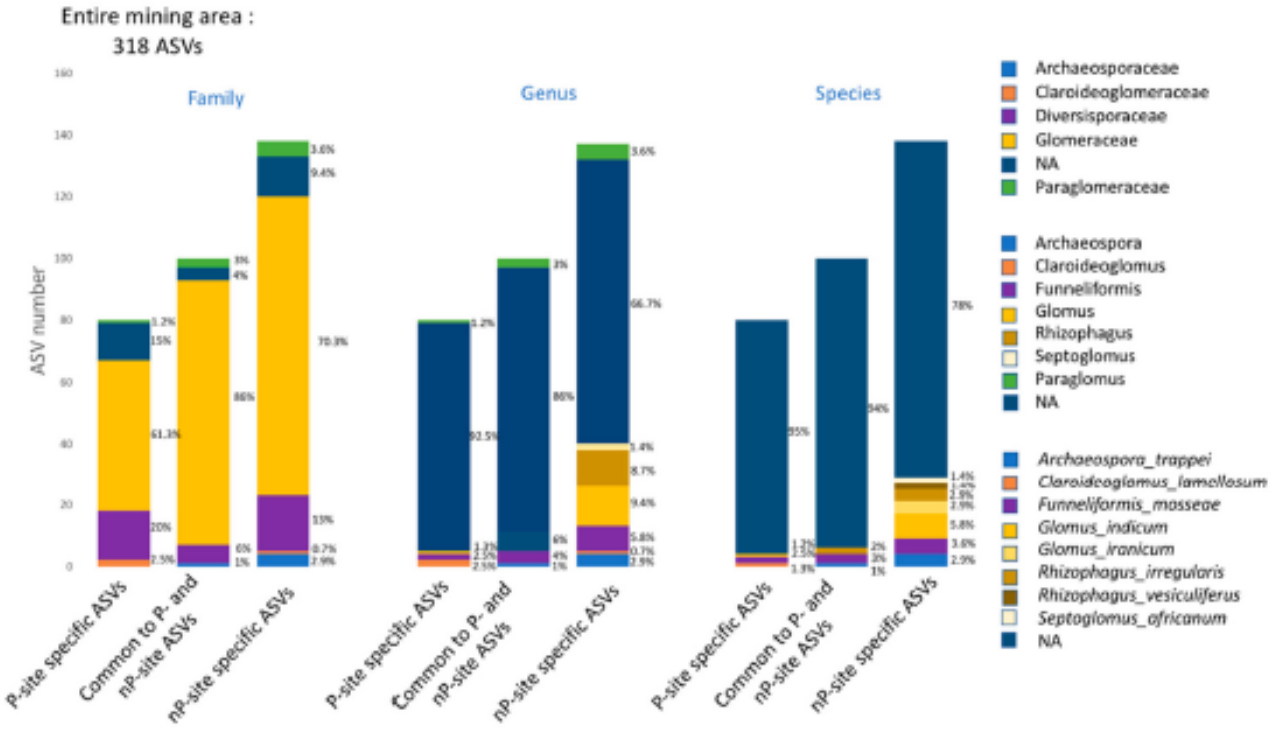

Figure 6. Distribution at the family, genus, and species level of the shared and specific ASVs identified in P and nP sites across the total mining area, NA: no-assigned ASVs.

In addition, comparisons between the three P sites were made. Different ASVs between the three P sites were identified with a higher number of site-specific ASVs and few shared ASVs, as shown in the Venn diagrams (Figure S4A). The same trend occurred comparing the nP sites (Figure S4B).

4. Discussion

In this study, we investigated AMF root colonization and taxonomic profiles of AMF communities in a former mining area comparing P and nP sites. These sites were characterized by total P concentrations ranging from 1067.1 to 13,927.9 mg·kg^{-1} while the values conventionally reported in the literature generally range from 40 to 3000 mg·kg^{-1} [65–67]. Additionally, the soluble P (P Olsen) concentrations ranged from 5 to 339.5 mg·kg^{-1}, which is largely above those displayed, for instance, in soils supplemented with mineral or organic fertilizers [67]. Such high concentrations in soluble P content are scarce and unexpected. Indeed, in the phosphatieres, soils are enriched with apatite, a form of highly insoluble soil P, and aluminous phosphate parageneses, clays, and sands [46]. As the soluble orthophosphate ions act as chemical ligands for soil compounds, P is generally considered to be highly unavailable in such soils and measured soil pH values (neutral). It is therefore appropriate to question the putative origin of such soluble P concentrations. Our work on the bacterial and fungal communities of the same sites revealed the presence of numerous taxa known to host phosphate-solubilizing microorganisms, and phosphate-solubilizing bacteria have also been successfully isolated (data not shown). These microorganisms may be factors of P release from its unavailable form. However, this would imply that there is no feedback to regulate microbial P solubilizing metabolic activity in the presence of high

phosphate substrate concentrations. However, to the best of our knowledge, there is no data in the literature on this point. In any case, such variations in P content provided the opportunity to examine the potential role of P content as a driver of AMF communities.

4.1. High P Contents in the Mining Area do Not Impact AMF Root Colonization

In this study, no difference in AMF colonization rates was observed when comparing P to nP soils, which therefore excluded a negative impact of high P levels on AMF colonization in the reclaimed soils studied.

Yet, a decrease in mycorrhizal colonization when P availability is high has been described by numerous authors [68–75]. Additionally, decreases in the abundance and richness of AMF communities were also observed in soil and roots after P fertilization [76–79]. However, some authors underlined that the decreased AMF colonization upon P fertilization was not systematic [80–84]. Moreover, a moderate amount of P fertilizer could improve AMF diversity while higher amounts of P reduce it [85,86], suggesting that the AMF response to soil P could be related to the P concentration in the soil [87]. On the contrary, Higo et al. [39] observed that the P fertilizer level did not impact either the AMF root colonization, diversity, or community structure.

Such data, therefore, highlight that the AMF response to P is a complex process and underline that the P level is not a reliable predictor of AMF occurrence, root colonization, or community patterns [88,89]. In particular, the AMF response to P may be strongly impacted by sampling time [90], agricultural management [39], or host plant species [28,42,83]. For instance, Tang et al., 2016 [83] showed that for faba bean, an excess of P fertilization did not significantly affect the mycorrhizal rates when compared to a low P and no P treatment. Conversely, the durum wheat root colonization was significantly lower at a high P level, and the arbuscular rate drastically decreased at both low and high P fertilization compared with the unfertilized treatment. In this line, we question the role of the site mining history possibly acting as a stronger driver of AMF communities compared to the P level.

4.2. AMF Community Characteristics across Sites: Consequences of Site Mining History Rather Than P Concentrations

In this report, we observed a large taxonomic similarity between AMF communities at the family level whether the soils were enriched in P or not. Glomeraceae was largely prevalent throughout the entire mining ecosystem whereas Archeosporaceae, Diversisporaceae, Paraglomeraceae, and Claroideoglomeraceae were under-represented. Paraglomeraceae and Gigasporaceae were not detected. In addition, we highlighted 26 ASVs shared by all sampling sites, three of which were very abundant.

Several hypotheses may be put forward to explain the low richness or diversity in terms of taxa at the family level: either the absence or under-representation of some AMF families and in contrast, the over-representation of Glomeraceae.

Firstly, Glomeraceae is classically recognized as the most widespread family in global, natural, and managed ecosystems [91,92]. In addition, anthropogenic activities are known to have considerable influence on AMF communities. Notably, intensive agricultural practices, such as soil disturbance by tillage [93] or P fertilization, can adversely affect AMF colonization and diversity [75,88,94,95]. Notably, some *Glomus* spp. are commonly found in soils subjected to fertilization and disturbance whereas others, especially *Scutellospora* spp., are indicative of minimally disturbed soils [96]. In the same way, some results interestingly suggest that soil AMF communities may differ according to land use history and time after land use conversion, suggesting that their diversity and composition may be a legacy of ancestral adaptations to historical habitat and exhibit niche conservatism [33,96]. It has also been suggested, that intensively managed agroecosystems are greatly simplified compared to natural ecosystems. For instance, comparisons between AMF communities in agrosystems managed in different ways showed that families such as Gigasporaceae or Acaulosporaceae are more frequently reduced or eliminated than Glomeraceae [97,98].

In theory, the AMF's life-history strategy (LHS) has been proposed to explain the dynamics, variations, and prevalence of some taxa among AMF communities [99,100]. This concept identifies K-strategists (i.e., AMF competitors that evolved traits to enhance survival in stable environments where competition is high) and r-strategists, or ruderals, that evolved traits to survive under stressful conditions and in disturbed environments, investing their energy mainly in the production of numerous offspring. Notably, Glomeraceae may show an opportunistic behavior similar to r-strategists [101–103]. Thus, taxa resilience and the prevalence of Glomerales in disturbed environments could be explained through abilities such as their higher sporulation, ability to produce high numbers of new hyphae from injured hyphae after mechanical disruptions with healing mechanisms, and anastomosis processes [103–105]. These properties are probably more suited to perturbed environments, and may enable them to spread and reestablish more quickly [106,107]. In addition, it has been shown that representatives of the *Glomus* genus promptly respond to carbon resource shortages with direct investment in the production of spores for reproduction, as is expected for r-strategists [108]. Conversely, Gigasporaceae may resemble the LHS of K-strategists; they mainly spread or colonize through spores from an intact mycelium within a long life cycle [109], which does not facilitate recovery and resilience from disturbance.

With regard to these theoretical assumptions, homogeneity across AMF communities as reported in our study might be explained partially by the landscape history, i.e., firstly by the drastic soil perturbation due to mining activities and secondly, by the restoration and revegetalization of soil during more than one century. Thus, we hypothesize that the soil recolonization by AMF would have taken place with the extensive development of AMF species functionally adapted to rapid recolonization and the stress of high P concentrations. Here, Glomeraceae could be relevant candidates while other AMF families could have been drastically or progressively suppressed, becoming low or nondetectable today under the influence of persistent high P concentrations in the soil for more than a century. In particular, no representative of Gigasporaceae has been identified in the sampled soils. Yet, it has been shown that Gigasporaceae provide functional complementarity to other AMF families in P uptake [110]. In our conditions, due to high soluble P concentrations in the sampling soils, we hypothesize that additional benefits might not have been supplied to the host by such taxa, resulting in their absence. Indeed, the assimilation of P by AMF-colonized plants reflects the sum of P uptake directly by plant cells and indirectly via the AMF pathway [69]. This, however, becomes subsidiary to plants when the P availability is high in the soil matrix and when direct plant uptake is sufficient to provide enough Pi without the help of the indirect mycorrhizal pathway. Consequently, AMF may no longer be essential for P uptake while their other functions (contribution to the nutrient or water supply as well as stress resistance) would remain major, hence, the persistent presence of AMF in P-rich soils. Therefore, we also hypothesize that, besides the predominance of Glomeraceae, the decline or loss of some AMF taxa, and the decrease in overall diversity and afferent mycorrhizal functioning, Glomeraceae recruited by plants may display a large functional diversity and complementation to gain the maximal benefits from the AMF communities in favor of the soil and host plant and for maintaining ecosystem functions.

In line, the occurrence of a representative of *Funneliformis* described in the literature as a ruderal and stress indicator taxon [100] and as a generalist AMF [111] may be compatible with the disturbed land story of sampling sites.

Following our results, the meaning and role filled by the set of persistent ASVs capable of coping with radically contrasting and altered environments can also be questioned. One could hypothetically suggest that this microbial core could correspond to taxa with basic functions (such as nutrient transfer, mainly C and N) and functional features (not yet identified) that confer some level of robustness, resistance, resilience, or functional redundancy in the face of high P content, environmental disturbances, and progressive soil restoration over time [112–114]. However, data about the taxonomic affiliation of the core members are insufficient to go further about their functionalities. Moreover, data in the

literature allowing functional inference to a given AMF species are still limited since the taxonomy and identification of AMF species are still to be refined.

4.3. A slight Impact of Soil P on AMF Assemblages Is Resolved at ASV Level

In this study, we identified shared and specific ASVs characterizing the P and nP sites; on the other hand, a lower richness and abundance of AMF communities were observed for the P profile compared to the nP profile. This was particularly pronounced in one site (the S3 site) which was furthermore characterized by the highest concentrations of total and soluble P.

Moreover, through an indicator species analysis, we statistically identified ASVs that are indicative of P and nP sites based on the indicator value calculation as a measure of specificity and fidelity of each ASV to the targeted clustering groups, i.e., P vs. nP site group [63]. Notably, both P- and nP-indicator ASVs were identified but with a lower number of P-indicator ASVs. As a whole, these results clearly differentiate the P and nP sites and therefore highlighted that the AMF community is responsive to P as an edaphic factor in driving AMF communities when analysis is performed with a stringent level of taxonomic resolution.

5. Conclusions

In this study, we identified a large similarity of AMF community compositions at the family rank as well as the persistence of a set of ASVs along the drastic P gradient inside the mining area sampled. This leads to the conclusion that AMF community profiles were robust across the contrasting soil P levels. Nevertheless, the shared and specific ASVs from the P vs. nP sites were also identified as well as indicator ASVs of the P vs. nP sites which, therefore, differentiated the P vs. nP sites. As a whole, these results highlighted the AMF community assemblages' responsivity to P but also showed that P is currently not a strong or prevalent ecological pressure exerted on the AMF community composition across the sampling area.

Conversely, our results suggest that contemporary AMF community assemblages may prevalently reflect life-history strategies and resilience of AMF communities, in response to mining history (i.e., land disturbance then ecosystem restoration), favoring Glomerales that behave as ruderals, able to rapidly respond to stressful conditions through their ability to disperse, colonize, and persist in soils and roots over time during reclamation of land-mined soils.

In conclusion, this work opens new opportunities to advance our in-depth understanding of the analysis of P's impact on AMF communities. It also improves the knowledge of AMF diversity in natural P-rich soil, thus providing novel perspectives for the design of AMF inoculants to be used in combination with RP fertilization and the development of more sustainable agroecosystems.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/microorganisms10122406/s1. Figure S1: A: The remaining material from past open-pit mines, B, C, D: Landscape in localization 1, 2, and 3 respectively. Figure S2: Rarefaction curves obtained from the 18S rRNA gene dataset A: Curves obtained from the 3,527,278 sequences yielded after quality filtering and chimera removal (24 soil samples and 24 root samples). B: Rarefaction curves obtained after a random resampling step, computed to compensate for the uneven sequencing efforts of different samples and to keep an adequate sequencing depth to capture most of the AMF diversity from soil and root samples. Thus, the curves were obtained for a standardized 6000 sequences per sample. Figure S3: taxonomic assignment and RaxML phylogenetic tree of mycorrhizal AVSs. Different colors differentiate each family assigned. Red to pink: Diversisporales; yellow to orange: Paraglomerales; dark orange: Archaeosporales; green: nonassigned; blue and purple: Glomerales; and grey: others. Figure S4: Numbers of specific and shared ASVs comparing (A) the P sites and (B) the nP sites of the three localizations: Low membership characterized by a high number of ASVs specific to one site and few overlapping ASVs. Table S1: Total P and available Pi contents in soils sampled across three localizations (L1, L2, L3); each harboring "P" site covered in the past by PR

deposits or "nP" sites without deposit. Table S2: Distribution of number of reads in each treatment and plant species. Table S3: Taxonomic distribution of the ASVs identified as AMF by the Silva database and those identified de novo by the phylogenetics analysis. Table S4: Taxonomic distribution patterns of the 318 ASVs identified as Glomeromycota at the genus (A) and species (B) taxonomic rank. Table S5: ASVs with assignment up to species taxonomic level (39 ASVs). Table S6: Shared ASVs (26) by the six sampling sites. Table S7: ASVs identified after indicator species analysis.

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CHAPITRE 2

B. Caractérisation taxonomique et détermination des traits fonctionnels PGP, d'isolats BSP issus de sols riches en RP

Synopsis

La sélection d'isolats racinaires et hyphosphériques PSB

L'exploitation des traits fonctionnels microbiens liés à la solubilisation du P est primordiale afin de proposer des stratégies de biofertilisation permettant d'augmenter l'efficacité de l'utilisation du RP, notamment dans de nombreux sols agricoles tropicaux à forte rétention de P (Kumar, 2016). Pour mémoire, l'analyse des données de la littérature montre que les choix expérimentaux constituent une opportunité et un challenge pour l'ingénierie d'inoculants synthétiques. Notamment, il est apparu qu'une pression environnementale pouvait permettre de sélectionner des isolats adaptés à cette pression.

Au sein du RP, la biodisponibilité du P étant faible comme expliqué au chapitre 1 de ce mémoire, nous avons donc posé pour hypothèse qu'un environnement hyper-riche en RP pouvait induire des mécanismes d'adaptation à cette contrainte/pression édaphique, et permettre d'isoler des bactéries utilisant le RP comme substrat pour leur croissance, et donc exprimant le trait solubilisateur de P.

L'originalité de notre recherche repose sur le choix des dispositifs d'isolement des souches bactériennes. Nous avons ainsi travaillé avec deux dispositifs expérimentaux qui intègrent des habitats singuliers, riches en RP, et à notre connaissance, peu explorés à travers les études déjà publiées. Le premier type d'habitat correspond aux racines de plantes herbacées (*Ranunculus bulbosus, Bromus sterilis, Taraxacum officinale* and *Dactylis glomerata*) échantillonnées dans les sols miniers riches en RP, pour la première partie de ce mémoire, et donc pour lesquels nous disposons de données écologiques de terrain. Le second type d'habitat correspond à l'hyphosphère de plantes, cultivées dans des parcelles expérimentales et supplémentées en RP par un apport très localisé au niveau des racines.

Ces travaux sont résumés ci-après sous forme d'un article scientifique (Article 4) dont la soumission est prévue dans la revue Microbiological Research : *Ducousso-Détrez, A., Lahrach, Z., Fontaine, J., Lounès-Hadj Sahraoui, A., & Hijri, M.*. Culturable Phosphate Solubilizing bacteria isolated from rhizospheric and hyphospheric habitats enriched in rock phosphate.

Principaux résultats obtenus :

Les deux procédures expérimentales retenues ont permis l'obtention d'une banque d'isolats bactériens racinaires et hyphosphériques, et d'identifier des PSB aptes à solubiliser in vitro le TCP avec des concentrations allant jusqu'à 259 et 175,50 $\mu g.ml^{-1}$, de P solubilisé respectivement parmi les isolats racinaires et hyphosphériques.

Le séquençage Sanger de l'ADNr 16S et l'analyse phylogénétique ont conduit à classer les PSB racinaires et hyphosphériques sélectionnées dans trois phyla bactériens : Proteobacteria, Firmicutes et, dans une moindre mesure, Actinobacteria. Les genres majoritairement identifiés sont *Bacillus* et *Pseudomonas*.

Les isolats PSB ont également été testés pour d'autres traits promoteurs de la croissance des plantes, telles que la production d'auxine, d'ammoniac, de sidérophores et de biofilm, la fixation de l'azote et la motilité bactérienne. Nos résultats montrent que les PSB sélectionnés sont dotés de capacités supplémentaires et multiples de promotion de la croissance des plantes. En particulier, deux isolats sont positifs pour quatre traits PGP et plus de 79,5 % des PSB présentaient plus d'un trait PGP.

La présence de PSB dans les sols riches en RP et P soluble est discutée, ainsi que la diversité taxonomique et fonctionnelle des isolats sélectionnés en tant que critère de sélection pour la formulation d'inoculants à usage agronomique, utilisables dans les sols enrichis en RP. Dans un cadre plus large, nous interrogeons également l'usage des PSB isolés depuis des environnements riches en RP, dans un contexte, non seulement agronomique, mais également de réhabilitation de sites miniers phosphatés abandonnés et leur restauration écologique.

ARTICLE 4 : Caractérisation taxonomique et détermination des traits fonctionnels PGP, d'isolats BSP issus de sols riches en RP

Ce paragraphe est présenté sous forme d'un article scientifique, en préparation pour une soumission.

Title: Culturable Phosphate Solubilizing bacteria isolated from rhizospheric and hyphospheric habitats enriched in rock phosphate

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Abstract:

Integration of Phosphate Solubilizing Bacteria (PSB) inoculants and rock-phosphate (RP) concentrate, two natural resources, is recognized as a cost-effective and environmental-friendly strategy compared to the use of conventional synthetic phosphate fertilizers. In this study, two experimental setups were used to isolate PSB, either from the root of native plants grown in a RP-mining area, or from the mycohyphosphere (zone of influence of arbuscular mycorhizal fungal hyphae) trapped in a membrane bag of 50 µm pore size containing RP concentrate, placed nearly crops grown in field conditions.

A subset of 24 bacterial isolates was selected out of 210 rhizospheric morphotypes, obtained from the rhizospheres of plants sampled from RP-mining, and subjected to an *in vitro* qualitative and quantitative P-solubilization assays using tricalcium phosphate (TCP) as the sole P source to test their inorganic P solubilizing activities. Another subset of 25 bacterial isolates out of 44 morphotypes that were obtained from mycohyphosphere of trapped fungal hyphae, was also tested for P solubilizing activities. These two bacterial subsets were then screened for some additional plant growth-promoting (PGP) traits, such as auxin, ammonia, siderophore and biofilm production, nitrogen fixation and bacterial motility. The bacterial isolates were identified using PCR and sequencing of 16S rDNA followed by phylogenetic analysis, which clustered these PSB isolates into three bacterial phyla, namely Proteobacteria, Firmicutes and in a less extend, Actinobacteria. *Bacillus* and *Pseudomonas* were the dominant genera. The two isolation experiments resulted on diverse PGP traits and phylogenetic affiliations of PSB bacterial collection that could be exploited to develop bioinoculant consortia and their integration into agricultural practices with the aim to increase phosphate-use efficiency in agroecosystems.

Keywords: Rock phosphate, Phosphate-solubilizing bacteria, bio-inoculants, rhizosphere, hyphosphere, mining area

INTRODUCTION

Stable agricultural production in conventional cultural systems relies on continual inputs of chemical P-fertilizers. However, their industrial production is extremely costly and may result in environmental injures and pollutions (Hudson-Edwards, 2016). In addition, a large fraction of soluble inorganic phosphate applied to the soil as chemical fertilizer can be rapidly sequestered and become unavailable for plant requirement. Therefore, to reduce environmental footprint of agricultural practices, sustainable alternatives that reduce over-reliance on chemical fertilizer applications while maintaining crop production, have been tested (Shen *et al.*, 2011). So, research efforts explored the multiple functions of the root-associated microbial resources to provide microbial-based agro-inputs that exhibit relevant effect on plant growth with low mineral inputs (Compant *et al.*, 2019; Elhaissoufi *et al.*, 2021). Indeed, the rhizosphere (i.e. the zone of soil surrounding plant roots where the biology and chemistry are influenced by root exudates) is regarded as a hot spot for microbial diversity (Richardson *et al.*, 2021) where neutral, positive, or harmful effects of microorganisms on plant fitness occurred (Barea, 2015; Rosenberg & Zilber-Rosenberg, 2016; Hassani *et al.*, 2018). In particular, free-living bacteria, commonly referred to as Plant Growth Promoting Rhizobacteria (PGPR) harbor diverse functional traits that promote plant growth and health (Richardson *et al.*, 2009; Vacheron *et al.*, 2013). They show a broad taxonomic diversity and interact cooperatively with a large range of host plant species (Hamedi & Mohammadipanah, 2015). The benefits to plant from host-PGPR interactions can be achieved by one or more mechanisms which have been described and discussed in several reviews (Vacheron *et al.*, 2013; Pérez-Montaño *et al.*, 2014; Bargaz *et al.*, 2018).

Among PGPR, phosphate solubilizing bacteria (PSB) mediate bioavailable soil P and play a critical role in soil P cycle, solubilizing in particular, inorganic P compounds through the release of low molecular weight organic acids which leads to the chelation of phosphate-bound cations (in particular the Al, Fe and Ca ions), thus converting P into soluble forms ($H_2PO_4^-$ and HPO_4^{2-}) (Goldstein, 1995). Therefore, PSB are considered as relevant drivers of biogeochemical P cycle (Tian *et al.*, 2021; Ducousso-Détrez *et al.*, 2022), having the potential to improve P availability in the rhizosphere, for their own need and potentially, for P uptake and nutrition of plants (Raymond *et al.*, 2021). Additionally, one PGPR/PSB strain can exhibit multiple PGP traits that act in synergy with P solubilization to promote plant growth (Shakeela *et al.*, 2017; Zhang *et al.*, 2017). Consequently, microbial biotechnology and biofertilization strategy are today considered to be among key agricultural components to improve soil P fertility and crop productivity, while contribute to more sustainable agro-ecosystems. (Verma *et al.*, 2015; Lobo *et al.*, 2019). And numerous bioinoculant products are available on the global market (Owen *et al.*, 2015; Koskey *et al.*, 2021).

Formulation of microbial-biofertilizers and inoculum engineering operate across several steps (Lawson *et al.*, 2019). The first one requires isolation, selection and identification beneficial bacterial isolates (Elhaissoufi *et al.*, 2021). Routinely, screenings for PSB are performed from rhizospheric soils or root where it is well admitted that PSB are abundant (Marschner *et al.*, 2004; Ashok *et al.*, 2012; Baliah *et al.*, 2016). PSB have also been screened from the soil environment along the extraradical hyphae of Arbuscular Mycorrhizal Fungi (AMF) : the hyphosphere (Johansson *et al.*, 2004; Toljander *et al.*, 2006). Indeed, in this microhabitat, it is well established that some bacteria can adhere to the surface of AMF hyphae (Ordonez *et al.*,

2016; Taktek *et al.*, 2017). And, even interactions between PSB and AMF are not well understood, it has been hypothesized that they may work synergistically in providing benefit to the plant (Toljander *et al.*, 2007). Thus, Cruz & Ishii (2011) successfully isolated probable endobacteria such as *Bacillus* and *Paenibacillus* isolates from *Gigaspora margarita* spores that exhibited multiple PGP traits including P solubilization. In accordance, Battini *et al.* (2017) evidenced positive effects of AMF and their associative endobacteria isolated from AMF spores, regarding facilitation of P uptake under P-limiting conditions. Benefits also occur for AMF and hyphae-associated PSB communities, interactions providing key resources to each other (Zhang *et al.*, 2016). In particular, AMF hyphae exudates (sugar, amino acids, carboxylates) may provide key nutriments for bacterial growth, while attachment of bacteria with P solubilizing capacity to the extraradical AMF hyphae, could allow the fungi to get additional soluble orthophosphate ions (Jansa *et al.*, 2013; Zhang *et al.*, 2018; Jiang *et al.*, 2021). Concomitantly, PSB can access to nutrients with limited diffusion in soils, inside of the mycorrhizosphere (Ordoñez *et al.*, 2016). Therefore, the speculations about joint PSB and AMF positive interactions on nutrient acquisition make isolation of hyphospheric PSB as promising to help the formulation of biofertilizers inoculants (Jansa *et al.*, 2013; Zhang *et al.*, 2016).

Furthermore, the dual use of microbial and mineral P resources has also increasingly gained attention as a promising approach. Indeed, used in combination, the two resources may synergistically co-interact to increase the agronomic efficiency of mineral fertilizer and improve the supply of nutriment and functions that plants need to thrive. Notably, the combinatory use of PSB-based inoculants and Rock phosphate (RP) had led to a successful "microbial-P mineral alliance" (Bargaz *et al.*, 2018; Tahir *et al.*, 2018). Indeed RP is a geological P-rich rock used alone in some agricultural systems to efficiently improve crop production with lower costs and environmental damages than water-soluble P chemical fertilizers (Sharma *et al.*, 2013; Ahemad & Kibret, 2014). However, due to its low solubility in water (largely linked to chemical, crystallographic and mineralogical composition of their apatites), RP reactivity (i.e. the rate of orthophosphate ion release when applied directly to soils under favorable soil conditions) and its agronomic effectiveness are generally lower than those of commercial fertilizers (Verma *et al.*, 2012). Nevertheless, a number of studies evidenced improved P availability in soils when RP is combined with PSB (Bargaz *et al.*, 2018; Elhaissoufi *et al.*, 2021), with significant increases in P uptake, shoot/root biomass and yield performances compared to inoculation treatments singly (Kaur & Reddy, 2015; Manzoor *et al.*, 2016; Adnan *et al.*, 2017; Ditta *et al.*, 2018; Elhaissoufi *et al.*, 2020).

Classically, the search for PSB is mainly performed in soils with low P-availability, with the assumption that in such environments, plants will attract in their rhizosphere and favor beneficial microbes (such as PSB) to cope with particular stress (for instance P starvation). This hypothesis, linked to the theory denominated "cry for help" proposed by (Liu & Brettell, 2019), therefore suggests that the probability of isolating PSB will be facilitated. In contrast, microbial communities, among them PSB, from RP mining environments are poorly investigated and described (Yang *et al.*, 2012). Nevertheless, exploration of diversity in microbes able to grow in alkaline P mining wastes, phosphogypsum has gained in interest in the last few years (Thabet *et al.*, 2017; Ye *et al.*, 2020; Mghazli *et al.*, 2021). Yet, their selection could be useful in developing bioinoculants better suited for agronomic purposes when used in combination with RP (Thavamani *et al.*, 2017), assuming that i) PSB communities in RP soils may have specific

metabolic profiles and molecular mechanisms that allow them, in response to RP, to adapt and thrive in RP enrichments, ii) RP is a relevant nutrient resource for beneficial microbes able to solubilize such ore characterized by high total P content but low reactivity leading to reduced P-availability in solution. Thus, in order to increase knowledge in this research area, and with the goal of developing a collection of PSB isolates adapted to RP-rich soils, we targeted two types of RP-rich environments for sampling PSB isolates: (1) the rhizosphere of herbaceous plants native of a former RP mining area, and (2) the hyphosphere of crop plants, grown in experimental plots supplemented with RP. The selected PSB were further screened for additional conventional plant growth-promoting traits and isolates were identified by 16S rDNA sequencing and phylogenetic analysis. The taxonomic and functional diversity of the selected isolates is discussed as a potential component in further inoculant formulation for increasing crop production, especially in RP-enriched soils.

EXPERIMENTAL PROCEDURES

Experimental setup for isolation of rhizospheric bacteria

- ***Sampling sites***

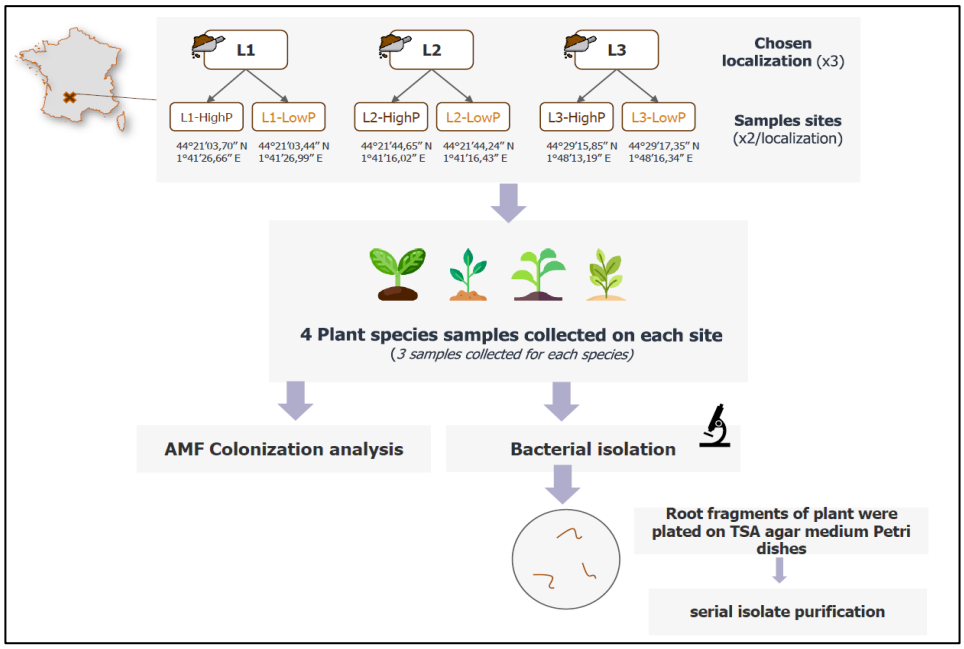
In order to isolate PSB from the rhizosphere, samplings were performed in a former RP mining area located in the National Nature Reserve of Geological Interest of the Lot department in France (44° 22′ 22″ N, 1° 41′ 16″ E). In this area, 3 locations (L1, L2 and L3) were identified, with for each, two sites characterized by contrasted P concentrations (referred to as high P to low P). Indeed, during mining operations, sedimentary PR-enriched soil fractions were exploited to manufacture P fertilizers, while the finer sediments were left in place. This resulted in surface soils locally enriched in phosphate deposits (hereinafter referred to as P soils), side by side with soils not enriched in phosphate (nP soils).

Briefly, the main soil characteristics were as follow: soil pH ranging between 6.9 and 7.3, total P concentrations ranged from 1 057,1 to 13 927,9 $mg.kg^{-1}$ while available Olsen P levels were 5,04 to 339, 5 $mg.kg^{-1}$. Total C contents were between 3.50 - 8.25% with total N ranged from 3.04 - 6.99% while C/N ratio varied from 11.2 to 14.04. Concomitantly, the percentage of organic matter (OM) varied between 6.04 - 14.22 %. Concentrations Al, Fe, Cu, Zn, and Ca are reported Table S1.

- ***Plant sampling***

A set of native plant species (*Bromus sterilis, Dactylis glomerata, Taraxacum officinalis* and *Ranunculus bulbosus*) were sampled across the mining sites, then placed in sterile Whirl-Pack bags, and stored at 4°C until bacterial isolation process.
Subsequently, the roots were separated from the aerial parts, and the bulk soil yet adhering to roots was eliminated by vigorously shaking the roots. Then, fresh root segments were washed by shaking in 90 mL of sterile saline solution (NaCl, 0.85% W/V) before to be plated on Tryptic Soy Agar (TSA) agar medium Petri dishes. Colonies were picked up after one week culture and again spread over the TSA nutrient plates. Serial subcultures were performed on the same medium until isolate purification, if required (Figure 1A).

A



B

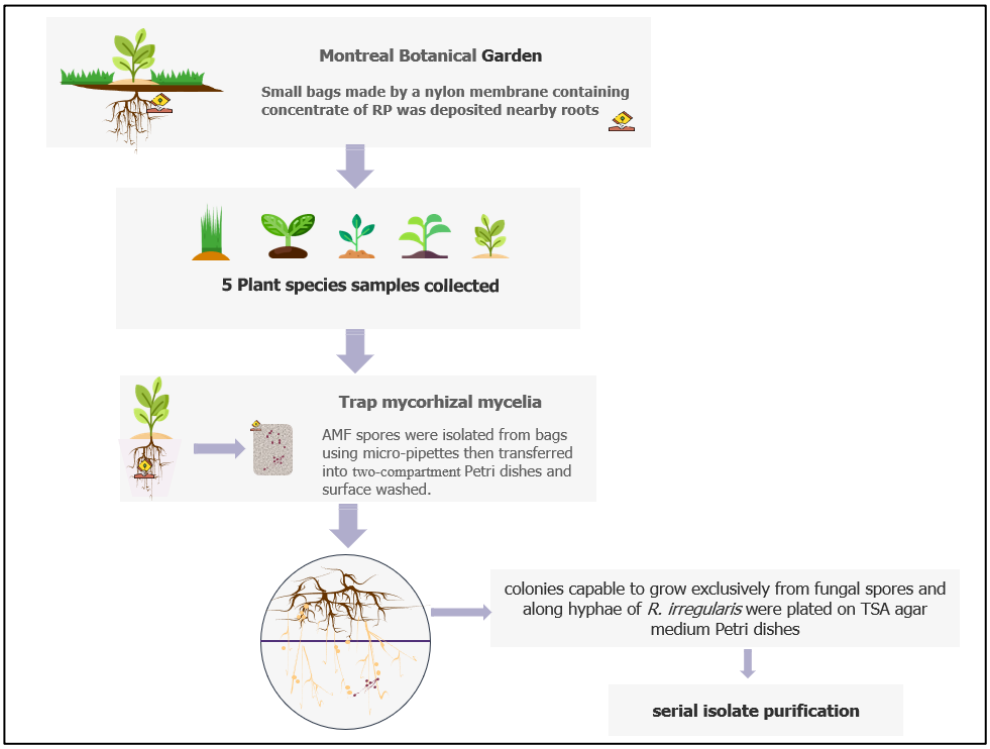


Figure 1: Experimental setup for root (A) and hyphosphere (B) bacteria isolation.

Experimental setup for isolation of hyphospheric bacteria

Hyphospheric bacterial isolates were selected throughout an experimental process consisting of three steps. The first was conducted with the objective of trapping mycorrhizal structures (spores and hyphae) of native AMF. It was performed at the Montreal Botanical Garden on June 2018, in a plot characterized by a Black Chernozem soil and used exclusively for organic farming practices. Thus, small bags (5cm width x10 cm length) made by a nylon membrane (opening size of 200 µm) containing igneous RP (Canada) or sedimentary RP (Morocco) were deposited nearby roots of the following plant species: potato, tomato, leek, eggplant and maize. After three months of culture, the RP bags were removed from the soil. A supplementary step was performed to extend the time required for the spore's development and increase their number inside the RP bags. It was carried out in a greenhouse (22°C with a photoperiod of 8h-16h) and RP bags were deposited near the roots of plants grown in pots. The soil substrate was watered to field capacity with tap water as needed. In addition, fertilization with a Hoagland solution without P, was performed weekly. After six months, bags were collected from each pot and AMF spore extraction from soil was performed using micro-pipettes. Collected spores were then surface washed with successive baths of sterile water with EDTA (0.5%).

The third step of the experimental setup aimed to isolate bacterial strains from AMF spores previously trapped and harvested from the RP bags. It was performed *in vitro*, using a two-compartment Petri dish design, as previously described by St-Arnaud *et al.* (1995) then taken over by Taktek *et al.* (2017). This design was used to isolate hyphospheric bacteria able to grow on the surface of AMF hyphae, utilizing the extraradical mycelium exudates as sole source of energy. Thus, the AMF *Rhizophagus irregularis* DAOM 197198 was grown on *Agrobacterium rhizogenes*-transformed carrot (*Daucus carota* L.) roots, in the first Petri dish compartment filled with 20 ml of M medium (Becard and Fortin, 1988). The second compartment received 20 ml of M medium without any carbon source or vitamins and was kept root free by trimming

the roots for exclusively facilitate the growth of AMF extraradical hyphae. Both media were solidified with 0.4% (W/V) of Phytagel (Sigma). Plates were incubated for approximately 5 weeks at 25ºC in the dark until hyphae colonized the second compartment. The spores previously trapped inside RP bags were then deposited onto hyphae. Petri dishes were then incubated for 5 days at 25ºC and observed daily to check the viability of hyphae. The bacterial colonies capable to grow along hyphae of *R. irregularis*, without any visible damage, were isolated and reinoculated repeatedly until single morphotypes were obtained on 10% TSA (QueLab Laboratories, Canada). Hereafter, they were referred as hyphospheric bacteria (Figure 1B).

***In vitro* screening for inorganic phosphate solubilizing bacteria**

After serial purification, the rhizospheric and hyphospheric bacterial isolates were spread on modified National Botanical Research Institute's Phosphate (NBRIP) agar medium, containing tricalcium phosphate (TCP) (Nautiyal, 1999). NBRIP plates were incubated at 28°C for 14 days and colonies forming a clear solubilization halo which result from their TCP solubilizing activity were taken as PSB.

For quantitative estimation of P-solubilization abilities in liquid medium, NBRIP medium in 250 ml flask were inoculated with an aliquot of an overnight bacterial culture (OD 600 nm =0.7) and incubated in shaking conditions for 7 days at 28°C. After centrifugation (for 10min, 10 000 rpm), 0.5 ml supernatant were added to trichloroacetic acid (10 % W/V) then with 4 ml ammonium molybdate solution. The flask was placed in dark for color reaction. Finally, optical absorbance was measured spectrophotometrically at 820 nm. Quantitative estimation of P solubilization in the bacterial culture supernatant was established from a standard curve obtained from a solution of KH_2PO_4.

Culture preservation and maintenance

The selected PSB isolates were stored in 25% glycerol at -80°C. After preservation, all subcultures were performed in 50 ml of 10% TSA (Difco Laboratories Inc. Detroit, Michigan, USA) at room temperature 25ºC, with continuous agitation at 150 rpm on a gyratory shaker, for 48h.

Identification of PSB and phylogenetic analysis

- ***DNA preparation***

Bacteria were grown overnight at 28°C, in 100 ml Erlenmeyer flaks containing TSA (150 rpm). Cells were then harvested by centrifugation (10,000 *g*, 10 min at 4˚C) and washed twice by centrifugation in saline solution. Washed cells were frozen in liquid nitrogen, lyophilized for 24 h, and resuspended in 1 ml of CTAB (2% hexadecyltrimethylammonium bromide, Sigma, 1.4-M NaCl, 0.2% 2-mercaptoethanol, 20-mM EDTA, 100-mM Tris-HCl pH 8.0). Total genomic DNA was extracted using the FastDNA® SPIN Kit (MP Biomedicals) and FastPrep® Instruments (MP Biomedicals). Following extraction, the DNA was re-suspended in TE (10-mM Tris-HCl, 1-mM EDTA, pH 7.4), quantified using the spectrophotometer NanoDrop, and diluted in TE to give a concentration of 50 ng μL^{-1}.

- ***PCR conditions***

The gene encoding the 16S rRNA was amplified by the polymerase chain reaction (PCR) using the combination of universal primers pA (AGAGTTTGATCCTGGCTCAG) and pH (AAGGAGGTGATCCAGCCGCA) (Edwards *et al.*, 1989). The PCR mix consisted of deoxynucleotides at 200 µM each, 0.25 µM of each primer, 2.5 µM MgCl2, 5 µl PCR buffer

and 0.25 U of Taq DNA polymerase (5 Prime GmbH). The following PCR conditions were used: 94°C for 2 min, followed by 30 cycles of 94°C for 30 sec, 58°C for 30 sec and 72°C for 1 min, and a final extension step at 72°C for 7 min.

- ***Purification and sequencing of PCR products***

The sizes of the PCR products were determined in 1% (W/V) Agarose TBE (Tris-Borate-EDTA, pH 8.3). The PCR products harboring an expected size of around 1500 bp were used for further process.

The PCR purification and sequencing was performed by the Genome Quebec Innovation Centre (Montreal, QC, Canada). Sequences were aligned with representative bacterial sequences from the NCBI GenBank database, using the algorithm BLASTN-NCBI, and then adjusted manually. The phylogenetic tree was inferred using the neighbor joining algorithms with bootstrap analysis of 1 000 replications. The FASTA sequences were deposited in GenBank with accession number reported in Table 1 and 2.

Table 1 : Taxonomic identifications of root PSB.

Root PSB isolates	Sample site	Phylum	Class	Order	Family/Genus/species
7	L1-HighP	Proteobacteria	γ-Proteobacteria	Pseudomonadales	Pseudomonadaceae/*Pseudomonas sp.*
8	L1-HighP	Proteobacteria	α-Proteobacteria	Sphingomonadales	Sphingomonadaceae/*Novosphingobium sp.*
19	L1-HighP	Proteobacteria	γ-Proteobacteria	Pseudomonadales	Pseudomonadaceae/*Pseudomonadales sp.*
23	L1-HighP	Proteobacteria	γ-Proteobacteria	Moraxellales	Moraxellaceae/*Acinetobacter sp.*
69	L1-LowP	Proteobacteria	β -Proteobacteria	Burkholderiales	Alcaligenaceae/*Achromobacter sp.*
82	L2-HighP	Proteobacteria	γ-Proteobacteria	Pseudomonadales	Pseudomonadaceae/*Pseudomonas sp.*
90	L2-HighP	Firmicutes	Bacilli	Bacillales	Paenibacillaceae/*Paenibacillus*
92	L2-HighP	Proteobacteria	γ-Proteobacteria	Pseudomonadales	Pseudomonadaceae/*Pseudomonas*
93	L2-HighP	Proteobacteria	γ-Proteobacteria	Pseudomonadales	Pseudomonadaceae/*Pseudomonas*
96	L2-HighP	Firmicutes	Bacilli	Bacillales	Paenibacillaceae/*Brevibacillus*
99	L2-HighP	Firmicutes	Bacilli	Bacillales	Paenibacillaceae/*Paenibacillus purispatii*
132	L2-LowP	Proteobacteria	γ-Proteobacteria	Pseudomonadales	Pseudomonadaceae/*Pseudomonas RU47*
133	L2-LowP	Firmicutes	Bacilli	Bacillales	Paenibacillaceae/*Paenibacillus amylolyticus*
136	L2-LowP	Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae/*Stenotrophomonas*
146	L3-HighP	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Bacillus cereus*
153	L3-HighP	Proteobacteria	γ-Proteobacteria	Pseudomonadales	Pseudomonadaceae/*Pseudomonas*
149	L3-HighP	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Bacillus*
164	L3-HighP	Proteobacteria	γ-Proteobacteria	Pseudomonadales	Pseudomonadaceae/*Pseudomonas fluorescens*
174	L3-HighP	Proteobacteria	γ-Proteobacteria	Pseudomonadales	Pseudomonadaceae/*Pseudomonas*
185	L3-LowP	Proteobacteria	γ-Proteobacteria	Pseudomonadales	Pseudomonadaceae/*Pseudomonas*
187	L3-LowP	Firmicutes	Bacilli	Bacillales	Paenibacillaceae/*Paenibacillus polymyxa*
196	L3-LowP	Actinobacteria	Actinomycetia	Micrococcales	Brevibacteriaceae/*Brevibacterium*
202	L3-LowP	Firmicutes	Bacilli	Bacillales	Paenibacillaceae/*Brevibacillus*
203	L3-LowP	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Bacillus*

Table 2 : Taxonomic identifications of hyphosphere PSB.

Hyphospheric PSB isolates	Sampled site	Phylum	Class	Order	Family/Genus/species
MI04	Maize/i	Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae/*Pseudoxanthomonas indica*
MI06	Maize/i	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Lysinibacillus fusiformis*
MI09	Maize/i	Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae/*Stenotrophomonas*
MI13	Maize/i	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Paenibacillus typhae*
MI14	Maize/i	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Paenibacillus xylanexedens*
MS03	Maize/s	Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae/*Pseudoxanthomonas sp.*
MS09	Maize/s	Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae/*Stenotrophomonas maltophilia*
MS11	Maize/s	Actinobacteria	Actinomycetia	Micrococcales	*/Microbacterium oxydans*
oMS04	Maize/s	Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae/*Stenotrophomonas maltophilia*
Ps*	Leeks/s	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Bacillus sp*
PS06	Leeks/s	Firmicutes	Bacilli	Bacillales	Bacillaceae/ *Lysinibacillus fusiformis*
PS09	Leeks/s	Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae/*Pseudoxanthomonas*
PS09b	Leeks/s	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Bacillus sp.*
Psi07	Leeks/s	Proteobacteria	α-Proteobacteria	Caulobacterales	*/Caulobacteraceae*
PTS08	Potatoes/s	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Bacillus*
TI01	Tomato/i	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Bacillus sp*
TI04	Tomato/i	Proteobacteria	α-Proteobacteria	Caulobacterales	Caulobacteraceae/ *Phenylobacterium sp*
TI05	Tomato/i	Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae/*Pseudoxanthomonas sp*
TS07	Tomato/s	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Bacillus sp*
TS11	Tomato/s	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Bacillus toyonensis*
TS12	Tomato/s	Proteobacteria	β -Proteobacteria	Burkholderiales	Burkholderiaceae/ *Cupriavidus necator*
TS18	Tomato/s	Actinobacteria	Actinomycetia	Micrococcales	*/Microbacterium sp*
PTS17	Potatoes/s	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Bacillus cereus*
PTS06	Potatoes/s	Proteobacteria	γ-Proteobacteria	Xanthomonadales	Xanthomonadaceae/*Pseudoxanthomonas*
PTS03	Potatoes/s	Firmicutes	Bacilli	Bacillales	Bacillaceae/*Priestia*

(i): Plant supplemented with igneous RP ; (s): Plant supplemented with sedimentary RP

Further *in vitro* screening of isolates for additional plant growth promoting traits

All the bacterial isolates identified as positive for phosphate solubilizing activity through at least, one subculture on NBRIP were referred to as PSB, considering they possess the metabolic capability to solubilize phosphate. They were further investigated for different conventional plant growth promoting traits, as described below. All bioassays were performed 3 times for each strain.

Screening for Indole-3-acetic acid (IAA)-related compound production

Qualitative and quantitative analysis of the production of IAA and IAA-related compounds by PSB isolates was determined by spectrophotometry in culture medium supplemented with tryptophan as IAA-precursor using the method of Salkowski (Biswas *et al.*, 2018). Firstly, bacterial isolates were overnight cultured in 5 ml of LB medium. Then, 20 µl of bacterial suspension (standardized to OD 600 nm =0.7) were inoculated into 15 ml Falcon tubes containing 5 ml 10 % LB liquid supplemented with tryptophan (5mM). The resulting cultures were incubated for 5 days at 28 ˚C on a rotary shaker (150 rpm) and cells from culture were

separated by centrifugation (10,000 *g*, 20 min, 4˚C). Salkowski reagent (2% of 0.5 M ferric chloride ($FeCl_3$) in 35% perchloric acid ($HClO_4$) were mixed with the culture supernatant (1/1 v/v) and the mixture was incubated in the dark at room temperature for 20 min. The development of a pink color indicating IAA production. Estimation of IAA production was performed spectrophotometrically at 530 nm using a standard IAA concentration curve prepared in LB 50% containing serial dilutions of the synthetic indol-3-acetic acid (Sigma Aldric, USA).

Siderophore production in solid medium

PSB isolates were tested for siderophores production in solid medium using the chromeazurol S (CAS) assay as described in Perez-Miranda *et al.* (2007). CAS solution and nutrient agar were prepared separately then both autoclaved. PSB isolates were firstly grown in 10% TSA at 28°C for 48 h on a rotary shaker (150 rpm). Then, bacterial cultures were spot-inoculated on CAS-agar plates and incubated 7 days at 28˚C. Positive siderophore production was indicated by a change of the medium color from blue to orange around the PSB colonies (Schwyn & Neilands, 1987).

Ammonia production

Estimation of ammonia production by PSB isolates was carried out in qualitative assays with peptone as described in Dutta *et al.* (2015). Aliquots of fresh overnight culture of isolates in TSA were inoculated into 10 ml tubes of 10% peptone water (peptone 10 $g.L^{-1}$; NaCl 5 $g.L^{-1}$; distilled water 1L), then incubated at 28°C for 72 h. After incubation, 0.5 ml Nessler's reagent (10% HgI2; 7%KI; 50% aqueous solution of NAOH 32%) was added to each tube. Appearance of brown to yellow color indicated positive test for ammonia production (Marques *et al.*, 2010).

Biofilm formation

Biofilm formation by isolates was assessed using the Crystal violet (CV) assay according to the method previously described by O'Toole (2011) with the following modifications proposed by (Taktek *et al.*, 2015). Inocula were prepared by growing bacteria overnight on a rotary shaker (180 rpm) at room temperature, in 25 ml of 10% TSB (Tryptic Soy Broth - Becton, Dickinson & Co., Franklin Lakes, N.J. USA). Cells were collected and washed twice in 10 ml sterile saline (SS) (0.85% NaCl) after centrifugation (10 000 g, 5 min, 4°C) and re-suspended in SS. Flat bottom wells of sterile polystyrene 96-well microtiter plates (Costar, Corning Inc. Tewksbury, MA, USA) were filled with 100µL of the modified NBRIP medium containing TCP. Then, each well was inoculated with 10 µL of a 48h primary culture adjusted at an OD_{600}= 0.7. For these experiments, a non-inoculated TSB medium was used as the negative control. After 72h of incubation at 28°C without agitation, the supernatant (i.e. medium and the non-adhering bacteria biofilm) were eliminated by simple inversion and each well was washed three times with 100 µL of sterile deionized water. The microplates were then air-dried for 30 min. The cells from the adhering biofilm were dyed with 100 µL of 0.1% crystal violet (CV) solution (30 min at room temperature). The excess of dye was removed by simple inversion and the biofilm was washed three times with running tap water at each wash. Then, the microplates were dried at room temperature during 10 min. Finally, bound CV was solubilized by adding 200 µL of 30% acetic acid solution and microtiter plates were incubated for 15 min. The absorbance in each well was sampled and the OD_{530} was read on a microplate reader. The results obtained were then transformed into a quantification of biofilm formation by calculating the average absorbance of three replicates and dividing by the average absorbance of the blank (without bacteria). The results expressed as this ratio can be considered as non-dependent from the non-specific coloring of the surface of the wells of the microplates by the CV. Following this

procedure, biofilm formation was considered to have occurred when the ratio is greater than two (Brian-Jaisson *et al.*, 2014).

In plate assays about Nitrogen fixation
The Burks medium (HiMedia Laboratories), containing inorganic salts along with carbon source but lacking nitrogen source, was used for detection of nitrogen fixing bacteria, able to fix nitrogen and grow when cultured on this nitrogen-free medium. Bacterial responses were observed after an incubation at 30°C for 7 days.

Motility tests
Flagellar and pilus (type IV) motility tests were performed on TSB 50% medium containing 0.3% and 1% agar, respectively (De Kievit *et al.*, 2001). Thus, for flagellar motility, an aliquot (1µL) of a fresh culture of the isolates was inoculated onto the agar and after 10h of incubation at 28°C, the diameter of the swarming zone was observed. For motility due to pilus, inoculation of bacteria was done at the bottom of the agar. After 48h of cultivation, the areas of slippage on the surface were observed (De Kievit et al., 2001).

RESULTS

Identification of phosphate solubilizing bacterial isolates and Additional plant growth promoting traits

Rhizospheric PSB
A collection of 210 isolates was obtained from root fragments, collected from different RP-rich soils of the mining area. Firstly, using the qualitative assay based on the formation of a clear TCP-solubilization halos around colonies plated on NBRIP solid medium, a total of 35 isolates (16.6%) were identified as PSB, generating halo with diameters of 0.5 to 4 cm (data not shown).

After Sanger sequencing of the 16S rRNA gene, 24 isolates, sampled from different soils with contrasted P contents (Table S1) were satisfactorily assigned to a genus or family rank as reported in Table 1, and were retained for further analysis. Among them, different P solubilizing abilities were highlighted using quantitative assays, with the concentrations of solubilized P released in liquid medium ranging from 39,13 (± 0,80) to 260,01 (±1,31) µg.mL-1 (Table 3). The highest value was recorded for isolate "99-*Paenibacillus purispatii*".

Table 3 : Plant Growth Promoting Traits of root isolates.

	Solubilization			Production					Fixation	Motility	
Rhizospheric PSB isolates	**Family/Genus/species**	**Phosphore (a)**	**Phosphore (µg.ml^{-1}) (b)**	**AIA**	**AIA (µg.ml^{-1}) (b)**	**NH3**	**Siderophore**	**Biofilm**	**N2**	**Flagelle**	**Pili**
7	*Pseudomonas sp.*	(+ + +)	70,91 ± 2,17	(-)	20,60 ± 0,75	(+)	(-)	(-)	(-)	(+)	(-)
8	*Novosphingobium sp.*	(+ - -)	60,87 ± 0,77	(-)	6,82 ± 0,97	(+)	(-)	(-)	(+)	(+)	(-)
19	Pseudomonadales	(+ + +)	70,01 ± 0,42	(+)	87,14 ± 0,84	(+)	(-)	(+)	(+)	(+)	(-)
23	*Acinetobacter sp.*	(+ + +)	49,75 ± 0,33	(+)	48,85 ± 1	(+)	(-)	(-)	(+)	(+)	(-)
69	*Achromobacter sp.*	(+ + +)	82,56 ± 0,64	(-)	15,89 ± 2,05	(+)	(-)	(-)	(-)	(+)	(-)
82	*Pseudomonas sp.*	(+ + +)	39,13 ± 0,80	(+)	34,19 ± 3,01	(+)	(+)	(-)	(-)	(+)	(-)
90	*Paenibacillus sp.*	(+ + +)	63,44 ± 0,66	(+)	69,56 ± 3,84	(+)	(-)	(-)	(+)	(+)	(+)
92	*Pseudomonas sp.*	(+ - -)	62,46 ± 0,03	(+)	51,80 ± 1,34	(+)	(-)	(-)	(-)	(+)	(-)
93	*Pseudomonas sp.*	(+ + +)	62,66 ± 0,02	(-)	11,52 ± 3,32	(+)	(-)	(-)	(-)	(+)	(-)
96	*Brevibacillus sp.*	(+ - -)	42,59 ± 0,45	(-)	6,15 ± 1,39	(+)	(-)	(-)	(-)	(+)	(-)
99	*Paenibacillus purispatii*	(+ - -)	260,01 ±1,31	(-)	14,79 ± 1,55	(+)	(-)	(-)	(-)	(+)	(-)
132	*Pseudomonas RU47*	(+ + +)	65,07 ±1,1	(+)	63,48 ± 1,33	(+)	(+)	(-)	(-)	(+)	(-)
133	*Paenibacillus amylolyticus*	(+ - -)	42,91 ± 0,45	(+)	330,27 ± 5,21	(+)	(-)	(-)	(-)	(+)	(-)
136	*Stenotrophomonas sp.*	(+ + +)	46,18 ± 0,87	(-)	10,14 ± 0,47	(+)	(-)	(-)	(-)	(+)	(-)
146	*Bacillus cereus*	(+ + +)	63,64 ± 0,65	(+)	48,79 ±2,41	(+)	(+)	(-)	(-)	(+)	(-)
153	*Pseudomonas sp.*	(++ +)	68,66 ± 0,96	(+)	32,52 ± 0,8	(+)	(-)	(-)	(-)	(+)	(-)
149	*Bacillus sp.*	(+ - -)	42,30 ± 0,29	(+)	53,30 ± 6,75	(+)	(-)	(-)	(-)	(+)	(-)
164	*Pseudomonas fluorescens*	(+ - -)	61,76 ± 0,54	(-)	3,23 ± 0,32	(+)	(-)	(-)	(-)	(+)	(-)
174	*Pseudomonas sp.*	(+ + +)	57,54 ± 0,6	(-)	7,25 ± 0,28	(+)	(-)	(-)	(-)	(+)	(-)
185	*Pseudomonas sp.*	(+ + +)	122,4 ± 0,44	(+)	39,90 ± 2,01	(+)	(-)	(+)	(-)	(+)	(-)
187	*Paenibacillus polymyxa*	(+ + +)	132,15 ± 0,21	(+)	251,58 ± 4,74	(+)	(-)	(-)	(+)	(+)	(-)
196	*Brevibacterium sp.*	(+ + -)	108,36 ± 0,9	(+)	242,03 ± 13,46	(+)	(-)	(-)	(-)	(+)	(-)
202	*Brevibacillus sp.*	(+ + +)	84,77 ± 0,11	(-)	10,35 ± 1,11	(+)	(-)	(-)	(-)	(+)	(-)
203	*Bacillus sp.*	(+ + +)	3,97 ± 0,06	(+)	35,50 ± 2,11	(+)	(+)	(-)	(-)	(+)	(-)

(+): Positive for the trait ; (-): Negative for the trait ; (a): Isolate's reponse after each of the three successive subculture ; (b): Values are the mean of 3 replicats independent assays ± standard error

BLAST and phylogenetic analysis placed the 24 PSB into three bacterial phyla, Proteobacteria, Firmicutes and Actinobacteria (Table 1, Figure 2a and b). Proteobacteria and Firmicutes phyla were prevalent (with 14 and 9 isolates respectively). PSB of Proteobacteria felt into alpha, beta and gamma subdivisions with similarities between 94.4-99.9%.

Most of them were assigned to the γ-Proteobacteria and were affiliated to four genera: *Pseudomonas* (nine isolates), *Stenotrophomonas* (two isolates) *Acinetobacter* (one isolate), and *Buttiauxella* (one isolate). One isolate was affiliated with the β-Proteobacteria and was associated to the genus *Achromobacter* with more 99% sequence identity. The remaining isolate felt among α-Proteobacteria and Burkholderiales order and was referred to *Novosphingobium.*

Among Firmicutes, the alignment of the sequences showed clustering of isolates with species of the genus *Paenibacillus* (four isolates), *Bacillus* (three isolates), or *Brevibacillus* (two

isolates). The Actinobacteria phylum was represented with only one isolate affiliated within the Micrococcales as *Brevibacterium* species.

Phenotypic characteristics of the 24 isolates were assessed for different PGP traits (Table 3). Thus, all the isolates were positive for ammonia production. The IAA production was observed for 13 PSB isolates. It ranged from 32,52 (± 0,8) to 330,27 (± 5,21) µg.mL^{-1}, with the isolates *"*133-*Paenibacillus amylolyticus"*, "187-*P. polymyxa"* and "196-*Brevibacterium"* showing the highest IAA production (330,27 (± 5,21) then 251,58 (± 4,74) and 242,03 (± 13,46) µg.mL^{-1}). Only five PSB were positively screened for N_2 fixations (among them: one *Novosphingobium*, two *Paenibacillus*, one *Acinetobacter*), and four for siderophore capability (two isolates assigned to *Pseudomonas* and two as *Bacillus*). Two PSB among which one *Pseudomonas*, formed a significant biofilm on an abiotic surface. The rhizospheric PSB mostly combined one additional PGP trait (nine isolates); six and eight PSB exerted respectively two and three additional capabilities. Phenotype of one PSB isolate (assigned to Pseudomonades) showed four additive PGP abilities.

Hyphospheric PSB

From a collection of 44 hyphospheric isolates, 25 isolates (56.8 %) sampled from pot cultures of maize, potato and tomato were identified as PSB from qualitative and quantitative TCP solubilization assays (Table 4). Different abilities for TCP solubilization were recorded, solubilized-P concentrations ranging from 24,15 (± 0,08) to 175,69 (± 0,61)µg.mL^{-1} and the isolate "MI07-*Lysinibacillus fusiformis*" showing the highest TCP solubilization activity.

Among the selected PSB, 10 were affiliated to the Proteobacteria phylum as reported in Table 1, Figure 2a, b and c: seven belong to the γ-Proteobacteria class with isolates displaying sequence identities with *Pseudoxanthomonas* (five isolates) or *Stenotrophomonas* (two isolates). One strain was classified as β-Proteobacteria and Burkholderiales, close to the referenced *Cupriavidus necator*. In addition, two isolates grouped inside the α-Proteobacteria class, related to Caulobacteraceae family and *Phenylobacterium* sp.

Besides, 12 isolates were affiliated to Firmicutes, all being assigned in the Bacilli class. These isolates were mainly related to *Bacillus* (seven isolates), PTS08 sharing more than 99.5 % identity with *Bacillus*. The other ones shared sequence identity with representatives of *Paenibacillus (two)*, *Priestia* (one) or *Lysinibacillus* (two) genus. The Actinobacteria phylum is represented with two *Microbacterium* isolates, one being related to *Microbacterium oxydans*.

Among the 25 hyphospheric PSB selected, all were identified as ammonia producer while 20 isolates representative of nine genera (among them, *Bacillus* being prevalent) displayed AIA production ranging from 41,40 (± 2,30) to 963,96 (± 8,05) µg.mL^{-1} (Table 4). Isolate "MI14-*P. xylanexedens*", "MI13-*P. typhae"* and "PTS08-*Bacillus*" exhibited the highest IAA production (963,96 ± (8,05)then 566,67 (± 2,61) and 425,97 (± 5,85) µg.mL^{-1} respectively). Only 12 isolates showed N_2 fixation, assigned to seven different genera. No isolate was identified as siderophore producer. Biofilm production was observed among 4 isolates assigned to *Pseudoxanthomonas* (two isolates), *Cupriavidus* (one isolate) and *Lysinibacillus* (one isolate).

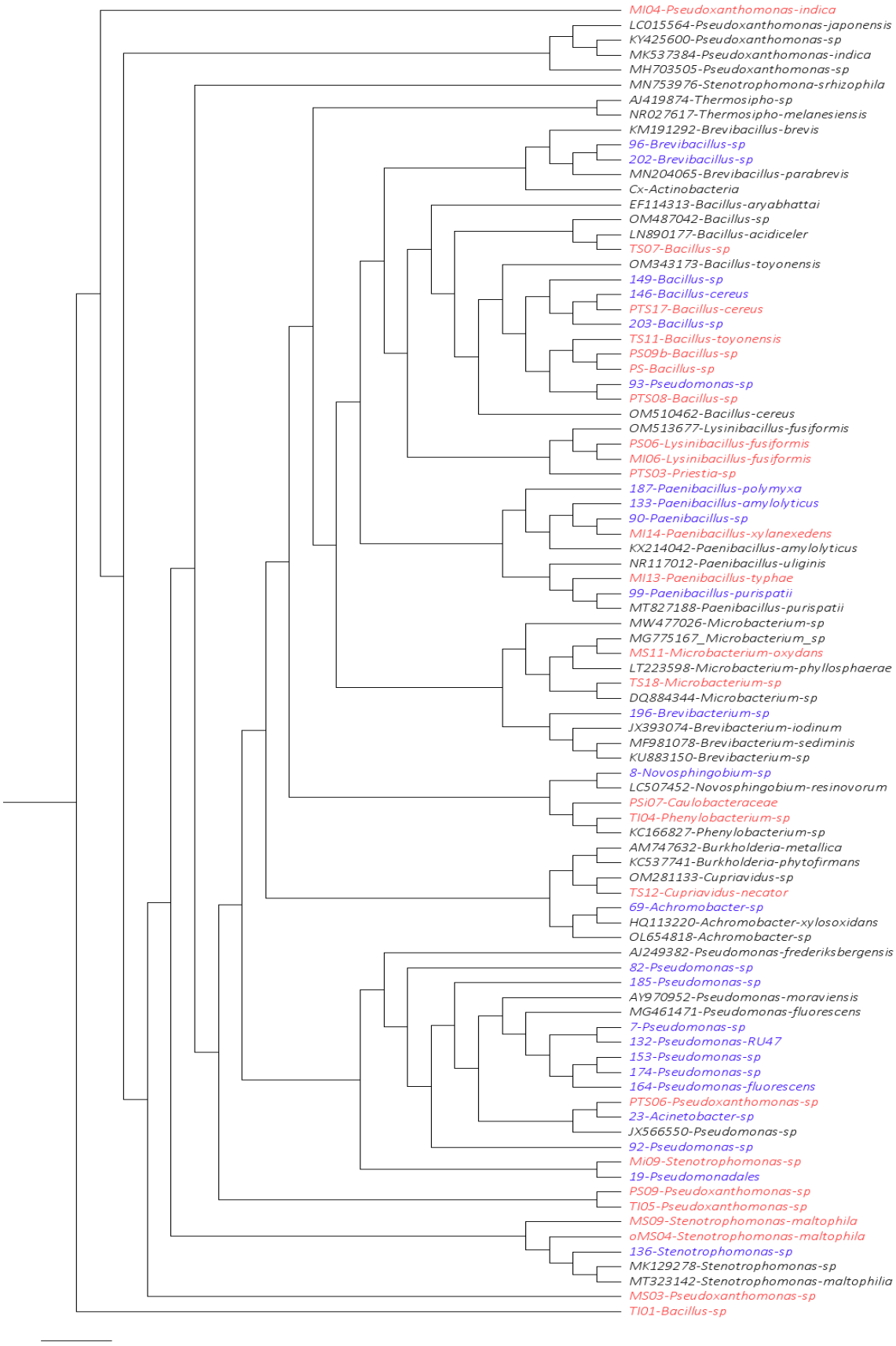
All rhizospheric and hyphospheric isolates were positive for swimming motility due to flagella. Pili motility was observed for one rhizospheric isolate (*Paenibacillus*) and three hyphospheric isolates assigned to *Stenotrophomonas*, *Pseudoxanthomonas*.

Table 4 : Plant Growth Promoting Traits of hyphosphere isolates.

	Solubilization				Production				Fixation	Motility	
Hyphospheric PSB isolates	**Family/Genus/species**	**Phosphore (a)**	**Phosphore ($\mu g.ml^{-1}$) (b)**	**AIA**	**AIA ($\mu g.ml^{-1}$) (b)**	**N H3**	**Siderophore**	**Biofilm**	**N2**	**Flagelle**	**Pili**
MI04	*Pseudoxanthomonas indica*	(+ + +)	59,46 ± 0,08	(+)	76,59 ± 6,5	(+)	(-)	(-)	(+)	(+)	(-)
MI06	*Lysinibacillus fusiformis*	(+ + +)	175,69 ± 0,61	(-)	7,61 ± 0,29	(+)	(-)	(-)	(-)	(+)	(-)
MI13	*Paenibacillus typhae*	(+ + +)	151,99 ± 0,22	(+)	566,67 ± 2,61	(+)	(-)	(-)	(-)	(+)	(-)
Mi14	*Paenibacillus xylanexedens*	(+ + +)	65,23 ± 0,25	(+)	963,96 ± 8,05	(+)	(-)	(-)	(+)	(+)	(-)
MS03	*Pseudoxanthomonas sp.*	(+ + +)	54,76 ± 0,22	(+)	139,11 ± 2,47	(+)	(-)	(-)	(-)	(+)	(+)
MS09	*Stenotrophomonas maltophilia*	(+ + +)	98,28 ± 3,17	(-)	3,96 ± 0,53	(+)	(-)	(-)	(+)	(+)	(-)
MS11	*Microbacterium oxydans*	(+ - -)	166,44 ±0,17	(+)	73,27 ± 0,96	(+)	(-)	(-)	(+)	(+)	(-)
oMS04	*Stenotrophomonas maltophilia*	(+ + +)	73,95 ±0,09	(+)	126,32 ± 1,13	(+)	(-)	(-)	(+)	(+)	(+)
Ps*	*Bacillus sp.*	(+ + -)	73,28 ±0,30	(+)	60,3 ± 3,74	(+)	(-)	(-)	(+)	(+)	(-)
PS06	*Lysinibacillus fusiformis*	(+ - -)	79,97 ±0,37	(+)	110,83 ± 2,93	(+)	(-)	(+)	(-)	(+)	(-)
PS09	*Pseudoxanthomonas sp.*	(+ + +)	64,16 ±0,61	(+)	88,74 ± 6,83	(+)	(-)	(-)	(-)	(+)	(-)
PS09b	*Bacillus sp.*	(+ + -)	67,41 ±0,50	(+)	46,75 ± 2,62	(+)	(-)	(-)	(-)	(+)	(-)
Psi07	Caulobacteraceae	(+ + +)	51,92 ± 0,53	(+)	80,21 ± 4,83	(+)	(-)	(-)	(-)	(+)	(-)
PTS08	*Bacillus sp.*	(+ + +)	24,15 ± 0,08	(+)	425,97 ± 5,85	(+)	(-)	(-)	(-)	(+)	(-)
TI01	*Bacillus sp.*	(+ + +)	64,73 ± 0,70	(+)	97,51 ± 1,16	(+)	(-)	(-)	(-)	(+)	(-)
TI04	*Phenylobacterium sp.*	(+ + -)	81,94 ± 0,36	(+)	107,09 ± 2,15	(+)	(-)	(-)	(+)	(+)	(-)
TI05	*Pseudoxanthomonas sp.*	(+ + -)	48,23 ± 0,22	(-)	18,41 ± 0,95	(+)	(-)	(+)	(+)	(+)	(-)
TS07	*Bacillus sp.*	(+ + +)	45,09 ± 0,15	(+)	49,74 ± 3,74	(+)	(-)	(-)	(+)	(+)	(-)
TS11	*Bacillus toyonensis*	(+ + +)	41,56 ± 0,22	(+)	51,5 ± 1,15	(+)	(-)	(-)	(+)	(+)	(-)
TS12	*Cupriavidus necator*	(+ + +)	133,59 ± 0,83	(-)	6,85 ± 2,37	(+)	(-)	(+)	(+)	(+)	(-)
TS18	*Microbacterium sp.*	(+ + -)	99,09 ± 0,32	(+)	75,98 ± 1,38	(+)	(-)	(-)	(+)	(+)	(-)
PTS17	*Bacillus cereus*	(+ + +)	44,68 ± 0,07	(+)	41,40 ± 2,30	(+)	(-)	(-)	(-)	(+)	(-)
PTS06	*Pseudoxanthomonas sp.*	(+ + -)	64,10 ± 0,77	(-)	21,12 ± 3,59	(+)	(-)	(+)	(-)	(+)	(-)
PTS03	*Priestia sp.*	(+ + +)	56,44 ± 0,77	(+)	128,2 ± 0,74	(+)	(-)	(-)	(-)	(+)	(-)
MI09	*Stenotrophomonas sp.*	(+ + -)	170,05 ± 0,24	(+)	246,7 ± 8,69	(+)	(-)	(+)	(+)	(+)	(+)

(+): Positive for the trait ; (-): Negative for the trait ; (a): Isolate's reponse after each of the three successive subculture ; (b): Values are the mean of 3 replicats independent assays ± standard error

A


MI04-Pseudoxanthomonas-indica
LC015564-Pseudoxanthomonas-japonensis
KY425600-Pseudoxanthomonas-sp
MK537384-Pseudoxanthomonas-indica
MH703505-Pseudoxanthomonas-sp
MN753976-Stenotrophomona-srhizophila
AJ419874-Thermosipho-sp
NR027617-Thermosipho-melanesiensis
KM191292-Brevibacillus-brevis
96-Brevibacillus-sp
202-Brevibacillus-sp
MN204065-Brevibacillus-parabrevis
Cx-Actinobacteria
EF114313-Bacillus-aryabhattai
OM487042-Bacillus-sp
LN890177-Bacillus-acidiceler
TS07-Bacillus-sp
OM343173-Bacillus-toyonensis
149-Bacillus-sp
146-Bacillus-cereus
PTS17-Bacillus-cereus
203-Bacillus-sp
TS11-Bacillus-toyonensis
PS09b-Bacillus-sp
PS-Bacillus-sp
93-Pseudomonas-sp
PTS08-Bacillus-sp
OM510462-Bacillus-cereus
OM513677-Lysinibacillus-fusiformis
PS06-Lysinibacillus-fusiformis
MI06-Lysinibacillus-fusiformis
PTS03-Priestia-sp
187-Paenibacillus-polymyxa
133-Paenibacillus-amylolyticus
90-Paenibacillus-sp
MI14-Paenibacillus-xylanexedens
KX214042-Paenibacillus-amylolyticus
NR117012-Paenibacillus-uliginis
MI13-Paenibacillus-typhae
99-Paenibacillus-purispatii
MT827188-Paenibacillus-purispatii
MW477026-Microbacterium-sp
MG775167_Microbacterium_sp
MS11-Microbacterium-oxydans
LT223598-Microbacterium-phyllosphaerae
TS18-Microbacterium-sp
DQ884344-Microbacterium-sp
196-Brevibacterium-sp
JX393074-Brevibacterium-iodinum
MF981078-Brevibacterium-sediminis
KU883150-Brevibacterium-sp
8-Novosphingobium-sp
LC507452-Novosphingobium-resinovorum
PSi07-Caulobacteraceae
TI04-Phenylobacterium-sp
KC166827-Phenylobacterium-sp
AM747632-Burkholderia-metallica
KC537741-Burkholderia-phytofirmans
OM281133-Cupriavidus-sp
TS12-Cupriavidus-necator
69-Achromobacter-sp
HQ113220-Achromobacter-xylosoxidans
OL654818-Achromobacter-sp
AJ249382-Pseudomonas-frederiksbergensis
82-Pseudomonas-sp
185-Pseudomonas-sp
AY970952-Pseudomonas-moraviensis
MG461471-Pseudomonas-fluorescens
7-Pseudomonas-sp
132-Pseudomonas-RU47
153-Pseudomonas-sp
174-Pseudomonas-sp
164-Pseudomonas-fluorescens
PTS06-Pseudoxanthomonas-sp
23-Acinetobacter-sp
JX566550-Pseudomonas-sp
92-Pseudomonas-sp
Mi09-Stenotrophomonas-sp
19-Pseudomonadales
PS09-Pseudoxanthomonas-sp
TI05-Pseudoxanthomonas-sp
MS09-Stenotrophomonas-maltophila
oMS04-Stenotrophomonas-maltophila
136-Stenotrophomonas-sp
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MT323142-Stenotrophomonas-maltophilia
MS03-Pseudoxanthomonas-sp
TI01-Bacillus-sp
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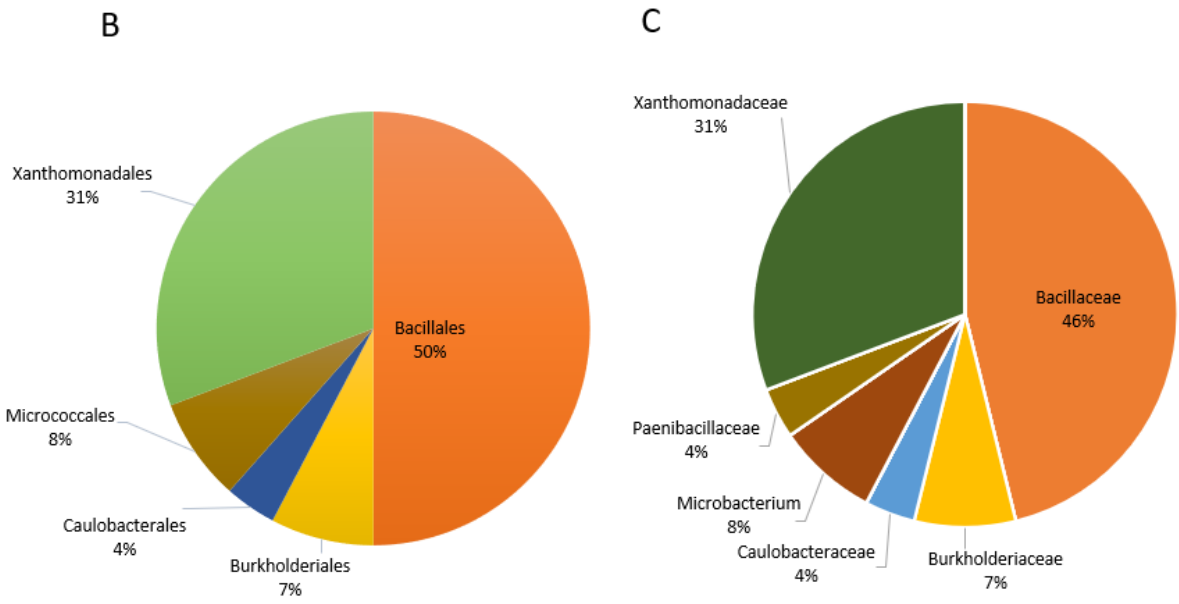

Figure 2: Taxonomic assignation (A) and taxonomic distribution of root bacteria (B) and hyphosphere (C). 16S based phylogenetic analyses showing the relationship between free living bacteria from two niches. For these analyses, 16S sequences were downloaded from NCBI, aligned using CLUSTAL-W and phylogenetic tree was generated using FigTree from CIPRES portal.

DISCUSSION

The current work is the first step of a project aiming to develop application of PSB as bioinoculants with potential ability to increase the agronomic effectiveness of the low-soluble RP ore which is sometimes used directly in soils in developing countries as a P fertilizer less expensive than industrial chemical fertilizer. Thus, two different experimental setups were developed to isolate rhizospheric and hyphospheric PSB from soil with RP.

From both experimental procedures, efficient PSB were identified for their abilities to solubilize in vitro TCP, some root and hyphosphere isolates looking promising by releasing solubilized-P concentrations up to 260,01 (±1,31) and 175,69 (± 0,61) $\mu g.mL^{-1}$ in 7 days, respectively. In light of these results, such PSB with high phosphate-solubilizing potential could be relevant as potential compound for inoculant engineering and subsequent use in combination with low-available P forms as RP to promote plants growth.

Rhizospheric PSB in RP-rich soils with high P availability from the mining area

About 16% of the rhizospheric isolates initially sampled were identified as culturable PSB, In their report, Khan *et al.* (2007) estimated that phosphate solubilizing microorganisms could represent up to 50 % of culturable populations, their occurrence, abundance and diversity being likely to be related to plant species. In line, estimations of Goswami *et al.* (2016) range from 20 to 40 %. Here, variation in percentage across studies may results from different factors. Notably, cultivable bacterial diversity can be analyzed differently by authors, some of them focusing on the occurrence of communities harboring preferential genes involved in P mobilization (PhoD for example), others using metabarcoding and sequencing. Similarly, selection and cultivation of PSB can be carried out from different step of the *in vitro* culture process and performed on different culture media (Pikovskaya, 1948; Nautiyal, 1999), thus

potentially favoring some taxa over others. For instance, in a first step, different nutrient media can be used to isolate the maximum possible culturable morphotypes; then PSB selection can occurred on the selective NBRIP medium. In opposite, culture can be plated firstly on the selective medium for P solubilization trait. Consequently, few isolates requiring specific media can be lost during the PSB screening and may reduce the culturable PSB fraction. In addition, abundance of PSB can vary with the sampled soil compartment. For instance, it has been found that the rhizosphere contains higher proportion of PSB than the surrounding root-free soil (Mander *et al.*, 2012; Nicolitch *et al.*, 2016). More extensively, Spohn *et al.* (2020) established the relative abundance of PSB amounted to 24.4% in the rhizoplane (root surface), and was significantly higher than in the rhizosphere or on saprolites (a chemically weathered bedrock) where plants have grown (9.5% and 2.2% respectively). Such ecological data can obviously impact the percentage of the cultivable faction among soil PSBs. Whatever, many questions remain unanswered regarding the achievement of a consensus allowing more rigorous comparison of PSB studies in a relative manner. The choice of culture techniques, the development of culture methods for a larger number of bacterial taxa, the standardization of protocols (from sampling design to characterization of isolates and their sequencing and data, the comprehensive description of soil microbiomes (notably to further characterize further the relation between taxonomy and functions), are still major challenges for the future of microbiome research and barriers to the replication of the same sampling strategies in different environments by different researchers.
Interestingly, Spohn *et al.* (2020) also showed enrichment of PSB in the rhizosphere of plants grown on saprolites with low P availability compared to saprolites with a higher soluble P fraction. In light of these results, it is important to note that in our work, a significant proportion of PSB were successfully screened from the RP-rich soils where P availability was high (up to 339.5 $mg.kg^{-1}$ P Olsen). Here, our results arise some questions for the future: are the mechanisms classically associated with P solubilization and on which the *in vitro* selection of PSB is based (i.e. release of organic acids) really contribute to soil P solubilization and high level of orthophosphate ions in the mining soils with elevated available P levels? Or do *in situ* PSB occurrence are linked to other functionalities in these soils (e.g. physicochemical mechanisms behind the phosphate solubilization trait could be involved in iron solubilization; indeed, in aerobic conditions and neutral pH, Fe is almost insoluble for plants (Schwab & Lindsay, 1983).

In addition to rhizospheric PSB, the current paper also reports success in PSB isolation from hyphospheric habitats. It has been reached using a three-step procedure including AMF spore culture with axenic hyphal culture in bi-compartmented Petri dishes with, upstream, a trapping procedure of AMF spores from RP-rich habitats of fields or pot cultures. Our experimental setup confirms the two-compartment Petri dishes as a good tool to target hyphospheric PSB, what is in accordance with previous reports (Ordonez *et al.*, 2016; Taktek *et al.*, 2017). Furthermore, while the process of selecting hyphospheric isolates could have significantly reduced the probability of selecting PSB isolates in vitro due to the multiplicity of steps in the procedure and their inherent selectivity, we obtained proportionally more hyphospheric PSB (25 PSB selected from 44 hyphospheric isolates) than rhizospheric PSB (24 PSB from 210 rhizospheric isolates). In a first analysis, one could conclude that the hyphospheric PSB isolation protocol is more efficient than the one developed for rhizospheric PSB isolation, as far as the proportion of PSB in the collection of cultivable PSB isolates is concerned.

Diversity of culturable root and hyphospheric PSB isolated from RP-rich habitats

Combining Sanger sequencing combined with phylogenetic analysis have revealed that both rhizospheric and hyphospheric PSB isolates were affiliated to only three bacterial phyla, namely

to Proteobacteria, Firmicutes and Actinobacteria. The cultivable isolates related to *Bacillus* and *Paenibacillus* as Firmicutes were prevalent in both rhizospheric and hyphospheric habitats. *Pseudomonas* and *Pseudoxanthomonas* as γ-Proteobacteria were dominant respectively in the rhizosphere and in the hyphosphere.

Proteobacteria, Firmicutes and Actinobacteria are omnipresent in numerous and various soils worldwide. In particular, exploration of taxonomic composition of bacterial communities from alkaline phosphate mine wastes has showed sequences belonging to Proteobacteria and Actinobacteria, and to genera *Pseudomonas* and *Bacillus are well represented* (Mghazli *et al.*, 2021). Similarly, Proteobacteria was the dominant phylum in root-associated soils and bulk soil associated to Chinese mining sites (Ye *et al.*, 2020). Besides, these three phyla are well-know in the literature to habit PGPR and effective P-solubilizers (Mghazli *et al.*, 2021). Notably, the plant growth promoting abilities of *Pseudomonas* and *Bacillus* genera, which are omnipresent in various soils worldwide, are well documented in the literature and have been reported to be effective and abundant P solubilizers (Sharma *et al.*, 2014; Kumar *et al.*, 2016; Vacheron *et al.*, 2016). So, some of them are included in the development of diverse microbial biofertilizer formulation, available in international markets for use by farmers (Goswami *et al.*, 2016; Kumari *et al.*, 2019; Lobo *et al.*, 2019). Interestingly our results are in accordance with the data of Yang *et al.* (2012) who placed 123 PSB, isolated from P-rich soils from a lake drainage area, into three bacteria phyla, namely Proteobacteria (with some *Pseudomonas representatives*), Actinobacteria and Firmicutes (among them, *Bacillus and Brevibacillus*).
However, we have to highlight an under representation of Actinobacteria: thus, only three Actinobacteria isolates were identified and referred to as genera *Microbacterium* (two hyphospheric isolates) or *Brevibacterium* (one root isolate). Yet, this phylum is ubiquitously distributed among a large range of soils (Pierzynski *et al.*, 2005; Qin *et al.*, 2016). In addition, a large number of Actinobacteria exhibited PGP traits, several being P-solubilizers (Hamedi & Mohammadipanah, 2015), with a free-living status or as endophytic bacteria (Qin *et al.*, 2017; Chen *et al.*, 2019). In accordance, Yang *et al.* (2012) only identified three isolates as Actinobacteria among 123 PSB they have selected. Here, we can hypothesize the culture-dependent procedures used were too discriminate, offering a low in-depth exploration of soil biodiversity to identify culturable Actinobacteria. For instance, the effectiveness of TCP-based medium to assess *in vitro* capability of bacteria for solubilization may be not optimal in some taxa (Bashan *et al.*, 2013; Bashan *et al.*, 2014).

Multifunctionality of PSB isolates for inoculant formulation

Our study showed that the selected PSB were endowed with additional and multiple plant growth promotion abilities. In particular, more than 79.5% of PSB exhibited more than one PGP trait and two isolates were positive for four PGP traits.

Ammonia production was the most common PGP trait observed among the isolates with all isolates tested positive. This trait is recognized to play a role in enhancing plant growth making nitrogen available to their host plant and thus promoting root and shoot elongation (Hayat *et al.*, 2010; Kandjimi *et al.*, 2015). Besides, about 58.3% and 80% of, rhizospheric and hyphospheric isolates respectively were able to produce IAA after incubation with tryptophan as auxin precursor. Increase in root elongation, lateral root formation and root hairs are classically associated with IAA production what can putatively enhanced the water and nutrients uptake efficiency of the plant root system. In line with these data, some reports

suggested that PSB may have a stronger effect on root traits (such as root biomass, root diameter, length, surface or volume) than rhizosphere P solubilization alone (Elhaissoufi et al. 2020), which make interesting to select isolates with both IAA production and PSB traits.

Siderophores production is another major factor for plant growth promotion. Indeed, siderophores are high affinity iron chelating compounds capable of acquiring ferric Fe^{3+}, from the mineral phases, to scavenge Fe and make it available gain as Fe^{2+}, a preferred form for uptake by plant roots (Vessey, 2003). In our studies, only 2 *Pseudomonas* and 2 *Bacillus* were siderophore producers, all being rhizospheric isolates. Here, it would have been interesting to have data not only on total Fe as shown in table S1, but also on about bioavailable Fe in mining soils to hypothesize about the possible relationship between soluble Fe content and low occurrence of siderophore producing PSB.

Interestingly, all isolated exhibited motility which is another interesting feature likely to promote colonization of rhizosphere.

CONCLUSIONS and PERSPECTIVES

In the current study, bacteria harboring the capacity for P solubilizing, were isolated from RP enriched habitats and analyzed for additional plant growth promoting traits. Such collection extends the knowledge on PSB from RP-rich environments and open new ways for a better in-depth understanding of their potential functional capabilities.

From these promising results, expected to contribute to the development of fertilization practices more environment friendly than those in current use, further investigations are now required. In particular, more favorable cultivation media should be included in further work to avoid some cultural bias and to putatively reach bacterial diversity closer to what it can be observed in natural habitats (Chen & Liu, 2019). Also, investigations about the structure and taxonomic diversity of bacterial communities within the mining area (using metabarcoding, sequencing and phylogenetic analysis of the 16 rRNA gene) in combination with identification of their potential metabolic function (using a functional prediction approach (Mghazli *et al.*, 2021) would be relevant to identify taxa and functions involved in P cycling in native in P mining environments, avoiding the culture-based method; this work is currently in progress.

Besides, we have hypothesized the advantage of using native PSB isolated from RP-rich environments is their possible ability to more easily adapt when inoculated into RP-rich soil. Therefore, further greenhouse and fields studies on testing and validating the dual use of the mineral nutrient and microbial resources (individually or in poly microbial formulations, multi-species and pluri-functional) will be performed for agronomic purposes in the future.

Also, extensive investigations on PSB from RP-rich soils could be the key for sustainable microbe-assisted rehabilitation strategies (i.e. the re-establishment of microbial soil properties and vegetative cover) of derelict RP mine lands in future. So, strategy of phytoextraction of excess P in soils (resulting from of over application of P, in excess of plant requirements, by a long-term farmer practices) by mining plant ecotypes of P-rich soils, assisted by chosen microbes (Ye *et al.*, 2020) might benefit of our data, using the most efficient soluble-P producer in this research. Alternatively, they might be tested to enhance phytoremediation of excess phosphorus pollution in phosphate mining wasteland soils. As well, analysis of molecular mechanisms behind the PSB evolution and adaptation to cope with a complex and extreme environment like the RP-rich mining soils would be relevant (Guo *et al.*, 2021).

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AUTHOR CONTRIBUTIONS

Conceived and performed the experiments; ADD and LZ. Contributed to the writing of the manuscript: ADD, MH, JF, ALS. Supervision of the project: MH, JF, ALS

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CHAPITRE 3

C. Etude des effets des inoculants à base de consortia bactériens solubilisatrice de P sur la croissance des plantes de la tomate

Synopsis

Au cours du chapitre précédent, nous avons sélectionné 49 isolats PSB, racinaires et hyphosphériques issus d'habitats riches en RP, et les avons caractérisés pour plusieurs traits d'intérêt, promoteurs de la croissance des plantes. Ce dernier chapitre a pour objectif de tester l'efficacité de deux consortia, l'un constitué d'isolats racinaires et l'autre d'isolats hyphosphériques, sur le développement d'une plante hôte.

Les inoculations ont été pratiquées en conditions contrôlées de serre, sur un sol non stérile et sur des plants de tomate (*Solanum lycopersicum* L.), une espèce agronomique d'importance majeure sur le plan économique mondial. Cultivée sous toutes les latitudes, dans des conditions très variées (climat, modes de production), la tomate est en effet le légume le plus consommé dans le monde, soit frais, soit après transformation ; sa production mondiale a progressé régulièrement au cours du XXe siècle et s'est accrue considérablement durant les dernières décennies. Ainsi, selon les données de la FAO, plus de 186 millions de tonnes de tomates ont été produites dans le monde en 2020, sur une superficie de 5 051 983 hectares. La variété choisie pour notre étude est le cultivar Tiny Tim.

Le choix des isolats constitutifs des consortia poly bactérien repose sur des hypothèses largement développées dans la littérature et explicité dans l'introduction de ce mémoire, à savoir : i) privilégier des inoculants en consortium pluri-bactériens et possédants différents traits PGP, promoteur de la croissance des plantes, associant des entités taxonomiques différentes, ii) optimiser l'efficacité et l'adaptabilité du mélange poly bactérien à un environnement d'inoculation enrichi en RP par le choix d'agents inoculants issus eux-mêmes d'habitats enrichis en RP.
L'influence de ces consortia sur la croissance et le développement de plants de tomate a été testée dans trois conditions de culture : inoculation des consortia seuls, ou en interaction avec un apport de RP, ou/et de l'inoculation par le champignon mycorhizien à arbuscules *Rhizophagus irregularis.*

Généralement, le bénéfice de l'inoculation par les PSB sur la plante est mis en évidence par l'examen de la croissance/développement des parties aériennes et de la fructification (principaux paramètres qui affectent le rendement des plantes de grandes cultures). Mais les PSB peuvent également moduler étroitement les traits fonctionnels des racines. Certains auteurs ainsi émis l'hypothèse que le bénéfice de l'inoculation par les PSB pouvait être associé, de manière plus marquée, au développement du système racinaire qu'au trait solubilisateur de P lui-même (Bakhshandeh *et al.*, 2015; Sarsan, 2016; Rezakhani *et al.*, 2019). De même, Elhaissoufi *et al.* (2020) ont montré chez le blé qu'une plus grande croissance des plantes après inoculation par des PSB, seraient associés à des changements dans les traits morphologiques des racines au fil du temps. Ainsi, nous avons tout particulièrement axé notre étude sur l'analyse de traits morphologiques racinaires, tels que la longueur totale de la racine, le diamètre moyen, le nombre de pointes racinaires, le taux de ramification racinaire et volume, mais également la longueur spécifique des racines et la densité tissulaire des racines (RTD).

Ces travaux sont présentés sous forme d'une publication en préparation : « Inoculation of phosphate-solubilizing bacterial consortia affects germination rate and root traits of tomato

plants in combinaison with amendment with an arbuscular mycorrhizal fungus and rock phosphate » *Ducousso-Détrez A., Lahrach Z., Lounès-Hadj Sahraoui A., Fontaine J., Hijri M.*

Une augmentation du taux de germination, un accroissement de la hauteur des jeunes plantules et des modifications au niveau du système racinaire des plants adultes, en particulier des variations de la densité du tissu racinaire, ont été observés en réponse à l'inoculation simple par les consortia ou à leur interaction double ou tripartite avec le RP et/ou le CMA. Le profil taxonomique du microbiote natif, sans ou avec apport des inoculants bactérien et mycorhizien et/ou apport de RP a été caractérisé. Des variations d'abondance des Glomerales et Paraglomerales ont été identifiés. L'apport des données environnementales acquises dans les chapitres précédents pour l'élaboration d'inoculants est discuté, mise en perspectives avec les contraintes liées i) aux méthodes d'acquisition et d'analyse des données de métabarcoding et ii) aux approches de culture in vitro. Les résultats acquis soulignent l'intérêt mais aussi la complexité de l'élaboration d'inoculants microbiens promoteurs de la croissance des plantes, solubilisateurs de P.

ARTICLE 5 : Etude des performances des inoculants à base de consortia bactériens solubilisatrices de P sur la croissance de la tomate

Ce paragraphe est présenté sous forme d'un article scientifique, article en préparation qui à terme sera soumis à la revue Microbiological Research.

Title : Inoculation of phosphate-solubilizing bacterial consortia affects germination rate and root traits of tomato plants in combinaison with amendment with an arbuscular mycorrhizal fungus and rock phosphate

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Abstract:

The application of microbial-based inoculants in crop production gained popularity in last decade due to its beneficial effects on plant nutrition and health while reducing the use of chemical fertilizers and pesticides. Phosphate (P)-solubilizing bacteria (PSB) have been utilized as an emerging solution for a higher rhizosphere P-availability and a better management of the plant-soil-microbial P cycle. In this study, we investigated in a greenhouse pot experiment, the effects of two bacterial consortia of PSB on germination and growth of tomato (*Solanum lycopersicum* L. cultivar Tiny Tim). These bacterial consortia were developed based on their plant growth promoting traits, isolation origin and phylogenetic affiliation. The first PSB consortium (RC) comprised five bacteria isolated from roots of plants grown in RP-mining sites, while the second one (HC) was made up of five PSB isolated from the myco hyphosphère of arbuscular mycorrhizal fungi trapped in bag containing Rock Phosphate (RP) in soil of cultivated crops. The inoculation treatments were: RC alone, HC alone; RC combined with *Rhizophagus irregularis,* HC combined *R. irregularis*; and amendment with RP. Each treatment was replicated five times in a randomized block design. Tomato plant growth parameters were measured after seed germination and at the harvesting time after four months in the greenhouse trail.

Our findings showed that inoculation by the consortium RC enhanced seed germination rate. Plantlet length of germinated seeds was increased by RC and HC. No significant differences were noticed regarding the aboveground plant growth parameters at harvesting stage. In contrast, variations in root traits were recorded, notably root tissue density. Modifications in diversity values, composition and abundance of indigenous bacterial and mycorrhizal communities were identified. Taken all together, our results underline the interest but also the complexity of developing microbial inoculants and of analyzing their impact on plant growth.

Key Words: Rock Phosphate, inoculation, consortium, Phosphate-solubilizing bacteria, Arbuscular Mycorrhizal Fungi, Tomato

INTRODUCTION

Low soil phosphorus (P) availability is a major constraint for plant growth in many agricultural soils which is a limiting factor for agricultural production. In response, P fertilizers have been widely used to increase P bioavailability. But due to natural chemical sorption and transformations, P fertilizers are usually used at high concentration to mitigate these physical interactions of P ions and soils particles, resulting on major environmental damages, such as eutrophication of continental and coastal waters, and heavy economic costs for agriculture. A growing demand then arose to develop an organic approach to more environmentally friendly agriculture, with less reliance on synthetic chemical-based inputs (Khan *et al.*, 2009; Sharma *et al.*, 2013). In this context, global efforts have been developed to advance in soil microbiome research and in the engineering of microbial inoculant, so-called bioinoculants, based on plant beneficial microorganisms. Indeed, the soil microbial component of the plant holobiont exhibit some important functions able to assist plant development and fitness, particularly in stressful conditions. Bacteria exhibiting plant growth promotion (PGP) traits are referred as Plant Growth-Promoting Rhizobacteria (PGPR) (Mendes *et al.*, 2013).

Among PGPR, the functional group of Phosphate Solubilizing Bacteria (PSB) are well-recognized to solubilize sparingly available P compounds into bioavailable orthophosphate, improving P availability in the rhizosphere for their own requirements and potentially, for P uptake by the plant (Kumar, 2016; Pérez *et al.*, 2016; Bargaz *et al.*, 2018).. Consequently, PSB are considered as an integral component of P cycling and as relevant candidates as P biofertilizers.

In addition, one PSB may exhibit multiple functional PGP traits which contribute to additional benefits for plants (Shakeela *et al.*, 2017; Zhang *et al.*, 2017). For instance, PSB are frequently described as producer of indole-3-acetic acid (IAA), a hormone which notably favors division, expansion and differentiation of plant cells and tissues, but also impacts root architecture. Even, it has been suggested that IAA-producer PSB effects on plant growth may be greater on root traits than the P bio-solubilization (Elhaissoufi *et al.*, 2020).

Beside the use of PSB as the sole input, the combination of PSB with P fertilizers has been proposed to obtain complementary and synergistic effects on plant growth (Bakhshandeh *et al.*, 2015; Adnan *et al.*, 2017). Notably, application of PSB inoculant together with RP as a natural and cheaper P fertilizer compared to water-soluble P chemical fertilizers, have been proved to increase P release capacity of insoluble RP (Gomes *et al.*, 2014; Giro *et al.*, 2015; Bargaz *et al.*, 2018). It has been also showed to improve plant nutrition in diverse legume and cereal plants (Ditta *et al.*, 2018; Tahir *et al.*, 2018; Elhaissoufi *et al.*, 2020) . Currently, various PGPR/PSB strains are used as commercial soil inoculants or as a seed coating to provide bioavailable P from fertilizers or soil stocks (Timmusk *et al.*, 2017; Koskey *et al.*, 2021).

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Arbuscular mycorrhizal fungi (AMF) are also known to supply P to their host plant through extensive plant root colonization and soil exploration, which allows access, in particular, to poorly mobile forms of P in soils. They also participate to P mobilization and solubilization from recalcitrant P compounds (Li *et al.*, 2006; Schnepf & Roose, 2006; Konvalinková *et al.*, 2017; Wang *et al.*, 2017). This has led to the development of mycorrhizal inoculants (Tabassum et al., 2017) with *Funneliformis mosseae* and *Rhizophagus irregularis* as the most commonly used AMF inoculants (Giovannini *et al.*, 2020; Musyoka *et al.*, 2020). Moreover, in the mycorrhizosphere or the hyphosphere (Andrade *et al.*, 1998), it has been shown that extraradical mycelia of mycorrhizal fungi support a wide microbial community which benefit of AMF exudates and carbon sources (Rambelli, 1973; Andrade *et al.*, 1998; Jansa *et al.*, 2013) . Also, a number of studies have investigated spore-associated microbiota, and AMF spores are recognized to harbor a wide diversity of bacterial taxa, living either intracellularly or associated with the spore wall (Battini *et al.*, 2016; Agnolucci *et al.*, 2019). In addition, evidences have been provided that some bacteria of the hyphal and spore microbial community harbor multifunctional traits, and among them the ability to mineralize and solubilize recalcitrant P-forms (Taktek *et al.*, 2015; Taktek *et al.*, 2017). Thus, it has been hypothesized that possible interactions could have evolved between PSB and AMF, with speculation that they work positively, in addition or synergy, eliciting in concert plant performances (Nacoon *et al.*, 2020). In particular, some authors suggest that PSBs could use the extraradical hyphae of AMFs as a pathway to new areas of the soil that could be rich in resources from plant exudates. Concomitantly, AMF could access to new additional soluble P sources due to solubilizing capacity of PSB (Toljander *et al.*, 2007; Ordoñez *et al.*, 2016; Nacoon *et al.*, 2020). Research has also led to identification of endobacteria closely associated with AMF exhibiting multiple PGP properties including P solubilization (Cruz & Ishii, 2011). Thus, Battini *et al.* (2017) highlighted positive effects of AMF and their associative endobacteria in improving P availability and hyphae elongation which were both attributed to phytohormone production. Despite the knowledges about mechanisms behind interactions between AMF and PSB are imprecise, isolation of AMF associated bacteria represent a relevant factor for plant growth promotion and also, as inoculant for the management of P cycling in soils.

Various studies in different plant species have shown that polymicrobial mixtures with several strains belonging to different functional and taxonomic groups, can induce increased plant growth promoting effect as compared to single inoculant (Compant *et al.*, 2019; Koskey *et al.*, 2021)for reviews). For instance, Braz & Nahas (2012) found that P solubilization was increased in co-inoculated cultures of *Aspergillus niger* and *Burkholderia cepacia* compared to single inoculants. Malusá *et al.* (2012) suggested mixed inocula could be more flexible and productive across contrasted abiotic and biotic environments. Some reports also hypothesized beneficial effect of microbial diversity towards the survival of introduced inoculants; for example, Hu *et al.* (2016) observed the survival of introduced *Pseudomonas* consortia was enhanced with increasing strain diversity. As well, the effects on plant performance, of co-inoculation of AMF and PSB in combination with RP have also been investigated. For instance, beneficial effects of dual inoculation of a PSB *Klebsiella variicola* and AMF *Rhizophagus intraradices* was observed in the growth promotion and inulin production of Jerusalem artichoke when combined with RP addition (Nacoon *et al.*, 2020).

However, it is evident that more academic and applied research on screening, designing, testing potential microbial resources for their impact on plant growth are required in order to thoroughly understand how to choose candidates to inoculant engineering. In particular, microbial ecology is not sufficiently integrated into microbial selection to make microbial inoculants more reliable (Kaminsky *et al.*, 2019). Yet, some authors highlighted the relevance of matching the environmental properties of the microbial collection site to those of the end-

use site to increase the success of microbial establishment and the probability of identifying microbes that exhibit a desired plant growth promoting traits (Compant *et al.*, 2019).
Based on the literature, we hypothesized that the selection of diverse bacterial consortia of PSP isolates from native habitats rich in PR, and harboring plant growth-promoting including phosphate-solubilizing abilities could be used as microbial inoculant to improve plant growth when combined with AMF and RP. Thus, we compared the effects of two different consortia of isolates with various plant growth promotion traits and taxonomic affiliations, on growth parameters of tomato plants (*Solanum lycopersicum* L. cultivar Tiny Tim. Bacterial and AMF communities of the tomato rhizosphere were also determined by amplicon sequencing on isolated DNA from samples of all culture conditions.

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MATERIAL AND METHODS

Root and hyphosphere bacteria with phosphate solubilizing capacities and additional plant growth promoting traits

Out of 254 bacteria captured either from root or from hyphosphere habitats rich in RP, *as* **previously described by Ducousso-Détrez et al. (2022 c),** 10 PSB isolates were selected based on their *in vitro* inorganic tri-calcium phosphate (TCP) solubilizing abilities using NBRIP's medium (Nautiyal, 1999) and for additional plant growth promoting traits such as IAA, siderophore, ammonia and biofilm production (Table S1). Thus, IAA and siderophore production were assayed qualitatively using respectively the Salkowski colorimetric reagent (Biswas *et al.*, 2018) and the chrome azurol S (CAS) assay (Perez-Miranda *et al.*, 2007). Ammonia production and biofilm formation by PSB strains were carried out in assays with peptone Dutta *et al.* (2015) and the Crystal violet (Taktek *et al.*, 2015) respectively. Accession numbers of the PSB sequences deposited in the GenBank database are reported in Table S1.

The selected PSB strains were stored in 25% glycerol at -80°C. All subcultures after preservation were performed in 50 ml of 10% Tryptone Soy Agar (TSA) (Difco Laboratories Inc. Detroit, Michigan, USA) at room temperature, with continuous agitation at 150 rpm on a rotary shaker, for 48h.

Design of multi-functional and pluri-species consortia for plant inoculation
Using the 10 PSB selected, two different consortia were used for plant inoculation. The first consortium was made up of root isolates (referred hereafter to as root consortium (RC); the second, of hyphosphere isolates (referred to as hyphospheric consortium RC). Both were consortia of 5 isolates exhibiting each solubilizing phosphate ability with additional PGP traits complementing each other to lead to a multifunctional consortium. Each consortium also combined isolates related to different phyla and genera (Table S1).

The RC consortium for plant inoculation was prepared as followed: as bacterial starter, each PSB isolate was prepared in the TSA medium at 28°C overnight. Then, the bacterial suspensions were diluted to DO_{600}= 0.7, and a same volume of the dilution was sampled for each isolate. Subsequently, the five aliquots were mixed together and the mix was diluted to obtain a DO_{600}= 0.7. This final dilution was used as consortium for plant inoculation. The same procedure has been followed to obtain HC.

AMF inoculant
Rhizophagus irregularis, isolate DAOM 197198, cultivated *in vitro* on carrot roots transformed with *A. rhizogenes,* was used for inoculations. The AMF inoculum was prepared from pieces of the culture media (Phytagel, SIGMA ALDRICH) containing extraradical mycelium and spores; the mixture was then solubilized, and filtered with 45 µm sieve. The soluble inoculum was then stored at 4°c until used. An estimation of the propagule number in solution was counted under the binocular lamp from an aliquot (10 µl) of the soluble inoculum, as a result of which the plant inoculation was carried out using 30 µl of mycorrhizal inoculum containing about 300 propagules.

Inoculation of tomato seeds
Seeds of *S. lycopersicum* L., variety Tiny Tim were firstly surface-sterilized using 70% ethanol for 1 min, 3% sodium hypochlorite for 1 min and three rinses in sterile distilled water. A group of five treatments (referred to as BAC treatment group in further statistical analysis) was then performed by soaking seeds (150 per treatment) for 1 h under gentle agitation in 20 ml of different solutions: the RC and HC consortia, the sterilized LB medium excluding any consortium (referred to as nC), the inactivated rhizospheric consortium (RCi) and the inactivated hyphospheric consortium (HCi), both sterilized by autoclaving (20 min, 120°C). Then, seeds were sown in Petri dishes on sterile filter paper, moistened by 2 ml of sterilized water. Germination rate of seeds and sprout length of seedlings (i.e. radicle and cotyledonary stem) were measured after 7 culture days from 200 seeds per treatment.

PSB inoculation, AMF and RP supplementations on tomato plants across greenhouse trials
After recording of germination data, seedlings were carefully removed from Petri dishes and the roots were gently washed with tap water. Subsequently, they were transplanted in germination cells. The potting substrate consisted of soil collected in the Botanical Garden of Montreal. Then, the BAC treatments were again performed, i.e. we reinoculated all seedlings with their respective BAC solution (10 ml RC, HC, RCi, HCi or nC). Plant growth then took place in a growth chamber under controlled conditions (28°C, 70% humidity, 16/8 h photoperiod), until the 2-leaf stage, what occurred around 3 weeks after seedling transplanting. In a second step, 2-leaf seedlings were transferred to 1L (4"x 6") pots filled with an unsterilized mixture of turface, sand, and Montreal soil. Then, for a third time, we reinoculated all plants

with their respective BAC solution (i.e. in the respect of the BAC treatment initially applied to the seeds and seedlings). In addition, each BAC treatments were performed in combination with RP and AMF supplementations, or not. Thus, the experimental design included 20 treatments, arranged in a complete randomized block design with five replicates per treatment (Figure S1), each replicate consisting of a pot with one plant (100 plants total). A schema of the complete experimental design is available in Figure 1.

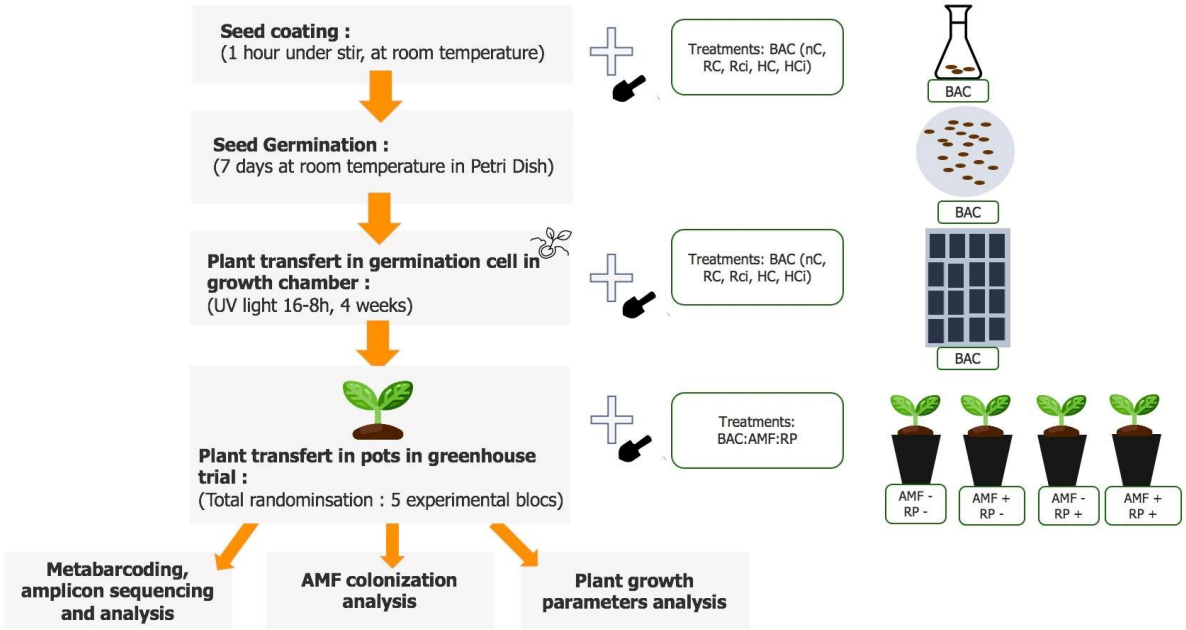


Figure 1: Experimental procedure - Seed and plant treatments and further analyses (3 groups of treatments: BAC (nC: LB medium; HC: Hyphosphere Bacteria; RC: Root Bacteria; HCi: Hyphosphere Bacteria inactivated; RCi: root Bacteria inactivated); RP (RP+ ; RP-) and AMF (AMF+ ; AMF-).

The experiments were conducted from June 2021 in a greenhouse of the Botanical Garden of Montreal with a photoperiod of 16/8h light/dark, at room temperature. Plants were watered once or twice a day if required.

Estimations of plant growth parameters

Plant growth and development were assessed four months after seed transplantation in germination cells, in October 2021. At harvesting, plants were separated into aerial and underground fractions.

From the underground fractions, the soils attached to the plant roots was gently shaken and collected as rhizospheric soil, then stored at 4°C. After carefully shaking off the rhizospheric soil, the roots from each pot were separately washed under tap water, blotted and fresh weights were determined. Then, the weighted root samples were divided into two parts of equal weight: the first one was oven dried for 2 d at 65°C for dry matter measures. From the second fraction, small root fragments were collected, then stored at -80°C for further DNA extraction and further molecular analysis. The remaining roots were stored in 30% ethanol (V/V) until further root morphological measurements and AMF colonization estimations.

For root growth analysis, root samples were evenly spread apart in a water layer on a Plexiglas transparent tray to be digitalized using the Epson flatbed scanner. Root images were then analyzed by the automated image analysis WinRhizo software (Regent Instruments Inc., Quebec City, Canada) for total root length (LengthRoot), average diameter (AvgDiam), number

of tips (Tips), branching root (BranchingRoot, i.e. ratio of tip number on length) and volume (RootVolume). Specific root length (SRL, i.e. ration of length on dry mass), root tissue density (RTD, i.e. ration of volume on dry mass) were subsequently calculated. After scanning, roots were dried for 2 d at 65°C. Therefore, including fresh weight (Root FW) and dry weight (Root DW) measures, eleven root traits were included in further analyses.
For the measurement of AMF colonization, root segments from each treatment were stained using non-vital Trypan blue (0.5 g. L^{-1}), following the method described by (Phillips & Hayman, 1970); microscopic observations were made according to the method of Trouvelot *et al.* (1986) (Nikon Eclipse E600, X100 magnification).
For shoot growth analysis, four traits were used: i) stem height measured from stem base to top and branching length which were both measured manually, ii) the fresh weight (Shoot FW), iii) the dry weight (Shoot DW) which was determined after 2d oven drying at 65°C, and v) leaf number.
A fraction of young leaves was ground into a fine powder with liquid nitrogen for analyses of C and N concentrations.

Soil properties
Elementary chemical composition of soils (a mixture of turface, sand and Montreal mixture) was performed using X-ray fluorescence spectrometry (XRF Titan 800 S X-Ray Fluorescence apparatus, CIRAD, Montpellier). For each of the 20 different culture conditions, rhizospheric soil samples were collected from 5 plants, then pooled into a composite fraction (table S2). Significantly, higher total P content in rhizospheric soil samples were recorded when tomato plants were supplemented with RP, as expected (Table S2).

DNA extraction, PCR amplification and sequencing
Genomic DNA was extracted from 250 mg of rhizospheric soil using the NucleoSpin™ Soil kit (Macherey-Nagel, Düren, Germany) following the manufacturer's protocol. Genomic DNA extraction was also operated from 250 mg of tomato roots initially frozen in liquid nitrogen, and then reduced in fine powder, using NucleoSpin™ plant mini kit (Détrez-Ducousso et al., 2022b). All extractions were performed in triplicate. DNA quality of root and soil extracts was assessed using 1% (w /v) agarose gel. All genomic DNA samples were then stored at −20 C until further treatments by the Genome Quebec Innovation Center (Montreal, QC, Canada) which performed PCR, barcoding and sequencing.

The primer pair 341(F): 5'-CCTACGGGNGGCWGCAG-3', and 805(R): 5'-GACTACHVGGGTATCTAATCC-3' targeting the V3–V4 hypervariable region of the bacterial 16S ribosomal RNA (rRNA) gene were used to generated the amplicon library for bacterial communities. A Nested-PCR was performed to target the 18S rRNA gene of AMF. Thus, the first-round PCR was performed using the AMF-discriminating primer pair AML1 (3'-ATCAACTTTCGATGGTAGGATAGA-5') and AML2 (3'-GAACCCAAACACTTTGGTTTCC-5'). The second PCR round was operated using the in-house set of internal primers nu-SSU-0595-5-F (ACACTGACGACATGGTTCTACACGGTAATTCCAGCTCCAATAG) and nu-SS0948-3-R (TACGGTAGCAGAGACTTGGTCTTTGATTAATGAAAACATCCTTGGC), complemented with CS1 and CS2 barcoded adapters (Stefani *et al.*, 2020). Sequencing was processed by using an Illumina MiSeq technology generating 2× 300 base-pair paired-end reads (Illumina, San Diego, CA, USA).

Bioinformatic and statistical analyses

Bioinformatic process and statistical analyses were operated in the R 4.0.2 software environment (R Core Development Team, 2019) as previously described (Ducousso-Détrez et al. 2022 b).

- **Amplicon sequencing, taxonomic inference and microbial community characterization**

The DADA2 pipeline (v. 1.16) (Callahan *et al.*, 2016) (https://benjjneb.github.io/dada2/tutorial.html) was used to process the sequencing data. Reads were grouped using 100% of sequence identity to infer amplicon sequence variants (ASV). Validity of sequencing depth was controlled from rarefaction curves, computed using the "rarecurve" function from the Vegan package in R (Figure S2). The ASVs obtained from the DADA2 pipelines were filtered for very low-abundance ASVs before any further analysis; thus, sequences present only once (singletons) in the whole data set were eliminated across the microbial profiles. The Silva v132 database formatted for the DADA2 (Callahan *et al.*, 2018) was used to assign bacterial 16S rRNA gene sequences. The taxonomic assignment of mycorrhizal ASVs was performed following a two-step approach (Stefani *et al.*, 2020; Raveau *et al.*, 2021). In the first step, assignment was obtained using the Silva v132 database formatted for DADA2 with assignTaxonomy() command (Callahan, 2018). ASV sequences were then entered in the MaarjAM database.

- **Analysis of plant growth traits**

Principal component analyses (PCA) were carried out to identify contribution of each root traits to total variation in the root trait matrice.

We assessed the effects of the different groups of treatments on tomato growth traits: 'BAC' treatment group (as five-level factor, i.e., RC, HC, RCi, HCi, nC), 'AMF' (as two-level factor, i.e., AMF+ *vs* AMF-) and 'RP' (as two-level factor, i.e., RP+ *vs* RP-), alone or in combination to all possible two-way (BAC/AMF, BAC/RP, AMF/RP) and three-way (BAC/AMF/RP) interactions. Thus we performed a mixed model analysis using the lmer() function in the lme4 package in R, as a model-fitting function to include both fixed-effects (i.e. BAC, AMF, RP, alone or in interaction) and random-effects terms (Bates et al. 2015). Our mixed effect model included “randomization bloc” as a random effect which allowed us to account for repeated measurements of plant variables. All variables were tested for normality and we assessed assumptions of homoscedasticity by comparing residuals to the fitted items. Tukey-adjusted post hoc pairwise comparisons of significant fixed effects were then performed on the reduced models using the emmeans functions in the emmeans package (Lenth 2018). Raw data from the growth assay are available in Supplementary dataset (Table S3).

- ***Bacterial and AMF community analysis***

The greenhouse experiment was also designed to explore the impact of the RC and HC consortia on soil bacterial and AMF communities. We examined their profile after tomato plant harvesting, i.e. four months after consortium inoculation, in interaction with AMF an RP. Analysis was performed from composite soil samples obtained by pooling rhizospheric soil of 5 plants from the same treatment.

The Chao 1 richness estimator and alpha diversity indices (Shannon and Simpson) (Shannon, 1948; Simpson, 1949; Kim *et al.*, 2017) were computed from each soil samples, for the bacterial and AMF community in soil, from the plot_richness () function using the phyloseq R package.

Variations in bacterial taxonomic composition was identified using the P package *metabarcoder*.

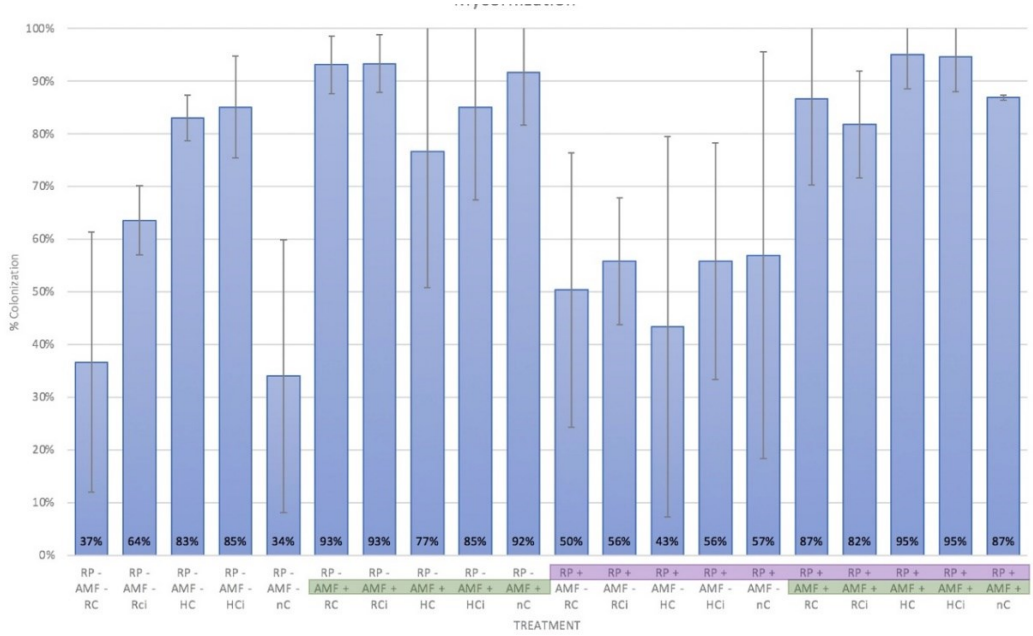
RESULTS

AMF colonization of plant roots

Colonization by AMF was observed in root samples for all treatments (consortia RC, HC, AMF and RP). A range from 34 to 95% of root colonisation was obtained among the different treatments, including the control (Figure 2A).

As excepted, inoculated plants with AMF, either alone or in combination, showed higher colonization percentages compared to non-inoculated plants (88.6% and 56.2% respectively for inoculated and non-inoculated plants with AMF). Data were significant as reported in Figure 2B.

A



B

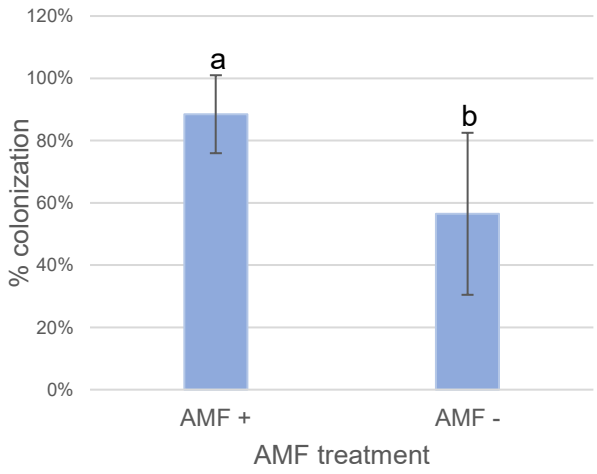


Figure 2: Percentage of root colonization by AMF: (A) For each treatment: control, RH or HC singly or in combination with AMF and/or RP. (B) After pooling data relative to samples with *versus* without AMF inoculation (AMF+ *versus* AMF-). Bar represent standard error and different letters represent statistically significant differences between the treatments (n=5 in A; n=50 in B).

Effects of treatments on seed germination

The consortium RC significantly improved germination compared to the control treatment (nC) (p-value 0.0219) (Figure 3A). Thus, germination rate reached 32.8% with consortium RC while it was 21.7 % in control treatment. However, no significant effect of consortium HC was observed compared to control treatment. On the contrary, HCi and RCi drastically reduced seed germination (9.1 and 11.1 % respectively).

Plantlet length was significantly increased both by RC and HC. Thus, these two treatments improved the length of 7-day-old seedlings by about 21 % and 19 % respectively, compared to seeds treated with nC. As it was observed for seed germination, treatments with bacterial consortia RC and HC had a drastic negative effect on length values (Figure 3B).

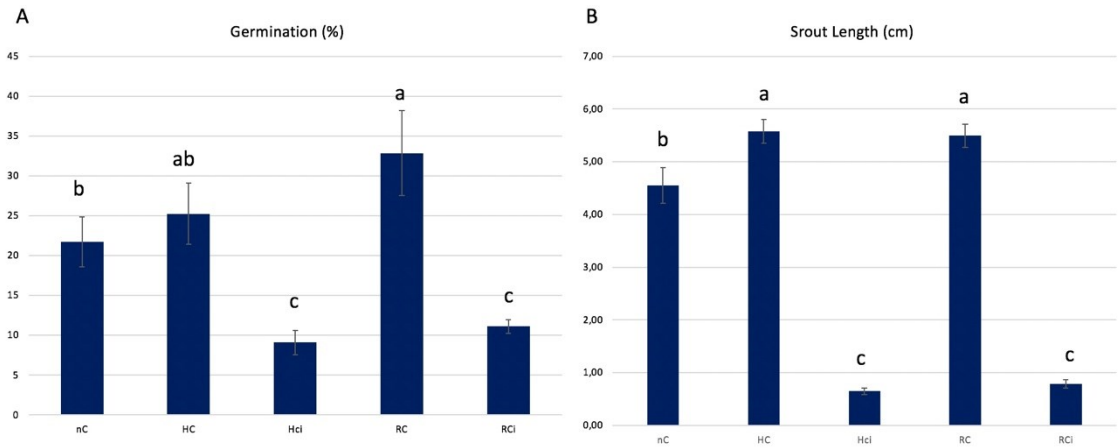


Figure 3: (A) Percentage of germination and (B) Plantlet length (expressed as mean length ± standard error) after BAC treatments (nC: LB medium; HC: Hyphosphere Bacteria; RC: Root Bacteria; HCi: Hyphosphere Bacteria inactivated; RCi: root Bacteria inactivated). Data (n=200) were analyzed by ANOVA. Bar represent standard error and different letters represent significant differences between the treatments.

Effect on shoot growth parameters of 4-month-old tomato plants

Analysis of the 100 plant samples showed large variations in shoot traits (Table S3). For example, stem height measured for each of the 20 treatments ranged from 68,4 to 139.3 cm, with branching length between 11,1 and 176.4 cm. However, no significant effect was noticed on any shoot traits observed when the "BAC", "AMF" or "RP" treatment were considered individually. Nevertheless, a significant interaction AMF: BAC indicated that AMF increased fresh shoot weight in presence of nC (p-values $1.562.\ 10^{-7}$).

Effect on root growth parameters of 4-month-old tomato plants

Well-developed root systems were observed with total root length ranging from 412,5 to 890,1 cm; root diameters from 0.23 to 0.42 mm and root volume from 0.29 to 1.04 cm^3 were recorded (Figure S3). The contribution of eight root traits to total variations observed among sampled tomato plants were analyzed using a Principal Component Analysis (PCA) and are reported in Figure S4. The first two axes maximized the variance, reaching together to more than 48%: the

maximum of contribution to PC1 was reached by average diameter and root volume, while the RTD and mycorrhization contributed dominantly to PC2.

Using image analysis of roots by WinRhizo software and a statistical mixed model analysis, no effect of HC or RC used alone was identified on any root traits studied. In contrast, a significant increase of RTD and root length was calculated when RP was input. The RTD values were also increased significantly by the AMF inoculation (Table S3, Figure S5).

In addition, significant variations for several root traits were identified when RC or HC were in dual interactions with RP or AMF. In particular, the effects of the BAC:AMF interaction were found significant for the root fresh weight, volume, length, RTD and average diameter. Thus, this significant interaction indicated that RC or HC produced significant effects in presence of AMF; interestingly, RC in combination with AM increased significantly RTD.
A significant interaction BAC:RP was also computed with respect to volume, length and RTD, with positive effect of RC in combination with RP regarding RTD.

We also observed a significant three way-interaction between RC or HC, AMF and RP regarding root length and RTD. Thus, RP:HC in interaction with AMF increased significantly root length. Besides, the interactions between HC:AMF and RC:AMF were intensified under RP supply, leading significantly, to higher RTD values, the highest values being obtained for the three-way interaction RC:AMF/RP.

Effect of bacterial Inocula on soil bacterial and AMF communities

- *Changes in AMF community*

After processing 16S rRNA amplicon sequences by Dada2, a total of 2.238.069 reads were obtained from soil samples and assigned to 365 ASV. After MarJaam taxonomic assignment step, 20 ASV were not classified as AMF ASV and removed for further analysis. The remaining ASV are then divided into four orders: Archaeosporales, Diversisporales, Glomerales and Paraglomerales; 62 ASV were without order-assignment. ASV distribution among them is provided in Figure S6.
At family level, eight families were identified, the most abundant being Claroideoglomeraceae (14 ASV; 276.413 reads), Glomeraceae (75 ASV; 235.292 reads) and Paraglomeraceae (80 ASV; 333.822 reads). At a lower taxonomic level, 137 genera were recorded, affiliated to *Acaulospora, Archaeospora, Diversispora, Claroideoglomus, Glomus, Paraglomus* and *Scutellospora*. The AMF *R. irregularis* was not identified among our assignated ASV dataset.

Computing the Shannon and Simpson alpha diversity indexes (Table 1), no variation was observed after RC or HC inoculation compared to the control. Even the experimental design did not permit statistical assessment, a reduction of the both indexes were identified for the following interactions: RC with AMF, RP with HC. As well, RP input, alone or with AMF, decreased the diversity values.

Comparing abundance of the two dominant orders (i.e. Glomerales and Paraglomerales), changes in AMF community were recorded as show in Figure 4. Thus, the relative abundance of Paraglomerales tend to be strongly increased by RC, but reduced with HC compared to the control. Besides, in presence of RP inputs, an increase in Paraglomerales and Glomerales abundances was observed with HC while no impact of RC seemed to occur, compared to the control+RP. Under the influence of *R. intraradices* AMF inoculation, relative abundances of

Glomerales and Paraglomerales were also reduced by over half by RC compared to the control+AMF. Reduction in abundance of both orders was also observed with HC, in particular for the Glomerales. Assessing the impact of the two consortia in interaction with AMF and RP, we observed increases in abundance of Paraglomerales and Glomerale with RC compared to the control+RP+AMF.

Table 1: Read number, ASV number and alpha diversity as computed for each treatment among: (A) the bacterial community and (B) the 18S rDNA community
Data obtained for RCi and HCi were removed from the study due to their negative impact on plant growth and mycorrhization; (+) with and (-) without AMF and RP (nC: LB medium; HC: Hyphosphere Bacteria; RC: Root Bacteria).

A – Bacterial community analysis

Sample	Read Number	ASV number	Shannon	Simpson
RP - AMF - Control	19781	848	6,360	0,997
RP - AMF - RC	19524	759	6,480	0,998
RP - AMF - HC	23930	929	6,207	0,996
RP - AMF + Control	19304	780	6,235	0,996
RP - AMF + RC	20077	904	6,318	0,997
RP - AMF + HC	21782	877	6,415	0,998
RP + AMF - Control	16860	719	6,147	0,997
RP + AMF - RC	18016	724	6,437	0,998
RP + AMF - HC	20442	861	6,340	0,998
RP + AMF + Control	17780	717	6,195	0,997
RP + AMF + RC	13008	758	6,140	0,996
RP + AMF + HC	13008	653	6,113	0,996

B - AMF community analysis

Sample	Read Number	ASV Number	Shannon	Simpson
RP - AMF - Control	112635	77	2,44	0,78
RP - AMF - RC	106618	48	2,13	0,70
RP - AMF - HC	120528	67	2,00	0,72
RP - AMF + Control	92986	57	2,75	0,89
RP - AMF + RC	105396	86	1,83	0,58
RP - AMF + HC	129805	64	2,07	0,67
RP + AMF - Control	129248	50	1,55	0,52
RP + AMF - RC	75638	56	2,89	0,90
RP + AMF - HC	149677	33	1,49	0,56
RP + AMF + Control	179098	54	1,51	0,56
RP + AMF + RC	101234	48	2,08	0,80
RP + AMF + HC	93736	66	2,20	0,69

Regarding Diversisporales, the percentage of reads assigned to this order reached 6.69 % when HC was in interaction with RP, what is the highest percentage recorded for this order across the overall experiment (% ranging from 0.82 to 6.69 with 3.92% for the control). Up to 16 ASV were identified AMF+RC).
Archaeosporales were reduced by each treatment compared to the control community (2.03%); up to five ASV were assignated to Archaeosporales with the culture condition RC+RP+AMF. The highest relative abundance of Diversisporales (6.69 %) was identified with HC in interaction with RP

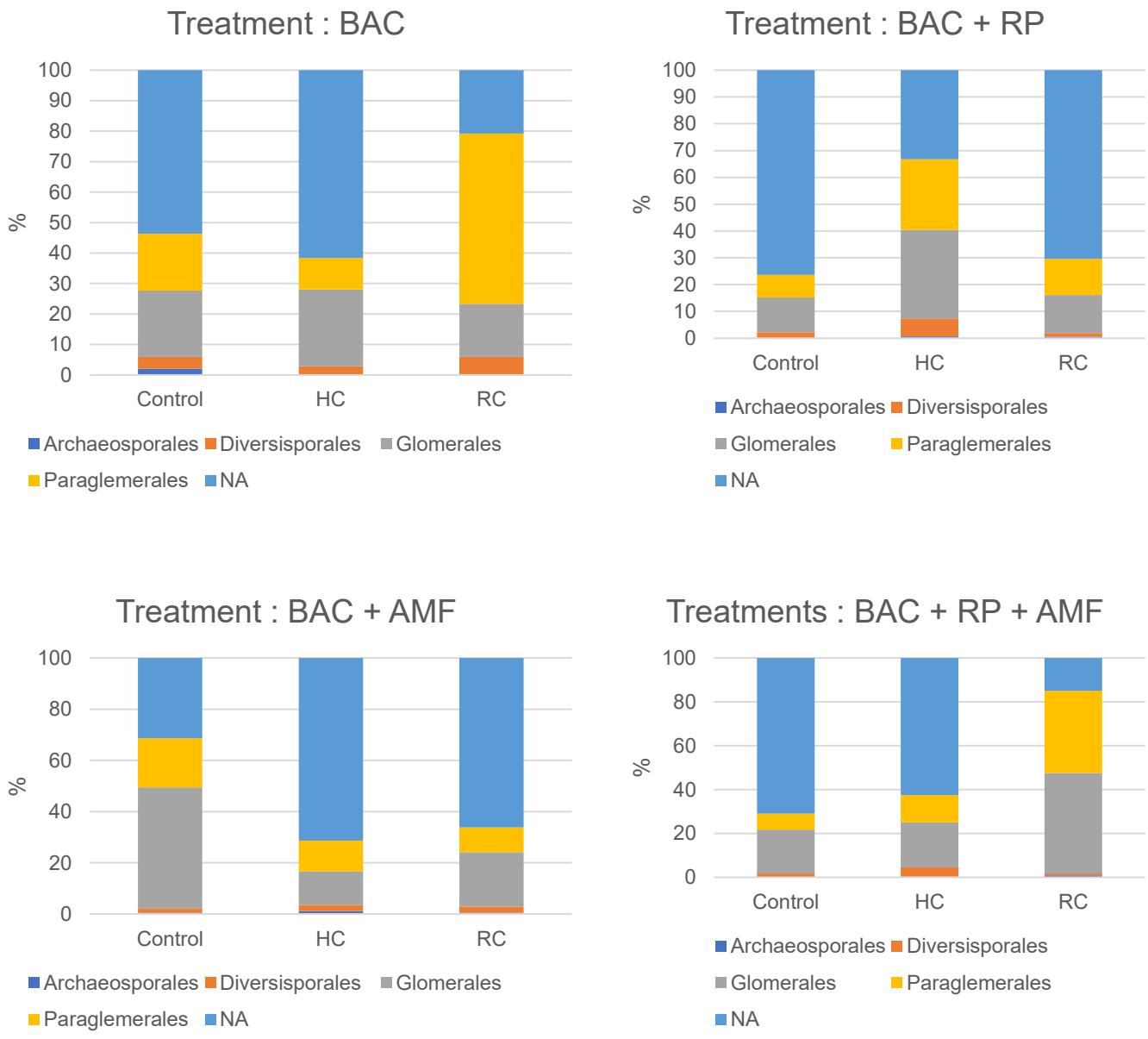


Figure 4: Relative abundance of the four AMF orders, in terms of read, according to the RC or HC inoculation in combination (+) or not (-) with AMF and RP (nC: LB medium; HC: Hyphosphere Bacteria; RC: Root Bacteria).

At genus taxonomic level, strong variations in relative abundance of *Glomus*, *Paraglomus* and *Claroideoglomus* were identified as reported in Table S4.

- *Changes in bacterial communities*

After Dada2 analysis, a total of 1.220.326 paired-end raw reads were obtained; removing the chimeric sequences, singletons and the rare ASV (ASV with fewer than 20), a total of 7.908 ASV from soil samples (373.408 reads) were kept.

Taxonomic assignation shown Actinobacteria and Proteobacteria were the dominant phyla, with Acidobacteria, Chloroflexi, Firmicutes and Gemmatimonadota in a lesser extent. As shown in Table 1, low variation in Shannon and Simpson bacterial diversity was computed across the different treatments. At the order level, Rhizobiales, Micrococcales, Micromonosporales, Propionibacteriales were identified. No variation in abundance could be robustly identified comparing the two consortia inoculation treatment in combination or not with AMF and RP (Figure S7).

CONCLUSION

In this study, we tested in a non-sterilized soil, the effects of two consortia developed based on their plant growth promoting traits, isolation origin and phylogenetic affiliation, on tomato plants. The first PSB consortium (RC) comprised five bacteria isolated from roots of plants grown in RP-mining sites, while the second one (HC) was made up of 5 PSB isolated from the hyphosphère of arbuscular mycorrhizal fungi trapped in bag containing Rock Phosphate (RP) in soil of cultivated crops. At the seedling stage, the growth promoting capabilities of RC and HC were documented; thus, increase in tomato germination rate was obtained with RC. Increases in length of 7-day old plantlets were also observed in response to HC and RC inoculation. Thus, at seedling stage, root and plantlet growth were improved by consortium inoculation which is likely consistent with in-plate findings about plant growth promoting abilities of isolates. Indeed, most of isolates displayed IAA production which is well-known to improve root elongation and growth.

In contrast, expression of plant growth promoting traits are less evident observing the 4-old plants: we did not find any benefit of using the two consortia to improve growth in aerial part of tomato plants and variations in root growth are subtle. Such data are in agreement with previous meta-analyses on rhizobacteria (Rubin *et al.*, 2017; Schmidt & Gaudin, 2018) that highlighted the variable effectivity of inoculants *in planta* when applied to soil systems; this, despite the rationale of applications of microbial consortia was the combination of microbes with different traits complementing each other, and tolerating specific environmental conditions.

Nevertheless, focusing in quantifying any changes in fine root morphology after RC, HC, AMF inoculations and RP supplementation, substantial variations in length root and RTD have been identified in response to the single RP or AMF treatments, as well as across dual and tripartite interaction of treatments with RC or HC. The RTD, defined as the amount of structural material invested by unit of volume, is regarded as a key functional trait to compare plant response to environmental conditions (Birouste *et al.*, 2014; Hogan *et al.*, 2020). It is an important predictor of plant resource use strategy, and inform about resource allocation within the plant, associated with many critical aspects of plant growth and survival (Kembel & Cahill Jr, 2011; Fortunel *et*

al., 2012). So, few hypothesis have emerged. Thus, a high RTD value would be associated to a higher investment on dry matter. Also, root tissue density and root diameter could be positively related to root lifespan and drought resistance but negatively related to nutrient uptake potential (Kramer-Walter *et al.*; Valverde-Barrantes & Blackwood, 2016). In our experiment, performed in greenhouse conditions in warm summer conditions, short drought period cannot been rule out and occurrence of drought resistance by changes in RTD could be questioned.

In addition, changes in profiles of native bacterial and AMF communities were also observed after RC or HC inoculation, alone or in combination with RP and AMF. In particular, we highlighted increase in AMF colonization rate and change in relative abundance of some taxa after RC or HC inoculation. All together, these results showed the AMF community is sensitive to RC and HC inoculation. Conversely, the PSB consortium had no impact on bacterial community structure of soils.

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AUTHOR CONTRIBUTIONS

Conceived, designed and performed the experiments: ADD with advises of MH. Contributed to the writing of the manuscript: ADD, MH, JF, ALS. Supervision of the project: MH, JF, ALS

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SUPPLEMENTARY MATERIAL

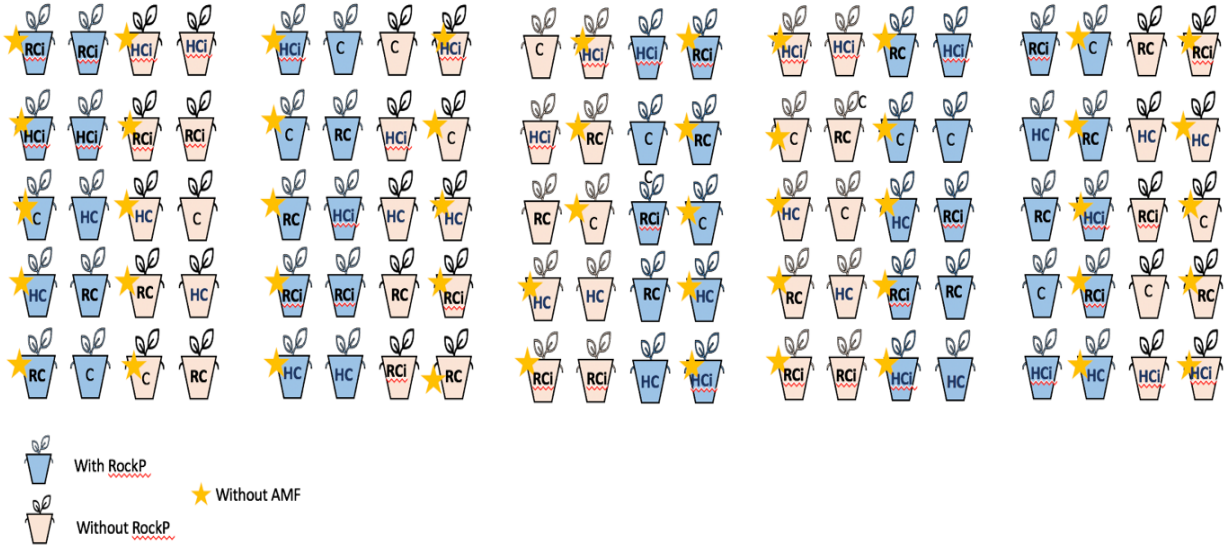


Figure S1. Experimental design performed to test the effects of two bacterial consortia on tomato growth and soil microbial communities, in interaction with or without RP or AMF supplementations: 20 treatments, arranged in a complete randomized block design with five replicates per treatment

HC: Hyphosphere Bacteria; RC: Root Bacteria; Hci: Hyphosphere Bacteria inactivated; RCi: root Bacteria inactivated
Total data: n=100; n=5 per treatment

A B

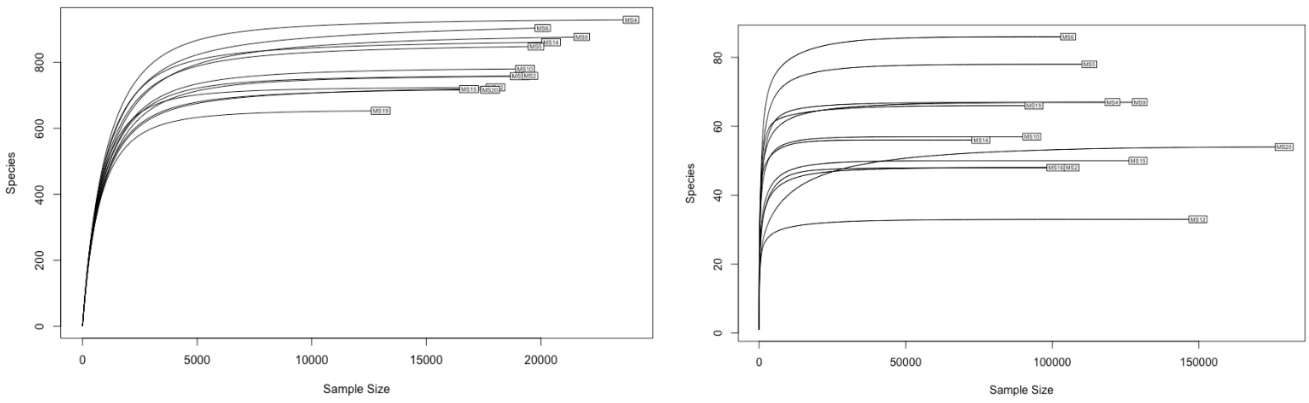
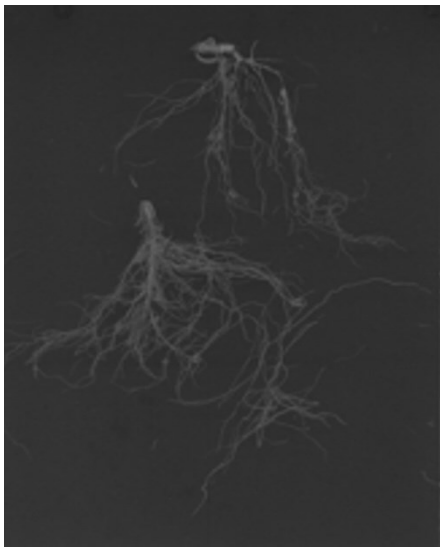
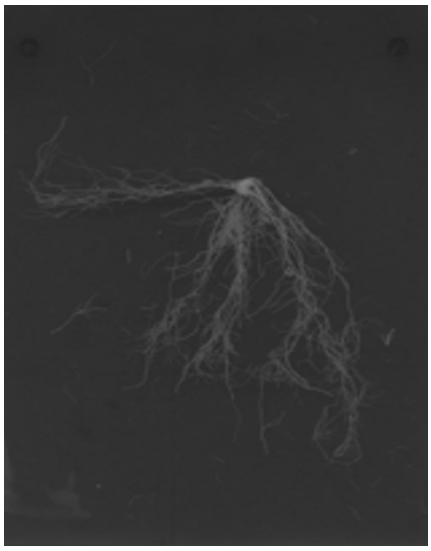


Figure S2: Rarefaction curves for (A) the 16S rRNA and (B) 18S rRNA amplicon libraries linked to each treatment: control, RH, HC, AMF and RP, singly or in combination with AMF and/or RP.

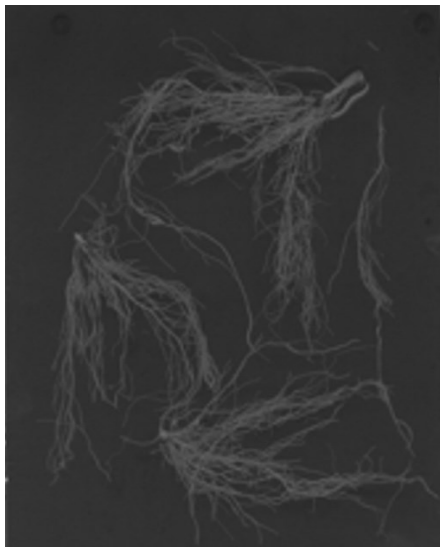
Control



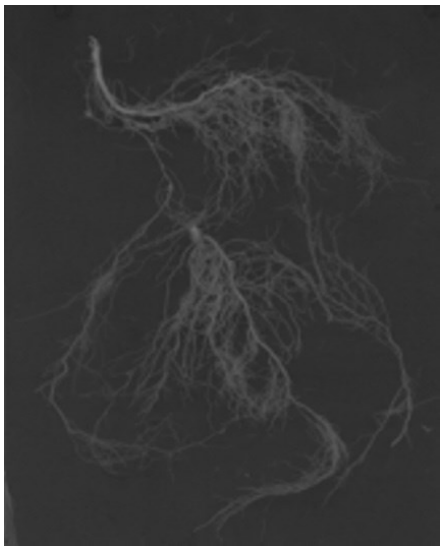
HC



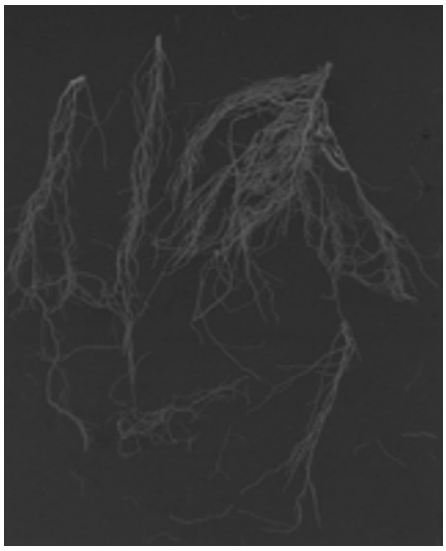
RC



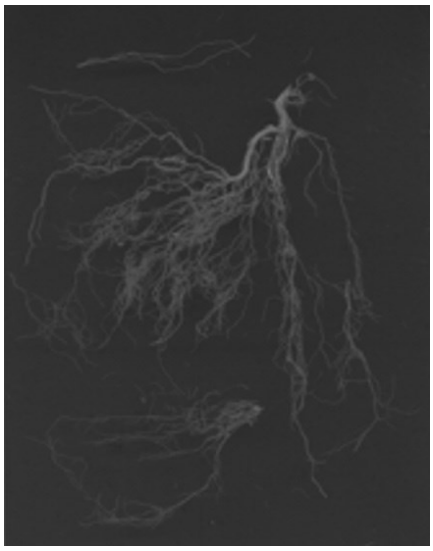
Control + AMF



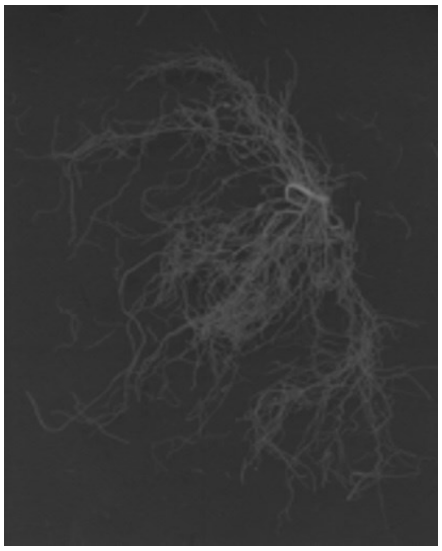
HC + AMF



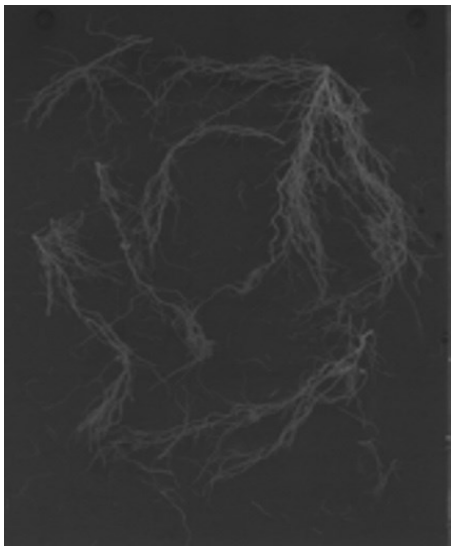
RC + AMF



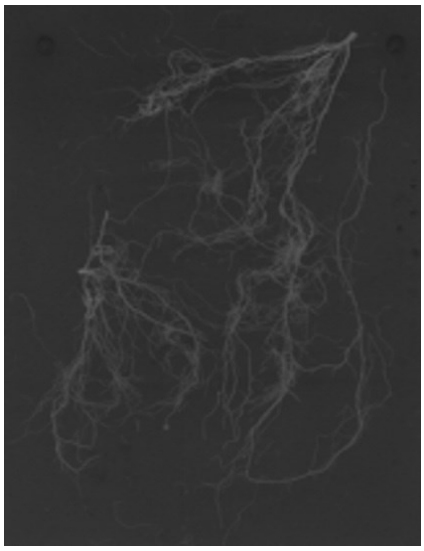
Control + RP



HC + RP



RC + RP



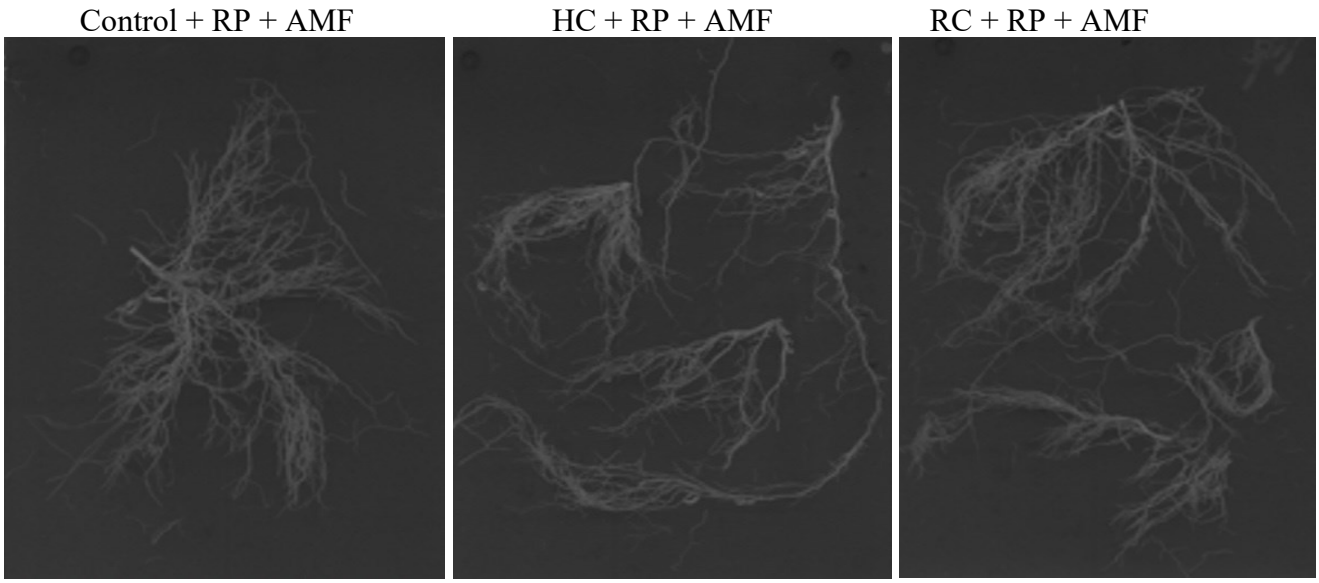
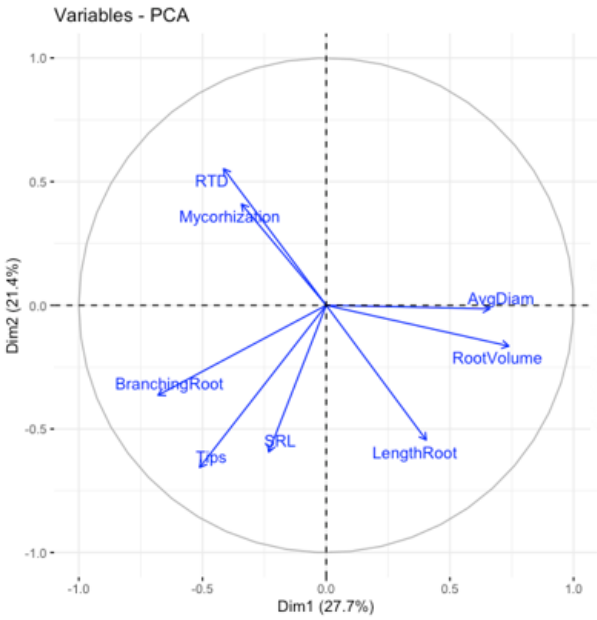


Figure S3: Root images as analyzed by the automated image analysis WinRhizo software after HC or RC consortium inoculation, combined with or without RP and AMF inputs.



	PC1	PC2	PC3
LengthRoot	0.2717051	-0.41647669	0.5435285
AvgDiam	0.4447834	-0.01087628	-0.3022553
RootVolume	0.4962933	-0.12603518	0.3604367
Tips	-0.3437331	-0.50128807	0.1074015
BranchingRoot	-0.4570258	-0.27815093	-0.2097890
SRL	-0.1560616	-0.45362841	0.1443898
RTD	-0.2788170	0.42214078	0.4340393
Mycorhization	-0.2300388	0.31289735	0.4674600

Figure S4: Principal components analysis showing contribution of 8 root traits to variations in root characterization as observed among 100 plants.
Data were first scaled and centered. The table shows contribution of each trait to the first three principal components for the analysis.

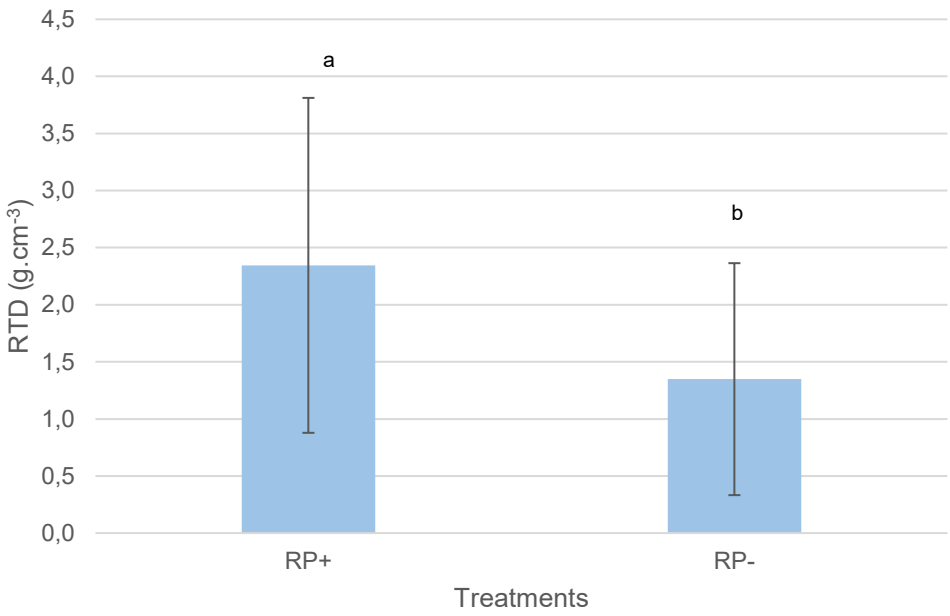


Figure S5: Impact of the RP treatment on Root Tissue Density (RTD). Bar represent standard error and different letters represent significant differences between the treatments (n=5).

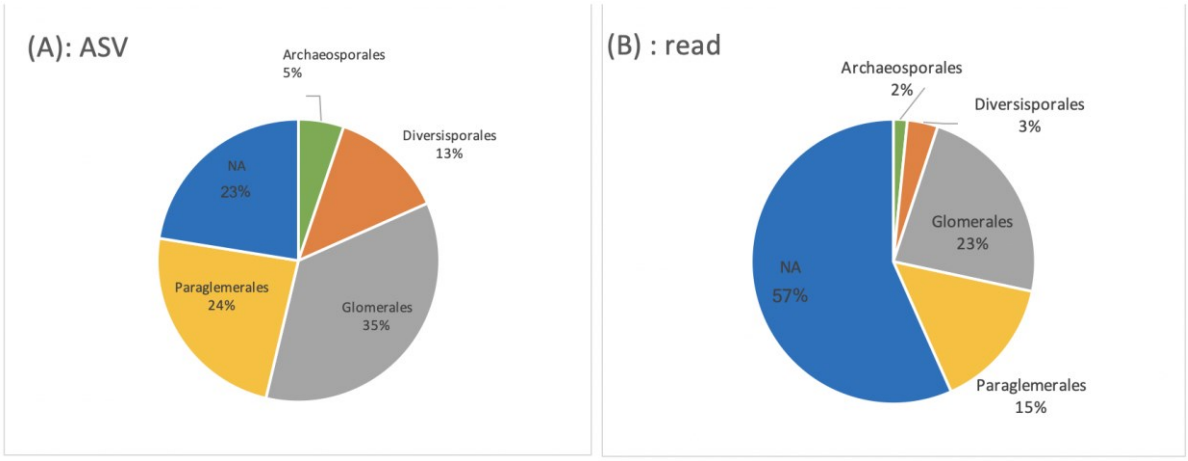


Figure S6: Order-level taxonomic distribution of the mycorrhizal ASV inside the overall data set, in terms of (A): ASV number and (B): read number.

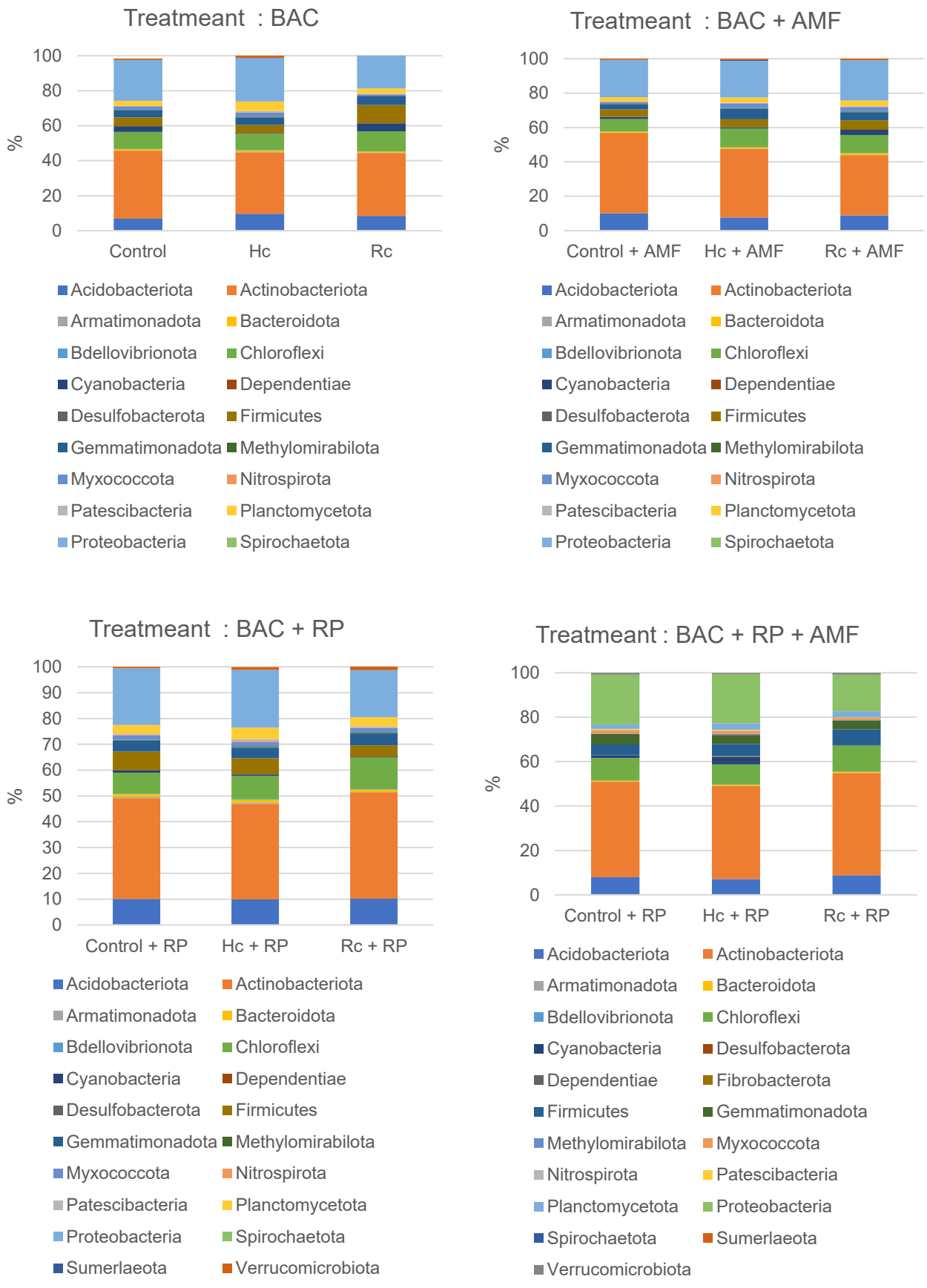


Figure S7: Relative abundance of the bacterial orders, in terms of read, according to the RC or HC inoculation in combination (+) or not (-) with AMF and RP (nC: LB medium; HC: Hyphosphere Bacteria; RC: Root Bacteria).

Table S1: Taxonomic affiliation and plant growth promoting traits of: (A) Root isolates and (B) Hyphosphere isolates

A		Solubilization		Production					Fixation	Motility	
Root Isolates	**Family/Genus /species**	**P (a)**	**P (µg.ml^{-1}) (a)**	**AIA**	**AIA (µg.ml^{-1}) (b)**	**NH3**	**Siderophore**	**Biofilm**	**N2**	**Flagelle**	**Pili**
19	Pseudomonadales	(+ + +)	70,01 ± 0,42	(+)	87,14 ± 0,84	(+)	(-)	(+)	(+)	(+)	(-)
82	*Pseudomonas sp.*	(+ + +)	39,13 ± 0,80	(+)	34,19 ± 3,01	(+)	(+)	(-)	(-)	(+)	(-)
99	*Paenibacillus purispatii*	(+ - -)	260,01 ±1,31	(-)	14,79 ± 1,55	(+)	(-)	(-)	(-)	(+)	(-)
187	*Paenibacillus polymyxa*	(+ + +)	132,15 ±0,21	(+)	251,58 ±4,74	(+)	(-)	(-)	(+)	(+)	(-)
196	*Brevibacterium sp.*	(+ + -)	108,36 ± 0,9	(+)	242,03 ±13,5	(+)	(-)	(-)	(-)	(+)	(-)

B

Hyphospheric isolates	**Family/Genus/species**	**P (a)**	**P (µg.ml^{-1}) (a)**	**AIA**	**AIA (µg.ml^{-1}) (b)**	**NH3**	**Siderophore**	**Biofilm**	**N2**	**Flagelle**	**Pili**
Mi14	*Paenibacillus xylanexedens*	(+ + +)	65,23 ± 0,25	(+)	963,96 ±8,05	(+)	(-)	(-)	(+)	(+)	(-)
MS11	*Microbacterium oxydans*	(+ - -)	166,44 ±0,17	(+)	73,27 ± 0,96	(+)	(-)	(-)	(+)	(+)	(-)
TS12	*Cupriavidus necator*	(+ + +)	133,59 ±0,83	(-)	6,85 ± 2,37	(+)	(-)	(+)	(+)	(+)	(-)
TS18	*Microbacterium sp.*	(+ + -)	99,09 ± 0,32	(+)	75,98 ± 1,38	(+)	(-)	(-)	(+)	(+)	(-)
Mi09	*Stenotrophomonas sp.*	(+ + -)	170,05 ±0,24	(+)	246,7 ± 8,69	(+)	(-)	(+)	(+)	(+)	(+)

(+): Isolate was positive for the trait ; (-): Negative for the trait ; (a): Isolate's response after each of the three successive subcultures ; (b): Values are the mean from 3 independent assays ± standard error

Table S2: Elementary chemical composition of soils analyzed by XRF spectrometry.

Sample	Length Root (cm)	AvgDiam (mm)	Root Volume (cm3)	Tips	Branchiness (tips cm^{-1})	SRL ($cm.g^{-1}$)	RTD ($g.cm^{-3}$)	Mycorhization %
RP - AMF - RCi	710,467 ± 38,24	0,36 ± 0,015	0,69 ± 0,07	2382,625 ± 237,86	3,36 ± 0,25	1138,69 ± 333,112	0,99 ± 0,22	63,61 ± 0,065
RP - AMF - RC	772,2 ± 63,70	0,37 ± 0,031	0,84 ± 0,13	2283,125 ± 228,67	3,17 ± 0,3	1090,49 ± 458,99	0,53 ± 0,38	36,66 ± 0,247
RP - AMF - HCi	593,18 ± 28,70	0,35 ± 0,016	0,62 ± 0,1	2166,25 ± 82,19	3,64 ± 0,18	1325,19 ± 402,03	1,05 ± 0,44	82,97 ± 0,096
RP - AMF - HC	568,26 ± 113,49	0,32 ± 0,013	0,48 ± 0,16	2207 ± 433,77	3,76 ± 0,54	397,43 ± 125,35	3,17 ± 1,4	85,05 ± 0,043
RP - AMF - nC	554,41 ± 30,61	0,33 ± 0,005	0,51 ± 0,04	1996,75 ± 317,96	3,62 ± 0,41	1149,58 ± 370,61	1,67 ± 1,02	34,04 ± 0,258
RP - AMF + RC	678,14 ± 24,17	0,33 ± 0,022	0,49 ± 0,1	2866,85 ± 967,82	3,53 ± 0,05	1979,89 ± 1012,03	0,62 ± 0,25	93,09 ± 0,054
RP - AMF + RCi	573,67 ± 29,68	0,36 ± 0,003	0,61 ± 0,05	2211,43 ± 268,6	3,74 ± 0,31	1109,67 ± 769,31	1,25 ± 0,73	93,33 ± 0,054
RP - AMF + HCi	643,6 ± 9,29	0,31 ± 0,064	0,52 ± 0,21	3326,68 ± 1149,39	4,31 ± 1,64	2099,31 ± 1167,79	1,42 ± 1,14	85 ± 0,175
RP - AMF + HC	820,97 ± 74,14	0,33 ± 0,002	0,73 ± 0,13	2475,19 ± 264,87	2,62 ± 0,16	2302,62 ± 1269,2	0,92 ± 0,38	76,66 ± 0,258
RP - AMF + nC	651,16 ± 32,21	0,33 ± 0,023	0,6 ± 0,14	2611,81 ± 611,53	3,50 ± 0,85	1300,54 ± 846,86	1,86 ± 0,96	91,66 ± 0,1
RP + AMF - RCi	688,53 ± 49,14	0,32 ± 0,022	0,48 ± 0,06	2774,25 ± 570,79	3,65 ± 0,66	2015,82 ± 738,15	0,79 ±0,28	55,83 ± 0,119
RP + AMF - RC	615,41 ± 53,71	0,36 ± 0,023	0,62 ± 0,09	2283,63 ± 238,7	4,11 ± 0,2	1761,28 ± 762,95	0,70 ± 0,44	50,38 ± 0,260
RP + AMF - HCi	684,19 ± 24,62	0,36 ± 0,019	0,65 ± 0,19	2760,81 ± 881,75	3,9 ± 1,1	1770,52 ± 1558,05	1,59 ± 0,78	55,83 ± 0,224
RP + AMF - HC	748,32 ± 32,03	0,32 ± 0,011	0,59 ± 0,07	2718,5 ± 225,67	3,74 ± 0,56	2795,71 ± 2423,75	0,86 ± 0,7	43,42 ± 0,360
RP + AMF - nC	703,62 ± 38,19	0,34 ± 0,015	0,61 ± 0,14	2748,44 ± 646,6	3,88 ± 1,15	1177,89 ± 722,725	1,73 ± 0,86	56,97 ± 0,385
RP + AMF + RC	635,79 ± 20,68	0,31 ± 0,02	0,64 ± 0,16	2246,75 ± 145,63	3,74 ± 0,59	2072,08 ± 758,81	4,80 ± 1,16	86,66 ± 0,163
RP + AMF + RCi	658,71 ± 39,67	0,35 ± 0,015	0,51 ± 0,05	2881,21 ± 793,58	4,58 ± 1,34	3430,94 ± 3403,73	3,42 ± 0,8	81,77 ± 0,101
RP + AMF + HCi	787,11 ± 53,9	0,35 ± 0,019	0,61 ± 0,04	2173,25 ± 493,96	3,41 ± 0,8	1193,51 ± 341,05	3,27 ± 0,11	94,58 ± 0,064
RP + AMF + HC	615,7 ± 27,76	0,33 ± 0,022	0,49 ± 0,08	1748,83 ± 176,07	3,05 ± 0,14	825,01 ± 208,42	2,96 ± 0,25	95 ± 0,066
RP + AMF + nC	734,88 ± 78,77	0,34 ± 0,021	0,76 ± 0,11	2498,04 ± 239,51	3,27 ± 0,11	1229,06 ± 672,41	3,34 ± 0,47	86,87 ± 0,004

Mean and standard error (n=3) obtained for each soil sample (i.e. a composite fraction of rhizospheric soil collected at the base of 5 plants).

Table S3: Effect of the HC or RC consortium inoculation, combined with or without RP and AMF inputs, on shoot and root growth traits of tomato plants under greenhouse pot experiment.

Sample	Stem Hight (cm)Stem Hight (cm)	Length Branching (cm)	Leaf (nb)	Shoot FW (g)	Shoot DW (g)	Root FW (g)	Root DW (g)	Root MS (DW/FW)	C	N	C/N
RP - AMF - nC	105,025 ± 33,41	76 ± 44,33	65,75 ± 10,11	37,17 ± 1,67	7,63 ± 1,17	12,37 ± 4,9	0,81 ± 0,49	0,06 ± 0,019	39,29 ± 2,95	2,89 ± 1,1	14,96 ± 5,14
RP - AMF - RC	84,225 ± 14,52	93,625 ± 40,1	53,75 ± 9,91	36,59 ± 12,13	7,68 ± 0,94	15,12 ± 9,64	0,78 ± 0,39	0,07 ± 0,048	38,73 ± 1,9	2,79 ± 0,91	15,43 ± 6,72
RP - AMF - RCi	81,225 ± 16,83	104,575 ± 29,8	69,25 ± 10,5	40,01 ± 9,2	8,22 ± 1,38	13,95 ± 3,14	1,02 ± 0,56	0,08 ± 0,053	39,35 ± 1,05	2,75 ± 0,82	15,73 ± 6,48
RP - AMF - HC	98,75 ± 16,82	84,275 ± 11,14	57,5 ± 16,05	35,49 ± 6,24	7,86 ± 0,78	9,46 ± 1,94	1,22 ± 0,48	0,13 ± 0,026	40,78 ± 1,51	3,11 ± 0,66	13,51 ± 2,6
RP - AMF - HCi	105,075 ± 26,4	95,875 ± 36,08	59 ± 17,26	34,62 ± 5,74	7,17 ± 1,75	11,40 ± 3,05	0,68 ± 0,17	0,06 ± 0,024	38,71 ± 1,44	3,08 ± 0,75	13,15 ± 3,42
RP - AMF + nC	93,85 ± 1,52	83,80 ± 22,25	60,75 ± 8,22	36,14 ± 5,7	7,19 ± 1,72	17,76 ± 5,51	1,02 ± 0,74	0,06 ± 0,038	39,44 ± 3,6	2,67 ± 0,86	16,04 ± 5,32
RP - AMF + RC	101,325 ± 17,46	93,95 ± 19,48	67,5 ± 14,39	37,27 ± 9,32	8,50 ± 1,64	11,98 ± 3,73	0,57 ± 0,62	0,07 ± 0,106	40,51 ± 3,4	2,79 ± 1,54	18,6 ± 10
RP - AMF + RCi	86,25 ± 10,22	55,5 ± 19,84	44,5 ± 21,76	34,91 ± 7,45	7,59 ± 1,4	10,64 ± 4,35	0,53 ± 0,44	0,05 ± 0,029	39,58 ± 2,86	2,78 ± 1,2	16,99 ± 6,84
RP - AMF + HC	97,95 ± 20,43	90,125 ± 58,87	55,75 ± 21,87	9,46 ± 9,46	8,33 ± 3,72	10,94 ± 4,91	0,48 ± 0,29	0,05 ± 0,048	41,13 ± 2,38	3,46 ± 2,21	14,97 ± 6,84
RP - AMF + HCi	96 ± 16,45	74,175 ± 26,11	53,25 ± 11,53	40,51 ± 2,29	7,50 ± 1,35	12,89 ± 1,83	0,54 ± 0,34	0,04 ± 0,022	40,39 ± 3,4	3,16 ± 0,7	13,39 ± 3,78
RP + AMF - nC	99,175 ± 11,8	74,55 ± 22,32	60 ± 17,22	40,84 ± 2,51	9,72 ± 1,03	12,95 ± 4,74	0,82 ± 0,49	0,09 ± 0,088	39,88 ± 3,96	3,29 ± 1,03	13,03 ± 4,04
RP + AMF - RC	98,2 ± 11,3	111,225 ± 35,32	58 ± 15,51	48,59 ±9,45	9,48 ± 1,76	13,34 ± 5,7	0,83 ± 1,06	0,05 ± 0,043	39,29 ± 0,77	3,17 ± 0,46	12,60 ± 1,78
RP + AMF - RCi	106,5 ± 29,74	100,75 ± 20,8	71,5 ± 10,72	43,42 ± 2,91	8,64 ± 1,428	11,64 ± 3,4	0,49 ± 0,34	0,05 ± 0,038	39,53 ± 5,61	3,17 ± 1,02	13,32 ± 4,17
RP + AMF - HC	71,6 ± 9,3	81,85 ± 38,54	55,25 ± 13,89	34,69 ± 4,02	7,34 ± 1,7	12,22 ± 5,51	1,01 ± 1,12	0,08 ± 0,084	39,66 ± 1,22	3,50 ± 1,4	11,98 ± 2,88
RP + AMF - HCi	115,525 ± 23,21	86,4 ± 22,2	54 ± 12,65	37,85 ± 2,67	7,74 ± 2,04	13,9 ± 6,86	0,81 ± 0,76	0,05 ± 0,024	41,04 ± 0,84	3,57 ± 0,67	11,80 ± 2,2
RP + AMF + nC	95,0075 ± 15,46	117,425 ± 29,46	73 ±37,89	40,66 ± 7,74	9,15 ± 1,91	12,63 ± 3,41	1,07 ± 0,55	0,09 ± 0,057	40,72 ± 0,68	2,84 ± 1,11	16,01 ± 5,82
RP + AMF + RC	95,925 ± 20,68	88,45 ± 55,15	54,5 ± 38,3	35,44 ± 18,34	7,59 ± 4,5	11,36 ± 6,67	0,33 ± 0,15	0,06 ± 0,082	40,76 ± 1,3	4,25 ± 1,65	10,44 ± 2,97
RP + AMF + RCi	86,7 ± 13,05	89,55 ± 59,73	60 ± 37,96	35,16 ± 6,33	6,00 ± 2,85	7,740 ± 5,92	0,56 ± 0,53	0,14 ± 0,14	40,96 ± 0,99	3,90 ± 1,58	11,85 ± 4,66
RP + AMF + HC	93,3 ± 12,44	81,675 ± 24,97	56,25 ± 18,15	40,49 ± 4,71	8,41 ± 1,81	15,31 ± 7,93	1,07 ± 0,52	0,08 ± 0,058	40,25 ± 1,5	2,69 ± 1,14	17,08 ± 7,12
RP + AMF + HCi	109,5 ±14,22	112,9 ± 18,54	75,25 ± 14,5	40,92 ± 5,91	8,62 ± 1	23 ± 10,99	0,69 ± 0,15	0,04 ± 0,02	40,29 ± 1,35	3,09 ± 0,61	13,38 ± 2,64

Sample	Length Root (cm)	AvgDiam (mm)	Root Volume (cm3)	Tips (nb)	Branchiness (tips cm^{-1})	SRL ($cm.g^{-1}$)	RTD ($g.cm^{-3}$)	Mycorrhizal rate %
RP - AMF - RCi	710,467 ± 38,24	0,36 ± 0,015	0,69 ± 0,07	2382,625 ± 237,86	3,36 ± 0,25	1138,69 ± 333,112	0,99 ± 0,22	63,61 ± 0,065
RP - AMF - RC	772,2 ± 63,70	0,37 ± 0,031	0,84 ± 0,13	2283,125 ± 228,67	3,17 ± 0,3	1090,49 ± 458,99	0,53 ± 0,38	36,66 ± 0,247
RP - AMF - HCi	593,18 ± 28,70	0,35 ± 0,016	0,62 ± 0,1	2166,25 ± 82,19	3,64 ± 0,18	1325,19 ± 402,03	1,05 ± 0,44	82,97 ± 0,096
RP - AMF - HC	568,26 ± 113,49	0,32 ± 0,013	0,48 ± 0,16	2207 ± 433,77	3,76 ± 0,54	397,43 ± 125,35	3,17 ± 1,4	85,05 ± 0,043
RP - AMF - nC	554,41 ± 30,61	0,33 ± 0,005	0,51 ± 0,04	1996,75 ± 317,96	3,62 ± 0,41	1149,58 ± 370,61	1,67 ± 1,02	34,04 ± 0,258
RP - AMF + RC	678,14 ± 24,17	0,33 ± 0,022	0,49 ± 0,1	2866,85 ± 967,82	3,53 ± 0,05	1979,89 ± 1012,03	0,62 ± 0,25	93,09 ± 0,054
RP - AMF + RCi	573,67 ± 29,68	0,36 ± 0,003	0,61 ± 0,05	2211,43 ± 268,6	3,74 ± 0,31	1109,67 ± 769,31	1,25 ± 0,73	93,33 ± 0,054
RP - AMF + HCi	643,6 ± 9,29	0,31 ± 0,064	0,52 ± 0,21	3326,68 ± 1149,39	4,31 ± 1,64	2099,31 ± 1167,79	1,42 ± 1,14	85 ± 0,175
RP - AMF + HC	820,97 ± 74,14	0,33 ± 0,002	0,73 ± 0,13	2475,19 ± 264,87	2,62 ± 0,16	2302,62 ± 1269,2	0,92 ± 0,38	76,66 ± 0,258
RP - AMF + nC	651,16 ± 32,21	0,33 ± 0,023	0,6 ± 0,14	2611,81 ± 611,53	3,50 ± 0,85	1300,54 ± 846,86	1,86 ± 0,96	91,66 ± 0,1
RP + AMF - RCi	688,53 ± 49,14	0,32 ± 0,022	0,48 ± 0,06	2774,25 ± 570,79	3,65 ± 0,66	2015,82 ± 738,15	0,79 ±0,28	55,83 ± 0,119
RP + AMF - RC	615,41 ± 53,71	0,36 ± 0,023	0,62 ± 0,09	2283,63 ± 238,7	4,11 ± 0,2	1761,28 ± 762,95	0,70 ± 0,44	50,38 ± 0,260
RP + AMF - HCi	684,19 ± 24,62	0,36 ± 0,019	0,65 ± 0,19	2760,81 ± 881,75	3,9 ± 1,1	1770,52 ± 1558,05	1,59 ± 0,78	55,83 ± 0,224
RP + AMF - HC	748,32 ± 32,03	0,32 ± 0,011	0,59 ± 0,07	2718,5 ± 225,67	3,74 ± 0,56	2795,71 ± 2423,75	0,86 ± 0,7	43,42 ± 0,360
RP + AMF - nC	703,62 ± 38,19	0,34 ± 0,015	0,61 ± 0,14	2748,44 ± 646,6	3,88 ± 1,15	1177,89 ± 722,725	1,73 ± 0,86	56,97 ± 0,385
RP + AMF + RC	635,79 ± 20,68	0,31 ± 0,02	0,64 ± 0,16	2246,75 ± 145,63	3,74 ± 0,59	2072,08 ± 758,81	4,80 ± 1,16	86,66 ± 0,163
RP + AMF + RCi	658,71 ± 39,67	0,35 ± 0,015	0,51 ± 0,05	2881,21 ± 793,58	4,58 ± 1,34	3430,94 ± 3403,73	3,42 ± 0,8	81,77 ± 0,101
RP + AMF + HCi	787,11 ± 53,9	0,35 ± 0,019	0,61 ± 0,04	2173,25 ± 493,96	3,41 ± 0,8	1193,51 ± 341,05	3,27 ± 0,11	94,58 ± 0,064
RP + AMF + HC	615,7 ± 27,76	0,33 ± 0,022	0,49 ± 0,08	1748,83 ± 176,07	3,05 ± 0,14	825,01 ± 208,42	2,96 ± 0,25	95 ± 0,066
RP + AMF + nC	734,88 ± 78,77	0,34 ± 0,021	0,76 ± 0,11	2498,04 ± 239,51	3,27 ± 0,11	1229,06 ± 672,41	3,34 ± 0,47	86,87 ± 0,004

Data expressed as mean values (n=5) with standard error.

Table S4: Number and relative abundance of the main genus identified among the 18S dataset in terms of read and ASV number, according to the RC or HC inoculation in combination or not with AMF and RP.

	Control				HC				RC			
Genre	**ASV**	**read**	**% ASV**	**% reads**	**ASV**	**read**	**% ASV**	**% reads**	**ASV**	**read**	**% ASV**	**% reads**
Acaulospora	1	33	1,30	0,03	2	96	2,99	0,08	4	1671	8,33	1,57
Archaeospora	2	2055	2,60	1,82	3	285	4,48	0,25	0	0	0,00	0,00
Diversispora	4	2592	5,19	2,30	5	2654	7,46	2,29	2	3802	4,17	3,57
Claroideoglomus	4	16939	5,19	15,04	4	730	5,97	0,63	2	14531	4,17	13,63
Glomus	13	7209	16,88	6,40	16	22223	23,88	19,15	3	978	6,25	0,92
Paraglomus	16	20577	20,78	18,27	10	11457	14,93	9,87	10	22068	20,83	20,70
Scutellospora	1	70	1,30	0,06	1	40	1,49	0,03	0	0	0,00	0,00
NA	36	63160	46,75	56,07	26	78569	38,81	67,70	27	63568	56,25	59,62
	AMF + Control				**AMF + HC**				**AMF + RC**			
Genre	**ASV**	**read**	**% ASV**	**% reads**	**ASV**	**read**	**% ASV**	**% reads**	**ASV**	**read**	**% ASV**	**% reads**
Acaulospora	1	63	1,75	0,07	2	414	3,13	0,32	2	220	2,33	0,20
Archaeospora	1	191	1,75	0,21	1	594	1,56	0,46	2	247	2,33	0,22
Diversispora	3	1324	5,26	1,42	2	1618	3,13	1,25	5	1171	5,81	1,04
Claroideoglomus	5	12796	8,77	13,76	4	7775	6,25	5,99	6	6196	6,98	5,51
Glomus	17	30635	29,82	32,95	15	8697	23,44	6,70	19	14967	22,09	13,31
Paraglomus	10	16969	17,54	18,25	12	11605	18,75	8,94	9	16969	10,47	15,09
Scutellospora	1	50	1,75	0,05	1	27	1,56	0,02	0	0	0,00	0,00
NA	19	30958	33,33	33,29	27	99075	42,19	76,33	43	72705	50,00	64,64

	RP + Control				RP + HC				RP + RC			
Genre	**ASV**	**read**	**% ASV**	**% reads**	**ASV**	**read**	**% ASV**	**% reads**	**ASV**	**read**	**% ASV**	**% reads**
Acaulospora	0	0	0,00	0,00	1	67	1,79	0,09	0	0	0,00	0,00
Archaeospora	0	0	0,00	0,00	2	546	3,57	0,72	2	880	6,06	0,59
Diversispora	3	1826	6,00	1,41	6	4066	10,71	5,38	1	433	3,03	0,29
Claroideoglomus	4	8634	8,00	6,68	4	8062	7,14	10,66	3	18195	9,09	12,16
Glomus	8	6466	16,00	5,00	16	16714	28,57	22,10	0	0	0,00	0,00
Paraglomus	8	7264	16,00	5,62	8	19847	14,29	26,24	6	19143	18,18	12,79
Scutellospora	0	0	0,00	0,00	0	0	0,00	0,00	1	687	3,03	0,46
NA	27	105058	54,00	81,28	19	26336	33,93	34,82	20	110339	60,61	73,72

	AMF + RP + Control				AMF + RP + HC				AMF +RP + RC			
Genre	**ASV**	**read**	**% ASV**	**% reads**	**ASV**	**read**	**% ASV**	**% reads**	**ASV**	**read**	**% ASV**	**% reads**
Acaulospora	0	0	0,00	0,00	2	211	3,03	0,23	0	0	0,00	0,00
Archaeospora	0	0	0,00	0,00	1	220	1,52	0,23	2	730	4,17	0,72
Diversispora	3	2875	8,11	2,23	6	3284	9,09	3,50	3	613	6,25	0,61
Glaroideoglomus	4	31265	10,81	24,27	5	10716	7,58	11,43	5	42252	10,42	41,74
Glomus	4	2984	10,81	2,32	12	6545	18,18	6,98	5	4105	10,42	4,05
Parglomus	5	13027	13,51	10,11	12	10877	18,18	11,60	8	36939	16,67	36,49
Scutellospora	1	130	2,70	0,10	0	0	0,00	0,00	1	104	2,08	0,10
NA	37	128817	2,70	0,10	28	61883	42,42	66,02	24	16491	50,00	16,29

SYNTHÈSE GÉNÉRALE

SYNTHESE GENERALE

La transition agro-écologique, visant une agriculture plus durable et écoresponsable requière une meilleure gestion du P dans les sols. Pour cela, plusieurs alternatives (synthétisées dans la Figure 17) peuvent être proposées dont la **valorisation de la diversité taxonomique et fonctionnelle des communautés microbiennes des sols et des racines**. Cette valorisation intègre notamment la sélection de pratiques culturales favorisant les microbiotes ayant des fonctions bénéfiques, la sélection variétale d'espèces végétales en tenant compte du microbe associé, ou la conception de consortia microbiens bénéfiques pour les plantes et utilisables comme bioinoculants en agriculture (Compant *et al.*, 2019).

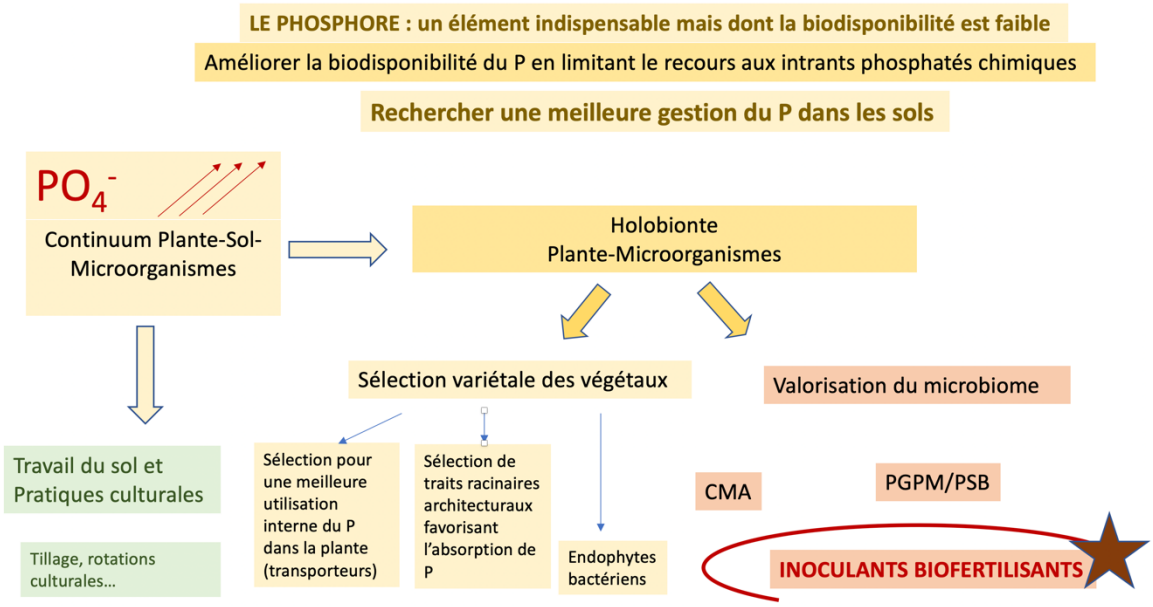


Figure 17 : Synthèse des diverses alternatives de recherche aptes à promouvoir une meilleure gestion du P dans les sols et contextualisant la thématique de recherche.

La production d'inoculants microbiens promoteurs de la croissance des plantes a déjà fait l'objet de nombreux travaux de recherche, évoqués dans la synthèse bibliographique de ce manuscrit. Cependant, la **conception des inoculants** rencontrent encore de nombreuses difficultés. En effet, les mécanismes qui régissent l'assemblage *in situ* du microbiome végétal, ses caractéristiques et son devenir en fonction des conditions environnementales et des propriétés du sol, sont extrêmement complexes et difficiles à analyser (**Article 1, Ducousso-Détrez *et al.* (2022)**. Or ces informations pourraient être pertinentes pour envisager des phytotechnologies raisonnées, aptes à proposer des bioinoculants adaptés à l'usage final et au site d'introduction et d'exploitation. **Des études plus approfondies sur les caractéristiques et le fonctionnement des communautés microbiennes naturelles ou introduites** sont donc indispensables.

Dans ce contexte général, mes travaux de thèse visaient à élargir les connaissances sur les communautés microbiennes des sols riches en RP pour envisager une **approche raisonnée concernant le choix d'un consortium poly bactérien, présentant des traits phytobénéfiques complémentaires et/ou synergiques, pour promouvoir la croissance des plantes d'intérêt agronomique en présence de RP.**

Notre questionnement a ainsi été le suivant : quelles informations pertinentes pour l'élaboration d'un tel inoculum bactérien, peuvent nous apporter l'étude des communautés naturelles de sols riches en RP et celle des caractéristiques *in vitro* d'isolats PGPR issus de sols riches en RP ? Peut-on identifier et exploiter certains groupes bactériens ou fongiques, fonctionnellement aptes à favoriser la nutrition phosphatée des plantes ?

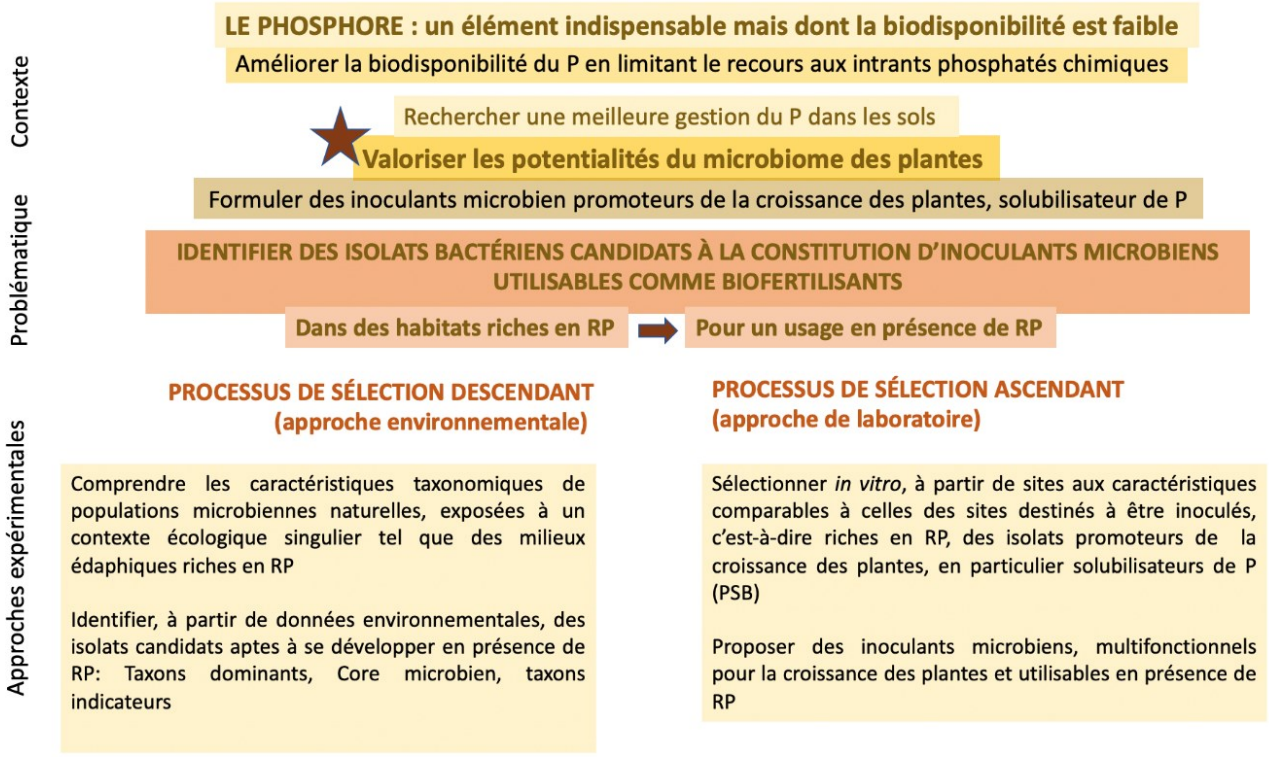


Figure 18 : Contexte général, problématique et approches retenues dans le cadre de l'étude menée.

Pour répondre à ces questions, nous avons adopté deux approches distinctes (Figure 18) :
i) **Un processus de sélection descendant** : cette première approche visait, à partir de données environnementales, à identifier *in fine* des taxons caractéristiques (dominants et/ou spécifiques) de milieux enrichis en RP. L'approche classique dans l'étude des communautés microbiennes en présence de RP mobilise généralement des sites agricoles. Dans notre projet, nous avons choisi de travailler sur des sites singuliers que sont d'anciens sites miniers, naturellement restaurés, et enrichis en P sous forme de RP (les phosphatières du Quercy). Les communautés bactériennes et fongiques, associées aux compartiments racinaires et rhizosphériques de plantes herbacées natives de ces sites *(Ranunculus bulbosus, Bromus sterilis, Taraxacum officinale* et *Dactylis glomerata*) ont alors été caractérisées par metabarcoding.

ii) **Un processus de sélection ascendant** : Cette seconde approche visait à caractériser des isolats PSB à partir d'environnements riches en RP (comme ceux destinés à être inoculés). Une collection *in vitro* de PSB a ainsi été réalisée, à partir d'isolats racinaires issus des sites miniers étudiés, ou à partir d'isolats issus de spores et d'hyphes mycorhiziens piégés au contact d'apports en RP déposés dans la rhizosphère de plantes d'intérêt agronomique. Les PSB racinaires ou hyphosphériques ainsi sélectionnés ont alors été testés pour plusieurs traits additionnels promoteurs de la croissance des plantes et leur affiliation taxonomique a été recherchée.

A partir de ces choix scientifiques et expérimentaux, les résultats originaux obtenus à partir des données environnementales ou à partir des données de culture *in vitro*, sont discutés ci-après.

A. Des avancées sur le plan écologique issues de l'analyse des données environnementales

1. Des profils taxonomiques dominants identifiés chez les champignons et les bactéries

Dans un premier temps, nos travaux ont contribué à étudier l'influence d'une contrainte édaphique liée au P et au RP sur la biodiversité et la structure des communautés microbiennes naturelles. Ainsi, à partir des séquences d'amplicons de gènes ribosomiques issues de l'ADN environnemental extrait de divers sites miniers, secondairement regroupées en ASV, des profils de dominance de certains groupes taxonomiques ont été identifiés, aussi bien chez les bactéries que chez les champignons (**Articles 2 et 3**).

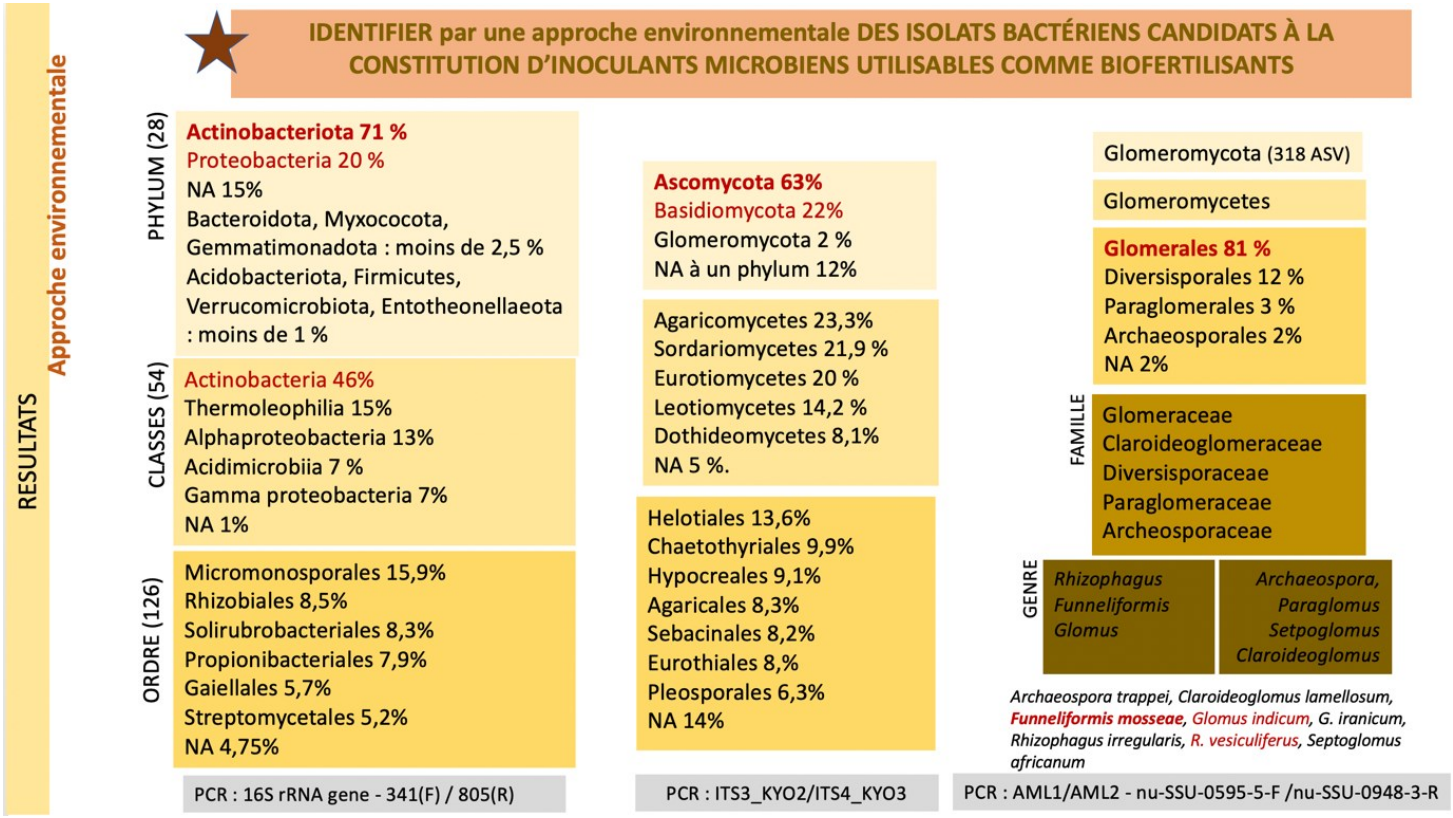


Figure 19 : Synthèse des principaux résultats obtenus par l'analyse des données de séquençage Illumina MiSeq issues des communautés bactériennes, fongiques et mycorhiziennes natives des sites miniers étudiés (approche environnementale) : présentation des taxa majoritaires sur l'ensemble de l'aire minière

Plus précisément, au niveau taxonomique des phyla, l'ensemble des sites miniers est dominé par les Actinobacteriota (71%) et Proteobacteriota (20%) ainsi que par les Ascomycota (63%) et Basidiomycota (22%). Au niveau des classes, les Actinobacteria (46%) Thermoleophilia (15%) et Alphaproteobacteria (13%) ainsi que les Agaricomycetes (23,3%), Sordariomycetes (21,9 %), Eurotiomycetes (20 %) et Leotiomycetes (14,2 %) sont prévalents. Parmi les Glomeromycota, les Glomerales sont largement représentés (81 % ; Diversisporales :12 %) (Figure 19).

Identifier de tels profils de dominance peut aider à élucider les relations entre les organismes et leur environnement, ainsi que les facteurs qui façonnent la dynamique de coexistence (Delgado-Baquerizo *et al.*, 2018) ; c'est un premier point que nous développerons dans les paragraphes qui suivent. En outre, les profils de dominance permettent également d'envisager des représentants des taxa bactériens dominants (que sont les Actinobacteriota et Proteobacteriota), potentiellement aptes, par ailleurs, à proliférer en présence de teneurs élevées en RP, comme candidats pertinents à l'élaboration d'inoculants bactériens utilisables en présence de RP. Nous confronterons cependant ces résultats et hypothèses avec les données acquises *in vitro* (Section B).

2. La concentration en P dans le sol : un moteur écologique mineur du profil des communautés microbiennes dans les sites étudiés

Nos résultats ont également mis en évidence des indices de richesse et de diversité significativement comparables entre les sites enrichis ou non en RP, et ce aussi bien chez les communautés bactériennes, fongiques que mycorhiziennes. De même, l'exploration des profils taxonomiques n'a pas été discriminante entre les sols enrichis *vs* non enrichis en RP, et cela en considérant les niveaux taxonomiques les plus élevés (phylum et famille) des communautés bactériennes ou fongiques dont les CMA. La cohérence de telles données permet de conclure sur une influence mineure des teneurs en RP et P sur le profil des communautés microbiennes. En d'autres termes, le P ne peut donc être considéré, dans nos conditions d'étude, comme un facteur édaphique dominant et prédicateur des profils microbiens, et ce pour les deux règnes bactériens et fongiques dont les Glomeromycetes. Pourtant, l'analyse des données de la littérature a souligné des effets sensibles liés au P, avec des modifications de l'alpha-diversité, de la bêta-diversité ou sur des profils d'abondance de certains phyla (Gomes *et al.*, 2018; Bodenhausen *et al.*, 2019). Nous avons donc été amené à envisager, dans les Articles 2 et 3 de ce manuscrit, d'autres facteurs susceptibles d'expliquer les profils microbiens homogènes obtenus malgré le caractère contrasté des sites échantillonnés, lié au large gradient de P identifié. L'importance de l'histoire des sols dans la médiation de l'effet de P sur le microbiome a ainsi été introduite comme un facteur structurant de la réponse au P.

3. Quand l'histoire d'usage des sols, la perturbation puis la résilience des écosystèmes médient la réponse des communautés microbiennes au P et leurs profils taxonomiques

Dans notre revue de synthèse, nous avons insisté sur la diversité des dispositifs expérimentaux utilisés pour comprendre l'influence du P en tant que moteur du profil des communautés microbiennes. Les modifications des indices de diversité ou des profils taxonomiques (composition et abondance des taxa) relevées par les auteurs correspondaient alors à une réponse quasi immédiate des communautés microbiennes à la perturbation de l'écosystème initial suite à l'apport de P. Aucun consensus ne permettant d'attribuer à P d'effets systématiques, Robbins *et al.* (2018) proposaient néanmoins la notion de résilience (c'est-à-dire la capacité d'un écosystème à s'adapter au changement pour ramener les effectifs populationnels vers un « état de repos » voisin de l'équilibre initial (Delettre & Korniliou, 2022)) pour expliquer des différences de profils des communautés microbiennes selon qu'elles avaient été soumises à des fertilisations réalisées à court *versus* long terme. Cette notion de résilience nous semble pertinente à développer pour notre étude. En effet, les sites miniers échantillonnés correspondent à des écosystèmes ayant subi une modification drastique et prolongée des paramètres biotiques ou

abiotiques initiaux, et donc une rupture majeure des équilibres écologiques. Sous l'influence de processus écologiques, de nouvelles modalités de compétition entre espèces se sont alors établies pour occuper la niche écologique modifiée. L'échantillonnage ayant été réalisé plus d'un siècle après la fin de l'exploitation minière, on peut considérer qu'il a été réalisé dans un environnement ayant atteint un nouvel état d'équilibre, correspondant à des réponses permanentes et stables, plutôt que transitoires, des communautés microbiennes, dans des écosystèmes toujours riches en P. En conséquence, si, comme nos résultats tendent à le démontrer, la réponse des communautés n'est pas déterminée de manière dominante par les teneurs en RP et P, il nous semble alors pertinent d'investir la notion de résilience : nous faisons l'hypothèse que le profil actuel des communautés microbiennes serait certes l'héritage de l'histoire d'usage passé des sols et de la réponse des communautés aux perturbations anciennes, mais résulterait aussi de la capacité de résilience de l'écosystème. Selon ce schéma hypothétique, les effets de P sur la diversité microbienne seraient alors variables en fonction de l'état d'avancement de l'écosystème vers un état d'équilibre abouti. En d'autres termes, le stade de résilience des communautés suite à une variation/perturbation de P doit donc être considéré comme un facteur important qui médie la réponse directe du microbiome au P.
Dans les écosystèmes agricoles, le maintien de la productivité de l'agrosystème implique des perturbations écologiques fréquentes telles que le travail du sol, les rotations culturales ou l'utilisation d'intrants. Face à la succession de ces perturbations, la réponse du microbiome s'opère sans qu'un équilibre stable ne puisse s'installer, ce qui laisse peu de place pour identifier l'impact de P vis-à-vis des communautés microbiennes dans un écosystème à l'équilibre. Au contraire, notre exploration des communautés microbiennes dans des écosystèmes résilients, constitue donc une approche originale et complémentaire. Elle est une opportunité exceptionnelle pour avancer dans l'interprétation des interactions complexes et multidimensionnelles entre P et les microorganismes, et statuer plus précisément sur le rôle de P dans la réponse des communautés microbiennes au P.

4. Des taxa bactérien et fongique dominants et un core microbien mycorhizien : quelle signification écologique ?

Comprendre pourquoi certains taxa sont dominants (plus grande distribution voire omniprésente, plus abondants) est un objectif majeur et une étape critique en écologie. En particulier, une meilleure compréhension des attributs écologiques de ces taxa dominants améliorerait par exemple notre capacité à les cultiver *in vitro* et à maitriser leur installation après introduction dans le sol en vue de promouvoir leurs capacités fonctionnelles. Elle nous permettrait également de mieux prédire la façon dont les communautés varient dans l'espace, dans le temps et en réponse aux changements environnementaux.

Concernant les bactéries, notre étude montre que les Actinobacteria sont largement dominantes : cosmopolites pour tous les sites échantillonnés, avec une abondance relative de 71% (contre 20% pour les Proteobacteria), et 13 représentants parmi le Top 20 des ordres les plus abondants en termes d'ASV. Les Micromonosporales (361 ASV ; abondance relative des lectures de séquençage :10.8%), Solirubrobacterales (539 ASV et 5.6% des lectures) et Propionibacteriales (235 ASV et 5.3% des lectures) constituent ainsi 3 des 4 ordres identifiés les plus abondants dans l'ensemble de l'aire minière. Pour les Proteobacteria, les ordres Rhizobiales (alpha Proteobacteria) et Burkholderiales (beta Proteobacteria) sont par ailleurs largement représentés en termes de nombres d'ASV (301 et 595 respectivement) et de nombres de lectures (5.6 et 3.1% respectivement). Ces distributions au niveau du phylum et de l'ordre

sont par ailleurs, à la fois cohérents avec la distribution combinée de tous les membres du phylum et déterminé par la distribution des genres dominants que sont, au sein du Top 20 des genres les plus abondants, *Solirubrobacter* (163 ASV ; 1.5% des lectures), *Nocardioides* (155 ASV ; 2% des lectures), *Gaiella* (96 ASV ; 1.6%), *Actinoplanes* (90 ASV ; 3%) pour les Actinobacteria. A noter que si le nombre d'ASV représentatives des genres *Streptosporangium* et *Kribbella* est moindre que pour les genres précédents (67 et 35 ASV respectivement), l'abondance du nombre de lectures qui leur sont associés est supérieur : respectivement 3.5 et 2.3%. Pour les autres phyla, les genres *Flavobacterium* (Bacteriodota), *Pseudomonas* et *Rhizobacter* (gamma Proteobacteria) ainsi que *Reyranella*, *Sphingomonas* (alpha Proteobacteria) sont les seuls représentés parmi les 20 genres les plus abondants.
L'étude de Delgado-Baquerizo *et al.* (2018) peut apporter un premier éclairage pour analyser les dominances observées dans nos échantillons. Elle documente ainsi la dominance des Actinobacteria à travers le globe, ce qui pourrait expliquer en partie nos résultats. Cependant, elle signale également la dominance des Proteobacteria (abondance relative voisine de 30% pour les deux phyla). Karimi *et al.* (2018) rapportent pour leur part, dans une biogéographie des communautés bactériennes des sols exclusivement français, une abondance relative dominante des Bacteroidetes puis des alpha-Proteobacteria et des Actinobacteria par ordre décroissant d'abondance relative (toutes voisines de 13 à 14%).
Les données de biogéographie des phyla microbiens décrites ci-dessus, au niveau du globe ou à l'échelle régionale de la France, ne suffisent donc pas à expliquer les schémas de dominance observés dans notre étude, à une échelle géographique plus basse. D'autres facteurs susceptibles d'expliquer la distribution des phyla bactériens telle que nous l'observons dans nos prélèvements de sols doivent alors être envisagés. Par exemple, les Actinobacteria, alpha Proteobacteria et beta Proteobacteria, sont identifiés pour être largement dispersés par les poussières de sol aérosolisées (Barberán *et al.*, 2014), et pour coloniser avec succès, de nouveaux environnements (Karimi *et al.*, 2018). Les Actinobactéries forment par ailleurs des stades physiologiques résistants qui leur permettent de survivre dans des environnements hostiles (Barberán *et al.*, 2014). Ainsi, Karimi *et al.* (2018) suggèrent que les points chauds d'Actinobacteria enregistrés dans les Landes (sud-ouest de la France) et le Centre de la France, pourraient être liés à la distribution de types particuliers de gestion des terres, en particulier liée à l'intensité de la culture et à la perturbation du sol (Dequiedt *et al.*, 2011). Les Actinobactéries sont également connus pour avoir un rôle important dans le renouvellement de la matière organique et la décomposition des molécules récalcitrantes, telles que la cellulose, ainsi que pour le caractère copiotrophe de certains taxa comme nous l'avons développé dans l'**Article 2**. Ils comprennent également des rhizobactéries favorisant la croissance des plantes, des symbiotes, des endophytes et des stimulateurs de la défense des plantes (Barka *et al.*, 2016). Ces compétences, ajoutées à leurs implications potentielles dans le cycle de l'azote (Nelson *et al.*, 2016) peuvent alors expliquer l'étendue de la distribution du phylum des Actinobactéries de manière générale, mais aussi de manière plus sensible, sur nos sites, où la destruction passée mais drastique, de l'écosystème en présence des activités minières, a pu leur laisser l'opportunité de coloniser plus rapidement que les autres phyla les sols, puis proliférer. Les alpha Proteobacteria, quant à elles, sont connues pour avoir des fonctions importantes dans le cycle de l'azote et pour être impliquées dans la décomposition des sous-produits de la lignine ; elles sont plus fréquemment abondantes là où les habitats riches en matière organique sont nombreux (Karimi *et al.*, 2018). Mais elles sont aussi là où la perturbation du sol est faible (Pascault *et al.*, 2013). On peut alors faire l'hypothèse que l'histoire minière des sols leur ait été initialement défavorable mais que la restauration progressive de l'écosystème ait permis leur implantation progressive.

Concernant les champignons, nous avons mis en avant la dominance des Ascomycota, et dans une moindre mesure celle des Basidiomycota. Ces résultats sont parfaitement cohérents avec les conclusions de Egidi *et al.* (2019), établies sur une large gamme de sols et de conditions environnementales au niveau du globe, et montrant que la plupart des phylotypes de champignons dominants identifiés appartenaient au phylum des Ascomycota, les Basidiomycota constituant le deuxième phylum en termes de dominance. Comme pour les bactéries, d'un point de vue mécanistique, plusieurs raisons peuvent expliquer la dominance de quelques taxa par rapport à d'autres. En particulier, une corrélation significative du pH avec certains groupes fonctionnels fongiques a été largement souligné dans le cadre d'analyses à l'échelle du globe (Tedersoo *et al.*, 2014; Soudzilovskaia *et al.*, 2015; Bahram *et al.*, 2018). Les paramètres climatiques (indice d'aridité, précipitation/évapotranspiration) sont également considérés comme des facteurs environnementaux dans la détermination de la composition des communautés fongiques et de la dynamique de l'assemblage des communautés de champignons à l'échelle mondiale (Tedersoo *et al.*, 2014; Bahram *et al.*, 2018). Dans **l'Article 2**, nous avons de plus discuté de l'importance des modes de vie des taxa dominants, notamment du caractère copiotrophe potentiel des Ascomycètes, Eurotiomycètes, Leotiomycètes et Dothideomycètes (Lundell *et al.*, 2014; Yao *et al.*, 2017) pour expliquer le caractère écologique dominant de ces taxa fongiques. Par ailleurs, Egidi *et al.* (2019) indiquent que les phylotypes fongiques distribués de manière transcontinentales incluent des genres connus pour leur dispersion passive par le vent, tels que *Penicillium* ou *Fusarium,* par ailleurs identifiés et largement représentés (59 et ASV respectivement) dans notre étude. En outre, étant donné la remarquable polyvalence des Ascomycètes, Egidi *et al.* (2019) ont recherché les gènes fonctionnels qui caractérisent les taxa Ascomycota dominants par rapport aux autres taxa, et ainsi identifié un certain nombre de traits génomiques qui variaient de manière significative : par exemple, un nombre plus élevé de gènes associés à la nutrition (par exemple transporteur de phosphate, immobilisation de l'azote) et au métabolisme des glucides (par exemple, CAZymes liés à la dégradation des glucides complexes, la synthèse des polysaccharides). Les auteurs ont alors proposé que la possession de capacités trophiques flexibles liées à un potentiel génomique plus élevé pour l'utilisation des ressources, pourrait permettre à certains taxons dominants d'occuper des niches environnementales multiples, ce qui pourrait être un facteur additionnel important dans la définition de l'étendue de l'habitat fongique, et donc de la dominance des Ascomycètes dans les sols. De plus, une fréquence significativement plus élevée de traits génomiques associés à la fois à la tolérance aux stress et aux capacités compétitives a pu être mis en évidence chez les Ascomycètes (Egidi *et al.* (2019). L'ensemble de ces caractéristiques conduirait alors à des stratégies plus généralistes qui pourraient à leur tour contribuer à leur dominance accrue dans les sols.
Notons par ailleurs pour les Basidiomycota (second phylum dominant dans notre étude, avec une abondance relative de 22%), la dominance des genres *Clavulinopsis*, *Tomentella* et *Sebacina*, des genres largement présents sur l'ensemble de la planète, vivant dans le sol ou dans les racines des plantes avec lesquelles elles entretiennent des symbioses ectomycorhiziennes couvrant un très large spectre tant en termes de types d'association que d'ordres et de familles de plantes (Weiss *et al.*, 2016; Lu *et al.*, 2022).

Au sein des communautés de CMA identifiées sur l'ensemble des sites miniers (**Article 3**), nous avons décrit une dominance des Glomerales, et un core microbien de 26 ASV partagé par les six sites d'échantillonnage. Ces ASV, omniprésents, représentaient environ 8,2 % du nombre total d'ASV, et correspondaient à environ 64 % du nombre total de lectures retenues après le traitement bio-informatique et l'assignation taxonomique et phylogénétique. Trois d'entre elles étaient prédominantes en termes d'abondance de séquence (ASV61, ASV62 et ASV208), contribuant à elles seules à 17,1, 11 et 8,4 % du nombre total de lectures, respectivement. Leur

signification écologique reste cependant non identifiable, faute de données taxonomiques conséquentes les concernant.

Pour les règnes bactérien et fongique, la mise en perspective de l'histoire des sols miniers et les traits des taxa dominants est donc compatible avec la dominance de ces taxa. Cependant, il est également intéressant de citer ici les travaux de Guerra *et al.* (2021). En effet, dans l'objectif de fournir des projections de la répartition de la distribution microbienne du sol à l'aide de plusieurs scénarios de changement global, ces auteurs ont utilisé une base de données mondiale des communautés microbiennes du sol pour estimer les tendances du microbiome du sol. Leurs résultats suggèrent qu'un processus d'homogénéisation généralisée des communautés affectant plus de 85% des écosystèmes terrestres s'opère, et s'opèrera au cours des décennies à venir ; les Ascomycota (*Venturia* spp, *Devriesi*a spp.) devenant en particulier, plus abondants dans leurs communautés. On peut s'interroger si l'homogénéité de l'ensemble des communautés microbiennes des sites étudiés tend à illustrer cette modélisation.

5. Etude de la biodiversité : limiter ou préserver dans le jeu de données les ASV de faible abondance ?

L'un des principaux constats associés à l'étude de la diversité microbienne par technologies de séquençage à haut débit réside dans l'augmentation importante du nombre de taxa caractérisés et donc de la richesse spécifique environnementale, permettant d'élargir la vision des scientifiques sur la diversité et la compréhension du monde microbien. Ainsi, les approches de métagénomique ont révélé une diversité microbienne largement sous-estimée, incluant la découverte de nouveaux phyla (Rinke *et al.*, 2013; Castelle *et al.*, 2015; Castelle & Banfield, 2018), et la redéfinition de certains groupes taxonomiques (Parks *et al.*, 2018; Keeling & Burki, 2019). Cet accroissement de la richesse spécifique résulte notamment d'une plus grande profondeur d'échantillonnage des milieux étudiés, ce qui permet d'accéder à des espèces microbiennes minoritaires dans les écosystèmes et non-encore décrites. Une proportion importante de taxa peut alors être représentée par un unique ou un faible nombre de lectures. Mais certaines d'entre elles peuvent aussi être la conséquence du traitement inapproprié des séquences ayant accumulées de multiples erreurs lors des étapes d'amplification et de séquençage inhérentes au metabarcoding (Sogin *et al.*, 2006; Quince *et al.*, 2009). Ces erreurs peuvent alors fausser l'image des communautés, la composition des groupes taxonomiques générés comme l'estimation de leurs tailles. Il convient donc de les limiter. Ce point critique nous a imposé d'avoir recours à des stratégies d'analyses moléculaires connues pour présenter des niveaux élevés de pertinence et de robustesse. Leur description nous semble essentielle car elle permet de donner une valeur expérimentale objective aux ASV, notamment à celles identifiées en faible abondance. Nous avons choisi de développer de manière plus précise certaines d'entre elles.

Ainsi, une première stratégie importante concerne le recours au pipeline Dada2 pour l'analyse de nos données de séquençage d'amplicons. Ce pipeline permet classiquement de visualiser la qualité des lectures de séquençage (les « reads ») et d'évaluer leurs caractéristiques. Il permet également de tronquer et de filtrer les séquences suivant leur taille et leur qualité. Ensuite, si nécessaire, les paires sont contiguées, en choisissant la longueur et le nombre d'erreurs dans l'overlap ; enfin une étape de suppression de chimères est effectuée. Mais plus singulièrement, Dada2 inclut un algorithme de débruitage (denoising) des données de séquençages qui permet de corriger les erreurs de séquençage en les modélisant et en appliquant une correction sur les

séquences d'amplicons identifiées potentiellement suspectes pour ensuite inférer des ASV (Callahan *et al.*, 2017). L'approche de denoising est inverse du clustering basé sur un taux d'homologie défini à l'avance (Callahan *et al.*, 2017). Ainsi, même si les ASV restent des unités de base imparfaites pour caractériser la diversité spécifique d'un écosystème, elles ont une résolution plus fine que les OTU (97% d'homologie de séquences) sur le plan génétique, et permettent de réduire le risque de perte de biodiversité potentiellement masquée par le seuil d'identité de séquence de 97% (Johnson *et al.*, 2019).
A l'issue de l'élaboration des ASV, une partie des ASV de faible abondance est en général supprimée du jeu de données : suppression des singletons, voire la suppression des ASV représentant moins d'une proportion définie du jeu de séquences sur l'ensemble des données initiales ou sur une fraction du jeu initial (Hugoni *et al.*, 2013). De plus, les ensembles de données peuvent être raréfiés à la même profondeur d'échantillonnage (c'est-à-dire un même nombre de lectures par échantillon), de sorte que les changements relatifs des niveaux de composition taxonomique microbienne peuvent être comparés entre les échantillons. Cependant l'application de ces filtres génère un échantillonnage de la biodiversité non exhaustif et s'opère au risque d'éliminer des ASV rares dont l'intérêt écologique peut d'avérer essentiel comme nous le développerons ci-après. Pour notre étude, nous avons donc fait le choix stratégique d'éliminer, certes les singletons, mais de garder toutes les autres ASV, même en présence d'un faible nombre de séquences afin de prendre en compte autant de diversité que possible. Ainsi, à l'issue de ce traitement des données de séquençage, plusieurs taxons rares, bactériens ou fongiques, ont été identifiés, notamment parmi les taxons indicateurs. Il serait intéressant d'identifier les fonctions particulières portées par ces taxons, leurs poids effectifs dans la caractérisation des écosystèmes, voire l'intérêt potentiel qu'ils pourraient apporter à l'élaboration d'un inoculum adapté au RP. Cependant, si leur importance est de plus en plus reconnue en tant que porteurs de fonctions essentielles pour l'écosystème, malgré leur rareté, l'étendue des rôles écologiques et fonctionnels des taxons rares ou satellites est encore mal identifiée. L'hypothèse largement admise est que ces organismes pourraient devenir dominants dans des conditions environnementales changeantes et que leur redondance fonctionnelle pourrait permettre le maintien des processus biogéochimiques (Bachy & Worden, 2014). Ainsi, il a été démontré que les taxons présents en faible abondance sont essentiels pour réduire les invasions microbiennes indésirables dans les communautés du sol. De même, les espèces bactériennes peu abondantes contribuent largement à la production de composés volatils antifongiques qui protègent la plante contre les pathogènes du sol. Hol *et al.* (2015) ont en outre constaté que la perte de microbes rares dans le sol peut avoir un impact négatif sur la productivité des plantes.

B. Exploiter les données environnementales pour l'élaboration d'un consortium bactérien : Pertinence et limites

1. La délicate identification taxonomique de candidats à l'élaboration d'inoculants bactériens

Dans notre étude, nous avons eu recours, pour caractériser la structure et la composition des communautés bactériennes et fongiques de sites miniers phosphatés, à une approche de metabarcoding et séquençage MiSeq d'amplicons des gènes marqueurs cible codant : i) pour l'ARNr 16S d'origine bactérienne et ii) pour l'ITS de l'ARNr 18S fongique, iii) pour la région V4 du 18S chez les CMA. Il est évident que le choix des amorces d'amplification de l'ADN environnemental conditionne la description ultérieure des communautés microbiennes, et donc la comparaison des données entre auteurs, de même que la comparaison avec les données dans

les bases de séquences de référence. Par la suite, nous avons eu recours au traitement des données de lecture issues du séquençage à partir du pipeline bio-informatique Dada2. Il nous a alors permis d'inférer, non pas le regroupement *de novo* des lectures de séquençage en unités taxonomiques opérationnelles (OTU avec 97% d'homologie), mais des ASV qui génèrent des séquences exactes sans erreur putative (Callahan *et al.*, 2016). Ces dernières sont alors des identificateurs stables, contrairement aux OTU qui peuvent ne pas être reproductibles d'une étude à l'autre, ou aux OTU de référence, qui sont également susceptibles d'être obsolètes (Callahan *et al.*, 2016). La recherche de similarité de séquences et l'assignation taxonomique des séquences a ensuite été réalisée par alignement de séquences (BLAST (Westcott & Schloss, 2015) à partir des bases de données de séquences de référence RDP (Ribosomal Database Project) UNITE et Silva. Cependant l'assignation taxonomique reste limitée par la richesse et la précision des bases de données actuellement disponibles et où il manque probablement des séquences de référence pleine longueur, nécessaires à classer de manière adéquate la biodiversité nouvelle qui est échantillonné par barcoding (Schloss *et al.*, 2016).
Ainsi, diverses études menées dans des environnements miniers ont mis en avant la présence d'entités non décrites (Altschul *et al.*, 1990); Liu *et al.*, 2019). (Bruneel *et al.*, 2017) ont pour leur part identifié environ 25 % de nouveaux génomes, qui n'auraient pas été détectés par le séquençage de l'amplicon de l'ADNr 16S. Les auteurs ont également remarqué que dans 55 % des échantillons de sol, plus de 95 % des lectures ne correspondaient à aucun génome de référence. La caractérisation taxonomique des communautés microbiennes est donc assujettie à diverses limites ou biais inhérents à chaque étape de l'étude, depuis la préparation des bibliothèques d'amplicons (extraction d'ADN et PCR), le séquençage (stratégies et profondeur) et le filtrage bio-informatique jusqu'à l'analyse des données de séquençage et l'inférence d'organismes à partir de données de bibliothèques (Davison *et al.*, 2015; Vasar *et al.*, 2017; Kajihara *et al.*, 2022). Cette situation constitue un point critique majeur pour nos travaux.

En effet, nos résultats suggèrent que les représentants des taxa bactériens dominants que nous avons identifiés dans les sols enrichis en RP et P, à savoir les Actinobacteriota et Proteobacteriota, sont potentiellement aptes à proliférer et se maintenir sous des teneurs élevées en RP. A ce titre, il serait pertinent de privilégier des représentants de ces phylum comme candidats à l'élaboration d'inoculants bactériens utilisables en présence de RP. Les taxa du core microbiome ou les taxa indicateurs spécifiques des habitats P pourraient de la même manière, constituer des candidats à la conception d'inoculants : les premiers, par leur caractère cosmopolite, pourraient permettre leur emploi dans une large gamme de milieux. Les seconds pourraient être plus spécifiquement adaptés à un milieu riche en RP. Ce sont là des résultats extrêmement intéressants car ils permettent de rationaliser le choix d'isolats pour l'élaboration d'inoculant. Cependant, nombre d'ASV de ces phyla n'ont pu obtenir d'assignation taxonomique précise, ce qui restreint de fait, dans l'état des connaissances et des techniques disponibles, le choix des inoculants parmi les candidats potentiels identifiés, sachant que les isolats doivent, pour entrer dans un processus industriel de production d'inoculum commercial, être parfaitement identifiés pour répondre aux contraintes de réglementation (traçage, protection sanitaire, …) (Lawson *et al.*, 2019; Nayfach *et al.*, 2021).

2. La nécessaire élaboration de techniques de culture dédiées à l'isolement des bactéries dominantes dans les sols

Outre être identifiables par une assignation taxonomiques, les candidats à l'élaboration d'inoculants doivent être cultivables. Or, alors que les données environnementales mettent en avant les Actinobacteriota et Proteobacteria comme taxa dominants dans les sites miniers

étudiés, l'approche par culture *in vitro* d'identification de PSB a conduit à isoler majoritairement des représentants des Proteobacteria et Firmicutes ; seuls deux des 45 isolats PSB ont été affiliés aux Actinobacteria, tant pour les isolats d'origine minière qu'hyphosphérique. Or les deux phyla sont connus pour héberger des PSB.
La distorsion entre les données acquises *in vitro* et les données environnementales est souvent retrouvée dans les études de microbiologie de l'environnement, l'approche de culture *in vitro* biaisant l'estimation de l'abondance des taxons. En effet, une grande partie de la diversité microbienne ne peut pas être maintenue en culture, par manque de connaissances des conditions de croissance d'un grand nombre de microorganismes (Legeay & Hijri (2022). Ainsi, à partir de données sur la diversité bactérienne des sols au niveau mondial, Schloss et al. (2016) ont conclu que seuls 16,9 % des OTU bactériennes identifiées au cours de leurs travaux avait au moins un représentant cultivé. Un fort biais de culture était par ailleurs relevé au sein des Firmicutes, associés à un pourcentage plus élevé de séquences générées par des représentants cultivés, reflétant probablement le nombre important de Firmicutes intéressants sur le plan biomédical (i.e. *Bacillus, Streptococcus, Lactobacillus, Staphylococcus*). À l'inverse, des phyla tels que les Actinobactéries ou les Bactéroïdètes présentaient un pourcentage plus faible de séquences appartenant à des représentants cultivés. Delgado-Baquerizo *et al.* (2018) estiment pour leur part que seuls 45% des 511 phylotypes bactériens dominants identifiés par leur étude à travers le globe sont liés à des isolats cultivés et que moins de 30% des phylotypes ont des souches types représentatives pour une similarité de séquence >97%. Le développement de méthodes de culture adéquates pour l'ensemble des microorganismes du sol est donc un défi actuel de la microbiologie du sol et une nécessité incontournable pour des valorisations agronomiques ou industrielles de microorganismes. Le développement de nouvelles techniques de culture et de génomique unicellulaire (Nichols *et al.*, 2010; Esteki *et al.*, 2015) ouvre ici une voie prometteuse, qui en outre pourraient permettre d'améliorer considérablement les données de séquence dans les bases de données publiques.

3. Les consortia : des inoculants idéaux ?

La seconde partie de notre étude s'est concentrée sur la formulation d'inoculant bactérien en consortium dont nous avons largement développé les enjeux dans l'introduction du mémoire. Dans un premier temps, il convient de revenir sur l'intérêt des isolats PSB sélectionnés au cours de notre étude et décrits dans **l'Article 4**. Ainsi, nos résultats élargissent le panel d'environnements à partir duquel des PSB ont été échantillonnés Kaur & Kaur (2020), en incluant des sites miniers naturellement revégétalisés ; ils enrichissent ainsi la base de données sur les PSB et peuvent contribuer à un choix plus large de souches pour la conception de biofertilisants susceptibles de répondre à la diversité de leurs conditions d'utilisation. Rappelons d'autre part, que parmi les PSB échantillonnées, plusieurs d'entre eux étaient issus de milieux hyper phosphatés (entre 2 880,0 to 13 927,9 $mg.kg^{-1}$ de P total alors que les sols contiennent classiquement entre 50 to 3000 mg kg^{-1} de P total) et riches en P biodisponible (jusqu'à 339.5 mg.kg-1 de P Olsen, ce qui représente jusqu'à 2.43% du P total). Et ce, bien que divers auteurs mettent en avant une répression du phénotype de solubilisation du P lorsque le P disponible est élevé, une augmentation de l'incidence des bactéries rhizosphériques de phénotype avec la baisse de la teneur en P du sol, ou un nombre de PSB corrélé négativement, avec l'augmentation des réserves de P du sol (Mander *et al.*, 2012; Kaur & Kaur, 2020).

Par ailleurs, les capacités de ces PSB à solubiliser le TCP ont été évaluées ; des concentrations en P solubilisé comprises entre 39.13 et 260.01 $\mu g.ml^{-1}$ pour les isolats racinaires, et 24.15 et 175.69 $\mu g.ml^{-1}$ pour les isolats hyphosphériques ont été relevées à 7 jours de culture. Selon la littérature, des valeurs comprises entre 115 et 716 $\mu g.ml^{-1}$ caractérisent les PSB ayant une bonne

capacité de solubilisation du P en conditions de culture en laboratoire (Chen *et al.*, 2006; Sarikhani *et al.*, 2019; Yu *et al.*, 2019; Kaur & Kaur, 2020; Ding *et al.*, 2021). La valeur la plus importante dans notre étude a été trouvée chez l'isolat "99-*Paenibacillus purispatii*" lequel a été inclus dans la formulation du consortium racinaire. Parmi les PSB hyphosphériques, deux isolats fortement solubilisateurs de TCP ont également été identifiés et inclus dans le consortium hyphosphérique : Mi09-*Stenotrophomonas sp (*170,05 µg.ml^{-1} de P solubilisé) et MS11-*Microbacterium oxydans (*166,44 µg.ml^{-1} P).

Ces résultats, combinés aux autres traits phytobénéfiques portés par les PSB (production d'auxine, d'ammoniac, de sidérophores et de biofilm, fixation de l'azote et motilité bactérienne), nous ont permis de proposer une sélection de PSB racinaires ou hyphosphériques, assignés majoritairement aux Proteobacteria et Firmicutes, pour la formulation de deux consortia avec différents traits PGP, intégrant une représentativité taxonomique spécifique élargie (Figure 20).

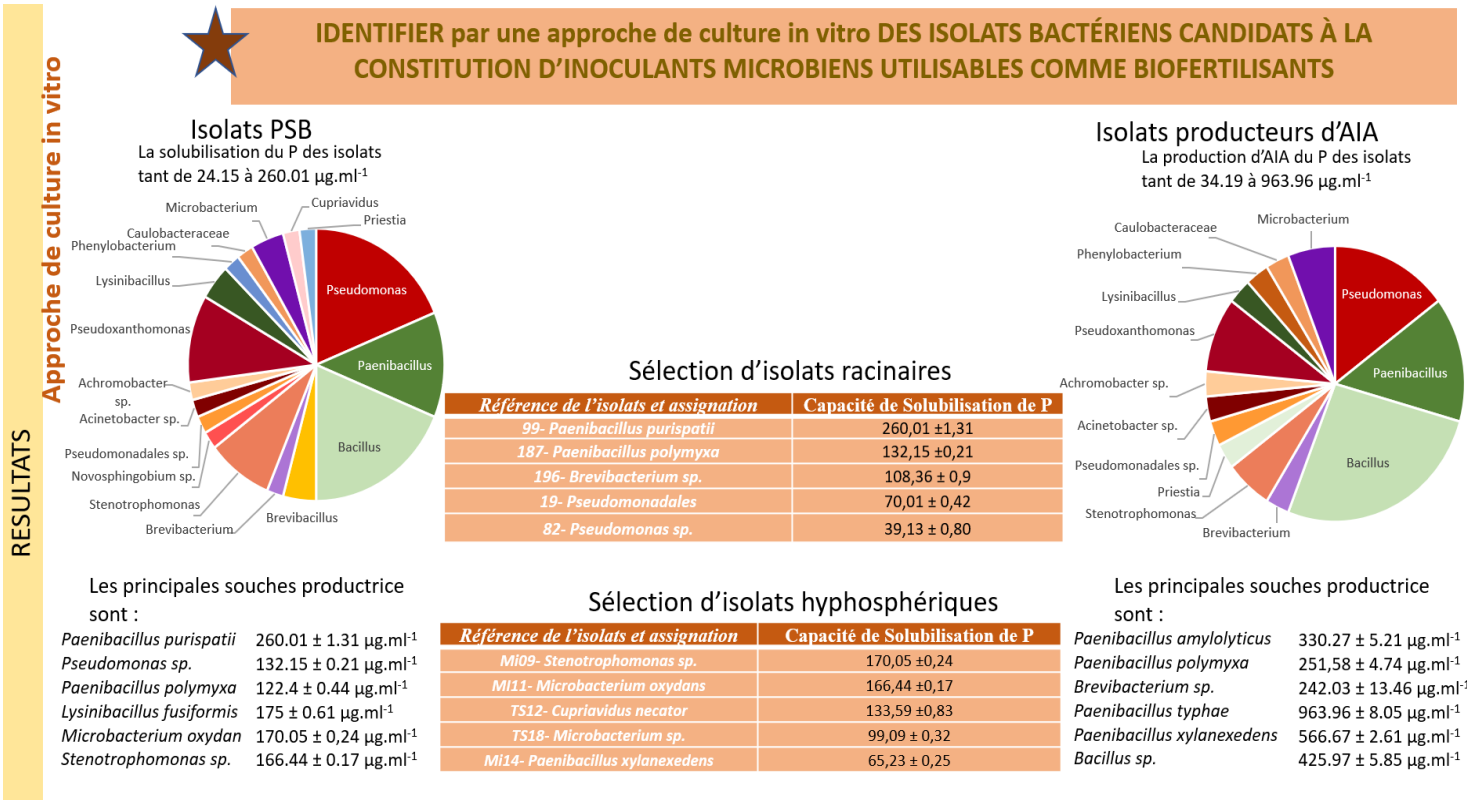


Figure 20 : Synthèse des principaux résultats obtenus par l'analyse des données de culture *in vitro* : Caractérisation des isolats solubilisateurs de P et producteurs d'AIA – Descriptif du profil taxonomique des inoculants sélectionnés pour la formulation en consortium.

Ces consortia ont alors été testés seuls ou en interaction avec l'apport de RP et/ou l'ajout d'un inoculum mycorhizien à base de *Rhizophagus irregularis*, sur la croissance de la tomate, espèce d'intérêt agronomique. Les résultats présentés dans **l'Article 5** ont souligné l'apport des consortia sur le taux de germination des graines de tomates et la croissance des jeunes plantules au cours de la première semaine de culture. En revanche, à l'issue de quatre mois de culture, aucun bénéfice sur la croissance des parties aériennes ou le rendement en fruits n'a été identifié. Néanmoins, des modifications au niveau du système racinaire des plants adultes, en particulier des variations de longueur des racines ont été relevées. De même, des augmentations de la densité du tissu racinaire (RTD) ont été observées en réponse à l'inoculation par les consortia seuls ou en interaction double ou tripartite avec le RP et/ou le CMA. Ces résultats corroborent

diverses recherches dans lesquelles des variations des traits morphologiques et physiologiques racinaires ont été identifiées en réponse à l'inoculation par des PSB (Wang & Chu, 2015; Suleman *et al.*, 2018; Liu *et al.*, 2019; Wang *et al.*, 2019; Elhaissoufi *et al.*, 2020). Ces auteurs ont démontré l'inoculation de PSB peut modifier le fonctionnement des racines par en modulant l'expression de gènes sensibles à l'auxine. Mais la mesure dans laquelle les PSB peuvent contribuer aux propriétés biophysiques des racines reste mal comprise. En particulier, l'étude de la RTD dans la littérature est rarement menée pour quantifier l'effet d'un inoculant microbien (Sarsan, 2016; Elhaissoufi *et al.*, 2020) bien que considérée comme un trait fonctionnel clé dans l'écologie fonctionnelle comparative (Birouste *et al.*, 2014). Il nous semble particulièrement intéressant pour l'avenir d'approfondir comment ce trait racinaire varie avec l'inoculation pour inférer des stratégies fonctionnelles chez la plante lors de l'inoculation.

L'expérience en serre a été réalisée sur sol non stérile. Nous nous sommes donc intéressés à l'impact des inoculants PSB sur le profil des communautés bactérienne et mycorhizienne indigènes au sol inoculé par le consortium. L'analyse des données de métabarcoding et de séquençage met en avant deux tendances. D'une part, des communautés bactériennes peu impactées au niveau de la diversité (indices de Simpson et Shannon très stables à travers les traitements) et de leur composition au niveau phylum suite à l'inoculation par les PSB, seules ou en interaction di- ou tripartite avec le RP et l'AMF. D'autre part, à l'inverse, une réactivité sensible des communautés mycorhizienne en réponse à l'inoculation des consortia lorsqu'ils sont en présence de RP et de l'AMF : des variations des indices de Simpson et Shannon sont relevées ainsi que des modifications des profils d'abondance des communautés mycorhiziennes indigènes. Par exemple, des variations d'abondance des Glomerales et Paraglomerales. Ces résultats mettent en évidence la complexité des interactions en jeu dans le sol, ce qui, en conséquence, ne permet d'établir si l'influence des consortia bactériens sur les modifications des traits de croissance de la plante est directe, liée à la présence de PSB, ou indirecte via l'ensemble du microbiome modifié.
Nos données soulignent donc la multiplicité des variables qui conditionne l'efficacité et l'analyse de l'efficacité d'un inoculum au bénéfice de la production agricole. L'élaboration d'un inoculant en consortium reste probablement une stratégie pertinente pour une application en conditions agronomiques ; mais elle est moins appropriée pour une approche scientifique mécanistique visant à rationalisation la formulation d'un microbiome synthétique. L'adoption d'approches interdisciplinaires dans le domaine des interactions microbiome/sol/plantes reste requise, appuyée par l'analyse bio-informatique et la modélisation informatique de ces interactions pour une interprétation impartiale des résultats et un criblage précis de candidats inoculants.

PERSPECTIVES

ET

CONCLUSION GÉNÉRALE

PERSPECTIVES

1. Etudier la diversité fonctionnelle des communautés microbiennes dans les sols miniers

L'analyse taxonomique par métabarcoding telle que nous l'avons menée renseigne sur la présence, passée ou présente, de séquences et de taxons microbiens dans les sols. En parallèle, l'analyse fonctionnelle permet d'élargir la caractérisation de la biodiversité microbienne en fournissant des informations complémentaires sur les fonctions portées par les séquences ou taxons (Bahram *et al.*, 2018)Gonzales et al. 2022, Legeay & Hijri (2022). Des outils bio-informatiques d'inférence fonctionnelle tels que PICRUSt (Langille *et al.*, 2013; Douglas *et al.*, 2020) ou tax4fun (Aßhauer *et al.*, 2015; Wemheuer *et al.*, 2018) peuvent alors aider à la prédiction fonctionnelle à partir des données de séquençage métagénomique ou d'amplicons. Ces outils attribuent à une séquence, la fonction du microorganisme le plus proche phylogénétiquement, donnant ainsi une approximation "la plus probable" de la fonction associée à cette séquence.
A partir des séquences du gène de l'ARNr 16S et des identifications ou affiliations taxonomiques obtenues au cours de nos travaux, les propositions de potentiel fonctionnel par inférence fonctionnelle pourraient ainsi apporter à brèves échéances des éléments de compréhension sur les mécanismes et modalités d'adaptation des taxons microbiens aux habitats miniers étudiés (Mghazli *et al.*, 2021). Cette démarche est actuellement en cours. Cependant, ces méthodes *in silico* présentent des lacunes. Ainsi les bases de données disponibles concernant les fonctions microbiennes référencées et les pipelines informatiques y afférents sont encore limités (Toju *et al.*, 2018). D'autre part, les inférences indiquent quelles sont les fonctions ou voies métaboliques que l'organisme ou la communauté est en mesure de réaliser. Or la composition génétique des communautés bactériennes peut évoluer plus rapidement que leur composition taxonomique, par exemple, par transfert horizontal de gènes notamment dans des environnements stressants. Des études "omiques" plus larges telles que la transcriptomique, ou la métabolomique pourraient combler ce vide en fournissant des données d'expression de gènes, preuve qu'une voie est activée plutôt qu'une autre ou par organisme plutôt qu'un autre ou une communauté plutôt qu'une autre. Ces approches permettent alors d'affiner le profil d'interactions réelles des microorganismes avec leur environnement et des comparaisons plus robustes entre écosystèmes. Il s'agit donc d'approches pertinentes qu'il serait intéressant de développer dans l'avenir afin de préciser l'influence de P sur les communautés microbiennes des sites miniers. Par exemple, l'expression de gènes codant pour des fonctions impliquées dans le prélèvement de P ou la solubilisation des phosphates pourraient être recherchées : notamment i) des gènes codant pour des phosphatases alkalines (*phoA*), phosphatases acides (*AP*), phytases (*phyC*) ou C-P lyases (*phn*) ; ou ii) des gènes impliqués dans la sécrétion d'acides organiques (gène *gdh* codant pour la glucose dehydrogenase (GDH) ; gènes *pqq* de synthèse de la pyrroloquinoline quinone (PQQ), ou encore iii) des gènes codant pour des transporteurs du P (*pht*) (Jacoby *et al.*, 2017; Zheng *et al.*, 2017; Liu *et al.*, 2020; Ding *et al.*, 2021).

2. Analyser les réseaux de co-occurrence microbienne

Une analyse des réseaux de co-occurrence microbienne dans les sols miniers que nous avons étudiés pourrait également être conduite afin de mieux caractériser l'importance des taxons dominants et d'affiner notamment notre analyse sur l'influence de P sur les communautés microbiennes. En effet, les analyses de réseau ont récemment été appliquées à l'écologie microbienne du sol pour révéler comment potentiellement, les taxons interagissent les uns avec

les autres. Elles permettent de visualiser des co-occurrences, et bien qu'elles n'indiquent pas *sensus stricto* de relations causales et d'interactions écologiques, elles ont aidé à explorer la structure des communautés, à visualiser les modèles de réponse microbienne aux variations environnementales ou aux pratiques agronomiques, et à identifier les membres individuels du microbiome qui influencent de manière significative la composition et l'assemblage de la communauté (de Menezes *et al.*, 2015; Banerjee *et al.*, 2016).
Construits à partir de comparaisons par paires entre les profils d'abondance de taxons individuels, les réseaux de co-occurrence s'organisent en modules (c'est-à-dire en groupes de taxa) et permettent ainsi d'identifier des réseaux d'association au sein de chaque domaine microbien, voire des interactions inter-domaines, même s'il existe des différences fondamentales dans la façon dont les bactéries et les champignons répondent aux signaux biotiques et abiotiques (Deveau *et al.*, 2018). Dans chaque réseau, les nœuds représentent ainsi les taxa et les arêtes représentent les relations de cooccurrence significatives. Plusieurs propriétés d'architecture et de topologie du réseau, considérées comme écologiquement pertinentes, sont alors calculées pour caractériser des communautés voire les comparer : par exemple, le nombre de nœuds et d'arêtes, la densité des modules, le coefficient de regroupement, la modularité et la centralité. L'analyse des réseaux microbiens permet alors d'identifier les microorganismes spécifiques qui occupent une position plus centrale dans le réseau, souvent définis comme des espèces "clés" (keystone) ou des "pivots" (hub) en coexistant fréquemment avec d'autres taxons et exerçant une forte influence sur la structure des communautés microbiennes. Il serait alors intéressant de comparer les données d'analyse des réseaux de co-occurrence avec les données relatives aux phylums dominants ou au groupe des ASV mycorhiziennes présentes de manière ubiquiste et abondantes que nous avons identifiés dans nos échantillons. L'association de prédiction déduite du réseau couplée avec des analyses fonctionnelles pourrait alors représenter un pas en avant important pour déchiffrer le fonctionnement des écosystèmes miniers étudiés.

3. **Mener des expérimentations au champ sur une large gamme de variétés de tomates**

L'analyse des données sur les modifications de croissance et développement des plants de tomates ne peut s'engager sans tenir compte de l'espèce végétale et son influence sur les communautés microbiennes (Thijs *et al.*, 2016; Jacoby *et al.*, 2017; Trivedi *et al.*, 2020). Nous avons ainsi rapidement évoqué en introduction l'impact majeur de la composition des exsudats racinaires sur les profils microbiens racinaire et rhizospérique. Certaines études ont même identifié des variations de profil entre variétés d'une même espèce agronomique interrogeant alors le rôle de la domestication et la sélection variétale dans la modification de l'exsudation et de l'architecture racinaires. Des différences sensibles dans les microbiotes de variétés anciennes et de cultivars modernes ont ainsi été rapportées (Martínez-Romero *et al.* (2020). Par exemple, chez le blé, la symbiose mycorhizienne semble profiter davantage aux cultivars anciens qu'aux modernes. De même pour les variétés traditionnelles de maïs vis-à-vis de bactéries bénéfiques et des anciennes variétés de soja avec les bactéries nodulaires (Martínez-Romero *et al.* (2020). Cependant, la description des propriétés d'un cultivar intègre encore très peu sa réceptivité aux microorganismes de leur environnement ou d'un inoculum. En conséquence, on peut faire l'hypothèse que la variété de tomates utilisées dans notre étude ne soit, que peu ou pas, réceptive aux inoculants utilisés, ne bénéficiant alors, que peu ou pas, des apports phytobénéfiques potentiels des isolats utilisés. Il serait donc intéressant de poursuivre l'étude des consortia promus dans cette recherche en explorant leurs impacts sur d'autres variétés de tomates.
Par ailleurs, l'expérimentation en serre d'un inoculum synthétique peut être considérée comme une étape préliminaire dans la perspective d'une utilisation agronomique mais

l'expérimentation au champ doit être une perspective rapidement envisagée pour juger de la pertinence de l'inoculum sur l'espèce végétale choisie en amont et dans les conditions de culture. Il conviendra alors de s'interroger également sur l'influence de la quantité d'inoculum apportée, et de la temporalité des apports afin de mettre en évidence les modifications "dans le temps et dans l'espace" qui permettront des interprétations précises des effets bactériens, la viabilité des consortia dans les sols inoculés et leur compétitivité par rapport aux communautés autochtones. La capacité de solubilisation du P pour laquelle les consortia ont été sélectionnés en premier lieu devra également être quantifiée.

4. Exploiter la diversité microbienne des sites miniers pour proposer des stratégies de biorestauration écologique : Revégétalisation et phytoremédiation

Le secteur minier, et notamment l'exploitation du phosphate, sont vitaux pour l'économie mondiale. Mais l'extraction produit également de vastes friches minières de phosphate. Elles sont principalement formées par les sols décapés en surface, ainsi que de grandes quantités de déchets de minerai (résidus ou sédiments) correspondant à des matériaux non rentables (Hudson-Edwards, 2016) souvent laissés sur place, comme dans les Phosphatières du Quercy. Les activités minières impactent ainsi drastiquement les sols et entraînent une incapacité plus ou moins longue de recolonisation par la végétation, voire une perte de la résilience de l'écosystème, qui engendre par ailleurs une accumulation de P et une eutrophisation des eaux de surface et des nappes phréatiques. Pour circonscrire ces impacts délétères et restaurer la résilience, le rétablissement progressif des groupes fonctionnels et des réseaux trophiques (Bruneel *et al.*, 2019) est incontournable, et nécessite la mise en place de stratégies de réhabilitation qui peuvent être médiées par les microorganismes (Schulz *et al.*, 2013; Thavamani *et al.*, 2017), par exemple dans le cadre de projets de revégétalisation facilitée par des organismes porteurs de traits promoteurs de la croissance des plantes (Hassani *et al.*, 2018; Kumar & Verma, 2018; Gupta & Pandey, 2019; van Bruggen *et al.*, 2019) . Quelques travaux ont également envisagé d'utiliser des microorganismes dans le cadre de la phytoremédiation des sols de friches minières de phosphate, considérées comme contaminées par un excès de P (Etesami *et al.*, 2015; Ye *et al.*, 2020). Par exemple, Guo *et al.* (2021) ont exploité les capacités solubilisatrices de P de *Trichoderma asperellum* et *Serratia sp*. dans le cadre de la phytoextraction du P par *Lolium perenne* L. et *Lactuca versicolor* dans un sol de friche minière de phosphate contaminé par un excès de P. Ils ont alors constaté que l'inoculation de ces deux espèces végétales améliorait considérablement leur croissance, la teneur en chlorophylle et la phytoextraction du P. Par conséquent, la phytoremédiation assistée par les PSB est aujourd'hui considérée comme une voie prometteuse pour la requalification des friches minières de phosphate, pour laquelle il serait intéressant de tester quelques PSB sélectionnées au cours de notre étude, à partir des sols miniers étudiés. Déployer les espèces cosmopolites identifiées dans les sites phosphatés ou les espèces centrales (core microbien) pourrait également permettre d'initier la dynamique de restauration des microbiomes dans d'autres des sites miniers dégradés afin d'engager une stratégie de revégétalisation assistée par les microorganismes (Toju *et al.*, 2018). Ainsi, alors que le criblage des microorganismes favorisant la croissance des plantes continuera à représenter une direction de recherche importante, nous proposons une autre direction de l'exploration de la diversité microbienne en se concentrant sur les microorganismes centraux susceptibles de jouer un rôle majeur dans les interactions microbiennes et d'exprimer des fonctions de recrutement et d'assemblage des microbiomes associés aux plantes hôtes.

CONCLUSIONS GENERALES

Face à l'exigence sociétale pour des pratiques agricoles plus durables, la production de bioinoculants à base de PSB est une biotechnologie prometteuse. Lorsque celle-ci est associée à l'apport de phosphate de roche (RP), elle pourrait constituer une alternative écoresponsable d'amélioration de la fertilité des sols rhizosphériques, notamment en termes de P biodisponible. Cependant, malgré l'intérêt croissant que suscite cette technologie, son développement auprès des agriculteurs est encore contraint par un manque de maîtrise dans la conception des inoculums, leurs conditions d'utilisation et leur devenir dans les sols en fonction des conditions édaphiques et de compétitivité en présence du microbiote autochtone. Une meilleure compréhension de la biodiversité microbienne des sols en interaction avec le RP est donc requise. Ainsi, dans ce cadre général, mes travaux de thèse ont permis, grâce à (i) une approche environnementale, de caractériser d'un point de vue taxonomique le microbiote naturel de sols hyper phosphatés, naturellement restaurés écologiquement après l'exploitation minière de RP, et (ii) une approche de culture *in vitro*, de caractériser pour les traits phytobénéfiques, dont la solubilisation du P, des communautés bactériennes échantillonnées à partir d'habitats enrichis en RP.
Malgré des teneurs fortement contrastées en RP et P des sites étudiés, une grande similarité entre les profils des communautés naturelles a été identifiée à haut niveau taxonomique des phyla. Nos résultats ont mis évidence une prévalence des phyla Actinobacteriota et Ascomycota, ainsi que les Proteobacteria et les Basidiomycota dans une moindre mesure, et de l'ordre des Glomerales pour les communautés des CMA. Cependant, l'analyse des espèces indicatrices a permis d'identifier des ASV spécifiques des environnements P- vs nP pour les bactéries, les champignons et notamment les CMA. Des variations dans les abondances relatives des ASVs mycorhiziennes persistantes à travers les différents sites d'échantillonnage, ainsi qu'une distribution des ASVs selon les sites P- vs nP- ont été enregistrés. L'ensemble de ces résultats apportent un nouvel éclairage sur la diversité microbienne dans les sols riches en RP. Ils augmentent également les connaissances sur l'importance relative du P en tant que prédicteur des modèles de ces communautés à la résolution taxonomique ASV, et offrent de nouvelles informations sur la restauration des écosystèmes en tant que moteur de l'assemblage des communautés de CMA dans les écosystèmes naturels.

Par ailleurs, la sélection *in vitro* de PSB nous a conduit à concevoir des consortia bactériens, en associant des souches différentes d'un point de vue taxonomique et présentant des traits complémentaires, promoteurs de la croissance des plantes, afin potentiellement d'optimiser l'efficacité et l'adaptabilité du mélange poly bactérien. Ces inoculums ont enfin été testés, seuls ou en combinaison avec l'apport de RP et/ou l'ajout d'un inoculum mycorhizien à base de *Rhizophagus irregularis*, sur la croissance de plants de tomate, et sur le profil des communautés bactériennes et mycorhiziennes autochtones du sol de culture. Des variations dans l'abondance relative des Paraglomerales et Glomerales ont notamment été identifiées après introduction des inoculums bactériens, en présence de RP et/ou CMA. A l'inverse, les communautés bactériennes résidentes des sols inoculé ont été peu impactées par les différentes conditions de culture des plants de tomate.

L'ensemble de nos travaux s'appuyant sur l'analyse des données environnementales, combinées aux données de laboratoire sur, notamment les isolats bactériens d'origine minière, soulignent l'intérêt, mais aussi la complexité de l'élaboration d'inoculants microbiens promoteurs de la croissance des plantes, solubilisateurs de P. Par exemple, les données de culture *in vitro* ont conduit à sélectionner des isolats PSB, d'origine racinaire ou

hyphosphérique, assignés à trois phyla : Proteobacteria, Firmicutes et, dans une moindre mesure, Actinobacteria ; *Bacillus* et *Pseudomonas* étaient les genres prédominants. Ces résultats soulignent donc une distorsion marquée entre les phyla dominants identifiés par chacune des deux approches, et l'étude soulève l'écart important entre les études réalisées en laboratoire « *in vitro* » et la réalité "*in situ*". De même, l'influence des inoculums sur les communautés microbiennes autochtones, rend l'analyse de l'effet de ces inoculums sur le développement des espèces végétales plus difficile : les effets sur la croissance des plantes ne sont pas nécessairement le résultat d'un effet direct des souches inoculées et peuvent être liés à l'induction ou à la répression de population microbiennes résidentes.

En conclusion, comme nos travaux l'ont montré, il est encore complexe de combiner les résultats obtenus sur la caractérisation des communautés microbiennes *in vitro* et en conditions naturelles. Cependant il demeure essentiel que la recherche actuelle persévère dans ces deux voies afin de nourrir la modélisation mathématique et les stratégies de recherche interdisciplinaires qui aideront réellement à identifier comment optimiser les fonctions du microbiome dans les agroécosystèmes et comment choisir les microorganismes qui entreront dans la composition d'un inoculant synthétique fiable destiné à l'inoculation agronomique.
Les données acquises dans ce travail de thèse ouvrent également la voie à plusieurs perspectives de recherche pour l'élaboration d'inoculants microbiens biofertilisants, notamment l'analyse de la fonctionnalité des communautés microbiennes ou l'analyse des interrelations biotiques existant entre les différents groupes microbiens par le biais de réseaux d'interactions. Plus largement, les environnements extrêmes, inexplorées sur le plan microbiologique, que constituent les anciens sites miniers du Quercy représentent également une opportunité importante pour la recherche sur la biorestauration écologique et la phytoremédiation assistée par les microorganismes, de sites miniers phosphatés.

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ANNEXES

Valorisation des travaux

PUBLICATIONS

Parues :

Amandine Ducousso-Détrez, Joël Fontaine, Anissa Lounès-Hadj Sahraoui, Mohamed Hijri (2022). Diversity of Phosphate Chemical Forms in Soils and Their Contributions on Soil Microbial Community Structure Changes. Microorganisms, 10, 609.https://doi.org/10.3390/microorganisms10030609

Zaremski, C., Ducousso-Détrez, A., Amusant, N., & Zaremski, A. (2019). Taxonomic study of French guiana fungi to identify and isolate pure cultured fungi for oleoresin production in Aquilaria: Use of sequences from the small ribosomal DNA (R) subunit (SSU) and the two primer pairs SR6/SR10R and SR7/SR1R.

Amandine Ducousso-Détrez, Robin Raveau, Joël Fontaine, Mohamed Hijri, Anissa Lounès-Hadj Sahraoui, Glomerales dominate arbuscular mycorrhizal fungal communities associated with spontaneous plants in phosphate-rich soils of former rock phosphate mining sites. Mircoorganisms,

Soumises :

Amandine Ducousso-Détrez, Simon Morvan, Joël Fontaine, Mohamed Hijri, Anissa Lounès-Hadj Sahraoui, How high phosphate concentrations affect soil microbial communities after a century of ecosystem self-reclamation ? Land Degradation & Development.

En préparation :

Amandine Ducousso-Détrez, Joël Fontaine, Anissa Lounès-Hadj Sahraoui, Mohamed Hijri, Culturable Phosphate Solubilizing bacteria isolated from rhizospheric and hyphospheric habitats enriched in rock phosphate.

Amandine Ducousso-Détrez, Joël Fontaine, Anissa Lounès-Hadj Sahraoui, Mohamed Hijri, Inoculation of phosphate-solubilizing bacterial consortia affects germination rate and root traits of tomato plants in combinaison with amendment with an arbuscular mycorrhizal fungus and rock phosphate. Microbiological Research.

COMMUNICATIONS ORALES

DUCOUSSO-DÉTREZ A., FONTAINE J., HIJRI M., LOUNÈS-HADJ SAHRAOUI A., 2022 - Les mines de phosphate du Quercy : une mine de champignons mycorhiziens arbusculaires. 7-9 juin 2022, Journées Francophones Mycorhizes 6, Dijon, France.

DUCOUSSO-DÉTREZ A., FONTAINE J., HIJRI M., LOUNÈS-HADJ SAHRAOUI A., 2021 - Microbial diversity in soils enriched in rock phosphate ores. 27-30 octobre 2021, AgriNov2021, Beni Mellall, Maroc.

DUCOUSSO-DÉTREZ A., 2021 - Microbiomes de sols enrichis en phosphore : Diversité et challenges. 1er juillet 2021, journée des doctorants, pôle MTE, Dunkerque, France.

ZAREMSKI C., DUCOUSSO-DÉTREZ A., AMUSANTN., ZAREMSKI A., 2019 - Biodiversity of wood-decaying fungi in French Guiana sequences of the small subunit (SSU) of ribosomal (r) DNA and the two primer pairs SR6/SR10R and SR7/SR1R. IRG50 Scientific Conference on Wood Protection, 12 - 16 May 2019, Québec City, Canada.

DUCOUSSO-DÉTREZ A., 2018 - Étude de la diversité fongique présente dans des écosystèmes forestiers contrastés de Guyane. Les Agapiades, Cirad, Montpellier

POSTERS

DUCOUSSO-DÉTREZ A., HIJRI M., FONTAINE J., LOUNÈS-HADJ SAHRAOUI A., 2019 - Influence de l'interaction plantes-bactéries-champignons mycorhiziens à arbuscules sur la dissolution biologique des phosphates. 2 et 3 avril 2019, Journées SFR Condorcet FR-CNRS 3417, Université de Reims Champagne-Ardenne, France

DUCOUSSO-DÉTREZ A., FONTAINE J., HIJRI M., LOUNÈS-HADJ SAHRAOUI A., 2019 - Isolement de rhizobactéries phytobénéfiques solubilisatrices de phosphate à partir des sites miniers phosphatés du Quercy. 25 et 26 juin 2019, Journées SFR Condorcet FR-CNRS 3417, Université de Reims Champagne-Ardenne, France. (sciencesconf.org:jc2019:279503)

PRODUITS destinés au grand public : (articles, interviews, éditions, vidéos, etc.)

DUCOUSSO-DÉTREZ A. : Concours « Ma thèse en 180 secondes » 2019. Apports d'engrais chimiques phosphatés versus dissolution biologique des phosphates ? Influence de l'interaction plantes-microbes-champignons mycorhiziens sur la dissolution biologique des phosphates : Quand le monde microbien permet d'envisager un progrès vers l'agriculture durable ! Participation aux Présélections du regroupement ComUE LNF et UPJV à Villeneuve d'Ascq, 11 mars 2019. Participation à la finale régionale Hauts de France à Amiens, 18 mars 2019. https://podcast.u-picardie.fr/download/4E2AE5029173-CFA104-D67E4A-84F9616A.mp4

FORMATIONS DOCTORALES

AU SEIN DE L'ED STS

Catégorie : Accompagnement de la thèse

17 crédits validés au sein de l'ED SMRE jusqu'à la fin de l'année 2019 avant le transfert vers l'EDSTS de l'A2U

- ☛ Initiation à la bioinformatique
  10+ heures enregistrées par : SMRE
- ☛ Atelier Voix
  7 heures enregistrées par : SMRE
- ☛ Ma thèse en 180 secondes
  15 heures enregistrées par : SMRE

Catégorie : Outils scientifiques et techniques

☛ Autoclaves (08 octobre 2019 - 8 octobre 2019) Université du Littoral Côte D'Opale - Calais

7 heures Crédits : 2 enregistrées par : Sciences, Technologie, Santé.

☛ Programmation sur R, Master FOGEM (20 septembre 2019) Université du Littoral Côte D'Opale - Boulogne

25 heures Crédits : 5 enregistrées par : Sciences, Technologie, Santé.

Catégorie : Éthique de la recherche et intégrité scientifique

☛ Éthique de la recherche (23 avril 2019 - 29 juin 2019) Université de Lyons

15 heures Crédits : 4 enregistrées par : Sciences, Technologie, Santé.

AU SEIN DE L'ED UNIVERSITE DE MONTREAL

Catégorie : Cours spécialisé

☛ BIO7009 Lectures dirigées en biologie (été 2020) – Université de Montréal

Crédits : 3

Catégorie : cours méthodologiques

☛ BIO6077 Analyse quantitative des données (Hivers 2020) – Université de Montréal

Crédits : 4

Catégorie : Étapes obligatoires

☛ Examen général de synthèse (Décembre 2020) – Université de Montréal

EXPERIENCE EN ENSEIGNEMENT

Poste d'ATER : Biologie Cellulaire, Méthodologies Biologiques, Physiologie Végétale, Biologie Végétale, Biochimie : TP et TD – Licence 1 et 2, ULCO, 2022 – 2023.

Diversité Fongique (cours BIO2350) : TD, TP - 1er cycle (équivalent licence en France) – Université de Montréal, 2021.