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# Caractérisation du partage des ressources en eau et en azote entre les arbres et les cultures au sein de jeunes peuplements agroforestiers

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# Avant-propos

Cette thèse a été réalisée du 9 septembre 2019 au 21 mars 2023, au sein de JUNIA-ISA, rattachée à l'Unité Mixte de Recherche transfrontalière (UMR-T) INRAE 1158 BioEcoAgro. Cette UMR-T regroupe des chercheurs français et belges travaillant dans le domaine de l'ingénierie biologique appliquée à l'agriculture, la biotechnologie, l'agro-alimentaire et l'environnement. Elle est structurée en 3 pôles, regroupant 9 équipes. Ce travail s'inscrit dans le cadre du pôle 1, « Systèmes de culture innovants pour la transition agro-écologique et bioéconomique dans le contexte du changement climatique » et de l'équipe 3, « Conduite, optimisation et conception de systèmes de culture répondant à des objectifs multicritères » (Figure i).

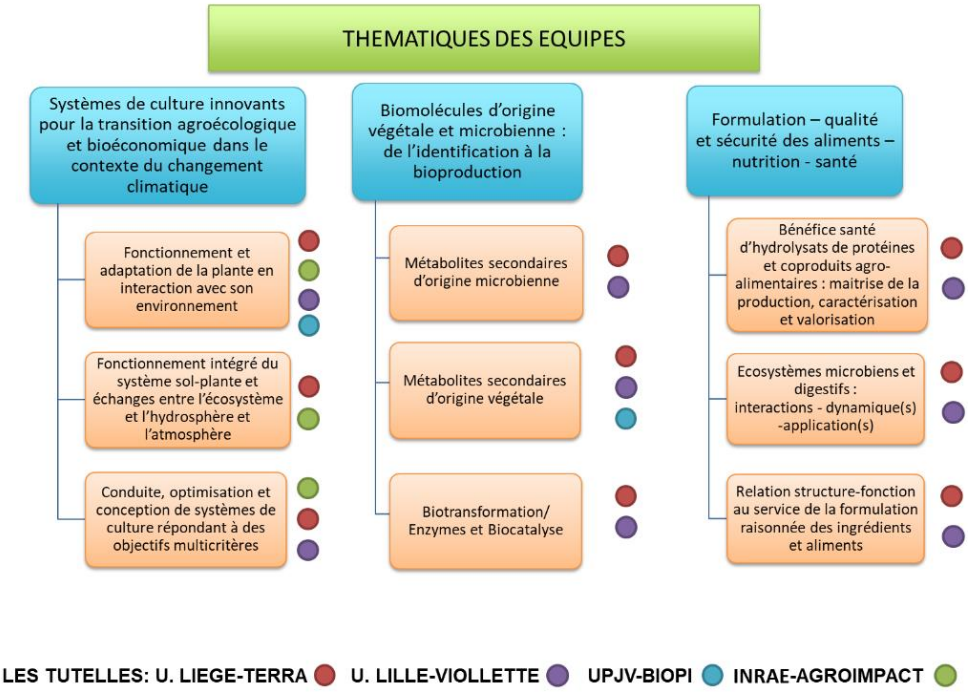

**Figure i :** Organisation des thématiques de recherche de l'UMRT 1158 BioEcoAgro (https://www.bioecoagro.eu/umrt-bioecoagro_fre/)

# Préambule

Cette thèse s'inscrit dans le cadre des projets de recherche AFRame (2018-2022) et JeuneAF (2019 – 2022).

Le projet de recherche AFRame vise à produire des références technico-économiques sur l'agroforesterie intraparcellaire en Hauts-de-France par la création d'un site expérimental vitrine. Il est piloté par JUNIA ISA avec Sitraka Andrianarisoa, Caroline Choma, François Delbende, Eric Manouvrier et Hélène Desmyttère. Ce projet compte cinq partenaires : les Planteurs Volontaires, le Carré de Ramecourt, l'Institut d'Hazebrouck, l'INRAE Grand Est - Nancy et la FREDON (Figure ii). Il a obtenu le concours financier de l'agence de l'eau Artois-Picardie, de l'ADEME, de la région Hauts-de-France et de la Fondation de France (Figure ii).

Le projet JeuneAF a été conçu pour pallier un manque de connaissances sur les interactions entre arbres et cultures pendant les phases d'installation des arbres en agroforesterie intraparcellaire. Il a été financé par la fondation de France et piloté par JUNIA ISA.

a)

b)

**Figure ii :** Partenaires (a) et co-financeurs du projet AFRame (b).

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# Résumé

L’introduction de lignes d’arbres dans les terres arables en agroforesterie intraparcellaire (AI) offre plusieurs services écosystémiques mais peut également induire une compétition pour l’espace et les ressources diminuant le rendement de la culture. Cependant, d’autres études montrent que grâce à une différenciation spatiale et temporelle des niches écologiques, l’AI permet une meilleure utilisation des ressources. Bien que la facilitation entre les arbres et les cultures associées ait largement été documentée dans les peuplements adultes, l’initialisation de ce processus lors des premières années de la phase d’installation des arbres en AI demeure peu étudiée. L’objectif de cette thèse était de caractériser la distribution spatiale et temporelle des racines fines, d’explorer le partage de l’eau et de l’azote et d’examiner l'activité microbienne globale pendant la phase d’installation des arbres sur les terres cultivées en système agroforestier intraparcellaire. L’abondance des racines fines des arbres a été quantifiée le long d’une chronoséquence de dix-sept peuplements agroforestiers intraparcellaires âgés entre 3 et 12 ans. Un suivi du partage de l’eau et de l’azote entre les arbres et la culture a été réalisé sur une parcelle en AI âgée de 4 ans sur le site expérimental de Ramecourt, par marquage des isotopes stables $^{2}$H et $^{15}$N. Enfin, le changement global de l'activité microbienne a été évalué à travers l'expérience de sachets de thé enterrés à 30, 50 et 100 cm de profondeur. La chronoséquence a révélé qu’avant 4 ans, les racines fines des arbres colonisent le premier horizon (0-30 cm) au niveau de la ligne, puis se développent principalement verticalement entre 4 et 6 ans, avant d’explorer latéralement les horizons profonds (1-2 m) au-delà de cet âge. L'âge du peuplement, la fréquence du travail du sol et la durée de la rotation des cultures ont expliqué 60 % de la variabilité de l'abondance des racines fines des arbres le long du profil du sol jusqu'à 2 m de profondeur. Sur le site expérimental de Ramecourt, la cohabitation entre le blé et les arbres de 4 ans a réduit l'abondance des racines fines du blé dans l’horizon supérieur à 2 m des lignes d'arbres. Les racines fines des jeunes saules et charmes ont cohabité avec le blé dans l’horizon supérieur, tandis que le merisier a développé des racines fines profondes sous la zone principale d'enracinement de la culture. Les jeunes arbres en AI ont contribué à valoriser l'azote des engrais sans impact significatif sur l'absorption globale de l'azote par le blé et ont favorisé sa valorisation dans les grains. Les jeunes arbres et le blé ont absorbé de l'eau dans l’horizon supérieur, cependant, les arbres en AI ont favorisé l'absorption d'eau par le blé dans l’horizon supérieur et ont été capables d’interchanger de manière flexible leur source d'eau en prélevant dans les horizons profonds en cas de faible disponibilité d'eau en surface. La teneur en eau du sol était négativement corrélée à l’abondance des racines fines des arbres, ce qui suggère qu'avec l'âge, les arbres ont asséché les horizons profonds sous la zone d'enracinement des cultures et augmenté la profondeur du sol capable de stocker les pluies d'automne et d'hiver. De même, une augmentation de la matière organique du sol a été observée dans les peuplements de 10-12 ans dans l’horizon supérieur au niveau de la ligne d’arbres, confirmant la capacité des systèmes en AI à stocker du carbone mais que ce processus est lent. Enfin, les racines fines des arbres ont modifié la dynamique de l'azote et du carbone en favorisant l'ammonification et en ralentissant la décomposition de la matière organique récalcitrante. Les changements se produisant lors de la transition d’un système grandes cultures en AI sont progressifs, après 4 ans de plantations, les effets des arbres sur les ressources du sol étaient encore faibles. Un suivi continu de l'initialisation de l'AI dans le temps permettra de quantifier l'étendue de la compétition ou de la complémentarité pour l'eau et l'azote entre les arbres et la culture, et d'examiner les changements microbiens avec l'âge du peuplement et la profondeur du sol.

**Mots clés :** Agroforesterie intraparcellaire, racines fines, partage d’eau entre les arbres et les cultures, décomposition de la matière organique, activité microbienne

# Abstract

The introduction of trees rows in arable land in alley cropping systems (AC) offers several ecosystem services but may also induce competition between tree and crop for space and resources leading to crop yield decrease. However, other studies showed that thanks to a spatial and temporal niche differentiation, intercropping allows to a better use of resources. Although facilitation between associated trees and crops was largely documented in adult stands, the initialization of this process during the early stage of trees establishment in AC was less investigated. The objective of this thesis was to characterize the spatial and temporal fine root distribution, to explore the sharing of water and nitrogen and to examine the overall microbial activity at the early phase of tree establishment in crop land. The tree fine roots abundance (TFRA) and distribution throughout the soil profile was measured along a chronosequence of seventeen AC stands ranging from 3 to 12 years old. The water and nitrogen (N) partitioning between trees and wheat was monitored on a 4-year-old AC at the Ramecourt experimental site, using $^{2}$H and $^{15}$N stable isotopes labeling. Finally, the overall change in microbial activity was evaluated using tea bags experiment buried at 30, 50 and 100 cm depth. The chronosequence revealed that before four years old, tree fine roots colonized the topsoil (0–30 cm) in rows and then mainly grew vertically from 4 to 6 years old, before laterally exploring deep soil layer (1–2 m) beyond this age. The stand age, the tillage frequency and the crop rotation duration accounted for 60 % of the variability of tree fine root abundance throughout soil profile down to 2 m depth. At the Ramecourt experimental site, the cohabitation between wheat and 4-year-old trees reduced the wheat fine roots abundance in upper soil layer at 2 m from tree rows. Young willow and hornbeam fine roots cohabited with wheat in topsoil whereas wild cherry already developed deep fine root under the main crop rooting zone. Young trees in AC contributed to valorize the N fertilizer without significant effect on global wheat N uptake and favored its valorization in grain. Young trees and wheat took up water in upper soil layer but in AC, trees favored wheat water absorption in topsoil and were able to flexibly shift their water source from deep layer in case of low water availability in upper soil layer. The SWC was negatively correlated to TFRA suggesting that as trees get older, they dried the deep soil layer below the crop rooting zone and increased the soil depth able to store autumn and winter rainfall. Similarly, an increase in soil organic matter was observed in the 10-12-year-old stands in topsoil and within the row, attesting the ability of AC to store carbon but this process is slow. Finally, the tree fine roots modified the nitrogen and carbon dynamics by promoting ammonification and slowing down recalcitrant organic matter decomposition. The changes occurring during the conversion from a cropland system to AC are gradual, after 4 years of establishment, trees still had faintly effects on soil resources. Continued monitoring of the AC initialization over time will allow us to quantify the extent of competition or complementarity for water and nitrogen between the trees and the crop, and to examine microbial change with stand age and soil depth.

**Keywords:** Alley-cropping, fine roots, tree and crop water and nitrogen, organic matter decomposition, microbial activity

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# Liste des abréviations

**%NO3** : pourcentage de nitrate

**AC** : Alley-cropping

**SAF** : Système agroforestier

**cm** : centimètre

**C** : carbon/*carbone*

**C/N** : ratio carbone sur azote

**COS** : Carbone organique du sol

**DM** : Dry biomass/*biomasse sèche*

**DNA** : Deoxyribonucleic acid

**$^2$H** : deutérium

**ha** : hectare

**kg N.ha$^{-1}$** : kg d'azote par hectare

**m** : mètre

**Mg ha$^{-1}$** : méga gramme par hectare

**ML** : Mass loss

**MO** : Matière Organique

**N** : Nitrogen/*azote*

**$^{15}$N** : isotope azote 15

**$NH_4^+$** : ammonium

**$NO_3^-$** : nitrate

**$R^2$** : coefficient de détermination/*determination coefficient*

**RG** : Ryegrass*/Ray-grass*

**RGFRA** : Ryegrass fine root abundance

**RGFRB** : Ryegrass fine root biomass

**RMSE** : Root mean square error/*Erreur quadratique moyenne*

**SAN** : Soil ammonium nitrogen

**SMN** : Soil mineral nitrogen

**SOC** : Soil organic carbon

**SOM** : Soil organic matter

**SNN** : Soil nitrate nitrogen

**STN** : Soil total nitrogen

**SWC** : Soil water content

**TFRA** : Tree fine root abundance

**TFRB** : Tree fine root biomass

**WFRA** : Wheat fine root abundance

**WFRB** : Wheat fine root biomass

# Introduction Générale

L’agriculture représente un enjeu international au regard de l’augmentation de la population. La production des denrées alimentaires doit augmenter chaque année pour subvenir à la demande mondiale. Au-delà de l’accroissement de la population, l’agriculture est parmi les secteurs les plus visés comme étant responsables du réchauffement climatique notamment par ses émissions de gaz à effets de serre. Par ailleurs, elle est également une des premières touchées par ce dérèglement climatique avec des épisodes climatiques extrêmes qui se multiplient et dévastent les cultures (inondations, ouragans, sécheresses…). Une transition agricole est alors nécessaire afin de faire face à la demande croissante des denrées alimentaires tout en diminuant l’impact de l’agriculture sur l’environnement. Des systèmes agricoles durables émergent avec la notion d’agroécologie, notamment la permaculture et l’agroforesterie. En grandes cultures, l’agroforesterie intraparcellaire permet de réintégrer les arbres dans les systèmes agricoles en s’adaptant à la mécanisation moderne.

L’importance et le rôle de l’arbre dans le paysage agricole questionnent les agriculteurs et des références scientifiques sur les systèmes agroforestiers (SAF) se multiplient à l’échelle mondiale. Néanmoins, il existe une importante diversité SAF et chaque parcelle est unique. Malgré les nombreuses études, il est parfois difficile d’extrapoler les résultats à l’échelle d’une exploitation agricole précise. En France, et plus particulièrement dans la région des Hauts-de-France, l’agriculture est ancrée dans le territoire et des références sont nécessaires pour que des changements soient possibles et acceptés. Par ailleurs, en plus de la surface amputée sur les terres arables pour planter les arbres, ceux-ci représentent une compétition directe avec les cultures pouvant diminuer les rendements. Il est donc indispensable d’entreprendre des études sur l’établissement des arbres durant les premières années des SAF afin que la transition soit efficace et rapide.

La compétition en eau et en azote a déjà été largement documentée dans différents agroécosystèmes associant cultures pérennes et cultures annuelles sur la même parcelle (van Noordwijk et al., 2015). Cependant, il a aussi été démontré que la culture et/ou le travail agricole (via le labour ou un sous solage) favorisent le développement des racines plus profondes chez les arbres agroforestiers en comparaison aux arbres forestiers (Mulia and Dupraz, 2006). Malgré les avancées scientifiques sur la modélisation de l’interaction entre arbres et cultures pour l’absorption d’eau et d’azote le long du profil du sol (van Noordwijk and Lusiana, 1999), peu de données sont disponibles sur la mise en place de ces interactions chez les jeunes peuplements. Ainsi, le travail réalisé durant cette thèse porte sur la caractérisation du partage de ces ressources et de la modification de l'activité microbienne pendant les phases d'installation des arbres dans les parcelles agroforestières. Le présent manuscrit débutera par un état de l'art présentant les connaissances actuelles sur le sujet et les objectifs visés par ce travail. Les principaux résultats obtenus seront ensuite décrits et discutés dans chaque chapitre écrit sous forme d'articles scientifiques. Enfin, une discussion générale clôturera ce manuscrit comprenant une synthèse des résultats obtenus et les perspectives.

# Chapitre 1 : Etat de l'art et problématique de la thèse

## 1. Synthèse bibliographique

### 1.1. L'agroforesterie

L'agroforesterie est une pratique agricole ancestrale avec une approche intégrée de l'utilisation des terres. Elle est définie comme l'association délibérée sur une même parcelle de plantes ligneuses pérennes avec une production agricole végétale ou animale afin de bénéficier des interactions écologiques et économiques qui en résultent (Nair, 1985). Le terme « agroforestry » a été évoqué la première fois en 1970 par les chercheurs canadiens pour définir des programmes de recherche agricole et forestière dans les pays en développement. L'agroforesterie de première génération était donc consacrée aux systèmes agricoles tropicaux. Dans les régions tempérées, l'agroforesterie "moderne" a émergé dans les années 1990, définissant d'abord des alignements d'arbres destinés à la production de bois d'œuvre au sein des parcelles agricoles, puis a évolué en incluant un grand nombre de pratiques associant les éléments en bordure de parcelles. Une première classification des différentes formes d'agroforesterie a été établie par Nair (1985), sur la base des composants du système donnant trois types fondamentaux : l'**agrosylviculture** (association des arbres et des cultures arables), le **sylvopastoralisme** (association des arbres et des prairies pâturées) et **l'agro-sylvopastoralisme** (association des arbres, prairies pâturées et des cultures arables).

Cette classification a ensuite évolué en se basant sur les objectifs du système agroforestier (SAF). Les critères de classification comprennent la structure du système (arrangement spatial et temporel des composants), la fonction (production de biens, rôle de protection, d'habitat), l'échelle socio-économique (système commerciaux, intermédiaires ou de subsistance) et la zone écologique (milieu d'implantation de la parcelle) (McAdam et al., 2008). Sinclair (1999) a largement approfondi cette classification en détaillant finement les pratiques agroforestières au sein de la parcelle. Une première classification départage les pratiques agroforestières selon le composant de base comme la classification de Nair (1985), puis cette classification est affinée selon l'usage principal des terres. Une deuxième classification détaille ensuite chaque famille selon les critères d'arrangement spatial, de densité et de diversité des essences présentes (Sinclair, 1999).

## 1.2. L'agroforesterie en Hauts-de-France

Dans les Hauts-de-France, les formes d'agroforesterie les plus rencontrées sont les vestiges des anciennes haies qui encadraient autrefois les parcelles agricoles. Il s'agit de haies basses ou hautes formant un paysage bocager. Il existe également de nombreux systèmes pré-vergers associés à la production de pommes, ainsi que des parcours volailles et des ripisylves bordants les cours d'eau. L'agroforesterie intraparcellaire, constituée d'alignements d'arbres au sein des parcelles agricoles, est très peu représentée dans la région. Des enquêtes réalisées en 2013-2014 auprès de 108 agriculteurs des départements du Nord et du Pas-de-Calais ont montré que 50 % d'entre eux ne connaissaient pas l'agroforesterie et seulement 25 % étaient favorables à l'implantation de l'agroforesterie intraparcellaire dans leur ferme (Andrianarisoa and Delbende, 2016). Les principales craintes des agriculteurs concernent la rentabilité du système, le fermage, la compétition entre les arbres et les cultures et le manque de connaissances sur ce type de système. Malgré l'augmentation des études scientifiques en agroforesterie ces dernières années, les Hauts-de-France manquent de références et d'exemples en agroforesterie intraparcellaire. Les agriculteurs sont donc parfois peu accompagnés dans leur démarche de conversion. Les analyses statistiques, réalisées sur les données des enquêtes, ont montré que la familiarisation avec le système est un des leviers permettant le développement de l'agroforesterie dans la région (Andrianarisoa and Delbende, 2016).

La région des Hauts-de-France est un territoire à dominance agricole avec une surface agricole utile totale de 2 127 879 ha, dont 57 % sont des terres arables (Agreste Hauts-de-France, 2022). Les principales orientations technico-économiques des exploitants sont les grandes cultures et la polyculture-élevage. En termes de production agricole, les départements du Nord et du Pas-de-Calais sont les principaux producteurs de pomme de terre, de chicorée et d'endives de France, et représentent une part importante de la production française de betterave sucrière, de blé et de lait. C'est aussi le territoire le moins boisé de France (Dereix et al., 2011) avec seulement 16 % de bois et forêts, le deuxième le plus artificialisé, et parmi le plus affecté par l'érosion (Biotope-GREET Nord Pas-de-Calais, 2008; DREAL, 2013; Le Bissonnais et al., 2002). De plus, presque l'ensemble du territoire est classé en zone vulnérable aux nitrates d'origine agricole dû aux fortes concentrations ($NO_3^- > 40$ mg $L^{-1}$) dans les eaux de surface et les nappes phréatiques (Chambre d'Agriculture Hauts-de-France, 2018). Une production agricole durable dans ce territoire nécessite de trouver des systèmes innovants, permettant de garder le même potentiel de productivité tout en limitant les impacts environnementaux.

La compatibilité de l'agroforesterie intraparcellaire avec l'agriculture moderne pourrait être un moyen de faire face à ces enjeux territoriaux. En effet, en janvier 2015, l'ADEME classait l'agroforesterie parmi les dix pratiques clefs permettant d'accompagner la transition agro-écologique du secteur agricole français. En 2030, le potentiel de développement à l'échelle nationale est de 400 000 ha de parcelles agroforestières et de 65 millions d'hectares à l'échelle européenne (ADEME, 2015). Toutefois, comme déjà mentionné ci-dessus, l'agroforesterie intraparcellaire peine à s'établir dans les systèmes agricoles, principalement dû aux manques de références locales et à la crainte de la compétition aérienne et souterraine entre arbres et cultures pour les ressources en lumière, en eau et en azote. L'étude menée dans cette thèse a été consacrée à l'agroforesterie **intraparcellaire** sur le partage des ressources souterraines entre les espèces associées.

### 1.3. Les bénéfices et contraintes de l'agroforesterie

L'agroforesterie offre plusieurs services écosystémiques tels que l'optimisation de la production agricole associée à un faible impact environnemental (Graves et al., 2007), la réduction de l'érosion des sols (Palma et al., 2007), la préservation ou restauration de la biodiversité (Jose, 2009) et l'atténuation du changement climatique (Lasco et al., 2014). En effet, la production totale de biomasse est plus élevée dans les SAF que la biomasse cumulée des espèces associées si elles étaient cultivées séparément sur une surface équivalente. Il a été démontré que 1 ha cultivé en agroforesterie est équivalent à la production de 1,22-1,45 ha de monoculture (Graves et al., 2007). Cannell et al. (1996) ont expliqué cette différence par la complémentarité de l'utilisation des ressources disponibles au-dessus et dans le sol. L'association des arbres avec une culture augmente l'efficience de l'absorption d'eau et de nutriments ainsi que la capture de la lumière grâce aux différences de leurs caractéristiques physiques ou phénologiques (Jose et al., 2009). Par ailleurs, les lignes d'arbres limitent l'érosion des sols en formant une barrière mécanique qui arrête le ruissellement et crée un brise-vent (Gul and Avciouglu, 2004; Torralba et al., 2016). Les racines des arbres et de la végétation spontanée se développant au sein des lignes retiennent les sols, réduisent le volume des ruissellements et les pertes de sédiments et de nutriments (Anderson et al., 2009; Udawatta et al., 2002). En plus de limiter la perte de nutriments, l'agroforesterie améliore leur disponibilité. En effet, la litière issue de la chute des feuilles d'arbres restitue à la surface du sol une part des éléments capturés par leurs racines et constitue un apport en nutriments dont l'azote pour la culture (Jose et al., 2000b; Partey et al., 2018; Pérez-Flores et al., 2018; Yengwe et al., 2018). Par ailleurs, les sols agroforestiers présentent une biomasse et une activité microbienne supérieure aux sols en monoculture (Beuschel et al., 2019; Clivot et al., 2020; Mungai et al., 2005; Zhang et al., 2018). Les microorganismes jouant un rôle déterminant dans la décomposition de la matière organique et dans les cycles biogéochimiques des nutriments, influencent ainsi les propriétés chimiques et physiques du sol. La richesse microbienne des sols agroforestiers améliore donc leur fertilité et santé biologique (Dollinger and Jose, 2018). L'augmentation de la biodiversité dans les SAF est un des services écosystémiques les plus documentés. Les lignes d'arbres fournissent des niches écologiques diverses et complexes dans les agroécosystèmes et peuvent attirer différentes espèces fauniques et floristiques, réduisant ainsi les problèmes de parasites (Stamps and Linit, 1997) et de ravageurs grâce à la présence d'ennemis naturels ou d'oiseaux insectivores (Maas et al., 2013; Schmidt and Tscharntke, 2005). Ces niches créent également des habitats et des corridors pour la faune, essentiels au maintien de la biodiversité (Udawatta et al., 2019). Enfin, l'agroforesterie contribue à l'adaptation et à l'atténuation du changement climatique par la séquestration du carbone dans les arbres et le sol (Cardinael et al., 2020, 2018a, 2017a; Hübner et al., 2021; Mayer et al., 2022). Les arbres génèrent un microclimat permettant de faire face aux événements extrêmes répétitifs tels que les sécheresses et les tempêtes associées à de fortes pluies et à des vents violents (Jose, 2009). En effet, le flux de vent à proximité des haies est modifié, les températures ont tendance à être légèrement plus élevées et l'évaporation est réduite (Brandle et al., 2004).

Malgré ces services, plusieurs contraintes sont observées dans les SAF. L'introduction d'arbres engendre du travail supplémentaire aux agriculteurs (Andrianarisoa and Delbende, 2016). En effet, afin de limiter l'ombrage et de faciliter le passage des engins agricoles, un entretien des arbres est indispensable notamment avec une taille régulière. Une formation sur l'élagage est parfois nécessaire aux agriculteurs n'ayant pas les notions liées la taille des arbres. L'entretien de la végétation se développant sur la ligne d'arbres est également requis afin d'éviter la propagation d'adventices dans la zone cultivée. L'augmentation de la biodiversité dans les SAF attirent également le gibier endommageant les arbres et cultures. Enfin, la perte de surface entraînant une diminution de la production et à terme la perte de rendement est l'une des principales contraintes. Pardon et al. (2018) ont observé de plus faibles rendements des cultures proche des lignes d'arbres par rapport aux plants au milieu du champ, en particulier pour le maïs et la pomme de terre dans des peuplements adultes. Des résultats similaires ont été constatés par Querné et al. (2017) avec une diminution du rendement de la luzerne à proximité de la ligne d'arbres. Cette diminution de rendement s'explique par une compétition aérienne et souterraine entre les arbres et les cultures. Dufour et al. (2013) ont mesuré une diminution du nombre de grains par épi de blé (35 % au maximum) et du poids des grains (16 % au maximum) dans un système en condition de lumière réduite. Les résultats de Bouttier et al. (2014) confirment l'impact négatif de la compétition pour la lumière sur le rendement du foin en association avec des chênes et des peupliers. Zhang et al. (2015) ont expliqué la diminution du rendement et de la biomasse du blé observé en SAF par rapport à un blé en monoculture par la compétition entre les racines.

## 1.4. La croissance racinaire des arbres

### 1.4.1. Le système racinaire des arbres

Selon la littérature, la croissance racinaire des arbres est principalement contrôlée par la génétique et les besoins physiologiques de chaque espèce (Gilman, 1990a). Cependant, elle peut être modifiée par des facteurs environnementaux physiques et biochimiques (Coutts, 1989, 1987) ou par les modes de conduite des peuplements. Cette interaction avec l'environnement permet aux racines de trouver localement des zones du sol riches en ressources ce qui conduit à des architectures racinaires diverses au sein et entre les espèces (Hirons and Thomas, 2017). Les systèmes racinaires des arbres diffèrent de ceux des plantes annuelles avec une structure de racines grossières ligneuses à longue durée de vie sur laquelle se développent des racines fines à courte durée de vie. Selon les espèces, les racines fines peuvent avoir une croissance lente et limitée avec de nombreuses ramifications ou à l'inverse une extension rapide et pourvue d'une croissance secondaire (Hirons and Thomas, 2017). Les racines les plus fines (< 0,5 mm) ont une longueur racinaire spécifique (LRS) très élevée et jouent un rôle dominant dans l'absorption de l'eau et des nutriments. Parmi 12 essences d'arbres de milieux tempérés, Mccormack et al. (2012) ont constaté que la LRS variait de 9 m $g^{-1}$ chez le *Liriodendron tulipifera* à m 90 $g^{-1}$ chez le *Carya glabra*. Les autres racines fines (entre 0,5 et 2 mm) sont principalement impliquées dans le transport de l'eau et des nutriments, le stockage des glucides et l'ancrage pour les plus épaisses, mais sont aussi capables d'absorber de l'eau (McCormack et al., 2015). La différence de fonction entre les racines induit une espérance de vie plus longue pour les racines grossières dont le diamètre augmente au cours de leur croissance pour améliorer le transport de l'eau, alors que les racines fines ont une dynamique de renouvellement élevée en proliférant rapidement dans les zones riches en nutriments (Hirons and Thomas, 2017; Wells and Eissenstat, 2002).

L'architecture du système racinaire des arbres se distingue selon quatre types fondamentaux (Köstler et al., 1968; Kutschera and Lichtenegger, 2002) :

a) **Systèmes traçants** dominés par des racines latérales poussant à une profondeur relativement constante, rencontrés chez les sapins (*Abies* spp.), épicéas (*Picea* spp.), peupliers (*Populus* spp.), hêtres (*Fagus* spp.) et robiniers faux-acacia (*Robinia pseudoacacia*) (Figure 1a) ;

b) **Systèmes à racines plongeantes** avec des racines latérales plongeantes. Ils se rencontrent chez certains chênes (*Quercus* spp.) et hêtres (*Fuscospora* et *Lophozonia* spp.) (Figure 1b) ;

c) **Systèmes mixtes (fasciculés, en cœur)** présentant un complexe central de racines plongeantes et obliques peu profondes donnant une structure compacte. Ils se rencontrent chez les bouleaux (*Betula* spp.), charmes (*Carpinus* spp.), chênes (*Quercus* spp.) et tilleuls (*Tilia* spp.) (Figure 1c) ;

d) **Systèmes pivotants** caractérisés par une racine pivot persistante et des racines latérales peu profondes, rencontrés chez de nombreux chênes (*Quercus* spp.) et pins (*Pinus* spp.) des climats méditerranéens (Figure 1d).

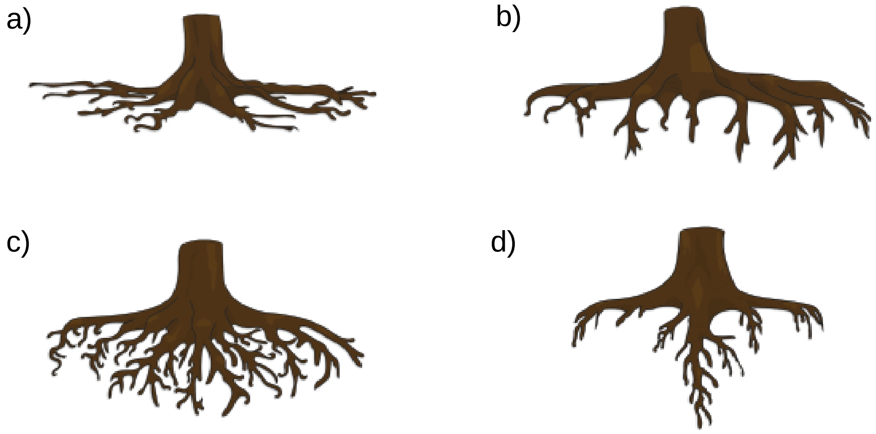

**Figure 1 :** Les quatre principaux types de racines identifiés chez les arbres tempérés : système traçant (a), système à racines plongeantes (b), système mixte (c) et système pivotant (d). Traduit d'après Hirons and Thomas (2017).

Chaque espèce d'arbres est caractérisée par son type de système racinaire, cependant, il existe une diversité morphologique entre les essences. Par exemple, le charme (*Carpinus betulus*, LRS

= 15 m $g^{-1}$, Kubisch et al., 2015) possède un système racinaire mixte avec la majeure partie de ses racines située dans les 30 premiers centimètres et une profondeur d'enracinement maximale d'environ 75 cm (Abdi et al., 2009). Le merisier (*Prunus avium,* LRS = 27 m $g^{-1}$, Weemstra et al., 2020) présente également un système racinaire mixte avec majoritairement des racines superficielles, certaines pouvant atteindre des profondeurs comprises entre 0,25 et 2,8 m. Les racines latérales du merisier sont peu profondes et très bien développées, avec plus de 50 % du système racinaire total poussant à moins de 1 m de la surface. Ces racines latérales s'entrelacent et se ramifient pour former un réseau racinaire plus ou moins parallèle à la surface du sol. Elles rétrécissent progressivement, s'étendant sur une distance relativement longue (Bibalani et al., 2008). Contrairement au charme et au merisier, les racines de l'aulne (*Alnus glutinosa*) présentent un système pivotant avec une croissance verticale, capables de pénétrer profondément dans les sols humides et anaérobies. Elles peuvent atteindre près de 5 m de profondeur (Claessens et al., 2010) et abritent des bactéries actinomycètes filamenteuses, du genre *Frankia*, pénétrant par les blessures des racines. Le saule (*Salix alba*, LRS = 67 m $g^{-1}$, McIvor et al., 2014; Weemstra et al., 2020) présente un profil intermédiaire aux espèces précédentes avec un système mixte profond avec des racines superficielles sur les 30 premiers centimètres constituant un support solide pour son ancrage dans le sol (Plante et al., 2014).

Ces différences morphologiques suggèrent un contrôle génétique important dans la mise en place de l'architecture racinaire (Rogers and Benfey, 2015). Cependant, certaines caractéristiques racinaires sont attribuées à des adaptations environnementales des plantes à leurs milieux et à la colonisation d'écosystèmes terrestres différents (Hutchings and John, 2004). Certaines études suggèrent que la profondeur maximale d'enracinement est essentiellement limitée par la nappe phréatique ou par les caractéristiques du sol empêchant l'enracinement (Bengough et al., 2011; Stone and Kalisz, 1991), alors que d'autres ont démontré que les arbres peuvent développer des racines dans la roche en altération (Forey et al., 2021; Schwinning, 2010) et/ou maintenir des racines actives sous la nappe phréatique (Laio et al., 2009) par libération d'oxygène dans des conditions hydromorphiques (Shimamura et al., 2007). En présence d'une nappe phréatique fluctuante, la saturation en eau du sol peut modifier la répartition spatiale des racines des arbres en raison d'un manque d'oxygène et d'une augmentation des concentrations de dioxyde de carbone et d'éthylène (Armstrong et al., 1994). Lorsque la nappe phréatique monte, les extrémités des racines pivotantes sont submergées ou asphyxiées et meurent. La régénération se produit lorsque la nappe phréatique baisse pendant les périodes plus sèches (Coutts, 1989). Cette succession de morts et de repousses produit des racines appelées « shaving brush » (blaireau) sur de nombreuses espèces d'arbres, caractérisées par le développement d'une quantité importante de racines latérales à la fin de chaque racine verticale (Nicoll and Coutts, 1998). Ainsi certaines plantes peuvent modifier les propriétés du sol dans leur proximité immédiate pour permettre l'enracinement profond. Les paramètres climatiques tels que le vent influencent également le développement des racines des arbres. Les mouvements fréquents des parties aériennes des arbres générés par le vent provoquent un stress mécanique perçu par les racines (Reubens et al., 2009). La croissance racinaire des arbres est alors stimulée afin d'améliorer leur ancrage au sol (Coutts et al., 1999; Stokes et al., 1995; Tamasi et al., 2005). La plasticité racinaire des arbres est aussi observée pour exploiter la distribution inégale des ressources en nutriments dans l'environnement du sol (Hodge, 2006). Selon Schenk and Jackson (2002), les racines se développent autant que possible en surface ou en profondeur en réponse à leur besoin et à la disponibilité en eau. En s'appuyant sur l'étude de 475 profils racinaires, ces auteurs ont montré que sur 90 % des profils, au moins 50 % des racines se trouvaient dans les 30 premiers centimètres du sol. Malgré cette plasticité racinaire, il n'existe actuellement pas de consensus sur la profondeur d'enracinement des arbres.

### 1.4.2. L'association avec la culture modifie la croissance racinaire des arbres en système agroforestier

#### *Profondeur racinaire des arbres en système agroforestier*

Dans les SAF tempérés, les arbres sont généralement plantés à une faible densité (entre 30 et 200 arbres ha$^{-1}$) afin de limiter la compétition directe avec la culture intercalaire pour les ressources naturelles (lumière, eau et nutriments). La compétition avec l'arbre voisin est alors réduite par rapport à un arbre forestier évoluant dans un espace dense (Khan and Chaudhry, 2007). Plusieurs études ont révélé un chevauchement des racines des arbres avec celles des plantes annuelles associées dans les horizons supérieurs des SAF (Schroth, 1995). Une forte proportion des racines fines est accumulée dans les couches supérieures du sol donnant aux arbres un accès de manière intermittente à l'eau et aux nutriments de cet horizon. A l'inverse, les racines de structure se développent en profondeur pour faciliter l'extraction de l'eau et de nutriments dans les couches du sol plus humides (Dhyani et al., 1990). L'accumulation de racines fines dans les couches supérieures du sol peut générer une compétition pour l'eau et les nutriments entre l'arbre et les cultures (Ravenek et al., 2016). Toutefois, les espèces associées peuvent s'adapter à leur environnement et modifier leur croissance racinaire. En effet, des arbres plantés en monoculture présentent des racines réparties en surface, alors que ceux plantés avec des cultures intercalaires ont davantage de racines se développant en profondeur (Andrianarisoa et al., 2016; Schroth, 1995). En SAF, les cultures annuelles présentent des racines peu profondes, tandis que les arbres ont des systèmes racinaires pouvant s'étendre plus profondément dans le sol. Forey et al. (2021) expliquent ce cloisonnement spatial des racines en raison de modèles de croissance racinaire différents entre les deux espèces. En effet, les arbres reprennent une croissance progressive chaque année alors que les cultures annuelles débutent leur croissance racinaire à chaque semis. Par conséquent, la capacité de l'arbre à explorer un volume croissant de sol augmente chaque année, tandis que la culture annuelle est intrinsèquement limitée, en particulier pour atteindre les couches profondes du sol.

La complémentarité de la distribution racinaire des arbres et des cultures peut minimiser la compétition tout en maximisant l'utilisation des ressources dans les SAF. Livesley et al. (2000) montrent une réduction de la longueur des racines fines de maïs en particulier à proximité de la ligne d'arbre, diminuant la compétitivité de la culture pour l'accès aux ressources. Toutefois, il est difficile de connaître de façon certaine la prédominance des phénomènes de compétition ou de facilitation dans une association agroforestière (Cannell et al., 1996). Il existe une grande variabilité des interactions au sein des SAF, notamment en fonction des espèces associées et des conditions pédoclimatiques du site étudié. De plus, l'intensité de la compétition varie dans le temps pour un même site en fonction de la croissance des arbres et des fluctuations annuelles du climat. A titre d'exemple, une association favorable une année pluvieuse peut se révéler défavorable une année sèche (Bargués Tobella et al., 2017). Les principales interactions entre les arbres et les cultures concernent l'eau, les éléments minéraux (prélèvements et restitutions), l'utilisation de la lumière, les maladies, les populations d'adventices et de ravageurs et le contrôle de certains paramètres du sol (érosion, compaction, taux de matière organique, lixiviation de l'azote) (van Noordwijk and Lusiana, 1999).

### *Période de croissance racinaire*

La profondeur racinaire des arbres en SAF peut être influencée par la période d'élongation des racines. Chez certaines essences d'arbres, l'élongation des racines se fait en deux phases. Une première élongation affectant principalement les racines situées dans les horizons supérieurs du sol commence au moment du débourrement au printemps et perdure jusqu'en été. Une seconde phase, dominée par la croissance des racines profondes, a lieu plus tard durant l'automne et l'hiver lorsque les feuilles tombent (Figure 2) (Germon et al., 2016). La période d'élongation racinaire des arbres est un facteur important à prendre en compte dans la compétition entre les arbres et les cultures en agroforesterie. En effet, les labours d'automne détruisent les racines en surface mais pas celles en profondeur qui se développent en hiver et continuent ainsi leur croissance dans le temps. Par ailleurs, selon Germon et al. (2016), les racines en profondeur ont un taux de mortalité beaucoup moins important que les racines présentes à la surface, ce qui permet à l'arbre de chercher de l'eau et des nutriments en dehors des zones d'enracinement de la culture.

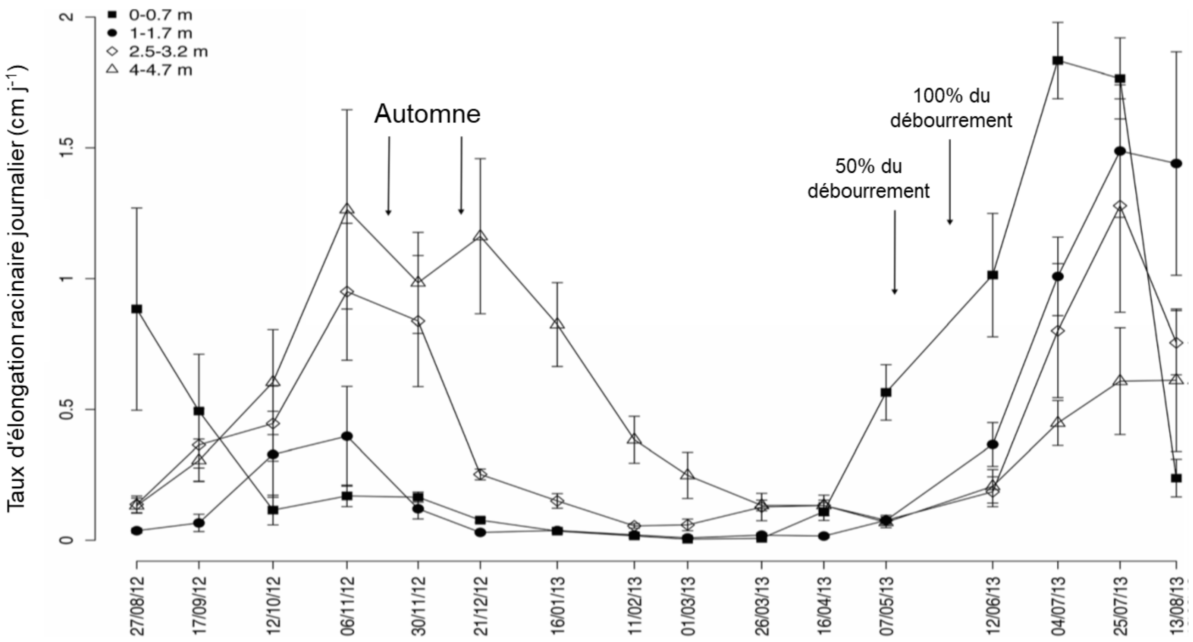

**Figure 2 :** Taux d'élongation racinaire journalier chez le noyer de 20 ans en fonction de la profondeur. Les données sont des moyennes. Les barres d'erreur représentent les écart-types. Traduit d'après Germon et al. (2016).

Il est possible de limiter la compétition entre les arbres et les cultures par différentes pratiques agricoles, notamment dès la conception de la parcelle agroforestière avec un large écartement et une orientation Nord-Sud des lignes (Dupraz and Liagre, 2008), mais aussi dans sa gestion afin d'orienter la stratégie de croissance racinaire des arbres.

### 1.4.3. Facteurs susceptibles d'induire une croissance racinaire des arbres en profondeur

*Essences d'arbres*

Dans un système forestier, la majorité des racines fines et absorbantes se trouvent dans la couche superficielle du sol, riche en humus, afin de récupérer les nutriments issus de la minéralisation de la matière organique (Andrianarisoa et al., 2017). En agroforesterie, la structure des racines et la D95 (profondeur d'enracinement à laquelle 95 % des racines sont situées) varient énormément selon les espèces d'arbres. Les espèces caduques telles que le chêne rouge et noyer noir adultes (25 ans d'âge) présentent un système racinaire pivotant profond avec une profondeur moyenne des racines de structure détectée à 0,29 m, une D95 de 0,95 m, ainsi qu'une faible densité racinaire dans les sols superficiels (Borden et al., 2017). Au contraire, les résineux tels que le thuya et l'épicéa possèdent la majorité de leurs racines dans les horizons supérieurs avec une profondeur moyenne des racines de structure détectée respectivement à 0,17 m et 0,21 m ainsi qu'une D95 de 0,52 m et 0,70 m, respectivement. Enfin le peuplier présente un système racinaire intermédiaire avec une profondeur moyenne des racines de structure détectée à 0,22 m, une D95 de 0,70 m ainsi qu'une concentration relativement élevée de racines près de la surface. En plus de leurs caractéristiques morphologiques, certaines essences d'arbres sont plus aptes à modifier leurs systèmes racinaires en fonction des conditions agropédoclimatiques dans lesquelles elles se développent. Par exemple, Mulia et Dupraz (2006) ont montré que les racines de peuplier de 9 ans restent en surface malgré l'association avec les cultures alors que celles du noyer se développent en profondeur sous la zone d'enracinement des cultures (Figure 3). Le choix des essences d'arbres introduites dans le système agroforestier est donc essentiel pour limiter la compétition racinaire entre les arbres et les cultures.

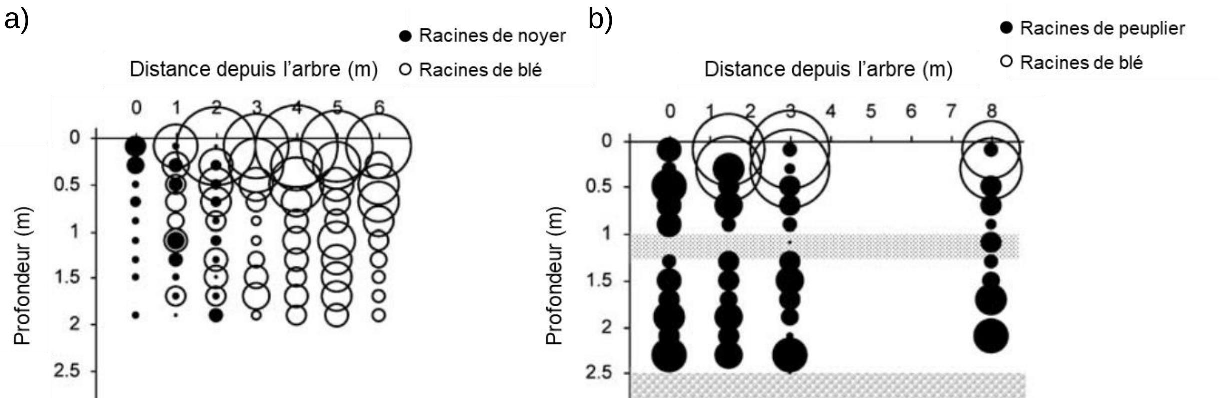

**Figure 3 :** Comparaison de la distribution des racines du blé dur et des arbres dans un peuplement agroforestier de noyers âgés de 7 ans (a) et dans un peuplement agroforestier de peupliers âgés de 9 ans (b). La taille des bulles est proportionnelle aux densités de longueurs racinaires (km m$^{-3}$) observées. Traduit d'après Mulia and Dupraz (2006).

### *Les facteurs pédoclimatiques*

L’influence des paramètres environnementaux sur le schéma de développement racinaire de l’arbre a été évoqué dans le paragraphe 1.4.1. Dans les SAF, malgré les caractéristiques racinaires de chaque essence, l’enracinement dépend aussi du type de sol. Un sol superficiel ne sera pas favorable à un enracinement profond des arbres et les racines risquent de se situer dans le même horizon que la culture. Un sol peu profond favorise donc la compétition entre les espèces associées en SAF. Cependant, Forey et al. (2021) ont observé la croissance de racines de pêchers à travers une discontinuité lithique, composée de cailloux dans une matrice calcaire poudreuse qui durcit en s'asséchant, et ont conclu que les arbres pouvaient accéder à une réserve d'eau seulement deux ans après la plantation, alors que les racines de couvert en association n'ont pas poussé au-delà de 0.5 m. Certains sols présentant ce type de discontinuité lithique peuvent donc convenir à l'agroforesterie en zone sèche, où de l'eau profonde est disponible pour les arbres, réduisant ainsi la compétition avec la culture. La saturation en eau d’un sol limite aussi la croissance racinaire des arbres. Les racines pivotantes gardent une orientation vers le bas malgré une mauvaise aération du sol (Coutts, 1989), mais changent de forme et se brunissent progressivement (Xu et al., 1997) entraînant un arrêt immédiat de la croissance. Ainsi, un sol avec une nappe phréatique peu profonde restreint la croissance racinaire des arbres dans les horizons supérieurs induisant une compétition avec les cultures associées. Enfin, des SAF sont parfois mis en place dans des couloirs venteux afin de protéger les cultures. Les racines étant sensibles au chargement mécanique induit par le vent, des essences résistantes avec un fort ancrage sont à privilégier dans ces situations (Coutts et al., 1999). Par ailleurs, les arbres faisant face à des vents réguliers développent un nombre réduit de petites feuilles (Niklas, 1996), sont plus compactes et présentent une croissance radiale plus importante que les arbres abrités (Gardiner et al., 2016; Mitchell, 2013). Les SAF en zones venteuses semblent donc réduire la compétition avec la culture associée en favorisant un enracinement profond et freinant la croissance aérienne des arbres.

### *Gestion de la parcelle*

Les racines des arbres et leur croissance peuvent être modulées par les pratiques culturales des agriculteurs utilisées au sein de leur parcelle en agroforesterie. En effet, une rotation avec des cultures de printemps peut favoriser un enracinement superficiel des arbres puisqu’au moment du débourrement, les racines des arbres et des cultures sont toutes les deux compétitives dans la recherche d’eau et de nutriments dans la zone d’enracinement des cultures. Au contraire, en présence de cultures d'hiver sur la parcelle au moment du débourrement, les racines d'arbres sont face à un appauvrissement du sol en eau et en nutriments dû à la présence des racines de culture et sont contraintes à orienter leur croissance en profondeur où les ressources sont encore disponibles (Cardinael et al., 2015b; Dupraz and Liagre, 2008).

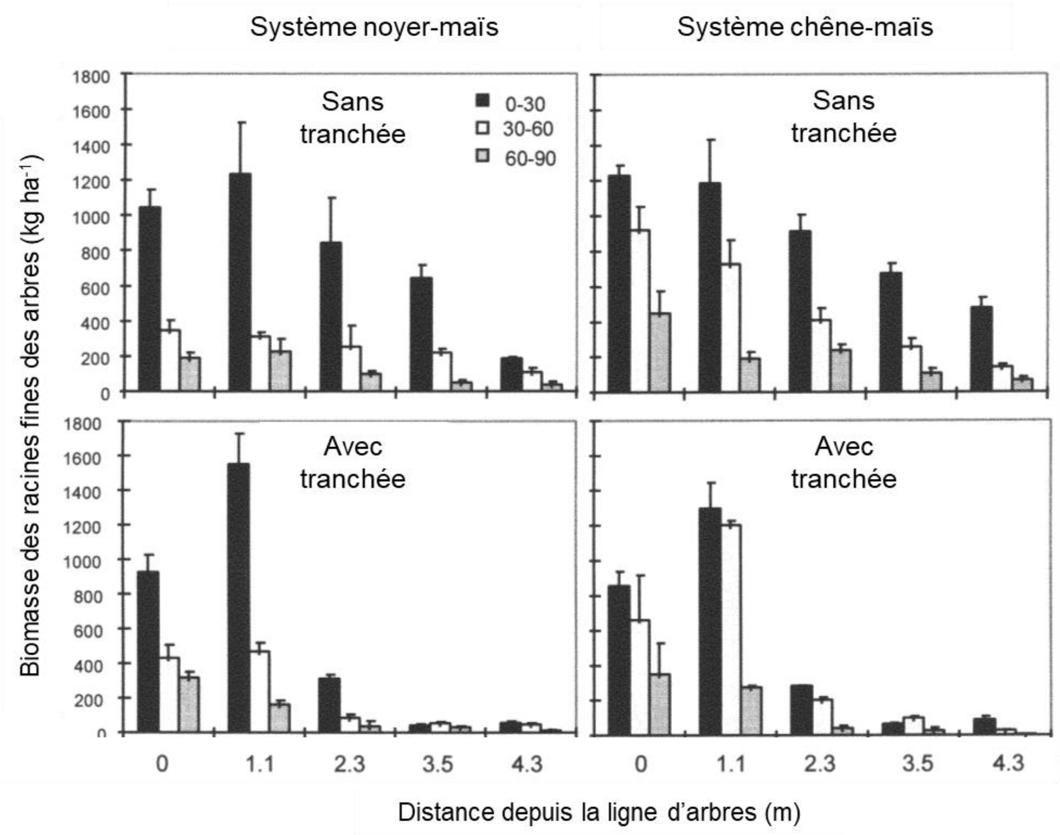

**Figure 4 :** Variation de la biomasse des racines fines de l'arbre (kg ha$^{-1}$) en fonction de la distance par rapport à l'arbre dans des peuplements agroforestiers de noyers noirs et de chênes rouges âgés de 15 ans avec ou sans creusement de tranchées. Les histogrammes sont des moyennes et les barres d'erreur représentent les écart-types. Traduit d'après Jose et al. (2000a).

L'itinéraire technique de la parcelle est également susceptible d'induire une croissance racinaire des arbres en profondeur. En effet, la fertilisation, le travail du sol, l'irrigation, l'utilisation de produits phytosanitaires mais aussi le cernage racinaire sont impliqués dans la stratégie de croissance des racines des arbres (Gilman, 1990b). Plusieurs études ont montré que la présence de cernage ou d'une tranchée (Jose et al., 2004, 2000b) proches des lignes d'arbres favorisent l'enracinement des racines d'arbres sous les racines des cultures (Figure 4). Le labour peut aussi être utilisé pour tailler les racines des arbres dans la couche arable avant le semis des cultures, évitant leur développement dans la couche superficielle et protégeant ainsi les cultures pendant un certain temps de la compétition racinaire avec les arbres (Korwar and Radder, 1994; Schroth, 1995). De même, l'utilisation répétée d'un déchaumeur à disques favorise l'enracinement des racines d'arbres (Jose et al., 2004). A l'inverse, l'irrigation et la fertilisation favorisent une croissance racinaire superficielle (Coleman, 2007). La présence d'eau et de minéraux disponibles rendent la couche arable plus attractive et favorisent la formation de systèmes racinaires peu profonds (Bakker et al., 2008; Gilman, 1990b).

La répartition spatiale du système racinaire des arbres et des cultures en agroforesterie est un facteur clé pour réussir la conduite des parcelles afin de limiter les compétitions entre les cultures associées mais également d'optimiser la valorisation des ressources naturelles disponibles dans l'air (lumière) et dans le sol (eau et nutriments).

### 1.5. Partage de l'eau

La compétition pour l'eau est apparue comme le facteur principal limitant le succès de l'agroforesterie intraparcellaire dans les régions tropicales semi-arides (Ong et al., 1991). Everson et al. (2009) ont observé que les arbres utilisaient davantage d'eau en profondeur (75-125 cm) lorsque l'espace interligne était étroit par rapport à un SAF avec un large espace entre les lignes. Une forte densité des arbres augmente donc la compétition pour l'eau en profondeur avec les cultures. Par ailleurs, Jose et al. (2000a) ont montré à travers leurs expériences que la compétition pour l'eau plutôt que pour la lumière semble être essentielle pour définir la productivité et la durabilité des systèmes de culture agroforestiers. Dans leur étude l'utilisation d'une barrière polyéthylène installée à 1,2 m de la ligne d'arbres sur 1,2 m de profondeur a empêché la compétition en eau entre le maïs et le noyer noir. L'absorption d'eau a été plus élevée pour le maïs cultivé dans le système avec la barrière par rapport au maïs cultivé sans barrière (Figure 5). Par ailleurs, la compétition pour l'eau entre les arbres et la culture est plus importante en période de faible pluviométrie, où une réduction de la quantité d'eau absorbée par la culture en place a été observée, principalement à la première ligne de maïs (Jose et al., 2000a). Livesley et al. (2004) ont également observé une augmentation de la teneur en eau du sol avec la distance depuis la ligne d'arbres suggérant une absorption préférentielle de l'eau sous la ligne et de la réduction de l'apport de pluie par l'interception de la canopée. Cette compétition pour l'eau est particulièrement intense lorsque les réserves en eau du sol s'épuisent (Miller and Pallardy, 2001). Ainsi, en période de fortes pluies, les arbres ne sont pas entrés en concurrence avec les cultures dû aux teneurs en eau du sol élevée (Everson et al., 2009).

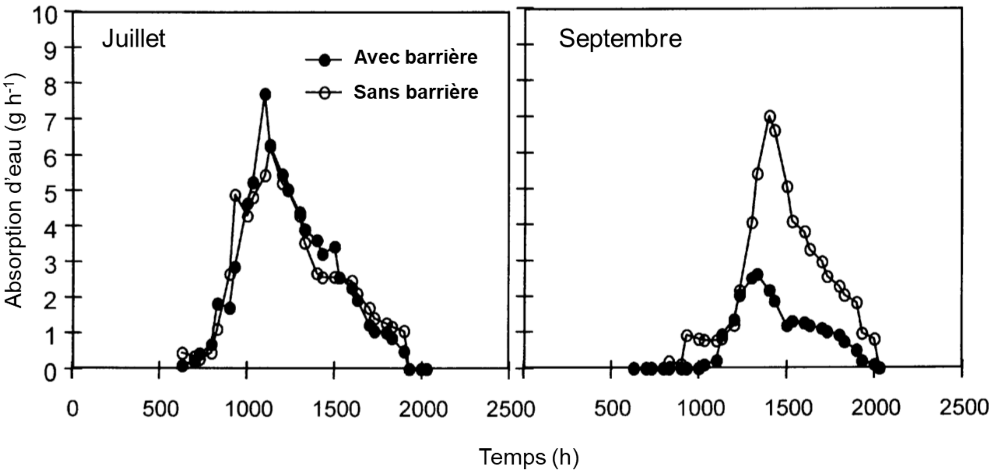

**Figure 5 :** Vitesse d'absorption d'eau par le noyer noir en système agroforestier en juillet (forte pluviométrie) et septembre (faible pluviométrie) dans les traitements avec ou sans barrière. Les barrières en polyéthylène ont été installées dans le sol à 1,2 m de distance de l'arbre et à 1,2 m de profondeur. Traduit d'après Jose et al. (2000a).

Dans certains SAF, une différenciation verticale de niche écologique entre la culture et les arbres en place pour l'utilisation d'eau est observée (Bargués Tobella et al., 2017). Les cultures ayant un enracinement peu profond utilisent l'eau des horizons supérieurs alors que les arbres exploitent les sources d'eau plus profondes. Cependant, en fonction de la saison (humide ou sèche), l'interaction entre les espèces associées peut être sous forme de compétition ou de complémentarité. En effet, les arbres adoptent généralement une stratégie opportuniste en prélevant la majeure partie de leur eau dans la partie supérieure du sol pendant la saison humide et en utilisant l'eau stockée plus profondément dans le sol, par exemple dans les eaux souterraines, pendant la saison sèche (Bargués Tobella et al., 2017). Enfin, une redistribution de l'eau le long du profil du sol par les arbres a également été mis en évidence. Grâce aux racines profondes, certains arbres sont capables de remonter l'eau et de la redistribuer aux horizons supérieurs par un processus connu appelé « ascenseur hydraulique » (Bayala et Pierto, 2020). Ce processus pourrait retarder le développement du stress hydrique et atténuer l'impact de l'assèchement du sol dans les horizons proches de la surface (Warren et al., 2007, 2005). Les cultures en association avec ces arbres bénéficient de cet apport d'eau et augmentent leur production de biomasse (Bogie et al., 2018; Izumi et al., 2018). Ce processus d'ascenseur hydraulique a été décrit pour de nombreuses espèces ligneuses utilisées dans les SAF en milieu semi-aride (Bayala et al., 2008) ou dans les forêts tropicales (Oliveira et al., 2005). Toutefois, il se retrouve généralement chez les espèces avec des systèmes racinaires dimorphiques (racines à la fois latérales et pivotantes, Lambers et al., 2008) ayant accès à l'eau dans les couches superficielles et profondes du sol (Scholz et al., 2008). Enfin, les effets nets de la redistribution de l’eau par les arbres sur leurs plantes voisines ne sont pas toujours clairs et restent à approfondir (Prieto et al., 2012) surtout pendant les phases de mise en place des arbres dans les parcelles agricoles. La disponibilité de l’eau étant indirectement liée à celle des nutriments, la compétition pour l’eau peut également engendrer une compétition pour les nutriments solubles dont l’azote.

### 1.6. Partage de l’azote

La coexistence des arbres et des cultures dans un SAF engendre une compétition inhérente pour l’azote. Les travaux de Jose et al. (2000b) ont montré que la présence d’une barrière polyéthylène, entre la ligne d’arbres et la culture sur 1,2 m de profondeur, empêche la compétition du maïs avec le noyer noir. En effet, un meilleur rendement et une meilleure qualité du grain ont été observés dans un système avec barrière par rapport à un SAF sans barrière (Tableau 1). Jose et al. (2000b) ont également observé que la compétition pour l'azote entre les racines des arbres et celles du maïs diminue l’efficacité de l'utilisation des engrais par la culture. En effet, 47,2 % de l’engrais utilisé (UFN) se retrouve dans les organes récoltés et principalement dans le grain cultivé avec une barrière alors que 38,9 % sont retrouvés lorsqu’il n’y a pas de séparation. En raison de leur croissance réduite, les plants de maïs cultivés sans barrière n'ont pas été en mesure d'utiliser efficacement l'engrais disponible. Au contraire, dans le système avec barrière, le maïs s’est mieux développé et ses racines ont pu explorer davantage la surface du sol, conduisant à une plus grande efficacité dans l'utilisation des engrais. Des résultats similaires ont été reportés par Zamora et al. (2009) en utilisant la même méthode pour observer la compétition des ressources en azote entre des pins de 5 ans et la culture de coton. Cependant, d’après les auteurs, la compétition pour l'azote provenant des engrais est minime puisque l'acquisition de nutriments n'est pas simultanée entre l’arbre et la culture.

La compétition pour l’azote entre les espèces en SAF est réduite lorsque l’absorption est séparée dans le temps ou dans l’espace. En effet, la complémentarité des systèmes racinaires des arbres et des cultures évoquée dans le paragraphe 1.4.2 contribue à une meilleure utilisation de l’azote du sol. Par ailleurs, l’enracinement profond des arbres sous la zone de culture constitue l'hypothèse du filet de sécurité, c’est-à-dire l'interception des nutriments mobiles qui sont lixiviés des zones racinaires des cultures (Rowe et al., 1999; Suprayogo et al., 2002). En effet, l'azote non absorbé par la culture peut ensuite être récupéré par les racines des arbres limitant ainsi la lixiviation. Bergeron et al. (2011) ont observé une réduction de la lixiviation de l'azote dans un SAF de peupliers ayant subi un cernage de 1 m de profondeur à 0,75 m de chaque côté de la ligne d'arbres en comparaison aux parcelles témoins sans cernage. Allen et al. (2004) ont également mis en évidence le rôle important des racines des arbres dans l'atténuation de la lixiviation des nitrates dans les eaux souterraines. Malgré la compétition que représente l'introduction d'arbres dans un systèmes agricole, la décomposition des feuilles et des racines fines des arbres peuvent améliorer le recyclage des nutriments du sol (Chifflot et al., 2006; Jose et al., 2000b). La matière organique produite par le système agroforestier joue un rôle essentiel dans le cycle des nutriments et de l'azote en particulier. Malgré les apports supplémentaires d'azote et de phosphore par les arbres, les quantités ne sont pas suffisantes pour maintenir la productivité des SAF, un apport d'engrais dans ces systèmes de cultures reste nécessaire (Jose et al., 2000b).

## 1.7. Stockage de carbone

L'introduction des arbres dans les parcelles agricoles offre un potentiel de stockage du carbone dans leurs parties aériennes, souterraines et dans le sol. En effet, la séquestration du carbone organique dans le sol (COS) est contrôlé par de nombreux paramètres dont les caractéristiques du sol, le type de culture, la gestion de la parcelle, l'âge, l'essence des arbres et la latitude (Cardinael et al., 2018b; Chatterjee et al., 2018). Cardinael et al. (2017) ont mesuré une quantité de COS plus élevée dans des SAF âgés entre 6 et 41 ans par rapport aux parcelles agricoles témoins. Dans cette étude, les jeunes plantations présentent des valeurs de COS plus élevé le long des lignes d'arbres et à 30 cm de profondeur, probablement en raison de la présence de végétation herbacée spontanée. La majorité du COS supplémentaire est donc stockée dans l'horizon supérieur du sol (0-30 cm) (Balesdent et al., 2018; Cardinael et al., 2018a, 2015a; Pardon et al., 2017; Peichl et al., 2006; Upson and Burgess, 2013) (Figure 6). Cardinael et al. (2017) ont observé sur deux SAF de 18 ans une quantité plus élevée de COS sous 30 cm de profondeur expliquée par une forte densité de racines fines d'arbres en profondeur. Dans des peuplements âgés entre 20 et 120 ans, Viaud and Kunnemann (2021) ont également montré que les concentrations et les stocks de COS étaient significativement plus élevés jusqu'à 1 m de la ligne d'arbres et jusqu'à 90 cm de profondeur. Selon Cardinael et al. (2018a), l'augmentation des apports de biomasse fraîche dans le sol (racines, litière foliaire) a favorisé le stockage supplémentaire de carbone organique dans la parcelle agroforestière. Néanmoins, dans certain

SAF, une diminution de la matière organique du sol au-delà de 60 cm de profondeur a parfois été constatée, en comparaison avec une parcelle témoin (Upson and Burgess, 2013). Les auteurs ont expliqué ces résultats par l'assèchement du sol entraînant une oxydation et le “priming effect”. En effet, un apport de matière organique fraîche dans l'écosystème rhizosphérique stimule la minéralisation microbienne de la matière organique stable en profondeur et modifie ainsi la dynamique du carbone et de l'azote du sol (Fontaine et al., 2007). Actuellement, il n'existe pas de consensus de la communauté scientifique sur la capacité des SAF à séquestrer le COS en profondeur. Toutefois, tous les auteurs s'accordent sur le fait que plusieurs années sont nécessaires pour détecter un changement dans les stocks de carbone organique (Clivot et al., 2020; Oelbermann et al., 2006; Peichl et al., 2006).

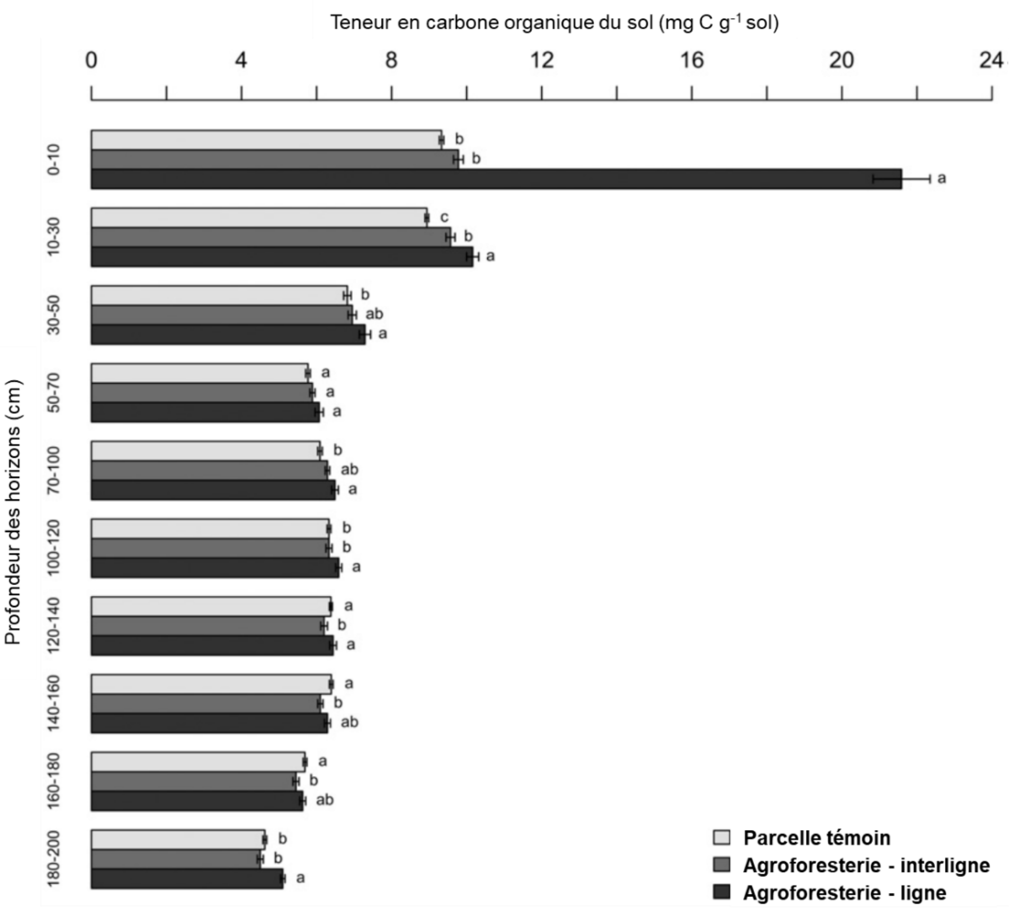

**Figure 6 :** Variation de la teneur en carbone organique le long du profil de sol jusqu'à 2 m de profondeur, dans la parcelle témoin et dans la parcelle agroforestière. Les histogrammes sont des moyennes et les barres sont les erreurs types (n = 40 pour la ligne d'arbres, n = 60 pour l'interligne et n = 93 pour le contrôle). Les moyennes avec les mêmes lettres ne sont pas significativement différentes pour une profondeur donnée ($p < 0.05$). Traduit d'après Cardinael et al. (2015a).

## 1.8. Effet des arbres sur l'activité microbienne dans les parcelles agroforestières intraparcellaires

L'introduction d'arbres dans un système agricole améliore la santé biologique du sol (Dollinger and Jose, 2018) en modifiant la taille, la diversité et l'activité microbienne du sol grâce aux apports de litière fraîche (feuilles et racines), à la création de microclimat, à la richesse floristique de la végétation spontanée poussant sur la ligne d'arbres et à l'exsudation racinaire par les arbres (Udawatta et al., 2019). Plusieurs études montrent que les SAF augmentent la biomasse microbienne par rapport à une parcelle agricole en monoculture (Beuschel et al., 2019; Udawatta et al., 2008; Zak et al., 2003), même dès les 4 années de plantation (Clivot et al., 2020). Certain taxons bactériens sont également favorisés en SAF (Banerjee et al., 2016) avec une diminution de l'abondance de certaines bactéries nitrifiantes dont celles qui oxydent l'ammonium (Ammonia Oxydizing Bacteria) et l'augmentation de bactéries dénitrifiantes (Beule et al., 2020, 2019). La présence des arbres impacte aussi la communauté fongique en favorisant leur diversité (Beule and Karlovsky, 2021; Chifflot et al., 2009; Zak et al., 2003; Zhang et al., 2018) et en augmentant le ratio $\frac{Champignons}{Bactéries}$ (Beuschel et al., 2019). Par exemple, Battie-Laclau et al. (2020) ont montré que la forte densité de racines de la végétation herbacée sous les arbres était favorable à l'installation de champignons mycorhiziens à arbuscules. Les SAF augmentent également l'activité enzymatique du sol (Beuschel et al., 2019; Udawatta et al., 2008), dont la ß-glucosidase (Mungai et al., 2005). Par ailleurs, les exsudats racinaires sécrétés par les racines des arbres augmentent les activités microbiennes de la rhizosphère (Colin-Belgrand et al., 2003; Yin et al., 2014) et favorisent la minéralisation de l'azote (Meier et al., 2017; Sun et al., 2021). Ainsi, les exsudats racinaires interviennent directement dans le cycle de l'azote en stimulant les processus de décomposition de la matière organique, mais également en influençant la composition du microbiote du sol. Les travaux de Broeckling et al. (2008) et Haichar et al. (2008) montrent que les plantes sont capables de réguler la population microbienne de leur environnement via leurs exsudats racinaires. Par exemple, l'exsudation d'acide salicylique favorisent les bactéries des familles Corynebacterineae, Pseudonocardineae et Streptomycineae alors que l'acide gamma-aminobutyrique (GABA) est positivement corrélé aux bactéries de la familles des Frankineae et Micromonosporineae (Badri et al., 2013). A travers ses exsudats, l'aulne inhibe la prolifération des microorganismes nitrifiants par rapport au peuplier (Gonzalez et al., 1995).

L'influence des arbres sur les communautés microbiennes dépend toutefois du site, des essences étudiées (Chander et al., 1998; Kooch et al., 2020; Myers et al., 2001) et de l'âge des arbres (Clivot et al., 2020; Mungai et al., 2005). Tedersoo et al. (2009) ont montré que certains champignons étaient spécifiques à l'aulne, lui conférant la capacité à attirer son propre cortège microbien.

### 1.9. Partage des ressources et évolution des communautés microbiennes chez les jeunes peuplements agroforestiers

Parmi les études menées au sein de jeunes peuplements agroforestiers, des différences ont été observées sur la distribution racinaires des arbres. Dans un SAF âgé de 10 ans, Swieter et al. (2022) ont observé des racines de peuplier jusqu'à 3 m de la ligne d'arbres alors que dans l'étude de Mulia et Dupraz (2006) sur un peuplement de 9 ans, les racines de peuplier atteignaient 10 m au-delà de la ligne d'arbres. Ces mêmes auteurs ont montré l'adaptabilité du noyer, dans un SAF de 7 ans, avec l'absence de racines d'arbres au-delà de 2 m de la ligne. De manière contrastée, les racines de noyers de 11 ans ont colonisé la zone de culture jusque 3,75 m de la ligne mais étaient restreintes à 1 m de la ligne dans un SAF de 5 ans (Battie-Laclau et al., 2020). Ces différences peuvent être dues à la variabilité des paramètres du sol, de la méthode d'échantillonnage et des itinéraires techniques (Swieter et al., 2022). Les résultats obtenus sur le partage en eau et azote du sol chez de jeunes peuplements agroforestiers dépendent également en partie des sites et des essences étudiés. Selon Huo et al. (2020), des arbres de 7 ans cultivés en SAF ont absorbé plus d'eau dans les horizons profonds que les arbres en monoculture en période sèche. Cette adaptation a permis aux arbres de faire face à la compétition avec la culture. Des résultats similaires ont été reportés par Forey et al. (2021) sur des pêchers de 2 ans en association avec un couvert composé de poacées et de légumineuses. Les auteurs ont montré que les arbres ont pu avoir accès à de l'eau située à 2 m de profondeur grâce à la croissance de leurs racines à travers une discontinuité lithique, alors que celles du couvert ne se sont pas développées au-delà de 50 cm de profondeur. Toutefois dans ces deux études, l'impact des arbres sur l'absorption d'eau de la culture n'a pas été évalué. Une compétition pour l'azote a été mise en évidence dans des SAF de 4 et 5 ans avec une diminution de l'efficacité d'utilisation des engrais des espèces associées (Zamora et al., 2009; Zhang et al., 2019). Bergeron et al. (2011) et Zamora et al. (2009) ont également montré que l'azote non absorbé par la culture a été intercepté par les arbres dans les horizons profonds. L'ensemble des travaux réalisés sur le partage des ressources dans les jeunes peuplements agroforestiers exposent les interactions entres les arbres et les cultures, mais peu de données précisent le caractère compétitif des arbres. Enfin, plusieurs études ont montré la rapidité des changements des communautés microbiennes dans les SAF. Une augmentation de la diversité fongique au niveau de la ligne d'arbres a été observée dès un an et demi après la plantation (Beule and Karlovsky, 2021). L'introduction d'arbres dans les parcelles agricoles favorise également le développement des réseaux mycéliens fongiques (Chifflot et al., 2009) et augmente la biomasse microbienne (Beuschel et al., 2019) dans des SAF âgés entre 5 et 8 ans. Des différences en fonction du type d'association ont été mises en évidence par Clivot et al. (2020), où au cours des 4 années suivant la plantation, la biomasse microbienne a été impactée dans le système peuplier-luzerne alors qu'aucune modification n'a été relevée dans le système aulne-poacées. Ces études confirment le rôle des arbres sur l'abondance et la diversité des populations microbiennes en système agricole, cependant, la variabilité des facteurs influençant cette communauté nécessite d'approfondir les recherches avec des données supplémentaires dans le temps et dans l'espace au sein d'un même SAF.

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## 2. Problématique et objectifs de thèse

Le développement de l'agroforesterie intraparcellaire représente un enjeu majeur pour l'agriculture. Ce système permet d'optimiser la production agricole tout en limitant l'impact sur l'environnement. L'importance et le rôle de l'arbre dans le paysage agricole interrogent les agriculteurs et des références se multiplient à l'échelle mondiale. Grâce à la plasticité des arbres, une complémentarité spatiale se dessine entre leurs racines et celles des cultures avec une différenciation dans la profondeur d'enracinement (Andrianarisoa et al., 2016; Schroth, 1995). Cette croissance racinaire des arbres peut être favorisée par plusieurs facteurs dès la conception de la parcelle et selon l'itinéraire technique. Malgré ces adaptations, une diminution du rendement des cultures proches de la ligne d'arbres est parfois observée (Artru et al., 2017; Pardon et al., 2018; Querné et al., 2017). Les communautés microbiennes sont diversifiées et le cycle de l'azote impacté (Beule and Karlovsky, 2021; Mungai et al., 2005). La production de biomasse des arbres et l'augmentation de matière organique dans les sols augmentent le stockage de carbone de l'écosystème (Cardinael et al., 2017a; Pardon et al., 2017; Peichl et al., 2006). La plupart de ces résultats sont constatés dans des peuplements matures, mais la caractérisation du partage des ressources et de la modification de l'activité microbienne du sol pendant les phases d'installation des arbres dans les parcelles agricoles reste assez peu documentée. L'initialisation de la croissance racinaire des arbres en profondeur demeure mal connue, alors même qu'il s'agit d'une phase déterminante pour une bonne conduite des parcelles agroforestières. Les questions posées dans cette thèse étaient :

- A quelle échéance les arbres commencent-ils à changer de stratégie et à développer des systèmes racinaires sous la zone d'enracinement des cultures ? Quels sont les facteurs qui contrôlent ce changement de stratégies ? Et quelles sont les conséquences sur le partage des ressources en eau et en azote entre les plantes associées ainsi que sur la répartition spatiale du carbone dans le sol ?

- Comment se met en place la compétition/complémentarité entre les cultures et les arbres pendant les phases d'établissement de ces derniers dans les parcelles agricoles ?

- Quelles sont les conséquences de la croissance racinaire des jeunes arbres sur l'activité microbienne du sol ?

**L'objectif de cette thèse était de caractériser la distribution spatiale et temporelle des racines fines, d'explorer le partage de l'eau et de l'azote et d'examiner l'activité microbienne globale pendant la phase d'installation des arbres les terres cultivées en système agroforestier intraparcellaire. Il a s'agi de suivre (i) l'évolution de la répartition spatiale des racines selon les espèces d'arbres, (ii) le profil d'absorption d'eau par les espèces associées, (iii) le devenir des engrais azotés apportés sur la culture, et (iv) la modification de l'activité microbienne du sol.**

Nos principales hypothèses de travail étaient les suivantes :

- La distribution racinaire des arbres en agroforesterie intraparcellaire est modifiée au cours des premières années après la plantation. Ce changement de stratégie est favorisé par des facteurs liés au travail du sol et au choix des cultures associées, et modifie la teneur en eau et en azote minéral le long du profil du sol. La teneur en carbone du sol augmente au moins dans les lignes d'arbres après 10 ans de plantations.

- La compétition pour l'eau et l'azote entre les plantes associées commence tôt, notamment dans les 30 premiers centimètres de profondeur et dans la zone de cohabitation des racines fines des cultures et des arbres.

- Les racines des arbres modifient l'activité microbienne du sol dès les premières années de plantation grâce aux activités racinaires de stimulation ou d'inhibition.

La thèse est divisée cinq chapitres écrits sous forme d'articles scientifiques. Le premier porte sur la caractérisation de la distribution racinaire le long d'une chronoséquence de jeunes peuplements agroforestiers et ses conséquences sur les ressources du sol. Le deuxième porte sur le partage de l'eau entre la culture et les arbres pendant la phase d'installation de ces derniers dans les parcelles agricoles. Le troisième décrit la distribution des racines fines des espèces associées avec l'utilisation de la méthode « core-break ». Le quatrième porte sur le partage de l'azote entre la culture et les arbres pendant la phase d'installation de ces derniers dans les parcelles agricoles. Enfin, le cinquième chapitre est consacré à l'étude de l'effet des activités racinaires des jeunes arbres sur la décomposition de la matière organique en surface ou en profondeur.

# 3. Méthodologie générale

## 3.1. Caractérisation de la distribution racinaire le long d'une chronoséquence de jeunes peuplements agroforestiers et ses conséquences sur les ressources du sol

Une chronoséquence de jeunes peuplements agroforestiers âgée de 3 à 12 ans a été établie sur dix-sept parcelles en agroforesterie intraparcellaire chez 11 agriculteurs en grandes cultures dans les Hauts-de-France. Une enquête a été réalisée auprès de ces agriculteurs afin de répertorier les informations relatives aux itinéraires techniques et à la conduite des parcelles choisies. Dans chaque parcelle, un arbre référent a été choisi : charme, merisier ou érable. A partir de cet arbre, une série de sept carottes de sol ont été prélevées à 0, 1, 3 et 10 m de part et d'autre de la ligne d'arbres, et jusqu'à 2 m de profondeur. Chaque carotte de sol a été divisée en tronçon de 20 cm, et le nombre de racines d'arbres par m² de sol a été compté selon la méthode de « core-break » (van Noordwijk et al., 2001) en cassant le tronçon en deux parties environ égales et en faisant la moyenne du nombre de racines fines observées sur chaque surface. Pour chaque carotte, trois échantillons de sol ont été prélevés à 0-30 cm, 30-100 cm et 100-200 cm. La teneur en eau, en carbone organique, en azote organique, en azote minéral, en CaCO3, le pH et la granulométrie ont été déterminés pour chaque échantillon de sol. L'abondance de racines fines des arbres a ensuite été corrélé avec les paramètres des sols et l'itinéraire technique de la parcelle.

## 3.2. Caractérisation du partage de l'eau et de l'azote entre les arbres et la culture pendant la phase d'installation des parcelles agroforestières

Ces deux chapitres s'appuient sur les expérimentations réalisées sur le site expérimental de recherche et de démonstration en agroforesterie à Ramecourt, mis en place en novembre 2018 sur une des parcelles de la SCEA Dequidt (Andrianarisoa et al., 2019). Le site a une superficie de 18 ha avec un dispositif en bloc aléatoire en trois répétitions. Les modalités comparées sont : trois types d'associations agroforestières (AF1, AF2 et AF3), un témoin forestier (TF) et un témoin agricole (TA) avec trois répétitions. Sur chaque ligne en AC et TF, les arbres sont espacés de 1 m, les essences de hautes tiges (charme, chêne, noyer, merisier, aulne, robinier) ont été plantées par paire et ont été intercalées tous les 7 m avec des essences de bourrage (châtaigner, cornouiller, érable, fusain, noisetier, tilleul, troène, saule, viorne). Les lignes d’arbres ont une largeur de 2 m. Dans les associations agroforestières, 36 m séparent les lignes d’arbres, et les interlignes sont cultivées de la même manière que les témoins agricoles. Dans les témoins forestiers, les lignes d’arbres sont espacées de 6 m, et les interlignes ne sont ni cultivées ni travaillées. Un mélange de couvert de poacées (fétuque, raygrass italien et fléole) et de plantes mellifères (sarrasin, bourrache, aneth, soucis, mauve, coquelicot, bleuet, radis fourrager, moutarde et phacélie) a été semé le 27 mars 2019. En 2020, le ray-grass (RG) a pris le dessus sur l’ensemble des plantes semées. Il est fauché mécaniquement tous les ans après les périodes de floraison. Sur les parcelles AC et TA, depuis le printemps 2019, la succession de cultures a été : orge de printemps2019/endives2020/blé tendre2021/lin2022/blé tendre2023. Un couvert a été semé lorsque la période entre chaque culture dépassait 60 jours.

Cette étude a été réalisée sur la culture de blé d’hiver semée en octobre 2020 et récoltée en août 2021. Elle a été fertilisée avec 220 kg N ha$^{-1}$ d’azote sous forme de granulés d’ammonitrate 27 fractionnés en quatre apports. Pour suivre le partage de l’azote entre les cultures et les arbres, un apport d’engrais enrichi en $^{15}$N ($^{15}NO_3$,$^{15}NH_4$ ; 10 % atomes) a été appliqué sur une zone de 4 m² à 1,5 m de distance des lignes d’arbres, en juin 2021 lors du 4$^{ème}$ apport d’engrais à raison de 30 kg N ha$^{-1}$. Des échantillons de feuilles d’arbres et de blé ont été prélevés tous les 15 jours suivant l’apport et l’enrichissement en $^{15}$N a été analysé par GC-IRMS. Pour caractériser l’absorption de l’eau par les arbres et la culture, un marquage avec une eau enrichie au deutérium ($^2H_2O$ ; 10 % atomes) a été réalisée en mai 2021 à 30, 50 et 100 cm de profondeur. Le devenir de cet isotope de l’hydrogène a été suivi dans les arbres et la culture avec un échantillonnage et des analyses tous les 15 jours, de la même manière que pour le suivi de l’azote. Parmi les essences d’arbres présentes sur le site expérimental, l’aulne, le charme, le merisier et le saule ont été choisis en fonction de leur vitesse de croissance et leur aptitude à fixer l’azote atmosphérique ou non. A 1,5 m de chaque arbre choisi, des carottes de sol de 120 cm de profondeur ont été prélevées à trois périodes de l’année (printemps, été, automne). L’humidité du sol et la teneur en azote minéral du sol ont été mesurées. La distribution racinaire a été évaluée selon la quantité de racines présentes dans les carottes de sol extraites en automne 2021.

### 3.3. Suivi de la décomposition de la matière organique sous des jeunes peuplements agroforestiers

Parallèlement au suivi du partage des ressources hydriques et azotées décrit dans les chapitres 3 et 4, la décomposition de la matière organique a été évaluée selon la méthode de « tea bag » sur le site expérimental de Ramecourt en mai 2021. Des sachets de thé vert et de rooibos ont été enterrés à 30, 50 et 100 cm et à 1,5 m de la ligne d'un arbre référent. Les sachets de thé ont été enterrés dans une carotte de sol de 15 cm de long et 60 mm de diamètre. Les carottes ont été enveloppées dans un filet en aluminium de 2 mm de maille et ont été déterrés à l'automne 2021 après une période d'incubation dans le sol entre 140 et 170 jours. La perte de masse du thé, l'ADN total du sol et la quantité de racines dans chaque sachet de thé ont été mesurés. Les détails de la méthodologie employée sont présentés dans le chapitre 5 ci-après.

# Chapitre 2

## Trees in temperate alley-cropping systems develop deep fine roots 5 years after plantation: What are the consequences on soil resources?

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### Abstract

Trees in alley-cropping systems (AC) were reported to develop deeper fine roots compared to forest trees and that they can modify soil water (SWC), mineral nitrogen (SMN) and organic matter (SOM) content. However, intercropping young trees has not been studied extensively. This study aimed to count tree fine roots abundance (TFRA) along a chronosequence of AC stands, to determine factors explaining its variability and to highlight its effects on soil resources. Seventeen alley-cropping plots ranging from 3 to 12 years old were chosen on farms in northern France. TFRA was measured by the core break method using soil samples collected at 0, 1, 3 and 10 m from a referent tree (a maple, a hybrid walnut or a hornbeam) down to 2 m depth. Before four years old, tree fine roots colonized the topsoil (0–30 cm) in rows and then mainly grew vertically from 4 to 6 years old, before laterally exploring deep soil layer (1–2 m) beyond this age. Stepwise analyses showed that stand age, tillage frequency and crop rotation duration explained 60 % of the variability of the sum of TFRA calculated for all soil layers at all distances from the tree row. The SWC was negatively correlated to TFRA suggesting that as trees get older, they dried the deep soil layer below the crop rooting zone and increased the soil depth able to store autumn and winter rainfall. No significant effect of either stand age or distance from tree rows was observed for SMN. It varied significantly with soil depth ($R^2 = 0.3^{***}$) and was strongly correlated with soil nitrate content ($R^2 = 0.97^{***}$). The soil ammonium content was significantly correlated with TFRA, suggesting that tree fine roots favor ammonium production or accumulation in soil, which may potentially allow for a reduction in the mineral nitrogen (N) mobility for leaching. Finally, we found a significantly high SOM correlated with TFRA only in topsoil on the tree rows at our oldest stands. No change of SOM was observed in the deep soil layer regardless of stand age. From this study, we concluded that fine root plasticity of intercropped trees occurred at early stage and may contribute with age to a better use of soil water, to managing the soil mineral N dynamic and to sequestrating carbon, at least in tree rows.

**Keywords**

Alley-cropping systems, tree fine roots abundance, soil water content, soil organic matter, soil mineral nitrogen, crop management, core-break, chronosequence

## 1. Introduction

Alley-cropping systems (AC), defined as the deliberate association of crops and rows of woody perennial plants within the same plot, are expanding in temperate regions and are recognized among sustainable agricultural practices as an alternative to intensive agriculture (Jose, 2009). They offer several ecosystem benefits, such as the optimization of agricultural production (Graves et al., 2007) with low environmental impacts, soil erosion reduction (Gul and Avciouglu, 2004, Palma et al., 2007, Udawatta et al., 2002), biodiversity preservation or restoration (Jose, 2009, Torralba et al., 2016) and climate change adaptation and mitigation (Cardinael et al., 2020, Cardinael et al., 2017, Hübner et al., 2021, Lasco et al., 2014, Mayer et al., 2022). Despite these numerous positive benefits, farmers are not enthusiastic about adopting AC due to the cohabitation between trees and crops which modifies the growth, development and yield of associated plants. In fact, depending on the design and management of the AC plot, a competition or a facilitation for resources (light, water and nutrients) use may occur between crops and trees (Cardinael et al., 2020, Isaac and Borden, 2019). Competition for nitrogen (N) was shown to decrease crop growth, biomass and grain N content (Jose et al., 2000b, Livesley et al., 2002) and was often associated with interspecific root overlap (Isaac and Borden, 2019). However, several studies also evidenced facilitation for nutrient acquisition in AC through enhanced chemical and microbial meditated processes (Isaac and Borden, 2019). For instance, Jose et al. (2000b) showed an improvement in crop efficiency through using N from fertilizers in AC. Zamora et al. (2009) attributed the potential of AC for nitrogen recycling efficiency to the ability of tree roots to intercept and uptake fertilizers from deeper soil layers as “safety-net” role (Rowe et al., 1999) and partially return it to soil surface via litterfall. In the same way, even though the competition for soil water between trees and crops was often observed in AC (Jose et al., 2000a, Miller and Pallardy, 2001, Bayala and Prieto, 2020), some authors showed that thanks to differences in the spatial root distributions along the soil profile (Andrianarisoa et al., 2016, Borden et al., 2020, Cardinael et al., 2015b, Isaac et al., 2014, Kumar and Jose, 2018, Mulia and Dupraz, 2006), the AC allow a better use of water (Fernández et al., 2008, Livesley et al., 2004) and/or promote water redistribution along the soil profile through hydraulic lift and shared mycorrhizal networks (Bayala and Prieto, 2020).

According to literature, tree root growth is mainly controlled by the genetics and physiological needs of each species (Gilman, 1990a, Pagès and Ariès, 1988), but it can be modified by external environmental factors (Coutts, 1987, Hutchings and John, 2004). In low input forest stands, tree fine roots are mainly found in the upper soil layer, composed of humus and organo-mineral elements, above 20 cm, in order to recover nutrients from the mineralization of soil organic matter (SOM) (Andrianarisoa et al., 2017, Andrianarisoa et al., 2016, Cardinael et al., 2015b, Mulia and Dupraz, 2006). It decreases more or less rapidly with soil depth according to tree species and becomes rare below 1 m. In AC, the fine root biomass of some trees species was shown to be uniformly distributed along the soil profile compared to forest trees thanks to their root plasticity (Andrianarisoa et al., 2016). For instance, Borden et al. (2017) showed that compared to coniferous species, *Juglans nigra* and *Quercus rubra* developed deeper root systems in AC. Mulia and Dupraz (2006) showed that roots of 10-year-old poplar remain on the surface despite the association with crops, whereas walnut develops deep roots below the crop rooting zone. Schroth (1995) and Cardinael et al. (2015b) observed a high amount of tree fine roots biomass below 2 m depth of intercropped trees.

In addition to specific morphological characteristics, the presence of crops in alleys may contribute to changing the tree fine root distribution along the soil profile (Cardinael et al., 2015b) and during different seasons (Huo et al., 2020) in AC. In shallow soils, as it is impossible for trees to go deeper, they inevitably develop root systems located in the same soil layer as the crop. In the presence of a fluctuating water table, the soil water saturation may change the spatial tree roots distribution due to a lack of oxygen and an increase in carbon dioxide and ethylene concentrations (Armstrong et al., 1994). Climatic parameters such as wind also influence tree root development. In response to frequent movements of the aerial parts by the wind, the tree root growth is stimulated to gain a better anchorage to the ground (Coutts et al., 1999, Stokes et al., 1995, Tamasi et al., 2005). Finally, tree fine roots distribution can be modulated by active management practices of crops and trees. Successive rotation of winter crops in alleys during the first years of trees establishment would reduce the colonization of upper soil layer by tree roots. At tree bud burst, as the topsoil is already explored by crops, tree roots are less competitive in this layer for water and nutrient uptake (Dupraz and Liagre, 2008). A deep rooting is then established by trees, in addition to those already developed during winter (Cardinael et al., 2015a). Other studies have shown that tree root pruning close to the rows promotes the tree rooting under the crop roots zone (Gilman and Yeager, 1988). Ploughing before crops sowing may limit tree root development in the upper soil layer and thus protect crops for a while from root competition with trees (Korwar and Radder, 1994, Schroth, 1995). Besides, autumn ploughing destroys shallow roots but not deep roots, which have developed during the winter and continue to grow over time. Similarly, periodical agronomic disking minimizes competition and maximizes niche separation (Jose et al., 2004). Conversely, irrigation and fertilization promote superficial root growth because they make the topsoil more attractive and stimulate the formation of tree shallow root systems (Bakker et al., 2008, Coleman, 2007, Gilman, 1990b). Finally, tree pruning was also shown to increase the depth at which trees in AC acquire nutrients (Rowe et al., 2006).

Lateral and vertical tree roots development and turnover in AC may provide an additional source of organic matter and may contribute to carbon sequestration in soil. Cardinael et al. (2017) showed higher soil organic carbon (SOC) contents in AC with ages varying from 6 to 41 years after tree planting by comparison with control crop plot. In their young stands, high SOC was observed along tree rows at 30 cm depth, possibly due to the presence of understory vegetation strips. They also observed a high amount of SOC below 30 cm depth in two 18-year-old silvoarable plots and explained it by a high density of tree fine roots measured at this depth. In most cases, the additional SOC observed in AC plot was measured at 0–30 cm depth (Cardinael et al., 2018, Cardinael et al., 2015a, Pardon et al., 2017, Peichl et al., 2006, Upson and Burgess, 2013).

Although the plasticity of the tree root system has been demonstrated in adult AC stands, the initialization of this deep root development remains poorly investigated. Studies reporting the evolution of lateral and vertical tree roots development during the first years of tree plantation in cropland and the induced changes on soil parameters are scarce, because often, no significant effects were observed (Bambrick et al., 2010, Pardon et al., 2017). Clivot et al. (2020) analyzed changes in soil parameters in the first 15 cm depth after 4 years of tree planting and concluded weak changes in soil organic matter. This was also confirmed by Chatterjee et al. (2018) for AC stands aged between 0 and 5 years. Nyberg and Högberg (1995) in western Kenya showed a significant change in soil carbon content 5 years after tree plantation. Wang et al. (2005) evidenced higher microbial activities in 0–10 cm depth after 5 years of Chinese fir. Bergeron et al. (2011) showed a decrease of nitrate and ammonium in soil solution in 5–8-year-old poplar AC with plantation at 70 cm depth, thanks to tree root uptake and to ammonification stimulation.

This study aimed to count tree fine roots abundance (TFRA) along a chronosequence of AC stands to determine factors explaining its variability and to highlight its consequences on soil water, organic matter and mineral nitrogen content. We assumed (i) that changes in tree roots distribution in AC occur early, during the first years after plantation; (ii) that this change of strategy is mainly driven by factors linked to soil and crop management and modifying the soil water and mineral nitrogen content along the soil profile; and (iii) that soil carbon content should change at least in tree rows after some years.

## 2. Materials and methods

### 2.1. Study territory description

The study was carried out in Hauts-de-France region in northern France (Appendix 1A). The climate is oceanic with an average (between 2010 and 2020) annual temperature and rainfall of

11.5 °C and 726 mm respectively at Lille-Lesquin station (https://www.infoclimat.fr/climatologie/globale/lille-lesquin/07015.html) and a maximum elevation of 295 m. The soil type throughout the region is dominated by cambisol, punctuated with luvisol and calcaric calcisol in some areas (Appendix 1A; Food and Agriculture Organization, 2015). The soil texture is mainly silt loam but zones with sand or clay loam with flint and limestone are also noted. The region is strongly dominated by agriculture with 2 131 503 ha of usable agricultural area, i.e. 67 % of the territory, including 57 % of arable land, and 26 093 farms (Agreste Hauts-de-France, 2020). Afforestation represents only 16 % of the territory. At the national scale, the region is the leading producer of wheat, sugar beet, potatoes, peas, carrots and witloof chicory. In this territory, agroforestry systems are mostly silvopastoral with apple orchards for poultry, cows or sheep, riparian wood land, shelterbelts, hedgerows or alley-cropping (Nair, 1985). Alley-cropping systems are rarely developed due to farmers' fears that trees within plot will be incompatible with the agro-pedoclimatic conditions (Andrianarisoa and Delbende, 2016).

### 2.2. Description of studied plots

Lists from the regional council and from the chamber of agriculture were used to identify and contact agroforestry farmers in the Hauts-de-France region. The selected farmers had alley-cropping plots between 3 and 12 years old mostly with deep (> 2 m depth) loamy soil (Appendix 1B). Twenty-six farmers were selected, corresponding to these criteria, and were contacted to participate in the study. Only 11 farmers with a total of 17 plots (Table 1 and Appendix 1A) positively responded. These plots were divided into 4 age groups to create a chronosequence of AC stands: < 4 years old (n = 4), [4–6] years old (n = 5), [7–9] years old (n = 4) and [10–12] years old (n = 4). The average of tree density, alley width and distance between trees within rows for all plots were 104 ± 57 trees ha$^{-1}$, 47 ± 41 m and 3.6 ± 2.2 m respectively (Table 1). For the whole farm, the type of farming was partly or exclusively arable land (Kempen et al., 2011) and the cultivated crops were mainly wheat, barley, rapeseed, corn, beet, potato. In all plots, the mean tillage frequency was 0.4 ± 0.3 y$^{-1}$ (i.e. twice every 5 years), the mean crop rotation duration was 4 years and the fertilization was mainly carried out with synthetic fertilizers (Table 1). The soil texture of selected plots was mainly silt loam in layer 1 (0–30 cm) and layer 2 (30–100 cm) and clay loam or silt clay loam in layer 3 (> 100 cm) (Appendix 1B). The average soil pH and soil organic matter was 7.8 ± 0.4 and 21.6 ± 3.01 g kg$^{-1}$, respectively

**Table 1:** Description of agroforestry sites (nd means not determined)

| [1]Site | Location | Age / planting date | [2]Tree row/alley width | Density | Tillage frequency | [3]Rotation | Rotation duration | [4]Percent winter crop |
|---|---|---|---|---|---|---|---|---|
| **Unit** | | **years /** | **m** | **trees ha$^{-1}$** | **$y^{-1}$** | | | **%** |
| BA | 49°51'38.3"N, 2°36'52.7"E | 8/2012 | 3/35 | 95 | 1/4 | W, meadow, W | 4 | 100 |
| BA2 | 49°51'38.4"N, 2°37'00.5"E | 8/2012 | 3/35 | 95 | 1/2 | lent./rye, lent./camelina, B, buck. | 4 | 50 |
| BE | 50°00'06.9"N, 3°19'47.7"E | 3/2017 | 6/50 | 33 | 0/1 | W, rap., W, beet | 4 | 75 |
| DO | 50°23'21.5"N, 3°04'17.1"E | 6/2014 | 1/200 | 50 | 2/5 | W, beet, C | 3 | 33 |
| FO | 50°15'22.2"N, 2°34'13.6"E | 3/2017 | 4/28 | 89 | 1/1 | F, endive, C | 3 | 33 |
| FO2 | 50°15'22.6"N, 2°34'15.1"E | 3/2017 | 4/28 | 89 | 1/1 | F, endive, C | 3 | 33 |
| GU | 50°51'31.3"N, 1°50'41.8"E | 6/2014 | 1/51 | 196 | 0/1 | W, W, B, flax | 4 | 50 |
| GU2 | 50°51'37.3"N, 1°51'10.3"E | 8/2012 | 1/51 | 196 | 0/1 | W, flax, W/B | 3 | 67 |
| GU3 | 50°51'04.6"N, 1°51'50.6"E | 7/2013 | 1/51 | 196 | 0/1 | W, B, P, W | 4 | 75 |
| LA | 49°57'08.8"N, 2°28'08.2"E | 11/2009 | 5/28 | 71 | 2/5 | W, P, W, B, rap. | 5 | 80 |
| LA2 | 49°57'12.8"N, 2°28'07.7"E | 11/2009 | 5/28 | 71 | 2/5 | W, P, W, B, rap. | 5 | 80 |
| LE | 49°55'03.8"N, 3°17'08.1"E | 3/2017 | 6/50 | 33 | 0/1 | W, beet, W, B | 4 | 75 |
| NE | 49°34'31.7"N, 2°01'19.8"E | 6/2014 | 2/30 | 167 | 3/8 | einkorn, oat, C, F, einkorn, spelt/lent. | 8 | 71 |
| RU | 50°00'59.6"N, 2°22'38.4"E | 4/2016 | 6/12 | 139 | 1/1 | W, B, legumes, P, W | 5 | 60 |
| SM | 49°36'40.5"N, 1°56'00.9"E | 12/2008 | 8/48 | 26 | 1/3 | rap., W, B | 3 | 100 |
| TH | 49°31'35.7"N, 2°19'46.8"E | 6/2014 | 2/45 | 111 | 3/7 | C, F, W, lent./rye, oat, carrot, B | 7 | 29 |
| VE | 49°40'04.5"N, 2°48'54.5"E | 11/2009 | 3/30 | 111 | 1/2 | W, beet, W, rap. | 4 | 75 |

| [1]Site | [5]Reference tree | [6]Crop management | [7]Temp. | [7]Precip. 2020 | Crop 2020 | Crop yield 2020 | [8]Fertilization | [9]Type of farming |
|---|---|---|---|---|---|---|---|---|
| **Unit** | | | **C°** | **mm yr$^{-1}$** | | **t ha$^{-1}$** | | |
| BA | Maple | Org., RT | 11.7 | 590 | temp. meadow | nd | none | mixed |
| BA2 | Hybrid walnut | Org., RT | 11.7 | 590 | buck. | 2 | none | mixed |
| BE | Hornbeam | Sus., RT | 12.3 | 749 | beet | nd | S+M | arable |
| DO | Hornbeam | Conv., tillage | 12.1 | 562 | beet/W | nd | S+M | arable & horticulture |
| FO | Maple | Org., RT | 11.6 | 908 | C | 2.5 | M | mixed |
| FO2 | Hybrid walnut | Org., RT | 11.6 | 908 | C | 2.5 | M | mixed |
| GU | Maple | No-tillage | 12.1 | 801 | flax | nd | S | arable |
| GU2 | Hybrid walnut | No-tillage | 12.1 | 801 | mix W/B | 8.5 | S | arable |
| GU3 | Maple | No-tillage | 12.1 | 801 | W | 8.5 | S | arable |
| LA | Hornbeam | Conv., tillage | 12.1 | 691 | W | 8.9 | S | arable |
| LA2 | Hybrid walnut | Conv., tillage | 12.1 | 691 | W | 8.9 | S | arable |
| LE | Maple | Sus., RT | 12.3 | 749 | B | 11.5 | S+M | arable |
| NE | Hybrid walnut | Org., RT | 11.5 | 622 | spelt/lent. | 2.5 | M | arable |
| RU | Maple | Org., RT | 12.1 | 839 | W | 2.5 | S+M | arable |
| SM | Maple | Conv., RT | 11.5 | 919 | W | 5 | S | arable |
| TH | Hornbeam | Org., tillage | 12.3 | 800 | B | 2.5 | none | arable & horticulture |
| VE | Maple | Sus., tillage | 12.1 | 653 | rap. | 2.5 | S | arable |

| [1]Site | Clay | | | Silt | | | Sand | | | SOM | | | pH | | | Limestone | | | Depth |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Unit | g kg$^{-1}$ | | | g kg$^{-1}$ | | | g kg$^{-1}$ | | | g kg$^{-1}$ | | | | | | g kg$^{-1}$ | | | cm |
| Layer | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 | |
| BA | 94 | 94 | 96 | 801 | 811 | 777 | 102 | 94 | 89 | 24.3 | 6.8 | 4.6 | 7.9 | 8.2 | 8.6 | 3 | 1 | 46 | >200 |
| BA2 | 89 | 146 | 103 | 795 | 771 | 808 | 115 | 83 | 88 | 22.8 | 6.7 | 3.8 | 7.8 | 8.0 | 8.3 | 2 | 1 | 1 | >200 |
| BE | 98 | 100 | 95 | 781 | 781 | 771 | 106 | 117 | 133 | 20.7 | 6.6 | 5.3 | 8.5 | 8.5 | 8.5 | 15 | 3 | 2 | >200 |
| DO | 91 | 118 | 105 | 766 | 751 | 680 | 141 | 130 | 156 | 28.8 | 5.7 | 3.7 | 7.7 | 8.0 | 8.7 | 2 | <1 | 59 | >200 |
| FO | 121 | 112 | 110 | 737 | 750 | 791 | 138 | 138 | 99 | 16.7 | 5.3 | 3.1 | 8.0 | 7.9 | 7.7 | 6 | <1 | <1 | >200 |
| FO2 | 111 | 124 | 150 | 743 | 747 | 744 | 139 | 129 | 105 | 20.3 | 5.2 | 3.1 | 8.1 | 7.7 | 7.5 | 7 | 1 | 1 | 60 |
| GU | 279 | 503 | nd | 581 | 393 | nd | 137 | 98 | nd | 24.0 | 14.4 | nd | 8.0 | 8.2 | nd | 3 | 7 | nd | 140 |
| GU2 | 117 | 169 | 347 | 697 | 673 | 344 | 185 | 158 | 305 | 19.9 | 7.3 | 5.7 | 6.9 | 7.6 | 8.0 | 1 | 2 | 5 | 120 |
| GU3 | 110 | 171 | nd | 710 | 648 | nd | 180 | 180 | nd | 23.8 | 10.1 | nd | 7.5 | 7.8 | nd | 1 | 1 | nd | 90 |
| LA | 152 | 267 | 329 | 457 | 297 | 182 | 388 | 436 | 393 | 22.4 | 6.8 | 4.5 | 7.9 | 8.0 | 8.0 | 4 | 1 | 2 | >200 |
| LA2 | 86 | 112 | 338 | 703 | 705 | 420 | 208 | 182 | 242 | 22.2 | 8.1 | 5.6 | 8.0 | 8.2 | 8.2 | 3 | 1 | 1 | >200 |
| LE | 109 | 139 | 110 | 804 | 738 | 207 | 72 | 57 | 13 | 17.8 | 9.2 | 5.1 | 8.5 | 8.6 | 8.8 | 15 | 66 | 511 | >200 |
| NE | 123 | 171 | 279 | 752 | 747 | 572 | 123 | 82 | 147 | 22.9 | 5.9 | 4.7 | 7.6 | 7.8 | 8.0 | 3 | 1 | 3 | >200 |
| RU | 118 | 143 | 362 | 773 | 759 | 502 | 107 | 97 | 136 | 16.9 | 8.3 | 4.8 | 7.4 | 7.4 | 7.5 | 2 | <1 | 1 | 150 |
| SM | 149 | 250 | 314 | 719 | 568 | 504 | 132 | 182 | 182 | 20.2 | 7.1 | 5.1 | 7.0 | 7.6 | 7.4 | <1 | <1 | <1 | 120 |
| TH | 153 | 203 | 251 | 772 | 731 | 632 | 72 | 64 | 118 | 22.9 | 5.9 | 4.1 | 8.0 | 8.1 | 8.3 | 4 | 5 | 1 | >200 |
| VE | 101 | 127 | 146 | 778 | 757 | 669 | 120 | 117 | 111 | 20.4 | 6.8 | 4.3 | 8.0 | 7.7 | 8.6 | 2 | <1 | 74 | >200 |

[1] Sites meaning: BA, Bayonvillers; BA2, Bayonvillers2; BE, Beaurevoir; DO, Douai; FO, Fosseux; FO2, Fosseux2; GU, Guînes; GU2, Guînes2; GU3, Guînes3; LA, Lahoussoye; LA2, Lahoussoye2; LE, Lehaucourt; NE, La Neuville-sur-Oudeuil; RU, Rubempré; SM, Saint-Maur; TH, Thieux; VE, Verpillières

[2] Tree row/alley width indicates the distance between trees intra and inter rows respectively. For instance, 3/35 means: the distance between two trees within each row is equal to 3 m and the distance between two tree rows is equal to 35 m

[3] Crop species meaning: B: barley, beet: sugar beet, buck.: buckwheat, C: corn, einkorn: einkorn wheat, F: faba bean, lent.: lentils, P: potato, rap.: rapeseed, temp. meadow: temporary meadow, W: wheat

[4] Percentage of winter crop in rotation: for instance W, rap., W, beet = 3 winter crops on the rotation mean ¾=75%

[5] tree species studied on the site from which the distance of soil cores position was calculated

[6] Crop management meaning: Conv.: conventional, Org.: organic, RT: reduced tillage, Sus.: sustainable

[7] Temp. and Precip. mean: temperature and precipitation

[8] Fertilization meaning: M: manure, S: synthetic fertilizer

[9] According to EU classification (Kempen et al., 2011)

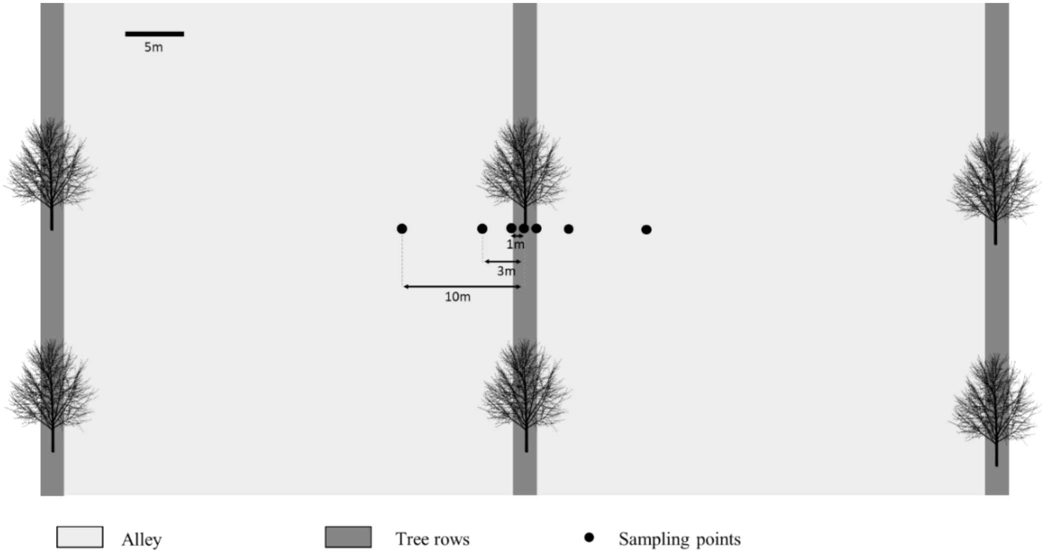


**Figure 1:** Description of soil cores sampling within each site.

in layer 1. Tree rows were composed of local tall standard mixed species within which spontaneous or sowed herbaceous vegetation (hereafter referred to as “understory vegetation strips”) grew on 1 m width on average on both sides. Weeds in alley were controlled chemically on the cropping area in plots from conventional farms and were scarce in plots from organic and sustainable farms. For all farmers, the understory vegetation strips were mowed, but the frequency of the cut varied according to weeds development. All information about the chosen alley-cropping plot (trees and crops): plot design, crop rotation, tillage, tree pruning, kind of N fertilization and irrigation were collected (Table 1).

### 2.3. Fine roots measurement

The tree and crop fine root abundance was measured according to “core-break” method (van Noordwijk et al., 2001). A referent tree was chosen within a given row selected in the middle of the plot. The referent tree was either a maple (*Acer pseudoplatanus*), a hybrid walnut (*Juglans regia x negra*) or a hornbeam (*Carpinus betulus*). Soil coring was carried out from July to November 2020 at 0, 1, 3 and 10 m distance from the referent tree on both sides perpendicular to the row (Fig. 1) totalizing 7 soil cores per site except at Guînes, Guînes2, Guînes3, Bayonvillers2 and Thieux (Table 1) due to the presence of flint. Soil cores were collected with portable electric core drill consisting of gouge connected to an electrical percussion hammer (BOSCH GSH 27 VC, Apageo). Two kinds of gouge with different dimensions were used: gouge 1 (60 cm length and 85 mm diameter) for soil cores from 0 to 120 cm depth and gouge 2 (85 cm length and 63 mm diameter) for soil cores from 120 to 200 cm depth. Due to the stand’s young age, soil cores were collected only down to 200 cm depth on the assumption that the number of tree fine roots were negligible beyond. When conditions did not allow 200 cm depth to be reached, for instance in the case of shallow calcaric soil developed on chalky bedrock at the Guînes site, the limit of coring corresponds to the soil depth.

Each 2 m collected soil core was divided into 20 cm long sub-cores. Each sub-core was broken by hand, close to the middle, and the number of living fine roots (diameter < 2 mm) visible on both horizontal surfaces was counted. Crop roots recognition was perfected thanks to soil core collected at 10 m from the tree row, whereas those for trees were carried out from soil core taken at the bottom of the trunk. In comparison with crops, tree roots were more lignified, hairy and often brownish. Despite our recognition experience, tree roots counting might be slightly over-estimated in rows due to the presence of weeds. A single person carried out root counting for all samples to avoid bias from the counter. The mean number of crop or tree fine roots counted on both sides of sub-cores was expressed on a square meter basis. It was called tree fine root abundance or TFRA ($m^{-2}$) for trees. Given the number of soil cores collected per stand age around the referent tree, we assumed that the error of TFRA extrapolation from soil core surface into square meter is reduced.

Because no soil cores were collected at 2 m distance from tree rows, the TFRA at this distance was estimated for each sub-core of 20 cm depth at a given site, assuming that it linearly decreased from tree row to 3 m distance.

### 2.4. Soil sampling and analyses

From each soil cores collected in section §2.3, soil samples were up taken according to the following layers: 0–30 cm (L1 or topsoil), 30–100 cm (L2) and 100–200 cm (L3 or deep soil layer). The collected soil samples for L1 were a mix between the first sub-core (0–20 cm) and the first half of the second sub-core (20–30 cm). For L2, they were the second half of the second sub-core and the 3 following sub-cores and for L3, all the remaining sub-cores. Fresh soil samples were sieved at 4 mm and stored at 4 °C. An aliquot of soil sample was directly frozen at −20 °C for further soil mineral nitrogen content (SMN) analyzes. Soil water content (SWC) was determined by oven drying an aliquot of sieved soil at 105 °C for 72 h. Soil physicochemical parameters were measured at the "Laboratoire Départemental d′Analyses et de Recherche" in Laon city using standard methods: soil particle size distribution (modified NF X 31–107), total $CaCO_3$ (NF EN ISO 10693), total Kjeldahl nitrogen (hereafter referred to as: soil organic nitrogen or SON), organic carbon content (NF ISO 14 235) (SOC), soil C/N ratio and pH. The soil organic matter content (SOM) was estimated by multiplying SOC by the Van Bemmelen coefficient of 1.724 (Rosell et al., 2001), assuming that the organic matter contains 58 % organic C. The nitrate ($NO_3$-N) and ammonium ($NH_4$-N) content of soil were determined by shaking 6 g of thawed soil with 30 mL of 0.5 M of $K_2SO_4$ solution for 1 h and then filtering. The $NO_3$-N and $NH_4$-N concentrations of extracts were measured using continuous flow colorimetry (SAN++, Skalar Analytical B.V., Breda, Neitherlands). The soil nitrate (SNN) and ammonium (SAN) content were expressed as mg N per kg of dry soil. The SMN was the sum of SNN and SAN and the percentage of nitrate in SMN (%$NO_3$) was the ratio between SNN and SMN multiplied by 100.

### 2.5. Statistical analyses

First, the variation of TFRA was analyzed using a simple linear model with stand age group, distance from tree row, soil layer and tree species as explanatory variable. Then bivariate linear models were run according to equation:

$$y = ax_1 + bx_2 + c + \beta \quad (1)$$

where "y" is TFRA, $x_1$ is a fixed factor such as the site, $x_2$ is either distance from tree row or soil layer, "a", "b" and "c" are coefficients and β the model error. For all regression models, the determination coefficient ($R^2$) and the *p*-value were estimated. Using the same model in Eq. 1, an ANOVA followed by a multiple comparison test was carried out using multcomp package of R software (Hothorn et al., 2008) to compare the mean value of TFRA for each $x_2$ variable with a post hoc Tukey's test ($p < 0.05$). For instance, the mean of TFRA was compared between soil layers for a given stand age group and a given distance from tree row and vice-versa. Soil layers were ordered from L1 to L3 within models with the function ordered of R to consider the possible interdependence between values. The ANOVA was validated after checking the normality of the model residual by using Shapiro test. Data at 10 m from tree rows were excluded to ANOVA analysis because no tree fine roots were found at this distance. Finally, a stepwise regression was carried out to select the best model explaining the variability of TFRA using stand age, distance, depth, clay, pH, limestone, silt, tillage frequency, tree density, crop rotation duration and the percentage of winter crop in the rotation as explanatory variables.

To analyze the variation of TFRA with variables collected at plot levels, a variable named sum of tree fine roots abundance (sTFRA) was calculated for each site in each side of the tree row with the following equations:

Equation 2:

For $l \in [1, 3]$ and for $d \in [0, 3] \not\subset 2$: $\text{sTFRA}_{l,d} = \Sigma_{s=1}^{n} TFRA_s$ (2)

Where *l* is the soil layer, *d* the distance from tree rows, s is the sub-core and n the number of sub-core within each layer

Equation 3

For $l \in [1, 3]$: $\text{sTFRA}_l = \Sigma_{d=1}^{3} \Sigma_{s=1}^{n} TFRA_{d,s}$ (3)

Equation 4:

For $d \in [0, 3] \not\subset 2$: $\text{sTFRA}_d = \Sigma_{l=1}^{3} \Sigma_{s=1}^{n} TFRA_{l,s}$ (4)

Equation 5:

$$\mathrm{sTFRA}_t = \Sigma_{d=0}^{3}\Sigma_{l=1}^{3}\Sigma_{s=1}^{n} TFRA_{d,l,s} \qquad (5)$$

In the results section, $\mathrm{sTFRA}_{l,d}$, $\mathrm{sTFRA}_{l}$, $\mathrm{sTFRA}_{d}$ and $\mathrm{sTFRA}_{t}$ were all called sTFRA but the concerned soil layer or distance is always specified. For sTFRA calculation, data at 10 m distance from rows were also excluded because no tree fine roots were observed. Simple regression analyses were performed between sTFRA and crop management or soil variables. The Spearman's rank correlation rho or the adjusted R-squared were calculated for quantitative or qualitative variable respectively. Stepwise regression models were also tested to select the variables allowing a better explanation of the variability of each sTFRA. The selected models are those with the first three significant explanatory variables but not those with the best AIC to limit the number of explanatory variables. The *p*-value of each model and the sign of coefficient retained for each explanatory variable are presented in Table 3.

For statistical analyses of SWC and SMN, only data collected before September 21th, 2020 was used because after this date, the soil was moistened by heavy and almost permanent rainfall. Multivariate regressions were used to explain SWC, SOM and SMN with site and distance from tree rows or soil layer as explanatory variables in the same way as the TFRA in Eq. 1. Regression analyses were followed by Tukey's tests for post hoc pairwise comparisons. One-way ANOVAs were performed to analyze the variability of SWC, SOM and SMN with stand age group as explanatory variable. The reduction of analyzed data due to samples collected after September rainfall limited the validity of some ANOVA analysis for SWC and SMN due to insufficient number of levels for some modalities (eg: stand age group, soil layer). Correlation analysis was performed to highlight the relationship between SWC, SOM and SMN and other soil and plant parameters including TFRA. Stepwise analysis was run to select the 2 or 3 best variables explaining the variability of SWC and SOM. Before running models, collinearity analysis was performed between quantitative variables using principal component analysis (PCA). Autocorrelated variables were graphically diagnosed and removed for stepwise regression analyses. For all regressions, a symbol *** was used to indicate a significant determination coefficient at $p < 0.001$ level, a symbol ** for $p < 0.01$ level and a symbol * for $p < 0.05$ level. The variable age of the plantation was used either as its numerical values (referred to as: "stand age") or as a group of stand age (referred to as: "stand age group"). In result section, all mean values for a given variable are followed by the standard deviation.

All statistical analyses were performed with R software version 4.0.4.

## 3. Results

### 3.1. Spatial fine roots distribution

The stand age group, the soil layer and the distance from tree rows accounted for 44 % of the tree fine roots abundance variability along the chronosequence of our AC stands ($p < 0,05$). Tree species accounted for only 4 %. During the first four years of plantation, trees developed abundant fine roots at 0–30 cm depth along tree rows and at 1 m distance (Fig. 2, Fig. 3).

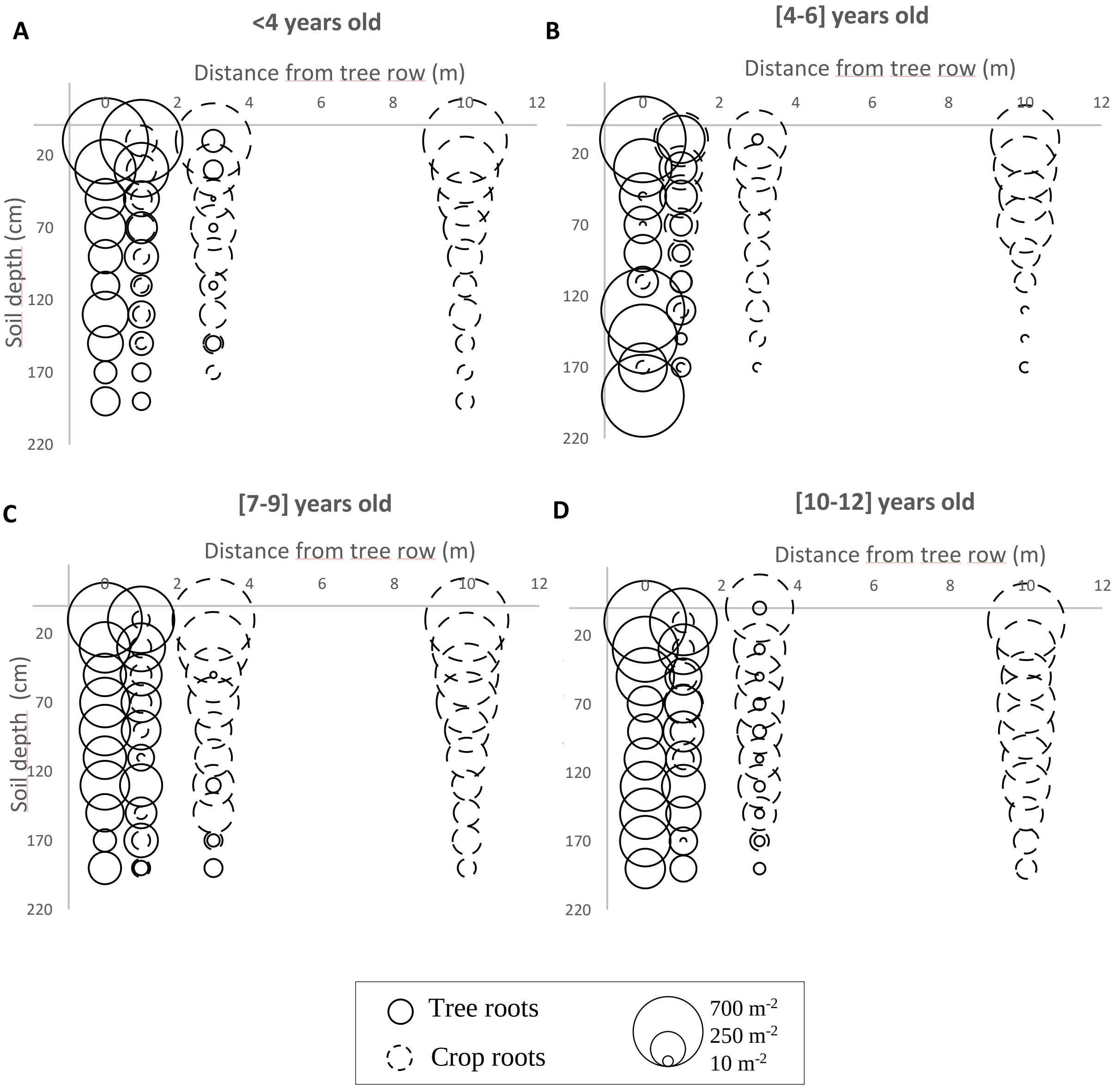
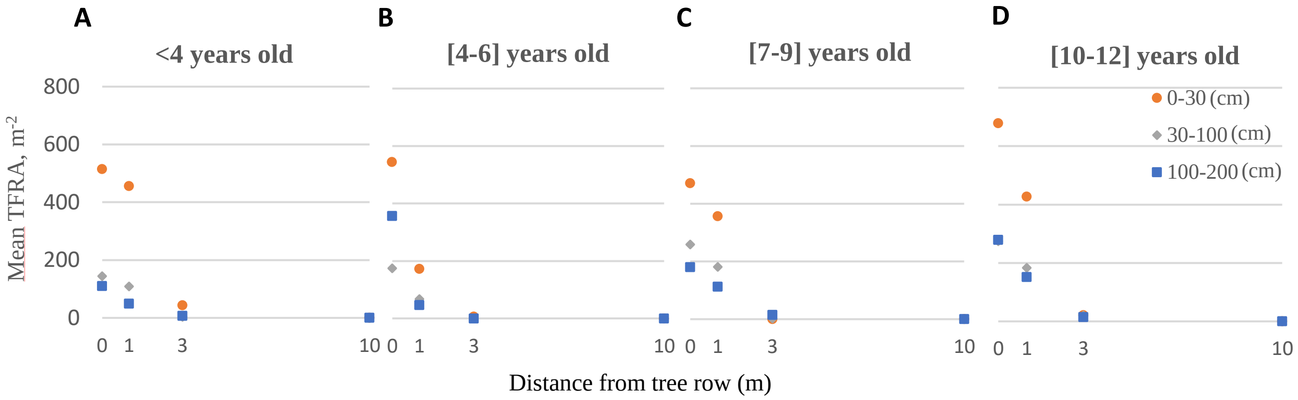


**Figure 2:** Lateral and vertical distribution of tree fine roots abundance (m$^{-2}$) down to 2 m depth at different distances from tree rows along a chronosequence of young agroforestry plots. Continuous and dotted circles represent tree and crop roots respectively. The size of circles is proportional to the tree fine root abundance.



**E**

| | <4 years | | | | 4-6 years | | | | 7-9 years | | | | 10-12 years | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Distance from tree row (m) | | | | | | | | | | | | | | | |
| Soil layer (cm) | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 |
| 0-30 → | a | a | b | - | a | b | c | - | a | a | b | - | a | b | c | - |
| 30-100 → | a | a | b | - | a | b | c | - | a | a | b | - | a | a | b | - |
| 100-200 → | a | b | c | - | a | b | b | - | a | a | b | - | a | b | c | - |

**F**

| | <4 years | | | | 4-6 years | | | | 7-9 years | | | | 10-12 years | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Distance from tree row (m) | | | | | | | | | | | | | | | |
| Soil layer (cm) | 0 ↓ | 1↓ | 3 ↓ | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 |
| 0-30 | B | B | nv | - | B | **A** | nv | - | B | C | nv | - | B | B | A | - |
| 30-100 | A | A | nv | - | A | A | nv | - | A | B | nv | - | A | A | A | - |
| 100-200 | A | A | nv | - | AB | A | nv | - | A | A | nv | - | A | A | A | - |

**G**

| Distance (m) | Layer 1: 0-30 cm | | | | Layer 2: 30-100 cm | | | | Layer 3: 100-200 cm | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stand age (years) | | | | | | | | | | | |
| | <4 | [4-6] | [7-9] | [10-12] | <4 | [4-6] | [7-9] | [10-12] | <4 | [4-6] | [7-9] | [10-12] |
| 0 → | a | a | a | a | a | a | a | a | a | b | ab | b |
| 1 → | b | a | ab | b | ab | a | b | b | a | a | ab | **b** |
| 3 → | nv | nv | nv | nv | nv | nv | nv | nv | ns | ns | ns | ns |

**Figure 3:** Evolution of tree fine roots abundance (TFRA, $m^{-2}$) at 0, 1, 3 and 10 m distance from the tree row and in different soil layers along a chronosequence of agroforestry stands (A, B, C, D) and results of Tukey's test ($p < 0.05$) to compare (E) distances from tree rows for a given soil layer and a given stand age; (F) soil layer for a given distance and a given stand age; (G) stand age for a given distance from tree row and a given soil layer. Data are means. Letters indicate homogeneous groups: means with same letters are not significantly different. "ns" means not significant, "nv" means not validated. Vertical or horizontal arrows show the direction of reading for statistical means comparisons. Bold letters are used to facilitate table reading.

However, at 3 m distance we also found more tree fine roots in topsoil than in L2 and L3 (Figs. 2A and 3A). From 4–6 years, trees significantly expanded deep fine roots (L3) in tree rows

(Figs. 2B, 3B, E and F) compared to other distances, evidencing a vertical exploration of soil profile. At the same time, the amount of tree fine roots in topsoil decreased at 1 m distance compared to young stands (Fig. 3B and G). From 7–9 years, trees continued to develop vertical deep fine roots on rows, but they started to colonize deep soil layers at 1 m distance (Figs. 2C and 3G), demonstrating both vertical and lateral fine roots expansion. No significant difference of TFRA was observed between stand age in deep soil layer at 3 m distance (Fig. 3G). Beyond 9 years old, we observed horizontal tree fine roots distribution at 1 m distance in layers 2 and 3 (Figs. 2D and 3G). In topsoil, we rediscovered high TFRA although they were rare between 4 and 9 years old as mentioned above. Regardless the age of the plantation, no tree fine roots were observed at 10 m from the rows.

Overall, crop fine roots were mainly concentrated in soil layer above 120 cm depth regardless the distance from rows (Fig. 2).

### 3.2. Relationships between tree fine roots distribution and different explanatory variables related to crop management, stand and soil parameters

Both in tree row and at 1 m distance, the sum of tree fine roots abundance (sTFRA) was positively correlated with stand age except in the topsoil (Table 2). The rho coefficient between the two variables was the highest in layer 3, testifying a deep tree rooting system for old stands. Considering all soil layers, the rho coefficient between sTFRA and stand age was the highest on tree rows and decreased at 1 m distance. This suggests that as trees grow, they developed fine roots mainly along the row and decreasingly away (Table 2). Finally, when data from all distances were analyzed excluding those from tree rows, the rho coefficient between stand age and sTFRA was not significant in topsoil whereas it became significant and increased from layer 2 to layer 3 confirming the deep and lateral tree fine roots development with stand age as already mentioned above.

The sum of tree fine roots abundance was positively correlated with tree density in tree rows only in layer 3 suggesting that close to rows, high tree density favored high fine roots abundance in the deep soil layer. Otherwise, negative rho coefficients were observed between tree density and sTFRA for other soil layers at different distances. We observed that globally high tillage frequency led to high sum of tree fine roots abundance (rho = 0,4***). At 1 m distance, the rho coefficient between the tillage frequency and sTFRA was high in deep layers (L2 and L3). The sTFRA was positively influenced by crop rotation duration in the tree rows in layer 3 only, suggesting that long crop duration rotation tended to concentrate tree fine rooting in rows (Table 2, column 6). Otherwise, the rho coefficient between sTFRA and crop rotation duration became negative at 1 m and 3 m distances. Positive correlations were also found between sTFRA and the percentage of winter crop in the rotation in tree rows on the whole soil profile.

In terms of soil and plant parameters, we observed no obvious conclusion about the effect of soil texture, limestone content, pH, tree row width and crop yield measured in 2020 on the vertical or lateral variation of sTFRA except in tree rows where a positive relationship was found between sTFRA and the soil sand content in the layer 3 (rho = 0,5***). However, as the high soil sand content was noted mainly in layer 3 of the oldest stands, this correlation may traduce an age effect instead of soil texture (Table 2). The sTFRA calculated for all distances and all depths was significantly affected by tree species ($R^2 = 0.2^{***}$): it was the highest for maple, the lowest for hornbeam and intermediate for hybrid walnut. Neither the type of fertilization used by farmers nor the crop management system (organic, sustainable, conventional or no tillage) presented a conclusive effect on sTFRA variability.

Stepwise analyses showed that stand age, tillage frequency and crop rotation duration explained 60 % of sTFRA variability calculated for all soil layers and all distances from the tree row (Table 3, last line). Among the seven tested variables included in each model, stand age was the most frequently selected by the stepwise method at the first rank (positive effect) followed by crop rotation duration (negative effect) and tillage frequency (positive effect). The soil sand content was also selected by the model with positive or negative effects according soil layers and distance from tree rows. The variable soil organic matter was rarely chosen but its influence was positive in layer 1 at 1 m distance and negative at 3 m distance considering all soil layers within the models. Tree density never appeared in the first three explanatory variables and percent winter crop only appeared as the third explanatory variable in tree row for layer 2. The determination coefficient of models explaining the variation of sTFRA calculated at each distance from tree row for all soil layers was highest in tree rows and at 1 m distance ($R^2 = 0.5$ ***) and lowest at 3 m distance ($R^2 = 0.3$ ***). Finally, regardless of the calculation method for sTFRA (per soil layer or distance), stand age, tillage frequency and crop rotation duration were the main variables explaining the variability of sTFRA (Table 3).

**Table 2:** Summary of Spearman’s rank correlation rho between sum of tree fine roots abundance and stand, soil characteristics and crop management variables. Values written in bold, italic and normal police were statistically significant at $p < \mathbf{0.001}$, $p < \mathit{0.01}$ and $p < 0.05$ respectively; ns means no significant. Colors classified the rho value from bright red (highest values) to dark blue (lowest values). L1, L2 and L3 means soil layer 1 (0-30 cm), soil layer 2 (30-100 cm) and soil layer 3 (100-200 cm) respectively.

| **Soil layer** | **Distance** | **Stand age** | **Tree density** | **Tillage frequency** | **Rotation duration** | **Percent winter crop** | **Clay** | **Sand** | **SOM** | **Limestone** | **Crop yield 2020** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Unit** | **m** | **years** | **trees ha$^{-1}$** | **y$^{-1}$** | **years** | **%** | **g kg$^{-1}$** | **g kg$^{-1}$** | **g kg$^{-1}$** | **g kg$^{-1}$** | **t ha$^{-1}$** |
| L1 | 0 | ns | ns | ns | ns | ns | ns | *-0.5* | ns | ns | ns |
| L2 | 0 | *0.4* | ns | ns | ns | 0.4 | ns | ns | *0.4* | ns | ns |
| L3 | 0 | **0.5** | 0.3 | ns | *0.4* | ns | ns | **0.5** | ns | ns | ns |
| L1 | 1 | ns | ns | ns | *-0.3* | ns | ns | *0.3* | **0.6** | ns | ns |
| L2 | 1 | 0.2 | ns | *0.3* | *-0.3* | ns | ns | ns | ns | **-0.4** | *-0.3* |
| L3 | 1 | **0.5** | ns | **0.3** | ns | 0.2 | *0.3* | ns | ns | ns | -0.3 |
| L1 | 3 | ns | ns | 0.3 | **-0.4** | ns | ns | ns | ns | ns | ns |
| L2 | 3 | ns | -0.3 | ns | **-0.4** | *0.3* | ns | *0.3* | ns | ns | 0.2 |
| L3 | 3 | ns | ns | ns | -0.2 | ns | *-0.3* | ns | ns | ns | -0.2 |
| All | 0 | **0.4** | ns | *0.3* | **0.3** | *0.3* | ns | ns | ns | 0.3 | ns |
| All | 1 | **0.3** | **-0.3** | **0.4** | **-0.3** | 0.1 | ns | ns | ns | ns | **-0.2** |
| All | 3 | ns | **-0.3** | *0.2* | **-0.6** | ns | *-0.2* | ns | *-0.2* | ns | -0.2 |
| L1 | 1, 2, 3 | ns | -0.2 | 0.2 | **-0.4** | ns | ns | **0.3** | *0.3* | 0.2 | ns |
| L2 | 1, 2, 3 | **0.3** | ns | **0.3** | **-0.4** | ns | ns | ns | ns | **-0.3** | **-0.3** |
| L3 | 1, 2, 3 | **0.5** | ns | 0.2 | ns | *0.3* | ns | ns | **0.3** | **0.3** | -0.2 |
| All | All | **0.4** | *-0.1* | **0.4** | **-0.2** | *0.1* | -0.1 | ns | **-0.1** | ns | **-0.2** |

**Table 3:** Results of stepwise regression analysis to model the sum of tree fine roots abundance at a given soil layer and at different distances from tree rows. Symbols '+' or '-' indicate the positive or negative effects of a given variable in the model: +++ (bright red) or --- (dark blue): $p < 0.001$; ++ (red) or -- (blue): $p < 0.01$; - (light blue): $p < 0.05$; . (light red or blue): $p < 0.1$. Rank indicates the position of the variable in the model chosen by the stepwise method. For each model, only the first two or three variables were kept for a best interpretation of results. R$^2$ represents the determination coefficient of the chosen model. Asterisks indicate *p*-value. L1, L2 and L3 means soil layer 1 (0-30 cm), soil layer 2 (30-100 cm) and soil layer 3 (100-200 cm) respectively.

| **Soil layer** | **Distance** | **Stand age** | rank | **Tree density** | rank | **Tillage frequency** | rank | **Rotation duration** | rank | **Percent winter crop** | rank | **Sand** | rank | **SOM** | rank | R$^2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Unit** | **m** | **years** | | **trees ha$^{-1}$** | | **y$^{-1}$** | | **years** | | **%** | | **g kg$^{-1}$** | | **g kg$^{-1}$** | | |
| L1 | 0 | +++ | 1 | | | | | | | | | --- | 2 | | | 0.6*** |
| L2 | 0 | +++ | 1 | | | | | | | . | 3 | ns | 2 | | | 0.3** |
| L3 | 0 | | | | | +++ | 1 | --- | 2 | | | | | | | 0.8*** |
| L1 | 1 | | | | | | | --- | 2 | | | | | +++ | 1 | 0.7*** |
| L2 | 1 | | | | | +++ | 1 | - | 2 | | | | | | | 0.2** |
| L3 | 1 | +++ | 1 | | | | | | | | | -- | 2 | | | 0.4*** |
| L1 | 3 | ++ | 2 | | | | | | | | | - | 3 | . | 1 | 0.2* |
| L2 | 3 | | | | | | | | | | | +++ | 1 | | | 0.3*** |
| L3 | 3 | + | 2 | | | | | - | 1 | | | ns | 3 | | | 0.1* |
| All | 0 | +++ | 3 | | | +++ | 1 | --- | 2 | | | | | | | 0.5*** |
| All | 1 | +++ | 1 | | | +++ | 3 | --- | 2 | | | | | | | 0.5*** |
| All | 3 | +++ | 1 | | | | | --- | 2 | | | | | --- | 3 | 0.3*** |
| L1 | 1, 2, 3 | | | | | | | | | | | +++ | 1 | ++ | 2 | 0.4*** |
| L2 | 1, 2, 3 | +++ | 1 | | | +++ | 2 | --- | 3 | | | | | | | 0.3*** |
| L3 | 1, 2, 3 | +++ | 1 | | | +++ | 3 | --- | 2 | | | | | | | 0.7*** |
| All | All | +++ | 1 | | | +++ | 2 | --- | 3 | | | | | | | 0.6*** |

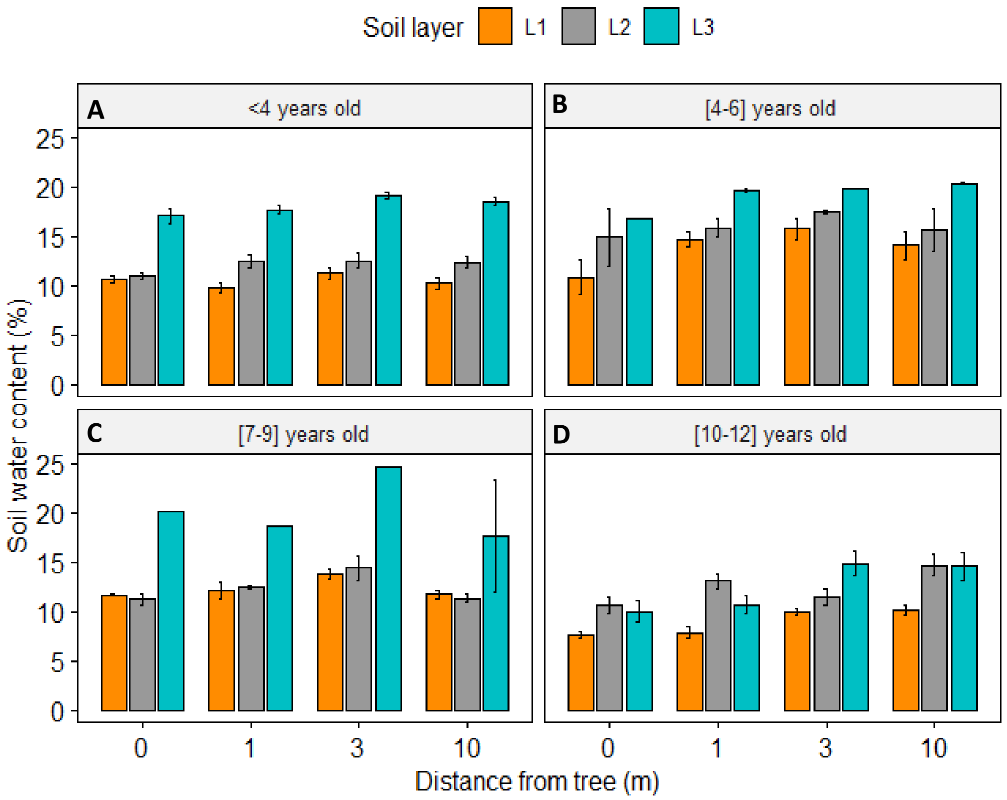
### 3.3. Relationship between root distribution and soil water content

Soil water content increased with depth in all stands ($R^2 = 0.29^{***}$) with an average of 11 ± 3 %, 13 ± 3 % and 17 ± 4 % in layers 1, 2 and 3, respectively. Multivariate regression analysis showed that the stand age group, the distance from tree row and the soil depth explained 49 % of the variation of soil water content. In young stands, soil moisture was significantly higher in deep soil layer (L3) compared to topsoil (L1) (Fig. 4A and F) regardless of the distance from the tree row. Soil water content significantly varied with the distance from the tree row in topsoil, whereas no effect of distance was observed in L2 and L3 (Fig. 4E).

In 4–6-year-old stands, SWC also increased with soil depth regardless of distance from tree row. It varied significantly with distance in topsoil whereas no effect was observed in layer 2. In deep soil layer (L3), the soil water content was lower in tree rows compared to other distances (Fig. 4B, E and F). In 7–9-year-old stands, soil water content was significantly higher in layer 3, but the two layers above were not significantly different (Fig. 4C and F). Finally, for old stands, SWC also increased significantly with depth (Fig. 4F) and with distance from tree row regardless of soil layer (Fig. 4E). Stepwise regression showed that the soil organic matter, clay and limestone content explained 22 % ($p < 0.001$) of the SWC variability.

Using a bivariate linear regression, our results showed that the site and the TFRA explained 40 % ($p < 0.001$) of the soil water content variability. The same model's determination coefficient was 0.65, 0.54 and 0.9 in topsoil, layer 2 and layer 3, respectively ($p < 0.001$). When the regression was run per distance from tree rows, the determination coefficient was highest at 1 m ($R^2 = 0.51^{***}$), intermediate in tree rows ($R^2 = 0.36^{***}$) and lowest at 3 m distance ($R^2 = 0.30^{***}$). Finally, the determination coefficient was highest in the oldest stands ($R^2 = 0.50^{***}$), intermediate in the youngest ($R^2 = 0.20^{***}$), lowest in 4–6 years old stands ($R^2 = 0.09^{*}$) and was not significant for 7–9-year-old stands.

Simple linear regression analyses showed a weak and negative relationship between TFRA and soil water content ($R^2 = 0.16^{***}$). The slope of the regression was the highest in deep soil layer ($R^2 = 0.25^{***}$), intermediate in topsoil ($R^2 = 0.12^{***}$) and the lowest in layer 2 ($R^2 = 0.03^{*}$; Fig. 7A). The same model was significant in tree rows ($R^2 = 0.12^{**}$), at 1 m distance ($R^2 = 0.26^{***}$; Fig. 7B), for young (<4 years; $R^2 = 0.14^{***}$) and for old (10–12 years) stands ($R^2 = 0.2^{***}$; Fig. 7C).



**E**

| Soil layer (cm) | <4 years | | | | 4-6 years | | | | 7-9 years | | | | 10-12 years | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Distance from tree row (m) | | | | | | | | | | | | | | | |
| | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 |
| 0-30 → | ab | a | b | ab | a | ab | b | ab | a | a | a | a | a | a | b | b |
| 30-100 → | a | a | a | a | a | a | a | a | a | ab | b | a | a | bc | ab | c |
| 100-200 → | a | a | a | a | **a** | b | b | b | nd | nd | nd | nd | a | a | b | b |

**F**

| Soil layer (cm) | <4 years | | | | 4-6 years | | | | 7-9 years | | | | 10-12 years | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Distance from tree row (m) | | | | | | | | | | | | | | | |
| | 0 ↓ | 1↓ | 3 ↓ | 10 ↓ | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 |
| 0-30 | nv | A | A | A | A | A | A | A | A | A | A | A | nv | A | A | nv |
| 30-100 | nv | B | A | B | B | A | A | A | A | A | A | A | nv | B | A | nv |
| 100-200 | nv | C | B | C | C | B | B | B | B | B | B | B | nv | B | B | nv |

**G**

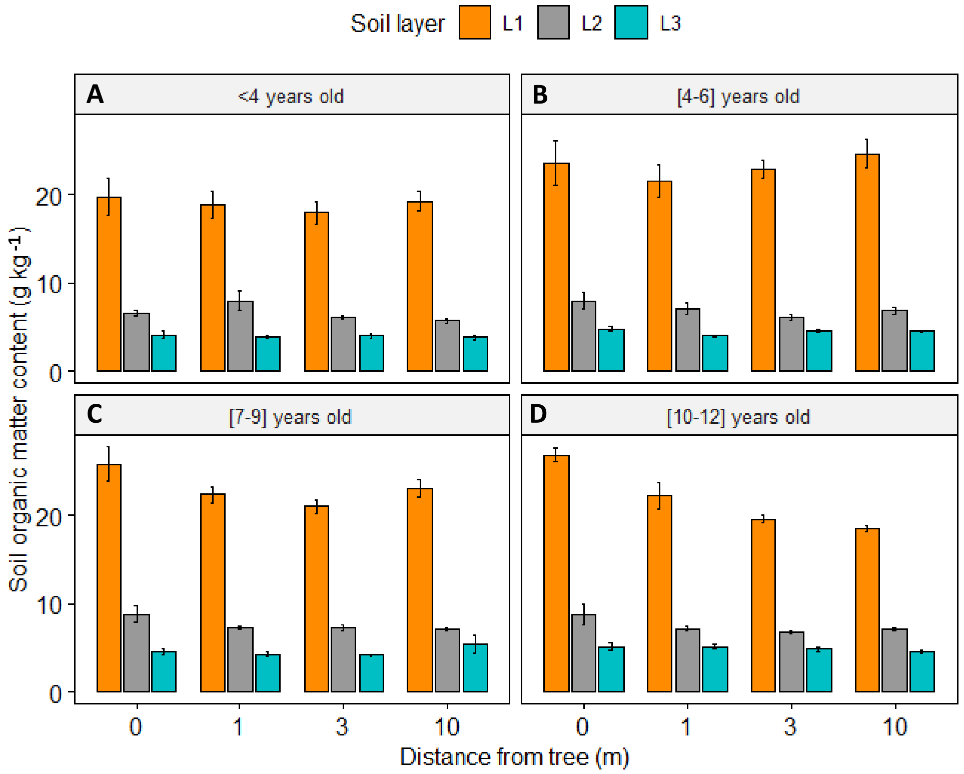
| Distance (m) | Layer 1: 0-30 cm | | | | Layer 2: 30-100 cm | | | | Layer 3: 100-200 cm | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stand age (years) | | | | | | | | | | | |
| | <4 | [4-6] | [7-9] | [10-12] | <4 | [4-6] | [7-9] | [10-12] | <4 | [4-6] | [7-9] | [10-12] |
| 0 → | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 1 → | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| 3 → | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |

**Figure 4:** Variation of the soil water content (%) at 0, 1, 3 and 10 m distance from tree rows and in different soil layers along a chronosequence of agroforestry stands (A, B, C, D) and results of Tukey's test ($p < 0.05$) to compare (E) distances from tree rows for a given soil layer and a

given stand age; (F) soil layer for a given distance and a given stand age; (G) stand age for a given distance from tree row and a given soil layer. Histograms are means and bars are standard errors. Letters indicate homogeneous groups: means with same letters are not significantly different. “nd” means no determined, “nv” means not validated. Vertical or horizontal arrows show the direction of reading for statistical means comparisons. Bold letters are used to facilitate table reading.

### 3.4. Relationship between root distribution and soil organic matter content

The principal component analysis (PCA) explaining 41.5 % of variability showed a first axis representing variables related to SOM and depth. A second axis represents soil physical and chemical parameters (Fig. 7A). The SOM decreased with depth in all stands ($R^2 = 0.82$ ***) and at all distances from tree rows (Figs. 5F and 7A). The stand age very weakly influenced the SOM ($R^2 = 0.01$**) and the distance from tree row did not have any effect. On average, the SOM was 21.5 ± 5.5 g $kg^{-1}$, 7.0 ± 2.4 g $kg^{-1}$ and 4.4 ± 1.4 g $kg^{-1}$ in layers 1, 2 and 3, respectively. It was strongly correlated with the SON ($R^2 = 0.95$ ***; Fig. 7A). The mean C/N ratio was 8.2 ± 1.5 and varied from 10 ± 1 in topsoil to 8 ± 1 in layer 2 and 7 ± 1 in layer 3. For young stands (<4 years and 4–6 years old), the SOM was not significantly different between distances in L1 (Fig. 5A and B). For 7–9-year-old stands, the SOM was significantly higher in tree rows compared to other distances only in L2. Finally in the oldest stands, the SOM significantly increased from 10 m distance to tree rows in topsoil and L2 (Fig. 5E). For instance, in topsoil the SOM was 27 ± 2 g $kg^{-1}$ in the tree row whereas it was 18 ± 1 g $kg^{-1}$ at 10 m distance, and in layer 2, the SOM was 9 ± 4 g $kg^{-1}$ in the tree row and 7 ± 1 g $kg^{-1}$ at 10 m distance (Fig. 5D). However, when comparison was made between different stand age groups in topsoil, the value of SOM in the tree row was not significantly different between the youngest and the oldest stands (Fig. 5G). In the deep soil layer L3, the SOM was higher in 10–12-year-old stands than in younger stands only at 1 and 3 m distance from the tree row (Fig. 5G). The SOM was positively correlated to TFRA ($R^2 = 0.2$***). The relationship between SOM and TFRA was statistically significant in layer 1 ($R^2 = 0.1$ ***), in tree rows ($R^2 = 0.3$***) and at 1 m distance ($R^2 = 0.3$ ***; Fig. 7C). The same relationship was the strongest for the oldest stands ($R^2 = 0.28$***) and the youngest stands ($R^2 = 0.26$***). Stepwise analyses carried out for layer 1 showed that TFRA, crop rotation duration and tillage frequency explained 30 % ($p < 0.001$) of SOM variability. In layer 3, 23 % ($p < 0.001$) of the SOM variability was explained by soil sand content, pH and tillage frequency. When stepwise analysis was performed per stand age group, the depth and the TFRA explained on average, 60 % of SOM variability, except for 4–6-year-old stand where the depth and soil sand content explained 62 % of the variability.



**E**

| | <4 years | | | | 4-6 years | | | | 7-9 years | | | | 10-12 years | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Soil layer (cm) | Distance from tree row (m) | | | | | | | | | | | | | | | |
| | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 |
| 0-30 → | a | a | a | a | a | a | a | a | b | ab | a | ab | **c** | b | ab | a |
| 30-100 → | ab | b | a | a | b | ab | a | ab | b | a | a | a | b | ab | a | a |
| 100-200 → | a | a | a | a | b | a | b | b | a | a | a | a | nv | nv | nv | nv |

**F**

| | <4 years | | | | 4-6 years | | | | 7-9 years | | | | 10-12 years | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Soil layer (cm) | Distance from tree row (m) | | | | | | | | | | | | | | | |
| | 0 ↓ | 1↓ | 3 ↓ | 10↓ | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 |
| 0-30 | B | C | B | C | B | B | B | nv | C | C | C | nv | C | B | C | C |
| 30-100 | A | B | A | B | A | A | A | nv | B | B | B | nv | B | A | B | B |
| 100-200 | A | A | A | A | A | A | A | nv | A | A | A | nv | A | A | A | A |

**G**

| Distance (m) | Layer 1: 0-30 cm | | | | Layer 2: 30-100 cm | | | | Layer 3: 100-200 cm | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stand age (years) | | | | | | | | | | | |
| | <4 | [4-6] | [7-9] | [10-12] | <4 | [4-6] | [7-9] | [10-12] | <4 | [4-6] | [7-9] | [10-12] |
| 0 → | a | a | a | a | nv | nv | nv | nv | nv | nv | nv | nv |
| 1 → | a | a | a | a | a | a | a | a | a | a | a | b |
| 3 → | a | b | ab | ab | a | a | b | ab | a | ab | ab | b |
| 10 → | a | b | ab | a | a | b | ab | b | a | ab | b | ab |

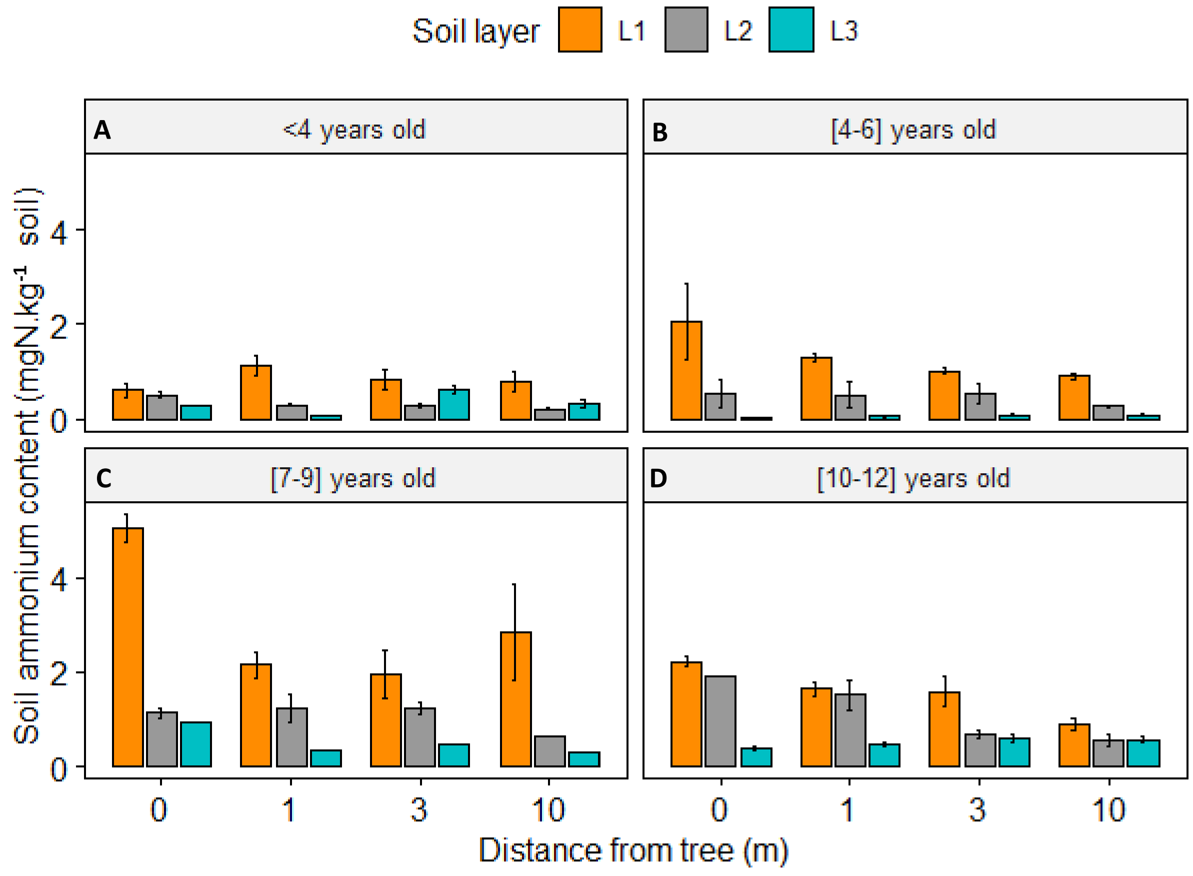
**Figure 5:** Variation of the soil organic matter content (g $kg^{-1}$) at 0, 1, 3 and 10 m distance from tree rows and in different soil layers along a chronosequence of agroforestry stands (A, B, C, D) and results of Tukey's test ($p < 0.05$) to compare (E) distances from tree rows for a given soil layer and a given stand age; (F) soil layer for a given distance and a given stand age; (G) stand age for a given distance from tree row and a given soil layer. Histograms are means and bars are standard errors. Letters indicate homogeneous groups: means with same letters are not significantly different. "nv" means not validated. Vertical or horizontal arrows show the direction of reading for statistical means comparisons. Bold letters are used to facilitate table reading.

### 3.5. Relationship between root distribution and soil mineral nitrogen content

The SMN was significantly higher in topsoil (8.0 ± 7.3 mg N $kg^{-1}$ soil) than in layer 2 (1.8 ± 1.4 mg N $kg^{-1}$ soil) and layer 3 (2.5 ± 2.6 mg N $kg^{-1}$ soil). The depth explained 30 % ($p < 0.001$) of the variation in SMN. The SMN was strongly correlated to the SNN ($R^2$ = 0.97***; Fig. 7A) indicating that the main form of mineral nitrogen in our soil was nitrate. The percentage of nitrate in SMN (%NO3) was on average 68 ± 24 % and was significantly different between soil layers: 77 ± 24 % in layer 3, 70 ± 25 % in topsoil and 60 ± 21 % in layer 2. No significant effect of the kind of N fertilizer in SMN was observed. The stand age affected very weakly the SMN ($R^2$ = 0.03**) and the distance not at all. There was a weak positive relationship between SMN and TFRA ($R^2$ = 0.03**) when all data were analyzed. This relationship was not significant when carried out per soil layer and per stand age group. No overall relationship was found between %NO3 and TFRA.

The SAN was, on average, 0.8 ± 0.8 mg $kg^{-1}$ and decreased significantly with soil depth ($R^2$ = 0.3***): 1.5 ± 1.1 mg N $kg^{-1}$ soil in topsoil, 0.7 ± 0.6 in layer 2 and 0.4 ± 0.3 mg N $kg^{-1}$ soil in layer 3. In average, it increased with stand age ($R^2$ = 0.2***) and decreased significantly with distance from tree rows ($R^2$ = 0.04***). In the youngest stands, the SAN was not significantly affected by the distance from tree row in topsoil (Fig. 6E). In the 4–6-year-old stand, the SAN decreased significantly from tree row to 10 m distance in the topsoil. The same effect was not observed in layer 2 (Fig. 6E). In 7–9-year-old stands, no significant change in SAN was observed for all distances and all soil layers. For the oldest stands, the SAN was significantly high close to tree rows until 3 m distance in topsoil and until 1 m distance in layer 2 (Fig. 6E). No significant change in SAN was observed for all distances in layer 3. Comparison between stand age groups in topsoil showed that in tree rows, the SAN was significantly lower in youngest stands compared to others (Fig. 6G).

The SAN was positively correlated to TFRA ($R^2 = 0.09^{***}$). The determination coefficient was the highest in layer 2 ($R^2 = 0.16^{***}$), in tree rows ($R^2 = 0.05^{*}$) and at 1 m distance ($R^2 = 0.08^{**}$). The same relationship was also observed for the 7–9-and 10–12-year-old stands ($R^2 = 0.27^{***}$ and $0.18^{***}$ respectively; Fig. 7D).



**E**

| Soil layer (cm) | <4 years | | | | 4-6 years | | | | 7-9 years | | | | 10-12 years | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Distance from tree row (m) | | | | | | | | | | | | | | | |
| | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 |
| 0-30 → | a | a | a | a | b | ab | ab | a | nv | nv | nv | nv | **b** | **b** | **b** | a |
| 30-100 → | b | ab | ab | a | a | a | a | a | a | a | a | a | **b** | **b** | a | a |
| 100-200 → | ab | a | b | a | nd | nd | nd | nd | nd | nd | nd | nd | a | a | a | a |

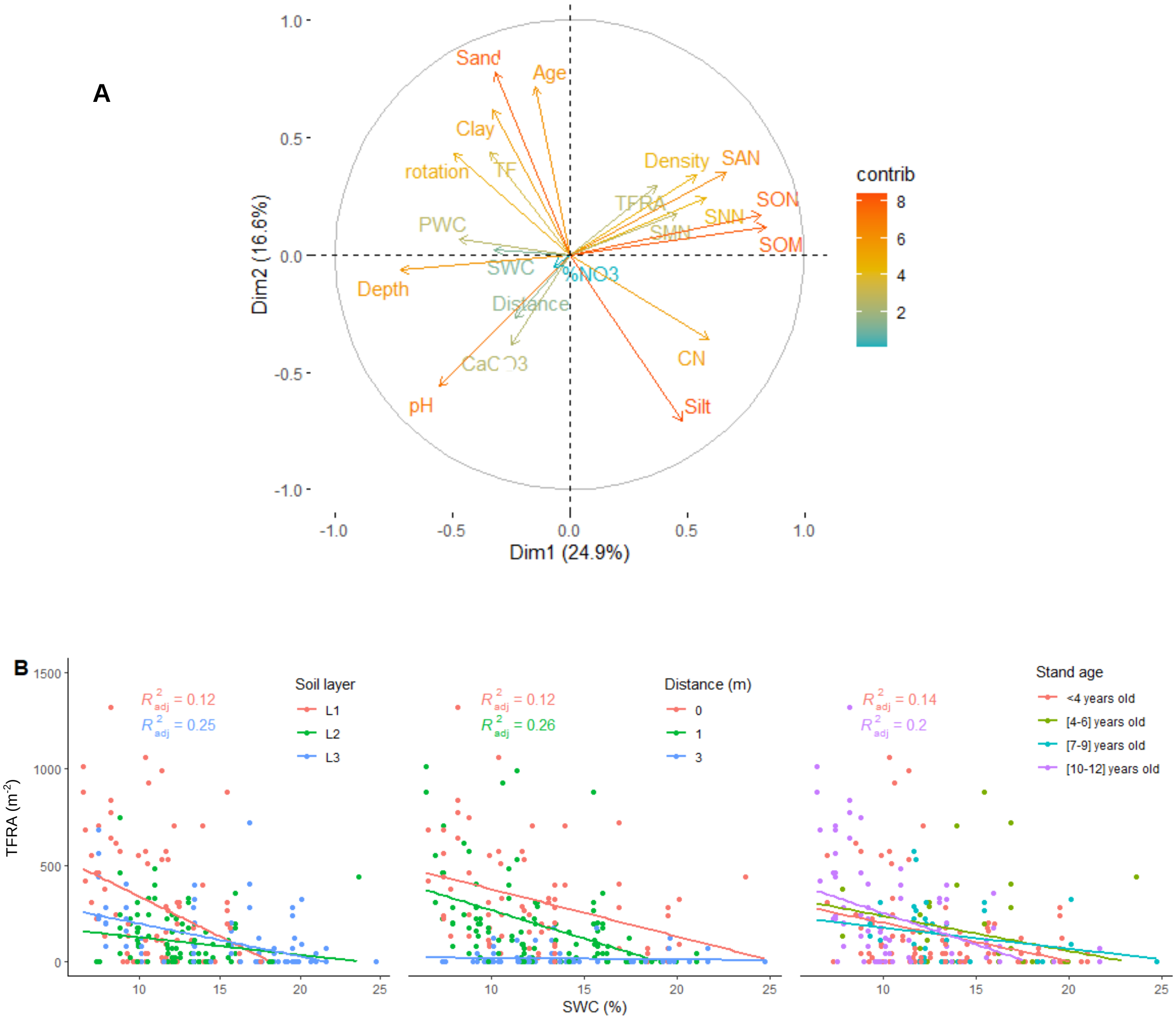
**F**

| Soil layer (cm) | <4 years | | | | 4-6 years | | | | 7-9 years | | | | 10-12 years | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Distance from tree row (m) | | | | | | | | | | | | | | | |
| | 0 ↓ | 1↓ | 3 ↓ | 10↓ | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 | 0 | 1 | 3 | 10 |
| 0-30 | B | B | B | B | B | B | B | C | B | nv | nv | nv | nv | B | B | nv |
| 30-100 | AB | A | A | A | AB | A | AB | B | A | nv | nv | nv | nv | B | A | nv |
| 100-200 | A | A | B | A | A | A | A | A | A | nv | nv | nv | nv | A | A | nv |

**G**

| Distance (m) | Layer 1: 0-30 cm | | | | Layer 2: 30-100 cm | | | | Layer 3: 100-200 cm | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Stand age (years) | | | | | | | | | | | |
| | <4 | [4-6] | [7-9] | [10-12] | <4 | [4-6] | [7-9] | [10-12] | <4 | [4-6] | [7-9] | [10-12] |
| 0 → | a | **b** | **c** | **b** | nv | nv | nv | nv | nv | nv | nv | nv |
| 1 → | a | ab | b | ab | a | ab | **b** | **b** | nv | nv | nv | nv |
| 3 → | a | a | a | a | nv | nv | nv | nv | b | a | ab | b |
| 10 → | a | a | b | a | a | ab | b | b | nv | nv | nv | nv |

**Figure 6:** Variation of the soil ammonium nitrogen content (SAN, mg N kg$^{-1}$ soil) at 0, 1, 3 and 10 m distance from tree rows and in different soil layers along a chronosequence of agroforestry stands (A, B, C, D) and results of Tukey's test ($p < 0.05$) to compare (E) distances from tree rows for a given soil layer and a given stand age; (F) soil layer for a given distance and a given stand age; (G) stand age for a given distance from tree row and a given soil layer. Histograms are means and bars are standard errors. Letters indicate homogeneous groups: means with same letters are not significantly different. "nd" means no determined, "nv" means not validated. Vertical or horizontal arrows show the direction of reading for statistical means comparisons. Bold letters are used to facilitate table reading.



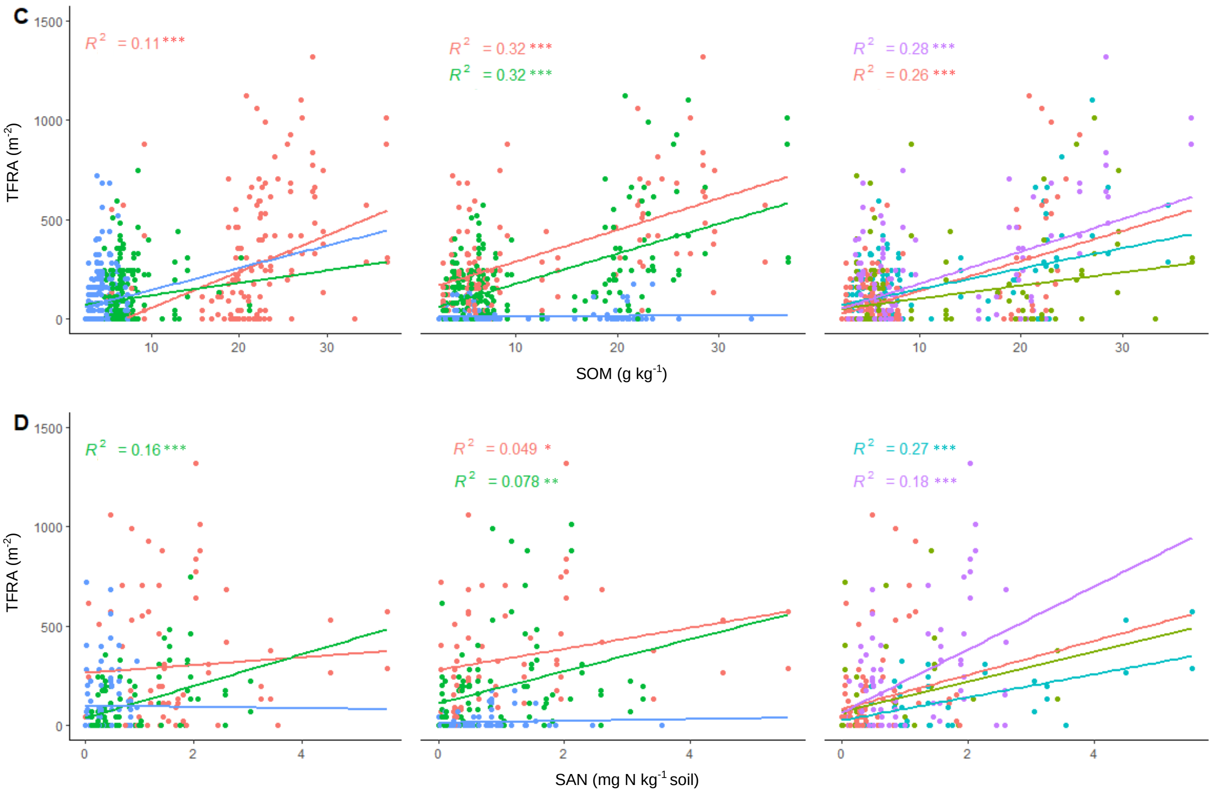


**Figure 7:** Correlation analysis between main studied variables: principal component analysis (A), simple linear regression between tree fine roots abundance (TFRA) and soil water content or SWC (%) (B), soil organic matter or SOM (g kg$^{-1}$) (C) and soil ammonium content or SAN (mg N kg$^{-1}$ soil) (D). $R^2$ is the adjusted coefficient of determination of each regression. A symbol *** was used to indicate a significant coefficient of determination at $p < 0.001$ level, ** for $p < 0.01$ level and * for $p < 0.05$ level. Abbreviations used in PCA graphic mean: Age = stand age (years); $CaCO_3$, Clay, Silt and Sand = soil limestone, clay, silt and sand content (g kg$^{-1}$); CN = ratio organic C to organic N; density = tree density (trees ha$^{-1}$); Depth = soil depth (cm); Distance = distance from tree row (m); PWC = percentage of winter crop in rotation (%); TF = tillage frequency (y$^{-1}$); SAN = soil ammonium content (mg N kg$^{-1}$ soil); SMN = soil mineral nitrogen content (mg N kg$^{-1}$ soil); SNN = soil nitrate content (mg N kg$^{-1}$ soil); SON = soil organic nitrogen (g kg$^{-1}$); SOM = soil organic matter (g kg$^{-1}$); %NO3 = percentage of nitrate in SMN (%).

## 4. Discussion

### 4.1. Agroforestry trees develop deep fine roots 5 years from intercropping

This study was, to our knowledge, one of the first papers to analyze the tree fine roots development in agroforestry systems during the ten first years of tree inclusion in cropland. We succeeded in showing that the deep tree fine rooting which was largely documented in old AC trees (Andrianarisoa et al., 2016, Borden et al., 2020, Cardinael et al., 2015b, Isaac et al., 2014, Kumar and Jose, 2018, Mulia and Dupraz, 2006) started from 5 years old. For stands younger than 4 years old, tree fine roots were observed in topsoil in tree rows spreading up to 3 m distance (Figs. 2A and 3A). The same results were shown by Zhang et al. (2015) on 1- and 2-year-old stands. This juvenile shallower fine root distribution resulting from tree establishment during their first years of growth was also observed in forest ecosystems (Claus and George, 2005). From 4–6 years old, trees expanded deep fine roots in rows accompanied by a decrease of tree fines roots in topsoil at 1 m and 3 m distance (Figs. 2B and 3B). Old stands continued to develop vertical roots on rows and started to colonize deep lateral soil layers up to 3 m distance from tree rows (Fig. 2, Fig. 3).

According to our findings, the tillage frequency and the crop duration partly explained this change in the root distribution along the chronosequence (Table 2, Table 3). Repetitive tree fine roots pruning with soil tillage can lead to a reduction of topsoil roots abundance (Gilman, 1990b, Schroth, 1998), to a proliferation of fine roots at a distance of 1 m probably due to new growth from pruned roots (Jose et al., 2000a, Jose et al., 2000b; Schroth, 1995), and to a forced development in depth to ensure the continuous nutrient and water supply. In fact, tree fine roots growing on upper soil layers during the spring (Germon et al., 2016) was shown to be vulnerable to soil tillage, unlike deep roots. In terms of the crop rotation duration effect, we speculate that the diversity of crop species within a long crop rotation should impact the crop rooting zone every year due to the diversity of crop management practices and the diversity of soil and plant treatment. It should disturb the lateral expansion of tree fine roots and mycorrhizae in this zone. The tree row or the adjoining zone and deep soil layer may offer a more stable and nutrient-rich environment for tree fine roots development. Moreover, we also observed that a high percentage of winter crop in rotation increases tree fine roots abundance along soil profile in tree rows and in layer 3 (Table 2). As suggested in other studies (Mulia and Dupraz, 2006, Zhang et al., 2015), winter crops can deplete upper soil layer before trees reach budbreak. This soil nutrient and water impoverishment may induce trees to develop fine roots in deep soil layer where they can compete for the remaining available resources. In contrast, Battie-Laclau et al. (2020) studied walnut-wheat alley-cropping systems and observed roots of 11-year-old trees in the cropping zone up to 3 m distance from tree rows, suggesting that our theory may not be verified everywhere because other parameters may be involved.

Despite our caution and rigor on tree fine roots recognition, it may be possible that we also counted some weed fine roots particularly in the tree rows and at 1 m distance as mentioned by Battie-Laclau et al. (2020). Very few weeds were noted in the alleys thanks to chemical or physical control by farmers. In the rows, the mechanical maintenance for young stands or trees development for old stands limited weeds growth thus only few were noticed, and their roots were easily recognizable by their color and softness. Finally, despite the different roots development patterns of our studied tree species in forest ecosystems (taproot system for walnut (Borden et al., 2017), fasciculate roots for maple (Köstler et al., 1968) and shallow horizontal roots for hornbeam (Abdi et al., 2009)), we observed that the association with crops modifies the tree fine roots distribution from their early age and the tree species explained only 4 % of the variability of the tree fine roots abundance. This demonstrated the fine root plasticity of these species shaped by agricultural work and/or crop interaction and may contribute to limiting nutrient loss outside the crop rooting zone (Bergeron et al., 2011).

Our results also showed that no tree roots reached distances farther than 10 m from the rows. As some of our old stands were up to 10 m height, we expected that tree fine roots could potentially explore an equivalent distance to their height (Danjon et al., 2020). Based on the Mulia and Dupraz (2006) study with 10 years old trees, poplar roots were found beyond 8 m from the tree row, whereas no walnut roots were found beyond 3 m from the tree row. A species and/or soil effect would explain the absence of tree roots at 10 m from the row in cropping area.

### 4.2. Tree fine roots distribution and soil water content

We showed that the stand age, the distance from tree rows and the soil depth explained almost half of soil water content variability but the abundance of tree fine roots at different depth and distance from tree row contributes to drying the soil along the profile. As soil samples were taken in summer period during which very low rainfall was observed (< 20 mm, in July 2020), the soil water content observed in topsoil was particularly low due to high soil evaporation. By comparison with deep soil layers, topsoil is the first interface with the atmosphere so that its water content fluctuates with events occurring in air (wind, solar radiation, rainfall, morning dew) independently of plant water uptake. Low soil water content in topsoil in agroforestry system in summer period was also shown by Anderson et al. (2009) for 6 years old trees but they attributed it to tree uptake. Indeed, the negative relationship observed between SWC and TFRA suggests that tree fine roots significantly dried the soil by up taking water. This relationship has been seen particularly: (i) in tree rows in topsoil; (ii) at 1 m distance where tree fine roots abundancy was high and cohabitation with crop fine roots was observed; (iii) in the layer 3 below the crop rooting zone; and (iv) in the oldest stands (Fig. 7B). These results show that during their first installation in cropland, intercropped trees mainly dry topsoil in rows, compete with crops at 1 m distance and valorize water not accessible for crops in deep soil. Jose et al. (2000a) highlighted a water competition between trees and maize at 1 m distance mainly due to a concentration of tree and crop roots in the top 30 cm soil layer. Huo et al. (2020) also found lower soil water content in alley-cropping compared to mono-cropped system, suggesting competition for water between trees and intercropped species. Unlike these authors, our tree fine roots contributed to decreasing soil water content also in deep soil layer as trees get older (Anderson et al., 2009, Bergeron et al., 2011).

These results evidenced the role of trees in facilitating water use optimization in AC plot thanks to root plasticity shaped by crop presence and management practiced on it. Deep layer drying may limit drainage and consequently nutrient leaching (Bergeron et al., 2011) during the autumn period thanks to the increase of the soil depth able to retain water. However, as we measured SWC only for one date, our conclusions need further confirmation from samples taken in spring and autumn to monitor the soil water dynamic during the tree vegetation growth period.

### 4.3. Spatial and temporal soil organic change along a chronosequence of young AC stands

Our results confirm the already largely documented theory that the SOM (Cardinael et al., 2017, Cardinael et al., 2015a) and their C/N ratio decreases with depth. Thanks to annual crop and plant residues, upper soil layer is rich in particulate and humified organic matter (Cardinael et al., 2015a). Some authors have shown that organic matter in deep soil is mainly composed of very old materials (Balesdent et al., 2018) with a very small size (Cardinael et al., 2015a) and a C/N ratio close to those of microbial communities. Although environmental conditions in deep layers are less favorable for microbial activities (Gill and Burke, 2002), some studies showed that the supply of fresh organic matter from root activities and turnover may stimulate microorganism activities, and may induce a priming effect that contributes to decomposing old organic matter (Fontaine et al., 2007).

Along our chronosequence of young AC stands, we found no significant change in soil organic matter content either at different distances from trees, or at different depths except in the topsoil of our oldest stands (10–12 years) in tree rows. The amount and the timing of carbon sequestration in AC system are still debated in the scientific literature but authors agreed that several years - often a decade - are necessary to detect changes in SOM (Smith, 2004). We confirmed the conclusion of Clivot et al. (2020), Oelbermann et al. (2006) and Peichl et al. (2006) showing that changes in SOC in temperate young alley-cropping are only expected to occur after at least 10 years of establishment. Pardon et al. (2017) observed higher SOC content in the 0–23 cm soil layer close to tree rows compared to crop plot control in mature AC stands (15–47 years old) whereas no significant SOC variation in relation to the distance from the tree row was found in young stands (< 5 years old). However, Thevathasan and Gordon (1997) found a 35 % relative increase in SOC (0–15 cm soil layer), within 2 m distance from poplar trees on an alley cropping field in southern Ontario (Canada) 8 years after establishment. Chatterjee et al. (2018) showed a significant higher SOM in 0–5-year-old stands compared to cropland and Beuschel et al. (2019) demonstrated that AC are able to enhance SOC at 0–5 cm depth in tree row within 5–8 years old. As our sampling in topsoil was carried out at 0–30 cm depth, our results did not evidence such changes. The relevance of tree rows in the organic matter sequestration was already reported in AC plots (Cardinael et al., 2015a). Authors often argue that the input of organic matter via tree (litter, fine roots and exudate) or understory vegetation strips is an important explanatory variable (Bambrick et al., 2010, Battie-Laclau et al., 2020, Cardinael et al., 2017, Oelbermann et al., 2004, Oelbermann and Voroney, 2007).

We also found that the SOM was positively correlated with tree fine roots abundance particularly for oldest stands in topsoil and in tree rows (Figs. 5D and 7C). This suggests that tree fine roots partially contribute to increased SOM. These results are consistent with the Sierra and Nygren (2005) studies, which demonstrate that carbon sequestration in AC is correlated with tree root biomass and with those of Germon et al. (2016) asserting that organic matter supply to the soil may result from tree roots inputs. The significant link between SOM and TFRA that we observed showed the capacity of old AC stand to favor carbon sequestration. The increase of SOM was not seen earlier in the chronosequence because the process may be slow, but root mortality and turnover contribute as trees get older. Cardinael et al. (2015a) found that soil organic C stocks were increased by 6 Mg C ha$^{-1}$ at 1 m depth in a 18-year-old AC stand compared to an agricultural plot. We did not detect any change in SOM at deep soil layers even in tree row probably because the amount of organic matter inputs from roots was not enough to induce a significant increase within a higher soil volume than the topsoil. Increase in soil organic matter in deep soil layer takes more time as described by authors above (Cardinael et al., 2015a). In other situations, a decrease in soil organic matter was even noticed due to increase of soil bulk density at deep soil layer in AC compared to monocrop and due to priming effect (Upson and Burgess, 2013).

From our findings, we conclude that thanks to supplies of fresh organic matter from trees and understory vegetation strips, the SOM may increase early in topsoil along tree rows in AC, but the sequestration in deep soil layer should take more time and is not systematic.

### 4.4. Tree fine roots distribution and soil mineral N content

We observed that regardless of the stand age group and the distance from tree rows, SMN decreased with soil depth and was mainly as nitrate. High values recorded in topsoil certainly came from residual fertilizers not valorized by crop and could be related to the amount of applied N. As samplings were taken in summer i.e. almost at crop harvest before the drainage period, most of the SMN has not yet been transferred to the deep soil layers. The dominance of nitrate form in SMN was already shown in cropland (Jeffery et al., 2010) and indicates that this ecosystem is favorable to the development of nitrifying microbial communities (Shen et al., 2008). In fact, alkaline and low C/N ratio soils are known to favor nitrification activities (Andrianarisoa et al., 2009, Falkengren-Grerup et al., 1998, Janssen, 1996). The high and continuous input of mineral N from fertilizers in cropland may favor the bacterial-pathways of organic matter decomposition (Jeffery et al., 2010), thus ammonium is quickly transformed into nitrate (Andrianarisoa et al., 2016).

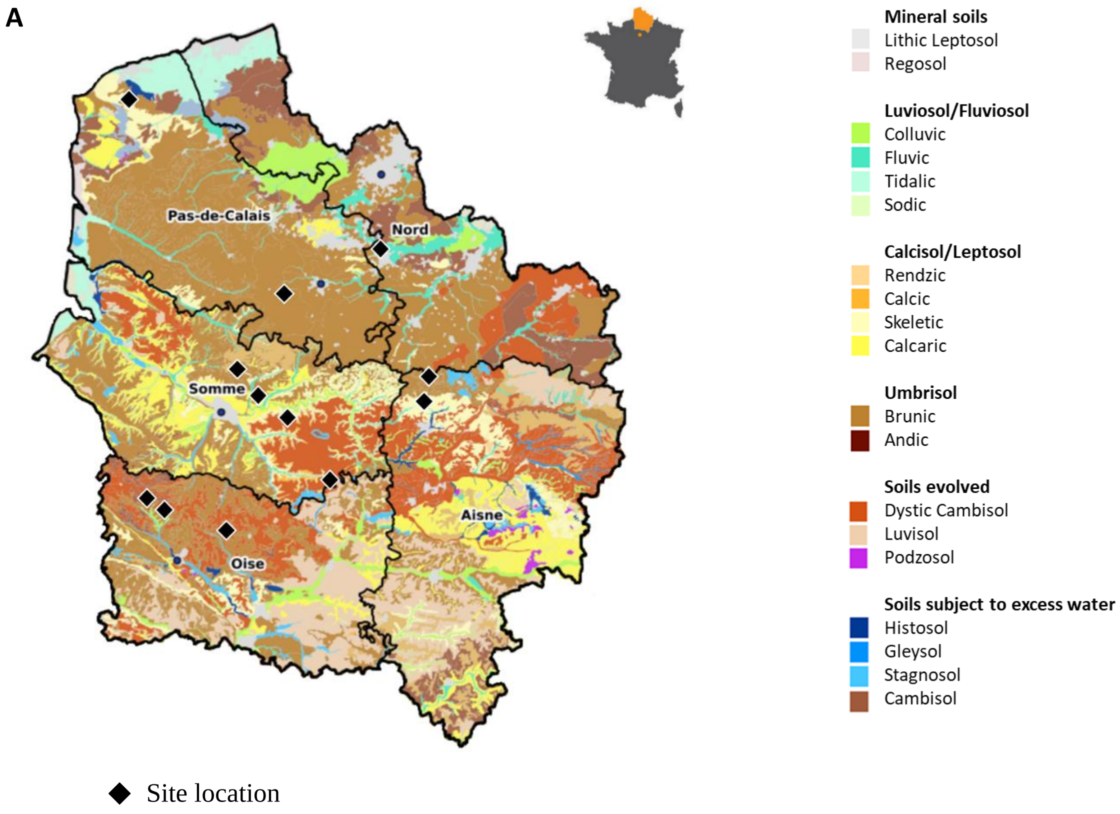
We did not observe significant variation of SMN with stand age group or distance from tree row. Beaudoin et al. (2005) showed that values of SMN measured in summer at crop harvest was almost explained by year and crop types. They explained that the excess of SMN observed at harvest are correlated to the excess of fertilizer. In our case, fertilizer falling on tree rows due to the absence of a barrier on the fertilizer spreader may favor excess of SMN in summertime because it is not valorized by trees and should exceed the need of understory vegetation strips. In the same way, the weak relationship between SMN and TFRA along the whole soil profile may be explained by the period of sampling. The impact of trees on SMN would be significant later in the season after a period of nitrate transfer in deep soil layer by autumnal rainfall. It was reported that the presence of trees in cropland within AC contributes to limiting N leaching (Bergeron et al., 2011, Rowe et al., 2001, Udawatta et al., 2002) because they are able to intercept soil nitrate that was not valorized by crops and transferred out of their rooting zone (Rowe et al., 1999). Andrianarisoa et al. (2016) showed the negative relationship between tree fine root biomass, distance from tree row and SMN but they measured SMN in late autumn during drainage period.

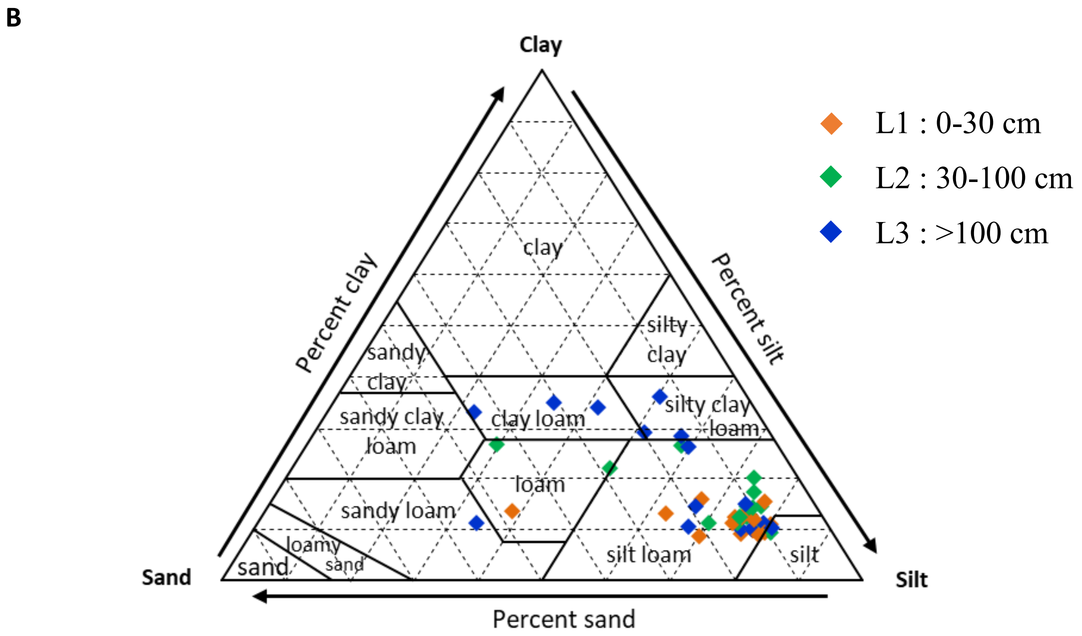
Unlike SMN, we found a significant effect of stand age group, distance from tree row and soil layer on SAN. It increased with stand age particularly close to trees rows in topsoil. The high presence of ammonium in soil may be explained by either a higher soil N ammonification, a lower soil N nitrification or a high microbial immobilization of nitrate favored by labile C compounds released by tree roots. Nevertheless, the process should be induced partially by the presence of tree fine roots in the soil because we found positive significant relationship between TFRA and SAN particularly in tree row, at 1 m distance, in old stand and at layer 2. We did not measure the microbial biomass nor diversity, but we speculate that by their activities, tree fine roots are able to change microbial composition by stimulating or inhibiting the activity of ammonifier or nitrifier populations (Andrianarisoa et al., 2017, Laffite et al., 2020) thanks to a specific compound released in rhizosphere. Tree fine roots also may imbalance the proportion of nitrate and ammonium by taking up more nitrate assuming that our three tree species have a preferential nitrate uptake. Jeffery et al. (2010) showed a more diverse microbial population in woody land compared to arable land where nitrifiers dominated. This microbial diversification may (i) either reduce nitrifier population, nitrification activity and favor $NH_4^+$ accumulation or (ii) promote ammonifier communities (Ribbons et al., 2016). Bradley and Fyles (1995) and Ehrenfeld et al. (1997) reported a positive effect of tree living roots on net soil ammonification. Conversely, a high soil nutrients availability may also induce a positive local response of tree fine root biomass evidenced by Mulia et al. (2010). Finally, with its positive charge, ammonium may be adsorbed on soil negative site from clay-humus complex and consequently reduces its mobility and transfer in groundwater.

## 5. Conclusion

From this study, we succeeded in evidencing that, from 5 years of intercropping, trees in AC develop lateral deep fine roots and start to explore zones below crop rooting most likely due to soil tillage and crop rotation. As trees get older, the fine root plasticity in AC may contribute to a best use of deep-water resources along profile. In the same way, their activities modified the mineral nitrogen dynamic, promoted the ammonification process and may reduce nitrate leaching caused by N fertilizer excess. We also observed an increase in soil organic content in topsoil in tree rows for old stands that favor soil carbon sequestration thanks in part to tree fine roots and plant residues. However, this process is slow particularly in deep layers. Unfortunately, our chronosequence was limited to 12-year-old stands but additional research with extending stand ages would allow us to validate whether the deep lateral tree fine root development that we began to evidence for young stands will be confirmed. Further studies are necessary to assess the extent and the timing of competition or facilitation between trees and crops for water and nitrogen, at which depth and which distance from tree rows. Finally, our results seem to highlight a shift from microbial communities in favor of ammonifier populations or an inhibition of nitrate production due to tree roots activity. It should improve soil health by increasing microbial diversity and reducing the mobility of N and its potential loss in groundwater. Further works are needed to confirm these assumptions.

## 6. Supplementary material





**Appendix 1:** Description of the study territory, sites sampling and soil texture: (A) Soil map with location of farmers (black diamonds) participating in the study (soil classification, FAO, 2015. Map is used with the courtesy of "*Groupement d'Intérêt Scientifique sur les Sols (GIS Sol) and Réseau Mixte Technologique Sols et Territoires", Pédologie – Les sols dominants de France métropolitaine, 2019*); (B) Representation of soil texture from seventeen plots on USDA triangle (L1 to L3 mean soil layers 1 to 3).

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Chapitre 3

## How do four-year-old intercropped trees share soil water with wheat in temperate alley-cropping experimental site: evidence from $^2H_2O$ artificial labeling?

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### Abstract

Despite numerous studies investigating competition and/or facilitation for soil water resources in alley-cropping systems (AC), share of water at the early stage of trees establishment in AC has been poorly examined. This work aimed to explore the water share between crops and trees after four years of tree establishment in AC at the Ramecourt block design alley-cropping experimental site. In mid-spring, we injected 300 mL of a 10 % deuterated water at 30, 50 and 100 cm soil depth at 1.5 m distance from a referent tree (alder, hornbeam or wild cherry) in AC, in pure-forest control plot with ryegrass (FC) and in a randomly chosen area in monocrop wheat control (CC) plots. The tracer uptake was monitored by collecting tree leaves and wheat and ryegrass (RG) whole-plant samples every two weeks in labeled and unlabeled area. For deuterium natural abundance analyses, the global mean of $\delta^2$H was significantly lower for wheat (- 44 ± 4 ‰) than RG (- 27 ± 6 ‰) and trees (- 20 ± 3 ‰), indicating that the most active sites of water absorption were different between these species. The mean wheat $\delta^2$H was 2481 ± 523 ‰, 715 ± 218 ‰, and 133 ± 68 ‰at 30, 50 and 100 cm labeling depth respectively. It was significantly higher in AC (2883 ± 585 ‰) compared to CC (1131 ± 274 ‰) only at 30 cm labeling depth. For trees, the $\delta^2$H of labeled samples remained negative unlike wheat. Particularly in AC, Alder and Wild cherry presented significant $\delta^2$H 15 and 45 days after labeling respectively from 50 and 100 cm labeling depth. We concluded that trees and wheat up take their water in upper soil layer but in AC, trees favored wheat water absorption in topsoil and were able to flexibly shift their water source from deep layer in case of low water availability in upper soil layer.

## Key words

Alley-cropping system, water sharing, deuterium labeling, cryogenic vacuum distillation, wheat

## 1. Introduction

In temperate alley-cropping systems (AC), the reintroduction of tree rows in arable land may be a way to cope with environmental issues such as climate change, soil erosion, biodiversity declining, water and air pollution while maintaining high agricultural productivity. Despite high overall biomass productivity (Arenas-Corraliza et al., 2018; Graves et al., 2007), it was often observed that due to above (Artru et al., 2017) and/or belowground interaction between associated plants, crop yield was lower especially close to tree rows in AC compared to monocrop systems (Pardon et al., 2018). The belowground effect on yield was primarily attributed to water competition (Gao et al., 2013; Hall et al., 2002; Miller and Pallardy, 2001; Unkovich et al., 2003) particularly during drought years (Kowalchuk and Jong, 1995) but competition for nutrient was also observed (Newman et al., 1998; Yun et al., 2012). For instance, Jose et al. (2000) compared soil water availability in AC between systems with and without root barrier installed close to tree row and showed that the presence of a barrier increased soil water availability in the crop alley. In absence of barrier, tree roots colonized the top 30 cm soil layer until the crop alley and shared water with maize. Comparative results were also obtained by Hou et al. (2003) where root pruning decreased tree water uptake and increased the crop biomass. They found higher soil water content (SWC) in the tree root-pruned plots compared to non-pruned plots. The extent of soil water depletion due to the sharing between trees and crops in AC systems was observed up to 1.5 m (Gao et al., 2013) from the tree row for young stands to the middle of alley for old stands (Jose et al., 2000) and until 1 m depth (Gao et al., 2013; Jose et al., 2000). Competition for water between trees and crop has been confirmed in adult AC stands, but studies for young stands are scarce. In a 7-year-old stand, Huo et al. (2020) found that trees absorbed high proportion of water up to 120 cm depth when SWC was high. It was shown that during their first year of establishment, trees in riparian zone depend on water in the upper soil layers, explaining their competition for water in topsoil, whereas mature trees used water from deeper strata (Dawson and Ehleringer 1991).

Despite the water competition between trees and crops, some authors showed that facilitation and complementarity for resources capture between species may be possible in AC (Cannell et al., 1996; Malézieux et al., 2009). Indeed, a spatial and temporal niche differentiation between trees and crop roots (Forey et al., 2021; Livesley et al., 2004), may not only limit competition but enhance water valorization (Everson et al., 2009; Fernández et al., 2008) throughout the soil profile. The improved water use in mixed systems is the result of root niche differentiation: one species with a shallow root distribution and another species with a deeper rooting system. Regardless of the type of agricultural system, Asbjornsen et al. (2008) showed that annual crops, herbaceous perennial plants, woody shrubs and trees extracted most of their water from the upper soil layer in early spring when the SWC was high. Then, later in the growing season, when rainfall declined and water became less available, the water uptake depth of these species increased with a maximum depth of 20 cm for the herbaceous species, 55 cm for the shrub species, and below 150 cm for the tall trees (Asbjornsen et al., 2008). The associated species extracted water from different depths in the soil profile, indicating complementarity in water uptake patterns. Similar results were observed by Everson et al. (2009) for good rainfall season, showing no significant difference of SWC in upper soil layer in maize area and in tree rows which indicated an absence of competition with crops. They suggested a complementary utilization of water between shallow-rooted and deep-rooted crops. However, Everson et al. (2009) highlighted the importance of tree species selection in the system. Indeed, among their two indigenous species, one of them was more competitive for water in topsoil. According to their root morphology and plasticity, some tree species are adapted to AC and others should be avoided. For example, the hybrid walnut developed deeper fine roots in AC than in forest systems (Andrianarisoa et al., 2016), and the ponderosa pine used less shallow water in AC than in monoculture (Fernández et al., 2008). Finally, thanks to tree deep roots, AC could also promote water redistribution along the soil profile through hydraulic lift or shared mycorrhizal networks (Bayala and Prieto, 2020).

Artificial labelling using a stable isotope of water ($^2$H or deuterium) as a tracer has proven utility for studying water uptake dynamic of plants from different soil layer (Beyer et al., 2016; Huo et al., 2020). Both analyses of artificial and natural water stable isotopes offer a reliable and efficient method allowing to identify the zone of trees and crops water uptake but also to characterize and quantify the share of water between species from natural or artificial isotopic signature (Beyer et al., 2018; Grossiord et al., 2014; Penna et al., 2020; Wang et al., 2023; Wu et al., 2017). For example, using artificial stable deuterated water, Huo et al. (2020) succeeded to show that compared to monocropped jujube trees in some extremely dry periods, intercropping resulted in jujube trees absorbing deeper water below the main root overlap layer whereas they obtained higher proportions of water in shallow layer during wet periods. Wu et al. (2017) studied water sharing in rubber-tea agroforestry using natural stable isotopes of water and evidenced that tea primarily took up water from shallow soil layer, whereas rubber mainly absorbed water from deep soil. Huo et al. (2018) analysed natural abundance of $^2$H and $^{18}$O of xylem water sap in jujube trees in China and revealed that 4-year-old jujube trees mainly used soil water from 0-20 cm and 20-60 cm layers, 8-year-old trees shifted flexibly their water sources between 0-20 cm, 20-60 cm, and 60-200 cm layers according to SWC in each soil layer, whereas 15- and 22-year-old trees primarily used water from the deep soil layers, especially when precipitation was low.

Despite numerous studies investigating competition and/or facilitation for soil water resources in alley-cropping systems (AC), share of water at the early stage of trees establishment in AC has been poorly examined. Early investigations of these mechanisms are necessary to provide optimum strategies for managing intercropping systems. The aim of this work was to explore the share of water between crops and trees after few years of tree establishment in AC. We assume (i) that the water sharing between associated plants in AC would start early following tree establishment in AC, especially in the topsoil close to the row, in a cohabitation zone of crop and tree fine roots, and (ii) that artificial $^2H_2O$ labeling allows to evidence and quantify this sharing.

## 2. Materials and methods

### 2.1. Description of the experimental site

The experiment was carried out on a plot of 18 ha of the SCEA Dequidt located in Ramecourt in northern France (50°22’N, 2°17’E) (Andrianarisoa et al., 2019). The climate is oceanic with an average annual temperature of 10.6°C and an average annual rainfall of 859 mm recorded from 2010 to 2020 at Humières weather station located at 6.7 km from Ramecourt. The site is windy with an annual average speed of 3.8 m $s^{-1}$ mainly from SW-NE direction. The soil is a deep luvic cambisol (Baize and Girard, 2009) with flint (< 3 % in upper layer) developed in loess deposits. The subsoil is composed of a red clay-with-flints itself resulting from the weathering of the underlying chalk with flints deposited from the upper cretaceous period (Sterckeman et al., 2006). The mean slope is about 4 % from NW-SE with a maximum level of 8 % in the middle of the plot. The chalk layer appeared at 2 m depth in the middle of the plot whereas it was noted at 3.7 m in the top (North) and in the bottom (South) of the plot. In 2018, before site set up, 20 soil cores down to 90 cm depth were carried out and physico-chemical soil parameters were determined according to standard methods of soil analysis (Table 1). The soil texture in upper layer was a silt loam with 73 % of silt, a pH of 7.9, and an organic matter content of 2.1 %. The soil clay content increased with depth from 15 % to 21 % at 30 cm and 90 cm depth respectively (Table 1) as well as the soil cation exchange capacity. Most of chemical soil parameters measured were the highest in upper soil layer and decreased with depth (Table 1).

Prior to the initiation of this study, the plot had always been an arable land cultivated with the following crop rotation: potato/wheat/sugar beet/barley/wheat/flax. About 12 Mg $ha^{-1}$ of green compost is applied before potato and 5 Mg $ha^{-1}$ of poultry manure before sugar beet. Soil tillage is done at 22 cm only before these last crops. A cover crop is sown between two main crops if the period between the harvest of the previous crop and the sowing of the next one is higher than 60 days. It is incorporated in the soil at the beginning of winter or before the winter crops sowing. Cereal straws are always exported whereas residues from other crops are ground and buried in soil. Due to the high amount of silt, the soil is highly affected by channel erosion, and sediments from runoff leave the plot in its corner SE (Figure 1A), especially during potato and sugar beet cropping.

**Table 1:** Some soil parameters measured before tree planting in July 2018 at Ramecourt alley-cropping experimental site.

| Soil parameters | Unit | Method of analysis | Soil layer (cm) | | |
|---|---|---|---|---|---|
| | | | 0-30 | 30-60 | 60-90 |
| **Physico-chemical parameters** | | | | | |
| Clay | % | NF X 31-107 | 15 (±2) | 19 (±3) | 21 (±5) |
| Silt | % | NF X 31-107 | 73 (±2) | 70 (±3) | 69 (±7) |
| Sand | % | NF X 31-107 | 12 (±2) | 11 (±3) | 11 (±3) |
| $CaCO_3$ | % | NF EN ISO 10693 | 2.8 (±) | 1.1 (±) | 0.75 (±) |
| pH | | NF ISO 10390 | 7.9 (±0.2) | 7.9 (±0.3) | 7.9 (±0.2) |
| | | | | | |
| $^{\#}\theta m$ at pF2.5 | % | (Wösten et al., 1999) | 21.4 | 21.7 | 22.1 |
| **Organic matter** | | | | | |
| SOM (2 x OC) | % | NF ISO 14 235 | 2.1 (±0.5) | 1.0 (±0.3) | 0.6 (±0.2) |
| Total N | % | NF ISO 11261 | 0.11 (±0.02) | 0.06 (±0.02) | 0.04 (±0.01) |
| C/N ratio | | | 9.8 (±0.4) | 8.9 (±0.7) | 8.1 (±0.6) |
| Olsen P | mg $kg^{-1}$ | NF ISO 11263 | 50 (±13) | 19 (±9) | 16 (±6) |
| CEC | cmol $kg^{-1}$ | NF X 31-130 | 9.8 (±1.6) | 10.4 (±1.4) | 10.5 (±2.2) |
| **Exchangeable cations and CEC** | | | | | |
| $K^+$ | mg $kg^{-1}$ | NF X31-108 | 155 (±48) | 120 (±42) | 103 (±20) |
| $Mg^{2+}$ | mg $kg^{-1}$ | NF X31-108 | 76 (±7) | 75 (±12) | 53 (±14) |
| $Ca^{2+}$ | mg $kg^{-1}$ | NF X31-108 | 2861 (±689) | 2487 (±642) | 2592 (±616) |
| **Oligoelements** | | | | | |
| B | mg $kg^{-1}$ | Boiling water extraction | 0.8 (±0.3) | 0.3 (±0.2) | 0.2 (±0.1) |
| $Cu^{2+}$ | mg $kg^{-1}$ | NF X31-120 | 2.7 (±0.6) | 1.4 (±0.3) | 1.1 (±0.3) |
| $Mn^{2+}$ | mg $kg^{-1}$ | NF X31-120 | 45.2 (±21) | 23.9 (±7) | 19.3 (±12) |
| $Zn^{2+}$ | mg $kg^{-1}$ | NF X31-120 | 5.2 (±2) | 2.2 (±1) | 3.0 (±4) |

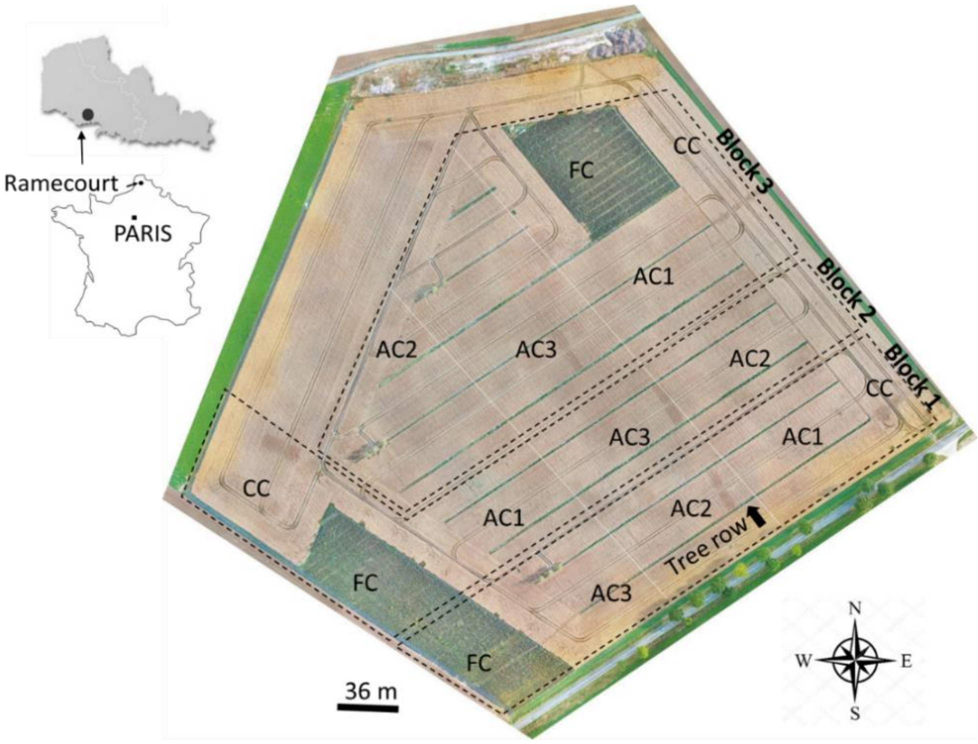
*$^{\#}\theta m$ means gravimetric soil moisture*

The experimental plot is a randomized block design with three replications. Within each block, three modalities of AC (AC1, AC2 and AC3) are compared with sole-crop (CC) and pure-forest (FC) control plots (Figure 1A). The size of subplots within each block was about 1 ha and 0.6 ha for AC and FC respectively. Throughout this manuscript, modalities AC, CC and FC were hereinafter referred to as "type of system". In November 2018, after the harvest of sugar beet, tree rows were delineated using an auto-guidance tractor equipped by a RTK-GPS-based system (Real Time Kinematic – Global Positioning system). They were oriented SW-NE and were spaced 38 m and 7 m apart in AC and FC, respectively. A single-tine subsoiler pulled by tractor was used to fracture the soil to 0.4 m depth on each tree row. Thereafter, one-year-old bare-root trees and shrubs were planted after digging holes with spades at 0.3 m depth. Landscape fabric (1 m by 1 m) made from organic materials was placed around each planted tree to limit weed proliferation and soil water evaporation. A 1.2 m-tall plastic tree shelter anchored with a wooden stake was installed to protect trees against wild animals and wind. Within each row, tall trees were planted 8 meters apart and were intercalated every 1 m by 9 species of shrubs (*Castanea sativa, Cornus sanguinea, Acer campestris, Euonymus europaeus, Corylus avelana, Tilia cordata, Ligustrum vulgaris, Salix alba and Viburnum lantana*) (Figure 1B).

The AC modalities are different from each other according to the presence or not of $N_2$-fixing tall tree species. The AC1 is an association of *Quercus robur* (oak) and *Carpinus betulus* (hornbeam), the AC2 is a mixture of *Juglans regia x regia* (hybrid walnut), *Alnus glutinosa* (alder) and *Prunus avium* (wild cherry) and the AC3 is a combination of *Alnus glutinosa* (alder) and *Robinia pseudoacacia* (black locust). The exact tree sequencing for each AC modality is presented in Figure 1B. Plant materials are from “La Cluse” nursery located 70 km from the site and their average height was between 80 and 100 cm except for willow (150 cm). Tall trees were planted two by two at 1 m apart but only one will be kept in the future. A strip of 2 m wide along the tree rows was not cultivated and spontaneous weeds were left to grow. In FC, each subplot was divided into 3 areas corresponding to the same 3 modalities of AC. A mixture of ten melliferous (*Anethum graveolens, Borago officinalis, Calendula officinalis, Centaurea cyanus, Fagopyrum esculentum, Malva sylvestris, Papaver rhoeas, Phacelia tanacetifolia, Raphanus sativus and Sinapis alba*) and three grass plants species (*Festuca arundinacea, Lolium multiflorum and Phleum pratense*) were sown between tree rows in FC in March 2019. Since 2020, *Lolium multiflorum* (hereinafter referred to as ryegrass or RG) dominated all sowed plants in FC. When considering only tall trees, the current density of plantation was 66 trees $ha^{-1}$ and 357 trees $ha^{-1}$ in AC and FC, respectively, but with shrubs, it became 263 trees $ha^{-1}$ and 1428 trees $ha^{-1}$ in AC and FC, respectively. Since 2019, the height of all trees was measured using a handmade measuring rod at the end of leaf fall in October.

In CC as well as in alleys of AC, the crop succession since tree plantation was: spring barley 2019/witloof chicory 2020/winter wheat 2021/flax 2022/winter wheat in 2023. This study was carried out during 2020-2021 crop cycle on winter wheat (*Triticum aestivum*) with the variety “Extase” (https://www.geves.fr/catalogue/). Winter wheat was sown in October 2020 following the chicory witloof harvest and was harvested in August 2021 with an average yield of 9.5 Mg ha$^{-1}$ on the whole cultivated area. Winter wheat in CC and AC received 220 kg N ha$^{-1}$ as solid mineral fertilizer N divided into four applications, 50 kg N ha$^{-1}$ (Sulfan 24% of N), 90 kg N ha$^{-1}$ (Ammonitrate 27%), 50 kg N ha$^{-1}$ (Ammonitrate 27%) and 30 kg N ha$^{-1}$ (Ammonitrate 27%) carried out on February 25th, March 23rd, April 24th and June 2nd, respectively. Fertilizer N was applied using solid fertilizer spreader. As no barrier was installed on spreader during application, part of fertilizer in AC falls on uncropped understory vegetation strip. No fertilizer was applied in FC treatment since tree plantation. While weeds and pests were chemically controlled in AC and CC, no pesticides were applied in FC but ryegrass on interrow space was cut twice a year using tow-behind lawn mowers. Finally, no irrigation was applied neither in cropped area nor in FC treatments. After wheat harvesting in summer, a cover crop composed of *Phacelia tanacetifolia*, *Eruca sativa* and *Trifolium* sp. was sown on August 25th, 2021, and was mechanically destroyed and buried in January 2022.

**A**



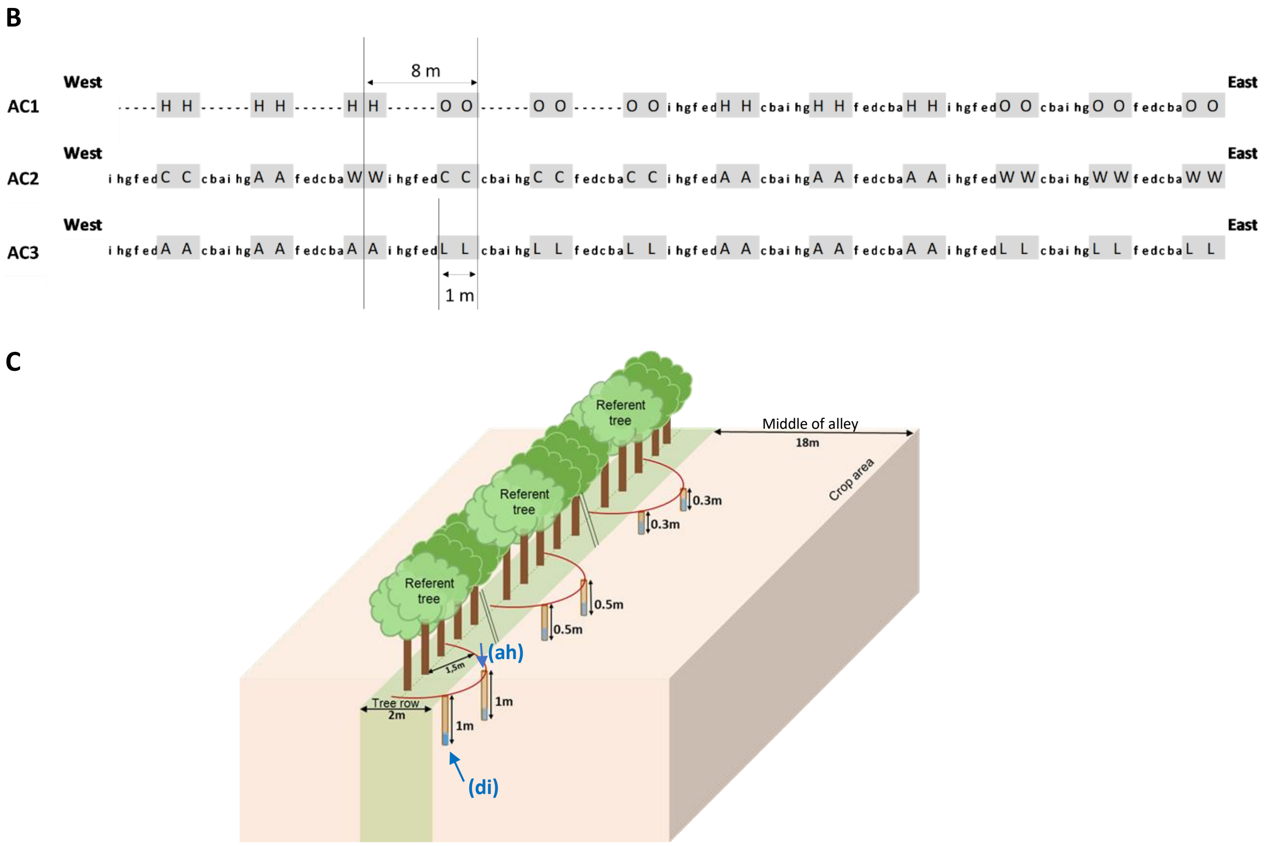


**Figure 1:** Schematic representation of ²H labeling experiment at the Ramecourt alley-cropping experimental site: (A) plot location and details of randomized experimental block design; (B) tree sequencing within rows in AC1, AC2, AC3 and FC and (C) example of $^2$H labelling experiment within a given block for one tree species. In each block, three alley-cropping modalities AC1, AC2 and AC3 are compared with pure-forest (FC) and sole-crop (CC) control plots. For tree sequencing, letters in upper and lower case represent the 6 tall trees (O = oak, H = hornbeam, L = black locust, A = alder, W = hybrid walnut, C = wild cherry) and 9 species of shrubs (***a.*** *Castanea sativa*, ***b.*** *Cornus sanguinea*, ***c.*** *Acer campestris*, ***d.*** *Euonymus europaeus*, ***e.*** *Corylus avellana*, ***f.*** *Tilia cordata*, ***g.*** *Ligustrum vulgare*, ***h.*** *Salix alba*, ***i.*** *Viburnum lantana*) respectively. The last scheme about labeling experiment is not in scale: (di) and (ah) mean deuterium injection and auger-drilled holes respectively.

## 2.2. Deuterium labeling experiment

Deuterium labeling experiment was done over 5 days from May 10th to 20th, 2021 during non-rainy days and when tree leaves were completely developed. Three tree species with contrasted growth rates (hornbeam, wild cherry and alder) were selected from woody type of system (AC and FC). For each species, three trees were randomly chosen within each block and were assigned to three labeling depths: 30, 50 and 100 cm (Figure 1C). As these tree species were planted in pairs at 1 m distance (Figure 1B), deuterium labeling was done with the easternmost one. To increase the probability of the presence of tree roots in the labeled zones, two holes instead of one at 1 m apart were drilled to the target depth at the day of $^2H_2O$ injection, using a hand auger (Φ = 6.3 cm) at 1.5 m radial distance from the south side of the tree trunk (Figure 1C). Then, a sufficiently long polyvinyl chloride pipe (PVC; Φ = 4 cm) was inserted into the hole at the target depth before injecting 300 mL of tracer solution (10 % atm $^2H_2O$, prepared by mixing 30 mL of 99.9 % atm $^2H_2O$ solution and 270 mL of deionized water). Deuterium enriched solution was provided by the Eurisotop (France). Considering that 300 mL would wet 400 $cm^3$ (Huo et al., 2020) for a soil at almost field capacity, the change in soil water content (SWC) caused by the added water should be negligible. After tracer injection, the PVC pipe was removed and soil cores that had been extracted during auger drilling were sieved at 4 mm and were replaced at the corresponding holes, by respecting the former different soil layers. In CC system, holes location were randomly chosen in the middle of the plot at the same depths (totalizing 6 holes per block for the 3 depths). A total of 126 holes were dug in the experimental site. Stakes were planted next to the holes to spatially locate them for further plant and soil sampling and their GPS coordinates were recorded. At labeling period, wheat was almost at the beginning of stem elongation (GS33; Zadoks et al., 1974).

Plant samples were taken at eight dates following the deuterium labeling experiment at almost 15 days of interval except in autumn: 28/05; 7/06; 17/06; 1/07; 16/07; 29/07;17/09; 08/10/2021. corresponding to 8; 18; 28; 42; 57; 70; 120; 141 days after labeling, from the last labeling day on 20 May. For trees, ten young leaves oriented toward the labeling area were taken from referent trees. For wheat and RG, tillers from four plants around each hole were collected. At each sampling date, leaves from unlabeled trees and wheat and RG tillers from unlabeled areas were also collected for control treatments. For sampling dates after wheat harvest, plant samples collected in cropped areas corresponded to the catch crop whole-plant. Plant samples were directly wrapped in cling film and aluminum foil to limit water loss and were stored at -20°C in laboratory before further analysis. A total of 156 samples were collected at each sampling date.

### 2.3. Soil sampling

Soil samples were collected at three sampling dates: in May before the labeling period, at the end of July before the wheat harvesting and in November at leaf fall. For woody type of system (AC and FC), soil cores were collected 1.5 m distance from the referent trees chosen in the labeling experiment down to 120 cm in the south side of tree row, using a manual auger ($\Phi$ = 6.3 cm). In CC, soil samples were randomly taken in the middle of each subplot. Soil samples were collected at the layers 0-30 (L1 or topsoil), 30-60 (L2) and 60-120 (L3) cm depth. They were sieved at 4 mm and stored at -20°C for samples collected in May and July and at 4°C for those collected in November. For each sampling date, soil water content (SWC) was determined by oven drying an aliquot of soil (20 g) at 105°C for 48 h. The soil water content at field capacity (pF2.5) was estimated using the pedotransfer function of Wösten et al. (1999). Values of soil bulk density was estimated from soil texture triangle as proposed by Jamagne et al. (1977).

## 2.4. Plant isotopic analyses

The natural and artificial deuterium abundance of tree leaves or wheat and RG whole-plant was determined by cryogenic vacuum distillation method. We first extracted water from 2 g of fresh plant through cold trapping with a custom-made static vacuum cryogenic distillation system (West et al., 2006). The water from plant materials was evaporated by heating the sampling tube in a water bath (65°C), then condensed with liquid nitrogen for 60 min in a collection tube. Plant samples were weighed before and after the extraction and again after oven-drying for 48 h (65°C) to calculate the extraction efficiency. Samples with an extraction efficiency below 97 % were discarded. The water content of plant samples was deduced from the mass balance between the mass of the remaining plant material after cryoextraction and oven-drying, and the extracted water. Then, the extracted water was analyzed for $^2H/^1H$ ratio using an elementary analyzer (EA, EuroPyrOH; EuroVector, Milano, Italy) coupled with an isotope-ratio mass spectrometry (Isoprime IRMS; Elementar, Manchester, UK) at the SILVATECH platform (SILVATECH, INRAE, 2018. Structural and Functional Analysis of Tree and Wood Facility, Nancy, France). The results were expressed as $\delta^2H$ (‰) relatively to the international Vienna standard mean ocean water (V-SMOW) following the equation 1:

$$\delta^2 H(‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000 \qquad (1)$$

where $R_{sample}$ and $R_{standard}$ represent the $^2H/^1H$ ratio of the sample and V-SMOW standard respectively (IAEA, 1995).

The mass spectrometer used in this study had a precision of ± 2.0 ‰ for $\delta^2H$ values. Deuterium analysis was started with unlabeled samples to avoid possible contamination during processes.

## 2.5. Statistical analyses

Bivariate linear models were performed to analyze the variability of SWC according to the equation 2:

$$y = ax_1 + bx_2 + c + \beta \quad (2)$$

where “y” is SWC, $x_1$ is the block, $x_2$ is either the type of system (AC, FC, CC), the sampling date, the tree species or the depth, “a”, “b” and “c” are coefficients and β the model error. An ANOVA followed by a multiple comparison test was carried out using the multcomp R software package (Hothorn et al., 2008) to compare the mean value of SWC for each $x_2$ variable using a post hoc Tukey’s test ($p < 0.05$).

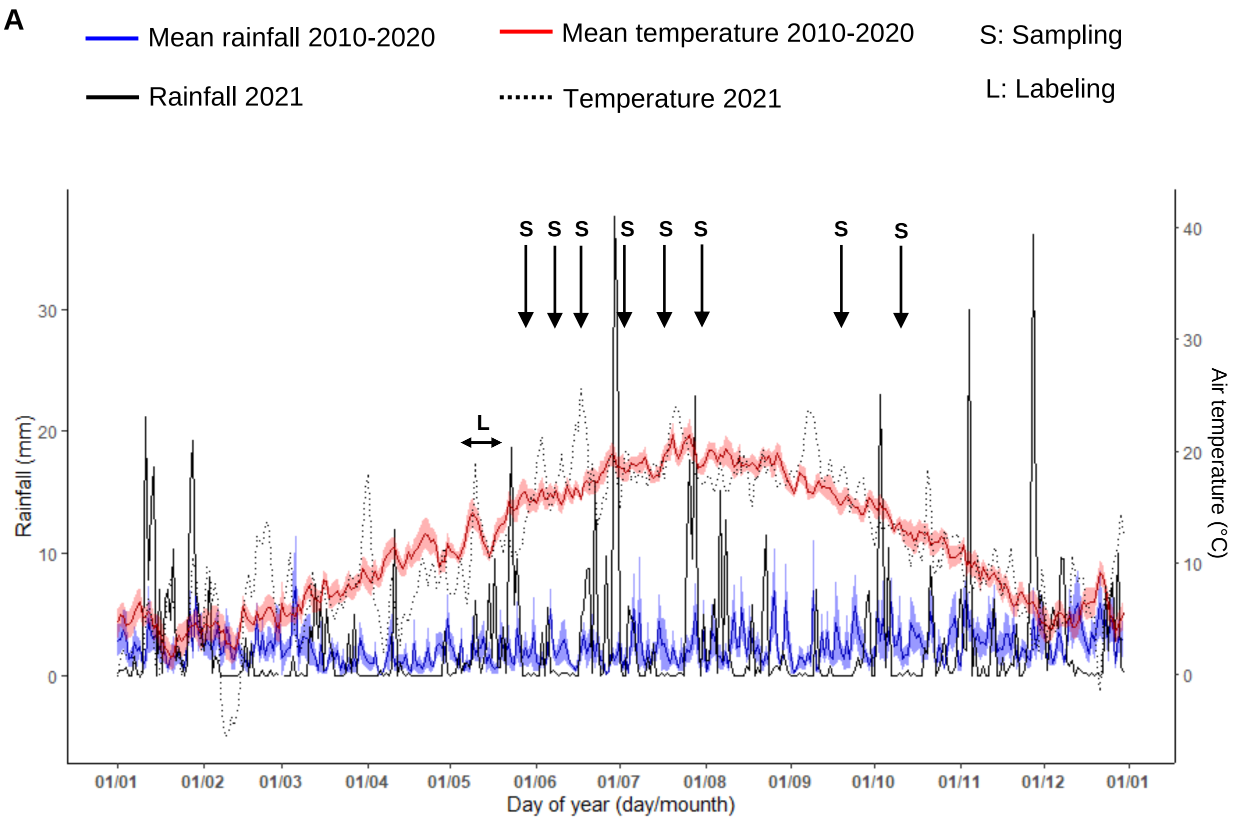
Then, to analyze the variation of tree foliar and wheat or RG whole plant $\delta^2H$ over time, between the type of systems or the associated tree species, the same model (equation 2) was run using $\delta^2H$ as the variable to be explained and the sampling date, the type of systems, or the associated tree species, respectively, as explanatory variables “$x_2$”. An ANOVA followed by a post hoc Tukey’s test ($p < 0.05$) was carried out to compare the sampling dates. For wheat and RG, labeled and unlabeled samples were analyzed separately. At each sampling date and each labeling depth, we compared the mean value of $\delta^2H$ between the type systems using an ANOVA, followed by a Dunnett’s test with CC as control reference ($p < 0.05$). Wheat in CC and wheat in all AC plot were referenced by the variables Wheat-CC and Wheat-AC, respectively. Then, for wheat in AC, at each sampling date and each labeling depth, differences in $\delta^2H$ between the associated trees were also tested with an ANOVA and subsequent Dunnett’s test with wheat in CC as control reference ($p < 0.05$). The wheat close to alder, to hornbeam or to wild cherry were referenced by the variables Wheat-AC-Alder, Wheat-AC-Hornbeam and Wheat-AC-Wild cherry, respectively. For RG, the same ANOVA was performed but using Tukey’s test for post hoc comparisons. The RG close to alder, to hornbeam or to wild cherry were referenced by the variables and RG-FC-Alder, RG-FC-Hornbeam, and RG-FC-Wild cherry. Finally, for trees, mean foliar $\delta^2H$ was compared between labeled and unlabeled samples in AC and FC using Student's t-test ($p < 0.05$) for a given tree species and a given labeling depth.

For all regressions, the symbol *** was used to indicate a significant determination coefficient at $p < 0.001$ level, the symbol ** for $p < 0.01$ level and the symbol * for $p < 0.05$ level. All statistical analyses were performed with R software version 4.0.4.

# 3. Results

## 3.1. Precipitation and soil water content evolution

By comparing with the average value of annual rainfall recorded from the last decade, the year 2021 was particularly wetter because the total amount of precipitation recorded was 966 mm (i.e 113 % higher than the mean of decade). In 2021, after a particularly wet start of year (200 % higher than last decade average precipitation) in January, several rain events occurred particularly from May to August compared to the mean of rainfalls between 2010 and 2020 (Figure 2). The monitoring of precipitation showed that the cumulative amount of rain fell on the plot was 50 mm from the end of the labeling to the first sampling (i.e. 8 days; Figure 2B). It decreased for samplings in June and was very high for samplings realized in July and September. During these four months (June-September), a total of 399 mm of rainfall was recorded in 2021, whereas the average for the last decades was 257 mm for the same period. By comparison with a normal year, summer 2021 was particularly wetter. The wheat harvest was delayed in August not because of grains immaturity but of the impossibility of combine harvester to access the plot due to a muddy soil.



**B**

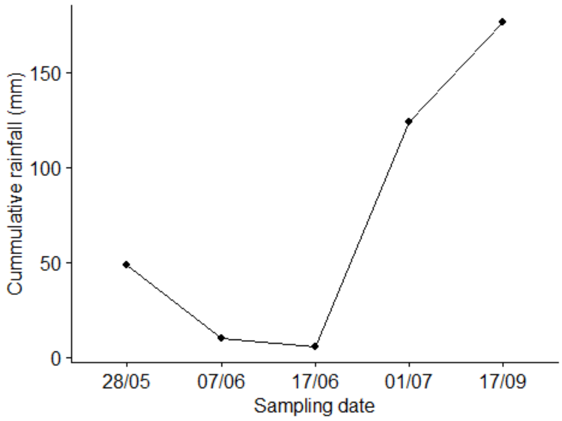
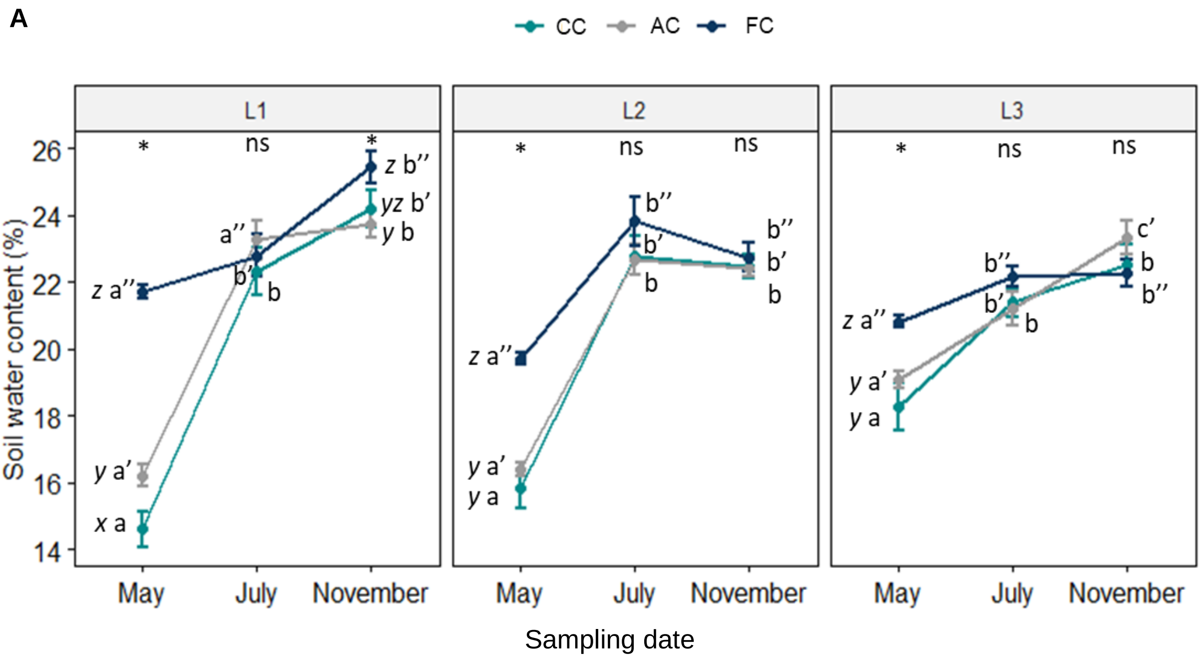


**Figure 2:** Mean daily fluctuation of rainfall (mm) and temperature (°C) during one year from Humières weather station located at 6.7 km of Ramecourt alley-cropping experimental site (A) and cumulative rainfall between sampling dates from May 20th, 2021 (B). Values in blue and red are means (± SE) of rainfall and temperature respectively, collected from 2010 to 2020. Continued and dotted black lines are rainfall and temperature respectively, collected in 2021. Horizontal arrow indicates the labeling (L) period and vertical arrows indicate the sampling (S) date.

Unlike the rainfalls, the average annual temperature in 2021 (10.3 ± 6°C) was close to the mean of decade (10.6 ± 5°C), even from May to August with a mean temperature of 15.4 ± 3 °C and 15.8 ± 2 °C on the 2010-2020 average. However, we noticed three heat peaks on June 2nd, 9th and 16th 2021, reaching 19.6, 18.1 and 23.5°C respectively, whereas at the same dates, the average temperatures over the last decade were 15.3 ± 3, 14 ± 3 and 14.5 ± 1°C, respectively. On average, the SWC in all soil profile down to 120 cm was significantly lower in May with a mean value of 18 ± 3 % compared to 22 ± 2 % and 23 ± 2 % in July and November respectively. In May, the mean value of SWC was equal to 18 ± 3 % in topsoil and in L2 and was significantly lower than in soil layer L3 (20 ± 1 %). Conversely, the mean value of SWC in July was higher in topsoil and in L2 (23 ± 2 %) than in L3 (22 ± 2 %). Finally, in November, SWC in topsoil (24 ± 2 %) was significantly higher than in L2 and L3 (23 ± 2 %).

In May, regardless of the depth, the mean value of SWC was significantly higher in FC (21 ± 2 %) compared to CC (16 ± 2 %), AC was intermediate (17 ± 2 %). When we considered separately soil layers, same differences were observed in topsoil. The SWC significantly increased with depth only in AC and CC (Figure 3B). In July and November, no significant differences in SWC were observed between the type of systems regardless soil layers with a mean value of 23 ± 2 %. Finally in November, the SWC was on average significantly higher in

L1 than in L2 and L3. Whether there was no significant difference of SWC between all soil layers in CC and AC, SWC was significantly higher in topsoil than soil L2 and L3 in FC.



**B**

| Sampling date | May | | | July | | | November | | |
|---|---|---|---|---|---|---|---|---|---|
| | Soil layer | | | | | | | | |
| | L1 | L2 | L3 | L1 | L2 | L3 | L1 | L2 | L3 |
| CC → | A | AB | B | A | A | A | A | A | A |
| AC → | A | A | B | B | AB | A | A | A | A |
| FC → | C | A | B | A | A | A | B | A | A |

**Figure 3:** Variation of the soil water content (A) in alley-cropping (AC), sole-crop (CC) and pure-forest (FC) control plots in soil layers L1 (0-30 cm), L2 (30-60 cm) and L3 (60-120 cm) and results of Tukey's test ($p < 0.05$) to compare soil layer for a given type of system and a sampling date (B). Data are means and bars are standard errors. Lowercase letters compare the sampling dates for a given type of system and soil layer; italic letters compare the type of systems for a given sampling date and soil layer ($p < 0.05$). Letters indicate homogeneous groups: means with same letters are not significantly different. Horizontal arrows show the direction of reading for statistical means comparisons.

The SWC values increased significantly between May and November in all systems and in all soil layers particularly between May and July in AC and CC (Figure 3A). There was no effect of tree species on the SWC regardless of type of system or different soil layer or the sampling date (data no shown).

### 3.2. Wheat and RG $\delta^2$H values

The mean $\delta^2$H for unlabeled samples of wheat and RG was negative, with a value of -37 ± 5 ‰. On average for all data, it was significantly lower for wheat (-44 ± 4 ‰) compared to RG (-27 ± 6 ‰). During the growing period, $\delta^2$H values for unlabeled wheat and RG were almost stable over time (Figure 4A) and were significantly higher for RG than wheat only at the first two sampling dates: 28/05 and 07/06 (Figure 4A and 5A). The mean value of $\delta^2$H natural abundance was not significantly different ($p < 0.05$) between wheat cultivated in AC and CC regardless the sampling date and the tree species (Figure 5A). In the same way, no significant difference was observed for whole-plant wheat $\delta^2$H in AC between tree species (Figure 5C). In FC for the natural abundance, the mean value of $\delta^2$H including all sampling dates was significantly higher in RG associated with wild cherry (-7 ± 6 ‰) than with hornbeam (-37 ± 3 ‰) and alder (-36 ± 4 ‰). These differences were due to high values observed in RG associated with wild cherry for samples collected at 17/06 (Figure 5E).

For labeled samples, the mean $\delta^2$H of whole-plant wheat and RG was positive for all sampling dates and regardless of the labeling depth. It was 1096 ± 400 ‰ and 378 ± 133 ‰ for wheat and RG respectively. The $\delta^2$H of labeled wheat and RG samples increased from 8 to 18 days after labeling and reached 1945 ‰ and 740 ‰ in wheat and RG respectively. Then, $\delta^2$H decreased until the 01/07. The mean value of $\delta^2$H for labeled wheat was significantly higher than RG only 28 days after labeling (Figure 4B).

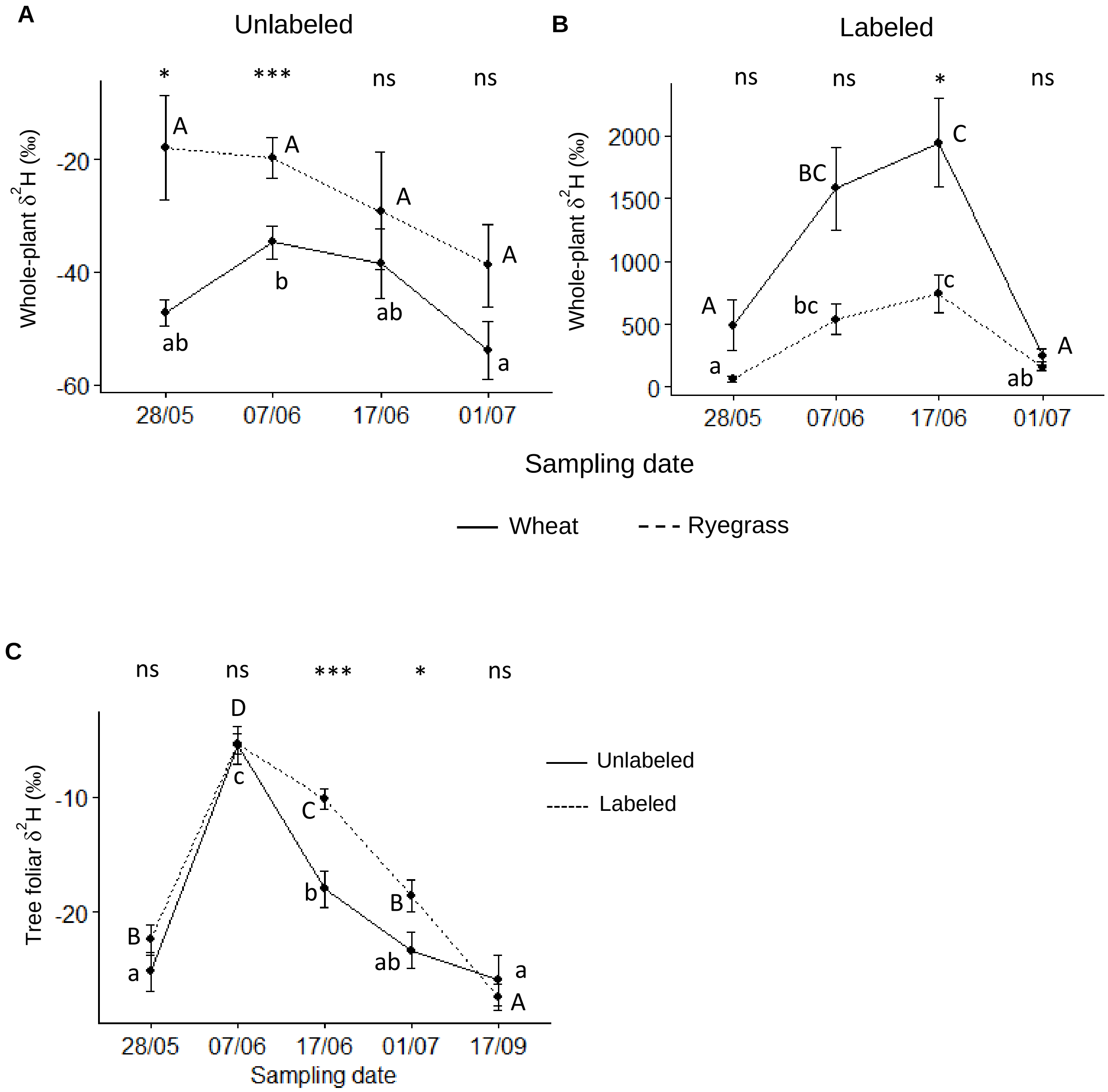


**Figure 4:** Evolution of mean values of $\delta^2$H (‰) over time in aerial tissues of unlabeled (A) and labeled (B) wheat and RG samples, and tree leaves (C). Data are means including both AC and CC for wheat, only FC for ryegrass and both AC and FC for tree leaves. Bars are standard errors. Letters indicates homogeneous groups from Tukey's test results ($p < 0.05$). Means with same letters are not significantly different. For graphics (A) and (C), symbols *, *** and ns indicate results of Student's test between unlabeled wheat and RG whole-plant $\delta^2$H and between labeled and unlabeled tree foliar $\delta^2$H at each sampling date ($p < 0.05$, $p < 0.001$ and not significant respectively); for graphic (B) they represent Mann-Whitney's test between labeled wheat and RG whole-plant $\delta^2$H at each sampling date.

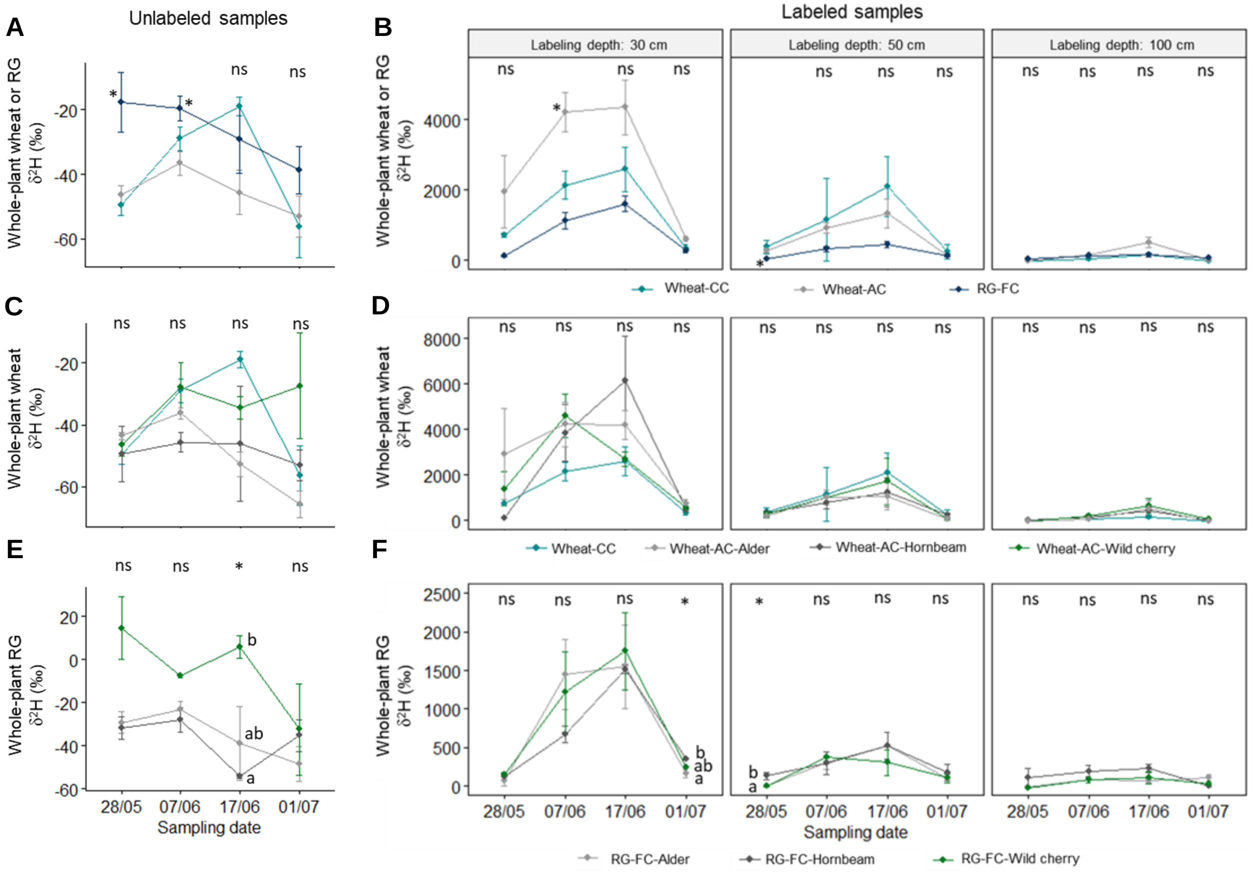


**Figure 5:** Evolution of whole-plant wheat and RG δ$^2$H (‰) in unlabeled (A, C, E) and labeled (B, D, F) experiment where $^2$H tracer was injected at 30, 50 and 100 cm in alley-cropping (AC), sole-crop (CC) and pure-forest control (FC) plots. Data are means (±SE). Lowercase letters indicate homogeneous groups for comparison between the tree species for a given sampling date and labeling depth (Tukey's test; $p < 0.05$). Means with same letters are not significantly different. In graphics (A) and (B), asterisks indicate significant differences between Wheat-CC and RG-FC or between Wheat-CC and Wheat-AC for a given sampling date (Dunnett's test; $p < 0.05$). "ns" means not significant. Wheat-CC, Wheat-AC, Wheat-AC-Hornbeam, Wheat-AC-Wild cherry and Wheat-AC-Alder mean the wheat in CC, in all AC plot, in AC close to hornbeam, in AC close to wild cherry and in AC close to alder respectively. Idem for RG-FC, RG-FC-Hornbeam, RG-FC-Wild cherry and RG-FC-Alder.

Regarding the artificial deuterium signature of labeled samples, results showed that regardless of labeling depth, the whole-plant wheat $\delta^2$H was positive and was significantly higher than the natural abundance. The mean whole-plant wheat $\delta^2$H was on average 2481 ± 523 ‰, 715 ± 218 ‰, and 133 ± 68 ‰ for samples corresponding to 30, 50 and 100 cm labeling depth respectively. These results testified that wheat has taken up the injected labeled water whatever the labeling depth, but water was mainly absorbed at 30 cm. The same results were observed for RG in FC but the $\delta^2$H was lower than wheat except at 100 cm labeling depth. The mean $\delta^2$H of RG was significantly higher at 30 cm labeling depth and decreased with increasing labeling depth.

For wheat samples collected above the 30 cm labeling depth, the whole-plant wheat $\delta^2$H was significantly higher in AC (2883 ± 585 ‰) compared to CC (1443 ± 274 ‰). This difference was mainly due to a high mean value observed in 07/06 when wheat was at the heading stage. For 50 and 100 cm labeling depths, no significant difference of $\delta^2$H was observed between the wheat cultivated in AC and CC. The mean value of $\delta^2$H in labeled wheat samples, significantly decreased with the labeling depth in both types of system AC and CC. At 100 cm labeling depths, the mean $\delta^2$H was equal to 171 ± 79 ‰ and 21 ± 20 ‰ in AC and CC respectively. For the wheat cultivated in CC, there was no significant differences between $\delta^2$H at 30 and 50 cm labeling depths, whereas the mean value of $\delta^2$H for wheat cultivated in AC was significantly higher at 30 cm than at 50 cm labeling depth. Finally, no significant differences of $\delta^2$H between the wheat in CC and wheat associated with alder, hornbeam or wild cherry were noticed for all sampling dates and all labeling depths.

In FC labeled samples, we only observed a higher $\delta^2$H for the RG associated with the hornbeam at 30 cm labeling depth on 01/07 and at 50 cm labeling depth on 28/05 compared to the RG associated with other tree species. No other significant differences of $\delta^2$H regardless of sampling dates and labeling depths were observed for RG.

### 3.3. Trees height, growth rate and $\delta^2$H values

In 2021, the tree height was on average 2.4 ± 0.6 m. They were 0.5 m taller in AC compared to FC (Table 2). Indeed, all tree species were taller in AC than in FC except for alder for which no significant difference of height was found between AC and FC. The same trend was found for the growth rate. Trees in AC grew faster (80 cm $y^{-1}$) compared to those in FC (50 cm $y^{-1}$). Whether difference of growth rate was particularly higher for wild cherry and hornbeam in AC than in FC, the growth rate of alder was not affected by the type of system (Table 2).

**Table 2:** Height (m) and growth rate (m $y^{-1}$) of referent tree species used for deuterium labelling experiment. Data are means (±SD). Letters are Tukey's test result for a multiple comparison between tree species for a given type of system.

| Tree species | | Height (m) | | | Growth rate (m $y^{-1}$) | | |
|---|---|---|---|---|---|---|---|
| | n | AC | FC | *#p-value* | AC | FC | *#p-value* |
| Alder | 9 | 3.0 (±0.3) b | 2.8 (±0.5) b' | ns | 0.9 (±0.2) b | 0.8 (±0.3) b' | ns |
| Hornbeam | 9 | 2.2 (±0.2) a | 1.9 (±0.2) a' | 0.02 | 0.5 (±0.1) a | 0.4 (±0.1) a' | 0.02 |
| Wild cherry | 9 | 3.2 (±0.3) b | 2.1 (±0.1) a' | <0.001 | 0.9 (±0.2) b | 0.4 (±0.05) a' | <0.001 |
| Mean | | 2.8 (±0.5) | 2.3(±0.5) | 0.001 | 0.8 (±0.2) | 0.5 (±0.3) | 0.001 |

# For a given tree species, *p-values* < 0.05 indicate a significant difference between AC and FC

The mean tree foliar $\delta^2$H of unlabeled samples was negative for all sampling dates and type of systems: -20 (± 3 ‰). It was significantly higher compared to the wheat $\delta^2$H but was not significantly different of values for RG. It increased significantly from 28/05 to 07/06 during the period of low rainfall, 18 days after the labeling experiment, and decreased until the end of our monitoring (Figure 4C). The type of system (AC and FC) did not significantly affect the natural abundance of tree foliar $^2$H. On average for all data, the mean $\delta^2$H was significantly higher for unlabeled hornbeam -15 ± 2 ‰ than wild cherry -22 ± 2 ‰ and alder -22 ± 3 ‰.

On average, the tree foliar $\delta^2$H for samples collected in labeled area was also negative (-17 ± 3 ‰) and was significantly higher than values for natural abundance. It highly increased between 28/05 and 07/06, during which values were not significantly different of unlabeled samples (Figure 4C). During the period between 17/06 and 01/07, i.e. 28 and 42 days after labeling, $\delta^2$H decreased for both unlabeled and labeled samples, but values were significantly higher for labeled samples compared to the natural abundance only for these dates (Figure 4C).

The artificial deuterium signature of tree leaves from labeling experiment significantly varied with tree species, with labeling depth and with sampling date. It was still significantly higher for hornbeam (-14 ± 3 ‰) compared to wild cherry (-19 ± 3 ‰), but was intermediate for alder (-17 ± 2 ‰). A significant higher $\delta^2$H was observed in labeled than in unlabeled samples only for alder.

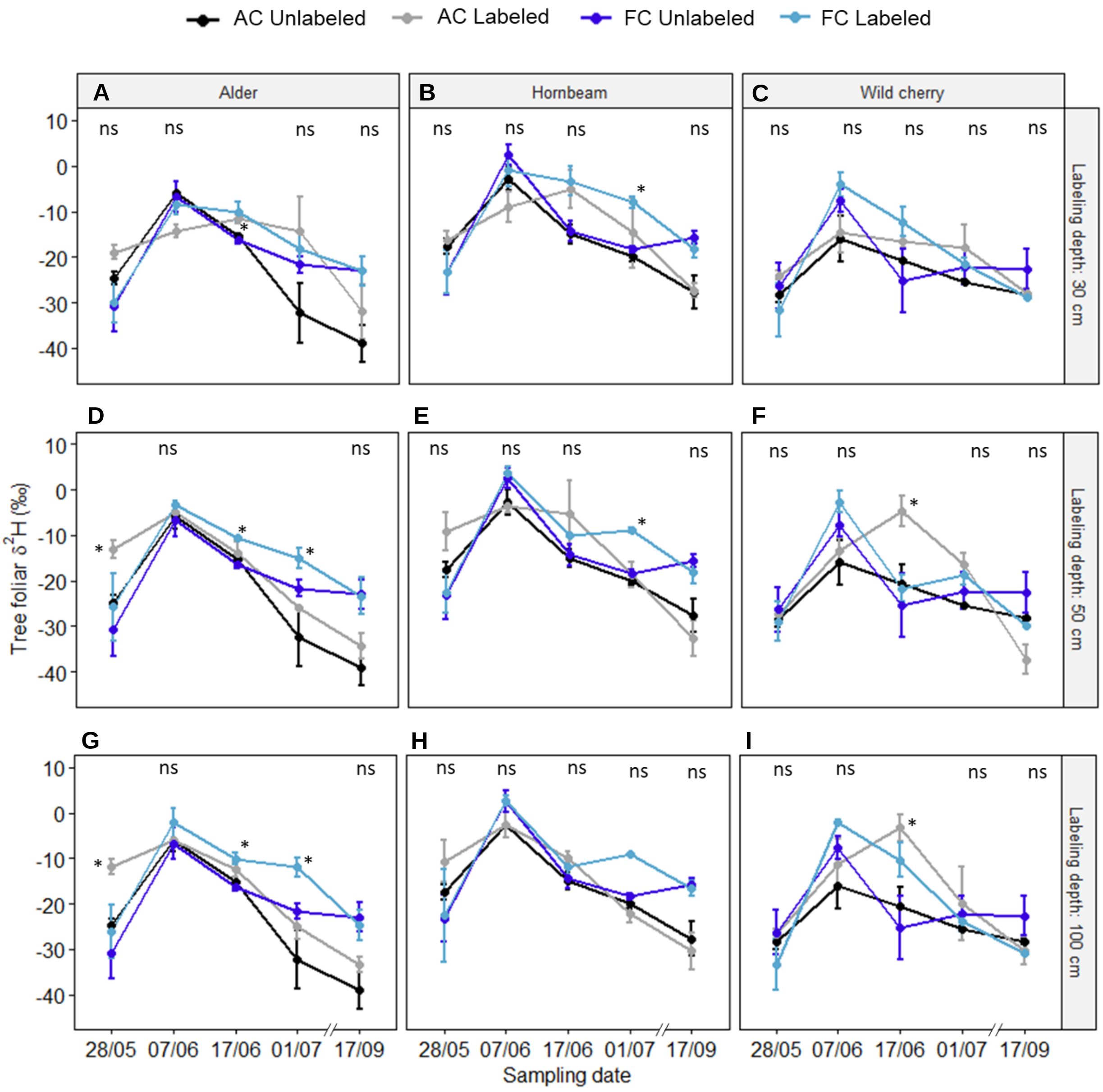


**Figure 6:** Evolution of tree foliar δ$^2$H (‰) in unlabeled and labeled experiment at 30, 50 and 100 cm of $^2$H labeling depth in alley-cropping (AC) and pure-forest control (FC) plots. Data are means (±SE). For each tree species and labeling depth, asterisks indicate significant differences between labeled and unlabeled samples for a given type of system and sampling date ($p < 0.05$), ns means not significant.

For alder, we noticed in AC that 8 days after labeling (on 28/05), the mean value of foliar δ$^2$H was higher for samples at 50 and 100 cm labeling depths compared to the $^2$H natural abundance (Figure 6D and 6G). A significant higher value of foliar δ$^2$H was observed in labeled samples at 30 cm labeling depth only on 17/06 i.e. after 28 days (Figure 6A). In FC, significant higher δ$^2$H in labeled samples compared to the $^2$H natural abundance were observed for 50 and 100 cm of labeling depths on 17/06 and 01/07 (i.e. 28 and 42 days after labeling). For hornbeam, regardless the labeling depth and the sampling date, trees in AC presented the same $\delta^2$H than natural abundance. However, a higher $\delta^2$H was noticed for the hornbeam in FC only on 01/07 at 30 and 50 cm labeling depths compared to the $^2$H natural abundance. Finally, for wild cherry in AC, a higher $\delta^2$H for labeled samples were observed on 17/06 (i.e. 28 days after labeling), at 50 and 100 cm labeling depths compared to the $^2$H natural abundance. Unlike other species for which the mean $\delta^2$H peaked in 07/06, we observed that for wild cherry, the peak of $\delta^2$H was shifted at 17/06 for 50 and 100 cm labeling depth in AC plot.

## 4. Discussion

### 4.1. Soil water content evolution

The crop year 2021 was particularly wet both at the beginning of the year in January and during spring and summer periods (Figure 2A). For the last decade, the amount of rain fell in 2021 was at a level equivalent to 2015. Monitoring of weather data at Lille-Lesquin station (https://www.infoclimat.fr/climatologie/annee/2021/lille-lesquin/valeurs/07015.html) revealed an excess of precipitation compared to the four last decades in January (+55 mm) and in May (+17 mm), June (+45 mm), July (+10 mm) and August (+14 mm) and a deficit of water in February (-12 mm), March (-26 mm) and April (-25 mm), i.e. three months before our labeling period. In May, as trees were just emerging from bud break and crop was at GS33 stage, plant water needs were not high enough to significantly dry the soil. Indeed, at the labeling period in May, the soil water content along profile was almost at field capacity even if values in topsoil were slightly lower compared to L2 and L3. It is often observed in temperate region that early in spring before the high cropping growth period, the soil water content is close to the field capacity (Coucheney et al., 2015; Eitzinger et al., 2004; Jégo et al., 2012). The lower value of SWC observed in May for topsoil, compared to L2 and L3, may be attributed to plant and soil evapotranspiration.

In May, we observed higher SWC in topsoil of FC compared to CC and AC because of the presence of dense RG that completely covered the soil in FC whereas the wheat cover in CC and AC was not complete. Almost all the rain falling a week before sampling was retained by the RG cover, contributing to limit runoff and keep water in the soil surface compared to AC and CC. After rain events, retained water may slowly infiltrate in soil from roots canal. In the rainy summer period of 2021, no effect of type of system on SWC along soil profile was observed. During July and November, due to heavy rains, neither tree species nor the type of system significantly affected SWC. Talbot (2011) modeled the repartition of water uptake between trees and wheat associated with walnut and showed that in July, wheat has completely satisfied its water need but trees are on their maximal level of water absorption. Due to high water availability with summer rainfall and the low water need of our young tree, no change in SWC was observed. In November, soil should be refilled by autumnal rainfall and as tree or crop need became negligible, no effect of type of systems was observed.

### 4.2. Variation of crop and trees deuterium natural abundance

Our result showed that for natural deuterium abundance, the mean value of wheat, RG and trees $\delta^2$H was negative and was particularly lower for wheat than other plants. Neither the type of system nor tree species significantly affected the natural deuterium abundance of wheat. Analysis of stable isotope composition provides a powerful, reliable and nondestructive method to study plant water source use (Dawson and Ehleringer, 1991) because there is no fractionation of isotopes by plant roots during water uptake (White et al., 1985). Negative values of $\delta^2$H for natural abundance were also observed in literature for trees (Dawson and Ehleringer, 1991; Huo et al., 2018; Thorburn and Ehleringer, 1995), crops and soil (Guo et al., 2016; Zhang et al., 2022) and precipitation (Dawson and Ehleringer, 1991). The difference in natural deuterium abundance between trees and wheat may indicate that these plants used water from different soil strata. Although wheat and trees may have roots distributed continuously throughout a soil profile, it seemed that the most active location of water absorption was different between these species. For wheat, very negative value of $\delta^2$H may indicate a high-water absorption from layer with high amount of water i.e. deeper layer. Zhang et al. (2022) showed a decrease of $\delta^2$H with soil depth and recorded very negative values of $\delta^2$H in deeper soil layer (from 75 to 200 cm). In spring, water in deep soil layer is less affected by evaporation but also results from infiltration of winter precipitation known to be depleted in deuterium. Since the natural deuterium abundance was not significantly different between RG and trees or between all tree species at each sampling date, we can conclude that all these species took up water at the same soil layer. Unfortunately, we did not measure the soil natural deuterium abundance, but it was shown that the soil-water isotope vertical gradient is mainly influenced by two simultaneous processes, i.e. evaporation and infiltration, with the mixing of stored water and infiltrating rainwater. Zhang et al. (2022) and Wang et al. (2023) showed a soil-water $\delta^2$H abundances declining as soil depth increased.

Our result also showed a significant increase in $\delta^2$H for trees and wheat at the 7/06 sampling date where very few amounts of rainfall were noted 15 days before. It suggests that trees and wheat took up water in zone where deuterium was most abundant such as upper soil layer which may be affected by strong evaporation fractionation. Soil surface typically experiences high levels of evaporation, which enriched the deuterium values in the soil water (Guo et al., 2016). When soil water evaporates, the remaining water in the soil becomes enriched with heavy isotopes because of the low molecular diffusivity in air of water heavy isotope (Gibson et al., 2005). Evaporation typically decreases with soil depth (Tang and Feng, 2001). However, we observed that the $\delta^2$H of trees and wheat decreased in 17/06 even though a very few amount of rainfall was also observed during the last 10 days preceding this sampling date. Due to low rainy event, wheat and trees may flexibly shift water source between soil layers. Decrease in $\delta^2$H in leaves suggested that trees accessed on less deuterium enriched water source.

From these findings, we concluded that as natural abundance of wheat was not significantly affected neither by the intercropping nor by the tree species, there was no effect of trees at this age on the wheat water uptake. However, intercropped 4-year-old trees, RG and wheat took up soil water from different layers undergoing high fluctuating natural deuterium abundance but little deeper for wheat.

### 4.3. Fate of artificial $^2$H tracer

#### *Artificial deuterium signature of wheat and RG*

Our results showed that unlike unlabeled wheat, the $\delta^2$H of labeled wheat was positive regardless of labeling depth and was the highest at 30 cm labeling depth. The high positive values of $\delta^2$H for wheat and RG samples evidenced the absorption of the artificial supplied water and validated the success of labeling experiment. As only a very few amount of heavy water (300 mL) was injected at a very precise location, we were not sure that plants would access it. Labeling experiment using deuterium isotopes, hand-auger holes and tracer injection at different depth has been validated elsewhere to characterize the share and the depth of water uptake between associated plant in agroforestry systems (Beyer et al., 2016; Huo et al., 2020; Kulmatiski et al., 2010; Wang et al., 2023) or to evidence water redistribution at dry season

(Priyadarshini et al., 2016). The higher values of artificial $\delta^2H$ observed at 30 cm labeling depth compared to other labeling depths confirmed that wheat taken up mainly water from soil layer around this depth. However, water was also taken up at 50 and 100 cm but with low proportion. Moreover, the increase of $\delta^2H$ in labeled samples between 28/05 and 17/06 (i.e. between 8 to 28 days after labeling) at 30 cm labeling depth, indicated a strong absorption of water by wheat and RG during this period. Zhao et al. (2018) also studied the contribution of different soil layers for winter wheat uptake and concluded that plants almost took up water from upper soil layer except on heading, flowering and milking where water was also taken in deep layer but less than 40 %. Unlike some authors showing that the absorption of labeled water occurred only few days after tracer injection (Wang et al., 2023), our results showed that the absorption of the artificial deuterium enriched water peaked at one month after labeling. Then, the decrease of $\delta^2H$ between 17/06 and 01/07 may be explained by a dilution of the heavy water due to the 130 mm of rainfall during this period or to the dissipation of the supplied artificial $^2H$ with time.

Trees did not affect water absorption of wheat, excepted in 07/06 at 30 cm of labeling depth where wheat in AC absorbed more water than the wheat in CC (Figure 5). Due to their young age (4 years old), the water need of tree should be largely lower than wheat (Talbot, 2011) and as the water resource was highly available, trees did not affect wheat water absorption. Everson et al. (2009) also did not find competition for water in good rainfall seasons. However, we noticed a beginning of a change in the water absorption profile of wheat in AC compared to the one in CC. Indeed, the presence of trees increased the water absorption of wheat in topsoil particularly at the heading stage. Ma and Song (2018) showed that wheat root water uptake was mainly between 20-70 cm depth during the jointing-heading stage. In our study, due to the lower SWC in CC compared to AC during the jointing stage, the wheat may have been forced to take up water in depth.

The lower $\delta^2H$ of labeled RG compared to wheat, particularly on June 17th (one month after labeling) at a peak of the water uptake, suggested a best distribution of water absorption along the soil profile for RG. The labeled water taken up by RG had to be diluted with all the water absorbed at different depths.

Finally, thanks to the wheat artificial deuterium abundance, we concluded that regardless of type of system, wheat absorbed mainly water at a depth around 30 cm all along the growing season. The high availability of water and the young age of trees limited the impact of trees on the wheat water uptake.

*Tree species accessed deep soil water*

Although natural deuterium abundance analysis revealed that trees should uptake water from upper soil layer undergoing fluctuate natural deuterium abundance value due to evaporation and infiltration, the labeling experiment showed no significant increase in deuterium abundance of tree leaves for 30 cm labeling depth except for alder only one month after labeling (corresponding to 17/06 sampling date). Contrary to the wheat for the labeling experiment, the negative deuterium abundance in tree leaves was almost at the same $\delta^2H$ level than the natural abundance. Many reasons may be evoked for these observations: (i) trees did not accessed the injected tracer due to the low probability that a feeder root meet the two depositions of 300 mL water at 1.5 m from their trunk; (ii) trees at this stage uptake soil water in the understory vegetation strip and there was no tree roots developed in the 1.5 m zone of deposition; (iii) wheat took up almost all applied artificial water before trees accessed it such as observed by Kulmatiski et al. (2010) for grass and trees or (iv) tree water absorption occurred during the few days after labeling (Wang et al., 2023) so that we missed the period during which they absorbed deuterated water. For all comparable labeling experiments that we explored, using the same method and often with older trees, the change in xylem and sap tree $\delta^2H$ remained low even though it was significantly different from natural abundance (Huo et al., 2020; Wang et al., 2023). For deuterium labeling at 1.5 m from tree, Kulmatiski et al. (2010) also found very few numbers of tree samples exceeding the control value after labeling. It should be noticed that soil tillage may have limited tree root expansion in upper soil layer, so that trees absorbed water in soil surface but within the understory vegetation strip.

A significant difference of $\delta^2H$ was observed between labeled and unlabeled at a specific period after labeling (17/06 and 01/07) (Figure 4C) when analyses were done without distinction between type of systems or labeling depth or tree species. These differences were mainly due to higher value of $\delta^2H$ for alder in FC and for wild cherry in AC at 50 and 100 cm labeling depth than unlabeled samples. Indeed, our results showed high values of $\delta^2H$ for 50 and 100 cm labeling depth for some tree species in AC or FC. We think that as trees were at their maximal growth period with leaves and twigs expansion in June (Etzold et al., 2022), the water absorption should be particularly high on 17/06 and 01/07 due to high demand of water coinciding with a particular dry and heat period (Figure 2B). Hence, tree may flexibly shift towards a deep water source to palliate the temporary lack of water in soil surface (Huo et al., 2018). Coners and Leuschner (2005) observed that the atmospheric saturation deficit was the environmental factor with largest influence on root water uptake in their three tree species studied. Therefore, due to the high temperature and the low rainfall around the 17/06, a low atmospheric saturation should increase the tree water uptake.

When focusing on tree species, we found that in AC, alder took up significantly water at 50 and 100 cm of labeled depth on 28/05 and at 30 cm depth on 17/06, and in FC, water absorption was detected for the alder at 50 and 100 cm depth on 17/06 and 01/07. These results suggest that alder should have a well-developed tree root system particularly in depth to be able to shift into different sources of water according to its availability. However, Lauteri et al. (2006) affirmed that in an 8-year-old mix forest system, the alder used a high proportion of shallow water sources. If tree fine root development in 30 cm depth at crop area was limited by soil tillage, their access to labeled water in this depth was limited favoring the shift in deeper layer thanks to deep fine roots. In FC, the cohabitation with ryegrass may have induced a deeper rooting of the alder compared to a forest system. For the wild cherry, water absorption was detected in AC at 50 and 100 cm depth suggesting that, like alder, this species certainly developed more deep and active tree roots in AC than other species allowing them to shift into the deep-water source at dry period. Soil tillage should limit the root development in crop area and may favor water uptake in the 30 cm depth but in the understory vegetation strip.

## 5. Conclusion

From natural and artificial deuterium analysis, we showed that four-year-old intercropped trees share water with wheat mainly at 30 cm depth in alley-cropping temperate system. We did not find a significant effect of trees on water uptake by wheat because of a very wet crop year, of the very young age of trees and probably of the fine root development limitation in crop area zone due to soil tillage. We found in AC that taller alder and wild cherry with higher growth rate than hornbeam, were able to flexibly shift their water source from deep layer in case of low availability of water in upper layer. Further works are needed to quantify exactly the amount of water taken up by two associated species and to calculate the proportion of water within trees from different soil layers according to the climate variation and water availability along crop and tree cycle. The monitoring should be continued all along tree growth to determine from what age trees would negatively impact the crop water uptake and at what stage of development this competition could be the most impacting or from what age trees uptake mainly water out of crop rooting zone and became complementary with them.

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# Chapitre 4

## Use of core-break method to characterize vertical fine root distribution of associated plants in young temperate alley-cropping experimental site

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### Abstract

The cohabitation between species in alley-cropping systems (AC) was shown to create competition between associated plants due to root overlaps, however, some authors showed a complementarity of roots distribution thanks to spatial and temporal niche differentiation. Here, we used the core-break and the soil coring method to quantify the vertical tree and wheat fine root abundance and biomass throughout a soil profile down to 120 cm in a 4-year-old temperate AC. Fine roots were measured on November 2021 at 2 m distance from a referent tree (hornbeam, wild cherry and willow) in tree-wheat AC, pure-forest associated with ryegrass (FC) and wheat sole-crop control (CC) plots. The mean wheat fine root abundance (WFRA, $m^{-2}$) was twice higher in CC (874 ± 152 $m^{-2}$) than in AC (437 ± 47 $m^{-2}$). It was significantly higher for wheat associated with hornbeam than willow particularly at 10 cm depth. The tree fine root abundance (TFRA, $m^{-2}$) was linearly correlated with tree fine root biomass (TFRB, kg DM $ha^{-1}$) for hornbeam ($R^2 = 0.79^{***}$), willow ($R^2 = 0.77^{***}$) and wild cherry ($R^2 = 0.54^{***}$). Using TFRA, the van Noordwijk's equation gave a satisfying prediction of TFRB for willow and wild cherry, but should be improved for hornbeam. The calibration factor for these conversions was X = 1.3, 2.1 and 8.1 for hornbeam, wild cherry and willow, respectively. The mean value of TFRA was seven times higher in FC (1116 ± 97 $m^{-2}$) than in AF (146 ± 24 $m^{-2}$) for all soil depths and all tree species. At 4 years old, willow and hornbeam fine roots cohabited with wheat in the upper soil layer whereas wild cherry already developed deep fine roots under the crop rooting zone. Further works are needed to see the evolution of tree and crop root distribution along soil profile with increasing age.

## Keywords

Tree fine root biomass, wheat fine root biomass, alley cropping, forest tree, core-break method, soil coring, soil depth

## 1. Introduction

In alley-cropping systems (AC) where tree rows are intercalated with annual crops in arable land, the competition or facilitation between associated plants are often determined by interspecific root overlap (Isaac and Borden, 2019) or niche differentiation along the soil profile, respectively. It was largely documented that intercropping systems modified the spatial roots distribution of trees. Andrianarisoa et al. (2016) showed in an adult walnut-wheat AC that tree fine roots grew deeper that in forestry control plot evidencing the plasticity of trees. Bouttier et al. (2014) also found an adaptation of oak roots distribution by reducing density in upper soil layer resulting in a spatial separation with the intercropped hay root system. Trees in AC develop deeper root systems than in monocrop stand and had a higher root density in the tree rows compared to the alleys (Cardinael et al., 2015). All of these studies showed a complementary spatial distribution between trees and crop fine roots in AC, however, it was not always observed. According to Mulia and Dupraz (2006), walnut was adapted to AC thanks to its deep fine roots, whereas poplar roots overlapped with those of wheat despite intercropping. Similar results were observed with poplar by Bouttier et al. (2014) and Swieter et al. (2022) who evidenced a cohabitation between tree and crop fine roots within a transition zone out of the row. It has been shown that from 5 years old, tree fine roots started to grow in depth (O'Connor et al., 2023), but additional studies are necessary to understand the effect of crop on tree fine roots distribution from their young age.

Different methods have been developed to study plant roots with the objective to obtain the root distribution as representative as possible of reality. The core-break method was proposed by van Noordwijk et al. (2001). It was used by Moreno et al. (2005), Mulia and Dupraz (2006) and Andrianarisoa et al. (2016) to characterize the spatial variability of roots in AC. In the soil coring method, volumetric soil samples are obtained using an auger and washed to separated roots from soil particles. This method was used by Coleman (2007), Ravenek et al. (2016) and Andrianarisoa et al. (2017). With the development of high resolution cameras, minirhizotron has been installed in some experiment sites to continuously monitor root growth (Germon et al., 2016; Jose et al., 2001). Then, a root profile can be determined by counting roots on a grid (Bouttier et al., 2014) or by root observation on a profile wall (Battie-Laclau et al., 2020). Finally, root mapping can be done using a plastic overlay (van Noordwijk et al., 2001). All these methods have advantages as well and disadvantages. The core-break method may be used thanks to the rapidity of measurements, but it presents the main disadvantages of: (i) the spatial variability of samples which is higher than the other methods, (ii) the effect of preferential root orientation, (iii) the random variation of number of roots intersecting the plane of observation and (iv) the counting errors (van Noordwijk et al., 2001). To deal with that, it may be compensated by multiplying the number of samples collected. In addition, the core-break method evaluates the tree fine root abundance on a surface basis but the conversion of tree fine root abundance into biomass per volume of soil is not always possible. An equation has been proposed by van Noordwijk et al. (2001) to obtain the tree fine root biomass from the number of roots counted with the core-break method, however, the calibration factor is not always available and its validity for young stands is questionable.

The aim of this work was to compare core-break and soil coring method to quantify the tree and crop fine root distribution within a very young temperate alley cropping system. We hypothesized that tree at their young age should develop fine roots in the crop rooting zone and share with them a transition zone; that vertical tree fine root distribution is significatively affected by type of system within which the tree grows and that core-break methods allows to well estimate tree fine root biomass.

## 2. Materials and methods

### 2.1. Experimental site description

This study was carried out at the alley-cropping experimental site of Ramecourt located 77 km from Lille city in Northern France (50°22'N, 2°17'E) (Andrianarisoa et al., 2019). The climate is oceanic with an average annual temperature of 10.6°C and rainfall of 859 mm, recorded from 2010-2020 at Humières weather station situated at 6.7 km from Ramecourt. The soil texture in the upper layer was a silt loam with 73 % of silt, a pH of 7.9 and an organic matter content of 2.1 %. Soil characteristics details are available in chapter 3 (section 2.1; Table 1).

The experimental plot is a randomized block design with three replications. Within each block, three modalities of AC (AC1, AC2 and AC3) are compared with sole-crop (CC) and pure-forest (FC) control plots. In November 2018, one-year-old trees were in SW-NE oriented rows spaced from 38 m and 7 m apart in AC and FC, respectively. Within each row, tall trees were planted two by two at 1 m apart, but only one will be kept in the future, and each pair were 8 m apart and intercalated every 1 m by 9 species of shrubs: *Castanea sativa* (chesnut), *Cornus sanguinea* (dogwood), *Acer campestris* (field mapple), *Euonymus europaeus* (European spindle), *Corylus avelana* (common hazel), *Tilia cordata* (linden), *Ligustrum vulgaris* (wild privet), *Salix alba* (willow) and *Viburnum lantana* (wayfarer). The AC modalities are different from each other according to the presence or not of nitrogen fixing tall tree species. The AC1 is an association of *Quercus robur* (oak) and *Carpinus betulus* (hornbeam), the AC2 is a mixture of *Juglans regia x regia* (or hybrid walnut), *Alnus glutinosa* (alder) and *Prunus avium* (wild cherry) and the AC3 is a combination of *Aulnus glutinosa* (alder) and *Robinia pseudoacacia* (black locust). In FC, a cover crop of *Lolium multiflorum* dominated the interrows space in FC and was mechanically cut twice a year. When considering only tall trees, the density of plantation was 66 trees ha$^{-1}$ and 357 trees ha$^{-1}$ in AC and FC, respectively. The detailed description of the experimental site can be found in chapter 3 (section 2.1. and Figure 1A; 1B).

In CC as well as in AC alleys, a winter wheat (variety "Extase") was sown in October 2020 following a previous chicory witloof crop and was harvested in August 2021.

## 2.2. Tree and crop fine root abundance and biomass measurement

Three tree species with contrasted growth rate: hornbeam, wild cherry and willow were selected from AC and FC. The willow grew faster and was taller than other species except in FC where its growth was not significantly different of other species. In 2021, the tree height was on average 2.7 ± 0.7 m. In AC, the tree height was significantly higher for willow (4. 9 ± 0.3 m) than wild cherry (3 ± 0.3 m) and hornbeam (2.3 ± 0.3 m) (Table 1). The growth rate was the same for all tree species in FC whereas it was significantly higher for willow (130 ± 30 cm $y^{-1}$) compared to wild cherry (80 ± 20 cm $y^{-1}$) and hornbeam (60 ± 10 cm $y^{-1}$) in AC (Table 1).

**Table 1:** Height (m) and growth rate (m $y^{-1}$) of referent tree species used for fine roots measurements. Data are means (±SD). Letters are Tukey's test result for a multiple comparison between tree species.

| | Height (m) | | | Growth rate (m $y^{-1}$) | | |
|---|---|---|---|---|---|---|
| Tree species | AC | FC | *#p-value* | AC | FC | *#p-value* |
| Hornbeam | 2.3 (±0.3) a | 1.9 (±0.2) a' | ns | 0.6 (±0.1) a | 0.4 (±0.1) a' | 0.04 |
| Wild cherry | 3 (±0.4) a | 2.1 (±0.1) a' | <0.001 | 0.8 (±0.2) a | 0.4 (±0.05) a' | <0.001 |
| Willow | 4.9 (±0.3) b | 2.9 (±0.3) b' | <0.001 | 1.3 (±0.03) b | 0.4 (±0.1) a' | <0.001 |
| Mean | 3.2 (±1.1) | 2.4 (±0.5) | 0.008 | 0.8 (±0.3) | 0.5 (±0.2) | <0.001 |

# For a given tree species, *p-values* < 0.05 indicate a significant difference between AC and FC

Tree and crop fine roots abundance and biomass were measured using both "core break" and "soil coring" methods, respectively (van Noordwijk et al., 2001). Soil coring was carried out from November 9th to 30th, 2021. Soil cores down to 1.2 m depth were collected 2 m distance from referent tree using a portable electric core drill consisting of gouge (60 cm length and 85 mm diameter) connected to an electrical percussion hammer (BOSCH GSH 27 VC, Apageo). A total of eleven soil cores were collected within each block (i.e two and one per tree species in AC and FC, respectively, and two in CC). In CC, soil cores were extracted in an area randomly chosen in the middle of the plot. Each 120 cm soil core was divided into 20 cm long sub-cores. Each sub-core was broken by hand, close to the middle, and the number of living fine roots (diameter < 2 mm) visible on both horizontal surfaces was counted. Crop roots recognition was perfected thanks to soil core collected in CC, whereas those for trees were carried out from soil core taken at the bottom of the trunk. For the RG, counter trained to recognize fine roots by uprooting some plants taken in the middle of FC interrow. In comparison with crops, tree roots were more lignified, hairy and often brownish. Despite our recognition experience, tree roots counting might be slightly over-estimated in rows due to the presence of weeds in AC. A single person carried out root counting for all samples to avoid bias from the counter. The mean of the number of fine roots counted on both sides of sub-cores was expressed on a square meter basis and was called tree fine root abundance (TFRA; $m^{-2}$), wheat fine root abundance (WFRA, $m^{-2}$) or ryegrass fine root abundance (RGFRA). All sub-cores were recovered, transported in the laboratory and stored at 4°C.

In the laboratory, fine roots within each sub-cores were manually collected, sorted according to their affiliation (trees, crops, RG and weeds), dried (65°C, 48h), cleaned and weighted for biomass measurement. Tree, crop and ryegrass fine roots biomass, hereafter referred to as TFRB, WFRB and RGFRB respectively, were expressed in kilograms of dry matter per hectare (kg DM $ha^{-1}$). For a given soil depth (*i*), the percentage of tree fine root biomass and wheat fine root biomass from the total biomass was calculated according to the formulae:

$$\%TFRB_i = \frac{TFRB_i * 100}{(TFRB_i + WFRB_i)} \qquad \text{(eq 1)}$$

and

$$\%WFRB_i = \frac{WFRB_i * 100}{(TFRB_i + WFRB_i)} \qquad \text{(eq 2)}$$

### 2.3. Statistical analyses

A Kruskal-Wallis’ test was used to analyze the wheat, tree and RG fine roots biomass according to the soil depth or the type of system, followed by post hoc Mann-Whitney’s tests. The comparison between AC and CC for WFRB and between AC and FC for TFRB was done using Mann-Whitney’s test. Wheat in CC and in all AC plots were referenced by the variables Wheat-CC and Wheat-AC, respectively. One-way ANOVAs were performed to analyze the variability of the wheat, tree and RG fine roots abundance with the soil depth, the associated tree species or the three type of system as explanatory variables and were followed by post hoc Tukey’s tests. The wheat close to hornbeam, to the wild cherry or to willow was referenced by the variables Wheat-AC-Hornbeam, Wheat-AC-Wild cherry and Wheat-AC-Willow, respectively. The comparison between AC and CC for WFRA was done using a Student t-test. Finally, a Kruskal-Wallis’ test was used to analyze TFRA, TFRB and WFRB variation according to the tree species, followed by multiple comparisons Dunn's test ($p < 0.1$).

Correlation analysis was performed between TFRA and TFRB for each tree species and a second-degree polynomial equation was calculated between WFRA and WFRB. Finally, the model proposed by van Noordwijk et al. (2001) was used to estimate the tree fine roots biomass using TFRA, according to equation 3:

$$Simulated\ tree\ fine\ root\ biomass\ (kg\ DM\ ha^{-1}) = \left[\frac{10*(0.002*\mathrm{X}*\mathrm{TFRA}*h)}{\mathrm{SRL}}\right]*1000 \qquad \text{(eq 3)}$$

where h is the height of the subsoil core in m (h = 0.2 m); SRL is the specific root length of tree species (SRL = 15 m $g^{-1}$ for the hornbeam (Kubisch et al., 2015); SRL = 27 m $g^{-1}$, for the wild cherry (Weemstra et al., 2020) and SRL = 66.8 m $g^{-1}$ for the willow (McIvor et al., 2014; Weemstra et al., 2020)) and X is a calibration factor depending on tree species. This factor was calculated using a nonlinear generalized reduced gradient in Excel solver. The retained X value is that which minimalizes the root mean square error (RMSE) between simulated TRFB and measured TFRB.

For all regressions, the symbol *** was used to indicate a significant determination coefficient at $p < 0.001$ level, the symbol ** for $p < 0.01$ level and the symbol * for $p < 0.05$ level.

## 3. Results

### 3.1. Wheat and RG fine roots abundance and biomass

The mean value of WFRA was twice higher in CC (874 ± 152 $m^{-2}$) than in AC (437 ± 47 $m^{-2}$) (Figure 1A). These differences were observed until 70 cm depth. In AC, the mean WFRA was higher for wheat associated with hornbeam than associated with wild cherry and willow (Figure 1B) particularly at 10 cm depth (Figure 1D et 1F). The WFRA significantly decreased with depth in both type of system CC and FC. From 30 cm depth, there was no longer significative difference in the WFRA between the tree species. Whereas the WFRA was significantly different between CC and AC until 30 cm depth for wheat associated with hornbeam and willow, it was observed until 70 cm for wheat associated with wild cherry. Finally, RGFRA in FC was significantly lower than WFRA. It was evenly distributed along the vertical soil profile until 110 cm depth.

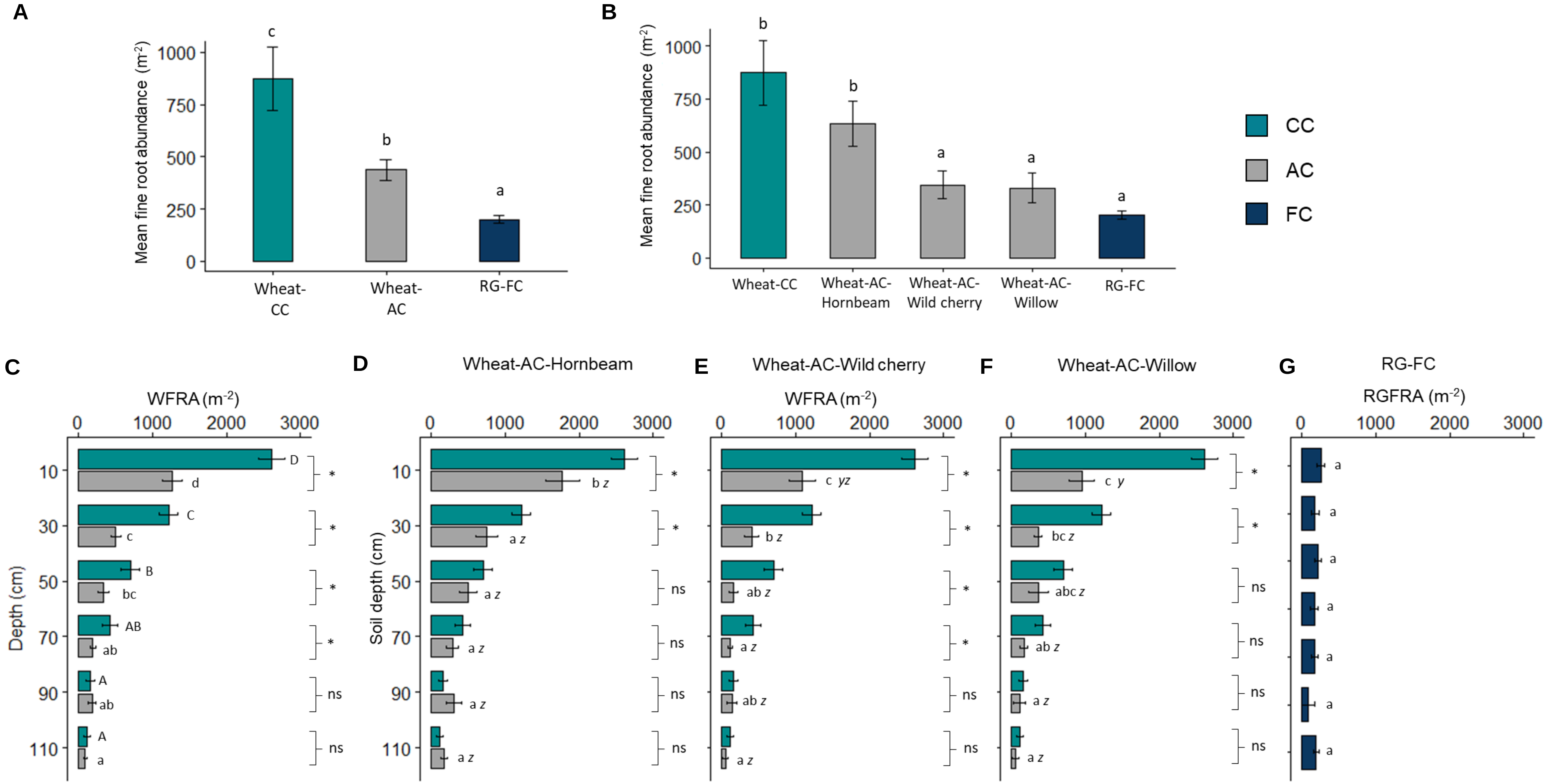


**Figure 1:** Mean values of wheat (WFRA, m$^{-2}$) and RG (RGFRA, m$^{-2}$) fine root abundance in sole-crop control (CC), alley-cropping (AC) and pure-forest control (FC) plots. (A) mean values for all data, (B) mean values according to the associated tree species, (C) mean values according to soil depth for Wheat-CC and Wheat-AC, (D) for Wheat-CC and Wheat-AC-Hornbeam association, (E) for Wheat-CC and Wheat-AC-Wild cherry association, (F) for Wheat-CC and Wheat-AC-Willow association and (G) for RG-FC association. Data are means (±SE). In graphics (A) and (B), lowercase letters indicate homogeneous group comparing the type of system and the associated species (Tukey's test; $p < 0.05$). (C) to

(F), uppercase and lowercase letters (a, b, c, d) indicate homogeneous groups for comparison between soil layer for Wheat-CC and Wheat-AC respectively (Tukey's test; $p < 0.05$). In graphics (E), (F) and (G), italic letters (y, z) indicate homogeneous groups to compare fine root of wheat in AC, associated with different tree species (Dunn's test; $p < 0.1$). Symbols * and "ns" are result of Student's test between type of system (AC, CC) on fine root abundance for a given soil layer. "ns" means not significant. In graphics (E), lowercase letters indicate homogeneous groups for comparison between soil layers (Tukey's test; $p < 0.05$).

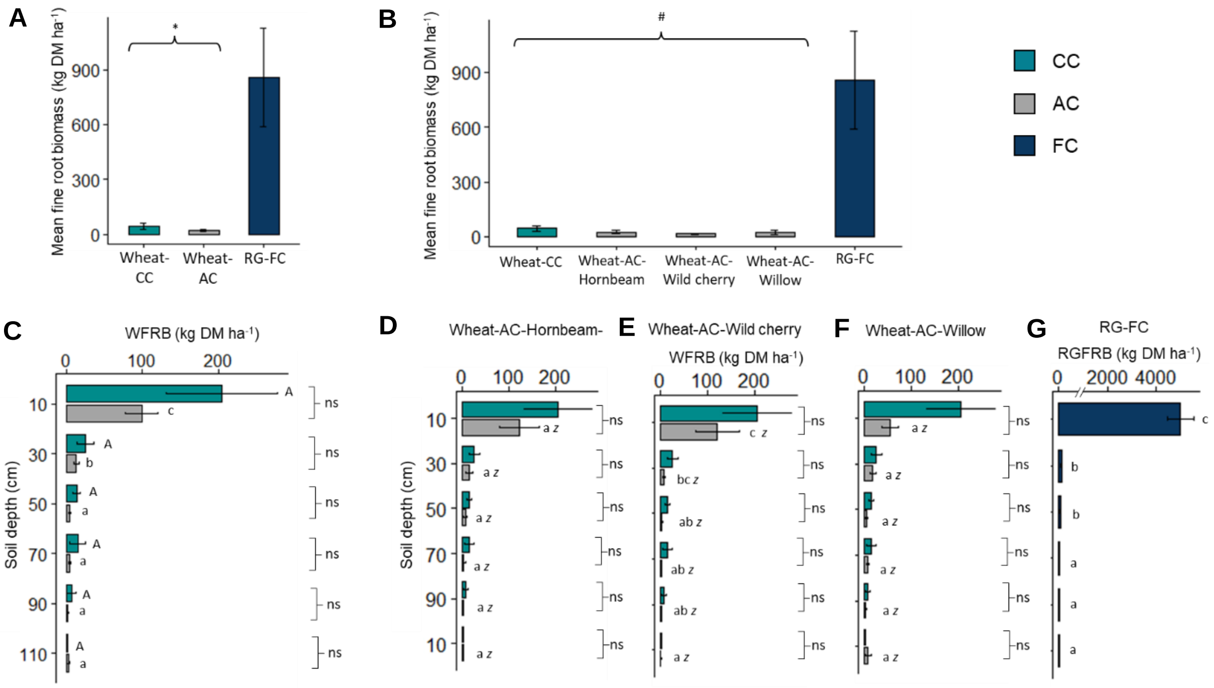


**Figure 2:** Mean values of wheat (WFRB, kg DM ha$^{-1}$) and RG fine roots biomass (RGFRB, kg DM ha$^{-1}$) in sole-crop control (CC), alley-cropping (AC) and pure-forest control (FC) plots. (A) Mean values for all data, (B) mean values according to the associated tree species, (C) mean values according to depth for Wheat-CC and Wheat-AC, (D) for Wheat-CC and Wheat-AC-Hornbeam association, (E) for Wheat-CC and Wheat-AC-Wild cherry association, (F) for Wheat-CC and Wheat-AC-Willow association and (G) for RG-FC association. Data are means (±SE). In graphic (A), Asterisk indicates significant difference between type of system (Kruskal-Wallis' test; $p < 0.05$). In graphic (B), symbol # indicates significant difference between wheat associated species and RG (Kruskal-Wallis' test; $p < 0.1$). In graphics from (C) to (F), uppercase and lowercase letters (a, b and c) indicate homogeneous groups for comparison between soil layer for Wheat-CC and Wheat-AC, respectively (Tukey's test; $p < 0.05$). In graphics (E), (F) and (G), italic letters (z) indicate homogeneous group to compare fine root of wheat in AC, associated with different tree species (Dunn's test; $p < 0.1$). Symbols "ns" are result of Student's test between the type of system (AC, CC) on fine root biomass for a given soil layer. "ns" means not significant. In graphic (E), lowercase letters indicate homogeneous groups for comparison between soil layer (Tukey's test; $p < 0.05$).

Unlike WFRA, no significant difference of WFRB was noticed between CC (45 ± 17 kg ha$^{-1}$) and AC (21 ± 5 kg ha$^{-1}$) regardless of the associated tree and the depth (Figure 2A and 2B). In AC, the WFRB significantly decreased with depth down to 50 cm depth and was stable beyond whereas in CC, WFRB was uniformly distributed along the soil profile. For wheat associated with the wild cherry, WFRB significantly decreased (Figure 2E). The same results were not observed for wheat associated with willow or hornbeam (Figure 2D and 2F). For RG, fine root biomass was particularly high at 10 cm depth (4983 ± 545 kg DM ha$^{-1}$) and sharply decrease at 30 cm depth with a mean value of (100 ± 21 kg DM ha$^{-1}$), continued to decrease at 70 cm depth and was stable down to 110 cm depth.

The WFRA and WFRB were significantly correlated ($R^2 = 0.58^{***}$) with a polynomial regression (Figure 5G). On average, along a soil profile down to 120 cm depth, WFRB represented 53 % of the total fine roots biomass in AC whereas it was almost 100 % in CC. The %WFRB was evenly distributed along vertical profile (Table 2).

**Table 2:** Vertical variation of percentage of TFRB and WFRB to total fine roots biomass and ratio between TFRB and WFRB. TFRB: tree fine roots biomass (kg DM ha$^{-1}$); WFRB: wheat fine roots biomass (kg DM ha$^{-1}$); AC: alley-cropping, FC: pure-forest control; CC: sole-crop control; H: hornbeam; WC: wild cherry; W: willow

| | | %TFRB | | %WFRB | | TFRB/WFRB | | | |
|---|---|---|---|---|---|---|---|---|---|
| Depth | Type of system | AC | FC | AC | CC | AC | AC | | |
| | Tree species | | | | | | H | WC | W |
| 10 | | 39 | 11 | 53 | 98 | 19 | 0.9 | 18 | 35 |
| 30 | | 29 | 87 | 49 | 100 | 3.8 | 0.2 | 4.3 | 5.7 |
| 50 | | 24 | 92 | 50 | 100 | 5.6 | 0.2 | 0 | 16 |
| 70 | | 27 | 100 | 63 | 100 | 0.5 | 0 | 0 | 0.8 |
| 90 | | 40 | 99 | 55 | 97 | 0.2 | 0 | 0.7 | 0 |
| 110 | | 46 | 98 | 42 | 100 | 0.5 | 0 | 1.9 | 0 |

### 3.2. Tree fine roots abundance and biomass

The mean value of TFRA was seven times higher in FC (1116 ± 97 m$^{-2}$) than in AC (146 ± 24 m$^{-2}$) for data including all soil depths (Figure 3A). This difference was observed at all soil depths down to 110 cm (Figure 3C) and for all tree species (Figure 3B). The TFRA was the highest at 10 cm depth and significantly decreased with depth in both AC and FC except for the hornbeam in AC where mean TFRA was evenly distributed all along soil profile (147 ± 33 m$^{-2}$) and became null beyond 70 cm depth (Figure 3D). In AC, the TFRA of wild cherry and willow, significantly decreased down to 50 cm depth (from 529 ± 87 m$^{-2}$ to 59 ± 33 m$^{-2}$) and then became null or stable beyond this depth. The TFRA was not significantly different between tree species at all depths in AC except at 10 cm where TFRA was significantly lower for hornbeam compared to willow (Figure 3D, attached table).

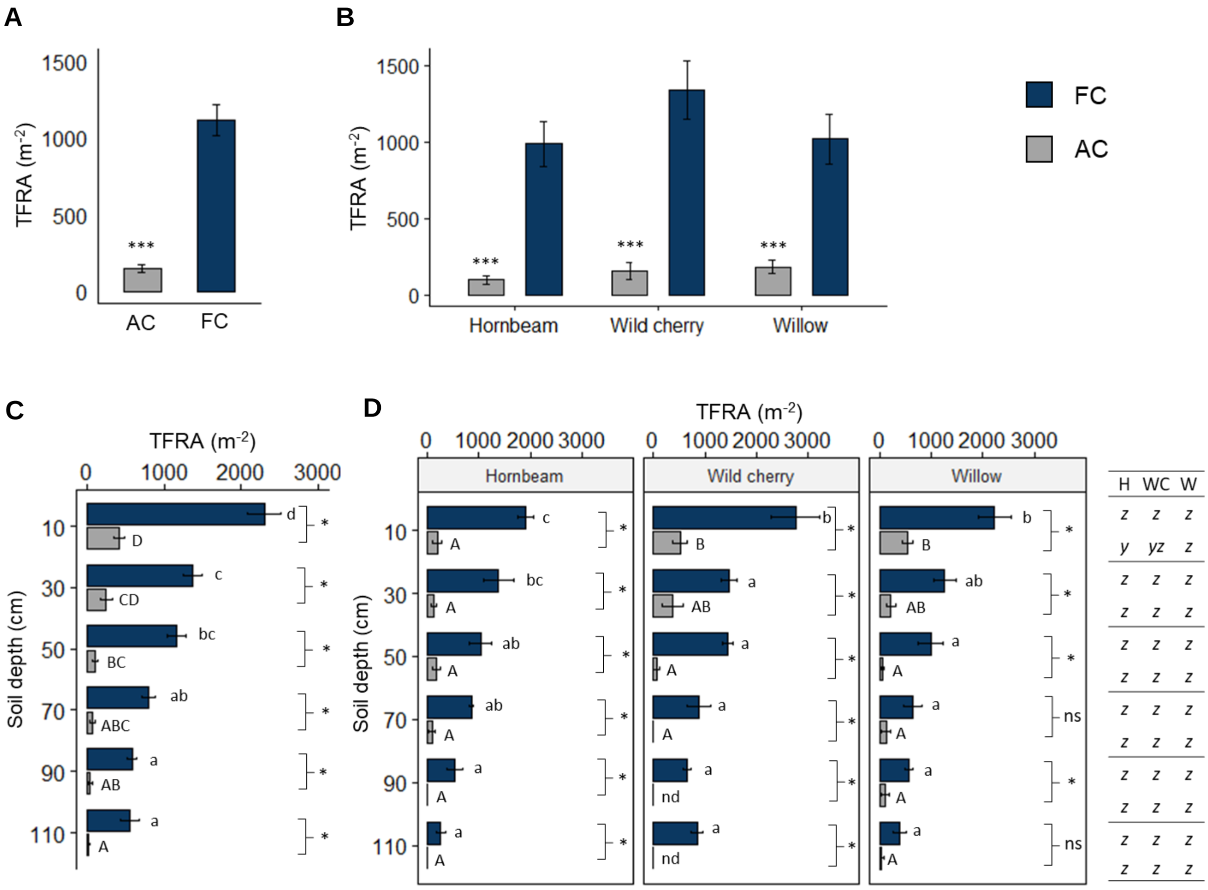
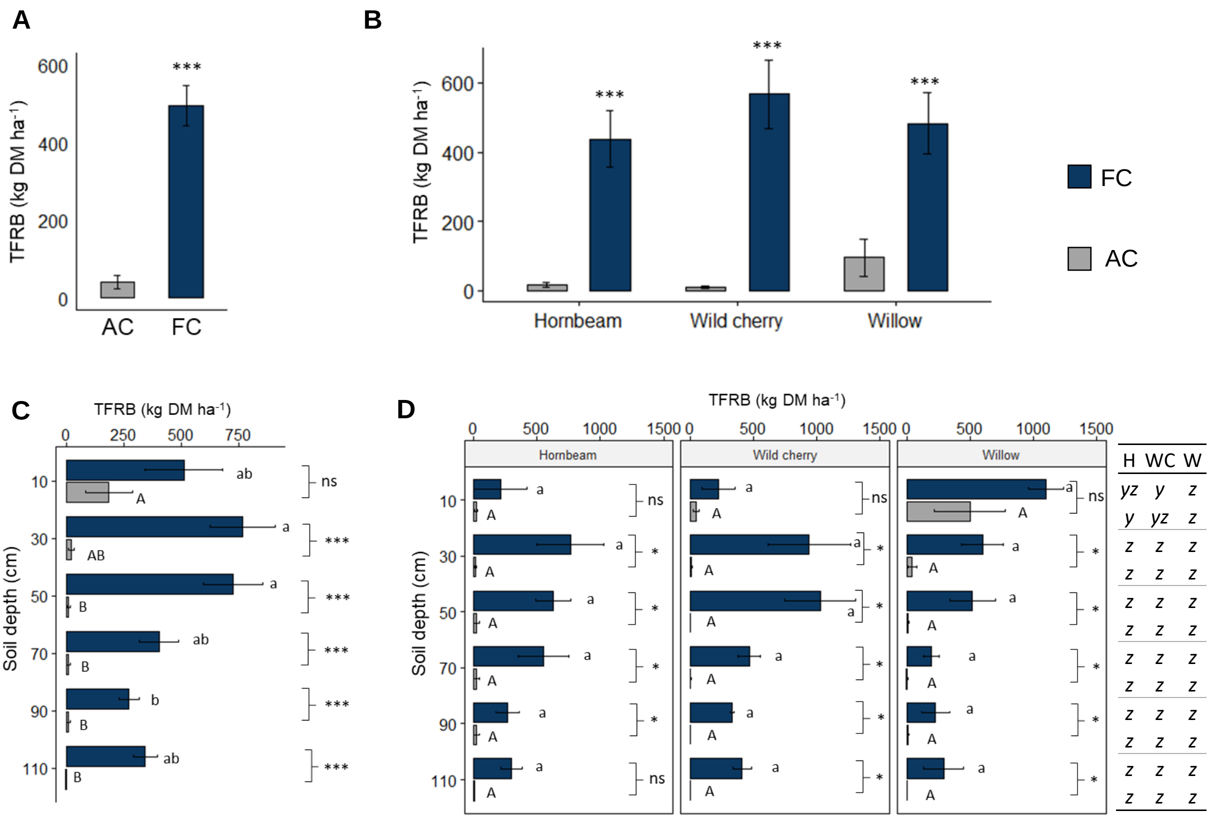


**Figure 3:** Mean values of tree fine root abundance (TFRA, m$^{-2}$) in pure-forest control (FC) and alley-cropping (AC) plots. (A) Mean values for all data, (B) mean values according to the associated tree species, (C) mean values according to soil depth, (D) mean values according to soil depth per tree species. Data are means (±SE). In graphics (A) and (B), symbols *** are result of Student's test between type of system (FC, AC) on fine root abundance ($p < 0.001$). In graphics from (C) and (D), lowercase (a, b, c, d) and uppercase letters indicate homogeneous groups for comparison between soil layers in FC and AC, respectively (Tukey's test; $p < 0.05$). Symbols * and "ns" are result of Student's test between type of system (FC, AC) on fine root abundance for a given soil layer. "ns" means not significant. In attached table, italic letters (y, z) indicate homogeneous groups to compare fine root of trees in AC according to species for a given type of system (Dunn's test; $p < 0.1$).

In FC, the TFRA was the highest at 10 cm and then globally decreased with depth (Figure 3C). For wild cherry, it was significantly higher at 10 cm compared to all other soil layers whereas for willow and hornbeam, it progressively decreased with depth (Figure 3D). No significant difference of the TFRA was observed between tree species at each soil layer of 20 cm (Figure 3D, attached table).



| H | WC | W |
|---|---|---|
| *yz* | *y* | *z* |
| *y* | *yz* | *z* |
| *z* | *z* | *z* |
| *z* | *z* | *z* |
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**Figure 4:** Mean values of tree fine root biomass (TFRB, kg DM ha$^{-1}$) in pure-forest control (FC) and alley-cropping (AC) plots. (A) Mean values for all data, (B) mean values according to the associated tree species, (C) mean values according to soil depth, (D) mean values according to soil depth per tree species. Data are means (±SE). In graphics (A) and (B), symbols *** are result of Student's test between type of system (FC, AC) on fine root biomass ($p < 0.001$). In graphics from (C) and (D), lowercase (a, b) and uppercase letters indicate homogeneous groups for comparison between soil layer in FC and AC, respectively (Tukey's test; $p < 0.05$). Symbols ***, * and "ns" are result of Student's test between type of system (FC, AC) on fine root biomass for a given soil layer. "ns" means not significant. In attached table, italic letters (y, z) indicate homogeneous group to compare fine roots of trees in AC according to species for a given type of system (Dunn's test; $p < 0.1$).

Regarding the TFRB, same trend as TFRA was observed: the mean value of TFRB was 12 times higher in FC (496 ± 52 kg ha$^{-1}$) than in AC (41 ± 18 kg ha$^{-1}$) (Figure 4A). The TFRB was significantly higher in FC than in AC for all tree species (Figure 4B), and for each soil layer of 20 cm except at 10 cm depth (Figure 4C). Comparison between tree species for TFRB showed a significant difference only at 10 cm: values for willow were significantly higher than hornbeam and wild cherry in AC and FC, respectively. The TFRB gradually decreased with depth in AC (Figure 4C).

The percentage of TFRB from total observed fine roots throughout soil profile was on average 35 % and 82 % in AC and FC respectively. In FC, the %TFRB was very low at 10 cm depth (11 %) and was on average 95 % beyond this depth. In AC, the %TFRB was evenly distributed along the soil profile (Table 2). Finally in AC, TFRB was 19 times greater than WFRB at 10 cm depth and on average 4.7 times higher from 30 to 50 cm depth. Finally, between 70 to 110 cm depth, WFRB was on average 2.5 times higher than TFRB. For hornbeam, WFRB was always higher than TFRB but beyond 70 cm depth, the TFRB to WFRB ratio was null. For wild cherry, there was more tree than wheat fine roots up to 30 cm depth, beyond the amount was low. Finally, TFRB of willow was higher than WFRB down to 50 cm depth, whereas at 70 cm depth, WFRB was 1.25 greater than TFRB and no more roots were observed bellow.

### 3.3. Modelling tree fine root biomass

Linear regression analyses showed that the TFRA (m$^{-2}$) measured with core-break method was linearly correlated to TFRB (kg DM ha$^{-1}$) measured with soil coring for each tree species (Figure 5A, 5B and 5C). The coefficient of determination of regressions was $R^2 = 0.79^{***}$, $0.77^{***}$ and $0.54^{***}$ for hornbeam, willow and wild cherry respectively. The calibration of the van Noordwijk et al. (2001) equation gave a satisfying prediction of TFRB for willow (RMSE = 253 kg DM ha$^{-1}$) and wild cherry (RMSE = 166 kg DM ha$^{-1}$) (Figure 5E and 5F), as linear regression between simulated and observed was almost confused with bisector but should be improved for hornbeam (RMSE = 211 kg DM ha$^{-1}$). With SRL values proposed in the literature, the calibration factor for the conversion of TFRA measured with core break method into fine root biomass was X = 1.3; 2.1 and 8.1 for hornbeam, wild cherry and willow, respectively.

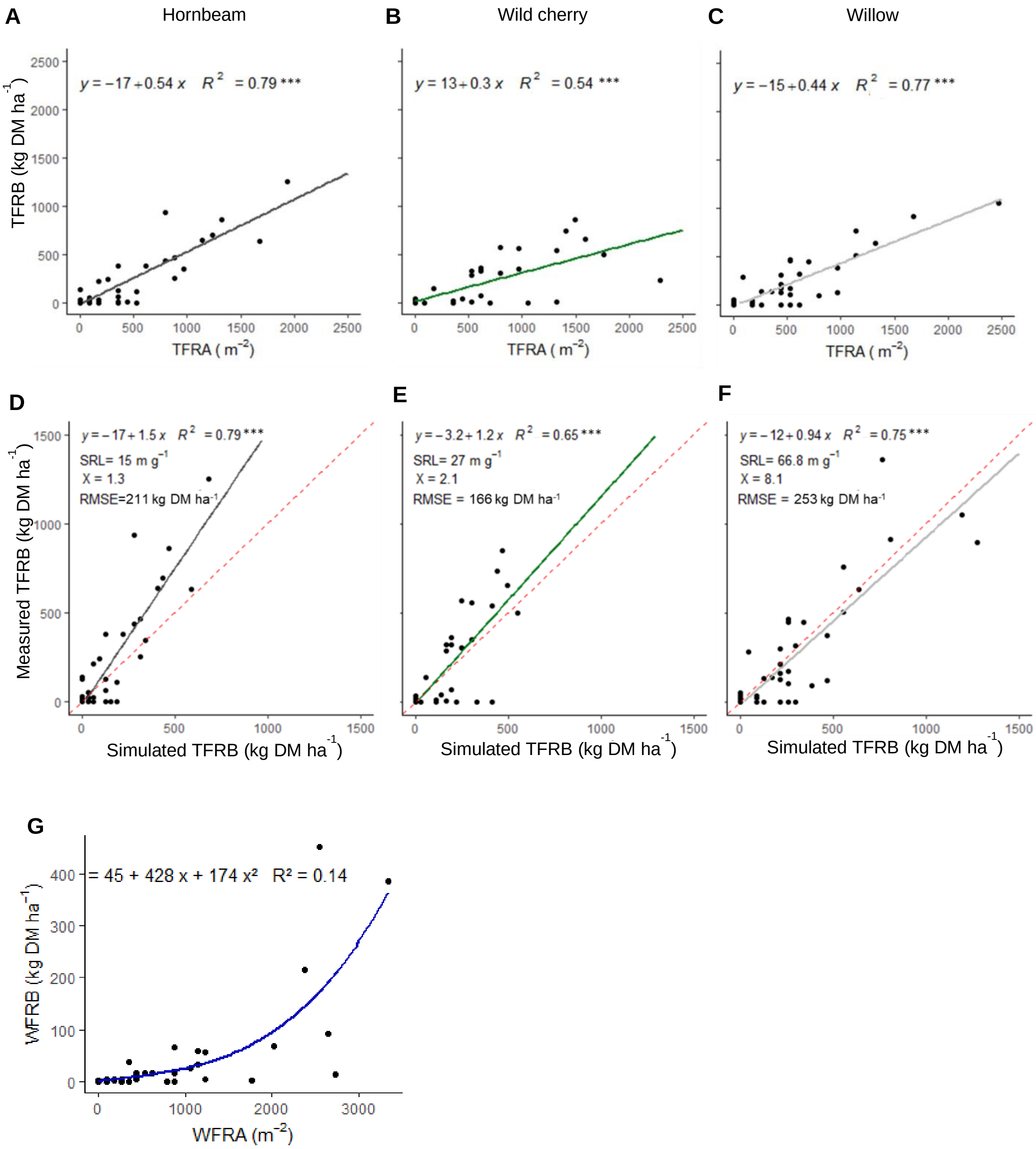

**Figure 5:** Relationship between fine roots measured with core-break method and fine root biomass measured with soil coring method. Graphics (A), (B) and (C) represent relationship between TFRA ($m^{-2}$) and TFRB (kg DM $ha^{-1}$) for hornbeam, wild cherry and willow. Graphics (D), (E) and (F) represent comparison between observed and simulated TFRB according to the equation of van Noordwijk et al. (2001) for hornbeam, wild cherry and willow, respectively. Graphic (G) represents the relationship between WFRA ($m^{-2}$) and WFRB (kg DM $ha^{-1}$). Solid lines are linear regression between variables and dotted lines are the bisector. R² represents the determination coefficient of each regression. A R² with symbols *** is significant at $p < 0.001$. SRL = specific root length, RMSE = root mean square error, X = calibration parameter.

# 4. Discussion

## 4.1. Spatial distribution of wheat and RG fine roots

*Wheat fine roots*

Although we counted on average more wheat fine root abundance in CC than in AC, we measured the same fine root biomass between the two systems. It should be due to an underestimation of WFRB. Indeed, soil coring were carried out in November when wheat fine roots started to decompose. They were visible to be counted but very light and breakable, therefore, after removing the soil particle sticking to wheat roots, some of them could not be recovered or were poorly detected by the precision weighing scale. This was consistent with the values of the %WFRB which was surprisingly low (50 %) whereas wheat roots should had dominate soil profile from March to July, according to Battie-Laclau et al. (2020), as trees are still young. We assume that during the time lapse between crop harvest and soil coring, a part of root was decomposed especially in the upper soil layer.

Regarding WFRA, the lower values observed in AC compared to CC may be due (i) to higher wheat roots decomposition close to understory vegetation strip through the activity of living tree or weed roots; (ii) to a lower wheat growth close to tree rows compared to CC caused by light competition with trees, or to nutrient competition with weeds, (iii) to a reduction of crop growth caused by border effect on this zone of tree/crop root cohabitation and (iv) to a cohabitation and competition between root growth. Indeed, due to the water absorption by trees in AC, there was a share of water between associated plants leading to a lower crop root abundance in AC compared to in CC. Trees and crop can overlap in shallow soils despite strong competition (Moreno et al., 2005). Many studies showed a reduction of crop roots close to tree row (Battie-Laclau et al., 2020; Cardinael et al., 2015; Duan et al., 2019; Livesley et al., 2000; Mulia and Dupraz, 2006; Zhang et al., 2013). Duan et al. (2019) found a low wheat root density close to tree rows of 1 or 2-year-old walnut and attributed it to the wheat and walnut roots overlap. Zhang et al. (2013) studied the root density in 2-, 4- and 6-year-old jujube tree/wheat intercropping, sole wheat and 2-, 4- and 6-year-old sole jujube down to 100 cm depth and concluded that intercropped wheat and jujube had less root length density at all soil depths than those of sole wheat and added that inhibition in wheat root growth increase with increasing jujube age. Contrary to our results, they showed that intercropped wheat had shallower root than sole crop. Competitiveness of roots in cohabitation zone are the main reason of this reduction of growth. Species with high root length density are more competitive than others. Zhang et al. (2013) showed that 6-year-old jujube trees were more competitive and contributed to decrease the crop root growth. Opposite results were found in dryland AC where the sorghum root system exploited a larger soil volume utilizing soil resources more efficiently than in monocrop (Lehmann et al., 1998).

However, even though a reduction of crop root development was observed close to tree rows compared to CC, the sum of total roots observed close to the tree row was higher than the total root in CC. This cohabitation may contribute to improve water resources in agroforestry systems compared to monocrop increasing the nutrient and water use efficiency.

Even though this increase of resource use efficiency was largely documented in agroforestry systems (Link et al., 2015; Rowe et al., 2001; Zhang et al., 2022) our result succeeded to show that same effect happened for very young trees. Cohabitation between tree and crop was more pronounced in topsoil (Duan et al., 2019), particularly when the wheat was associated with the willow than with the hornbeam. From 50 to 70 cm depth, the wheat in CC was as much developed as in association with the hornbeam whereas the wheat associated with the wild cherry was less developed than those in CC. These results showed that beyond 50 cm depth, the hornbeam had no longer impact on the wheat fine roots growth unlike the wild cherry which still reduced wheat fine roots growth. These results suggested that the hornbeam fine roots were mainly developed in the upper soil layer whereas those of the wild cherry grew deeper. These observations were consistent with the shallow fine roots distribution of the hornbeam found by Abdi et al. (2009), and to the ability of the wild cherry roots to develop in depth by Bibalani et al. (2008). Moreover, throughout soil profile, the proportion of the hornbeam fine roots was particularly lower than those of wheat (Table 2), supporting that the wheat was dominant over the tree. This shallow root system of the hornbeam was more exposed to the ploughing destruction than the other tree species.

### *RG fine roots*

In FC, we found a very high value of RGFRB whereas a very low RGFRA in the first 10 cm. We think that such difference may be due to an underestimation of RG fine root counting by observer in the field due to a very high number of roots to count on a small surface. Hence, some RG roots were confused with tree roots and should overestimate them. Otherwise, RG fine root abundance or biomass was evenly distributed along soil profile. Moreno et al. (2005)

also showed a shallower herbaceous root development in grazed or intercropped systems. A high and dense root system in the first centimeters of soil is a common pattern of herbaceous plants (Jackson et al., 1996). To reduce competition with RG for below-ground resources, trees should develop a deep root system, thanks to their ability to access to deep layers (Schroth, 1995), and little root proliferation near topsoil. This different spatial root distribution allowed RG to use resources from upper soil layer. Fernández et al. (2008) showed a complementary use of soil water between pines and grasses in a temperate semiarid AC. Besides, the nutrient competition with trees may have promoted RG growth and high root density (Ravenek et al., 2016). Indeed, Cheng and Bledsoe (2004) evidenced the competitiveness of grass for nitrogen against blue oak seedlings due to their high rooting density and rapid growth rate.

### 4.2. Spatial fine root distribution of sole and intercropped trees

Globally, the tree fine roots were more developed in FC compared to AC for all tree species and throughout profile. Andrianarisoa et al. (2016) also found more fine roots in forest system than in AC up to 1 m depth. Indeed, due to the absence of fertilization, trees in FC need to develop roots to acquire nutrient whereas trees in AC benefited from fertilizers falling in tree row during spreading. Moreover, trees in FC competed with the RG fines roots particularly developed in topsoil (Figure 3D and 4D, Table 2). This finding corroborates with conclusion of Moreno et al. (2005) about the high presence of herbaceous roots in topsoil when associated with trees. Unlike trees in FC where fine roots completely dominated the deep soil layer (< 20 cm), trees fine roots in AC were evenly distributed along soil profile (Table 2). Species like hornbeam were completely dominated by wheat along soil profile, whereas willow dominated the shallow soil until 50 cm and wild cherry both shallow (< 40 cm) and deep soil (>100 cm). Duan et al. (2019) also showed that intercropping significantly influenced root density of walnut roots both vertically and horizontally. All tree species presented different root plasticity when intercropped due to crop management and stand age (O'Connor et al., 2023) and due to ecophysiological trait of each species (Mulia and Dupraz, 2006). Zhang et al. (2013) showed that young jujube dominated over wheat root plants in AC. Duan et al. (2019) found that wheat reduced the root density of young walnut tree by comparison with sole-cropped 2-year-old walnut trees. Still in young wheat-walnut AC, Zhang et al. (2015) showed that root of intercropped trees remained in shallow soil in the same layer than wheat. Finally, O'Connor et al. (2023) confirmed that below 5 years old, trees mainly colonized first the rows and nearby areas in upper soil layer.

Finally, with increasing stand age, competition with crop may force tree roots to explore deeper soil layers where nutrients and water should be available (Cardinael et al., 2015; Mulia and Dupraz, 2006; O'Connor et al., 2023).

### 4.3. Comparison between core-break and soil coring method

Core-break and soil coring are two methods largely used in agroforestry (Moreno et al., 2005; van Noordwijk et al., 2001) and arable land (Mulia and Dupraz, 2006; van Noordwijk et al., 1995) to quantify the fine root abundance, biomass or length density for trees and crop. We showed linear regression between TFRA estimated with core-break method and TFRB evaluated with soil coring for each tree species and a polynomial regression for wheat. These regressions attest the coherence and the reliability of our data for the two methods. From core-break method, Moreno et al. (2005) also showed linear relationship between root abundance counted in small soil surface and root length density (km $m^{-3}$) measured after root washing and length. Unlike them, the intercept of our linear regression was positive for wild cherry and negative for hornbeam and willow. Positive intercept indicates that observer in core-break method counted zero tree fine root on soil surface whereas fine root existed within cores, and conversely, negative intercept means that observer counted tree fine roots on soil surface whereas they did not exist within cores. This is illogical and contradictory to the observations; in most cases of low root biomass obtained with the soil coring method no roots could be counted on the broken faces. To deal with this error, Kücke et al. (1995) used a quadratic regression as $y = (c+dx)^2$ where $c >= 0$, however, in our case the regression obtained for trees species was not representative of data measured (results no shown).

Difficulties for both methods concerned the recognition of fine root during counting in core-break or during root sorting after recovering in soil coring method. For fine root biomass measurement, tree and wheat fine roots recognition was perfected by cutting an aliquot of tree fine root from standing trees and by uprooting a wheat plant. For counting, root recognition was based on root coloration, softness and flexibility.

Using the equation proposed by van Noordwijk et al. (2001) and Mulia and Dupraz (2006), we succeeded to propose calibration factors varying from X = 8.1, 2.1 to 1.3 for willow, wild cherry and hornbeam, respectively. Factor of calibration for wheat was not calculated. As suggested by van Noordwijk et al. (2001), X should differ from 1 for preferentially oriented roots. Our values are not in the range of values proposed by Bennie et al. (1987) or van Noordwijk et al.

(1995) but error may be from values of SRL = 15 m $g^{-1}$ for hornbeam (Kubisch et al., 2015), SRL = 27 m $g^{-1}$ for the wild cherry (Weemstra et al., 2020) and SRL = 66.8 m $g^{-1}$ for the willow (McIvor et al., 2014; Weemstra et al., 2020). The values that we proposed are close to those of Drew and Saker (1980) but X value for willow remained surprisingly high. Our equation allowed a better prediction of fine root biomass for willow and wild cherry but an underestimation of hornbeam. Finally, for our fine root biomass estimation, roots were not washed contrary to advised in the method, but clay or silt particle stuck on fine roots was hand removed after oven drying. Soil particles were carefully removed but despite our relentlessness some might escape to our vigilance.

## 5. Conclusion

From our findings, we showed that intercropping change root distribution of associated plants due to belowground competition. Young trees reduced the crop root abundance and biomass close to tree rows and cohabited with them in the upper soil layer. This was particularly true for willow and hornbeam whereas wild cherry already developed deep fine root under the wheat rooting zone. At 4 years old, different growth rates between species were evidenced with taller and more developed fine roots in topsoil for willow than hornbeam in AC. In pure-forest control plots where no fertilizer was applied since plantation, tree root development along 1 m depth was very high compared to AC trees, certainly due to the lack of nutrients. The core-break method allowed to an accurate estimation of crop and tree root abundance but suffer from difficulties in root recognition especially when roots were abundant or when they were already in decomposition at the counting time. Further works are needed to see the evolution of tree and crop root distribution along soil profile with increasing age. It will indicate if the fine root growth of each tree species will continue in the same direction as at 4 years old, or if as getting older they will spread in topsoil, particularly for willow and hornbeam. Analyses of water and nitrogen content could also be investigated to quantify exactly the effect of trees on water and nutrient competition.

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# Chapitre 5

## Early share of $^{15}$N-labelled fertilizer between trees and crop in young temperate alley-cropping system

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### Abstract

Nitrogen partitioning between trees and crop in alley cropping systems (AC) may increase the fertilizer nitrogen (N) use, but more investigation is needed to elucidate it for young trees. Using $^{15}$N labeling, we traced the fate of fertilizer N in wheat associated with four-years old trees at Ramecourt alley-cropping experimental site. In mid-spring, fine powder of $^{15}NH_4$,$^{15}NO_3$ with 10 atm% was applied in soil surface in a 2 m * 2 m area located 1.5 m distance from a referent tree (hornbeam, wild cherry and willow) in AC and in sole-crop control (CC) plots. The $^{15}$N abundance of tree leaves and crop whole-plant was compared with unlabeled samples from unfertilized pure-forest control plot (FC). The wheat $\delta^{15}$N increased from 0.7 ± 0.6 ‰ to 994 ± 474 ‰ before and 12 days after labeling and remained stable until harvest. No significant difference was observed between AC and CC or between associated tree species. The catch crop following wheat recovered a part of unused tracer as revealed by high δ$^{15}$N value (172 ± 129 ‰) compared to natural abundance (5.8 ± 1.8 ‰). Tree foliar δ$^{15}$N was multiplied by 1.5, 1.8 and 4 for willow, hornbeam and wild cherry respectively compared to unlabeled trees in AC. Unlike wheat, tree foliar $^{15}$N enrichment was observed only 30 and 40 days after labeling. At wheat harvest, the artificial δ$^{15}$N was significantly higher in ears (1181 ± 111 ‰) than in stems + leaves (714 ± 86 ‰) and in roots (429 ± 42 ‰). The wheat associated with trees allocated significantly more tracer N to ears than wheat in monocrop. Young trees in alley cropping systems contributed to valorize the N fertilizer without significant effect on global wheat uptake and favored its valorization in grain.

**Key words:** Alley-cropping system, tree and wheat nitrogen sharing, $^{15}$N labelling, tree foliar δ$^{15}$N, N and C content, whole-plant wheat δ$^{15}$N, wheat ears δ$^{15}$N

## 1. Introduction

Since the discovery of ammonia synthesis from hydrogen and nitrogen (N) under high pressure by Haber and Bosch (Bosch, 1932; Haber, 1920; Leigh, 2004; Paull, 2009), the use of synthetic mineral N to fertilize crops has intensified agricultural production, resulting in highly increased crop yields. In parallel, the problem of N leaching (Roth and Fox, 1990) and high nitrate concentration in both groundwater and surface water (Datta et al., 1997) has emerged. At the French national level, N input for a given crop is often calculated from the balance sheet method (COMIFER, 2013; Meynard et al., 1997) and the recommended N fertilizer dose is split into several applications according to the crop development stage, based on farming current practices. For wheat, the last N fertilizer is applied almost at the heading stage (GS51-59, Zadoks et al., 1974) to increase the final grain proteins content (T. Fuertes-Mendizábal et al., 2010). However, as the wheat N needs are almost satisfied at this period and if no rainfall follows the application, most of this last N fertilizer remains in the soil at harvest and may potentially leach into groundwater during the autumnal period with water drainage (Beaudoin et al., 2005).

Alley-cropping systems (AC) may be a lever to mitigate negative impacts of agriculture on environment (Smith et al., 2012) and are considered better than conventional agriculture in long term-productivity and sustainability (Thevathasan and Gordon, 2004). These systems may alleviate water quality issues arising from intensive agricultural practices (Jose, 2009) by improving N fertilizer use efficiency and reducing losses (Palma et al., 2007; Udawatta et al., 2002; Zhu et al., 2020). Some authors showed that trees are able to capture N below the crop root-zone, thanks to their deep roots and may limit N leaching (Allen et al., 2004; Bergeron et al., 2011; Lehmann and Schroth, 2003; Zamora et al., 2009). This mechanism is known as the safety-net hypothesis. In addition to excess nutrient uptake, trees recycle N into the system through root turnover and litterfall, increasing the nutrient use efficiency (Thevathasan and Gordon, 2004; van Noordwijk et al., 2015; Zamora et al., 2009).

Despite the ability of trees to recycle N in AC, their roots may also enter into competition for N with the associated plants, resulting in a crop yield decrease (Arenas-Corraliza et al., 2022). Jose et al. (2000) showed that the presence of a root barrier prevented the competition between corn and adult black walnut and thus provided a better yield and grain quality. They also evidenced that competition for N between maize and tree roots decreased the fertilizer use by the crop. Indeed, 47.2 % of the fertilizer used was found in the harvested tissues (grain and stover) grown with a barrier, while only 38.9 % was found when there was no tree roots separation. They explained this decrease by an inability to use the available fertilizer due to the reduced growth of the crop in competition with trees. These results were also confirmed by Zamora et al. (2009) using the same method to observe nitrogen resource competition between 5-year-old pine trees and cotton crop. However, in the absence of a barrier, the percentage of N from fertilizer in the crop was higher than in the barrier treatment (Gillespie et al., 2000; Zamora et al., 2009). Thus, the competition with trees induced a high N fertilizer use by the crop.

The success of AC partly depends on the balance between competition and complementarity of the associated species (Schroth et al., 2003). The choice of tree species may limit the competition and promote the complementarity thanks to temporal and/or spatial separation of nutrient uptake with crops. Indeed, the competition for N fertilizer is minimal when N acquisition is not simultaneous in time and space (Rowe et al., 2001; Zamora et al., 2009). Cardinael et al. (2015) evidenced that walnut fine roots grew in depth when trees were intercropped with winter wheat. This ability of trees to adapt their root disrtibution to their environment reduce N competition with crops and may improve its use efficiency throughout the soil profile (Allen et al., 2004; Rowe et al., 1999). Artificial stable isotope like $^{15}$N-enriched fertilizer or natural $^{15}$N abundance of trees, crop and soil has been used to examine the competition for N between trees and crop in AC (Zamora et al., 2009; Allen et al., 2004), to trace movement of applied N (Rowe et al., 1999. ; Jose et al., 2000; Allen et al., 2004a,b) at different component of agroecosystems, to quantify the different flux of N recovered or not by crop and to study the N dynamics.

Wheat is the most cultivated crop in the Hauts-de-France region of France and represents a considerable economic challenge especially for proteins content of grains for exportation. The 3rd or 4th application of N fertilizer at the heading period is relevant to obtain such high proteins content but also increases the risk of N excess in soil in case of low valorization. Tree inclusion in arable land is progressively gaining interest in farmers in the territory and may be a lever to recover this potential N excess as well as all agronomical innovative practices, but more information is needed about the interactive dynamics of N in tree-crop systems to maximize fertilizer-use efficiency. The competition for nitrogen between trees and crops and the safety-net role of trees has been demonstrated in adult AC stands (Allen et al., 2004; Jose et al., 2000; Suprayogo et al., 2002). Until now, few data are available about the beginning of root cohabitation and N partitioning between trees and crops for young stands, whereas the installation phase of AC is a key step to understanding the belowground interactions between tree and crop roots. Bergeron et al. (2011) and Zamora et al. (2009) investigated the N dynamics in 5-year-old AC and showed belowground interactions between crops and tree roots, competition for N and reduction of N leaching.

The aim of this work was to trace the fate of the fourth fertilizer N supply in wheat crop in the presence of four-years-old trees in alley-cropping systems using $^{15}$N labeling experimentation. The objectives were (i) to determine the $^{15}$N tracer enrichment of trees and crop after labeling, (ii) to characterize its distribution within plants and (iii) to quantify the labeled N remaining in the soil. We hypothesized that competition between associated plants would start early especially in the topsoil and in the cohabitation zone close to the tree row, that the N labeling tool may testify this competition, that intercropped trees change the uptake of N fertilizer by wheat, and that the wheat-AC association can improve N uptake.

## 2. Materials and methods

### 2.1. Description of the experimental site

This work was conducted at the Ramecourt alley-cropping experimental site (18 ha) located 77 km from Lille in northern France (50°22’N, 2°17’E) (Andrianarisoa et al., 2019) (see chapter

2). The climate is oceanic with an average annual temperature, rainfall and wind of 10.6°C, 859 mm and 3.8 m $s^{-1}$ respectively (years 2010-2020). The soil is a deep luvic cambisol (Baize and Girard, 2009) with a silt loam texture in the upper soil layer and locally silty with flint and alkaline pH (7.9). Soil characteristics details are available in chapter 3 (section 2.1; Table 1).

The experimental plot is a randomized block design with three replications where three types of alley-cropping (AC1, AC2 and AC3) were compared with sole-crop (CC) and pure-forest (FC) control plots. One-year-old trees were planted in SW-NE oriented rows spaced from 38 m and 7 m apart in AC and FC respectively in 2018. Within each row, tall trees were planted two by two at 8 m apart and were intercalated every 1 m by 9 species of shrubs (including *Salix alba*). The AC1 is an association of *Quercus robur* (oak) and *Carpinus betulus* (hornbeam), the AC2 a mixture of *Juglans regia x regia* (hybrid walnut), *Alnus glutinosa* (alder) and *Prunus avium* (wild cherry) and the AC3 a combination of *Alnus glutinosa* (alder) and *Robinia pseudoacacia* (black locust). A strip of 2 m wide along the tree rows was not cultivated and spontaneous weeds were left to grow. The same trees associations were repeated in FC but the interrow was occupied by a sowed ryegrass plant (*Lolium multiflorum*), cut twice a year and left on the ground. Since 2019, the height of all trees was measured using a handmade measuring rod at the end of leaf fall in October.

## 2.2. Experimental material and design

In the AC alley, as well as in the CC plot, a winter wheat (*Triticum aestivum*), cultivar “Extase” (https://www.geves.fr/catalogue/), was sown on October 15th, 2020. The previous crop harvested at the beginning of October was chicory witloof. A superficial soil disking was done to prepare the seedbed before wheat sowing. Winter wheat received 220 kg N $ha^{-1}$ as solid mineral N fertilizer divided into four applications: 50 kg N $ha^{-1}$ (Sulfan 24% of N), 90 kg N $ha^{-1}$ (Ammonitrate 27%), 50 kg N $ha^{-1}$ (Ammonitrate 27%) and 30 kg N $ha^{-1}$ (Ammonitrate 27%) carried out on February 25th (GS26; Zadok et al., 1974), March 23rd (GS30), April 24th (GS31) and June 2nd (GS55) respectively. Fertilizer N was applied using solid fertilizer spreader. As no barrier was installed on the spreader during application, part of the fertilizer fell on the uncropped understory vegetation strip. Weeds and pests were chemically controlled. After wheat harvesting in summer, a cover crop composed of *Phacelia tanacetifolia*, *Eruca sativa* and *Trifolium* sp. was sown on August 25th, 2021, and was mechanically destroyed and buried in January 2022. No fertilizer and no pesticides were applied in FC plots.

For this study, the tested treatments were the "type of system" (AC, FC and CC), the "sampling date" (indicates all dates of plant sampling), the "tree species" (hornbeam, wild cherry and willow) and the associated species: sole-cropped wheat (Wheat-CC), wheat intercropped with hornbeam (Wheat-AC-Hornbeam), wheat intercropped with wild cherry (Wheat-AC-Wild cherry) and wheat intercropped with willow (Wheat-AC-Willow). Tree species were chosen according to their contrasted aerial growth rate.

### 2.3. $^{15}$N labeling experiment

The $^{15}$N labeling experiment was caried out on June 2nd, 2021, at the fourth N fertilizer application on wheat. An area of 2 m x 2 m was delimited within the crop alley 1.5 m distance from the referent tree in the south side of the row in AC (Figure 1), or randomly located in CC. The $^{15}$N tracer used was ammonium nitrate powder ($^{15}NH_4$,$^{15}NO_3$) applied at the dose of 30 kg N ha$^{-1}$. The day of application, 12 g of ammonium nitrate enriched in $^{15}$N at 10 atm% with 98 % of purity (Cambridge Isotope Laboratories, Inc., Andover, MA, USA) was mixed with 500 g of sieved soil (4 mm) from the upper soil layer and was evenly spread by hand on the soil surface in the dedicated zone, avoiding wheat leaves. To ensure the homogeneity of hand $^{15}$N tracer application, only one operator did the application, and a preliminary test was done on an equivalent surface. In the rest of plot, the equivalent N fertilizer dose as ammonium nitrate form (27 % of N) was applied but to avoid double input of fertilizer on the labeled areas, plastic tarpaulins (2.5 m x 3 m) were attached to four stakes before the ammonium nitrate application with spreader. Fertilizer granules falling on the tarpaulins were thrown away from the labeled area. As no fertilizer was applied in FC, no $^{15}$N labeling was done.

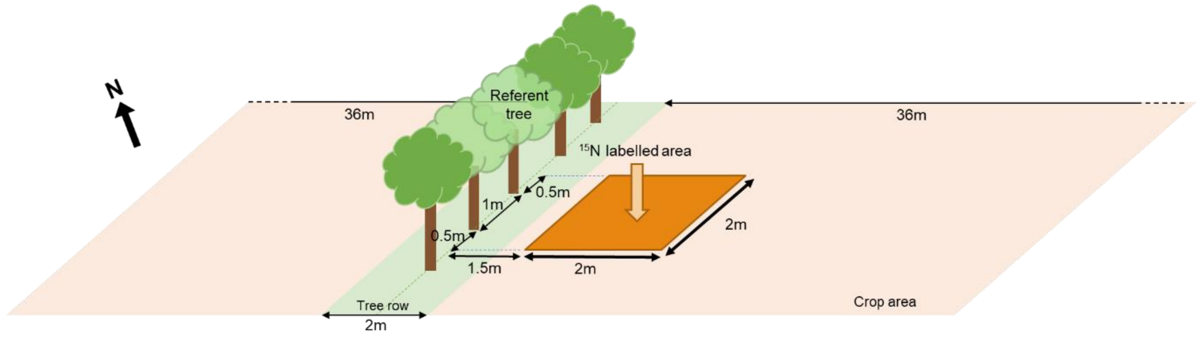


**Figure 1:** Schematical representation of $^{15}$N labeling design for one tree species within AC in block 1. Tree rows were at 36 m apart. The scheme is not in scale.

### 2.4. Wheat biomass measurement

Wheat biomass was measured 3 m distance from tree rows only in unlabeled area to limit disturbance within our labeled area. As the trees are still young, we assumed that there is no effect of tree species on wheat biomass measured at this distance. Therefore, we did not measure the wheat biomass of wheat close to each chosen tree species but in a random area within the AC plot. In each block, wheat plants within a frame of 50 m x 50 cm were cut at soil level (n = 6 for AC and n = 3 for CC). The wheat biomass was calculated from samples collected on May 3rd, June 2nd and July 21st which corresponded to the second node (GS32), the heading (GS55) and the ripening (GS89) stages respectively (Zadoks et al., 1974). Samples collected on the three sampling dates were oven dried at 65°C for 72 h and the dry biomass was expressed per unit area.

### 2.5. Plant and soil sampling

Plant samples were taken at seven sampling dates following the $^{15}N$ labeling experiment at almost 15 days of interval except in autumn: 27/05; 16/06; 30/06; 15/07; 29/07; 16/09; 07/10/2021. Additionally, tree leaves were also sampled on 10th May 2022 to evaluate the foliar $\delta^{15}N$ from remobilization. Twenty young tree leaves oriented toward the labeling area were sampled from the referent tree and twenty wheat tillers in the labeling area. At each sampling date, leaves from unlabeled trees and wheat and ryegrass (RG) tillers from unlabeled areas were also collected in AC, CC and FC for control treatments. Among samples collected at wheat maturity on 29th July, 3 or 4 plants were uprooted from $^{15}N$ labeled and unlabeled zones. Roots, stems and ears were hand separated using a pair of scissors. Roots were hand shaken to remove soil particles and were washed.

For sampling dates after wheat harvest, the collected plant samples in cropped areas corresponded to the catch crop whole-plant. Samples were placed in perforated bags and were transported to laboratory where they were oven dried in an oven at 65°C for 72 h. A total of 60 samples were collected at each sampling date (n = 24, 27 and 9 for wheat, trees and RG respectively).

Soil samples were collected in November 2021 in labeled and unlabeled areas. Soil cores were taken 2 m from the referent trees down to 120 cm in AC and FC, using a manual auger ($\Phi$ =

6.3 cm). In CC, soil cores were taken both in the middle of the labeled and in random unlabeled areas. Soil samples were collected according to the following layers: 0-30 cm (L1 or topsoil), 30-60 cm (L2) and 60-120 cm (L3) and were stored at 4°C for further analysis.

### 2.6. Soil and plant chemical analyses

For soil $^{15}N$ analysis, an aliquot was oven dried at 65°C for 5 days. Dried plant and soil samples were finely ground to 200 µm. The $^{15}N/^{14}N$ ratio, the nitrogen (N) and the carbon (C) content were measured using an elementary analyzer (EA, EuroPyrOH; EuroVector, Milano, Italy) coupled with an isotope-ratio mass spectrometry (Isoprime IRMS; Elementar, Manchester, UK) at the SILVATECH platform (SILVATECH, INRAE, 2018. Structural and Functional Analysis of Tree and Wood Facility, Nancy, France). The $^{15}N$ abundance were expressed as $\delta^{15}N$ (‰) relatively to the international standard $^{15}N_2$ in atmosphere (Mariotti et Lquid, 1983) following the equation (1):

$$\delta^{15}N(‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000 \qquad (1)$$

where $R_{sample}$ and $R_{standard}$ represent the $^{15}N/^{14}N$ ratio of the sample and $^{15}N_2$ in atmosphere standard respectively.

### 2.7. Statistical analyses

Bivariate linear models were performed to analyze the variability of plant and soil parameters according to equation 2:

$$y = ax_1 + bx_2 + c + \beta \qquad (2)$$

where “y” is the interest variables (soil $\delta^{15}N$, plant $\delta^{15}N$, C, N, C/N), $x_1$ is the block, $x_2$ is the explanatory variable (sampling date, type of system, associated tree species, interactions between these variables, wheat tissues), “a”, “b” and “c” are coefficients and $\beta$ the model error. For all tested models, the coefficient of determination ($R^2$) and the *p-value* was noted. The labeled and unlabeled samples was first separately analyzed. Multiple comparison tests were carried out using the multcomp R software package (Hothorn et al., 2008) to compare the mean value of interest variables for each $x_2$ variable using a post hoc Tukey’s test ($p < 0.05$). For a given quantitative variable (soil $\delta^{15}N$, plant $\delta^{15}N$, C, N, C/N), the comparison between two means for a qualitative variable (e.g. between AC and CC in wheat and catch crop samples, between sampling dates of the catch crop, between labeled or unlabeled) were compared using Student's t-test ($p < 0.05$).

For trees, comparison between the type of system and the species were also performed using the same model in equation (2), with either $\delta^{15}N$ or N and C content, or C/N as interest variable, and type of system or tree species as the explanatory variable $x_2$. Then, $\delta^{15}N$ significant differences between samples collected in labeled and unlabeled areas were identified using the Kruskal-Wallis nonparametric test ($p < 0.05$). A bivariate linear model was also performed to analyze tree height and growth, with the block and type of system or tree species as explanatory variables, followed by a Tukey's tests for post hoc pairwise comparisons ($p < 0.05$).

A Pearson correlation coefficient was calculated between sampling date and N, C content and C/N ratio in the wheat, the RG and the tree samples. For tree and wheat samples, simple linear regression analyses were used to investigate the relationships between $\delta^{15}N$, N, C content, or C/N and explanatory variables such as type of system, tree species and sampling date.

One-way ANOVAs were performed to analyze the variability of soil $\delta^{15}N$ in soil with type of system, soil layers or tree species as explanatory variables followed by a Tukey's test for post hoc pairwise comparisons ($p < 0.05$), labeled and unlabeled samples were analyzed separately. For each tree species and soil layer, significant differences of $\delta^{15}N$ between labeled and unlabeled soil samples in CC and AC were identified using the Kruskal-Wallis nonparametric test ($p < 0.05$), and a Student (or a Mann-Whitney test when the conditions of validity of the Student test were not verified) was run to evidence significant difference between AC and FC in unlabeled soil samples ($p < 0.05$).

Along the text, the symbol *** was used to indicate a significant correlation coefficient at $p < 0.001$ level, the symbol ** for $p < 0.01$ level and the symbol * for $p < 0.05$ level. In the results section, all mean values for a given variable are followed by the standard deviation. All statistical analyses were performed with R software version 4.0.4.

## 3. Results

### 3.1. Evolution of crops and trees N, C content and C/N ratio

*Wheat, catch crop and RG*

**Table 1:** Coefficient of determination and *p-value* of linear regression model relating the variables type of system, tree species, sampling date and their interaction with foliar and whole-plant with $\delta^{15}$N, N and C content and C/N ratio of trees and wheat respectively, collected in unlabeled and labeled area at Ramecourt alley-cropping experimental site. "ns" means not significant.

| Tested variable | Plant species | Unlabeled samples | | | | | | | | Labeled samples | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Foliar $\delta^{15}$N (‰) | | Foliar N content (%) | | Foliar C content (%) | | Foliar C/N | | Foliar $\delta^{15}$N (‰) | | Foliar N content (%) | | Foliar C content (%) | | Foliar C/N | |
| | | *p* value | R² | *p* value | R² | *p* value | R² | *p* value | R² | *p* value | R² | *p* value | R² | *p* value | R² | *p* value | R² |
| Type of system | Tree | ns | - | <0.001 | 0.39 | <0.001 | 0.09 | <0.001 | 0.41 | - | - | - | - | - | - | - | - |
| Tree species | | 0.002 | 0.08 | 0.015 | 0.05 | ns | - | ns | - | <0.001 | 0.20 | 0.002 | 0.15 | ns | - | 0.002 | 0.15 |
| Sampling date | | <0.001 | 0.13 | <0.001 | 0.21 | <0.001 | 0.29 | <0.001 | 0.23 | 0.009 | 0.17 | <0.001 | 0,4 | <0.001 | 0,3 | <0.001 | 0,38 |
| Type of system*tree species | | <0.001 | 0.18 | <0.001 | 0.46 | 0.004 | 0.09 | <0.001 | 0.42 | - | - | - | - | - | - | - | - |
| Type of system*sampling date | | 0.002 | 0.16 | <0.001 | 0.67 | <0.001 | 0.44 | <0.001 | 0.76 | - | - | - | - | - | - | - | - |
| Tree species*sampling date | | <0.001 | 0.22 | <0.001 | 0.27 | <0.001 | 0,34 | <0.001 | 0,22 | 0.008 | 0,31 | <0.001 | 0,66 | <0.001 | 0.58 | <0.001 | 0,64 |
| Type of system*tree species*sampling date | | <0.001 | 0.31 | <0.001 | 0.85 | <0.001 | 0.53 | <0.001 | 0.86 | - | - | - | - | - | - | - | - |

| | Plant species | Whole-plant $\delta^{15}$N (‰) | | Whole-plant N content (%) | | Whole-plant C content (%) | | Whole-plant C/N | | Whole-plant $\delta^{15}$N (‰) | | Whole-plant N content (%) | | Whole-plant C content (%) | | Whole-plant C/N | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *p* value | R² | *p* value | R² | *p* value | R² | *p* value | R² | *p* value | R² | *p* value | R² | *p* value | R² | *p* value | R² |
| Type of system | Wheat | ns | - | ns | - | ns | - | ns | - | ns | - | ns | - | ns | - | ns | - |
| Tree species | | ns | - | ns | - | ns | - | ns | - | ns | - | ns | - | ns | - | ns | - |
| Sampling date | | <0.001 | 0.41 | <0.001 | 0.38 | <0.001 | 0.19 | <0.001 | 0.24 | <0.001 | 0.34 | <0.001 | 0.37 | 0.003 | 0.15 | <0.001 | 0.25 |
| Type of system*tree species | | ns | - | ns | - | ns | - | ns | - | 0.162 | ns | 0.519 | ns | ns | - | ns | - |
| Type of system*sampling date | | <0.001 | 0.42 | <0.001 | 0.37 | 0.017 | 0.15 | 0.003 | 0.20 | <0.001 | 0.36 | <0.001 | 0.33 | ns | - | 0.003 | 0.20 |
| Tree species*sampling date | | <0.001 | 0.48 | 0.002 | 0.29 | 0.409 | ns | ns | - | <0.001 | 0.46 | 0.005 | 0.25 | ns | - | ns | - |
| Type of system*tree species*sampling date | | <0.001 | 0.48 | 0.002 | 0.29 | 0.409 | ns | ns | - | <0.001 | 0.46 | 0.005 | 0.25 | ns | - | ns | - |

**Table 2:** Temporal evolution of aerial N, C content and C/N ratio of wheat, catch crop and ryegrass (RG) samples collected in unlabeled and labeled area at Ramecourt alley-cropping experimental site. The r represents Pearson correlation coefficient between the sampling date and N, C content and C/N ratio. The symbol * indicate significant correlation coefficient; *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. "ns" means not significant. The color shades vary according to the value for each interested variables (N, C content and C/N ratio), from dark blue to bright red corresponding to the lowest and the highest value respectively. AC: alley-cropping, FC: pure-forest control, CC: sole-control crop

| Sampling date | Crop type | Aerial crop N content (%) | | | | | Aerial crop C content (%) | | | | | Aerial crop C/N ratio | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Unlabeled | | Labeled | | mean | Unlabeled | | Labeled | | mean | Unlabeled | | Labeled | | mean |
| | | AC | CC | AC | CC | | AC | CC | AC | CC | | AC | CC | AC | CC | |
| 27/05/2021 | Wheat | 1.9 | 1.8 | 1.9 | 1.8 | 1.8 | 44 | 44 | 44 | 44 | 44 | 24 | 25 | 24 | 25 | 24 |
| 16/06/2021 | | 1.3 | 1.4 | 1.3 | 1.4 | 1.4 | 44 | 44 | 45 | 45 | 45 | 35 | 32 | 34 | 31 | 33 |
| 30/06/2021 | | 1.0 | 1.1 | 1.1 | 1.2 | 1.1 | 44 | 43 | 44 | 44 | 44 | 44 | 38 | 43 | 36 | 41 |
| 15/07/2021 | | 1.1 | 1.4 | 1.1 | 1.0 | 1.2 | 44 | 44 | 44 | 44 | 44 | 40 | 31 | 42 | 43 | 39 |
| 29/07/2021 | | 0.8 | 0.9 | 0.8 | 0.9 | 0.9 | 37 | 39 | 40 | 41 | 39 | 66 | 56 | 67 | 59 | 62 |
| r | | -0.5<br>*** | ns | -0.5<br>*** | -0.5<br>* | -0.5<br>*** | -0.7<br>*** | -0.5<br>* | -0.3<br>* | ns | -0.6<br>*** | 0.5<br>*** | 0.5<br>* | 0.5<br>*** | 0.5<br>* | 0.5<br>*** |
| 16/09/2021 | Catch crop | 4.8 | 5.1 | 5.1 | 4.7 | 4.9 | 40 | 42 | 42 | 40 | 41 | 9 | 8 | 8 | 9 | 8 |
| 07/10/2021 | | 3.8 | 3.7 | 4.1 | 3.5 | 3.8 | 41 | 42 | 41 | 43 | 42 | 11 | 11 | 10 | 13 | 11 |

Table 2 (*continued*)

| Sampling date | Aerial RG N content (%) | Aerial RG C content (%) | Aerial RG C/N ratio |
|---|---|---|---|
| 27/05/2021 | 1.41 | 42.4 | 31.0 |
| 16/06/2021 | 1.07 | 43.4 | 41.7 |
| 30/06/2021 | 0.97 | 41.8 | 44.4 |
| 15/07/2021 | 1.29 | 42.2 | 33.9 |
| 29/07/2021 | 1.51 | 39.6 | 26.4 |
| 16/09/2021 | 1.29 | 40.4 | 31.6 |
| 07/10/2021 | 1.15 | 41.4 | 36.3 |
| r | ns | -0.6*** | ns |

The monitoring of aerial wheat biomass over the crop growing cycle showed no significant difference between wheat biomass measured at 3 m from tree rows in AC and CC, whether in second node, in heading or in ripening stage (data not shown).

Globally, the N content of aerial wheat gradually decreased over time (r = -0.5***, Table 2). The mean N content of aerial wheat varied from 1.8 % at the labeling period on May 27th 2021, to 0.9 % at the harvest. The sampling date accounted for 38 % and 37 % of wheat N content in unlabeled and labeled samples respectively (Table 1). When all data was analyzed including all sampling date, the mean N content of aerial wheat was equal to 1.3 ± 0.3 %, and no significant difference was observed between the type of system, and between labeled and unlabeled samples (Table 2). The C content of aerial wheat also significantly decreased over time (r = -0.6***) with few exceptions. The mean C content of aerial wheat varied from 44 % on May 27th 2021, to and 39 % at the harvest. The sampling date accounted for 19 % and 15 % of wheat C content in unlabeled and labeled samples respectively (Table 1). When data for all sampling dates were analyzed, the C content of aerial wheat was equal to 43 ± 2.1 %, and no significant difference was observed between the type of system, and between labeled and unlabeled samples (Table 2). Finally, the C/N ratio of aerial wheat significantly increased over time for all samples (r = 0.5***). The mean value of aerial wheat C/N ratio varied from 24 in spring to 62 at the harvest, i.e. 2.6 times higher. When data for all sampling date were analyzed, the C/N ratio of aerial wheat was equal to 40 ± 13, and no significant difference was observed between the type of system, and between labeled and unlabeled samples.

Regarding the catch crop, the mean value of N and C content and the C/N ratio of the aerial tissues were 4.3 %, 41 % and 10, respectively. For these three parameters, no significant difference was observed between the type of system, and between labeled and unlabeled samples. The C content of aerial RG significantly decreased from spring to autumn 2021 (r = -0.6***, Table 2), however, during the same period, the N content and the C/N ratio of aerial RG were stable. There was no significant difference of the aerial N content and the C/N ratio between the RG and the wheat regardless of the type of system, the labeled and the unlabeled samples. The mean value of aerial C content was significantly lower for RG than wheat.

### *Tree growth and foliar N and C content*

In 2021, the tree height was on average 2.7 ± 0.7 m. They were 0.8 m taller in AC compared to FC (see chapter 4, Table 1). Indeed, all tree species were taller in AC than in FC except

**Table 3:** Temporal evolution of foliar N, C content and C/N ratio of tree samples collected in unlabeled and labeled area at Ramecourt alley-cropping experimental site. The r represents Pearson correlation coefficient between the sampling date and N, C content and C/N ratio. The symbol * indicate significant correlation coefficient; *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. "ns" means not significant. The color shades vary according to the value for each interested variables (N, C content and C/N ratio), from dark blue to bright red corresponding to the lowest and the highest value respectively. H: Hornbeam, WC: Wild cherry, W: Willow, AC: alley-cropping, FC: pure-forest control, CC: sole-control crop

| Sampling date | Foliar N content (%) | | | | | | | | | | Foliar C content (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Unlabeled | | | | | | Labeled | | | | Unlabeled | | | | | | Labeled | | | |
| | H | | WC | | W | | H | WC | W | mean | H | | WC | | W | | H | WC | W | mean |
| | AC | FC | AC | FC | AC | FC | AC | AC | AC | | AC | FC | AC | FC | AC | FC | AC | AC | AC | |
| 27/05/2021 | 3.1 | 2.5 | 3.4 | 2.8 | 4.2 | 3.3 | 3.1 | 3.4 | 4.2 | 3.3 | 45 | 44 | 46 | 43 | 46 | 43 | 45 | 46 | 46 | 45 |
| 16/06/2021 | 2.3 | 1.7 | 3.3 | 1.6 | 3.5 | 1.8 | 2.5 | 3.0 | 3.3 | 2.6 | 46 | 46 | 48 | 46 | 47 | 44 | 45 | 48 | 46 | 46 |
| 30/06/2021 | 1.9 | 1.5 | 2.6 | 1.4 | 3.4 | 1.5 | 1.9 | 2.4 | 3.1 | 2.2 | 45 | 44 | 46 | 44 | 47 | 45 | 45 | 46 | 47 | 45 |
| 15/07/2021 | 2.4 | 1.4 | 2.5 | 1.5 | 3.7 | 1.5 | 2.2 | 2.2 | 3.3 | 2.3 | 46 | 46 | 48 | 46 | 47 | 46 | 46 | 47 | 47 | 47 |
| 29/07/2021 | 2.4 | 1.3 | 2.6 | 1.6 | 3.3 | 1.4 | 2.2 | 2.5 | 2.6 | 2.2 | 46 | 45 | 45 | 44 | 45 | 45 | 47 | 46 | 46 | 45 |
| 16/09/2021 | 2.5 | 2.3 | 1.9 | 2.0 | 2.5 | 1.7 | 2.7 | 2.3 | 2.5 | 2.2 | 47 | 46 | 46 | 48 | 46 | 45 | 48 | 46 | 47 | 46 |
| 07/10/2021 | 3.1 | 2.0 | 2.2 | 1.5 | 2.5 | 1.7 | 2.5 | 2.2 | 2.4 | 2.2 | 45 | 46 | 46 | 47 | 46 | 47 | 48 | 47 | 47 | 47 |
| r | ns | ns | -0.7<br>*** | ns | -0.9<br>*** | -0.6<br>** | ns | -0.8<br>*** | -0.8<br>*** | -0.4<br>*** | ns | 0.5<br>* | ns | 0.5<br>* | ns | 0.7<br>*** | 0.9<br>*** | ns | 0.5<br>* | 0.3<br>*** |
| 10/05/2022 | 2.9 | 2.3 | 3.4 | 2.3 | 3.6 | 2.9 | 3.0 | 3.4 | 3.6 | 3.0 | 45 | 45 | 45 | 45 | 44 | 43 | 46 | 45 | 44 | 45 |

Table 3 (*continued*)

| Sampling date | Foliar C/N ratio | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Unlabeled | | | | | | Labeled | | | mean |
| | H | | WC | | W | | H | WC | W | |
| | AC | FC | AC | FC | AC | FC | AC | AC | AC | |
| 27/05/2021 | 15 | 17 | 14 | 16 | 11 | 13 | 15 | 14 | 13 | 14 |
| 16/06/2021 | 20 | 27 | 15 | 29 | 13 | 24 | 19 | 16 | 14 | 20 |
| 30/06/2021 | 23 | 30 | 18 | 33 | 14 | 32 | 24 | 19 | 15 | 23 |
| 15/07/2021 | 20 | 33 | 20 | 31 | 13 | 31 | 21 | 21 | 14 | 23 |
| 29/07/2021 | 19 | 36 | 17 | 27 | 14 | 33 | 21 | 19 | 18 | 23 |
| 16/09/2021 | 19 | 20 | 20 | 25 | 18 | 28 | 18 | 21 | 19 | 21 |
| 07/10/2021 | 15 | 23 | 21 | 31 | 19 | 27 | 19 | 21 | 20 | 22 |
| r | ns | 0.8 *** | 0.7 *** | ns | 0.9 *** | 0.5 * | ns | ns | 0.8 *** | 0.3 *** |
| 10/05/2022 | 16 | 20 | 13 | 20 | 12 | 15 | 16 | 14 | 13 | 15 |

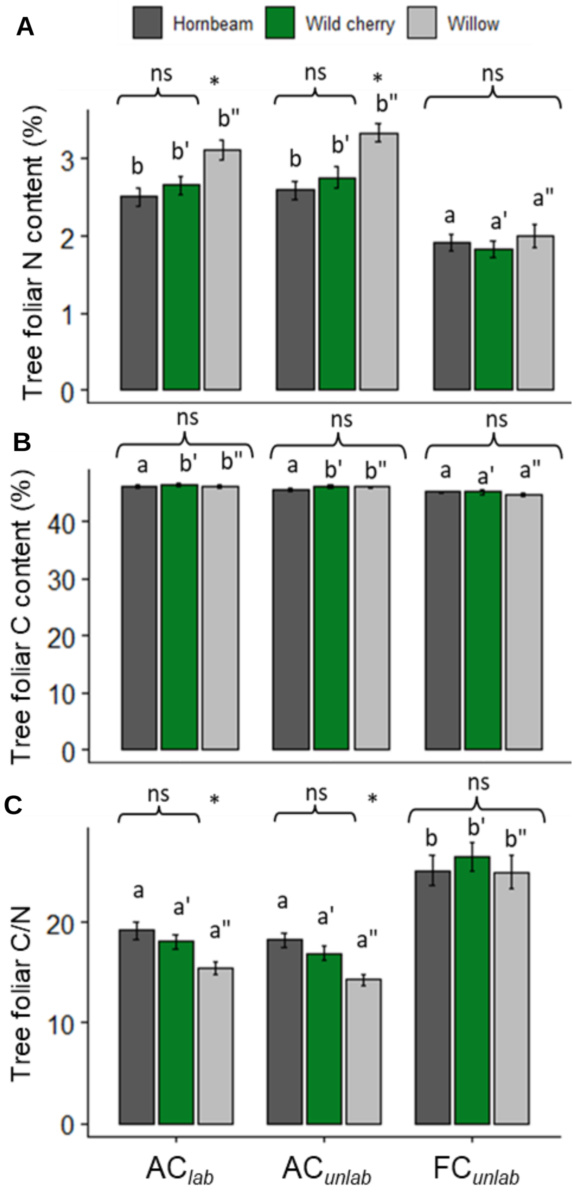


**Figure 2:** Mean values of N (%), C content (%) and C/N ratio in tree leaves collected in labeled (AC*lab*) and unlabeled (AC*unlab* and FC*unlab*) area at Ramecourt alley-cropping experimental site. Histograms are means (±SE). Letters compare type of systems (AC*lab*, AC*unlab* and FC*unlab*) for a given tree species; means with same letters are not significantly different (Tukey's test; $p < 0.05$). Asterisks indicate significant difference between tree species for a given type of systems (Tukey's test; $p < 0.05$). "ns" means not significant. AC: alley-cropping, FC: pure-forest control plot.

hornbeam. In both AC and FC, the mean tree height was significantly higher for willow compared to wild cherry and hornbeam. The same trend was found for the growth rate. Trees in AC grew faster (80 ± 30 cm $y^{-1}$) compared to those in FC (50 ± 20 cm $y^{-1}$). The growth rate was the same for all tree species in FC whereas it was significantly higher for willow (130 ± 30 cm $y^{-1}$) compared to wild cherry (80 ± 20 cm $y^{-1}$) and hornbeam (60 ± 10 cm $y^{-1}$) in AC. The willow grew faster and was taller than other species except in FC where its growth was not significantly different of other species.

The mean value of tree foliar N content significantly decreased from spring to autumn 2021 except for all hornbeam samples and wild cherry unlabeled samples in FC (Table 3). Indeed, the mean value of tree foliar N content varied from 3.3 %, in May 27th 2021 to 2.2 % in October 10th 2021. Then, in spring 2022, the tree foliar N content was high again for all samples (3 %). In FC, the mean value of tree foliar N content was significantly lower than in AC for all tree species (Figure 2A). In AC, the tree foliar N content of willow was significantly higher than hornbeam and wild cherry for both labeled and unlabeled samples, whereas no significant difference between tree species was observed in FC. The interaction between type of system and tree species accounted for 46 % of tree foliar N content variability (Table 1).

The mean value of tree foliar C content significantly increased from spring to autumn 2021 except in AC for unlabeled samples for all tree species and for labeled wild cherry (Table 3). The next spring in 2022, tree foliar C content was low for all samples. In FC, the mean value of tree foliar C content was significantly lower than in AC for wild cherry and willow (Figure 2B). For all types of system, labeled or unlabeled, no significant difference in tree foliar C content was observed between tree species.

As it did for the C content, the mean value of tree foliar C/N ratio significantly increased from spring to autumn 2021 with some exception (Table 3). On average, C/N ratio ranged from 14 to 22 between May and October 2021. Then, for all samples tree foliar C/N ratio was low in spring 2022. Finally, the mean value of tree foliar C/N ratio was significantly lower in FC than in AC for all tree species (Figure 2C). Consistent with N content results, no significant difference in tree foliar C/N ratio was observed between tree species in FC, and it was significantly lower for willow than hornbeam and wild cherry for both labeled and unlabeled samples in AC.

## 3.2. Wheat and trees $^{15}$N natural abundance

### *Whole-plant wheat, catch crop and RG $^{15}$N natural abundance*

For the $^{15}$N natural abundance of wheat, the $\delta^{15}$N was positive with a mean value of $\delta^{15}N = 1.4 \pm 2.5$ ‰. It reached a maximum value just after the fourth N fertilizer, sharply decreased until the heading stage and stabilized until the harvest (Figure 3A). The sampling date significantly explained wheat $\delta^{15}$N natural abundance at 41 % ($p < 0.001$; Table 1). For $^{15}$N natural abundance of RG, the $\delta^{15}$N was also positive with a mean value of $\delta^{15}N = 2.6 \pm 2.3$ ‰ which remained stable over time (Figure 3A). The $^{15}$N natural abundance was on average the highest for the catch crop ($\delta^{15}N = 5.8 \pm 1.8$ ‰), intermediate for RG and the lowest for the wheat. No significant difference of $\delta^{15}$N was observed for wheat and for catch crop between associated tree species at all sampling date (Figure 3B). After the fourth N fertilizer application, the $\delta^{15}$N of unlabeled samples significantly increased during the bolting stage for wheat associated with hornbeam and wild cherry, then it decreased at the heading stage and stabilized until harvest.

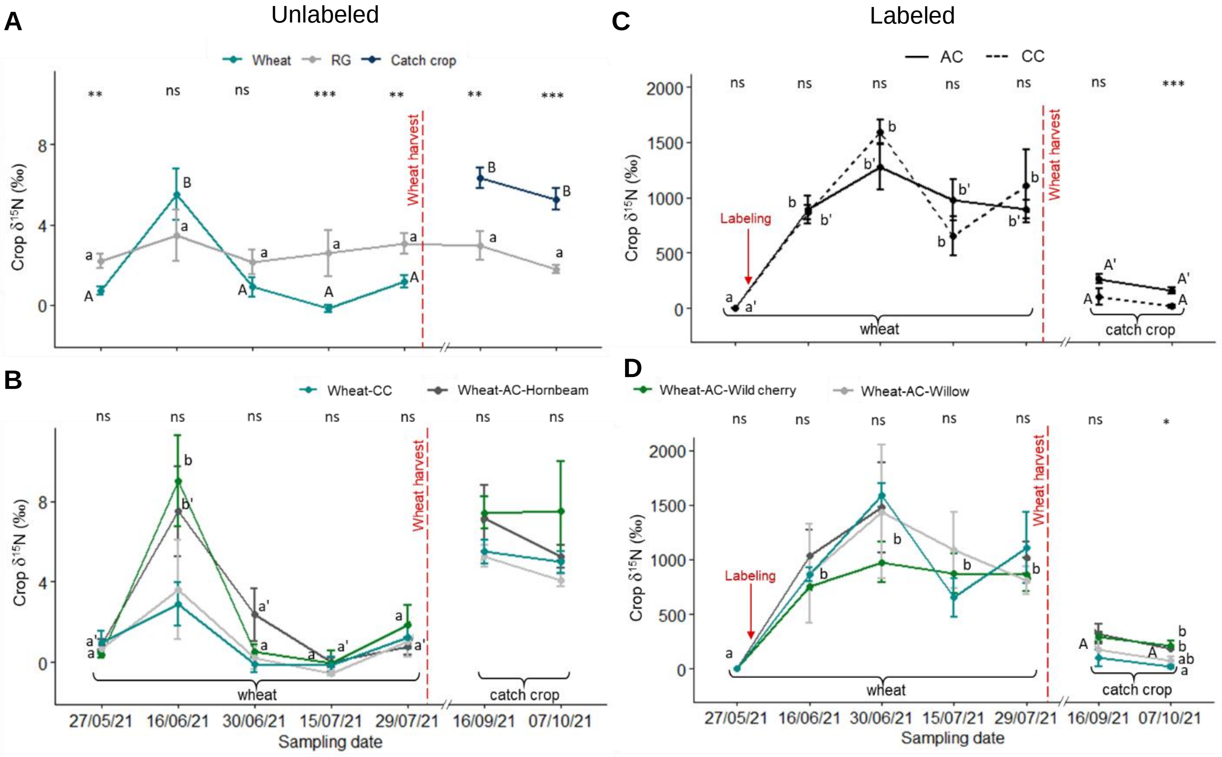


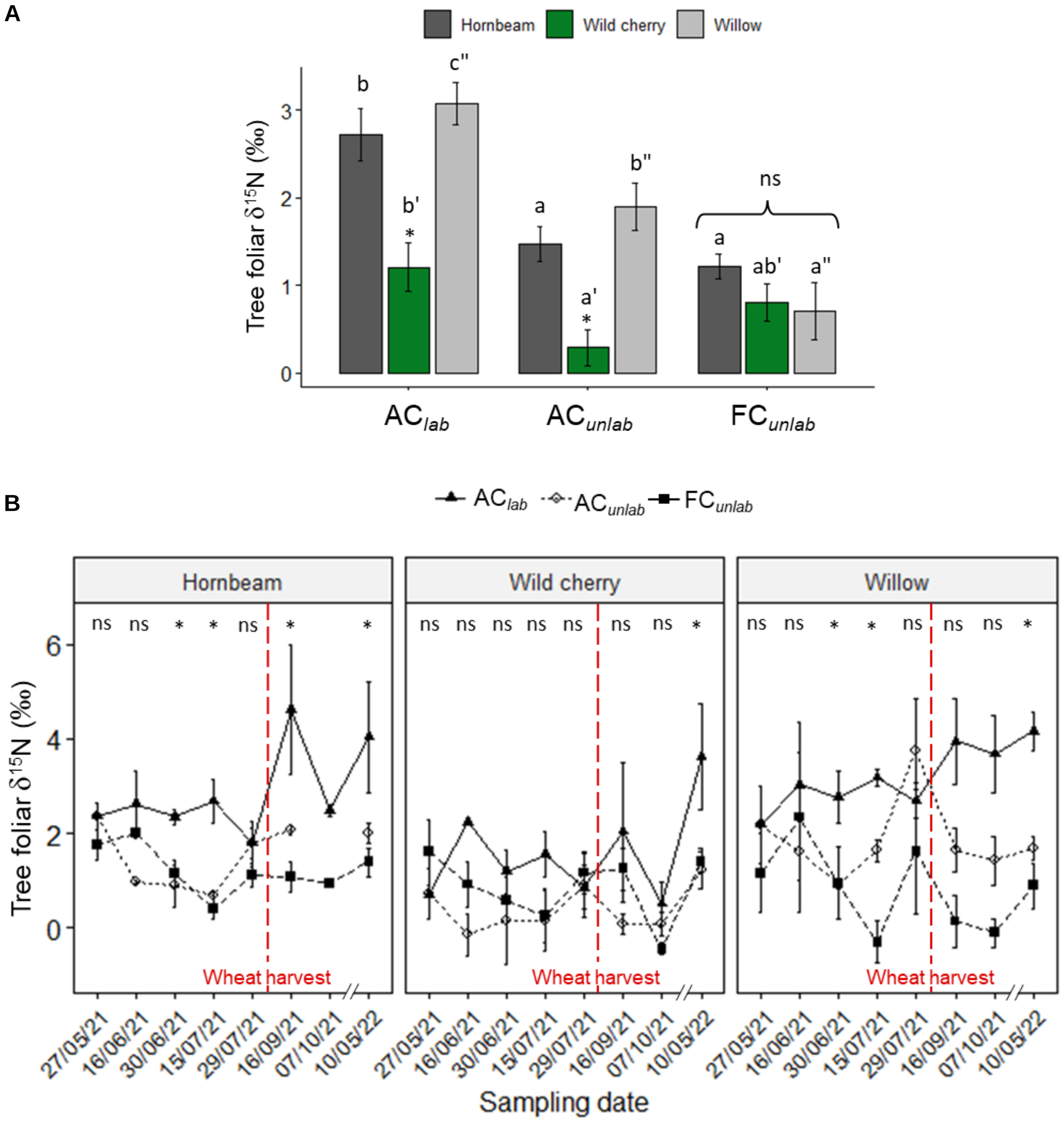
**Figure 3:** Evolution of mean aerial $\delta^{15}$N (‰) in wheat, catch crop or ryegrass (RG) in unlabeled (A and B) and labeled (C and D) area at Ramecourt alley-cropping experimental site. Graphics (B) and (D) show $\delta^{15}$N in wheat and catch crop according to its associated tree species. Data are means (±SE). Letters compare sampling date; means with same letters are not significantly different ($p < 0.05$). In graphic (D), same results were obtained for the comparison between

sampling date for all associated tree species, thus only one letter is indicated per sampling date. Asterisks indicate significant difference between species (graphic A), between alley-cropping (AC) and sole-crop control (CC) plots (graphic C) or between species associated with wheat or catch crop (graphic D) for a given sampling date; *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. “ns” means not significant.

The same results were not observed for wheat associated with willow and wheat in CC for which, the $\delta^{15}N$ was stable over time. The interaction between sampling date, type of system and tree species significantly explained 48 % ($p < 0.001$) of the wheat $^{15}N$ natural abundance variability (Table 1). The $^{15}N$ natural abundance of the RG remained constant over time regardless of the associated species, and at each date, no significant difference of $^{15}N$ natural abundance in RG was observed between the associated species (data not shown). Finally, no significant relationship was observed between $^{15}N$ natural abundance of wheat and its total N and C content, nor C/N ratio for all data and for both wheat in CC and AC.

*Tree foliar $^{15}N$ natural abundance*

For the tree foliar $^{15}N$ natural abundance, the mean value of $\delta^{15}N$ was positive for all collected samples ($\delta^{15}N = 1.1 \pm 1.1$ ‰). It was not significantly different to the whole-plant wheat $\delta^{15}N$. The $^{15}N$ natural abundance of tree leaves was not significantly different between AC ($\delta^{15}N = 1.2 \pm 1.3$ ‰) and FC ($\delta^{15}N = 0.9 \pm 1.1$ ‰) (Table 1). However, when tree species were separately analyzed, the average $\delta^{15}N$ of the willow was significantly higher in AC than in FC, whereas no significant difference of $\delta^{15}N$ was observed between AC and FC for hornbeam and wild cherry (Figure 4A). In AC, the mean value of $\delta^{15}N$ natural abundance for the wild cherry (0.3 ± 0.9 ‰) was lower than for hornbeam and willow (1.5 ± 0.8 ‰ and 1.9 ± 1.3 ‰ respectively, Figure 3A). In FC no significant difference of $^{15}N$ natural abundance was observed between tree species. The interaction between the type of system and tree species accounting for 18 % of tree foliar $\delta^{15}N$. When data for AC and FC were gathered, the mean value of $^{15}N$ natural abundance was significantly lower for wild cherry ($\delta^{15}N = 0.5 \pm 1.0$ ‰) than for hornbeam and willow ($\delta^{15}N = 1.3 \pm 0.7$ ‰ and 1.3 ± 1.4 ‰ respectively). The tree species and the sampling date accounted only for 8 % and 13 % of $^{15}N$ natural abundance respectively (Table 1). However, when data in AC and FC were analyzed separately, the sampling date accounted for 21 % of $^{15}N$ natural abundance in FC ($p < 0.01$), whereas no significant variations were observed in AC (data no shown). A significant increase of $^{15}N$ natural abundance was evidenced between the last sampling date in 2021 and May 2022 in AC and FC (data no shown).



**C**

| Sampling date | Hornbeam | | | Wild cherry | | | Willow | | |
|---|---|---|---|---|---|---|---|---|---|
| | AC$_{lab}$ | AC$_{unlab}$ | FC$_{unlab}$ | AC$_{lab}$ | AC$_{unlab}$ | FC$_{unlab}$ | AC$_{lab}$ | AC$_{unlab}$ | FC$_{unlab}$ |
| 16/09/2021 | c | ns | ns | ns | a’ | ns | ns | ns | a’’ |
| 07/10/2021 | a | ns | ns | ns | a’ | ns | ns | ns | a’’ |
| 10/05/2022 | b | ns | ns | ns | b’ | ns | ns | ns | b’’ |

**Figure 4:** Tree foliar $\delta^{15}$N (‰) of hornbeam, wild cherry and willow collected in labeled (AC$_{lab}$) and unlabeled (AC$_{unlab}$ and FC$_{unlab}$) area at Ramecourt alley-cropping experimental site : (A) Mean tree foliar $\delta^{15}$N values between type of system, (B) temporal evolution of tree foliar $\delta^{15}$N and (C) results of Tukey’s test ($p < 0.05$) to compare the last three sampling dates for a given tree species and type of system (AC$_{lab}$, AC$_{unlab}$ and FC$_{unlab}$). No significant difference

was observed between the previous sampling dates. Data are means (±SE). Letters compare type of systems ($AC_{lab}$, $AC_{unlab}$ and $FC_{unlab}$) for a given tree species; means with same letters are not significantly different (Tukey's test; $p < 0.05$). In graphic (A), asterisks indicate significant difference between tree species for a given type of systems (Tukey's test, $p < 0.05$). In graphic (B), asterisks indicate significant difference in AC between labeled ($AC_{lab}$,) and unlabeled ($AC_{unlab}$,) samples for a given sampling date (Kruskal-Wallis' test, $p < 0.05$). "ns" means not significant. AC: alley-cropping, FC: pure-forest control plot.

Then, regarding tree species separately, the mean value of $^{15}N$ natural abundance did not significantly vary over time in AC and FC for all tree species (Figure 4B).

Finally, a significant positive and negative relationship was observed between $^{15}N$ natural abundance of tree leaves and their total N content ($R^2 = 0.06^{**}$) and C/N ratio ($R^2 = 0.08^{***}$), respectively, whereas no significant relationship was observed with total C content. In FC, we also noticed a negative relationship between tree foliar $^{15}N$ natural abundance and total C content ($R^2 = 0.05^{*}$) and C/N ratio ($R^2 = 0.08^{*}$) but none with total N content. In AC, there was no significant correlation between tree foliar $^{15}N$ natural abundance and total N and C content, nor C/N ratio.

### 3.3. Wheat and trees $\delta^{15}N$ from labeling experiment

#### *Whole-plant wheat and catch crop $\delta^{15}N$ from labeling*

The mean value of $\delta^{15}N$ for labeled wheat samples increased from 0.7 ± 0.6 ‰ before labeling, to 994 ± 474 ‰ 12 days after labeling, and remained stable until the harvest both in CC and AC and for all associated tree species (Figure 3C and 3D). For labeled samples, the sampling date accounted for 34 % of whole-plant wheat $\delta^{15}N$ variability (Table 1). Regardless of the sampling dates, no significant difference in whole-plant wheat $\delta^{15}N$ was observed between type of system and between associated tree species (Figure 3C and 3D). The interaction between type of system, tree species and sampling date accounted for 46 % of whole-plant wheat $\delta^{15}N$ variability (Table 1). The mean value of $\delta^{15}N$ for catch crop samples collected in labeled area was also very high compared to the $^{15}N$ natural abundance ($\delta^{15}N = 172 \pm 129$ ‰). For the catch crop, no significant difference of $\delta^{15}N$ was observed between the two sampling dates both in CC and AC (Figure 3C) and regardless of the associated tree species (Figure 3D). On 19/09/21, no significant difference of catch crop $\delta^{15}N$ value was noticed between the type of system or the tree species associated, whereas on 07/10/21, the δ$^{15}$N of catch crop samples in AC was significantly higher than in CC. Focusing on this last sampling date, the δ$^{15}$N of the catch crop was significantly higher when associated with hornbeam and wild cherry compared to the catch crop δ$^{15}$N associated with willow or in CC (Figure 3D).

*Tree foliar δ $^{15}$N from labeling experiment*

The mean value of tree foliar δ$^{15}$N for labeled samples was significantly higher than unlabeled samples for all tree species. In AC trees, the foliar δ$^{15}$N of labeled samples was multiplied by 1.5, 1.8 and 4 for willow, hornbeam and wild cherry respectively compared to unlabeled samples. Then, for hornbeam and willow, the mean value of δ$^{15}$N was significantly higher in labeled samples than in unlabeled samples 30 and 40 days after labeling (Figure 4B). This difference was not observed for wild cherry. After wheat harvest in autumn, significant difference in the mean value of δ$^{15}$N between labeled and unlabeled samples was observed only for hornbeam, for which δ$^{15}$N was significantly higher for labeled than natural abundance. A significant decrease of δ$^{15}$N mean value was then observed for hornbeam labeled samples, but not for wild cherry and willow. For labeled samples, the type of system and tree species explained 17 % and 20 % of the δ$^{15}$N variability (Table 1). The interaction between the sampling date and the tree species accounted for 31% of the tree foliar δ$^{15}$N of labeled samples (Table 1). Finally, we checked the foliar δ$^{15}$N of trees in May 2022, i.e. almost 1 year after labeling and showed that for each tree species, the foliar δ$^{15}$N was significantly higher in labeled samples than in unlabeled samples.

### 3.4. δ$^{15}$N, N, C content, C/N ratio of wheat tissues at harvest

*Results for unlabeled samples*

The $^{15}$N natural abundance of wheat tissues was significantly higher in ears (δ$^{15}$N = 2.1 ± 1.2 ‰) and in roots (δ$^{15}$N = 3.0 ± 1.4 ‰) than in stems and leaves (δ$^{15}$N = 0.2 ± 1.1 ‰) regardless of the associated tree species. At the same time, in unlabeled samples, N content was significantly higher in ears (1.5 ± 0.2 %) compared to stems and leaves (0.4 ± 0.1 %) and roots (0.4 ± 0.1 %) (Figure 5A). The C content was significantly lower in roots (23 ± 5 %) than for ears and for stems and leaves (43 ± 0.6 % and 43 ± 0.4 % respectively) (Figure 5B). Finally, the C/N ratio was significantly different depending on wheat tissues: it was the lowest in ears (28 ± 3 %), intermediate in roots (58 ± 8 %) and the highest in stems and leaves (107 ± 26 %) (Figure 5C). Assuming that proteins contain 16 % of N, the mean protein content of the collected wheat ears was 9.4 %. There was a negative significant relationship between $^{15}N$ natural abundance and C/N ratio of wheat tissues at the harvest ($R^2 = 0.31$***, n = 37).

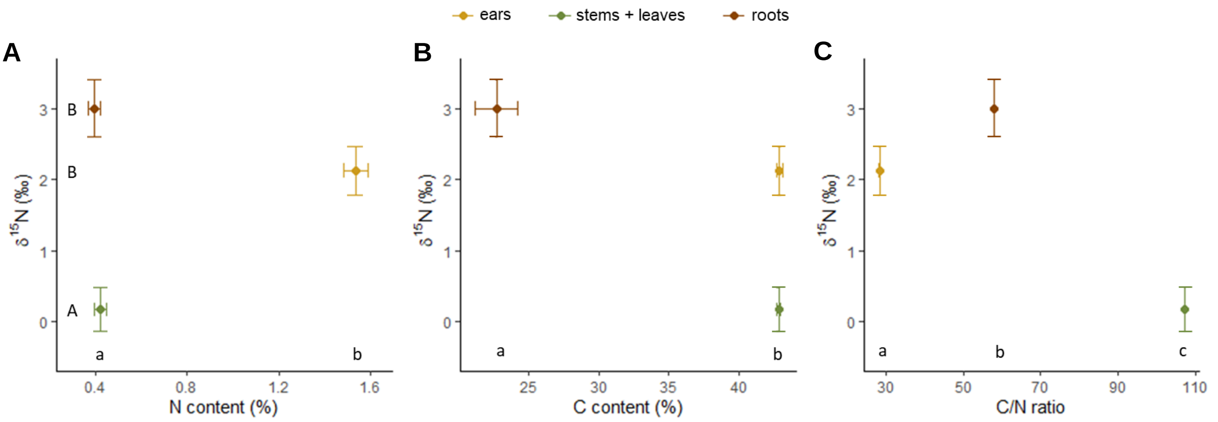


**Figure 5:** Relationship bewteen $^{15}N$ natural abundance of wheat ($\delta^{15}N$, ‰) and N content (%), C content and C/ ratio of different tissues samples at harvest within each wheat tissues samples collected in unlabeled area at Ramecourt alley-cropping experimental site. Data are means (±SE, n=3). Uppercase letters compare $\delta^{15}N$ in wheat tissues, lowercase letters compare N, C content and C/N ratio in wheat tissues. Means with the same letters are not significantly different (Kruskal-Wallis' test, $p < 0.05$).

### *Results from labeling experiment*

In labeled samples, the mean value of $\delta^{15}N$ was significantly higher in ears (1181 ± 111 ‰) than in stems and leaves (714 ± 86 ‰) and in roots (429 ± 42 ‰) (Figure 6A). In CC, no significant difference of $\delta^{15}N$ mean value between wheat tissues was noticed (Figure 6B). In AC, the mean value of wheat $\delta^{15}N$ was significantly higher in ears than in roots, regardless of associated tree. However, the mean value of $\delta^{15}N$ in stems and leaves was not significantly different to ears $\delta^{15}N$ when wheat was associated with hornbeam and was not significantly different to roots $\delta^{15}N$ when the wheat was associated with wild cherry and willow (Figure 6B). The distribution of N and C content in wheat tissues in labeled samples at the harvest was the same as in unlabeled samples. We only observed significantly more N content in roots (0.5 ± 0.1 %) compared to unlabeled samples, and more C content in all wheat tissues than unlabeled samples particularly for roots. No significant difference was observed in C/N ratio between labeled and unlabeled samples for all wheat tissues. As in unlabeled samples, the C/N ratio was the lowest in ears (28 ± 3 %), intermediate in roots (63 ± 14 %) and the highest in stems and leaves (104 ± 28 %).

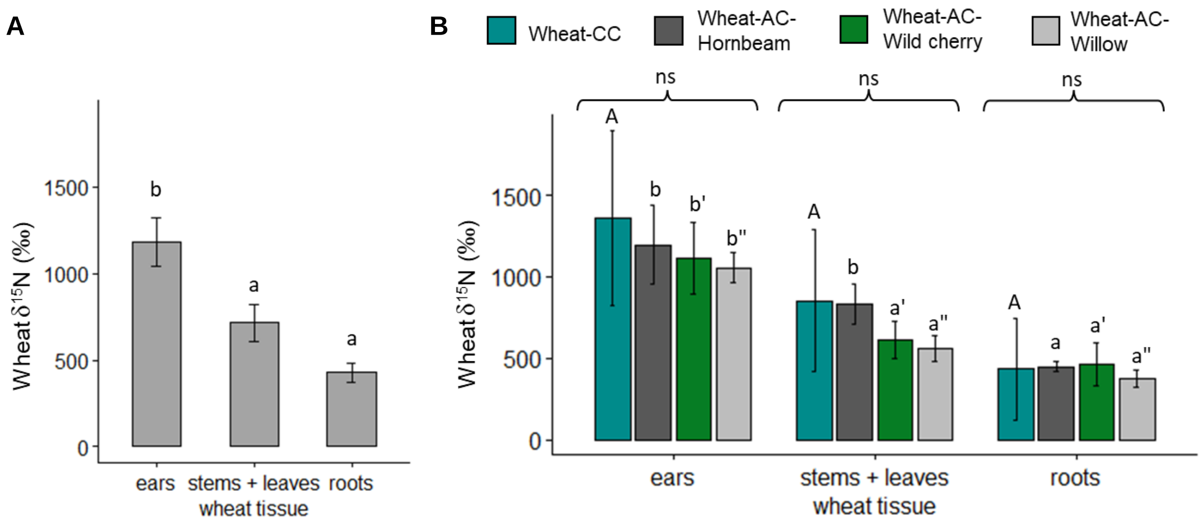


**Figure 6:** Mean value of wheat $\delta^{15}$N (‰) at harvest within each wheat tissues (A) and according to its associated tree species in AC and CC (B) in samples collected in labeled area at Ramecourt alley-cropping experimental site. Data are means (±SE). Letters indicate homogeneous groups and compare wheat tissues for a given associated tree species. Means with same letters are not significantly different (Tukey's test, $p < 0.05$). "ns" means not significant for comparison between associated tree species for a given wheat tissues.

### 3.5. Soil $\delta^{15}$N, C, N and C/N ratio measured at the end of tree growth cycle in autumn 2021

On average the soil total N down to 120 cm depth was 0.07 %. It was 0.12 % from 0 to 30 cm depth, 0.06 % from 30 to 60 cm depth and 0.04 % from 60 to 120 cm depth. The soil total C was on average 0.57 % down to 120 cm depth. It was 1.1 % from 0 to 30 cm depth, 0.4 % from 30 to 60 cm depth and 0.2 % from 60 to 120 depth. There was a positive relationship between total N and total C in the soil profile ($R^2 = 0.98$***). Regarding soil samples without $^{15}$N labeling, a positive correlation was also observed between $^{15}$N natural abundance and soil total N ($R^2 = 0.56$***). The $^{15}$N natural abundance was significantly higher in topsoil (5.3 ± 0.4 ‰) compared to deep soil layers L2 and L3 where $\delta^{15}$N was equal to 4.5 ± 0.5 ‰ and 4.7 ± 0.7 ‰ respectively. However, when the type of system was analyzed separately, this difference was no longer significant in AC and CC, but only in FC (Table 4). Furthermore, in topsoil and in L2, $\delta^{15}$N was significantly higher in AC than in FC. There was no significant difference of $\delta^{15}$N between tree species both in AC and FC. Then, only one significant difference of $\delta^{15}$N was noticed between AC and FC for hornbeam in topsoil where $\delta^{15}$N was higher in AC than in FC (Table 4).

**Table 4:** Mean values of soil $\delta^{15}$N (‰) collected in alley-cropping (AC), pure-forest control (FC) and sole-crop control (CC) plots in unlabeled and labeled area at Ramecourt alley-cropping experimental site at 0-30 cm (L1), 30-60 cm (L2) and 60-120 cm (L3) depth. Data are means (n = 6, 9 and 18 for CC, FC and AC respectively). Letters indicate significant difference between type of system for a given soil layer (lowercase and uppercase letters compared unlabeled and labeled samples respectively). Means with same letters are not significantly different (Tukey and Student's test for unlabeled and labeled samples respectively; $p < 0.05$). Italic letters indicate significant difference between soil layer for a given type of system (Tukey's test; $p < 0.05$). Asterisks indicate significant difference between labeled and unlabeled samples in AC and CC (Kruskal-Wallis' test; $p < 0.05$). The symbol # indicate significant difference between AC and FC in unlabeled samples (Student's test; $p < 0.05$). The absence of symbols * and # means that the comparison was not significant. Bold letters are used to facilitate table reading. H: Hornbeam, WC: Wild cherry, W: Willow.

Unlabeled soil $\delta^{15}$N (‰)

| Soil layer | AC | | | | FC | | | | CC |
|---|---|---|---|---|---|---|---|---|---|
| | H | WC | W | mean | H | WC | W | mean | Wheat |
| L1 | 5.7# | 5.5 | 5.3 | 5.5**b** *z* | 5.1 | 5.2 | 5.1 | 5.1**a** ***y’*** | 5.3ab *z’’* |
| L2 | 5.2 | 4.8 | 4.6 | 4.8**b’**z | 4.0 | 4.3 | 4.4 | 4.2**a’** *z’* | 4.5ab’ *z’’* |
| L3 | 4.6 | 5.3 | 4.8 | 4.9a’’z | 4.4 | 4.4 | 4.2 | 4.3a’’*z’* | 5.0a’’ *z’’* |

Table 4 (*continued*)

Labeled soil $\delta^{15}$N (‰)

| Soil layer | AC | | | | FC | | | CC |
|---|---|---|---|---|---|---|---|---|
| | H | WC | W | mean | H | WC | W | Wheat |
| L1 | 26.6* | 9.4* | 14.2* | 16.7**B*Y*** | - | - | - | 6.4* **A** *Z’* |
| L2 | 6.0* | 5.6 | 5.5 | 5.7 **B’**Z | - | - | - | 4.2**A’***Z’* |
| L3 | 8.0 | 5.8 | 5.5 | 6.4A’’Z | - | - | - | 5.0A’’*Z’* |

Regarding labeled soil samples, $\delta^{15}$N was significantly higher in topsoil (14.6 ± 3.3 ‰) compared to L2 and L3 (5.3 ± 1.1 ‰ and 6.1± 2.7 ‰ respectively). It was significantly higher in topsoil and in L2 than in unlabeled samples. However, no significant difference between soil layers was observed in CC, whereas in AC, $\delta^{15}$N in topsoil was almost three times higher than in L2 and L3 (Table 4). Then, $\delta^{15}N$ in topsoil and in L2 was significantly higher in AC than in CC, whereas in L3, no significant difference of $\delta^{15}N$ was observed between type of system. Moreover, in topsoil, $\delta^{15}N$ was significantly higher in labeled than in unlabeled samples for all tree species in AC and in CC. In layer 2, there was no significant difference of $\delta^{15}N$ between labeled and unlabeled soil samples, except for hornbeam where $\delta^{15}N$ was significantly higher in labeled than in unlabeled samples. There was no significant difference of $\delta^{15}N$ between tree species nor between CC and tree species in all soil layers (data not shown).

## 4. Discussion

### 4.1. Evolution of biomass, N, C content and C/N ratio of plants

Even if we did not measure a difference in wheat biomass between CC and at 3 m from tree rows in AC, we observed a significant decrease of aerial total N content in wheat and trees throughout the cropping season and attributed it to the plant growth N diluting within the aerial biomass (Justes et al., 1994). The decrease of N content in wheat as it grew also resulted in the redistribution of N compounds from lower to upper leaves and then from upper leaves to grains (Neumann et al., 2004). The same results were found in tree leaves, also obtained by Mei et al. (2015) with a continuous decrease of N content from May to September in larch and ash. In old oaks, leaf N content was high at the beginning of leaf unfolding in April, then declined progressively until full expansion in June (El Zein et al., 2011). This decline in N content could result from the synthesis of protein in leaves necessary for photosynthesis during the growing season (Evans, 1989). An exception should be noted for RG for which N content was stable from spring to autumn 2021 because it is a perennial crop and never stops growing.

For tree species, the higher total N content of tree leaves in AC compared to FC and the significant increase of N content in AC were due to fertilization which accelerates tree growth and N assimilation. In FC, the presence of the RG in interrows may intensify the competition with trees and slow their growth unlike in AC. Cheng and Bledsoe (2004) and Gargaglione et al. (2014) reported in their study that due to the higher root density and growth rate, grasses were more competitive for N uptake than trees. Moreover, the higher total N content in willow, for both labeled and unlabeled samples in AC, compared to hornbeam and wild cherry attested its greater development than other tree species (Table 3). Finally, in spring 2022, the N remobilization occurring at bud break explained the high total N content in all tree species (El Zein et al., 2011; Grassi et al., 2003).

The plant growth along the cropping season also explained the significant total C content increase in tree species. Indeed, the aerial biomass was formed by the assimilation of C in tissues (Mei et al., 2015). This was consistent with the decline in leaf N concentration during leaf expansion which essentially resulted from the dilution of N by increasing C biomass (Chapin, 1980). However, in wheat and RG, the total C content was stable until the middle of July and decreased at the end of that month, indicating the end of the growth cycle. In FC, trees were less developed than in AC and thus contained less C particularly in spring (Table 3).

The significant increase in C/N ratio in both aerial wheat tissues and tree leaves was consistent with the aerial and foliar total N and C content evolution over time. For the catch crop, aerial C/N ratio was low due to its high total N content and to its early growth stage. Finally, as for the total N content, aerial C/N ratio of RG was stable over time due to its uninterrupted growth.

## 4.2. $^{15}$N natural abundance

### *$^{15}$N natural abundance of wheat, catch crop and RG aerial tissues*

The positive value of $\delta^{15}$N observed for wheat, RG and catch crop for $^{15}$N natural abundance were consistent with values found in literature for arable land (Sierra and Daudin, 2010; Zhou et al., 2013). The positive $\delta^{15}$N of plant species is partly explained by the fertilization (Choi et al., 2017). Indeed, the successive synthetic and organic N fertilizer applications may increase $^{15}$N content in soil (Choi et al., 2003) and therefore in plants. As selection of light $^{14}$N isotope is given priority by successive crops, the soil $^{15}$N/$^{14}$N ratio increase together with annual and perennial crops in the long term (Kriszan et al., 2009). The higher $^{15}$N natural abundance in the catch crop compared to the wheat and the RG that we observed may be explained by the development stage of plants. As the catch crop was sown in late August, it was just starting to grow whereas wheat and RG were sown seven months and two years before the first sampling date, respectively. When they were collected, the $^{15}$N natural abundance should be concentrated in the catch crop whereas it was diluted in the wheat and the RG samples. It should progressively decrease with catch crop growth thank to dilution from $^{14}$N isotope absorption as observed by Høgh-Jensen and Schjoerring (1994). In FC, the stable value of RG $^{15}$N natural abundance over time reflected $^{15}$N enrichment of the absorbed soil N (Høgh-Jensen and Schjoerring, 1994). Due to the absence of fertilization, there was no external input of $^{15}$N or $^{14}$N isotopes, thus $^{15}$N/$^{14}$N ratio in soil was stable throughout the RG growing cycle. Similar results were found by Høgh-Jensen and Schjoerring (1994) on a low fertilized RG whereas with 400 kg N ha$^{-1}$, they observed a decrease of $\delta^{15}$N natural abundance over the growing season.

We observed that soon after the fourth N fertilizer application in June, the $^{15}$N natural abundance of wheat increased especially for those associated with hornbeam and wild cherry (Figure 3A and 3B). Then, it decreased over time until harvest. We assume that this sudden increase of $^{15}$N natural abundance was related to fertilizer application inducing an imminent N absorption including $^{15}$N isotope. Similar variations with time were found by other authors who attributed increasing $\delta^{15}$N values in fertilized crops to the decreasing availability of synthetic fertilizer with time due to plants uptake (Choi et al., 2002; Flores et al., 2007). Additionally, in a study carried out by Fuertes-Mendizábal et al. (2018) testing different fertilizer management strategies, an increase of $^{15}$N natural abundance was observed only on wheat which received amendment. Watzka et al. (2006) explained that mineral N fertilizer may stimulate soil processes which discriminate $^{15}$N (e.g. nitrification, denitrification, ammonia volatilization) by the increased supply of readily available N, leading to loss of the $^{14}$N enriched compounds and subsequent $^{15}$N enrichment of soils. Therefore, the fertilization positively influenced the plant $\delta^{15}$N in the short-term. Indirectly, our results suggest that an isolated high loss of $^{14}$N occurred in wheat associated with hornbeam and wild cherry compared to monocrop or to wheat associated with willow. Over time, the wheat growth induced a $^{15}$N dilution in all plant tissues and therefore a decrease of $\delta^{15}$N natural abundance along crop cycle. However, Fuertes-Mendizábal et al. (2018) attribute this impoverishment in $^{15}$N along the plant lifecycle to the $^{15}$N-enriched ammonia losses from the wheat to the atmosphere when N remobilization occurs. Several authors have reported that the ammonia emissions peak related to N remobilization takes place during late leaf senescence (Wang et al., 2011; Wang and Schjoerring, 2012).

Overall, the tree species associated with wheat in AC did not affect its $^{15}$N natural abundance. However, the high increase of $^{15}$N natural abundance for wheat associated with hornbeam and wild cherry suggested that heavy $^{15}$N should be more available next to these species than close to willow. Indeed, among the three tree species studied, the willow had the fastest growth. Additionally, results about tree fine roots distribution described in chapter 4, showed that willow had a more developed root system than hornbeam in topsoil. Therefore, the willow should be more competitive for $^{15}$N uptake than the hornbeam.

### $^{15}N$ *natural abundance of tree leaves*

In forest ecosystems, the $^{15}N$ natural abundance of trees is often represented with a negative value of $\delta^{15}N$ (Andrianarisoa et al., 2009; Pardo et al., 2002). However, within agricultural plots, plant $^{15}N$ natural abundance is usually positive even for agroforestry trees (Rowe et al., 1999). In our study, the positive value of $\delta^{15}N$ for tree leaves natural abundance was explained by the plantation on a former agricultural plot with a positive soils $\delta^{15}N$ (Table 4). Watzka et al. (2006) showed a positive relationship between soil and plant $\delta^{15}N$ values and showed on average a difference of 3 ‰ between them. Craine et al. (2009) also confirmed the linear regression between the two values and even showed that values are close to bisector for high soil $\delta^{15}N$.

We showed that on average for all sampling dates, the $\delta^{15}N$ for unlabeled samples was higher in AC than in FC for willow whereas it was not the case for other species (Figure 4A). Due to N fertilizer in AC, the high $^{15}N$ natural abundance of willow may be attributed to $^{15}N$ enrichment of soil N pools available for plants after N losses through processes involving N isotope fractionation (ammonia volatilization, nitrification followed by leaching and denitrification, and denitrification in itself) (Högberg et al., 2011). Furthermore, Högberg et al. (2011) explained the high value of tree foliage $^{15}N$ natural abundance in fertilized forests to the loss of ectomycorrhizal fungi and their function in tree N uptake, which involves redistribution of N isotopes in the ecosystem. Meanwhile, they showed that *Pinus sylvestris* needles growing in N-limited control plots became depleted in $^{15}N$, reflecting high retention of $^{15}N$ by mycorrhizal fungi. Craine et al. (2009) also confirmed that arbuscular mycorrhizal, ectomycorrhizal and ericoid mycorrhizal plants were depleted in foliar $\delta^{15}N$ by 2 ‰, 3.2 ‰ and 5.9 ‰, respectively, relative to nonmycorrhizal plants. The lower $^{15}N$ natural abundance in wild cherry compared to hornbeam and willow observed in AC may correspond to the isotopic signature of each tree species, assuming that their low growth in FC was not representative of their isotopic signature. In their study, Craine et al. (2009, 2012) described that globally the foliar $\delta^{15}N$ varies by more than 35 ‰. Through more than 12 000 worldwide leaves collected, they established an average $\delta^{15}N$ of 0.9 ‰ with 95 % of the samples between -7.8 ‰ and 8.7 ‰. As mentioned above, mycorrhizal symbioses may influence the plant $^{15}N$, therefore, different interactions between wild cherry and fungi in AC compared to FC could also interfered its $^{15}N$ natural abundance. We also think that change in $\delta^{15}N$ between AC and FC for willow was due to its faster growth compared to other species. As observed by Craine et al. (2009), we showed a positive relationship between foliar $\delta^{15}N$ and N content. Therefore, the high biomass developed by the willow resulted in more $^{15}N$ assimilation compared to willow in FC. While for hornbeam and wild cherry, the $^{15}N$ natural abundance was the same for trees in AC and FC maybe because of their slow growth and low foliar N content. This was consistent with results on the wheat $^{15}N$ isotope absorption which was higher in association with hornbeam and wild cherry than with willow after the fourth fertilizer application.

### 4.3. Fate of $^{15}N$ tracer in crop and trees

*$^{15}N$ artificial signature of wheat and catch crop*

We found that compared to unlabeled samples, the $\delta^{15}N$ of wheat and catch crop was multiplied by 1000 and 30 respectively, for labeling samples, testifying the absorption of $^{15}N$ tracer by both crops over time (Figure 3C). As tracer was applied on wet soil and was followed by 7 mm of rainfall during the 10 days after application, it increased its availability for wheat. It suggested that at this stage, wheat took up N in the upper soil layer and well valorized this last N fertilizer application (Fuertes-Mendizábal et al., 2010; 2018).

The $\delta^{15}N$ of catch crop growing in a labeled zone was lower compared to wheat because as they were sown after wheat harvest, most of $^{15}N$ tracer was already taken up or leached out by rainfall summer and autumn. However, this higher $\delta^{15}N$ compared to unlabeled samples testifies that wheat did not valorize all the supplied $^{15}N$ tracer and that catch crop allowed the recovery of part of it in autumn (Liang et al., 2013; Raun et al., 1999; Shi et al., 2012). Justes et al. (2012) and Tribouillois et al. (2015) showed the ability of a catch crop during the intercrop period to recover the mineral N unused by crop, reducing the potential N leaching during the drainage autumnal period.

There was no effect of either the type of system (CC or AF) or the associated tree species on $^{15}N$ uptake by wheat and its biomass. This absence of tree disturbance on the wheat growth and N uptake may be explained by their young age, especially by the weakness of their N needs compared to wheat (Talbot 2011) and by the limit of their root exploration. O'Connor et al. (2023) showed that before 5 years old, tree fine roots in AC were mainly developed in the topsoil close to the row. Similar results were found by Livesley et al. (2000) in a three year-old AC stand where tree roots were greatest in topsoil and decreased exponentially with depth. In their study, the maize roots dominated the area 450-525 cm from the tree rows in the upper 120 cm soil, whereas 75-150 cm from the tree rows, tree roots dominated except in the upper 30 cm. Despite of the beginning of tree fine roots colonization in the crop area, they did not affect wheat N uptake.

At the beginning of autumn, the catch crop associated with hornbeam and wild cherry absorbed more $^{15}N$ than in CC, whereas the same amount of $^{15}N$ was found in catch crop associated with willow and in CC (Figure 3D). Similarly for the unlabeled wheat samples, the $^{15}N$ tracer should be more available next to hornbeam and wild cherry than close to willow. This result confirmed that hornbeam and wild cherry were less competitive than willow. Our results about wheat fine roots distribution in chapter 4 also showed fewer wheat fine roots when associated with willow than with hornbeam, attesting the low competitiveness of this species. Even if trees did not affect the wheat growth, despite their young age, a difference in competitiveness between species was beginning to emerge. In a three year-old AC stand, Livesley et al. (2000) also distinguished different competitive effects for N among their two tree species even if the total amount of N taken up by the both AC was the same. They explained their results by the amount of N removed through pruning and by the different inherent characteristics of the two species influencing mechanisms for internal N retranslocation and denitrification or leaching. At this young stage of development, our studied tree species did not affect the agricultural system, but local differences related to the specificities of each species were observed.

### *$^{15}N$ artificial signature of trees*

The significant higher tree foliar $\delta^{15}N$ for labeled than unlabeled trees evidenced that all our young tree species absorbed N from topsoil. However, the increase in $\delta^{15}N$ between labeled and unlabeled trees (multiplied by 2.2) was lower than for wheat or the catch crop. Unfortunately, we failed to calculate the percentage of $^{15}N$ tracer absorbed by trees, but our result confirmed that, at this stage of tree development (after four years), trees compete with wheat and allow the recovery of part of the fourth N fertilizer application. Unlike wheat for which $\delta^{15}N$ directly increased only a few days after labeling, the tree leaves were significantly enriched in $^{15}N$ almost 30 days after labeling in early summer only for hornbeam and willow (Figure 4B). This observation may be explained by the fact that these sampling dates coincided with the active growth of trees (Etzold et al., 2022; Singh et al., 2017) increasing their N need and uptake, whereas after budbreak N found in tree leaves mainly came from previous-year N stored in woody tissues (Dyckmans and Flessa, 2001; Muñoz et al., 1993). Another explanation was that $^{15}N$ tracer was absorbed only a few days after labeling such as for wheat, but a delay was required to observe $^{15}N$ enrichment in tree leaves due to the application of labeled fertilizer carried out in the crop area 1.5 m from the referent tree. If $^{15}N$ tracer was absorbed by trees in its ammonium form, it should be assimilated directly within roots as proteins form due to ammonia toxicity and should take time to be transported to leaves, but if $^{15}N$ tracer is absorbed in nitrate form, it should be transported by sap flow into leaves. The preferential uptake of mineral N as ammonium form rather than nitrate form is known particularly for hornbeam and wild cherry (Jacob and Leuschner, 2015; Reuter et al., 2021) unlike willow (Neuschütz and Greger, 2010; Sommer et al., 2017) and is consistent with the delay of $^{15}N$ increase in tree leaves. After the wheat harvest, we also observed a significant $^{15}N$ absorption by hornbeam. As this tree species grew slowly, was not very tall and was less competitive, the $^{15}N$ tracer should be more available for hornbeam after wheat harvest.

At the beginning of autumn, the decrease of $\delta^{15}N$ in labeled samples (Figure 4C) could be due to the senescence of leaves with a reduction of the foliar N content (Table 3). In unlabeled samples, because of the lower values of $\delta^{15}N$ compared to labeled samples, the decrease of $\delta^{15}N$ in autumn should be low and therefore not significant. Finally, for all tree species, the significant high value of $\delta^{15}N$ in labeled samples in spring 2022, evidenced the remobilization of N stored in the woody tissues during winter (El Zein et al., 2011; Kolb and Evans, 2002). When the shoot growth of trees was completed, Frak et al. (2002) found that around 54 % of total N to new shoots was provided by N remobilization. This N remobilization was also seen in unlabeled samples with significant increase of $\delta^{15}N$ between October 2021 and May 2022 (Figure 3C).

### 4.4. Analysis of $\delta^{15}N$, N and C content of wheat tissues at harvest

At wheat harvest, the low value of $\delta^{15}N$ in stems and leaves of unlabeled samples (Figure 5) suggests a redistribution of heavy N in ears and roots, regardless of the associated tree species. The same trend was also observed by Zhou et al. (2013). The heavy isotopes in roots had to remain in roots and those in stems and leaves remobilized in grains. Indeed, at the end of its growth cycle, N was concentrated in the wheat grains (Figure 5A) to ensure necessary reserves for the development of the future plants. About 60-95 % of the grain N at harvest came from the remobilization of N stored in shoots and roots before anthesis (Kichey et al., 2007; Palta and Fillery, 1995). Zhou et al. (2013) also found a strong decrease of the N contents in stems and leaves at the grain filling stage with the beginning of the N transfer to the wheat grains. During the grain filling period, ears were the main sink of N and other tissues behaved as sources organ remobilizing N during the senescence process (Fuertes-Mendizábal et al., 2018). It was also possible that N located in the lower part of the wheat stem was relocated to roots, rather than in grains, which would be less energy-greedy.

In wheat labeled samples, the high value of $\delta^{15}N$ in ears attested that the fourth N fertilizer application was valorized in ears (Figure 6A). It defends the farmer's hypothesis that the fourth N fertilizer application is used to fill the grains for protein production. Wheat associated with trees in AC allocated more N from this last fertilizer application to ears than wheat in monocrop (Figure 6B). These results may be explained by the shade effect of trees. Dufour et al. (2013) evidenced an increase of protein grain content in shady conditions. Similar results were obtained by Artru et al. (2017) who found a higher grain protein content in wheat exposed to periodic and continuous shade compared to wheat which received 100 % of the available light. At the same time, they observed less biomass in grains of wheat in shade treatment than those in light. They explained this difference by a dilution of N in the grain. The protein content of the grain resulting from the remobilization of N accumulated by the plant was negatively related to final grain yield.

### 4.5. Soil $\delta^{15}N$

Regarding $^{15}N$ natural abundance in soil, the high value of $\delta^{15}N$ in topsoil is explained by the fertilization which added external $^{15}N$ to the system and which remained in the upper soil layers. Watzka et al. (2006) also observed $^{15}N$ isotope from fertilizer in agricultural soil. In fact, a surplus of N resulted in high $\delta^{15}N$ values of soil because most of the light $^{14}N$ was lost from the agricultural system (Kriszan et al., 2009). Then, thanks to fertilizers, the nitrification rate was higher than in forest and grassland, which may have contributed to the higher $\delta^{15}N$ (Yang et al., 2008). However, this was particularly observed in FC where $^{15}N$ remained on the topsoil due to a $^{15}N$ immobilization in the FC rooting soil layer. Indeed, several studies showed that no-tilled management reduced the amount of nitrate in soil and its leaching because N was less available for mineralization (Couto-Vázquez and González-Prieto, 2016; Thomas et al., 2019). This result was related to the higher C/N ratio found in FC compared to AC and CC (results shown in chapter 4, Table 1) (Kristensen et al., 2000). Moreover, the $\delta^{15}N$ in topsoil and in soil layer 2 was higher in AC than in FC suggesting that $^{15}N$ would be less valued by trees.

By comparison with soil in the labeling area, we saw that labeled $^{15}N$ remained in upper soil layers. The labeled fertilizer did not migrate in depth despite of rainfalls. Several authors using $^{15}N$ fertilizer labeling showed that the $^{15}N$ tracer migrated slowly down the soil profile and $^{15}N$ was still present in the top 30 cm (Chantigny et al., 2004; Muñoz et al., 2003; Rowe et al., 1999). Moreover, in AC, the high $\delta^{15}N$ in topsoil showed that trees took up less N compared to the wheat in CC. The labeled $^{15}N$ provided at the fourth N fertilizer application was not totally absorbed by trees. Then, we saw a migration of N in soil layer 2 in association with hornbeam, maybe due to a small amount of N uptake by this tree species inducing leaching of the remaining $^{15}N$ through the soil layer 2. This was consistent with the less developed fine roots of hornbeam compared to wild cherry and willow.

## 5. Conclusion

From $^{15}N$ natural abundance and labeling, we were able to examine the dynamics of N and followed the fate of the fourth N supply destined to increase grain proteins in young alley-cropping systems. From $^{15}N$ natural abundance analysis, we found a high availability of N for wheat close to hornbeam and wild cherry and speculated about a potential high loss of $^{15}N$ depleted in this area after N fertilizer application. We also showed that the decrease of $^{15}N$ natural abundance of the crop may be due to the loss of $^{15}N$ during N remobilization before leaf senescence. Regarding the $^{15}N$ tracer, we observed no effect of tree species on the valorization of the fourth N application by wheat and identified that catch crop may partially recover it after harvest. We also confirmed the higher availability of tracer close to hornbeam and wild cherry even after wheat harvest. All tree species significantly absorbed the $^{15}N$ tracer testifying that they compete with wheat and allow a better use of this fourth N application especially in summer when wheat needs start to decline. However, unlike wheat for which the $^{15}N$ tracer was observed few days after labeling, there was a shift of 30 days after labeling before observing significant increase of $^{15}N$ tracer in tree leaves. This study showed that in a 4-year-old alley-cropping stand, trees and wheat started to compete for N in topsoil, particularly in association with willow, which grew faster than hornbeam and wild cherry. After wheat harvest, tree species like hornbeam continue to recover $^{15}N$ tracer as did the catch crop. However, we surprisingly do not observe that for willow or wild cherry, which both presented high growth.

Some of the $^{15}$N tracer absorbed by trees was stored in branches or roots and was remobilized at budburst, showing a valorization of N fertilizer by trees in the long-term.

At harvest, our result showed that, as often mentioned by farmers, the N absorbed from the fourth N fertilizer application contributes to grains filling and protein production. It seems that association with trees improves this phenomenon, probably due to tree shade.

Biomass analyses and quantification of wheat and trees should have allowed us to quantify more accurately the proportion of $^{15}$N tracer absorbed by trees and crops relative to the $^{15}$N applied. Further work is needed over time to see the evolution of this competition of N between associated species with tree growing. We evoked a potential loss of N close to low growth tree species, but we failed to demonstrate it from this study.

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# Chapitre 6

## Young trees slow down the decomposition of recalcitrant litter organic matter in temperate alley-cropping systems

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### Abstract

Litter decomposition is poorly investigated in young temperate alley cropping systems (AC) whereas it may be an integrative indicator to explore the early effect of trees on overall soil microbial activity. In this study, we evaluate the effect of four-year-old trees on relative mass loss (ML) of low-quality rooibos and high-quality green tea at different soil depths at the Ramecourt bloc design alley-cropping experimental site. In May 2021, tea bags were inserted within aluminum ingrowth 2 mm mesh bags and were buried at 30, 50 and 100 cm depth 1.5 m distance from a referent tree (alder, hornbeam and wild cherry) for 6 months. The ML and fine roots (FRB) growing within tea bags were quantified and compared between tree-wheat AC plot, sole-crop wheat control (CC) plot and unfertilized pure-forest control plot with ryegrass (FC). Soil mineral N (SMN), ammonium N (SAN), percentage of nitrate in SMN (%NO3), organic matter (SOM) and total DNA content were measured at different periods. On average, the ML was significantly higher for green tea (65 ± 15 %) compared to rooibos tea (47 ± 10 %). It significantly decreased with depth as well as all measured soil parameters. For green tea, no effect of trees was observed on its late-stage decomposition, but fine roots proliferated within it in FC, probably stimulated by nutrient release. The SOM and FRB accounted for 50 % of ML variability in topsoil. By contrast, tree species negatively affected the ML of rooibos particularly in FC and at 100 cm depth in AC. The availability of nitrate was the main driver of rooibos decomposition at 30 cm depth whereas soil total DNA explained 33 % of its variability at 100 cm depth only for woody plots. We highlight the ability of trees to slow down the decomposition of recalcitrant litter in AC which might favor carbon sequestration in soil depth.

## Key words

Alley-cropping system, litter decomposition, soil mineral nitrogen, soil ammonium nitrogen, percentage of nitrate, tea-bag method, fine roots, soil DNA, soil water content

## 1. Introduction

Litter organic matter decomposition is among most relevant processes allowing to recycle carbon (C), nitrogen (N) and other macro and microelements in terrestrial ecosystems (Bradford et al., 2016; Gessner et al., 2010; Handa et al., 2014). The organic matter is at the heart of biogeochemical nutrients contributing to improve chemical, physical and biological soil fertility. The balance between the organic matter input and its decomposition determines its increase or decrease in soil (Lal, 2009). A better understanding of how they decompose, and what factors control this process, is essential for global climate models that try to integrate C cycle responses under environmental change scenarios.

The introduction of tree rows within arable land in temperate farming system may induce physicochemical and biological changes in soil (Beuschel et al., 2020, 2019). In alley-cropping system (AC), an increase in soil organic matter (SOM) content and its quality was evidenced compared to arable land at different depths and distances from tree rows (Beuschel et al., 2019; Guillot et al., 2021; Udawatta et al., 2008). For instance, after 5 to 8 years of poplar plantation, Beuschel et al. (2019) measured an increase of soil organic C in tree rows at 5 cm depth but observed no change elsewhere. The increase of soil organic C in AC is a slow process and several years are necessary to detect changes in SOM (Oelbermann et al., 2006; Peichl et al., 2006; Smith, 2004). Tree fine roots development and turnover provide an additional source of organic matter both in the upper and deep soil layers (Cardinael et al., 2017a; Germon et al., 2016). O'Connor et al. (2023) found a positive relationship between tree fine roots and SOM in 10-12-year-old AC in tree row, attesting that fine roots activity, deposition and turnover may contribute to SOM increasing in AC. Udawatta et al. (2008) also evidenced that in a 10-year-old AC, the establishment of trees in agricultural system increased soil N content.

Changes in activity and composition of the soil organism community as well as carbon and nutrient availability have been observed in AC (Beule et al., 2019; Beuschel et al., 2020, 2019; Cardinael et al., 2017b). For instance, Banerjee et al. (2016) showed that trees in AC promoted soil bacterial abundance and species richness. Other authors found higher microbial biomass, diversity and activity in AC compared to monoculture plots (Beuschel et al., 2019; Clivot et al., 2020; Mungai et al., 2005; Udawatta et al., 2008). These changes were partly explained by new species in the agricultural system. Zak et al. (2003) showed that the microbial community biomass and the fungal abundance significantly increased with high plant diversity. From their young age, trees implementation in arable systems shifted the composition of main microbial groups towards a higher fungal abundance and functional diversity (Beuschel et al., 2019), particularly with a gradual diversification of the fungal communities under tree rows (Beule and Karlovsky, 2021).

Trees may also stimulate the organic matter decomposition in AC, thanks to the exudation of C-rich compounds. Exudates released from crop roots indirectly stimulate the assimilation of soil organic matter by a diversified bacterial community (Haichar et al., 2008). Some authors showed that tree exudation rates are positively correlated with rhizosphere microbial activities such as the increase of N mineralization (Sun et al., 2021) and the stimulation of the SOM decomposition by microorganisms (Colin-Belgrand et al., 2003; Meier et al., 2017; Sun et al., 2021; Yin et al., 2014). Higher decomposition rates of litter have been observed under trees in AC than in arable alleys (Wachendorf et al., 2020) and have been attributed to a result of a longer period of tree root growth and, thus, higher rhizodeposition inducing priming effects (Pausch et al., 2013). Priming effects in the presence of living roots may not only increase the decomposition rate of SOM (Kuzyakov, 2002), but also increase the decomposition of plant residues (Wang et al., 2015). Finally, dead fine roots may be a source of organic matter and were shown to play a more significant role in nutrient cycling within the alley cropping system because of their faster release of both N and P after decomposition as compared to leaves (Gillespie et al., 2000).

As the level of mineral N in soils under trees and crops may differ, due to the frequent applications of N fertilizer to crops in alleys, an effect of mineral N on litter decomposition in transects of AC may be observed. Ågren et al. (2001) mentioned that the major causes of observed changes in decomposition rate after N fertilization are: an increase in decomposer efficiency, a more rapid formation of recalcitrant material, and, although less pronounced, a decrease of decomposers growth rate. A negative effect of mineral N on litter decomposition was observed for litter with a high C/N ratio (Janssens et al., 2010). Nitrogen addition frequently leads to greater stabilization into humus through a combination of chemical reactions and enzyme inhibition (Prescott, 2010). However, N fertilization accelerates early-stage but slows late-stage decomposition (Gill et al., 2021). Within a particular substrate, early-stage N-stimulation of decomposition was associated with reduced rates of late-stage decay. As the products of early- and late-stage decomposition are stabilized in soils through distinct chemical and physical mechanisms, changes induced by N fertilizer in litter decomposition process may influence the formation and cycling of soil C. Zhang et al. (2020) showed that N addition decreased litter decomposition rates by 15-40 % due to soil bacterial biomass reduction (Li et al., 2015). Grandy et al. (2013) showed that N fertilization led to an increase in activities of three hydrolase enzymes involved in simple carbohydrate and N metabolism (β-D-cellobiohydrolase and β-1,4-glucosidase, β-1,4-N-acetylglucosaminidase), but had no effects on enzymes regulating the breakdown of aromatic compounds (phenol oxidase). Finally, the fate of fresh organic matter should vary according to soil depth in which they are deposited (Hicks Pries et al., 2018), due to a decrease of microbial activities and the lack of labile organic carbon (Fontaine et al., 2007).

The Tea Bag Index was introduced by Keuskamp et al. (2013) to measure litter decomposition rate, based on the determination of mass loss from commercial tea bags. Two kinds of tea bags representing fast (green tea) and slow (rooibos tea) decomposition substrates as standard materials are used. Studies on tea bag experiments confirm that the main drivers of litter decomposition are litter quality, site and plot, climate, incubation time, tree age and species (Desie et al., 2023; Djukic et al., 2018; Duddigan et al., 2020).

Processes like litter decomposition have been investigated less intensively in temperate AC whereas it may be an integrative indicator for estimating overall biological activity. In these systems, litter decomposition may be affected by inherent spatial heterogeneity as well as by modifications of soil habitat due to the implementation of trees. The effect of trees may vary depending on distance from trunk, height and age of plants, species, soil depth, spatial distribution of root and mineral N availability.

In this study, we evaluate the effect of four-year-old trees on relative mass loss (ML) of low-quality rooibos and high-quality green tea at different soil depths at the Ramecourt alley-cropping experimental site. We hypothesized that (i) from tea bag experiment we should evidence the effect of young tree species on fresh organic matter decomposition in alley cropping system close to row, (ii) that through their root growth, trees should modify the bio-physicochemical soil parameters and impact the tea decomposition and (iii) that tea decomposition should vary with soil depth and with the soil mineral N availability.

## 2. Materials and methods

### 2.1. Description of experimental site

This experiment was conducted at Ramecourt alley-cropping experimental site in northern France (50°22'N, 2°17'E) (Andrianarisoa et al., 2019). The climate is oceanic with an average annual temperature and precipitation of 10.6°C and 859 mm respectively. The soil is an alkaline deep luvic cambisol (Baize and Girard, 2009) with flint (< 3 % in upper layer) developed in loess deposits. The soil texture in the upper layer was a silt loam with an organic matter content of 2.5 %. The 18 ha-experimental plot is a randomized block design with three replications where three modalities of AC (AC1, AC2 and AC3) are compared with sole-crop (CC) and pure-forest (FC) control plots (see chapter 3). One-year-old bare root trees and shrubs were planted in SW-NE oriented rows spaced 38 m and 7 m apart in AC and FC respectively. Within each row, tall trees were planted 8 m apart and were intercalated every 1 m by 9 species of shrubs (see chapter 3; Figure 1). The AC1 is an association of *Quercus robur* (oak) and *Carpinus betulus* (hornbeam), AC2 is a mixture of *Juglans regia x regia* (hybrid walnut), *Alnus glutinosa* (alder) and *Prunus avium* (wild cherry) and AC3 is an association of *Aulnus glutinosa* and *Robinia pseudoacacia* (black locust). Tall trees were planted two by two at 1 m apart. A strip of 2 m wide along the tree rows was not cultivated and spontaneous weeds were left to grow. In FC, ryegrass (*Lolium multiflorum)* was sown in spring 2019 in inter-rows and was mechanically cut twice a year. When considering only tall trees, the density of plantation was 66 trees ha$^{-1}$ and 357 trees ha$^{-1}$ in AC and FC respectively but with shrubs, it became 263 trees ha$^{-1}$ and 1428 trees ha$^{-1}$ in AC and FC respectively.

### 2.2. Experimental design

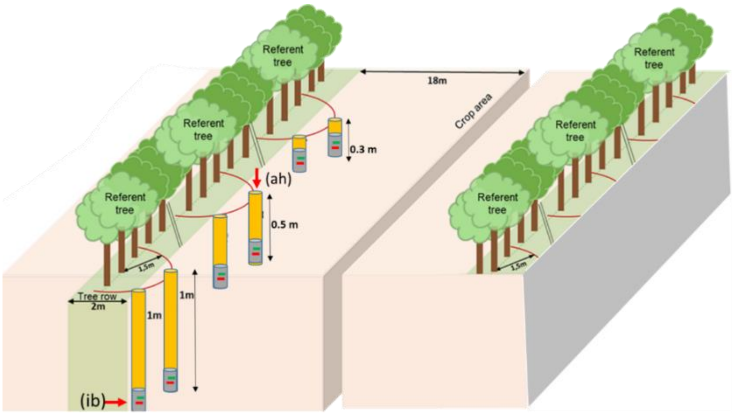
Three tree species (alder, hornbeam and wild cherry) were chosen according to their contrasted aerial growth rate and to their ability to fix $N_2$ from atmosphere. In 2021, the mean tree height was significantly higher in AC (2.8 ± 0.5 m) than in FC (2.3 ± 0.5 m). It was the highest for wild cherry in AC (3.2 ± 0.3 m) and the lowest for hornbeam in FC (1.9 ± 0.2 m) (see chapter 3; Table 2). In CC as well as in alleys of AC, a winter wheat cultivar "Extase" (https://www.geves.fr/catalogue/) was sown in October 2020 following a chicory witloof harvest and was harvested in August 2021. Winter wheat received 220 kg N $ha^{-1}$ as solid mineral fertilizer N, divided into four applications and was applied on February 25th (50 kg N $ha^{-1}$; Sulfan 24% of N), March 23rd (90 kg N $ha^{-1}$; Ammonium nitrate 27%), April 24th (50 kg N $ha^{-1}$; Ammonium nitrate 27%) and June 2nd (30 kg N $ha^{-1}$; Ammonium nitrate 27%). No fertilizer was applied in FC treatment since tree plantation. After wheat harvesting in summer, a cover crop composed of *Phacelia tanacetifolia*, *Eruca sativa* and *Trifolium* sp. was sown on August 25th, 2021, and was mechanically destroyed and buried in January 2022.

### 2.3. Tea bag experiment

The tea bag method developed by Keuskamp et al. (2013), was used in this study to examine the potential effect of trees on organic matter decomposition and overall microbial activity at different soil depths. Following the Tea Bag Index protocol (Teatime 4 Science; https://www.teati me4science.org/), green (EAN: 8714100770542; Lipton, Unilever) and rooibos tea bags (EAN: 5014328045570; Tetley, Tata) were used. Tea bags were from commercially available synthetic tea bags containing 1.3 g and 1.8 g of fresh green and rooibos tea respectively. The mesh size of tea bags was 0.25 mm allowing microorganisms and mesofauna to enter the bags, but excluded macrofauna (Setälä et al., 1996).

Rooibos and green tea decomposition and fine roots production were measured using cylindrical ingrowth bags constructed from aluminum with a mesh size of 2 mm, a diameter of 6 cm and 15 cm length. In AC and FC, two ingrowth bags were buried in 2 holes at 1 m apart, previously dug at 1.5 m from three referent tree per species and per bloc and at 3 depths (30, 50 and 100 cm) (Figure 1). In CC, two ingrowth bags were randomly buried within the plot for each depth. For each burial, the hole was drilled using a hand auger ($\Phi$ = 6.3 cm) and the extracted soil was sieved at 4 mm by respecting soil pedological horizons. The ingrowth bag was filled according to the following layer: 5 cm of sieved soil from the corresponding depth, the rooibos tea bag, again 5 cm of soil, the green tea bag and 5 cm of soil (Figure 1B). Each 5 cm soil layer was compacted using a wooden stake to have almost the same bulk density as the surrounding soil. The ingrowth bag was tied up and knotted at the top with string and wire, 20 cm longer than the depth of the hole, and girdled with two cable ties for further extraction. After ingrowth bag installation, the hole was backfilled with the sieved soil, respecting the different soil layers. A total of 126 ingrowth bags were buried in May 2021, representing 252 tea bags. They were carefully retrieved after 6 months of field incubation in November 2021. At retrieving period, holes were re-opened using a hand auger at the right depth taking care not to break the attached wire in the top of bag. Bags were recovered by hand, transported in the laboratory and stored at 4°C for further processing.

**A**



**B**

**Figure 1:** Schematic representation of tea bag experiment at the Ramecourt alley-cropping experimental site: (A) scheme example of tea bag experiment within a given block for one tree species (scheme is not in scale; "ib" and "ah" means ingrowth bag and auger-drilled hole) and (B) steps of tea bag burial within ingrowth bag.

## 2.4. Tea chemical analyses, decomposition measurement and fine root biomass quantification

The initial composition of rooibos and green tea was analyzed, from 150 g of control samples (about 100 and 120 bags for rooibos and green tea respectively), at the "Laboratoire Départemental d'Analyses et de Recherche" in Laon city. The carbon fractions were determined using internal methods (FD U 44-162). Four fractions were determined by sequential extraction: soluble organic compound (SOL), hemicellulose (HEM), cellulose (CEL) and lignin and cutin (LIC). The other measured parameters were: the water content at 105 °C (NF U44 171), the organic matter content by calcination (internal method VAL-372), the total carbon (Dumas method), the $CaCO_3$ content (NF U 44-001), the mineralization coefficient at 3 days (FD U 44-163) (or ct3), the organic matter stability index (FD U 44-162) (or OMSI) and total nitrogen (internal method VAL-216) and the loss on ignition (550°C) (Table 1).

**Table 1:** Quality parameters of rooibos and green tea used in this study. Data are from chemical analyses of one composite sample from 150 g of tea bags per type except for variables: loss on ignition (%), dry and fresh weight, ash weight and empty bag weight which are mean values (± SD; n = 10). SOL: soluble organic compound, HEM: hemicellulose, CEL: cellulose, LIC: lignin and cutin, ct3: mineralization coefficient at 3 days, OMSI: organic matter stability index.

| Parameters | Rooibos tea | Green tea |
|---|---|---|
| SOL fraction (%OM) | 36.6 | 66.6 |
| HEM fraction (%OM) | 13.1 | 15.1 |
| CEL fraction (%OM) | 31.0 | 11.5 |
| LIC fraction (%OM) | 19.3 | 6.8 |
| ct3 (%OC) | 10.2 | 18.8 |
| OMSI (%OM) | 46.7 | 37.0 |
| Water content (%) | 5.4 | 7 |
| Organic matter (OM; g $kg^{-1}$) | 975 | 941 |
| Total carbon (OC; g $kg^{-1}$) | 505 | 495 |
| Total nitrogen (ON; g $kg^{-1}$) | 9.6 | 32.3 |
| C/N ratio | 52.6 | 15.5 |
| $CaCO_3$ content (g $kg^{-1}$) | < 1.06 | < 1.08 |
| Fresh weight (g tea $bag^{-1}$) | 1.8 ± 0.4 | 1.3 ± 0.05 |
| Dry weight (WD; g tea $bag^{-1}$) | 1.7 ± 0.4 | 1.2 ± 0.05 |
| Loss on ignition (%) | 2.3 ± 0.001 | 4.7 ± 0.004 |
| Empty bag weight (g tea $bag^{-1}$) | 0.15 ± 0.002 | 0.20 ± 0.006 |

After field incubation, ingrowth aluminum bags were opened using pair of scissors. The remaining rooibos and green tea materials were carefully recovered by hand. As the remaining tea materials were colonized by numerous fine roots, they were separated from the unmineralized rooibos and green tea. Both dead and living roots were recovered without any distinction between those belonging to trees, wheat or ryegrass roots. Fine roots dry biomass (FRB) within each rooibos and green tea bag were determined by oven drying them at 65°C for 48 h.

To determine tea decomposition rate, the unmineralized rooibos and green tea from ingrowth bags were first dried at 65°C for 48h, then burned at 550°C for 4 h in a muffle furnace. Tea samples and ashes were weighed after drying (WD) and calcination (WB) respectively. Ten control rooibos and green tea bags which were not buried underwent the same process to obtain the mean value of rooibos and green tea dry weight (Wi) and ashes (Wa) contained in a tea bag. For each sample of rooibos and green tea from ingrowth bags, a final weight (Wf) was calculated by subtracting the ash weight (WB) from the dry weight (WD) to eliminate the clay traces that may have penetrated the tea bag during field incubation and by adding the average weight of the control tea ashes (Wa) to account for the mineral compounds initially present.

$$Wf = WD - WB + Wa \qquad \text{(Eq 1)}$$

Then, the percentage of rooibos and green tea mass lost (ML) and remaining (MR) were calculated as following:

$$ML\ (\%) = \left(\frac{Wi - Wf}{Wi}\right) \times 100 \qquad \text{(Eq 2)}$$

$$MR\ (\%) = \frac{Wf}{Wi} \times 100 \qquad \text{(Eq 3)}$$

Finally, fine roots within the ingrowth bags were also recovered by hand and were frozen at -20°C for further analysis (data not shown).

### 2.5. Soil sampling and analyses

Soil samples were collected three times during the year: in May when ingrowth bags were buried, at the end of July before the wheat harvest and in November when ingrowth bags were retrieved. Soil samples were collected at the layers 0-30, 30-50 and 50-100 cm depth according to soil in each ingrowth bag. In AC and FC, soil cores were collected at 1.5 m from the referent trees down to 120 cm using a manual auger (Φ = 6.3 cm; n = 6 par tree species). In CC, soil samples were randomly taken in the middle of each subplot (n = 6). Soil samples were sieved at 4 mm, transported in the laboratory and frozen at -20°C for further analysis.

The soil water content (SWC) was determined by oven drying an aliquot of 20 g soil at 105°C for 48 h. Soil total Kjeldahl nitrogen (STN) and organic carbon content (NF ISO 14 235) (SOC) were measured at the "Laboratoire Départemental d'Analyses et de Recherche" in Laon city. The soil organic matter content (SOM) was estimated by multiplying SOC by the Van Bemmelen coefficient of 1.724 (Rosell et al., 2001), assuming that the organic matter contains 58 % organic C. The soil C/N ratio was the ratio between SOC and STN. The soil nitrate ($NO_3^-$) and ammonium ($NH_4^+$) were extracted by 1 M KCl solution. The $NH_4^+$ and $NO_3^-$ concentrations of extracts were determined using continuous flow colorimetry. The SMN was the sum of soil nitrate N (SNN) and soil ammonium N (SAN) expressed as mg N per kg of dry soil. The percentage of nitrate in SMN (%NO3) was the ratio between SNN and SMN multiplied by 100.

Total DNA of soil samples within ingrowth bag retrieved in November was extracted from 0.6 g soil using total genomic soil extraction kit "Macherey-Nagel™ Sol NucleoSpin™" (Fisher Scientific SAS, Illkirch, France). Soil samples were mixed with the bead and extraction buffer; were vortexed for 5 min and centrifugated at 11,000 x *g* for 120 s; the supernatant was transferred to a NucleoSpin® inhibitor removal Column and was centrifugated (11,000 x *g*; 60 s). The supernatant was purified by passing through a NucleoSpin® Soil Column and re-centrifugated at 11,000 x *g* for 60 s followed by three successive washes. The DNA concentration in each purified extract was quantified from 1 µL aliquot by spectroscopy with Thermo Scientific™ NanoDrop™ One UV-Vis Spectrophotometer (Fisher Scientific SAS, Illkirch, France) and expressed as ng $\mu L^{-1}$. The total extracted DNA was stored at -20°C until metagenomic analyses.

The SNN, SAN, SMN, %NO3 and SWC was measured for soil samples collected in May, July and November and respectively referred with the mention "_ini" "for "initial", "_int" for "intermediate" and "_f" for "final". The SOC, SOM, STN, C/N and DNA were measured only in soil samples from ingrowth bag retrieved in November (Table 1). Along this chapter, SMN, SNN, SAN and %NO3 were referred as mineral N variables.

## 2.6. Statistical analyses

Bivariate linear models were performed to analyze SMN and SAN in soil samples according to equation (Eq 4):

$$y = ax_1 + bx_2 + c + \beta \qquad \text{(Eq 4)}$$

where "y" is the SMN or SAN, $x_1$ is the block, $x_2$ is either the sampling date or the type of system (CC, AF or FC), "a", "b" and "c" are coefficients and β the model error. An ANOVA followed by a multiple comparison test was carried out using the multcomp R software package (Hothorn et al., 2008) to compare the mean value of SMN or SAN for each $x_2$ variable with a post hoc Tukey's test ($p < 0.05$). The test of Kruskal-Wallis was used to analyze the %NO3 according to the sampling date or the type of system, followed by post hoc Mann-Whitney's tests. Simple linear models were also used to evaluate the soil depth effect on SMN, SAN and %NO3. The significant determination coefficients were indicated with symbol *** for $p < 0.001$, ** for $p < 0.01$ and * for $p < 0.05$.

The comparison between the rooibos and green tea mass loss (ML), remaining mass (MR) and the FRB was done with a Student's t-test. Then, One-way ANOVAs were performed to analyze the variability of ML with the species or the soil layer as explanatory variables. Differences in ML between AC and FC for each tree species were identified with a test of Mann-Whitney. The test of Kruskal-Wallis was used to analyze the FRB variability according to the tree species, the soil layer or the type of system, followed by a post hoc Mann-Whitney's test.

Multivariate regressions were used to explain the variability of SWC, SOM, C/N and DNA with the block and the type of system, the tree species or the soil layer as explanatory variables in the same way as the SMN or SAN in Eq. 1. Regression analyses were followed by Tukey's tests for post hoc pairwise comparisons. The Spearman's rank correlation rho was calculated between all soil and N variables. Stepwise regression models were also used to select the variables allowing a better explanation of the ML variability in rooibos and green tea, using SMN, SAN, %NO3, SOM, C/N, DNA and FRB as explanatory variable. The *p-value* of each model and the sign of coefficient retained for each explanatory variables are presented in Table 4.

All statistical analyses were performed with R software version 4.0.4.

**Table 2:** Mean values of soil water content (SWC, %), soil organic matter (SOM, g kg-1 soil), soil total N (STN, g kg$^{-1}$ soil), soil C/N ratio and soil total DNA (ng µl$^{-1}$) in samples collected in November 2021 according to type of system, depth and tree species at Ramecourt alley-cropping experimental site. Data are means (n = 12). For each variable, lowercase letters compare the mean of the variable between the type of system (AC, CC, FC) for a given soil layer; vertical uppercase letters compare the mean of the variable between the soil layers. The italic lowercase letters compare the species for a given type of system and a given depth and the italic uppercase letters compare the mean of the variable between species for a given depth. In the last line, horizontal uppercase letters compare the mean of the variable according to the type of system regardless of the depth. Bold characters are used to facilitate table reading.

| | | SWC | | | | SOM | | | | STN | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Unit | | % | | | | g kg$^{-1}$ | | | | g kg$^{-1}$ | | | |
| Depth (cm) | Species | CC | AC | FC | mean | CC | AC | FC | mean | CC | AC | FC | mean |
| 30 | Alder | | 26*z* | 26*z’* | 26*Z* | | 25*z* | 24*z’* | 25*Z* | | 1.2*z* | **1.2*y*’** | 1.2*Z* |
| | Hornbeam | | 26*z* | 26*z’* | 26*Z* | | 24*z* | 26*z’* | 25*Z* | | 1.2*z* | **1.3*z*’** | 1.2*Z* |
| | Wild cherry | | 26*z* | 25*z’* | 26*Z* | | 23*z* | 26*z’* | 25*Z* | | 1.2*z* | 1.2*yz’* | 1.2*Z* |
| | Wheat | 26 | | | 26*Z* | 24 | | | 24*Z* | 1.2 | | | 1.2*Z* |
| Mean | | 26a | 26a | 26a | 26B | 24a | 24a | 25a | **25C** | 1.2a | 1.2a | 1.2a | **1.2C** |
| 50 | Alder | | 26*z* | 24*z’* | 25*Z’* | | 13*z* | **18*z*’** | 15*Z’* | | 0.7*z* | **0.9*z*’** | 0.8*Z’* |
| | Hornbeam | | 26*z* | 25*z’* | 25*Z’* | | 11*z* | 16*yz’* | 14*Z’* | | 0.6*z* | 0.8*yz’* | 0.7*Z’* |
| | Wild cherry | | 25*z* | 25*z’* | 25*Z’* | | 13*z* | **15*y*’** | 14*Z’* | | 0.7*z* | **0.7*y*’** | 0.7*Z’* |
| | Wheat | 25 | | | 25*Z’* | 14 | | | 14*Z’* | 0.7 | | | 0.7*Z’* |
| Mean | | 25a’ | 26a’ | 25a’ | 25A | **14b’** | **13a’** | **16c’** | **14B** | 0.7b’ | **0.6a’** | 0.8b’ | **0.7B** |
| 100 | Alder | | 25*z* | 25*z’* | 25*Z’’* | | 9*z* | 8*z’* | 9*YZ’’* | | 0.5*z* | 0.5*z’* | 0.5*Z’’* |
| | Hornbeam | | 26*z* | 24*z’* | 25*Z’’* | | **6*y*** | 9*z’* | **8*Y*’’** | | **0.4*y*** | 0.5*z’* | 0.4*Z’’* |
| | Wild cherry | | 25*z* | 23*z’* | 24*Z’’* | | 11*z* | 9*z’* | **10*Z*’’** | | 0.6*z* | 0.5*z’* | 0.5*Z’’* |
| | Wheat | 24 | | | 24*Z’’* | 9 | | | 9*YZ’’* | 0.5 | | | 0.5*Z’’* |
| Mean | | 24a’’ | 25a’’ | 24a’’ | 25A | 9a’’ | 8a’’ | 9a’’ | **9A** | 0.5a’’ | 0.4a’’ | 0.5a’’ | **0.5A** |
| Mean | | **25B’** | **26A’** | **25B’** | 25 | **16AB’** | **15A’** | **17B’** | 16 | 0.8A’ | 0.8A’ | 0.8A’ | 0.8 |

**Table 2** (*continued*)

| | | C/N | | | | DNA | | | |
|---|---|---|---|---|---|---|---|---|---|
| Unit | | | | | | ng µl$^{-1}$ | | | |
| Depth (cm) | Species | CC | AC | FC | mean | CC | AC | FC | mean |
| 30 | Alder | | **10.2*z*** | 10.5*z’* | 10.4*Z* | | 246z | 232*z’* | 238*Z* |
| | Hornbeam | | **9.8*y*** | 10.2*z’* | 10.0*Y* | | 214z | 155*z’* | 182*Z* |
| | Wild cherry | | 10.0*yz* | 10.6*z’* | 10.3*Z* | | 236z | 196*z’* | 216*Z* |
| | Wheat | 10.2 | | | 10.2*Z* | 194 | | | 194*Z* |
| Mean | | 10.2ab | 10.0a | 10.4b | **10.2C** | 194a | 232a | 194a | **209B** |
| 50 | Alder | | 10.0z | 10.3*z’* | 10.1*Z’* | | 80z | 114*z’* | 97*Z’* |
| | Hornbeam | | 9.6z | 10.4*z’* | 10.0*Z’* | | 73z | 67*z’* | 70*Z’* |
| | Wild cherry | | 9.8z | 10.3*z’* | 10.1*Z’* | | 74z | 62*z’* | 68*Z’* |
| | Wheat | 9.6 | | | 9.6*Y’* | 57 | | | 57*Z’* |
| Mean | | 9.6a’ | 9.8a’ | **10.3b’** | **10.0B** | 57a’ | 76a’ | 81a’ | **75A** |
| 100 | Alder | | 9.3*y* | 8.9*z’* | 9.2*YZ’’* | | 61z | 134*z’* | 89*Z’’* |
| | Hornbeam | | 9.2*y* | 9.7*z’* | 9.5*Z’’* | | 87z | 60*z’* | 73*Z’’* |
| | Wild cherry | | **9.9z** | 9.6*z’* | 9.7*Z’’* | | 61z | 55*z’* | 57*Z’’* |
| | Wheat | 9.0 | | | 9.0*Y’’* | 43 | | | 43*Z’’* |
| Mean | | 9.0a’’ | 9.4b’’ | 9.5ab’’ | **9.4A** | 43a’’ | 71a’’ | 75a’’ | **67A** |
| Mean | | **9.6A’** | **9.8A’** | **10.0B’** | 10.0 | 98A’ | 126A’ | 119A’ | 120 |

## 3. Results

### 3.1. Variation of soil parameters between soil depth, type of system and tree species

The mean value of SWC was 25 ± 2 % along the soil profile in November: it was slightly higher in topsoil than in soil layers 2 and 3 (Table 2). On average, the SWC was higher in AC compared to CC and FC, however, per soil layer, there was no significant difference between the type of system. No significant difference in SWC between tree species was observed in November. The mean value of SOM was 16 ± 7 g kg soil$^{-1}$ on the whole profile. On average, the SOM was equal to 25 ± 2 g kg soil$^{-1}$, 14 ± 2 g kg soil$^{-1}$ and 9 ± 2 g kg soil$^{-1}$ at 30, 50 and 100 cm depth respectively. It significantly decreased with the depth ($R^2 = 0.7^{***}$). The SOM was positively correlated to the STN ($R^2 = 0.9^{***}$) and to the C/N ratio ($R^2 = 0.3^{***}$). The SOM content was not significantly different between AC, CC and FC at 30 cm and 100 cm depth, whereas it was significantly higher in FC, than in AC and in CC at 50 cm depth. In the same way, the mean value of SOM in the whole profile was significantly higher in FC than in AC, CC was intermediate (Table 2). At 50 cm depth in FC, the SOM was significantly higher for the alder than for the wild cherry. When all data for all types of system were gathered, the mean value of the SOM at 100 cm depth, was significantly higher for the wild cherry compared to the hornbeam. In AC, a significant higher SOM was observed for the alder than for the hornbeam at 100 cm, whereas no significant difference between species was observed in FC. No significant difference between species was noticed at 30 cm depth.

The mean of the STN was 0.8 ± 0.3 g kg soil$^{-1}$ in whole soil profile (Table 2). It significantly decreased with depth ($R^2 = 0.7^{***}$): 1.2 ± 0.1 g kg soil$^{-1}$, 0.7 ± 0.2 g kg soil$^{-1}$ and 0.5 ± 0.1 g kg soil$^{-1}$ at 30, 50 and 100 cm respectively. No significant difference between the type of system was observed except at 50 cm depth where the STN in AC was significantly lower than in CC and in FC. In FC at 30 cm depth, the STN was significantly lower for the alder than for the hornbeam and at 50 cm depth the STN was significantly higher for the alder than for the wild cherry. Finally, at 100 cm depth in AC, the STN was significantly lower for the hornbeam compared to the alder and the wild cherry. In the whole profile, the C/N ratio was on average 10 ± 0.6. It also decreased with depth ($R^2 = 0.3^{***}$). The mean value of C/N was 10.2 ± 0.4, 10 ± 0.5, and 9.4 ± 0.6 at 30, 50 and 100 cm respectively. It was significantly higher in FC compared to AF and CC, particularly at 50 cm depth. In AC, at 30 cm depth the C/N was significantly higher for the alder than for the hornbeam and at 100 cm depth, it was significantly higher for the wild cherry than for the hornbeam and the alder.

Finally, the amount of DNA was the highest in topsoil (209 ± 94 ng µl$^{-1}$) (Table 2). It significantly decreased with depth with on average 75 ± 60 ng µl$^{-1}$ and 67 ± 53 ng µl$^{-1}$ in soil layers 2 and 3, respectively. No significant difference was observed either between the type of system or between tree species.

### 3.2. Evolution of soil mineral N

The mean value of SMN significantly decreased along the soil profile and was equal to 10 ± 5 mg N kg soil$^{-1}$, 4 ± 3 mg N kg soil$^{-1}$ and 1 ± 2 mg N kg soil$^{-1}$ at 30, 50 and 100 cm respectively. This negative correlation between SMN and soil depth was observed at all sampling dates and for all types of system. The SMN was positively correlated to the STN ($R^2 = 0.7^{***}$). The mean value of SMN was significantly higher in May (8 ± 6 mg N kg soil$^{-1}$) than in July (3 ± 3 mg N kg soil$^{-1}$) and in November (4 ± 3 mg N kg soil$^{-1}$) for all types of system (Figure 2A). No significant difference between the type of system was observed when all soil layers were gathered (Figure 2A) for each sampling date. In topsoil, the difference in SMN between the type of system was significant only in November: the mean value was lower in FC compared to CC and AF (Figure 2B). In soil layer 2, there was no significant difference between the type of system. In soil layer 3, the SMN in FC was always lower or equal to other type of system at each sampling depth. In FC, values measured in May at this layer were always lower than on other sampling dates.

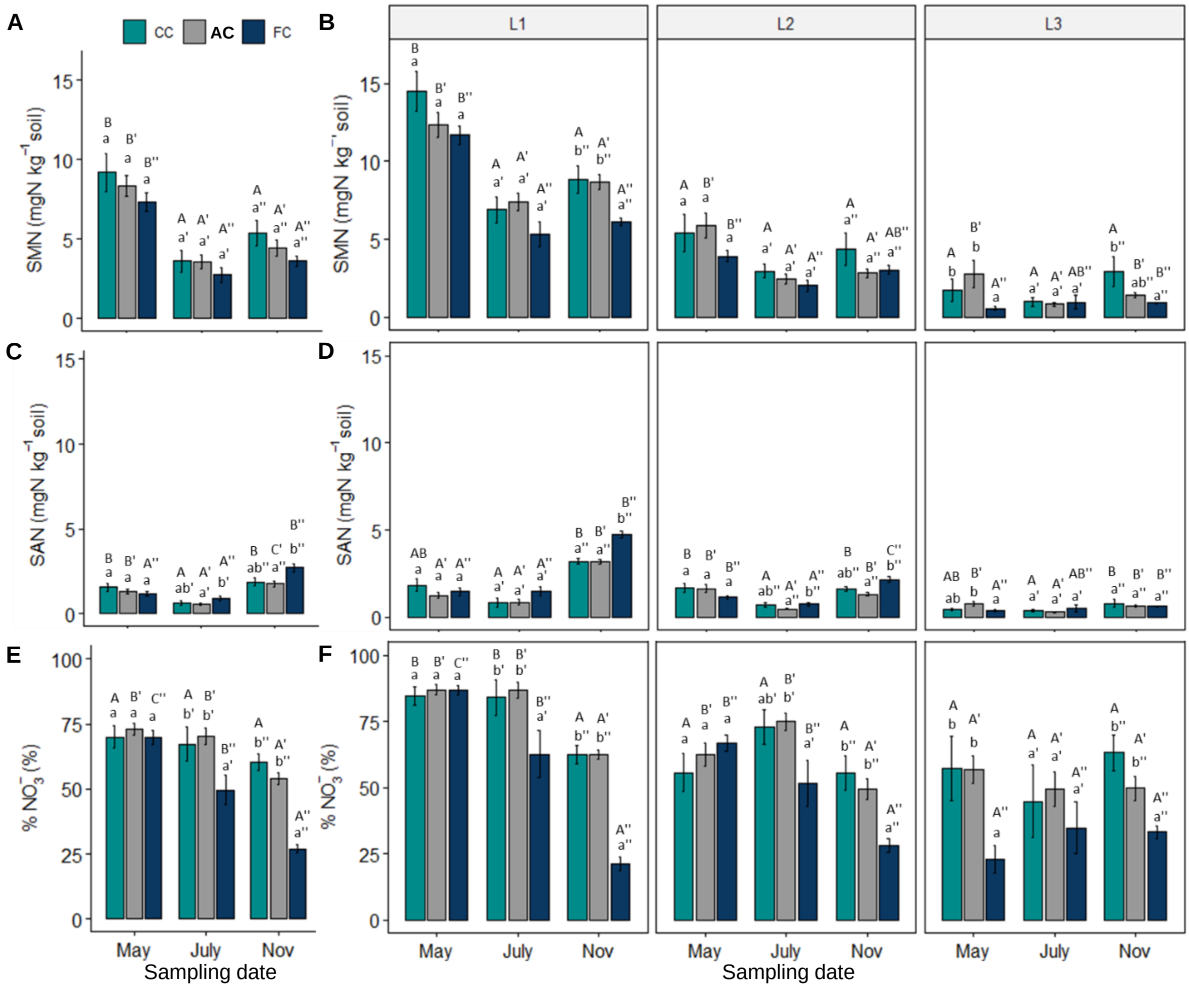


**Figure 2:** Evolution of soil mineral N (SMN, mg N kg$^{-1}$ soil), soil ammonium N (SAN, mg N kg$^{-1}$ soil) and %NO3$^-$ (%) at different soil layers during the 2021 crop season at Ramecourt alley-cropping experimental site in sole-crop control (CC), alley-cropping (AC) and pure-forest control (FC) plots. The graphics (A), (C), (E) represent the mean values from all sampling dates and (B), (D), (F) represent details for each variable at different soil layers. Data are means and bars are standard errors. Letters are homogeneous groups for multiple comparisons of means.

Lowercase letters compare the type of system (CC, AC and FC) for a given sampling date ($p < 0.05$). Uppercase letters compare the sampling date for a given type of system ($p < 0.05$). L1, L2 and L3 mean soil layer 1 (0-30 cm), soil layer 2 (30-50 cm) and soil layer 3 (50-100 cm) respectively. Nov means November.

Regarding the SAN, the mean value was significantly higher in topsoil (1.9 ± 1.5 mg N kg soil$^{-1}$) than in soil layer 2 (1.3 ± 0.9 mg N kg soil$^{-1}$) and soil layer 3 (0.5 ± 0.3 mg N kg soil$^{-1}$). The SAN was stable along the soil profile in May for all types of system whereas it decreased with depth in July (except in CC) and November, particularly in FC ($R^2 = 0.73$***). For all types of system, the SAN was significantly higher in November, (2.2 ± 1.5 mg N kg soil$^{-1}$) than in July (0.6 ± 0.7 mg N kg soil$^{-1}$) and was intermediate in May (1.2 ± 1.3 mg N kg soil$^{-1}$) (Figure 2C). Again, in July and November, the SAN was significantly higher in FC compared to AC and intermediate in CC. In topsoil, the SAN was particularly high in November in FC (Figure 2D). In soil layer 2, such as in topsoil a significant high value of SAN was also observed in November in FC. Finally, in soil layer 3, very low values of SAN were observed. No significant difference between the type of system was observed except in May where the SAN was significantly lower in FC than in AC.

The mean value of the %NO3 was 62 ± 27 % in whole soil profile. It was higher in topsoil (76 ± 24 %) than in soil layer 2 (58 ± 23 %) and soil layer 3 (44 ± 26 %). On average, the %NO3 was negatively correlated with the depth in May and July. Conversely in November, %NO3 increased with soil depth particularly in FC ($R^2 = 0.15$**). The mean value of the %NO3 was significantly higher in May (71 ± 26 %) than in July (64 ± 28 %) and in November (43 ± 20 %) (Figure 2E). In FC, the mean value of the %NO3 gradually decreased from May to November. In topsoil, the %NO3 was significantly higher on May and July than on November in both CC and AF whereas in FC, the %NO3 gradually decreased from May to November (Figure 2F). In soil layer 2, there was no significant difference in %NO3 between the sampling date in CC whereas in AF and FC, the %NO3 was significantly higher in May and July than in November. In soil layer 3, the %NO3 was significantly lower in FC than in other type of system except in July.

No significant difference between species was observed for SMN, SAN and %NO3 (data not shown).

### 3.3. Chemical composition of rooibos and green tea

The initial fresh mass of tea including the mass of synthetic container bag was lower for green (1.50 ± 0.04 g tea bag$^{-1}$) than for rooibos (1.95 ± 0.02 g tea bag$^{-1}$) (Table 1). The green tea contained almost twice more soluble organic compounds and three times less cellulose than the rooibos. There was also less lignin and cutin in green tea than in rooibos. The total C and the $CaCO_3$ content were the same for both tea whereas the total N was higher in rooibos than in green tea. Then, the mineralization coefficient at 3 days was almost twice higher for green tea than for rooibos consisting with the lower organic matter stability index. Finally, the green tea contained more water than the rooibos and its loss of ignition was twice higher than rooibos. The C/N and LIC/N ratio were higher for green than rooibos (Table 1).

### 3.4. Mass loss of tea after field incubation and ingrowth fine roots

The tea mass loss was significantly higher for the green tea (65 ± 15 %) compared to the rooibos (47 ± 10 %) (Figure 3A) even in all types of system. The type of tea accounted for 35 % of the tea mass loss variability. Conversely, the percentage of the tea mass remaining at the end of field incubation was significantly lower for the green tea (35 ± 15 %) compared to the rooibos (53 ± 10 %) (Figure 3B). For rooibos tea, the mass loss significantly decreased with depth ($R^2 = 0.1^{**}$) beyond 50 cm depth: 49 ± 10 %, 49 ± 7 % and 42 ± 10 % at 30, 50 and 100 cm depth respectively (Figure 4A). It was significantly higher for wheat-CC than for hornbeam and wild

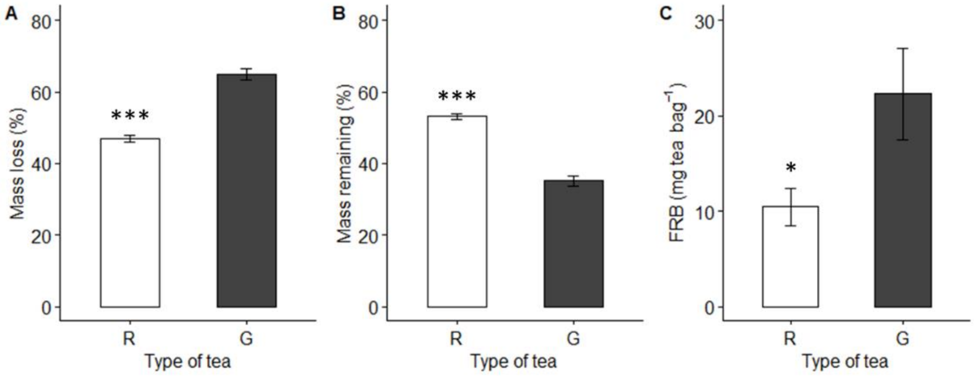


**Figure 3:** Percentage of mass loss (A), mass remaining (B) and mean values of fine roots biomass (FRB, mg tea bag$^{-1}$) within tea materials at retrieving (C) from tea bag experiment. R: rooibos tea, G: green tea. Data are means and bars are standard errors. Asterisks indicate a significant difference between rooibos and green tea; *: $p < 0.05$ (Tukey's test); ***: $p < 0.001$ (Mann-Whitney's test).

cherry (Figure 4B), and significantly higher in CC than in FC, AC was intermediate (Figure 4C). For green tea, the mass loss decrease with soil depth was also significant ($R^2 = 0.2^{***}$) and was observed from 30 cm depth. It was equal to 75 ± 18 %, 65 ± 11 % and 57 ± 10 % at 30, 50 and 100 cm depth respectively. No significant difference in tea mass loss was observed between tree species and type of system for the green tea (Figure 4B and 4C).

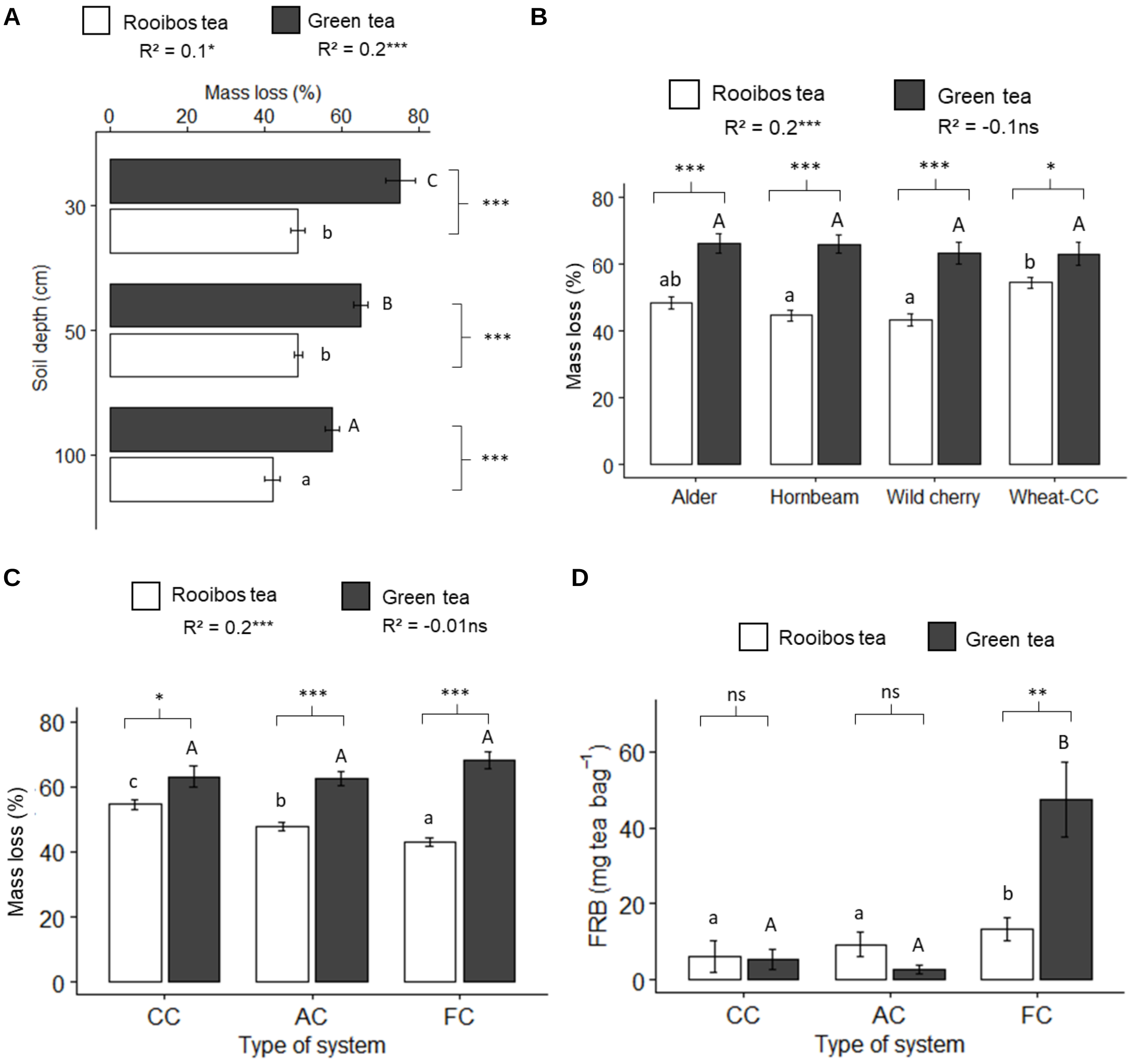


**Figure 4:** Variation of tea mass loss (ML, %) of rooibos and green tea according to (A) soil depth, (B) species and (C) type of system, and (D) variation of ingrowth fine root biomass (FRB, mg tea bag$^{-1}$). Histograms are means and bars are standard errors. In graphic (A), uppercase and lowercase letters compare ML between soil layers for green tea and rooibos, respectively. In graphics (B), (C) and (D), uppercase and lowercase letters indicate significant differences between type of system for green tea and rooibos, respectively. Asterisks indicate a significant different between rooibos and green tea. *: $p < 0.05$; **: $p < 0.01$; 'ns' means not significant.

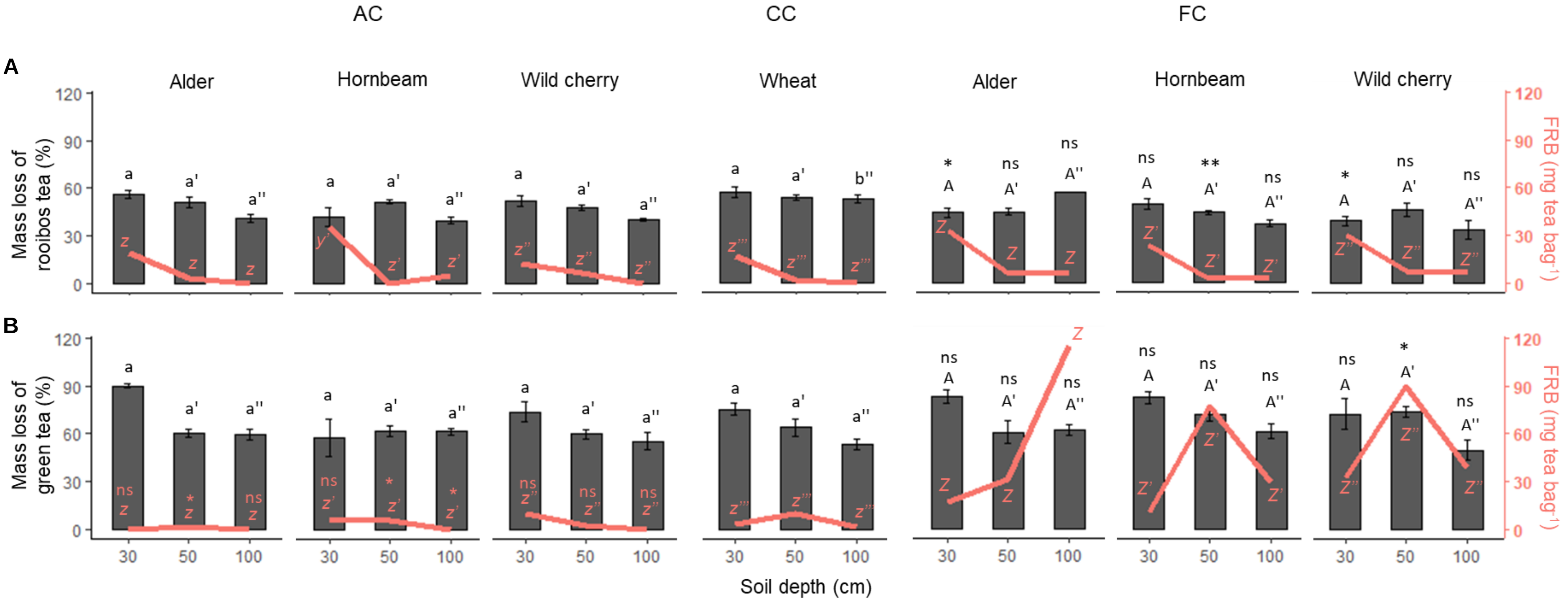


**Figure 5:** Variation of tea mass loss (ML, %) of rooibos (A), green tea (B) and ingrowth fine root biomass (FRB, mg tea bag$^{-1}$) according to soil depth, to type of system and to species. Histograms are means and bars are standard errors. Black lowercase letters compare ML between species in AC and CC for a given soil layer and black uppercase letters compare ML between tree species in FC for a given soil layer. Black asterisks indicate significant different ML between AC and FC for a given tree species. Red lowercase letters compare FRB between soil depth in AC and CC for a given species and red uppercase letters compare FRB between soil depth in FC for a given tree species. Red asterisks indicate significant difference FRB between AC and FC for a given tree species. *: $p < 0.05$; **: $p < 0.01$; 'ns' means not significant.

We did not observe significant difference in the rooibos mass loss between tree species in AC and wheat except at 100 cm depth where it was significantly higher for the wheat compared to all tree species (Figure 5A). No significant difference between tree species was noticed in FC for all soil depths. The rooibos tea mass loss was significantly lower in FC than in AC, particularly for alder and wild cherry at 30 cm depth and for hornbeam at 50 cm depth. For green tea, no significant difference was observed between type of system regardless of soil depth, except for wild cherry where tea mass loss was significantly higher in FC than in AC at 50 cm depth (Figure 5B).

Regarding the ingrowth fine roots recovered within tea bag samples, we observed that on average, the amount of FRB was twice higher for the green tea (22 ± 4 mg) compared to the rooibos (10 ± 2 mg) (Figure 3C). Then, for rooibos, the FRB was significantly higher in topsoil (24 ± 4 mg) than in soil layer 2 (4 ± 1 mg) and soil layer 3 (3 ± 1 mg). Same trend was observed for all species and in all types of system, but it was significant only for hornbeam in AC (Figure 5A). For green tea, no significant difference in FRB was observed between the soil depth. We noticed very few FRB at all depths for trees in AC and wheat, whereas in FC, FRB was significantly higher than in AC, particularly at 50 and 100 cm depth (Figure 5A). For both rooibos and green tea, there was higher FRB in FC than in CC and AC (Figure 4D) and no significant difference between tree species in AC and FC.

### 3.5. Relationship between soil parameters, tea mass loss and ingrowth fine roots biomass

The mineral N variables (SMN, SNN, SAN and %NO3) measured at different sampling dates were positively correlated with each other, particularly SNN and SMN ($r \geq 0.9$***), SNN and %NO3 ($r \geq 0.7$***) (Table 3). The mineral N variable which was the less correlated with others was the %NO3_f measured in November. We also observed positive relationships between SOM, STN and C/N variables ($r \geq 0.5$***). The total DNA was positively correlated with SOM and mineral N variables and negatively correlated with the depth ($r = -0.6$***). Moreover, all variables were negatively correlated with depth except %NO3_f, SWC_i and the FRB. The variable ML was positively correlated with SOM, SOC, STN, C/N, SMN, SNN_i/int, SAN_f and negatively correlated with depth. Finally, the FRB was negatively correlated with %NO3_f and positively correlated with SWC_i.

**Table 3**: Spearman's rank correlation rho between studied variables. The symbol *** indicate a significant rho at $p < 0.001$ level, ** for $p < 0.01$ level and * for $p < 0.05$. The absence of data means that rho was not significant. Abbreviations mean: SOM = soil organic matter (g kg$^{-1}$); STN = soil total nitrogen (g kg$^{-1}$); C/N = ratio organic C to organic N; DNA = total DNA content (ng µL$^{-1}$ soil); SMN = soil mineral nitrogen (mg N kg$^{-1}$soil); SNN = soil nitrate N (mg N kg$^{-1}$soil); SAN = soil ammonium N (mg N kg$^{-1}$soil); %NO3 = percentage of SNN in SMN (%); SWC = soil water content (%); ML = mass loss (%); FRB = fine roots biomass; Depth = soil depth (cm); Tea = type of tea (1 = green and 2 = rooibos tea). All variables with « _ini », «_int » or « _f » extensions indicate the sampling date in May, July and November respectively.

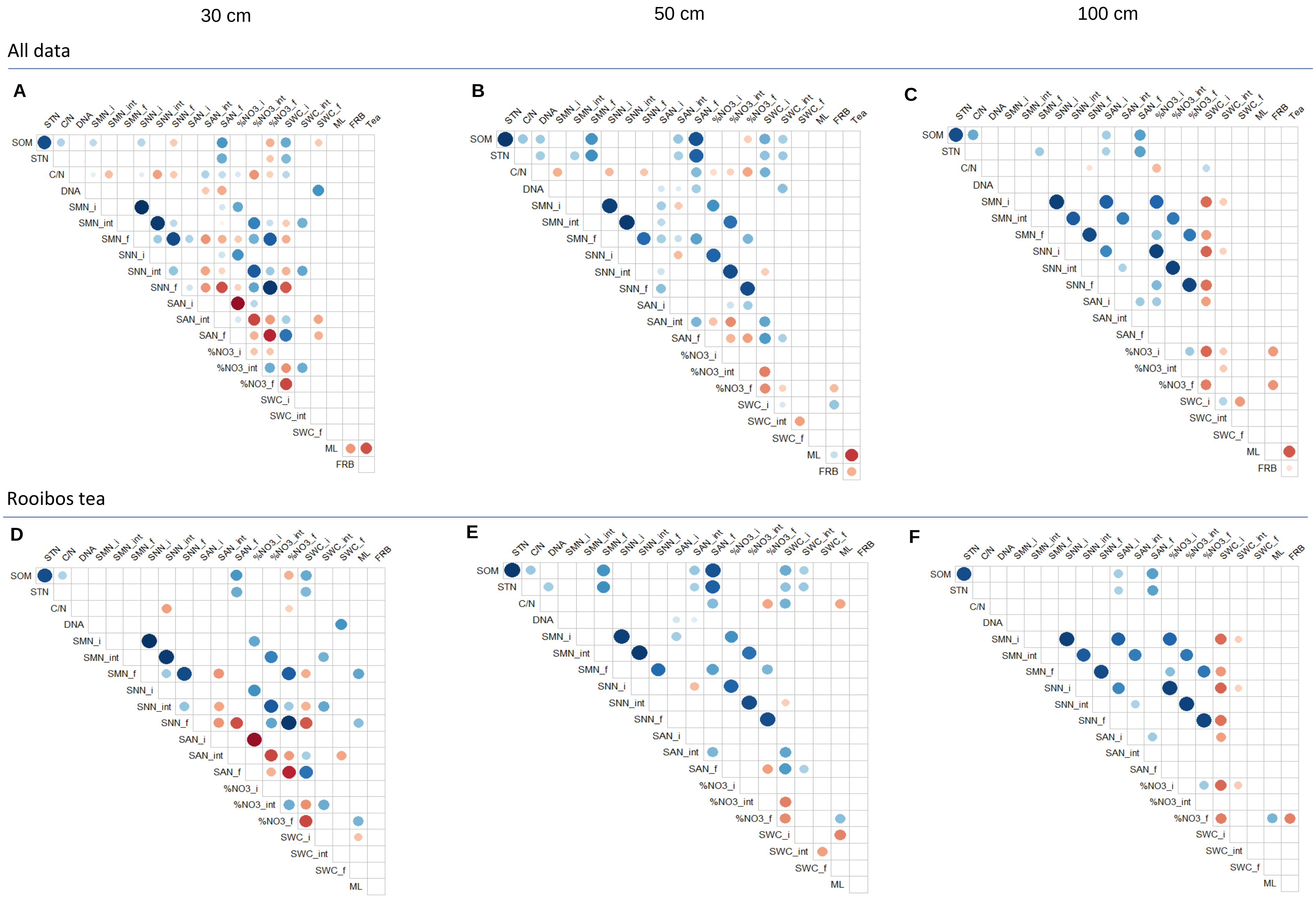
| | STN | C/N | DNA | SMN_i | SMN_int | SMN_f | SNN_i | SNN_int | SNN_f | SAN_i | SAN_int | SAN_f | %NO3_i | %NO3_int | %NO3_f |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Unit | g kg$^{-1}$ | | ng µL$^{-1}$ | mg N kg$^{-1}$ soil | mg N kg$^{-1}$ soil | mg N kg$^{-1}$ soil | mg N kg$^{-1}$ soil | mg N kg$^{-1}$ soil | mg N kg$^{-1}$ soil | mg N kg$^{-1}$ soil | mg N kg$^{-1}$ soil | mg N kg$^{-1}$ soil | % | % | % |
| SOM | 0.99*** | 0.6*** | 0.6*** | 0.8*** | 0.8*** | 0.8*** | 0.8*** | 0.7*** | 0.5*** | 0.3* | 0.5*** | 0.9*** | 0.7*** | 0.4*** | |
| STN | | 0.5*** | 0.6*** | 0.8*** | 0.8*** | 0.9*** | 0.8*** | 0.7*** | 0.6*** | 0.3* | 0.5*** | 0.9*** | 0.7*** | 0.4*** | |
| C/N | | | 0.3*** | 0.4*** | 0.3*** | 0.4*** | 0.4*** | 0.3*** | | | 0.4*** | 0.6*** | 0.3*** | 0.1*** | -0.3*** |
| DNA | | | | 0.6*** | 0.5*** | 0.6*** | 0.6*** | 0.5*** | 0.5*** | | 0.2*** | 0.6*** | 0.5*** | 0.3*** | |
| SMN_i | | | | | 0.7*** | 0.8*** | 1.0*** | 0.6*** | 0.6*** | 0.4*** | 0.4*** | 0.8*** | 0.9*** | 0.4*** | |
| SMN_int | | | | | | 0.7*** | 0.6*** | 1.0*** | 0.5*** | 0.3*** | 0.6*** | 0.7*** | 0.6*** | 0.8*** | |
| SMN_f | | | | | | | 0.8*** | 0.7*** | 0.9*** | 0.4*** | 0.4*** | 0.8*** | 0.7*** | 0.4*** | 0.3*** |
| SNN_i | | | | | | | | 0.6*** | 0.5*** | 0.4*** | 0.4*** | 0.8*** | 0.9*** | 0.4*** | |
| SNN_int | | | | | | | | | 0.6*** | 0.4*** | 0.4*** | 0.7*** | 0.5*** | 0.9*** | 0.1** |
| SNN_f | | | | | | | | | | 0.3*** | | 0.5*** | 0.5*** | 0.4*** | 0.7*** |
| SAN_i | | | | | | | | | | | | 0.3* | | 0.3*** | |
| SAN_int | | | | | | | | | | | | 0.5*** | 0.4*** | | -0.2*** |
| SAN_f | | | | | | | | | | | | | 0.7*** | 0.3*** | -0.2*** |
| %NO3_i | | | | | | | | | | | | | | 0.3*** | |
| %NO3_int | | | | | | | | | | | | | | | 0.3*** |
| %NO3_f | | | | | | | | | | | | | | | |

**Table 3** *(continued)*

| | SWC_i | SWC_int | SWC_f | ML | FRB | Depth | Type of tea |
|---|---|---|---|---|---|---|---|
| Unit | % | % | % | % | mg | cm | |
| SOM | 0.2** | 0.3*** | 0.2** | 0.1** | | -0.9*** | |
| STN | 0.2** | 0.4*** | 0.2** | 0.1** | | -0.9*** | |
| C/N | 0.3*** | 0.1* | | 0.1* | | -0.5*** | |
| DNA | | 0.3** | 0.3*** | | | -0.6*** | |
| SMN_i | | 0.2* | 0.3** | 0.1* | | -0.8*** | |
| SMN_int | | 0.3*** | 0.2** | 0.1* | | -0.8*** | |
| SMN_f | | 0.2* | 0.3** | 0.2** | | -0.9*** | |
| SNN_i | | | 0.3** | 0.1* | | -0.8*** | |
| SNN_int | | 0.4*** | 0.2** | 0.1* | | -0.8*** | |
| SNN_f | -0.3*** | | 0.2* | | | -0.6*** | |
| SAN_i | -0.3*** | 0.2* | | | | -0.4*** | |
| SAN_int | 0.2*** | | | | | -0.5*** | |
| SAN_f | 0.2*** | 0.3*** | 0.2* | 0.2** | | -0.9*** | |
| %NO3_i | | | 0.2** | | | -0.7*** | |
| %NO3_int | -0.3*** | 0.3*** | 0.2* | | | -0.5*** | |
| %NO3_f | -0.5*** | | | | -0.3** | | |
| SWC_i | | | | | 0.3** | | |
| SWC_int | | | -0.1* | | | -0.3*** | |
| SWC_f | | | | | | -0.3*** | |
| ML | | | | | | -0.2** | -0.6*** |
| FRB | | | | | | | -0.1* |
| Depth | | | | | | | |

The positive correlations between SNN, %NO3 and SMN were always observed at each soil depth (Figure 6A, 6B, 6C). We also noticed positive relationships between SOM, STN, C/N and SAN_f along the soil profile. When analyses were done on all data, the ML was correlated with only FRB at 30 cm (negative rho) and 50 cm depth (positive rho) and with no variables at 100 cm. The FRB was correlated with only ML at 30 cm depth, with %NO3_f (negative rho), SWC_i (positive rho) and ML (positive rho) at 50 cm depth and only with %NO3_f at 100 cm.

For the rooibos, ML was correlated with SMN_f, SNN_f and %NO3_f (positive rho) and SMN_i (negative rho) at 30 cm depth (Figure 6D). At 50 cm depth, it was correlated with C/N and SMN_i (negative rho) and %NO3_f positive rho). It was positively correlated with %NO3_f at 50 and 100 cm depth (Figure 6E and 6F). Stepwise analysis showed that SMN_f and %NO3_f was the main variable explaining ML accounting for 12 to 23 % of variability according to the soil depth (Table 4A). Regarding the FRB, it was correlated with any measured variables at 30 and 50 cm but was negatively correlated with %NO3_f at 100 cm depth.


30 cm
50 cm
100 cm
All data
A
B
C
SOM
STN
C/N
DNA
SMN_i
SMN_int
SMN_f
SNN_i
SNN_int
SNN_f
SAN_i
SAN_int
SAN_f
%NO3_i
%NO3_int
%NO3_f
SWC_i
SWC_int
SWC_f
ML
FRB
Tea
Rooibos tea
D
E
F

## Green tea

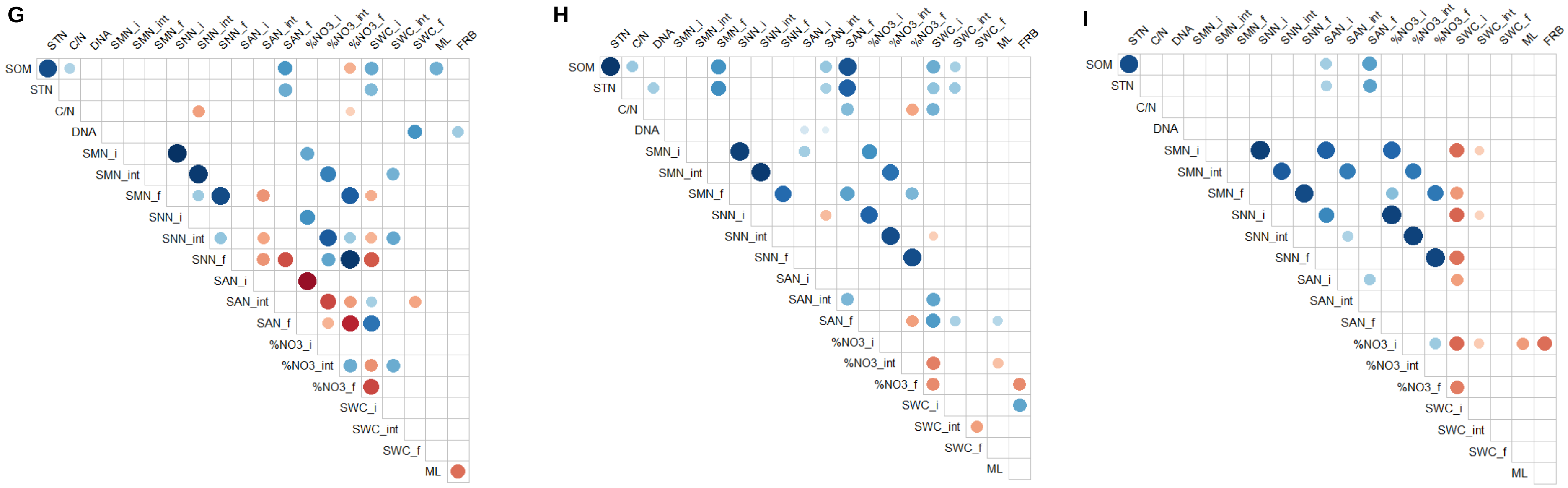


**Figure 6:** Matrix of Spearman's rank correlation rho between studied variables at 30, 50 and 100 cm depth. For graphics (A), (B) and (C) rho was calculated from all data; for (D), (E), (F) rho was calculated only from data about rooibos and for (G), (H), (I) rho was calculated only from data about green tea. Abbreviations mean: SOM = soil organic matter (g kg$^{-1}$); STN = soil total nitrogen (g kg$^{-1}$); C/N = ratio organic C to organic N; DNA = DNA content (ng µl$^{-1}$ soil); SMN = soil mineral nitrogen (mg N kg$^{-1}$soil); SNN = soil nitrate N (mg N kg$^{-1}$soil); SAN = soil ammonium N (mg N kg$^{-1}$soil); %NO3 = percentage of SNN in SMN (%); SWC = soil water content (%); ML = mass loss (%); FRB = fine roots biomass; Depth = soil depth (cm); Tea = type of tea (1 = green tea and 2 = rooibos tea). All variables with « _ini », «_int » or « _f » extensions indicate the sampling date in May, July and November respectively. The size of circles is proportional to the correlation coefficient rho, blanks indicate no significant correlations.

**Table 4:** Results of stepwise regression analysis to model the tea mass loss at a given soil layer in rooibos and green tea. (A) regression was done from all data, (B) regression was done only with data from AC and CC and (C) regression was done only with data from AC and FC. Symbols '+' or '-' indicate the positive or negative effects of a given variable in the model: +++ or ---: $p < 0.001$; ++ or --: $p < 0.01$; + or -: $p < 0.05$. $R^2$ represents the determination coefficient of the chosen model. Asterisks denote significance levels (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Abbreviations mean: SMN = soil mineral N, SAN = soil ammonium N, %NO3 = percentage of SNN in SMN, SOM = soil organic matter, FRB = fine roots biomass. All variables with « _f », «_int » or « _ini » extensions indicate the sampling date in November, July and May respectively.

**A**

| Depth (cm) | Rooibos tea | | | Green tea | | | | |
|---|---|---|---|---|---|---|---|---|
| | SMN_f | %NO3_f | $R^2$ | SOM | FRB | %NO3_int | %NO3_ini | $R^2$ |
| | mg N $kg^{-1}$ soil | % | | g $kg^{-1}$ | mg | % | % | |
| 30 | ++ | | 0.23** | +++ | -- | | | 0.50*** |
| 50 | | + | 0.13* | | | - | | 0.12* |
| 100 | | + | 0.12* | | | | -- | 0.20** |

**B**

| Depth (cm) | Rooibos tea | | | Green tea | | | | |
|---|---|---|---|---|---|---|---|---|
| | %NO3_f | SAN_ini | $R^2$ | SAN_int | SMN_ini | %NO3_ini | C/N | $R^2$ |
| | % | mg N $kg^{-1}$ soil | | mg N $kg^{-1}$ soil | mg N $kg^{-1}$ soil | % | | |
| 30 | ++ | | 0.23* | ++ | ++ | ++ | ++ | 0.79** |
| 50 | | | ns | | | | | ns |
| 100 | | | ns | | + | --- | | 0.46** |

**Table 4** *(continued)*

**C**

| Depth (cm) | Rooibos tea | | | | Green tea | | |
|---|---|---|---|---|---|---|---|
| | SMN_f | %NO3_f | DNA_f | R² | SOM | FRB | R² |
| | mg N kg$^{-1}$ soil | % | ng µl$^{-1}$ | | g kg$^{-1}$ | mg | |
| 30 | + | | | 0.17* | ++ | -- | 0.50** |
| 50 | | + | | 0.14* | | | ns |
| 100 | | | ++ | 0.33** | | | ns |

For the green tea, the ML was correlated with SOM (positive rho) and FRB (negative rho) at 30 cm depth, and with SAN_f (positive rho) and %NO3_int (negative rho) at 50 cm depth. Stepwise analysis showed that SOM and FRB accounted for 50 % of ML variability in topsoil (Table 4A). For FRB, it was correlated with DNA (positive rho) and ML (positive rho) at 30 cm depth) (Figure 6G) and with %NO3_f and SWC_i at 50 cm (Figure 6H). Finally, at 100 cm depth, ML and FRB were both negatively correlated with %NO3_i ) (Figure 6I).

Stepwise analyses, done on a dataset concerning only AC and CC (i.e. in type of systems receiving fertilizer N) revealed that for rooibos, the %NO3_f and the SAN_ini explained 23 % of ML variability at 30 cm depth (Table 4B). The availability of nitrate compared to that of ammonium was the main driver of rooibos decomposition at 30 cm depth. No variables were selected by the statistical method to explain the variability of ML at deep layer. For green tea, the SMN_ini, %NO3_ini, SAN_int and soil C/N ratio explained 79 % of ML variability at 30 cm depth (Table 4B). At 100 cm, the SMN_ini, %NO3_ini accounted for 46 % of ML variability.

Finally, stepwise analyses done on a dataset concerning only woody systems AC and FC revealed that for rooibos, the SMN_f, %NO3_f and DNA_f explained the ML variability at 30, 50 and 100 cm depth respectively, accounting for 14 to 33 % (Table 4C). For green tea, the SOM and FRB accounted for 50 % of ML variability in topsoil whereas no variable among those measured was selected by stepwise regression method to explain the variation of ML at 50 and 100 cm depth (Table 4C).

# 4. Discussion

## 4.1. Evolution of the mineral N along crop and tree growth cycle

The evolution of soil mineral N in agroecosystem is mainly rhythmed by the inputs from fertilization and soil mineralization and the outputs from plant uptake, gaseous loss, leaching and microbial immobilization (Beaudoin et al., 2005). The high values of SMN observed in May certainly reflect the effect of N fertilizer brought at the end of April in AC and CC and the high spring N mineralization in FC. As fertilizer N was applied to soil surface in AC and CC and N mineralization mainly occurred in ploughed and organo-mineral horizon in agroecosystem and forest ecosystem respectively (Andrianarisoa et al., 2010; Brisson et al., 1998; Cassman and Munns, 1980), our topsoil contained more SMN than other soil layers regardless of the type of systems. Thereafter, the SMN decreased at harvest in July testifying the optimal valorization of SMN by plant thanks to a favorable wet weather. Values observed in July were of the same order of magnitude as those recommended for SMN at harvest by balance sheet method (COMIFER, 2013; Meynard et al., 1997) which was not valorized by plants. The SMN remained stable between harvest and the start of drainage period in November thanks to catch crop in CC and AC. Beaudoin et al. (2005) also showed a significant relationship between SMN measured at harvest and at the end of autumn and highlight the role of catch crop on the N recovery between the two periods.

Globally, we did not observe differences between CC and AC in the SMN value regardless of sampling date and soil depth. The effect of sampling date and the soil depth on the variability of SMN was stronger than those of type of system and even more than for tree species. However, we surprisingly observed that the same values of SMN for fertilized (AC and CC) and unfertilized system (FC) in topsoil and layer 2 during the crop growth cycle, again testifying the best valorization of SMN by wheat. Nevertheless, in deep layer in May, the lower value of SMN observed in FC than in AC and CC was probably due to late leaching of N inputs favored by the heavy rainfalls or thanks to high fine roots counted in FC which were able to capture the soil mineral N. In November, as ryegrass and trees in FC continued to uptake mineral N, by comparison with catch crop/trees association, a significantly lower amount of SMN was observed in FC in topsoil and in layer 3 compared to other types of system.

Regarding SAN content, values measured at a given period and soil depth result on the balance between inputs from fertilizer, mineralization or leaching from soil layer above, and outputs due to nitrification, volatilization, microbial immobilization, leaching and plant uptake. In agroecosystems with alkaline soil pH, as nitrification is promoted, the amount of ammonium in soil is almost low (Andrianarisoa et al., 2016). As fertilizer ammonium input was done on soil surface and the ammonification processes occurred in topsoil, it was not surprising that we observed high values of SAN in topsoil compared to deep layer. We observed that SAN was particularly higher in November compared to other sampling dates mainly due to values measured in FC in topsoil and layer 2. Consequently the %NO3 was lower in FC compared to fertilized type of system in November especially at deep soil layer 3. For AC and CC, high values of SAN in November in topsoil suggest (i) a high ammonification during autumn or (ii) low ammonium loss by nitrification, microbial immobilization, volatilization or plant uptake. Indeed, the catch crop sown in CC and AC was poorly developed and took up small amount of the mineralized ammonium.

The first soil layer was rich in SOM and total DNA evidencing a dynamic microbial activity in topsoil which were consistent with the SMN decrease along soil profile. Ribbons et al. (2016) showed a high microbial gene abundance associated to N transformation rate such as nitrification and denitrification in topsoil. Moreover, still in November, trees and/or ryegrass induced more SAN in FC compared to CC and AF systems. This high amount of SAN could be due to (i) an increase in SAN production probably with a diversification of the microbial community through tree and ryegrass roots at low input ecosystem, or (ii) to a low ammonium uptake in FC. Indeed, in our study, FC was associated with high SAN and low %NO3 which evidenced a change in the mineralization processes with the stimulation of ammonium production, or an inhibition of the nitrification or an acceleration of denitrification. Beule et al. (2020) showed that denitrifiers community were more abundant in tree rows than in crop alleys and monocrop systems, suggesting a greater potential for nitrate removal through denitrification close to tree rows. Some authors evidenced that trees may inhibit nitrification through knock out of the nitrite oxidizers *Nitrobacter* (Laffite et al., 2020). On the one hand, trees enhanced the $N_2$ formation from $NO_3^-$ and on the other hand, they avoided the $NO_3^-$ formation from $NH_4^+$ which *in fine* decreased the $NO_3^-$ form in SMN. Higher ammonium concentrations than nitrate were also found in Atlantic forest and cocoa-cabruca agroforestry system by de Souza et al. (2018).

Finally, we retained that there was no effect of type of systems on SMN but FC promoted ammonium production especially during the autumnal period when mineral N is susceptible to be transferred into groundwater. As ammonium is less mobile than nitrate and thanks to its positive charge, its transfer toward groundwater should be reduced.

### 4.2. Tea bag decomposition and fine root biomass

Regardless of soil depth, the mass loss of tea was mainly explained by the type of tea with a faster decomposition of the green tea than the rooibos. These results were consistent with conclusions of other studies examining the decomposition of both tea types in agroecosystems (Desie et al., 2023; Djukic et al., 2018; Duddigan et al., 2020; Fanin et al., 2020; MacDonald et al., 2018). They explained this difference by the more labile material (Keuskamp et al., 2013) and a lower C/N ratio (Duddigan et al., 2020) in green tea compared to rooibos, as we also observed (Table 2). Thanks to an ample supply of N in green tea (C/N ratio ∼16), decomposers have been able to metabolize the tea C whilst satisfying their N demand from N also contained within the tea. For the decomposition of rooibos tea (C/N ratio ∼ 53), decomposing microbes will have drawn N from the surrounding environment. According to Prescott, (2010), litter with low lignin to N ratio as our green tea should decompose faster than those with high lignin to N ratio like rooibos.

At all soil depths of field incubation, the decomposition of green tea was higher than rooibos. As we incubated green tea over longer periods compared to the recommended delay (Andriamampianina et al., 2018; Keuskamp et al., 2013), we certainly missed the probable effects of type of system or species on the early-stage decomposition of this kind of litter. Indeed, Sievers and Cook (2018) showed that the decomposition curve of green tea rapidly decreases then stabilizes from 4 weeks after field incubation. In our case, the green tea decomposition was probably reached the stabilized rate for each sample especially in surface, and the soil depth remained the only significant explanatory variable among those tested. As we did not find significant effect either of the type of system or tree species, our result suggested that at this stage of tree growth, no effect on slow-stage decomposition of green tea was observed.

The slow litter decomposition that we observed at depth suggests that microbial capacity becomes limited probably because the pool of labile litter C and N are depleted after the early-stage decomposition. The initial microbial litter decay should be supported by the labile content of litter inputs at all depths, but decay rates should diverge once this initial pulse of labile C has been depleted. Labile substrates are more limiting to microbial activity in subsoils (Fontaine et al., 2007; Heitkötter et al., 2017; Tian et al., 2016). In topsoil, continuing fresh inputs of labile C support faster decomposition. Microorganisms may use the energy provided by root exudates to synthesize enzymes needed to decompose the remaining litter-derived compounds that have higher energetic barriers to decay (Blagodatskaya and Kuzyakov, 2008). Breaking down litter-derived organic matter remaining after the initial phase of decomposition in deeper soils is not energetically favorable given the low availability of labile compounds, thus, decomposition rates are very slow. Moreover, in our case, microbial N limitations due to a lower soil mineral N availability in deep layer may also be a major limiting factor for subsoil C decay as suggested by Preusser et al. (2017) and Tian et al. (2016). Decreasing C/N ratios with increasing depth as we observed in this study should not be necessarily associated with higher N availability for microorganisms (Rumpel and Kögel-Knabner, 2011).

In contrast to green tea, the decomposition of rooibos was poorly explained by the soil depth whereas the type of system affected the relative mass loss. As argued above, the recalcitrance of this kind of litter limited its decomposition as well as the decrease of N availability with depth. Indeed, regardless of the soil depth, the wheat in CC promoted the rooibos decomposition while ryegrass and trees limited it. In opposite to green tea, comparison between CC and AC revealed a negative effect of all tree species on rooibos decomposition at 100 cm depth (Figure 5A). The explanation can be biotic with root activity. Indeed, even if the same wheat fine root abundance was found between AC and CC beyond 70 cm depth (see chapter 4; Figure 1), the mix of wheat and tree fine roots in AC limited the litter decomposition and reduced priming effect compared to 100 % of wheat fine roots in CC. It also can be explained by abiotic process with the low availability of N in depth, particularly in AC. We noticed in Figure 2B for soil layer 3 in November that SMN in AC was intermediate between CC and FC, however, when we compare only CC and AC, SMN was significantly higher in CC than in AC. One last explanation can be a higher C retention in AC due to the lower decomposition rate of tree roots compared to wheat fine roots, increasing labile C compounds and litter decomposition in AC (Poirier et al., 2018).

We also observed that compared to AC, some tree species in FC reduced rooibos litter decomposition in topsoil (alder and wild cherry) and in 50 cm depth (hornbeam). These results attested that wheat accelerated the rooibos mineralization while the ryegrass reduced it. This may partly be explained by a high N microbial immobilization in FC resulting from the cessation of tillage (Couto-Vázquez and González-Prieto, 2016; Thomas et al., 2019). The decrease of the rooibos decomposition may also be due to changes in the system caused by the introduction of the trees. The soil in FC was formerly agricultural and no more fertilizer was applied since the plantation of trees inducing a low N availability and low soil net N mineralization. The system was diversifying, a decrease in biological activity could have taken place contrary to the rest of the plot always enriched in fertilizer. Indeed, enzyme activities were very sensitive to land-use change (Dollinger and Jose, 2018). After 1 year of tree establishment in an alfalfa system, Clivot et al. (2020) observed a decrease in microbial biomass N and enzyme activities, whereas after 4 years, the differences between AC and the crop control disappeared. The high microbial diversity in FC may have contrasted C and N needs compared to AC. Then, a high competition between ryegrass, trees and microorganism for mineralized N and a low labile C compound content of tree and ryegrass exudates could explain the lower litter decomposition in FC than in AC.

Fine roots were mainly found in the green tea compared to the rooibos, especially in FC plot, suggesting that in absence of fertilization, roots were present where there was nutrient release from organic matter in decomposition. Indeed, in green tea, a negative correlation was observed between fine root biomass and the relative mass loss of tea. This particular fine roots attraction towards a specific place where organic matter is still in decomposition was shown by Mulia et al. (2010), as a positive local response to a high soil nutrients availability. Then, for both kinds of tea, fine roots were more abundant in FC than in CC and AC probably due to the ryegrass established since 2019 that has been able to develop a dense root system.

We retained from this discussion the negative effect of young tree fine roots on the decomposition of recalcitrant organic matter probably due to low C and N labile compound limitation at different depths. Fine root locally responded to nutrient enrichment in tea litter still in decomposition.

### 4.3. Relationship between tea decomposition and soil characteristics

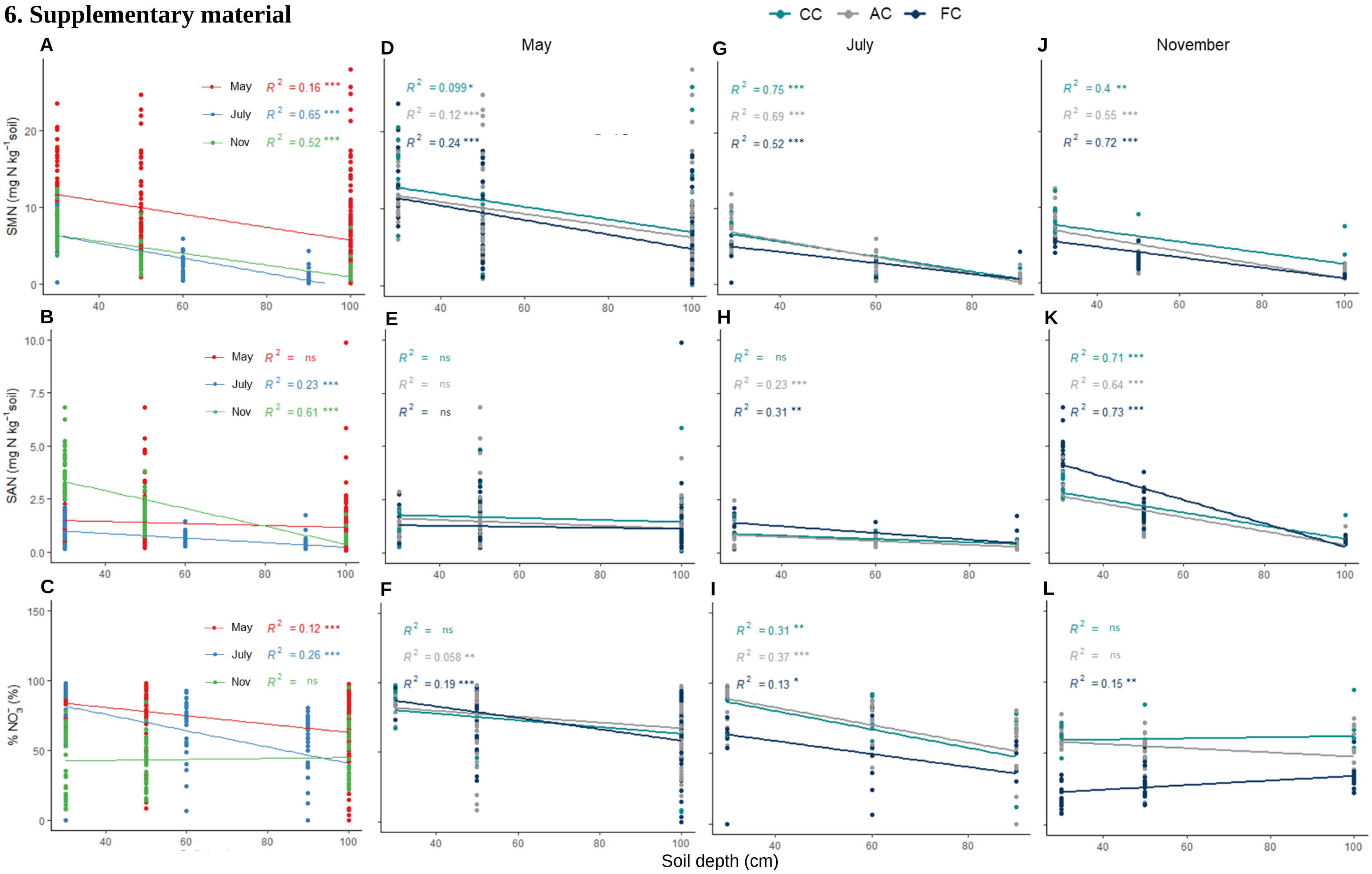
The decomposition of rooibos was mainly correlated and explained by the amount of SMN, particularly in topsoil suggested that an input of mineral nitrogen was necessary to stimulate its decomposition. Due to its recalcitrance, the rooibos decomposition through microorganisms was progressive and seemed to require an external mineral N input to be activated. Conversely, the green tea decomposition was mainly explained by the SOM in topsoil. The labile material in the green tea allowed the microbial community to begin the decomposition process. This was consistent with Bonanomi et al. (2017) study, evidencing that plant organic debris with low N content and high C/N ratio, which correspond to rooibos characteristics, had a greater response to the exogenous N availability, whereas little or no effects on N rich compound (as the green tea) were observed. Then, the negative correlation between ML and %NO3_int and %NO3_ini at 50 and 100 cm depth suggest that ammonium promoted the green tea decomposition in depth. Indeed, material with high N and low recalcitrant carbon content may be readily mineralized, while the decomposition of material compound with low N biomass and low lignin are stimulated with exogenous N in subsoils (Liang et al., 2018). As soil water was homogeneous along soil profile, it is not surprising that there was no effect of soil water content in the litter decomposition at different soil depths.

## 5. Conclusion

We examined the effect of young trees on litter decomposition of two kinds of tea with fast (green tea) and low (rooibos) decomposition and showed that the chemical characteristic of litter was the main factor explaining their decomposition rate. It decreased with increasing soil depth regardless of the type of tea and the type of system within which they were incubated. No trees effect was observed on the late-stage decomposition of green tea but tree roots proliferated within green tea still in decomposition probably stimulated by the nutrient release from high quality organic matter. By contrast, we found a negative effect of trees on the relative mass loss of rooibos at 100 cm depth in AC and at 30 cm depth in FC for alder and wild cherry. As the amount of labile C and availability of N are the two main limiting factors to recalcitrant litter decomposition at different soil depths, we believe that tree species contribute to reduce these parameters compared to monocrop. These findings confirm the ability of alley-cropping system to limit the decomposition of recalcitrant dead roots injected along soil profile from tree roots mortality and favor carbon sequestration in soil depth. Alongside that, trees in FC favored ammonium N formation probably due to diversification of microbial community and may potentially contribute to limit the N loss by leaching in groundwater thanks to its low mobility in soil.

Soil modifications were occurring in young AC due to the presence of new perennial species, further studies on microbial communities should be conducted to identify which microorganisms were impacted in tree row and in alley and related it to their function. Then, the fine root growth will be evaluated from roots collected in root ingrowth bags. These data will be connected to rooibos and green tea mass loss to see if there was a link between the fine root growth and the SOM decomposition. Indeed, the strong relationship between fine roots, mineralization and microorganisms through root exudate should be deepened with analyses of rhizospheric and distant soil communities.

## 6. Supplementary material



**Appendix 1:** Variation of soil mineral N (SMN, mgN $kg^{-1}$ soil), soil ammonium N (SAN, mgN $kg^{-1}$ soil) and percentage of nitrate in SMN (%$NO3^{-}$, %) with depth in spring (May), at harvest (July) and in autumn (November) during the 2021 crop season at Ramecourt alley-cropping experimental site. CC = sole-crop control, AC = alley-cropping, FC = pure-forest control. The (A), (B), and (C) graphics represent data from all sampling dates; (D), (E), (F) are data from May sampling date, (G), (H), (I) are from July and (J), (K), (L) are from November 2021. Lines are linear regression between SMN, SAN and %NO3 and soil depth. A symbol *** was used to indicate a significant coefficient of determination ($R^2$) at $p < 0.001$ level, ** for $p < 0.01$ level and * for $p < 0.05$ level. "ns" means not significant.

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# Chapitre 7 : Discussion générale et perspectives

## 1. Discussion générale sur la méthodologie employée

### *Etude de la distribution racinaire le long d’une chronoséquence*

Les difficultés rencontrées sur l’étude de la distribution racinaire le long d’une chronoséquence de peuplements agroforestiers âgés entre 3 et 12 ans, ont été dans un premier temps de trouver des jeunes parcelles en agroforesterie intraparcellaire. Les exploitations des départements du Nord et du Pas-de-Calais étaient visées au début du projet de thèse. Le secteur de recherche a été élargi à l’ensemble de la région des Hauts-de-France, afin d’avoir entre quatre et cinq parcelles d’étude par classe d’âge, ce qui comptabilisait l’ensemble des contacts de agriculteurs disponibles. De plus, parmi les différents sites étudiés, nous avons réussi à couvrir les différentes pratiques rencontrées dans l’agriculture (conventionnelle, raisonnée, biologique, conservation). Par ailleurs, chaque parcelle ayant des essences d’arbres différentes, nous avons sélectionné les trois les plus représentées et de manière à ce qu’au moins une de chaque soit présente par classe d’âge. L’étude d’une unique espèce aurait donnée des résultats plus robustes sur sa distribution racinaire au cours de sa croissance, cependant, nous avons ainsi introduit la variable « essence » dans notre étude qui explique 4 % de la variabilité de l’abondance des racines fines des arbres le long de la chronoséquence. En intégrants plus de parcelles dans l’étude, nous aurions eu une meilleure représentation de l’évolution de la distribution racinaire des arbres en agroforesterie intraparcellaire et une diminution de l’impact des facteurs inhérents à chaque site. Nous avons également rencontré quelques difficultés de carottage dans les sols caillouteux. Dans ces situations, nous avons essayé à plusieurs reprises les carottages autour de la zone ciblée afin de contourner le caillou. Toutefois dans les sols très caillouteux, il fut parfois impossible d’atteindre les 2 m de profondeur, notre matériel n'étant pas assez puissant pour briser successivement les silex rencontrés.

La méthode “core-break” a été choisie pour évaluer la distribution des racines fine car elle est rapide et permet de couvrir des zones étendues. Pour faire face à la forte variabilité spatiale induite par cette méthode, nous avons réalisé des carottages des deux côtés de la ligne d’arbres. Ainsi, il y a eu deux répétitions pour chaque zone de prélèvement (excepté au niveau du tronc) par parcelle étudiée. Un quadrillage de la zone étudiée en multipliant les carottages dans différentes directions par rapport à la ligne d'arbres aurait permis d'obtenir une meilleure représentation de la distribution racinaire de l'arbre référent. Nous avons choisi de multiplier le nombre de sites dans le territoire pour un âge de peuplement donné en supposant que cela puisse compenser la variabilité spatiale des racines. A travers cette méthode, nous avons également extrapolé les données de l'abondance des racines fines obtenue sur une faible surface (correspondant au diamètre des gouges) à l'échelle de la parcelle. Nous avons fait l'hypothèse que l'erreur induit par cette extrapolation était compensée par le nombre de carottages collectés par âge de peuplement autour d'un arbre référent. Cette méthode ne prend pas non plus en compte l'orientation des racines, nous aurions dû faire une inspection de leur orientation mais nous avons choisi d'en considérer qu'une. Enfin, nous n'avons pas pu nous affranchir de la dépendance entre les valeurs obtenues pour chaque horizon au sein d'une même carotte. Certains auteurs ont compensé cette possible interdépendance entre les données par l'utilisation de modèles linéaires mixtes en définissant pour chaque modèle un facteur aléatoire et un facteur fixe (Beuschel et al., 2019; Cardinael et al., 2015b).

D'autres méthodes auraient pu être utilisées telles que la détermination de la biomasse racinaire en prélevant l'ensemble des carottes de sol et en procédant à un lavage des racines avant de les sécher et peser (Andrianarisoa et al., 2017). Un profil racinaire aurait aussi pu être fait en creusant un transect et en dénombrant les racines à l'aide d'une grille (Bouttier et al., 2014). Enfin le suivi de l'isotope 13 du carbone ($^{13}C$) est parfois utilisé pour estimer la biomasse des racines (February and Higgins, 2010).

### *Etude du partage de l'eau*

Des sondes capacitives basées sur la transmission (Time Domain Transmission ou TDT) ont été installées dans les 15 sous parcelles constituant le site expérimental agroforestier de Ramecourt dans le but d'avoir un suivi continu de la teneur en eau du sol à différentes distances de la ligne d'arbres et sur trois profondeurs. Malheureusement, nous n'avons pas réussi à générer les données de ces sondes qui auraient pu nous aider à comprendre la dynamique de l'eau dans le sol au cours de la saison culturale. En effet, l'année 2021 ayant été très pluvieuse, la solution enrichie en deutérium ($^{2}H$) a pu être dissipée au cours de l'expérimentation. Huo et al. (2020) ont réinjecté du $^{2}H$ trois fois à un mois d'intervalle entre juillet et septembre. Par ailleurs, les fortes teneurs en eau du sol, dues à la pluviométrie élevée, ont certainement masqué l'effet des arbres qui ont eu suffisamment d'eau à proximité de leur tronc et en surface pour couvrir leur besoin. Il serait pertinent de refaire cette expérience dans des conditions plus sèches et de comparer les résultats. Les arbres étant également encore jeunes, il aurait été judicieux de faire l'expérimentation une année plus tard afin d'augmenter les possibilités de compétitions avec la culture en place.

Il aurait également été possible d'analyser l'eau du xylème en échantillonnant des branches d'arbres (Grossiord et al., 2014; Stahl et al., 2013). Les jeunes feuilles des arbres étant aux extrémités, il est possible que leur source d'eau soit pauvre en $^{2}$H due à la distribution en amont dans les organes inférieurs. Le suivi de l'abondance naturelle du $^{2}$H aurait pu être complété avec celui de l'oxygène 18 ($^{18}$O), et ainsi permettre d'estimer la proportion d'eau absorbée à différentes profondeurs du sol par un modèle mixte (Huo et al., 2020; Wang et al., 2023; Zhang et al., 2022). Aussi, nous aurions dû mesurer la teneur en $^{2}$H du sol afin d'identifier la provenance de l'eau des différentes espèces. Cette donnée aurait été complémentaire aux résultats obtenus pour les dépôts de $^{2}$H faits à trois profondeurs différentes. Par ailleurs, même si nous avons fait deux dépôts de $^{2}$H par arbre référent, la multiplication d'injections aurait augmenté la probabilité de tomber sur des racines d'arbres. Enfin, des mesures de biomasse des arbres auraient permis de suivre leur croissance aérienne, de la corréler avec l'absorption de l'eau et d'observer l'effet de la compétition sur cette croissance aérienne.

### *Etude de la distribution racinaire*

La méthode "core-break" a été utilisée pour déterminer la distribution racinaire des arbres, du blé et du ray-grass dans le site expérimental agroforestier de Ramecourt, cependant, elle a induit un biais en particulier lorsque la densité de racines était élevée. En plus des méthodes citées ci-dessus pour caractériser les systèmes racinaires, il existe aussi l'insertion de « ingrowth bags » permettant d'évaluer la croissance des racines sur une période choisie (Germon et al., 2018; Silva et al., 2011), ainsi que la mise en place de minirhizotrons, filmant en continu le développement des racines (Jose et al., 2001; McCormack et al., 2012). Dans notre étude, l'installation d'un plus grand nombre d'« ingrowth bags » avec des périodes d'enfouissement différentes aurait donné un suivi de la croissance du système racinaire au cours de la saison culturale. Nous aurions pu également mesurer la longueur spécifique des racines (SRL) de chaque espèce étudiée en les lavant, scannant et en mesurant la longueur.

### *Etude du partage de l'azote*

Dans l'expérience du suivi de l'azote entre les arbres et les cultures, nous avons choisi de mesurer l'azote 15 ($^{15}$N) dans les feuilles des arbres mais les analyses auraient pu être réalisées dans les parties ligneuses. Nous aurions pu également analyser indépendamment les différents tissus du blé en séparant les feuilles des tiges et les grains des épis afin d'avoir des données précises de la répartition de l'azote dans la plante au cours de son cycle de croissance. Par ailleurs, nous avons apporté de l'engrais marqué uniquement lors du 4$^{ème}$ apport mais il existe des études où le marquage a été fait sur plusieurs apports (Fuertes-Mendizábal et al., 2018; Shi et al., 2012) ce qui permet d'évaluer l'efficacité d'absorption des engrais par la plante au cours de sa croissance et ainsi de déterminer quels apports sont les mieux valorisés. Nous n'avons pas calculer la biomasse des arbres ni des cultures qui nous auraient permis de réaliser un bilan des teneurs en $^{15}$N des plantes, il aurait également été pertinent de calculer le pourcentage d'azote provenant des engrais (%NDF) (Jose et al., 2000a; Zamora et al., 2009). Enfin, un bilan total des flux d'azote aurait pu être réalisé pour appuyer nos hypothèses émises d'après les résultats du suivi du $^{15}$N.

### *Etude de la décomposition du thé*

Les résultats obtenus sur l'expérimentation sur la décomposition du thé ont été plus variés concernant le rooibos que le thé vert. Un temps d'incubation plus court des sachets de thé aurait peut-être apporté davantage de données significatives (Andriamampianina et al., 2018; Keuskamp et al., 2013). Cette expérimentation devait être complétée par des mesures microbiologiques pour caractériser l'activité microbienne que nous n'avons pas pu mettre en place. Par ailleurs, les racines retrouvées dans les « ingrowth bags » n'ont pas pu être quantifiées, il aurait été intéressant de tester la relation entre la décomposition des thés et le nombre de ces racines. Cela a été fait pour les racines récupérées dans les sachets de thé décomposés, néanmoins, il était difficile des distinguer à quelle espèce elles appartenaient. Nous avons donc quantifié les racines dans leur ensemble sans différencier celles du blé, du raygrass et des arbres. Enfin, nous n'avons pas réussi à récupérer quelques « ingrowth bags » enfouis à 1 m de profondeur dont les ficelles et fils de fer se sont cassées lors de l'extraction, des cordes plus solides aurait pu éviter ces pertes.

## 2. Discussion générale sur les principaux résultats de cette thèse

### 2.1. Croissance racinaire et effets sur la teneur en eau, carbone et azote dans les sols

Les systèmes agroforestiers apportent une diversification de leur environnement. En effet, l'introduction d'autres espèces dans une parcelle agricole induit des changement physiques et biologiques afin qu'un nouvel équilibre se mette en place. Les arbres complexifient notamment leur milieu en modifiant la structure du sol (Udawatta et al., 2008). Contrairement aux cultures annuelles, les arbres s'établissent progressivement dans leur environnement. Il est donc important pour les agriculteurs de bien gérer cette installation afin que les racines des arbres n'envahissent pas la zone de culture en particulier lorsqu'elle n'est pas exploitée. De même que pour la majorité des espèces, les racines des arbres se développent préférentiellement dans la partie supérieure du sol où les ressources sont disponibles. Pour la plupart des arbres, les 50 premiers centimètres du sol abritent 80 à 90 % de la biomasse racinaire et 90 à 99 % se trouvent dans les 1 m supérieurs (Hirons and Thomas, 2017). Cependant, en réponse à la contrainte d'immobilité due à la vie fixée, les végétaux ont développé une forte capacité d'adaptation à leur milieu notamment par la plasticité de leur système racinaire (Bradshaw, 1965).

En système agroforestier intraparcellaire, nos résultats ont montré qu'à partir de 5 ans, les arbres développent des racines verticales en profondeur, limitant la compétition avec la culture (O'Connor et al., 2023). Dès 7 ans de plantation, la croissance des racines des arbres se poursuit en profondeur et horizontalement sous la zone d'enracinement des cultures. Par ailleurs, il est possible de moduler la croissance des arbres en y exerçant des contraintes physiques. En effet, les coupes successives des racines superficielles des arbres dues au labour ont favorisé leur croissance en profondeur. Une spatialisation entre les racines des arbres et des cultures se mets donc en place lors des 10 premières années d'installation d'une parcelle d'agroforesterie intraparcellaire. Toutefois, dans certaines études, des racines d'arbres ont été relevées jusque 3 et 3.75 m de la ligne d'arbres dans des SAF peuplier-blé de 10 et noyer-blé de 11 ans respectivement (Battie-Laclau et al., 2020; Swieter et al., 2022). Ces disparités peuvent être dues aux différentes essences d'arbres étudiées, à la méthode d'échantillonnage, aux caractéristiques du sol ainsi qu'à l'itinéraire technique (labour, élagage…). Un suivi de la croissance racinaire des arbres du site expérimental de Ramecourt apporterait de nouvelles données sur le développement des racines lors de la phase d'installation d'un SAF. Ainsi, nous pourrions comparer si le processus de croissance racinaire suit le même développement déterminer par la chronoséquence sur nos 17 sites.

L'établissement des racines des arbres dans une terre arable augmente la porosité du sol et ainsi sa capacité à retenir l'eau (Udawatta and Anderson, 2008). Cependant, la forte densité des racines des arbres dans la ligne et sur les 30 premiers centimètre du sol peut diminuer localement la teneur en eau du sol (O'Connor et al., 2023; Swieter et al., 2022). L'abondance des racines fines des arbres a contribué à l'assèchement le long du profile du sol augmentent la capacité des sols à stoker l'eau (O'Connor et al., 2023). Anderson et al. (2009) ont également montré une meilleure infiltration de l'eau à travers le système racinaire des arbres en agroforesterie et ainsi un stockage d'eau du sol plus élevé qu'en monoculture. Un assèchement plus profond du profil de sol en début de l'automne grâce aux arbres permet d'augmenter la profondeur de sol pouvant stocker les pluies hivernales et donc d'optimiser l'utilisation des ressources en eau.

La présence continue des racines des arbres modifient également la fertilité des sols. Plusieurs études ont montré une augmentation de la matière organique (MO) du sol en milieu agroforestier (Cardinael et al., 2017, 2015a; Oelbermann et al., 2006; Pardon et al., 2017; Peichl et al., 2006). Cependant la séquestration du carbone organique du sol (COS) est un processus lent, une décennie est généralement nécessaire pour en observer une augmentation au niveau de la ligne d'arbre (O'Connor et al., 2023). Nous n'avons pas pu mettre en évidence de différence de COS entre les classes d'âge, notamment dû aux différents facteurs pédoclimatiques inhérents à chaque site et qui interviennent dans sa séquestration.

Une relation positive entre les racines fines des arbres et la MO a été également mise en évidence, principalement au niveau de la ligne d'arbres chez des peuplements âgés entre 10-12 ans (O'Connor et al., 2023). Les racines fines des arbres, en parti par leur turnover, contribuent à l'augmentation de la teneur en MO des sols agroforestiers, et leur décomposition libère également de l'azote au système (Jose et al., 2000b; Swieter et al., 2022). Ces résultats montrent que les arbres modifient la dynamique de la minéralisation de l'azote. En effet l'augmentation de la teneur en ammonium en milieu agroforestier (O'Connor et al., 2023) indique une stimulation des processus d'ammonification et/ou une diminution de la nitrification. Enfin, contrairement au nitrate, l'ammonium est plus facilement adsorber sur le complexe argilo-humique grâce à sa charge positive, réduisant son transfert vers les nappes souterraines et la pollution des eaux.

Dans cette étude, nous avons montré qu'à partir de 5 ans, les arbres en agroforesterie intraparcellaire commençaient à développer des racines verticales en profondeur, puis explorent horizontalement la zone sous l'enracinement des cultures. Cette stratégie de croissance racinaire est en partie favorisée par le labour et la durée de rotation des cultures. La croissance des racines fines des arbres a contribué à l'assèchement de l'horizon supérieur proche de la ligne d'arbres, à l'augmentation des teneurs en ammonium et en carbone organique du sol, dans cette même zone, pour les peuplements les plus vieux.

### 2.2. Mise en place de la compétition en eau et en azote lors des phases juvéniles des arbres

La réussite d'une parcelle en agroforesterie dépend particulièrement de la gestion de la cohabitation aérienne et racinaire entre les espèces. En effet, malgré les nombreux services écosystémiques apportés par les arbres sur une parcelle agricole tels que l'augmentation de la biodiversité, la réduction de l'érosion et l'enrichissent les sols, la diminution de la productivité des cultures due à la compétition pour les ressources est l'une des principales craintes des agriculteurs. Une complémentarité pour l'utilisation des ressources peut avoir lieu grâce à la séparation spatiale des racines des arbres et des cultures et au décalage temporel des besoins en ressources en eau et azote. Cependant, il existe toujours une compétition entre les racines, en particulier à l'interface entre la ligne d'arbres et la zone de culture (Gao et al., 2013; Lehmann et al., 1998; Swieter et al., 2022), entrainant une diminution du rendement.

Le suivi de l'abondance naturelle du $^2$H des arbres et du blé d'un système en agroforesterie intraparcellaire de 4 ans a mis en évidence une absorption de l'eau provenant de profondeurs différentes pour ces deux espèces. Le blé et les arbres semblent majoritairement avoir prélevé de l'eau dans les horizons superficiels, soumis à des fluctuations de l'abondance naturelle en $^2$H par la succession des évènements d'évaporation et d'infiltration, mais les arbres ont adapté leur profil d'absorption en fonction de la disponibilité de l'eau dans les horizons. Cette stratégie opportuniste des arbres a aussi été montré par Bargués Tobella et al. (2017). L'utilisation d'une solution enrichie en $^2$H pour tracer l'absorption d'eau entre les espèces a confirmé cette capacité des arbres à prélever de l'eau en profondeur en cas de déficit en surface, en particulier pour l'aulne et le merisier, alors que le charme a absorbé majoritairement de l'eau en surface. Huo et al. (2020) ont observé les même conclusions avec des proportions d'eau provenant des horizons supérieurs et en profondeur, plus élevées lors des périodes humides et sèches respectivement. Le marquage isotopique a révélé une forte absorption d'eau en surface pour le blé, en particulier lorsqu'il est en association avec les arbres. Cependant, les arbres n'ont pas impacté la quantité d'eau prélevée par le blé. Cela peut être dû à leur jeune âge, à une faible compétitivité pour l'eau, mais aussi aux conditions météorologiques. En effet, l'année 2021 fût très humide, les teneurs en eau du sol étaient élevées et similaires dans les trois systèmes comparés : agroforestier, témoin agricole et témoin forestier. Cette forte disponibilité de l'eau a pu masquer la compétition entre les arbres et le blé.

L'étude du partage de l'azote, au sein d'un système en agroforesterie intraparcellaire de 4 ans, a montré une absorption d'azote du blé imminente après le 4$^{ème}$ apport d'engrais azotés. Cette absorption a été observée sur l'abondance naturelle en $^{15}$N, en particulier pour le blé associé au charme et au merisier, suggérant une disponibilité plus importante d'azote à proximité de ces espèces. Les analyses de $^{15}$N du blé cultivé dans les zones marquées avec de l'engrais enrichi en $^{15}$N, ont montré une forte absorption d'azote en témoin agricole et système agroforestier. Les arbres n'ont pas impacté le prélèvement d'azote du blé, quelle que soit l'essence, mais un début de compétition pour l'azote absorbé en surface a été observée. En effet, malgré les faibles teneurs en $^{15}$N mesurées dans les feuilles des arbres, une augmentation significative du $\delta^{15}$N a été perçue par rapport aux arbres situés dans les zones non marquées, en particulier pour le charme et le saule. Aucune étude réalisée en système agroforestier de moins de 4 ans sur les mêmes espèces d'arbres permettent de confirmer ces résultats. Toutefois, après deux ans de plantation, la comparaison de deux systèmes grevillea-maïs et senna-maïs a montré une compétitivité plus élevé du senna par rapport au grévilla, induisant une réduction de l'absorption d'azote du maïs associé au senna (Livesley et al., 2002). Zamora et al. (2009) ont également observé une compétition pour l'azote dans un système pin-coton âgé de 5 ans.

L'étude de la distribution racinaires des différentes espèces associées confirme l'impact des arbres sur l'absorption d'eau et d'azote du blé. En effet, une diminution de l'abondance des racines du blé a été observée lorsqu'il était cultivé en système agroforestier par rapport aux témoins agricoles. De nombreuses études ont montré une réduction des racines des cultures à proximité des rangées d'arbres (Battie-Laclau et al., 2020; Cardinael et al., 2015b; Duan et al., 2019). Cette diminution a été plus importante en surface pour le blé en association avec le saule et plus faible avec le charme, mais aussi observée jusque 70 cm de profondeur pour le blé associé avec le merisier. Ces résultats confirment la plus forte compétitivité du saule pour l'absorption de l'azote en surface, l'absorption d'eau majoritairement dans l'horizon supérieur pour le charme, et la capacité du merisier à absorber l'eau en profondeur. Par ailleurs, les racines fines des arbres étaient plus développées en surface, en particulier pour le saule par rapport au charme, le merisier ayant une distribution intermédiaire. Cela confirme que le cohabitation entre les racines fines des arbres et des cultures était plus prononcée dans l'horizon supérieur (Duan et al., 2019).

Dans cette étude, les arbres âgés de 4 ans n'ont pas impacté la croissance ni les quantités d'eau et d'azote prélevées par la culture de blé en place. A ce stade de développement, les arbres et le blé ont partagé les ressources en eau et azote sans impact significatif sur leur croissance respective. Cependant, la présence des arbres a modifié le profil d'absorption de l'eau et de l'azote par le blé, qui a prélevé davantage d'eau en surface et avec un début de compétition pour l'azote absorbé en surface. Par ailleurs, des différences entre les essences d'arbres commencent à émerger. En effet, due à sa croissance rapide, le système racinaire du saule était plus développé que celui du charme et du merisier. L'association avec les arbres a diminué le développement des racines fine du blé, particulièrement en surface et avec le saule. De même, le merisier et l'aulne apparaissent plus compétitifs pour l'eau par rapport au charme, indiquant que la compétition entre les arbres et les cultures dépend en partie des espèces associées.

### 2.3. Conséquence des arbres sur la décomposition de la matière organique

La conversion d'un système agricole en agroforesterie modifie les caractéristiques physiques et biologiques du sol (Udawatta et al., 2008). En effet une augmentation de la biomasse et de l'activité microbienne est perçue dans les systèmes agroforestiers intraparcellaires même dès les premières années suivant la plantation (Beuschel et al., 2019; Clivot et al., 2020; Mungai et al., 2005; Udawatta et al., 2008). Nous avons observé que les arbres stimulent la production d'ammonium, particulièrement dans la ligne d'arbres et en profondeur. Dans notre site d'étude âgé de 4 ans, la production d'ammonium a été perçue dans les témoins forestiers mais pas dans les systèmes en agroforesterie. Les racines des arbres et du ray-grass des témoins forestiers n'ont pas connu de perturbations en 4 ans et ont pu ainsi se développer densément. De la même manière, nous supposons que les microorganismes ont colonisé le système progressivement.

L'étude de la décomposition de thé vert et de rooibos a montré que la rapidité de dégradation de la matière organique était principalement due à sa composition. Des matières contenant des composés labiles et riches en azote se dégradent plus rapidement que celles contenant majoritairement des composés carbonés récalcitrants (Prescott, 2010). La décomposition des matières organiques diminue aussi avec la profondeur. Cela peut être dû à la diminution de composés carbonés labiles et à la faible disponibilité de l'azote en profondeur, qui sont nécessaires à la stimulation de l’activité microbienne (Preusser et al., 2017; Rumpel and Kögel-Knabner, 2011; Tian et al., 2016). Les arbres n’ont pas eu d’effet sur la phase rapide de décomposition, alors qu’une une diminution de la décomposition de la matière organique récalcitrante a été observée dans les systèmes agroforestiers et les témoins forestiers par rapport aux témoins agricoles. L’augmentation du ratio C/N notée dans les témoins forestiers témoigne de la plus faible disponibilité de l’azote, pouvant expliquer la diminution de décomposition des matières récalcitrantes comme détaillé ci-dessus. Par ailleurs, le ratio C/N est un facteur principal déterminant la structure des communauté microbiennes (Wan et al., 2015). Le ralentissement de la décomposition des matières récalcitrantes et l’augmentation du ratio C/N suggèrent qu’une diversification microbienne est en train de s’opérer dans le milieu. En effet, la composition des communautés fongiques du sol réagit rapidement à l'intégration d'arbres dans les terres arables. Un an et demi après la plantation, Beule and Karlovsky (2021) ont observé une dispersion des communautés fongiques dans la ligne d’arbres. Dans les systèmes agroforestiers l’impact négatif des arbres est observé seulement à 100 cm de profondeur où la teneur en azote minéral est plus faible qu’en témoins agricoles. Par ailleurs, une relation positive a été observée entre la décomposition des matières récalcitrante et le teneur en azote minéral du sol. Ces observations confirment le besoin d’azote disponible pour stimuler la décomposition des matières récalcitrantes.

La différence des temps de décomposition observée pour la litière récalcitrante entre les témoins forestiers et les systèmes agroforestiers peut être due au travail du sol (Couto-Vázquez and González-Prieto, 2016; Thomas et al., 2019), aux rotations de cultures, ainsi qu’aux intrants qui imposent un temps plus long pour que la diversification des communautés microbiennes ait lieu. L’apport d’azote par la fertilisation diminue le ratio C/N du sol et la biomasse microbienne (Sun et al., 2020) pouvant retarder la transition des communautés microbiennes. Les teneurs en carbone et azote organique du sol, intermédiaires entre les valeurs des systèmes agricoles et forestiers, témoignent du changement qui se produit. Par ailleurs, la relation positive entre les racines des arbres et la teneur en ammonium du sol est particulièrement élevée pour les peuplements âgés de 10-12 ans, confirmant le temps nécessaire à la diversification du système.

## 2.4. Schémas de synthèse

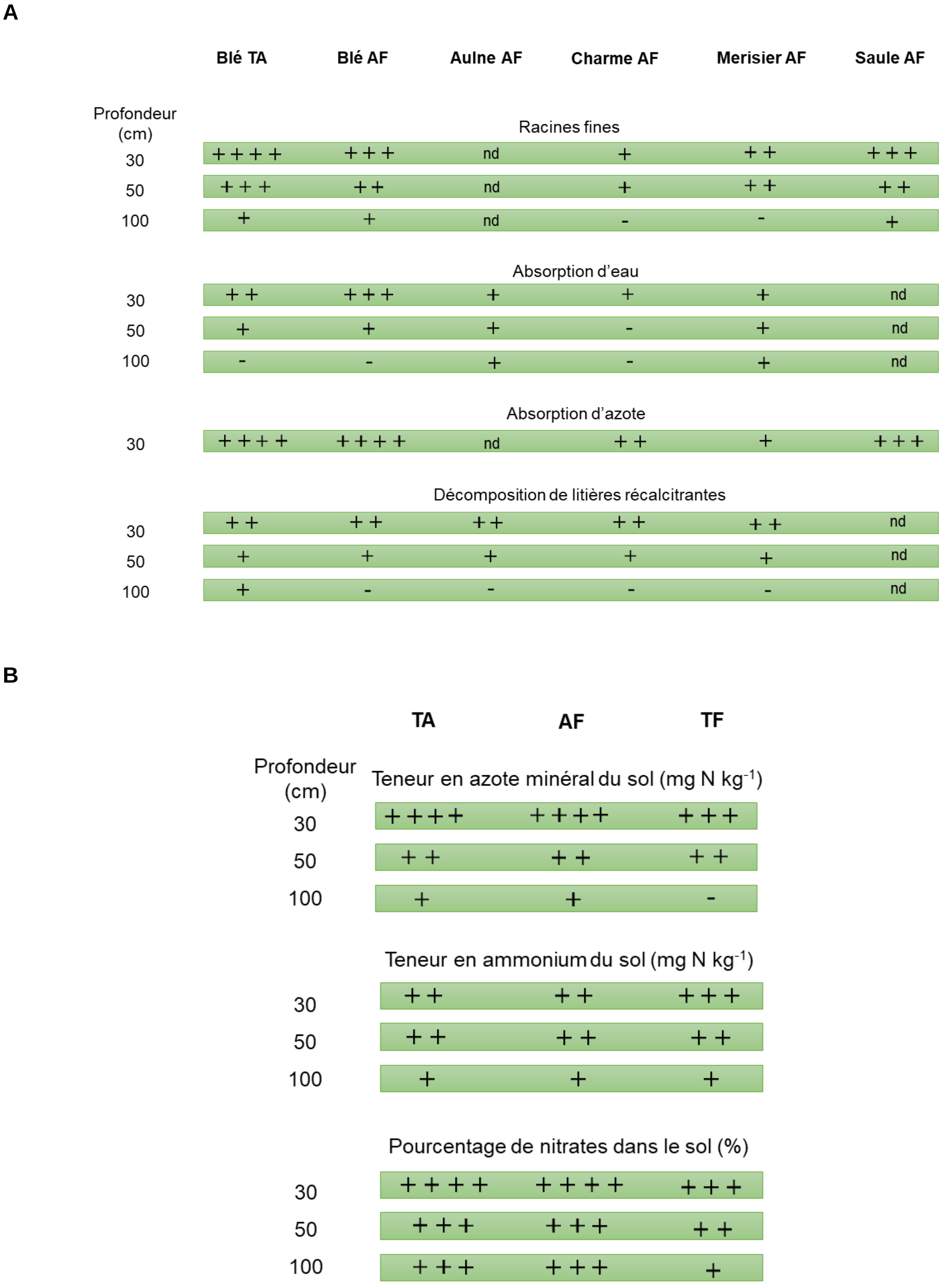
Nous avons montré sur le site expérimental en agroforesterie intraparcellaire à Ramecourt, que :

- Le blé en témoin agricole avait développé plus de racines fines jusque 70 cm de profondeur et a prélevé plus d'eau en surface par rapport au blé en système agroforestiers mais que leur quantité d'azote absorbé en surface était la même ; que la décomposition des matières organiques récalcitrantes était plus importante en profondeur dans les témoins agricoles qu'en systèmes agroforestiers (Figure 1A).

- Les racines fines du charme étaient encore peu développées et majoritairement situées en surface, expliquant son absorption d'eau et d'azote dans l'horizon supérieur (Figure 1A). Le développement des racines fine du merisier était entre celui du charme et du saule (Figure 1A), lui permettant d'adapter la profondeur d'absorption d'eau en fonction de sa disponibilité le long du profil de sol, de même que pour l'aulne (Figure 1A). Le saule avait développé d'importante racines fines, en particulier dans l'horizon supérieur le rendant compétitif pour l'absorption de l'azote en surface (Figure 1A).

- Les essences d'arbres n'ont pas eu d'effet sur la décomposition des matières organiques récalcitrantes (Figure 1A).

- Les teneurs en azote minérale du sol et le pourcentage de nitrates avaient diminué le long du profil du sol dans les témoins forestiers par rapport aux témoins agricoles et systèmes agroforestiers (Figure 1B), mais que la teneur en ammonium dans l'horizon supérieur des témoins forestiers était plus élevée par rapport aux témoins agricoles et systèmes agroforestiers (Figure 1B).



**A**

Racines fines

| Profondeur (cm) | Blé TA | Blé AF | Aulne AF | Charme AF | Merisier AF | Saule AF |
|---|---|---|---|---|---|---|
| 30 | ++++ | +++ | nd | + | ++ | +++ |
| 50 | +++ | ++ | nd | + | ++ | ++ |
| 100 | + | + | nd | - | - | + |

Absorption d’eau

| Profondeur (cm) | Blé TA | Blé AF | Aulne AF | Charme AF | Merisier AF | Saule AF |
|---|---|---|---|---|---|---|
| 30 | ++ | +++ | + | + | + | nd |
| 50 | + | + | + | - | + | nd |
| 100 | - | - | + | - | + | nd |

Absorption d’azote

| Profondeur (cm) | Blé TA | Blé AF | Aulne AF | Charme AF | Merisier AF | Saule AF |
|---|---|---|---|---|---|---|
| 30 | ++++ | ++++ | nd | ++ | + | +++ |

Décomposition de litières récalcitrantes

| Profondeur (cm) | Blé TA | Blé AF | Aulne AF | Charme AF | Merisier AF | Saule AF |
|---|---|---|---|---|---|---|
| 30 | ++ | ++ | ++ | ++ | ++ | nd |
| 50 | + | + | + | + | + | nd |
| 100 | + | - | - | - | - | nd |

**B**

Teneur en azote minéral du sol (mg N kg$^{-1}$)

| Profondeur (cm) | TA | AF | TF |
|---|---|---|---|
| 30 | ++++ | ++++ | +++ |
| 50 | ++ | ++ | ++ |
| 100 | + | + | - |

Teneur en ammonium du sol (mg N kg$^{-1}$)

| Profondeur (cm) | TA | AF | TF |
|---|---|---|---|
| 30 | ++ | ++ | +++ |
| 50 | ++ | ++ | ++ |
| 100 | + | + | + |

Pourcentage de nitrates dans le sol (%)

| Profondeur (cm) | TA | AF | TF |
|---|---|---|---|
| 30 | ++++ | ++++ | +++ |
| 50 | +++ | +++ | ++ |
| 100 | +++ | +++ | + |

**Figure 1** : Bilan du comportement des différentes espèces (A) et des teneurs en azote minéral (B) selon les systèmes de culture. Le signe − indique les valeurs très faibles ou nulles, + pour les valeurs faibles, ++ pour les valeurs moyennes, +++ pour les valeurs fortes et ++++ pour les valeurs très fortes ; nd signifie non déterminé. AF = agroforesterie intraparcellaire ; TA = témoin agricole et TF = témoin forestier.

## 5. Perspectives et questions subsistants à la suite à cette thèse

L'introduction des arbres dans les différentes parcelles étudiées a pu induire des changements de la population microbienne. Il a manqué dans cette étude une vision précise de la population microbienne fongique, bactérienne, des mycorhizes et des changements engendrés par les arbres sur ces paramètres. Il aurait été pertinent de savoir comment varient ces populations en fonction de la distance, de la profondeur, de l'âge, des essences, des pratiques agricoles et du temps. Un projet Interreg sur l'évolution des indicateurs biologiques du sol à travers des peuplements d'âges et de systèmes agroforestiers différents, a été soumis pour étudier ces questions.

Dans les chapitres 3 et 5, nous n'avons pas réussi à quantifier l'eau et l'azote réellement partagé par les plantes associées, nous avons simplement tracé le devenir de l'eau et des engrais marqués. Par ailleurs, il serait intéressant d'installer des barrières dans le sol à l'interface entre la ligne d'arbres et la culture afin de restreindre le développement des racines des arbres au niveau de la ligne et de comparer l'absorption d'eau et de nutriments avec une modalité sans barrière (Allen et al., 2004; Ong et al., 1991; Zamora et al., 2009). La quantification de biomasse des arbres et des cultures apporterait aussi des données supplémentaires de l'impact de la compétition en eau et/ou en azote sur la croissance des espèces associées. En continuant l'étude du partage des ressources entre les arbres et la culture sur le site expérimental d'agroforesterie intraparcellaire de Ramecourt, une nette compétition sera peut-être visible au cours des années à venir avec la croissance des arbres (Jose et al., 2000b, 2000a). Il serait également intéressant d'étudier la gestion et l'évolution de cette compétition par différentes techniques de gestion de la parcelle, notamment en fonction de l'élagage et du labour (Jones et al., 1998; Schroth and Zech, 1995).

Les sondes capacitives (TDT) installées sur le site de Ramecourt permettront d'avoir un suivi temporel des teneurs en eau du sol, il faudrait en parallèle quantifier le partage de l'eau entre les arbres et la culture. Un suivi des flux de sève pourrait être fait en remplacement ou pour compléter les analyses sur les feuilles d'arbres. Enfin, des analyses de la teneur en $^{2}$H du sol ainsi que des mesures de $^{18}$O compléteraient nos données et permettraient d'utiliser un modèle mixte Bayesian (MixSIAR, SIAR, IsoSource) afin de connaitre l'origine de l'eau absorbée par les différentes plantes (Huo et al., 2020; Parnell et al., 2013; Wang et al., 2023; Zhang et al., 2022).

De même que pour l’eau, il faudrait quantifier la compétition pour l’azote et connaitre les quantités exactes d’azote prélevées par les arbres et les cultures à différentes profondeurs. Un bilan complet des pertes de l’azote devrait être fait en évaluant ses différents flux. Un suivi dans le temps du $^{15}$N du sol permettrait de voir si les arbres ont un impact sur la diffusion de l’azote en profondeur. Un projet de thèse a notamment été déposé à la région Hauts-de-France sur l’étude du partage de l’azote au sein de peuplements agroforestiers d’âges différents.

Dans le chapitre 6, le travail peut être compléter par la mesure de l’effet des arbres sur la santé biologique des sols et ses indicateurs en mesurant l’activité enzymatique, la respiration microbienne, la biomasse du microbiote du sol, les activités de minéralisation de l’azote et le quotient métabolique (Allen et al., 2011; Clivot et al., 2020; Udawatta et al., 2008). Il faudrait aussi quantifier les populations rhizosphériques ainsi que leur diversité et leurs fonctions. Afin de suivre les changements se produisant dans les modalités agroforestières, il serait pertinent de mesurer régulièrement ces paramètres et de vérifier cette diversification microbienne le long d’une chronoséquence. En effet, des prélèvements dans la ligne d’arbres et à 2 m de la ligne ont été réalisés ainsi que des analyses métagénomiques identifiant les phylum et genres des populations bactériennes et fongiques présentes. Les résultats ont montré que les arbres ont influencé l’abondance relative de certains phylums bactériens et fongiques, mais la diversité et les biomasses bactériennes et fongique n’ont pas été affectées par la présence des arbres (Willemetz, 2022). Une réitération de cette étude de la population microbienne sur plusieurs années permettrait de suivre son évolution et d’identifier les différentes fonctions biologiques mises en place au sein de la parcelle au long du développement des arbres. Enfin, une simulation de la production d’ammonium en laboratoire permettrait de vérifier l’effet de réduction de la lixiviation de l’azote par les arbres. Un projet de thèse a été déposé à la région Hauts-de-France en cotutelle avec Gembloux Agro-Bio Tech pour étudier la santé biologique des sols en système agroforestier en évaluant la taille, la diversité, les fonctions et la variation spatio-temporelle de la population microbienne.

# Conclusion

L'objectif principal de cette thèse était de caractériser la distribution spatiale et temporelle des racines fines, d'explorer le partage de l'eau et de l'azote et d'examiner l'activité microbienne globale lors de la phase d'installation des arbres dans les terres cultivées en système agroforestier intraparcellaire.

Nous avons montré à travers l'étude de dix-sept peuplements en agroforesterie intraparcellaire âgés entre 3 et 12 ans, qu'à partir de 5 ans, les arbres développaient des racines fines verticales profondes et que leur croissance se poursuivait horizontalement sous la zone d'enracinement des cultures. Ce changement de stratégie de la croissance racinaire a été favorisé par le travail du sol et la durée de rotation des cultures. L'évolution du développement des racines fines des arbres au cours du temps a induit un assèchement des horizons supérieurs proches de la ligne pour les jeunes peuplements et plus profonds pour les peuplements plus âgés pendant la période estivale. Ce qui permet alors d'augmenter le volume de sol capable de stocker les eaux de pluie automnales et hivernales, de contribuer à une meilleure utilisation de l'eau située dans les horizons profonds et de limiter le drainage de l'eau hors de la zone d'exploration racinaires. L'activité des racines a modifié la dynamique de l'azote minéral en favorisant le processus d'ammonification pouvant réduire la lixiviation des nitrates causée par les excès d'engrais azotés. Nous avons également observé une augmentation significative de la teneur en carbone organique du sol dans l'horizon supérieur au niveau des lignes d'arbres seulement pour les peuplements âgés de 10-12 ans, grâce en partie à l'abondance des racines fines des arbres mais également aux différents résidus provenant de la végétation spontanée poussant sur la ligne ainsi qu'aux litières provenant de la chute des feuilles des arbres.

Sur le site expérimental en agroforesterie intraparcellaire de Ramecourt, où un focus a été mis sur un peuplement très jeune âgé de 4 ans, nous avons confirmé la croissance en surface des racines fines des arbres qui cohabitent avec la racine du blé à 2 m de la ligne. Les jeunes arbres favorisent l'enracinement superficiel du blé alors que certaine espèce comme le merisier ont déjà développé des racines profondes. Les arbres forestiers du même âge qui n'ont pas reçu de fertilisation ont cependant colonisé tout le profil du sol jusqu'à 1 m de profondeur, certainement à la recherche de nutriment disponible. Nous avons observé un début de compétition pour l'eau et l'azote entre la culture et les arbres âgés de 4 ans. La présence des jeunes arbres a induit une absorption d'eau du blé davantage en surface, ainsi qu'une compétition pour l'azote en surface. Cependant, à ce stade de développement, la quantité d'eau absorbée par la culture n'a pas été impactée par les jeunes arbres. Les arbres et les cultures prélèvent leur eau principalement dans les horizons de surface mais certaines espèces ayant développé un système racinaire profond ont été capables de décaler leur prélèvement d'eau en profondeur lorsque l'horizon supérieur était moins humide. En ce qui concerne l'azote, les arbres contribuent à valoriser le dernier apport d'engrais tard dans la saison culturale du blé, mais n'affectent pas à ce stade son absorption en azote, certainement due à la faible quantité prélevée par les arbres. Cependant, par comparaison aux blés en monoculture, ceux associés avec les arbres ont plus valorisé ce dernier apport d'azote dans les grains. Certaines espèces ayant un enracinement superficiel comme le charme, contribuent à absorber l'excès d'azote non valorisé par le blé après la récolte au même titre que les cultures intermédiaires pièges à nitrates et remobilisent cet azote au moment du débourrement au prochain cycle végétatif.

Nous avons mis en évidence que les jeunes arbres ralentissaient la décomposition de matières organiques récalcitrantes en profondeur, probablement dû à une diminution de l'azote minéral disponible et à l'apport limité de composés carbonés labiles pouvant favoriser leur décomposition. Ce qui signifie que par leur présence, les arbres malgré leur jeune âge modifient l'activité microbienne globale du sol et le cycle global du carbone et de l'azote à différentes profondeurs. Ces résultats confirment également la capacité des systèmes agroforestiers à limiter la décomposition de matières organiques fraiches récalcitrantes et à favoriser leur séquestration en profondeur. Une augmentation de la formation d'ammonium dans les témoins forestiers a également été observée reflétant probablement une diversification des communautés microbiennes.

Nous n'avons pas pu mesurer exactement la quantité d'eau et d'azote absorbés par les arbres et la culture pour quantifier plus précisément la compétition pour ces ressources entre les espèces associées car nous n'avons pas mesuré leur biomasse respective mais ce sera certainement la prochaine étape, d'autant plus que les arbres deviennent de plus en plus grands et que leurs besoins augmentent. L'évaluation de la croissance racinaire dans les zones d'incubation des sachets de thé aurait permis de mieux déterminer la relation entre les racines, la décomposition de la matière organique et l'activité microbienne. Enfin, des mesures plus pointues sur la taille, la biomasse, la diversité et l'activité microbienne nous auraient permis d'explorer l'effet de ces jeunes arbres sur le changement des communautés microbiennes assez tôt dans la phase d'installation des arbres dans les parcelles, ainsi que leurs fonctions écologiques et les services qu'elles apportent.

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