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Thèse de Doctorat

*Mention Sciences Ecologiques
Spécialité Agroécologie et Ecophysiologie*

présentée à l'Ecole Doctorale en Sciences Technologie et Santé (ED 585)

de l'Université de Picardie Jules Verne

par

Nicolas Honvault

pour obtenir le grade de Docteur de l'Université de Picardie Jules Verne

***Rôle des traits fonctionnels des plantes dans le cycle du phosphore et sa disponibilité au sein des agroécosystèmes
Agroécologie des cultures intermédiaires***

Soutenue le 18 Novembre 2020, après avis des rapporteurs, devant le jury d'examen :

M. Sylvain PELLERIN, Directeur de recherches HDR, INRAE	Président
M ^{me} Catherine PICON-COCHARD, Directrice de recherches HDR, INRAE	Rapporteur
M ^{me} Claude PLASSARD, Directrice de recherches HDR, INRAE	Rapporteur
M. Jérôme DUCLERCQ, Maître de Conférences, UPJV	Examineur
M. Michel-Pierre FAUCON, Directeur de recherches HDR, Unilasalle	Directeur de thèse
M. David HOUBEN, Enseignant-chercheur, Unilasalle	Co-encadrant
M. Pierre MARIOTTE, Scientific collaborator, Agroscope (Switzerland)	Membre invité
M ^{me} Savine OUSTRAIN, Directrice Recherche et innovation, VIVESCIA	Membre invité





Doctoral thesis

*Honours Ecological Sciences
Speciality Agroecology and Ecophysiology*

Presented to the *Doctoral school in Sciences, Technology and Health (ED 585)*

of the University of Picardie Jules Verne

by

Nicolas Honvault

to obtain the degree of Doctor from the University of Picardie Jules Verne

***Role of plant functional traits in phosphorus cycling and
availability in agroecosystems
The case of cover crops***

Defended on the 18th of November 2020, after the reviewer's opinion, in front of the
examination jury:

M. Sylvain PELLERIN, Head of Research HDR, INRAE	President
Ms. Catherine PICON-COCHARD, Head of Research HDR, INRAE	Reviewer
Ms. Claude PLASSARD, Head of Research HDR, INRAE	Reviewer
M. Jérôme DUCLERCQ, Lecturer, UPJV	Examiner
M. Michel-Pierre FAUCON, Head of Research HDR, Unilasalle	Supervisor
M. David HOUBEN, Associate Professor, Unilasalle	Co-supervisor
M. Pierre MARIOTTE, Scientific collaborator, Agroscope (Switzerland)	Guest member
Ms. Savine OUSTRAIN, Head of Research, VIVESCIA	Guest member



AVANT-PROPOS

Ce projet de doctorat a commencé en novembre 2017 dans le cadre d'une collaboration CIFRE (Convention Industrielle de Formation par la Recherche) subventionnée par l'ANRT (Association Nationale de la Recherche et de la Technologie) entre l'équipe de recherche AGHYLE (Agroécologie, Hydrogéochimie, Milieux et Ressources) d'UniLaSalle Beauvais et VIVESCIA.

Le manuscrit de thèse est rédigé sous forme d'une thèse sur articles en anglais. Le premier chapitre a été publié dans un journal international et le second est en cours de correction pour publication dans un journal international. La liste des publications associées à ce projet de doctorat est disponible en annexe.

FOREWORD

This PhD project started in November 2017 and is a CIFRE collaboration (Industrial research training agreements) funded by the ANRT (National Association of Research and Technology) between research unit AGHYLE from UniLaSalle and VIVESCIA.

The PhD thesis is in the form of a PhD on articles redacted in English. The first chapter has been published in an international journal and the second is awaiting corrections for publication an international journal. A list of the publications associated with this PhD project is available in the appendices.

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RESUME ETENDU

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Rôle des traits fonctionnels des plantes dans le cycle du phosphore et sa disponibilité au sein des agroécosystèmes

Agroécologie des cultures intermédiaires

Nicolas HONVAULT

Directeur de thèse :

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Introduction

Enjeux agroécologiques de la disponibilité du phosphore au sein des agrosystèmes

L'utilisation durable du phosphore (P) dans les agrosystèmes fait l'objet de nombreuses préoccupations en raison de l'épuisement progressif des ressources non-renouvelables en roches phosphatées et des tensions géopolitiques dans les régions où elles sont concentrées. La biodisponibilité limitée du P dans la plupart des sols requiert de plus des apports importants de fertilisants phosphatés pour maintenir une productivité élevée. Assurer une meilleure efficacité d'utilisation du phosphore dans les systèmes de culture est par conséquent un défi majeur pour l'agriculture moderne. Plusieurs solutions ont été proposées pour répondre à ce défi, notamment réduire les quantités d'engrais apportées, augmenter le recyclage du phosphore ou son efficacité d'utilisation. Dans ce contexte les pratiques et systèmes dit « agroécologiques » peuvent offrir une opportunité d'améliorer la disponibilité du phosphore dans les sols cultivés et rendre son utilisation plus efficace. Mettant notamment en avant la diversité d'espèces au sein du système de culture, l'intensification agroécologique de l'agriculture pourrait en effet être l'occasion d'intégrer des espèces plus efficaces vis-à-vis du P. De part leur capacité à accéder aux divers stocks de P des sols et les rétroactions positives qu'elles engendrent, certaines espèces peuvent ainsi augmenter la disponibilité du phosphore dans les sols. Cependant les effets des pratiques agroécologiques sur le cycle du phosphore au sein des agrosystèmes restent peu connus, soulignant l'importance de nouvelles études sur le potentiel de celles-ci d'augmenter la disponibilité du P dans les sols.

Rôle des plantes et leurs traits dans le cycle du phosphore

Les plantes sont les principaux moteurs de nombreux mécanismes impliqués dans le cycle biogéochimique du P. Via l'ensemble de stratégies qu'elles possèdent celles-ci peuvent en effet accéder aux stocks de phosphore organique et inorganique présents dans les sols, altérer leurs disponibilités et conjointement avec les micro-organismes du sol mener à des rétroactions positives ou négatives sur son acquisition future par d'autres plantes. Les plantes influencent le cycle du phosphore dans les agrosystèmes principalement via trois mécanismes : (i) L'acquisition de phosphore depuis les divers stocks de phosphore des sols ; (ii) Leurs interactions avec les micro-organismes du sol altérant la disponibilité du phosphore pour elles-mêmes ainsi que pour les autres espèces en rotation ; (iii) La libération du phosphore contenu dans leurs résidus après récolte ou destruction. Davantage que les espèces en présence, l'impact des plantes sur ces mécanismes est lié à leurs caractéristiques ou traits. L'approche fonctionnelle permet ainsi d'examiner les effets des plantes sur les processus impliqués dans le cycle du phosphore par leurs traits et donc sans à priori sur les espèces ou milieux d'intérêt. Mettre en évidence les traits impliqués dans l'acquisition du phosphore par les plantes, directement ou via interactions avec les micro-organismes du sol, ainsi que les traits impliqués dans la libération du phosphore depuis leurs résidus est essentiel pour approfondir nos connaissances sur le cycle du phosphore dans les agrosystèmes et concevoir des systèmes et pratiques augmentant la disponibilité du phosphore. En outre la complexe association de ces traits au sein de compromis formant autant de stratégies reste également à explorer.

Augmenter la disponibilité du phosphore dans les agrosystèmes via l'approche des traits : Potentiel et défis des cultures intermédiaires

Les cultures intermédiaires sont des cultures non récoltées implantées pendant la période d'inter-culture. Ces cultures peuvent fournir un grand nombre de services écosystémiques tels que la réduction de l'érosion, la fixation de l'azote atmosphérique ou l'augmentation du stock de carbone dans les sols. Cependant malgré un intérêt croissant pour leurs potentiels bénéfiques vis-à-vis de la disponibilité du phosphore dans les agrosystèmes, leurs impacts ont rarement été explorés. Des quantités importantes de phosphore peuvent être acquises par les cultures intermédiaires, de 1 à 30 kg ha⁻¹, pour être ensuite libérées pendant leur décomposition contribuant à la disponibilité du phosphore pour les cultures suivantes. Outre ce premier processus, plusieurs espèces de cultures intermédiaires entraînent des modifications importantes de la rhizosphère via leurs stratégies d'acquisition de phosphore. Ces modifications peuvent en retour augmenter durablement la disponibilité du phosphore. Enfin les cultures intermédiaires interagissent avec les communautés microbiennes du sol, pouvant mener à d'importantes augmentations de l'abondance de plusieurs groupes microbiens comme par exemple les champignons mycorhiziens arbusculaires. Ceux-ci peuvent à leur tour conduire à des rétroactions positives sur l'acquisition de phosphore par les cultures suivantes. Via l'ensemble de ces mécanismes les cultures intermédiaires peuvent contribuer significativement à la disponibilité du phosphore dans les agrosystèmes. Cependant, la multiplicité des mécanismes impliqués couplée à leur forte variabilité pose de multiples défis qui doivent être pleinement étudiés afin de maximiser leurs bénéfices. Les multiples stratégies d'acquisition de phosphore des cultures intermédiaires restent par exemple largement à explorer. Un large éventail de valeurs est aussi rapporté pour les facteurs influençant la dynamique de libération du phosphore à partir des résidus de cultures intermédiaires, suggérant des investigations complémentaires. L'élucidation ciblée de ces points pourrait offrir d'optimiser l'effet des cultures intermédiaires sur la disponibilité du P.

Objectifs

Ce projet de doctorat vise à mieux comprendre le rôle des traits fonctionnels des cultures intermédiaires et leurs interactions dans les processus biogéochimiques influant sur la disponibilité du phosphore au sein des agrosystèmes. Pour répondre à cet objectif général, quatre chapitres ont été produits pour examiner les questions centrales et toujours en suspens.

Le chapitre 1 a examiné les stratégies d'acquisition de phosphore dans les cultures intermédiaires avec pour objectif de : (i) Caractériser les relations entre les traits fonctionnels pour explorer les compromis entre traits et les principales stratégies d'acquisition du phosphore des cultures intermédiaires ; (ii) Examiner si les formes de phosphore et le type de sol interviennent dans l'expression de ces compromis et stratégies d'acquisition de phosphore.

Le chapitre 2 a ensuite examiné la contribution des interactions entre micro-organismes du sol et plantes pour l'acquisition du phosphore avec pour buts : (i) D'examiner l'influence des traits fonctionnels des plantes sur les communautés microbiennes et ses conséquences sur l'acquisition du phosphore ; (ii) De mieux comprendre la médiation des interactions plante-sol-micro-organismes pour l'acquisition du phosphore par le type de sol.

Le chapitre 3 vise à mieux comprendre l'influence des traits des résidus de cultures intermédiaires sur la disponibilité du phosphore dans les agroécosystèmes via leur influence sur la libération de phosphore pendant leur décomposition.

Enfin, le chapitre 4 a pour objectif d'estimer les effets de rétroaction de la disponibilité du phosphore dans le sol, sur les effets des cultures intermédiaires sur la disponibilité du phosphore pour les cultures suivantes via la modélisation de la dynamique de libération du phosphore à partir de leurs résidus.

Approche méthodologique

Afin d'étudier le rôle des traits fonctionnels des plantes et leurs interactions dans les processus impliqués dans la disponibilité du phosphore dans les agroécosystèmes, trois approches complémentaires ont été employées. Le rôle des cultures intermédiaires dans les processus et interactions plante-sol-micro-organismes impliqués dans le cycle du phosphore dans les agroécosystèmes a ainsi d'abord été examiné avec une approche par les traits fonctionnels ex situ, avant de modéliser les impacts attendus sur la disponibilité du phosphore.

Caractérisation des traits végétaux impliqués dans les interactions plant-sol-microbes pour l'acquisition du phosphore, leurs relations et agrégation en stratégies.

Malgré notre compréhension croissante du cycle du phosphore dans les agrosystèmes, le rôle des traits et des stratégies des cultures intermédiaires dans les multiples processus impliqués reste sous-exploré. Afin d'examiner les différentes stratégies d'acquisition de phosphore au sein des cultures intermédiaires mais également leurs interactions avec les communautés microbiennes du sol, un dispositif expérimental ex situ commun a été conçu. Les traits morphologiques, architecturaux et physiologiques impliqués dans la capacité des plantes à explorer le sol et mobiliser le phosphore ont été caractérisés au sein de treize espèces de cinq familles phylogénétiques variées. Les communautés microbiennes dans la rhizosphère d'un sous ensemble de ces espèces ont également été mesurées pour étudier les interactions plantes micro-organismes impliquées dans l'acquisition du phosphore. Une approche multivariée a ensuite été utilisée pour identifier les stratégies d'acquisition du phosphore des cultures intermédiaires basées sur cette gamme de traits ainsi que les interactions entre traits végétaux et communautés microbiennes de sol.

Examen du rôle des traits des résidus de cultures intermédiaires sur le devenir du phosphore.

Après avoir examiné les stratégies des plantes pour l'acquisition du phosphore, nous avons cherché à comprendre la libération de phosphore capturé via ces diverses stratégies et son impact sur la disponibilité du phosphore. Pour étudier l'effet des traits des résidus de cultures intermédiaires sur le devenir du phosphore dans les sols, les résidus de six espèces aux traits et stratégies d'acquisition du phosphore variés ont été examinés lors d'une expérience en serre et d'une incubation simultanée. Une approche de marquage isotopique permettant de suivre le devenir du phosphore issu de résidus via le rapport entre les isotopes radioactifs et stables du phosphore a été utilisée. L'impact des résidus sur la disponibilité du phosphore a été mesuré à plusieurs reprises via l'extraction du phosphore disponible contenu dans les sols incubés mais aussi la mesure de la biomasse et du contenu de phosphore d'un ray-grass grandissant sur les sols amendés. Pour examiner les dynamiques microbiennes impliquées le phosphore contenu dans la biomasse microbienne a également été mesuré.

Modélisation de la dynamique de libération du phosphore depuis les résidus et des effets de rétroaction de la disponibilité du phosphore dans le sol.

Afin d'étudier les effets de rétroaction de la disponibilité du phosphore dans le sol sur la dynamique de libération de phosphore à partir des résidus de cultures intermédiaires et leur impact sur la disponibilité du phosphore, l'approche de modélisation développée par Damon et al. (2014) a été employée. La conception simple d'utilisation du modèle nécessite peu d'entrées et a ainsi permis l'exploitation de données largement disponibles dans la littérature tout en ajustant les paramètres du modèle basés sur les résultats obtenus dans les chapitres précédents. Deux scénarios destinés à représenter la biomasse moyenne la teneur en phosphore des cultures intermédiaires sous une disponibilité élevée ou faible de phosphore dans le sol ont été produits et comparés afin d'offrir une base pour mieux comprendre la médiation des bénéfiques des cultures intermédiaires par la disponibilité du phosphore dans les sols.

Chapitre 1 : Compromis entre les traits racinaires d'acquisition du phosphore des espèces cultivées pour l'intensification agroécologique

Les stratégies d'acquisition des plantes sont dictées par de multiples traits morphologiques et physiologiques souterrains ainsi que par les interactions entre ces traits. Ce chapitre visait à caractériser les relations entre les caractères impliqués dans l'acquisition de P pour explorer les compromis et les principales stratégies d'acquisition de P. Des compromis entre le diamètre racinaire et les traits physiologiques ont été observés, les racines à diamètre élevé présentant une plus grande libération de carboxylate et/ou une activité de phosphatase dans la rhizosphère plus intense. L'analyse multivariée des traits fonctionnels impliqués dans l'acquisition de P a mis en évidence quatre stratégies principales d'acquisition de P reposant principalement sur des traits morphologiques, des traits physiologiques ou une combinaison de ceux-ci. La diversité des stratégies démontre un potentiel pour des effets positifs de la diversité fonctionnelle dans les communautés végétales cultivées via un accès préférentiel à différents pools de P conduisant à des complémentarités pour l'acquisition de ressources. Dans l'ensemble, ces résultats peuvent contribuer à la conception de cultures multi-espèces fonctionnellement complémentaires, améliorant la disponibilité de P dans les agrosystèmes.

Chapitre 2 : Interactions entre les traits fonctionnels souterrains et les micro-organismes rhizosphériques pour l'acquisition du phosphore

Malgré le rôle central des interactions plante-sol-micro-organismes dans le fonctionnement de l'écosystème et l'acquisition des éléments nutritifs par les plantes, l'influence des traits souterrains des plantes sur les communautés microbiennes du sol et leurs conséquences sur l'acquisition du phosphore (P) restent sous-explorées. Dans ce chapitre, de multiples corrélations ont été observées entre les traits fonctionnels souterrains et les communautés fongiques et bactériennes rhizosphériques. Les taux de libération de malate et de malonate en particulier étaient fortement liés aux indicateurs de bactéries à Gram négatives, elles-mêmes corrélés au contenu en P des plantes. Ces résultats suggèrent que les traits souterrains liés aux interactions plante-sol microorganisme peuvent jouer un rôle important dans les stratégies d'acquisition de P et soulignent le rôle plausible des carboxylates dans ces interactions.

L'intégration de ces interactions dans des modèles biogéochimiques conduirait à une meilleure compréhension du cycle P et du fonctionnement des écosystèmes.

Chapitre 3 : Rôle des traits végétaux dans la dynamique de libération du phosphore à partir de résidus de cultures intermédiaires

La libération du P contenu dans les résidus de culture peut augmenter la disponibilité de P dans les sols et contribuer de manière significative à la nutrition en P des cultures suivantes. Les résidus des cultures intermédiaires présentent cependant des effets contrastés sur la disponibilité du phosphore, allant d'augmentations importantes à des diminutions suite à l'immobilisation microbienne du phosphore. Mieux comprendre le rôle des traits des résidus de cultures intermédiaires dans la dynamique de libération de P pendant leur décomposition pourrait offrir un aperçu des principaux traits à gérer pour maximiser leurs effets sur la disponibilité du P. Les effets des résidus de cultures intermédiaires aux traits contrastés ont été étudiés via deux expériences simultanées examinant l'influence de l'ajout de résidus sur l'acquisition de P par une culture suivante ainsi que sur le P disponible et microbien. Le rôle central du ratio C/P dans les résidus pour le devenir du P issus de résidus a été mis en évidence. En effet, les résidus avec un rapport C/P élevé ont eu un impact négatif marqué sur la disponibilité du P, probablement lié à des phénomènes d'immobilisation microbienne du P suite à l'ajout de carbone. L'ajout de résidus de cultures intermédiaires a cependant eu peu d'influence sur l'acquisition cumulée de P par le ray-grass au cours des quatre mois d'expérience. Les faibles effets des résidus de cultures intermédiaires sur la disponibilité du P et son acquisition par une culture ultérieure dans des sols à disponibilité modérée de P, suggèrent que les pratiques et compositions de couvert optimales pour améliorer la disponibilité du P, devraient être adaptées en fonction de la disponibilité du P du sol, en s'appuyant notamment sur les multiples autres services fournis par des cultures intermédiaires.

Chapitre 4 : Modélisation des effets de rétroaction de la disponibilité du phosphore sur la dynamique de libération du phosphore depuis les résidus de cultures intermédiaires et leurs conséquences pour la disponibilité du P

Les cultures intermédiaires se sont révélées être une pratique de gestion prometteuse pour augmenter la disponibilité de P dans de nombreux agrosystèmes, avec cependant des effets importants mais aussi très variables. Comprendre les facteurs expliquant cette variabilité pourrait offrir l'opportunité de concevoir des solutions localement adaptées. En utilisant une approche de modélisation, nous avons étudié les effets de rétroaction de la disponibilité du P dans les sols sur les effets des cultures intermédiaires sur la disponibilité du phosphore par le biais la libération de phosphore contenu dans leurs résidus. Les prévisions du modèle soutiennent une contribution plus élevée de la libération de P contenu dans les résidus de cultures intermédiaires, à la disponibilité du P dans les sols où le P est fortement disponible. Cependant, quelle que soit la disponibilité initiale en P dans le sol, une diminution de la disponibilité du P a été projetée pendant un à deux mois après la destruction de la culture intermédiaire en raison de phénomènes d'immobilisation microbienne du P. La médiation des effets des cultures intermédiaires par la disponibilité du P dans les sols suggère des compositions de couvert différentes en fonction de celle-ci. L'immobilisation microbienne et ses effets négatifs souvent négligés sur la disponibilité du P doivent être intégrés dans notre compréhension des effets de la culture intermédiaire sur le cycle du P et dans les outils d'aide à la décision afin d'adapter les pratiques de gestion pour maximiser les avantages des cultures intermédiaires.

Discussion générale et perspectives

Compromis entre traits et stratégies pour l'acquisition du phosphore dans les cultures intermédiaires

Caractériser l'association entre les traits impliqués dans l'acquisition du P par les plantes est un défi majeur à relever pour comprendre les impacts des plantes sur le cycle du P. De multiples compromis entre les traits morphologiques et physiologiques des plantes ont été mis en évidence, notamment entre la proportion de racines fines et l'activité de la phosphatase dans la rhizosphère. Ce compromis suggère des stratégies d'acquisition du P divergentes au sein des cultures intermédiaires, reposant sur une acquisition du P par exploration du sol et capture du P disponible ou par mobilisation du P non directement disponible. Des relations négatives ont également été observées entre le pourcentage de racines fines et les indicateurs bactéries Gram négatives dans la rhizosphère, suggérant un axe de compromis supplémentaire. Des relations négatives entre le diamètre racinaire et la colonisation des racines par les champignons mycorhiziens arbusculaires ont également été observées dans les écosystèmes naturels, ce que nos résultats tendent à étendre aussi à l'association avec des communautés bactériennes. L'analyse de la convergence des traits des plantes dans un espace multi-traits nous a en outre permis de souligner le caractère multidimensionnel des interactions entre traits racinaires ainsi que la diversité des stratégies d'acquisition du P dans les cultures intermédiaires. Un spectre économique racinaire multidimensionnel a été proposé dans la littérature pour expliquer les diverses stratégies des plantes pour l'acquisition d'eau et de nutriments. La médiation des compromis ainsi que des stratégies d'acquisition de P par le type de sol observée dans nos résultats semble renforcer cette théorie, démontrant des ajustements multidimensionnels des stratégies végétales selon des axes multiples face aux contraintes environnementales. Ce constat pourrait en retour fournir des éléments de compréhension face à l'expression parfois inégale des compromis entre traits racinaires dans différents contextes environnementaux.

Effets de rétroaction des traits des cultures intermédiaires sur la disponibilité du phosphore

Comprendre et quantifier les impacts des cultures intermédiaires sur la disponibilité du P nécessite d'étudier les mécanismes de rétroaction par lesquels les stratégies et les caractéristiques des plantes ont un impact sur le cycle du P. L'examen de la dynamique de libération du P à partir des résidus de cultures intermédiaires et du rôle des traits des plantes dans ces dynamiques a mis en évidence les effets du rapport C/P des résidus. Celui-ci offre dans nos résultats le meilleur indicateur de la dynamique de libération de P. Le rôle central du rapport résidu C/P dans nos résultats est probablement dû à une immobilisation microbienne importante du P, entraînant des effets principalement neutres voir négatifs des résidus de cultures intermédiaires sur la disponibilité du P. Les travaux de modélisation réalisés semblent plutôt renforcer cet impact significatif de l'immobilisation microbienne du P sur la disponibilité du P après l'ajout de résidus de cultures intermédiaires, avec la prédiction d'effets négatifs à neutres de l'ajout de résidus pendant un à deux mois après la destruction de celles-ci. Ceci est cohérent avec les effets négatifs des cultures intermédiaires sur la disponibilité de P parfois observés dans la littérature

Éléments pour la sélection d'espèces et de stratégies pour une meilleure disponibilité du phosphore dans les agroécosystèmes

Une implication majeure de cette thèse est l'opportunité d'adapter la composition des cultures intermédiaires pour une meilleure disponibilité du phosphore dans les agrosystèmes via des stratégies et des traits fonctionnels adaptés. Les multiples compromis observés dans nos résultats suggèrent l'absence de stratégie « idéale » ou d'espèce offrant simultanément un fort potentiel de mobilisation de P peu disponible, une forte capacité à explorer le sol pour acquérir le P disponible, tout en assurant simultanément des rétroactions microbiennes positives. Poursuivre un processus spécifique se fera probablement au détriment des autres, tandis que les mélanges multi spécifiques de cultures intermédiaires, une perspective majeure de ce projet, peuvent offrir l'opportunité d'associer ces différents processus et donc potentiellement leurs bénéfices. La contribution relative d'un processus spécifique est en outre influencée par les conditions du sol et en particulier la disponibilité du P, ce qui suggère des compositions de cultures intermédiaires reposant sur des processus

différents en fonction de la disponibilité du P dans les sols. Dans les contextes à haute disponibilité de P, l'acquisition de P et la libération ultérieure à partir des résidus peuvent être le principal mécanisme impliqué dans les effets des cultures intermédiaires sur la disponibilité de P. Les stratégies et traits présentant une capacité d'acquisition élevée du P (et à une concentration élevée de P dans la biomasse) telles qu'observées chez les Brassicacées, ou éventuellement des Poacées à forte concentration en P, pourraient ainsi être recommandées dans ces contextes. Le ratio C/P, identifié comme critique pour la disponibilité du P issus de résidus dans notre étude, devra cependant être adapté notamment via la sélection d'espèces avec un cycle de vie adapté. En effet le ratio C/P a tendance à augmenter avec le stade de développement.

En cas de faible disponibilité du P dans les sols, la moindre contribution du P libéré par les résidus de cultures intermédiaires à la disponibilité de P renforce le rôle d'autres processus tels que la mobilisation du P ou les rétroactions microbiennes positives. Les stratégies et les traits impliqués dans la mobilisation du P et favorisant les associations microbiennes devraient par conséquent être d'un intérêt particulier dans ces contextes, tels que ceux identifiés dans la famille des Fabacées. Le spectre de stratégies mises en évidence au sein de la famille des Fabacées dans nos résultats propose d'aller plus loin en sélectionnant au sein de cette famille des espèces de Fabacées avec les stratégies souhaitées. Des espèces comme la féverole peuvent par exemple mobiliser fortement le P peu disponible tandis que la vesce pourrait offrir de mobiliser du P tout en explorant également un large volume de sol pour y acquérir le P disponible.

Nos résultats peuvent donc offrir des indications pour la sélection de stratégies et de traits végétaux afin d'améliorer la disponibilité du P dans les agrosystèmes en fonction de la disponibilité du P du sol. La recherche d'une plus grande disponibilité de P dans les agrosystèmes grâce à des compositions de cultures intermédiaires adaptées peut cependant se faire au détriment d'autres services de cultures intermédiaires, comme leur capacité d'acquérir l'eau en profondeur. Ces compromis doivent également être pris en compte lors du choix de leur composition et réfléchis dans le cadre des services souhaités par l'agriculteur. Enfin, pour assurer l'adoption de couverts adaptés par les agriculteurs, il est nécessaire d'aller plus loin et de modéliser les bénéfices attendus, notamment pour la disponibilité du P, et d'incorporer ces modèles dans des systèmes d'aide à la décision.

Les défis de l'intégration du rôle des traits des cultures intermédiaires dans la disponibilité du P dans des modèles et systèmes d'aide à la décision

Une implication majeure de l'identification des traits et des stratégies impliqués dans l'acquisition de P au sein des cultures intermédiaires ainsi que l'étude de la dynamique de libération de P et sa médiation par la disponibilité de P du sol est l'intégration des connaissances produites par ces approches dans des modèles. Modéliser l'effet ces processus pourrait en effet permettre d'évaluer et de quantifier les bénéfices fournis par les cultures intermédiaires pour la disponibilité du P et son acquisition par les cultures suivantes. L'approche développée dans le projet, sur la base des travaux par Damon et al, nous a permis de renforcer les observations indiquant une forte médiation de la contribution de la libération du P contenu dans les résidus de cultures intermédiaires à la disponibilité du P dans les sols par la disponibilité initiale en P des sols.

L'approche de modélisation développée a également eu l'avantage supplémentaire de mettre en évidence les manques de connaissances à combler dans l'évaluation des effets des culture intermédiaires sur la disponibilité du P. La spéciation du P dans les résidus est notamment une cause majeure d'incertitude avec des différences prononcées rapportées dans la littérature entre les espèces mais aussi entre stades de développement. Les impacts complexes des micro-organismes sur la dynamique de la libération du P restent également très incertains. Plusieurs processus mal quantifiés pourraient par ailleurs jouer un rôle majeur dans la détermination des exigences microbiennes et donc du devenir du P libéré par les résidus. Le rapport C/P de la biomasse microbienne est par exemple central pour la détermination des exigences microbiennes en P mais souvent très instable, conditionné en retour par la disponibilité en P du sol. Les changements dans la composition des communautés microbiennes et leur activité induits par les cultures intermédiaires pourraient aussi avoir des effets rétroactifs sur la dynamique de libération de P à partir de leurs résidus, qui restent également des sources plausibles d'incertitudes peu considérées dans les approches de modélisation. Une élucidation ciblée de ces questions techniques et scientifiques dans une gamme de contextes variés pourrait mener à des estimations plus précises des effets des cultures intermédiaires sur la disponibilité de P via la libération de P à partir des résidus.

Produire des modèles capables de prédire avec précision les effets des cultures intermédiaires nécessite cependant d'intégrer également les autres processus par lesquels les cultures intermédiaires influencent le cycle du P dans les agrosystèmes. En effet les impacts incertains des rétroactions microbiennes et les effets durables de la mobilisation du phosphore par les cultures intermédiaires, pendant leur croissance sur la disponibilité du P, restent largement à évaluer. Gagner une meilleure compréhension de ces processus pourrait en retour offrir de produire des modèles plus précis. Une telle compréhension, intégrée dans des approches de modélisation elles-mêmes associées dans des cadres prenant en compte l'ensemble des autres services produits par les cultures intermédiaires et les compromis entre ces services, offrirait une base solide pour la construction de systèmes d'aide à la décision permettant une gestion optimale des cultures intermédiaires pour l'intensification agroécologique.

Table of content

RESUME ETENDU	11
LIST OF FIGURES	31
LIST OF TABLES	33
1. INTRODUCTION	35
1.1. Potential and challenges of agroecological intensification	35
1.1.1. Sustainable intensification.....	35
1.1.2. Influence of agroecological practices on ecosystems services	36
1.2. Phosphorus, a key limiting nutrient.....	37
1.2.1. Phosphorus availability in soil	38
1.2.2. Phosphorus use efficiency in agroecosystems	40
1.3. Role of plants in phosphorus cycling in agroecosystems	41
1.3.1. Phosphorus uptake by plants.....	42
1.3.2. Role of plant – soil microorganisms interactions for phosphorus acquisition.	43
1.3.3. Phosphorus release from residue	45
1.4. Trait based approach of phosphorus cycling in agroecosystems	47
1.4.1. Functional approach	47
1.4.2. Effects of plant traits on phosphorus cycling in agroecosystems	50
1.5. Increasing phosphorus availability and cycling via trait-based agroecological practices: Potential and challenges of cover crops	54
1.5.1. Roles and benefits of cover crops in phosphorus cycling	54
1.5.2. Challenges in designing cover crops for increased phosphorus availability in agroecosystems.....	56
2. PhD OBJECTIVES	59
3. PhD METHODS.....	63
3.1. Characterisation of plant traits involved in plant soil microbes interactions for phosphorus acquisition, their relationships and aggregation in strategies.	63
3.1.1. Identifying tradeoffs and co-variation between phosphorus acquisition traits.	64
3.1.2. Examining plant soil microorganisms interactions for phosphorus-acquisition	66

3.2. Examining the role of residue traits on the fate of residue phosphorus in the plant-soil-microbe system	67
3.3. Modelling cover crop residue phosphorus release dynamics impact on phosphorus availability and feedback effects of soil phosphorus availability.	69
4. TRADEOFFS AMONG PHOSPHORUS-ACQUISITION ROOT TRAITS OF CROP SPECIES FOR AGROECOLOGICAL INTENSIFICATION	91
4.1. Introduction.....	92
4.2. Material and methods	94
4.2.1. Greenhouse experiment	94
4.2.2. Plant traits measurement	94
4.2.3. Morphological traits.....	95
4.2.4. Nutrient uptake.....	96
4.2.5. Rhizosheath properties	96
4.2.6. Data analysis	96
4.3. Results	98
4.3.1. Variation in P-acquisition traits and rhizosphere processes.....	98
4.3.2. Comparison of P-acquisition traits among different clusters	100
4.3.3. Modelling trait-combination effects on P acquisition	102
4.3.4. Differences among trait variation and tradeoffs per soil type	104
4.4. Discussion	106
4.4.1. Tradeoffs among functional traits to understand P acquisition of crop species.....	106
4.4.2. Phosphorus-acquisition strategies to design multispecies crops	107
4.4.3. Mediation of tradeoffs and P-acquisition strategies according to P forms in soil	109
4.5. Conclusions.....	111
5. INTERACTIONS BETWEEN BELOWGROUND TRAITS AND RHIZOSHEATH FUNGAL AND BACTERIAL COMMUNITIES FOR PHOSPHORUS ACQUISITION	121
5.1. Introduction.....	123
5.2. Material and methods	126
5.2.1. Experimental design	126
5.2.2. Plant measurements	128
5.2.3. Soil analyses.....	129
5.2.4. Phosphorus availability	129

5.2.5.	PLFA and NLFA determination	130
5.2.6.	Statistical analyses	130
5.3.	Results	132
5.3.1.	Differences among belowground traits, microbial communities and P acquisition indicators between Brassicaceae and Fabaceae	132
5.3.2.	Interaction between PLFA/NLFA, P acquisition traits and P acquisition	136
5.3.3.	Modelling P-acquisition via interaction between PLFA and NLFA and root traits	140
5.4.	Discussion	142
5.4.1.	Interaction between root morphology and rhizosheath microbial communities for P acquisition	142
5.4.2.	Carboxylate exudation role in plant-microorganism interactions for P-acquisition	143
5.4.3.	Soil type mediate plant-soil microorganism interactions involved in plant P-acquisition	145
5.5.	Conclusion.....	147
6.	ROLE OF COVER CROP RESIDUE TRAITS IN PHOSPHORUS RELEASE DYNAMICS FROM COVER CROP RESIDUES.....	155
6.1.	Introduction.....	157
6.2.	Material and methods	161
6.2.1.	Soil collection and preparation	161
6.2.2.	Preparation of crop residues	161
6.2.3.	Experimental design	164
6.2.4.	Statistical analysis.....	167
6.3.	Results	169
6.3.1.	Dry matter and phosphorus uptake by ryegrass	169
6.3.2.	Nutrition indexes in ryegrass.....	172
6.3.3.	Specific activity in ryegrass	172
6.3.4.	Phosphorus dynamics in amended soils.....	175
6.3.5.	Relation between residue traits and phosphorus uptake	178
6.4.	Discussion	180
6.4.1.	Influence of cover crop residues on ryegrass biomass, P uptake and soil P availability	180
6.4.2.	Residue traits influence on phosphorus availability and uptake	181

6.4.3.	Implication of cover crop residues effect on P availability	183
6.5.	Conclusion.....	184
7.	MODELLING APPROACH TO FEEDBACK EFFECTS OF SOIL PHOSPHORUS AVAILABILITY ON COVER CROPPING INFLUENCE ON PHOSPHORUS CYCLING IN AGROSYSTEMS	193
7.1.	Introduction.....	194
7.2.	Material and methods	197
7.2.1.	Modelling approach.....	197
7.2.2.	Data sources and scenarios.....	199
7.3.	Results	200
7.3.1.	Effect of soil P availability on P release from cover crop residues	200
7.4.	Discussion	201
7.4.1.	Contrasted feedback effects of soil P availability on P release dynamics from cover crop residues.....	201
7.4.2.	Benefits and limits of the modelling approach.....	202
7.5.	Conclusions.....	204
8.	DISCUSSION	211
8.1.	Tradeoffs and functional strategies for phosphorus acquisition in cover crops	211
8.2.	Feedback effects of cover crop traits on P availability	213
8.2.1.	Effects of cover crop residues traits on plant-soil-microbe mechanisms involved in P availability.....	214
8.2.2.	Implications of plant-soil-microbe interactions for phosphorus acquisition in subsequent crops	214
8.2.3.	Perspectives for the study of cover crop feedback effects	215
8.3.	Study perspectives on the effects of cover crop functional diversity on P availability in agroecosystems.....	216
8.4.	Trait based approach to cover crop designs for improved phosphorus availability in agroecosystems.....	221
8.4.1.	Functionally optimal cover crop design for improved phosphorus availability in agroecosystems: Insight into species and strategies selection ..	221
8.4.2.	Designing for improved phosphorus benefits of cover crops: Implications of trade-offs and association between ecosystem services produced by cover crops	225
8.5.	From theory to practice: Integrating the role of cover crop traits in P availability in models and decision support systems for adapted cover crop and fertilizer management.....	227

9. APPENDICES	243
9.1. APPENDIX A – Tradeoffs among phosphorus-acquisition root traits of crop species for agroecological intensification	244
9.2. APPENDIX B – Supplementary information second chapter	258
9.3. APPENDIX C – List of publications and communications.....	263

LIST OF FIGURES

Figure 1 : Phosphorus pools in soil.....	38
Figure 2 : Impact of soil pH on phosphorus availability (Penn and Camberato 2019).	39
Figure 3 :Technologies to increase phosphorus use in agroecosystems (Menezes-Blackburn et al. 2018).....	41
Figure 4 : Colonization of flax roots by arbuscular mycorrhizal fungi (MS Turmel) ...	44
Figure 5 : Diagram of phosphorus release from residue. Adapted from Prescott (2005).	46
Figure 6 : Example of plant functions and functional traits (Garnier and Navas 2012).	48
Figure 7 :Example of trade-offs between leaf phosphorus concentration and leaf nitrogen concentration (Wright et al. 2004).....	50
Figure 8 : Role of plant traits in phosphorus acquisition. Adapted from Li et al. (2014).	51
Figure 9 : Pathway of cover cropping effects on phosphorus availability for subsequent crops. Adapted from (Hallama et al. 2019).....	55
Figure 10 : PhD objectives.....	61
Figure 11 : Greenhouse experiment setting.....	64
Figure 12 : Model of liquid scintillation analyser used to measure specific activity in the soils and plant samples	68
Figure 13 :Schematic representation of the conceptual model developed by Damon et al. (2014) and adapted in the PhD project.	70
Figure 14 : Principal component analysis (PCA) of functional traits and rhizosphere processes involved in phosphorus acquisition in Retisol (a) and Calcaric Cambisol (b)	98
Figure 15 : Partial least square- path model predicting phosphorus uptake	103
Figure 16 : Correlations between rhizosheath fungal and bacterial NLFA concentrations, belowground traits and plant phosphorus (P) content in the Retisol.	138
Figure 17 : Correlations between rhizosheath fungal and bacterial NLFA concentrations, belowground traits and plant P-content in the Calcaric Cambisol..	139
Figure 18 : Partial least square-path models predicting phosphorus (P) acquisition.	141
Figure 19 : Ryegrass biomass and phosphorus uptake (\pm standard error) after amendment with cover crop residues.	170
Figure 20: Phosphorus acquired by ryegrass (\pm standard error) per harvest after amendment with cover crop residues.	171
Figure 21 : Relationship between phosphorus uptake in ryegrass and cover crop residues C:P ratio.	179
Figure 22 : Model structure.....	197
Figure 23 :Predicted P release from cover crop residue grown in soils with different P availability	200

Figure 24 : Summarized framework for addressing the effect of functional diversity on ecosystem functioning. Adapted from Diaz et al. (2007)..... 220

Figure 25 : Radar chart summarizing the properties of the cover crop families and their effects on soil (Hallama et al. 2019) 222

Figure 26: Predicted P release from Fabaceae (a) and Brassicaceae (b) cover crop based on model by Damon et al. (2014)..... 224

LIST OF TABLES

Table 1 : Chemical and physical soil characteristics.....	94
Table 2 : List of abbreviations and associated units	95
Table3 : Mean values \pm standard error (n= 4 to 11) and analyses of variance of phosphorus- acquisition traits and rhizosphere processes per cluster	101
Table 4 : Selected models fitted to phosphorus uptake	102
Table 5: Average trait value \pm standard error and analyses of variance per species and per soil.....	105
Table 6 : Chemical and physical soil characteristics.....	127
Table 7 : Change in rhizosheath soil P concentration and shoot P concentration \pm standard error and analyses of variance per family and species	133
Table 8 : Mean rhizosheath PLFA and NLFA concentration \pm standard error and analyses of variance per family and species	134
Table 9 : Mean belowground traits \pm standard error and analyses of variance per family and species	135
Table 10 : Chemical and physical soil characteristics.....	162
Table 11 : Cover crop residues traits and inputs for each residue treatment (all inputs designed to add 15 mg P kg ⁻¹ soil)	163
Table 12 : Nitrogen nutrition index in ryegrass (\pm standard error) per harvest after amendment with cover crop residues.	173
Table 13 : Phosphorus nutrition index in ryegrass (\pm standard error) per harvest after amendment with cover crop residues.	174
Table 14 : Phosphorus concentration in resin extracts (\pm standard error) after amendment with cover crop residues.	176
Table 15 : Microbial phosphorus (\pm standard error) after amendment with cover crop residues.....	177

INTRODUCTION

1.INTRODUCTION

1.1. Potential and challenges of agroecological intensification

Modern agriculture is faced with numerous challenges such as population and income growth, depletion of mineral resources and climate change. Many thus stress the importance of rethinking and adapting agricultural systems toward more sustainable systems (Lampkin et al. 2015; Cristofari et al. 2018; Mockshell et al. 2018). “Agroecology” first defined in the 1920s as an application of the field of ecology to agricultural systems (Wezel and Soldat 2009) proposes addressing these major challenges via practices managing and enhancing ecosystems services in order to ensure increased food production while reducing externalities and environmental impacts (Cristofari et al. 2018). Employing ecological concepts and principles to the study of sustainable agricultural systems design and management, agroecology aims at proposing an alternative to conventional farming (Mockshell et al. 2018). Agroecological concepts such as sustainable intensification have been gaining increasing interest, with several associated practices being set as sustainable development goals by the United Nation (GA 2015). Investigating the effects of agroecological practices could lead to a better understanding of the underlying processes involved and ecosystem functioning and draw conclusions for agricultural systems designs. In turn approaches such as sustainable intensification based on these agroecological principles and practices could lead to reduced external inputs, increased productivity and environmental benefits (Lampkin et al. 2015; Mockshell et al. 2018).

1.1.1. Sustainable intensification

The concept of “sustainable intensification” was first defined and promoted in 1997 (Pretty 1997), as a vision of agriculture “relying on integrated use of a wide range of technologies to manage pest, soil and water” (Lampkin et al. 2015). With time numerous interpretation of that concept appeared, largely overlapping with sustainable intensification but presenting major nuances such as the concepts of “ecological

intensification of agriculture” or “agroecological intensification” (Wezel et al. 2015). These concepts all advocate practices and systems making a better use of resources notably via better management of input and outputs but also enhanced ecosystems processes and services reducing the adverse effect of food production while maintaining or increasing productivity.

Several farming systems such as organic agriculture or conservation agriculture fit into the framework and objectives set by sustainable intensification (Knowler and Bradshaw 2007; Cristofari et al. 2018; Mockshell et al. 2018). Via diverse agroecological practices these systems offer to influence ecosystem processes such as nutrient cycling or microbial dynamics in order to ensure enhanced resource use efficiency and enhanced ecosystem resources and services (Mockshell et al. 2018). Both systems have been gaining increased interest and adoption in Europe and worldwide (Knowler and Bradshaw 2007). Multiple factors however limit their expansion, notably their knowledge intensive aspect requiring a fine understanding of ecosystem functioning. Gaining a better understanding of how these systems and practices influence ecosystem functioning and services could help addressing some of the major challenges facing modern agriculture and provide precious insights into agricultural system functioning also potentially transposable to natural ecosystems

1.1.2. Influence of agroecological practices on ecosystems services

A wide range of practices can be considered agroecological such as mulching, intercropping, crop rotations, integrated soil, nutrient and water management, integrated pest management, biological control strategies and the judicious and efficient use of pesticides, organic inputs and of fertilizers (Mockshell et al. 2018). These practices offer to produce a vast array of ecosystem services, defined as benefits to humans of ecosystem processes (Faucon et al. 2017), via influencing ecosystems processes and in turn soil physical, biological and chemical properties. Soil physical properties can be influenced directly or indirectly by practices such as reduced tilling, cover cropping or intercropping via effect of roots and vegetation on processes involved in erosion like water infiltration or via reliance on enhanced soil biota increasing soil aggregation and improving soil structure (Liu et al. 2005; Carof et al. 2007; Sharaiha and Ziadat 2008). Soil biological properties are often the main focus

of agroecological practices due to their contribution to multiple ecosystems processes such as nutrient cycling, carbon storage or pathogen suppression (Brown and Morra 1997; Chavarria et al. 2018; Chahal et al. 2020). Several practices such as crop rotation, cover cropping or field margins can be used to suppress soil pathogens or attract beneficial biota (Médiène et al. 2011). Cover cropping and crop rotation can lead to lasting shifts in microbial communities potentially contributing to beneficial plant soil-microorganisms improving plant health and nutrient cycling (Zhu and Morel 2019). Several agroecological practices can also exploit plant allelopathic properties to suppress weeds (Jabran et al. 2015).

The impacts of agroecological practices on soil chemical properties and more generally soil fertility are the most documented along with their role in erosion. Incorporation of Fabaceae in the cropping system for their capacity to fix atmospheric nitrogen is amongst the most adopted practices be it in crop rotations, intercropping or cover cropping. Several agroecological practices also contribute to carbon storage, increasing the stocks of organic carbon in soils (Chahal et al. 2020). Increased use of organic inputs, cover crops and crop rotations can strongly influence the processes involved in nutrient cycling notably for major limiting elements such as nitrogen, phosphorus or potassium (Aronsson et al. 2016; Abdalla et al. 2019) leading to improved nutrient availability. Agroecological practices introducing diverse plant species in the cropping system can influence plant soil interactions modifying nutrient speciation and thus availability, as observed for P and N under cover cropping or intercropping (Kuo and Jellum 2002; Betencourt et al. 2012; Lambers et al. 2013). Effects on fertility may also be indirect via influence on soil microbial communities having feedback effects on nutrient uptake capacity in subsequent crops as observed for P after cover cropping (Njeru et al. 2014). Through these multiple ecosystems processes agroecological practices can offer the opportunity to produce numerous ecosystems services and in particular may offer the opportunity to improve the availability and cycling efficiency of key limiting elements such as N or P.

1.2. Phosphorus, a key limiting nutrient

Sustainable phosphorus use in agroecosystems is the subject of many concerns due to progressive depletion of the non renewable resources in phosphate rocks and geopolitical tensions in the regions where they are concentrated (Cordell et al. 2010; Cordell and White 2014). Understanding the processes involved in phosphorus

availability in agroecosystems and the challenges to efficient phosphorus use could help addressing these concerns via adapted designs and practices. In the context of agroecological intensification, plant could help bridge the gaps in phosphorus cycling and designed optimal practices.

1.2.1. Phosphorus availability in soil

Phosphorus is a limiting element in most of the soil worldwide. Even in primarily nitrogen limited soils often occurring in Western Europe, phosphorus is often the second most limiting element for plant growth (Vance 2001). Phosphorus is essential for life, playing a central role in plant energy production via molecules of Adenosine triphosphate (ATP), but also being a key building element for proteins or even DNA. Phosphorus in soil is present in a multitude of different inorganic and organic forms (Figure 1).

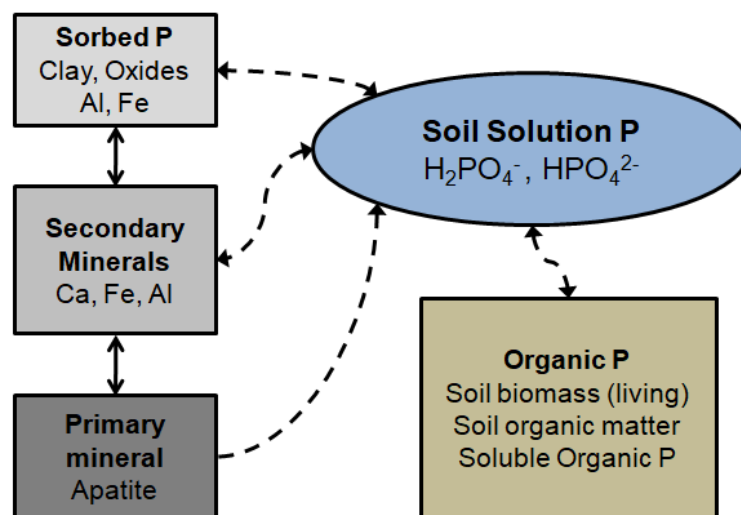


Figure 1 : Phosphorus pools in soil

Only inorganic phosphorus in solution is directly available for plant uptake. Most of soil phosphorus is however present as insoluble precipitated or fixed forms, with only a small fraction of this pool of insoluble phosphorus being labile and in equilibrium with the soil solution (Stutter et al. 2012; Pierzynski et al. 2015). Phosphorus is strongly and quickly adsorbed to soil constituents, especially in soil with high iron and aluminium oxides, clay or organic matter content. Phosphorus may also precipitate with cations (Al, Fe, Ca), forming insoluble, poorly plant available phosphorus forms. Phosphorus is thus very immobile in soil due to reactions with multiple soils constituents.

Phosphorus sorption/desorption dynamics and precipitation with soil constituents are influenced by multiple soil properties, especially soil pH (Figure 2).

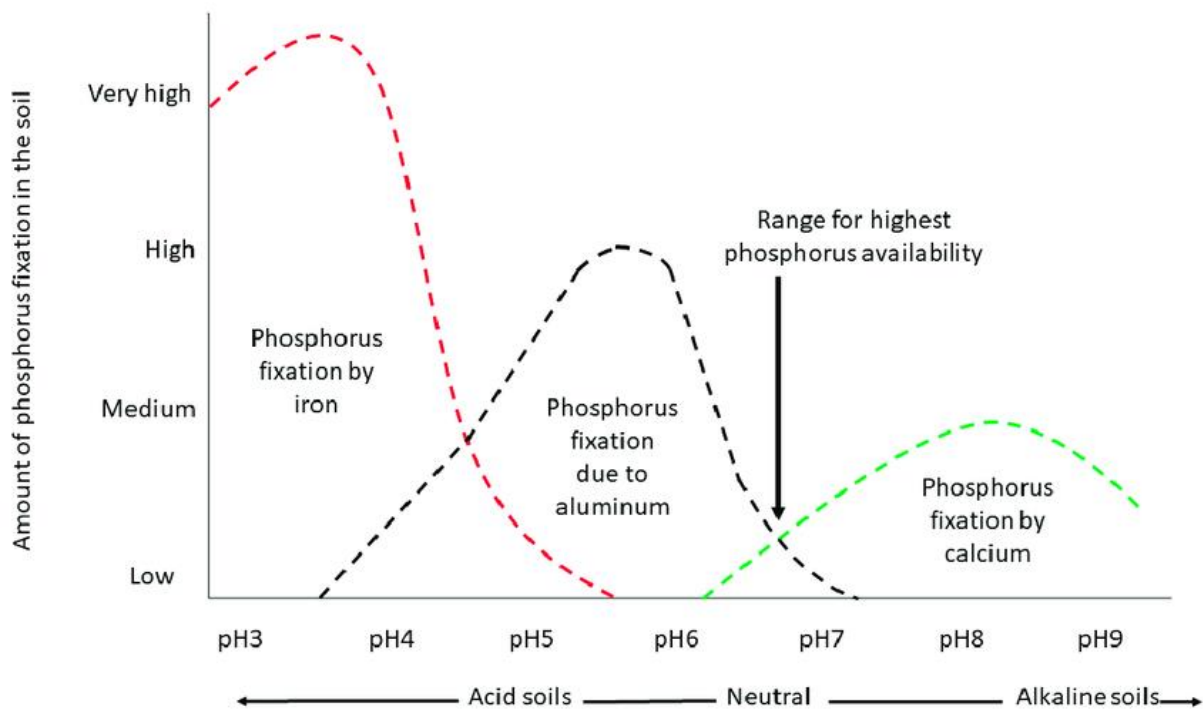


Figure 2 : Impact of soil pH on phosphorus availability (Penn and Camberato 2019).

Phosphorus tends to be the most available in soils with neutral pH. In acid soils increased concentration of aluminium then iron react phosphorus decreasing soluble phosphorus. Soils rich in calcium are generally basic and precipitation with calcium also decrease phosphorus availability in these soils. Precipitates of calcium and phosphorus are less recalcitrant than precipitates of phosphorus and aluminium or precipitates of phosphorus and aluminium, although their solubility depends of the proportion of calcium in the precipitates (Mengel et al. 2001). A significant amount of soil phosphorus can be present in organic forms, 30 to 65 % of total phosphorus (Condron et al. 1990). Organic phosphorus as for inorganic phosphorus can be strongly fixed to soil constituents. Organic phosphorus is not directly plant available requiring prior mineralization by enzymes released by plants or microorganisms. Soil pH also strongly mediates organic phosphorus mineralization. Due to poor phosphorus mobility in soil phosphorus pools tend to be very uneven not only chemically but also spatially. Managing phosphorus chemical, temporal and spatial availability in soil is a major challenge of modern agriculture.

1.2.2. Phosphorus use efficiency in agroecosystems

Due phosphorus limited availability in most contexts, important phosphorus inputs are required to ensure productivity in cultivated systems (Menezes-Blackburn et al. 2018). Most historical agriculture practices relied on saturating soil phosphorus fixing capacity in order to maintain optimum level of available phosphorus concentration (Fox and Kamprath 1970). Fertilization in these systems originates from non renewable, geographically uneven resources of rock phosphate, the source of many concerns for sustainable and equitable use of these resources (Richardson et al. 2011). Over-fertilization to compensate for soil phosphorus fixation led overtime to build up of a stock of poorly available “legacy” phosphorus pools in soils (Liu et al. 2015; Menezes-Blackburn et al. 2018). In turn, this resulted in poor use efficiency, defined as the ratio of phosphorus outputs over phosphorus inputs (Weaver and Wong 2011). Phosphorus balance efficiencies of around 50 % have been reported for cropping systems in southern Australia for example (McLaughlin et al. 1992; Weaver and Wong 2011). In Western Europe less than 0.3 t ha^{-1} of phosphorus have been estimated to be exported on average between 1965 and 2007 as compared to average inputs of 1.1 t ha^{-1} during the same period (Sattari et al. 2012). Ensuring better phosphorus use efficiency in cropping systems is a major challenge to modern agriculture (Menezes-Blackburn et al. 2018). Improving phosphorus efficiency requires reducing fertilizer loads, increasing phosphorus recycling and use efficiency but also exploiting the stock of legacy phosphorus in soils (Simpson et al. 2011). As summarized in Menezes-Blackburn et al. (2018), they are multiple strategies and technologies that could ensure better soil phosphorus use efficiency, centred around three main axes: Biofertilizers, Management practices and phosphorus efficient plants (Figure 3).

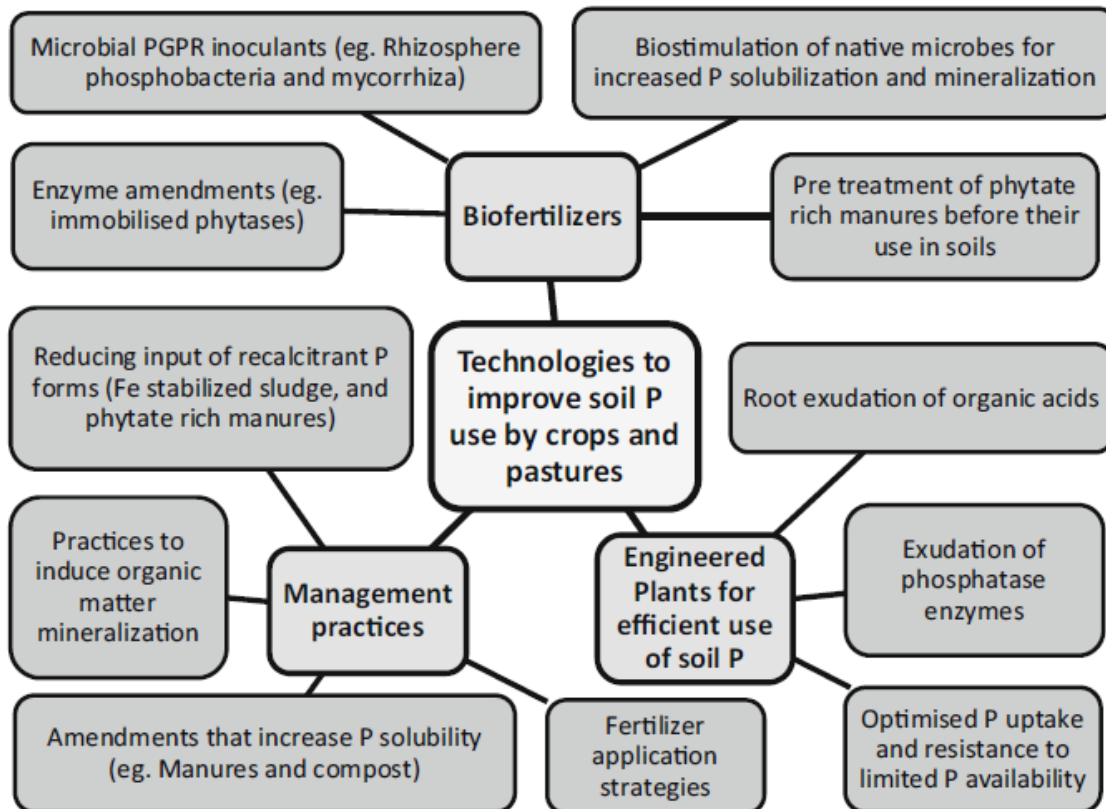


Figure 3 :Technologies to increase phosphorus use in agroecosystems (Menezes-Blackburn et al. 2018).

Improved phosphorus use efficiency in cropping systems can be achieved via: i) Adapted management practices enhancing organic phosphorus mineralization and adapting fertilizer application. ii) Biofertilizers improving microbial activity and benefiting plant growth. Use of specific bacteria mixtures, along with their grazers could significantly increase soil availability and uptake (Irshad et al. 2012). iii) Improved phosphorus use efficiency in plants. Plants can also be selected for their capacity to mobilize recalcitrant phosphorus through specific root characteristics and strategies. Overall achieving better phosphorus use efficiency in cropping systems requires gaining a better understanding of phosphorus biogeochemical cycling in agroecosystems (Bünemann et al. 2010) starting with the effects of plants, practices and biofertilizers (Menezes-Blackburn et al. 2018).

1.3. Role of plants in phosphorus cycling in agroecosystems

Plants are the central drivers of many mechanisms involved in the biogeochemical cycling of phosphorus in agroecosystems. Via efficient use of the diverse pools of phosphorus in soil, plants can offer to increase phosphorus cycling efficiency in

agroecosystems (Lynch 2015; Menezes-Blackburn et al. 2018). Plants influence phosphorus cycling via three main mechanisms: i) Phosphorus acquisition by plants via phosphorus foraging and mobilization; ii) Interactions with soil microorganisms mediating phosphorus availability in the plant soil microbes system; iii) Release of phosphorus contained in plants residues after destruction or harvest.

1.3.1. Phosphorus uptake by plants

Only inorganic phosphorus in solution is directly plant available (Schachtman et al. 1998). However due to low phosphorus availability in many soil contexts, plants have developed several strategies to acquire phosphorus from the diverse soil phosphorus pools which remain to be fully explored (Lynch 2011). These strategies can be grouped in two main phosphorus acquisition strategies: phosphorus foraging strategies and phosphorus mining strategies. Phosphorus foraging strategies rely on maximised soil scavenging for phosphorus acquisition, i.e. exploring a large soil volume to capture available phosphorus. This can be achieved through different plant strategies. In order to forage a larger soil volume plants can invest more in their root biomass (Hermans et al. 2006). When faced with low phosphorus availability plants with higher root/shoot ratio tend to perform better (Nielsen et al. 2001) and acquire more phosphorus (Steingrobe et al. 2001). Enhanced soil foraging capacity can also be achieved through reducing the cost of soil exploration. Fine, short lived roots can explore larger root volume at reduced costs allowing plants to maintain high productivity at lower level of available phosphorus (Lynch 2007). Plant phosphorus foraging capacity can also be increased via strategies increasing area of contact per root length. Plants in phosphorus limiting conditions tend to develop more root abundance and longer root hairs (Lynch 2007). As phosphorus is very immobile in soil, root hairs not only increase root area but also allow access to pools of phosphorus that would not diffuse to roots (Fort 2013). In barley, a linear relationship has been identified between root hair length and phosphorus uptake (Gahoonia and Nielsen 2004). A final plant foraging strategy can be through preferential exploration of more phosphorus rich patches. Surface horizons tend to be more concentrated in phosphorus in agroecosystems which can be exploited by plants increasing their root biomass in these horizons, as observed in peas (Liao et al. 2001). Reliance on a specific phosphorus foraging strategy is in part genetic however environmental factors such as soil type and properties also play a still unclear role in defining plant P foraging strategy (Müller and Schmidt 2004). Root hair

length was for example observed to be highly responsive to soil strength and porosity in Barley (Haling et al. 2014).

Phosphorus mining strategies rely on mobilizing chemically unavailable phosphorus, thus allowing plant to access pools of fixed inorganic phosphorus or organic phosphorus. In order to mobilize this phosphorus plants can impact several processes involved in phosphorus availability. Phosphorus sorption/desorption equilibrium can be influenced by plant exudations competing for phosphorus sites in order to decrease sorption to mineral surfaces (Lambers et al. 2006; Richardson et al. 2011; Wang and Lambers 2020). Phosphorus precipitates can be dissolved via proton release by plants, which is especially relevant for phosphorus and calcium precipitates. The mineralization of organic phosphorus can also be enhanced by plant leading to the release of plant available inorganic phosphorus. Enzyme exudation by roots can significantly enhance organic phosphorus mineralization, strongly contributing to total plant uptake (Kroehler and Linkins 1988). Finally, a last phosphorus mining strategy consists in the formation of cluster roots. Cluster roots combine phosphorus foraging strategies and phosphorus mining strategies. As such they tend to express high fine roots, surface area, root hair length and abundance, as well as high root exudations to access pools of hardly available soil phosphorus. As for phosphorus foraging strategies, P mobilization by plants can be affected by multiple environmental factors such as phosphorus availability, pH or soil type (Pearse et al. 2006; Li et al. 2007) the effects of which needs to be fully investigated to gain a better understanding of plant phosphorus acquisition.

1.3.2. Role of plant – soil microorganisms interactions for phosphorus acquisition

Soil microorganisms are key drivers of phosphorus cycling in agroecosystems, strongly contributing to several processes affecting phosphorus availability such as organic matter mineralization or phosphorus mobilization (Richardson et al. 2011). Soil microbial communities in the rhizosphere of plants are not directly under plant control, with soil properties (pH, clay content, organic matter, ...) and native soil microbial communities being important determining factors (Hunter et al. 2014). However plants may have considerable impact on soil microbial community and functions, especially in their rhizosphere (Hunter et al. 2014). Association or colonization of roots by

Arbuscular Mycorrhizal Fungi (AMF) is the best known plant soil-microorganisms interaction for phosphorus acquisition (Figure 4). As much as 80 % of plant species are involved in association with AMF (Schüßler 2002). Through this association, fungi and plants interact with plants providing sugar to the fungi in exchange for water and nutrients (Willis et al. 2013). Association with AMF allows plant to forage phosphorus from an important soil volume, up to 25 cm away from the roots (Smith and Read 2010). Up to 75 % of annual phosphorus uptake by plants can be through association with AMF (van der Heijden et al. 2008). In addition to foraging for available phosphorus, there is recent evidence that AMF can contribute to mobilizing soil phosphorus, either by increasing organic phosphorus mineralization or via their own interactions with bacterial communities (Sato et al. 2015; Zhang et al. 2016, 2018). AMF contribution to plant phosphorus uptake is however strongly impacted by soil phosphorus availability (Grman and Robinson 2013). Maximum benefits from association with AMF are observed in contexts with low available phosphorus concentrations, and negative impacts on plant growth have been reported in contexts with high available phosphorus concentrations due to the carbon cost of this association for the plant (Johnson et al. 1997).

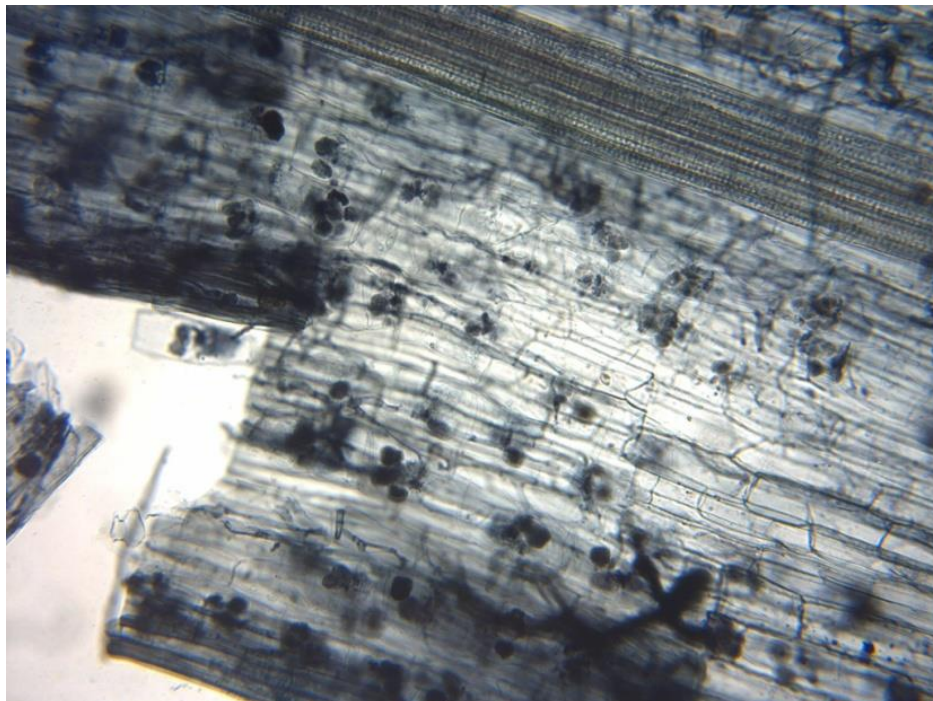


Figure 4 : Colonization of flax roots by arbuscular mycorrhizal fungi (MS Turmel)

Interactions with bacterial communities can also strongly contribute to phosphorus uptake. Many plant exudates including carboxylates are quickly metabolised in soil and

play the role of a carbon source for rhizosphere bacteria possibly influencing bacterial functions and communities (Marschner et al. 2002; Paterson et al. 2007; Adeleke et al. 2017). In turn, rhizosphere bacteria subsequently influence phosphorus availability in the rhizosphere. Between 1 and 50 % of rhizosphere bacteria are phosphorus solubilising bacteria (Kalayu 2019), potentially mobilizing phosphorus from poorly available sources and benefiting plant phosphorus uptake. Several genus of rhizobacteria are known to release phosphatase and phytase, contributing to mineralization of organic phosphorus (Krey et al. 2011; Mukhametzyanova et al. 2012; Miao et al. 2013). Precise microbial components associated with phosphorus solubilisation are however not well known and likely to vary depending on environmental conditions and other factors (Hunter et al. 2014). Rhizosphere microbial communities are influenced by soil type and plant age for example (Marschner 2001; Smalla et al. 2001). In addition, microbial communities can also influence phosphorus uptake by plants through eliciting plant defence pathways (Hammond 2004). Phosphorus deficiency responses in roots are indeed regulated via general stress response pathways as well as phosphorus specific responses (Hammond 2004). Rhizosphere microbial communities via eliciting plant defence pathways may thus increase plant phosphorus uptake (Yao et al. 2011). Overall plant microbial interactions for phosphorus acquisition are highly complex and quickly evolving leading to still not fully understood consequences on phosphorus cycling (Hunter et al. 2014). Exploring these interactions and selecting for beneficial plant characteristics could improve phosphorus uptake capacity (Richardson et al. 2011).

1.3.3. Phosphorus release from residue

Phosphorus release from crop residues is a complex process involving multiple steps and influenced by many factors such as climate, soil type and properties, residue quality and microbial communities (Prescott 2005). As for carbon, the release of phosphorus from residues and its effects on phosphorus availability in soil follows a two stage pattern : a rapid initial release of labile phosphorus then followed by a longer phase of slow phosphorus release from recalcitrant organic phosphorus sources in biomass (Umrit and Friesen 1994; Jalali and Ranjbar 2009; Damon et al. 2014) (Figure 5).

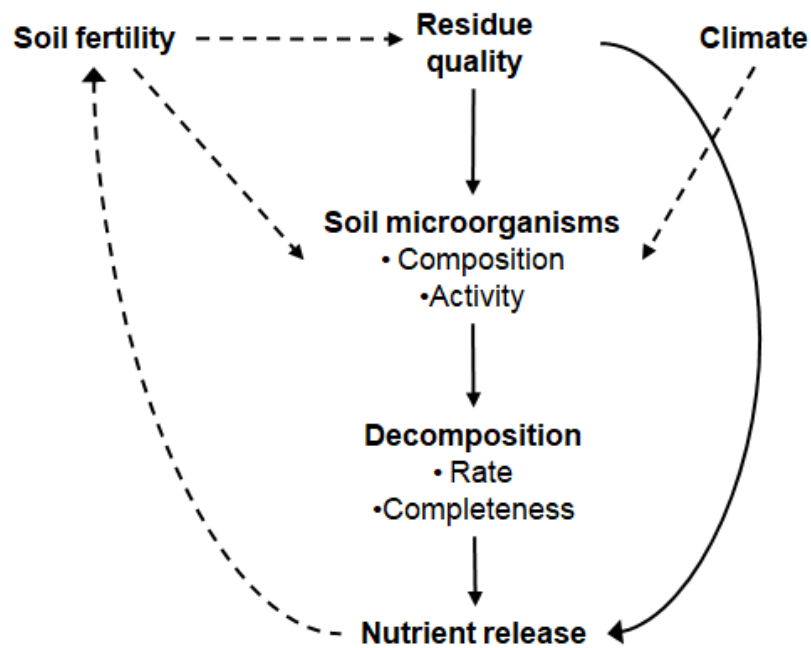


Figure 5 : Diagram of phosphorus release from residue. Adapted from Prescott (2005).

Fast initial release of phosphorus is generally in the form of inorganic phosphorus (orthophosphate) even though, to a lesser extent, some easily decomposed organic phosphorus forms can also be quickly released. Between 35 to 75 % of the total phosphorus in crop can be present as orthophosphate and is easily leached to soil during residue decomposition (Noack et al. 2012). Phosphorus speciation in residue however can change considerably with vegetative stage (Noack et al. 2012; Damon et al. 2014), resulting in different phosphorus release dynamics. Soil microorganisms play a central role in phosphorus release from residue, not only due to their implication in residue mineralization but also by being more competitive than plants for soil phosphorus uptake. Depending on residue quality, phosphorus can either be quickly mineralized by microorganisms or immobilized in microbial biomass. Residue where quality meets microbial demands are quickly mineralized whereas if microbial demands are not met, important microbial immobilization of phosphorus can occur. Phosphorus immobilized in microbial biomass is then released by microbial turnover. Microbial turnover typically is relatively fast, with turnover times ranging from days to months (Spohn and Widdig 2017). However, microbial turnover rates tend to be very variable depending on soil type and climatic factors (Oberson and Joner 2005; Achat et al. 2010). Recalcitrant phosphorus contained in residue is slowly mineralized over time during the second phase of residue phosphorus release. A significant proportion of the phosphorus involved in this process can however be only partly degraded and

converted to recalcitrant organic phosphorus forms (Oliveira et al. 2017). Phosphorus captured by plants and later released could significantly contribute to phosphorus availability in cropping systems. However, our understanding of phosphorus release dynamics remains limited with wide uncertainty on the key parameters involved (Damon et al. 2014).

Although the main mechanisms influencing plants role in phosphorus cycling in agroecosystems have been identified, many unknowns remain on the underlying processes and their relative contribution and agronomical significance across different contexts or species, which can prove to be challenging when attempting to design agroecosystems for improved phosphorus availability (Simpson et al. 2011; Hunter et al. 2014; Menezes-Blackburn et al. 2018). Further investigations are needed to quantify the functions ensured by plants in P cycling and highlight the key plant characteristics involved irrespective of local context or species.

1.4. Trait based approach of phosphorus cycling in agroecosystems

Investigating the role and potential benefits of plants in phosphorus cycling in agroecosystems requires approaches able to highlight and evaluate the influence of plant characteristics on ecosystem processes. Functional ecology offers to examine the response and effects of plant characteristics on ecosystem processes (Lavorel and Garnier 2002). Highlighting the plant characteristics or traits involved in plant phosphorus acquisition, directly or via interactions with soil microorganisms, and later release from plant residues and quantifying their effects is key to deepen our knowledge of phosphorus cycling in agroecosystems and design functionally efficient cropping systems and practices (Richardson et al. 2011; Faucon et al. 2017; Schneider et al. 2019).

1.4.1. Functional approach

1.4.1.1. Functional traits

Use of plant characteristics as indicators of ecosystem processes and assembly pre-dates the definition of functional traits with notions such as life history strategies (Southwood 1988) or functional effects and functional response groups (Woodward and Cramer 1996). Approaches based on plant characteristics or “traits” have proven

to be more efficient in predicting ecosystems processes and structure as compared to relying on species identity or richness (Lavorel and Garnier 2002). Based on this observation, functional ecology offer to examine plant role or functions within ecosystems as products of their traits. Plant traits can be defined as “any morphological, physiological or phonological feature measurable at the individual level, from the cell to the whole organism, without reference to the environment or any level of organisation“ (Violle et al. 2007) (Figure 6).

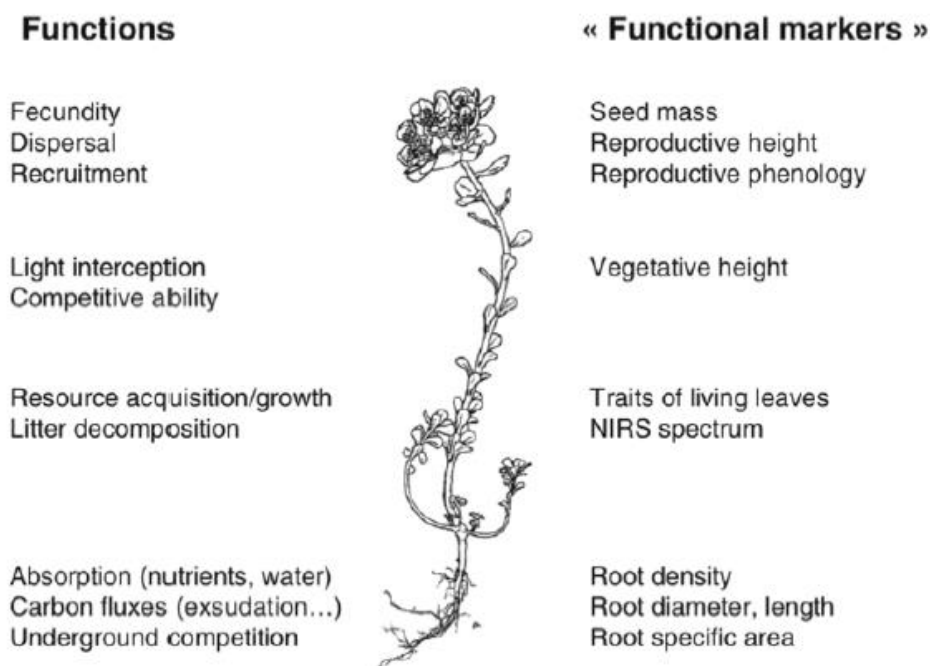


Figure 6 : Example of plant functions and functional traits (Garnier and Navas 2012).

The value of a trait in a specific biotic and abiotic context is called an attribute. Plant attributes are susceptible to change with time (Violle et al. 2007) or in different environmental conditions. Species thus present different attributes along environmental gradients. Plant functional traits constitute a subset of traits that directly or indirectly influence plant fitness (Lavorel et al. 2007; Violle et al. 2007). Traits influencing plant photosynthetic activity, nutrient capture or growth rate in turn directly and indirectly influencing individual performance via vegetative biomass or reproductive output are thus considered functional (Lavorel et al. 2007; Violle et al. 2007; Garnier and Navas 2012). Plants functional traits can be employed to investigate ecosystem functioning at multiple scales from intra-specific variation to landscape wide change in plant community composition (Lavorel et al. 2011; Albert et al. 2011; Gámez-Virués et al. 2015). Functional traits allow the investigation of the effects of plant on

ecosystem processes irrespective of local species composition and thus allow drawing conclusions applicable to multiple systems. Functional traits also reflect the relationships between plants and their environment (Lavorel et al. 2007). Plant responses to environmental conditions but also effects on their environment can thus be predicted based on plant traits. This led to the creation of two distinct but complementary notions: response traits and effect traits.

Response traits are influenced in their attributes by environmental conditions, biotic or abiotic (Lavorel and Garnier 2002; Garnier et al. 2015). Climate (Moles et al. 2014) or inter-specific competition (Pierce et al. 2017) can for example be drivers of response traits attributes in a specific context. They provide useful information on plant adaptation to a local context. Effects traits drive ecosystem processes such as nutrient cycling (Diaz et al. 2004; De Deyn et al. 2008; Hobbie 2015). They are responsible for plant impact on ecosystem function and can be selected for in order to achieve desired ecosystem services such as increased phosphorus cycling efficiency (Richardson et al. 2011; Faucon et al. 2017). Response and effects traits however tend to largely overlap depending on the processes considered (Lavorel and Garnier 2002), and due to coordination and tradeoffs between plant functional traits.

1.4.1.2. Tradeoffs and functional trait-based strategies

Plant functional attributes are the results of plant adaptation to a specific abiotic and biotic context (Reich et al. 2003). However, due to carbon costs associated with trait expression (Lynch and Ho 2005; Ryan et al. 2012), plant functional traits are involved in multiple tradeoffs and associations influencing traits response to environmental conditions (Lavorel and Grigulis 2012). These tradeoffs can be highlighted via co-variation between traits, highlighting multiple negative relationships between functional traits such as between Specific Leaf Area (SLA), leaf nutrient concentrations and Leaf Dry Matter Content (LDMC) as part of the leaf economic spectrum (Wright et al. 2004) (Figure 7). Similar observations have been made at root level for resource acquisition as part of the root economic spectrum, notably between Specific Root Length (SRL), Root Tissue Density (RTD) and Root Dry Matter Density (RDMC) (Roumet et al. 2016). Tradeoffs are the results of environmental or physiological constraints or competitive advantages associated with an expression of trait ensembles (Poisot et al. 2011; Lavorel and Grigulis 2012). Groups of functional traits can be identified based on independency or co-variation between traits, underlining the existence of a spectrum

of functional strategies for resource acquisition in plants (Wright et al. 2004; Lavorel and Grigulis 2012; Roumet et al. 2016).

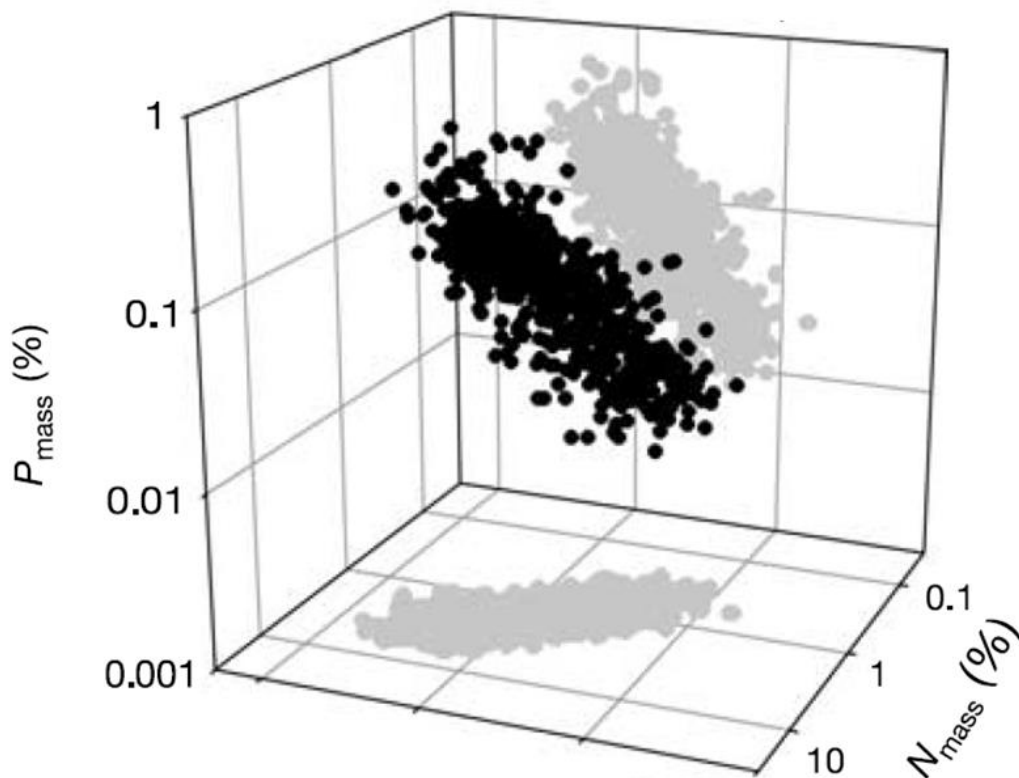


Figure 7 :Example of trade-offs between leaf phosphorus concentration and leaf nitrogen concentration (Wright et al. 2004).

Traits and tradeoffs based approaches for characterising plant functional strategies have shown their utility in understanding ecosystem processes such as phosphorus acquisition by plants and the tradeoffs (Lyu et al. 2016) and strategies involved (Wendling et al. 2016). These approaches however face numerous challenges such as non linearity of the relationships between traits (Kong et al. 2019) or inconsistent tradeoffs expression in different contexts (Sgrò and Hoffmann 2004). Addressing this challenges and gaining a better understanding of the effects of plant traits and tradeoffs on phosphorus cycling thus requires further investigations across different contexts and at varied scales (Lynch 2015; Weemstra et al. 2016).

1.4.2. Effects of plant traits on phosphorus cycling in agroecosystems

Plant functional traits influence on phosphorus cycling has been receiving more and more attention recently, with efforts focused on identifying plant traits and strategies

leading to improve phosphorus use efficiency in agroecosystems (Richardson et al. 2011; Menezes-Blackburn et al. 2018; Hallama et al. 2019). Gaining a better understanding of the effects of plant functional traits and strategies on soil properties is indeed required in order to achieve desired ecosystem services (Faucon et al. 2017). Highlighting traits or strategies of interest could offer agronomical levers for agroecological intensification.

1.4.2.1. Effects of plant functional traits on plant phosphorus acquisition

Multiple plants morphological, architectural and physiological traits have been highlighted to offer proxy to plant phosphorus acquisition strategies (Figure 8).

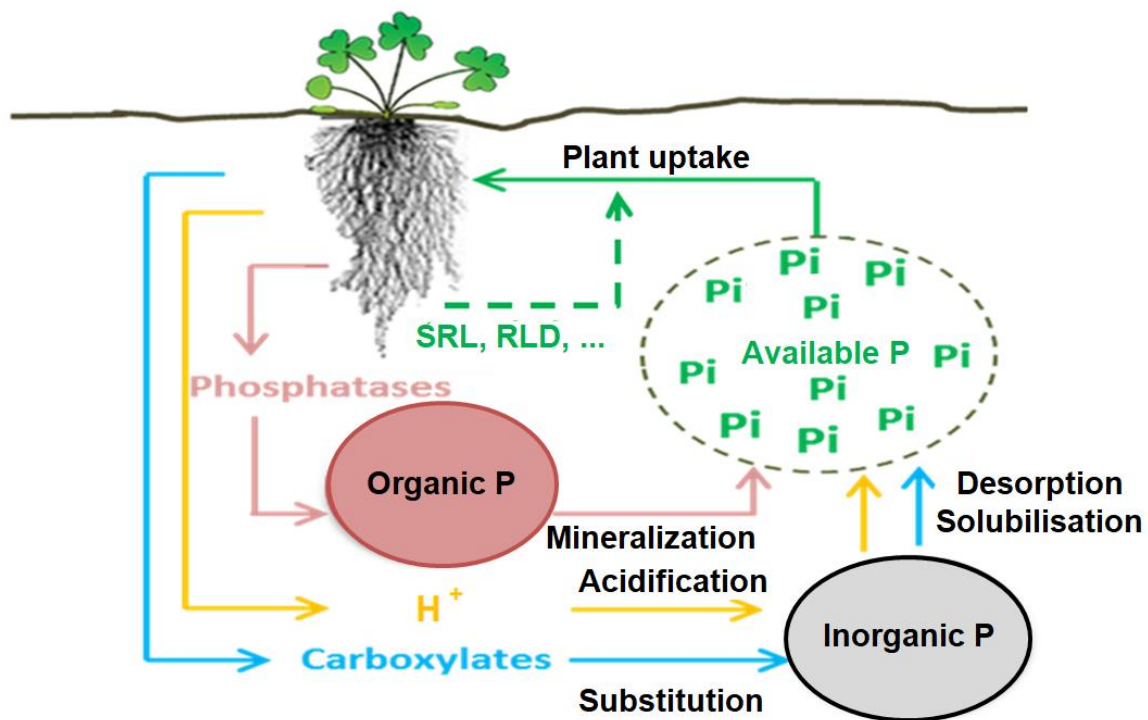


Figure 8 : Role of plant traits in phosphorus acquisition. Adapted from Li et al. (2014).

Plant morphological and architectural traits are the main drivers of plant phosphorus foraging capacity. Plants with high SRL, low root diameter, RDMC and RTD can forage a large soil volume at a relatively lower cost per volume explored (Vance et al. 2003; Schroeder and Janos 2004; Lugli et al. 2020). SRL was for example observed to increase in *Brassica napus* when faced with phosphorus deficiency (Lyu et al. 2016). Preferential foraging of a certain soil volume can be achieved through higher RLD or root branching in this volume (Nanzyo et al. 2002; Postma et al. 2014). Under

phosphorus limited condition *Trifolium subteraneum* was observed to adapt by increasing its RLD (Haling et al. 2018).

Plant capacity to mine unavailable phosphorus is driven by multiple physiological traits, mostly involved plant exudation. Root carboxylate exudation, while being challenging to measure in the fields (Oburger and Jones 2018), offers a proxy for strategies influencing phosphorus sorption/desorption dynamics. Malate and citrate are the two most abundant carboxylates released by plants (Neumann and Römheld 1999; Pearse et al. 2006). Their release rate has been observed to increase in phosphorus efficient plants under phosphorus limiting condition (Hoffland et al. 1989; Yan et al. 2004; Shahbaz et al. 2006). Plants can influence soil pH through another physiological trait, that is root proton emission which aims at dissolving precipitated phosphorus such as calcium phosphate precipitates. Fabaceae are known to strongly influence rhizosphere pH (Maltais-Landry 2015) leading to dissolution of precipitated phosphorus in basic soils (Hinsinger et al. 2011). Plant capacity to access organic phosphorus in soil and contribute to its mineralization can be measured via traits such as phosphomonoesterase release (PME) (Lambers et al. 2006). Species exhibiting higher PME release or activity in the rhizosphere such as *Brassica napus* have been observed to benefit more from organic fertilizer such as sewage sludge (Nobile et al. 2019).

Morphological and physiological traits in turn tend to be part of tradeoffs due to their carbon cost for the plant (Lynch and Ho 2005; Walk et al. 2006; Ma et al. 2018). Plants with high morphological traits denoting high phosphorus foraging capacity thus tend to have lower physiological traits and thus phosphorus mining potential (Lyu et al. 2016). While the effects of individual plant functional traits on phosphorus acquisition in crops (Lyu et al. 2016; Nobile et al. 2019) and natural ecosystems (Fort 2013) have been more and more investigated recently, the effects of tradeoffs and plant functional phosphorus acquisition strategies on phosphorus acquisition and cycling remain underexplored (Wen et al. 2019).

1.4.2.2. Effects of plant traits on soil microbial communities and phosphorus availability

Relationships between plant traits and soil microbial communities for plant phosphorus acquisition remain uncertain and poorly understood (Hunter et al. 2014). Plant carboxylate exudation rate has been identified to indicate root presence to microbial

partners. Many soil-borne microorganisms such as bacteria and AMF follow gradient of exudates including carboxylates to roots (Whipps 2001). Several carboxylates released by roots have been observed to result in specific shifts in rhizosphere microbial communities. Citrate release by plant was shown to induce shifts in microbial and bacterial communities while malate more specifically influenced bacterial communities (Marschner et al. 2002). In *Arabidopsis thaliana*, malate produced by roots proved to select for the presence of plant growth promoting rhizobacteria (Rudrappa et al. 2008). In turn interactions between plant and microorganisms can also be identified via their feedback on plant traits such as the reduced carboxylate exudation observed in root colonized by AMF (Ryan et al. 2012; Del-Saz et al. 2017). Plant morphological traits also can play an important role in plant-microorganism interactions. Roots with lower diameter have for example been observed to be less associated with AMF. Shifts in microbial community structure and activity due to interactions with plant traits in turn influence phosphorus availability for plant uptake. Increasing evidence point toward an important role of plant traits in plant soil microorganisms interactions, however contrasting and unclear effects of plant traits and their consequences for phosphorus cycling and availability remain to be fully explored (Hunter et al. 2014; Sasse et al. 2018). Gaining a better understanding of plant traits effects on microbial communities is a major challenge but also an opportunity to potentially increase phosphorus acquisition efficiency (Richardson et al. 2011; Hunter et al. 2014).

1.4.2.3. Effects of litter traits on phosphorus availability

Several litter functional traits drive litter quality for decomposition and phosphorus release. Leaf phosphorus concentration in particular plays a central role in phosphorus release from litter. Concentrations above 3 mg P g⁻¹ C lead to fast release and mineralization of litter phosphorus (Kwabiah et al. 2003; Iqbal 2009). On the contrary low concentrations below 2 mg phosphorus g⁻¹ C tend to favour microbial phosphorus immobilization (Kwabiah et al. 2003; Alamgir et al. 2012). Other leaf chemical traits similarly influence the fate of residue during microbial decomposition such as leaf C:N ratio, leaf N:P ratio and leaf C:P ratio. Low attributes of these traits, below 24, 15 and 200 respectively for C:N ratio, N:P ratio and C:P ratio tend to favour fast mineralization and phosphorus release while higher values favour microbial immobilization (Prescott 2005). Leaf structural components such as lignin also strongly impact residue

decomposition and nutrient release (Prescott 2010). Morphological and physical leaf traits influence on phosphorus release from litter have received less attention except for SLA (Zukswert and Prescott 2017). These traits could play an important role in phosphorus release due to their implication in defining litter accessibility to decomposers (Zukswert and Prescott 2017). Leaves with high toughness for example decompose and release nutrient slower than leaves with low toughness (Pérez-Harguindeguy et al. 2013). Our understanding of the role of plant traits in phosphorus release from litter remains limited despite many factors being identified (Damon et al. 2014).

1.5. Increasing phosphorus availability and cycling via trait-based agroecological practices: Potential and challenges of cover crops

Incorporating more phosphorus efficient plant in cropping systems could increase phosphorus availability and cycling efficiency (Menezes-Blackburn et al. 2018). Increased phosphorus efficiency can be achieved via genetic modification or breeding (Richardson et al. 2011). However, some agroecological practices such as intercropping or cover cropping also offer the potential to incorporate phosphorus efficient plant in cropping systems.

1.5.1. Roles and benefits of cover crops in phosphorus cycling

Cover crops also sometimes named intermediate crops are non harvested crops grown during the off season and providing a range of ecosystems services such as reduced erosion, fixation of atmospheric nitrogen or increased carbons storage (Schipanski et al. 2014; Daryanto et al. 2018). These crops are generally terminated either mechanically, chemically or via freezing before being left to decompose in the field. The effects of cover crops on nitrogen dynamics are well documented. However despite gaining increased interest for their potential to improve phosphorus availability and cycling efficiency, cover crops effects on phosphorus dynamics have been rarely addressed (Hallama et al. 2019). Cover crops can significantly increase phosphorus availability and phosphorus uptake in subsequent crop through three main mechanisms: i) Phosphorus acquisition by cover crops and later release during decomposition; ii) Shifts in microbial communities and activity leading to improved

phosphorus availability in subsequent crops; iii) Lasting mobilization of soil phosphorus pools (Figure 9).

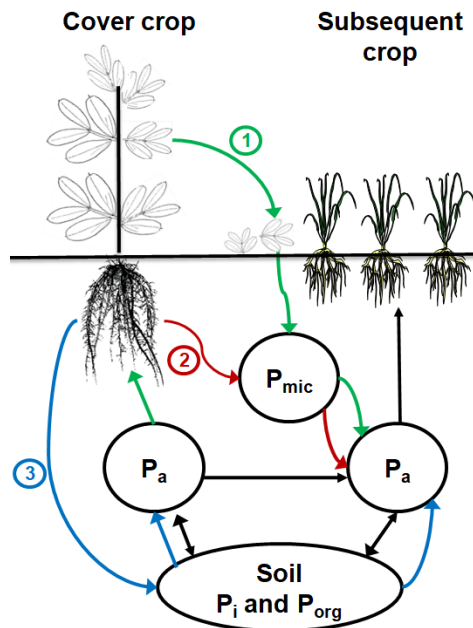


Figure 9 : Pathway of cover cropping effects on phosphorus availability for subsequent crops. Adapted from (Hallama et al. 2019).

1 Phosphorus capture by cover crops and later release of phosphorus dynamics of phosphorus stored in cover crop residues; 2 Shifts in microbial communities, activity and phosphorus content; 3 Modification of phosphorus availability via phosphorus mobilization by plants. P_{mic} microbial communities and phosphorus contained in microbial biomass. P_a available phosphorus during and after cover cropping. P_i unavailable inorganic phosphorus P_{org} Organic phosphorus.

Cover crops can acquire phosphorus pools unavailable to cash crops in rotation (Nuruzzaman et al. 2005; Lambers et al. 2013; Dube et al. 2014). Cover crops species present a variety of phosphorus acquisition traits forming multiples strategies to forage for available soil phosphorus (Wendling et al. 2016) but also mobilize poorly available phosphorus. Through these strategies, between 1 and 30 kg phosphorus ha^{-1} can be acquired in cover crops and then released during decomposition contributing to phosphorus uptake by subsequent crops (Damon et al. 2014; Maltais-Landry and Frossard 2015; Wendling et al. 2016). Several cover crops species especially Fabaceae can strongly modify rhizosphere properties through their phosphorus mobilization strategies (Maltais-Landry 2015). In turn, rhizosphere modifications by cover crops such as increases in phosphatase activity by around 20 % after cover cropping, can increase phosphorus availability influencing P uptake in subsequent crop (Hallama et al. 2019). Cover crops phosphorus acquisition traits and strategies can thus strongly influence the mechanisms by which they influence phosphorus

availability. Living cover crops interact with soil microbial communities potentially leading to positive feedbacks increasing phosphorus availability in the crop-rhizobiome-system (Oberson et al. 2006; Njeru et al. 2014). Shifts in microbial communities and increases in AMF populations under cover cropping can then play the role of inoculums for the subsequent crops indirectly influencing their phosphorus uptake with reports of increases of 50 % in AMF abundance and 25 % microbial phosphorus after cover cropping (Hallama et al. 2019). Despite significant cover cropping benefits via these processes, several factors limit their use and lead to uncertain influence on phosphorus availability. Water scarcity and short growing season can reduce or negate cover cropping effects. Soil phosphorus availability is especially influential with greater effects overall in systems with lower phosphorus availability and different mechanisms and strategies being of interest in systems with high phosphorus availability (Hallama et al. 2019). Mechanisms involved in phosphorus cycling in cover crops also present complex tradeoffs with other cover cropping benefits underlining the need for comprehensive approaches balancing these multiple benefits at the cost of reduced performance in individual functions (Schipanski et al. 2014). Overall cover cropping effect on phosphorus cycling depends on multiple mechanisms and factors offering opportunities for locally functionally optimal designs. However the multiplicity of mechanisms involved in the effect of cover crops on phosphorus availability, coupled with their high variability and changing relative contributions poses complex challenges that need to be fully investigated and elucidated in order to maximise cover cropping benefits.

1.5.2. Challenges in designing cover crops for increased phosphorus availability in agroecosystems

Achieving functionally optimal cover crop designs increasing phosphorus availability and cycling efficiency requires fully understanding the factors involved in the multiple mechanisms contributing to cover cropping impact on phosphorus availability, starting with cover crops phosphorus acquisition strategies. Cover crop phosphorus acquisition strategies indeed play a major role in defining the mechanisms of phosphorus benefits (Hallama et al. 2019). To date, one of the most complete descriptions of cover crops phosphorus acquisition strategies was proposed in Wendling et al (2016) who

identified five main strategies based on above and belowground functional traits. However phosphorus mining traits such as carboxylate exudation, phosphomonoesterase emission and association with soil microorganisms such as AMF were not measured in this study and require further investigation due to their potentially large role, especially in contexts with low phosphorus availability (Smith and Smith 2011). Moreover phosphorus mining traits have been observed to form tradeoffs with phosphorus foraging traits (Lyu et al. 2016) which still need to be fully explored in cover crops (Wen et al. 2019). Evidence also points to similar tradeoffs between plant traits involved in phosphorus mining or foraging and association with microorganisms (Ryan et al. 2012; Chen et al. 2016). Understanding the diverse phosphorus acquisition strategies in cover crops and the tradeoffs involved is key to maximise cover cropping benefits (Wendling et al. 2016). However the relevance of specific strategies could also be strongly influenced by soil characteristics and especially soil phosphorus availability (Hallama et al. 2019). Maltais-Landry and Frossard (2015) highlighted the low influence of phosphorus mobilization on phosphorus cycling efficiency in systems with high phosphorus availability and highlighted the importance of phosphorus release from cover crop residues in that context. The main factors influencing phosphorus release dynamics from cover crop residues, namely phosphorus concentration and speciation in residue, but also residue C:N and C:P ratio as well as SLA have in turn mostly been identified. However a wide range of values have been reported for these factors (Prescott 2005; Pérez-Harguindeguy et al. 2013; Damon et al. 2014), suggesting limited consensus between studies. Maximising cover crops benefits for phosphorus uptake in the subsequent crops requires fully understanding phosphorus release dynamics in order to adapt cover crops management and design to ensure that phosphorus release dynamics from residue match subsequent crops requirement (Wendling et al. 2016). A few models such as the Agricultural Production Systems Simulation or the CENTURY model propose to address this issue however their data intensive design make them challenging to use at farm level (Parton et al. 1988; Keating et al. 2003; Damon et al. 2014). Moreover, the multiple mechanisms involved in cover cropping effects on phosphorus cycling still remains to be fully integrated in these models and decisions tools, suggesting further investigations of the relative contribution of processes such as rhizosphere chemical modification or interactions soil microorganisms and associated legacy effects (Hallama et al. 2019).

OBJECTIVES

2. PhD OBJECTIVES

This PhD project aims at getting a better understanding of the role of plant functional traits in the plant-soil-microorganisms processes influencing phosphorus availability in agroecosystems. To address this general objective four chapters were produced to examine the central and still pending questions involved (Figure 10).

Chapter 1 examined the strategies and factors involved in plant phosphorus acquisition. The aims of this chapter were to (1) Characterize the relationships among functional traits involved in phosphorus acquisition to explore tradeoffs and main phosphorus-acquisition strategies in cover crops; (2) Examine whether phosphorus forms and soil type mediate expression of these tradeoffs and phosphorus-acquisition strategies in cover crops. Gaining insight in these strategies and their mediation by soil types would underpin potentially functionally complementary cover crop design enhancing phosphorus cycling efficiency.

Chapter 2 inquired the contribution of plant soil microorganism interactions to phosphorus acquisition in cover crops. The objectives of this chapter were to (1) Examine the influence of belowground functional traits involved in phosphorus acquisition on microbial communities and its consequence for phosphorus acquisition; (2) Gain a better understanding of the mediation of plant-soil microorganisms interactions for phosphorus acquisition by soil type and properties. These aims would lead to an improved understanding of the influence of plant trait soil microorganisms interactions for phosphorus acquisition on phosphorus cycling and availability potentially offering elements to select for beneficial interactions in cover-crops.

Chapter 3 aimed at gaining a better understanding of the influence of cover crops residue traits on phosphorus availability in agroecosystems. The chapter focused on identifying the key residue functional traits involved in phosphorus release during decomposition and their influence on phosphorus availability dynamics. The results of this chapter could help designing and managing cover-crops for optimised transfer of phosphorus acquired to soil and subsequent crops.

Chapter 4 aimed at estimating the influence of cover crops on phosphorus availability for subsequent crops. The objective of this chapter was to examine via modelling the feedback effects of soil P availability on phosphorus release dynamics from cover crop residues and their effect on phosphorus availability for subsequent crops. This chapter, based on data extracted from literature, could provide insights into the expected benefits of cover cropping on phosphorus availability across soil with varying levels of phosphorus.

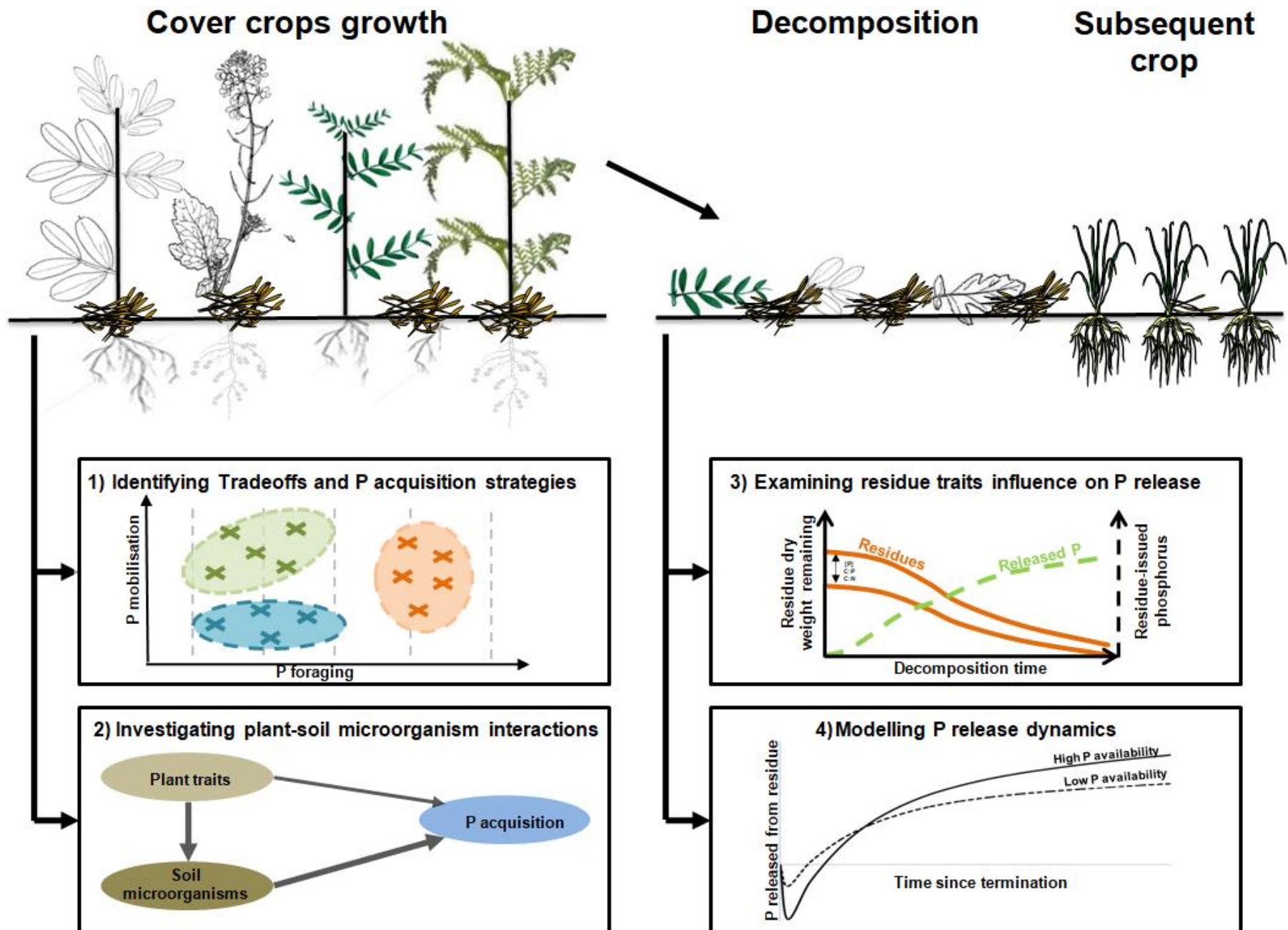


Figure 10 : PhD objectives

Phd METHODS

3. PhD METHODS

In order to investigate the role of plant functional traits and interactions thereof in the plant-soil-microorganisms processes involved in phosphorus cycling and availability in agroecosystems, we designed three complementary approaches. The first approach, addressing the objectives described for chapters 1 and 2 (Figure 10) aimed at characterizing the plant traits involved in plant-soil-microbe interactions for phosphorus acquisition, their relationships and aggregation in plant phosphorus acquisition strategies. The second approach, addressing the objectives described for chapter 3, examined the role of residue traits on the fate of residue phosphorus in the plant soil microbe system. The third and final approach, addressing the objectives described for chapter 4, aimed at modelling phosphorus release dynamics from cover crop residues influence on phosphorus availability and feedbacks effects of soil phosphorus availability.

The role of cover crops in the plant soil microbe processes involved in phosphorus cycling in agroecosystems was first examined with trait-based approaches *ex situ*, before modelling expected impacts on phosphorus availability at field level.

3.1. Characterisation of plant traits involved in plant soil microbes interactions for phosphorus acquisition, their relationships and aggregation in strategies.

Despite our increasing understanding of phosphorus cycling in agrosystems (Bünemann et al. 2010), the role of cover crops traits and strategies in the multiple plant soil microbe processes involved in phosphorus cycling remains underexplored (Hallama et al. 2019). Highlighting key plant traits and strategies involved in the plant soil microbe interactions involved in phosphorus acquisition offers to gain a better understanding of these processes and opportunities to increase phosphorus availability in cropping systems (Menezes-Blackburn et al. 2018).

In order to examine cover crop phosphorus acquisition, both via different plant phosphorus acquisition strategies but also via interactions with microbial communities, a common *ex situ* experimental device was designed (Figure 11). Plant trait attributes and their relationships were measured in two soil types, a Retisol and a Calcaric

Cambisol selected for their relevance to the local context of north eastern France and contrasted phosphorus fractionation (Chapter 1). Soil phosphorus availability strongly mediates traits attributes involved in phosphorus acquisition (Lyu et al. 2016). We thus selected soil from fields with low to moderate phosphorus availability (Olsen phosphorus < 20 mg phosphorus kg⁻¹) and further decreased phosphorus availability via removing topsoil (0-5 cm) prior to collection and mixing with washed sand (22 %) in order to examine trait attributes in soil with low phosphorus availability where cover cropping may be particularly efficient in increasing phosphorus availability (Hallama et al. 2019).



Figure 11 : Greenhouse experiment setting.
Plants were grown at two individuals per 4.5 L pots for approximately 2.5 months.

3.1.1. Identifying tradeoffs and co-variation between phosphorus acquisition traits

Plants phosphorus acquisition traits have been observed to be involved in multiple tradeoffs and co-variation, forming multidimensional phosphorus-acquisition strategies which remain poorly understood and characterized (Weemstra et al. 2016; Wen et al. 2019). In order to examine the multiple traits interactions and strategies involved in plant phosphorus uptake, a wide range of traits were measured for their recognized role in phosphorus acquisition (Lynch 2011). Measured traits included morphological

and architectural traits (root length density, root surface area, specific root length, finer root percentage) involved in plant phosphorus foraging capacity (Lynch 2011; Haling et al. 2018; Ma et al. 2018) and physiological traits or indicator thereof (phosphomonoesterase activity in the rhizosheath, change in rhizosheath pH, carboxylate exudation rate) involved in plant phosphorus mobilizing capacity (Richardson et al. 2011; Sun et al. 2019; Wang and Lambers 2020). Aboveground traits such as leaf phosphorus and manganese concentration were included along with belowground traits as they have been shown to correlate with belowground strategies, especially for leaf manganese concentration which was observed to be an indicator of phosphorus mobilisation by plants via carboxylate release (Lambers et al. 2015; Pang et al. 2018). The indirect indications provided by leaf manganese concentration were complemented via direct root carboxylate exudation rate measurements, allowing us to gain insight into the efficiency of released carboxylate in mobilizing phosphorus via leaf manganese.

Species selection is central in order to observe enough diversity in trait attributes to produce generalisable results (Duarte et al. 1995). Species from five different phylogenetic families (Poaceae, Brassicaceae, Polygonaceae, Fabaceae and Hydrophyllaceae) were selected in order to ensure we would observe varied trait values and phosphorus acquisition strategies. Fabaceae have been observed to have high phosphorus mining capacity (Maltais-Landry 2015) and be a key functional group to ensure complementary effect in mixtures (Díaz and Cabido 2001; Fox 2005), often incorporated in cover crops for their ability to fix atmospheric nitrogen. A variety of Fabaceae species was thus selected to further investigate the diversity of strategies within that family of interest (6 out of the 13 species examined). Brassicaceae were selected based on both their common use as cover crops and their different phosphorus acquisition strategies and notably their absence of association with AMF. Few Poaceae species were selected due to the potential issues associated with managing pathogens and regrowth with a subsequent cereal cash crop. Finally Polygonaceae and Hydrophyllaceae were selected for their capacity to mine phosphorus and capacity to forage large soil volumes respectively.

A multivariate clustering approach was employed to identify plant phosphorus acquisition strategies based on the array of phosphorus acquisition trait measured. Simultaneously including multiple traits may in part be redundant due the multiple co-

variation and tradeoffs between root traits (Roumet et al. 2016; Ma et al. 2018; Wen et al. 2019). However given the suspected multidimensional relationships between root traits (Weemstra et al. 2016), this approach allowed us to investigate convergence toward common phosphorus acquisition traits in a multi-trait space further expanding upon previous observations of tradeoffs between traits involved phosphorus acquisition.

3.1.2. Examining plant soil microorganisms interactions for phosphorus-acquisition

Plant soil microorganisms play an important but underexplored role in plant phosphorus acquisition, notably due to difficulties in relating rhizosphere microbial community composition and its functions for plants (Jansson and Hofmockel 2018). To investigate plant soil microorganisms interactions involved in phosphorus acquisition, microbial communities were assessed indirectly through bioindicators on a subset of species whose traits and strategies were previously analysed in chapter 1. Relationships between these microbial indicators, plant phosphorus acquisition traits and plant phosphorus uptake were then examined.

Phospholipid fatty acids (PLFA) and neutral lipid fatty acid (NLFA) concentration in the rhizosphere were measured to highlight shifts in microbial communities in the rhizosphere. PLFA and NLFA analyses were selected in order to gain a general understanding of the type of organisms present in the rhizosphere without precisely identifying the genus involved. These bioindicators provided information on the abundance of Gram negative or positive bacteria, fungi and more precisely arbuscular mycorrhizal fungi. Relationships between shifts in PLFA and NLFA concentration in the rhizosphere and shifts in microbial phosphorus content in the rhizosphere were examined in order to reinforce the function of shifts in rhizosphere microorganisms communities in phosphorus cycling. Consequences for phosphorus acquisition by plants were also indirectly assessed via shifts in phosphorus availability in the rhizosphere measured by Olsen and resin extraction.

A partial least square path modelling approach was employed in order to investigate the combined influence of plant traits, soil microbial communities and their interactions on phosphorus uptake by plants. Three latent variables, reflecting respectively microbial communities, root traits and phosphorus uptake were defined with the

objective of predicting the “phosphorus uptake” latent variable based on root traits and microbial communities. The model structure was designed to identify interactions between root traits and microbial communities for phosphorus acquisition via a specified interaction between these latent variables. Alongside the identified effect of the “root traits” latent variable on the latent variable representing microbial communities, indirect effects of the “root traits” on phosphorus uptake via its effects on the microbial component allowed us to examine the contribution of plant-soil microorganisms interactions for phosphorus uptake.

3.2. Examining the role of residue traits on the fate of residue phosphorus in the plant-soil-microbe system

After examining plant strategies for phosphorus acquisition via tradeoffs and aggregation between phosphorus acquisition traits and interactions with microorganisms, we aimed at understanding the release of phosphorus captured via these diverse strategies and its impact on phosphorus availability. To investigate the fate of residue phosphorus residues of six species with varied phosphorus-acquisition traits and strategies (Brassicaceae: *Brassica carinata* A. Braun; Polygonaceae: *Fagopyrum esculentum* Moench.; Fabaceae: *Lens culinaris* Medik., *Vicia faba* L., *Vicia villosa* Roth.; Hydrophyllaceae: *Phacelia tanacetifolia* Benth.) were employed in a greenhouse experiment and a simultaneous incubation experiment.

Tracking the fate of residue phosphorus in amended soils can be complex due to the confounding effects of several processes stimulated by residue addition such as priming effects, change in soil pH during decomposition or shifts in microbial communities due to C addition. We thus elected to use an isotopic labelling approach, which can allow tracking the fate of residue phosphorus via ratio between radioactive and stable phosphorus isotopes. Due to the short half-life of radioactive phosphorus isotopes however, an indirect approach labelling the pools of phosphorus in soil and based on isotopic dilution due to unlabelled phosphorus contained in residues was selected (Frossard et al. 2011) (Figure 12).



Figure 12 : Model of liquid scintillation analyser used to measure specific activity in the soils and plant samples

To observe the fate of residue phosphorus in the soil microbe system soil microbial phosphorus and resin extractable phosphorus were measured after 10 days and 59 days after residue addition. Impacts on phosphorus availability for subsequent crops were assessed via phosphorus content and biomass of a *Lolium multiflorum* grown in amended soils. Phosphorus content in *Lolium multiflorum* was measured 35, 47, 70, 91 and 110 days after amendments to investigate phosphorus release dynamics and their impact on phosphorus availability. Isotopic dilution was measured in plant biomass and soil extracts in order to differentiate between soil-issued phosphorus and residue-issued phosphorus. In order to examine whether changes observed in ryegrass biomass and phosphorus content were indeed due to change in phosphorus availability due to residue addition and not confounding effects of the nitrogen contained in the residue biomass, the nitrogen nutrition index and phosphorus nutrition index (Duru and Ducrocq 1996; Lemaire et al. 2008) were calculated to measure nitrogen and phosphorus limitation in the *Lolium multiflorum*. Moreover 120 mg kg⁻¹ of N was added after each harvest to further exclude possible interference of nitrogen dynamics.

Residue morphological traits are often ignored in decomposition studies due to use of milled residues (Noack et al. 2014). In order to investigate the still unclear role of morphological traits, alongside the chemical traits commonly measured, residues were

cut in 0.25 to 0.40 cm² pieces before being mixed with the soil to maintain morphological ratios. This allowed us to investigate the role of multiple residue traits such as carbon, nitrogen and phosphorus content but also ratio between these elements as well as specific leaf area on phosphorus release dynamics from residues and their consequences for phosphorus availability. Phosphorus release dynamics both from leaves residues only and aboveground residues encompassing both leaves and stems were examined to investigate the effects of traits often defined at leaf level but also simultaneously estimate phosphorus released by cover crops in more realistic conditions

3.3. Modelling cover crop residue phosphorus release dynamics impact on phosphorus availability and feedback effects of soil phosphorus availability.

Several models can potentially offer mechanistic predictions of phosphorus release from residues such as the APSIM model or CENTURY model (Parton et al. 1988; Keating et al. 2003). In turn their complex design and multiple data inputs can limit their use to detailed case studies. In order to investigate feedbacks effects of soil phosphorus availability on phosphorus release dynamics from cover crops residues and their impact on phosphorus availability, the modelling approach developed by Damon et al. (2014) was employed (Figure 13). The choice of this model was supported by (i) The easy-to-use design of the model requiring few inputs and thus allowing the exploitation of data largely available in the literature (ii) The mechanistic structure of the model allowing model adjustments based on the results obtained in the precedent chapters.

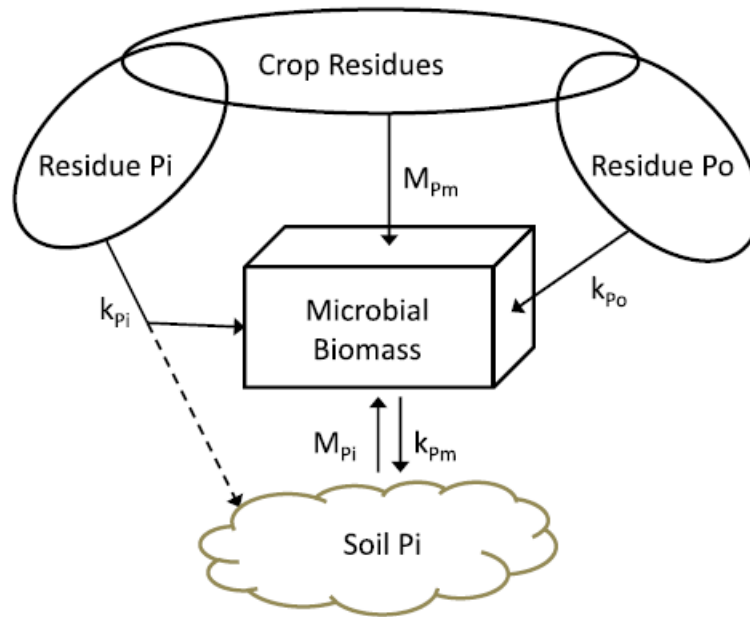


Figure 13 :Schematic representation of the conceptual model developed by Damon et al. (2014) and adapted in the PhD project.

Residue Pi' represents water-soluble phosphate and 'Residue Po' represents the organically bound component of P in 'Crop Residues'. 'Soil Pi' represents Pi that is associated with the mineral component of soil that is potentially exchangeable with the soil solution. ' k_{Pi} ' and ' k_{Po} ' represent the decay constants for the rate of release of 'Residue Pi' and 'Residue Po', respectively. ' M_{Pm} ' represents the amount of Pi assimilated by the 'Microbial Biomass' as it proliferates in response to the availability of C substrate from 'Crop Residues', whereas ' M_{Pi} ' represents the uptake of 'Soil Pi' by the stimulated 'Microbial Biomass' where P released from crop residues is less than ' M_{Pm} '. ' k_{Pm} ' represents the decay constant for the rate of release of Pi from the 'Microbial Biomass' as it decays in response to diminishing availability of C substrate from 'Crop Residues'.

Two scenarios meant to represent average cover crop biomass and phosphorus content under high or low soil phosphorus availability were produced based on data extracted from literature in temperate climates. Model predictions under these scenarios were then compared in order to offer a basis for addressing the complex mediation of cover cropping benefits by phosphorus availability and gain insight into possible consequences for cover crops management and designs

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CHAPTER 1

4. TRADEOFFS AMONG PHOSPHORUS-ACQUISITION ROOT TRAITS OF CROP SPECIES FOR AGROECOLOGICAL INTENSIFICATION

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Abstract

Plant P acquisition strategies are driven by multiple belowground morphological and physiological traits as well as interactions among these traits. This study aimed to characterize the relationships among traits involved in P acquisition to explore tradeoffs and the main P-acquisition strategies and their mediation by soil type.

Ten morphological and physiological traits involved in P acquisition were measured across 13 species grown in controlled conditions in two contrasting soils with moderate P limitation.

Tradeoffs between thicker and thinner roots were observed, with thicker roots exhibiting greater carboxylate release or phosphatase activity in the rhizosheath. Tradeoffs and coordination amongst traits were strongly mediated by soil type. Multivariate analysis of functional traits involved in P acquisition highlighted four main P-acquisition strategies relying primarily on morphological traits, physiological traits or a combination thereof.

The diversity of strategies demonstrates a potential for functional diversity benefits in cultivated plant communities via preferential access to different P pools leading to complementarities and reduced competition for resource acquisition. Overall, our results underpin functionally-complementary multispecies crop designs, enhancing P availability and cycling efficiency

Key words: agroecology, cover crops, phosphorus acquisition, root functional trait, tradeoffs

4.1. Introduction

Phosphorus (P) is a limiting nutrient in many natural and managed ecosystems due to its strong sorption with soil particles which limits its availability for plants. To overcome low P availability and ensure productive agriculture, P fertilisers are applied, albeit often with very low efficiency (Richardson et al. 2011). In highly-productive fertilized systems, there is a significant gap in P exports and inputs, leading to the accumulation of poorly available organic and inorganic P in the soil (Simpson et al. 2011; Bouwman et al. 2017). In these systems, improving P availability can be achieved by selecting for plant traits and strategies that allow access to the legacy P pool (Menezes-Blackburn et al. 2018). Plants have developed a range of morphological, architectural and physiological traits, granting access to the diverse pools of soil P. Morphological traits such as specific root length (SRL) and architectural traits such as root length density (RLD) allow plants to increase their P-foraging capacity (Pang et al. 2010; Haling et al. 2018; Ma et al. 2018). While only inorganic P in solution is taken up by plants, roots can also mobilize both inorganic and organic P by secreting protons (H^+) to dissolve Ca-phosphate, and carboxylate, which compete with P for binding sites, and thus decrease sorption on mineral surfaces (Wang and Lambers 2020). Plants can also hydrolyze organic P through the release of acid phosphatases, both monoesterases and diesterases (Richardson et al. 2011).

Overall, plant P-acquisition strategies are defined by the expression and association of P-acquisition traits. However, as the expression of these traits has a carbon cost, plants tend to rely mainly on one or a few P-acquisition traits (Pearse et al. 2006; Raven et al. 2018). There may thus be interactions among P-acquisition traits such as root morphological traits being negatively correlated with physiological traits (Zhang et al. 2016; Lyu et al. 2016; Wen et al. 2017). This indicates potential tradeoffs among diverse P-acquisition traits. However, trait interactions and tradeoffs that are central in gaining insight into plant P acquisition, are poorly understood, especially for fast-growing plants such as cover crops (Wen et al. 2019). Understanding tradeoffs and trait-combination effects would allow us to unravel the complexity of the P-acquisition strategies of species and bring new knowledge to design cultivated communities (i.e. multi-species crops) such as cover crops or intercrops to improve P acquisition and availability. Furthermore, trait expression and associated processes are strongly influenced by soil conditions, especially P availability (Raven et al. 2018; Wen et al.

2019). Tradeoffs among life history traits and nutrient-acquisition traits are inconsistent across environmental conditions (Sgrò and Hoffmann 2004; Kong et al. 2019), warranting further investigations as for the consistency of tradeoffs involved in P acquisition.

Intermediate crops, often referred to as cover crops, would offer an opportunity to use a high plant species diversity to enhance P recycling in agroecosystems (Hallama et al. 2019). They can accumulate a large amount of nutrients including P during their growing period (Wendling et al. 2016), which is then released at termination to play a major role in maintaining and improving P availability (Dube et al. 2014; Damon et al. 2014). Moreover, through plant traits associated with a high P-foraging capacity and P mobilization, intermediate crops could acquire P from P pools that are unavailable to the cash crop (Nuruzzaman et al. 2005). A promising opportunity to improve soil P availability for crops can thus be developed by enhancing P cycling in intermediate crops on the basis of their potential to acquire P from poorly-available sources (Richardson et al. 2011). Cover crops present a wide range of P-acquisition traits, therefore potentially presenting different P-acquisition strategies exploiting different P pools (Wendling et al. 2016; Lyu et al. 2016). Examining these strategies and the factors conditioning their expression by characterizing root trait relationships in plant species with diverse root traits would result in a better understanding of P-acquisition in crop species and insights for the design of more P-efficient systems. In this study, a greenhouse experiment was carried out to measure morphological, architectural and physiological traits in 13 intermediate crops species with diverse phylogenetic lineages in two contrasting soil types, in order to:

- i) Characterize the relationships among traits involved in P-acquisition to explore tradeoffs and the main P-acquisition strategies;
- ii) Examine whether P forms and soil type mediate expression of tradeoffs and P-acquisition strategies in intermediate crops.

4.2. Material and methods

4.2.1. Greenhouse experiment

Soils used in the greenhouse experiment were collected at 5 to 20 cm depth from two fields from north-eastern France, after topsoil (0-5 cm) removal to further decrease P availability. Fields with a known P deficiency were selected to ensure low P availability. A Retisol (formerly called Albeluvisol) and a Calcaric Cambisol (FAO 2014) were selected for their contrasting soil characteristics, mainly their different P forms, in particular their apatite P concentration (Table 1). Prior to use, the soils were dried at ambient temperature, sieved at 2 mm and then mixed with washed sand (22% mass).

Table 1 : Chemical and physical soil characteristics

	Clay % (<0.002mm)	Sand % (>0.05mm)	pH KCl	Ca ^a (gkg ⁻¹)	N tot (%)	CEC (cmol _c kg ⁻¹)	Olsen P ^c (mg kg ⁻¹)	Ca -P ^d (mg kg ⁻¹)
Calcaric Cambisol	30.7	22.0	7.9	44.7 ^b	0.17	9.3	19.8	542
Retisol	22.3	5.4	7.4	2.60	0.10	10.5	16.3	76.7

^aExtractant : Ammonium acetate 0.5 M, EDTA 0.02 M pH 4.65

^bCaCO₃ 56.4%

^cAccording to Olsen (1954)

^dApatite phosphorus as defined in García-Albacete et al. (2012)

Thirteen (sub)species of diverse phylogenetic lineages (Poaceae: *Avena nuda* L., *Avena strigosa* Schreb; Brassicaceae: *Brassica carinata* A. Braun, *Raphanus sativus* L., *Sinapis alba* L.; Polygonaceae: *Fagopyrum esculentum* Moench.; Fabaceae: *Lens culinaris* Medik., *Pisum sativum* subsp. *arvense* L., *Trifolium alexandrinum* L., *Vicia faba* L., *Vicia sativa* L., and *Vicia villosa* Roth.; Hydrophyllaceae: *Phacelia tanacetifolia* Benth.) were selected for their diverse morphologies, P-acquisition traits and relevance for the local context. The experimental design included two soil types, 13 species and a control (bare soil) with four replicates. Plants were sown mid-January 2018 at two individuals per pot per species in 4.5 L fully filled pots and watered twice a week. Pots were arranged in a random design and greenhouse temperatures were maintained at 22°C during the day (14 hours) and at least 18 °C at night.

4.2.2. Plant traits measurement

At harvest (77 days) plants were manually separated from the bulk soil with special care given to ensure minimum damage. Rhizosheath adhering to the roots up to a

maximum of 2 mm after shaking was collected and stored at -20°C for further analysis. Roots were then immersed in 0.20m M CaCl₂ for 1 hour, after removing any remaining soil particles by quickly rinsing with CaCl₂. The CaCl₂ volume was adjusted to ensure a complete immersion of the root system. The solution was then sampled and stored at -20°C before measuring carboxylic acid exudation by reversed-phase column liquid chromatography (RPLC) (Cawthray 2003; Yacoumas et al. 2020). Briefly, an acid mobile phase (93% 25 mM KH₂PO₄ at pH 2.5 and 7% methanol) allowed a good resolution of carboxylates (citric, fumaric, maleic, malic, malonic) on a C18 column with a 15 min elution time and a 1 mL min⁻¹ flow rate. Total carboxylate release rate was later calculated as the sum of all previously mentioned acids (see Table 2 for abbreviations).

Table 2 : List of abbreviations and associated units

Variable	Abbreviation	Unit
Change in rhizosheath pH	ΔpH	-
Fine root percentage (<0.5mm ∅)	FR	-
Phosphomonoesterase activity	PME	μg nitrophenol g ⁻¹ hour ⁻¹
Root length density	RLD	cm cm ⁻³
Root surface area	RSA	cm ²
Specific leaf area	SLA	mm ² mg ⁻¹
Specific root length	SRL	m g ⁻¹
Total carboxylate release rate	TCE	μmol g root ⁻¹ hour ⁻¹

4.2.3. Morphological traits

Aboveground biomass and roots were separated by cutting the stem 1 cm above the first visible root. Roots were then scanned while being immersed in deionised water using an Epson Scanner perfection V800 (Regent Instruments Inc., Québec, Qc, Canada) to produce a 600 dpi image. The image was analysed using WinRHIZO Regular software V.2016a (Regent Instruments Inc., Québec, Qc, Canada) to determine root traits including root surface area (RSA), the percentage of fine root (FR), defined here as length of roots with a diameter < 0.5 mm, and root length density (RLD). After 48 hours drying at 55°C, scanned roots were weighed to calculate specific root length (SRL). Three young but fully-developed leaves per replicate were scanned at 600 dpi, and then dried at 60°C for 48 hours to determine specific leaf area (SLA). All aboveground biomass was dried at 60 °C for 48 hours and weighed.

4.2.4. Nutrient uptake

Dried leaves and stems were digested via acid digestion and a microwave heating treatment (Lange et al. 2016). Between 0.1 and 0.2 g of biomass was introduced in Teflon vessels with 8 mL of 65% (v/v) HNO₃ and 2 mL of 36% (v/v) HCl and heated to 185°C for one hour. Digests were then filtered and P and manganese (Mn) concentrations (as a proxy for rhizosheath carboxylate concentration) determined by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Scientific XSERIES2, Beauvais, France). SRM1573, a certified reference material (tomato leaves) was used as a standard.

4.2.5. Rhizosheath properties

After defrosting, acid phosphomonoesterase activity in the rhizosheath (PME) was measured with a modified buffer at pH 6.5 (Tabatabai and Bremner 1969). Briefly, phosphatase activity was assessed via production of *p*-nitrophenol from sodium *p*-nitrophenyl phosphate during a 1 hour incubation at 37°C with 0.5 g dry soil, 0.2 mL toluene, 4 mL modified buffer and 1 mL substrate. *p*-Nitrophenol release was determined spectrophotometrically at 410 nm after stopping the reaction with 4mL 0.5 M NaOH and 1mL 0.5 M CaCl₂ and filtering. Rhizosheath pH was measured on 2 g equivalent dry soil with a 1:10 soil to solution ratio. ΔpH between rhizosheath and bulk soil from unplanted pots post-growth was then calculated.

4.2.6. Data analysis

Linear mixed models (LMM) were used in order to test for differences among plant P uptake and traits among soil types, with species as fixed factors and soil types as random effect. Differences in traits between soils were then investigated with non-parametric tests (Kruskall-Wallis test + post hoc test of Mann Whitney). As multiple differences were observed between soils, further analysis was performed separately for each soil type. To identify the main covariation in P-acquisition traits and rhizosphere processes, a principal component analysis (PCA) was performed using the “FactomineR” package on log-transformed data (Lê et al. 2008). The number of components was selected to represent more than 75% of the total variability. Tradeoffs between traits were confirmed with Spearman correlation tests as data did not fulfil the condition of normality. Hierarchical classification on principal components (HCPC) was then performed to define groups with similar patterns of P-acquisition traits and

influence on the rhizosphere. Species were attached to the group including most of their replicates. A multi response permutation procedure (MRPP) confirmed a clear differentiation between clusters in multivariate space. Differences between clusters for each individual factor were then investigated, either with tests of variance (ANOVA and post-hoc test of Tukey) or non-parametric tests (Kruskall-Wallis test + post-hoc test of Mann Whitney).

To investigate the importance of the factors leading to group formation, their influence on P uptake was modelled via generalised linear models (GLM) using the “FactomineR” package. Briefly, all factors were individually tested as predictors of P uptake as well as combinations of the best fitting factors. Models were compared based on second-order Aikake’s information (AICc) with the lowest relative value considered the best fit. Differences between models were tested with ANOVA as well as the criteria $\Delta AICc > 2$. Complementary to GLM regression, partial square path modelling (PLS–PM) was performed to underline the relative ability of trait combinations and type for predicting P uptake. Three clusters of variables, or “latent variables” were defined, respectively, the “root morphology” variables encompassing root surface area, SRL, fine root percentage, root length density, the “root physiology” variables encompassing PME activity, carboxylate release and change in rhizosheath pH and the “aboveground traits” variables encompassing SLA, foliar [P] and [Mn]. Verifications were made to ensure model quality, notably unidimensionality of latent variables and cross-loadings between traits associated with a latent variable and other variables as suggested in Sanchez (2013). To ensure the condition of positively correlated variables in a latent variable, the sign of some variables was changed. Variables and components were selected based on their loading and correlation as suggested in Sanchez (2013). Overall model quality was evaluated with the Goodness of Fit (GoF) index. Analyses were performed with the package “plsrm” version 0.4.9. All tests were performed in R version 3.6.0 with a significance level of 0.05.

4.3. Results

4.3.1. Variation in P-acquisition traits and rhizosphere processes

In the Calcaric Cambisol, the PCA summarised 84% of the total variability, with the first two components representing 50.8% of the total variability (Figure 14). In the Retisol 82% of the total variability was summarised by the PCA, with the first two components representing 51.3% of the total variability (Figure 14). Overall, we observed moderate to strong correlations between morphological indicators such as fine root percentage, RLD, SRL and root surface area ($|r|$ between 0.30 and 0.88). We observed similar patterns between physiological indicators such as change in rhizosheath pH, PME activity and carboxylate release, albeit more differentiated by soil type.

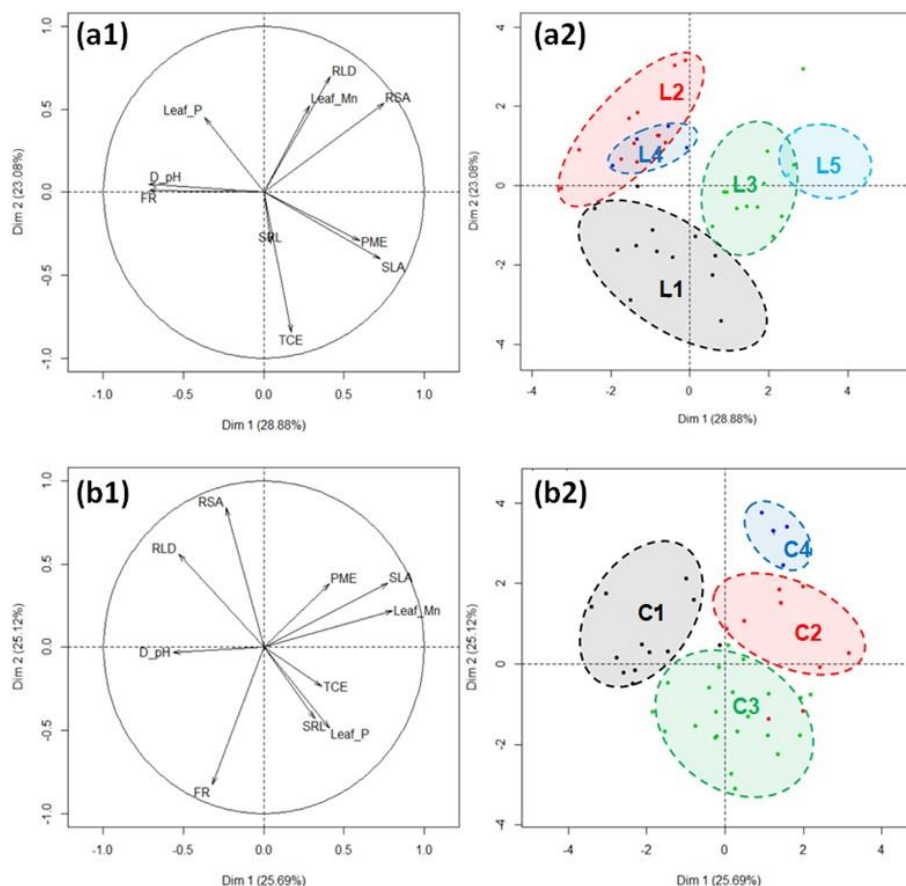


Figure 14 : Principal component analysis (PCA) of functional traits and rhizosphere processes involved in phosphorus acquisition in Retisol (a) and Calcaric Cambisol (b)

(1) Variable covariation along first two components, **(2)** clusters formed with Hierarchical Classification on Principal Components (HCPC). Abbreviations: D_pH: change in rhizosheath pH; FR: percentage roots with diameters less than 0.5 mm; Leaf_Mn: foliar manganese concentration; Leaf_P: foliar phosphorus concentration; PME: acid phosphomonoesterase activity; RLD: root length density; RSA: root surface area; SLA: specific leaf area; SRL: specific root area; TCE: total carboxylate release rate.

In the Retisol, the first component was formed based on all modifications in the rhizosphere properties measured (pH, PME activity) and carboxylate release, while also presenting a gradient of fine root percentage and root surface area. Aboveground, SLA and leaf P concentration were also important contributors to the first component. The component presented a gradient with strong negative correlations between fine root percentage and PME activity ($r=-0.65$) and negative correlation between fine root percentage and rhizosphere acidification ($r=-0.32$). PME activity, rhizosphere acidification and carboxylate release were positively correlated. At the leaf level, SLA was negatively correlated with fine root percentage. The second component was mostly formed based on root physiology and morphology (root length density and surface area). Physiological traits important for this axis were carboxylate release as well as to a lesser extent leaf Mn concentration. The component presented a gradient with a negative correlation between carboxylate release and RLD ($r=-0.36$). Root length density was also positively correlated with root surface area.

In the Calcaric Cambisol, the first component was based on aboveground characteristics such as SLA and foliar Mn concentration. To a lesser extent, the first component was also based on morphological characteristics such as RLD, and rhizosphere modification (rhizosphere acidification). SLA was strongly positively correlated with leaf Mn concentration ($r=0.66$). Root length density and root surface area were positively correlated. The second axis mostly represented morphological traits such as root surface area, fine root percentage and root length density with a smaller influence of leaf traits such as foliar P concentration and marginal modifications to the rhizosphere such as PME activity. Foliar P concentration was positively correlated with fine root percentage ($r=0.33$) and SRL ($r=0.43$), and negatively with root surface area ($r=-0.30$).

4.3.2. Comparison of P-acquisition traits among different clusters

Based on the PCA scores, we identified four groups via HCPC in the Calcaric Cambisol, five in the Retisol (Figure 14, Table 3). In the Retisol, the first “physiological/exudation” group L1 encompassed lentil, common vetch and white mustard. It showed the greatest exudation of carboxylic acids, significant activity of PME in the rhizosheath, as well as the lowest RLD observed in all groups. The second “intermediate/morphological” group L2 encompassed common and naked oat, Indian mustard, buckwheat and forage radish. It presented a low carboxylate release and PME activity in the rhizosheath, while presenting a small increase in rhizosheath pH and a high RLD, fine root percentage and foliar P concentration compared with other groups. The third “physiological/morphological” group L3 had a strong to intermediate expression of all physiological traits as well as SRL, RLD and SLA and a low foliar P concentration. It grouped forage pea, clover and hairy vetch. The fourth “morphological” group L4, encompassing phacelia, presented low expression of P-mining traits, except for an intermediate carboxylate release, a high fine root percentage and the highest RLD and SRL observed in all groups. Finally the “physiological/mining” group L5, encompassing faba bean, had the lowest SRL and fine root percentage associated with a strong expression of all physiological traits with in particular a strong decrease in rhizosheath pH. Overall the different strategies resulted in similar levels of total P uptake.

In the Calcaric Cambisol, the first “morphological” group C1 was mainly characterised by a high root length density and surface area, an intermediate SRL and a low expression of physiological traits. It encompassed naked oat, forage pea and forage radish. The second “morphological/physiological” group C2, encompassing hairy and common vetch had a significantly higher SLA, stronger rhizosheath acidification, intermediate PME activity in the rhizosheath and higher SRL. The third “intermediate” group C3 encompassed common oat, buckwheat, lentil, phacelia, white mustard and clover. It presented the highest percentage of fine roots, intermediate values of SRL, SLA and the highest foliar P. It also presented the highest value of carboxylate release, while not significantly different from the chemical and morphological groups. Finally the fourth “physiological” group C4, encompassing faba bean, presented the highest PME activity in the rhizosheath, and second-highest carboxylate release. It also presented the lowest fine root percentage and lowest SRL. Overall, these different strategies once again resulted in similar levels of total P uptake.

Table 3 : Mean values \pm standard error (n= 4 to 11) and analyses of variance of phosphorus- acquisition traits and rhizosphere processes per cluster

Soil type	Cluster	TCE	PME	Δ pH	RLD	FR	SRL	SLA	Leaf [P]	Leaf [Mn]
Retisol	L1:Physiological/exudation	41.6 \pm 5.3	0.026 \pm 0.004	-0.04 \pm 0.04	0.09 \pm 0.01	0.81 \pm 0.02	29.0 \pm 4.2	46.1 \pm 4.2	2.14 \pm 0.19	47 \pm 5
	L2:Intermediate/morphological	10.8 \pm 1.3	0.017 \pm 0.001	0.11 \pm 0.04	0.20 \pm 0.02	0.85 \pm 0.01	14.4 \pm 1.6	31.5 \pm 2.7	3.20 \pm 0.20	166 \pm 53
	L3:Physiological/morphological	24.1 \pm 4.8	0.036 \pm 0.007	-0.16 \pm 0.05	0.27 \pm 0.03	0.77 \pm 0.02	38.2 \pm 5.4	62.6 \pm 2.6	1.78 \pm 0.17	88 \pm 9
	L4:Morphological	19.5 \pm 2.9	0.020 \pm 0.002	0.03 \pm 0.09	0.33 \pm 0.02	0.87 \pm 0.03	65.4 \pm 8.8	24.6 \pm 4.3	4.17 \pm 0.66	36 \pm 10
	L5 : Physiological/mining	26.5 \pm 5.1	0.038 \pm 0.007	-0.29 \pm 0.05	0.21 \pm 0.02	0.38 \pm 0.03	6.8 \pm 1.4	60.5 \pm 8.8	3.35 \pm 0.23	75 \pm 4
	p value	<.0001	0.0002 ^b	<.0001	<.0001	0.0013 ^b	<.0001	<.0001 ^b	<.0001	0.0053
Calcaric Cambisol	C1 : Morphological	9.6 \pm 2.1	0.011 \pm 0.001	-0.05 \pm 0.01	0.46 \pm 0.02	0.77 \pm 0.02	17.3 \pm 2.4	31.2 \pm 3.9	1.90 \pm 0.22	52 \pm 9
	C2:Morphological/Physiological	17.0 \pm 3.2	0.019 \pm 0.004	-0.22 \pm 0.02	0.29 \pm 0.05	0.72 \pm 0.03	35.4 \pm 3.5	71.4 \pm 7.3	2.16 \pm 0.25	125 \pm 15
	C3:Intermediate	25.0 \pm 7.1	0.010 \pm 0.001	-0.07 \pm 0.01	0.22 \pm 0.02	0.82 \pm 0.01	28.9 \pm 3.9	40.8 \pm 3.9	2.91 \pm 0.22	135 \pm 15
	C4:Physiological	24.8 \pm 13.9	0.022 \pm 0.001	-0.05 \pm 0.03	0.29 \pm 0.05	0.32 \pm 0.02	8.1 \pm 1.1	57.6 \pm 5.1	1.87 \pm 0.34	295 \pm 34
	p value	0.0169	0.0079 ^b	<.0001 ^b	<.0001 ^b	0.0001 ^b	0.0003	0.0002 ^b	0.0161	<.0001

^b:Kruskall-Wallis test; FR: Fine root percentage (<0.5 mm diameter), Leaf [Mn]: Leaf manganese concentration ($\mu\text{g Mn g}^{-1}$), Leaf [P]: Leaf phosphorus concentration (mg P g^{-1}), PME : Phosphomonoesterase activity ($\mu\text{g nitrophenol g}^{-1} \text{hour}^{-1}$), RLD: Root length density (cm cm^{-3}), SLA: Specific leaf area ($\text{mm}^2 \text{mg}^{-1}$), SRL: Specific root length (m g^{-1}), TCE: Total carboxylate release rate ($\mu\text{mol g root}^{-1}\text{hour}^{-1}$), ΔpH : Change in rhizosphere pH.

4.3.3. Modelling trait-combination effects on P acquisition

As we observed a similar P uptake among strategies, we investigated overall relationships between P uptake and factors involved in group formation with GLM (Table 4). PLS PM were also used to highlight the combinations of traits and type of trait best predicting P uptake (Figure 15). In the Retisol, the best model fit was obtained with fine root percentage, leaf Mn concentration and PME activity as predictors, while a similar fit was also achieved without PME activity. Fine root percentage was the best predictor when used alone, however, it performed worse than models incorporating multiple traits. While offering a lower fit when incorporating multiples traits, SLA was the second-best predictor, being in itself correlated with multiple morphological and physiological traits. The PLS PM approach ($R^2 = 0.5047$) produced similar results, with PME activity in the rhizosheath being the best single predictor in the physiological component, followed by rhizosheath acidification, and fine root percentage being the best predictor in the morphological component, followed by root surface area. Similar weights were associated with the physiological (correlation value =0.37) and morphological (correlation value =0.32) components in this soil with a low predicting ability of the aboveground component leading to its removal.

Table 4 : Selected models fitted to phosphorus uptake

Soil type	Models	AICc	Δ AICc
Retisol	FR + Leaf [Mn] + PME	-18.8	0.0
	FR + Leaf [Mn]	-17.4	1.4
	FR	-16.5	2.3
	SLA	-14.5	4.3
	PME	-12.5	6.2
	Leaf [Mn]	-8.2	10.6
Calcaric Cambisol	FR + Δ pH + SLA + RSA	-43.4	0.0
	Δ pH + SLA + RSA	-39.5	3.9
	FR + SLA + RSA	-39.5	3.9
	SLA	-35.1	8.4
	RSA	-30.7	12.7
	FR	-25.8	17.6
	Δ pH	-23.4	20.0

Sorted from best fit to worst. AICc: second order Aikake's information, Δ AICc: Difference between AICc model and lowest AICc observed. FR: Fine root percentage (<0.5mm ϕ), Leaf [Mn]: Leaf manganese concentration, PME: Phosphomonoesterase activity, RSA: Root surface area, SLA: Specific leaf area, Δ pH: change in rhizosheath pH

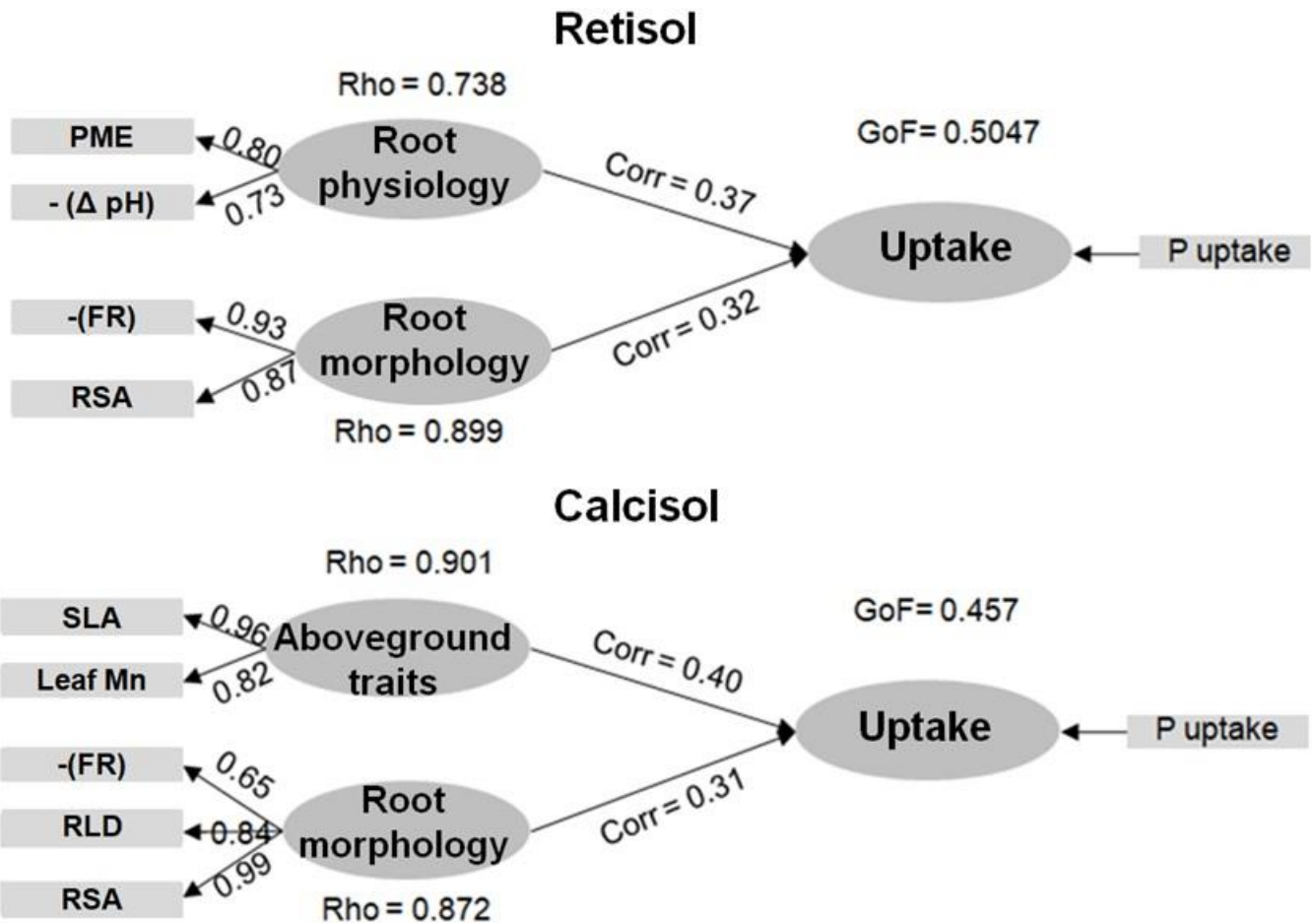


Figure 15 : Partial least square- path model predicting phosphorus uptake

Based on three latent variables, namely root physiology, root morphology and aboveground traits in two soils. Corr is the correlation effect, Rho the Dilon-Golstein coefficient. Variables negatively transformed indicated by - (variable). GoF= Goodness of Fit of the model. ΔpH: change in rhizosheath pH; FR: percentage roots with diameters less than 0.5 mm; Leaf Mn: foliar manganese concentration; PME: acid phosphomonoesterase activity; RLD: root length density; RSA: root surface area; SLA: specific leaf area.

In the Calcaric Cambisol, the best model fit was achieved with the combination of SLA, root surface area, fine root percentage and pH modification in the rhizosheath. Purely morphological models had a significantly poorer fit than models incorporating pH, while rhizosheath acidification alone was a poor predictor. Excluding correlated traits, the best predictors were SLA and root surface area (while not significantly different from SLA+root surface area+change in rhizosheath pH). SLA was the best predictor for single-trait models. PLS PM ($R^2 = 0.457$) underlined the importance of the morphological component (correlation value =0.31) and aboveground component (correlation value =0.40), while the physiological one was removed due to its poor predicting ability. SLA was the most impactful contributor for the aboveground component. For the morphological component it was root surface area.

4.3.4. Differences among trait variation and tradeoffs per soil type

Trait expressions were significantly different between soil types, with very uneven trait plasticity among species (Table 5). PME activity and carboxylate release were overall significantly lower in Calcaric Cambisol. In contrast, root length density and surface area were higher overall in this soil, especially for Fabaceae and Brassicaceae. Covariation between morphological P-acquisition traits and rhizosphere processes were observed less in Calcaric Cambisol such as the correlation between root surface area and PME and rhizosheath acidification or RLD and carboxylate release observed in Retisol which were not observed in this soil. Positive covariations such as between PME activity in the rhizosheath, carboxylate release and rhizosheath acidification were also not observed in this soil. Similarly, SLA was correlated with change in rhizosheath pH, PME activity and carboxylate release in Retisol, but only with change in rhizosheath pH in Calcaric Cambisol.

Table 5: Average trait value ± standard error and analyses of variance per species and per soil

Soil	Species	FR	Leaf [Mn]	Leaf [P]	PME	RLD	RSA	SLA	SRL	TCE	Δ pH
Retisol	<i>Avena nuda</i>	0.79±0.02	48±16	3.58±0.10	0.020±0.002	0.14±0.03	60±11	32.9±2.3	10.8±3.1	24.2±15.0	0.19±0.03
	<i>Avena strigosa</i>	0.79±0.02	144±31	2.90±0.17	0.016±0.001	0.27±0.09	103±29	33.0±2.6	14.7±1.9	10.4±2.6	0.01±0.03
	<i>Brassica carinata</i>	0.83±0.02	72±4	2.14±0.19	0.015±0.002	0.18±0.02	70±9	27.4±4.9	13.9±1.4	17.1±2.4	0.01±0.05
	<i>Fagopyrum esculentum</i>	0.88±0.01	456±121	3.79±0.50	0.020±0.001	0.21±0.04	66±12	46.3±5.8	17.2±7.0	6.8±2.3	0.10±0.10
	<i>Lens culinaris</i>	0.78±0.01	47±8	2.52±0.16	0.025±0.001	0.07±0.02	38±11	54.9±2.8	42.8±4.5	48.2±10.1	-0.04±0.09
	<i>Pisum sativum</i>	0.74±0.02	45±10	1.16±0.07	0.032±0.005	0.22±0.07	126±42	66.2±3.1	24.9±7.9	29.5±12.5	-0.13±0.03
	<i>Phacelia tanacetifolia</i>	0.87±0.03	40±12	4.79±0.32	0.020±0.002	0.27±0.07	96±29	25.3±4.0	65.4±8.9	23.7±5.0	-0.06±0.02
	<i>Raphanus sativus</i>	0.68±0.22	36±9	3.05±0.40	0.013±0.001	0.17±0.07	66±15	22.7±3.6	19.5±7.5	13.0±2.4	0.27±0.04
	<i>Sinapis alba</i>	0.9±0.020	34±2	2.04±0.13	0.018±0.001	0.11±0.03	34±5	32.1±7.1	17.5±5.2	25.1±5.4	-0.08±0.05
	<i>Trifolium alexandrinum</i>	0.86±0.02	106±12	2.13±0.22	0.027±0.002	0.23±0.04	92±19	59.1±3.3	48.3±11.1	22.8±2.9	-0.29±0.03
	<i>Vicia faba</i>	0.38±0.02	75±4	3.35±0.23	0.037±0.005	0.21±0.01	191±19	55.2±8.2	6.5±1.1	25.2±3.8	-0.31±0.04
	<i>Vicia sativa</i>	0.79±0.01	64±7	1.82±0.25	0.053±0.021	0.12±0.03	66±19	54.6±5.4	34.6±9.0	33.0±7.4	-0.04±0.06
	<i>Vicia villosa</i>	0.72±0.02	100±15	1.74±0.24	0.033±0.004	0.32±0.06	183±45	63.8±7.3	38.1±5.3	32.5±13.9	-0.11±0.09
	<i>p value</i>	0.0002 ^a	0.0006 ^a	<0.0001	0.0026 ^a	0.0020	<0.0001	0.0004 ^a	<0.0001	0.0126	<0.0001
Calcaric Cambisol	<i>Avena nuda</i>	0.74±0.02	33 ±6	1.63±0.28	0.013±0.001	0.47±0.05	247±37	29.67±1.0	9.5±2.9	7.9±4.5	-0.03±0.01
	<i>Avena strigosa</i>	0.82±0.03	148±17	2.69±0.32	0.013±0.001	0.30±0.06	127±35	39.6±2.1	28.7±9.3	13.8±4.6	-0.12±0.03
	<i>Brassica carinata</i>	0.83±0.01	92±31	2.03±0.18	0.008±0.001	0.37±0.03	146±14	21.8±3.4	15.4±1.2	12.0±2.3	-0.01±0.01
	<i>Fagopyrum esculentum</i>	0.84±0.01	67±3	3.56±0.27	0.009±0.001	0.27±0.09	105±38	44.4±8.7	16.1±3.3	6.2±1.9	-0.10±0.01
	<i>Lens culinaris</i>	0.72±0.01	252±30	2.91±0.15	0.013±0.002	0.22±0.06	127±42	58.7±7.4	34.6±4.7	23.1±1.4	-0.09±0.02
	<i>Pisum sativum</i>	0.66±0.01	93±27	0.99±0.22	0.009±0.003	0.34±0.07	223±46	64.9±6.2	24.5±3.1	14.6±3.8	-0.07±0.05
	<i>Phacelia tanacetifolia</i>	0.87±0.01	53±10	3.71±0.40	0.012±0.001	0.19±0.06	67±22	18.6±2.9	53.1±11.0	78.4±32.8	-0.03±0.02
	<i>Raphanus sativus</i>	0.85±0.02	47±12	1.99±0.29	0.012±0.001	0.44±0.06	163±28	17.5±2.3	27.8±2.7	9.9±1.9	-0.07±0.01
	<i>Sinapis alba</i>	0.86±0.01	91±22	1.71±0.33	0.009±0.003	0.12±0.03	44±9	31.8±2.8	10.9±2.1	17.6±3.3	-0.08±0.01
	<i>Trifolium alexandrinum</i>	0.84±0.02	180±30	4.19±0.36	0.010±0.001	0.13±0.02	57±11	60.4±1.8	28.2±8.8	12.8±2.1	-0.10±0.02
	<i>Vicia faba</i>	0.32±0.02	295±34	1.87±0.34	0.022±0.001	0.29±0.05	287±53	57.6±5.1	8.1±1.1	24.8±13.9	-0.05±0.03
	<i>Vicia sativa</i>	0.67±0.04	123±7	2.39±0.27	0.009±0.001	0.30±0.08	198±57	76.0±14.3	32.4±5.7	23.5±5.0	-0.26±0.04
	<i>Vicia villosa</i>	0.74±0.04	141±27	2.51±0.23	0.028±0.008	0.36±0.08	205±49	67.2±10.7	38.8±4.0	14.0±3.6	-0.15±0.02
	<i>p value</i>	<0.0001 ^a	<0.0001	<0.0001	0.0123 ^a	0.0276 ^a	0.0051 ^a	<0.0001	<0.0001	0.0169	0.0012 ^a

^aKruskal-Wallis test. Analysis performed on log transformed data. FR: Fine root percentage (<0.5 mm diameter), Leaf [Mn]: Leaf manganese concentration (µg Mn g⁻¹), Leaf [P]: Leaf phosphorus concentration (mg P g⁻¹), PME : Phosphomonoesterase activity (µg nitrophenol g⁻¹ hour⁻¹), RLD: Root length density (cm cm⁻³), RSA: Root surface area (cm²), SLA: Specific leaf area (mm² mg⁻¹), SRL: Specific root length (m g⁻¹), TCE: Total carboxylate release rate (µmol g root⁻¹hour⁻¹), ΔpH: Change in rhizosheath pH.

4.4. Discussion

4.4.1. Tradeoffs among functional traits to understand P acquisition of crop species

Multiple studies have demonstrated the importance of a single trait/few traits for P uptake such as root length density and surface area (Pang et al. 2010; Haling et al. 2018) or PME activity, carboxylate release or rhizosphere acidification (Lambers et al. 2006; Rose et al. 2010; Li et al. 2017; Nobile et al. 2019). However despite increased observations of coordination and tradeoffs among belowground resource-acquisition traits (Roumet et al. 2016; Ma et al. 2018), our understanding of trait interactions and tradeoffs that are central to P acquisition remains limited, especially in crop species (Wen et al. 2019). Our results highlight multiple covariation and tradeoffs between root morphological and physiological traits across a range of fast-growing crop species. Fine root percentage was negatively correlated with PME activity, rhizosheath acidification, and foliar Mn concentration, and marginally with carboxylate release. As foliar Mn can be used as a proxy for rhizosheath carboxylate concentration (Lambers et al. 2015; Pang et al. 2018; Yu et al. 2020), this suggests a greater relevance of physiological strategies associated with P mining for root systems with less fine root percentage. Our results are consistent with recent findings showing that roots with a large diameter present high expression of P-mining traits, while species with thinner fibrous roots express higher levels of morphological traits for P acquisition (Lyu et al. 2016; Wen et al. 2019), extending this observation to a different range of species (intermediate crops species) and context (north-western European soils with moderate P deficiency). Under P stress, plants can modify their root morphology through higher fine root percentage, increased SRL, root hair density and root length density to increase soil foraging at lower cost (Lambers et al. 2006; Shen et al. 2011; Haling et al. 2018). Root physiology can also be modified to increase P availability in the rhizosheath via increased carboxylate, enzyme and proton exudation. Synergistic action of these physiological traits is common, as shown for phytase activity and carboxylate release (Giles et al. 2017) and reinforces the coordination between PME activity in the rhizosheath, carboxylate release and rhizosheath acidification. A possible explanation for this observation is the release of organic P sorbed to soil particles by carboxylates for subsequent mineralisation (Clarholm *et al.*,2015). While

both physiological and morphological strategies should benefit P acquisition, tradeoffs between both are suggested to form along a cost / benefit balance due to the important investment they can represent for the plant (Pearse et al. 2006; Raven et al. 2018). Overall our results confirm and extend previous findings in other systems demonstrating multiple coordination and tradeoffs among morphological and physiological traits involved in P acquisition in crop species (Zhang et al. 2016; Giles et al. 2017; Li et al. 2017). Because physiological traits tend to allow greater access to legacy P pools compared with morphological traits, which allow access to available P pools (Lyu et al. 2016), this morphological/physiological tradeoff offers insight into how to enhance P acquisition and to increase exploitation of the diverse pools of P. Our results demonstrate a potential to select for species at different ends of the tradeoffs spectrum in multispecies systems to increase P acquisition, while reducing competition for resources. Our results constitute a novel contribution toward understanding P-acquisition in crop species, and also underline the need for a better understanding of how these trait combinations and tradeoffs are structured in a multi-traits space to form P-acquisition strategies. Such an understanding would offer the opportunity to better design multispecies cropping systems, notably with the main functions of improving P cycling and availability.

4.4.2. Phosphorus-acquisition strategies to design multispecies crops

Plants present very uneven trait plasticity when exposed to different levels of available P (Pearse et al. 2006; Haling et al. 2018; Wen et al. 2019). Uneven plasticity along tradeoffs and covariations suggests both a convergence toward common resource-acquisition strategies and divergence among strategies (Wendling et al. 2016; Li et al. 2017). Plants under P stress rely on resource-acquisitive strategies, either enhancing expression of root morphological traits associated with soil foraging, or modifying their physiological traits to mobilise poorly-available P (Rose et al. 2010; Teng et al. 2013; Lyu et al. 2016). A more complex association of morphological and physiological traits along a cost-benefit axis has also been suggested (Lynch 2015; Weemstra et al. 2016; Wen et al. 2019), prompting further investigations. A certain dichotomy was indeed observed between P-acquisition strategies relying more on physiological or more on morphological traits, corroborating the tradeoffs we observed. However, multivariate analysis highlighted a diversity of intermediate strategies, rather supporting the

hypothesis of a complex association of traits along a cost-benefit continuum for P acquisition. Our results offer important new insights into the complex and diverse range of P-acquisition strategies occupying a different trait space, and thus offering opportunities for complementarities in resource acquisition. In both soils, similar extreme strategies could be identified on both ends of a morphological/physiological spectrum. On the morphological end, a strategy could be identified, presenting a low expression of physiological indicators and high root length density/fine roots percentage, mainly encompassing Brassicaceae and Poaceae. This strategy is probably oriented toward soil scavenging, as an important proportion of fine roots can be a way to forage soil at a low cost, while important root length density similarly denotes an important exploration of a given soil volume (Lynch 2015; Yuan et al. 2016; Ma et al. 2018). Similar groupings of species constituting this group (notably Indian mustard, radish, and oat) have been observed before, based on morphological traits, and proposed to be an intermediate strategy between very resource-acquisitive species such as phacelia, and more conservatives ones such as faba bean (Wendling et al. 2016). On the other end of the spectrum, the several physiological groups, mainly comprising Fabaceae, presented high expressions of all physiological traits with overall lower fine root percentage and SRL. High rhizosphere phosphatase activity, carboxylate release and rhizosphere acidification indicated strategies potentially increasing P availability via mineralisation of organic P, ligand exchange and dissolution of precipitated phosphates, respectively (Hoffland et al. 1989; Jones et al. 2003). While our results confirm the greater effect of Fabaceae on rhizosphere properties compared with other phylogenetic groups (Maltais-Landry 2015), a diverse spectrum of strategies was observed, also within Fabaceae. Two to three clusters per soil expressed intermediate to high values of physiological indicators, and were comprised mainly, but not only of Fabaceae. These groups differed in their expression of morphological traits, especially RLD, fine roots percentage and SRL. This corroborates the existence of a spectrum of P-acquisition strategies combining scavenging and mining processes in intermediate crops. These contrasting strategies, interestingly, did not differ significantly in their P uptake, indicating a potential for maximising P acquisition in multispecies systems via mixing strategies accessing diverse P pools in soils (Lyu et al. 2016; Li et al. 2017) and as such less competition for resources. Such complementarities in resource acquisition among plant species has been suggested as an explanation for the positive effects of functional diversity

(Petchey and Gaston 2006; Faucon et al. 2017). Functionally more diverse systems indeed outperform single species for P uptake (Li et al. 2014; Xue et al. 2016). Overall, our results could lead to enhanced P cycling efficiency and P availability in multispecies cropping systems via better designs exploiting more of the total P stock including the pool of legacy P (Richardson et al. 2011). Functional diversity effects on P availability and uptake are not well known however (Huang et al. 2019), and variable shifts in root functional traits and plant P-acquisition strategy should be expected in multispecies conditions (Li et al. 2014). Hence, there is a need for further studies of functional diversity effects on P availability and P cycling. Perspectives include examining functional diversity effect on P uptake and P availability in systems combining different P-acquisition strategies and in different soil and climate contexts. Other traits potentially involved in P acquisition such as root hair length and density (Haling et al. 2013; Yuan et al. 2016), association with mycorrhizal fungi (Smith and Smith 2011; Campos et al. 2018) or rhizosphere bacteria (Richardson et al. 2011) should also be included in future studies to further refine the strategies identified.

4.4.3. Mediation of tradeoffs and P-acquisition strategies according to P forms in soil

Although expression of both P-mining traits and morphological traits are essential for P-acquisition, their costs in a specific context may define how plants express and mix their functional traits (Raven et al. 2018; Zhou et al. 2018). Phosphorus-mining traits in particular have proven to have their efficiency strongly impacted by soil type and soil P forms such as proposed for carboxylates in strongly P-sorbing soils (Wang and Lambers 2019). Indeed, we observed greater relevance and coordination of physiological traits in Retisol, while morphological traits were more expressed in Calcaric Cambisol. The modulation of P-acquisition strategies was further evidenced when modelling P uptake via GLM and PLS PM, providing insight into the relative importance of trait type. Phosphorus uptake was best predicted by root morphological traits and aboveground traits in Calcaric Cambisol, and a mixture of root morphological and physiological traits in Retisol. While several single trait correlations with P uptake were previously observed (Pearse et al. 2006; Lambers et al. 2006; Rose et al. 2010; Wen et al. 2017) our results complement our incomplete knowledge of coordination between physiological and morphological responses as well its mediation by soil type and soil P forms (Lyu et al. 2016). At low concentration, exudates may not be in a range

relevant to significantly impact P uptake in Calcaric Cambisol, or they may have their efficiency impacted by soil characteristics. Adverse effects impacting P mobilisation via carboxylate exudation have indeed been observed, because of “Ca-aided co-adsorption” when released at low concentration, with low amounts of carboxylates increasing adsorption of calcium ions, in turn increasing adsorption of P (Duputel et al. 2013). The higher acid buffering capacity of the Calcaric Cambisol might also have impacted the efficiency of rhizosphere acidification as a P-acquisition trait, further decreasing the potential benefits of physiological traits in this soil. Overall, the strong soil mediation observed offers precious insights into P acquisition. As mentioned, morphological and physiological traits benefit P-acquisition from different P pools, offering opportunities for enhanced P cycling in multispecies systems, notably by exploiting the pool of legacy P. However, by reinforcing the importance of plant-soil interactions, our results suggest that improved designs for enhanced P benefits should rely on different processes and strategies in different soils. Some processes such as P mobilisation should be less relevant in some contexts (Maltais-Landry et al. 2014), rather suggesting reliance on a mixture of resource-acquisitive strategies (Wendling et al. 2016). To be able to improve P benefits of intermediate crops, further efforts are needed to investigate the stability and relevance of the tradeoffs and strategies involved in P-acquisition for a range of soil types and associated P forms and availability.

4.5. Conclusions

Enhancing P cycling and availability in cultivated systems via ecological intensification requires a better understanding of tradeoffs and strategies involved in P acquisition. Morphological and physiological traits comprised diverse P-acquisition strategies structured along an axis of trait covariation and tradeoffs. Our results demonstrate tradeoffs between thicker and thinner roots, exhibiting more physiological traits and morphological traits, respectively. Reliance on either morphological or physiological traits and combinations thereof for P acquisition were underlined by model approaches, and strongly mediated by soil P forms. The multiple P-acquisition strategies with similar P uptake observed indicates the potential for positive effects of functional diversity via complementarities in resource acquisition, and thus offers insights into mixture designs for improved P benefits. Finally, varied tradeoffs and strategy plasticity amongst soil with different P forms highlighted the importance of considering soil-plant interactions when attempting to improve our understanding of resource-acquisition strategies. Further studies should investigate trait and strategy plasticity across a gradient of functional diversity and various soil properties to help us select for locally optimized crop services.

Authors' Contributions

Nicolas Honvault carried out the experiment and wrote the manuscript with support from Michel-Pierre Faucon, David Houben and Hans Lambers. Stéphane Firmin and Cécile Nobile helped process the data and perform the analyses. All authors contributed to the final version of the manuscript.

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CHAPTER 2

5. INTERACTIONS BETWEEN BELOWGROUND TRAITS AND RHIZOSHEATH FUNGAL AND BACTERIAL COMMUNITIES FOR PHOSPHORUS ACQUISITION

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Abstract

1. Despite the central role of plant-soil microorganism interactions in ecosystem functioning and plant nutrient acquisition, the influence of plant belowground traits on soil microbial communities and their consequences for phosphorus (P) acquisition remain underexplored.
2. Interactions between belowground functional traits involved in P acquisition and rhizosheath soil microbial communities that are quantified using PLFA (phospholipid fatty acid) and NLFA (neutral lipid fatty acid) analyses were investigated across eight herbaceous species in controlled conditions. Changes in rhizosheath microbial, resin-extractable and Olsen P concentrations were quantified.
3. Multiple correlations were observed between belowground functional traits and rhizosheath fungal and bacterial communities. The release rates of malate and malonate in particular were strongly linked with indicators of Gram-negative bacteria, which were correlated with changes in rhizosheath soil P concentration and plant P content.
4. Our results suggest that belowground traits related to plant-soil microorganism interactions may play an important role in plant P acquisition strategies, and underline the plausible role of carboxylates in these interactions. Incorporating these interactions in biogeochemical models would lead to better predicting power and understanding of P cycling and ecosystem functioning.

Key words: NLFA, phosphorus acquisition, plant-microorganism interactions, PLFA

List of abbreviations:

AMF: Arbuscular mycorrhizal fungi

AMF NLFA: Concentration of C16:1 ω 5 neutral lipids

Gram-negative NLFA: Sum of concentrations of cy17:0, C18:1 ω 7, cy:19:0 neutral lipids

Gram-positive NLFA: Sum of concentrations of i15:0, a15:0, i16:0, i17:0, a17:0 neutral lipids

Fungi NLFA: Concentration of C18:2 ω 6,9 neutral lipid

Bacterial NLFA: Sum of Gram-negative NLFA and Gram-positive NLFA

NLFA: Neutral lipid fatty acid

N/P AMF: Ratio of concentration of C16:1 ω 5 neutral lipid and C16:1 ω 5 phospholipid

Gram-negative PLFA: Sum of concentrations of cy17:0, C18:1 ω 7, cy:19:0 phospholipid

Gram-positive PLFA: Sum of concentration of i15:0, a15:0, i16:0, i17:0, a17:0 phospholipid

Fungi PLFA: Concentration of C18:2 ω 6,9 phospholipid

Bacterial PLFA: Sum of concentration of Gram-negative PLFA and Gram-positive PLFA

PLFA: Phospholipid fatty acid

PME: Phosphomonoesterase activity in the rhizosheath

RLD: Root length density

SRL: Specific root length

Δ microbial P: Difference in microbial P concentration between rhizosheath and bulk soil

Δ Olsen P: Difference in Olsen P concentration between rhizosheath and bulk soil

Δ resin P: Difference in resin P concentration between rhizosheath and bulk soil

Δ pH: Difference in pH between rhizosheath and bulk soil

5.1. Introduction

Phosphorus (P) is a key limiting nutrient in most ecosystems, playing a central role in ecosystem functioning via its influence on net productivity and carbon cycling (Nottingham et al. 2015). Understanding and managing natural ecosystems and agroecosystems functioning thus requires fully grasping the factors involved in P cycling, and notably the influence of plants on P cycling via plant P acquisition and later release. Multiple factors influence plant P acquisition such as plant P acquisition traits and strategies (Wendling et al. 2016), soil microbial communities (Smith and Smith 2011; Richardson et al. 2011), climate (Hou et al. 2018) and soil properties. Interactions between these factors, and especially between plant P acquisition traits and soil microorganisms are not fully understood (Legay et al. 2014; Bardgett et al. 2014; Sasse et al. 2018). Gaining a better understanding of the complex relationships between plants and soil-microorganisms is central in order to gain insights into plant P acquisition and in turn its effects on P cycling and ecosystem functioning.

A vast array of morphological, architectural, physiological and biotic traits can allow plants to acquire P from the multiple pools of P present in soils, either directly or indirectly through interactions with microbial communities (Campos et al. 2018). Spatially uneven pools of available inorganic P can be acquired by plant through exploring large soil volumes via P foraging traits such as specific root length (SRL), root length density (RLD) or root hair length and abundance (Fort 2013; Haling et al. 2018; Ma et al. 2018). P-mobilisation traits can also allow plants to acquire hardly available P pools. Precipitated inorganic P forms such as Ca/Mg-phosphate can be dissolved via proton release in the rhizospheric soil (White and Hammond 2008; Gómez-Suárez et al. 2020). Plant-released carboxylates, by competing with P for binding sites and complexing cations (e.g., Al, Fe and Ca), can also promote P desorption from soil constituents (Wang and Lambers 2020). Finally, organic P can be taken up after its mineralization by phosphomonoesterase, phosphodiesterase or phytases released by roots (Richardson et al. 2011; Nobile et al. 2019). In addition to plant P foraging and P mobilizing traits, the activity and composition of soil microbial communities also strongly influences plant P acquisition (Smith and Smith 2011). A considerable amount of P taken up by plants can indeed be through association with microbial groups such as arbuscular mycorrhizal fungi (AMF) (van der Heijden et al.

2008). Several microbial groups such as phosphate solubilising bacteria or AMF can contribute to the mobilisation of organic P and unavailable inorganic P present in soils, in turn influencing plant P uptake (Menezes-Blackburn et al. 2018). Plant P acquisition traits also play an important role in shaping these bacterial and fungal communities (Legay et al. 2014; Bardgett 2017), jointly influencing plant P-acquisition. Much, however, remains unknown on how belowground functional traits influence fungal and bacterial communities and the contribution of plant-soil microorganism interactions to P acquisition (Bardgett et al. 2014; Bardgett 2017; Sasse et al. 2018).

Several studies have underlined the considerable influence of plants on rhizospheric soil microbial communities' composition and activity, which in turn play a central role in P acquisition (Parniske 2008; Smith and Smith 2011; Richardson and Simpson 2011). At plant community level, grasses tend to be associated with Gram-positive bacteria, while legumes can influence Gram-negative bacteria abundance and fungal/bacterial ratio (Habekost et al. 2008; Lange et al. 2014). At individual level, root exudates and species identity have been observed to influence the abundance and composition of the bacterial and fungal community (Marschner et al. 2002; Micallef et al. 2009; Harrison and Bardgett 2010; Sasse et al. 2018). Carboxylates in particular can provide a carbon source for bacterial growth, and may therefore influence rhizospheric soil microbial community composition (Marschner et al. 2002; Paterson et al. 2007; Adeleke et al. 2017). Changes in rhizospheric soil microbial community composition can in turn influence plant P acquisition as between 1 and 50% of rhizosphere soil bacteria are P-solubilising bacteria (Kalayu 2019), potentially mobilising poorly-available P forms. Root morphology also interacts with microbial communities, such as observed in the case of plant growth promoting rhizobacteria synthesizing growth regulators influencing P foraging traits and plant P uptake (Spaepen and Vanderleyden 2011). Plants with fine, fibrous roots have moreover been observed to be less colonized by mycorrhizal fungi (Wen et al. 2019) while this association can strongly contribute to plant P acquisition in coarser roots (Smith and Smith 2011).

Due to the carbon cost associated with traits expression and microbial associations however, multiple trade-offs and interactions exist between P acquisition traits as well as their efficiency in acquiring P and rhizosphere soil microbial community composition and activity that remain to be fully explored. Lower carboxylate release rate has been

observed in mycorrhized roots for instance, although inconsistencies have also been noted (Ryan et al. 2012). The P-mobilizing efficiencies of carboxylates have moreover been proposed to be linked negatively with their metabolization rate by soil microorganisms (Wang and Lambers 2020), while increased microorganism abundance and activity can also contribute to P mobilisation, highlighting the need to better understand plant and microbial mechanisms for P mobilisation and their interactions in the rhizosphere (Richardson et al. 2011). Discrepancies among studies about the trade-off between root diameter and association with arbuscular mycorrhizal fungi and their contribution to P acquisition, also warrants further examination (Maherali 2014). Overall, the root functional traits playing a role in shaping fungal communities and their role in the complex plant-soil-microorganisms interactions involved in P acquisition remain unclear (Legay et al. 2014; Bardgett et al. 2014; Bardgett 2017).

The objective of this study was to examine the interactions between plant P-acquisition traits and microbial communities, and to assess their role and contribution to plant P acquisition. In order to examine plant-soil microorganism interactions, multiple belowground functional traits of eight cover crop species of two families were measured together with fungal and bacterial bio-indicators in their rhizosphere in two soil types. The interactions of plants P acquisition strategies with rhizosphere microbial communities via specific morphological and physiological traits and their consequences for P acquisition, as mediated by soil type, were investigated. We specifically investigated correlations between carboxylate release rate and fungal and bacterial communities to gain a better understanding of the role of carboxylate release in the complex plant-soil microorganism interactions involved in P-acquisition. We also examined trade-offs between root morphology and, in particular, fine root percentage and association with microbial communities and their consequences for P acquisition.

5.2. Material and methods

5.2.1. Experimental design

In order to examine the interactions between belowground functional traits involved in P-acquisition and fungal and bacterial communities, eight species (Brassicaceae: *Brassica carinata* A. Braun, *Raphanus sativus* L., *Sinapis alba* L.; Fabaceae: *Lens culinaris* Medik., *Pisum sativum* subsp. *Arvense* L., *Trifolium alexandrinum* L., *Vicia faba* L. and *Vicia sativa* L.) were grown under greenhouse conditions in two soil types. We selected species of two contrasting phylogenetic lineages with different traits in order to assess a gradient of belowground morphological and physiological P acquisition traits and understand how their diverging P acquisition strategies interact with microbial communities for P acquisition. Brassicaceae were selected alongside Fabaceae to assess their different P acquisition strategies and notably interactions with soil microorganisms, because Brassicaceae are predominantly non-mycorrhizal. Soils with contrasting characteristics (Table 6), a Retisol (formerly called Albeluvisol) and a Calcaric Cambisol (characterized by the presence of an A horizon developed over a calcareous substrate) (FAO 2014), were collected from cultivated fields from north-eastern France. In order to further reduce P availability, soils were collected after topsoil (0-5 cm) removal and mixed with washed sand (22% of the final weight). After mixing, soil P concentrations were of 19.8 mg Pkg⁻¹ and 16.7 mg kg⁻¹. Shoot P concentration of 0.8 to 3 mg P kg⁻¹ might indicate moderate P limitation (Table 7). Pots were randomly arranged with two individuals per species per pot (4.5 L pots), and 2.5 months growing period. Greenhouse conditions were 22°C during the day (14 hours) and 18°C at night. The experiment was carried out in triplicate, with eight species and two soil types for a total of 48 pots.

Table 6 : Chemical and physical soil characteristics

	% Clay (<0.002mm)	% Sand (>0.05mm)	pH KCl	Ca ^a (gkg ⁻¹)	N tot (mg kg ⁻¹)	CEC (cmol _c kg ⁻¹)	Olsen P ^c (mg kg ⁻¹)	Ca-P ^d (mg kg ⁻¹)
Calcaric Cambisol	30.7	22.0	7.9	44.7 ^b	1700	9.3	19.8	542
Retisol	22.3	5.4	7.4	2.60	1000	10.5	16.7	76.7
p value							NS	0.03

p value: Analysis of variance between soil for replicated conditions. NS: Not significant. Abbreviations: Ca: exchangeable calcium; N tot: total nitrogen; CEC: cation exchange capacity.

^aExtractant: Ammonium acetate 0.5 M, EDTA 0.02 M pH 4.65

^bCaCO₃ 56.4 %

^cAccording to Olsen (1954)

^dApatite phosphorus as defined in García-Albacete et al. (2012)

5.2.2. Plant measurements

5.2.2.1. Carboxylate release

At harvest, plants were carefully separated from the bulk soil, with care taken to avoid damage to the root system. Rhizosheath adhering to the root after shaking was collected (Pang et al. 2017) and stored at -20°C for further analysis. Carboxylate-release rate was measured by immersing the entire root system in 0.20 mM CaCl₂ for 1 hour under the same greenhouse conditions as used for plant growth after rinsing off any remaining soil (Yacoumas et al. 2020). Reversed-phase column liquid chromatography was then used to measure carboxylate concentrations in the extract, offering a good resolution for five carboxylates (citrate, fumarate, maleate, malate, malonate). Briefly, a C18 column with a 15 min elution time and a 1 mL min⁻¹ flow rate was used with an acid mobile phase of 93% 25 mM KH₂PO₄ at pH 2.5 and 7% (v/v) methanol (Cawthray 2003; Yacoumas et al. 2020). The total carboxylate-release rate was calculated as the sum of all mentioned carboxylates.

5.2.2.2. Morphological traits

The plants were severed at the root-shoot junction for further measurements on the root system. All Fabaceae were nodulated. The root systems were scanned while being immersed in pure water using an Epson Scanner perfection V800 (Regent Instruments Inc., Québec, Qc, Canada) to produce a 600 dpi image. Root length, root surface area and root length per diameter class were determined using WinRHIZO Regular software V.2016a (Regent Instruments Inc., Québec, Qc, Canada). Root length density (RLD) was calculated based on 4.5 L pot volume, and the percentage of fine roots was calculated based on the percentage of root length of roots with a diameter less than 0.5 mm (Elshafie et al. 2013; Wang et al. 2015). After drying at 60°C for 48 hours, root dry weight was measured and specific root length (SRL) was calculated.

5.2.2.3. Phosphorus content

All aboveground biomass was dried at 60°C for 48 hours and weighed, before being digested via acid digestion and a microwave heating treatment (Lange et al. 2016). Briefly, 0.1 to 0.2 g of dry biomass was digested for one hour in a Teflon vessel with 8 mL of 65% (v/v) HNO₃ and 2 mL of 36% (v/v) HCl. After filtration, digest P and manganese (Mn) concentration, a proxy for rhizosheath carboxylate concentration

(Pang et al. 2018), were determined by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Scientific XSERIES2, Beauvais, France). A certified reference (SRM1573) was used as external standard.

5.2.3. Soil analyses

Acid phosphatase activity in the rhizosphere soil (PME) was determined by measuring the production of *p*-nitrophenol from sodium *p*-nitrophenyl phosphate, as described in Tabatabai and Bremner (1969). Briefly, three sub-samples of 0.5 g dry soil per replicate were added to a modified universal buffer at pH 6.5 with 0.2 mL toluene and 1 mL substrate (*p*-nitrophenyl phosphate). After one hour at 37°C, the reaction was stopped with 4 mL 0.5M NaOH and 1 mL 0.5 M CaCl₂. After filtering to remove the soil from the solution, *p*-nitrophenol release was determined spectrophotometrically at 410 nm. Soil pH was measured in the rhizosphere and bulk soil on 2 g equivalent dry soil with a 1:10 water to soil ratio. Δ pH between rhizosphere and bulk soil was then calculated.

5.2.4. Phosphorus availability

Microbial P, resin P and Olsen P in the rhizosphere were extracted before inorganic P determination on filtered extracts using malachite green colorimetry at 610 nm (Ohno and Zibilske 1991). Olsen P was extracted with a 1:20 soil to solution ratio and a solution of NaHCO₃ at pH=8.5 (Olsen 1954). Microbial P was determined via a differential method between hexanol-fumigated and non-fumigated extracts (Bunemann et al. 2007). After initial preparation with 0.5 M NaHCO₃, anion-exchange strips were shaken for 16 h with 2 g equivalent dry soil and 30 mL deionised water. For each sample, three portions of soil were extracted, with or without addition of 1 mL hexanol as fumigant and with spiking of a known amount of inorganic P (KH₂PO₄). The anion-exchange strips (VWR, 551642S) were then rinsed with deionised water before P was extracted by shaking 16 h in 20 mL 0.5 M HCl. To compensate for sorption of the P released after fumigation, P recovery was calculated in spiked samples otherwise extracted identically to non-fumigated samples. Microbial P was calculated as the difference between fumigated and non-fumigated samples, corrected by the recovery after spiking. To examine change in rhizosphere P concentrations, Δ resin P, Δ microbial P and Δ Olsen P were calculated based on differences of concentration of resin P, microbial P and Olsen P between rhizosphere and bulk soil.

5.2.5. PLFA and NLFA determination

Frozen rhizosphere soil was freeze-dried for 48 hours. Any remaining root fragments were then manually removed before milling with an MM 300 Mixer Mill (Qiagen, City, USA). Phospholipid fatty acids (PLFAs) were then extracted and analysed by the procedure of Frostegård et al. (1993). The total lipid extraction procedure followed that described by Frostegård et al. (1991), which is based on the method by Bligh & Dyer (1959) as modified by White et al. (1979). For detailed information on the PLFA and NLFA extraction see supplementary material S1 (Appendix B). Specific PLFA and NLFA biomarkers, as highlighted in He et al. (2007), were summarised per main microbial group, that is bacteria, Gram-positive bacteria, Gram-negative bacteria, fungi and arbuscular mycorrhizal fungi. The NLFA/PLFA ratio of C16:1 ω 5 was calculated to account for possible bacterial interference when interpreting NLFA C16:1 ω 5 concentration as an indicator of AMF storage over growth, with possible interference being noted for a ratio less than 1. NLFA were used as indicators of recently dead bacterial necromass (Malosso 2004; Amir et al. 2008; Rinnan and Bååth 2009). The sum of Gram-negative NLFA and Gram-positive NLFA and the sum of Gram-negative PLFA and Gram-positive PLFA were calculated as indicators of total bacterial abundance (necromass and living biomass respectively). A fungal:bacterial biomass ratio was also calculated based on NLFA data, with the sum of the fungal NLFA C18:2 ω 6,9 and C16:1 ω 5 divided by the sum of the bacterial NLFAs.

5.2.6. Statistical analyses

All tests were performed with R v 3.6.0 at a significance level of 0.05. Differences between PLFA and NLFA and associated bacterial and fungal communities in the rhizosphere of species in the two soils were first examined via a principal component analysis (PCA). As two distinct clusters regrouping all species per soil type were identified on the PCA, further analysis was performed separately per soil type (supplementary material S2, Appendix B).

Interactions between traits, PLFA, NPLA and P-acquisition, as measured via total P content and change in rhizosphere P concentration (Resin P, Olsen P, Microbial P), were examined via either Pearson or Spearman correlation, according to the normality of the data. In order to identify the differences between plant belowground P acquisition traits and rhizosphere microbial indicators between the two families examined and their

contribution to the plant soil microorganisms interactions identified, comparison of variance and Kruskal-Wallis tests were performed between families for PLFA/NLFA indicators, belowground traits and P acquisition indicators. Differences between species per family were then examined via either comparison of variance or Kruskal-Wallis tests. When significant differences were observed at the family and species per family level interactions between traits, PLFA, NFLA and P-acquisition were investigated via either Spearman or Pearson correlation within the Fabaceae family.

To understand the combined influence of root traits and PLFA/NFLA on P acquisition and synthesise the multiple correlations observed to select the key variables involved, we used a partial least square path model (PLS PM) (Sanchez 2013). Three clusters of variables, or “latent variables” were defined: “belowground traits”, “PLFA/NFLA” and “P acquisition”. “Belowground traits” grouped change in rhizosphere pH, total carboxylate-release rate, citrate-release rate, fumarate release rate, maleate-release rate, malate-release rate, malonate-release rate, leaf Mn concentration, phosphomonoesterase activity in the rhizosphere, root surface area, specific root length, root length density and fine root percentage. “PLFA/NFLA” grouped Fungi PLFA, Gram-positive PLFA, Gram-negative PLFA, bacterial PLFA, AMF NLFA, Fungal NLFA, Gram-positive NLFA, Gram-negative NLFA, bacterial NLFA, NLFA fungal/bacterial ratio, total PLFA and total NLFA. “P-acquisition” grouped total P content, Δ Olsen P, Δ resin P and Δ microbial P. The “belowground traits” and “PLFA/NFLA” latent variables were then used to predict the “P acquisition” latent variable. To account for a possible influence of belowground traits on microbial communities, an effect of belowground traits on the NLFA/PLFA component was specified in the model structure. Inverting the structure with an effect of NLFA/PLFA on belowground traits yielded the same results. To avoid dependency effects, replicates were averaged per species. Unidimensionality of latent variables, as well as cross-loadings of variables within each latent variable were verified. To ensure positive correlations in a latent variable, the sign of some variables was modified. A first model incorporating all variables was used to select the most representative variables. Variables were selected based on their loadings (greater than 0.7) (Sanchez 2013). The PLFA/NFLA component was thus renamed as NFLA, after all PLFA indicators were eliminated when selecting variables. Models were produced with the “plsrm” package version 0.4.9 and evaluated based on their goodness of fit (GOF).

5.3. Results

5.3.1. Differences among belowground traits, microbial communities and P acquisition indicators between Brassicaceae and Fabaceae

5.3.1.1. Differences between rhizosheath microbial communities between families

Multiple rhizosheath PLFA and NLFA concentration were significantly different between Brassicaceae and Fabaceae (Table 8). AMF NLFA rhizosheath concentration was higher for Fabaceae, while Brassicaceae had lower AMF NLFA rhizosheath concentration with ratios of concentration of C16:1 ω 5 neutral lipid and concentration of C16:1 ω 5 phospholipid inferior to one in both soils. In the Retisol Gram-negative NLFA concentration and bacterial NLFA concentration were higher in the rhizosheath of Fabaceae than Brassicaceae.

5.3.1.2. Differences between belowground traits between families

Malonate and malate carboxylate exudation rates were higher for the Fabaceae family in both soils, while the total carboxylate exudation rate was higher for Fabaceae in the Retisol (Table 9). Leaf manganese concentration was also higher overall for Fabaceae. Brassicaceae had higher fine root percentage than Fabaceae in both soils.

5.3.1.3. Differences between P acquisition indicators between families

Fabaceae had higher P content overall, with shoot P concentration between 0.8 mg P kg⁻¹ and 3 mg P kg⁻¹ (Table 7). In both soils the Δ Resin P and the Δ Olsen P were higher for Brassicaceae.

Table 7 : Change in rhizosheath soil P concentration and shoot P concentration \pm standard error and analyses of variance per family and species

Soil	Species	Δ Resin P	Δ Microbial P	Δ Olsen P	Shoot [P]		
Calcaric Cambisol	Brassicaceae	<i>Brassica carinata</i>	-8.1 \pm 2.0	11.6 \pm 4.0	1.2 \pm 2.4	2.3 \pm 0.1	
		<i>Raphanus sativus</i>	-7.6 \pm 1.4	-7.3 \pm 3.4	4.0 \pm 1.3	2.1 \pm 0.3	
		<i>Sinapis alba</i>	-3.1 \pm 4.7	6.2 \pm 1.7	2.2 \pm 1.5	1.7 \pm 0.1	
		<i>p value</i>	NS	<0.01	NS	0.04	
	Fabaceae	<i>Lens culinaris</i>	-9.8 \pm 1.9	1.8 \pm 6.5	3.8 \pm 1.9	2.3 \pm 0.0 ¹	
		<i>Pisum sativum</i>	-12.3 \pm 3.7	4.3 \pm 7.1	-6.2 \pm 1.6	1.3 \pm 0.0 ¹	
		<i>Trifolium alexandrinum</i>	-10.4 \pm 3.0	10.8 \pm 14.3	2.4 \pm 2.9	3.0 \pm 0.1	
		<i>Vicia faba</i>	-13.6 \pm 1.9	13.9 \pm 8.2	0.3 \pm 2.6	1.8 \pm 0.1	
		<i>Vicia sativa</i>	-16.8 \pm 2.1	3.3 \pm 5.3	-7.6 \pm 1.2	1.2 \pm 0.0 ¹	
		<i>p value</i>	NS	NS	<0.01	<0.01 ^a	
	<i>p value</i>		<0.01	NS	0.04	NS	
	Retisol	Brassicaceae	<i>Brassica carinata</i>	0.4 \pm 4.5	-2.0 \pm 2.6	-3.8 \pm 1.7	2.3 \pm 0.1
			<i>Raphanus sativus</i>	-0.7 \pm 1.4	-0.7 \pm 4.1	-1.6 \pm 2.0	3.0 \pm 0.2
			<i>Sinapis alba</i>	-2.3 \pm 1.2	2.9 \pm 4.4	-2.4 \pm 3.5	1.4 \pm 0.0 ¹
<i>p value</i>			NS	NS	NS	<0.01	
Fabaceae		<i>Lens culinaris</i>	-4.3 \pm 2.5	3.9 \pm 1.0	-5.5 \pm 1.6	2.3 \pm 0.0 ¹	
		<i>Pisum sativum</i>	-8.6 \pm 1.4	3.5 \pm 3.1	-10.3 \pm 0.9	0.8 \pm 0.0 ¹	
		<i>Trifolium alexandrinum</i>	0.9 \pm 0.7	-4.5 \pm 3.9	-2.5 \pm 3.2	1.4 \pm 0.0 ¹	
		<i>Vicia faba</i>	-8.4 \pm 1.6	5.4 \pm 3.9	-8.2 \pm 1.5	2.1 \pm 0.1	
		<i>Vicia sativa</i>	-10.8 \pm 1.4	5.2 \pm 4.9	-11.6 \pm 0.3	2.0 \pm 0.0 ¹	
		<i>p value</i>	<0.01	NS	0.02 ^a	<0.01 ^a	
<i>p value</i>		<0.01	NS	<0.01	NS		

1 : Inferior to 0.1. Analyses of variance and a Kruskal-Wallis test per species within families and between families. NS : not significant; Abbreviations: Δ Resin P: Change in rhizosheath resin extracted P concentration (mg kg⁻¹); Δ Microbial P: Change in rhizosheath microbial P concentration (mg kg⁻¹); Δ Olsen P: Change in rhizosheath Olsen P concentration (mg kg⁻¹); Shoot [P] : P concentration in the shoot biomass (mg P g⁻¹).

Table 8 : Mean rhizosheath PLFA and NLFA concentration ± standard error and analyses of variance per family and species

Soil	Family	Species	PLFA					NLFA					N/P
			Fungi	Gram +	Gram -	Bacterial	AMF	Fungi	Gram +	Gram -	Bacterial	AMF	
Calcaric Cambisol	Brassicaceae	<i>Brassica carinata</i>	1.4± 0.1	4.8± 0.2	4.3± 0.2	9.1± 0.4	0.4± 0.1	1.3± 0.1	1.2± 0.1	3.3± 0.2	4.5± 0.3	0.4± 0.1	
		<i>Raphanus sativus</i>	1.3± 0.3	4.1± 0.5	5.0± 0.1	9.1± 0.5	0.3± 0.0*	1.0± 0.1	1.1± 0.1	2.5± 0.2	3.6± 0.3	0.4± 0.1	
		<i>Sinapis alba</i>	1.6± 0.3	4.8± 0.6	4.3± 0.6	9.1± 1.1	0.6± 0.2	1.3± 0.2	1.4± 0.2	3.6± 0.6	5.1± 0.7	0.7± 0.3	
		<i>p value</i>	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
	Fabaceae	<i>Lens culinaris</i>	1.5± 0.1	4.7± 0.2	5.0± 0.2	9.8± 0.4	9.2± 1.0	2.0± 0.4	1.2± 0.1	7.6± 1.6	8.9± 1.6	7.1± 0.8	
		<i>Pisum sativum</i>	1.5± 0.1	4.5± 0.3	4.5± 0.4	9.0± 0.7	5.8± 0.4	1.4± 0.2	1.2± 0.1	4.9± 0.6	6.1± 0.6	4.6± 0.3	
		<i>Trifolium alexandrinum</i>	3.5± 1.2	4.5± 0.3	6.9± 1.0	11.3± 1.2	1.8± 1.0	1.3± 0.1	1.4± 0.2	4.0± 1.1	5.3± 1.2	1.4± 0.5	
		<i>Vicia faba</i>	1.1± 0.1	4.3± 0.4	4.8± 0.9	9.2± 1.2	4.9± 1.9	1.7± 0.2	1.1± 0.1	5.5± 0.9	6.5± 1.0	4.3± 1.2	
		<i>Vicia sativa</i>	1.6± 0.3	4.7± 0.3	3.7± 0.3	8.4± 0.2	7.8± 0.7	1.3± 0.1	1.3± 0.1	4.0± 0.7	5.3± 0.7	5.3± 0.6	
		<i>p value</i>	NS	NS	NS	NS	<0.01	NS	NS	NS	NS	<0.01	
		<i>p value</i>	NS	NS	NS	NS	0.02	NS	NS	NS	NS	0.03	
	Retisol	Brassicaceae	<i>Brassica carinata</i>	1.1± 0.1	3.7± 0.3	3.2± 0.3	6.9± 0.6	0.4± 0.0*	1.6± 0.1	1.9± 0.3	3.4± 0.2	5.2± 0.5	0.6± 0.0*
			<i>Raphanus sativus</i>	1.4± 0.2	3.7± 0.1	3.0± 0.2	6.8± 0.2	0.3± 0.0*	1.5± 0.3	1.1± 0.2	2.2± 1.0	3.3± 1.0	0.4± 0.0*
			<i>Sinapis alba</i>	1.0± 0.1	3.4± 0.3	2.9± 0.2	6.3± 0.4	0.4± 0.1	1.0± 0.4	1.5± 0.1	2.3± 0.7	3.8± 0.8	0.6± 0.1
<i>p value</i>			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
Fabaceae		<i>Lens culinaris</i>	1.2± 0.2	3.5± 0.2	3.5± 0.4	6.9± 0.6	1.1± 0.3	1.8± 0.2	1.8± 0.1	5.1± 0.4	6.8± 0.3	1.5± 0.3	
		<i>Pisum sativum</i>	4.4± 2.7	4.7± 0.0*	7.1± 2.9	11.8± 2.9	3.8± 0.9	1.4± 0.1	1.3± 0.1	5.1± 1.0	6.4± 1.0	3.1± 0.5	
		<i>Trifolium alexandrinum</i>	2.8± 1.7	3.9± 0.2	6.6± 2.2	10.5± 2.3	0.5± 0.2	1.3± 0.2	1.1± 0.2	3.5± 0.4	4.6± 0.5	0.6± 0.1	
		<i>Vicia faba</i>	1.3± 0.1	4.1± 0.2	3.8± 0.2	7.9± 0.4	4.5± 0.1	1.9± 0.1	1.7± 0.1	6.7± 0.4	8.4± 0.5	4.2± 0.1	
		<i>Vicia sativa</i>	1.4± 0.1	3.2± 0.1	2.9± 0.4	6.1± 0.4	2.9± 0.2	1.3± 0.0*	1.6± 0.1	4.9± 0.3	6.6± 0.3	3.7± 0.2	
		<i>p value</i>	NS	<0.01	NS	0.04 ^a	<0.01	NS	<0.01	0.03	0.01	<0.01	
<i>p value</i>	NS	NS	NS	NS	0.02 ^a	NS	NS	0.02	0.03	NS			

Concentration in µg g⁻¹ dry soil. *: inferior to 0.05. Analyses of variance and ^aKruskall-Wallis test per species within families and between families. NS : not significant; Abbreviations : PLFA : Phospholipid fatty acid; NLFA : Neutral lipid fatty acid; AMF: Concentration of C16:1ω5 neutral lipid; Fungi: Concentration of C18:2ω6,9 phospholipid or neutral lipid; Gram + : Sum of concentration of i15:0, a15:0, i16:0, i17:0, a17:0 phospholipids or neutral lipids; Gram - : Sum of concentration of cy17:0, C18:1ω7, cy:19:0 phospholipids or neutral lipids; Bacterial : Sum of Gram-positive and Gram-negative bacteria phospholipids or neutral lipids concentration; N/P AMF : Ratio of concentration of C16:1ω5 neutral lipid and concentration of C16:1ω5 phospholipid.

Table 9 : Mean belowground traits ± standard error and analyses of variance per family and species

Soil	Family	Species	Δ pH	TCR	Citrate	Malate	Malonate	Leaf [Mn]	PME	RSA	RLD	FR
Calcaric Cambisol	Brassicaceae	<i>Brassica carinata</i>	-0.01±0.01	12.0±2.3	10.8±2.0	1.1±0.3	0.0±0.0	92±31	0.008±0.001	146±14	3700±300	83±1
		<i>Raphanus sativus</i>	-0.07±0.01	9.9±1.9	7.7±1.4	2.0±1.4	0.0±0.0	47±12	0.012±0.001	163±28	4400±600	85±2
		<i>Sinapis alba</i>	-0.08±0.01	17.6±3.3	16.3±3.0	1.2±0.3	0.0±0.0	91±22	0.009±0.003	44±9	1200±300	86±1
		<i>p value</i>	<0.01	NS	NS	NS	<0.01 ^a	NS	NS	<0.01	<0.01	NS
	Fabaceae	<i>Lens culinaris</i>	-0.09±0.02	23.1±1.4	11.9±2.7	3.9±1.0	7.2±2.2	252±30	0.013±0.002	127±42	2200±600	72±1
		<i>Pisum sativum</i>	-0.07±0.05	14.6±3.8	9.5±2.0	4.3±1.8	0.4±0.1	93±27	0.009±0.003	223±46	3400±700	66±1
		<i>Trifolium alexandrinum</i>	-0.1±0.02	12.8±2.1	10.3±2.3	0.7±0.1	1.8±0.4	180±30	0.010 ±0.001	57±11	1300±200	84±2
		<i>Vicia faba</i>	-0.05±0.03	24.8±13.9	13.0±9.1	5.6±2.6	5.9±2.7	295±34	0.022±0.001	287±53	2900±500	32±2
		<i>Vicia sativa</i>	-0.26±0.04	23.5±4.9	14.3±4.9	3.6±1.1	5.4±0.8	123±7	0.009±0.001	198±57	3000±800	67±4
		<i>p value</i>	<0.01	NS	NS	NS	0.01 ^a	<0.01	<0.01	0.02 ^a	NS	<0.01 ^a
	<i>p value</i>	NS	NS	NS	<0.01 ^a	<0.01 ^a	<0.01 ^a	NS	NS	NS	<0.01 ^a	
Retisol	Brassicaceae	<i>Brassica carinata</i>	0.01±0.05	17.1±2.4	16.1±2.3	0.8±0.1	0.0±0.0	72±4	0.015±0.002	70±9	1800±200	83±2
		<i>Raphanus sativus</i>	0.27±0.04	13.0±2.4	11.5±1.8	1.3±0.7	0.0±0.0	36±9	0.013±0.001	66±15	2300±500	91±0*
		<i>Sinapis alba</i>	-0.08±0.05	25.1±5.4	23.6±5.1	1.4±0.3	0.0±0.0	34±2	0.018±0.001	34±5	1100±300	90±2
		<i>p value</i>	<0.01	NS	NS	NS	<0.01 ^a	0.01	NS	NS	NS	Ns
	Fabaceae	<i>Lens culinaris</i>	-0.04±0.09	48.2±10.1	25.6±4.2	9.5±2.4	12.7±4.9	47±8	0.025±0.001	38±11	700±200	78±1
		<i>Pisum sativum</i>	-0.13±0.03	29.5±12.5	9.5±2.4	18.0±9.2	1.5±0.7	45±10	0.032±0.005	126±42	2200±700	74±2
		<i>Trifolium alexandrinum</i>	-0.29±0.03	22.8±2.9	14.0±4.4	3.0±0.2	5.6±1.4	106±12	0.027±0.002	92±19	2300±400	86±2
		<i>Vicia faba</i>	-0.31±0.04	25.2±3.8	9.9±4.8	8.9±3.3	6.0±1.8	75±4	0.037±0.005	191±19	2100±100	38±2
		<i>Vicia sativa</i>	-0.04±0.06	33.0±7.4	16.1±2.4	8.3±4.4	8.3±3.3	64±7	0.053±0.021	66±19	1200±300	79±1
		<i>p value</i>	<0.01	NS	NS	NS	NS	<0.01	NS	0.01 ^a	NS	<0.01 ^a
	<i>p value</i>	<0.01	<0.01 ^a	NS	<0.01 ^a	<0.01 ^a	0.04	<0.01 ^a	NS	NS	<0.01 ^a	

*: inferior to 1%. Analyses of variance and ^aKruskall-Wallis test per species within families and between families. NS : not significant; Abbreviations: Δ pH : Change in rhizosphere pH; TCR: Total carboxylate release rate (μmol g root⁻¹ hour⁻¹); Citrate : Citrate-release rate (μmol g root⁻¹ hour⁻¹); Malate : Malate-release rate (μmol g root⁻¹ hour⁻¹); Malonate : Malonate-release rate (μmol g root⁻¹ hour⁻¹); Leaf [Mn] : Leaf manganese concentration (μg Mn g⁻¹); PME : Phosphomonoesterase activity in the rhizosphere (μg nitrophenol g⁻¹ hour⁻¹); RSA: Root surface area (cm²); RLD : Root length density (mm⁻³); FR: Fine root percentage (<0.5 mm diameter).

5.3.2. Interaction between PLFA/NLFA, P acquisition traits and P acquisition

5.3.2.1. Correlation between carboxylate release rate and PLFA/NLFA indicators

Significant correlations were observed between root functional traits, PLFA and NLFA fungal and bacterial indicators, with most correlation coefficients between 0.7 and 0.9 (Figures 16 and 17, supplementary Table S3, Appendix B). Carboxylate-release rate was strongly correlated with NLFA and PLFA concentrations in the rhizosheath, with malate release rate in particular being positively correlated with multiple indicators such as AMF NLFA concentration in both soils (Figures 16a1,17a1) but also Gram-negative NLFA concentration in the Retisol (Figure 16 c3). In the Calcaric Cambisol, malonate-release rate was positively correlated with Gram-negative NLFA concentration (Figure 17 b3), AMF NLFA concentration and bacterial NLFA concentration. Citrate-release rate was negatively correlated with the NFLA fungal/bacterial ratio (Figure 16 b1). Despite correlations between individual carboxylates and PLFA or NLFA concentrations in the rhizosheath, total carboxylate-release rate was not correlated with any single PLFA or NFLA concentration in the Retisol, while it was positively correlated with AMF NLFA, fungi NLFA and Gram-negative NLFA concentrations in the Calcaric Cambisol. Gram-negative NLFA concentrations, bacterial NLFA and AMF NLFA were positively correlated with leaf Mn concentration in the Calcaric Cambisol (Figure 17 b4), and leaf Mn concentration was also positively correlated with total carboxylate-release. Within the Fabaceae family correlations between carboxylate release rate and Gram-negative NLFA concentration, AMF NLFA concentration and bacterial NLFA were not significant.

5.3.2.2. Correlation between root morphological traits and PLFA and NLFA indicators

Fewer correlations were observed between root morphological traits and bacterial and fungal communities. Root surface area and root length density were both negatively correlated with Gram-positive NLFA concentration. The percentage of fine roots was negatively correlated with Gram-negative NLFA concentration in both soils (Figures 16 c2, 17 b2), AMF NLFA and bacterial NLFA concentrations in the Retisol. The correlation between Gram-negative NLFA concentration and the percentage of fine

roots was maintained within the Fabaceae family in the Retisol, while the other correlations observed were not significant within the Fabaceae family only.

5.3.2.3. Correlation between changes in rhizosphere soil P concentration, plant P content and PLFA/NLFA indicators

We observed multiple correlations between P content or changes in the rhizosphere P concentration and PLFA and NLFA concentrations. Phosphorus content was strongly positively correlated with Gram-negative NLFA concentration (Figures 16 c1,17 b2) and bacterial NLFA concentration in both soils, and also with AMF NLFA concentration in the Retisol. Δ resin P and Δ Olsen P were similarly correlated with Gram-negative PLFA, AMF NLFA, Gram-negative NLFA and bacterial NLFA concentrations. The negative correlation between these variables indicates higher decreases in P availability in the rhizosphere associated with higher value of these indicators. Correlations between P-acquisition indicators and PLFA/NLFA indicators were no longer significant within the Fabaceae family only.

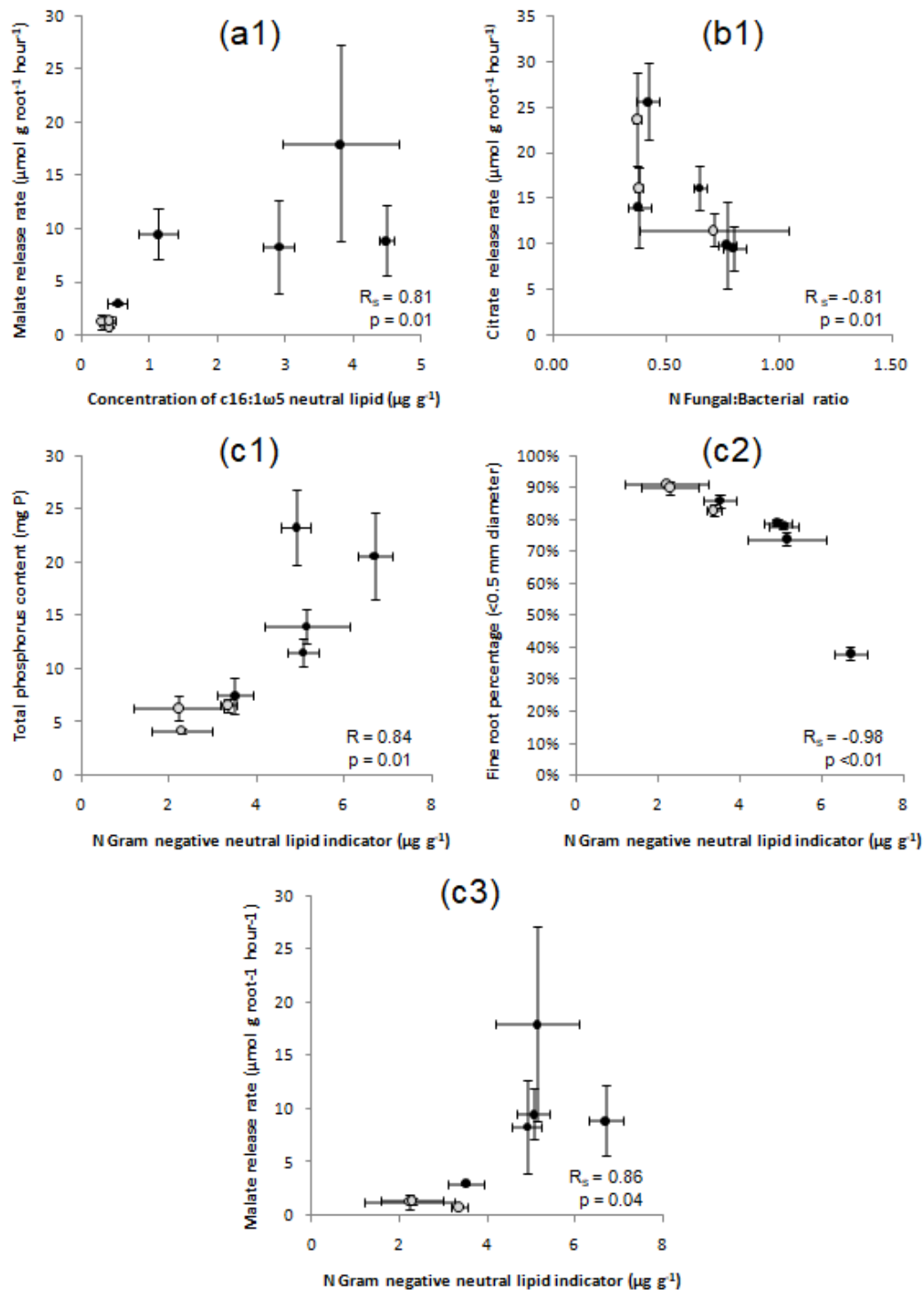


Figure 16 : Correlations between rhizosheath fungal and bacterial NLFA concentrations, belowground traits and plant phosphorus (P) content in the Retisol.

a Correlations between AMF NLFA indicator and **1** Malate-release rate; **b** Correlations between the NLFA Fungal bacterial ratio and **1** Citrate release rate; **c** Correlations between Gram-negative NLFA indicator and **1** Total plant P content; **2** Fine root percentage; **3** Malate-release rate.. R: Pearson correlation coefficient. R_s : Spearman correlation coefficient. p : p value of the corresponding correlation test. Brassicaceae are represented in light gray, Fabaceae in black.

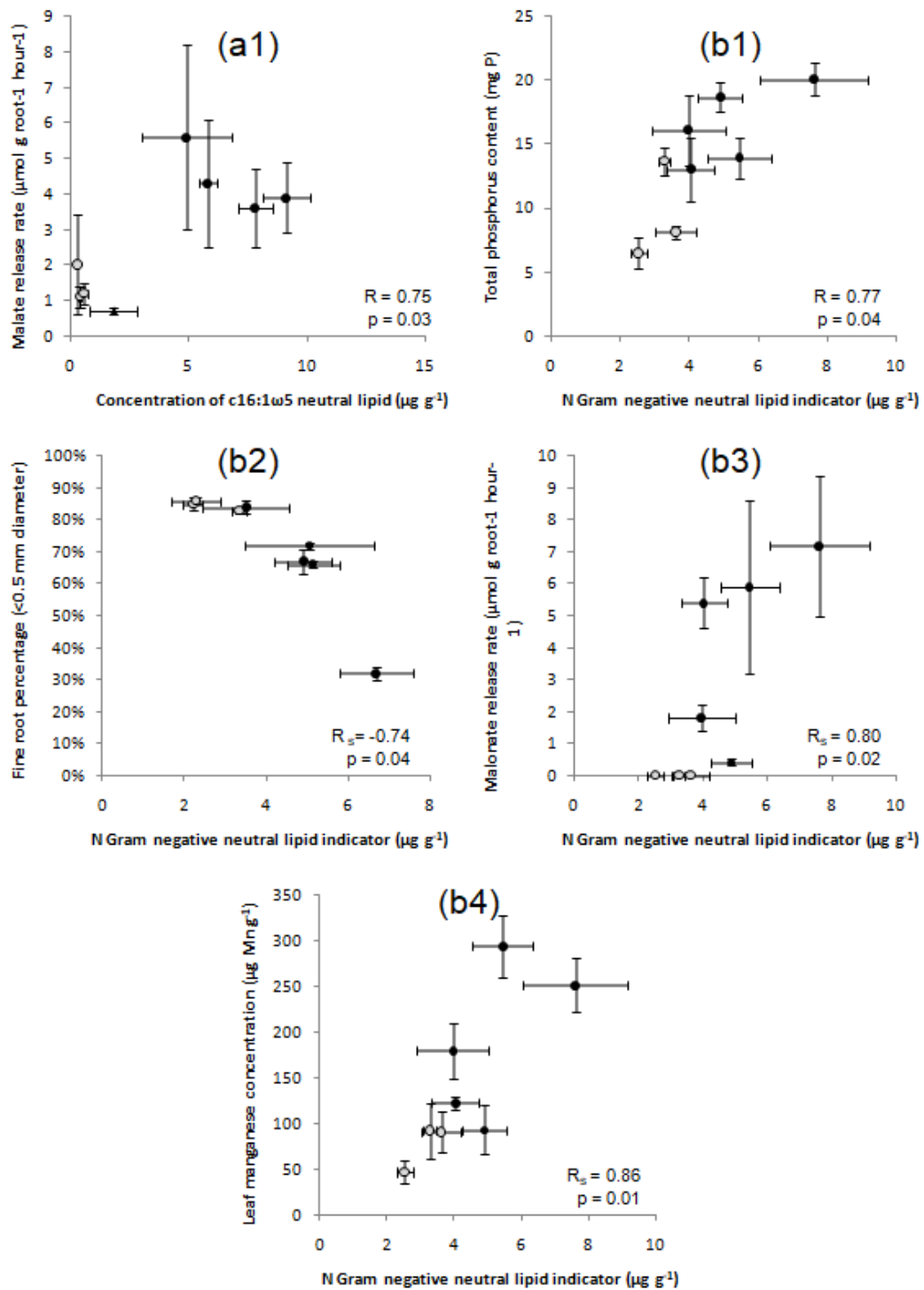


Figure 17 : Correlations between rhizosphere fungal and bacterial NLFA concentrations, belowground traits and plant P-content in the Calcaric Cambisol.

a Correlations between AMF NLFA and 1 Malate-release rate; rhizosphere. **b** Correlations between the Gram-negative NLFA indicator and 1 Total plant P content; 2 Fine root percentage; 3 Malonate-release rate; 4 Leaf manganese (Mn) concentration. R: Pearson correlation coefficient. R_s : Spearman correlation coefficient. p: p value of the corresponding correlation test. Brassicaceae are represented in light gray, Fabaceae in black.

5.3.3. Modelling P-acquisition via interaction between PLFA and NLFA and root traits

5.3.3.1. Interactions between belowground traits and NLFA indicators in predicting P acquisition

PLS-PM offered a good fit when predicting P-acquisition as measured via total P content and Δ P concentrations, with a GOF between 0.60 and 0.82 (Figure 18). We observed strong correlations between the “belowground traits” latent variable and the “NLFA” latent variable (Corr= 0.68 and 0.88 in the Calcaric Cambisol and Retisol, respectively). Correlation between the “belowground traits” latent variable and the “NLFA” latent variable resulted in a slightly lower indirect effect of belowground traits on P-acquisition via their effect on NLFA (Corr= 0.34) than the direct effect of the “NLFA” latent variable (Corr= 0.38) in the Retisol. In the Calcaric Cambisol, the latent variables “belowground traits” and “NLFA” presented equal correlations (Corr= 0.37) with the latent variable “P acquisition”. In the Retisol the latent variable “belowground traits” presented a stronger correlation (Corr= 0.54), while the latent variable “NLFA” had a similar correlation coefficient to the one observed in the Calcaric Cambisol (Corr= 0.38).

5.3.3.2. Contribution of microbial indicators to the “NLFA” latent variable

The “NLFA” latent variable was accurately described (ρ = 0.96 and 0.98) by similar variables in both soils, namely AMF NLFA, Gram-negative NLFA and bacterial NLFA. The NLFA latent variable was mainly represented by the Gram-negative NLFA variable, followed by the bacterial NLFA variable indicating that most of the variation in NLFA concentrations was observed in these variables.

5.3.3.3. Contribution of root traits to the “belowground traits” latent variable

The traits best describing the “belowground traits” latent variable ($\rho = 0.84$ and 0.91) differed between soils. Two morphological traits, fine root percentage and root surface area, were selected in the Calcaric Cambisol while none were in the Retisol. Malate-release rate strongly contributed to the “belowground traits” latent variable in both soils, while total carboxylate-release rate described the latent variable well in Calcaric Cambisol, but not in Retisol, where another physiological indicator, phosphomonoesterase activity best described it.

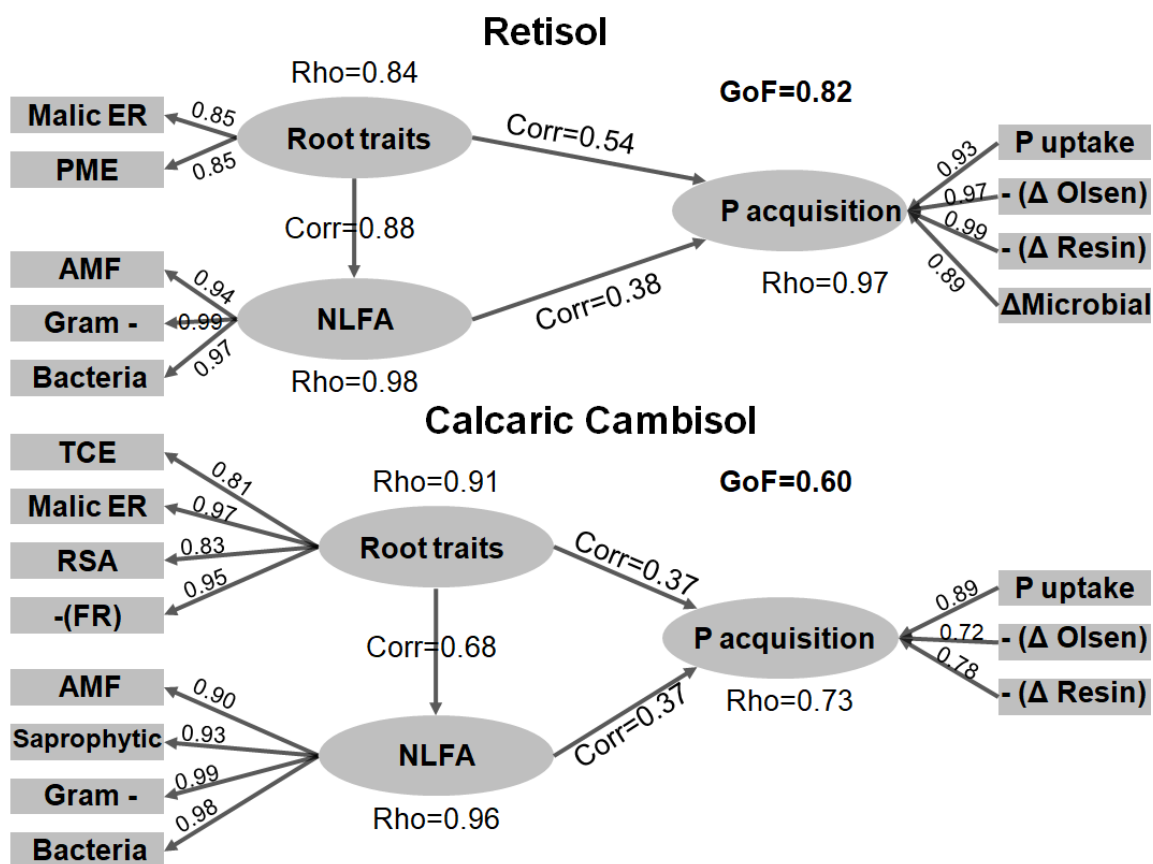


Figure 18 : Partial least square-path models predicting phosphorus (P) acquisition.

Corr is the correlation effect, Rho the Dillon-Golstein coefficient. Variables negatively transformed indicated by - (variable). GoF= Goodness of Fit of the model. Based on three latent variables “Belowground traits”, “NLFA” and “P acquisition”. Individual factors loading presented per latent variable. Abbreviations: Δ Microbial: difference in microbial P concentration between rhizosphere and bulk soil; Δ Olsen: difference in Olsen P concentration between rhizosphere and bulk soil; Δ Resin: difference in resin P concentration between rhizosphere and bulk soil; FR : % fine roots (<0.5 mm); Malate : malate-release rate; PME : phosphomonoesterase activity; RSA : root surface area; TCE : total carboxylate-exudation rate; AMF: Concentration of C16:1ω5 neutral lipid; Gram - : Sum of concentrations of cy17:0, C18:1ω7, cy:19:0 neutral lipid; Fungi : Concentration of C18:2ω6,9 neutral lipid; Bacteria: Sum of N Gram-negative and N Gram-positive; NLFA: Neutral lipid fatty acid.

5.4. Discussion

5.4.1. Interaction between root morphology and rhizosphere microbial communities for P acquisition

Plant-soil microorganism interactions play a major role in nutrient cycling and ecosystem functioning (Richardson and Simpson 2011; Richardson et al. 2011; Sunita 2017; Nobile et al. 2019). Despite recognition of the importance of such plant-soil microorganism interactions the influence of specific plant functional traits on soil fungal and bacterial communities involved in P acquisition remain unclear (Bardgett et al. 2014; Bardgett 2017). Our study shows evidence for strong correlations between several P acquisition traits and fungal and bacterial rhizosphere communities, with potentially important consequences for plant P uptake. Fine root percentage was negatively correlated with Gram-negative NLFA indicators in both soils, and with AMF NLFA indicators in the Retisol. The several Brassicaceae species examined in this study, which are non-mycorrhizal, might in part explain these correlations as Brassicaceae were observed to have higher fine root percentage overall in our data and as expected low concentration of AMF NLFA indicators. Examining these correlations only within the Fabaceae family to avoid interferences of phylogeny similarly highlighted a negative yet not significant tendency (p -value = 0.08) between fine root percentage and AMF NLFA indicators in the Retisol. The negative correlation between fine root percentage and Gram-negative NLFA indicators was however maintained within the Fabaceae family only but only in the Retisol. Plants with thinner roots in turn presented smaller decrease in rhizosphere resin P or Olsen P compared to the bulk soil (in the Calcaric Cambisol and the Retisol, respectively). While potentially exploiting a large soil volume at a reduced cost, they captured less of the available P in their rhizosphere, possibly due to reduced bacterial associations. Plants with thin roots tend to rely less on associations with arbuscular mycorrhizal fungi for P-acquisition, and rather rely on enhanced soil P foraging through higher SRL or root branching (Zhou et al. 2018; Wen et al. 2019), which is in part confirmed by our findings showing a negative tendency between fine root percentage and AMF NLFA indicators. Tradeoffs between root diameter and other root functional traits involved in P-acquisition such as colonization by arbuscular mycorrhizal fungi, but also carboxylate

release or phosphomonoesterase activity in the rhizosphere, have been reported in numerous species as a consequence of the carbon cost associated with these diverging P-acquisition strategies (Lyu et al. 2016; Wen et al. 2019). Here, the lower concentration of bacterial indicators in the rhizosphere of thinner roots suggests that these tradeoffs could also extend to associations with rhizosphere bacteria. Bacterial NLFA have, however, been suggested to be indicators of recently dead rather than living bacterial biomass (Rinnan and Bååth 2009; Malosso 2004; Amir et al. 2008), suggesting important bacterial turnover. In summary, we report plausible tradeoffs between root diameter and associations with bacteria in the rhizosphere, and observed a smaller decrease of available P in the rhizosphere of thinner roots, which are less associated with soil microbial communities. Further studies incorporating other root morphological traits such as root-hair length or abundance and measuring pools of less plant-available soil P could provide valuable insights into the complex and still unclear interactions between root morphology and rhizosphere microbial communities for P-acquisition from multiple soil P pools.

5.4.2. Carboxylate exudation role in plant-microorganism interactions for P-acquisition

Differential exudation may play a major role in modulating plant interactions with soil microorganisms, as recently reviewed in Sasse et al. (2018). Carboxylates, a major fraction of total root exudates, can act as carbon source for microorganisms (Sasse et al. 2018), while also promoting P-acquisition via P mobilisation (Wang and Lambers 2020). Understanding the still enigmatic role of carboxylate release in shaping microbial communities and its implications for P-acquisition is as such central to unravelling plant P acquisition strategies. Our results demonstrate relationships between the release of carboxylates and rhizosphere community structure and could expand our understanding of the plausible role of specific carboxylates in plant-microorganism interactions for P-acquisition. Specifically, we observed significant links between the release rate of malate, malonate and citrate and rhizosphere fungal and bacterial community composition. Both Gram-negative and AMF NLFA indicators increased in concentration with increasing malate-release rate and malonate-release rate, while citrate-release rate appeared to more specifically be negatively related with the fungal/bacterial ratio. These correlations between microbial communities and carboxylate release might have been in part driven by the phylogenetic families

examined. Fabaceae were indeed observed to release higher amounts of malate and malonate than Brassicaceae overall, and presented higher Gram-negative NLFA indicators in their rhizosheath. Brassicaceae, as expected, had lower AMF NLFA indicators, with ratios of concentration of C16:1 ω 5 neutral lipid and concentration of C16:1 ω 5 phospholipid indicating bacterial interference. Higher abundance of Gram-negative bacteria have been observed before for Fabaceae (Habekost et al. 2008), which could in part be due to their association with rhizobia which are Gram-negative for nitrogen fixation. The extent to which this may affect rhizosheath microbial communities remains unclear as rhizobia should be concentrated in non-harvested nodules rather than evenly in the rhizosheath. As proposed in Habekost et al. (2008), the greater abundance of Gram-negative bacteria in the rhizosheath of Fabaceae may be the result of more root exudation including exudation of carboxylates. The abundance of Gram-negative bacteria indeed tends to be favoured by low-molecular-weight carbon sources (Prashar et al. 2014), and carboxylates have been proposed to be key drivers of rhizosheath soil microbial community composition (Hunter et al. 2014). This observation, coupled with ours of strong correlations between carboxylate release and Gram-negative bacteria indicators could indicate that irrespectively from the phylogenetic effects of the families and their exudates other than carboxylates, plants may have influenced their rhizosheath microbial communities for P-acquisition, via bacterial consumption of the carboxylates released. Several carboxylates have indeed been observed to have considerable effects on rhizosheath microbial communities. Citrate release for instance influences bacterial and fungal community structure, while malate affects bacterial community structure (Marschner et al. 2002). In the present study, the correlation between citrate-release rate and fungal/bacterial ratio could strengthen these observations, while correlations between malate or malonate and bacterial and fungal NLFA appear to expand them to other carboxylates. Shifts in microbial communities were in turn strongly related to plant P uptake, as indicated by positive correlations between plant P content and both Gram-negative NLFA indicators. A significant fraction of rhizosheath bacteria comprises P-solubilising bacteria or plant-growth-promoting bacteria (Kalayu 2019). As such, shifts in microbial community structure possibly due to carboxylate release by roots may increase P-acquisition. For instance, carboxylates promote root colonization by plant-growth-promoting bacteria (Yuan et al. 2015). Positive correlations between indicators of Gram-negative bacteria and plant P content, as well as with decrease in rhizosheath

P concentration could thus suggest the modulation of rhizosheath bacterial communities by plants for P acquisition, notably via carboxylate release. Fabaceae tended to have greater P content and result in higher decrease in rhizosheath concentration while also releasing more carboxylates, which might also have contributed to these correlations. By highlighting interactions between carboxylate release by plants and rhizosheath fungal and microbial communities, driven in part by phylogenetic effects while being also coherent with the current understanding of plant-soil microorganisms interactions, our new findings support the sparsely-reported role of carboxylate release in shaping microbial communities and its implications for P acquisition. As our results show that these interactions depend on soil type and properties future studies examining how soil biotic and abiotic factors drive the influence of specific carboxylates on soil microorganisms are needed.

5.4.3. Soil type mediate plant-soil microorganism interactions involved in plant P-acquisition

The efficiency of several P acquisition traits such as carboxylate release or rhizosheath acidification as well as plant associations with mycorrhizal fungi is highly dependent on soil properties, especially P availability (Wen et al. 2019; Wang and Lambers 2020). In order to investigate the influence of soil types and properties on trait responses and interactions with microorganisms for P-acquisition we examined two contrasting soil types in our study. The results highlight relationships between different belowground traits and microbial communities and P uptake per soil type. Most notably, leaf Mn concentration, an indicator of P mobilisation involving carboxylate release (Pang et al. 2018), was correlated with AMF NLFA, Gram-negative NLFA and bacterial NLFA in the Calcaric Cambisol, but not in the Retisol. Leaf Mn concentration was also correlated with total carboxylate release rate in the Calcaric Cambisol, but not in the Retisol. Interestingly, we observed no correlation between leaf Mn concentration and total carboxylate release rate corrected for the number of carboxylic groups per carboxylate. Our results may thus indicate a different role of released carboxylates in plant-soil microorganism interactions for P-acquisition dependent on soil properties, although an influence of soil Mn, not measured in our experiment, can not be ruled out. In the Retisol, the lack of correlation between microbial indicators and leaf Mn concentration could indicate rapid microbial consumption upon release, as shown before (D'Angioli et al. 2017). Increased microbial abundance due to carboxylate

consumption would, in turn, increase plant P-acquisition through positive plant-microorganisms feedback, explaining the slightly higher correlation coefficient between microbial indicators and indicators of plant P acquisition in this soil, although higher P content in Fabaceae may affect this observation. In the Calcaric Cambisol, correlations between leaf Mn concentration and bacterial NLFA indicators suggest that the released carboxylates were effective at mobilising P which is reinforced by the correlation between total carboxylate release and leaf Mn concentration, but also partly metabolized by bacteria. The complex combination of carboxylate release for P mobilisation and microbial consumption of exudates has indeed been suggested to depend on the prevailing soil conditions and microbial community (Hunter et al. 2014). The increased P availability in soil with high Ca-P concentration can be explained by the weathering effect of carboxylate on minerals such as apatite as well as the mobilisation of Ca from the Ca-P surface and the Ca chelation by carboxylates, which, in turn, promotes the dissolution of Ca-P (Jones and Darrah 1994; Ryan et al. 2001; Rosling et al. 2007). We report how soil properties may affect plant-microorganism interactions for P-acquisition, with the carboxylates released most likely playing a different role in shaping the soil microbial communities and mobilizing P dependent on soil type.

5.5. Conclusion

Examining interactions between belowground functional traits and fungal and bacterial communities by measuring PLFA and NLFA concentrations in the rhizosheath allowed us to gain a new understanding of the role of plant-soil microorganism interactions in P-acquisition and the factors involved. We demonstrate that plant functional traits are strongly linked with rhizosheath fungal and bacterial communities, which are in return strongly involved in P acquisition, although some interactions are constitutive of Brassicaceae and Fabaceae. Our results mainly highlighted specific links between bacterial communities in the rhizosheath and the release of carboxylates, namely malate, malonate and citrate, with the microbial groups affected seemingly playing an important role in P-acquisition. Differences in plant trait interactions with PLFA and NLFA between soil types suggested that soil type may mediate the role of carboxylates for P-acquisition. Perspectives include examining the plant-soil microorganism interactions for P acquisition over a large pool of species within several phylogenetic lineages to clarify the relative contribution of differential exudation of carboxylate in these interactions. The mechanisms involved in the relationships between soil properties and plant-soil microorganism interactions should moreover be investigated over a wide soil gradient where pH and P forms vary to elucidate their role in P acquisition by plants and ecosystem functioning.

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CHAPTER 3

6.ROLE OF COVER CROP RESIDUE TRAITS IN PHOSPHORUS RELEASE DYNAMICS FROM COVER CROP RESIDUES

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Abstract

P release from crop residues can increase P availability and significantly contribute to the P nutrition of subsequent crops. Some cover crops residues could contribute to the increase of P availability. Cover crops residues however present contrasting effects on P availability, ranging from important increases to decreases due to immobilization in the microbial biomass. Gaining a better understanding of the role of cover crops residues traits involved in P release dynamics could offer insight into the key plant traits to manage and select for to ensure optimal P cycling efficiency in cropping systems.

We examined the influence of cover crops residues traits on P availability and P uptake in a subsequent crop. The effects of cover crop residues of six different species with contrasting traits were investigated via two simultaneous experiments examining the influence of residue addition on P uptake by ryegrass and on soil microbial P as well as available P in a soil with moderate P availability.

Residue C:P ratio was highlighted to play an important role in the fate of residue P due to suspected microbial P immobilization negatively impacting P availability for residue with high C:P ratio. Cover crops residues addition however had little influence on P uptake by ryegrass over the course of four months.

Cover crop C:P ratio should be managed in order to ensure minimized adverse effects of P immobilization. Little effects of cover crops residues on P availability and uptake in a subsequent crop in soils with moderate P availability suggests that optimal management practices and cover crop designs for improved P nutrition in subsequent crops should be adapted according to soil P availability relying on the multiple other services provided by cover crops such as P mobilization.

Abbreviations

BraC: *Brassica carinata*

FagE: *Fagopyrum esculentum*

LenC: *Lens culinaris*

PhaC: *Phacelia tanacetifolia*

P_{res}: Resin extractable phosphorus

P_{mic}: Microbial phosphorus

VicF: *Vicia faba*

VicV: *Vicia villosa*

SLA: Specific leaf area

6.1. Introduction

Low bioavailability of soil phosphorus (P) in agroecosystems often requires the addition of P fertilizer. One problem with this is that a large portion of the added P is sorbed onto soil mineral surfaces, which reduces plant P uptake and results in low P fertilizer use efficiencies (Richardson et al. 2011). Overtime, the regular addition of P fertilizer results in the accumulation of sparingly-soluble forms of soil P (Bouwman et al. 2017). Exploiting this stock of legacy P could lead to increased P cycling efficiency and reduced dependency on declining resources of phosphate rock (Richardson et al. 2011; Simpson et al. 2011; Faucon et al. 2015). Several plant architectural, physiological and morphological traits can allow plants to utilize pools of sparingly-soluble soil P and reduce the accumulation of legacy P stocks in cultivated soils, which can be grouped in three main strategies : Strategies improving P foraging to support higher yield at lower plant available concentration (Pang et al. 2010; Haling et al. 2018; Ma et al. 2018); Strategies mobilizing poorly available P by the release of exudates such as carboxylates to decrease P desorption on mineral surfaces or by the release of acidifying agents to solubilize Ca-P in calcareous soils (White and Hammond 2008; Li et al. 2017; Wang and Lambers 2020); Strategies favouring the mineralization of slowly mineralizing organic P pools (Richardson et al. 2011; Nobile et al. 2019). The use of these plant strategies and their inclusion in cropping systems, either via selection for targeted traits in crops or selection of suitable species in multispecies systems, could improve P cycling efficiency in agroecosystems (Honvault et al. 2020).

Cover Crops can be designed to offer many ecosystem services, such as carbon sequestration, enhanced nutrient cycling or atmospheric nitrogen fixation (Daryanto et al. 2018) and could play an important role in enhancing and maintaining P availability. In a recent meta-analysis, P uptake in the subsequent crop after cover-cropping was reported to increase by up to 50 % (Hallama et al. 2019). Through a vast array of traits cover crop species can acquire P from soil P pools of varied spatial and chemical availability, accumulating different amounts of P in the same soil context. With average yields of 2 to 7 t ha⁻¹, significant amounts of P (5-30 kg P ha⁻¹) can be acquired by cover crops (Damon et al. 2014; Maltais-Landry and Frossard 2015; Wendling et al. 2016; Ruis et al. 2019). Starting at termination and during decomposition of the cover crop biomass left on the field, acquired P is then released, potentially resulting in increased P nutrition to subsequent crops and increased P availability (Dube et al.

2014). Other processes may explain this increased P uptake after cover-cropping such as positive plant soil-soil microorganisms feedbacks due to shifts in soil microbial communities under cover cropping (Hallama et al. 2019) or suppression of soil pathogens (Brown and Morra 1997; Olivier et al. 1999). Benefits of cover cropping for P availability and cycling are thus multi-factorial and tend to be very variable across environmental conditions (Hallama et al. 2019). The contribution of P release from cover crops residues to P availability in particular may be significant only in conditions where large amounts of P is acquired and later released by cover crops (Thibaud et al. 1988; Damon et al. 2014). The large variability in the potential contributions of cover crop residues to P availability thus suggests further investigations of the P dynamics from residue and the factors involved in order to optimize cover crops designs for enhanced benefits on P availability (Faucon et al. 2015).

Phosphorus release from crop residues during their decomposition is a complex process generally involving a pattern of rapid initial P leaching followed by slower release of P (Prescott 2005). Between 35 to 75 % of the total P in crop residue is in an inorganic, easily leached form (orthophosphate) (Noack et al. 2012). Upon release this orthophosphate can either remain plant available, be adsorbed onto soil particles, or be immobilized in soil microorganisms (Prescott 2005; Simpson et al. 2011; Alamgir et al. 2012; Damon et al. 2014). Residue P can be either immobilized in microbial biomass if the amount of soluble P does not meet microbial requirements or mineralized if in excess (Damon et al. 2014). Much uncertainty however remains as to how much P is taken up by microbial biomass with values ranging from 1.5 to 3 mg P g⁻¹ C added (Kwabiah et al. 2003). Immobilized P is then released by microbial turnover typically within days to a month after residue addition (Spohn and Widdig 2017), while longer P immobilisation for up to a year have been reported (Damon et al. 2014). More recalcitrant residue P forms, not mineralised by soil microorganisms are converted into soil organic P of low plant availability (Oliveira et al. 2017). Many factors influence P release from residue and transfer to subsequent crops that have yet to be fully investigated for cover crop residues (Espinosa et al. 2017).

There is increasing evidence that leaf functional traits play a central role in residue decomposition and nutrient release from residue at both local and global scale (Freschet et al. 2013; Zekker and Prescott 2017). Functional traits allow characterizing plant responses and effects on soil and functioning ecosystem via

morphological, physiological, morphological or architectural characters of a species measurable at individual level (Lavorel and Garnier 2002; Violle et al. 2007). Multiple leaf functional traits such as leaf N concentration, leaf P concentration but also specific leaf area (SLA) have been found to correlate with residue decomposition rates and nutrient release dynamics (Zuikswert and Prescott 2017). Positive correlations have been observed between SLA, leaf nutrient content and decomposition rate for instance, while negative correlations have been observed between nutrient ratios such as C:N or C:P and decomposition (Freschet et al. 2013; de la Riva et al. 2019; Lin et al. 2020). Despite this however our understanding of plant functional traits involved in P release dynamics from residue remains limited, as does its impact on P nutrition on the subsequent crop (Damon et al. 2014). Most studies investigating residues functional traits effect on P release focused on residue from mature crops (Alamgir et al. 2012; Noack et al. 2014), thus possibly underestimating the effects of vegetative stage residues such as cover crop residues, and the different range of trait values associated (Maltais-Landry and Frossard 2015). Moreover, despite correlations between leaves, stems and belowground functional traits, understanding how residues affect nutrient cycling requires going beyond leaf traits and investigating the processes and traits affecting P release from whole-plant residues (Hobbie 2015).

The effects of chemical functional traits such as leaf P concentration or nutrient ratios such as C:N or C:P for P release dynamics from residue are the most documented. In general, crop residues with P concentrations greater than 3 g kg⁻¹ result in a rapid P release into the soil solution, whereas crop residues with P concentrations less than 2.4 g kg⁻¹ lead to a net microbial P immobilization (Kwabiah et al. 2003; Alamgir et al. 2012; Damon et al. 2014). Residue C:N ratio plays a major role in residue decomposition, with residues with low and narrow C:N ratios (inferior to 25 to 30) decomposing faster and releasing nutrients faster, although P release is also influenced by N:P ratios with residues with N:P >15 likely to release N and retain P (Prescott 2005). Residue C:P ratio also plays an important role for P release from residues due to simultaneous microbial P uptake when adding C. Residues with C:P higher than 300 tend to lead to net P microbial immobilization, while much uncertainty remains on this critical value for with reports of values between 60 and 700 (Espinosa et al. 2017). C:P ratio effects on P immobilization in turn may be misleading (Umrit and Friesen 1994),

prompting more investigation especially in immature residues with high orthophosphate content such as cover crop residues.

The effects of morphological and physical functional traits such as SLA on P release from residue are less documented despite evidence of correlation between SLA and residue mass loss (Pérez-Harguindeguy et al. 2013). Due to the use of milled residues in most studies the influence of SLA and other morphological traits on P release from residues is largely ignored despite evidence of lower release rate for large residues pieces (Noack et al. 2014; Damon et al. 2014). Leaf morphological traits may play an important yet underestimated role in P release dynamics due to their role in determining the accessibility of residue material to decomposers (Zuikswert and Prescott 2017).

Contribution from residue P to the subsequent crops P uptake have been reported within the range of 1 to 45 % (Thibaud et al. 1988; Nachimuthu et al. 2009; Noack et al. 2014), with residue quality and thus residue functional traits being important factors (Maltais-Landry and Frossard 2015). Further investigations are required to better understand the role of residue functional traits in P release dynamics and potential availability to the subsequent crop in order to select for plant traits and strategies enhancing P cycling efficiency in agrosystems (Faucon et al. 2015; Espinosa et al. 2017).

In this study, we investigated the influence of cover crop residues traits, on the fate of residue P in the plant-soil-microbe system during a greenhouse and an incubation experiment. The effects on P acquisition by the following crop were investigated in controlled condition with six cover crops species selected for their gradient of traits. The effects on soil phosphorus availability (resin extractable and microbial P pools) was investigated with an incubation experiment. We tested the hypotheses that i) residues with higher P concentration or SLA will release more phosphorus faster, contributing more to soil P availability and subsequent plant uptake, and ii) residues with higher C:P ratio will result in P immobilization and delayed and reduced contribution to soil P availability and subsequent plant uptake.

6.2. Material and methods

6.2.1. Soil collection and preparation

A Retisol soil (formerly called Albeluvisol) (FAO 2014) at a depth of 5 to 20 cm was collected from a cultivated field in north-eastern France (48° 54' 37" N, 3° 43' 57" E) . The surface 0 to 5 cm layer was removed prior to collection in order to obtain soil with low concentrations of 'plant-available' P. The soil was dried at ambient temperature for 60 days and then passed through a 2mm sieve. See Table 10 for some chemical and physical properties of the soil.

6.2.2. Preparation of crop residues

Residues from six cover crop species were selected for their diverse phylogenetic lineages and functional traits (Brassicaceae: *Brassica carinata* (BraC) A. Braun; Polygonaceae: *Fagopyrum esculentum* (FagE) Moench.; Fabaceae: *Lens culinaris* (LenC) Medik., *Vicia faba* (VicF) L., *Vicia villosa* (VicV) Roth.; Hydrophyllaceae: *Phacelia tanacetifolia* (PhaC) Benth.) (Table 11). Cover crop residues were obtained from crops grown in the glasshouse on the aforementioned Retisol soil diluted with washed sand (22% mass) to further decrease concentrations of plant-available soil P. Plants were sown in several batches of approximately 2.5 months each in a replicated random design. Basal nutrients were not added to mimic field conditions. At harvest plants were mostly toward the end of their vegetative stage with a first onset of flowering. Growth conditions were set at a photoperiod of 14 hour day⁻¹, with 22 ° C at day and 18 ° C at night and pots were watered twice a week. The biomass of each batch was combined and homogenized. Due to lacking biomass, biomass grown on the same soil in field conditions was mixed with biomass produced in the glasshouse when necessary before thoroughly homogenizing. Leaves from several individuals were separated from the above ground biomass at harvest and used in the leaves only conditions. Said individuals were excluded from the pool of biomass used for the leaves and stems conditions.

The specific leaf area (SLA) of crop residues was determined via scanning using an Epson Scanner perfection V800 to produce a 600 dpi image. The image was then analyzed using imageJ software to determine leaf area. After 48 hours drying at 55°C, scanned leaves were weighed to calculate its SLA. The residues were then manually cut into pieces 0.25 cm² to replicate shredding while also conserving mass to area ratio (SLA) and were sieved at 0.49 cm². This was done to examine the effect of SLA on the fate of residue P in the plant-soil system.

Table 10 : Chemical and physical soil characteristics

	Clay % (<0.002mm)	Sand % (>0.05mm)	pH KCl	Organic C (gkg ⁻¹)	Exchangeable Ca ^a (gkg ⁻¹)	N tot (g kg ⁻¹)	CEC (cmol _c kg ⁻¹)	Olsen P ^b (mg kg ⁻¹)	Ca -P ^c (mg kg ⁻¹)
Retisol	22.3	5.4	7.4	9	2.60	1	10.5	16.3	76.7

^aExtractant : Ammonium acetate 0.5 M, EDTA 0.02 M pH 4.65

^bAccording to Olsen (1954)

^cApatite phosphorus as defined in García-Albacete *et al.* (2012)

Table 11 : Cover crop residues traits and inputs for each residue treatment (all inputs designed to add 15 mg P kg⁻¹ soil) .

Type of residues	Species	P g kg ⁻¹	N	C	C:N	C:P	N:P	SLA mm ² mg ⁻¹	Weight added g kg ⁻¹ soil	N added mg kg ⁻¹ soil	C added
Leaves	BraC	1.86	25.24	404	16.0	217	13.6	21.7	8.07	203	3257
	FagE	2.41	24.43	777	15.5	156	10.1	47.4	6.23	152	2347
	LenC	2.71	35.14	23	12.0	156	13.0	31.3	5.53	194	2340
	PhaC	2.54	18.43	52	19.2	139	7.2	24.2	5.91	109	2082
	VicF	3.07	49.94	22	8.5	137	16.2	38.5	4.88	244	2060
	VicV	2.04	35.14	14	11.8	204	17.2	38.8	7.37	259	3054
Leaves and stems	BraC	2.44	18.64	00	21.5	164	7.6	21.7	6.14	114	2456
	FagE	2.49	9.2	403	43.9	162	3.7	47.4	6.04	55	2434
	LenC	2.26	23.04	29	18.7	189	10.1	31.3	6.62	152	2842
	PhaC	2.73	8.6	379	44.0	139	3.2	24.2	5.49	47	2083
	VicF	1.85	26.34	15	15.8	224	14.2	38.5	8.10	213	3362
	VicV	1.28	22.54	04	18.0	316	17.6	38.8	11.72	264	4739

BraC: *Brassica carinata*; FagE: *Fagopyrum esculentum*; LenC: *Lens culinaris*; PhaC :*Phacelia tanacetifolia*; VicF : *Vicia faba*; VicV : *Vicia villosa*

6.2.3. Experimental design

To investigate the influence of residue traits, namely residue P concentration, N concentration C:P ratio, C:N ratio, N:P ratio and SLA, on the fate of residue P in the plant-soil-microbe system a greenhouse and an incubation experiment were set up. Relationships between residue traits and the fate of residue P in the plant-soil-microbe were examined separately for leaves biomass only and leaves and stems biomass in order to investigate whether key traits differed between leaves and whole plant above ground biomass. Experimental treatments included the addition of two types of plant residues to the soil (i.e. the residues of (i) leaves and that of (ii) stems and leaves) from six cover crops species, the addition of mineral P fertilizer (15P), and an unfertilized treatment (0P). All plant residues and the mineral fertilizer were added to supply 15 mg P kg⁻¹soil. This rate corresponds to a field productivity of 6.5 t DM ha⁻¹and a residue P concentration of 3 mg P kg⁻¹, and is similar to that reported in previous studies (Noack et al. 2014; Maltais-Landry and Frossard 2015). The experiment was arranged in a factorial randomized complete design and replicated four times.

6.2.3.1. Pot experiment set up

The aforementioned soil was rewetted at gravimetric water content (GWC) of 156 g H₂O kg⁻¹ soil, which is equivalent to 41 % of the soil's maximum water holding capacity (WHC), in order to increase microbial activity. After 10 days of incubation, the soil was then labelled with a carrier free ³³P H₃PO₄ radiotracer at 1.9 MBq kg⁻¹ soil. Briefly, 1 kg of dry-weight equivalent soil was weighed and a 10 mL aliquot of 190 MBq L⁻¹ carrier free ³³P H₃PO₄ solution was added evenly across the soil. The soil was then mixed for 2 minutes, adjusted to 60 % of its WHC, and then left to equilibrate for 18 days.

After the incubation with the radiotracer, the P treatments were added to the soil and mixed for 2 minutes. This included the aforementioned crop residues or 10 mL of a solution of 1.5 g/L KH₂PO₄. The 0P treatment was mixed for 2 minutes to apply the same amount of disturbance as the fertilized treatments. Lastly, all soils simultaneously received basal nutrients except for P at the following rates (mg kg⁻¹ soil): 120 N, 250 K, 40 Ca, 50 Mg, 1 Fe, 150 Cl, 1 B, 2 Mn, 1 Zn, 2 Cu, 1 Mo.

Italian ryegrass (*Lolium multiflorum* L.) seed (0.5 g) were added to each pot and placed 10 mm below the soil surface. This corresponded to an input of 1.6 mg P kg⁻¹ soil, which is approximately 10 % of the P added by residue addition. Italian ryegrass was

chosen as a model specie primarily acquiring available soil P with low access to less bio-available P forms such as organic P or adsorbed P. Soils were kept between 50 and 60 % of their WHC during ryegrass growth with distilled water. Growth conditions were set at a photoperiod of 14 hours day⁻¹, with 24 ° C at day and 18 ° C at night. Pots were randomized weekly.

Ryegrass shoots were harvested 2 cm above the soil surface on day 35, 47, 70, 91 and 110 after the addition of P. After each harvest, the plants were supplied with all nutrients except for phosphorus at the following rates (mg kg⁻¹ soils) : 120 N, 250 K, 40 Ca, 50 Mg, 1 Fe, 150 Cl, 1 B, 2 Mn, 1 Zn, 2 Cu, 1 Mo. Nitrogen was added split into two equal doses.

6.2.3.2. Plant analyses

All ryegrass shoots were dried at 45 °C for 72 hours, weighed, and then ground to powder using an MM 300 Mixer Mill (Qiagen, USA). Concentrations of total P in shoot material were determined using the method of Hoenig (2001). Briefly, 0.2 g of shoot material was digested with 2 mL HNO₃ 69 % (v/v) for 1 hour at 200 °C with a MLS Turbowave (MWS GmbH, Heerbrugg, Switzerland). Concentrations of P in digests were then determined via colorimetry using malachite green (Ohno and Zibilske 1991). Concentrations of total C and N in shoot material were determined via dry combustion.

³³P activity in plant material were determined via scintillation counting after mixing 2 mL of plant digests with 5 mL scintillation liquid (Ultima Gold AB, Packard Instrument Co.). The specific activity (SA) was then calculated based on measured P concentration in biomass and dry biomass weight. As no significant ³³P dilution was observed between ryegrass grown on labelled soil amended with 15 mg P kg⁻¹ soil as compared to unfertilized control, P derived from fertilizer as defined by the ratio between specific activity in biomass from amended pot to specific activity in biomass from unfertilized pots could not be estimated.

To assess the limitation of nitrogen and P in plant shoots, the nitrogen nutrition index (NNI) and P nutrition index (PNI) were calculated according to the methods described in Lemaire et al. (2008) and Duru and Ducrocq (1996). NNI was calculated as follow:

$$NNI = \frac{Na}{Nc} = \frac{Na}{4.8 \times DM^{-0.32}}$$

With

Na: Actual N uptake

Nc: Critical N uptake

DM: Plant dry biomass

And PNI as follow:

$$PNI = \frac{Pa}{Pc} = \frac{Pa}{0.15 + 0.065 \times Na}$$

With

Pa: Actual P uptake

Pc: Critical P uptake

Na: Actual N uptake

6.2.3.3. Soil incubation design and set up

Soil in the incubation experiment was amended as for the greenhouse experiment. Experimental treatments thus included the addition of two types of plant residues to the soil (i.e. the residues of (i) leaves and that of (ii) stems and leaves) from six cover crops species, the addition of mineral P fertilizer (15P), and an unfertilized treatment (0P). Soil was labelled with a carrier free ^{33}P H_3PO_4 radiotracer at 1.9 MBq kg^{-1} soil and mixed for 2 minutes. Soil then was adjusted to 60 % of its WHC, and left to equilibrate for 18 days before amendment with P treatments. Simultaneously with amendments with P treatments soil received basal nutrients except for P at the following rates (mg kg^{-1} soil): 120 N, 250 K, 40 Ca, 50 Mg, 1 Fe, 150 Cl, 1 B, 2 Mn, 1 Zn, 2 Cu, 1 Mo. Soil was then mixed for 2 minutes before being kept in the dark under identical experimental conditions as for the greenhouse experiment. Soil was then sampled at 10 and 59 days after amendment. Any remaining visible residue pieces were separated from the soil using a pair of tweezers before P_{res} and P_{mic} measurement and measurement of specific activity in the corresponding extracts. A subsample of soil from every treatment was used for dry matter determination.

6.2.3.4. Soil analyses

Microbial P was determined via a difference method between hexanol fumigated and non-fumigated resin extracts (Bunemann et al. 2007). After initial preparation with 0.5 M NaHCO_3 anion-exchange strips were shaken for 16 H with 2 g equivalent dry soil and 30 mL deionised water. For each sample 3 portions of soils were extracted, with

or without addition of 1 mL hexanol as fumigant and with spiking of a known amount of inorganic P (15 mg P kg⁻¹ as KH₂PO₄). The anion-exchange strips (VWR, 551642S) were then rinsed with deionised water before having their phosphate extracted by shaking 1 h in 30 mL 0.5 M HCl. At day 59 to ensure measurable amount of radioactivity resins were rinsed with 10 mL 0.5 M HCl instead. To compensate for sorption of the phosphate released after fumigation, phosphate recovery (60 % of the added 15 mg P kg⁻¹ as KH₂PO₄ on average) was calculated based on spiked samples otherwise extracted identically to non-fumigated samples. Extracted phosphorus in the three extracts was measured via malachite green colorimetry as for plant digests (Ohno and Zibilske 1991). Microbial phosphorus was calculated as the difference between fumigated and non-fumigated samples, corrected by the recovery after spiking:

$$P_{mic} = \frac{(P_{fum} - P_{res})}{P_{recovery}}$$

With

P_{mic}: Microbial P

P_{fum}: P in fumigated extract

P_{res}: P in resin extract

P_{recovery}: Fraction of P recovered in spiked extracts

Radioactivity was measured by scintillation counting after mixing 1 ml of extracts with 5 ml of scintillation liquid (Ultima Gold AB, Packard Instrument Co.). SA in microbial extracts was calculated as follow: SA_{microbial} = SA_{fumigated} - SA_{resin}. Prior to calculation SA in the resin extract was corrected for P sorption with the same factor as for microbial P, assuming similar sorption between ³¹P and ³³P. As for ryegrass biomass no significant isotopic dilution was detected not allowing for determination of P derived from fertilizer.

6.2.4. Statistical analysis

One way ANOVA and Kruskal-Wallis tests were used in order to test differences in ryegrass biomass and P uptake per harvest among residue type and residues species. As differences between residue types were observed at some harvests further analysis and comparison was performed separately per type of biomass for comparison of residue effect per harvest and cumulative effects. Residue traits effects were first examined separately per type of biomass and then conjointly on both residue types as

correlations between the same factors and P uptake and P availability were identified for both residue types. This allowed assessing the relationships between factors over a wider trait-range. Differences between species per harvest were tested with one way ANOVA and post hoc tests of Tukey or Kruskal-Wallis test + post hoc test of Mann Whitney depending on normality of the data. Cumulative data was tested with Generalized Linear Mixed Models via Penalized Quasi-Likelihood (GlimmPQL). Linear models were produced to investigate traits effects on P availability and subsequent phosphorus release. Briefly, all residue traits, as well as phosphorus availability in the incubation experiment were individually tested as predictors of P uptake in ryegrass as well as combinations of the best fitting factors. Models were compared based on adjusted R^2 with the highest relative value considered the best fit. All tests were performed in R version 3.6.0 with a significance level of 0.05.

6.3. Results

6.3.1. Dry matter and phosphorus uptake by ryegrass

Cumulative ryegrass shoots were largely similar across all treatments, which was on average 4 g per pot (Figure 19). However, some significant differences were observed between treatments. Cumulative ryegrass shoots for VicV was significantly lower than that for 15P of the leaves and stems treatment (19.9 g DM for VicV compared to 21.3 g for 15P). Cumulative ryegrass biomass for BraC and VicF were significantly higher than that for VicV of the leaves and stems treatment. In general, cumulative ryegrass biomass for crop residues in the leaves and stems treatment was similar to that of the leaves treatment. However, there was a significant difference for the VicF compared to 0P of the leaves treatment. There was also a significant difference between PhaC and VicF.

Total cumulative P uptake by ryegrass after most treatments was also mostly similar across all treatments (Figure 19). Residue amendment resulted in lower cumulative P uptake than 0P, lower on average by 2.6 mg P, although mostly not significantly. Amendments with leaves and stems residues of VicV resulted in the most pronounced decrease in P uptake (-7.8 mg P), while equivalent mineral fertilization resulted in an increase of cumulative P uptake of about a third of the quantity of P applied (+ 5.7 mg P for a 15 mg P kg⁻¹ input). Leaves and stems residues resulted in a greater decrease in P uptake overall than leaves only residues with leaves and stems residues of VicV resulting in a cumulative P uptake 3.3 mg P lower compared to leaves residues of the same specie.

Differences in P uptake mostly occurred in early harvests up to 70 days after amendment after which no significant differences were observed (Figure 20). While most treatments resulted in similar P uptake, differences in P uptake were observed with some treatments resulting in larger decreases in P uptake than other and some treatments resulting in similar uptake compared to mineral fertilization faster than other. A gradient of P uptake between amendments was observed for leaves and stems residues with P uptake being lower after amendment with VicV than with FagE and PhaC after 35 or 70 days. Ryegrass amended with residues of leaves and stems of FagE and PhaC and leaves of PhaC and VicF did not differ significantly from equivalent mineral fertilization from 70 days after amendment onward.

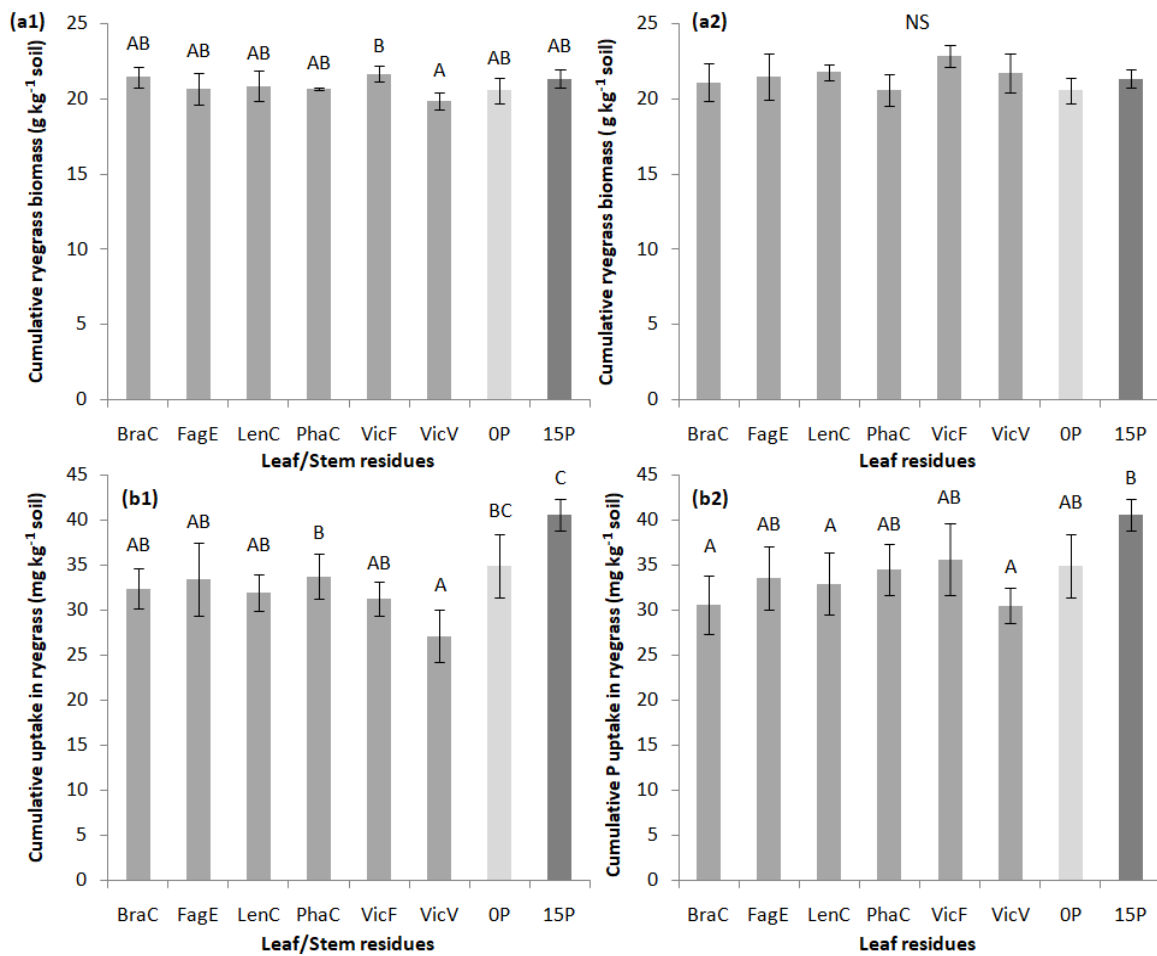


Figure 19 : Ryegrass biomass and phosphorus uptake (\pm standard error) after amendment with cover crop residues.

a: cumulative biomass over 110 days; b: cumulative phosphorus uptake over 110 days; 1: Leaves and stems residue; 2: Leaf residue. Letters above the bars represent statistically significant differences among treatments within the same residue type. BraC: *Brassica carinata*; FagE: *Fagopyrum esculentum*; LenC: *Lens culinaris*; PhaC: *Phacelia tanacetifolia*; VicF: *Vicia faba*; VicV: *Vicia villosa*.

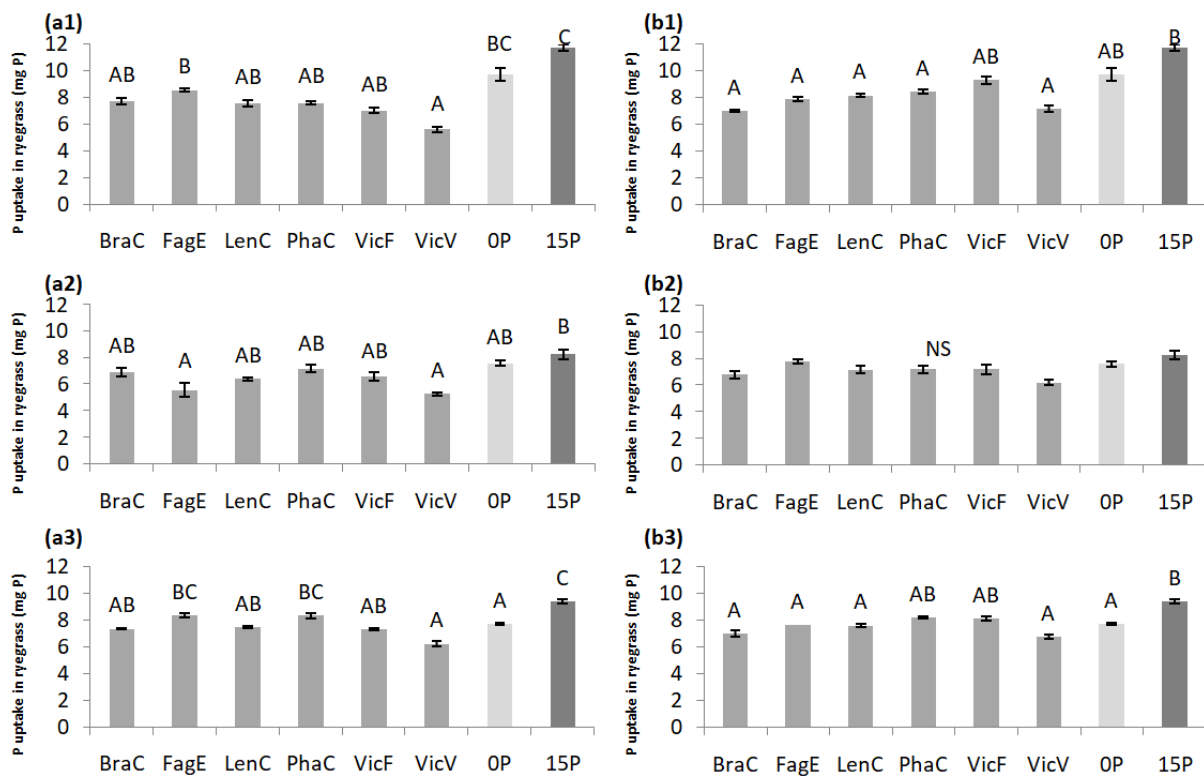


Figure 20: Phosphorus acquired by ryegrass (\pm standard error) per harvest after amendment with cover crop residues.

a: P uptake after amendment with leaves and stems residues; b: P uptake after amendment with leaves residues; 1: P uptake 35 days after amendment; 2: P uptake 47 days after amendment; 3: P uptake 70 days after amendment. Letters above the bars represent statistically significant differences among treatments within the same residue type. BraC: *Brassica carinata*; FagE: *Fagopyrum esculentum*; LenC: *Lens culinaris*; PhaC: *Phacelia tanacetifolia*; VicF: *Vicia faba*; VicV: *Vicia villosa*.

6.3.2. Nutrition indexes in ryegrass

NNI in ryegrass averaged at 84 % across all harvests. Differences in NNI were especially pronounced in the ryegrass harvested after 35 days, after which NNI was mostly similar across treatments (Table 12). NNI progressively decreased with time up till 70 days after amendment (102 % on average after 35 days, 70 % on average after 70 days) and increased thereafter (86 % on average after 110 days). Amendments with VicF, VicV and LenC resulted in the highest values of NNI after 35 days (109-125% for leaves and stems residues, 115% to 127 % for leaves residues), while FagE and PhaC resulted in the lowest values of NNI (75-80% for leaves and stems residues, 115% to 127 % for leaves residues). No significant correlation was observed between NNI and ryegrass biomass.

PNI in ryegrass averaged at 50 % across all harvests, decreasing over time from 53% to 43% on average (Table 13). Differences in PNI between treatments mostly occurred during early harvests up till 70 days after amendment, after which PNI was not significantly different between treatments (including controls). Amendment with leaves and stems residues resulted in a gradient of PNI in ryegrass, with PNI in ryegrass amended with VicF, VicV and LenC residues being significantly lower than after amendment with FagE and 0P control after 35 days. Leaves residues of VicV resulted in significantly lower PNI in ryegrass than PhaC and 0P up till day 70 after amendment. Most residue amendments resulted in lower PNI in ryegrass than the mineral treatment after 35 days, while in later harvests no significant differences were observed. PNI in ryegrass amended with leaves and stems residues of FagE however did not differ from PNI in ryegrass amended with mineral P. PNI was overall strongly correlated to ryegrass P uptake but not to biomass. PNI was also correlated with NNI but only for the first harvest.

6.3.3. Specific activity in ryegrass

Specific activity (SA) in ryegrass biomass did not differ significantly between treatments, neither was isotopic dilution observed between 0P and 15P controls, not allowing for estimation of P derived from residues.

Table 12 : Nitrogen nutrition index in ryegrass (\pm standard error) per harvest after amendment with cover crop residues.

Type of biomass	Days since addition	NNI after BraC addition	NNI after FagE addition	NNI after LenC addition	NNI after PhaC addition	NNI after VicF addition	NNI after VicV addition	NNI after OP addition	NNI after 15P addition
Leaves	35	1.05 \pm 0.03 BC	0.9 \pm 0.08 A	1.22 \pm 0.05 D	0.99 \pm 0.07 AB	1.15 \pm 0.08 CD	1.27 \pm 0.05 D	0.91 \pm 0.05 A	0.88 \pm 0.06 A
	47	0.85 \pm 0.05	0.8 \pm 0.04	0.87 \pm 0.06	0.79 \pm 0.04	0.84 \pm 0.07	0.91 \pm 0.07	0.78 \pm 0.04	0.78 \pm 0.05
	70	0.72 \pm 0.03	0.68 \pm 0.04	0.69 \pm 0.02	0.73 \pm 0.04	0.72 \pm 0.04	0.73 \pm 0.04	0.68 \pm 0.03	0.72 \pm 0.05
	91	0.82 \pm 0.04	0.81 \pm 0.04	0.79 \pm 0.02	0.83 \pm 0.04	0.8 \pm 0.03	0.82 \pm 0.04	0.77 \pm 0.04	0.78 \pm 0.03
	110	0.88 \pm 0	0.86 \pm 0.05	0.88 \pm 0.02	0.89 \pm 0.04	0.85 \pm 0.02	0.87 \pm 0.03	0.84 \pm 0.03	0.83 \pm 0.03
Leaves and stems	35	0.93 \pm 0.06 C	0.75 \pm 0.04 A	1.15 \pm 0.06 DE	0.81 \pm 0.02 AB	1.09 \pm 0.04 D	1.25 \pm 0.06 E	0.91 \pm 0.05 BC	0.88 \pm 0.06 BC
	47	0.8 \pm 0.05 AB	0.72 \pm 0.06 A	0.8 \pm 0.04 AB	0.76 \pm 0.07 A	0.76 \pm 0.04 A	0.9 \pm 0.03 B	0.78 \pm 0.04 A	0.78 \pm 0.05 AB
	70	0.67 \pm 0.05	0.66 \pm 0.05	0.68 \pm 0.01	0.71 \pm 0.02	0.72 \pm 0.03	0.74 \pm 0.03	0.68 \pm 0.03	0.72 \pm 0.05
	91	0.77 \pm 0.02 A	0.8 \pm 0.05 AB	0.77 \pm 0.03 A	0.79 \pm 0.03 AB	0.8 \pm 0.02 AB	0.85 \pm 0.04 B	0.77 \pm 0.04 AB	0.78 \pm 0.03 AB
	110	0.86 \pm 0.03 AB	0.83 \pm 0.02 A	0.86 \pm 0.04 AB	0.87 \pm 0.03 AB	0.86 \pm 0.01 AB	0.9 \pm 0.04 B	0.84 \pm 0.03 AB	0.83 \pm 0.03 A

Letters represent statistically significant differences among treatments within the same residue type and time since amendment. BraC: *Brassica carinata*; FagE: *Fagopyrum esculentum*; LenC: *Lens culinaris*; PhaC : *Phacelia tanacetifolia*; VicF : *Vicia faba*; VicV : *Vicia villosa*.

Table 13 : Phosphorus nutrition index in ryegrass (\pm standard error) per harvest after amendment with cover crop residues.

Type of biomass	Days since addition	PNI after BraC addition	PNI after FagE addition	PNI after LenC addition	PNI after PhaC addition	PNI after VicF addition	PNI after VicV addition	PNI after OP addition	PNI after 15P addition	
Leaves	35	0.45 \pm 0.02 AB	0.57 \pm 0.04 AC	0.46 \pm 0.02 AB	0.57 \pm 0.06 BC	0.52 \pm 0.07 AC	0.4 \pm 0.06 A	0.66 \pm 0.14 CD	0.76 \pm 0.08 D	
	47	0.53 \pm 0.1 AB	0.65 \pm 0.06 AB	0.56 \pm 0.08 AB	0.61 \pm 0.08 AB	0.54 \pm 0.1 AB	0.47 \pm 0.05 A	0.65 \pm 0.06 AB	0.68 \pm 0.12 B	
	70	0.48 \pm 0.07 AB	0.51 \pm 0.01 AB	0.51 \pm 0.04 AB	0.57 \pm 0.02 B	0.52 \pm 0.05 AB	0.46 \pm 0.04 A	0.56 \pm 0.03 B	0.69 \pm 0.05 C	
	91	0.37 \pm 0.03	0.42 \pm 0.1	0.37 \pm 0.11	0.44 \pm 0.01	NS	0.43 \pm 0.05	0.4 \pm 0.03	0.45 \pm 0.04	0.49 \pm 0.02
	110	0.43 \pm 0.03	0.4 \pm 0.01	0.45 \pm 0.03	0.44 \pm 0.02	NS	0.44 \pm 0.03	0.43 \pm 0.07	0.41 \pm 0.02	0.45 \pm 0.02
Leaves and stems	35	0.53 \pm 0.06 BC	0.69 \pm 0.05 CD	0.48 \pm 0.06 AB	0.56 \pm 0.04 BC	0.42 \pm 0.05 AB	0.35 \pm 0.05 A	0.66 \pm 0.14 CD	0.76 \pm 0.08 D	
	47	0.57 \pm 0.1 AB	0.51 \pm 0.19 AB	0.54 \pm 0.03 AB	0.63 \pm 0.1 AB	0.54 \pm 0.09 AB	0.42 \pm 0.04 A	0.65 \pm 0.06 AB	0.68 \pm 0.12 B	
	70	0.51 \pm 0.02 AB	0.61 \pm 0.04 CD	0.54 \pm 0.02 BC	0.59 \pm 0.07 BC	0.51 \pm 0.01 AB	0.44 \pm 0.06 A	0.56 \pm 0.03 BC	0.69 \pm 0.05 D	
	91	0.41 \pm 0.03	0.45 \pm 0.04	0.43 \pm 0.04	0.46 \pm 0.05	NS	0.44 \pm 0.02	0.4 \pm 0.06	0.45 \pm 0.04	0.49 \pm 0.02
	110	0.44 \pm 0.03	0.45 \pm 0.04	0.44 \pm 0.03	0.43 \pm 0.03	NS	0.4 \pm 0.03	0.45 \pm 0.04	0.41 \pm 0.02	0.45 \pm 0.02

Letters represent statistically significant differences among treatments within the same residue type and time since amendment. BraC: *Brassica carinata*; FagE: *Fagopyrum esculentum*; LenC: *Lens culinaris*; PhaC : *Phacelia tanacetifolia*; VicF : *Vicia faba*; VicV : *Vicia villosa*.

6.3.4. Phosphorus dynamics in amended soils

Residue addition resulted in a gradient of P_{res} from 6.7 mg P kg⁻¹ to 16.6 mg P kg⁻¹ as compared to 12.6 mg P kg⁻¹ in the unfertilized conditions (Table 14). 10 days after amendment leaves and stems residue of VicV resulted in significantly lower P_{res} compared to the unfertilized control (- 6.7 mg P kg⁻¹). For leaves and stems residues, BraC and FagE resulted in similar P_{res} as for mineral fertilization (17.1 mg P kg⁻¹), significantly higher than the unfertilized control (13.4 mg P kg⁻¹). The increase in P_{res} after amendment with leaves and stems residues of FagE was of 3.2 mg P kg⁻¹ about a fifth of the P added, while equivalent mineral fertilization resulted in an increase of 3.7 mg P kg⁻¹ about a quarter of the P added. Other leaves and stems amendments resulted in similar P_{res} as the unfertilized condition. Leaves residues of VicV also resulted in a lower P_{res} (10.2 mg P kg⁻¹) although not significantly so compared to the unfertilized condition (13.38 mg P kg⁻¹). Pots amended with leaves residues of FagE, PhaC and VicF had similar concentration as 15P (17.1 mg P kg⁻¹), significantly higher than P_{res} after amendment with leaves residues of VicV.

Fewer differences in P_{res} between treatments were observable after 59 days than after 10 days. Differences in P_{res} between the mineral condition and soil amended with leaves and stems residues of VicV decreased overtime from -10.3 mg P kg⁻¹ after 10 days to -5.3 mg P kg⁻¹ after 59 days. Residue amendment however still resulted in a gradient of P_{res} after 59 days with leaves and stems of VicV resulting in 9.4 mg P kg⁻¹ and leaves and stems of PhaC in 14.4 mg P kg⁻¹. Leaves residues of BraC similarly resulted in lower P_{res} after 59 days than leaves residues from FagE (respectively 12.0 mg P kg⁻¹ and 15.3 mg P kg⁻¹). Overtime P_{res} decreased for most treatments except unfertilized control.

High intra-species variability was observed for microbial P, with little to no significant differences observed (Table 15). Amendment with leaves and stems of VicV resulted in a significant increase of P_{mic} of about 9.2 mg P kg⁻¹ compared to unfertilized control, about two third of the P added. While other biomass amendments did not significantly differ from VicV, amendment with leaves and stems of BraC did result in significantly lower P_{mic} (12.6 mg P kg⁻¹ comparable to the unfertilized condition 11.8 mg P kg⁻¹). P_{mic} significantly decreased overtime for all residue treatments.

SA in the microbial and resin extracts biomass did not differ significantly between treatments, neither was isotopic dilution observed between 0P and 15P controls, not allowing for estimation of P derived from residues.

Table 14 : Phosphorus concentration in resin extracts (\pm standard error) after amendment with cover crop residues.

Type of biomass	Day since addition	P _{res} after BraC addition	P _{res} after FagE addition	P _{res} after LenC addition	P _{res} after PhaC addition	P _{res} after VicF addition	P _{res} after VicV addition	P _{res} after 0P addition	P _{res} after 15P addition
Leaves	10	11.1 \pm 1.6 AB	15.7 \pm 0.3 C	14.3 \pm 0.8 AC	14.6 \pm 1.2 BC	16.5 \pm 0.5 C	10.2 \pm 0.9 A	13.4 \pm 0.6 AC	17.1 \pm 1.1 C
	59	12 \pm 0.7 AB	15.3 \pm 0.5 B	14.8 \pm 1.1 AB	14.7 \pm 0.9 AB	15.2 \pm 0.5 AB	13.8 \pm 0.3 AB	11.9 \pm 0.6 A	14.7 \pm 0.8 AB
Leaves and stems	10	16.1 \pm 0.4 B	16.6 \pm 0.7 B	14.2 \pm 1.1 AB	15.2 \pm 0.6 AB	16 \pm 1.1 ABC	6.8 \pm 0.8 C	13.4 \pm 0.6 A	17.1 \pm 1.1 B
	59	11.6 \pm 1.1 AB	14.1 \pm 1.9 AB	11.2 \pm 0.7 AB	14.4 \pm 0.4 B	10.4 \pm 1.5 AB	9.4 \pm 0.4 A	11.9 \pm 0.6 AB	14.7 \pm 0.8 B

Letters represent statistically significant differences among treatments within the same residue type and time since amendment. BraC: *Brassica carinata*; FagE: *Fagopyrum esculentum*; LenC: *Lens culinaris*; PhaC : *Phacelia tanacetifolia*; VicF : *Vicia faba*; VicV : *Vicia villosa*.

Table 15 : Microbial phosphorus (\pm standard error) after amendment with cover crop residues.

Type of biomass	Day since addition	P _{mic} after BraC addition	P _{mic} after FagE addition	P _{mic} after LenC addition	P _{mic} after PhaC addition	P _{mic} after VicF addition	P _{mic} after VicV addition	P _{mic} after 0P addition	P _{mic} after 15P addition
Leaves	10	24.1 \pm 4.8	15 \pm 2.2	16.8 \pm 3	14.3 \pm 2.8	16 \pm 1.3	20.8 \pm 1	11.8 \pm 1.8	14.2 \pm 2.1
		NS							
	59	7.6 \pm 4	8.8 \pm 1.2	7.1 \pm 1.3	8.1 \pm 2	11.7 \pm 2	8.7 \pm 2	9.3 \pm 1.7	10.7 \pm 2.1
		NS							
Leaves and stems	10	12.6 \pm 2.1	16.2 \pm 0.9	14.6 \pm 1	13 \pm 1.6	16.2 \pm 3	21.1 \pm 0.3	11.8 \pm 1.8	14.2 \pm 2.1
		A	AB	AB	AB	AB	B	A	AB
	59	10.7 \pm 4.5	9.8 \pm 1.3	8.3 \pm 1	5.5 \pm 1.6	7.8 \pm 1.2	6.2 \pm 1.7	9.3 \pm 1.7	10.7 \pm 2.1
		NS							

Letters represent statistically significant differences among treatments within the same residue type and time since amendment. BraC: *Brassica carinata*; FagE: *Fagopyrum esculentum*; LenC: *Lens culinaris*; PhaC : *Phacelia tanacetifolia*; VicF : *Vicia faba*; VicV : *Vicia villosa*

6.3.5. Relation between residue traits and phosphorus uptake

Correlations between the same residue traits and P availability and P uptake in ryegrass were identified for leaves and stems residues and leaves residues. Cumulative P uptake in ryegrass was correlated positively with residue P concentration ($r = 0.88$, $p < 0.0001$) and negatively with residue C:P ($r_s = -0.95$, $p < 0.0001$). C:N, N:P and residue N and C concentration as well as residue SLA were not correlated with cumulative P uptake by ryegrass. C:P was the single best predictor when predicting both cumulative P uptake and per harvest P uptake in ryegrass. Overtime the negative relationship between residue C:P and P uptake by ryegrass decreased in significance up to reaching no significant correlation at 91 days (Figure 21). The slope coefficient of the linear models also decreased in absolute value from -0.016 on day 35 to -0.011 on day 70. Incorporating incubation results in the model improved fit, with P_{res} after 10 days and after 59 days being positively correlated with cumulative P uptake by ryegrass ($r_s = 0.78$, $p < 0.001$ and $r = 0.73$, $p < 0.01$ respectively). P_{mic} 10 days after amendment was negatively correlated with cumulative P uptake by ryegrass ($r_s = -0.63$, $p < 0.05$). Best model fit was achieved with residue C:P and P_{res} as predictors.

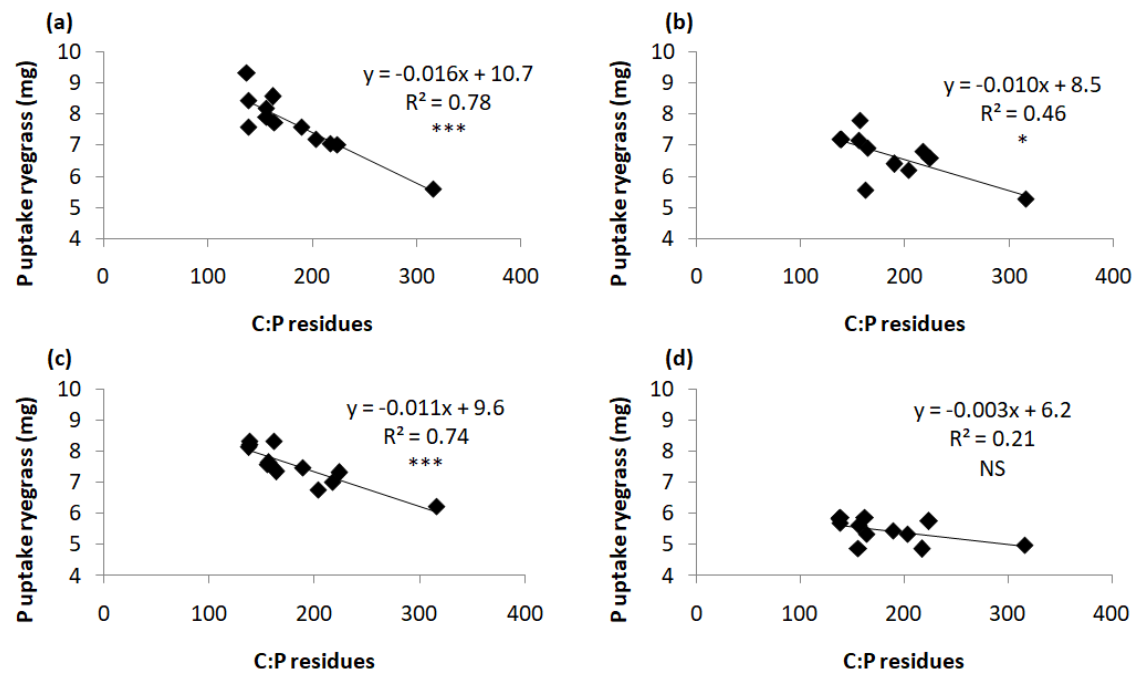


Figure 21 : Relationship between phosphorus uptake in ryegrass and cover crop residues C:P ratio.

a: 35 days after amendment; b: 47 days after amendment; c: 70 days after amendment; d: 91 days after amendment. No significant model after 91 onward days after amendment.

6.4. Discussion

6.4.1. Influence of cover crop residues on ryegrass biomass, P uptake and soil P availability

Multiple processes affect the release of residue P during decomposition and its contribution to P availability and subsequent uptake, namely rapid P leaching, microbial mineralization of residue P and/or microbial P immobilization of residue P and subsequent microbial turnover, sorption dynamics of released P on soil particles and transformation of residue P to recalcitrant P forms (Prescott 2005; Simpson et al. 2011; Alamgir et al. 2012; Damon et al. 2014). In our study, residue addition mostly decreased or maintained P availability over the course of the 4-month experiment. Ryegrass biomass, P uptake, PNI and soil P availability mostly did not differ between conditions amended with different cover crop residues, with few amendments resulting in significant decreases or increases. Vetch residues in particular resulted in a strong decrease in cumulative uptake of 50 % of the residue P, as well as a decrease in P_{res} and an increase in P_{mic} concentrations, consistent with reports in Alamgir et al. (2012) or Noack et al. (2014). Other residues such as BraC and FagE increased P_{res} as much as mineral fertilization. Our results seem to indicate simultaneous microbial P immobilisation and P release and leaching from residue. Overall, residue P release and microbial P immobilization appeared to be mostly balanced, resulting in little influence of residue P on P availability and subsequent uptake (Alamgir et al. 2012). Shifts in P_{mic} concentration support early microbial P immobilization with higher values after 10 days than 59 days, and vetch residues resulting in higher microbial P concentration. Shifts in P_{res} concentration reinforce this hypothesis with significantly lower P_{res} in soils amended with vetch residues. Decreases in P availability and P uptake were temporary as no more difference in P uptake was observed after three months onward, which is consistent with microbial P turnover. It would however indicate a longer microbial P turnover time than could be expected, within days to a month (Spohn and Widdig 2017), while similar decreases in P uptake up to 80 days after amendment were reported in Noack et al. (2014). Despite slightly higher P uptake in ryegrass (not significantly) in amended soils compared to unfertilized control after 110 days, microbial P turnover did not appear to contribute to P availability and thus P uptake in later harvests. Phosphorus released during microbial turnover is often

considered to be plant available (Oehl et al. 2001; Bünemann et al. 2004). However, our results tend to reinforce other findings suggesting quick transformation of microbe-derived P to more stable, less available P forms (Ha et al. 2007; Alamgir et al. 2012). Longer term benefits of residues for P availability are also plausible as a significant proportion of residue P may be transformed to less plant available P forms, with report of 2-6 fold higher increases in NaOH-EDTA extracted organic P following residue addition compared to the increase in microbial P (Maltais-Landry and Frossard 2015). Overall our results suggest seemingly neutral P balance between early microbial P immobilization and release of residue P for most amendments, as proposed in Alamgir et al. (2012), as well as little contribution of microbial turnover issued P to P availability and subsequent P uptake. A better understanding of the factors involved in these processes and their mediation by soil P availability could offer an opportunity to optimize residue P release for enhanced cover cropping benefits. Examining these processes across a gradient of soil P availability could thus help better understand the uncertain effects of cover crop residues on P availability and help ensuring positive plant-soil feedbacks.

6.4.2. Residue traits influence on phosphorus availability and uptake

Trait-based approaches have proven to offer reliable information in understanding and in predicting residue decomposition (Makkonen et al. 2012), thus highlighting their potential to also address the nutrient release during residue decomposition. Several residue functional traits such as leaf P content, leaf C:N ratio or leaf C:P ratio have proven to be pivotal in understanding P release dynamic from residue and its contribution to P availability, while also having very inconsistent effects, prompting further investigations (Umrit and Friesen 1994; Kwabiah et al. 2003; Damon et al. 2014; Maltais-Landry and Frossard 2015; Oliveira et al. 2017). Residue C:P ratio in particular plays a central role in understanding the fate of residue P due to microbial P immobilization following C addition (Tate 1985; Prescott 2005), but is also considered as misleading (Umrit and Friesen 1994). Our results highlighted that residue C:P ratio has a strong influence on the fate of residue P regardless of residue type (ie leaves or leaves and stems), corroborating thereby the importance of residues C:P to understand and manage P availability. The most pronounced decrease was observed for a C:P ratio > 300, which agrees with previous observations by Tate (1985), while

linear models show poor fit for residue with C:P ratio lower than 190. Similar P microbial immobilization after amendment with residue C:P ratio >200 were reported by Alamgir et al. (2012), while Prescott (2005) reported a range of critical C:P ratios from 230 to 480. Residue P concentration also presented strong correlation with P_{res} , PNI in ryegrass and ryegrass P uptake, while C:P ratio remained the best single predictor of ryegrass P uptake. Likely due to P limited growth condition for cover crop growth, cover crop residues P concentrations were all inferior to 3 mg P kg⁻¹ indicated in Damon et al. (2014) as the critical value for fast P release. Most of the concentrations were below the value of 2.4 mg P kg⁻¹ reported as the threshold for microbial P immobilization (White and Ayoub 1983). Despite the transitory nature of C:P ratio influence on P availability via microbial P immobilization, C:P ratio was also observed to be correlated with the cumulative P uptake in ryegrass, strengthening the central and still uncertain importance of residue C:P ratio in understanding P availability for plant uptake. Overall our results offer a better understanding of the processes and residue traits involved in P availability dynamics after amendment with residues and notably residue C:P ratio as well as references for modelling these processes. Managing residue C:P ratio, notably via adapted termination date to limit flowering as is common practice for residue C:N ratio (Dabney et al. 2010), could help ensuring optimized cover cropping impacts. C:P ratio influence on P release from residue is however influenced by microbial requirements and microbes C:P ratio which was in turn proposed to vary according to the soil P availability. Further efforts are thus needed to understand the complex impacts of soil P availability on the influence of residue C:P on P release from residues and integrate them for enhanced and optimized cover cropping benefits.

Morphological traits, such as SLA, have been observed to play an important role in residue decomposition and nutrient dynamics across natural ecosystems (Santiago 2007; Zekwert and Prescott 2017; Liu et al. 2018). A probable influence of morphological traits on P release has been proposed to explain the slower release of residue P observed from large residue pieces due to reduced surface area (Noack et al. 2014). SLA in particular was observed to be positively correlated with leaf decomposition rate and thus potentially nutrient release rate (Garnier et al. 2004; Pérez-Harguindeguy et al. 2013). Contrary to our hypothesis, no correlation was however observed between residues SLA and P uptake in ryegrass or P_{mic} or P_{res} .

These findings, which contrast with the multiple correlations observed in natural ecosystems, might possibly be explained by the lack of correlation between SLA and leaf P concentration or other residue traits. As cover crops species are primarily selected for their fast growing characteristics and easy termination (Hallama et al. 2019), the use of a reduced number of cover crop residues might have restricted the range of SLA values, resulting in no visible effect. Overall, residue SLA did not offer a proxy to understand and possibly model the contribution of cover crop residues to availability.

6.4.3. Implication of cover crop residues effect on P availability

Potential benefits of cover cropping on P availability have recently been reviewed across a wide range of contexts with reports increases of up to 50 % in P uptake in subsequent crops (Hallama et al. 2019). Cover cropping benefits remain very inconsistent however with reports of negative or neutral impacts on P availability (Hallama et al. 2019). Our results showed similar cumulative P uptake after crop residue amendment as compared to the unfertilized condition, with little differences between species except for vetch. Comparable observation of little differences between residue species of immature residues were previously reported by Maltais-Landry and Frossard (2015), while other studies highlighted more pronounced differences (El Dessougi et al. 2003; Eichler-Löbermann et al. 2008). Vetch residues (VicV) were previously observed to contribute less to P uptake compared to other residues in Maltais-Landry and Frossard (2015), despite a different P concentration and trait range in this study as compared to ours. This suggests that other traits such as phenols, or secondary metabolites may have conjointly played a role in the results observed for vetch (Gougoulis 2011). Early decrease or at best maintained P availability in amended soils in our experiment contrasts with reports of increased P availability and uptake after cover crop residues amendment (Maltais-Landry and Frossard 2015; Hallama et al. 2019), seemingly due different experimental conditions. Cover crop residues presented low P concentration (between 1.3 and 3 mg P kg⁻¹) and high C:P ratio (between 137 and 316) in our study, favouring microbial P immobilization over P mineralization. Paired with the P limited condition for ryegrass growth (average PNI = 50 %), the effects of microbial P immobilization may have been amplified by the soil low P availability. The momentary decrease in P availability due to microbial P

immobilization may have had a larger impact due to the reduced pool of available P. Co-limitation with N (average NNI= 85%) might have also amplified the effects as treatments with higher NNI tended to have lower PNI in early harvest. No significant correlation were however observed between P cumulative uptake and NNI, contrary to PNI. Higher contribution of residue P to P availability is most likely to occur in contexts with high P availability (Thibaud et al. 1988; Damon et al. 2014), suggesting that cover crops benefits in moderate P availability may be related to cover crops capacity to forage for P unavailable to the main crops (Alamgir et al. 2012). Agricultural practices either avoiding the temporary decrease in P availability or enhancing P availability during decomposition via mineral fertilization (Baggie et al. 2004) could ensure optimized cover cropping benefits in more P limited contexts. Further perspectives include maximising P acquisition and mobilization by cover crops in order to maximise P concentration and residue P contribution to P availability while also managing cover crops for reduced C:P ratios via termination date and selected species.

6.5. Conclusion

Understanding the factors involved in the fate of residue P in the plant-soil-microbe system is central in order to manage and potentially enhance P availability and P cycling efficiency in cropping systems via agroecological intensification. Our study reinforced the role of residue C:P ratio due to microbial P immobilization when adding C (Tate 1985; Prescott 2005). Residue with high C:P resulted in decreased P availability for up to two months after addition. Our results however highlighted mostly neutral effects of cover crop residues grown in a P limited soil on P availability and P uptake by subsequent crops, seemingly due to neutral balance between microbial P immobilization and P release from residues. Patterns of initial P decreases due to microbial immobilization underlined the importance of adapted cover crop management, notably influencing residues C:P ratio for minimal adverse effects. Neutral effects of P release from cover crops residues on P availability suggested that in context with low to moderate P availability cover cropping benefits for P availability may be achieved via other pathways such as P mobilization by cover crops or shifts in microbial communities under cover cropping (Hallama et al. 2019). Exploring the relative contribution of these pathways and especially P release to P availability in contexts with varied soil P availability constitute a major perspective of this work.

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CHAPTER 4

7. MODELLING APPROACH TO FEEDBACK EFFECTS OF SOIL PHOSPHORUS AVAILABILITY ON COVER CROPPING INFLUENCE ON PHOSPHORUS CYCLING IN AGROSYSTEMS

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Abstract

Cover crops have proved to be a promising management practice for increasing P availability in agrosystems, with recent reports of significant yet very variable cover cropping benefits for P availability and cycling efficiency (Hallama et al. 2019). Gaining insight into the factors mediating the uncertain benefits of cover cropping on P availability offers the opportunity to design for locally adapted solutions optimizing cover crops services.

Here, using a modelling approach, we investigated feedback effects of soil P availability on cover cropping effects on P cycling in agrosystems. Based on data extracted from literature, we examined average projected effects of cover crops on P availability through P release from residues grown in soil with low or high P availability.

Model predictions support higher contribution of cover crop residue P release to P availability in contexts with high soil P availability. However, irrespective of the initial soil P availability, a one to two-month P decrease of available P concentrations was projected after cover crop termination due to microbial immobilization.

Mediation of cover crops effects by soil P availability suggests different locally adapted designs, which require further investigations to quantify and model the multiple processes involved in cover crops effects on P cycling and their relevance under varying soil P availability. Microbial immobilization and its often neglected negative effects on P availability needs to be integrated in our understanding of cover cropping effects on P cycling and in decision support tools in order to adapt managements practices for maximised cover cropping benefits.

7.1. Introduction

Phosphorus (P) is a major limiting nutrient in most of the soils worldwide due to its strong sorption to soil particles. To overcome P limitation and ensure sufficient P availability in highly productive agroecosystems, significant P inputs are needed. These inputs are often largely superior to exports leading to poor P use efficiency (Menezes-Blackburn et al. 2018). Overtime low P use efficiency led to accumulation of poorly available legacy P in cultivated soils (Sattari et al. 2012). Technologies and practices exploiting these pools of legacy P, such as cover cropping, could lead to enhanced P cycling efficiency and availability in agroecosystems (Richardson et al. 2011; Simpson et al. 2011). Despite our increasing understanding of P cycling in cropping systems (Bünemann et al. 2010), P dynamics in cover crops have sparsely been addressed (Hallama et al. 2019; Honvault et al. 2020).

Cover crops can serve multiple purposes, from reducing erosion to fixating atmospheric nitrogen (Daryanto et al. 2018), and have recently been suggested to play a major a major role in P cycling in agroecosystems (Hallama et al. 2019). Through multiple morphological and physiological traits, cover crops can acquire P from pools of varied chemical and spatial availability (Wendling et al. 2016; Wen et al. 2019; Honvault et al. 2020), thus potentially mobilizing legacy P and increasing P cycling efficiency (Menezes-Blackburn et al. 2018). Cover crops also influence soil microbial communities which can in turn contribute to increasing crop capacity to access poorly available P (Oberson et al. 2006). At termination, which typically occurs within one to a few weeks before implanting the next crop except for winter killed cover crops, P captured by cover crops during the growing period (1 to 30 kg P ha⁻¹) is released during decomposition. The resulting increase in soil P availability may supply up to 50% of the P taken up by the subsequent crops (Damon et al. 2014; Maltais-Landry and Frossard 2015; Wendling et al. 2016; Hallama et al. 2019). Transfer of P contained in cover crops residues to the subsequent crop may be the main pathway of cover cropping influence on P availability (Maltais-Landry et al. 2014). The relative contribution of the multiple pathways of cover crops influence on P availability however remains unclear, with soil P availability being a key mediating factor (Hallama et al. 2019). Phosphorus release from cover crop residues in particular was proposed to be higher in contexts with high soil P availability (Thibaud et al. 1988), while it may contribute less to the overall effects of cover cropping in contexts with low soil P

availability. Cover cropping influence on P availability and P cycling efficiency thus tends to be very variable across soil contexts and especially soil P availability but also across cover crop compositions (Talgre et al. 2012; Hallama et al. 2019). Gaining a better understanding of the mediation by soil P availability of the mechanisms involved in cover crops effects, starting with P release dynamics from cover crop residue, could offer insights into optimal cover crops designs for improved benefits.

The transfer of P from cover crop residues to pools of available soil P depends on three main processes: i) Leaching of labile P contained in residues in inorganic and easily degradable organic forms; ii) Release of recalcitrant organic-bound P contained in residues after mineralization; iii) P immobilization in soil microorganisms and later release during microbial turnover (Baggie et al. 2004; Prescott 2005; Jalali and Ranjbar 2009). The P release during rapid initial leaching depends on the labile P content in residue, which usually ranges from 60 to 80 % of total P content in cover crop residues (Damon et al. 2014). The proportion of labile P in residues however strongly varies according to vegetative stage and species (Noack et al. 2012). Release of recalcitrant P contained in residues can be adequately described via mineralization of stable C in residue (Jalali and Ranjbar 2009), thus depending on similar factors such as cellulose, hemi-cellulose and lignin content in residue (Freschet et al. 2013; de la Riva et al. 2019). As soil microorganisms are primarily C-limited (Oberson et al. 2011), a significant proportion of P released from residues is immobilized in microbial biomass following C addition, ranging between 1.5 mg P g⁻¹ C and 3 mg P g⁻¹ C (Kwabiah et al. 2003). When microbial P requirements following C addition are not met by residue-derived P, an important amount of soil P can be immobilized by soil microorganisms, leading to decrease in soil P availability especially in contexts with low soil P availability. Along with the decay of the C added to the soil, this immobilized P is then released to the soil during microbial turnover. Although the main processes and factors involved in P release have been identified, our understanding of the influence of P release from residue on P availability in agrosystems remains limited, with projected effects of P release ranging from significant and lasting decreases in P availability to immediate increases (Damon et al. 2014). Managing agrosystems for maximized P benefits from cover cropping thus requires investigating P release dynamics from cover crop residues and their effect on P availability but also integrating that understanding

in models and decision support tools to ensure effective cover crops management and P fertilization.

Multiple models and tools have been developed to predict P release from crop residues. Models such as the Agricultural Production Systems Simulation (APSIM) or the CENTURY model can offer mechanistic predictions of P release from residue and its impact on subsequent crops (Parton et al. 1988; Keating et al. 2003). These models are however designed for scientist use and require detailed information that may not be available for agricultural advisers. Other approaches such as mass balances can be used at farm level and allows oversimplified estimations of the services produced by cover crops with 100 % of the P contained in residues often being assumed to be released and contribute to P uptake in subsequent crops. An alternative approach was proposed in Damon et al. (2014), designed for easy and general use with few data entries and based on mechanistic modeling of the three main processes involved in P release from crop residues and transfer to pools of available soil P. This approach, via focusing on P release and excluding the influence of site soil conditions presents a basis for producing general estimates of the contribution of crop residues to soil P availability to a subsequent crop (Damon et al. 2014). Phosphorus release dynamics are estimated in this approach via two components exponential decay functions which have been widely used to describe nutrient release from organic residues (Jalali and Ranjbar 2009): a flat rate of microbial P immobilization due to C addition and an exponential decay function describing release of P in microbial biomass along with the decay of carbon sources in soil and microbial turnover. The framework presented in the Soil Organic Carbon Reserves And Transformations in EcoSystems or SOCRATES (Grace et al. 2006) is largely employed in the model, due to similarities observed between C and P release and mineralization (Jalali and Ranjbar 2009).

Applying the model presented by Damon et al. (2014) to cover crop residues could provide precious insights into cover crops effects on P availability and on P uptake by subsequent crops as well as help addressing the main factors involved in the inconsistent P benefits of cover cropping such as soil P availability. In order to examine P release dynamics from cover crop residues and their control by P availability, we employed the modeling approach developed by Damon et al. (2014) on average cover crop biomass and P content extracted from literature, adjusting model parameters based on previous results and new references on cover crops effects on P availability.

Feedback effects of soil P availability on cover crops influence on P cycling in agrosystems were examined across scenarios designed to represent average cover crops biomass and P content in context with high or low soil P availability.

7.2. Material and methods

7.2.1. Modelling approach

In order to decipher the release of P contained in cover crop residues, a modelling approach was implemented to predict P release dynamics based on cover crop residue P content and biomass. Estimation of P released from residue was performed based on a two-phase P release pattern of labile and recalcitrant P contained in residues (Umrit and Friesen 1994; Prescott 2005), adjusting for P microbial immobilization and microbial P turnover (Kwabiah et al. 2003). The model structure thus included three P pools: i) P content in cover crop residue in labile inorganic forms or in recalcitrant organic forms; ii) P content in the soil microbial biomass; iii) Exchangeable inorganic P content in soil (Figure 22).

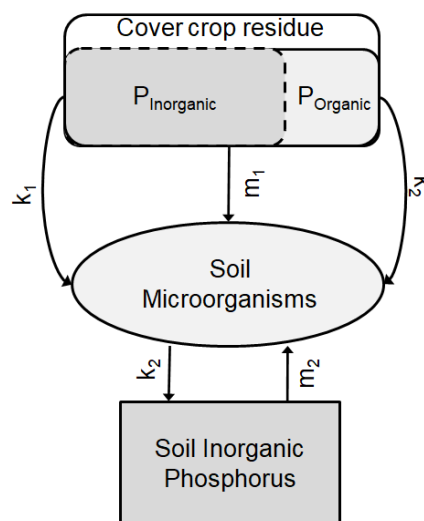


Figure 22 : Model structure

'Cover crop residue' encompasses both labile inorganic P forms and recalcitrant organic P. k_1 and k_2 are the decay constants for the pools of inorganic P in residue and organic P in residue respectively. Soil microorganisms accumulate an amount of P m_1 in response of residue C addition and an amount m_2 from the pools of exchangeable inorganic P in soil if P supplied from cover crop residue does not meet microbial requirements. Microbial turnover and release of P immobilized in microbial biomass occurs at the same rate k_2 (Damon et al. 2014).

Release of labile and recalcitrant P forms from residues was estimated via a two component exponential decay function (Baggie et al. 2004; Jalali and Ranjbar 2009; Damon et al. 2014) (Equation 1). Two decay constants k_1 and k_2 were defined

respectively for the pools of labile and recalcitrant P in cover crop residues (Shammas et al. 2003). Decays constants were estimated based on values reported for decay of labile and stable C in the SOCRATES model (Grace et al. 2006). This approach was found to offer good fit for estimating P released from residues (Jalali and Ranjbar 2009; Damon et al. 2014) notably because of correlations between mineralisation of C and P (Baggie et al. 2004).

$$P_t = P_0 \exp(-kt)$$

P_t : P remaining at time t (week)

P₀ : Initial P content in the labile or recalcitrant fraction (kg P ha⁻¹)

k : Decay constant (week⁻¹)

Multiplicative temperature and moisture factors were calculated as in Grace et al. (2006) to correct for the climate of north-eastern France (Based on mean annual temperature = 10.2 ° C, mean annual precipitation = 620 mm) (Equations 2 and 3). The adjusted decay constants k₁ for the labile fraction was 0.11 week⁻¹ and k₂ for the recalcitrant fraction was 0.01 week⁻¹. Proportion of labile to recalcitrant P in residues was estimated at 70 % based on averages for vegetative stage green residues (White and Ayoub 1983; Ha et al. 2008).

$$\text{Temperaturefactor} = 0.177 \exp^{(0.069 T)}$$

$$\text{Moisturefactor} = 0.0598 P_r^{0.279}$$

Where T = mean annual temperature (°C), P_r = mean annual precipitation (mm).

Microbial P immobilization due to addition of carbon in residues was assumed to occur at the rate of 2 mg P g⁻¹C added by residue based on previous observations (Kwabiah et al. 2003; Damon et al. 2014, Chapter 3). Microbial P immobilization was assumed to occur within the first week after addition. A flat rate of 450 g C kg⁻¹ residue dry matter was assumed for predicting carbon addition based on residue biomass (Baldock 2009). Microbial turnover and release of P immobilized in microbial biomass was estimated occur at the same k₂ rate as mineralization of recalcitrant P, as the two P pools were shown to be adequately described by the same rate in Damon et al. (2014). Phosphorus released from residues was assumed to be fully transferred to the soil P pools when in excess of microbial requirements (0.9 mg P g⁻¹ dry residue). When microbial requirements were not met, P was assumed to be immobilized both from P

released from residue and from soil inorganic P pools, resulting in negative P releases early in the decomposition.

7.2.2. Data sources and scenarios

The influence of soil P availability on P release from cover crop residues was assessed via comparing model predictions for two scenarios defined to represent average cover crop biomass and P content in systems with either high or low soil P availability. Data used for model run were extracted from a recent worldwide review of the role of cover cropping in P cycling in agrosystems (Hallama et al. 2019). The dataset was filtered to only include results of experiments performed in a temperate climate with the objective of examining P release dynamics in similar context to that of North-Eastern Europe. Covers crops with biomass inferior to 0.9 t DM ha⁻¹ and superior to 10 t DM ha⁻¹ were considered as non-representative of cover crops in these contexts and were therefore excluded from the dataset. Only data including both cover crop biomass and P content and only phylogenetic families present in both soil available P conditions were retained for analysis. The dataset was completed with addition of the results reported in Aronsson et al. (2016). Phosphorus availability levels were defined as described in the data sources as in Hallama et al. (2019). The model was run on median values for all cover crop species that is P content = 7.95 kg ha⁻¹, biomass = 3.1 t ha⁻¹ for high available soil P (n=14) and P content = 5.2 kg ha⁻¹, biomass = 1.4 t ha⁻¹ for low available soil P (n=20).

7.3. Results

7.3.1. Effect of soil P availability on P release from cover crop residues

Model predictions indicated short term decreases in P availability after cover cropping due to microbial P immobilisation irrespective of the scenario (Figure 23). Microbial P immobilization was more pronounced for cover crops grown in soils with high P availability than in soils with low P availability (-2 kg ha^{-1} as compared to less than -1 kg ha^{-1}). The projected decreases in P availability lasted till 6 weeks after cover crops termination in soil with high P availability as compared to around 4 weeks in soils with low P availability. Cover crops grown in soils with low availability as such resulted in higher P release to the soil than cover crops grown in soils with high P availability up till nearly three months after destruction. Four months after cover crop termination cover crop residues grown in soils with high P availability resulted in 15 % more P released than cover crop residues grown soils with low P availability ($+ 0.3 \text{ kg P ha}^{-1}$), 35 % after a year ($+ 1.2 \text{ kg P ha}^{-1}$). After one year 56 % of P contained in cover crop residue grown in soils with high P availability was released to the soil (4.5 kg P ha^{-1}), 63 % of P contained in cover crop residue grown soils with low P availability (3.3 kg P ha^{-1}).

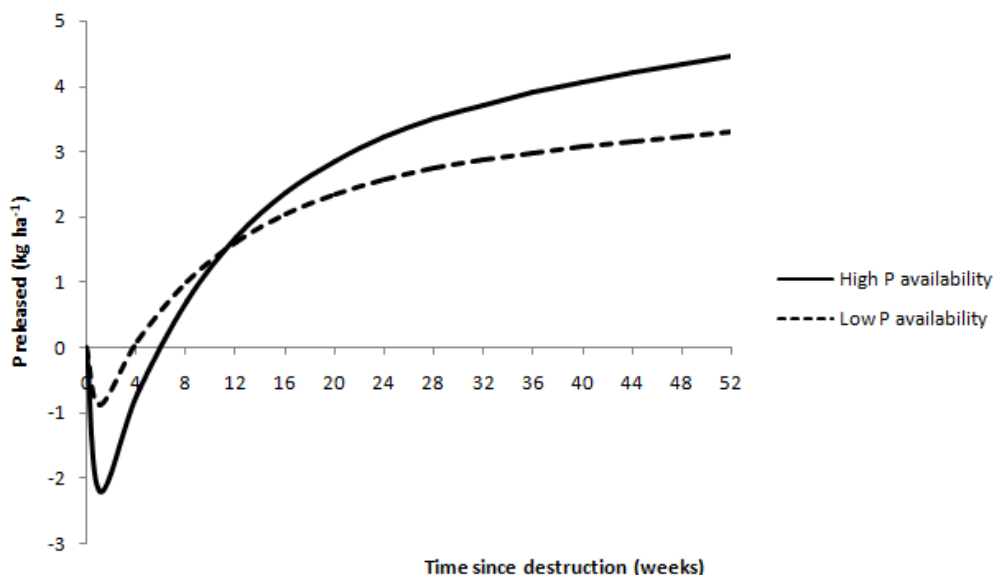


Figure 23 :Predicted P release from cover crop residue grown in soils with different P availability

Negative P release indicates microbial P immobilization from the soil inorganic P pool.

7.4. Discussion

7.4.1. Contrasted feedback effects of soil P availability on P release dynamics from cover crop residues.

Cover crops can lead to important increases in P availability and uptake in following cash crops. However cover cropping influence on P availability tends to be very inconsistent according to site conditions and especially soil P availability. Several studies report increases of P uptake by subsequent cash crops by up to 50% while others show decreases by up to 20 % (Hallama et al. 2019). Contribution of cover cropping to soil P availability via P release from residues is expected to be higher in systems with high P availability (Thibaud et al. 1988). In order to examine the effect of soil P availability on P release dynamics we produced two different scenarios based on average biomass production and P concentration reported in literature in soils with high or low P availability and compared model predictions. Our estimates showed a larger contribution of cover crop residue to soil P availability and potentially P uptake by subsequent crops in systems with higher soil P availability, while also demonstrating significant microbial P immobilization leading to longer lasting decrease in P availability in these systems. Higher biomass production coupled with lower P concentration in cover crops grown in soils with high P availability contributed to predictions of higher early decrease in P availability due to microbial P immobilization in soils with high P availability, with average P concentrations below the threshold favouring mineralisation (i.e. 3 mg P g^{-1}) (Damon et al. 2014). Up till three months after destruction, cover crop residues from systems with low P availability thus were projected to contribute more to soil P availability. Our scenarios might however have accentuated this observation, with lower biomass production than average for temperate agroecozones (3.1 t ha^{-1} in the high available soil P scenario as compared to 3.4 t ha^{-1} on average) (Ruis et al. 2019) and lower P content (8 kg P ha^{-1} as compared to reports of 5 to 30 kg P ha^{-1}) (Damon et al. 2014; Maltais-Landry and Frossard 2015; Wendling et al. 2016). Early decrease in P availability due to microbial P immobilization requires adapted management to fully benefit from P released from cover crops residue and especially adapted termination date to ensure sufficient delay before the main crop growth (Wendling et al. 2016; Hallama et al. 2019). Overall our results strengthened the

expected stronger contribution of cover crops to P availability via P release from residues in systems with higher soil P availability, while also highlighting significant early decreases in P availability that need to be managed to ensure maximised P benefits from cover cropping. While P benefits from cover cropping via P release from residues are projected to be lower in systems with low P availability, multiple mechanisms contribute to the influence of cover cropping on P cycling efficiency. Perspectives thus include examining how P benefits from cover cropping can be differently optimized according to soil P availability and explore in particular the potential for maximising other mechanisms such as P mining or positive interactions with microbial communities.

7.4.2. Benefits and limits of the modelling approach

While the main factors influencing P release from residue have been identified, the contribution of P release from residue to P availability remains unclear and largely unquantified (Damon et al. 2014). By adapting the modelling approach developed by Damon et al. (2014), we aimed at examining P release dynamics from cover crop residues and their influence on P availability. Based on a few readily available parameters, this approach predicted similar amounts of P released from residue to that observed in literature, 70 % after six months as compared to the 70-80 % observed in Talgre et al. (2012). Around 80 % of the P contained in residue was predicted to be released after one year with approximately 60 % being released to pools of inorganic soil P as opposed to the 100 % often assumed in mass balance approaches. The extent and duration of decreases in P availability due to microbial P immobilization could also be predicted, complementing our understanding of the negative effects of cover crop residues in some contexts (Thibaud et al. 1988; Hallama et al. 2019; Chapter 3). However the datasets used for model run did not allow for model validation, thus suggesting cautious interpretation of the results and consideration of model limitations. A previous sensitivity analysis demonstrated high sensitivity of the model to P concentration and speciation in residues as well as P uptake by the microbial biomass following carbon addition (Damon et al. 2014). In our approach P concentration in residues was a model input while P speciation in residue was assumed to be 70 % labile P, 30 % recalcitrant P (Damon et al. 2014). Phosphorus speciation in residue however can vary significantly between species and vegetative stage with reports of 25 to 75 % P in residue being orthophosphate in different crop

species and approximately 20 % less phosphorus being labile in mature residue as compared to vegetative stage residues (Noack et al. 2012; Damon et al. 2014). Moreover, due to very uncertain partitioning between root and shoot with 16 to 65 % of total plant P being contained in roots (Franchini et al. 2004), P contained in roots was not considered in the model, underestimating P content in cover crop residues. Microbial P immobilization due to carbon addition is also a central point of uncertainty, with reported values ranging from 1.5 mg P g⁻¹ C to 3 mg P g⁻¹ C added (Iqbal; Kwabiah et al. 2003; Baggie et al. 2004; Alamgir et al. 2012; Damon et al. 2014). Two mg P g⁻¹ C were assumed to be immobilized by microbes in our model based on previous work by Honvault et al. (Chapter 3), larger than the 1 mg P g⁻¹ C assumed in Damon et al. (2014) possibly overestimating microbial P immobilization. Carbon content in residue was also assumed to be 45 % as in Damon et al. (2014), which is higher than some reports (Ma et al. 2018) and possibly accentuates this overestimation of microbial P immobilization. Decay factors were adjusted to local climate based on 620 mm annual precipitation and 10.2 ° C annual temperature. However with cover crops usually being destroyed and decomposing during winter, finer adjustments with monthly temperature and precipitation as in the CENTURY model could provide more accurate estimates. Moreover the approach developed, based on the work of Damon et al. (2014), while allowing for general estimations exclude the considerable influence that soil properties can have on these processes. Soil P availability in particular should be analysed in the context of P fractionation in the soil rather than just based on extractions of “available” P via Olsen or Bray extraction methods. Overall, model estimates can help understanding and estimating cover crop residue contribution to P availability under different scenarios, yet the model parameters chosen may either underestimate this contribution for P speciation and microbial P immobilization or slightly overestimate for climate information. Further work should focus on investigating the remaining uncertainties in parameter values as well as validating the model based on empirical observations.

7.5. Conclusions

Ensuring efficient cover crops designs and management for improved P availability and cycling efficiency requires tools to guide farmer decisions (Damon et al. 2014), in turn calling for a fine understanding of the mechanisms involved and the main factors mediating these mechanisms (Hallama et al. 2019). In order to investigate the effects of cover cropping on P availability, we examined P release dynamics from cover crop residues across two scenarios designed to address their mediation by soil P availability, as predicted based on a modelling approach. Our results strengthen the stronger contribution of cover crops to P availability via P release from residues in systems with higher P availability (Thibaud et al. 1988), while also highlighting important projected decreases in P availability after cover crop destruction up till one or two months. Negative impacts of microbial immobilization of P after cover crop destruction needs to be integrated in our understanding and management of cover crops on P cycling in agrosystems, notably by adapting delays between cover crop destruction and subsequent crop implantation. In order to optimize cover cropping effects on P availability, cover crops designs should also be adapted according to P availability, which require further investigation of the other pathways of cover crop influence on P availability especially in contexts where P availability is low and P release from residue may contribute less to the overall impacts of cover crops.

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DISCUSSION

8. DISCUSSION

Facing depletion of rock phosphate resources, unstable fertilizer prices and adverse environmental effects of over-fertilization, practices and technologies offering to increase P cycling efficiency in agroecosystems have gained increasing interest (Richardson et al. 2011; Menezes-Blackburn et al. 2018) and adoption (Knowler and Bradshaw 2007). Cover crops offer the opportunity for farmer to adapt their cropping system and deliver multiple ecosystems services (Schipanski et al. 2014; Daryanto et al. 2018) including increased P availability and cycling efficiency (Hallama et al. 2019). Designing cover crops for improved P cycling efficiency in cropping systems however requires gaining a better understanding of the biogeochemical cycling of P in agroecosystems and the complex plant-soil feedbacks involved (Bünemann et al. 2010). The objective of this PhD thesis was to understand the role of cover crops traits in the multiple plant-soil-microorganisms processes governing P availability in agrosystems. The functional approach adopted here offers precious insights into the relevant plant traits and strategies which are very useful for designing and managing cover crop for increased P availability in cropping systems. Moreover, the references produced and new knowledge gained on the influence of plant traits on P cycling in agroecosystems could contribute to the improvement of existing models and their integration in decision support systems. These systems would in turn allow the evaluation of the effects of cover crops on P availability and support management decisions by farmers such as adapted P fertilization.

8.1. Tradeoffs and functional strategies for phosphorus acquisition in cover crops

Characterizing the complex association between the multiple traits involved in P acquisition by plants is a major challenge to overcome in order to understand the impacts of plants on P cycling. A vast array of chemical, physiological, biological and morphological traits indeed contribute to the capacity of plants to access the various pools of P in soils (Lynch 2011; Richardson et al. 2011; Wang and Lambers 2020). These traits are associated among complex tradeoffs due to the carbon cost associated with their expression (Lynch and Ho 2005; Raven et al. 2018), which need to be understood in order to unravel the complex balance between the ecosystems services to which they contribute.

Multiple tradeoffs between plant morphological and physiological P acquisition traits were expectedly highlighted when examining thirteen cover crops species (**Chapter 1**). Tradeoffs between the proportion of fine roots (with diameters inferior to 0.5 mm) and PME activity indicated diverging P acquisition strategies relying either on increased P foraging or P mobilizing for P uptake, as previously reported for crop species (Lyu et al. 2016). These results were reinforced by recent reports of lower carboxylate exudation and PME activity in the rhizosphere of plants with lower root diameters (Wen et al. 2019). Investigating plant-soil microorganisms interactions allowed us to identify a probable additional tradeoff axis (**Chapter 2**). Negative relationships were indeed observed between fine root percentage and indicators Gram negative bacteria. While negative relationships between root colonization by AMF and root diameter have been observed in natural ecosystems (Chen et al. 2016; Li et al. 2017; Zhou et al. 2018a), this observation extended to bacterial indicators in our study, pointing toward the role of plant traits in shaping bacterial communities for P acquisition (Sasse et al. 2018).

Going further and analysing the convergence of plants traits toward common P acquisition strategies in multi-trait space allowed us to underline multidimensional root trait interactions and a diversity of P acquisition strategies in cover crops (**Chapter 1**). Root traits have been proposed to form a root economic spectrum, analogue to the leaf economic spectrum, ranging from resource conservative traits to resource acquisitive traits (Roumet et al. 2006). The tradeoffs axes highlighted in our approach (**Chapter 1 and 2**) could in part reinforce this observation. A more complex understanding of the root economic spectrum has however been proposed, highlighting its multidimensional character due to the complex interactions between root environmental constraints and soil biota (Weemstra et al. 2016). Mediation of tradeoffs as well as P acquisition strategies by soil types (**Chapter 1 and 2**) appears to reinforce multidimensional adjustment of plant strategies along multiple axes when faced with environmental constraints. In turn it could provide elements of understanding toward reports of inconsistent tradeoffs expression in different environmental contexts (Sgrò and Hoffmann 2004; Kong et al. 2019).

The multidimensionality of root traits relationships poses a major challenge when investigating plant P acquisition strategies. Multi-dimensional adjustments of root traits when exposed to varied environmental constraints indeed requires examining a wide range of traits simultaneously in order to gain a comprehensive understanding of plant P acquisition strategies. Including other traits involved in P acquisition such as root hair length or abundance (Fort 2013) or root branching intensity (Wen et al. 2019) would thus be recommended alongside the physiological and morphological traits considered in this study. Similar grouping were however noted in our approach as compared to a precedent approach by Wendling et al. (2016), hinting toward central traits capturing most of the information. Root diameter, reflected by the percentage of fine root in our study, especially could offer a good proxy to plant P acquisition strategies (Wen et al. 2019). Investigating this trait, alongside many other P-acquisition traits across a wide gradient of soils would offer precious insight into the role of plants in P cycling in agrosystems and the opportunity to design more efficient systems via adapted P acquisition strategies and traits.

8.2. Feedback effects of cover crop traits on P availability

Plant P acquisition strategies are the main drivers of the effects of cover crops on P availability in agroecosystems, determining the mechanisms involved and conjointly with soil and climate conditions their relative contribution to the overall effects (Hallama et al. 2019). Understanding and quantifying the impacts of cover crops on P availability but also P uptake in subsequent crops however require investigating the feedbacks mechanisms through which these plant strategies and traits impact P cycling. Feedbacks effects of cover crops on P availability mostly occur through three pathways: Release of P contained in cover crop biomass after termination and associated effects on P availability; Microbial feedbacks due to shifts in microbial communities under cover cropping; Legacy effects of P mobilization by cover crops on P pools of soil.

8.2.1. Effects of cover crop residues traits on plant-soil-microbe mechanisms involved in P availability

Examining P release dynamics from cover crop residues and the role of plant traits in these dynamics highlighted the effects of residue chemical traits, and in particular C:P ratio (**Chapter 3**). Linear models showed poor fit for C:P ratio inferior to 190, showing decreased P availability and uptake in subsequent crop for superior values (**Chapter 3**). Although the effects of C:P ratio have been identified before (Kwabiah et al. 2003; Prescott 2005; Espinosa et al. 2017), a wide range of values have been reported from 60 to 700 and its effects have been considered misleading (Umrit and Friesen 1994). In our study, it appeared to be the single best predictor of the fate of residue P, offering a good proxy to plausible P release dynamics. The central role of residue C:P ratio in our results was proposed to be due to important P microbial immobilization, in turn mostly resulting in neutral if not negative effects of cover crop residues in P availability (**Chapter 3**, Alamgir et al. 2012). The modelling approach developed in **Chapter 4** rather appeared to reinforce the central role of P microbial immobilization. Negative to neutral effects of residue addition were indeed projected for one to two months after destruction based on average cover crops biomass and P content. This is coherent with the negative effects of cover cropping on P availability sometimes observed in literature (Eichler-Löbermann et al. 2008; Hallama et al. 2019). Model projections provided elements of understanding toward the mostly neutral effects observed in **Chapter 3** with only a reduced proportion of P being projected to be transferred to pools of inorganic P in soils after four months (30 to 39 %). Lower, but not significantly, P uptake in the unfertilized conditions after 4 months could then be attributed to the delayed release of immobilized P.

8.2.2. Implications of plant-soil-microbe interactions for phosphorus acquisition in subsequent crops

Microbial feedbacks for P acquisition can prove challenging to measure at field level notably due to fast turnover rate of the communities involved (Schmidt et al. 2007). Important effects of native soil microbial communities, soil properties and management history on soil microbial communities' composition all constitute possible confounding factors reducing result generalization (Oehl et al. 2004; Richardson et al. 2011; Hunter

et al. 2014). As such directly measuring the role of plant traits in these feedbacks may prove challenging, and produce site-specific results. We could observe that microbial NLFA and PLFA indicators were negatively associated with proportion of fine roots and positively with carboxylate exudation rate (**Chapter 2**). Increases in C16:1 ω 5 an indicator of AMF storage forms, i.e. spores (Ngosong et al. 2012) could be of interest. As AMF may strongly contribute to P uptake by plants (Whipps 2001), this appears to indicate that selecting for plants with important root exudations and intermediate to low proportions of fine roots could potentially provide the basis for positive microbial feedbacks. Fabaceae cover crops which are known for their important impact on soil properties via exudations (Maltais-Landry 2015), have notably been observed to significantly contribute to P uptake in subsequent crop through positive microbial feedbacks which appears to reinforce that suggestion (Hallama et al. 2019).

8.2.3. Perspectives for the study of cover crop feedback effects

While not measured here additional traits such as cellulose, hemi-cellulose and lignin content may be very influential for P release dynamics from residue (Sjöberg et al. 2004) due to microbial P immobilization and simultaneous mineralization of C and P contained in residue (Baggie et al. 2004). Phenols may also be important traits to include in future approaches (Gougoulas 2011). The main perspective remains to explore the microbial dynamics involved in P release and especially how residue C:P ratio drives these dynamics alongside microbial requirements across different conditions. Microbial C:P, while flexible, plays an important role in determining microbial requirement and microbial P immobilization. Microbial C:P was moreover proposed to vary across soil with different P availability (Parton et al. 1988). As such further investigations across a range of P availability are warranted to examine and quantify microbial dynamics in order to optimize P release from residue.

8.3. Study perspectives on the effects of cover crop functional diversity on P availability in agroecosystems.

The tradeoffs and P acquisition strategies shown by our approach were observed in monospecific conditions, as for traits interactions on microbial community and effects on P availability via P release from residues (**Chapter 1 to 4**). However, cover crops are often multispecific, allowing the farmer to pursue multiple services simultaneously (Schipanski et al. 2014) and generally being more productive than monospecific crops (Wortman et al. 2012; Finney et al. 2016). Understanding the effects of the diversity of plants traits and strategies in multispecific mixtures and their influence on the feedbacks mechanisms involved in P availability in agrosystems is thus a central perspective of our work in order to exploit our findings for the design of P efficient cover crops.

Rather than species diversity, functional diversity has proven to be more efficient in explaining the services produced by plant communities (Lavorel et al. 1997; Cadotte et al. 2011). The effects of functional diversity on ecosystem functioning can follow two main hypotheses: i) Dominance effects where the most abundant traits attributes present in the community drive its functioning, leading to additive effects of species attributes weighted by their abundance (Grime 1998; Lavorel et al. 2007); ii) Diversity effects where the diversity or divergence between trait attributes in the community drive its functioning leading to non-additive effects of species attributes, often synergic (Tilman et al. 1997; Gartner and Cardon 2004; Petchey and Gaston 2006). Divergence in trait attributes in functionally diverse communities can induce complementarities due to niche differentiation and facilitation between species, increasing resource use efficiency and decreasing competition for resource acquisition (Petchey and Gaston 2006; Turner 2008; Li et al. 2014). With the example of residue decomposition, functionally diverse mixtures composition has been observed to result in synergistic effects, negative effects or additive effects (Redin et al. 2014). Functional diversity effects on ecosystem functioning thus remain uncertain, notably due to the influence of environmental factors and idiosyncratic effects, that is the effect of a specific specie or individual on the global response of a mixture (Hättenschwiler et al. 2005; Makkonen et al. 2013; Laureto et al. 2015). Inconsistent effects of functional diversity on ecosystem functioning thus represent a major challenge that needs to be addressed in order to design multi-crops system (Ehrmann and Ritz 2014; Laureto et al. 2015).

In turn gaining a better understanding of the effects of functional diversity on ecosystems functioning could, in the case of cover crops, lead to increased P uptake and later release (Li et al. 2014), improved microbial feedbacks from more functionally diverse communities (Qin et al. 2017) and P release kinetics more synchronous with P requirement of subsequent crops (Faucon et al. 2015). They are thus three main processes by which cover crops functional diversity can benefit P availability in agroecosystems : i) Functional divergence and ecological complementarities between species with different strategies efficiently exploiting the different P pools in soils; ii) Plant functional diversity shaping the microbial communities composition and functions differently in mixtures leading to enhanced P uptake and improved feedback effects on subsequent crop P acquisition; iii) Non-additive effects of diverse litter traits on P release dynamics. Investigating these processes require adapted experimental designs allowing to separately estimate their incidence on P availability for subsequent crops as well as their relative contribution to the overall benefits of cover crop functional diversity.

The effects of functional diversity on P acquisition in species mixtures received the most attention in the context of intercropping (Hinsinger et al. 2011; Li et al. 2014; Tang et al. 2016). Several associations between species acquire more P than their individual components such as associations between chickpea and wheat, white lupine and wheat, or maize and faba bean (Horst 1987; Li et al. 2003, 2007). Diverging P-acquisition strategies in the species used in these associations, relying more on mining or foraging for P acquisition, suggest that their benefits are based on plant-plant facilitation (Li et al. 2014). The different rooting depths observed in these species also could indicate niche differentiation (Hector et al. 2002; Turner 2008). The spectrum of P acquisition strategies identified in cover crops (**Chapter 1**) thus offers an opportunity for beneficial effects of functional diversity via associating diverging P acquisition strategies in mixtures. Diverse cover crops mixtures could indeed exploit different soil P pools leading to plant-plant facilitation and ensuring reduced competition for resource acquisition. Associating diverse strategies in multispecific experiments could in turn offer precious insights into the potential benefits of cover crops functional diversity on P availability in agroecosystems. In order to distinguish between identity effects and diversity effects, several species of each key functional groups, and notably Fabaceae, should be examined and associated in mixtures. Ideal mixture designs for investigating the effects of cover crop functional diversity on P availability would then integrate species with diverging strategies, that is : Species heavily relying on P mining for P acquisition, as can be the case for several Fabaceae species and in particular

white lupine (Lambers et al. 2013); Species heavily relying on P foraging such as *Phacelia tanacetifolia* (**Chapter 1**) or *Guizotia abyssinica* (Wendling et al. 2016); Species with intermediate strategies relying on both foraging and mining to varied extents according to soil properties such as *Vicia villosa* (**Chapter 1**). Due to the central role of microbial associations for P acquisition, as reinforced in **Chapter 2**, non-mycorrhizal species such as Brassicaceae should also be included in ideal designs to fully investigate the effects of species mixtures with diverse P acquisition strategies. In order to observe a gradient of functional diversity in the experimental design, functional diversity could be projected based on observed attributes under similar conditions which could be extracted from trait databases such as the TRY database (Kattge et al. 2011). To avoid confounding effects of species richness the gradient of functional diversity should conserve the same number of species in mixtures. Finally, the number of conditions should be adapted in order to allow for modelling approaches, projecting the effects of diversity while also incorporating the environmental factors potentially mediating them.

Investigating plant functional diversity interactions with microbial communities and their effects on P uptake in subsequent crops can be challenging, especially under field conditions, notably due to the indirect influence of soil properties (Jansson and Hofmockel 2018; Li and Wu 2018). Functionally diverse plant mixtures are likely to result in functionally diverse rhizosphere microbial communities (Zhang et al. 2011; Yang et al. 2016; Qin et al. 2017) although rhizosphere microbial communities composition may also be dominated by the specific microbial community of the most competitive species (Song et al. 2007; Hortal et al. 2017). In turn as microbial communities tend to have high functional redundancy (Louca et al. 2018), whether diverse microbial community composition results in positive feedbacks for P acquisition and productivity in subsequent crops remains to be fully explored (Wang et al. 2017). Examining the effects of plant functional diversity on the plant-soil-microbe interactions involved in P-acquisition and their feedbacks effects on subsequent crops thus require investigating the effects of functionally diverse cover crop mixtures on microbial functional diversity and the influence of increased microbial diversity on P-acquisition by cover crops and subsequent crops. **Chapter 3** highlighted the relationships between microbial communities, root diameter and carboxylate exudation, thus suggesting that these traits should be central axes for the study of the effect of plant functional diversity on microbial functional diversity. While direct measurements can be long and tedious, indirect bioindicators such as PLFA and NLFA but also root DNA extracts could be used to assess rhizosphere microbial communities during growth along a gradient of

plant functional diversity such as the one previously proposed. Feedback effects could then be examined via measuring the microbial communities associated with subsequent crops. Furthermore, as functionally diverse cover crop residues will also increase microbial functional diversity during their decomposition (Drost et al. 2020), separating the effects of cover crops traits on microbial communities during their growth and the effects of cover crop residues traits on microbial communities during their decomposition may require experimental designs exporting the residue produced.

Functional diversity effects on residue decomposition and associated phosphorus release tend to be very variable with reports of non additive effects (Gartner and Cardon 2004) but also additive effects of community weighted traits in some contexts (Tardif 2013). The gradient of functional diversity previously proposed in order to assess functional complementarities in P acquisition could offer a basis for evaluating these effects. Due to the numerous challenges in examining the fate of P released from residues, a litter bag or incubation approach could be necessary. Litter bags filled with cover crops residues could offer the opportunity to measure at different stages the release of phosphorus from residues. As environmental factors may strongly contribute to the non additive effects or additive effects of residue traits on residue decomposition (Makkonen et al. 2013), modelling approaches should be used to examine the influence of these environmental factors on residue decomposition conjointly with the effects of functional diversity (Tardif 2013). There are many challenges to the development of models addressing the effects of diverse species mixtures as reviewed in Ehrmann and Ritz (2014). However, applying conceptual modelling frameworks such as the iterative approach proposed by Diaz et al. (2007) (Figure 24) could contribute to the understanding of the respective influence of residue functional diversity and abiotic conditions on residue decomposition and nutrient release dynamics.

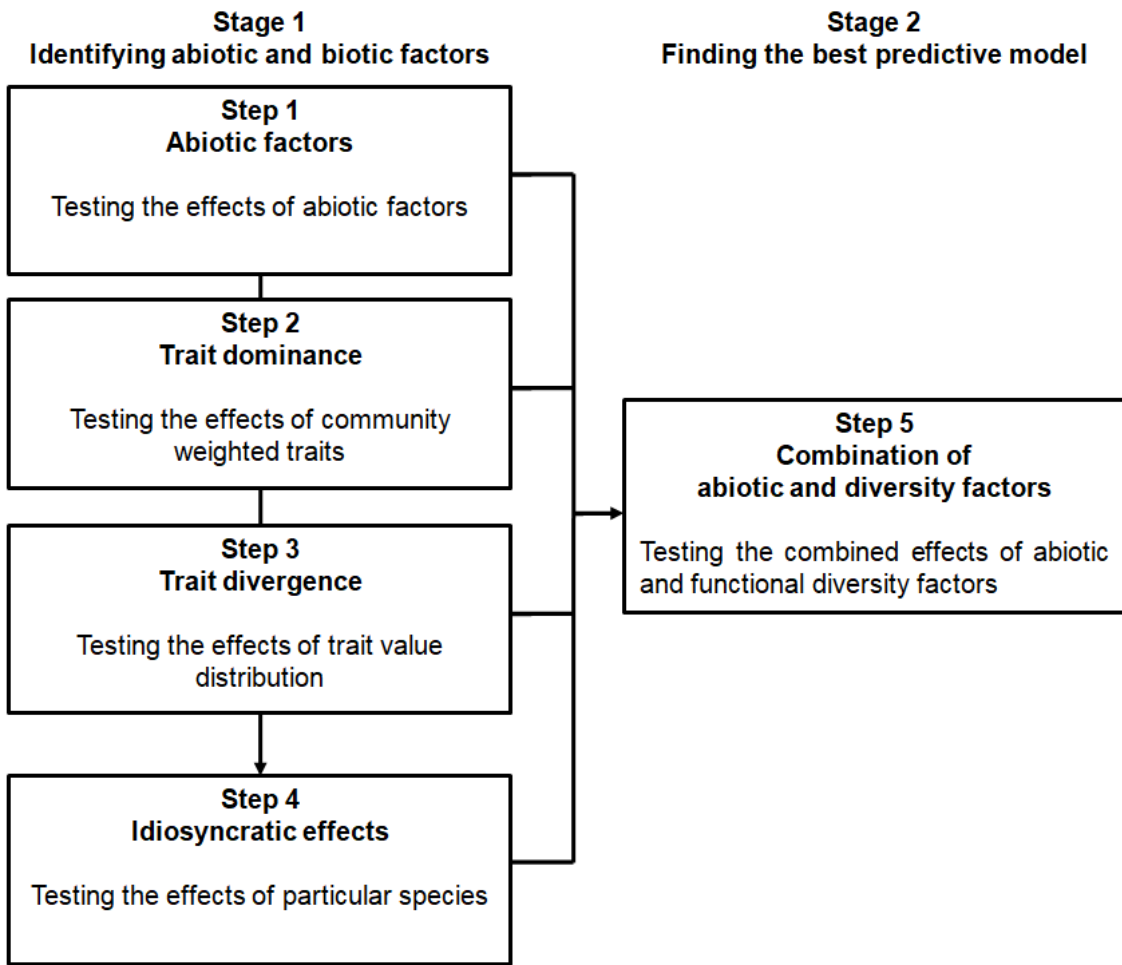


Figure 24 : Summarized framework for addressing the effect of functional diversity on ecosystem functioning. Adapted from Diaz et al. (2007)

8.4. Trait based approach to cover crop designs for improved phosphorus availability in agroecosystems

8.4.1. Functionally optimal cover crop design for improved phosphorus availability in agroecosystems: Insight into species and strategies selection

A major implication of this PhD is the opportunity to adapt cover crops composition for improved P availability in agrosystems via beneficial plant functional strategies and traits. Examining thirteen cover crops species highlighted a spectrum of strategies structured along tradeoffs between traits involved in P foraging and traits involved in P mobilization (**Chapter 1**). A potential second tradeoffs axis was also underlined between foraging traits and association with soil microorganisms for P-acquisition (**Chapter 2**). Our results thus suggests the lack of an “ideal” strategy or specie offering high potential for mobilising hardly available P, high capacity to forage available P while simultaneously ensuring positive microbial feedbacks (Richardson et al. 2011). Pursuing a specific process via plant traits and strategies is likely to be at the expense of the others, while diverse cover crops mixtures, a major perspective of this project, may offer the opportunity to associate these different processes. The relative contribution of a specific process is moreover influenced by soil conditions and especially P availability (**Chapter 4**), suggesting cover crops designs relying on different processes according to soil P availability notably via employing the contrasted services offered by the diverse cover crop families (Figure 25).

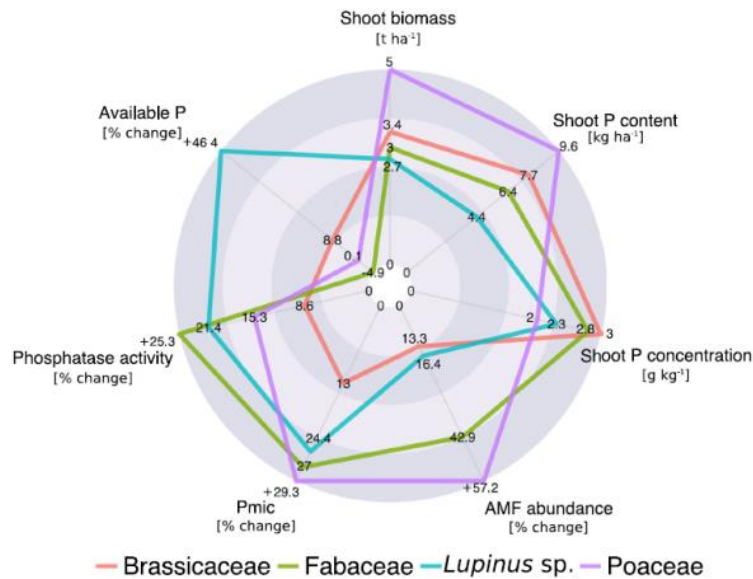


Figure 25 : Radar chart summarizing the properties of the cover crop families and their effects on soil (Hallama et al. 2019)

In contexts with high P availability, P acquisition and later release from residue may be the main pathway involved in the effects of cover crops on P availability (Thibaud et al. 1988; Maltais-Landry 2015; Hallama et al. 2019). Microbial association or P mobilization have indeed been observed to be less efficient in soil with high P availability (Grman and Robinson 2013). Strategies and traits associated with high P foraging capacity (and high P concentration in biomass) such as the “morphologically inclined strategies” observed in **Chapter 1** may thus be the most adapted to ensure maximised benefits of cover cropping on P availability in these contexts (Wendling et al. 2016). Brassicaceae, or eventually Poaceae with high P concentration, could thus be recommended in contexts with high P availability. The strong contribution of the “residue pathway” in contexts with high P availability would however reinforce the need to select for species with adapted life cycle, as C:P ratio tends to increase with development stage (Wendling et al. 2016), in turn potentially leading to reduced or even adverse effects of cover cropping on P availability (**Chapter 3**, Hallama et al. 2019).

The lower contribution of P released from cover crops residues to P availability projected under low P availability (**Chapter 4**), reinforces the higher relative contribution of the other processes such as P mobilization or positive microbial feedbacks in these contexts (Hallama et al. 2019). As such strategies and traits involved in P mobilization and favouring microbial associations should be of particular interest under low P availability, such as the ones identified in the Fabaceae family

(Chapter 1 and 2). Fabaceae have indeed been observed to be the most efficient cover crop family for improving P availability (Hallama et al. 2019). The diverse spectrum of strategies highlighted within the Fabaceae family (**Chapter 1**) thus offers to go further by selecting within the Fabaceae family species with the desired strategies either heavily relying on P mobilization (such as Faba bean for example) or balancing P foraging and P mobilization (such as Hairy vetch for example). While potential benefits of cover crops via positive microbial feedbacks remain to be fully explored and quantified, mycorrhizal species should also be preferred over non mycorrhizal species such as Brassicaceae in contexts with low P availability (Cosme et al. 2018) (Box A). Brassicaceae have moreover been proposed to mainly contribute to P cycling via P released from residues, further decreasing their benefits under low P availability (Hallama et al. 2019).

Our results can offer general guidelines for selecting plant strategies and traits in order to improve P cycling efficiency and availability in agrosystems according to soil P availability. The functional approach chosen can potentially be employed to generalize the results obtained in vastly differing ecosystems rather than recommending species adapted to a specific phytogeographical territory. However, mediation of strategies and tradeoffs by soil types (**Chapter 1 and 2**) suggest that further investigations are needed to evaluate the relevance of plant strategies and traits in different soil contexts. Some processes such as rhizospheric acidification via plant proton release may be less efficient in acid soils for example (Hinsinger 2001). The effects of diverse association of plant strategies also largely remain to be fully explored as previously developed, although maximised cover cropping benefits should be achieved by diverging strategies exploiting different phosphorus pools with limited competition for resources. Incorporating these aspects with the precedent guidelines could offer a sound basis for pursuing more efficient P cycling in cropping systems. Pursuing higher P availability in agrosystems via adapted cover crop designs may however come at the cost of other services of cover crops (Schipanski et al. 2014), which should be considered when designing cover crops composition.

Box A: Insight into species selection: Which cover crops family for what services ?

Highlighting the relevant plant strategies and associated services according to soil P availability constitute a first step toward designing cover crops composition for enhanced P availability in agroecosystems. The next logical step is then to select the adapted cover crop families and species in order to produce these ecosystems services.

In order to compare the services produced by different cover crop families, the modelling approach described in **Chapter 4**, based on the work of Damon et al. (2014), was employed on data extracted from a field experiment. Multiple species of two commonly used cover crop families, Brassicaceae and Fabaceae, were compared based on their estimated influence on P availability via P release from residues

Brassicaceae residues were estimated to contribute more to P availability (Figure 26) via P release due to higher biomass and similar P concentrations. High microbial immobilization was however expected with negative effects lasting for up to 2 months. Lower contribution of Fabaceae may be unexpected as they have been observed to be the most effective family for improving P availability (Hallama et al. 2019). Fabaceae are however known to provide many other services which could explain their lower contribution via P release.

Brassicaceae could consequently be preferred in contexts where P release from residue strongly contributes to P availability such as soil with high P availability while Fabaceae may be preferred in contexts with lower P availability. However recent evidence points toward high potential complementarities of legume-brassica mixtures for nutrient acquisition (Zhou et al. 2008b, 2019), suggesting their association in cover crops mixtures.

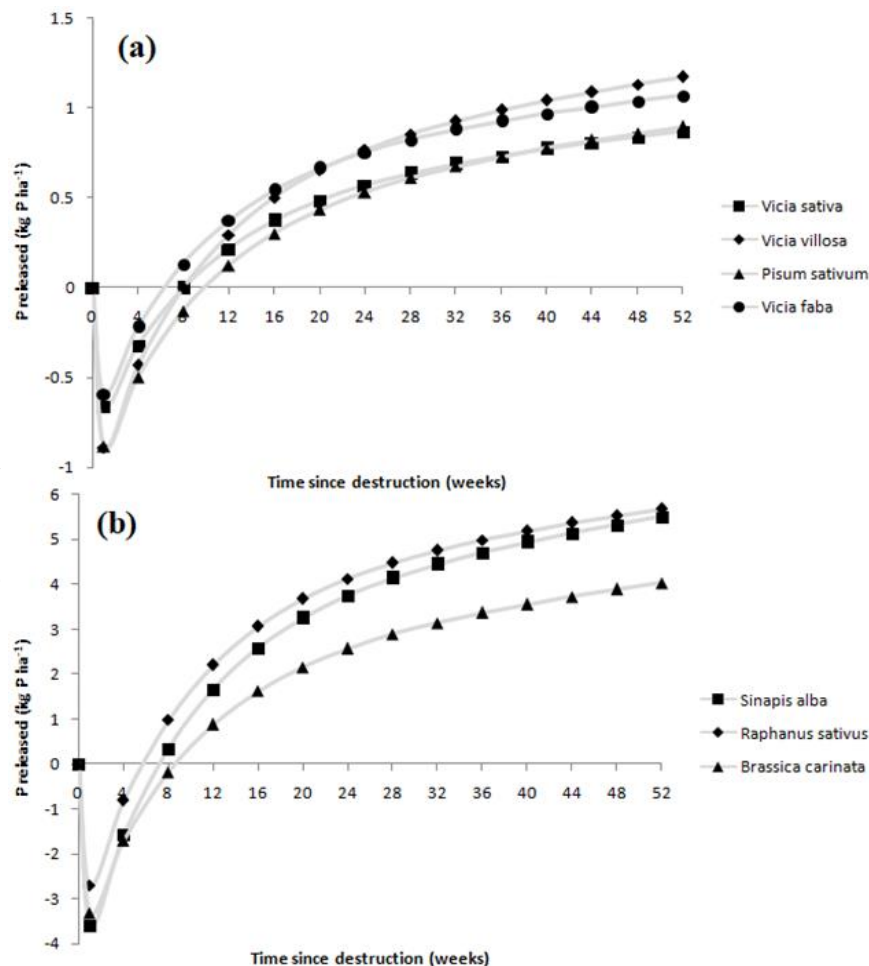


Figure 26: Predicted P release from Fabaceae (a) and Brassicaceae (b) cover crop based on model by Damon et al. (2014).

8.4.2. Designing for improved phosphorus benefits of cover crops: Implications of trade-offs and association between ecosystem services produced by cover crops

In addition to their role in P cycling in agrosystem, cover crops and their traits can produce many ecosystem services such as promoting nitrogen fixation from the atmosphere, reducing nutrient leaching and erosion or increasing carbon storage in soil (Daryanto et al. 2018). The cover crops traits and strategies highlighted previously, associated with increased P availability in agroecosystems, may positively influence other services produced by cover crops. For example, P mining strategies in cover crops can contribute to increased organic P mineralization and thus to better use efficiency of organic fertilizers from waste sources such as sludge or compost. Designing for improved P availability in agroecosystems via cover cropping may thus help addressing major challenges such as the increase used of recycled fertilizers (Faucon et al. 2015). However, several other services produced by cover crops present trade-offs with efficient use of P by cover crops, suggesting careful consideration when designing cover crops composition (Schipanski et al. 2014; Hallama et al. 2019).

Improved P foraging can be ensured by plant strategies with low root diameter and important root length density (Haling et al. 2018, **Chapter 1**). However important investment in shallow roots to forage the topsoil while effectively improving P acquisition due to higher P concentration in that layer (Lynch and Brown 2001), presents major tradeoffs with the plant capacity to acquire water (Ho et al. 2005). Shallow rooting in beans for example induced higher productivity under P stress, but also significantly increased sensibility to drought stress (Ho et al. 2005). This trade-off may be especially pronounced for cover crops which are typically implanted during mid to late summer in relatively dry soils (Thomas and Archambeaud 2013). In turn drought stress, especially at emergence, can significantly reduce cover crop growth and biomass, which remains the best proxy to estimate cover crop impact on P availability, inducing negative feedback on the desired service.

Fabaceae species are of particular interest in pursuing improved P availability in agrosystems via cover crops due to their ability to mine P (**Chapter 1**), their positive

feedback effects on soil microbial communities (**Chapter 2**) and the effects of nitrogen enrichment due to atmospheric nitrogen fixation. Fabaceae cover crops are however less efficient at reducing nitrate leaching and can lead to increased N₂O emission (Jensen et al. 2012).

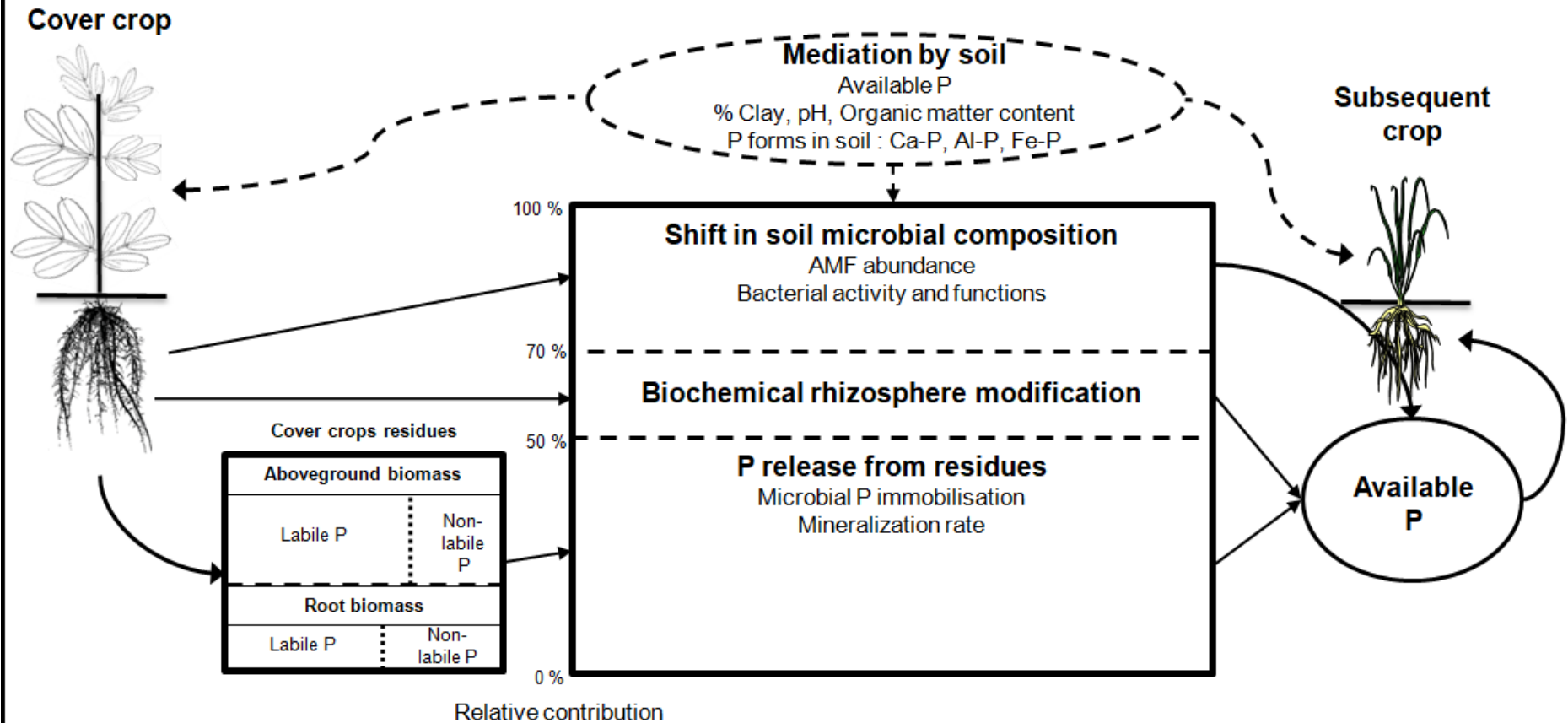
Due to the relatively recent interest of designing cover crops to improve P availability in agroecosystems, tradeoffs between improving this ecosystem service and maintaining the numerous others delivered by cover crops remain unclear (Lynch 2015). Designing for improved benefits of cover cropping on P availability should integrate a more comprehensive understanding of the tradeoffs with other ecosystems services. Integrating the service of “improving P availability and uptake in subsequent crops” in conceptual frameworks quantifying the ecosystems services produced by cover crops, their temporality and relationships, such as the one developed by Schipanski et al. (2014), could offer precious insights into cover crops designs optimizing the multiple services produced (Schipanski et al. 2014; Daryanto et al. 2018; Hallama et al. 2019). Ensuring optimal cover crops designs would also require adapting the multiple services produced according to the requirements of the cropping systems, i.e. crop rotation and practices. Going a step further and modelling the expected benefits of cover crops services and incorporating these models into decision support systems for farmer use might in turn be required for these designs to be adopted by farmers.

8.5. From theory to practice: Integrating the role of cover crop traits in P availability in models and decision support systems for adapted cover crop and fertilizer management

A major implication of the identification of traits and strategies involved in P acquisition in cover crops (**Chapter 1 and 2**), investigation of P release dynamics from cover crop residues (**Chapter 3**) and their mediation by soil P availability (**Chapter 4**) is the incorporation of the knowledge produced in modelling approaches. Modelling the role of cover crops functional traits in P acquisition and later release by cover crops, mobilization of soil P and in positive microbial feedbacks allows evaluating and quantifying the potential benefits of cover crops for P availability and uptake by subsequent crops. Attempts at modelling these processes, even partially, could provide a basis for the integration of cover cropping effects on P availability in decision support systems, allowing farmers to optimize their cover crops designs and management and adapt their P fertilization (Damon et al. 2014).

While the contribution of P mobilization by cover crops to subsequent crop P uptake remains uncertain (Damon et al. 2014), as does the contribution of positive microbial feedbacks (Hallama et al. 2019), several models have been developed to evaluate P release dynamics from residues. The APSIM and CENTURY models notably integrate a mechanistic understanding of the processes involved in P release from residues (Parton et al. 1988; Keating et al. 2003). Their designs however require detailed information not readily available at farm level (Damon et al. 2014). The approach developed by Damon et al. (2014) was selected in our project for its mechanistic modelling of the processes involved, allowing us to integrate the knowledge produced in previous chapters but also its simple, readily available variables and easy use which are advantageous in order to implement this model as part of a larger decision support system accessible to farmers.

Box B : Processes and challenges in modelling cover crops effect on P availability



Most approaches attempting to model cover cropping impacts on P availability focused on P release dynamics (Keating et al. 2003). Characterizing the relative contribution of shifts in microbial communities and their feedback on P acquisition capacity, or biochemical modification of the rhizosphere and their effect on P availability could offer more comprehensive approaches. Key model limitation highlighted included P speciation in residue, as well as proportion of P allocated to roots.

Microbial dynamics driving P release are also not fully understood (Maltais-Landry and Frossard 2015). Accounting for the effect of soil properties on the different processes involved is also challenging, requiring systematic approaches across varied contexts. Integrating the effects of plant traits as drivers influencing these processes, alongside with elucidation of the challenges underlined would provide a more precise predictions and a basis for decision making.

Model predictions allowed us to reinforce observations of shifts in overall contribution of P release dynamics across soil with different P availability (**Chapter 4**, Thibaud et al. 1988; Hallama et al. 2019) but also had the added benefit of highlighting the remaining knowledge gaps to address in evaluating the effects of cover cropping on P availability (BOX B). Our current use of the model indeed underlined several technical and scientific gaps. Due to poor characterisation of P content in roots, the root compartment was ignored in our approach despite potentially significant effects with 16 to 65 % of total plant P being contained in roots (Franchini et al. 2004). Similar approaches evaluating the effects of cover crops on nitrogen cycling propose correction factors for the root compartment typically 10-15 % of total plant N (Kumar and Goh 2000), that have yet to be defined for P. Phosphorus speciation in residue is similarly a major cause for uncertainty with pronounced differences reported in literature across species but also development stage (Noack et al. 2012). The influence of soil P availability in this speciation (Noack et al. 2014) moreover complicates model parameterisation. The complex impacts of microorganism in P release dynamics also remain very uncertain (Maltais-Landry and Frossard 2015). Multiple values have been reported for the simplified parameter expressing microbial requirement due to C addition used in the model (Kwabiah et al. 2003). Several poorly quantified processes could moreover play a major role in determining microbial requirements and thus the fate of microbial P, notably microbial C:P ratios, themselves conditioned by soil P availability (Parton et al. 1988). Shifts in microbial communities and activity under cover cropping potentially feeding back into P release dynamics from cover crop residues (Dinesh et al. 2009) or impacts of soil biota and in particular microbial grazers on the availability of microbial P for plants (Irshad et al. 2012) also remain underexplored.

Targeted elucidation of these technical and scientific questions across a range of contexts could provide more accurate estimations of the effects of cover crops on P availability via P release from residue. However, as reinforced in **Chapter 4**, the relative contribution of a specific process to cover cropping effects on P availability is likely to be very dependent on soil conditions and in particular P availability.

Next steps in developing fully comprehensive models of cover crops effects on P availability should thus focus on addressing the uncertain effects of microbial feedbacks, such as increases in AMF abundance (Boglaienko et al. 2014), and the legacy effects of P mobilization by cover crops (Teboh and Franzen 2011) and evaluate their relative contribution to the overall effects of cover crops. Gaining a better understanding of these processes should contribute to more precise and comprehensive models. Incorporating these models into frameworks considering the multiple services produced by cover crops and the tradeoffs among these services, would in turn offer a sound basis for the development of decision support systems. Such systems could then contribute to the efficient management and designs of cover crops for increased P availability in agroecosystems and more generally, agroecological intensification.

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APPENDICES

9. APPENDICES

APPENDIX A – TRADEOFFS AMONG PHOSPHORUS-ACQUISITION ROOT TRAITS OF CROP SPECIES FOR AGROECOLOGICAL INTENSIFICATION

APPENDIX B – SUPPLEMENTARY INFORMATION SECOND CHAPTER

APPENDIX C – LIST OF PUBLICATIONS AND COMMUNICATIONS

9.1. APPENDIX A – Tradeoffs among phosphorus-acquisition root traits of crop species for agroecological intensification

Plant Soil

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REGULAR ARTICLE



Tradeoffs among phosphorus-acquisition root traits of crop species for agroecological intensification

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Abstract

Aims Plant P acquisition strategies are driven by multiple belowground morphological and physiological traits as well as interactions among these traits. This study aimed to characterize the relationships among traits involved in P acquisition to explore tradeoffs and the main P-acquisition strategies and their mediation by soil type.

Methods Ten morphological and physiological traits involved in P acquisition were measured across 13 species grown in controlled conditions in two contrasting soils with moderate P limitation.

Results Tradeoffs between thicker and thinner roots were observed, with thicker roots exhibiting greater carboxylate release or phosphatase activity in the rhizosheath. Tradeoffs and coordination amongst traits were strongly mediated by soil type. Multivariate analysis of functional traits involved in P acquisition highlighted four main P-acquisition strategies relying

primarily on morphological traits, physiological traits or a combination thereof.

Conclusions The diversity of strategies demonstrates a potential for functional diversity benefits in cultivated plant communities via preferential access to different P pools leading to complementarities and reduced competition for resource acquisition. Overall, our results underpin functionally-complementary multispecies crop designs, enhancing P availability and cycling efficiency.

Keywords Agroecology · Cover crops · Phosphorus acquisition · Root functional trait · Tradeoffs

Abbreviations

Δ pH Change in rhizosheath pH
PME Phosphomonoesterase activity
RLD Root length density
SLA Specific leaf area
SRL Specific root length

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Introduction

Phosphorus (P) is a limiting nutrient in many natural and managed ecosystems due to its strong sorption with soil particles which limits its availability for plants. To overcome low P availability and ensure productive agriculture, P fertilisers are applied, albeit often with very low efficiency (Richardson et al. 2011). In highly-productive fertilized systems, there is a significant gap in P exports and inputs, leading to the accumulation of

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poorly available organic and inorganic P in the soil (Simpson et al. 2011; Bouwman et al. 2017). In these systems, improving P availability can be achieved by selecting for plant traits and strategies that allow access to the legacy P pool (Menezes-Blackburn et al. 2018). Plants have developed a range of morphological, architectural and physiological traits, granting access to the diverse pools of soil P. Morphological traits such as specific root length (SRL) and architectural traits such as root length density (RLD) allow plants to increase their P-foraging capacity (Pang et al. 2010; Haling et al. 2018; Ma et al. 2018). While only inorganic P in solution is taken up by plants, roots can also mobilize both inorganic and organic P by secreting protons (H^+) to dissolve Ca-phosphate, and carboxylate, which compete with P for binding sites, and thus decrease sorption on mineral surfaces (Wang and Lambers 2019). Plants can also hydrolyze organic P through the release of acid phosphatases, both monoesterases and diesterases (Richardson et al. 2011).

Overall, plant P-acquisition strategies are defined by the expression and association of P-acquisition traits. However, as the expression of these traits has a carbon cost, plants tend to rely mainly on one or a few P-acquisition traits (Pearse et al. 2006; Raven et al. 2018). There may thus be interactions among P-acquisition traits such as root morphological traits being negatively correlated with physiological traits (Zhang et al. 2016; Lyu et al. 2016; Wen et al. 2017). This indicates potential tradeoffs among diverse P-acquisition traits. However, trait interactions and tradeoffs that are central in gaining insight into plant P acquisition, are poorly understood, especially for fast-growing plants such as cover crops (Wen et al. 2019). Understanding tradeoffs and trait-combination effects would allow us to unravel the complexity of the P-acquisition strategies of species and bring new knowledge to design cultivated communities (i.e. multi-species crops) such as cover crops or intercrops to improve P acquisition and availability. Furthermore, trait expression and associated processes are strongly influenced by soil conditions, especially P availability (Raven et al. 2018; Wen et al. 2019). Tradeoffs among life history traits and nutrient-acquisition traits are inconsistent across environmental conditions (Sgrò and Hoffmann 2004; Kong et al. 2019), warranting further investigations as for the consistency of tradeoffs involved in P acquisition.

Intermediate crops, often referred to as cover crops, would offer an opportunity to use a high plant species diversity to enhance P recycling in agroecosystems

(Hallama et al. 2019). They can accumulate a large amount of nutrients including P during their growing period (Wendling et al. 2016), which is then released at termination to play a major role in maintaining and improving P availability (Dube et al. 2014; Damon et al. 2014). Moreover, through plant traits associated with a high P-foraging capacity and P mobilization, intermediate crops could acquire P from P pools that are unavailable to the cash crop (Nuruzzaman et al. 2005). A promising opportunity to improve soil P availability for crops can thus be developed by enhancing P cycling in intermediate crops on the basis of their potential to acquire P from poorly-available sources (Richardson et al. 2011). Cover crops present a wide range of P-acquisition traits, therefore potentially presenting different P-acquisition strategies exploiting different P pools (Wendling et al. 2016; Lyu et al. 2016). Examining these strategies and the factors conditioning their expression by characterizing root trait relationships in plant species with diverse root traits would result in a better understanding of P acquisition in crop species and insights for the design of more P-efficient systems. In this study, a greenhouse experiment was carried out to measure morphological, architectural and physiological traits in 13 intermediate crops species with diverse phylogenetic lineages in two contrasting soil types, in order to:

- 1 Characterize the relationships among traits involved in P acquisition to explore tradeoffs and the main P-acquisition strategies;
- 2 Examine whether P forms and soil type mediate expression of tradeoffs and P-acquisition strategies in intermediate crops.

Materials and methods

Greenhouse experiment

Soils used in the greenhouse experiment were collected at 5 to 20 cm depth from two fields from north-eastern France, after topsoil (0–5 cm) removal to further decrease P availability. Fields with a known P deficiency were selected to ensure low P availability. A Retisol (formerly called Albeluvisol) and a Calcaric Cambisol (FAO 2014) were selected for their contrasting soil characteristics, mainly their different P forms, in particular their apatite P concentration (Table 1). Prior to use,

the soils were dried at ambient temperature, sieved at 2 mm and then mixed with washed sand (22% mass).

Thirteen (sub)species of diverse phylogenetic lineages (Poaceae: *Avena nuda* L., *Avena strigosa* Schreb; Brassicaceae: *Brassica carinata* A. Braun, *Raphanus sativus* L., *Sinapis alba* L.; Polygonaceae: *Fagopyrum esculentum* Moench.; Fabaceae: *Lens culinaris* Medik., *Pisum sativum* subsp. *arvense* L., *Trifolium alexandrinum* L., *Vicia faba* L., *Vicia sativa* L., and *Vicia villosa* Roth.; Hydrophyllaceae: *Phacelia tanacetifolia* Benth.) were selected for their diverse morphologies, P-acquisition traits and relevance for the local context. The experimental design included two soil types, 13 species and a control (bare soil) with four replicates. Plants were sown mid-January 2018 at two individuals per pot per species in 4.5 L fully filled pots and watered twice a week. Pots were arranged in a random design and greenhouse temperatures were maintained at 22 °C during the day (14 h) and at least 18 °C at night.

Plant traits measurement

At harvest (77 days) plants were manually separated from the bulk soil with special care given to ensure minimum damage. Rhizosheath adhering to the roots up to a maximum of 2 mm after shaking was collected and stored at -20 °C for further analysis. Roots were then immersed in 0.20 mM CaCl₂ for 1 h, after removing any remaining soil particles by quickly rinsing with CaCl₂. The CaCl₂ volume was adjusted to ensure a complete immersion of the root system. The solution was then sampled and stored at -20 °C before measuring carboxylic acid exudation by reversed-phase column liquid chromatography (RPLC) (Cawthray 2003; Yacoumas et al. 2020). Briefly, an acid mobile phase (93% 25 mM KH₂PO₄ at pH 2.5 and 7% methanol) allowed a good resolution of five acids (citric, fumaric, maleic, malic, malonic) on a C18 column with a 15 min elution time and a 1 mL min⁻¹ flow rate. Total carboxylate release rate was later calculated as the sum of all previously mentioned acids (see Table 2 for abbreviations).

Morphological traits

Aboveground biomass and roots were separated by cutting the stem 1 cm above the first visible root. Roots were then scanned while being immersed in deionised water using an Epson Scanner perfection V800 (Regent Instruments Inc., Québec, Qc, Canada) to produce a 600 dpi image. The image was analysed using WinRHIZO

Regular software V.2016a (Regent Instruments Inc., Québec, Qc, Canada) to determine root traits including root surface area (RSA), the percentage of fine root (FR), defined here as length of roots with a diameter < 0.5 mm, and root length density (RLD). After 48 h drying at 55 °C, scanned roots were weighed to calculate specific root length (SRL). Three young but fully-developed leaves per replicate were scanned at 600 dpi, and then dried at 60 °C for 48 h to determine specific leaf area (SLA). All aboveground biomass was dried at 60 °C for 48 h and weighed.

Nutrient uptake

Dried leaves and stems were digested via acid digestion and a microwave heating treatment (Lange et al. 2016). Between 0.1 and 0.2 g of biomass was introduced in Teflon vessels with 8 mL of 65% (v/v) HNO₃ and 2 mL of 36% (v/v) HCl and heated to 185 °C for one hour. Digests were then filtered and P and manganese (Mn) concentrations (as a proxy for rhizosheath carboxylate concentration) determined by inductively coupled plasma mass spectrometry (ICP-MS) (Thermo Scientific XSERIES2, Beauvais, France). SRM1573, a certified reference material (tomato leaves) was used as a standard.

Rhizosheath properties

After defrosting, acid phosphomonoesterase activity in the rhizosheath (PME) was measured with a modified buffer at pH 6.5 (Tabatabai and Bremner 1969). Briefly, phosphatase activity was assessed via production of *p*-nitrophenol from sodium *p*-nitrophenyl phosphate during a 1 h incubation at 37 °C with 0.5 g dry soil, 0.2 mL toluene, 4 mL modified buffer and 1 mL substrate. *p*-Nitrophenol release was determined spectrophotometrically at 410 nm after stopping the reaction with 4 mL 0.5 M NaOH and 1 mL 0.5 M CaCl₂ and filtering. Rhizosheath pH was measured on 2 g equivalent dry soil with a 1:10 soil to solution ratio. ΔpH between rhizosheath and bulk soil from unplanted pots post-growth was then calculated.

Data analysis

Linear mixed models (LMM) were used in order to test for differences among plant P uptake and traits among soil types, with species as fixed factors and soil types as random effect. Differences in traits between soils were

Table 1 Chemical and physical soil characteristics

	Clay % (< 0.002 mm)	Sand % (> 0.05 mm)	pH KCl	Ca ^a (g kg ⁻¹)	N tot (%)	CEC (cmol _c kg ⁻¹)	Olsen P ^c (mg kg ⁻¹)	Ca-P ^d (mg kg ⁻¹)
Calcaric Cambisol	30.7	22.0	7.9	44.7 ^b	0.17	9.3	19.8	542
Retisol	22.3	5.4	7.4	2.60	0.10	10.5	16.3	76.7

^a Extractant : Ammonium acetate 0.5 M, EDTA 0.02 M pH 4.65

^b CaCO₃ 56.4%

^c According to Olsen (1954)

^d Apatite phosphorus as defined in Garcia-Abacete et al. (2012)

then investigated with non-parametric tests (Kruskall-Wallis test + post hoc test of Mann Whitney). As multiple differences were observed between soils, further analysis was performed separately for each soil type. To identify the main covariation in P-acquisition traits and rhizosphere processes, a principal component analysis (PCA) was performed using the “FactomineR” package on log-transformed data (Lê et al. 2008). The number of components was selected to represent more than 75% of the total variability. Tradeoffs between traits were confirmed with Spearman correlation tests as data did not fulfil the condition of normality. Hierarchical classification on principal components (HCPC) was then performed to define groups with similar patterns of P-acquisition traits and influence on the rhizosphere. Species were attached to the group including most of their replicates. A multiresponse permutation procedure (MRPP) confirmed a clear differentiation between clusters in multivariate space. Differences between clusters for each individual factor were then

investigated, either with tests of variance (ANOVA and post-hoc test of Tukey) or non-parametric tests (Kruskall-Wallis test + post-hoc test of Mann Whitney).

To investigate the importance of the factors leading to group formation, their influence on P uptake was modelled via generalised linear models (GLM) using the “FactomineR” package. Briefly, all factors were individually tested as predictors of P uptake as well as combinations of the best fitting factors. Models were compared based on second-order Aikake’s information (AICc) with the lowest relative value considered the best fit. Differences between models were tested with ANOVA as well as the criteria $\Delta AICc > 2$. Complementary to GLM regression, partial square path modelling (PLS-PM) was performed to underline the relative ability of trait combinations and type for predicting P uptake. Three clusters of variables, or “latent variables” were defined, respectively, the “root morphology” variables encompassing root surface area, SRL, fine root percentage, root length density, the “root physiology” variables encompassing PME activity, carboxylate release and change in rhizosheath pH and the “above-ground traits” variables encompassing SLA, foliar [P] and [Mn]. Verifications were made to ensure model quality, notably unidimensionality of latent variables and cross-loadings between traits associated with a latent variable and other variables as suggested in Sanchez (2013). To ensure the condition of positively correlated variables in a latent variable, the sign of some variables was changed. Variables and components were selected based on their loading and correlation as suggested in Sanchez (2013). Overall model quality was evaluated with the Goodness of Fit (GoF) index. Analyses were performed with the package “plsrm” version 0.4.9. All tests were performed in R version 3.6.0 with a significance level of 0.05.

Table 2 List of abbreviations and associated units

Variable	Abbreviation	Unit
Change in rhizosheath pH	Δ pH	-
Fine root percentage (< 0.5 mm ϕ)	FR	-
Phosphomonoesterase activity	PME	μ g nitrophenol g ⁻¹ h ⁻¹
Root length density	RLD	cm cm ⁻³
Root surface area	RSA	cm ²
Specific leaf area	SLA	mm ² mg ⁻¹
Specific root length	SRL	m g ⁻¹
Total carboxylate release rate	TCE	μ mol g root ⁻¹ h ⁻¹

Results

Variation in P-acquisition traits and rhizosphere processes

In the Calcaric Cambisol, the PCA summarised 84% of the total variability, with the first two components representing 50.8% of the total variability (Fig. 1). In the Retisol 82% of the total variability was summarised by the PCA, with the first two components representing 51.3% of the total variability (Fig. 1). Overall, we observed moderate to strong correlations between morphological indicators such as fine root percentage, RLD, SRL and root surface area ($|r|$ between 0.30 and 0.88). We observed similar patterns between physiological indicators such as change in rhizosphere pH, PME activity and carboxylate release, albeit more differentiated by soil type.

In the Retisol, the first component was formed based on all modifications in the rhizosphere properties measured (pH, PME activity) and carboxylate release, while also presenting a gradient of fine root percentage and root surface area. Aboveground, SLA and leaf P concentration were also important contributors to the first component. The component presented a gradient with strong negative correlations between fine root percentage and PME activity ($r=-0.65$) and negative correlation between fine root percentage and rhizosphere acidification ($r=-0.32$). PME activity, rhizosphere acidification and carboxylate release were positively correlated. At the leaf level, SLA was negatively correlated with fine root percentage. The second component was mostly formed based on root physiology and morphology (root length density and surface area). Physiological traits important for this axis were carboxylate release as well as to a lesser extent leaf Mn concentration. The component presented a gradient with a negative correlation between carboxylate release and RLD ($r=-0.36$). Root length density was also positively correlated with root surface area.

In the Calcaric Cambisol, the first component was based on aboveground characteristics such as SLA and foliar Mn concentration. To a lesser extent, the first component was also based on morphological characteristics such as RLD, and rhizosphere modification (rhizosphere acidification). SLA was strongly positively correlated with leaf Mn concentration ($r=0.66$). Root length density and root surface area were positively correlated. The second axis mostly represented

morphological traits such as root surface area, fine root percentage and root length density with a smaller influence of leaf traits such as foliar P concentration and marginal modifications to the rhizosphere such as PME activity. Foliar P concentration was positively correlated with fine root percentage ($r=0.33$) and SRL ($r=0.43$), and negatively with root surface area ($r=-0.30$).

Comparison of P-acquisition traits among different clusters

Based on the PCA scores, we identified four groups via HCPC in the Calcaric Cambisol, five in the Retisol (Fig. 1; Table 3). In the Retisol, the first “physiological/exudation” group L1 (Fig. 1) encompassed lentil, common vetch and white mustard. It showed the greatest exudation of carboxylic acids, significant activity of PME in the rhizosphere, as well as the lowest RLD observed in all groups. The second “intermediate/morphological” group L2 encompassed common and naked oat, Indian mustard, buckwheat and forage radish. It presented a low carboxylate release and PME activity in the rhizosphere, while presenting a small increase in rhizosphere pH and a high RLD, fine root percentage and foliar P concentration compared with other groups. The third “physiological/morphological” group L3 had a strong to intermediate expression of all physiological traits as well as SRL, RLD and SLA and a low foliar P concentration. It grouped forage pea, clover and hairy vetch. The fourth “morphological” group L4, encompassing phacelia, presented low expression of P-mining traits, except for an intermediate carboxylate release, a high fine root percentage and the highest RLD and SRL observed in all groups. Finally the “physiological/mining” group L5, encompassing faba bean, had the lowest SRL and fine root percentage associated with a strong expression of all physiological traits with in particular a strong decrease in rhizosphere pH. Overall the different strategies resulted in similar levels of total P uptake.

In the Calcaric Cambisol, the first “morphological” group C1 was mainly characterised by a high root length density and surface area, an intermediate SRL and a low expression of physiological traits. It encompassed naked oat, forage pea and forage radish. The second “morphological/physiological” group C2, encompassing hairy and common vetch had a significantly higher SLA, stronger rhizosphere acidification, intermediate PME

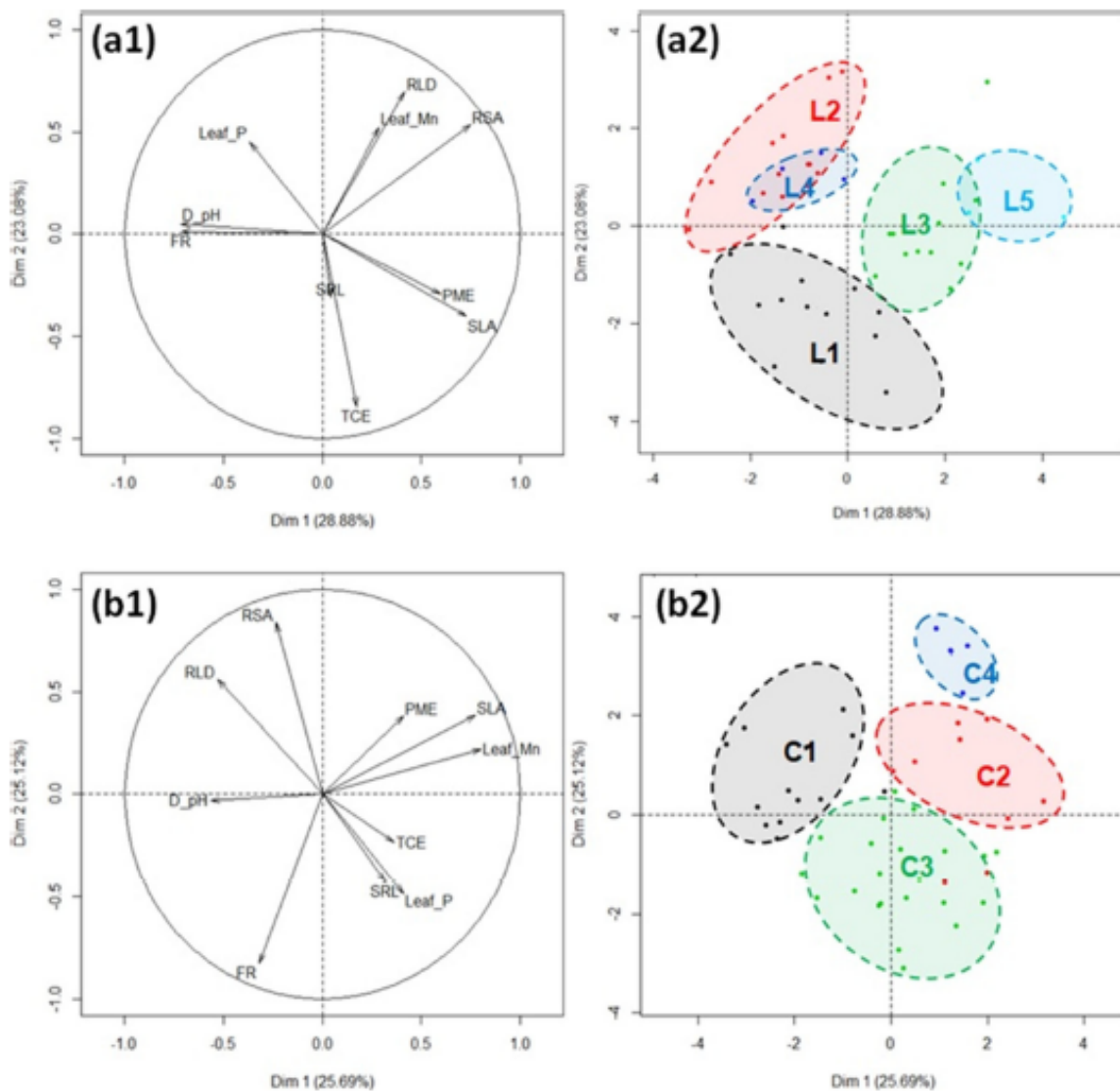


Fig. 1 Principal component analysis (PCA) of functional traits and rhizosphere processes involved in phosphorus acquisition in retisol (a) and Calcaric Cambisol (b). (1) Variable covariation along first two components, (2) clusters formed with Hierarchical Classification on Principal Components (HCPC). Abbreviations: D_pH: change in rhizosphere pH; FR: percentage roots with

diameters less than 0.5 mm; Leaf_Mn: foliar manganese concentration; Leaf_P: foliar phosphorus concentration; PME: acid phosphomonoesterase activity; RLD: root length density; RSA: root surface area; SLA: specific leaf area; SRL: specific root area; TCE: total carboxylate release rate

activity in the rhizosphere and higher SRL. The third “intermediate” group C3 encompassed common oat, buckwheat, lentil, phacelia, white mustard and clover. It presented the highest percentage of fine roots, intermediate values of SRL, SLA and the highest foliar P. It also presented the highest value of carboxylate release, while not significantly different from the chemical and

morphological groups. Finally the fourth “physiological” group C4, encompassing faba bean, presented the highest PME activity in the rhizosphere, and second-highest carboxylate release. It also presented the lowest fine root percentage and lowest SRL. Overall, these different strategies once again resulted in similar levels of total P uptake.

Table 3 Mean values \pm standard error ($n=4$ to 11) and analyses of variance of phosphorus-acquisition traits and rhizosphere processes per cluster

Soil type	Cluster	TCE	PME	Δ pH	RLD	FR	SRL	SLA	Leaf[P]	Leaf [Mn]
Retisol	L1: Physiological/exudation	41.6 \pm 5.3	0.026 \pm 0.004	-0.04 \pm 0.04	0.09 \pm 0.01	0.81 \pm 0.02	29.0 \pm 4.2	46.1 \pm 4.2	2.14 \pm 0.19	47 \pm 5
	L2: Intermediate/-morphological	10.8 \pm 1.3	0.017 \pm 0.001	0.11 \pm 0.04	0.20 \pm 0.02	0.85 \pm 0.01	14.4 \pm 1.6	31.5 \pm 2.7	3.20 \pm 0.20	166 \pm 53
	L3: Physiological/-morphological	24.1 \pm 4.8	0.036 \pm 0.007	-0.16 \pm 0.05	0.27 \pm 0.03	0.77 \pm 0.02	38.2 \pm 5.4	62.6 \pm 2.6	1.78 \pm 0.17	88 \pm 9
	L4: Morphological	19.5 \pm 2.9	0.020 \pm 0.002	0.03 \pm 0.09	0.33 \pm 0.02	0.87 \pm 0.03	65.4 \pm 8.8	24.6 \pm 4.3	4.17 \pm 0.66	36 \pm 10
	L5: Physiological/mining	26.5 \pm 5.1	0.038 \pm 0.007	-0.29 \pm 0.05	0.21 \pm 0.02	0.38 \pm 0.03	6.8 \pm 1.4	60.5 \pm 8.8	3.35 \pm 0.23	75 \pm 4
	p value	<.0001	0.0002 ^a	<.0001	<.0001	0.0013 ^a	<.0001	<.0001 ^b	<.0001	0.0053
	Calcaric Cambisol	C1: Morphological	9.6 \pm 2.1	0.011 \pm 0.001	-0.05 \pm 0.01	0.46 \pm 0.02	0.77 \pm 0.02	17.3 \pm 2.4	31.2 \pm 3.9	1.90 \pm 0.22
C2: Morphological/-Physiological	17.0 \pm 3.2	0.019 \pm 0.004	-0.22 \pm 0.02	0.29 \pm 0.05	0.72 \pm 0.03	35.4 \pm 3.5	71.4 \pm 7.3	2.16 \pm 0.25	125 \pm 15	
C3: Intermediate	25.0 \pm 7.1	0.010 \pm 0.001	-0.07 \pm 0.01	0.22 \pm 0.02	0.82 \pm 0.01	28.9 \pm 3.9	40.8 \pm 3.9	2.91 \pm 0.22	135 \pm 15	
C4: Physiological	24.8 \pm 13.9	0.022 \pm 0.001	-0.05 \pm 0.03	0.29 \pm 0.05	0.32 \pm 0.02	8.1 \pm 1.1	57.6 \pm 5.1	1.87 \pm 0.34	295 \pm 34	
p value	0.0169	0.0079 ^a	<.0001 ^a	<.0001 ^a	0.0001 ^a	0.0003	0.0002 ^a	0.0161	<.0001	

^a:Kruskal-Wallis test; *FR* Fine root percentage (<0.5 mm diameter), *Leaf [Mn]* Leaf manganese concentration ($\mu\text{g Mn g}^{-1}$), *Leaf [P]* Leaf phosphorus concentration (mg P g^{-1}), *PME* Phosphomonoesterase activity ($\mu\text{g nitrophenol g}^{-1} \text{hour}^{-1}$), *RLD* Root length density (cm cm^{-3}), *SLA* Specific leaf area ($\text{mm}^2 \text{mg}^{-1}$), *SRL* Specific root length (m g^{-1}), *TCE* Total carboxylate release rate ($\mu\text{mol g root}^{-1} \text{hour}^{-1}$), ΔpH Change in rhizosphere pH

Modelling trait-combination effects on P acquisition

As we observed a similar P uptake among strategies, we investigated overall relationships between P uptake and factors involved in group formation with GLM (Table 4). PLS PM were also used to highlight the combinations of traits and type of trait best predicting P uptake (Fig. 2). In the Retisol, the best model fit was obtained with fine root percentage, leaf Mn concentration and PME activity as predictors, while a similar fit was also achieved without PME activity. Fine root percentage was the best predictor when used alone, however, it performed worse than models incorporating multiple traits. While offering a lower fit when incorporating multiple traits, SLA was the second-best predictor, being in itself correlated with multiple morphological and physiological traits. The PLS PM approach ($R^2 = 0.5047$) produced similar results, with PME activity in the rhizosphere being the best single predictor in the physiological component, followed by rhizosphere

acidification, and fine root percentage being the best predictor in the morphological component, followed by root surface area. Similar weights were associated with the physiological (correlation value = 0.37) and morphological (correlation value = 0.32) components in this soil with a low predicting ability of the above-ground component leading to its removal.

In the Calcaric Cambisol, the best model fit was achieved with the combination of SLA, root surface area, fine root percentage and pH modification in the rhizosphere. Purely morphological models had a significantly poorer fit than models incorporating pH, while rhizosphere acidification alone was a poor predictor. Excluding correlated traits, the best predictors were SLA and root surface area (while not significantly different from SLA + root surface area + change in rhizosphere pH). SLA was the best predictor for single-trait models. PLS PM ($R^2 = 0.457$) underlined the importance of the morphological component (correlation value = 0.31) and aboveground component

(correlation value = 0.40), while the physiological one was removed due to its poor predicting ability. SLA was the most impactful contributor for the aboveground component. For the morphological component it was root surface area.

Differences among trait variation and tradeoffs per soil type

Trait expressions were significantly different between soil types, with very uneven trait plasticity among species (Table 5). PME activity and carboxylate release were overall significantly lower in Calcaric Cambisol. In contrast, root length density and surface area were higher overall in this soil, especially for Fabaceae and Brassicaceae. Covariation between morphological P-acquisition traits and rhizosphere processes were observed less in Calcaric Cambisol such as the correlation between root surface area and PME and rhizosphere acidification or RLD and carboxylate release observed in Retisol which were not observed in this soil. Positive covariations such as between PME activity in the rhizosphere, carboxylate release and rhizosphere acidification were also not observed in this soil. Similarly, SLA was correlated with change in rhizosphere pH, PME activity and carboxylate release in Retisol, but only with change in rhizosphere pH in Calcaric Cambisol.

Discussion

Tradeoffs in functional traits to understand P acquisition of crop species

Multiple studies have demonstrated the importance of a single trait/few traits for P uptake such as root length density and surface area (Pang et al. 2010; Haling et al. 2018) or PME activity, carboxylate release or rhizosphere acidification (Lambers et al. 2006; Rose et al. 2010; Li et al. 2017; Nobile et al. 2019). However despite increased observations of coordination and tradeoffs among belowground resource-acquisition traits (Roumet et al. 2016; Ma et al. 2018), our understanding of trait interactions and tradeoffs that are central to P acquisition remains limited, especially in crop species (Wen et al. 2019). Our results highlight multiple covariation and tradeoffs between root morphological and physiological traits across a range of fast-growing

crop species. Fine root percentage was negatively correlated with PME activity, rhizosphere acidification, and foliar Mn concentration, and marginally with carboxylate release. As foliar Mn can be used as a proxy for rhizosphere carboxylate concentration (Lambers et al. 2015; Pang et al. 2018; Yu et al. 2020), this suggests a greater relevance of physiological strategies associated with P mining for root systems with less fine root percentage. Our results are consistent with recent findings showing that roots with a large diameter present high expression of P-mining traits, while species with thinner fibrous roots express higher levels of morphological traits for P acquisition (Lyu et al. 2016; Wen et al. 2019), extending this observation to a different range of species (intermediate crops species) and context (north-western European soils with moderate P deficiency). Under P stress, plants can modify their root morphology through higher fine root percentage, increased SRL, root hair density and root length density to increase soil foraging at lower cost (Lambers et al. 2006; Shen et al. 2011; Haling et al. 2018). Root physiology can also be modified to increase P availability in the rhizosphere via increased carboxylate, enzyme and proton exudation. Synergistic action of these physiological traits is common, as shown for phytase activity and carboxylate release (Giles et al. 2017) and reinforces the coordination between PME activity in the rhizosphere, carboxylate release and rhizosphere acidification. A possible explanation for this observation is the release of organic P sorbed to soil particles by carboxylates for subsequent mineralisation (Clarholm et al. 2015). While both physiological and morphological strategies should benefit P acquisition, tradeoffs between both are suggested to form along a cost / benefit balance due to the important investment they can represent for the plant (Pearse et al. 2006; Raven et al. 2018). Overall our results confirm and extend previous findings in other systems demonstrating multiple coordination and tradeoffs among morphological and physiological traits involved in P acquisition in crop species (Zhang et al. 2016; Giles et al. 2017; Li et al. 2017). Because physiological traits tend to allow greater access to legacy P pools compared with morphological traits, which allow access to available P pools (Lyu et al. 2016), this morphological/physiological tradeoff offers insight into how to enhance P acquisition and to increase exploitation of the diverse pools of P. Our results demonstrate a potential to select for species at different ends of the tradeoffs spectrum in multispecies systems to increase P

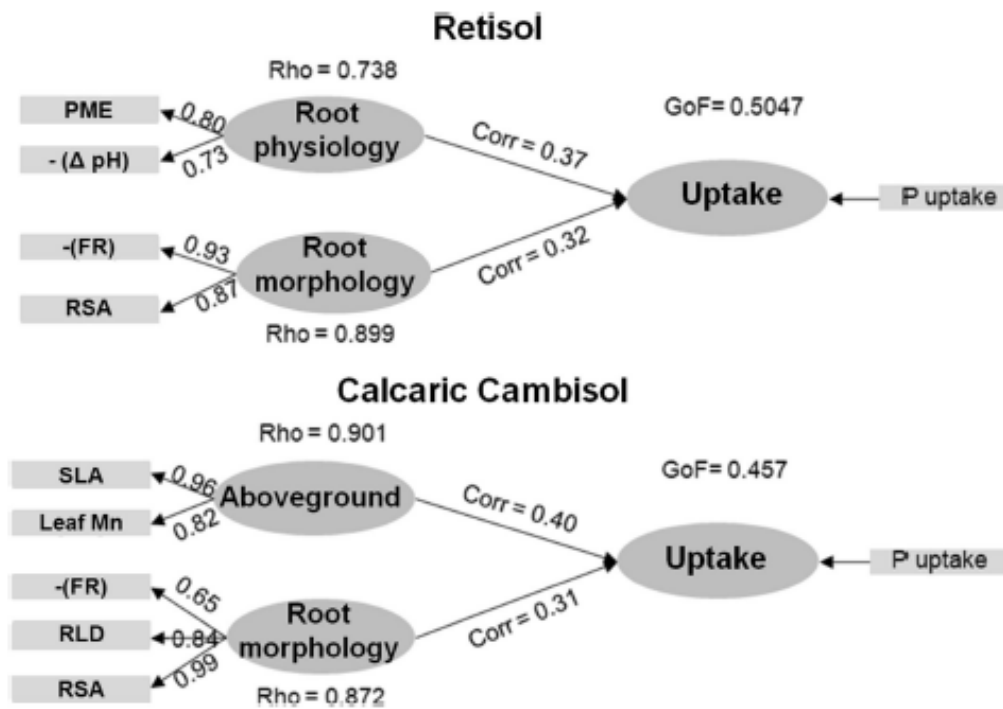


Fig. 2 Partial least square-path models predicting phosphorus uptake. Based on three latent variables, namely root physiology, root morphology and aboveground traits in two soils. Corr is the correlation effect, Rho the Dilon-Golstein coefficient. Variables

negatively transformed indicated by -(variable). GoF = Goodness of Fit of the model. Δ pH: change in rhizosphere pH; FR: percentage roots with diameters less than 0.5 mm; Leaf Mn: foliar manganese concentration; PME: acid phosphomonoesterase activity; RLD: root length density; RSA: root surface area

Table 4 Selected models fitted to phosphorus uptake

Soil type	Models	AICc	Δ AICc
Retisol	FR + Leaf [Mn] + PME	-18.8	0.0
	FR + Leaf [Mn]	-17.4	1.4
	FR	-16.5	2.3
	SLA	-14.5	4.3
	PME	-12.5	6.2
	Leaf [Mn]	-8.2	10.6
Calcaric Cambisol	FR + Δ pH + SLA + RSA	-43.4	0.0
	Δ pH + SLA + RSA	-39.5	3.9
	FR + SLA + RSA	-39.5	3.9
	SLA	-35.1	8.4
	RSA	-30.7	12.7
	FR	-25.8	17.6
	Δ pH	-23.4	20.0

Sorted from best fit to worst. AICc second order Aikake's information, Δ AICc Difference between AICc model and lowest AICc observed, FR Fine root percentage (<0.5 mm ϕ), Leaf [Mn] Leaf manganese concentration, PME Phosphomonoesterase activity, RSA Root surface area, SLA Specific leaf area, Δ pH change in rhizosphere pH

acquisition, while reducing competition for resources. Our results constitute a novel contribution toward understanding P acquisition in crop species, and also underline the need for a better understanding of how these trait combinations and tradeoffs are structured in a multi-traits space to form P-acquisition strategies. Such an understanding would offer the opportunity to better design multispecies cropping systems, notably with the main functions of improving P cycling and availability.

Phosphorus-acquisition strategies to design multispecies crops

Plants present very uneven trait plasticity when exposed to different levels of available P (Pearse et al. 2006; Haling et al. 2018; Wen et al. 2019). Uneven plasticity along tradeoffs and covariations suggests both a convergence toward common resource-acquisition strategies and divergence among strategies (Wendling et al. 2016; Li et al. 2017). Plants under P stress rely on resource-acquisitive strategies, either enhancing

Table 5 Average trait value ± standard error and analyses of variance per species and per soil

Soil	Species	FR	Leaf [Mn]	Leaf [P]	PME	RLD	RSA	SLA	SRL	TCE	Δ pH	
Retisol	<i>Avena nuda</i>	0.79 ± 0.02	48 ± 16	3.58 ± 0.10	0.020 ± 0.002	0.14 ± 0.03	60 ± 11	32.9 ± 2.3	10.8 ± 3.1	24.2 ± 15.0	0.19 ± 0.03	
	<i>Avena strigosa</i>	0.79 ± 0.02	144 ± 31	2.90 ± 0.17	0.016 ± 0.001	0.27 ± 0.09	103 ± 29	33.0 ± 2.6	14.7 ± 1.9	10.4 ± 2.6	0.01 ± 0.03	
	<i>Brassica carinata</i>	0.83 ± 0.02	72 ± 4	2.14 ± 0.19	0.015 ± 0.002	0.18 ± 0.02	70 ± 9	27.4 ± 4.9	13.9 ± 1.4	17.1 ± 2.4	0.01 ± 0.05	
	<i>Fagopyrum esculentum</i>	0.88 ± 0.01	456 ± 121	3.79 ± 0.50	0.020 ± 0.001	0.21 ± 0.04	66 ± 12	46.3 ± 5.8	17.2 ± 7.0	6.8 ± 2.3	0.10 ± 0.10	
	<i>Lens culinaris</i>	0.78 ± 0.01	47 ± 8	2.52 ± 0.16	0.025 ± 0.001	0.07 ± 0.02	38 ± 11	54.9 ± 2.8	42.8 ± 4.5	48.2 ± 10.1	-0.04 ± 0.09	
	<i>Pisum sativum</i>	0.74 ± 0.02	45 ± 10	1.16 ± 0.07	0.032 ± 0.005	0.22 ± 0.07	126 ± 42	66.2 ± 3.1	24.9 ± 7.9	29.5 ± 12.5	-0.13 ± 0.03	
	<i>Phaseola tanacetifolia</i>	0.87 ± 0.03	40 ± 12	4.79 ± 0.32	0.020 ± 0.002	0.27 ± 0.07	96 ± 29	25.3 ± 4.0	65.4 ± 8.9	23.7 ± 5.0	-0.06 ± 0.02	
	<i>Raphanus sativus</i>	0.68 ± 0.22	36 ± 9	3.05 ± 0.40	0.013 ± 0.001	0.17 ± 0.07	66 ± 15	22.7 ± 3.6	19.5 ± 7.5	13.0 ± 2.4	0.27 ± 0.04	
	<i>Sinapis alba</i>	0.9 ± 0.020	34 ± 2	2.04 ± 0.13	0.018 ± 0.001	0.11 ± 0.03	34 ± 5	32.1 ± 7.1	17.5 ± 5.2	25.1 ± 5.4	-0.08 ± 0.05	
	<i>Trifolium alexandrinum</i>	0.86 ± 0.02	106 ± 12	2.13 ± 0.22	0.027 ± 0.002	0.23 ± 0.04	92 ± 19	59.1 ± 3.3	48.3 ± 11.1	22.8 ± 2.9	-0.29 ± 0.03	
	<i>Vicia faba</i>	0.38 ± 0.02	75 ± 4	3.35 ± 0.23	0.037 ± 0.005	0.21 ± 0.01	191 ± 19	55.2 ± 8.2	6.5 ± 1.1	25.2 ± 3.8	-0.31 ± 0.04	
	<i>Vicia sativa</i>	0.79 ± 0.01	64 ± 7	1.82 ± 0.25	0.053 ± 0.021	0.12 ± 0.03	66 ± 19	54.6 ± 5.4	34.6 ± 9.0	33.0 ± 7.4	-0.04 ± 0.06	
	<i>Vicia villosa</i>	0.72 ± 0.02	100 ± 15	1.74 ± 0.24	0.033 ± 0.004	0.32 ± 0.06	183 ± 45	63.8 ± 7.3	38.1 ± 5.3	32.5 ± 13.9	-0.11 ± 0.09	
	<i>p value</i>	0.0002*	0.0006*	< 0.0001	0.0026*	0.0020	< 0.0001	0.0004*	< 0.0001	0.0126	0.0126	< 0.0001
	Calcane Cambisol	<i>Avena nuda</i>	0.74 ± 0.02	33 ± 6	1.63 ± 0.28	0.013 ± 0.001	0.47 ± 0.05	247 ± 37	29.67 ± 1.0	9.5 ± 2.9	7.9 ± 4.5	-0.03 ± 0.01
<i>Avena strigosa</i>		0.82 ± 0.03	148 ± 17	2.69 ± 0.32	0.013 ± 0.001	0.30 ± 0.06	127 ± 35	39.6 ± 2.1	28.7 ± 9.3	13.8 ± 4.6	-0.12 ± 0.03	
<i>Brassica carinata</i>		0.83 ± 0.01	92 ± 31	2.03 ± 0.18	0.008 ± 0.001	0.37 ± 0.03	146 ± 14	21.8 ± 3.4	15.4 ± 1.2	12.0 ± 2.3	-0.01 ± 0.01	
<i>Fagopyrum esculentum</i>		0.84 ± 0.01	67 ± 3	3.56 ± 0.27	0.009 ± 0.001	0.27 ± 0.09	105 ± 38	44.4 ± 8.7	16.1 ± 3.3	6.2 ± 1.9	-0.10 ± 0.01	
<i>Lens culinaris</i>		0.72 ± 0.01	252 ± 30	2.91 ± 0.15	0.013 ± 0.002	0.22 ± 0.06	127 ± 42	58.7 ± 7.4	34.6 ± 4.7	23.1 ± 1.4	-0.09 ± 0.02	
<i>Pisum sativum</i>		0.66 ± 0.01	93 ± 27	0.99 ± 0.22	0.009 ± 0.003	0.34 ± 0.07	223 ± 46	64.9 ± 6.2	24.5 ± 3.1	14.6 ± 3.8	-0.07 ± 0.05	
<i>Phaseola tanacetifolia</i>		0.87 ± 0.01	53 ± 10	3.71 ± 0.40	0.012 ± 0.001	0.19 ± 0.06	67 ± 22	18.6 ± 2.9	53.1 ± 11.0	78.4 ± 32.8	-0.03 ± 0.02	
<i>Raphanus sativus</i>		0.85 ± 0.02	47 ± 12	1.99 ± 0.29	0.012 ± 0.001	0.44 ± 0.06	163 ± 28	17.5 ± 2.3	27.8 ± 2.7	9.9 ± 1.9	-0.07 ± 0.01	
<i>Sinapis alba</i>		0.86 ± 0.01	91 ± 22	1.71 ± 0.33	0.009 ± 0.003	0.12 ± 0.03	44 ± 9	31.8 ± 2.8	10.9 ± 2.1	17.6 ± 3.3	-0.08 ± 0.01	
<i>Trifolium alexandrinum</i>		0.84 ± 0.02	180 ± 30	4.19 ± 0.36	0.010 ± 0.001	0.13 ± 0.02	57 ± 11	60.4 ± 1.8	28.2 ± 8.8	12.8 ± 2.1	-0.10 ± 0.02	
<i>Vicia faba</i>		0.32 ± 0.02	295 ± 34	1.87 ± 0.34	0.022 ± 0.001	0.29 ± 0.05	287 ± 53	57.6 ± 5.1	8.1 ± 1.1	24.8 ± 13.9	-0.05 ± 0.03	
<i>Vicia sativa</i>		0.67 ± 0.04	123 ± 7	2.39 ± 0.27	0.009 ± 0.001	0.30 ± 0.08	198 ± 57	76.0 ± 14.3	32.4 ± 5.7	23.5 ± 5.0	-0.26 ± 0.04	
<i>Vicia villosa</i>		0.74 ± 0.04	141 ± 27	2.51 ± 0.23	0.028 ± 0.008	0.36 ± 0.08	205 ± 49	67.2 ± 10.7	38.8 ± 4.0	14.0 ± 3.6	-0.15 ± 0.02	
<i>p value</i>		< 0.0001*	< 0.0001	< 0.0001	0.0123*	0.0276*	0.0051*	< 0.0001	< 0.0001	0.0169	0.0169	0.0012*

* Kruskal-Wallis test. Analysis performed on log transformed data. FR Fine root percentage (< 0.5 mm diameter), Leaf [Mn]/ Leaf manganese concentration (μg Mn g⁻¹), Leaf [P]/ Leaf phosphorus concentration (mg P g⁻¹), PME Phosphomonoesterase activity (μg nitrophenol g⁻¹ h⁻¹), RLD Root length density (cm cm⁻³), RSA Root surface area (cm²), SLA Specific leaf area (mm² mg⁻¹), SRL Specific root length (m g⁻¹), TCE Total carboxylate release rate (μmol g root⁻¹ h⁻¹), ΔpH Change in rhizosphere pH

expression of root morphological traits associated with soil foraging, or modifying their physiological traits to mobilise poorly-available P (Rose et al. 2010; Teng et al. 2013; Lyu et al. 2016). A more complex association of morphological and physiological traits along a cost-benefit axis has also been suggested (Lynch 2015; Weemstra et al. 2016; Wen et al. 2019), prompting further investigations. A certain dichotomy was indeed observed between P-acquisition strategies relying more on physiological or more on morphological traits, corroborating the tradeoffs we observed. However, multivariate analysis highlighted a diversity of intermediate strategies, rather supporting the hypothesis of a complex association of traits along a cost-benefit continuum for P acquisition. Our results offer important new insights into the complex and diverse range of P-acquisition strategies occupying a different trait space, and thus offering opportunities for complementarities in resource acquisition. In both soils, similar extreme strategies could be identified on both ends of a morphological/physiological spectrum. On the morphological end, a strategy could be identified, presenting a low expression of physiological indicators and high root length density/fine roots percentage, mainly encompassing Brassicaceae and Poaceae. This strategy is probably oriented toward soil scavenging, as an important proportion of fine roots can be a way to forage soil at a low cost, while important root length density similarly denotes an important exploration of a given soil volume (Lynch 2015; Yuan et al. 2016; Ma et al. 2018). Similar groupings of species constituting this group (notably Indian mustard, radish, and oat) have been observed before, based on morphological traits, and proposed to be an intermediate strategy between very resource-acquisitive species such as phacelia, and more conservative ones such as faba bean (Wendling et al. 2016). On the other end of the spectrum, the several physiological groups, mainly comprising Fabaceae, presented high expressions of all physiological traits with overall lower fine root percentage and SRL. High rhizosphere phosphatase activity, carboxylate release and rhizosphere acidification indicated strategies potentially increasing P availability via mineralisation of organic P, ligand exchange and dissolution of precipitated phosphates, respectively (Hoffland et al. 1989; Jones et al. 2003). While our results confirm the greater effect of Fabaceae on rhizosphere properties compared with other phylogenetic groups (Maltais-Landry 2015), a diverse spectrum of strategies was observed, also within Fabaceae. Two to three clusters

per soil expressed intermediate to high values of physiological indicators, and were comprised mainly, but not only of Fabaceae. These groups differed in their expression of morphological traits, especially RLD, fine roots percentage and SRL. This corroborates the existence of a spectrum of P-acquisition strategies combining scavenging and mining processes in intermediate crops. These contrasting strategies, interestingly, did not differ significantly in their P uptake, indicating a potential for maximising P acquisition in multispecies systems via mixing strategies accessing diverse P pools in soils (Lyu et al. 2016; Li et al. 2017) and as such less competition for resources. Such complementarities in resource acquisition among plant species has been suggested as an explanation for the positive effects of functional diversity (Petchey and Gaston 2006; Faucon et al. 2017). Functionally more diverse systems indeed outperform single species for P uptake (Li et al. 2014; Xue et al. 2016). Overall, our results could lead to enhanced P cycling efficiency and P availability in multispecies cropping systems via better designs exploiting more of the total P stock including the pool of legacy P (Richardson et al. 2011). Functional diversity effects on P availability and uptake are not well known however (Huang et al. 2019), and variable shifts in root functional traits and plant P-acquisition strategy should be expected in multispecies conditions (Li et al. 2014). Hence, there is a need for further studies of functional diversity effects on P availability and P cycling. Perspectives include examining functional diversity effect on P uptake and P availability in systems combining different P-acquisition strategies and in different soil and climate contexts. Other traits potentially involved in P acquisition such as root hair length and density (Haling et al. 2013; Yuan et al. 2016), association with mycorrhizal fungi (Smith and Smith 2011; Campos et al. 2018) or rhizosphere bacteria (Richardson et al. 2011) should also be included in future studies to further refine the strategies identified.

Mediation of tradeoffs and P-acquisition strategies according to P forms in soil

Although expression of both P-mining traits and morphological traits are essential for P acquisition, their costs in a specific context may define how plants express and mix their functional traits (Raven et al. 2018; Zhou et al. 2018). Phosphorus-mining traits in particular have proven to have their efficiency strongly impacted

by soil type and soil P forms such as proposed for carboxylates in strongly P-sorbing soils (Wang and Lambers 2019). Indeed, we observed greater relevance and coordination of physiological traits in Retisol, while morphological traits were more expressed in Calcaric Cambisol. The modulation of P-acquisition strategies was further evidenced when modelling P uptake via GLM and PLS PM, providing insight into the relative importance of trait type. Phosphorus uptake was best predicted by root morphological traits and aboveground traits in Calcaric Cambisol, and a mixture of root morphological and physiological traits in Retisol. While several single trait correlations with P uptake were previously observed (Pearse et al. 2006; Lambers et al. 2006; Rose et al. 2010; Wen et al. 2017) our results complement our incomplete knowledge of coordination between physiological and morphological responses as well its mediation by soil type and soil P forms (Lyu et al. 2016). At low concentration, exudates may not be in a range relevant to significantly impact P uptake in Calcaric Cambisol, or they may have their efficiency impacted by soil characteristics. Adverse effects impacting P mobilisation via carboxylate exudation have indeed been observed, because of “Ca-aided co-adsorption” when released at low concentration, with low amounts of carboxylates increasing adsorption of calcium ions, in turn increasing adsorption of P (Duputel et al. 2013). The higher acid buffering capacity of the Calcaric Cambisol might also have impacted the efficiency of rhizosphere acidification as a P-acquisition trait, further decreasing the potential benefits of physiological traits in this soil. Overall, the strong soil mediation observed offers precious insights into P acquisition. As mentioned, morphological and physiological traits benefit P acquisition from different P pools, offering opportunities for enhanced P cycling in multispecies systems, notably by exploiting the pool of legacy P. However, by reinforcing the importance of plant/soil interactions, our results suggest that improved designs for enhanced P benefits should rely on different processes and strategies in different soils. Some processes such as P mobilisation should be less relevant in some contexts (Maltais-Landry et al. 2014), rather suggesting reliance on a mixture of resource-acquisitive strategies (Wendling et al. 2016). To be able to improve P benefits of intermediate crops, further efforts are needed to investigate the stability and relevance of the tradeoffs and strategies involved in P acquisition for a range of soil types and associated P forms and availability.

Conclusions

Enhancing P cycling and availability in cultivated systems via ecological intensification requires a better understanding of tradeoffs and strategies involved in P acquisition. Morphological and physiological traits comprised diverse P-acquisition strategies structured along an axis of trait covariation and tradeoffs. Our results demonstrate tradeoffs between thicker and thinner roots, exhibiting more physiological traits and morphological traits, respectively. Reliance on either morphological or physiological traits and combinations thereof for P acquisition were underlined by model approaches, and strongly mediated by soil P forms. The multiple P-acquisition strategies with similar P uptake observed indicates the potential for positive effects of functional diversity via complementarities in resource acquisition, and thus offers insights into mixture designs for improved P benefits. Finally, varied tradeoffs and strategy plasticity amongst soil with different P forms highlighted the importance of considering soil/plant interactions when attempting to improve our understanding of resource-acquisition strategies. Further studies should investigate trait and strategy plasticity across a gradient of functional diversity and various soil properties to help us select for locally optimized crop services.

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Authors' contributions Nicolas Honvault carried out the experiment and wrote the manuscript with support from Michel-Pierre Faucon, David Houben and Hans Lambers. Stéphane Firmin and Cécile Nobile helped process the data and perform the analyses. All authors contributed to the final version of the manuscript.

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9.2. APPENDIX B – Supplementary information second chapter

Supplementary Material S1: Detailed protocol for PLFA and NLFA extraction and interpretation

Lipid extraction and fractionation

The total lipid extraction procedure followed that described by Frostegård et al. (1991), which is based on the method by Bligh & Dyer (1959) as modified by White et al. (1979). Briefly, 3 g (dry weight) portions of soil were extracted in a one-phase mixture consisting of chloroform, methanol and citrate buffer (1: 2:0.8, v/v/v). After splitting the extracts into two phases by adding chloroform and citrate buffer, the lipid-containing phase was dried under a stream of nitrogen. The lipid material was fractionated on a SPE silica column (Solid Phase Extraction, Hypersep SILICA 500mg from Thermo Fisher Scientific, Waltham, MA, USA) into neutral, glycolipid- and phospholipid-containing polar lipids (Frostegård et al. 1991). The phospholipid fraction was dried under a stream of nitrogen, and redissolved in toluene/methanol (1:1) and subjected to a trans-esterification using a base solution (KOH, 0.2 M prepared in methanol) at 37°C for 15 min to release free fatty acid methyl esters (FAMES) from the PLFA and the NLFA.

Mild alkaline methanolysis and GC analysis

A known amount of methyl nonadecanoate was added to the phospholipid fractions as an internal standard. The samples were then subjected to a mild alkaline methanolysis (Dowling et al. 1986), and the resulting fatty acid methyl esters were separated by gas chromatography–mass spectrometry (GC-MS) SHIMADZU QP2010 ULTRA equipped with a single quadrupole mass detector simultaneously coupled with a flame ionization detector. The samples were analyzed on a (10m x 0.1mm x 0.1µm) ZB-1MS (100 % diméthyl polysiloxane, Zebron, Phenomenex, Torrance CA, USA) fast capillary column using helium as a carrier gas, and injections were made in a split mode (ratio 100:1). The injector temperature was 280°C, and the detector temperature was 330°C. The temperature program was: initial temp. 175°C for 1, increasing 25°C min⁻¹, final temp. 275°C for 0.5 min. Relative retention times of assessed fatty acid methyl esters were compared with those of standards.

Identifications using GC-MS.

Electron energy in electron impact was 70 eV. Identifications of fatty acid methylesters were based on comparison with spectra that were either obtained from standards or reported in the literature (NIST Standard Reference Database).

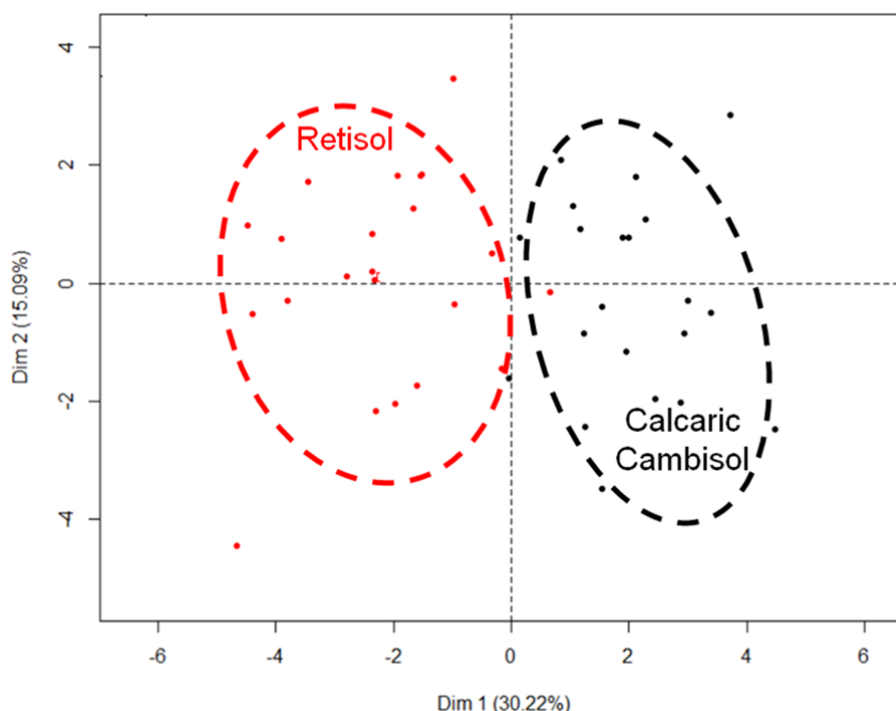
Interpretation of PLFA and NLFA indicators

The amount of NLFA C16:1 ω 5 in the soil was determined and used as indicator of arbuscular mycorrhizal fungal biomass. The NLFA/PLFA ratio of C16:1 ω 5 was calculated to account for possible bacterial interference when interpreting NLFA C16:1 ω 5 concentration as an indicator of AMF storage over growth, with possible interference being noted for ratio inferior to 1. PLFA and NLFA C18:2 ω 6,9 were used as an indicator of fungal biomass (except arbuscular mycorrhizal fungi) (Olsson et al. 1997; Schnoor et al. 2011). Gram-positive living bacterial biomass was estimated by the quantification of the PLFA: i15:0, a15:0, i16:0, i17:0, a17:0 amounts and Gram-negative living bacterial biomass by the quantification of the PLFA: cy17:0, C18:1 ω 7 and cy19:0 amounts in the soil. NLFA were used as indicators of recently dead bacterial necromass (Malosso 2004; Amir et al. 2008; Rinnan and Bååth 2009). Gram-positive recently dead bacterial necromass was estimated by the quantification of the NLFA: i15:0, a15:0, i16:0, i17:0, a17:0 amounts and Gram-negative recently dead bacterial necromass by the quantification of the NLFA: cy17:0, C18:1 ω 7 and cy19:0 amounts in the soil. The sum of Gram-negative NLFA and Gram-positive NLFA and the sum of Gram-negative PLFA and Gram-positive PLFA were calculated as indicators of total bacterial abundance (necromass and living biomass respectively). A fungal:bacterial biomass ratio was also calculated based on NLFA data, with the sum of the fungal NLFA C18:2 ω 6,9 and C16:1 ω 5 divided by the sum of the bacterial NLFAs.

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Supplementary Material S2: Principal component analysis of rhizosheath PLFA and NLFA concentration per soil type



Individual factor map, with individuals coloured per cluster and corresponding soil types. Principal component analysis performed with the following variables : Concentration of a15.0 neutral lipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of a17.0 neutral lipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of C16.1 ω 5 neutral lipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of C18.1 ω 7 neutral lipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of C18.2 ω 6.9 neutral lipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of cy.19.0 neutral lipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of cy17.0 neutral lipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of i15.0 neutral lipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of i16.0 neutral lipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of i17.0 neutral lipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of a15.0 phospholipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of a17.0 phospholipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of C16.1 ω 5 phospholipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of C18.1 ω 7 phospholipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of C18.2 ω 6.9 phospholipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of cy.19.0 phospholipid ($\mu\text{g g}^{-1}$ dry soil); Pcy17.0 : Concentration of cy17.0 phospholipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of i15.0 phospholipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of i16.0 phospholipid ($\mu\text{g g}^{-1}$ dry soil); Concentration of i17.0 phospholipid ($\mu\text{g g}^{-1}$ dry soil).

Supplementary Material S3 : Correlation between PLFA, NFLA, belowground traits, phosphorus (P) content and changes in rhizosheath P concentration

Soil type	PLFA NFLA		Correlation coefficient	P-value
Retisol and Calcaric Cambisol	AMF NLFA	Malate	0.81 ^a ; 0.76	0.01; 0.03
	Gram-negative NLFA	Fine roots	-0.98 ^a ; -0.74 ^a	<0.01; 0.04
		Total P content	0.84; 0.77	0.01; 0.04
	Bacterial NLFA	Total P content	0.82; 0.78	0.01; 0.02
Retisol	AMF NLFA	Fine roots	-0.95 ^a	<0.01
		PME	0.88 ^a	<0.01
		Total P content	0.90 ^a	<0.01
		Δ microbial P	0.71 ^a	<0.05
		Δ Olsen P	-0.88 ^a	<0.01
	Bacterial NLFA	Fumarate	0.71	<0.05
		Fine roots	-0.93 ^a	<0.01
		Δ Olsen P	-0.77	0.03
	Fungal:bacterial NLFA	Δ resin P	-0.73	0.04
		Citrate	-0.81 ^a	0.01
	Fungi PLFA	Citrate	-0.71 ^a	<0.05
	Gram-negative NLFA	Malate	0.86 ^a	0.04
Total P content		0.84	0.01	
Δ Olsen P		-0.78	0.02	
Δ resin P		-0.75	0.03	
Gram-negative PLFA		RSA	0.76 ^a	0.03
Gram-positive PLFA	RLD	0.71	<0.05	
Calcaric Cambisol	AMF NLFA	Leaf [Mn]	0.71 ^a	0.05
		Malonate	0.80 ^a	0.02
		TCR	0.76	0.03
	Bacterial NLFA	Leaf [Mn]	0.88 ^a	<0.01
		Malonate	0.83 ^a	0.01
		Δ Olsen P	0.90 ^a	<0.01
	Fungal:bacterial ratio NLFA	Δ resin P	-0.82	0.01
		Malonate	0.75 ^a	0.03
		TCR	0.76	0.03
	Fungi NLFA	Malate	0.71 ^a	0.05
		TCR	0.79 ^a	0.02
	Gram-negative NLFA	Leaf [Mn]	0.86 ^a	0.01
Malonate		0.85 ^a	0.01	
TCR		0.79	0.02	
Gram-negative PLFA	Δ Olsen P	0.71 ^a	<0.05	
Gram-positive NLFA	RLD	-0.83	0.01	
	RSA	-0.81	0.01	

Pearson correlation coefficients, except for factors labelled with ^a in which case Spearman correlation coefficients. Abbreviations : Citrate: citrate-release rate; Fine roots : % fine roots (<0.5 mm \varnothing); Fumarate: fumarate-release rate; Leaf [Mn] : leaf manganese concentration; Malate: malate-release rate; Malonate: malonate-release rate; PME: phosphomonoesterase activity; RLD: root length density; RSA: root surface area; TCR: total carboxylate-release rate; Total P content: total P content in shoot biomass; Δ microbial P: difference in microbial P concentration between rhizosheath and bulk soil; Δ Olsen P: difference in Olsen P concentration between rhizosheath and bulk soil; Δ pH : difference in pH concentration between rhizosheath and bulk soil; Δ resin P: difference in resin P concentration between rhizosheath and bulk soil. AMF NLFA: Concentration of C16:1 ω 5 neutral lipid; Gram-negative NLFA: Sum of concentrations of cy17:0, C18:1 ω 7, cy:19:0 neutral lipids; Gram-positive NLFA: Sum of concentration of i15:0, a15:0, i16:0, i17:0, a17:0 neutral lipids; Fungi NLFA: Concentration of C18:2 ω 6,9 neutral lipid; Bacterial NLFA: Sum of Gram-negative NLFA and Gram-positive NLFA; Fungal:bacterial ratio NLFA : Sum of the fungal NLFA C18:2 ω 6,9 and C16:1 ω 5 divided by the sum of the bacterial NLFAs. NLFA: Neutral lipid fatty acid; Gram-negative PLFA: Sum of concentration of cy17:0, C18:1 ω 7, cy:19:0 phospholipids; Gram-positive PLFA: Sum of concentration of i15:0, a15:0, i16:0, i17:0, a17:0 phospholipids; Fungi PLFA: Concentration of C18:2 ω 6,9 phospholipid.

9.3. APPENDIX C – List of publications and communications

Articles published in international peer-reviewed journals

Honvault N, Houben D, Nobile C, et al (2020) Tradeoffs among phosphorus-acquisition root traits of crop species for agroecological intensification. *Plant Soil*. <https://doi.org/10.1007/s11104-020-04584-3>

Yacoumas A, **Honvault N**, Houben D, et al (2020) Contrasting Response of Nutrient Acquisition Traits in Wheat Grown on Bisphenol A-Contaminated Soils. *Water, Air, & Soil Pollution* 231:23. <https://doi.org/10.1007/s11270-019-4383-7>

Manuscript submitted in international peer-reviewed journals

Honvault N, Houben D, Firmin S, Meglouli H, Laruelle F, Fontaine J, Lounès-Hadj Sahraoui A, Lambers H, Faucon M.P. Interactions between belowground traits and rhizosheath fungal and bacterial communities for phosphorus acquisition. *Functional Ecology*.

Poster in national and international congresses

Honvault N, Houben D, Lambers H, Nobile C, Firmin S, Faucon M.P. Highlighting phosphorus-acquisition strategies in intermediate crops, a functional approach. *Phosphorus in Soils and Plants* 6, 10/09/2018-13/09/2018. Leuven, Belgique.

Oral communications in national and international congresses

Honvault N, Houben D, Lambers H, Nobile C, Firmin S, Faucon M.P. Highlighting phosphorus-acquisition strategies in intermediate crops, a functional approach. *International Phosphorus Workshop* 9, 08/07/2019-12/07/2019. Zurich, Suisse.

Honvault N, Houben D, Oberson A, McLaren T, Frossard E, Lambers H, Faucon M.P. Trait based approach of phosphorus cycling to unravel multispecies systems functioning. *2020 ESA Annual Meeting*, 03/08/2020-06/08/2020. Online.

Rôle des traits fonctionnels des plantes dans le cycle du phosphore et sa disponibilité au sein des agroécosystèmes

Agroécologie des cultures intermédiaires

Le phosphore (P) est l'un des principaux facteurs limitant dans la plupart des sols du monde. Améliorer sa disponibilité dans les agrosystèmes et assurer son utilisation efficace est donc un défi majeur de l'agriculture moderne. Plusieurs pratiques, telles que les couverts intermédiaires, peuvent potentiellement augmenter la disponibilité du P dans les agrosystèmes. Cependant, les effets de ces pratiques sur la disponibilité du P ont rarement été étudiés. Ce projet de doctorat vise à mieux comprendre le rôle des traits fonctionnels des cultures intermédiaires dans les processus influençant la disponibilité du P dans les agrosystèmes. La caractérisation d'un large éventail de traits d'acquisition de P a mis en évidence de multiples compromis racinaires, notamment entre la capacité des plantes à mobiliser le P et explorer un large volume de sol pour capturer le P disponible, tout en soulignant la contribution considérable des interactions plantes-microbes à l'acquisition de P par les plantes. Les multiples stratégies d'acquisition de P identifiées offrent l'opportunité de concevoir des cultures intermédiaires multi-espèces complémentaires dans leur utilisation du P. L'examen du rôle des traits des cultures intermédiaires sur la libération de P depuis leurs résidus et son impact sur la disponibilité du P a offert des indications précieuses sur les dynamiques microbiennes impliquées et le rôle central du ratio C:P. Les nouveaux éléments de compréhension apportés sur les effets des cultures intermédiaires sur la disponibilité du P pourraient constituer une base solide pour la modélisation des effets des cultures intermédiaires sur la disponibilité du P.

Mots clés:

Traits fonctionnels souterrains, cultures intermédiaires, écologie fonctionnelle, acquisition du phosphore, interactions plante-microbe, rétroaction plante-sol, interactions plante-sol, compromis racinaires

Role of plant functional traits in phosphorus cycling and availability in agroecosystems

The case of cover crops

Phosphorus (P) is a key limiting element in most of the soils worldwide. Overcoming poor P availability and ensuring productive agriculture requires significant P inputs. Improving P use efficiency and availability is thus a major challenge of modern agriculture. Several agroecological practices such as cover cropping can increase P cycling efficiency and availability in agrosystems. However the effects of these practices on P availability have rarely been addressed. This PhD project aims at getting a better understanding of the role of cover crops functional traits in the plant-soil-microbes processes influencing P availability in agrosystems. Characterising a wide range of P acquisition traits highlighted multiple tradeoffs, notably between P mining traits and P foraging traits, while also underlining the considerable contribution of plant-soil-microbes interactions to P acquisition. The spectrum of diverging P acquisition strategies identified could underpin complementary multispecies cover crops designs. The examination of the role of cover crop residue traits on P release from residues and its impact on P availability offered precious insights into the soil microbial dynamics involved and the role of residue C:P ratio. The effects of cover crop residues on P availability via P release were however projected to be highly mediated by soil P availability. The new understanding gained on the effects of cover crops on P availability via their diverse traits and strategies for P acquisition, the impact of their traits on P release from residue, and its mediation by soil properties could constitute a sound basis for modelling cover crops effects on P availability.

Key words:

Belowground functional traits, Cover crops, Functional ecology, Phosphorus acquisition, Plant-microorganism interactions, Plant-soil feedback, Plant-soil-interactions, Tradeoffs