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Remi Cardinael

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Par

Rémi Cardinael

Stockage de carbone et dynamique des matières organiques des sols en agroforesterie sous climat méditerranéen et tempéré

Thèse présentée et soutenue à Montpellier, le 27 novembre 2015.

Composition du Jury :

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Résumé

Le carbone organique des sols (COS) joue un rôle majeur dans le maintien des propriétés des sols, il constitue un important réservoir de carbone à l'échelle planétaire et est très sensible au mode de gestion des terres. L'agroforesterie caractérise un mode d'occupation des sols qui associe des arbres et des cultures et/ou des animaux au sein d'une même parcelle. On parle respectivement d'agrosylviculture et de sylvopastoralisme. Les systèmes agroforestiers pourraient jouer un rôle dans l'atténuation mais aussi dans l'adaptation au changement climatique. Cette thèse avait pour objectifs de (1) quantifier le stockage et les formes de COS dans les sols de parcelles agroforestières, (2) expliquer le stockage de COS observé et (3) proposer un modèle de dynamique du COS à l'échelle pluriannuelle.

Nous avons mesuré le stockage de COS dans cinq parcelles agrosylvicoles et dans une parcelle sylvopastorale en France. Nous avons eu recours à une approche synchronique, c'est-à-dire à une comparaison entre une parcelle agroforestière et une parcelle agricole adjacentes. Les stocks de COS ont été quantifiés de 20 cm à 2 m de profondeur selon les sols étudiés, avec une prise en compte de la distance aux arbres. Sur le site du domaine expérimental de Restinclières (INRA) que nous avons particulièrement étudié, un stockage de COS a été observé jusqu'à 1 m de profondeur. Les taux de stockage de COS ont été estimés à 0.25 ± 0.03 t C ha^{-1} an^{-1} sur 0-30 cm et à 0.35 ± 0.04 t C ha^{-1} an^{-1} sur 0-100 cm. Sur le réseau de parcelles étudiées, ce taux de stockage est de 0.24 (0.09 – 0.46) t C ha^{-1} an^{-1} sur 0-30 cm. Dans l'inter-rang, aucun effet de la distance à l'arbre sur les stocks de COS n'a été mis en évidence. Les lignes d'arbres, occupées par une végétation herbacée, représentent les plus forts stocks de carbone.

Nous avons également cherché à expliquer les processus de stockage de COS en agroforesterie. Pour cela, nous avons quantifié toutes les entrées de matières organiques au sol. Les biomasses des racines fines ont été estimées à l'aide de fosses profondes, jusqu'à 4 m de profondeur. La mortalité des racines fines des arbres a été étudiée à l'aide de minirhizotrons installés à différentes profondeurs et distances des arbres. Nous avons estimé que le sol de la parcelle agroforestière recevait environ 40% de matière organique en plus que la parcelle agricole (3.80 t C ha^{-1} an^{-1} par rapport à 2.69 t C ha^{-1} an^{-1}) sur une profondeur de 2 m. Les racines fines des arbres et les racines des cultures représentent chacun environ 30% des apports de MO en agroforesterie. Un fractionnement granulo-densimétrique de la matière

organique a été utilisé pour identifier le COS additionnel. La majorité du carbone additionnel est fait de matières organiques particulaires (MOP) de la taille des sables (50-200 µm et 200-2000 µm), et est majoritairement localisé dans les horizons de surface. Les potentiels de minéralisation du COS des deux parcelles ont été mesurés par des incubations de sols issus de différentes profondeurs et par des incubations de sols ayant subi différents niveaux de déstructuration. Les taux de minéralisation du COS diminuent exponentiellement avec la profondeur du sol et de façon identique dans la parcelle agricole et dans l'inter-rang de l'agroforesterie. La destruction des macro-agrégats des sols incubés n'a pas entraîné de sur-minéralisation, indiquant que ces MOP ne sont pas protégées au sein de ces agrégats.

Afin de mieux comprendre la dynamique du COS en agroforesterie, nous avons développé un modèle de dynamique du carbone en deux dimensions, discrétisé en fonction de la profondeur et représentant différentes distances à l'arbre. Les sorties issues de la modélisation suggèrent que le stockage de carbone en agroforesterie est le résultat d'un apport accru de MO au sol plutôt que d'une diminution de la minéralisation du COS. Cette étude démontre l'intérêt et le potentiel des systèmes agroforestiers pour augmenter les stocks de COS des sols agricoles. Elle pose cependant la question de la durabilité de ce stockage.

Mots clés : agroforesterie, matière organique des sols, fractionnement granulo-densimétrique, stockage de carbone, carbone profond, biomasse racinaire, renouvellement des racines, modélisation, géostatistique

Abstract

Soil organic carbon (SOC) plays a major role in maintaining soil properties. Globally, it represents an important carbon pool that is particularly sensitive to the land use type. Agroforestry is a land use type where trees are associated with crops and/or animals within the same field. In this case, it is commonly termed silvoarable agroforestry and silvopastoralism, respectively. Agroforestry systems could play a role in climate change mitigation, but also adaptation. In this thesis, we aimed to (1) quantify SOC stocks and forms in agroforestry systems, (2) explain the additional storage and (3) develop a model simulating SOC dynamics in agroforestry systems.

We measured SOC storage in five silvoarable agroforestry systems and in one silvopastoral system. We used a synchronic approach, i.e., a comparison between an agroforestry plot and an adjacent agricultural control plot. SOC stocks were quantified from 20 cm to 2 m depth depending on the studied soil. Soil samples were taken up at different distances from the trees. At the Restinclières agroforestry experimental site – near Montpellier – which is monitored by the INRA since the tree planting in 1995, a significant SOC storage was observed down to 1 m depth. SOC accumulation rates were 0.25 ± 0.03 t C ha^{-1} yr^{-1} at 0-30 cm and 0.35 ± 0.04 t C ha^{-1} yr^{-1} at 0-100 cm. This rate reached 0.24 (0.09 – 0.46) t C ha^{-1} yr^{-1} at 0-30 cm for all the silvoarable plots. No effect of the distance to the trees on SOC stocks was observed within the inter-row. The highest SOC stocks were observed in the tree rows, which are occupied by a herbaceous vegetation.

We quantified all organic matter inputs to the soil. Fine root biomass was assessed using deep pits, down to 4 m soil depth. Tree fine root mortality was studied with minirhizotrons tubes installed at different depths and distances from the trees. We estimated that the agroforestry plot received about 40% more organic matter to the soil than the agricultural plot (3.80 t C ha^{-1} yr^{-1} compared to 2.69 t C ha^{-1} yr^{-1}) to 2 m depth. Tree fine roots and crop fine roots contributed equally to about 30% of organic inputs in the agroforestry. Particle size fractionation was used to identify the additional SOC and we found that most of additional SOC was made of particulate organic matter (POM) of the size of sands (50-200 µm and 200-2000 µm), and was mainly located in top soil layers. We measured SOC mineralisation potentials of the two plots by incubating soil samples from different depths and also with incubation of soils with different levels of disruption. SOC mineralisation rates decreased

exponentially with increasing soil depth, without any difference between the control plot and the inter-row. Macro-aggregate disruption did not increased SOC mineralisation, suggesting that POM were not physically occluded and protected within these aggregates.

We developed a model of carbon dynamic in two dimensions, vertically spatialized and taking into account different distances from the tree. Model outputs suggest that SOC storage in agroforestry systems is the result of higher organic inputs to the soil, more than a reduction of SOC mineralisation. This study demonstrates the interest and the potential of agroforestry systems in enhancing SOC stocks of agricultural soils. However, it raises concerns about the stability of this storage.

Keywords: agroforestry, soil organic matter, particle-size fractionation, carbon storage, deep soil carbon, root biomass, root turnover, modeling, geostatistics

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Introduction

Agroforesterie ou agroforesterieS

L'agroforesterie est un mode d'utilisation des terres associant des arbres et des cultures et/ou des animaux sur une même parcelle. Le terme « agroforesterie » englobe en fait une multitude de systèmes agroforestiers, chaque système étant adapté au contexte pédoclimatique local et aux objectifs de l'agriculteur (Nair 1985; Somarriba 1992; Nair 1993). Le centre mondial d'agroforesterie (ICRAF) propose cette définition : « L'agroforesterie est un terme général pour désigner des systèmes d'utilisation des terres et des pratiques où des plantes pérennes ligneuses sont délibérément intégrées avec des cultures et/ou des animaux sur une même unité de surface. L'intégration peut se faire en mélange dans l'espace ou dans une séquence temporelle. Il y a normalement à la fois des interactions écologiques et économiques entre les composants ligneux et non ligneux ». Différentes méthodes de classification des systèmes agroforestiers ont été proposées, la plupart basées sur la nature et l'arrangement des composantes du système (Fig. 0.1). D'autres méthodes de classification existent (Fig. 0.2), basées par exemple sur les interactions dans le temps et l'espace entre les composantes arborées et les cultures (Torquebiau 2000) ou encore sur les services écosystémiques rendus (Nair 1985).

L'agroforesterie est une pratique ancestrale, utilisée dans la plupart des pays du monde. Encore aujourd'hui, on estime que 1.2-1.5 milliards de personnes pratiquent l'agroforesterie sur leur ferme ou dans leur communauté, et dépendent des produits fournis par ces systèmes (Garrity 2004; Garrity et al. 2006; Zomer et al. 2009). En Europe, les systèmes agroforestiers représentaient également une composante importante des paysages agricoles (Eichhorn et al. 2006; Rigueiro-Rodríguez et al. 2006), mais l'intensification agricole, le remembrement et la mécanisation ont provoqué un arrachage massif des arbres et des haies. Aujourd'hui, le plus vaste système agroforestier d'Europe (plus de 5 millions d'ha) se trouve entre l'Espagne et le Portugal – la Dehesa - (Joffre et al. 1999). Il s'agit d'un système sylvo-pastoral associant des chênes et du bétail, principalement des porcs ibériques qui permettent la fabrication des jambons Pata Negra.

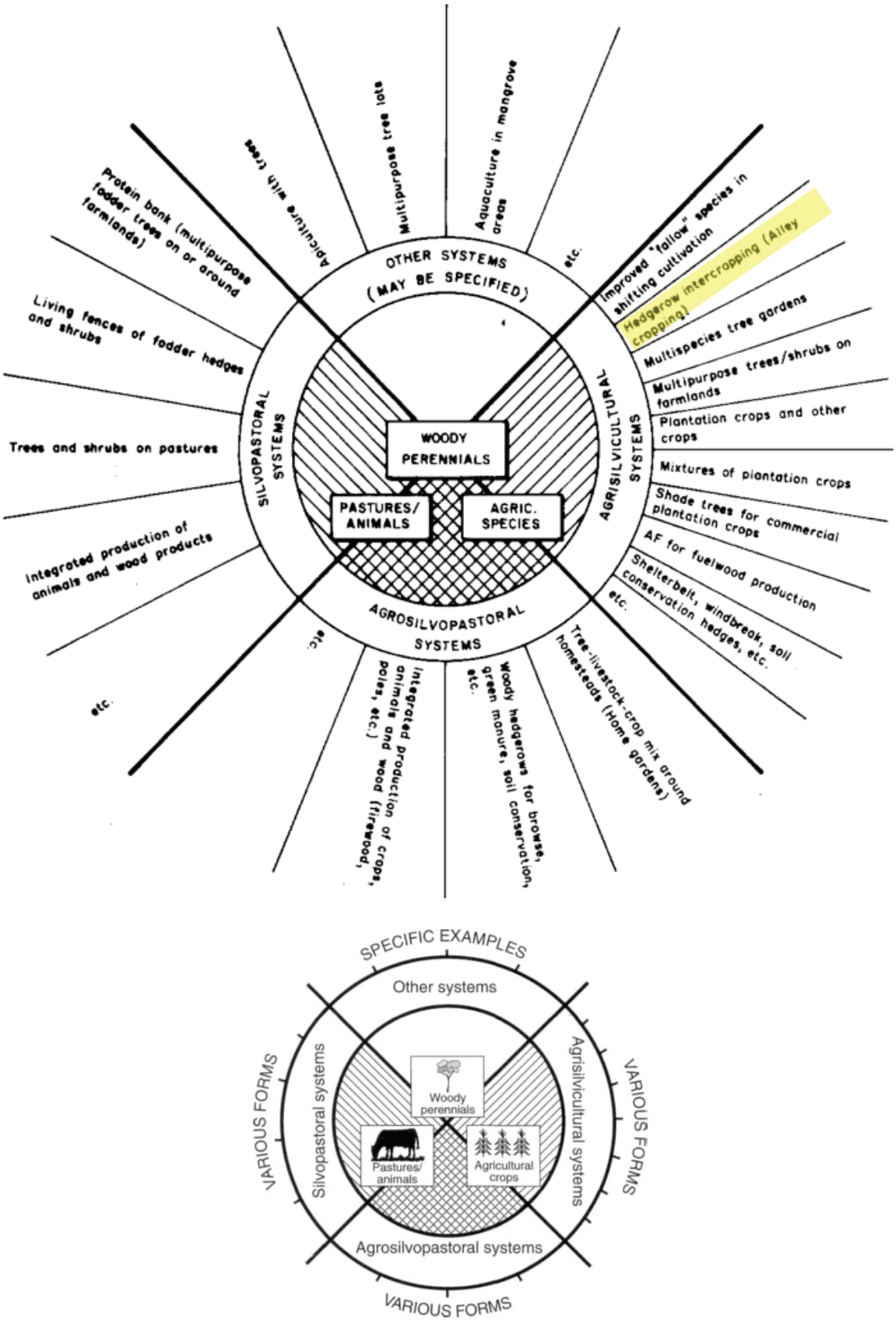

Figure 0.1. Classification des systèmes agroforestiers basée sur le type de composantes (issu de Nair 1985, et Nair 1993). En jaune : type de système agroforestier étudié dans cette thèse.

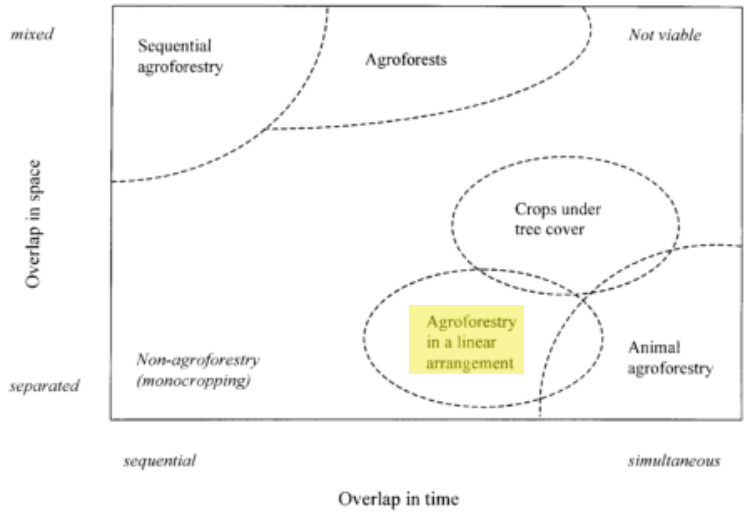

Figure 0.2. Classification des systèmes agroforestiers en fonction du chevauchement dans le temps et l'espace des arbres, cultures et animaux (issu de Torquebiau 2000). En jaune : type de système agroforestier étudié dans cette thèse.

En France, le système agroforestier le plus répandu est le pré-verger, surtout présent en Normandie, il associe des arbres fruitiers (pommiers, poiriers) et des prairies pâturées (Dupraz and Liagre 2008). Ces dernières années, on assiste cependant à un regain d'intérêt pour les systèmes agroforestiers dans tous les pays dits développés, pour des raisons environnementales et économiques (Kort et al. 2009).

Agroforesterie et services écosystémiques

Les services écosystémiques sont « les bénéfices que les humains retirent des écosystèmes sans avoir à agir pour les obtenir » (Millennium Ecosystem Assessment 2005). Ces services incluent des services d'auto-entretien (ou de support) comme la formation des sols, la photosynthèse, le cycle des nutriments, des services d'approvisionnement (oxygène, nourriture, eau, fibres…), des services de régulation qui affectent par exemple le climat, les inondations, les maladies, les déchets, et des services culturels qui procurent des bénéfices récréatifs, esthétiques, et spirituels (Fig. 0.3).

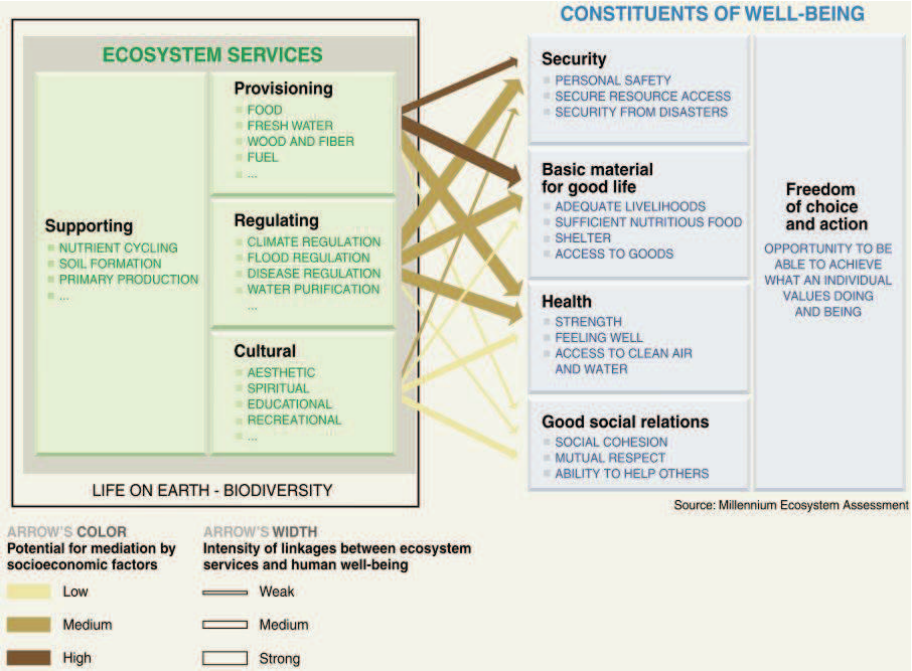

Figure 0.3. Liens entre services écosystémiques et composantes du bien-être de l'Homme (Millennium Ecosystem Assessment 2005).

De nombreux travaux montrent le rôle crucial de la biodiversité sur le fonctionnement des écosystèmes et sur les services qu'ils rendent (Hooper et al. 2005; Tilman et al. 2006; Balvanera et al. 2006). Comparativement aux parcelles agricoles classiques, les systèmes agroforestiers apportent une composante arborée supplémentaire (qui peut être un mélange d'essences), mais également tout un ensemble de plantes herbacées qui se développent au pied des arbres, sur les lignes d'arbres. Cette association avec des plantes pérennes induit généralement une augmentation de la biodiversité à l'échelle de la parcelle, notamment pour les insectes, oiseaux, arachnides et chauves-souris (Burgess 1999; Bhagwat et al. 2008). Plusieurs études ont montré une meilleure régulation des populations de ravageurs dans les systèmes agroforestiers grâce à la présence accrue d'ennemis naturels – araignées, hyménoptères, coccinelles (Dix et al. 1995; Stamps and Linit 1997; Schmidt and Tscharntke 2005) ou encore d'oiseaux insectivores et chauves-souris (Maas et al. 2013). De plus, cette biodiversité accrue peut également avoir un rôle sur la pollinisation des cultures, et ainsi impacter directement les rendements (Varah et al. 2013).

Les arbres agroforestiers peuvent également réduire le ruissellement et ainsi limiter l'érosion des sols (Udawatta et al. 2002; Udawatta et al. 2010), mais également capter une partie de l'azote lixivié par les cultures (Bergeron et al. 2011; Tully et al. 2012), grâce au « filet

racinaire » des arbres (van Noordwijk et al. 1996; Rowe et al. 1999) qui se développe sous les cultures, contribuant ainsi à préserver la qualité de l'eau.

Les systèmes agroforestiers sont souvent évoqués pour leur rôle dans l'adaptation et l'atténuation du changement climatique. En termes d'adaptation, les arbres peuvent procurer une protection aux cultures face aux fortes chaleurs, limitant les stress thermiques et l'échaudage, mais aussi une protection aux animaux (Nair 1993). Ils atténueraient l'effet de serre en stockant du carbone dans les arbres, mais aussi dans le sol (Verchot et al. 2007; Schoeneberger et al. 2012). **L'impact des systèmes agroforestiers sur le stockage de carbone organique dans le sol est l'objet de cette thèse**. Les différentes inconnues actuelles et les enjeux associés seront développés dans la suite de cette introduction.

Matières organiques et carbone organique des sols

« Les matières organiques des sols (MOS), dans leur acception la plus large, correspondent à l'ensemble des matériaux organiques, vivants et morts, présents dans le sol, ce qui comprend à la fois les organismes vivants et les résidus décomposés et non décomposés. La matière organique du sol est donc un continuum de matières plus ou moins complexes en perpétuel renouvellement » (Bernoux et al. 2011). Dans une définition un peu plus restreinte, les matières organiques sont le produit de la décomposition des plantes et des substances animales (Manlay et al. 2007). **La teneur en matière organique (MO) d'un sol résulte d'un équilibre entre les entrées de MO (résidus de cultures, racines…) et les pertes (décomposition, érosion, lessivage).** Elle dépend des conditions pédoclimatiques, de l'utilisation et de la gestion des sols.

Les matière organiques sont un élément essentiel de la fertilité des sols (Tiessen et al. 1994). Elles sont une source de nutriments pour les plantes et leur recyclage est un facteur clé de la productivité d'un écosystème. Elles affectent aussi la structure et la porosité du sol, ainsi que l'infiltration de l'eau et la réserve utile du sol. Elles sont source de nourriture pour tout un ensemble d'organismes du sol qui jouent un rôle majeur dans le fonctionnement biologique du sol (Bardgett and van der Putten 2014).

La matière organique du sol est composée en moyenne de 58% de carbone organique (Stevenson 1994). Ce dernier étant plus facilement déterminable au laboratoire, c'est cette

variable que l'on utilise couramment pour quantifier les MO du sol. Cette variable présente également l'avantage de relier facilement carbone du sol et dioxyde de carbone (CO_2) atmosphérique (1 t C = 3.67 t CO_2). Mais il faut garder à l'esprit que **lorsque l'on parle de carbone organique du sol, on parle en fait de matières organiques du sol**.

Rôle des sols dans le bilan de carbone global

Les stocks de carbone organique du sol représentent environ 1560 milliards de tonnes (Gt C) sur une profondeur de 1 m, et environ 2300 Gt C sur une profondeur de 3 m (Jobbagy and Jackson 2000; Houghton 2007). Cela correspond à 2 ou 3 fois le pool de carbone contenu dans l'atmosphère (environ 800 Gt C) et à 4-5 fois le pool de carbone contenu dans la végétation (environ 600 Gt C). Au niveau français, les stocks de carbone du sol sont estimés à 3.1 – 3.3 Gt C sur les 30 premiers centimètres du sol (Arrouays et al. 2001; Martin et al. 2011).

Les émissions de gaz à effet de serre (GES) provenant de l'utilisation de carbone fossile représentent 8.9 Gt C an^{-1} (Le Quéré et al. 2014). A l'échelle globale, les émissions de GES représentent donc environ 4‰ du stock de carbone organique. Ainsi, une faible variation des stocks de carbone du sol peut avoir un impact majeur sur les émissions de GES et l'atténuation du changement climatique. Actuellement, les écosystèmes terrestres (sols + végétation) compensent un peu plus de 30% des émissions de GES anthropiques (Fig. 0.4).

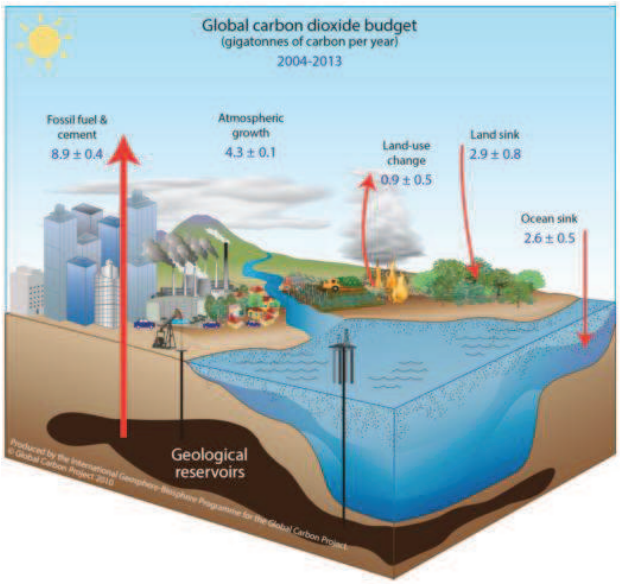

Figure 0.4. Bilan de carbone global pour les années 2004-2013 (issu de Le Quéré et al. 2014).

Stockage de carbone dans les sols – définition et évaluation

Il est **nécessaire de distinguer la séquestration de carbone dans les sols du stockage de carbone dans les sols**. Ces notions sont couramment confondues, y compris dans la littérature scientifique où l'expression « soil carbon sequestration » remplace souvent « soil carbon storage » (Krna and Rapson 2014). Le processus du stockage de carbone dans les sols peut être défini comme le prélèvement de CO_2 de l'atmosphère et son stockage sous une forme organique dans le compartiment sol (Bernoux et al. 2005).

La séquestration de carbone est une notion plus globale, qui intègre toutes les émissions de GES à l'échelle d'une parcelle ou d'un territoire. En effet, le stockage de carbone ne s'intéresse qu'aux flux de CO_2, mais un bilan positif peut être contrebalancé en partie par des émissions de méthane (CH_4) ou de protoxyde d'azote (N_2O). Or, ces gaz ont un pouvoir de réchauffement global respectivement 34 et 298 fois plus élevé que le CO_2 (IPCC 2013). Ainsi, Bernoux et al. (2005) proposent la définition suivante : « La séquestration du carbone dans le sol ou plutôt « séquestration du carbone dans le système sol-plante » pour un agro-écosystème donné, en comparaison avec un agro-écosystème de référence, et pour une période et une surface données, doit être considérée comme le résultat du bilan net, exprimé en équivalents $C\text{-}CO_2$ ou en équivalents CO_2, de tous les flux directs de GES à l'interface sol-plante-atmosphère, mais aussi de tous les flux indirects (combustibles, émissions animales, . ..). **Dans le cadre de cette thèse, nous nous sommes intéressés uniquement au processus de stockage de carbone dans les sols sous agroforesterie**.

Le stockage de carbone – ou déstockage – est toujours déterminé par rapport à un système de référence et à un temps donné. L'année t_0 utilisée correspond à l'année de mise en place de la pratique stockante ou déstockante. Deux approches sont couramment utilisées : l'approche diachronique, et l'approche synchronique (Bernoux et al. 2005; Olson et al. 2014b; Olson et al. 2014a). L'**approche diachronique** consiste à mesurer au cours du temps, **sur une même parcelle**, l'évolution des stocks de C dans le sol depuis un temps t_0 qui correspond à la mise en place du nouveau système. Cette approche est considérée comme la plus fiable (Costa Junior et al. 2013) car elle limite les biais liés à l'utilisation de parcelles différentes (texture, historique…), mais en pratique elle reste peu utilisée car il faut attendre de nombreuses années avant de pouvoir observer des différences de stocks de C (Smith 2004). L'**approche synchronique** consiste à comparer, **à un instant donné**, le stock de C d'une parcelle correspondant à la pratique stockante menée depuis plusieurs années à celui d'une parcelle de

référence (gestion conventionnelle). Il faut cependant s'assurer que la parcelle de référence n'ait pas subit de modifications majeures (érosion).

Si le stock de carbone organique d'un sol dépend du bilan entre les entrées et les sorties de MO, la quantité de C stockée et la durée de ce stockage dépendent aussi de processus de stabilisation du carbone complexes.

Stockage de carbone dans les sols – processus de stabilisation

La composition chimique des matières organiques a longtemps été considérée comme le facteur clé contrôlant leur décomposition et leur stabilisation dans les sols. La teneur en lignine, molécule polyphénolique complexe et supposée résistante à la décomposition par les microorganismes, a souvent été utilisée comme un indicateur de la « **récalcitrance chimique** » des matières organiques à la décomposition (Rasse et al. 2006). Il est maintenant avéré que la récalcitrance ne contrôle que les premières phases de la décomposition, mais pas la stabilisation à l'échelle pluri-décennale (von Lützow et al. 2006). D'autres processus majeurs de stabilisation ont été mis en évidence. L'**adsorption** de la MO sur les minéraux faiblement cristallins, notamment sur les argiles, semble être un processus majeur protégeant la MO de la décomposition (Torn et al. 1997; Mikutta et al. 2006; Marschner et al. 2008; Schrumpf et al. 2013). Kleber et al. (2007) proposent une organisation en « pelures d'oignon » autour des minéraux, les molécules organiques étant directement en contact avec les minéraux étant plus stabilisés que les composés organiques qui se superposent sur ces premières couches. La **protection physique** au sein de macro et micro agrégats est également un processus impliqué dans cette stabilisation du carbone (Puget et al. 2000; Chenu and Plante 2006; Virto et al. 2010). Les agrégats limitent en effet l'accès à la MO pour les microorganismes, et limitent la diffusion de l'oxygène essentiel à la décomposition aérobie. L'accessibilité des microorganismes à la MO à décomposer est un facteur déterminant (Dungait et al. 2012). Enfin, la **complexation de métaux** comme le fer ou l'aluminium avec la MO peut également être un processus important de préservation de la MO vis-à-vis de la décomposition (Sollins et al. 1996; Fransson et al. 2004; Kleber et al. 2005). Les récents travaux menés dans ce domaine semblent montrer que les processus de stabilisation ne sont pas les mêmes dans les horizons superficiels et profonds, avec généralement un rôle plus important de l'agrégation en surface, alors que la majorité du carbone profond semble être

adsorbé sur des minéraux ou complexé à des métaux (Moni et al. 2010; Salomé et al. 2010; Rumpel et al. 2015). De plus, le carbone profond et ancien semble être protégé de la décomposition du fait d'un manque d'énergie disponible dans le milieu pour les microorganismes. En effet, l'apport de matière organique fraîche (racines, exsudats…) entraîne une minéralisation du carbone stabilisé, on parle de « priming effect » (Fontaine et al. 2004; Fontaine et al. 2007). Ces travaux récents montrent les limites des connaissances actuelles concernant les processus de stabilisation du C dans les sols et d'autres travaux sont nécessaires afin de d'identifier les facteurs clés de ces processus, qu'ils soient pédologiques (texture, pH, teneurs en métaux…), climatiques, qu'ils concernent la nature des MO, ou encore qu'ils soient de nature écologique et concernent les interactions entre les différents organismes du sol. Récemment, il a par exemple été démontré le rôle des mycorhizes dans la stabilité des agrégats du sol et dans le stockage de carbone (Rillig et al. 2002; Rillig and Mummey 2006; Clemmensen et al. 2013), mais peu de choses sont encore connues.

Etant donné le rôle prépondérant des particules fines (argiles et limons fins < 20 μm) dans la stabilisation du carbone des sols, Hassink (1997) a proposé d'utiliser cette caractéristique afin d'estimer le potentiel d'un sol à stocker du carbone. Pour cela il a établi une relation linéaire entre le pourcentage de particules fines d'un sol et leur teneur en carbone organique, pour des sols saturés en carbone (sous prairie ou forêt depuis longtemps). Ainsi, en comparant la **saturation** théorique d'un sol avec la saturation réelle, on peut avoir une idée du potentiel d'un sol à stocker du carbone organique (Angers et al. 2011; Wiesmeier et al. 2014). Mais cette approche présente des limites. Elle n'a été pour l'instant qu'appliquée sur les horizons de surface (0-20 cm), et il semble qu'elle doive être précisée en prenant par exemple en compte la surface spécifique des minéraux, et pas seulement leur abondance (Feng et al. 2011). De plus, des travaux récents semblent montrer qu'une faible proportion des surfaces de la taille des argiles contribue à la stabilisation du carbone (Vogel et al. 2014), où encore qu'il faut tenir compte de l'altération des minéraux dans le temps (Basile-Doelsch et al. 2015).

Stockage de carbone dans les sols – pratiques agronomiques

Le mode d'occupation des sols a un fort impact sur les stocks de COS. Ainsi, la conversion de forêts en parcelles cultivées entraîne généralement une forte diminution de stocks de COS, alors que ces stocks sont conservés ou même améliorés quand une prairie est installée (Murty

et al. 2002; Fujisaki et al. 2015). A l'inverse, la conversion de terres agricoles en prairies ou en forêts augmente fortement les stocks de carbone, mais cette augmentation se fait beaucoup plus lentement que la perte de COS (Conant et al. 2001).

Une même parcelle agricole peut présenter des stocks de carbone très différents selon la gestion qui en est faite. Ainsi, différentes pratiques agronomiques permettent d'améliorer les teneurs en MOS des sols agricoles, et ainsi d'augmenter les stocks de COS, en augmentant les entrées de MO au sol, ou/et en réduisant leur minéralisation dans le sol. Des rotations plus longues et plus complexes, avec notamment présence de légumineuses ont un impact positif sur le COS, et permettent d'accumuler du carbone à un taux de 0.2 t C ha^{-1} an^{-1} sur 0-21 cm (West and Post 2002; Jarecki and Lal 2003). Une restitution accrue des résidus de culture au sol (Jarecki and Lal 2003) ou des apports de fumiers, de compost ou de boues de station d'épuration sont également bénéfiques, avec des taux de stockage allant de 0.1 à 0.7 t C ha^{-1} an^{-1} sur les horizons de surface (Smith et al. 2000; Smith et al. 2005; Favoino and Hogg 2008; Diacono and Montemurro 2010). L'introduction de cultures intermédiaires, initialement destinées à piéger les nitrates excédentaires dans le sol en automne et donc limiter leur lixiviation, permet également d'augmenter les stocks de carbone du sol, à un taux moyen de 0.32 ± 0.08 t C ha^{-1} an^{-1} sur 0-22 cm (Poeplau and Don 2015). En effet, ces cultures ne sont pas récoltées, et sont restituées au sol avant le semis ou la plantation suivante. Les associations de culture au sein d'une même parcelle, notamment les associations céréales-légumineuses permettent d'accumuler du carbone dans le sol à un taux de 0.18 ± 0.09 t C ha^{-1} sur 0-20 cm (Cong et al. 2014). Enfin, certains auteurs suggèrent de sélectionner des cultures à enracinement profond afin de favoriser le stockage de carbone en profondeur, mais peu de données sont encore disponibles sur ce sujet (Smith et al. 2005; Kell 2011; Kell 2012).

De très nombreuses études ont porté sur le non labour ou le non travail du sol. Ces études semblaient montrer un effet très bénéfique de ces pratiques sur les stocks de COS (West and Post 2002; Metay et al. 2007). Les principales hypothèses étaient que le labour et le travail du sol entraînaient une déprotection de la MO (destruction des macro-agrégats du sol) et une meilleure oxygénation du sol, conduisant à une minéralisation accrue de la MO. Mais des études récentes ont montré que cet effet positif du non labour sur le stockage de carbone avait été surestimé, notamment à cause de la méthode de comparaison, souvent de type synchronique (Costa Junior et al. 2013), mais surtout à cause de prélèvements de sols trop superficiels masquant les effets en profondeur. En effet, le non labour entraîne une redistribution du carbone dans le profil de sol, avec une accumulation en surface, mais une

diminution relative en profondeur par rapport à un labour (Baker et al. 2007; Angers and Eriksen-Hamel 2008). Ainsi, les études plus récentes montrent des taux de stockage plus faibles que précédemment estimés – 0.23 t C ha^{-1} an^{-1} sur 0-30 cm (Virto et al. 2012), certaines études montrent même qu'il n'y a pas d'effet sur le stock total de COS (Luo et al. 2010; Dimassi et al. 2013).

Stockage de carbone dans les sols et agroforesterie

Les systèmes agroforestiers peuvent être très productifs, dans certains cas jusqu'à 30% plus productif qu'un assolement avec des parcelles agricoles d'un côté, et un reboisement de terres agricoles de l'autre (Graves et al. 2007; Dupraz et al. 2010). Cette performance est mesurée par la Surface Equivalente d'Assolement (SEA) ou Land Equivalent ration (LER) en anglais (Mead and Willey 1980) et est définie par la somme des rendements relatifs des cultures associées. Cette performance s'explique notamment par une meilleure utilisation des ressources du milieu. En effet, en milieu tempéré il n'y a en général qu'une culture par an, le sol reste donc improductif après la récolte. Un bon choix des espèces associées permet de produire de la biomasse tout au long de l'année. Ainsi, un système agroforestier associant une culture d'hiver (par exemple du blé) à un arbre à débourrement tardif (par exemple du noyer) permet d'utiliser l'eau, les nutriments et l'énergie lumineuse qui arrivent sur la parcelle tout au long de l'année. De plus, les arbres agroforestiers, plantés à faible densité, subissent moins de compétition pour la lumière qu'en forêt (Khan and Chaudhry 2007), et bénéficient des apports en fertilisants destinés aux cultures, ce qui explique leur croissance rapide (Balandier and Dupraz 1999; Chaudhry et al. 2003; Chifflot et al. 2006). En parallèle, une réduction du rendement des cultures peut être observée dans les systèmes à forte densité d'arbres (Yin and He 1997; Dufour et al. 2013). Une partie de cette biomasse produite dans le système agroforestier va retourner au sol sous forme de matière organique, c'est notamment le cas des feuilles et des racines fines des arbres, des résidus de culture, et parfois des résidus d'élagage (Jordan 2004; Peichl et al. 2006). Ces apports de MO pourraient contribuer à augmenter les stocks de carbone organique du sol.

Les arbres agroforestiers ont un enracinement très profond à cause de la compétition exercée par la culture intercalaire (Mulia and Dupraz 2006), et du carbone est ainsi apporté en profondeur par la mortalité et l'exsudation des racines. Or, le carbone issu des racines a un

temps de résidence plus important dans le sol que le carbone issu des parties aériennes, et constitue une part importante du COS (Balesdent and Balabane 1996; Rasse et al. 2005; Kätterer et al. 2011). La récalcitrance des racines fines à la décomposition n'explique que très peu cette différence, la protection physico-chimique par adsorption (Oades 1995; Chenu and Plante 2006) et la protection physique au sein de micropores et microagrégats du sol (Gale et al. 2000) semblent jouer un rôle important, ainsi que le manque d'oxygène. Comparativement à une parcelle agricole, les apports additionnels de C issus des racines des arbres pourraient être stockés à très grande profondeur dans le sol. Cependant, ils pourraient également accélérer la minéralisation du carbone ancien, par le « priming effect » (Fontaine et al. 2007).

La plupart des études portant sur l'impact des systèmes agroforestiers sur les stocks de COS ont été réalisés dans les pays tropicaux, car ces systèmes sont très répandus dans ces zones (Albrecht and Kandji 2003; Soto-Pinto et al. 2010; Somarriba et al. 2013). Etant donné la diversité des systèmes agroforestiers en termes d'espèces associées, de pratiques agronomiques, de gestion des résidus et tailles, le tout dans des contextes pédoclimatiques très différents, il est difficile de donner une estimation moyenne des taux de stockage de COS. Les estimations varient également selon l'âge des parcelles et le précédent cultural. La très grande majorité des articles scientifiques indiquent des stocks de COS plus important dans les systèmes agroforestiers que dans les systèmes agricoles. Les stocks de carbone dans la biomasse aérienne des arbres sont également extrêmement variables selon les sites, et peuvent varier de 10 à plus de 150 t C ha^{-1} (Nair et al. 2009). Des fourchettes pour les taux de stockage de COS sont proposées pour les systèmes tropicaux, avec un stockage de 100 à 200 t C ha^{-1} sur une période de 10 ans et sur 1 m de profondeur pour les systèmes agroforestiers à ombrage (cacao, café), ou encore un stockage de 30 à 120 t C ha^{-1} pour les systèmes agroforestiers avec cultures annuelles intercalaires sur 10 ans et 1 m de profondeur et par rapport à une parcelle agricole (Nair et al. 2009; Nair et al. 2010). Deux synthèses récentes (Stavi and Lal 2013; Lorenz and Lal 2014) résument les effets des systèmes agroforestiers sur le sol, en systèmes tropical et tempéré.

En milieu tempéré, les données sont beaucoup plus succinctes, proviennent en grande majorité d'Amérique du Nord et concernent des systèmes agroforestiers linéaires ou des haies brise-vent. Les taux de stockage de COS pour ces systèmes varient de 0.3 à 1.04 t C ha^{-1} an^{-1} sur 0-20 cm (Peichl et al. 2006; Oelbermann et al. 2006; Bambrick et al. 2010). Une récente expertise de l'INRA visant à évaluer les pratiques permettant de réduire les émissions de gaz à effet de serre de l'agriculture française, a estimé un taux moyen de stockage de COS de 0.3 t

C ha^{-1} an^{-1} (Pellerin et al. 2013). Une étude récente a cependant été menée en Grande-Bretagne sur un système agroforestier âgé de 19 ans. Les auteurs ont trouvé un taux de stockage de COS de 1 t C ha^{-1} an^{-1} sur 0-60 cm, mais pas de stockage additionnel par rapport à une parcelle agricole sur 0-150 cm (Upson and Burgess 2013). Les stocks de COS dans le témoin agricole étaient en effet supérieurs à ceux de la parcelle agroforestière en dessous de 60 cm. Les auteurs ont fait l'hypothèse qu'il s'agissait d'une hétérogénéité initiale, antérieure à l'implantation du dispositif. Quelques études ont également été réalisées sous climat méditerranéen (Muñoz et al. 2007; Howlett et al. 2011) ou sous climat aride (Takimoto et al. 2008), mais sans parcelles de référence pour pouvoir estimer un stockage additionnel de carbone.

Très peu d'études ont porté sur l'impact des systèmes agroforestiers sur les stocks de carbone organique profond du sol (Haile et al. 2010; Howlett et al. 2011; Upson and Burgess 2013). La majorité des études ont évalué cet effet sur les horizons de surface du sol, en général jusqu'à 50 cm de profondeur (Oelbermann et al. 2004; Sharrow and Ismail 2004; Peichl et al. 2006; Oelbermann and Voroney 2007; Bambrick et al. 2010). Ce manque de données concernant les horizons profonds du sol est principalement lié aux difficultés d'échantillonnage et au coût d'analyses des échantillons. Mais, des méthodes récentes, comme la spectrométrie dans le visible et le proche infrarouge ont été développées (Brown et al. 2006; Stevens et al. 2013). Elles permettent des estimations rapides et à bas coûts de la concentration en carbone organique du sol (Gras et al. 2014), et pourraient être utilisées pour augmenter le nombre d'échantillons prélevés en profondeur.

Dans les systèmes agroforestiers mécanisés associant des lignes d'arbres à des bandes de cultures intercalaires, de la végétation herbacée, spontanée ou semée, se développe sur ces lignes. Le sol des lignes d'arbres n'est de plus pas travaillé, ce qui pourrait favoriser le stockage de carbone dans le sol (Virto et al. 2012). De plus, les arbres affectent la distribution spatiale et en profondeur de la matière organique. Celle-ci n'est pas distribuée de façon homogène dans la parcelle, notamment en fonction de la distance aux arbres, ce qui peut se traduire par une hétérogénéité des stocks de COS (Bambrick et al. 2010). Certains auteurs ont mesuré des stocks de carbone organique du sol à 1 m de profondeur plus importants sous la canopée des arbres qu'à 5 m des arbres (Howlett et al. 2011), d'autres ont montré que cette répartition spatiale des stocks de COS pouvait varier avec l'âge des arbres (Bambrick et al. 2010). D'autres encore ont montré qu'il n'y avait pas d'effet distance à l'arbre, juste un effet ligne/inter-rang (Peichl et al. 2006; Upson and Burgess 2013).

De plus, on connaît très peu de choses concernant les formes du carbone additionnel entre une parcelle agroforestière et agricole. Ce carbone peut être dans des fractions de sol à turnover rapide, comme les matières organiques particulaires (qui sont des matières organiques en décomposition, de la taille des sables), ou associé à des argiles ou limons, et ainsi stabilisé dans le sol pour une période plus longue. Takimoto et al. (2008) et Howlett et al. (2011) ont montré que les fractions grossières du sol étaient enrichies en carbone, à différentes profondeurs. Mais Haile et al. (2010) ont montré que le carbone issu des arbres plantés dans un système sylvopastoral contribuait principalement aux fractions limons et argiles du sol à 1 m de profondeur.

Agroforesterie et modélisation de la dynamique du carbone du sol

Plusieurs modèles de dynamique du COS existent, les plus couramment utilisés étant le modèle CENTURY (Parton et al. 1987) et le modèle RothC (Jenkinson 1990). Ces modèles représentent différents compartiments du COS en fonction de leur stabilité (pools labiles, passifs,…), l'évolution de chacun de ces pools étant représentée par des cinétiques de premier ordre. D'autres modèles ont également été développés, comme le modèle MOMOS (Sallih and Pansu 1993; Pansu et al. 2004; Pansu et al. 2010), le modèle SN-SIM (Petersen et al. 2005), le modèle Yasso (Liski et al. 2005), ou encore le modèle DAISY (Hansen et al. 1991). Tous ces modèles simulent une couche de sol superficielle, et ne permettent pas de modéliser la dynamique du carbone sur l'ensemble d'un profil de sol. Ainsi, Oelbermann and Voroney (2011) ont testé le modèle Century sur des systèmes agroforestiers au Costa Rica et au Canada en utilisant une couche de sol de 20 cm de profondeur, et avec des entrées de MO au sol dépendantes de la distance à la ligne d'arbres. Le modèle CO2FIX développé pour les écosystèmes forestiers puis agroforestiers (systèmes agroforestiers à base de plantes pérennes uniquement, typiquement café/cacao sous arbres d'ombrage) n'est pas non plus discrétisé en fonction de la profondeur (Masera et al. 2003). Cependant, son module sol étant basé sur le modèle Yasso, la profondeur maximale du sol simulée est ajustable, mais les horizons ne sont pas différenciés (Negash and Kanninen 2015).

Les stocks de carbone profonds représentent des quantités de carbone importantes (Jobbagy and Jackson 2000) et peuvent se renouveler partiellement à l'échelle décennale (Baisden and Parfitt 2007; Koarashi et al. 2012). Ces dernières années, une prise de conscience de

l'importance de ce sujet de la part de la communauté des modélisateurs a entraîné le développement de différents modèles discrétisés en fonction de la profondeur (Braakhekke et al. 2011; Braakhekke et al. 2013; Guenet et al. 2013; Koven et al. 2013; Taghizadeh-Toosi et al. 2014). Cependant, à notre connaissance, aucun de ces modèles n'a été testé sur les systèmes agroforestiers, qui nécessitent également de prendre en compte un effet distance à l'arbre sur les entrées de carbone au sol.

Problématique, hypothèses générales et objectifs

Les questions posées dans cette thèse sont nombreuses et variées :

Les systèmes agroforestiers permettent-ils de stocker du carbone dans les sols ? Si oui, à quelle profondeur, et jusqu'à quelle distance des arbres ? A quel taux ? Qu'est-ce qui explique ce stockage de carbone additionnel : des apports de MO plus importants ou une minéralisation réduite ? Sous quelle forme est ce carbone additionnel, est-il stabilisé ?

Nos hypothèses principales sont les suivantes :

- Les systèmes agroforestiers permettent d'augmenter les stocks de COS, en surface et en profondeur, mais avec une distribution spatiale hétérogène de ces stocks, les stocks les plus importants se trouvant près des lignes d'arbres

- les entrées de matière organique au sol sont plus importantes dans un système agroforestier que dans un système agricole, et expliquent le stockage de carbone

- une partie de ce carbone additionnel est stabilisé par adsorption sur des argiles, notamment en profondeur.

Les objectifs de la thèse sont les suivants:

1- Quantifier le stockage de C additionnel dans le sol en système agroforestier, en prenant en compte le profil de sol dans son ensemble et la variabilité latérale potentielle de ces systèmes
2- Expliquer les variations observées de stocks par :
    a. une quantification des entrées de C au sol, avec un focus particulier sur les entrées racinaires au sol
    b. une évaluation des sorties par minéralisation
3- Evaluer les formes du stockage de carbone additionnel, et ainsi estimer sa stabilité sur le long terme
4- Proposer un modèle de dynamique de carbone du sol adapté aux systèmes agroforestiers

On se propose donc dans ce travail de contribuer significativement à la connaissance sur les possibilités de stockage de C dans les sols en agroforesterie, par une quantification des stocks de C dans des parcelles agroforestières et par une analyse des mécanismes sous-jacents. Cette analyse permettra une meilleure compréhension de la dynamique du C dans les systèmes agroforestiers. Les enjeux scientifiques sont multiples: contribuer à améliorer les connaissances sur les potentialités de stockage de carbone de ces systèmes, comprendre comment et pourquoi ces systèmes sont intéressants en quantifiant les entrées de carbone dans le sol, mais également étudier les processus de stabilisation du carbone qui ont lieu dans les horizons profonds du sol.

Démarche générale et sites d'étude

Dans le cadre de cette thèse, nous nous sommes intéressés aux systèmes agroforestiers dits « en arrangements linéaires » ou agroforesterie linéaire. Il s'agit d'une **forme d'agrosylviculture associant des alignements d'arbres avec des cultures intercalaires**. Ce sont les systèmes agroforestiers actuellement les plus plantés en zones méditerranéenne et tempérée. La littérature anglo-saxonne fait référence à ces systèmes par les termes « silvoarable agroforestry », « alley cropping » ou encore « tree-based intercropping systems » (Fig. 0.1). Cette forme d'agroforesterie est parfois qualifiée – à tort –

d' « agroforesterie moderne » pour signifier qu'elle est compatible avec la mécanisation des cultures. Le terme « agroforesterie mécanisée » semble plus adapté. Dans cette thèse, nous utiliserons simplement le terme « agroforesterie » pour faire référence à notre sujet d'étude.

Pour répondre à nos objectifs, nous avons principalement utilisé des **essais en plein champ**, notamment un **essai d'agroforesterie de longue durée** mis en place en 1995 à Prades-le-Lez, à 15 km au Nord de Montpellier. C'est un dispositif expérimental exceptionnel et unique en Europe, où des études sont menées par l'INRA depuis la plantation des arbres. La température moyenne est de 15.4°C et la pluviométrie moyenne de 873 mm. Le sol est un Fluvisol (IUSS Working Group WRB 2007) profond et carbonaté, avec des teneurs en argiles et limons augmentant avec la profondeur. Le site est constitué d'une parcelle agroforestière, d'un témoin agricole, et d'un témoin forestier (Fig. 0.5). Les arbres sont plantés en lignes orientées Est-Ouest, et ont une densité actuelle de 110 arbres ha^{-1}. Les lignes ont une largeur de 2 m, sont espacées de 13 m, et sont occupées par de la végétation spontanée et herbacée. Les inter-rangs sont cultivés par du blé dur, tout comme le témoin agricole, et le sol est labouré à 20 cm avant semis.

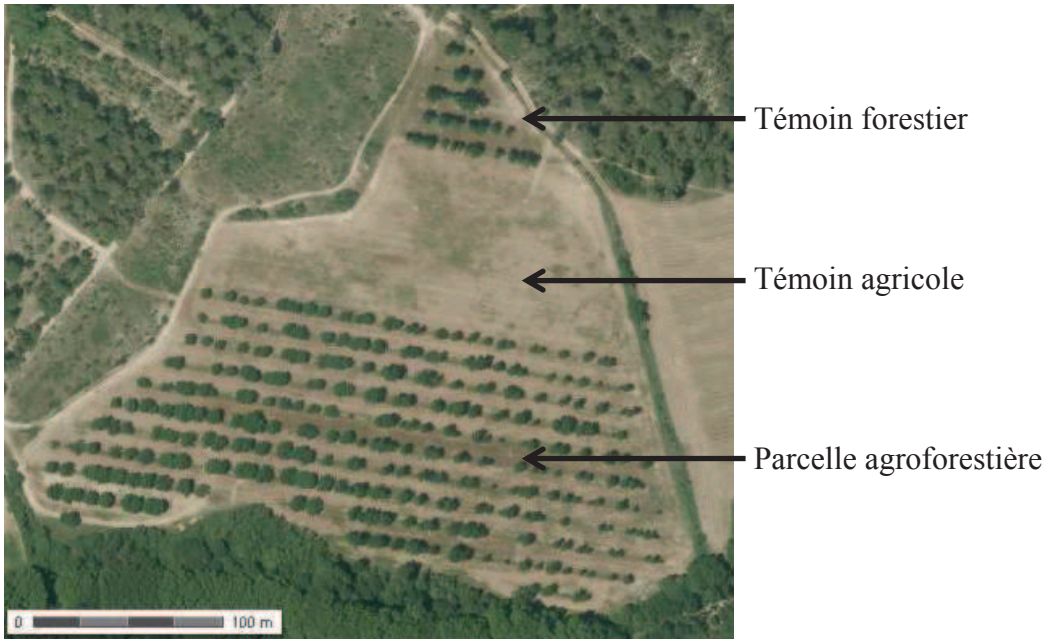

Figure 0.5. Vue aérienne de la parcelle expérimentale de Prades-le-Lez (photo géoportail).

Pour quantifier le stockage de carbone additionnel en agroforesterie, nous avons utilisé une approche de type synchronique. Les sites expérimentaux en agroforesterie tempérée et méditerranéenne sont rares, et leur mise en place a été réalisée dans un contexte où les thématiques « carbone » et « matières organiques » n'étaient pas les thématiques prioritaires. Il s'agissait avant tout d'étudier l'impact de ces systèmes sur les rendements agricoles et la

rentabilité économique du système. Les questions environnementales sont arrivées plus tard. Si bien que sur les sites expérimentaux à disposition, l'état initial (t_0) des stocks de COS avant la plantation des arbres n'est en général pas connu. En revanche, ces systèmes ont souvent été mis en place sur seulement une partie de la parcelle agricole existante. C'est le cas sur le domaine expérimental de Prades-le-Lez, ainsi que chez les agriculteurs visités pendant cette thèse. Ainsi, une partie a été conservée en parcelle agricole, qui sert maintenant de parcelle de référence ou parcelle témoin. Cela permet d'avoir un historique identique avant la mise en place du système agroforestier, et de limiter les différences de texture même si cette propriété du sol peut être variable à faible distance.

Dans le cadre du projet AGRIPSOL (AGroforesteRIe pour la Protection des SOLs) financé par l'ADEME, une campagne de carottage de sol a été réalisée dans la parcelle agricole et agroforestière en 2013, afin de quantifier les stocks de carbone du sol jusqu'à 2 m de profondeur. Des outils innovant comme la spectrométrie dans le visible et proche et infrarouge, et les géostatistiques ont été utilisés. Afin d'essayer de généraliser les résultats obtenus sur ce site de Prades-le-Lez concernant les stocks de carbone du sol en agroforesterie, 5 autres parcelles agroforestières ont été étudiées en France: 1 dans le Puy-de-Dôme, 1 en Eure-et-Loir, 1 dans les Deux-Sèvres, 1 en Charente-Maritime, et 1 dans le Gard. Ces parcelles, présentant aussi un témoin agricole, ont la particularité d'appartenir à des agriculteurs (sauf le site dans le Puy-de-Dôme, géré par l'INRA de Clermont-Ferrand). Sur chacun de ces sites, des quantifications des stocks de COS ont été réalisées, de 20 à 60 cm de profondeur selon les sites, ainsi que des analyses de texture et des mesures de biomasses aériennes des arbres.

Le site expérimental d'agroforesterie de Prades-le-Lez a été utilisé dans le cadre du projet ECOSFIX (Ecosystem Services of Roots - Hydraulic Redistribution, Carbon Sequestration and Soil Fixation, ANR-2010-STRA-003-01), où différentes fosses mises en place en 2012 dans la parcelle agroforestière, agricole et forestière ont permis de mesurer les biomasses des racines fines des arbres et des cultures. Des minirhizotrons installés jusqu'à 4 m de profondeur ont permis d'étudier le renouvellement des racines des arbres. Embauché en tant qu'ingénieur d'étude dans le cadre du projet ECOSFIX, j'ai été responsable de ce site, et j'ai contribué à la mise en place de ces fosses, assuré le suivi des dispositifs durant la durée du projet. Ces différents dispositifs mis en place juste avant le début de la thèse ont été repris et valorisé durant cette thèse. Ils ont permis la quantification des entrées de carbone au sol par voie racinaire. Les estimations des entrées de carbone dans le sol réalisées pendant la thèse

(végétation herbacée sur la ligne, racines des arbres et des cultures…) ont été complétées par des mesures réalisées par l'UMR System depuis la plantation (évolution des rendements, croissance des arbres…).

Afin d'étudier les formes du carbone additionnel, du fractionnement granulo-densimétrique de la matière organique a été réalisé au laboratoire. Cela a été fait sur des carottes prélevées sur le site de Prades-le-Lez, sur la ligne d'arbre, l'inter-rang et le témoin agricole, et à 4 profondeurs différentes. Concernant les potentiels de minéralisation du carbone du sol, des incubations de sols ont été réalisées, à 4 profondeurs, et avec différents niveaux de destruction de la structure du sol. La part du CO_2 provenant du carbone organique et inorganique a été différenciée par des analyses isotopiques (^{13}C) du gaz émis lors des incubations.

Un modèle discrétisé en fonction de la profondeur a été développé en se basant sur le modèle proposé par Guenet et al. (2013). Le modèle en 2D prend en compte des entrées de carbone différentes selon la profondeur et la distance aux arbres. Nous avons calibré le modèle avec les entrées de MO et les stocks de COS mesurés sur le site de Prades-le-Lez. Ce modèle pourra par la suite être validé et testé sur d'autres sites.

Ces différentes parties sont organisées de la façon suivante dans la thèse (Fig. 0.6) :

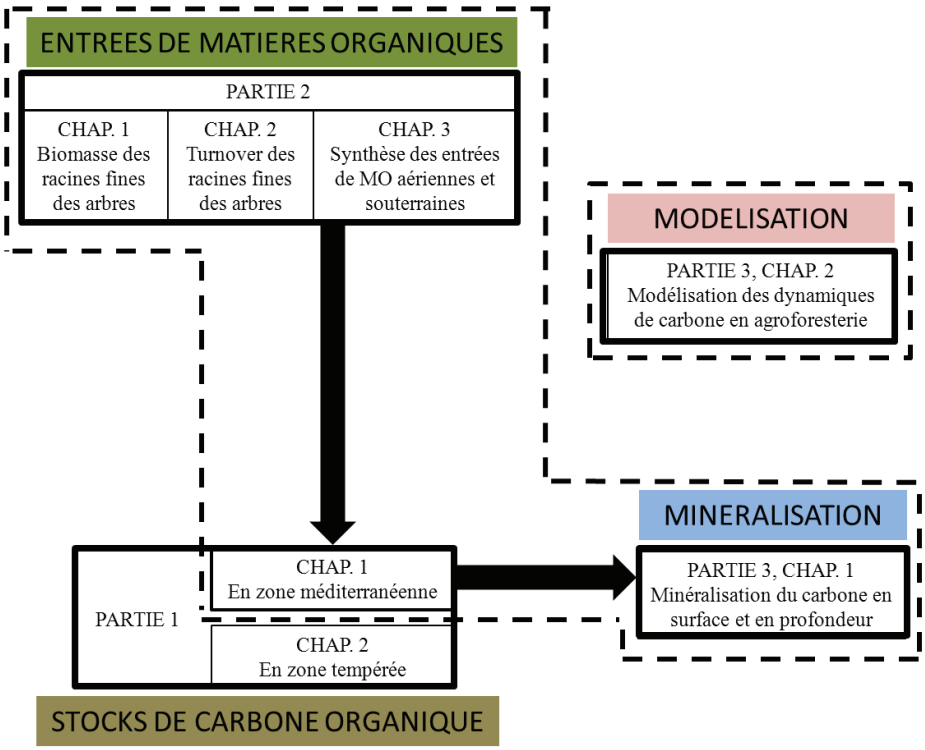

Figure 0.6. Organisation générale de la thèse.

Partie 1:

Stockage de carbone dans les sols sous agroforesterie en zone méditerranéenne et tempérée

Chapitre 1

Impact de l'agroforesterie sur les stocks, la forme et distribution spatiale du carbone organique du sol – un cas d'étude en contexte méditerranéen

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Impact of alley cropping agroforestry on stocks, forms and spatial distribution of soil organic carbon - A case study in a Mediterranean context

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Abstract

Agroforestry systems, i.e., agroecosystems combining trees with farming practices, are of particular interest as they combine the potential to increase biomass and soil carbon (C) storage whilst maintaining an agricultural production. However, most present knowledge on the impact of agroforestry systems on soil organic carbon (SOC) storage comes from tropical systems. This study was conducted in southern France, in an 18-year-old agroforestry plot, where hybrid walnuts (*Juglans regia* × *nigra* L.) are intercropped with durum wheat (*Triticum turgidum* L. subsp. *durum*), and in an adjacent agricultural control plot, where durum wheat is the sole crop. We quantified SOC stocks to 2.0 m depth and their spatial variability in relation to the distance to the trees and to the tree rows. The distribution of additional SOC storage in

different soil particle-size fractions was also characterised. SOC accumulation rates between the agroforestry and the agricultural plots were 248 ± 31 kg C ha^{-1} yr^{-1} for an equivalent soil mass (ESM) of 4000 Mg ha^{-1} (to 26-29 cm depth) and 350 ± 41 kg C ha^{-1} yr^{-1} for an ESM of 15700 Mg ha^{-1} (to 93-98 cm depth). SOC stocks were higher in the tree rows where herbaceous vegetation grew and where the soil was not tilled, but no effect of the distance to the trees (0 to 10 m) on SOC stocks was observed. Most of additional SOC storage was found in coarse organic fractions (50-200 and 200-2000 μm), which may be rather labile fractions. All together our study demonstrated the potential of alley cropping agroforestry systems under Mediterranean conditions to store SOC, and questioned the stability of this storage.

1.1 Introduction

Agroforestry systems are defined as agroecosystems associating trees with farming practices (Somarriba 1992; Torquebiau 2000). Several types of agroforestry systems can be distinguished depending on the different associations of trees, crops and animals (Torquebiau 2000). In temperate regions, an important part of recently established agroforestry systems are alley cropping systems, where parallel tree rows are planted in crop lands, and designed to allow mechanization of annual crops. Agroforestry systems are of particular interest as they combine the potential to provide a variety of non-marketed ecosystem services, defined as the benefits people obtain from ecosystems (Millennium Ecosystem Assessment 2005; Power 2010) whilst maintaining a high agricultural production (Clough et al. 2011). For instance, agroforestry systems can contribute to water quality improvement (Bergeron et al. 2011; Tully et al. 2012), biodiversity enhancement (Schroth et al. 2004; Varah et al. 2013), and erosion control (Young 1997). But agroforestry systems are also increasingly recognized as a useful tool to help mitigate global warming (Pandey 2002; Verchot et al. 2007; Stavi and Lal 2013). Trees associated to annual crops store the carbon (C) assimilated through photosynthesis into their aboveground and belowground biomass. The residence time of C in the harvested biomass will depend on the fate of woody products, and can reach many decades especially for timber wood (Profft et al. 2009; Bauhus et al. 2010). Agroforestry trees also produce organic matter (OM) inputs to the soil (Jordan 2004; Peichl et al. 2006), and could thus enhance soil organic carbon (SOC) stocks. Leaf litter and pruning residues are left on the soil, whereas OM originating from root mortality and root exudates can be incorporated much deeper into the soil as agroforestry trees may have a very deep rooting to minimize the competition with the annual crop (Mulia and Dupraz 2006; Cardinael et al. 2015c). Moreover,

several studies showed that root-derived C was preferentially stabilized in soil compared to above ground derived C (Balesdent and Balabane 1996; Rasse et al. 2005), mainly due to physical protection of root hairs within soil aggregates (Gale et al. 2000), to chemical recalcitrance of root components (Bird and Torn 2006), or to adsorption of root exudates or decomposition products on clay particles (Oades 1995). Compared to an agricultural field, additional inputs of C from tree roots could therefore be stored deep into the soil, but could also enhance decomposition of SOM, i.e., due to the priming effect (Fontaine et al. 2007).

Although it is generally assumed that agroforestry system have the potential to increase SOC stocks (Lorenz and Lal 2014), quantitative estimates are scarce, especially for temperate (Peichl et al. 2006; Nair et al. 2010; Pellerin et al. 2013; Upson and Burgess 2013) or Mediterranean (Howlett et al. 2011) agroforestry systems combining crops and tree rows. Most studies concern tropical regions where agroforestry is a more widespread agricultural practice (Albrecht and Kandji 2003; Somarriba et al. 2013).

Moreover, as pointed out by Nair (2012), very few studies assessed the impact of agroforestry trees deep in the soil (Haile et al. 2010; Howlett et al. 2011; Upson and Burgess 2013). Most of them considered SOC at depths above 0.5 m (Oelbermann et al. 2004; Sharrow and Ismail 2004; Peichl et al. 2006; Oelbermann and Voroney 2007; Bambrick et al. 2010). This lack of knowledge concerning deep soil is mainly due to difficulties to attain profound soil depths, and to the cost of analyzing soil samples from several soil layers. Recently, new methods such as visible and near infrared reflectance (VNIR) spectroscopy have been developed (Brown et al. 2006; Stevens et al. 2013). They allow time- and cost-effective determination of SOC concentration, in the laboratory but also in the field (Gras et al. 2014). Additionally to the lack of data for deep soil, reference plots were not always available, preventing from estimating the additional storage of SOC due specifically to agroforestry (Howlett et al. 2011).

In alley cropping systems, spaces between trees in tree rows are usually covered by natural or sowed herbaceous vegetation, and the soil under tree rows is usually not tilled, which may favor SOC storage in soil (Virto et al. 2012). Moreover, while trees strongly affects the depth and spatial distribution of OM inputs to soils (Rhoades 1997), distribution of SOC stocks close and away from trees was seldom considered. Some authors reported higher SOC stocks under the tree canopy than 5 m from the tree to 1 m soil depth (Howlett et al. 2011), others found that spatial distribution of SOC stocks could vary with the age of the trees (Bambrick et

al. 2010). Some authors reported that spatial distribution of SOC stocks to 20 cm depth was not explained by the distance to the trees but by the design of the agroforestry system, tree rows having higher SOC stocks than inter-rows whatever the distance to the trees (Peichl et al. 2006; Upson and Burgess 2013). To our knowledge, geostatistical methods (Webster and Oliver 2007) have never been used to describe the spatial distribution of SOC stocks in alley cropping agroforestry system although they have been recognized to be very powerful to map and understand spatial heterogeneity at the plot scale (Philippot et al. 2009) especially when dealing with more diverse and heterogeneous systems.

In addition, it is not known whether additional SOC (compared to an agricultural field) due to the presence of trees and tree rows, corresponds to soil fractions with a rapid turnover, such as particulate organic matter (POM), or to clay and silt associated OM, likely to be stabilized in soil for a longer period of time (Balesdent et al., 1998). Takimoto et al. (2008) and Howlett et al. (2011) found that carbon content of coarse organic fractions was increased at different depths under agroforestry systems. But, Haile et al. (2010) found that trees grown in a silvopastoral system contributed to most of the SOC associated to the fine silt + clay fractions to 1 m depth. The potential of a soil for SOC storage in a stable form is limited by the amount of fine particles (clay + fine silt) and can estimated by the difference between the theoretical SOC saturation (Hassink 1997) and the measured SOC saturation value for the fine fraction (Angers et al. 2011; Wiesmeier et al. 2014).

In this study, we aimed to assess the effect of introducing rows of timber trees into arable land on SOC storage. For this i) we quantified SOC stocks to a depth of 2.0 m in an agroforestry plot and in an adjacent agricultural control plot, ii) we assessed the spatial distribution of SOC stocks in a geostatistical framework taking into account the distance to the trees and to the tree rows, iii) we studied the distribution of SOC in different soil particle-size fractions.

We hypothesized that SOC stocks would be higher in the agroforestry plot compared to the control plot, also at depth, and that SOC stocks would decrease with increasing distance to the trees at all depths. Moreover, our hypothesis was that additional SOC in the agroforestry plot compared to the control plot would enrich all particle-size fractions.

1.2 Materials and methods

1.2.1 Site description

The experimental site was located in Prades-le-Lez, 15 km North of Montpellier, France (Longitude 04°01' E, Latitude 43°43' N, elevation 54 m a.s.l.). The climate is sub-humid Mediterranean with an average temperature of 15.4°C and an average annual rainfall of 873 mm (years 1995–2013). The soil is a silty and carbonated deep alluvial Fluvisol (IUSS Working Group WRB 2007). From 1950 to 1960, the site was a vineyard (*Vitis vinifera* L.), and from 1960 to 1985 the field was occupied by an apple (*Malus* Mill.) orchard. The apple tree stumps were removed in 1985. Then, durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) was cultivated. In February 1995, a 4.6 hectare agroforestry alley-cropping plot was established after the soil was ploughed to 20 cm depth, with the planting of hybrid walnuts (*Juglans regia* × *nigra* cv. NG23) at 13 × 4 m spacing, with East–West tree rows (Fig. I-1.1). The remaining part of the plot (1.4 ha) was kept as a control agricultural plot. The walnut trees were planted at an initial density of 200 trees ha^{-1}. They were thinned in 2004 down to 110 trees ha^{-1}. In the tree rows, the soil was not tilled and spontaneous herbaceous vegetation grew. The cultivated inter-row was 11 m wide. Since the tree planting, the agroforestry inter-row and the control plot were managed in the same way. The annual crop was most of the time durum wheat, except in 1998, 2001 and 2006, when rapeseed (*Brassica napus* L.) was cultivated, and in 2010 and 2013, when pea (*Pisum sativum* L.) was cultivated. The durum wheat crop was fertilized as a conventional crop (120 kg N ha^{-1} yr^{-1}), and the soil was ploughed annually to 20 cm depth, before durum wheat was sown.

Figure I-1.1. Hybrid walnut-durum wheat agroforestry system. Left panel: in November 2013; Right panel: in June 2014.

1.2.2 Soil core sampling

The experimental site was not designed as traditional agronomical experiments with blocks and replicates, but with two large adjacent plots. First, soil texture was analyzed for 24 profiles down to 2 m soil depth, following a random sampling design within the two plots. In May 2013, a sub-plot of 625 m^2 was sampled in both plots, following an intensive sampling scheme (Fig. I-1.2). In the agroforestry plot, this sub-plot included two tree rows, two inter-rows and nine walnut trees. Walnut trees had a mean height of 11.21 ± 0.65 m, a mean height of merchantable timber of 4.49 ± 0.39 m and a mean diameter at breast height of 25.54 ± 1.36 cm. Soil cores (n=36) were sampled on a regular grid, every 5 m (Fig. I-1.2). Around each tree, a soil core was collected at 1 m, 2 m and 3 m distance from the tree (n=57), in the tree row and perpendicular to the tree row. Seven soil cores were sampled additionally in the middle of the inter-row to study short scale (1 m distance) spatial heterogeneity of SOC stocks far from the trees (Fig. I-1.2). The same sampling scheme was followed in the control plot without these seven additional soil cores. Thus, 100 soil cores were sampled in the agroforestry sub-plot (40 in tree rows, 60 in inter-rows) and 93 in the agricultural sub-plot (Fig. I-1.2). All cores were sampled down to 2 m depth using a motor-driven micro caterpillar driller (8.5-cm diameter and 1-m long soil probe). The soil probe was successively pushed two times into the soil, to get 0-1 m and 1-2 m cores at each sampling point. Each soil core was then cut into ten segments, corresponding to the following depth increments: 0-10, 10-30, 30-50, 50-70, 70-100, 100-120, 120-140, 140-160, 160-180, and 180-200 cm.

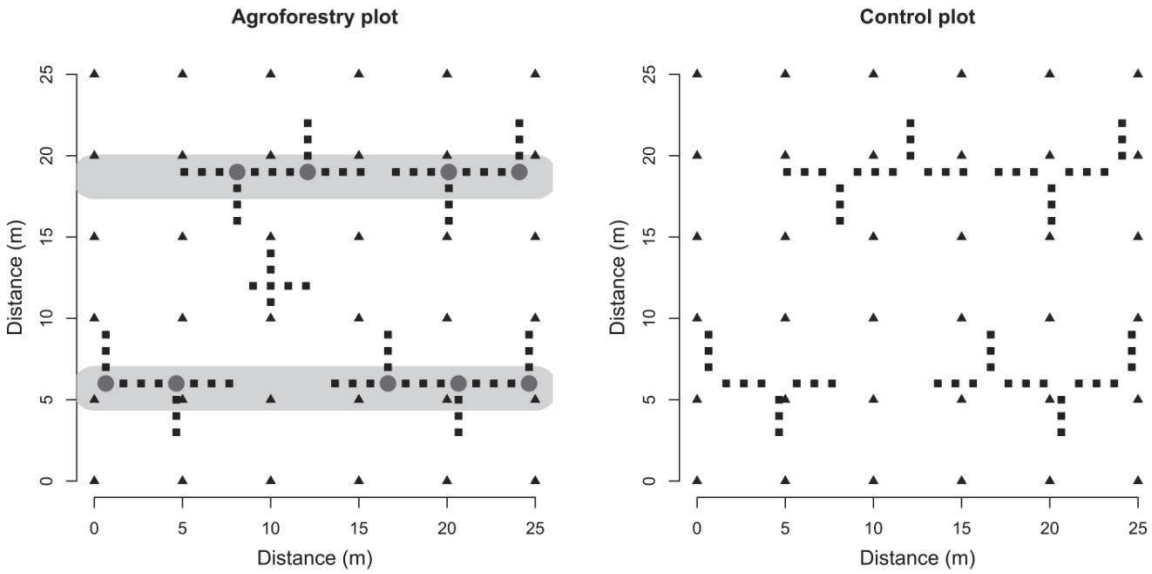

Figure I-1.2. Description of the intensive sampling scheme in the agroforestry and in the control sub-plots. Circles represent hybrid walnuts, the grey strips represent the tree rows, triangles are for soil cores on the regular grid (every 5 m), squares are for soil cores on transects (every 1 m).

1.2.3 Use of field visible and near infrared spectroscopy to predict SOC

As core surface had been smoothed by the soil probe, each segment was refreshed with a knife before being scanned, in order to provide a plane but un-smoothed surface. Then, four VNIR spectra (from 350 to 2500 nm at 1 nm increment) were acquired in the field at different places of each segment, using a portable spectrophotometer ASD LabSpec 2500 (Analytical Spectral Devices, Boulder, CO, USA), and were then averaged. Reflectance spectra were recorded as absorbance, which is the logarithm of the inverse of reflectance. The whole spectrum population was composed of 1908 mean spectra (i.e. 193 cores with 10 segments per core but a few samples were lost due to mechanical problems). In topsoil (0-30 cm), the soil was dry and crumbled whereas in deeper soil horizons, it was moister and had higher cohesion. Thus, two different predictive models were built: one for topsoil samples, the other for subsoil (30-200 cm) samples. The "topsoil model" for predicting SOC was built using the 116 most representative topsoil samples, out of 380 samples, and the "subsoil model", using the 142 most representative subsoil samples, out of 1488 samples. The procedure to select the most representative samples is presented below. The 0-10 cm soil layer from the tree rows (40 samples) was not used for the topsoil model as it contained abundant plant debris (roots, leaves, etc.) and a PCA revealed that these VNIR spectra were different from the whole spectra population. SOC concentration of these samples was therefore determined with a CHN elemental analyzer, and, thus, not predicted by VNIR. The SOC concentration of the 258 samples selected for building the VNIR prediction models was also analyzed using a CHN elemental analyzer.

1.2.4 VNIR spectra analysis and construction of predictive models

VNIR spectra analysis was conducted on topsoil and subsoil samples separately, using the WinISI 4 software (Foss NIRSystems/ Tecator Infrasoft International, LLC, Silver Spring, MD, USA) and R software version 3.1.1 (R Development Core Team 2013). The most representative samples, from a spectral viewpoint, were selected using the Kennard-Stone algorithm, which is based on distance calculation between sample spectra in the principal component space (Kennard and Stone 1969). For the topsoil model, the calibration subset included 104 samples (90%) selected as the most representative spectrally, and the validation subset 12 samples (10%). For the subsoil model, the calibration subset included 128 samples (90%), and the validation subset 14 samples. Fitting the spectra to the SOC concentrations

determined with a CHN elemental analyzer was performed using partial least squares regression (PLSR; Martens and Naes, 1989). We tested common spectrum preprocessing techniques including first and second derivatives, de-trending, standard normal variate transformation and multiplicative scatter correction, but the best models were obtained when no pre-treatment was applied on the spectra (data not shown). Then cross-validation was performed within the calibration subset, using groups that were randomly selected (10 groups), in order to build the model used for making predictions on the samples not analyzed in the laboratory. No outlier was removed. The number of components (latent variables) that minimized the standard error of cross-validation (SECV) was retained for the PLSR. The performance of the models was assessed on the validation subsets using the coefficient of determination (R^2) and the standard error of prediction (SEP) between predicted and measured values, and also the ratio of standard deviation to SEP, denoted RPD, and the RPIQ, which is the ratio of performance to IQ (interquartile distance), i.e. IQ/SEP = (Q3 – Q1)/SEP, where Q1 is the 25th percentile and Q3 is the 75th percentile (Bellon-Maurel et al. 2010). Then all sub-set samples (i.e., calibration and validation samples) were used to build models that were applied on the samples not analyzed in the laboratory. The performance of these models was also assessed according to R^2, SECV, RPD and RPIQ.

Subsoil models performed better than topsoil models (Table I-1.1, Fig. I-1.S1). In external validation, RPD was higher than 2 for the subsoil, which has been considered a threshold for accurate NIRS prediction of soil properties in the laboratory (Chang et al. 2001). This RPD threshold was not achieved for the topsoil model, but SOC concentrations were predicted for less than 60% of topsoil samples, the rest was directly analyzed with a CHN elemental analyzer. It is worth noting that cross-validation on the whole set (for making prediction on the samples not analyzed in the lab) yielded better results than external validation (on 10% of analyzed samples) in the subsoil, but the opposite was observed in the topsoil.

Topsoil														
External validation on 10% samples after calibration using 90% samples								Prediction model using 100% samples (10-group cross-validation)						
N	Mean mg g^{-1}	SD mg g^{-1}	SEP mg g^{-1}	Bias mg g^{-1}	R^2	RPD	RPIQ	N	Mean mg g^{-1}	SD mg g^{-1}	SECV mg g^{-1}	R^2	RPD	RPIQ
12	9.71	2.09	1.04	-0.59	0.78	1.75	2.60	116	9.18	1.99	1.20	0.63	1.66	4.35

Subsoil														
External validation on 10% samples after calibration using 90% samples								Prediction model using 100% samples (10-group cross-validation)						
N	Mean mg g^{-1}	SD mg g^{-1}	SEP mg g^{-1}	Bias mg g^{-1}	R^2	RPD	RPIQ	N	Mean mg g^{-1}	SD mg g^{-1}	SECV mg g^{-1}	R^2	RPD	RPIQ
14	6.19	1.80	0.83	0.01	0.74	2.03	3.03	142	6.06	1.86	0.77	0.83	2.40	4.85

Table I-1.1. External validation and prediction model results for soil organic carbon. N: numbers of samples; SD: standard deviation (mean and standard deviation of the conventional determinations); R^2: coefficient of determination; RPD is the ratio of performance to deviation, i.e. the ratio of SD to SEP or SECV. RPIQ is the ratio of performance to IQ (interquartile distance), i.e. IQ/SEP (or SECV) = (Q3 - Q1)/SEP (or SECV).

1.2.5 Bulk densities determination

Each segment was weighed in the field to determine its humid mass. Following this step, each segment was crumbled and homogenized, and a representative sub-sample of about 300 g was sampled. Sub-samples were sieved at 2 mm to separate coarse fragments such as stones and living roots. Coarse fragments represented less than 1% of each soil mass and were considered as negligible. Moisture contents were determined for 23 soil cores (i.e. 230 samples) after 48 h drying at 105°C, and were used to calculate the dry mass of all samples. Bulk density (BD) was determined for each sample by dividing the dry mass of soil by its volume in the soil corer tube.

1.2.6 Reference analysis measurements

After air drying, soil samples were oven dried at 40°C for 48 hours, sieved at 2 mm, and ball milled until they passed a 200 µm mesh sieve. Carbonates were removed by acid fumigation, following Harris et al., (2001). For this, 30 mg of soil was placed in open Ag-foil capsules. The capsules were then placed in the wells of a microtiter plate and 50 µL of demineralized water was added in each capsule. The microtiter plate was then placed in a vacuum desiccator with a beaker filled with 100 mL of concentrated HCl (37%). The samples were exposed to HCl vapors for 8 hours, and were then dried at 60°C for 48 hours. Capsules were then closed in a bigger tin capsule. Decarbonated samples were analyzed for organic carbon concentration with a CHN elemental analyzer (Carlo Erba NA 2000, Milan, Italy). Isotopic measurements were performed on a few samples to check that decarbonation was well performed ($\delta^{13}C$ OM = -25 ‰).

1.2.7 Soil organic carbon stock calculation

In most studies comparing SOC stocks between treatments or over time periods, SOC stocks have been quantified to a fixed depth as the product of soil bulk density, depth and SOC concentration. However, if soil bulk density differs between the treatments being compared, the fixed-depth method has been shown to introduce errors (Ellert et al. 2002). A more accurate method is to use an equivalent soil mass (ESM) (Ellert and Bettany 1995). We defined a reference soil mass profile that was used as the basis for comparison, based on the lowest soil mass observed at each sampling depth and location. For this reference, soil mass

layers (0-1000, 1000-4000, 4000-7300, 7300-10700, 10700-15700, 15700-18700, 18700-21900, 21900-25100, 25100-28300, 28300-31500 Mg ha^{-1}) corresponded roughly to soil depth layers (0–10, 10–30, 30–50, 50-70, 70-100, 100-120, 120-140, 140-160, 160-180, 180-200 cm, respectively). For the different treatments (control, tree row, inter-row), SOC stocks were calculated on this basis, soil mass was the same, whereas depth layer varied. The effect of the ESM correction can be seen in Table I-1.S1. SOC stocks in the agroforestry plot were calculated with tree rows representing 16% of the plot surface area and inter-rows 84%:

$$\text{SOC stock}_{Agroforestry} = 0.16 \times \text{SOC stock}_{Tree\ row} + 0.84 \times \text{SOC stock}_{Inter\ row} \quad (1)$$

We defined delta SOC stock as the difference between SOC stock in the agroforestry plot and in the control plot:

$$\Delta\ \text{SOC stock} = \text{SOC stock}_{Agroforestry} - \text{SOC stock}_{Control} \quad (2)$$

All SOC stocks were expressed in Mg C ha^{-1}. SOC accumulation rates (kg C ha^{-1} yr^{-1}) were calculated by dividing delta stocks by the number of years since the tree planting (18 years):

$$\text{SOC accumulation rate} = \frac{\Delta\ \text{SOC stock}}{18} \times 1000 \quad (3)$$

1.2.8 Particle-size fractionation

Particle-size fractionation was performed for five soil cores from the inter-rows, five from the tree rows and six from the control plot, and at four depths: 0-10, 10-30, 70-100 and 160-180 cm. Thus, 64 soil samples were fractionated, as described in Balesdent et al. (1998) and Gavinelli et al. (1995). Briefly, 20 g of 2-mm sieved samples were soaked overnight at 4°C in 300 mL of deionized water, with 10 mL of sodium metaphosphate (HMP, 50 g L^{-1}). Samples were then shaken 2 h with 10 glass balls in a rotary shaker, at 43 rpm. The soil suspension was wet-sieved through 200-µm and 50-µm sieves, successively. The fractions remaining on the sieves were density-separated into organic fractions, floating in water, and remaining mineral fractions. The 0-50 µm suspension was ultrasonicated during 10 min with a probe-type ultrasound generating unit (Fisher Bioblock Scientific, Illkirch, France) having a power output of 600 watts and working in 0.7:0.3 operating/interruption intervals. This 0-50 µm suspension was then sieved through a 20-µm sieve. The resulting 0-20 µm suspension was transferred to l-L glass cylinders, which were then shaken by hand and 50 mL of the

suspension were withdrawn immediately after. They constituted an aliquot of the entire 0-20 µm fraction. After a settling time of 8 h approximately, a second aliquot of 50 mL was removed by siphoning the upper 10 cm of the suspension left after the first sampling. This represented an aliquot of the 0-2 µm fraction. A third aliquot was also collected in the upper 10 cm, and centrifuged two times 35 min, at 4000 rpm. This aliquot was then filtered at 2 µm to get the hydrosoluble fraction. Fractions were then dried at 40°C, finely ground, decarbonated and analyzed with a CHN elemental analyzer. A binocular microscope was used to check if separation of coarse mineral fractions and of light organic coarse fractions (200-2000 and 50-200 µm) was well done. Organic carbon contents of coarse mineral fractions were then assumed to be 0 mg C g^{-1}. A sub-sample of each of the 64 selected samples was used to perform a classical textural analysis after destruction of organic matter. These texture analyses were used to evaluate the quality of the dispersion for soil particle size fractionation.

1.2.9 Calculation of SOC saturation

The theoretical value of SOC saturation was calculated according to the equation proposed by (Hassink 1997):

$$SOC_{sat-pot} = 4.09 + 0.37 \times \text{particles} < 20\ \mu\text{m} \quad (4)$$

where $SOC_{sat-pot}$ is the potential SOC saturation (mg C g^{-1}) and where particles < 20 µm represents the proportion of fine soil particles <20 µm (%).

To calculate the SOC saturation deficit (Angers et al. 2011; Wiesmeier et al. 2014), the estimated current SOC concentrations of the fine fraction were subtracted from the potential SOC saturation:

$$SOC_{sat-def} = SOC_{sat-pot} - SOC_{cur} \quad (5)$$

where $SOC_{sat-def}$ is the SOC saturation deficit (mg C g^{-1}) and SOC_{cur} is the current mean SOC concentration of the fine fraction <20 µm (mg C g^{-1}). The total amount of the SOC storage potential ($SOC_{stor-pot}$, Mg C ha^{-1}) was calculated multiplying $SOC_{sat-def}$ by soil bulk density and soil layer thickness.

These calculations were performed for the four depths where particle-size fractionation was done (0-10, 10-30, 70-100 and 160-180 cm).

1.2.10 Statistical analyses

The observed variability in a soil property Z such as SOC concentration results from complex processes operating over various spatial scales. A simple but useful statistical model for Z at a set of observations that could be spatially located, $\boldsymbol{s}_i = \boldsymbol{s}_1, \boldsymbol{s}_2, \cdots, \boldsymbol{s}_n$ is

$$Z(\boldsymbol{s}_i) = \mu(\boldsymbol{s}_i) + \varepsilon(\boldsymbol{s}_i) \quad (6)$$

where $\mu(\boldsymbol{s}_i)$ is a deterministic component and $\varepsilon(\boldsymbol{s}_i)$ is a correlated random component that can include a pure noise random one. A soil property can be correlated with other environmental variables such as, in this work, the distance to the closest tree. This can be represented in Equation 6 by assuming that $\mu(\boldsymbol{s}_i)$ comprises an additive combination of one or more fixed effect:

$$\mu(\boldsymbol{s}_i) = \beta_0 + \sum_{j=1}^{q} \beta_j x_j(\boldsymbol{s}_i) \quad (7)$$

where $x_j (j = 1,2,\cdots,q)$ are q auxiliary variables and $\beta_0, \ldots, \beta_q$ are the associated fixed effects. This model is referred as a Mixed Effects Model which offers a flexible framework by which to model the sources of variation and correlation that arise from grouped data (Pinheiro and Bates 2000; Lark et al. 2006). In this work, we fitted two different linear mixed models (LMM).

We first fitted a LMM using the whole set of the bulk densities, SOC concentrations, and SOC stocks observations at the different depths. We used the *nlme* package (Pinheiro et al. 2013). Soil core ID was considered as a random effect to take into account a sample effect. These soil properties were then compared by depth and per location (control, tree row, inter-row). An ANOVA was performed on these models. We then used the *multcomp* package (Hothorn et al. 2008) to perform a post hoc analysis and determine which means differed significantly between the control, tree rows and inter-rows, using the Tukey-Kramer test, designed for unbalanced data. To study spatial influence on SOC stocks, "distance to the closest tree" was added to the LMM model, and an ANOVA was performed.

Secondly, we fitted a LMM in a geostatistical framework using the cumulated SOC stock observations for 3 depths (0-30 cm, 0-100 cm and 0-200 cm). In a spatial context, the random effects of the LMM describe spatially-correlated random variation. The LMM model is then parameterized by a global vector, called **Θ**, of model parameters which include the parameters of the covariance function and the fixed effects coefficients. These can be fitted to the data by

a likelihood method. Lark et al. (2006) described how the maximum likelihood estimator is biased in the presence of fixed effects and suggested that the restricted maximum likelihood estimator (REML) should be applied. Following Villanneau et al., (2011) we have tested the assumption that the random effects are spatially correlated by comparing the quality of the model-fit for spatially correlated and spatially independent models (usually called pure nugget model). Webster and McBratney, (1989) suggested that the Akaike information criterion (AIC, Akaike, 1974) should be used to compare different spatially correlated models. Once the parameters of the LMM have been fitted, they may be plugged into the best linear unbiased predictor to form the empirical best linear unbiased predictor (E-BLUP) of the property at unsampled sites (Lark et al. 2006). The error variance of the E-BLUP can also be computed at any unsampled site. For this, the value of fixed effects covariates must be known at each prediction site. We therefore calculated several grids of the fixed effects with a 25 cm cell size. The use of any model of spatial variation implies that assumptions have been made about the type of variation the data exhibit. Once the model has been fitted, cross-validation can be used to confirm that these assumptions are reasonable and that the spatial model appropriately describes the variation. We therefore computed a 'leave-one-out cross-validation'. For each sampling location, $\boldsymbol{s}_i$ $(i = 1,2,\cdots,q)$, the value of the property at $\boldsymbol{s}_i$ was predicted by the E-BLUP upon the vector of observations excluding $Z\ \boldsymbol{s}_i$, in order to compute the standardized squared prediction error (SSPE: the squared difference between the E-BLUP and the observed value divided by the computed prediction error variance (PEV)). Under an assumption of normal prediction errors, the expected mean SSPE is 1.0 if the PEVs are reliable (which requires an appropriate variogram model), and the expected median SSPE is 0.455. The spatial analysis package *GeoR* (Ribeiro and Diggle 2001) was used for REML fitting and kriging.

Finally, a Kruskal-Wallis test (Kruskal and Wallis 1952) was performed to analyze SOC concentration in soil fractions per depth and per location (5 or 6 replicates). This test was followed by a post hoc analysis using Dunn's test (Dunn 1964) with a Bonferroni correction (p-value=0.017).

All the statistical analyses were performed using R software version 3.1.1 (R Development Core Team 2013), at a significance level of <0.05.

1.3 Results

1.3.1 Changes in soil texture with depth

Clay, silt and sand profiles were very similar at both plots (Fig. I-1.3). Soil texture was homogeneous in the first 50 cm. Clay and silt contents linearly increased till 100 cm soil depth to reach about 325 g kg^{-1} and 575 g kg^{-1} respectively, while sand content decreased. Soil texture did not change between 100 and 200 cm soil depth. Below 140 cm depth, clay and sand content were significantly different ($F=71.31$, $P<0.001$) in both plots, but the maximum difference was less than 20 g kg^{-1}.

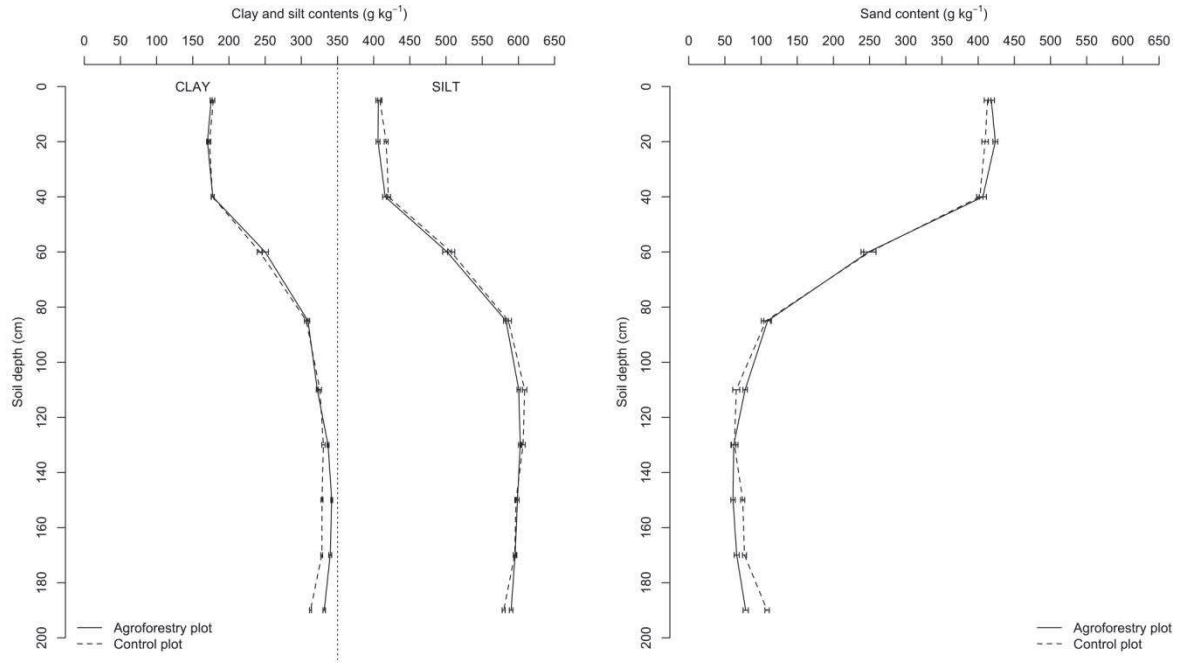

Figure I-1.3. Changes in soil texture with depth in the control plot and in the agroforestry plot. Error bars represent standard errors (n=100 in the agroforestry, n=93 in the control).

1.3.2 Soil bulk densities

Soil bulk densities were significantly higher in the control plot than in the tree row at all depths except for 30-50 and 140-160 cm, and higher than in the inter-row, except for 10-30 and below 140 cm depth (Table I-1.2). In the agroforestry system, soil bulk densities were higher in the inter-row than in the tree row for 0-10 and 10-30 cm.

Depth (cm)	Agroforestry – tree row	Agroforestry – inter-row	Control plot
0-10	1.10 ± 0.02 c	1.23 ± 0.03 b	1.41 ± 0.01 a
10-30	1.49 ± 0.01 b	1.60 ± 0.02 a	1.61 ± 0.00 a
30-50	1.71 ± 0.01 ab	1.67 ± 0.02 b	1.73 ± 0.00 a
50-70	1.73 ± 0.01 c	1.77 ± 0.01 b	1.80 ± 0.00 a
70-100	1.68 ± 0.00 c	1.71 ± 0.00 b	1.74 ± 0.00 a
100-120	1.55 ± 0.01 b	1.55 ± 0.01 b	1.61 ± 0.00 a
120-140	1.63 ± 0.00 b	1.64 ± 0.01 b	1.65 ± 0.00 a
140-160	1.64 ± 0.00 a	1.64 ± 0.01 a	1.65 ± 0.00 a
160-180	1.62 ± 0.01 b	1.65 ± 0.01 a	1.65 ± 0.00 a
180-200	1.64 ± 0.00 b	1.65 ± 0.00 a	1.65 ± 0.00 a

Table I-1.2. Mean soil bulk densities (g cm^{-3}). For a given depth, means followed by the same letters do not differ significantly at p = 0.05. Associated errors are standard errors (40 replicates for the tree-row, 60 replicates for the inter-row, and 93 replicates for the control plot).

1.3.3 Soil organic carbon concentrations

An ANOVA performed on the LMM model revealed that soil depth (F-value=270, $P<0.0001$) and location, i.e., tree row vs. inter-row (F-value=171, $P<0.0001$), were the only variables affecting significantly SOC concentrations. Distance to the closest tree had no significant effect (F-value=1.3, $P=0.28$). As shown in Fig. I-1.4, for 0-10 cm, SOC concentration doubled in the tree row (21.6 ± 0.8 mg C g^{-1}) compared to the inter-row (9.8 ± 0.1 mg C g^{-1}) and to the control (9.3 ± 0.1 mg C g^{-1}), whereas the latter two were not significantly different. SOC concentration was significantly higher in the tree row than in the control plot to 120 cm soil depth, except in the 50-70 cm soil layer where no difference was observed. SOC concentration was significantly higher in the tree row than in the inter-row to 30 cm soil depth.

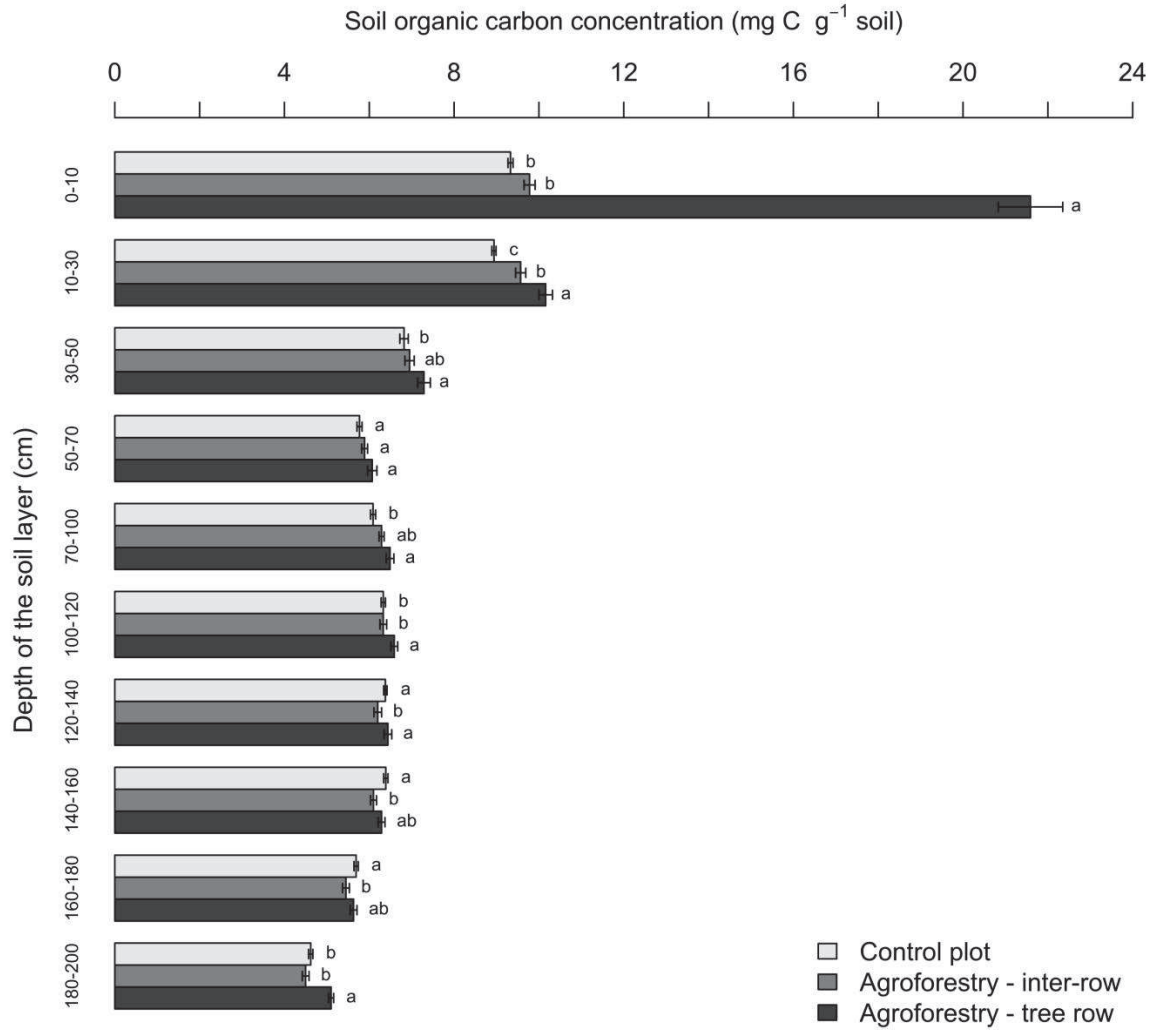

Figure I-1.4. Soil organic carbon concentration (mg C g^{-1} soil) of soil layers to 2-m depth in the control plot and in the agroforestry plot. Error bars represent standard errors (n=40 for the tree row, n=60 for the inter-row, and n=93 for the control). Significantly (p-value<0.05) different SOC concentrations per depth are followed by different letters.

1.3.4 Soil organic carbon stocks

Fig. I-1.5 represents SOC stocks in the agroforestry plot as a function of soil depth, location and distance to the closest tree. For a given depth and distance to the closest tree, variability of SOC stocks was high, and there was no effect of the distance to the closest tree on SOC stocks (Fig. I-1.5). An ANOVA performed on the LMM model confirmed that SOC stocks were significantly influenced by soil depth (F-value=483, P<0.0001) and location, i.e., tree row vs. inter-row (F-value=66, P<0.0001), but not by the distance to the closest tree (F-value=1.5, P=0.22).

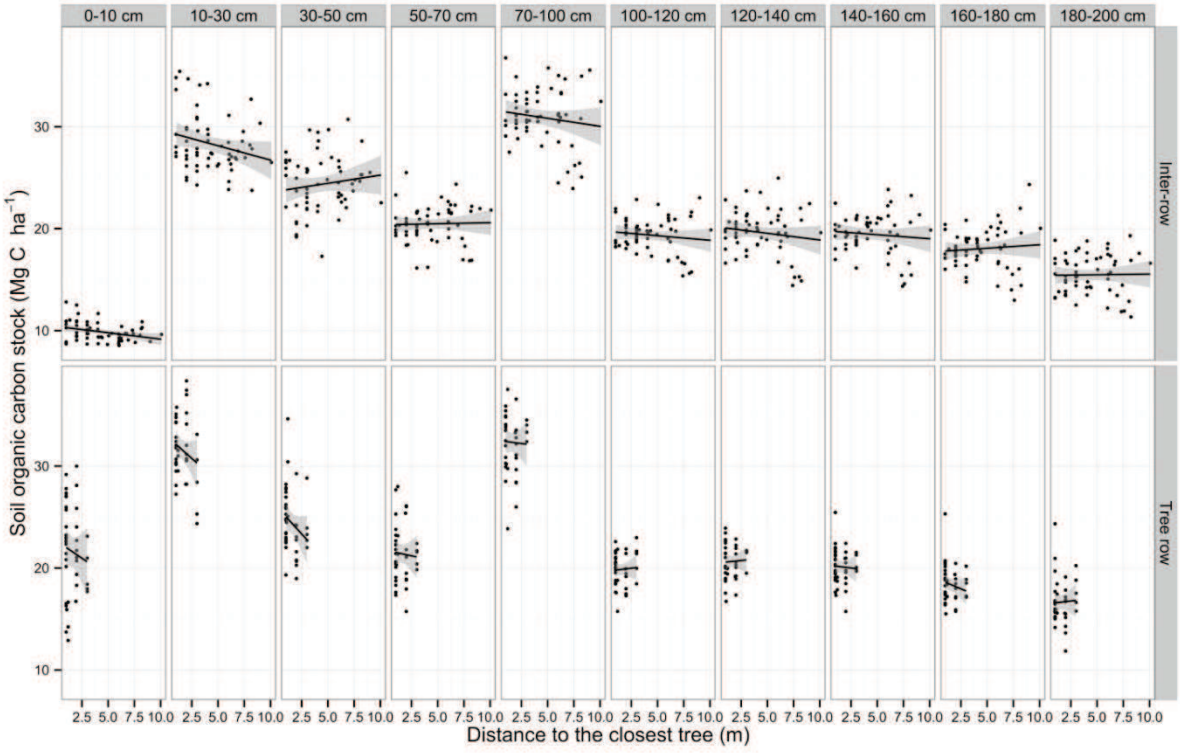

Figure I-1.5. Soil organic carbon stocks (Mg C ha^{-1}) in the agroforestry plot as a function of depth, location (tree row vs. inter-row) and distance to the closest tree. The lines represent the regression lines fitted using soil samples per investigated depth. The gray shades display the prediction confidence interval at the 0.95 level.

For an equivalent soil mass (ESM) of 4000 Mg ha^{-1} (to 26-29 cm depth), SOC stocks were significantly higher in the tree row than in the inter row and in the control (Table I-1.3). For an ESM of 31500 Mg ha^{-1} (to 189-196 cm depth), SOC stocks were about 20 Mg C ha^{-1} higher in the tree rows compared to the inter-rows or to the control. Cumulated SOC stocks were significantly higher in the inter-row than in the control plot to an ESM of 18700 Mg ha^{-1} (to 112-115 cm depth), except for an ESM of 1000 Mg ha^{-1} where not difference was found (Table I-1.3).

At the plot scale, cumulated SOC stocks in the agroforestry plot were significantly higher than in the control plot at all depths (Table I-1.3). For an ESM of 4000 Mg ha^{-1} (to 26-29 cm depth), SOC stocks were 40.3 ± 0.5 Mg C ha^{-1} and 35.8 ± 0.2 Mg C ha^{-1} in the agroforestry and in the control, respectively. For a soil mass of 15700 Mg ha^{-1} (to 93-98 cm depth), delta SOC stock between the agroforestry and the control was 6.3 ± 0.7 Mg C ha^{-1}.

Cumulated ESM (Mg ha^{-1})	Cumulated calculated depth to ESM (cm)			Cumulated SOC stocks (Mg C ha^{-1})				Δ SOC stocks (Mg C ha^{-1})	SOC accumulation rates (kg C ha^{-1} yr^{-1})	
	Tree-row	Inter-row	Control	Tree-row	Inter-row	Agroforestry	Control	Δ (Agroforestry – Control)	Agroforestry vs Control	Inter-row vs Control
1000	0-9	0-8	0-7	21.6 ± 1.0 a	9.8 ± 0.4 c	11.7 ± 0.3 b	9.3 ± 0.1 c	2.3 ± 0.4	129 ± 20	24 ± 21
4000	0-29	0-27	0-26	52.8 ± 1.4 a	37.9 ± 0.6 c	40.3 ± 0.5 b	35.8 ± 0.2 d	4.5 ± 0.6	248 ± 31	115 ± 33
7300	0-49	0-47	0-45	77.1 ± 1.5 a	62.0 ± 0.7 c	64.4 ± 0.6 b	59.4 ± 0.2 d	5.0 ± 0.6	276 ± 36	141 ± 39
10700	0-69	0-66	0-64	98.1 ± 1.5 a	82.4 ± 0.7 c	84.9 ± 0.6 b	79.7 ± 0.3 d	5.1 ± 0.7	286 ± 39	147 ± 43
15700	0-98	0-95	0-93	130.4 ± 1.5 a	113.7 ± 0.7 c	116.4 ± 0.7 b	110.1 ± 0.3 d	6.3 ± 0.7	350 ± 41	202 ± 45
18700	0-118	0-115	0-112	150.3 ± 1.5 a	133.1 ± 0.8 c	135.9 ± 0.7 b	129.3 ± 0.4 d	6.5 ± 0.8	363 ± 43	210 ± 46
21900	0-137	0-134	0-131	170.9 ± 1.5 a	152.8 ± 0.8 c	155.7 ± 0.7 b	149.5 ± 0.4 c	6.2 ± 0.8	345 ± 44	185 ± 48
25100	0-157	0-154	0-150	191.0 ± 1.6 a	172.4 ± 0.8 c	175.4 ± 0.7 b	169.9 ± 0.4 c	5.5 ± 0.8	306 ± 45	140 ± 49
28300	0-176	0-173	0-170	209.5 ± 1.6 a	190.5 ± 0.8 c	193.5 ± 0.7 b	189.3 ± 0.4 c	4.3 ± 0.8	238 ± 47	69 ± 51
31500	0-196	0-193	0-189	226.1 ± 1.6 a	206.0 ± 0.84 c	209.2 ± 0.7 b	205.9 ± 0.4 c	3.3 ± 0.9	183 ± 48	5 ± 53

Table I-1.3. Soil organic carbon stocks (Mg C ha^{-1}) and SOC accumulation rates (kg C ha^{-1} yr^{-1}). Associated errors are standard errors (40 replicates for the tree-row, 60 replicates for the inter-row, and 93 replicates for the control plot). ESM=Equivalent Soil Mass. Significantly (P-value < 0.05) different SOC stocks are followed by different letters.

1.3.5 Soil organic carbon accumulation rates

Compared to the control, inter-rows accumulated 115 ± 33 kg C ha^{-1} yr^{-1} for an ESM of 4000 Mg ha^{-1} (26-29 cm) (Table I-1.3), and 202 ± 45 kg C ha^{-1} yr^{-1} for an ESM of 15700 Mg ha^{-1} (93-98 cm). SOC accumulation rates in the agroforestry plot compared to the control were 248 ± 31 kg C ha^{-1} yr^{-1} for an ESM of 4000 Mg ha^{-1}, 350 ± 41 kg C ha^{-1} yr^{-1} an ESM of 15700 Mg ha^{-1}, and 183 ± 48 kg C ha^{-1} yr^{-1} an ESM of 31500 Mg ha^{-1} (Table I-1.3). The SOC accumulation rates for 0-10 cm and 10-30 cm were respectively explained at 80% and 60% by the tree rows.

1.3.6 Spatial distribution of SOC stocks

The AIC (Table I-1.4) of the spatially correlated model were less than that of the spatially uncorrelated model for 2 depths (0-100 cm and 0-200 cm for the agroforestry and the control plots), indicating that spatial correlation should be included in the model of variation. We tested several models of spatial variation and retained the spherical model (Webster and Oliver 2007). For top soil depth of the two plots (0-30 cm), the AIC of the spatially uncorrelated model was slightly the smallest indicating that the residual variation could be independent once fixed effects had been included in the model. But the difference was very small so we considered the spatially correlated model for the rest of the study. The cross-validation results confirmed the validity of the fitted LMM. The nugget to sill ratio measures the unexplained part of the observed variability. The smallest value was observed for the 0-200 cm depth in the control plot and the higher was observed for the 0-30 cm depth in both plots. When mapping the SOC stocks for three fixed depths with the BLUP in the two plots, a clear pattern can be observed in the agroforestry plot, with high SOC stocks in the tree rows (Fig. I-1.6). The fitted fixed effects indicate that, in average, the SOC stocks were 15 to 20 Mg C ha^{-1} higher in the tree rows to 30 to 200 cm depth (Table I-1.4). At the opposite, the control plot did not exhibit any spatial pattern.

	Depth (cm)	Mean SSPE	Median SSPE	ME	RMQSE	AIC	AIC.ns	β_0	β_1	Nugget	Sill	Range	Nugget to Sill ratio
Agroforestry	0-30	0.99	0.36	-0.004	20.7	585	**583**	38.1	14.8	19.7	1.3	15.2	0.94
	0-100	0.99	0.45	-0.010	43.3	**662**	665	114.1	16.4	36.0	16.3	12.8	0.69
	0-200	0.98	0.39	0.055	123.1	**769**	780	207.1	19.4	97.8	79.2	12.9	0.55
Control	0-30	1.01	0.33	0.000	2.6	361	**357**	35.9	-	2.4	0.2	19.4	0.93
	0-100	1.01	0.50	0.061	25.7	**578**	579	111.2	-	20.2	11.0	12.6	0.65
	0-200	0.98	0.40	0.519	57.5	**665**	681	208.9	-	16.4	85.8	6.3	0.16

Table I-1.4. Summary of selected models fitted to the data on cumulated soil organic carbon stocks at 3 depths (0-30 cm, 0-100 cm and 0-200 cm) for the 2 plots, and cross validation. SSPE, standardized squared prediction errors; ME, mean error (Mg C ha^{-1}); RMSQE, root mean squared error (Mg C ha^{-1}); AIC, AIC of the spatially correlated model; AIC.ns, AIC of the non-spatially correlated model; β_0 and β_1 the fixed effects (Mg C ha^{-1}). Bold characters represent the smallest AIC for each depth. The medians and the mean of the cross validation statistics are within the 95% confidence interval.

	$SOC_{sat\text{-}pot}$ (mg C g^{-1})	SOC_{cur} (mg C g^{-1})		$SOC_{sat\text{-}def}$ (mg C g^{-1})		$\frac{SOC_{cur}}{SOC_{sat-pot}}$		$SOC_{stor\text{-}pot}$ (Mg C ha^{-1})
Depth (cm)	Agroforestry	Tree row	Inter-row	Tree row	Inter-row	Tree row	Inter-row	Agroforestry
0-10	18.0 ± 0.4	7.2 ± 0.3	5.4 ± 0.3	10.3 ± 0.4	13.1 ± 0.4	40%	30%	15.3 ± 0.4
10-30	18.7 ± 0.4	6.1 ± 0.1	5.4 ± 0.1	12.6 ± 0.3	13.3 ± 0.3	33%	29%	41.8 ± 0.9
70-100	32.9 ± 0.8	5.6 ± 0.1	5.6 ± 0.1	26.9 ± 0.7	27.6 ± 0.4	17%	17%	140.7 ± 1.9
160-180	32.0 ± 1.1	4.6 ± 0.2	4.6 ± 0.3	26.8 ± 0.7	28.1 ± 0.9	14%	14%	91.9 ± 2.4

Table I-1.5. Soil organic carbon saturation of the fractionated soil samples in the agroforestry plot. $SOC_{sat\text{-}pot}$, potential SOC saturation (mg C g^{-1}); SOC_{cur} , current mean SOC concentration of the fine fraction <20 µm (mg C g^{-1}); $SOC_{sat\text{-}def}$, SOC saturation deficit (mg C g^{-1}); $SOC_{stor\text{-}pot}$, total amount of the SOC storage potential (Mg C ha^{-1}). Associated errors are standard errors (n=5). Values of SOC saturation for deep soil layers are only indicative.

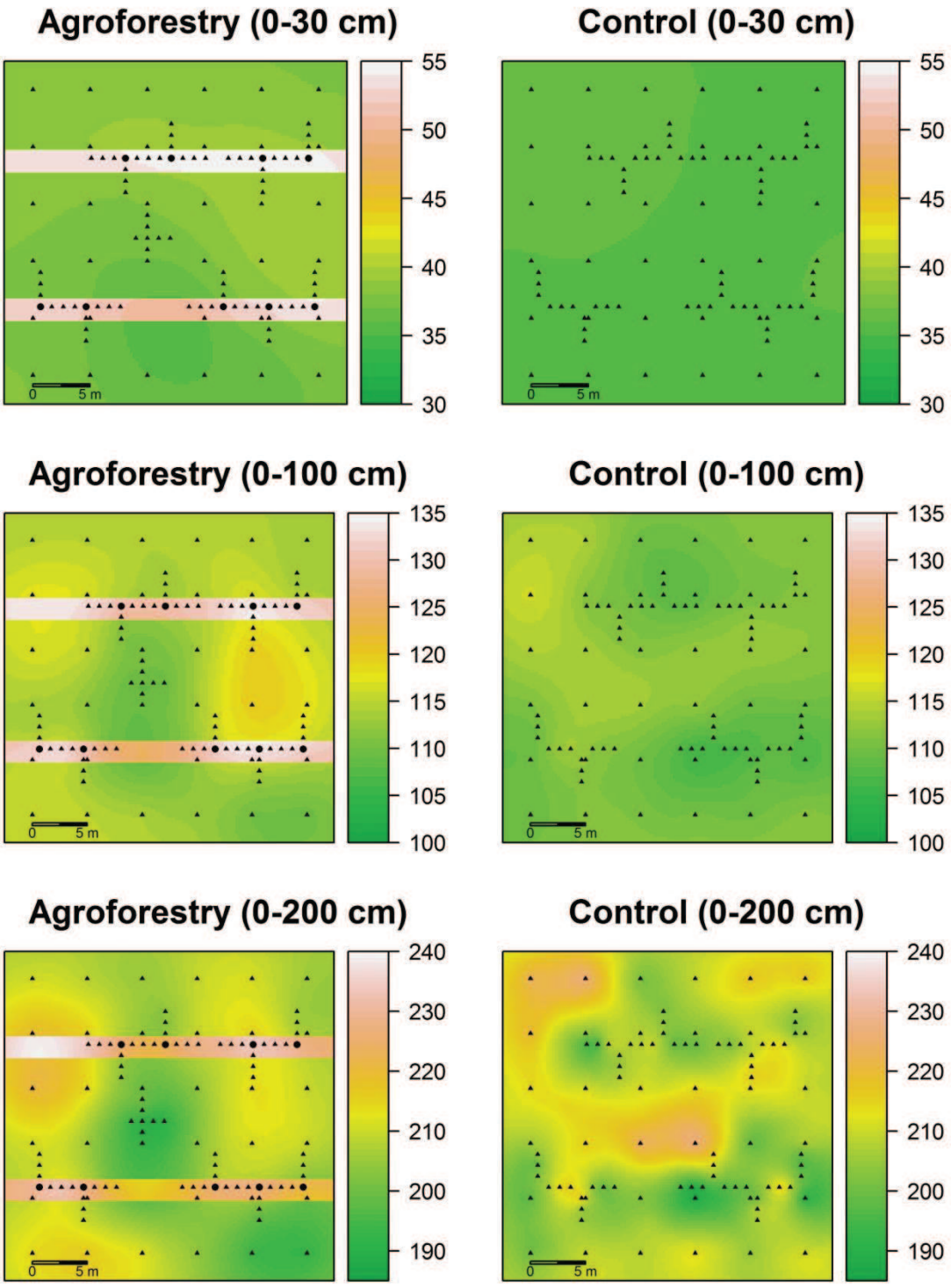

Figure I-1.6. Kriged maps of cumulated soil organic carbon stocks (Mg C ha^{-1}) in the agroforestry and in the control plot.

1.3.7 Organic carbon distribution in soil fractions

An average mass yield of 98% and an average carbon yield of 96% were obtained, showing the quality of the particle size fractionation. Furthermore, the variation between soil texture and soil fractionation was only 5-6% (data not shown). Soil segments used for soil fractionation had similar total SOC concentrations compared to mean SOC concentrations at the same depth (Fig. I-1.S2). However, the small differences found between SOC concentrations in the inter row and in the control was not visible with the soil segments used for fractionation.

For 0-10 cm depth, the distribution of OC in particle size fractions was strongly modified in the tree rows, with an important increase of C in particulate organic matter (POM) fractions (50-200 µm and 200-2000 µm) compared to the inter-row and to the control (Fig. I-1.7). An increase of C in silt size fractions (2-20 µm and 20-50 µm) of the tree rows compared to the inter row and to the control was also observed. Significantly higher C concentrations in the clay fraction were observed in the tree row than in the inter-row (Fig. I-1.S3), but it was not the case for the amount of C in the clay fraction per gram of soil (Fig. I-1.7).

Similar trends in C distribution in fractions were observed at 10-30 cm depth compared to 0-10 cm, although with much smaller differences (Figs. I-1.7, I-1.S3). At deeper depths (70-100 and 160-180 cm) there were no differences between the three locations (tree row, inter-row and control) except a lower amount of C in the soluble fraction in the tree row. The potential SOC saturation of particles <20 µm was not reached at any depths (Table I-1.5), and the SOC deficit was high, especially for 70-100 and 160-180 cm.

1.3.8 Distribution of additional OC in soil fractions

For 0-10 cm depth, the additional OC stored between the tree row and the inter-row was explained at 80% by POM fractions, at 15% by silt size fractions, and at 5% by clay fraction, whereas the additional OC stored between the tree row and the control was explained at 80% by POM and at 20% by silt size fractions (Fig. I-1.7). For 10-30 cm, the additional SOC storage between the tree row and the inter-row was explained at 50% by POM fractions, at 25% by coarse and fine silt fractions, and at 25% by clay fraction (Fig. I-1.7), whereas when comparing the tree row and the control these numbers were of 50% (POM) and 50% (silt).

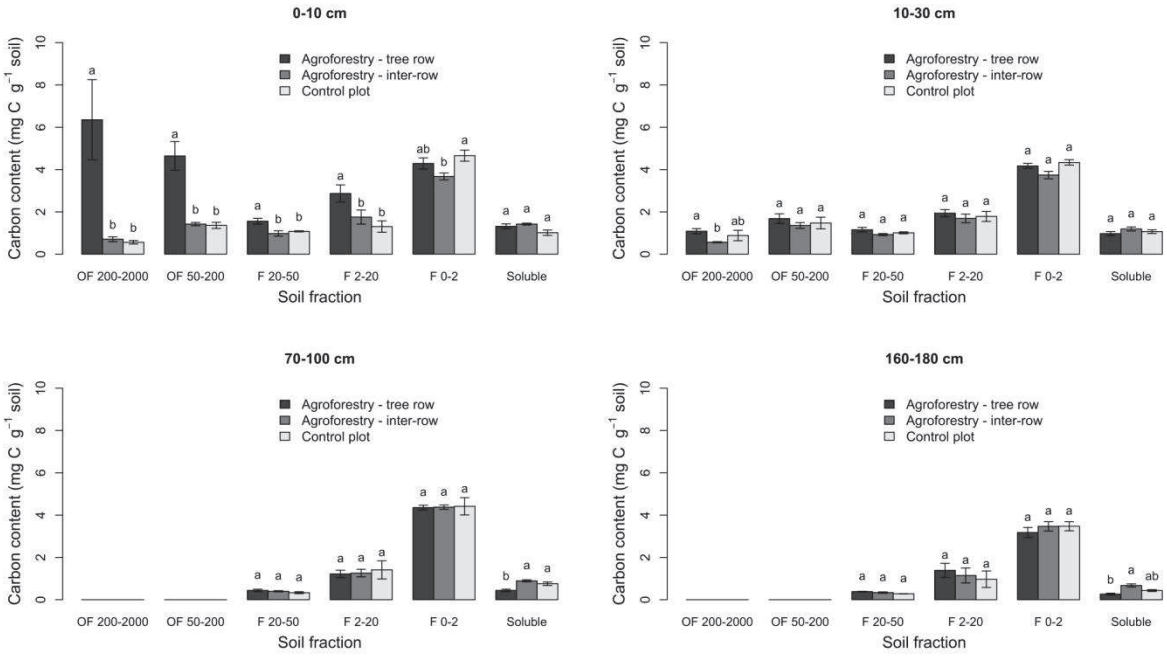

Figure I-1.7. Organic carbon contents in each soil fraction (mg C g^{-1} soil). Error bars represent standard errors (n=6 in the control, n=5 in the inter-row and in the tree row). OF = Organic fraction, F = organo-mineral fraction. 0-2, 2-20, 20-50, 50-200 and 200-2000 represent particle size (µm). Means followed by the same letters do not differ significantly at p=0.017 (Dunn's test with Bonferroni correction).

1.4 Discussion

1.4.1 A shallow additional SOC storage

Sampling to 2-m soil depth indicated that the 0-30 cm soil layer contained less than 20% of total SOC stocks to 2-m depth, demonstrating the importance of deeper soil layers for storing SOC (Jobbagy and Jackson 2000; Harper and Tibbett 2013). SOC stocks observed in 0-30 cm, from 36 to 41 Mg C ha^{-1}, were comparable to reported values for the Mediterranean region, i.e., 25 to 50 Mg C ha^{-1} (Martin et al. 2011; Muñoz-Rojas et al. 2012). Additional SOC storage in the agroforestry system compared to the agricultural system was mainly observed up to 30 cm soil depth in the inter-row and up to 50 cm in the tree row. A companion study at the same site indicated that 60% of additional OM inputs (leaf litter, aboveground and belowground biomass of the natural vegetation in the tree row, tree fine roots) to 2 m depth in the agroforestry plot compared to the control plot were located in the first 50 cm (unpublished data). Even if 50% of tree fine root density was found between 1 and 4 m soil depth (Cardinael et al. 2015c), it was also proven at this site (Germon et al., submitted) and at other sites (Hendrick and Pregitzer 1996) that the turnover rate of fine roots

decreased with increasing depth, resulting in low OM inputs in deep soil layers. Time since the tree planting (18 years) is probably not long enough to detect changes in SOC stocks at deeper soil depths considering low organic inputs below 1 m depth. For 2012, organic C input due to tree fine root mortality was estimated to be less than 150 kg C ha^{-1} for 100-200 cm soil depth. Below 1.2 m soil depth, delta of cumulated SOC stocks between the agroforestry and the control plot decreased, due to higher SOC concentrations and stocks in the control at these depths. These higher SOC concentrations were linked to higher SOC in the clay fraction. This difference may be due to pre-experimental soil heterogeneity, the soil in the agroforestry plot may have had a lower level of SOC below 1.2 m depth before tree planting. An initial heterogeneity was also proposed by Upson and Burgess (2013) who found higher SOC stocks at depth in a control plot compared to an agroforestry plot in an experimental site in England . This shows the limit of paired comparisons - or synchronic studies - to evaluate SOC changes after land use change (Costa Junior et al. 2013; Olson et al. 2014a), and pleads for long-term diachronic studies in agroforestry systems. An alternative explanation could be a positive priming effect, i.e., the acceleration of native SOC decomposition by the supply of fresh organic carbon (Fontaine et al. 2004; Fontaine et al. 2007) from the trees. However, this seems highly unlikely since positive priming effect could not explain such a high C loss of about 3.2 Mg C ha^{-1} between 1.2 and 2.0 m soil depth in 18 years, i.e., about 180 kg C ha^{-1} yr^{-1}. Another hypothesis to explain higher SOC stocks below 1.2 m depth in the control plot is a different belowground water regime between the two plots. Water table depth at this site is known to be very variable (between 5 to 7 m). A shallower water table in the agroforestry plot compared to the control plot may promote capillary action, and therefore cause wetting-drying cycles that could enhance SOM decomposition in deep soil layers (Borken and Matzner 2009).

1.4.2 Tree rows and SOC storage in agroforestry systems

The high SOC stocks observed in tree rows accounted for an important part of SOC stocks of the agroforestry plot even though tree rows only represented 16% of the surface area. In a poplar (*Populus* L.) silvoarable agroforestry experiment in England, Upson and Burgess, (2013) also found that the SOC concentration was greater in the top 40 cm under the tree row (19.6 mg C g^{-1}) in the agroforestry treatment than in the cropped alleys (17 mg C g^{-1}), or the arable control (17.1 mg C g^{-1}). Tree rows are comparable to a natural permanent pasture with trees, given that spontaneous vegetation grows and that the soil is not tilled. Conversion of

arable lands to permanent grasslands is recognized as an efficient land use for climate change mitigation (Soussana et al., 2004). Grasslands can accumulate SOC at a very high rate. For instance, it was estimated on about 20 years old field experiments that conversion from crop cultivation to pasture stored SOC at a rate of 1.01 Mg C ha^{-1} yr^{-1} in 0-30 cm (Conant et al. 2001). In our case, SOC accumulation rate in the tree rows was 0.94 ± 0.09 Mg C ha^{-1} yr^{-1} in 0-30 cm. Management of tree rows could therefore have an important role in improving agroforestry systems in terms of SOC storage. Improved grass species could be sown in the tree rows, as well as shrubs between trees. Further research should focus on this aspect to evaluate benefits in terms of SOC storage and biodiversity for instance.

1.4.3 Homogeneous distribution of SOC stocks in the cropped alley

There was no significant effect of the distance to the trees on SOC stocks at all depths, either in the tree row or in the inter-row. This was also indicated by the maps of the SOC stocks. Tree density was high at this site, and walnuts were about 13 m in height, which is also the distance between two tree rows. This could explain the homogeneous distribution of leaf litterfall observed in the plot (personal observation). In a similar agroforestry system in terms of tree density in Canada, Bambrick et al., (2010) and Peichl et al., (2006) also found no effect of the distance to the trees on SOC stocks to 20 cm depth. They also suggested that the 18 m high poplar trees distributed litterfall equally in the cropped alleys. Close to the tree rows (1 to 2 m distance), the intercrop had a lower yield (15% less in 2012) compared to the middle of the inter-row at the study site (Dufour et al. 2013). On the contrary, tree fine root density was higher close to the tree rows (2.79 t DM ha^{-1} between 0 and 1.5 m from the tree row in the inter row, and to 4-m soil depth) than in the middle of the inter-rows (1.32 t DM ha^{-1} between 3 and 4.5 m from the tree row in the inter row, and to 4-m soil depth) (Cardinael et al. 2015c). Thus, lower carbon inputs from crop residues close to the tree rows may be counterbalanced with higher inputs from tree fine root mortality, explaining homogeneous SOC stocks within the inter-row (Peichl et al. 2006; Bambrick et al. 2010). In the tree row, homogeneous distribution of SOC stocks may be explained by the short distance between trees and by the presence of abundant herbaceous vegetation.

1.4.4 Agroforestry systems: an efficient land use to improve SOC stocks

Compared to other agroforestry systems having about the same tree density, a lower SOC accumulation rate in 0-30 cm (0.25 Mg C ha^{-1} yr^{-1}) was observed at our site. Peichl et al. (2006) reported a SOC accumulation rate of 1.04 Mg C ha^{-1} yr^{-1} (0-20 cm) in a 13-year old temperate barley (*Hordeum vulgare* L.)-poplar intercropping system (111 trees ha^{-1}). In a 21-year old agroforestry system in Canada where poplars were intercropped with a rotation of wheat (*Triticum aestivum* L.), soybean (*Glycine max* (L.) Merr.) and corn (*Zea mays* L.), Bambrick et al. (2010) estimated a SOC accumulation rate of 0.30 Mg C ha^{-1} yr^{-1} (0-20 cm). Our lower accumulation rate may be explained by warmer climate, higher temperatures enhancing OM decomposition (Conant et al. 2011; Hamdi et al. 2013). Moreover, valuable hardwood species like walnut trees have a slower growing rate than fast growing species like poplar (Teck and Hilt 1991), and therefore for a same tree age, the amount of OC inputs (leaflitter, fine roots) to the soil is lower for slow growing species.

Together with other climate-smart farming practices (Lipper et al. 2014), alley-cropping agroforestry systems have the potential to enhance SOC stocks and to contribute to climate change mitigation (Nair et al. 2010; Pellerin et al. 2013). No-till farming is a commonly cited agricultural practice supposed to have a positive impact on SOC stocks. But recent meta-analyses showed this practice had no effect on SOC stocks to 40 cm depth (Luo et al. 2010) or a smaller one (0.23 Mg C ha^{-1} yr^{-1} to 30 cm depth) than previously estimated (Virto et al. 2012). A meta-analysis also revealed that the inclusion of cover crops in cropping systems could accumulate SOC at a rate of 0.32 ± 0.08 Mg C ha^{-1} yr^{-1} to a depth of 22 cm (Poeplau and Don 2015). At our site, we found a mean SOC accumulation rate of 0.12 in 0-30 cm in the inter-rows compared to the control. This rate reached 0.25 Mg C ha^{-1} yr^{-1} for the whole agroforestry system. A companion study at this site estimated that the tree aboveground C stock was 117± 21 kg C $tree^{-1}$ (unpublished data). With 110 trees ha^{-1}, total organic carbon (SOC to 1 m soil depth + aboveground tree C) accumulation rate was 1.11 ± 0.13 Mg C ha^{-1} yr^{-1}, making agroforestry systems a possible land use to help mitigating climate change (Lal 2004a; Lal 2004b; Lorenz and Lal 2014).

1.4.5 A long-term SOC storage?

Most of additional SOC in the agroforestry plot compared to the control plot was located in coarse soil fractions (50-200 µm and 200-2000 µm). These soil fractions are assumed to

contain labile fractions (Balesdent et al. 1998), that are not stabilized by interaction with clays and thus prone to be decomposed by soil microorganisms. Our site might not be old enough to observe a difference in the fine soil fractions as changes in the clay fractions are often long-term processes (Balesdent et al. 1988; Balesdent 1996). For example, Takimoto et al., (2008) found in a 35-year-old *Faidherbia albida* parkland in Mali, that the silt + clay soil fraction (< 53 μm) was enriched in C at depth compared with treeless systems. But on the other hand, Howlett et al., (2011) did not observe any difference for the same soil fraction in a 80 year-old Dehesa cork oak (*Quercus suber* L.) silvopasture, but they found that C storage in the macroaggregate fraction (250–2000 mm) was 68% greater underneath *versus* away from the tree canopy (in 0-25 cm). Several studies have demonstrated that protection of C within the macroaggregate size class was affected by afforestation (Del Galdo et al. 2003; Denef et al. 2013) and cessation of tillage (Tan et al. 2007). The fractionation method that was used in this study disrupted macroaggregates (von Lützow et al. 2007), and part of this labile fractions could be located within them and therefore be physically protected from decomposition by soil microorganisms (Puget et al. 2000; Six et al. 2000a). Further work will focus on this aspect in order to estimate the amount of particulate organic matter located in soil aggregates. Calculation of SOC saturation revealed a high deficit of SOC of this soil compared to the theoretical value, especially at depth, suggesting that accumulation of SOC due to the agroforestry system could continue for decades before reaching saturation.

1.5 Conclusion

This study showed the potential of agroforestry systems to increase SOC stocks. However, despite a deep tree rooting system, additional SOC was mainly located in topsoil layers, and in labile organic fractions, making this C storage vulnerable. Tree rows were shown to be a key factor for SOC storage in alley cropping systems. Combining agroforestry systems with no-till or permanent cover systems could be a very efficient way to increase SOC stocks, but more research is needed on this aspect. To fully estimate the impact of agroforestry systems on SOC sequestration, other aspects should be taken into account. For instance, higher SOC stocks in the inter-rows could increase soil fertility and reduce the need for chemical fertilizer, contributing indirectly to a reduction of greenhouse gases emissions; further work should therefore focus on nutrient cycling in these systems.

1.6 Supplementary material

	Cumulated SOC stocks (Mg C ha^{-1})		Δ SOC stocks (Mg C ha^{-1})	SOC accumulation rates (kg C ha^{-1} yr^{-1})
Soil depth (cm)	Agroforestry	Control	Δ (Agroforestry – Control)	Agroforestry vs Control
0-10	13.9 ± 0.3	13.2 ± 0.1	0.7 ± 0.4	41 ± 20
0-30	44.5 ± 0.5	41.9 ± 0.2	2.5 ± 0.6	139 ± 32
0-50	67.9 ± 0.7	65.5 ± 0.4	2.5 ± 0.8	137 ± 42
0-70	88.8 ± 0.7	86.3 ± 0.4	2.6 ± 0.8	143 ± 46
0-100	121.2 ± 0.7	118.0 ± 0.5	3.1 ± 0.9	173 ± 54
0-120	140.9 ± 0.8	138.4 ± 0.6	2.5 ± 1.0	138 ± 56
0-140	161.4 ± 0.8	159.4 ± 0.6	1.9 ± 1.0	106 ± 58
0-160	181.5 ± 0.8	180.5 ± 0.6	0.9 ± 1.0	53 ± 60
0-180	199.5 ± 0.9	199.3 ± 0.6	0.2 ± 1.1	11 ± 60
0-200	214.6 ± 0.9	214.5 ± 0.6	0.1 ± 1.1	7 ± 62

Table I-1.S1. Soil organic carbon stocks (Mg C ha^{-1}) and SOC accumulation rates (kg C ha^{-1} yr^{-1}) without the equivalent soil mass (ESM) correction. Associated errors are standard errors (100 replicates for the agroforestry plot, 93 for the control plot).

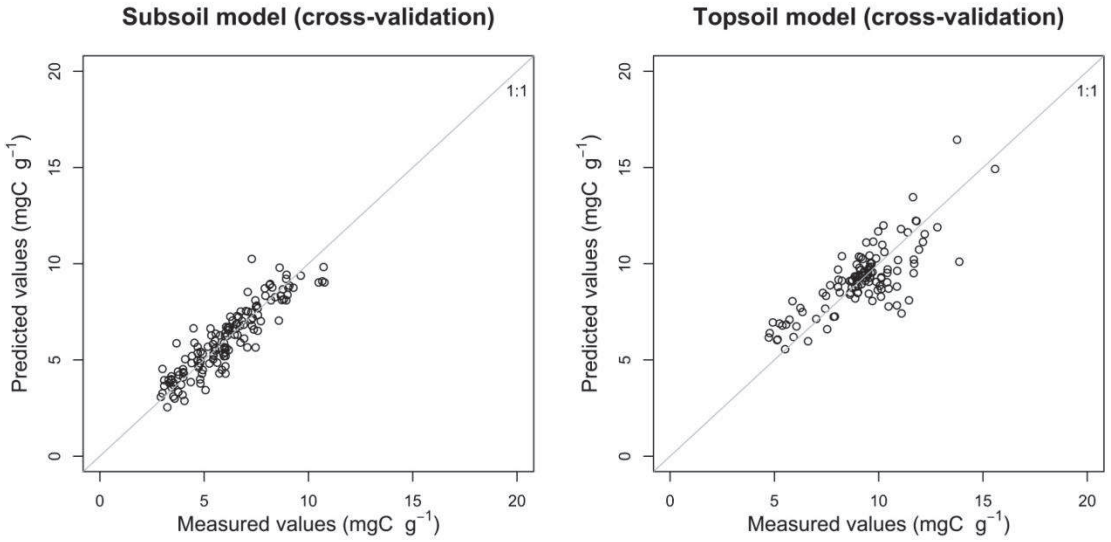

Figure I-1.S1. Measured and cross-validation predicted values of soil organic carbon concentrations for the topsoil and subsoil models.

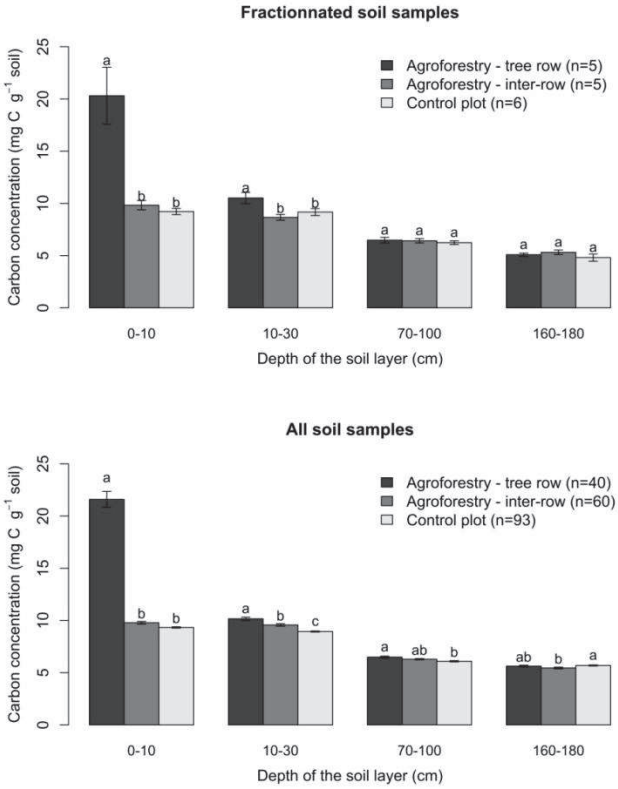

Figure I-1.S2. Carbon concentration of bulk fractionated samples. Error bars represent standard errors (n=6 in the control, n=5 in the inter-row and in the tree row).

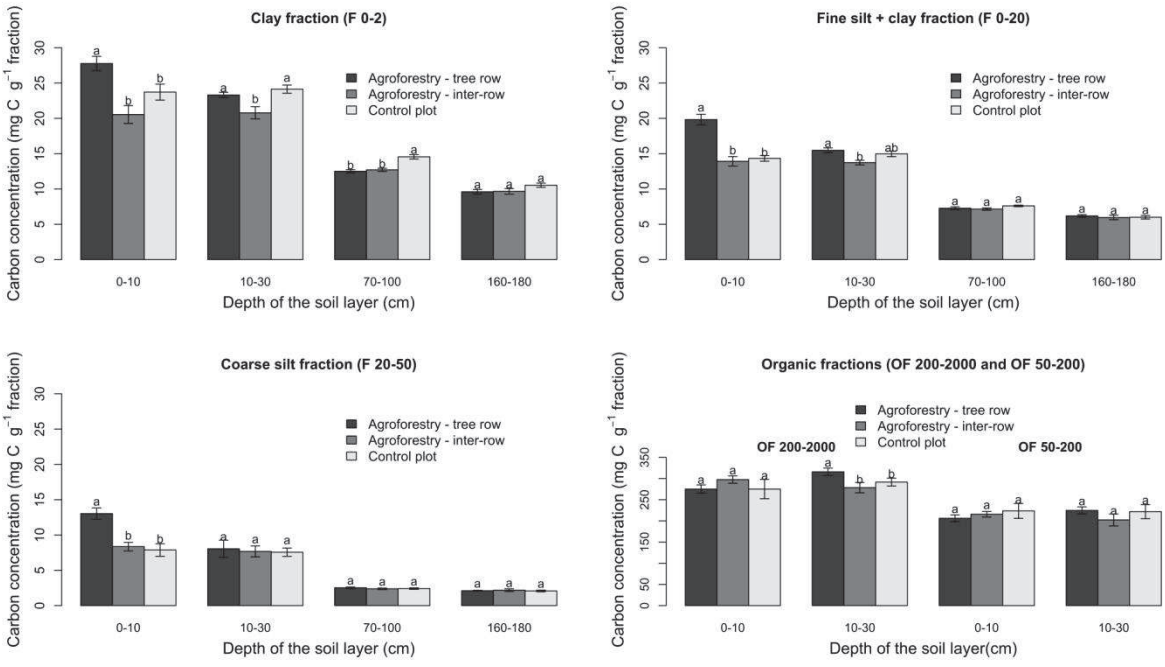

Figure I-1.S3. Carbon concentration (mg C g^{-1} fraction) of each soil fraction. Error bars represent standard errors (n=6 in the control, n=5 in the inter-row and in the tree row). OF = Organic fraction, F = organo-mineral fraction. Means followed by the same letters do not differ significantly at p=0.017 (Dunn's test with Bonferroni correction).

Chapitre 2

Stockage de carbone dans les sols et dans la biomasse des arbres de différents systèmes agroforestiers en France

Article en préparation pour *Agriculture, Ecosystems & Environment*

Soil organic and biomass carbon stocks under different agroforestry systems in France

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Abstract

Agroforestry systems are agroecosystems where farmers grow trees and crops or animals in temporal or spatial associations in the same field. Besides producing food and wood, these systems provide a variety of ecosystem services, such as erosion control, water quality improvement, biodiversity enhancement, and climate change mitigation. They can store a lot of carbon (C) in both the aboveground and belowground biomass of trees, and increase soil organic carbon (SOC) stocks. However, few studies have assessed the impact of agroforestry systems on carbon storage under temperate climates, as most of them were performed in tropical regions. This study was conducted in 5 alley cropping agroforestry systems and in 1 silvopastoral system in France. All sites included an agroforestry system and an agricultural control plot. The age of the study sites ranged from 6 to 41 years, with an average age of 19

years since the tree planting. SOC stocks were measured on an equivalent soil mass basis on more than 1500 soil cylinders. Sampling depth ranged from 20 to 100 cm, with an average sampling depth of 48 cm. Tree aboveground biomass was measured in all sites, and belowground biomass was estimated using an allometric equation. The mean SOC accumulation rate in alley cropping agroforestry systems was 239 ± 67 kg C ha^{-1} yr^{-1} in 0-30 cm. Young systems can also store SOC quickly after tree establishment, not through the trees, but through the herbaceous vegetation growing in the tree rows, a permanent grassland-like land use introduced as an indirect consequence of tree rows. In old plantations, C stocks in the aboveground biomass reached up to 35 Mg C ha^{-1}. All together our study demonstrated the general potential of agroforestry systems to store SOC in temperate regions.

2.1 Introduction

Soils are of crucial importance in the global carbon budget (Houghton 2007). Currently, the land sink (soil + vegetation) absorbs about 30% of the carbon emitted to the atmosphere through the burning of fossil fuel and cement production (Le Quéré et al. 2014). But since 1850, the depletion of SOC pool in cultivated lands has contributed to about 70 Gt C to the atmosphere (Amundson 2001; Lal 2004b). These SOC depleted areas are now seen as an opportunity for future C sinks through SOC sequestration (Paustian et al. 1997; Freibauer et al. 2004). In France, SOC stocks are 3.1-3.3 Gt C in the first 30 cm of soils (Arrouays et al. 2001; Martin et al. 2011). Using the concept of SOC saturation of soil (Hassink 1997), based on the hypothesis that the quantity of stable SOC in a soil is limited by the amount of fine particles, Angers et al., (2011) found that the median saturation deficit of French arable topsoils was 8.1 g C kg^{-1}. About 70% of French agricultural topsoils would then be unsaturated in SOC and have thus a potential for additional SOC storage. Increasing SOC stocks is often seen as a win-win strategy (Janzen, 2006; Lal, 2004) as it allows the transfer of CO_2 from the atmosphere to the soil while enhancing soil quality and fertility (Lal, 2004).

Several agricultural practices enhancing SOC stocks have been identified. For instance, introduction of cover crops (Constantin et al. 2010; Poeplau and Don 2015), and of grasslands (Conant et al. 2001; Soussana et al. 2004) in the cropping sequence have shown to be effective. The effect of no-till farming on SOC stocks is highly variable (Luo et al. 2010; Virto et al. 2012; Dimassi et al. 2013) and relies on the amount of the crop C inputs to the soil (Virto et al. 2012). Agroforestry systems, i.e., agroecosystems associating trees with crops

(Nair 1993), are recognized as a possible land use to maintain and increase SOC stocks, in tropical regions (Albrecht and Kandji 2003) and under temperate climates (Peichl et al. 2006; Bambrick et al. 2010). However, many of the available studies only report on surface layers of soil (< 20 or 30 cm) and a true control is often lacking. A recent study in the Mediterranean region of France revealed that an alley cropping agroforestry system increased SOC stocks by 248 ± 31 kg C ha^{-1} yr^{-1} in 0-30 cm and by 350 ± 41 kg C ha^{-1} yr^{-1} in 0-100 cm compared to an agricultural plot (Cardinael et al. 2015a). In Europe, estimates of SOC storage under agroforestry systems are scarce (Howlett et al. 2011; Upson and Burgess 2013) and thus it remains difficult to evaluate the impact of this practice (Pellerin et al. 2013).

The objectives of this study were i) to quantify SOC stocks in agroforestry systems and in adjacent agricultural plots in six different sites in France, ii) to measure OC stocks in the aboveground biomass of the agroforestry systems, iii) to estimate SOC accumulation rates for different agroforestry systems under different pedoclimatic conditions in France.

2.2 Materials and methods

2.2.1 Description of the 6 sites

Each study site comprised an alley cropping agroforestry system and an adjacent agricultural control plot, with the same historical land use and the same current management. All sites were owned and managed by farmers, except the Restinclières (RE) and Theix (TH) sites that are research experimental sites.

The first site (CH) was located in Châteaudun (Fig. I-2.1), in the department of Eure-et-Loir (Longitude 1°17'58'' E, Latitude 48°06'08'' N, elevation 147 m a.s.l.). The average temperature is 11.1°C and the average annual rainfall is 595 mm (years 2001-2013, INRA CLIMATIK). The soil is a silty loam (Table I-2.1) Luvisol (IUSS Working Group WRB 2007). Hybrid walnut (*Juglans regia* × *nigra* cv. NG23) trees were planted in February 2008 at a density of 34 trees ha^{-1}. The distance between two tree rows was 26 m and trees were planted every 10 m along the tree rows. A mix of ray-grass (*Lolium perenne* L.) and of tall fescue (*Festuca arundinacea* Schreb.) was sown in the tree rows before tree planting (in August 2007), on a width of 2 m. In the control plot and in the inter rows, a rotation of wheat (*Triticum aestivum* L. subsp. *aestivum*) and rapeseed (*Brassica napus* L.) was cropped (Table I-2.2) since the tree planting. The mean yield was 7.5-8 t ha^{-1} for wheat, and 3.8 t ha^{-1} for

rapeseed. All crop residues were left in the field. Ploughing occurred every three years to 22 cm soil depth, and superficial tillage (8 cm) was performed the other years.

The second site (ME) was located in Melle (Fig. I-2.1), in the department of Deux-Sèvres (Longitude 0°10'37'' W, Latitude 46°11'54'' N, elevation 107 m a.s.l.). The average temperature is 11.7°C and the average annual rainfall is 810 mm (years 1990-2013, INRA CLIMATIK). The soil is a silty loam (Table I-2.1) Luvisol (IUSS Working Group WRB 2007). Hybrid walnut (*Juglans regia* × *nigra* cv. NG23) trees were planted in 2008 at a density of 35 trees ha^{-1}. The distance between two tree rows was 29 m and trees were planted every 8 m along the tree rows. Sheep fescue (*Festuca ovina* L.) was sown in the tree rows before tree planting, on a width of 2 m. In the control plot and in the inter rows, a rotation of wheat (*Triticum aestivum* L. subsp. *aestivum*), rapeseed (*Brassica napus* L.), wheat and sunflower (*Helianthus annuus*) was cropped (Table I-2.2) since the tree planting. The mean yield was 8-8.5 t ha^{-1} for wheat, 3.3 t ha^{-1} for rapeseed and 2.5 t ha^{-1} for sunflower. Crop residues were most of the time collected, but counterbalanced by manure. Before crops were sown in spring (sunflower), a cover was established during winter to prevent soil erosion and nitrate leaching. This cover crop was a mix of radish (*Raphanus sativus* L.), phacelia (*Phacelia tanacetifolia* Benth.) and mustard (*Sinapis alba* L.). The soil was ploughed every year down to 20 cm soil depth. It has to be noted that at this site, the agroforestry system was established in a weak sloppy part of the field, while the control plot was in a flat area. The area where the agroforestry plot was established could have been eroded compared to the control plot.

The third site (SJ) was located in Saint-Jean-d'Angély (Fig. I-2.1), in the department of Charente-Martime (Longitude 0°13'57'' W, Latitude 46°00'39'' N, elevation 152 m a.s.l.). The average temperature is 12.9°C and the average annual rainfall is 850 mm (years 1990-2013, INRA CLIMATIK). The soil is a carbonated silty clay (Table I-2.1) Luvisol (IUSS Working Group WRB 2007). Black walnut (*Juglans nigra* L.) trees were planted in 1973 at a density of 102 trees ha^{-1}. The distance between two tree rows was 14 m and trees were planted every 7 m along the tree rows. Tree rows were 2 m wide and were covered by a natural herbaceous vegetation. In the control plot and in the inter rows, a rotation of sunflower (*Helianthus annuus* L.), wheat (*Triticum aestivum* L. subsp. *aestivum*) and barley (*Hordeum vulgare* L.) was cropped (Table I-2.2) since the tree planting. Crop residues were left in the field. The soil was ploughed every three years down to 10-20 cm soil depth.

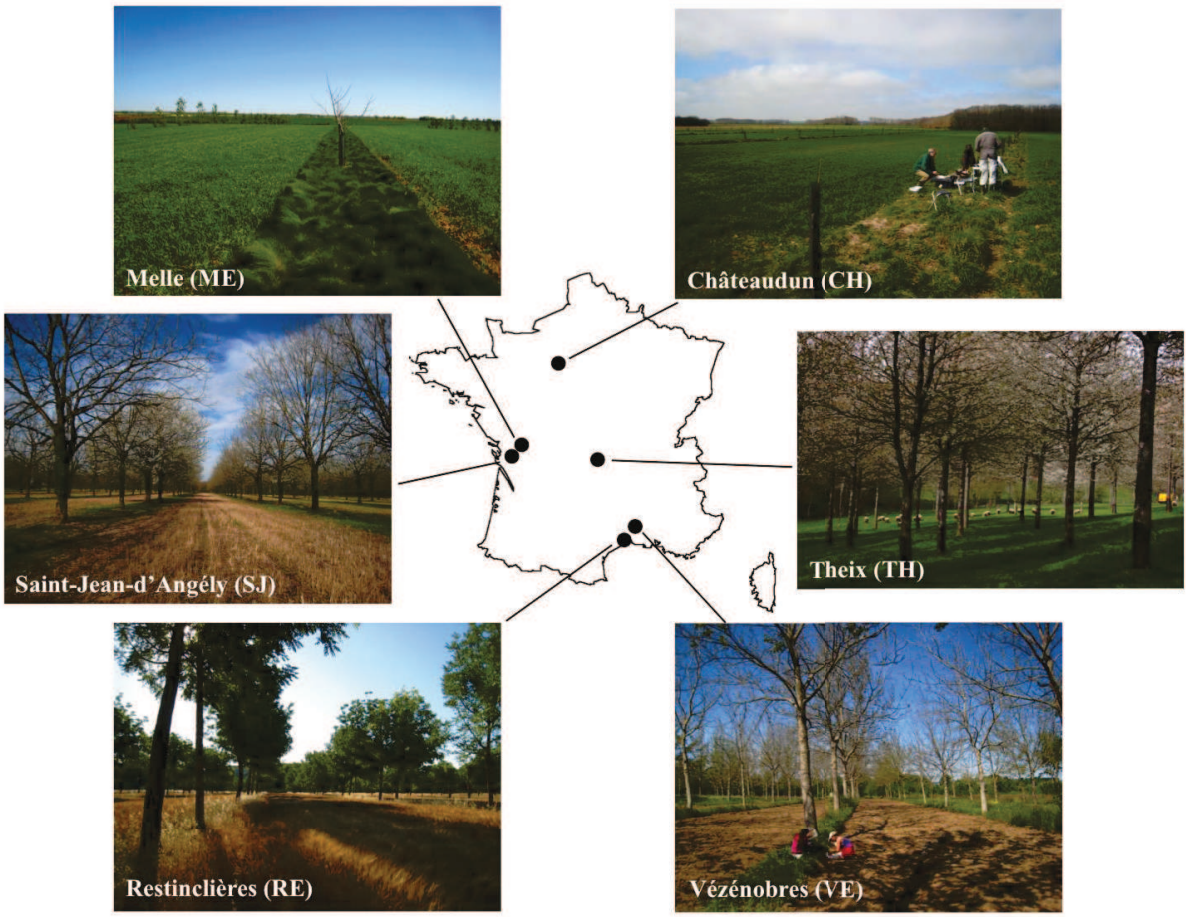

Figure I-2.1. Location and description of the six study cases under agroforestry systems sampled in France.

The fourth site (TH) was located in Theix (Fig. I-2.1), in the department of Puy-de-Dôme (Longitude 3°01'39'' E, Latitude 45°42'58'' N, elevation 829 m a.s.l.). The average temperature is 7.7°C and the average annual rainfall is 800 mm (years 1990-2013, INRA CLIMATIK). The soil is a clay loam (Table I-2.1) Andosol (IUSS Working Group WRB 2007). Wild cherry (*Prunus avium* L.) trees were planted in 1988 at a density of 200 trees ha^{-1} on a natural permanent pasture. The distance between two tree rows was 7 m and trees were planted every 7 m along the tree rows. There was no difference between tree rows and inter rows in terms of soil cover, permanent pasture occupied both areas (Table I-2.2). The permanent pasture was mainly composed of ryegrass (*Lolium perenne* L.) and fescue (*Festuca* L.) in both the control and in the agroforestry plots, and was regularly grazed by sheeps.

Site	Mean annual temperature (°C)	Mean annual pluviometry (mm)	Soil type (FAO)	Soil depth (cm)	Soil texture clay/silt/sand (g kg^{-1})		Soil pH
					Agroforestry	Control	
CH	11.1	595	Luvisol	0-30	200/700/100	190/710/100	7.0
ME	11.7	810	Luvisol	0-30	240/660/100	260/630/110	-
SJ	12.9	850	Luvisol	0-20	560/370/70	500/410/90	7.7
TH	7.7	800	Andosol	0-20	340/300/360	380/360/260	6.5
				20-50	320/280/400	360/380/260	6.5
VE	14.5	1037	Fluvisol	0-30	110/410/480	90/370/540	8.3
				30-60	100/440/460	80/370/550	8.3
RE	15.4	873	Fluvisol	0-30	173/406/421	176/413/411	8.0
				30-50	178/416/406	177/421/402	8.1
				50-70	250/501/249	243/507/250	8.2
				70-100	309/582/109	307/586/107	8.3

Table I-2.1. Site characteristics.

Site	Tree species	Age	Density (trees ha^{-1})	Distance between trees in tree rows (m)	Width of inter-rows (m)	Width of tree rows (m)	Area occupied by tree rows in the AF plot (%)	Crops
CH	*Juglans regia* × *nigra* cv. NG23	6	34	10	24	2	8	*Triticum aestivum; Brassica napus*
ME	*Juglans regia* × *nigra* cv. NG23	6	35	8	27	2	7	*Triticum aestivum; Brassica napus; Helianthus annuus*
SJ	*Juglans nigra*	41	102	7	12	2	14	*Helianthus annuus; Hordeum vulgare; Triticum aestivum*
TH	*Prunus avium*	26	200	7	-	-	-	*Lolium perenne; Festuca*
VE	*Juglans regia* × *nigra* cv. NG23	18	100	10	9	1	18	*Brassica napus; Triticum aestivu; Solanum tuberosum; Allium sativu; Helianthus annuus*
RE	*Juglans regia* × *nigra* cv. NG23	18	110	4-12	11	2	16	*Triticum durum; Brassica napus; Cicer arietinum*

Table I-2.2. Description of the agroforestry plots.

The fifth site (VE) was located in Vézénobres (Fig. I-2.1), in the department of Gard (Longitude 4°06'37'' E, Latitude 46°00'39'' N, elevation 102 m a.s.l.). The climate is sub-humid Mediterranean with an average temperature of 14.5°C and an average annual rainfall of 1037 mm (mean 1995-2007). The soil is a deep sandy loam (Table I-2.1) alluvial Fluvisol (IUSS Working Group WRB 2007). Hybrid walnut (*Juglans regia* × *nigra* cv. NG23) trees were planted in 1995 at a density of 100 trees ha^{-1}. The distance between two tree rows was 10 m and trees were planted every 10 m along the tree rows. Tree rows were 1 m wide and were covered by a natural herbaceous vegetation. In the inter rows, a rotation of rapeseed (*Brassica napus* L.) and wheat (*Triticum aestivum* L. subsp. *aestivum*) was cropped till 2010 (Table I-2.2). In 2011, the farm turned to an organic farm, and potatoes were planted (*Solanum tuberosum* L.). In 2012, inter rows were occupied by garlic (*Allium sativum* L.), in 2013 they were left in fallow, and in 2014 sunflower (*Helianthus annuus* L.) was sown. In the control plot, the same crops were grown, except in 2011 and 2012 where the field was occupied by wheat (*Triticum aestivum* L. subsp. *aestivum*) and a fallow, respectively. The soil was occasionally ploughed down to 20 cm soil depth.

The sixth site (RE) was located in Prades-le-Lez, at the Restinclières experimental site (Fig. I-2.1), in the department of Hérault (Longitude 04°01' E, Latitude 43°43' N, elevation 54 m a.s.l.). This site was fully described and studied by Cardinael et al. (2015a). Briefly, the climate is sub-humid Mediterranean with an average temperature of 15.4°C and an average annual rainfall of 873 mm (years 1995–2013). The soil is a deep carbonated sandy loam (Table I-2.1) Fluvisol (IUSS Working Group WRB 2007). Hybrid walnut (*Juglans regia* × *nigra* cv. NG23) trees were planted in 1995 at a current density of 110 trees ha^{-1} (Table I-2.2), and intercropped with durum wheat (*Triticum turgidum* L. subsp. *durum*). The distance between two tree rows was 13 m. Tree rows were 2 m wide and were covered by a natural herbaceous vegetation.

2.2.2 Soil sampling protocol

Agroforestry designs varied between sites, i.e., there were different spacing between tree rows and between trees along tree rows. Therefore, an adaptive sampling protocol was necessary, but at the same time it had to remain consistent to allow comparisons between sites. A sampling pattern was repeated three times in the agroforestry plots of all sites. The sampling pattern was defined by transects of sampling positions around one tree (Fig. I-2.2). Soil

samples were taken at fixed positions in all sites, at 1, 2 and 3 m from the tree, in the tree row, in the inter row in front of the tree, and in the inter row between two trees. Depending on the distance between two tree rows (L), soil samples were also taken at mid-distance $\frac{L}{2}$ and at $\frac{L}{4}$. If the distance between two trees in the tree row (d) was equaled or higher than 8 m, a soil sample was taken at mid-distance $\frac{d}{2}$. In the control plots, a sampling pattern was repeated three times. The sampling pattern was a rectangle with the dimensions $\frac{L}{2} \times \frac{d}{2}$. Soil samples were taken at each corner of the rectangle. Maximum sampling depth varied between sites, depending on soil depth. Soil samples were taken at least down to 30 cm soil depth, except for the SJ site where the soil was only 20 cm thick.

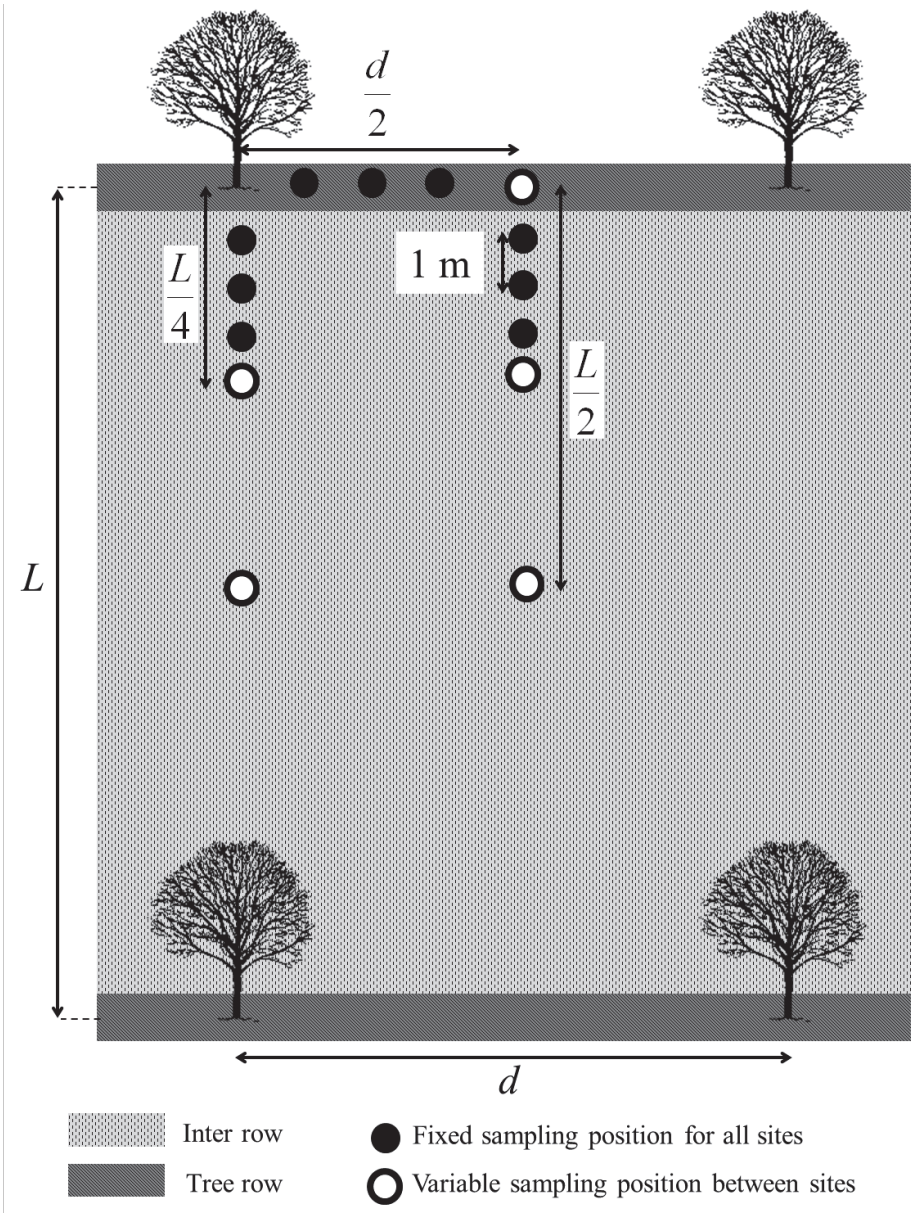

Figure I-2.2. Sampling pattern for the agroforestry sites.

2.2.3 Bulk densities determination

Soil samples were taken using a cylinder (500 cm^3), every 10 cm. Each soil sample was weighed in the field to determine its humid mass. After air-drying in the lab, soil samples were oven dried at 40°C for 48 hours and sieved at 2 mm, and weighed again without stones > 2 mm. A sub-sample of each soil sample (< 2 mm) was weighed and oven dried at 105°C for 48 hours. Soil moistures were calculated for each sub-sample as well as the dry mass (g) of soil samples. Bulk density was calculated as the ratio of dry mass of fine soil and cylinder volume.

2.2.4 Organic carbon analysis

Soil samples were ball milled until they passed a 200 µm mesh sieve. If soil contained inorganic carbon, carbonates were removed by acid fumigation, following Harris et al., (2001). It was the case for samples from SJ and RE sites. For this, 30 mg of soil was placed in open Ag-foil capsules. The capsules were then placed in the wells of a microtiter plate and 50 µL of demineralized water was added in each capsule. The microtiter plate was placed in a vacuum desiccator with a beaker filled with 100 mL of concentrated HCl. The samples were exposed to HCl vapors for 8 hours, and were then dried at 60°C for 48 hours. Capsules were then closed in a bigger tin capsule. All samples were analyzed for organic carbon concentration with a CHN elemental analyzer (Carlo Erba NA 2000, Milan, Italy).

2.2.5 SOC stock calculation

For each site, SOC stocks were calculated on an equivalent soil mass (ESM) basis (Ellert and Bettany 1995). This method allowed us comparing locations (control, tree row, inter-row) with different soil bulk densities within the same site. SOC stocks in the agroforestry plot were calculated multiplying tree row carbon stocks and inter-row carbon stocks by their respective surface area:

$$\text{SOC stock}_{Agroforestry} = \text{p} \times \text{SOC stock}_{Tree\ row} + 1 - \text{p} \times \text{SOC stock}_{Inter\ row} \quad (1)$$

where p is the percentage of the agroforestry plot occupied by tree rows (%, Table I-2.2), and SOC stocks are expressed in Mg C ha^{-1}.

SOC accumulation rates were calculated by dividing delta stocks between the agroforestry and the control plot by the number of years since the tree planting.

2.2.6 Tree aboveground and belowground biomass

At each site, 10 to 20 trees were measured to estimate the aboveground biomass. As it was not possible to chop down trees in farmers' fields, aboveground biomass was approximated multiplying the volume of trunk and branches by the wood density, using the global wood density database (Zanne et al. 2009; Chave et al. 2009). The volume of the trunk was approximated as the sum of the volume of three truncated cones (Fig. I-2.S1). For this purpose, diameter of the trunk was measured 5 cm above soil surface, at 1.30 m (Diameter at Breast Height, DBH) and beneath the first branch. Total height (H_{tot}) and merchantable height (H) of the trees were also measured. The volume of first order branches, i.e., directly inserted on the trunk, was also estimated. For this purpose, diameters at the insertion on the trunk and the length of the branches were measured, and volume was calculated as the volume of a cone. For the RE site, it was possible to chop down three trees to directly measure trunk and branches biomass. Carbon concentrations of trunk and branches were measured for *Juglans regia* × *nigra* cv. NG23. They were considered to be the same for *Prunus avium* and *Juglans nigra*.

To our knowledge, no allometric equation is available for temperate agroforestry trees (Tumwebaze et al. 2011). To have an estimate of the total belowground biomass of the trees at our sites, we used an equation proposed by Cairns et al., (1997), where the authors estimated a mean root:shoot ratio of 0.26 for temperate forests:

$$\mathrm{RB} = e^{-1.3267+0.8877*\ln AB +0.1045*\ln(Age)} \quad (2)$$

where RB is the total root biomass (Mg C ha^{-1}), AB is the aboveground biomass (Mg C ha^{-1}) and Age is the age of the plantation (years).

2.2.7 Statistical analysis

First, we analyzed the influence of sampling in the inter row in front of a tree or between two trees, on SOC concentrations, bulk densities and SOC stocks. For this purpose, we fitted a mixed effects model for each site, using the set of SOC concentrations, bulk densities and SOC stocks at the two locations in the inter row. We used the *nlme* package (Pinheiro et al. 2013). Soil core ID was considered as a random effect to take into account a sample effect. An ANOVA was performed on these models and revealed that sampling in the inter row in front of a tree or between two trees had no influence on the results (Table I-2.S1). Secondly,

we fitted a mixed effects model for each site, using the whole data set of SOC concentrations, bulk densities and SOC stocks. These soil properties were compared by depth, by location (control, tree row, inter row) and by distance to the closest tree. An ANOVA was performed on these models. All the statistical analyses were performed using R version 3.1.1 (R Development Core Team 2013), at a significance level of <0.05.

2.3 Results

2.3.1 Soil bulk densities

At all sites, bulk density increased with increasing soil depth (Table I-2.3, I-2.S2). The ME and RE sites had a lower topsoil bulk density under the tree row than under the inter row. In the first 30 cm, bulk density ranged from 0.7 to 1.6 g cm^{-3} depending on the site.

2.3.2 Soil organic carbon concentrations

SOC concentrations decreased with increasing soil depth, except in the ploughed layer where SOC concentration was homogeneous (Table I-2.3, I-2.S2). At all site, SOC concentrations were significantly higher in 0-10 cm in the tree row than in the inter row. For half of the sites (CH, SJ, RE), SOC concentrations in 0-10 cm were also higher in the inter row than in the control. At the VE and TH sites, the increase between the inter row and the control was only observed below 30 and 40 cm, respectively (Table I-2.3). SOC concentrations were only significantly dependent on the distance to trees in the oldest experimental site (SJ), this effect was not observed at any other site (Table I-2.S2).

2.3.3 Soil organic carbon stocks

SOC stocks in 0-10 cm were generally higher in the tree row than in the inter-row and in the control plot, except in TH site were not difference was found (Fig. I-2.3). SOC stocks decreased with increasing depth. Delta stocks between the agroforestry and the control ranged from 0 to 19 Mg ha^{-1} in 0-30 cm (Table I-2.4). In the grassland site (TH), no additional SOC storage was found in topsoil, the only difference was observed below 40 cm. A loss of SOC was found at the 6-year-old site ME.

		Bulk densities (g cm^{-3})			Soil organic carbon concentration (mg C g^{-1})		
Site	Soil depth (cm)	Tree-row	Inter-row	Control	Tree-row	Inter-row	Control
CH	0-10	1.09 ± 0.03 (12)	1.10 ± 0.02 (24)	1.18 ± 0.02 (12)	19.44 ± 1.00 (12)	16.44 ± 0.26 (24)	14.88 ± 0.38 (12)
	10-20	1.12 ± 0.02 (12)	1.13 ± 0.02 (24)	1.16 ± 0.03 (12)	13.58 ± 0.31 (12)	14.39 ± 0.34 (24)	14.56 ± 0.48 (12)
	20-30	1.15 ± 0.02 (12)	1.20 ± 0.01 (24)	1.25 ± 0.02 (12)	11.76 ± 0.65 (12)	12.07 ± 0.48 (24)	11.78 ± 0.35 (12)
ME	0-10	1.04 ± 0.03 (12)	1.28 ± 0.02 (24)	1.34 ± 0.02 (12)	21.30 ± 0.63 (12)	12.80 ± 0.19 (24)	13.23 ± 0.38 (12)
	10-20	1.28 ± 0.02 (12)	1.31 ± 0.02 (24)	1.31 ± 0.03 (12)	13.14 ± 0.26 (12)	12.02 ± 0.38 (24)	13.76 ± 0.48 (12)
	20-30	1.21 ± 0.01 (12)	1.34 ± 0.01 (24)	1.42 ± 0.01 (12)	10.35 ± 0.21 (12)	8.68 ± 0.41 (24)	8.95 ± 0.35 (12)
SJ	0-10	0.67 ± 0.03 (8)	0.76 ± 0.02 (16)	0.78 ± 0.01 (12)	58.60 ± 1.88 (8)	49.49 ± 1.28 (16)	32.89 ± 0.33 (12)
	10-20	0.84 ± 0.03 (8)	0.78 ± 0.03 (16)	0.88 ± 0.04 (12)	35.60 ± 0.82 (8)	32.01 ± 0.67 (16)	24.86 ± 1.12 (12)
TH	0-10	0.76 ± 0.03 (9)	0.75 ± 0.02 (18)	0.69 ± 0.02 (10)	61.32 ± 3.82 (9)	65.34 ± 3.08 (18)	67.83 ± 2.45 (10)
	10-20	0.78 ± 0.02 (9)	0.80 ± 0.02 (18)	0.75 ± 0.01 (10)	48.48 ± 2.09 (9)	46.22 ± 1.37 (18)	49.31 ± 0.89 (10)
	20-30	0.83 ± 0.04 (9)	0.79 ± 0.02 (18)	0.73 ± 0.02 (10)	39.58 ± 1.48 (9)	38.44 ± 1.12 (18)	40.56 ± 0.86 (10)
	30-40	0.84 ± 0.02 (9)	0.80 ± 0.02 (18)	0.78 ± 0.02 (10)	33.81 ± 0.86 (9)	32.45 ± 0.96 (18)	29.92 ± 0.75 (10)
	40-50	0.78 ± 0.02 (6)	0.81 ± 0.02 (13)	0.79 ± 0.03 (10)	29.23 ± 1.10 (6)	28.38 ± 1.24 (13)	22.69 ± 1.25 (10)
VE	0-10	1.06 ± 0.04 (12)	0.98 ± 0.03 (18)	0.91 ± 0.02 (10)	17.25 ± 0.49 (12)	15.95 ± 0.37 (18)	15.00 ± 1.11 (10)
	10-20	1.12 ± 0.02 (12)	1.18 ± 0.02 (18)	1.24 ± 0.03 (10)	13.72 ± 0.40 (12)	13.50 ± 0.49 (18)	13.19 ± 0.70 (10)
	20-30	1.16 ± 0.03 (12)	1.25 ± 0.01 (18)	1.31 ± 0.02 (10)	11.38 ± 0.30 (12)	10.83 ± 0.25 (18)	10.89 ± 0.68 (10)
	30-40	1.29 ± 0.04 (12)	1.39 ± 0.02 (18)	1.47 ± 0.04 (10)	10.82 ± 0.27 (12)	10.31 ± 0.29 (18)	8.55 ± 0.78 (10)
	40-50	1.30 ± 0.05 (12)	1.37 ± 0.03 (18)	1.34 ± 0.03 (10)	10.52 ± 0.33 (12)	8.25 ± 0.35 (18)	5.79 ± 0.69 (10)
	50-60	1.36 ± 0.04 (12)	1.39 ± 0.04 (18)	1.37 ± 0.06 (10)	9.74 ± 0.35 (12)	7.16 ± 0.62 (18)	5.28 ± 0.86 (10)
RE	0-10	1.10 ± 0.02 (40)	1.23 ± 0.03 (60)	1.41 ± 0.01 (93)	21.59 ± 0.76 (40)	9.78 ± 0.13 (60)	9.33 ± 0.06 (93)
	10-30	1.49 ± 0.01 (40)	1.60 ± 0.02 (60)	1.61 ± 0.00 (93)	10.16 ± 0.16 (40)	9.57 ± 0.12 (60)	8.94 ± 0.05 (93)
	30-50	1.71 ± 0.01 (40)	1.67 ± 0.02 (60)	1.73 ± 0.00 (93)	7.29 ± 0.15 (40)	6.95 ± 0.11 (60)	6.82 ± 0.10 (93)
	50-70	1.73 ± 0.01 (40)	1.77 ± 0.01 (60)	1.80 ± 0.00 (93)	6.07 ± 0.11 (40)	5.89 ± 0.07 (60)	5.77 ± 0.06 (93)
	70-100	1.68 ± 0.00 (40)	1.71 ± 0.00 (60)	1.74 ± 0.00 (93)	6.49 ± 0.16 (40)	6.29 ± 0.06 (60)	6.09 ± 0.06 (93)

Table I-2.3. Mean soil bulk densities (g cm^{-3}) and mean soil organic carbon concentrations (mg C g^{-1}) with associated standard errors. Numbers in brackets represent the number of replicates.

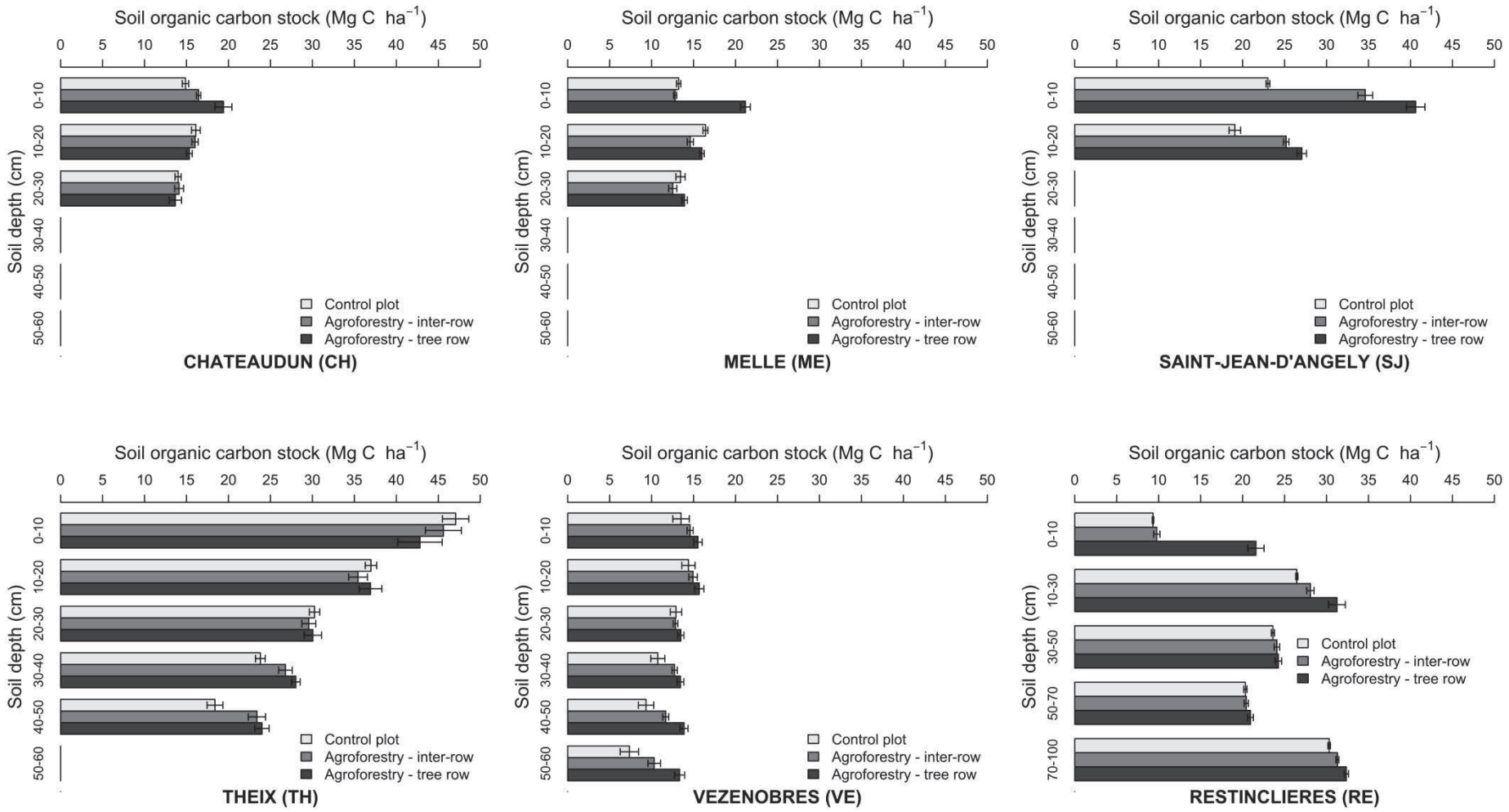

Figure I-2.3. Soil organic carbon stocks (Mg C ha^{-1}) at the different sites.

Site	Cumulated ESM (Mg ha^{-1})	Cumulated SOC stocks (Mg C ha^{-1})				Δ SOC stocks (Mg C ha^{-1})	SOC accumulation rates (kg C ha^{-1} yr^{-1})		
		Tree-row	Inter-row	AF	Control	AF – Control	AF/Control	Tree-row/Control	Inter-row/Control
CH	1000	19.4 ± 1.0	16.4 ± 0.3	16.7 ± 0.3	14.9 ± 0.4	1.8 ± 0.5*	301 ± 76*	756 ± 179*	261 ± 77*
	2100	34.8 ± 1.2	32.5 ± 0.5	32.7 ± 0.5	31.0 ± 0.9	1.7 ± 1.0*	276 ± 169*	627 ± 250*	245 ± 172*
	3250	48.4 ± 1.7	46.6 ± 1.0	46.7 ± 1.0	45.0 ± 1.1	1.7 ± 1.4*	290 ± 241*	573 ± 330*	265 ± 249*
ME	1000	21.2 ± 0.6	12.8 ± 0.2	13.4 ± 0.2	13.2 ± 0.3	0.2 ± 0.3	26 ± 53	1326 ± 106*	-72 ± 54*
	2200	37.2 ± 0.6	27.4 ± 0.5	28.1 ± 0.4	29.7 ± 0.5	-1.6 ± 0.7*	-259 ± 111*	1256 ± 135*	-373 ± 115*
	3500	51.1 ± 0.8	39.9 ± 0.8	40.7 ± 0.8	43.1 ± 0.8	-2.4 ± 1.1*	-397 ± 189*	1335 ± 192*	-528 ± 196*
SJ	700	40.6 ± 1.1	34.6 ± 0.9	35.5 ± 0.8	23.0 ± 0.2	12.4 ± 0.8*	303 ± 20*	429 ± 28*	283 ± 22*
	1450	67.7 ± 1.1	59.8 ± 1.0	60.9 ± 0.9	42.1 ± 0.8	18.8 ± 1.2*	459 ± 28*	624 ± 33*	432 ± 31*
TH	700	42.8 ± 2.7	45.6 ± 2.1	44.2 ± 3.4	47.1 ± 1.6	-2.9 ± 3.8	-110 ± 144	-	-
	1450	79.8 ± 3.8	81.1 ± 3.2	80.4 ± 5.0	84.1 ± 1.9	-3.7 ± 5.3	-140 ± 204	-	-
	2200	109.8 ± 4.7	110.6 ± 3.8	110.2 ± 6.1	114.3 ± 2.3	-4.1 ± 6.5	-157 ± 248	-	-
	3000	137.8 ± 4.9	137.4 ± 4.2	137.6 ± 6.5	138.2 ± 2.3	-0.5 ± 6.9	-18 ± 264	-	-
	3800	169.3 ± 4.9	169.3 ± 4.3	169.3 ± 6.5	156.5 ± 2.7	12.8 ± 7.0*	492 ± 271*	-	-
VE	900	15.5 ± 0.5	14.6 ± 0.4	14.8 ± 0.3	13.5 ± 1.0	1.3 ± 1.0*	69 ± 58*	112 ± 62*	60 ± 59*
	2000	31.2 ± 0.8	29.5 ± 0.8	29.8 ± 0.6	27.9 ± 1.5	1.9 ± 1.6*	107 ± 90*	183 ± 93*	90 ± 93*
	3150	44.7 ± 1.0	42.4 ± 0.9	42.8 ± 0.8	40.8 ± 2.0	2.0 ± 2.2	110 ± 119	214 ± 124*	87 ± 123
	4400	58.1 ± 1.2	55.1 ± 1.2	55.7 ± 1.0	51.8 ± 2.5	3.9 ± 2.7*	216 ± 151*	353 ± 155*	187 ± 155*
	5700	72.0 ± 1.5	66.8 ± 1.3	67.7 ± 1.1	61.2 ± 3.2	6.5 ± 3.4*	362 ± 190*	598 ± 197*	310 ± 194*
	7050	85.3 ± 1.9	77.1 ± 1.6	78.6 ± 1.4	68.6 ± 4.1	10.0 ± 4.3*	557 ± 241*	931 ± 251*	475 ± 246*
RE	1000	21.6 ± 1.0	9.8 ± 0.4	11.7 ± 0.3	9.3 ± 0.1	2.3 ± 0.4*	129 ± 20*	681 ± 54*	24 ± 21*
	4000	52.8 ± 1.4	37.9 ± 0.6	40.3 ± 0.5	35.8 ± 0.2	4.5 ± 0.6*	248 ± 31*	947 ± 78*	115 ± 33*
	7300	77.1 ± 1.5	62.0 ± 0.7	64.4 ± 0.6	59.4 ± 0.2	5.0 ± 0.6*	276 ± 36*	984 ± 81*	141 ± 39*
	10700	98.1 ± 1.5	82.4 ± 0.7	84.9 ± 0.6	79.7 ± 0.3	5.1 ± 0.7*	286 ± 39*	1017 ± 84*	147 ± 43*
	15700	130.4 ± 1.5	113.7 ± 0.7	116.4 ± 0.7	110.1 ± 0.3	6.3 ± 0.7*	350 ± 41*	1131 ± 86*	202 ± 45*

Table I-2.4. Soil organic carbon stocks (Mg C ha^{-1}) and additional SOC storage rates (kg C ha^{-1} yr^{-1}). Associated errors are standard errors. Agroforestry SOC stock = p×Tree-row SOC stock + (1-p)×Inter-row SOC stock, with p is the percentage of the agroforestry plot occupied by tree rows (%). ESM = Equivalent Soil Mass. Significantly (p-value<0.05) different SOC stocks are followed by different letters. Additional SOC storage rates significantly different from zero are followed by *.

2.3.4 Carbon stocks in the tree aboveground biomass

Carbon concentrations of the trunk and branches of *Juglans regia* × *nigra* cv. NG23 were 44.57 ± 0.10 and 42.86 ± 0.17 mg C g^{-1}, respectively. Organic carbon stocks in the trees ranged from 0.5 to 266 kg C $tree^{-1}$ between the different sites (Table I-2.5). Total carbon stocks in the tree aboveground biomass, depending on the tree density and the age since planting, ranged from 0.02 to about 37 Mg C ha^{-1}.

2.3.5 Soil and total organic carbon accumulation rates

SOC accumulation rates ranged from 0 to 290 kg C ha^{-1} yr^{-1} in 0-30 cm, and was 460 kg C ha^{-1} yr^{-1} in 0-20 cm for the SJ site (Table I-2.4). Tree rows contributed for about 20 to 50% of these SOC accumulation rates while they represented only 7 to 18% of the agroforestry plot. The mean SOC accumulation rate was 142 ± 146 kg C ha^{-1} yr^{-1} in 0-30 cm for alley cropping systems (all sites except the grassland site, TH). In young plantations, biomass C accumulation rate was negligible, and became important in old and dense plantations, reaching more than 1.5 Mg C ha^{-1} yr^{-1} in TH and VE sites (Table I-2.5).

2.3.6 Influence of site and system characteristics on SOC accumulation

When comparing tree rows to the control plots, a clear positive relationship between delta SOC stock and plantation age and clay content was observed (Fig. I-2.4a), as well as with total aboveground biomass except for the VE site (sandy soil). When comparing inter-rows to the control plots and agroforestry plots to the control plots, relationships were weaker (Fig. I-2.4b, I-2.S2), but still positive for the plantation age.

Site	DBH (cm)	Height of merchantable timber (m)	Total height (m)	C stock of merchantable timber (kg C $tree^{-1}$)	ABG C stock (kg C $tree^{-1}$)	ABG C stock (Mg C ha^{-1})	Estimated BEG C stock (Mg C ha^{-1})	ABG accumulation rates (kg C ha^{-1} yr^{-1})	Estimated total biomass accumulation rates (kg C ha^{-1} yr^{-1})
CH	2.6 ± 0.2	1.45 ± 0.04	2.12 ± 0.11	0.44 ± 0.06	0.49 ± 0.07	0.017 ± 0.002	0.01 (0.01-0.01)	3 ± 0	4 ± 0
ME	5.5 ± 0.3	1.13 ± 0.03	3.18 ± 0.13	1.18 ± 0.12	2.07 ± 0.19	0.073 ± 0.007	0.03 (0.03-0.04)	12 ± 1	17 ± 1
SJ	29.9 ± 1.3	3.11 ± 0.23	13.18 ± 0.10	41.44 ± 2.36	194.56 ± 14.94	19.85 ± 1.52	5.55 (3.28-9.38)	484 ± 37	619 ± 101
TH	30.7 ± 1.4	4.10 ± 0.23	14.70 ± 0.32	53.80 ± 1.76	183.46 ± 2.66	36.69 ± 0.53	9.13 (5.34-15.63)	1411 ± 20	1762 ± 251
VE	31.7 ± 1.5	4.17 ± 0.18	15.52 ± 0.36	56.85 ± 3.77	266.44 ± 19.90	26.64 ± 1.99	6.61 (4.00-10.95)	1480 ± 111	1848 ± 265
RE	25.5 ± 1.4	4.49 ± 0.39	11.21 ± 0.65	46.23 ± 2.47	98.93 ± 7.80	10.88 ± 0.86	2.99 (1.89-4.72)	604 ± 48	770 ± 107

Table I-2.5. Tree characteristics, aboveground and belowground carbon stocks at the different sites. ABG = Aboveground, BEG = Belowground. Errors represent standard errors. Number of measured trees: CH=24, ME=20, SJ=10, TH=10, VE=10, and RE=9 except for biomass measurements where n=3. Values in brackets represent the 95% prediction interval for the estimation of the belowground biomass (Cairns et al. 1997).

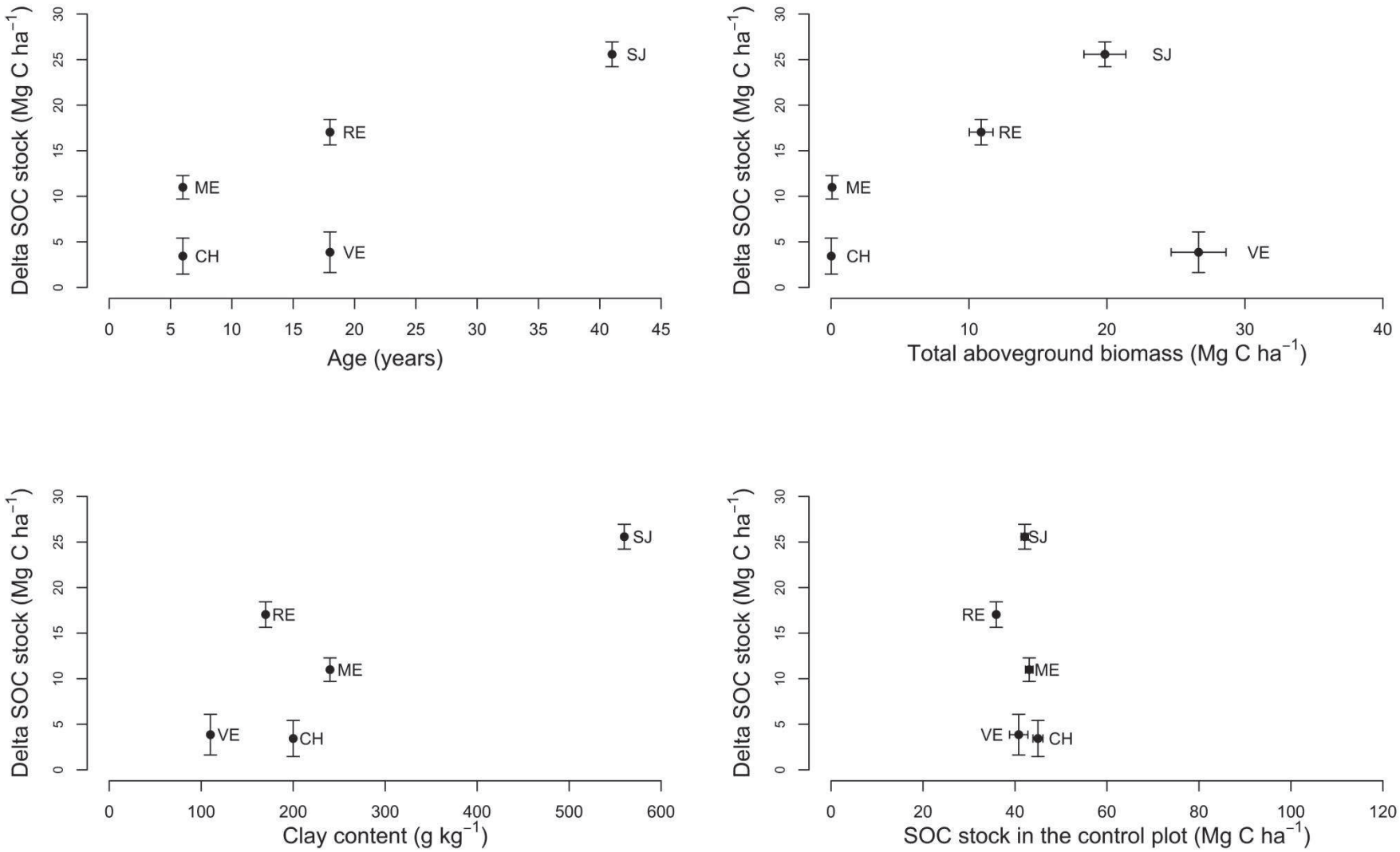

Figure I-2.4a. Difference of SOC stock (Mg C ha^{-1}) between the tree row and the control plot as a function of different variables. Values are for 0-30 cm, except for the SJ site (0-20 cm, maximum soil depth).

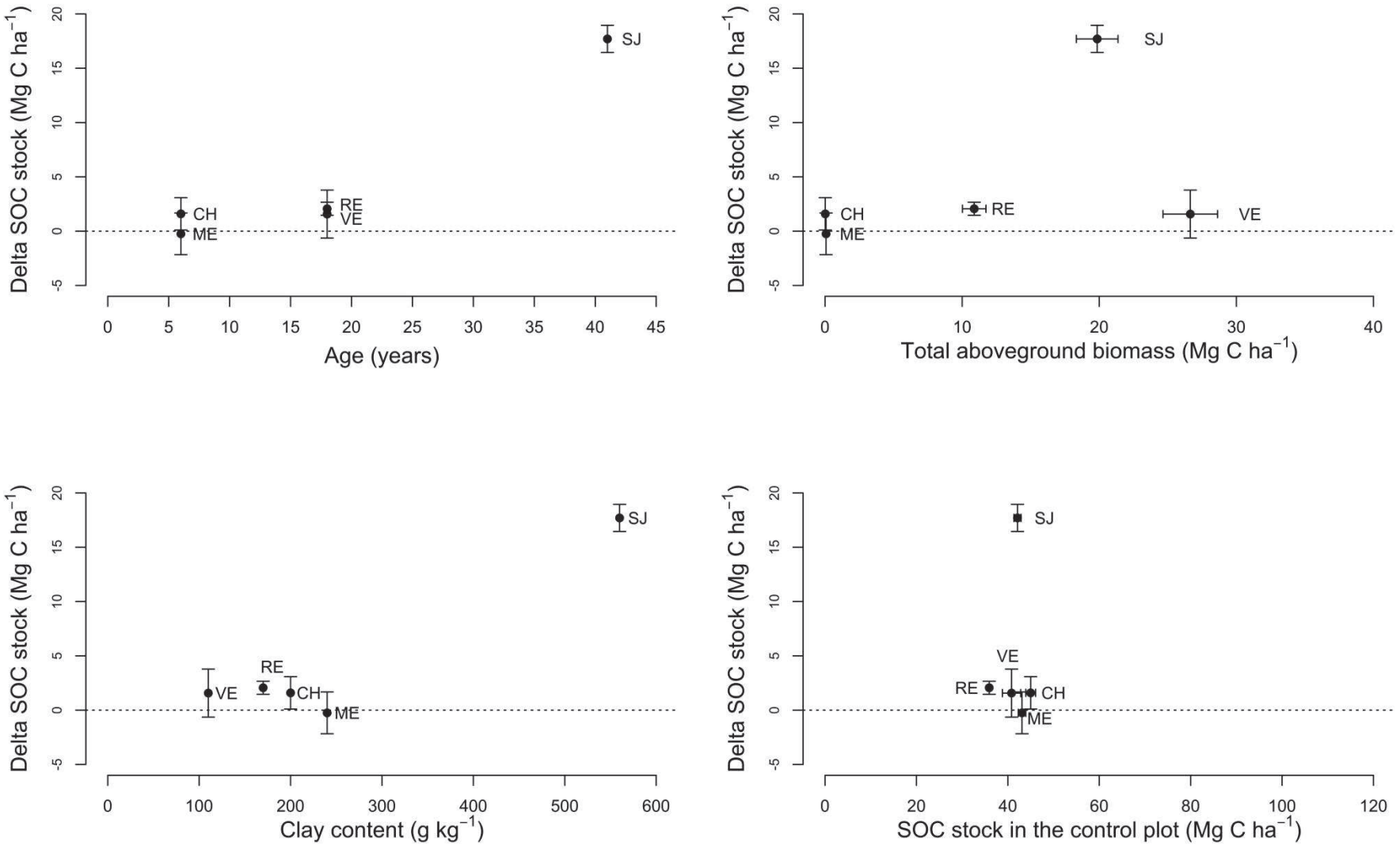

Figure I-2.4b. Difference of SOC stock (Mg C ha^{-1}) between the inter-row and the control plot as a function of different variables. Values are for 0-30 cm, except for the SJ site (0-20 cm, maximum soil depth).

2.4 Discussion

2.4.1 SOC accumulation rates

The negative difference of SOC stocks between the agroforestry and the agricultural system at the ME site is without doubt due to a bad choice for the control plot. The area where the agroforestry plot was established was probably eroded compared to the control plot before the establishment of the experiment. Tree rows were spaced of 29 m, and trees were 3 m height and were 6 year old. We can therefore assume that the middle of the inter-row is not yet affected by the presence of the trees. Using soil samples taken in the middle of the inter-row as controls, we observe a significant additional storage in 0-20 cm, with an accumulation rate of 331 ± 166 kg C ha^{-1} yr^{-1} and a positive but not significant accumulation rate of 90 ± 304 kg C ha^{-1} yr^{-1} in 0-30 cm, mainly due to SOC storage in the tree rows. With this correction, we have a mean SOC accumulation rate of 239 ± 67 kg C ha^{-1} yr^{-1} in 0-30 cm instead of 142 ± 146 kg C ha^{-1} yr^{-1} previously estimated. This re-evaluation appears more correct if we consider the control plot in ME was not adequate. This estimate is a bit lower than previously suggested for agroforestry systems in France (0.3 Mg C ha^{-1} yr^{-1}) by Pellerin et al., (2013) but is in the same order of magnitude. This estimate is also a bit lower than reported by Oelbermann et al. (2006) in a Canadian alley cropping system associating hybrid poplars (*Populus deltoides* × *nigra* DN-177) and a wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), and maize (*Zea mays* L.) rotation. These rates were 0.30 and 0.39 Mg C ha^{-1} yr^{-1} in 0-20 and 0-40 cm, respectively. Globally, our estimate is lower than most of published research on this topic (Lorenz and Lal 2014). For instance, Peichl et al. (2006) reported a SOC accumulation rate of 1.04 Mg C ha^{-1} yr^{-1} in 0-20 cm in a hybrid poplar-barley (*Hordeum vulgare* L) agroforestry system. But together with Cardinael et al. (2015a), we provide the first estimation of SOC using an equivalent soil mass basis, a more accurate method when soil bulk density is modified with land use change (Ellert and Bettany 1995; Ellert et al. 2002), which is the case in agroforestry systems, especially in tree rows.

2.4.2 Tree rows and SOC storage

The permanent cover in the tree rows had an important impact on SOC accumulation rates, contributing up to 50% of additional SOC storage in the agroforestry plot compared to agricultural plot. This herbaceous vegetation can be compared to a permanent grassland, a type a land use that was showed to be very efficient in terms of SOC storage (Conant et al. 2001; Soussana et al. 2004). No clear difference was observed between sowed and natural

vegetation, even if the highest SOC accumulation rate was a sowed grass (ME site, 1.3 Mg ha^{-1} yr^{-1}). However, management of these tree rows seems to be a key factor to increase the ability of agroforestry systems to store carbon. Several studies showed that increasing species richness and functional composition of grasslands had a positive impact on productivity, and on SOC storage (Tilman et al. 2001; Steinbeiss et al. 2008; Marquard et al. 2009; Lange et al. 2015; Prieto et al. 2015b). Compared to grassland, vegetation in the tree rows is usually not harvested due to difficulties of mechanization around the trees, and therefore not economically productive. But it should be possible for instance to introduce grazing animals in tree rows during a given season in the year, enhancing productivity and economical returns of these systems while contributing to SOC storage and biodiversity enhancement. Some farmers in France already experiment hazel or red berries production between timber trees in the tree rows, or short rotation woody crops, or even multispecific flower covers in order to produce honey. In any case, agroforestry systems can store SOC fast after the establishment of the system through the permanent cover in the rows, an indirect consequence due to the plantation of trees in parallel rows. Trees will take over in the C storage process on the long term, both in soils and in the biomass. This characteristic of alley cropping agroforestry systems has to be taken into account for the assessment of the potential of these systems to store C, but also for modeling purposes.

2.4.3 No additional SOC storage in C-saturated soils

The agroforestry system established in an andosol on a permanent grassland showed no additional SOC in the first 40 cm compared to a treeless grassland. This site had been a pasture for decades, SOC contents are very high and the soil was probably saturated in C, meaning that the soil could not store additional carbon (Hassink 1997). In an andosol, Dube et al., (2012) also found no effect of a silvopastoral system compared to a natural pasture on SOC to 40 cm depth. At our site, we only found a significant effect of agroforestry on SOC stocks between 40 and 50 cm. We suggest that deep soil layers in grasslands are less C-saturated than topsoil layers, and tree roots can therefore contribute to additional storage at depth. Haile et al., (2010) also found an impact of trees at depth in silvopastoral systems. Besides SOC, pastures grown in silvopastoral systems are less sensitive to shade than annual crops grown in agroforestry systems, and can accommodate a higher tree density (Benavides et al. 2009; Devkota et al. 2009), resulting in high C stocks in the tree biomass.

2.4.4 Carbon storage in the tree biomass

Carbon stocks in the aboveground biomass were high, and reached up to 35 Mg C ha^{-1} at one of our sites. The residence time of C in the harvested biomass will depend on the fate of woody products, but can reach many decades for timber wood (Profft et al. 2009; Bauhus et al. 2010). Branches that are not suited for timber production could instead be used as a substitution of fossil fuel to produce energy (Marland and Schlamadinger 1997). Replacing fossil fuels by renewable biomass produced in agroforestry has a great potential, providing that bioenergy is used locally to avoid transport emissions (Kürsten and Burschel 1993; Pandey 2002; Cardinael et al. 2012).

2.4.5 A long-term SOC storage?

In a parallel study performed at the RE site, Cardinael et al., (2015) found that additional SOC storage was mainly due to particulate organic matter (50–200 and 200–2000 μm), which are rather labile fractions (Balesdent et al. 1998). But particle-size fractionation should be performed in other agroforestry systems to allow a generalization of this phenomenon. Stabilization process may be different depending on the soil type, especially in andosols rich in allophanes (Chevallier et al. 2010). However, calculation of SOC saturation at the RE site revealed a high deficit of SOC compared to the theoretical value, suggesting that accumulation of SOC due to the agroforestry system could continue for decades before reaching saturation (Cardinael et al. 2015a). The location of an important part of additional SOC storage in the tree rows and in the topsoil layers make the storage vulnerable to future land use change. Additional carbon will be stored as long as agroforestry systems are maintained, but will be lost if tree rows are cultivated after the tree harvest. At the RE site, it was possible to sample at depth and additional SOC storage was found up to 1 m depth (Cardinael et al. 2015a), making this storage less vulnerable than in topsoil. Further work is needed on this aspect on a wide range of agroforestry systems and in different pedo-climatic conditions.

2.5 Conclusion

This study showed the general potential of agroforestry systems to increase SOC stocks as well as storing a lot of C in wood products under temperate regions. However, recent studies

demonstrated that inorganic nutrient availability was critical to store SOC in a stable form (Kirkby et al. 2013; Kirkby et al. 2014; de Vries 2014). Studied agroforestry systems were managed in conventional farming, i.e, using chemical fertilizers. Future experimental sites in agroforestry should be implemented using nitrogen fixing trees. These trees may have the potential to reduce the need for N fertilizer for the crop while enhancing the formation of stable SOC, and improving the global carbon budget of the field. Further work should therefore study the coupling of C, N but also P cycles in agroforestry systems.

2.6 Supplementary material

	Soil organic carbon content		Bulk density		Soil organic carbon stock	
Site	F-value	Pr(>F)	F-value	Pr(>F)	F-value	Pr(>F)
CH	0.0509	0.8223	1.8220	0.1824	0.1901	0.6645
ME	0.4870	0.4880	4.9490	0.0301*	1.1160	0.2951
SJ	0.4000	0.5339	0.0231	0.8807	0.1402	0.7118
TH	1.0188	0.3167	0.6800	0.4127	0.6969	0.4070
VE	0.0932	0.7609	0.0390	0.8439	0.1027	0.7494

Table I-2.S1. Comparison between transects in the inter-row, in front of a tree or between two trees. ANOVA on the LME model for SOC content, bulk density and SOC stock as a function of the location in the inter-row (in front of a tree vs. between two trees). Asterisks indicate significance levels where * P=0.05, ** P=0.01 and *** P <0.001

		Soil organic carbon content		Bulk density		Soil organic carbon stock	
Site		F-value	Pr(>F)	F-value	Pr(>F)	F-value	Pr(>F)
CH	Depth	64.982	<0.0001***	10.956	0.0001***	22.341	<0.0001***
	Location	2.246	0.137	3.153	0.079	1.890	0.173
	Distance	0.394	0.532	0.266	0.607	0.379	0.540
	Depth×Location	8.078	0.0006***	0.672	0.513	6.908	0.002**
	Depth×Distance	0.576	0.564	0.296	0.744	0.570	0.568
	Location×Distance	0.227	0.635	0.226	0.636	0.472	0.494
ME	Depth	140.956	<0.0001***	20.473	<0.0001***	24.004	<0.0001***
	Location	130.363	<0.0001***	78.246	<0.0001***	116.989	<0.0001***
	Distance	0.012	0.911	7.257	0.008**	0.016	0.900
	Depth×Location	51.699	<0.0001***	15.888	<0.0001***	45.731	<0.0001***
	Depth×Distance	1.627	0.202	1.910	0.154	2.895	0.063
	Location×Distance	0.004	0.949	0.162	0.688	0.144	0.705
SJ	Depth	370.623	<0.0001***	7.285	0.0104*	284.905	<0.0001***
	Location	35.543	<0.0001***	0.356	0.554	33.719	<0.0001***
	Distance	15.183	0.0004**	0.691	0.411	8.827	0.005**
	Depth×Location	6.719	0.014*	6.305	0.017*	9.250	0.004**
	Depth×Distance	4.101	0.0501	7.985	0.008**	10.264	0.002**
	Location×Distance	0.987	0.327	1.534	0.223	0.728	0.399
TH	Depth	89.206	<0.0001***	2.739	0.033*	59.624	<0.0001***
	Location	0.040	0.842	0.577	0.449	0.032	0.859
	Distance	1.511	0.222	6.966	0.010**	0.446	0.506
	Depth×Location	0.673	0.612	0.817	0.517	0.622	0.648
	Depth×Distance	0.225	0.924	0.750	0.560	0.341	0.850
	Location×Distance	0.235	0.629	1.663	0.200	0.001	0.975
VE	Depth	110.547	<0.0001***	39.920	<0.0001***	19.071	<0.0001***
	Location	24.017	<0.0001***	5.956	0.016*	23.272	<0.0001***
	Distance	0.001	0.980	0.674	0.413	0.083	0.773
	Depth×Location	2.801	0.019	1.998	0.082	2.243	0.053
	Depth×Distance	0.086	0.994	0.917	0.472	0.151	0.980
	Location×Distance	0.278	0.599	0.095	0.758	0.075	0.785

Table I-2.S2. ANOVA on the LME model for SOC content, bulk density and SOC stock in the agroforestry plots as a function of the depth, location (inter-row or tree row), distance to the closest tree, and interactions. Asterisks indicate significance levels where * P=0.05, ** P=0.01 and *** P <0.001

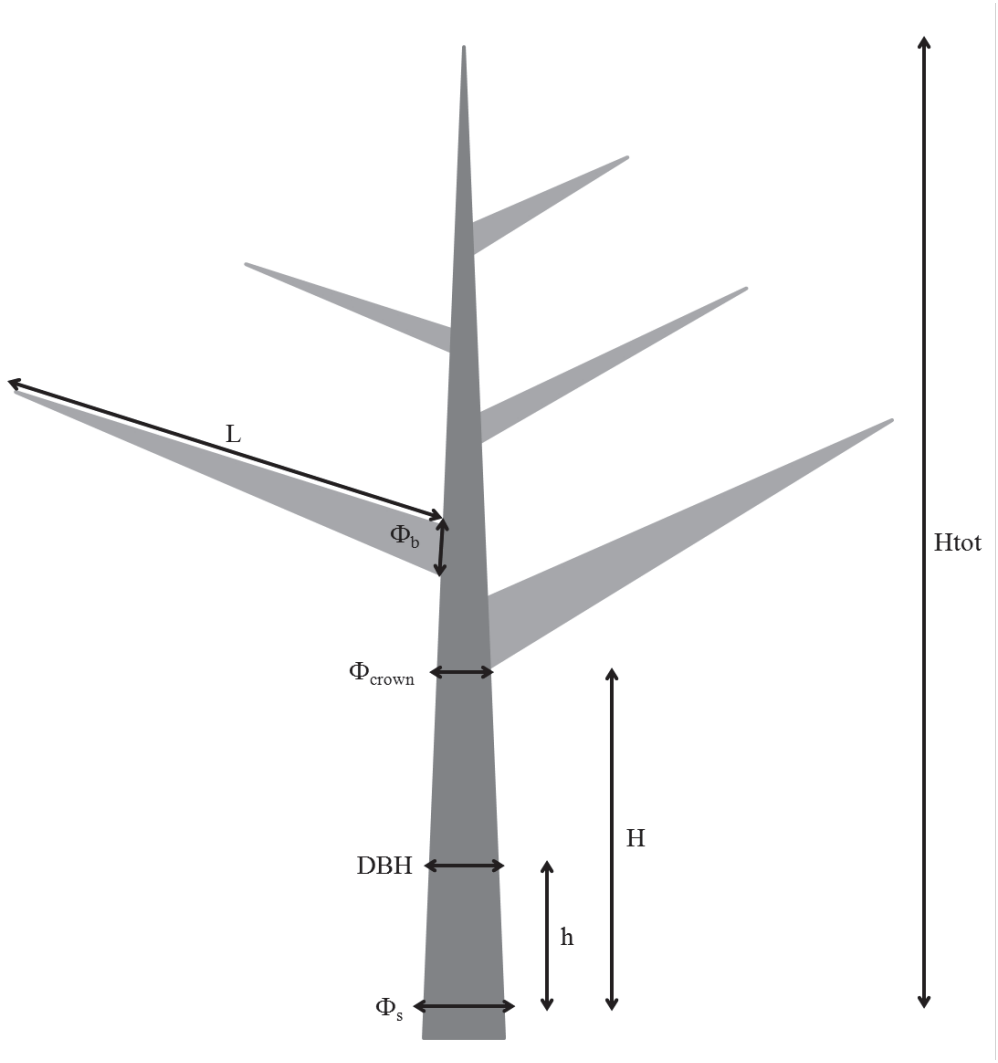

Figure I-2.S1. Schematic representing the method used to estimate aboveground tree biomass. Htot: total tree height (m); H: height of merchantable timber (m); h: height at diameter at breast height (m). Φ_s: trunk diameter (m) 5 cm above soil ground. DBH: diameter at breast height (m); Φ_{crown}: trunk diameter below the first branche (m). Φ_b: diameter of first order branches (m); L: length of first order branches (m).

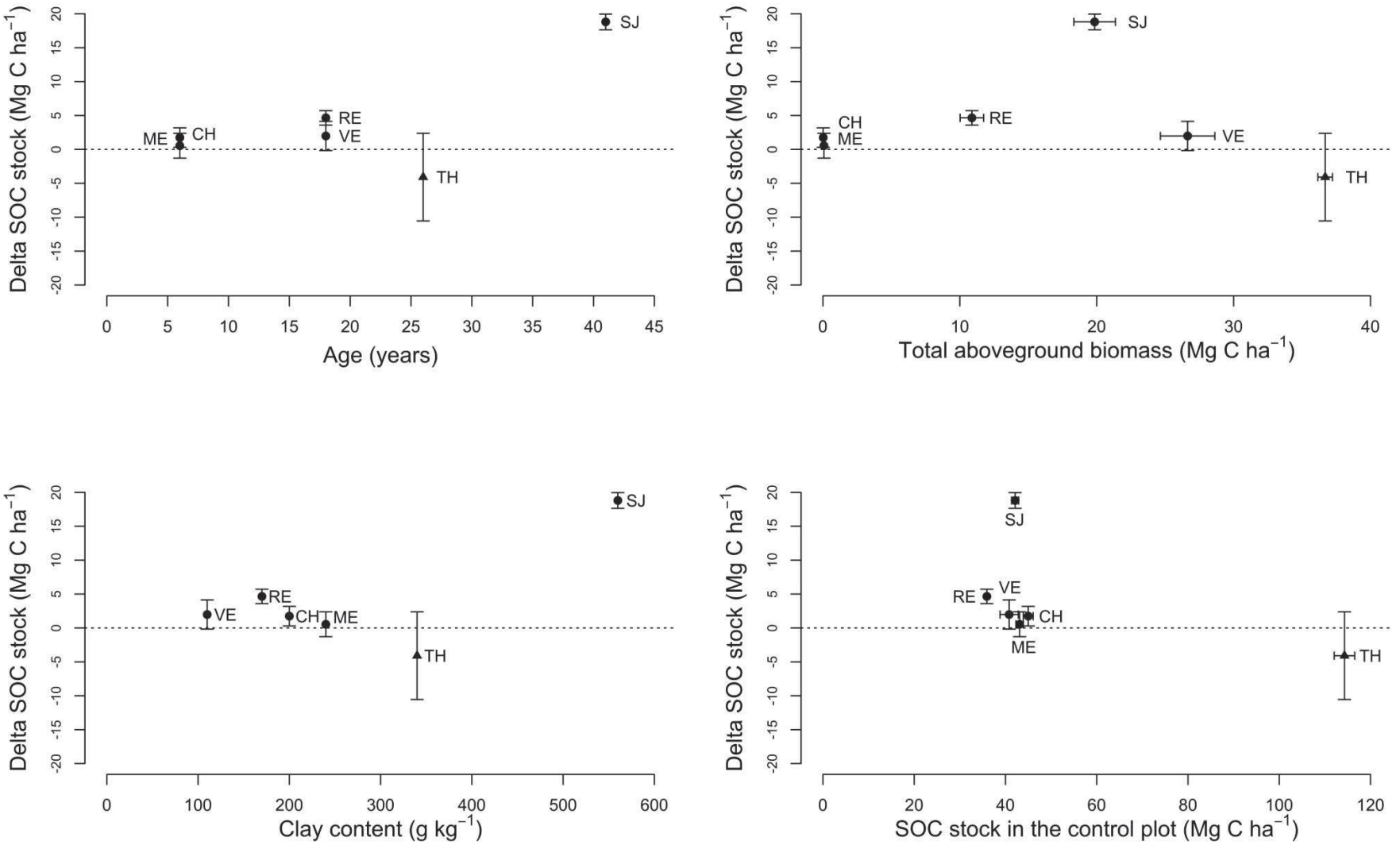

Figure I-2.S2. Difference of SOC stock (Mg C ha^{-1}) between the agroforestry and the control plot as a function of different variables. Values are for 0-30 cm, except for the SJ site (0-20 cm, maximum soil depth). Circles: alley cropping systems; Triangles: silvopastoral system (TH).

Partie II :

Apports de matières organiques au sol dans un système agroforestier sous climat méditerranéen

Chapitre 1

Distribution des racines fines des arbres dans un système agroforestier

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Competition with winter crops induces deeper rooting of walnut trees in a Mediterranean alley cropping agroforestry system

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Abstract

Characterising the spatial distribution of tree fine roots (diameter ≤ 2 mm) is fundamental for a better understanding of belowground functioning when tree are grown with associated crops in agroforestry systems. Our aim was to compare fine root distributions and orientations in trees grown in an alley cropping agroforestry stand with those in a tree monoculture. Fieldwork was conducted in two adjacent 17 year old hybrid walnut (*Juglans regia* × *nigra* L.) stands in southern France: the agroforestry stand was intercropped with durum wheat (*Triticum turgidum* L. subsp. *durum*) whereas the tree monoculture had a natural understorey. Root intercepts were mapped to a depth of 150 cm on trench walls in both stands, and to a depth of 400 cm in the agroforestry stand in order to characterise tree root distribution below the crop's maximum rooting depth. Soil cubes were then extracted to assess three dimensional root orientation and to establish a predictive model of root length densities (RLD) derived from root intersection densities (RID). In the tree monoculture, root mapping demonstrated a very high tree RID in the top 50 cm and a slight decrease in RID with increasing soil depth.

However, in the agroforestry stand, RID was significantly lower at 50 cm, tree roots colonized deeper soil layers and were more vertically oriented. In the agroforestry stand, RID and RLD were greater within the tree row than in the inter-row. Fine roots of intercropped walnut trees grew significantly deeper, indicating a strong plasticity in root distribution. This plasticity reduced direct root competition from the crop, enabling trees to access deeper water tables not available to crop roots.

1.1 Introduction

Fine roots of trees, usually defined as those with a diameter ≤ 2 mm (Trumbore and Gaudinski 2003), play a fundamental role in the provision of multiple services in tree-based agroecosystems. The absorptive function of fine roots for water and nutrients (Hinsinger 2001; Newman and Hart 2006) is closely associated with aboveground tree performance, and is thus essential for wood and fruit production in mixed intercropping systems (i.e. trees grown in association with an annual crop). Fine roots are also the most active part of tree root systems with regard to carbon dynamics, mainly through production, respiration, exudation and decomposition (Norby et al. 1987; Desrochers et al. 2002; Marsden et al. 2008; Hobbie et al. 2010), and thus can play a major role in carbon sequestration (Kuzyakov and Domanski 2000; Rasse et al. 2005), especially in agroforestry systems (Nair et al. 2010; Haile et al. 2010).

To better evaluate the services of tree-based agroecosystems, characterising the spatial distribution of fine roots is vital (Moreno et al. 2005; Mulia and Dupraz 2006; Upson and Burgess 2013). In mixed trees and crop systems, the aboveground performance of trees is linked to the amount of competition experienced, especially with regard to root systems. This competition depends largely on the spatial distribution of roots which is modified if competition is high (Mulia and Dupraz 2006). Spatial root distribution and density have been studied considerably in monocultural tree stands using both observational and modelling approaches (Hoffmann and Usoltsev 2001; Zianis et al. 2005). By using simple statistical tools and establishing allometric equations, several studies found that root density increases significantly with greater tree size and decreases with distance to tree stem and increasing soil depth (see Hoffmann and Usoltsev 2001; Zianis et al. 2005). We hypothesize that root competition between trees and crops in mixed systems will lead to differences in horizontal and vertical root distributions.

In temperate agroforestry systems, crop species are usually cultivated between parallel tree rows in strips (Torquebiau 2000). This sort of system is described as an alley cropping agroforestry system, and has become increasingly popular in Europe as it has the capacity to optimize nutrient and water cycles and provide multiple ecosystem services (Quinkenstein et al. 2009). However, in alley cropping systems, tree root distributions may be constrained both vertically and horizontally, due to competition with crop roots (Casper and Jackson 1997; Fernández et al. 2008), that could reduce the availability of water and nutrients in the soil (Schroth 1995; van Noordwijk et al. 1996). Belowground competition of roots from different species have been described in intercropped agricultural fields with two or more herbaceous species (Ozier-Lafontaine et al. 1998, Li et al. 2001, Li et al. 2006, Gao et al. 2010 and Neykova et al. 2011), but has been seldom examined between trees and crops (but see Mulia and Dupraz 2006; Wang et al. 2014). This knowledge gap may hinder our understanding of ecological interactions between species and their consequences for providing ecosystem services, as well as developing sustainable management strategies in the context of climate change. By considering the fine root distribution of trees as a proxy of root competition, existing studies on alley cropping agroforestry systems have found the root interaction between trees and annual crops to be very complex both in time and space (Mulia and Dupraz 2006; Wang et al. 2014). In particular, trees intercropped with annual crops tend to have deeper root systems and greater root length densities (RLD) beneath the root systems of the neighbouring crop (Mulia and Dupraz 2006). We hypothesize that deeper roots will permit trees to obtain nutrients and water not available to crops. However, a better quantification of tree root distribution is needed to understand the complex interactions between trees and crops.

Traditionally, studies on tree root distribution related to crops are usually based on data obtained from soil coring. Considered as the most routine approach to detect root spatial distribution (van Noordwijk et al. 2000), root coring is not very laborious and can attain profound soil depths (van Noordwijk et al. 2000; Saint-André et al. 2005; Christina et al. 2011). However, coring is difficult to carry out when soils are extremely dry and stony such as is usually the case in Mediterranean climates. Another negative aspect of root coring is that it cannot be used determine the spatial variability of root patches in soil, since this method is discontinuous in the horizontal space (several cores and extrapolation techniques would be needed to do this). Root-profiling methods have therefore been developed to complement or replace coring techniques. Using root-profiling techniques, root maps can be created by

manually counting roots intersecting the soil profile in a trench and the distribution of root patches on these maps can be characterised using geo-statistical methods (Laclau et al. 2013).

The study of fine root spatial distributions has been limited mainly to shallow soil depths, but the distribution of roots in deep soils, defined as those located at depths below 1.0 m (Maeght et al. 2013), has rarely been studied (but see Christina et al. 2011; Laclau et al. 2013). The lack of data concerning deep root spatial distributions can be explained by the difficulties associated with sampling in the field, especially when using root-profiling techniques (Maeght et al. 2013). When soil depth exceeds 1.0 m, excavation of a root profile becomes tedious and even dangerous, as soil walls are more prone to collapse. Using these and other methods, it has been shown that tree roots can extend to depths below 20 m (Haase et al. 1996; Hubble et al. 2010; Bleby et al. 2010), but these studies remain descriptive and lack a detailed characterisation of deep root distribution. Roots in deep soils perform important functions in particular with regard to mechanical anchorage, carbon sequestration, water uptake and transport (Stokes et al. 2009; Prieto et al. 2012; Maeght et al. 2013). Thus, it is important to characterise deep root distributions in contrasting ecosystems to understand the potential implications for ecosystem functioning and services.

Similarly to root density, root orientation is considered as an important trait related to the plant capacity to absorb water and nutrients (Nobel and Alm 1993; Ho et al. 2004). Root orientation is influenced by gravity, distribution of water (Cassab et al. 2013) and nutrients in the soil (Bonser et al. 1996). Compared to shallow roots, deep roots are more likely to uptake soil water supplied by water tables (Chen and Hu 2004) if not too deep. We therefore hypothesize that deep fine roots will be more vertically oriented than shallow roots due to the hydrotropism (Cassab et al. 2013). The spatial variability of preferential root orientation, or anisotropy, has been rarely studied, especially in field (Chopart et al. 2008; Maurice et al. 2010). Estimating root orientation also allows to determine RLD via root intersection density (RID, defined as the number of root tips counted on a given soil surface) which is an important trait defining the utilization of resources (Gregory 2006; Markesteijn and Poorter 2009). As the measurement of RLD is more time-consuming than that for RID (Chopart and Siband 1999), a series of studies has attempted to explore the relationship between RLD and RID by introducing the effect of root orientation (Chopart and Siband 1999; Chopart et al. 2008; Maurice et al. 2010). To our knowledge, no such relationship is as yet available for walnut, an economically valuable species for wood production, especially in agroforests. Establishing this relationship would allow a better characterisation and quantification of root

biomass and when combined with data for root turnover and decomposition rates, can be used to quantify carbon sequestration.

Our aim was to characterise the spatial distribution of fine roots of hybrid walnut (*Juglans regia* × *nigra* L.) trees in a Mediterranean alley cropping agroforestry system mixed with durum wheat (*Triticum turgidum* L. subsp. *Durum)* in southern France. We hypothesized that tree and crop roots are in competition and that this will be reflected in the distribution of tree roots. To do this, we used six root profiles excavated to a depth of 150 cm in an agroforestry (hybrid walnut trees × durum wheat) stand and in a tree monoculture (walnut trees × natural understorey). To study tree root spatial distribution beneath the maximum rooting depth of durum wheat, a 400 cm deep trench was also dug in the intercropped stand (four additional root profiles). To characterise the spatial variability of root distribution, we measured the RID and RLD, and calculated the orientation of roots along the soil profile. Our hypotheses were that (i) trees in the agroforestry stand would have lower RID and RLD near the soil surface compared to trees in the monocultural stand but have a greater root density deeper in the soil, (ii) RID and RLD would decrease with increasing distance from the trees and this effect would be greatest in the agroforestry stand, (iii) the orientation of roots would change with soil depth from isotropic to anisotropic, i.e. from a uniform root growth in all orientations to a preferential growth orientation. At the same time, we sought to highlight new methodologies for analysing root data by using (i) geo-statistical methods to better characterise and visualize root spatial heterogeneity and (ii) a segmented linear model to better describe deep root distribution (Qian and Cuffney 2012).

1.2 Materials and methods

1.2.1 Study site

The study was conducted at the Restinclières experimental site, 15 km north of Montpellier, France (43°43' N, 4°1’ E, 54 m a.s.l.). The climate is sub-humid Mediterranean with an average temperature of 14.5°C and an average annual rainfall of 951 mm (years 1996–2003). Soils are silty deep alluvial fluvisols (IUSS Working Group WRB 2007), with 25% clay and 60% silt (Dupraz et al. 1999) with a slope < 1° within the site. The site is near the Lez River watershed and the depth from the soil surface to the water table usually oscillates between 5 m in winter and 7 m in the summer.

Root sampling was conducted in two adjacent types of hybrid walnut (*Juglans regia* × *nigra* L. *cv. NG23*) plantations. All the walnut trees at the site were planted in February 1995 in parallel tree rows with an east–west orientation (Fig. II-1.1). The site comprised:

- an alley cropping agroforestry stand (walnut trees at 13 × 4 m tree spacing) where walnut trees were usually intercropped with durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.). However, rapeseed (*Brassica napus* L.) was also grown in 1998, 2001 and 2006, and pea (*Pisum sativum* L.) in 2010.
- a tree monoculture with natural vegetation in the understorey (walnut trees at 7 x 4 m tree spacing). Understorey vegetation was dominated by *Vicia lutea* L. and composed mainly of herbaceous species, including *Medicago* sp., *Avena* sp. and *Papaver* sp.

Figure II-1.1. a) Hybrid walnut trees intercropped with durum wheat in the agroforestry stand and b) 400 cm deep pit in the agroforestry stand.

In the agroforestry stand, the annual crop was fertilized with approximately 150 kg N ha^{-1} yr^{-1}, whereas the tree monoculture did not receive any fertilization. The soil in the inter-row was usually ploughed to 20 cm every year before the winter crop was sown. Durum wheat is sown in late October and harvested in late June. Exceptionally, the soil was tilled to 10-15 cm but not ploughed before durum wheat was sown in 2011. The last ploughing was performed in October 2010, i.e. 18 months before this study was performed. The soil has never been tilled along the tree rows in the intercropped stand or in the tree monoculture, and natural spontaneous vegetation was present in these areas.

1.2.2 Sampling locations

Two 200 (length, i.e. perpendicular to tree row) × 200 (width, i.e. parallel to tree row) × 160 cm (depth, i.e. vertical) trenches were dug in March 2012 in both the agroforestry (AF) stand

and the tree monoculture (M) (Fig. II-1.2). Tree root mapping and soil sampling were performed in April 2012. The trenches were located on the edge of the tree row, i.e. in the inter-row between two trees (Fig. II-1.2). The distance of the nearest edge of the pits to a tree was 100 cm. The two trees surrounding the AF pit were 13.80 and 11.60 m tall, respectively, and had a diameter at breast height (DBH) of 29.0 and 25.2 cm, respectively. In the tree monoculture, the two surrounding trees were 14.00 and 13.30 m tall, respectively, and had a DBH of 31.5 and 26.4 cm, respectively.

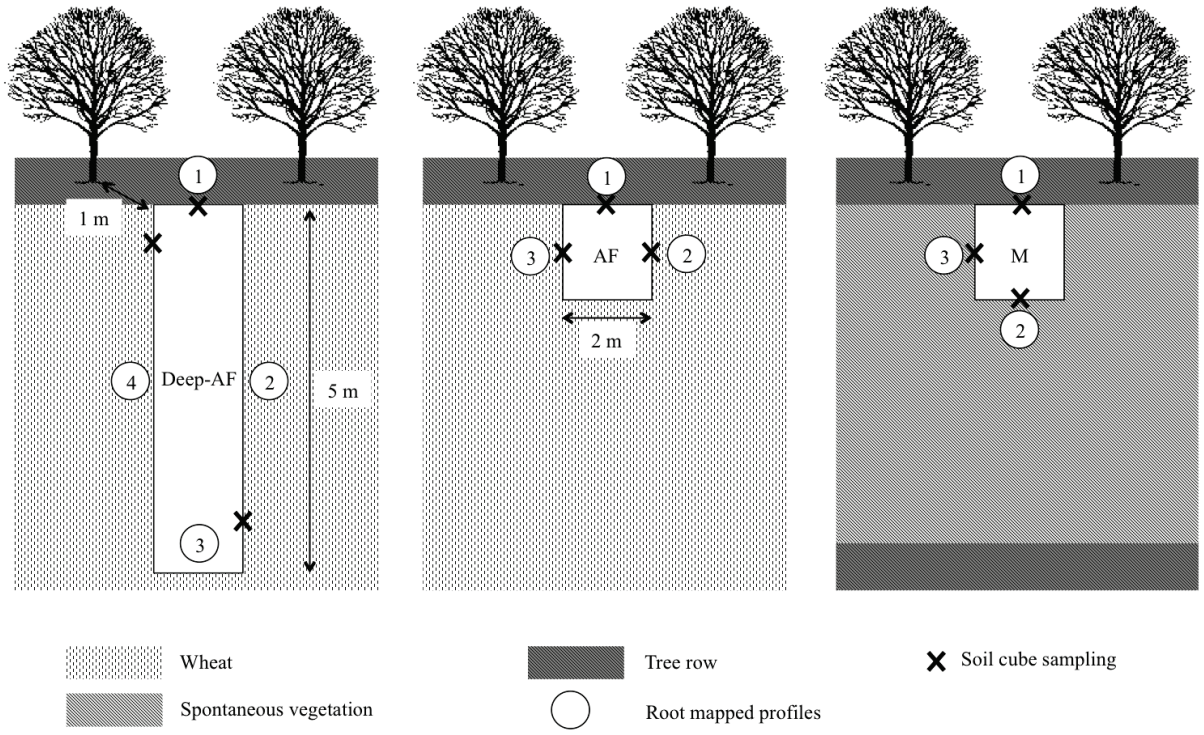

Figure II-1.2. Schematic figure of the sampling protocol in both agroforestry (AF) stand and tree monoculture (M), seen from above. AF and M pits were 150 cm deep and the Deep-AF pit was 400 cm deep.

In order to study tree root spatial distribution beneath the maximum rooting depth of wheat (i.e. 150 cm), an additional trench was dug in the agroforestry stand (deep-AF). This pit was 500 (length) × 150 (width) × 400 cm (depth), and as the AF pit described previously, perpendicular to the tree row (Figs. II-1.1, II-1.2). For safety reasons, before the deep-AF pit was dug, six iron posts were pushed 500 cm deep in the soil with a mechanical shovel corresponding to each corner of the future trench. Two additional posts were inserted in the middle of each of the longest trench walls. The pit was dug between the posts to a depth of

400 cm. A wooden framework was then built using the iron posts at different depths in order to secure access to the bottom of the pit and to leave spaces between wooden posts to perform measurements. This trench started on the tree row and ended in the middle of the intercropped row. The two trees surrounding the deep-AF pit were 13.80 and 11.70 m tall and had a DBH of 26.1 and 30.5 cm, respectively.

1.2.3 Tree root and soil cube sampling

In both the AF and M trenches (Fig. II-1.2), fine roots (diameter ≤ 2 mm) of walnut were mapped using a grid with regular squares (10 × 10 cm) along three soil profiles of 150 (length) × 150 (width) × 150 cm (depth). Live fine roots were determined visually and their number per square was counted. It was easy to distinguish tree roots from the arable crop and the herbaceous understorey roots. Walnut roots are black whereas wheat and herbaceous roots were whitish/yellowish. A precision calliper was used to measure precisely root diameter when roots were considered to be close to 2 mm in diameter. We then calculated the mean root intersection density (RID, roots cm^{-2}) for each square in the grid by dividing the number of roots counted in each square by the surface area (100 cm^{-2}). Mean RID profiles were calculated per depth interval (10 cm) along a width of 150 cm (the width of the grid).

In order to predict RLD from RID and to assess root anisotropy, we used a similar technique to that of Maurice et al. (2010). Two soil cubes (10 × 10 × 10 cm, Fig. 3) per profile were taken at depths of 10, 40, 70, 100 and 150 cm. Cubes of soil were extracted in the middle of each soil profile (Fig. II-1.2). Cubes had one horizontal face (H) parallel to the soil surface and perpendicular to the profile; one lateral face (L) perpendicular to the soil surface and to the profile, and one transversal face (T) perpendicular to the soil surface and parallel to the profile (Fig. II-1.3). The transversal face of these cubes corresponded to the plane of the soil profile walls where root impacts were mapped. This face is the most accessible for studying root spatial distribution but does not allow the three dimensional (3D) distribution of fine roots to be mapped. Therefore, two additional faces (H and L faces) were also sampled. Two cubes per depth and per profile were sampled close to each other, one in position A (lateral face on the right), one in position B (lateral face on the left, Fig. II-1.3). Overall, a total of 30 cubes were sampled per pit (n=2 positions of cube × 5 soil depths × 3 root profiles).

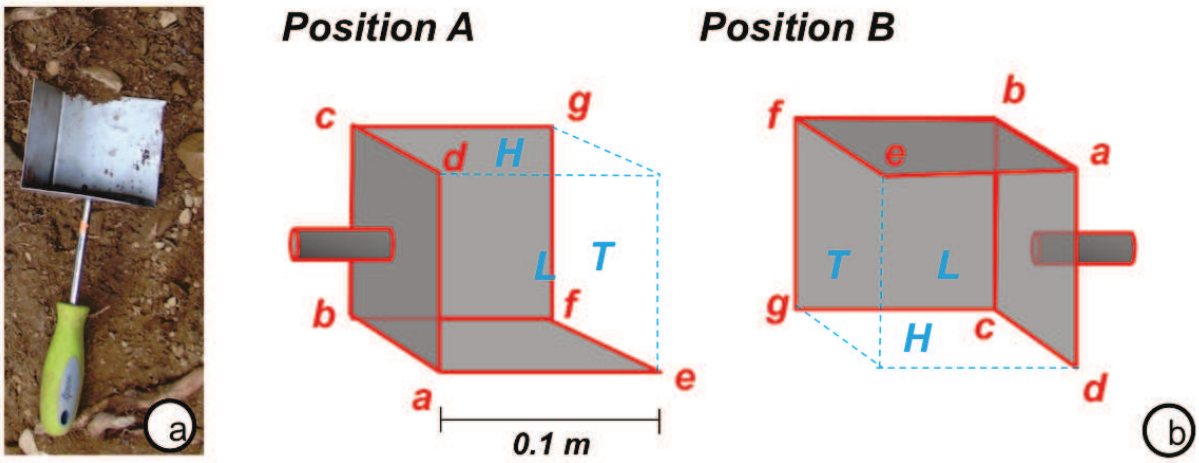

Figure II-1.3. Sampling of cubes. a) sampling device; b) sampling positions. In b), the grey faces, solid red lines and red letters at vertices represent the sampling device. The hollow faces dashed blue lines and blue letters in the middle of a plan represent the sampled soil cube; H: horizontal, T: transversal; L: lateral.

In the deep-AF trench, fine root impacts were mapped on all four lateral soil profiles (Fig. II-1.2). Following the same protocol mentioned above, soil cubes were sampled at depths of 10, 40, 70, 100, 150, 200, 300 and 400 cm. Two replicates (cubes) were sampled in the middle of the tree row profile (see profile 1 in Fig. II-1.2) and in the inter-row 100 cm from the tree row (see profile 4 in Fig. II-1.2). Cubes were also sampled at 500 cm from the tree row (see profile 2 in Fig. II-1.2). Overall, a total of 48 cubes were sampled in this trench (2 positions of cube × 8 soil depths × 3 root profiles). Cubes were taken to the laboratory and stored at 4ºC before measurements were performed, which were carried out within a few days after sampling.

1.2.4 Root counts on soil cubes

Live fine roots were determined visually and the number of fine root intercepts was counted on each of the three cube faces. The RIDs were then calculated for each cube face as the number of roots per cm^{-2} and were named RID_H, RID_L and RID_T respectively for the H, L and T faces. The average number of fine root intercepts for each soil cube (RID, roots cm^{-2}) was calculated as follows:

$$RID = \frac{RID_H + RID_L + RID_T}{3} \qquad \text{(Eq. 1)}$$

1.2.5 Root traits

After roots were counted, all roots from each cube were carefully extracted by gently washing the cube with tap water using a 0.2 mm mesh sieve. Coarse roots (>2 mm diameter) were removed from the analysis. Remaining roots were then rinsed, spread out onto a mesh tray and scanned at 400 dpi with a scanner (Epson Expression © 10000 XL, Japan). The resulting image was then processed using an image analysis software (WinRHIZO v. 2005b ©, Regent Instruments Inc., Québec, Canada) to determine the total fine root length (L, cm) and mean root diameter per diameter class, i.e. 0.0-0.5 mm, 0.5-1.0 mm, 1.0-1.5 mm, and 1.5-2.0 mm. We then calculated the proportion of root length in each diameter class, with regard to total root length for all diameter classes combined. Total RLD (cm cm^{-3}) was then calculated as L/V, where L is the total root length in the cube and V is the volume of the soil cube (1000 cm^3). Roots were then dried at 65°C for 48 hours and weighed to determine their dry mass (DM, g). We calculated the specific root length (SRL, m g^{-1}) as the ratio between L/DM.

1.2.6 Root anisotropy

Root anisotropy (*A*) is considered one of the most important and commonly used metrics of root orientation (Lang and Melhuish 1970). Root distribution is fully isotropic when root growth is uniform in all orientations, whilst root distribution is fully anisotropic, when root growth is toward only one orientation. However, anisotropy is almost impossible to estimate in the field, as measuring the orientation of individual fine roots is extremely painstaking and time-consuming. Therefore, *A* was interpreted as the deviation degree from a random orientation of roots within a soil cube (Chopart and Siband 1999) and can be expressed as:

$$A = \sqrt{\frac{(RID_T - RID)^2 + (RID_L - RID)^2 + (RID_H - RID)^2}{6RID^2}} \quad \text{(Eq. 2)}$$

where, RID_T, RID_L and RID_H are the root intersection densities (roots cm^{-2}) on the T, L and H faces of a given soil cube, respectively. The denominator term in the equation allows for normalization of *A* (dimensionless) so that it ranges between 0 and 1. When $A = 0$, i.e. $RID_T = RID_L = RID_H$, there is isotropy (i.e. root distribution is isotropic) and there is no specific orientation for fine root growth. When $A = 1$ (in a fully anisotropic status), this indicates that roots are counted only on one face but do not penetrate the other two faces of the cube ($RID = 0$ on these faces).

1.2.7 Data analysis

Separate generalized linear models (GLM) were used with either the proportion of root length in each diameter class, RLD, DM, or SRL as the dependent variables and the soil cube position (A or B), the stand (AF or M), the distance to the tree row (quantitative factor), and soil depth (quantitative factor) as factors and all interactions between factors. When a maximum soil depth of 150 cm was considered for analysis, soil profiles from the AF and deep-AF pits were considered as individual replicates. A Shapiro-Wilk test was performed before each GLM to guarantee that the investigated indicator followed a normal or quasi-normal distribution. These analyses were followed by a one-way analysis of variance (ANOVA) for each factor.

Root vertical profiles are usually described using logarithmic or exponential models (Jackson et al. 1996; Hartmann and Wilpert 2014). However, we used a hockey stick model (Qian 2009; Qian and Cuffney 2012) to compare rooting patterns between the different stands (AF and M). We applied this model to the mean RID between the profiles of the inter-row and of the tree row for each stand in order to determine if the vertical rooting pattern was distributed smoothly throughout the soil profile and compare rooting depths. Compared to these conventional models, the advantage of the hockey stick model is that the significant breakpoints in a series of data can be found. The hockey stick model can have several breakpoints and segments, but adding extra-breakpoints might not always be biologically meaningful, and would make the model less robust mathematically due to the high number of parameters. Therefore we decided to use this model with only one breakpoint:

$$\mathrm{RID} = \begin{cases} \beta + \alpha_1(z-\delta), & z < \delta \\ \beta + (\alpha_1+\alpha_2)(z-\delta), & z \geq \delta \end{cases} \qquad \text{(Eq. 3)}$$

where, z is soil depth (cm), α_1, α_2, δ and β are coefficients (see Fig. II-1.4 for geometrical definitions, and supplementary material for the R code). If δ is statistically significant, i.e. there is a significant breakpoint along soil depth, and at a soil depth of $z = \delta$, there is a root vertical distribution with RID = β. For the agroforestry stand, profiles from the AF and deep-AF pits were averaged for values to a depth of 150 cm and a distance of up to 150 cm from the tree row.

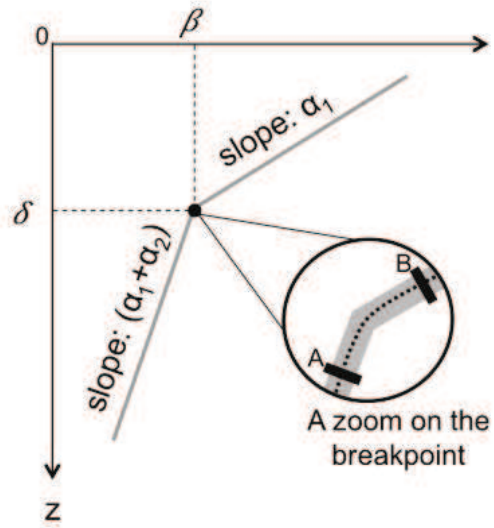

Figure II-1.4. Geometric meanings of all the coefficients of the four-parameter hockey stick model, which comprises two linear segments. RID is the root intersection density (roots cm^{-2}), z is soil depth (cm). β *and* δ are the breakpoint coordinates, α_1, α_2 are the slope coefficients. Around the breakpoint, a quadratic curve was estimated between point A and B, which are sufficiently close to the breakpoint, so that the whole hockey stick model becomes smooth and derivable, and therefore can be fitted using non-linear regression approaches (Qian 2009).

The spatial distribution of roots was also analyzed using univariate geostatistical methods including kriging (Webster and Oliver 2007), that was performed using GS+ (Gamma Design Software 2004).

Ordinary least square regressions (OLS) between RLD and RID_T or RID were performed for the cubes of each pit (local model) and for the cubes of all pits at the same time (global model). Since A and B cubes were spatially correlated for each soil depth (taken next to each other but with opposing orientations), these cubes were not considered as individual replicates. Due to the chosen sampling method (Chopart and Siband 1999), the horizontal face of the B-type cube was 10 cm deeper than the horizontal face of the A-type cube (Fig II-1.3). Therefore, we considered both A- and B-type cubes at each sampling position (depth) as one sample using mean values between A- and B-type cubes as no significant differences were found between them (F=0.31, P=0.58). Since the intercepts of the OLS models were not significantly different from zero, we performed additional OLS forcing the intercept through the origin. This method is based on the assumption that if the mean number of root intercepts for a given cube equals zero there are no roots inside the cube. The slope of the regression line (α) as well as the 95% confidence interval for the slope were calculated. Slopes were compared using an analysis of covariance (ANCOVA) (Andrade and Estévez-Pérez 2014). We performed a Fisher's (F) test to determine which model should be chosen between a local

model (containing a series of equations, one equation per level of factor) and a global model (containing one equation for all levels mixed).

With regard to anisotropy (*A*), a GLM and ANOVA were also applied in the same way as for the analysis of root traits. As *A* possesses no information on the preferential orientation of roots (Chopart and Siband 1999), when we detected anisotropy, we also analysed the proportion of root impacts per cube face (H, L, T) using the same methodology to determine the preferential orientation of roots at each depth.

All calculations were carried out with the R software, Version 2.15.3 (R Development Core Team 2013) at a significance level of <0.05.

1.3 Results

1.3.1 Root traits from soil cubes

In both stands, fine roots from walnut trees were mainly constituted of roots ≤ 0.5 mm in diameter that represented almost 80% of the total root length (Fig. II-1.5). The only significant variable that impacted the proportion of root length in each diameter class in the upper 150 cm of soil was stand type. The finest roots (≤ 0.5 mm) represented 85% of the total root length in the tree monoculture, but only 77% in the agroforestry plot (F=9.15, *P*=0.003, df=81). Within the additional trench dug in the agroforestry stand (deep-AF), there was a higher proportion of very fine roots (77%) in the upper 150 cm of soil compared to the deeper soil (69%) although the result was not significant (F=3.61, *P*=0.065, df=41). There were no significant differences in the proportion of root length within each diameter class with regard to soil depth or distance to the tree.

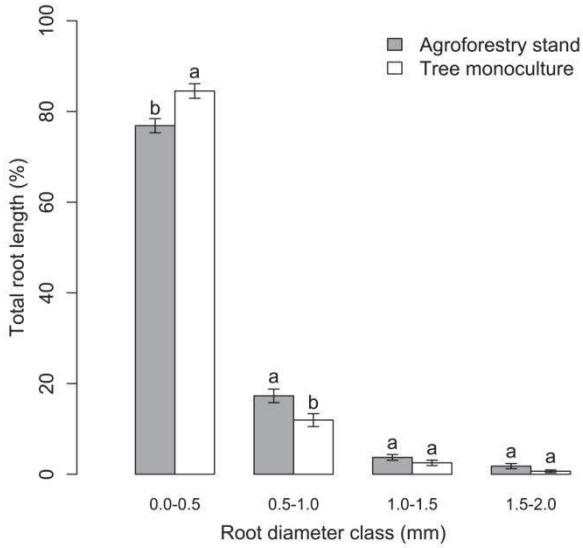

Figure II-1.5. Histogram of the proportion of walnut fine root length per class of diameter. Data represent means ± standard errors. Number of replicates: n=60 for the agroforestry stand, n=30 for the tree monoculture.

The stand type, the distance to the tree, soil depth and interaction between distance to the tree and depth, had a significant effect on RLD and DM of fine roots from soil cubes (Table II-1.1). The interaction between distance to the tree and stand type was not significant. RLD and DM were greater in the tree monoculture (0.26 ± 0.03 cm cm^{-3} and 0.17 ± 0.02 g, respectively) compared to the agroforestry stand (0.10 ± 0.01 cm cm^{-3} and 0.10 ± 0.02 g, respectively), and decreased with increasing distance from the tree row and depth.

The SRL of walnut roots to a depth of 150 cm did not differ significantly between the agroforestry stand (17.29 ± 1.84 m g^{-1}) and the tree monoculture (17.19 ± 1.02 m g^{-1}). SRL was not significantly different between soil cubes, depth or distance to the tree (Table II-1.1).

	RLD		DM		SRL	
	F-value	Pr(>F)	F-value	Pr(>F)	F-value	Pr(>F)
Cube	0.31	0.58	0.47	0.50	0.002	0.97
Stand type	41.61	< 0.001***	9.00	0.004**	0.001	0.97
DTR	9.29	0.003**	10.56	0.002**	0.20	0.66
Depth	17.17	< 0.001***	16.35	< 0.001***	1.13	0.29
DTR × depth	14.47	< 0.001***	17.10	< 0.001***	3.14	0.08
DTR × stand	0.40	0.53	0.31	0.58	0.26	0.61

Table II-1.1. ANOVA on the GLM model for walnut fine root length density (RLD), dry mass (DM) and specific root length (SRL) as a function of the cube (A or B), stand type, distance to tree row (DTR), and soil depth. Number of cubes: n=60 for the agroforestry stand, n=30 for the tree monoculture. Asterisks indicate significance levels where * P=0.05, ** P=0.01 and *** P <0.001.

1.3.2 Mapping tree fine root impacts

In the tree monoculture (Fig. II-1.6a and Fig. II-1.S1a for raw data), most tree fine roots were concentrated in shallower depths compared to the agroforestry stand (Fig. II-1.6b and Fig. II-1.S1b for raw data), and appeared to be more homogeneously distributed along the horizontal plane. Using a hockey stick model, it was shown that the soil depth above which most root impacts were counted was > 150 cm (no breakpoint detected when a depth of 150 cm was considered) in the tree row in the agroforestry stand and 104 cm in the tree monoculture. In the inter-row, this depth was 122 cm in the agroforestry stand and 64 cm in the tree monoculture (Fig. II-1.7a). Wheat roots were not mapped but we visually determined a maximum rooting depth of 150 cm.

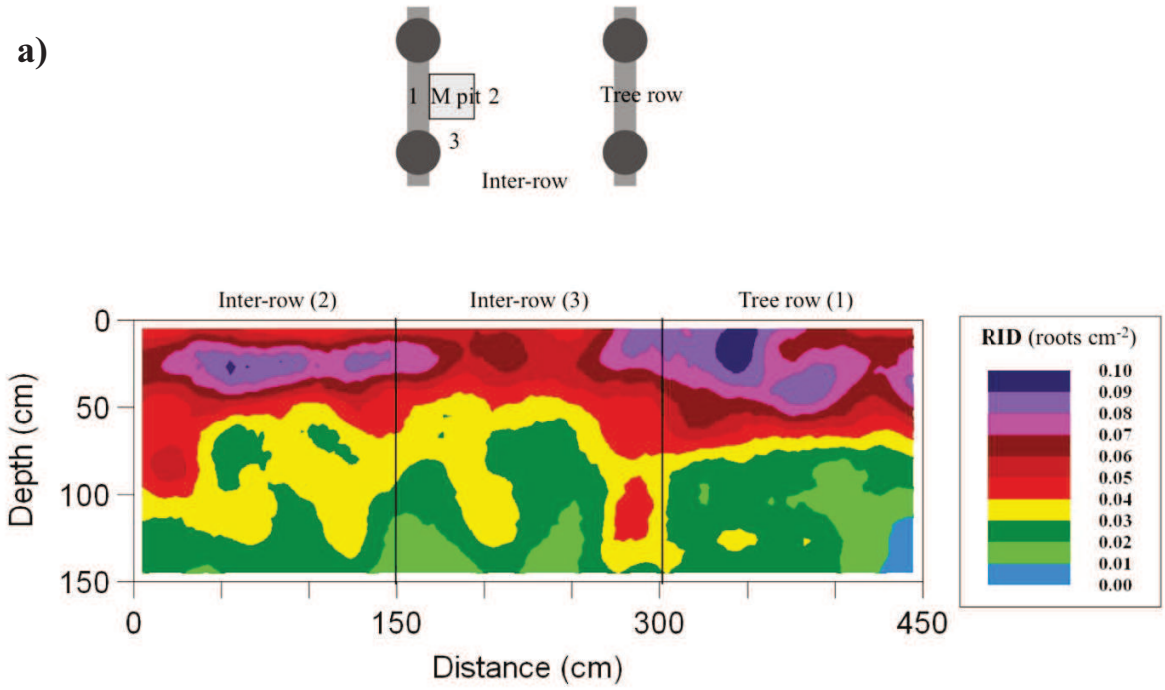

Figure II-1.6. a) Kriged map of walnut fine root intersection densities (RID) within the pit in the tree monoculture.

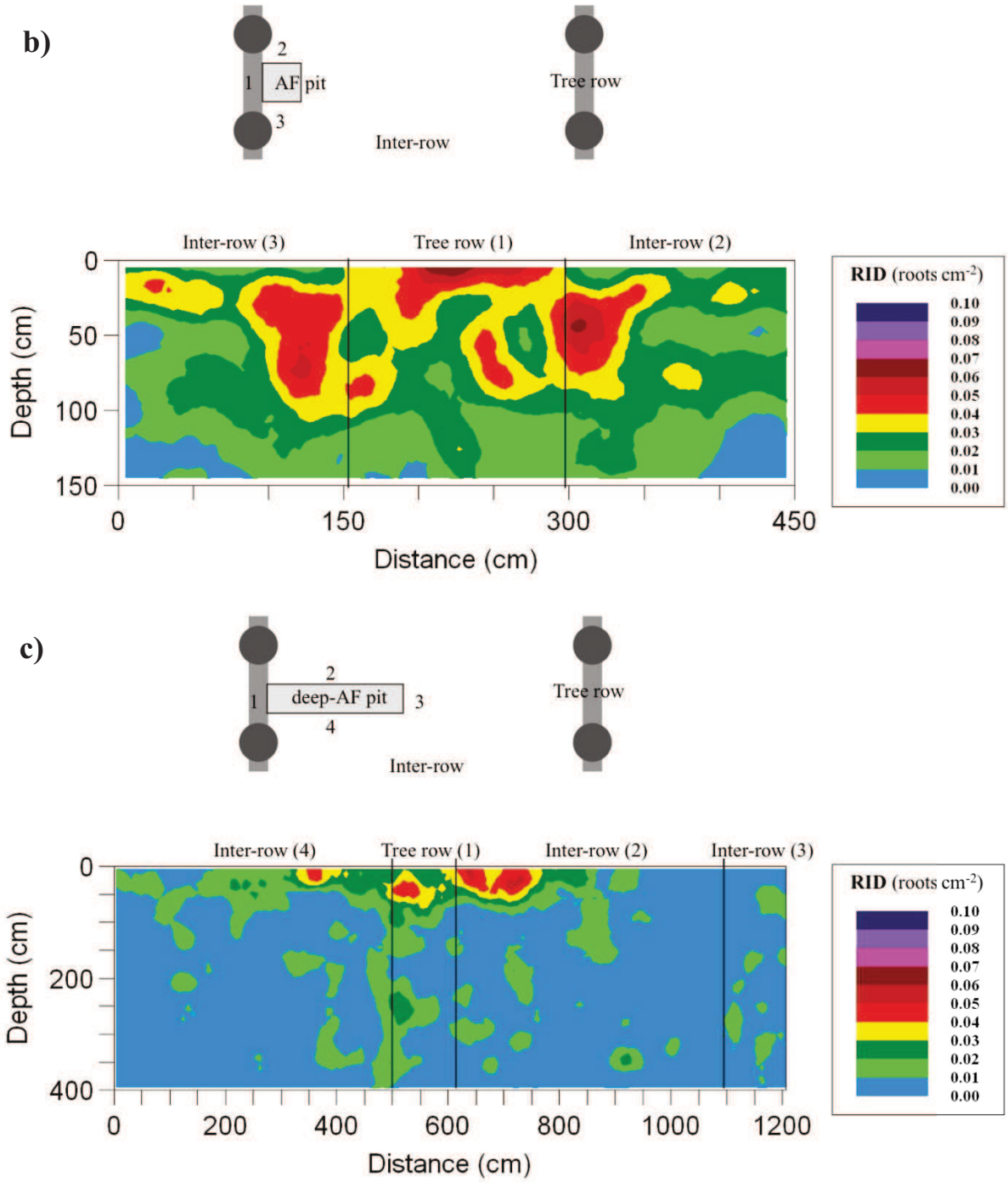

Figure II-1.6 b) Kriged map of walnut fine root intersection densities (RID) within the agroforestry pit and c) Kriged map of walnut fine root intersection densities (RID) within the 400 cm deep agroforestry pit.

In the deep-AF pit, tree fine roots colonized the whole soil profile both vertically (400 cm deep) and horizontally (500 cm long) (Fig. II-1.6c and Fig. II-1.S1c for raw data). In the inter-row, 2 m away from the tree, and to a depth of 75 cm, the RID of fine roots was still high (0.04 to 0.05 roots cm^{-2}), and comparable with that under the tree row. Further away from the

tree row, RID started to decrease in topsoil layers. Below 100 cm deep in the inter-row, no clear spatial pattern was observed, and fine roots appeared to be randomly distributed regardless of increasing soil depth and tree distance. In deeper layers, RID was generally lower but in the tree row it remained high (0.02 to 0.03 root cm^{-2}) at depths < 150 cm. Fine roots tended to grow in clusters at depths greater than 150 cm (Fig. II-1.6c). Consistent with results from the AF pit, an estimate with hockey stick models of the soil depth above which most root impacts occurred showed that, in the deep-AF pit, this depth was around 150 cm for the tree row (Fig. II-1.7b) whereas it decreased to 79 cm and 104 cm in the inter-rows, respectively between 0 and 150 cm, and between 150 and 300 cm from the tree row. From 50 to 100 cm soil depth, the mean RID in the tree row was 0.028 roots cm^{-2}, whereas it was 0.012 roots cm^{-2} in the inter-row at a distance of 150 cm from the tree row. Three meters away from the tree row, RID was low and constant along the whole profile so that the hockey stick model failed to detect a breakpoint.

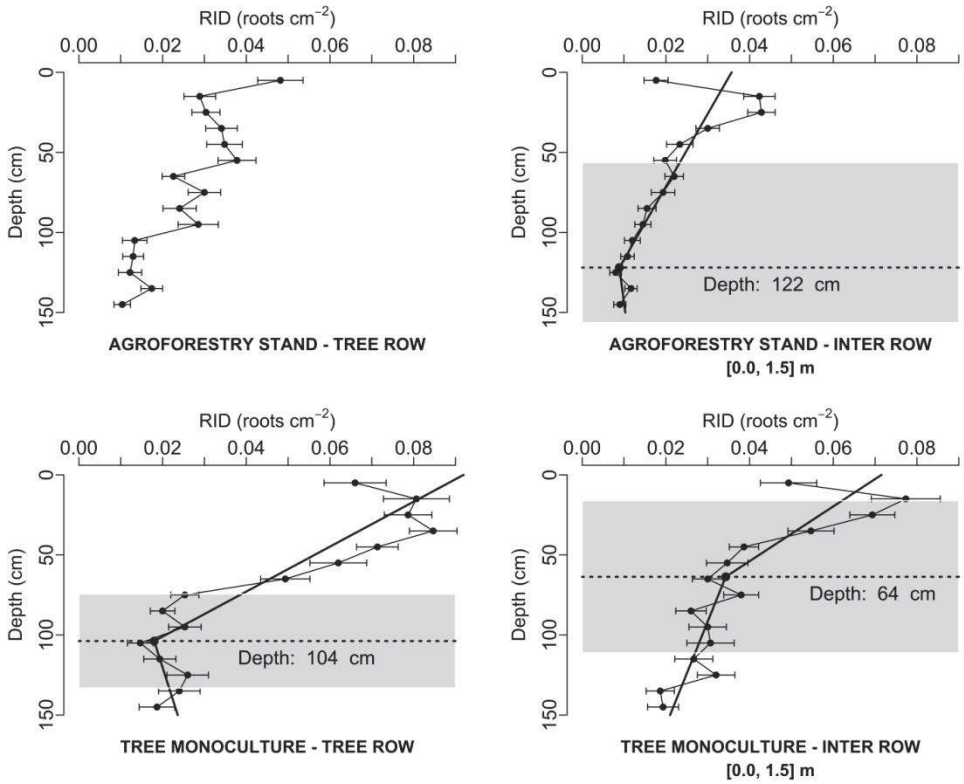

Figure II-1.7. a) Walnut fine root intersection density (RID) profiles in the agroforestry stand and in the tree monoculture to a depth of 150 cm. For the agroforestry stand, profiles from the AF and deep-AF pits were combined for the values down to 150 cm.

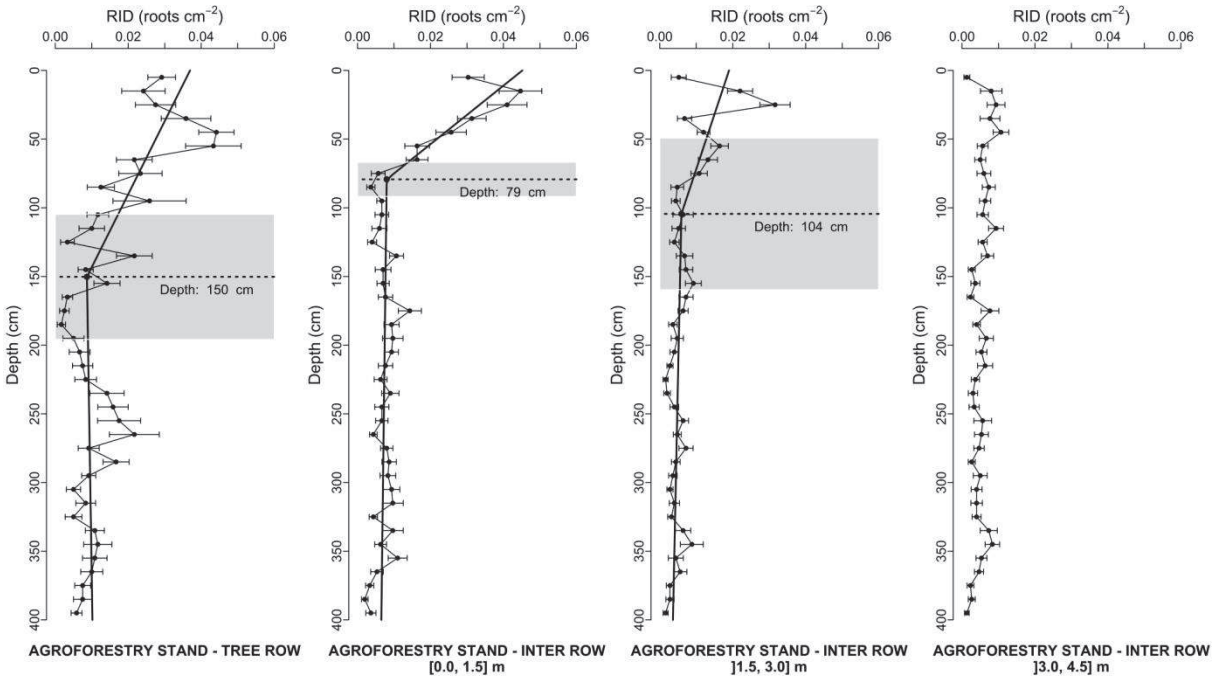

Figure II-1.7. b) Walnut fine root intersection density (RID) profiles in the agroforestry stand to a depth of 400 cm as a function of distance to the tree row.

1.3.3 Root anisotropy

GLM and ANOVA analysis revealed that the stand type and the distance to the tree row had a significant impact on root anisotropy where soil depth ≤ 150 cm (Table II-1.2).

	All pits, soil depth ≤ 150 cm		deep-AF pit, soil depth ≤ 400 cm	
	F-value	Pr(>F)	F-value	Pr(>F)
Cube	1.27	0.26	1.04	0.31
Stand type	6.89	0.011*	-	-
DTR	9.61	0.003**	0.09	0.77
Depth	3.44	0.067	12.96	< 0.001***
DTR × depth	1.65	0.20	0.13	0.72
DTR × stand	1.43	0.24	-	-

Table II-1.2. ANOVA on the GLM model for walnut fine root anisotropy as a function of the cube (A or B), stand type, distance to tree row (DTR), and soil depth. Number of cubes: n=60 for the agroforestry stand, n=30 for the tree monoculture. Asterisks indicate significance levels where * P=0.05, ** P=0.01 and *** P <0.001.

Tree roots in the tree monoculture (A=0.30) were significantly (P<0.05) more isotropic than in the agroforestry stand (A=0.45) (Fig. I-3.8). Fine roots in the tree row were significantly more isotropic (A=0.28) than in the inter-row (A=0.46).

In the deep-AF pit, GLM and ANOVA analysis revealed that depth had a significant impact on root anisotropy (Table 2). Shallow fine roots were more isotropic (A=0.26 at a depth of 10

cm) than deep fine roots (A=0.71 at an average depth of 400 cm) (Fig. II-1.8). An analysis of the proportion of fine root counts on each cube face showed that the horizontal face of cubes had a higher proportion of root intercepts with increasing depth (F=16.59, P<0.001). About 24% of root intercepts were counted on the horizontal face of cubes from the soil surface to a depth of 150 cm, but this proportion reached 62% at a depth of 200 to 400 cm. Tree fine roots were preferentially vertically oriented with increasing depth.

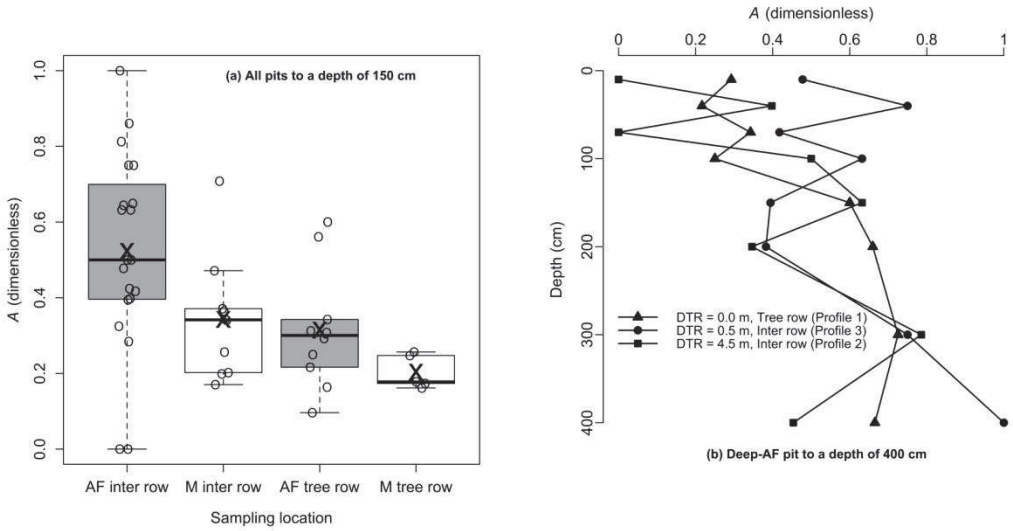

Figure II-1.8. Variation of walnut fine root anisotropy according to sampling location (a, for all the pits) and soil depth (b, only for the 400 cm deep agroforestry pit). In a), "AF" and "M" represent agroforestry stand and tree monoculture, respectively. Black crosses on each boxplot represent the mean value of anisotropy (A).

1.3.4 Prediction of Root Length Density (RLD)

The slopes of the OLS regressions between RLD and either RID_T or RID, respectively, were not significantly different from each other (Table II-1.3). The confidence interval was generally slightly narrower when RID was used (Table II-1.3, Fig. II-1.S2). The F test revealed that a local model (i.e. one model for each pit) was not more significant than a global model for all pits (F=1.67, P=0.185). Thus, we were able to link the mean number of root impacts and RLD for hybrid walnut trees as follows:

$$\text{RLD}\ \ \text{cm cm}^{-3}\ = 4.47 \times \text{RID (number of roots cm}^{-2}) \qquad \text{(Eq. 5)}$$

This equation was then used to predict RLD in both the agroforestry stand and the tree monoculture, in the tree row and in the inter-row (Fig. II-1.S3a, b).

Pit	Variable	Slope (α)	Confidence Interval (P=0.95)	R^2
Deep agroforestry	RID_T	3.86 a	2.91 - 4.80	0.46
	RID	4.20 a	3.33 - 5.08	0.59
Agroforestry	RID_T	4.39 a	2.76 - 6.02	0.42
	RID	4.60 a	3.71 - 5.49	0.65
Tree monoculture	RID_T	4.21 a	3.12 - 5.30	0.54
	RID	4.30 a	3.34 - 5.26	0.65
All pits	RID_T	4.32 a	3.75 - 4.89	0.61
	RID	4.47 a	3.97 - 4.97	0.71

Table II-1.3. Linear regressions between walnut fine root length density (RLD, cm cm^{-3}) and walnut fine root intersection density on the transversal face (RID_T, roots cm^{-2}) or the mean of the three faces (RID, roots cm^{-2}), for the different pits. Shown are mean values between A-type and B-type cubes. Slope coefficients followed by the same letter did not differ significantly at P=0.05 (ANCOVA).

1.4 Discussion

We showed that agroforestry practices promoted deeper rooting of walnut trees as root densities were much smaller near the soil surface in the agroforestry stand compared to the tree monoculture. Roots were also more heterogeneously distributed horizontally in the agroforestry stand, with larger root densities deeper in the soil in the tree row compared to the inter-row.

1.4.1 Plasticity of root distribution

The tree monoculture had a root distribution typical of that found in a forest stand, i.e. with a vertical heterogeneous root distribution and very high root densities in the top 0.5 m of soil (López et al. 2001; Yuan and Chen 2010; Hartmann and Wilpert 2014). In the agroforestry stand, root distribution was more vertically homogeneous and RLD was much smaller than in the tree monoculture, but roots occupied a higher volume of soil. These results confirm our first hypothesis that trees in the AF stand would have deeper root distributions induced by a greater belowground competition from the crop. This disparity between the two types of systems demonstrates the highly plastic behaviour of walnut trees in that their vertical root distribution was modified, likely in response to crop competition (Mulia and Dupraz 2006), even though soil type and environmental conditions were the same for both stands. This phenomenon has been commonly observed in other economically important tree species. For example, Dupraz and Liagre (2008) showed that poplars (*Populus* L.) possessed completely

different rooting patterns when grown in a tree monoculture compared to an agroforestry stand, with significantly deeper rooting for the latter. Livesley et al. (2000) and Wang et al. (2014) found that *Grevillea robusta* L. grown with *Zea mays* L. and Jujube (*Ziziphus jujuba* L.) trees grown together with wheat (*Triticum aestivum* L.) in an arid climate, had a lower RLD than trees grown in a monospecific stand due to competition from the crops. However, the belowground interaction between trees and crops can be more complex and roots of both trees and crop can be overlapped in shallow soils despite strong competition (Moreno et al. 2005). Further support to our hypothesis comes from the fact that annual crop species often have high SRL near the soil surface and are thus able to absorb nutrients and shallow soil water faster than trees, which usually have lower SRL (Moreno et al. 2005). The SRL of walnut trees at our field site was lower than that of wheat, both at the surface (20 cm) and in deep soil layers (150 cm) (Prieto et al. 2014). Therefore, we suggest that walnut trees in the agroforestry stand had minimal competition from wheat plants through the production of deeper fine roots. Deep roots will enable trees to access water from the water table not available to root crops, and to benefit from nutrients leached beneath the crop root systems. On the contrary, in the tree monoculture, walnut trees laid down roots in shallow soil because the understorey herbaceous species were mostly leguminous (Prieto et al. 2015a) and less competitive than winter wheat, mainly because of a lower root density. A parallel study estimated that root biomass of wheat was about 4.5 t DM ha^{-1} in 0-50 cm, whereas the root biomass of herbs was less than 1.5 t DM ha^{-1}. Herbaceous herbs in the tree monoculture were brown/green in colour during the winter months and very short. As herbaceous roots are less active in the winter (Steinaker and Wilson 2008) compared to winter wheat roots, the surface soil may contain less roots in the tree monoculture compared to the agroforestry stand. Therefore, in the spring, tree root in the monoculture could rapidly occupy the neighbouring superficial soil poorly colonized by herbaceous species.

Another factor potentially affecting the vertical root distribution in the AF stand is soil tillage (Korwar and Radder 1994; Sinclair 1995). In our system, the soil in the inter-row of the agroforestry stand was regularly ploughed to a depth of 20 cm and coarse roots in the soil surface were frequently damaged, affecting tree fine root production in these layers. In this sense, tillage might also induce deeper rooting in trees. However, soil disturbances can also stimulate root growth through root pruning (Joslin and Wolfe 1999) and by releasing soil micronutrients (Balesdent et al. 2000). Whatever the case, this explanation would be valid only for the top 20 cm of soil, the maximum tillage depth. Below this depth, only root

competition between durum wheat and walnut trees can explain the contrasting rooting patterns observed between the AF and M stands.

Temporal differences between the root growth of durum wheat and walnut trees may also influence the root distribution of walnut trees. Durum wheat is sown in late October at our field site, and is fully developed before walnut bud break, which occurs between late April and early May at the site (Mulia and Dupraz 2006). This period coincides with the peak fine root production for walnut trees (unpublished data). By this date, durum wheat, with a maximum rooting depth of 150 cm, will have already captured most of the nutrients and water contained in the topsoil (Burgess et al. 2004). We propose that temporal differences in growing periods between annual crops and tree species is therefore a key parameter for certain agroforestry systems to be successful (i.e. in Mediterranean ecosystems), and must be considered if new mixtures of crops and trees are to be successful (Schroth 1995; van Noordwijk et al. 1996; Burgess et al. 2004).

In the AF stand, the horizontal root distribution was heterogeneous and dependent on the distance from the tree, with higher root densities in the tree row or close to the tree row. The tree monoculture did not exhibit such a drastic decline in their root density, confirming our second hypothesis.

This unusual fine root distribution in the AF stand may promote carbon storage in the tree row and deep in the soil. Several studies have shown that carbon stocks in agroforestry systems were heterogeneously distributed, with more carbon in the tree row than in the inter row (Bambrick et al. 2010; Howlett et al. 2011; Lorenz and Lal 2014). A parallel study at this site confirmed that soil organic carbon (SOC) stocks were significantly higher in the AF stand (116.7 ± 1.5 Mg C ha^{-1}) in the upper metre of soil compared to that in a control agricultural plot (110.4 ± 0.6 Mg C ha^{-1}). SOC stocks were also significantly higher in the tree rows than in inter-rows (Cardinael et al., submitted). This additional SOC will not only be due to leaf litter from trees, but will also originate from fine root exudation and turnover (Haile et al. 2010).

1.4.2 Shallow roots and deep roots

We found smaller proportions of very fine roots in the soil 200 to 400 cm deep. These results are in accordance with Prieto et al. (2014) who found that fine roots deep in the soil were not only thicker than those near the surface, but presented traits associated to a more conservative strategy (i.e. lower root nitrogen and higher lignin concentrations). Prieto et al. (2014)

attributed this result to thinner, more acquisitive roots in shallow soil layers being more efficient for absorbing nutrients, which usually accumulate near the soil surface (Jobbagy and Jackson 2000). Our third hypothesis was confirmed since we found that tree fine roots down to 150 cm showed no clear orientation patterns but that deeper roots (200-400 cm) were preferentially vertically orientated.

This result suggests that, once a certain depth threshold is achieved, deep roots are preferentially oriented to access more stable water resources (i.e. the water table). Groundwater is present in this agroforestry stand at a depth of 500-700 cm, depending on the season, and having access to groundwater will enable walnut trees to overcome the summer drought period (Rambal 1984; Bréda et al. 1995; Bréda et al. 2006). Deep roots may be able to reach and take up nitrate leached from fertilizers beneath the wheat crop rooting depth, which may also explain the deep rooting observed in the AF stand. This "tree root safety net" (Cadisch et al. 1997; Rowe et al. 1999) could also contribute to reducing groundwater nitrate levels (Bergeron et al. 2011; Tully et al. 2012) and therefore improve ecosystem services provided by agroforestry systems.

In spite of the disparity in the trait distribution and functioning between shallow and deep roots (Prieto et al. 2015a), few studies have aimed at determining their distinct roles (Laclau et al. 2013; Maeght et al. 2013). Here, we show that a sharp breakpoint exists between two populations of roots within the soil profile: roots from upper soil layers, where root density declines sharply with increasing soil depth, and where roots have no determined spatial orientation, and roots in deeper soil layers (200-400 cm), where root density remains quite stable regardless of soil depth and roots are preferentially vertically oriented. Although we do not yet know the mechanisms behind this distribution, the breakpoints and identification of these thresholds in different ecosystems with deep-rooted species seem important to determine competition and foraging behaviours. These can be statistically estimated using the hockey stick model, which might be a promising tool to better define root distribution patterns than conventional linear, exponential or logarithmic functions.

1.5 Conclusion

Using deep soil profiles, we evidenced how tree fine root density can be both horizontally and vertically modified by the belowground competition from understory crops. Trees in the agroforestry stand rooted deeper in the soil than trees in the monocultural stand and had a

higher root density in the tree rows compared to the cropped inter-rows. These results enrich our understanding of the functioning of agroforestry systems. The plasticity in tree root distribution seems to be an important feature to achieve efficient agroforestry systems. This may also have implications concerning carbon and nutrient cycling in these systems as exploration of deep soil layers by roots is favoured. Methodologically, we highlighted the interest of using the hockey stick model. This model has a strong potential for use in future studies when attempting to define shallow and deep rooting profiles and distribution.

1.6 Supplementary material

Supplementary Material 1: an example of R code to fit the hockey stick model (see Qian (2009) Environmental and ecological statistics with R. 440p.)

```
# The hockey stick model
# Reference: Qian (2009) Environmental and ecological statistics with R. 440p.

hockey = function(x,alpha1,beta1,beta2,brk){
  eps=diff(range(x))/100
  x = x-brk
  delta = T
  if(delta == T){
    beta2 = beta1+beta2}
  x1 = -eps
  x2 = +eps
  b = (x2*beta1 -x1*beta2)/(x2-x1)
  cc = (beta2-b)/(2*x2)
  a = alpha1 + beta1 *x1-b*x1-cc*x1^2
  alpha2 = -beta2*x2+(a+b*x2+cc*x2^2)
  lebrk =(x<=-eps)
  gebrk =(x>=eps)
  eqbrk =(x>-eps & x<eps)
  result = rep(0,length(x))
  result[lebrk] = alpha1+beta1*x[lebrk]
  result[eqbrk] = a+b*x[eqbrk]+cc*x[eqbrk]^2
  result[gebrk] = alpha2+beta2*x[gebrk]
  return(result)
}

# Model fit
 HCK1 = nls(impacts_fine_roots ~ hockey(x = z, alpha = alpha0, beta1 = beta11, beta2 =
beta22, brk = brk0),
          start = list(alpha0 = 1, beta11=-0.01, beta22=0.01, brk0 = 200), na.action=na.omit,
trace = T,
          data = RID)
```

```
# Retrieving model parameter
myCoef = coef (summary( HCK1))
depth_pred = seq (0, 400, 0.1)
RID_pred = hockey(depth_pred, myCoef[1, 1], myCoef[2, 1], myCoef[3, 1], myCoef[4, 1])

# End
```

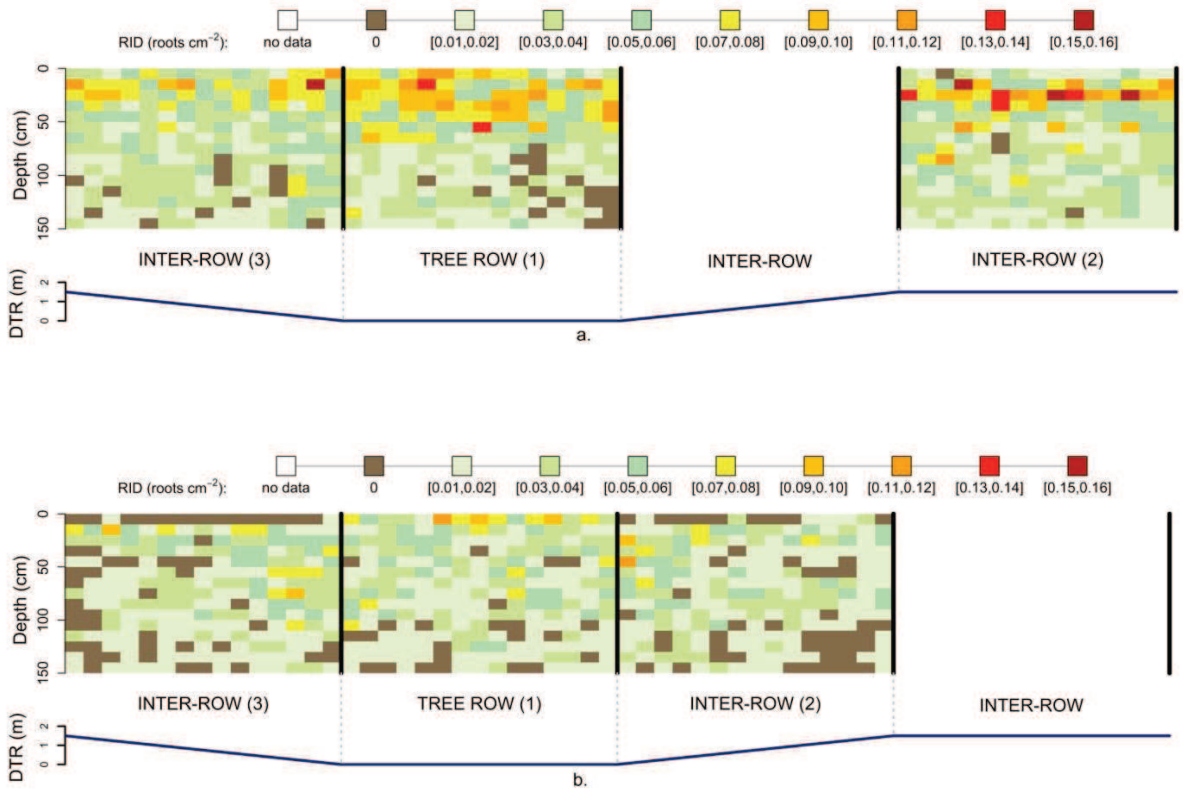

Figure II-1.S1. a) Raw data of walnut fine root intersection densities (RID) within the pit in the tree monoculture. DTR = Distance to the tree row. b) Raw data of walnut fine root intersection densities (RID) within the agroforestry pit. DTR = Distance to the tree row.

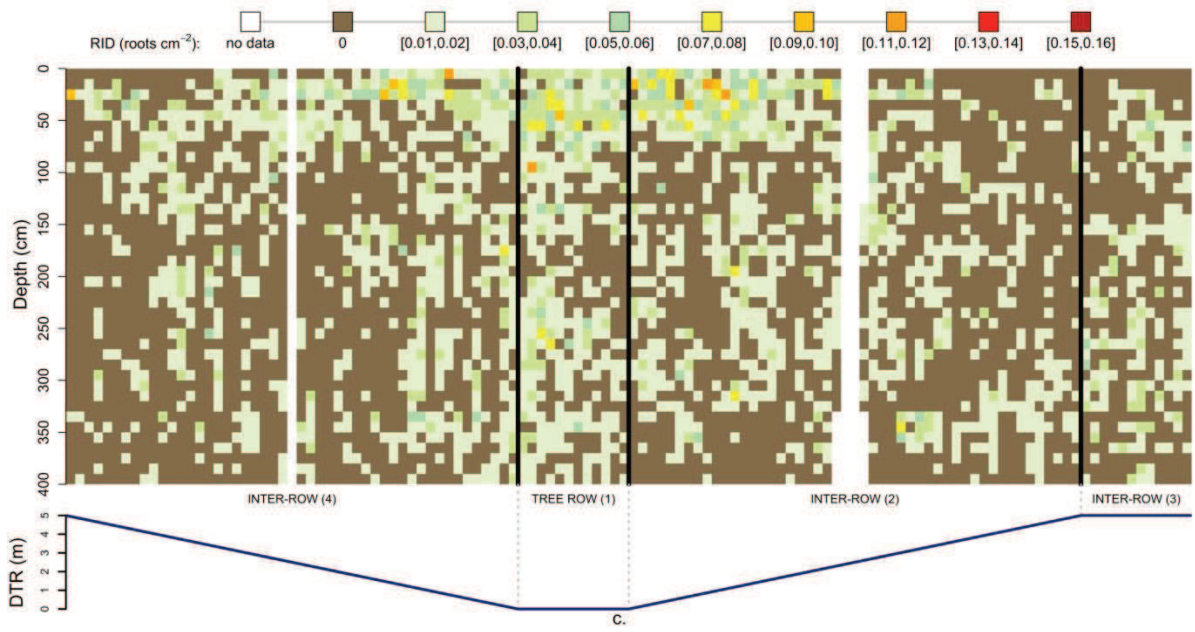

Figure II-1.S1. c) Raw data of the walnut fine root intersection densities (RID) within the 400 cm deep agroforestry pit. DTR = Distance to the tree row.

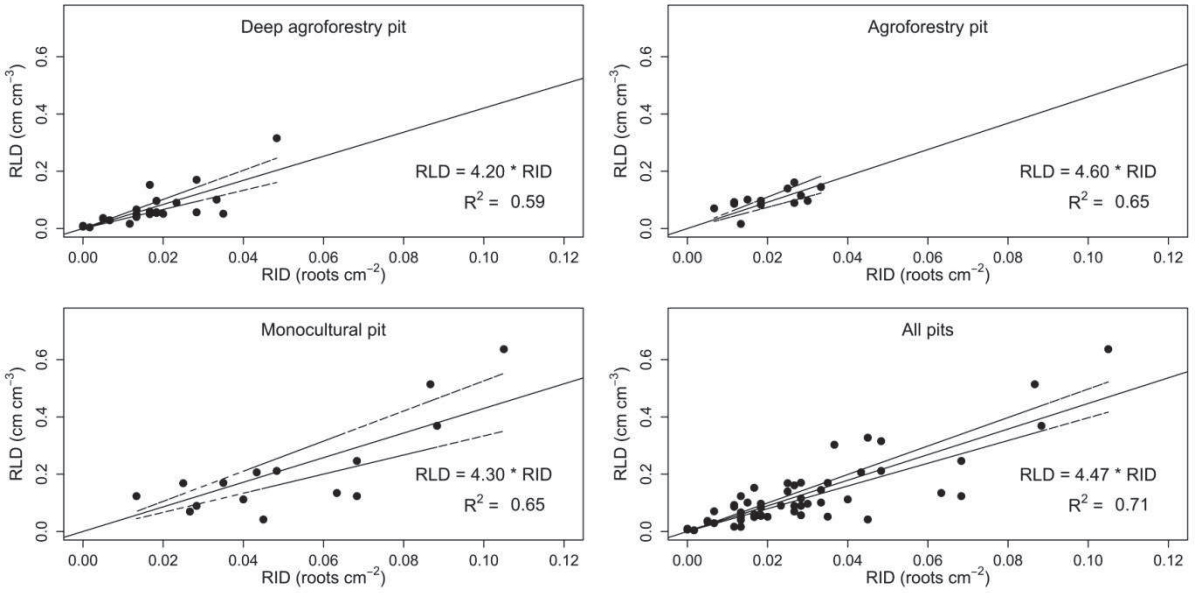

Figure II-1.S2. Linear regressions between walnut fine root length density (RLD) and the mean fine root intersection density (RID) for cubes, for the different pits. Dotted lines: confidence interval of the regression line.

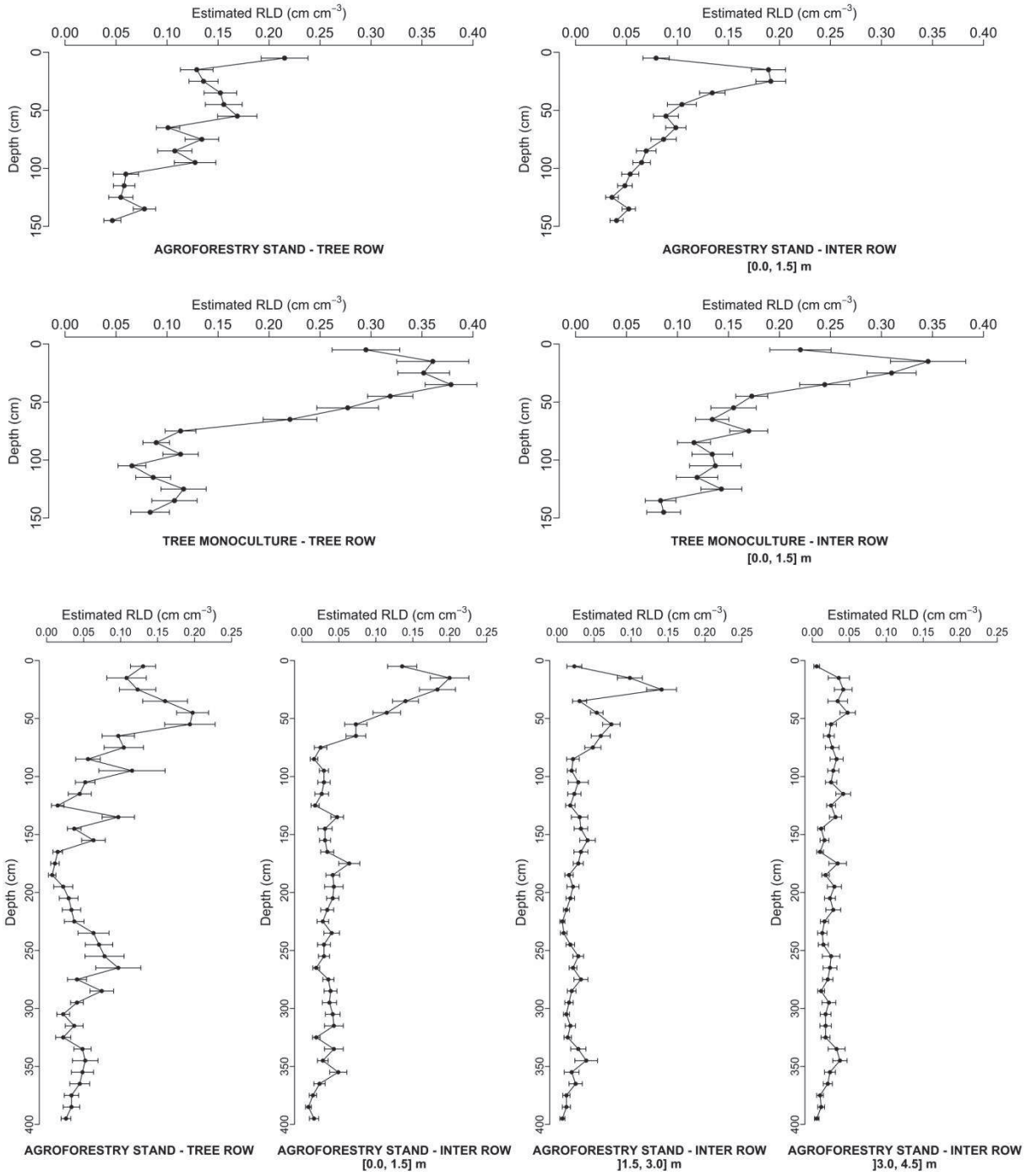

Figure II-1.S3. a) Estimated walnut fine root length density (RLD) profiles in the agroforestry and in the tree monoculture to a depth of 150 cm. For the agroforestry stand, profiles from the AF and deep-AF pits were combined for values to a depth of 150 cm. b) Estimated walnut fine root length density (RLD) profiles in the agroforestry stand to a depth of 400 cm as a function of distance to the tree row.

Chapitre 2

Renouvellement des racines fines des arbres superficielles et profondes dans un système agroforestier

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Unexpected phenology and lifespan of shallow and deep fine roots of walnut trees grown in a silvoarable Mediterranean agroforestry system

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Abstract

Fine roots play a major role in the global carbon cycle through respiration, exudation and decomposition processes, but their dynamics are poorly understood. Current estimates of root dynamics have principally been observed in shallow soil horizons (< 1 m), and mainly in forest systems. We studied walnut (*Juglans regia* × *nigra* L.) fine root dynamics in an agroforestry system in a Mediterranean climate, with a focus on deep soils (down to 5 m), and root dynamics throughout the year. Sixteen minirhizotron tubes were installed in a soil pit, at depths of 0.0-0.7, 1.0-1.7, 2.5-3.2 and 4.0-4.7 m and at two distances from the nearest trees (2

and 5 m). Fine root (diameter ≤ 2 mm) dynamics were recorded across three diameter classes every three weeks for one year to determine their phenology and turnover in relation to soil depth, root diameter and distance from the tree row. Deep (> 2.5 m) root growth occurred at two distinct periods, at bud break in spring and throughout the winter i.e., after leaf fall. In contrast, shallow roots grew mainly during the spring-summer period. Maximum root elongation rates ranged from 1 to 2 cm day^{-1} depending on soil depth. Most root mortality occurred in upper soil layers whereas only 10% of fine roots below 4 m died over the study period. Fine root lifespan was longer in thicker and in deeper roots with the lifespan of the thinnest roots (0.0-0.5 mm) increasing from 129 days in the topsoil to 190 at depths > 2.5 m. The unexpected growth of very deep fine roots during the winter months, which is unusual for a deciduous tree species, suggests that deep and shallow roots share different physiological strategies and that current estimates based on the shortest root growth periods (i.e. during spring and summer) may be underestimating root production. Although high fine root turnover rates might partially result from the minirhizotron approach used, our results help gain insight into some of the factors driving soil organic carbon content.

2.1 Introduction

Tree growth is highly dependent on the absorptive function of fine roots (with a diameter ≤ 2 mm) for water and nutrients (Leuschner 1998; Hinsinger 2001). Fine roots also play a major role in the global carbon (C) cycle, mainly through production, respiration, exudation and decomposition processes (McClaugherty et al. 1982; Desrochers et al. 2002; Berg and McClaugherty 2008; Strand et al. 2008). Fine roots generate a rapid return of C to the atmosphere through decomposition and respiration processes, but conversely represent a significant C input to the soil by the incorporation of dead root material and exudates (Balesdent and Balabane 1996; Kuzyakov and Domanski 2000). Fine roots therefore have a significant impact on soil C sequestration (Matamala et al. 2003; Rasse et al. 2005), that could be especially relevant in deep soil layers (Kell 2012). Several studies have shown that root lifespan generally increases with soil depth (Hendrick and Pregitzer 1996; Baddeley and Watson 2005), root order (Eissenstat and Yanai 1997; Guo et al. 2008a; Guo et al. 2008b) and root diameter (Wells and Eissenstat 2001; Joslin et al. 2006). However, most of the studies dealing with fine root dynamics have been performed in relatively shallow soil horizons (< 1 m), in forest ecosystems and in temperate regions (Hendrick and Pregitzer 1993a; Brunner et al. 2013). Root dynamics below a depth of 1 m still remain poorly studied despite their crucial

role for supplying water to plants during dry periods (Tian and Doerner 2013; Maeght et al. 2013; Binkley 2015) and their possible influence on C sequestration (Rasse et al. 2005; Maeght et al. 2013).

Silvoarable agroforestry systems are simultaneous or sequential associations of woody perennial species and crop production systems in the same area (Somarriba 1992; Torquebiau 2000). In addition to maintaining a high agricultural production (Dupraz and Liagre 2008; Clough et al. 2011), agroforestry systems provide many ecosystem services, e.g., biodiversity enhancement relative to intensive agriculture (Varah et al. 2013), protection against soil erosion (Young 1997) and might contribute to buffering climate change through high C sequestration capacities (Oelbermann et al. 2004; Lorenz and Lal 2014). However, the belowground component of agroforestry systems still remains poorly understood (Mulia and Dupraz 2006; Cardinael et al. 2015c). This lack of knowledge is particularly important for root dynamics since, to our knowledge, only a few studies have been performed in these systems (Schroth and Zech 1995; Livesley et al. 2000; Muñoz and Beer 2001) and data on root dynamics at depths > 2 m are extremely scarce (Richter and Billings 2015).

In tree-based intercropping systems, trees are usually grown at low densities (30 to 200 trees ha^{-1}) to minimize direct competition on the intercrop for natural resources (light, water, nutrients). Competition with neighbouring trees is therefore low in agroforestry systems compared to trees growing in denser forest ecosystems (Khan and Chaudhry 2007). Besides genetic factors and climatic conditions, the growth rate of a given tree species depends mainly on its capacity to explore the soil volume and extract soil resources (mainly water and nutrients) through its roots (Tian and Doerner 2013). In low competition systems, as is the case for agroforestry systems, root exploration is maximized and thus tree growth is also maximized in respect to forest trees, explaining why some authors have found faster growing rates in trees from agroforestry systems compared to trees from plantation forests (Balandier and Dupraz 1999; Chaudhry et al. 2003; Gavaland and Burnel 2005). In forest systems, it has been found that root elongation rates are directly related to tree growth (Graefe et al. 2008). We would therefore expect greater root elongation rates in agroforestry stands.

Little is known about the seasonality of fine root phenology as a function of soil depth and resource availability. Generally, root production is synchronized with leaf production (Burke and Raynal 1994; McCormack et al. 2014), but this is not always the case and root production may be decoupled from leaf production in some tree species (McCormack et al. 2015). In

Mediterranean ecosystems, with a relatively long drought period during the summer, root growth in shallow soil layers can be very constrained (López et al. 1998). In these ecosystems, leaf production occurs mainly during the spring when water availability is high (Dufour et al. 2013). Moreover, fine root elongation rates and lifespan are in directly dependent on soil water availability and soil temperature (Gill and Jackson 2000), and thus subject to seasonal variations in these variables (e.g., drought periods) and on variations with soil depth (Mainiero and Kazda 2006; Meier and Leuschner 2008). Since leaf production in Mediterranean deciduous species takes place during the spring (Dufour et al. 2013), we expect that root and leaf production would be in synchrony with a peak production in spring. We would then expect a winter rest period in the belowground compartment as found in the aerial compartment (Dufour et al. 2013).

Recent studies have shown that temporal patterns of resource availability and competition may shape root turnover rates (Majdi and Andersson 2005; McCormack and Guo 2014). Due to their specific phenology and development, winter crops take up water and nutrients before bud break occurs in trees in late spring (Burgess et al. 2004; Dufour et al. 2013). Topsoil layers are therefore commonly impoverished with regard to water and nutrients, forcing agroforestry trees to develop deep root systems (Mulia and Dupraz 2006; Dupraz and Liagre 2008; Cardinael et al. 2015c). It has also been shown that nitrogen (N) fertilization, a common practice in agroforestry systems, decreases the lifespan of shallow fine roots (McCormack and Guo 2014). Additionally, environmental conditions such as soil moisture and temperature are buffered in deep soil layers, fluctuating less at daily and seasonal scales, usually resulting in lower fine root turnover rates (Anderson et al. 2003; Baddeley and Watson 2005). We therefore hypothesize that tree fine roots growing at similar depths to crop roots would have a shorter root lifespan than deeper tree roots, due to the competition with crop roots and more fluctuating environmental conditions.

The distance to tree trunk is another potential factor influencing root dynamics. The most distal lateral roots usually have the highest rates of metabolism, i.e the highest rates of respiration, associated to short lifespans (Pregitzer et al. 1998; Pregitzer et al. 2002; Xia et al. 2010). However, very few studies have compared whether fine root phenology and turnover rates are different in roots growing close to and far away from the tree trunk. Photosynthates have to be transported a longer distance to reach roots far from the trunk. Considering long-distance C transport theory, based on the transport-resistance model (Minchin and Lacointe

2005), we hypothesize that over one year, growth would be initiated in root near the trunk, but that these same roots would die last.

Using a large and deep (4 m) soil pit and the minirhizotron technique, we quantified tree fine root dynamics in a Mediterranean agroforestry system where hybrid walnut (*Juglans regia* × *nigra* L.) trees are grown intercropped with durum wheat (*Triticum turgidum* L. subsp. *durum*). Our objectives were to examine the phenology, elongation, and turnover rates of walnut fine roots and to estimate the influence of soil depth, root diameter and distance to the trunk of nearby trees. We hypothesize that i) fine root dynamics are in synchrony with leaf growth peaking in late spring and are arrested during the dry months and in autumn and winter after leaf fall, ii) fine root turnover rates and mortality are lower in deep soil layers compared to top soil layers and decrease with increasing root diameter, and iii) deep root elongation and turnover rates are higher in this agroforestry system than commonly reported in other studies carried out in Mediterranean forest ecosystems regardless of their observation methods and iv) fine root mortality would be initiated far from the trees and would be higher than that close to the trees.

2.2 Materials and methods

2.2.1 Study site

Measurements were conducted at the Restinclières farm estate, located 15 km North of Montpellier, France (Longitude 4°01'E, Latitude 43°43'N, 54 m a.s.l.). The climate is sub-humid Mediterranean with an average annual rainfall of 873 mm (mean from 1995 to 2013), which is lowest in July and highest in September to December. The mean temperature (mean from 1995 to 2013) is 15.4°C, with a maximum monthly mean in July (24.9 °C), and minimum in January (7.1 °C) (Fig. II-2.S1). The soil is a silty clay (25% clay and 60% silt) deep alluvial Fluvisol (IUSS Working Group WRB 2007) and the average pH is approximately 8.0 (Dupraz et al., 1999).

The study site is an alley-cropping plot where hybrid walnut trees (*Juglans regia* × *nigra* cv. NG23) were planted in parallel east-west oriented tree rows spaced at 13 m. Trees were planted every 4 m along the tree rows. In the inter-rows, crops are planted 1 m away from the tree trunks so that cropped inter-rows are 11 m in width. All walnut trees were planted in 1995 at an initial density of 192 trees ha^{-1}, and were thinned to the actual 96 trees ha^{-1} in 2004.

The associated crop is mainly durum wheat (*Triticum turgidum* L. subsp. *durum*). However, rapeseed (*Brassica napus* L.) was also cultivated in 1998, 2001 and 2006, and pea (*Pisum sativum* L.) in 2010. The soil is either ploughed to a depth of 0.2 m or surface tilled with disks before sowing, and the crop is fertilized with an average of 120 kg N ha^{-1} yr^{-1}.

2.2.2 Opening of the deep pit

In March 2012, a pit 5 m (length) × 1.5 m (width) × 4 m (depth) was dug perpendicular to the tree rows, starting close to the tree row and ending in the middle of the inter-row (Cardinael et al. 2015c). This pit was located between two walnut trees belonging to the same tree row (Fig. II-2.1). Trees were 13.80 and 11.70 m tall and had a diameter at breast height (DBH) of 26.1 and 30.5 cm, respectively.

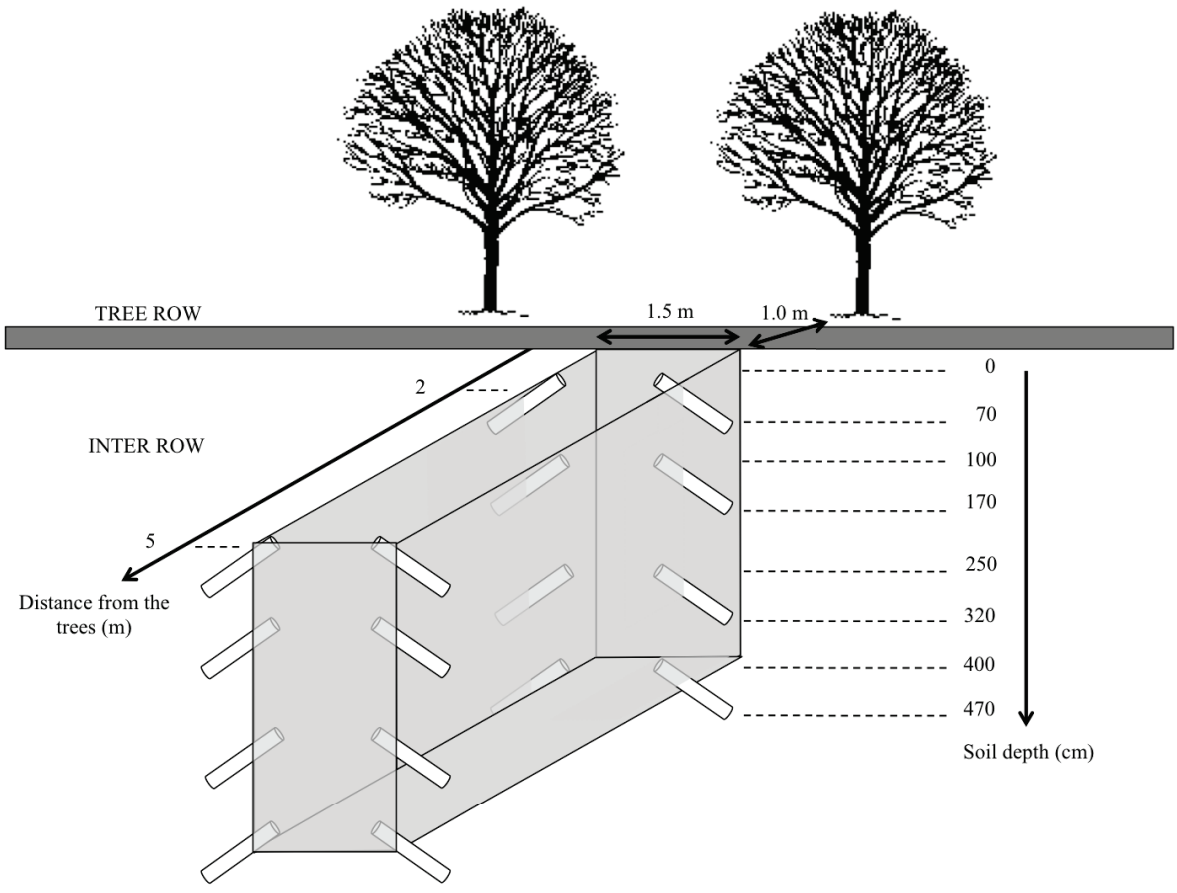


Figure II-2.1. Experimental setup showing the observation pit with the position of the 16 minirhizotrons tubes depending on soil depth and the distance to the nearest tree.

In order to reduce potential disturbances of the root-soil system and soil collapse, a wooden frame was set up on the walls of the whole pit, at the end of the digging phase. Whenever large gaps between soil and the wooden planks were found, these were filled with the original soil of the site and depth. The pit was covered by a light-coloured metallic roof, which prevented both radiation and rain from entering the pit. The rain pooling on the roof was evacuated laterally (5 m away from the pit) through gutters pipes in order to avoid excess water ponding around the pit.

2.2.3 Minirhizotron set up and monitoring of root dynamics

Fine root (diameter < 2 mm) dynamics were studied using the minirhizotron technique (Majdi et al. 2001; Tierney and Fahey 2002; Withington et al. 2003). In June 2012, 16 transparent polyvinylchloride tubes (105 cm in length and 7.6 cm in diameter) were installed into the lateral pit walls using a drill, at depths of 0.0, 1.0, 2.5 and 4.0 m. Wooden planks were cut as closely as possible, to fit around the tubes and avoid soil evaporation. Eight tubes were located at a distance of 2 m from the trees and eight additional tubes at 5 m, in the middle of the inter-row (Fig. II-2.1). Tubes were inserted with a 45° degree angle, and thus reached a depth of 0.7 m below the depth at which they were installed. Even if measurements of root growth in minirhizotrons are likely to be biased due to the disturbance of intact soils (Strand et al. 2008), minirhizotrons are the most commonly used devices to measure root dynamics because they allow direct, continuous, non-destructive *in situ* observations. Fine roots were monitored from the first month after minirhizotron installation. Nevertheless, as a precaution and to allow for a stabilization period following the soil disturbance associated with the installation, the root dynamics data in the first 2 months after minirhizotron installation were excluded from analyses (Graefe et al. 2008). We ensured this stabilization period was long enough by comparing results from these minirhizotrons with results from previously installed minirhizotrons in the site (see below).

Outside the pit, six other minirhizotrons were installed in November 2011 in the same plot, i.e., six months before those installed inside the pit (8 months before the first scan), and measurements were carried out over a 19 months period (from January 2012 to July 2013). Minirhizotrons were set up in the tree row at a distance of 2.3 m from the trunk and down to a depth 0.7 m close to three representative trees (two tubes per tree). Results of root elongation rate are presented in supplementary material (Fig. II-2.S2), and were used to check if root

growth observed in 0.0-0.7 m tubes in the pit (two months of stabilization period), was similar to that observed in these tubes (eight months of stabilization period).

Root dynamics were recorded using a scanner system (CI-600 Root Growth Monitoring System, CID, USA). Five images (21.59 cm × 19.56 cm) per tube were taken every three weeks during a one year period (from August 2012 to August 2013).

In April 2013, eight volumetric soil moisture sensors (Campbell CS 616) and eight temperature sensors (Campbell 107) were installed in the pit near the minirhizotrons tubes at four depths (0.5 m, 1.2 m, 3 m and 4 m) and at two distances from the tree row (2 m and 5 m). Holes made within the wooden planks to insert moisture and temperature probes were sealed up around the cable with adhesive sealant to avoid soil water evaporation. Soil temperature and volumetric moisture were recorded every hour using a Campbell dataloger (CR1000).

2.2.4 Root digitizing and image analysis

Root images were analysed using the WinRHIZO Tron software (Régent, Canada) to trace roots while marking their length and diameter (Graefe et al. 2008). For the analysis, only walnut roots were digitized, and were given unique identification numbers. It was easy to distinguish walnut roots from durum wheat roots based on their morphological characteristics. Walnut roots are cream coloured during the first days of growth, before turning black, and have few absorbent root hairs. Lateral branches are perpendicular and the tip of their apex is yellowish-brown.

Before analysing a new dataset, the most recent images were superimposed with those from the previous dataset to characterize the evolution of the traced roots and report their new characteristics and state: "*live*" if they had lengthened or remained cream in colour, "*dead*" if they were dead or presented obvious signs of decay (shrivelled, transparent, faint, or turning black), or "*new*" if they were observed for the first time (Cheng et al. 1991; Satomura et al. 2007). To check the dead status of roots, successive images recorded in later periods were analysed on the same location of the tube to ensure they remained black and no growth occurred. For all images, root diameter (mm), the length (mm) of *live* and *dead* roots, and appearance of every *new* root were recorded.

2.2.5 Root length production and mortality

The following metrics were used to describe root length production and mortality:

(i) *live* length production ($LLP_{t\text{-}1,t}$, in cm m^{-2}) and *dead* length loss ($DLL_{t\text{-}1,t}$, in cm m^{-2}) through mortality, were defined as the length of *live* or *dead* fine roots during a given time period from t_{-1} to t (where t is the time of root inventory) per unit of observed soil area (A, in m^2), respectively:

$$LLP_{t_{-1},t} \text{ (or } DLL_{t-1,t}) = \sum_{n=1}^{N} \frac{l_{n,t} - l_{n,t_{-1}}}{A} \qquad (1)$$

where, $l_{n,t\text{-}1}$ and $l_{n,t}$ are the length of the *live* (or *dead*) root n at inventory time t_{-1} and t, respectively; $n \in [1, N]$, N is total number of *live* (or *dead*) roots included for calculating *live* or *dead* length loss; A is unit of observed soil area through scanned images.

(ii) Cumulative *live* length production ($CLLP_{t\text{-}1,t}$, in cm m^{-2}) and cumulative *dead* length loss ($CDLL_{t\text{-}1,t}$, in cm m^{-2}) were defined as the total length of *live* or *dead* roots until the time period T, respectively (Hendrick and Pregitzer 1996; Majdi and Andersson 2005; Graefe et al. 2008):

$$CLLP_T \text{ or } CDLL_T = \sum_{t=1}^{T} LLP_{t_{-1},t} \text{ (or } DLL_{t_{-1},t}) \qquad (2)$$

(iii) By subtracting C$LLP_{t\text{-}1,t}$ and C$DLL_{t\text{-}1,t}$, we obtain the net root production (NRP_T, in cm m^{-2}).

$$NRP_T = CLLP_T - CDLL_T \qquad (3)$$

(iv) To estimate the number of roots that died ($DN_{t\text{-}1,t}$, in No. of roots m^{-2}) during a given time interval, the difference in the number of *dead* roots between t and t_{-1} was calculated (Withington et al. 2003) and standardized per unit soil area A.

$$DN_{t_{-1},t} = \frac{DN_t - DN_{t_{-1}}}{A} \qquad (4)$$

where, $DN_{t\text{-}1}$ and DN_t are the number of *dead* roots at inventory time t_{-1} and t, respectively.

Individual root growth was evaluated by calculating the difference between the root length at t_{-1} and at t. To determine the daily root elongation rate (RER) (cm day^{-1}), the mean of all

individual root lengths produced between time t and t_{-1} was divided by the duration of the corresponding period:

$$RER_{t_{-1},t} = \frac{1}{N} \sum_{n=1}^{N} \frac{l_{n,t} - l_{n,t_{-1}}}{p_{t_{-1},t}} \quad (5)$$

where, $RER_{t-1,t}$ is the average daily root elongation rate (in cm d^{-1}); $l_{n,t-1}$ and $l_{n,t}$ are the length of the root n at inventory time t_{-1} and t, respectively; $n \in [1, N]$; N is total number of roots included for calculating average daily RER; $p_{t-1,t}$ is the period between inventory time t_{-1} and t (d).

All these variables were calculated for each root diameter class (0.0-0.5 mm; 0.5-1.5 mm; 1.5-2.0 mm), distance to tree trunk (proximal: 2 m and distal: 5 m) and soil depth (0.0-0.7 m, 1.0-1.7 m, 2.5-3.2 m and 4.0-4.7 m).

Mean soil temperature and volumetric moisture at t was calculated, as the mean of the temperature and volumetric moisture between t and t_{-1}. Soil temperature and soil volumetric moisture were only measured simultaneously with the image acquisition during 5 months.

2.2.6 Root lifespan and turnover

Individual root lifespan was calculated as the number of days between the first observation of the root (birth date) and the day it was declared dead (Anderson et al. 2003; Graefe et al. 2008). Median lifespan (MLS, days) of fine roots was estimated as the median of elapsed time between root initiation and death. Turnover ($year^{-1}$) was calculated as the inverse of median lifespan:

$$T = \frac{365}{MLS} \quad (6)$$

where T is the renewal rate (y^{-1}); MLS is the median lifespan (d)

We used the non-parametric Kaplan-Meier method to determine the survivorship rate (S) of roots that were growing over a given period of time (Majdi et al. 2001; Tierney and Fahey 2002), as well as the MLS (Goel et al. 2010; Crawley 2012). Each individual root had an equal weight and was classified as still *live* (censored, not dead yet) or *dead* (uncensored) at the end of the study. The hazard function estimated the probability for a root to die at time t,

knowing that it was live at t_{-1}. As a result, it could be considered as the instantaneous probability of mortality (Anderson et al. 2003; Withington et al. 2003; Majdi and Andersson 2005). The Kaplan-Meier survivorship function is:

$$S = \prod_{t<T} \frac{r\ t\ - d\ t}{r\ t} \qquad (7)$$

where, *r(t)* is the number of roots still *live* at time *t* (those that have not yet died); *d(t)* is the number of *dead* roots at time *t*; *T* is the whole period of the root monitoring time.

We used the semi-parametric Cox Proportional Hazard Model (Cox 1972) to analyse the effects of different factors on root dynamics during the growing span (Wells and Eissenstat 2001). This model assumes that the hazard of mortality of a root *i* at time period *t* (noted as $h_i(t)$) is a multiple of a baseline hazard (noted as $h_0(t)$) and an exponential linear function of a group of *k* covariates:

$$h_i\ t\ = h_0\ t\ e^{\beta_1 x_{i,1} + \beta_2 x_{i,2} + \cdots + \beta_k x_{i,k}} \qquad (8)$$

The factors acting as co-variables include distance to tree trunk, soil depth and root diameter class. The Cox's proportional hazards regression approach was validated ($p < 0.05$) by pooling the depth classes in two subsets, in order to increase the number of data in each class: 0.0-0.7 m with 1.0-1.7 m and 2.5-3.2 m with 4.0-4.7 m.

2.2.7 Data processing

Separate generalized linear models (GLM) were used with RER, NRP and CDLL as the dependent variables, and root diameter, soil depth or distance to tree trunk as independent factors and all interactions between factors. We used each minirhizotron tube as a single replicate (i.e. n=4 for each depth). To account for the non-independency among the four tubes within a single depth (e.g. 20 cm), we included a covariance matrix (corMatrix function in R software) into the GLM. A Shapiro-Wilk test was performed before each GLM to guarantee that the investigated indicator followed a normal or quasi-normal distribution. Homogeneity of variances was checked and a log-transformation used when data did not comply with normality of the residuals in the model. These analyses were followed by a one-way analysis of variance (ANOVA) for each factor. Post-hoc differences between root diameters were analysed using Tukey's post-hoc Honest Significant Difference (HSD) test at $p<0.05$. To analyse the effects of soil depth and root diameter on root survivorship we used the Kaplan-

Meier and Cox Proportional Hazard methods using the 'Survival' package in R (Therneau 2014). Wilcoxon tests were used to determine significance in root survivorship and lifespan. All calculations and analyses were performed using the R software, Version 2.15.3 (R Development Core Team 2013).

2.3 Results

2.3.1 Influence of soil depth and distance to tree trunk on fine root phenology

Two distinct periods of root growth were observed (Figs. II-2.2; II-2.3a), one during the winter period (November-January) and one during late spring and summer (April-August). Winter growth was dominated by deep roots (> 2.5 m), whereas summer growth occurred at all soil depths but was highest in the upper horizons. In spring 2013, root growth initiated in April in the topsoil layers (0.0-0.7 m) and in May at a depth of 1.0-1.7 m. However, in deeper layers (2.5-3.2 m and at 4.0-4.7 m) root growth did not start until two months later, in June (Fig. II-2.2). Root growth in the upper layers (0-0.7 m and 1-1.7 m) was in synchrony with leaf growth as 50% and 100% of the bud burst took place in April and May, respectively.

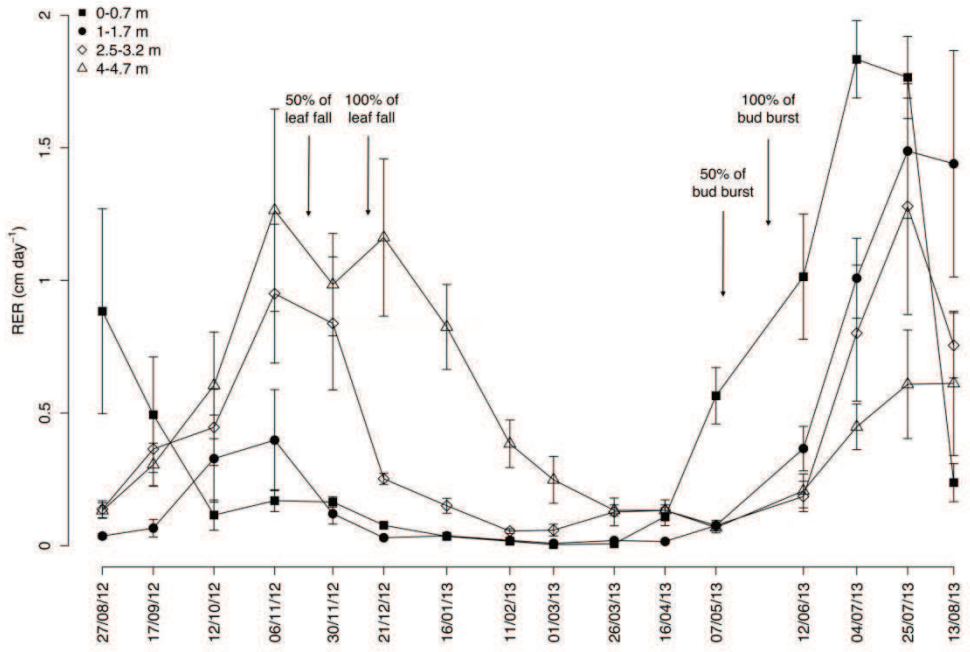


Figure II-2.2. Mean daily root elongation rate (RER, cm day^{-1}) in the 0.0-0.7 m, 1.0-1.7 m, 2.5-3.2 m and 4.0-4.7 m soil layers from August 2012 to August 2013. Vertical arrows indicate when 50% and 100% of bud burst and leaf fall were observed in *Juglans regia* tree branches. Vertical bars represent standard deviations (not shown when smaller than the symbol size).

The intensity (measured as the maximum RER) and the duration of the root growth period also differed with soil depth; growth flushes were more intense and of shorter duration in the top 0.7 m than at 4.0-4.7 m (significant depth effect; F = 4.14, P = 0.007; Table II-2.1). In topsoil layers (0.0-0.7 m), RER started to decrease before the winter period and almost ceased in the winter. In deep soils (4.0-4.7 m), the maximum RER (>1.3 cm day^{-1}) was observed in November after leaf fall. In spring, the maximum RER occurred in topsoil layers (0.0-0.7 m) reaching 1.8 cm day^{-1} (Fig. II-2.2). Mean RER are presented in Table II-2.2.

There was no significant effect (F = 1.72, P = 0.19) of distance to tree trunk on RER (Table 1). However, the interaction between soil depth and distance to tree trunk was highly significant (F = 4.40, P = 0.005) (Table II-2.1). Root growth tended to occur mainly close to the tree than further away from it (Figs. II-2.4 a,b) and in shallow layers (0.0-0.7 m). Root growth started in April in distal roots but in proximal roots it occurred one month later, in May, which was not the case in deeper roots.

	RER		NRP		CDLL	
	F-value	Pr(>F)	F-value	Pr(>F)	F-value	Pr(>F)
Diameter	64.65	<0.001***	155.77	<0.001***	64.34	< 0.001***
Depth	4.14	0.007**	26.73	<0.001***	71.49	< 0.001***
Distance	1.72	0.19	0.15	0.70	0.98	0.32
Diameter × Depth	2.84	0.01**	13.27	<0.001***	27.34	< 0.001***
Diameter × Distance	0.32	0.72	1.28	0.28	4.91	0.008**
Depth × Distance	4.40	0.005**	13.77	<0.001***	25.62	< 0.001***

Table II-2.1. ANOVA on the GLM model for root elongation rate (RER), cumulative net root production (CNRP) and cumulative dead length loss (CDLL) as a function of the fine root diameter, soil depth, distance to the tree, and interactions between factors. Asterisks indicate significance levels where * P=0.05, ** P=0.01 and *** P <0.001

2.3.2 Effect of depth and distance to tree trunk on fine root mortality

Root mortality (CDLL) and root production (CLLP) followed a similar pattern to RER (Fig. II-2.3b). There was a significant effect ($F = 71.49$, $P < 0.001$) of soil depth on CDLL (Table II-2.1). Two periods of fine root mortality were observed for roots in the topmost layer (< 0.7 m), one in the winter and one in the summer, but for deep roots, no preferential period of mortality was observed. Mortality was thus concentrated near the soil surface (0.0-0.7 m), whereas roots below 1 m contributed relatively little to the total length of dead roots (Fig. II-2.3b).

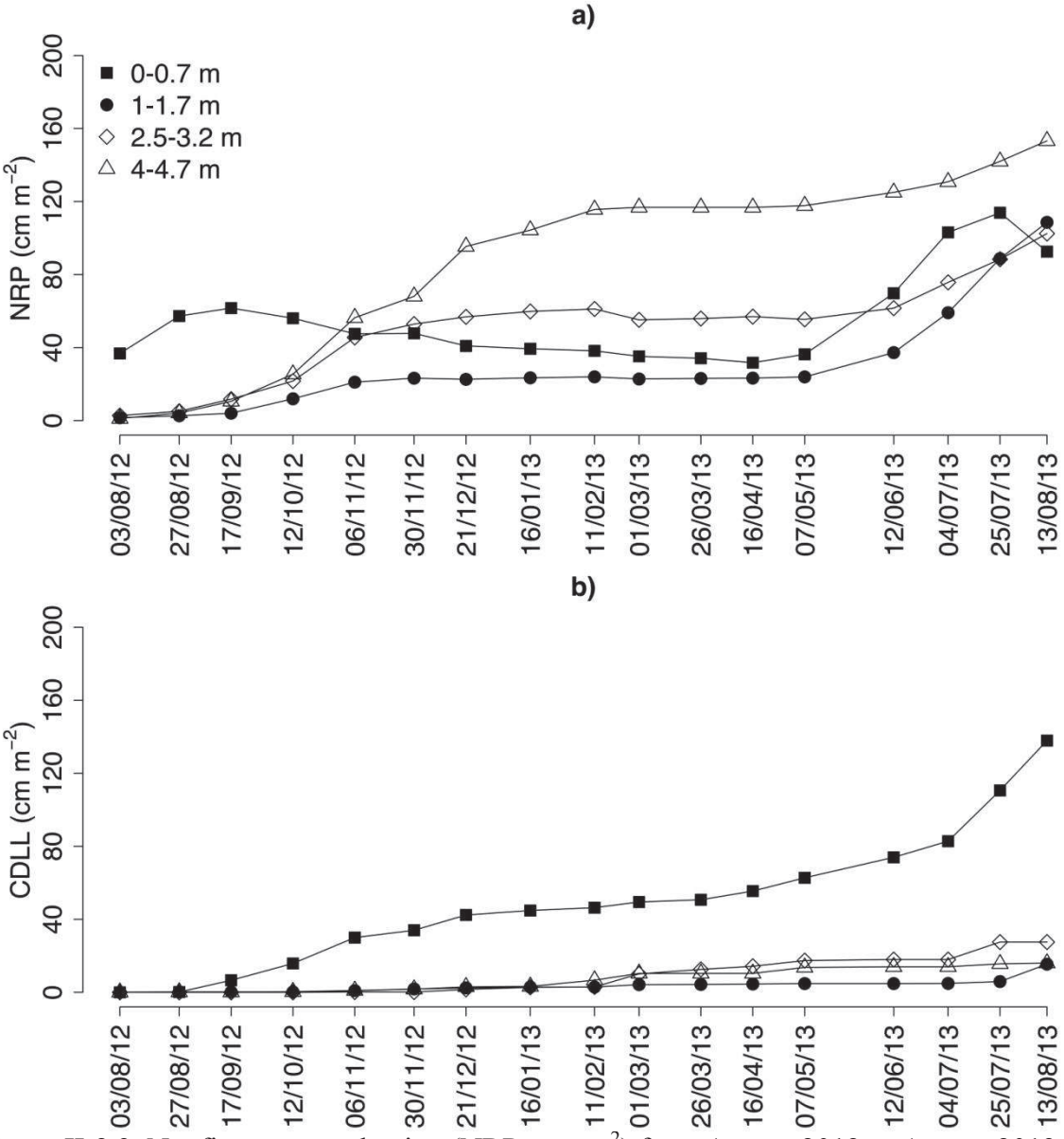


Figure II-2.3. Net fine root production (NRP, cm m^{-2}) from August 2012 to August 2013 a) in the 0.0-0.7 m, 1-1.7 m, 2.5-3.2 m and 4.0-4.7 m soil layers, b) and cumulative dead length loss (CDLL, cm m^{-2}) in the same soil layers.

The cumulative dead root length and number of dead roots in the 0.0-0.7 m soil horizon represented more than 60% of the total dead root length and the total number of dead roots through the year of the study (Table II-2.2). Nearly 80% of all fine roots produced at a depth of 0.0-0.7 m were dead by the end of the study whereas only 10% were dead at 4.0-4.7 m.

Soil Depth (m)	0.0-0.7	1.0-1.7	2.5-3.2	4.0-4.7
CLLP (cm m^{-2})	228	145	150	198
CDLL (cm m^{-2})	162	18	33	19
% in each soil layer of the total root length produced over 1 year	31.7	20.1	20.7	27.5
% of root mortality	70	12	22	10
Mean RER over 1 year (cm day^{-1})	0.46 ± 0.59	0.34 ± 0.52	0.40 ± 0.36	0.46 ± 0.34
N (total number of roots per m^2 of minirhizotron appeared over 1 year)	387	133	164	218

Table II-2.2. Cumulative live length production (CLLP) and cumulative dead length loss (CDLL) over 1 year, estimated per class of depth, % of root length produced in each soil layer relative to the total root length produced, % of the total root length modality per each soil layer and total number of roots per m^2 of minirhizotrons tube. Mean root elongation rates (cm day^{-1}) calculated over 1 year in each soil layer.

No significant relationship ($F = 0.98$, $P = 0.32$) was found between CDLL and distance to tree (Table II-2.1). However, the interaction between soil depth and distance to tree on CDLL was significant ($F = 25.62$, $P = < 0.001$) (Table II-2.1). Far from the tree (5 m), a period of high mortality was observed from October to December (Fig. II-2.4d). In contrast, in proximal roots, at a distance of 2 m from the tree trunk, no root mortality was observed within the same period (Fig. II-2.4c) and in proximal roots to the trunk mortality started in June (Fig. II-2.4c). Finally, similar amounts of dead root length occurred at 2 and 5 m from the trees.

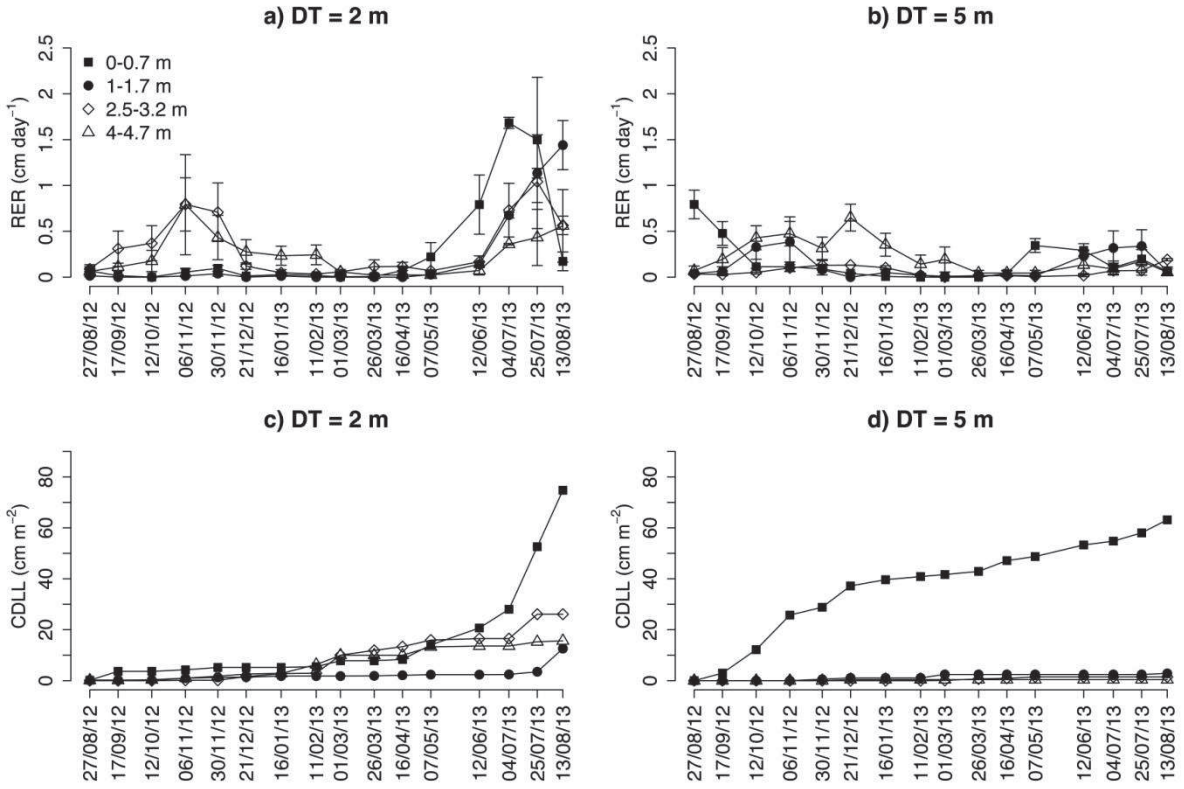


Figure II-2.4. Mean daily root elongation rate (RER, cm day^{-1}) from August 2012 to August 2013 in the 0.0-0.7 m, 1.0-1.7 m, 2.5-3.2 m and 4.0-4.7 m soils layers at a distance of a) 2m from the nearest trees, and at b) 5 m from the nearest trees. Cumulative *dead* length loss (CDLL, cm m^{-2}) for each soil depth at a distance of c) 2 m from the nearest tree, and d) 5 m from the nearest tree. Vertical bars represent standard deviations (not shown when smaller than the symbol size).

2.3.3 Effect of root diameter and soil edaphic conditions on root elongation rates

A significant effect of root diameter class ($F = 64.65$, $P < 0.001$) and soil depth ($F = 2.84$, $P = 0.01$) on RER was found (Table II-2.1). The finest roots (0.0-0.5 mm) had a growth peak in spring with a maximum elongation rate of 0.3 cm day^{-1} in June. These very fine roots were the first to appear and also the first to die (Fig. II-2.5). Roots with a diameter between 0.5-1.5 mm flushed one month later at all soil depths (Fig. II-2.5b). This diameter class contributed the most to elongation rate estimates, as it included the majority of fine roots. Fine roots with a diameter between 1.5-2.0 mm started to grow in October for the winter flush and in June for the summer flush. At depths greater than 1 m, this fine root growth occurred one month after that of fine roots with a diameter 0.5-1.5 mm. For all diameter classes, fine root elongation was delayed by 1 month between the topsoil and the deeper horizons.

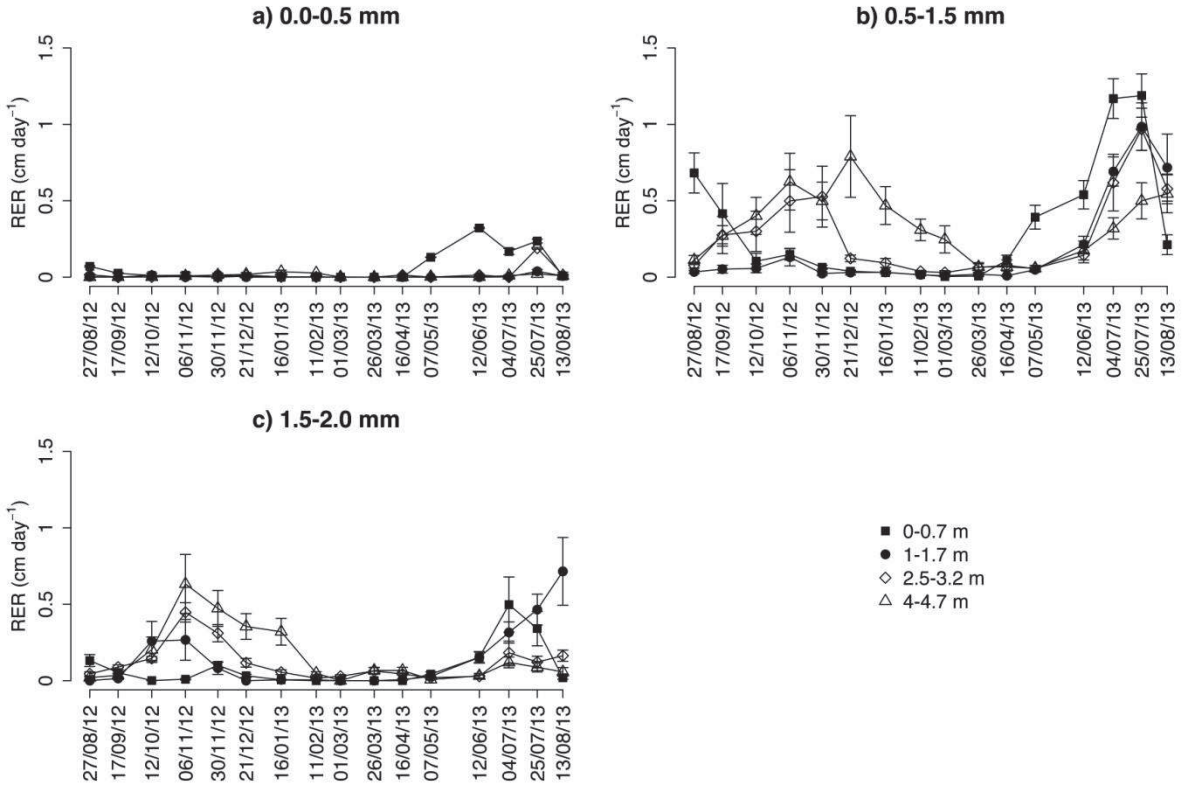


Figure II-2.5. Mean daily root elongation rate (RER, cm day^{-1}) from August 2012 to August 2013 in the 0.0-0.7 m, 1.0-1.7 m, 2.5-3.2 m and 4.0-4.7 m soil layers for roots with a diameter between a) 0.0-0.5 mm, between b) 0.5-1.5 mm, and c) 1.5-2.0 mm. Vertical bars represent standard deviations (not shown when smaller than the symbol size).

There was a positive relationship (r^2=0.86, F = 121, P < 0.001) between RER and mean soil temperature (Fig. II-2.6). A weaker, but significant negative effect of soil volumetric humidity on RER was found (r^2=0.39, F = 13, P = 0.002). The interaction of soil temperature and soil volumetric moisture was not significant. Soil moisture measurements were not recorded during the first summer dry period of the study year (August-September 2012). There were not enough data to separate the effects of depth and distance to tree from the soil temperature or soil volumetric humidity on RER.

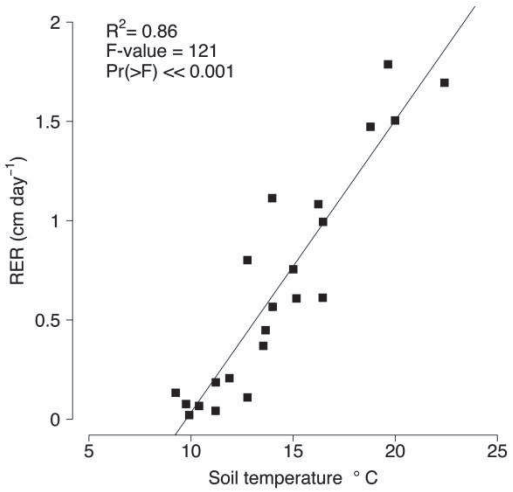


Figure II-2.6. Relationship between daily root elongation rates (RER, cm day^{-1}) and daily soil temperatures (°C).

2.3.4 Fine root turnover as a function of diameter class and depth

Median root lifespan estimates differed between diameter classes (Fig. II-2.7). The survival probability estimated with these approaches was not smooth but followed a series of steps due to the predictive model (Fig. II-2.7). Root diameter, soil depth and their interaction had a significant influence on CDLL (Table II-2.1). The survival probability of extremely fine roots (0.0-0.5 mm) decreased faster over time than that of fine roots within the other diameter classes (0.5-1.5 mm and 1.5-2.0 mm; Fig. II-2.7a). No roots survived longer than the 1-year study period. After 350 days, fine roots had an estimated survival probability of 0% regardless of their diameter class (Fig. II-2.7a). Fine root median lifespan increased with increasing diameter class and was estimated in the range 142-243 days (Table II-2.3). Fine root turnover significantly decreased with increasing diameter and was estimated in the range 1.5-2.6 yr^{-1}.

Deep roots lived longer (2.5 months on average) than shallow roots and fine root turnover averaged 2.2 and 1.8 yr^{-1}, for the depths 0.0-1.7 m and 2.5-4.7 m, respectively (Table II-2.3). Within the same diameter class, deep roots always had a significantly longer lifespan compared to shallow roots (Table II-2.4).

	Diameter classes (mm)			Soil layer (m)	
	0.0-0.5	0.5-1.5	1.5-2.0	0.0-1.7	2.5-4.7
Median lifespan (days)	142 ± 13.8 c	195 ± 25.1 b	243 ± 9.34 a	167 ± 11.4 b	208 ± 9.76 a
Turnover ($year^{-1}$)	2.6	1.9	1.5	2.2	1.8
N (Number of roots)	640	2131	295	1769	1297

Table II-2.3. Median lifespan of individual roots estimated using proportional hazard regressions per diameter class and soil layer. Standard deviations are indicated. Different letters in the same line denote significant differences ($p < 0.05$) between diameter classes or depth classes.

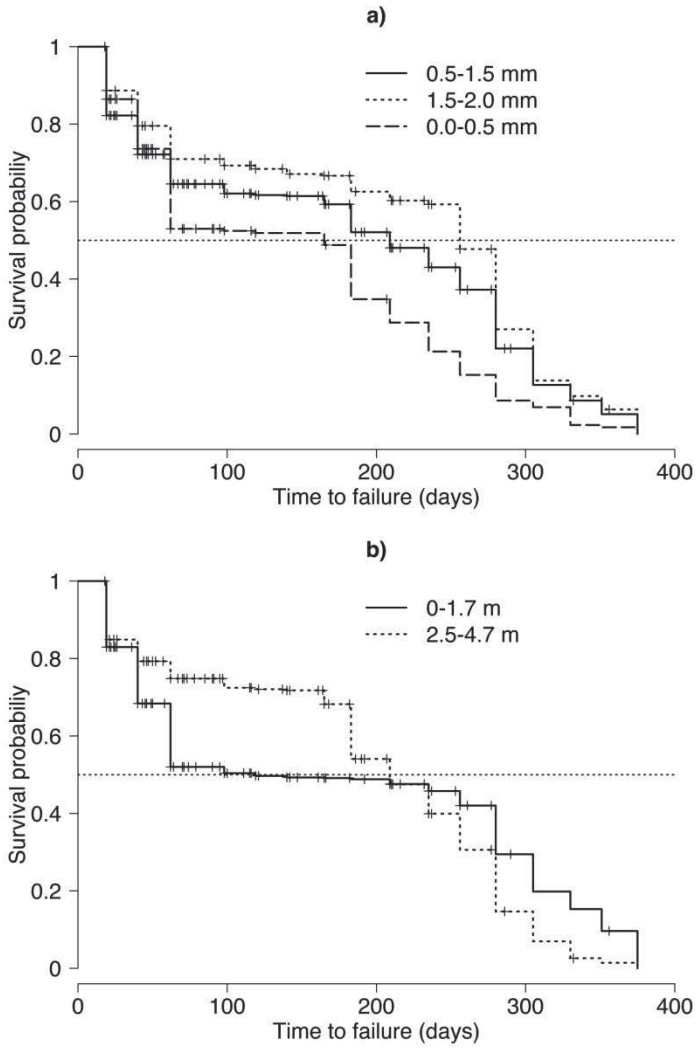


Figure II-2.7. Root survivorship a) for each root diameter class (0.0-0.5 mm, 0.5-1.5 mm and 1.5-2.0 mm), and b) each soil layer (0.0-0.7 m and 2.5-4.7 m) from August 2012 to August 2013. Survivorships were estimated using a proportional hazards regression. See Table II-2.1 for detailed statistical results.

	Depth classes (m)					
	0.0-1.7			2.5-4.7		
	Diameter classes (mm)			Diameter classes (mm)		
	0.0-0.5	0.5-1.5	1.5-2.0	0.0-0.5	0.5-1.5	1.5-2.0
Median lifespan (days)	129 ± 9.85 f	175 ± 15.6 e	221 ± 6.45 b	190 ± 6.38 d	207 ± 14.7 c	255 ± 4.66 a
Turnover (yr^{-1})	2.8	2.1	1.7	1.9	1.8	1.4
N (Number of roots)	451	1125	193	189	1006	101

Table II-2.4. Median lifespan of individual roots estimated using proportional hazard regressions per diameter class and soil layer. Standard deviations are indicated. Different letters in the same line denote significant differences ($p < 0.05$) between diameter classes and depth classes.

There was an effect of soil depth on the survival probability of fine roots (Fig. II-2.7b). The survival probability was high (> 80%) at all depths during the first month but then started to differ between soil depths (Fig. II-2.7b). After 150 days, fine roots in upper soil horizons had a 30% lower survival probability than roots from deeper soils (50% versus 80% respectively). These differences disappeared after 200 days as survival probability at all depths dropped to nearly 0% after 350 days (Fig. II-2.7b).

2.4 Discussion

Our results showed that, surprisingly, root growth occurred deep in the soil during the autumn and winter months and after leaf fall had taken place and that consistent differences in RER and survival were found between soil depths. We also demonstrate major differences in root turnover and survival rates between fine root diameter classes, as commonly reported in forest ecosystems, which suggests that the longevity of fine roots in agroforestry systems is also dependent on their functional role (Guo et al. 2008b).

2.4.1 Key role of deep roots

Deep roots, and in particular deep fine roots, are crucial organs for plant growth and survival especially in Mediterranean ecosystems, as they can provide a reliable source of water to the tree during the drought period (Padilla and Pugnaire 2007). While many studies in temperate forests have shown that the majority of annual fine root production occurs in the topsoil (Joslin and Henderson 1987; Mao et al. 2013a; Mao et al. 2013b), we demonstrated that fine roots below a depth of 4 m can account for more than one fourth of the total root production in the whole soil profile (to a depth of 4.7 m). At the same site, Cardinael et al. (2015b) found that 35% of total fine root intersection density occurred below a depth of 2 m in the spring. We also found a lower survivorship and a higher turnover rate of shallow roots compared to deep roots, thus validating the first part of our second hypothesis. Joslin and Henderson (1987) and Hendrick and Pregitzer (1996) found similar results in a *Quercus alba L*. forest and in a northern hardwoods, respectively. Deep roots in our study had relatively high growth rates and survival probabilities compared to roots in shallow soil layers. Root longevity has been found to increase with soil depth down to 0.5 m, for Concord grape (*Vitis labruscana* B., Anderson et al. 2003) and down to 1.6 m for peach trees (*Prunus persica* L., Wells et al. 2002). In shallow soil horizons, temperature and moisture have larger fluctuations than deeper in the soil, which was the case in our study, and this may affect root lifespan (Gill and Jackson 2000; Hendricks et al. 2006; McCormack and Guo 2014). In addition, walnut roots may suffer from competition with the annual intercrop in topsoil (Mulia and Dupraz 2006; Cardinael et al. 2015c), where dry soil conditions might contribute to reduce the lifespan of walnut roots. Thus, deep roots may act as a reliable and longer term C sink (Kell 2012), but their impact on deep soil organic C has to be assessed in the long term, as these roots are renewed more slowly and have a lower density than shallow roots. At the same site, Cardinael et al. (2015a) found that soil organic C stocks were increased by 6 Mg C ha^{-1} to a depth of to 1 m in the agroforestry plot compared to an agricultural plot, of which 75% of this increase was located in the top 0.3 m. Eighteen years after tree planting, no additional storage was found below 1 m. This result may be due to a lower C input at depth than in the topsoil, indicating the possibility that a longer period may be required before being able to detect a change in the organic C stock of deep soil horizons.

2.4.2 Root mortality influenced by distance to tree and root diameter

Our fourth hypothesis was only partially confirmed as root mortality occurred earlier for distal roots compared to proximal roots, but the rate of mortality decreased closer to the tree. The earlier root mortality in distal roots may be due to a lower photosynthate supply from shoots situated at a long distance (Radin et al. 1978; Keel et al. 2012).). Trees might not be able to sustain photosynthate supply to these roots because additional energy is required for such long distance transport. Hence, root mortality is triggered earlier.

Fine root turnover decreased with increasing root diameter class, which fully confirms our second hypothesis. This phenomenon has been observed for many species (Gill and Jackson 2000; Majdi et al. 2001; Wells and Eissenstat 2001; Tierney and Fahey 2002; Beyer et al. 2013). Trees likely preserve thicker fine roots because they are essential for resource transport and are the origin of thinner lateral roots, whose role is crucial in the uptake of nutrients and water (Richter and Billings 2015). Roots differing in functional role have different turnover rates (Anderson et al. 2003) and thicker roots usually have lower concentrations of N, but higher concentrations of carbohydrate, lignin and cellulose compared to thinner roots (Guo et al. 2004; Prieto et al. 2015a). Thicker roots are then more expensive to construct in terms of C cost, and tree investment is likely to favour long-term organs, with a reduced turnover (Eissenstat 1992).

2.4.3 Unexpected fine root phenology

The observed synchronous growth of fine roots with leaf growth in spring could reflect the need to meet the fast growing demand for transpiration during leaf emergence (Hendricks et al. 2006). There is still a lively debate concerning the synchronism between the emergence of leaves and fine roots: which appears first? (Willaume and Pagès 2006; Chantereau et al. 2012; McCormack et al. 2015). Our measurements at monthly intervals are not frequent enough to accurately answer this question but our study suggests that root and shoot production was synchronous, since they occurred within the same month. This synchronism only happened in topsoil layers, whereas root growth was delayed in deeper soil layers indicating a degree of asynchrony within the root system. We hypothesize that this lag in root growth with increasing soil depth might be due to a rapid exhaustion of the resources, especially water, in topsoil layers, inducing tree roots to start growing deeper in the soil to take up water and nutrients (Richter and Billings 2015).

Our results were surprising in that RER was high in deep soil layers during the winter months, i.e. after leaf fall. Many previous studies (Hendrick and Pregitzer 1993b; Hendrick and Pregitzer 1996) showed that fine root production in temperate forest trees occurred mainly in spring and early summer at all soil depths. Nonetheless, winter growth was also reported for shallow roots in a similar study performed in natural and managed forests in the French Alps where shallow root growth occurred during the winter in high altitude spruce (*Picea abies* L.) and fir (*Abies alba* Mill.) dominated mixed forests (Mao et al. 2013b). Shallow root growth has also been observed during the winter for grape plants (*Vitis vinifera* L.) in Mediterranean southern France (Jourdan, unpublished data) and for conifer species in Mediterranean ecosystems (Leshem 1970; Waisel et al. 2002). In our study, we report winter root growth also in deep soil layers, which may be due to edaphic soil conditions, as temperature and moisture were less variable than in the topsoil. Such stable environmental conditions in deep soil layers may make root growth possible. In Mediterranean ecosystems, the soil surface is usually humid during the winter whereas in summer it becomes very dry, preventing root growth (Leshem 1970; Waisel et al. 2002). Although remobilization of non-structural C (NSC) and nutrients stored in woody organs can be essential for early leaf development in spring (Millard and Grelet 2010), our results suggest that roots are able to utilize local stores of NSC for fine root growth in the winter. However, while both the favourable soil conditions and adequate NSC stocks may well explain the ability of trees to develop deep roots in winter, the reason for which the trees develop these roots remains unclear. We hypothesize that deep root growth in walnut trees in winter is not driven by the need to provide water or nutrients to shoots during this period, as it is a deciduous species, but that it may be simply a consequence of superfluous NSC plus favourable environmental conditions. With this mechanism, trees could extend their roots as far and deep as possible, which can be interpreted as a biological strategy for maximizing their survivorship if environmental conditions deteriorate.

2.4.4 Root elongation and environmental conditions

Walnut tree RER in our study was highly correlated with soil temperature (Fig. II-2.6), but the relationship between RER and soil volumetric moisture was rather poor. The high sensitivity of fine root growth to soil temperature is well documented in forest ecosystems under boreal and temperate climates (Gill and Jackson 2000; Hendricks et al. 2006; Mao et al. 2013a; McCormack and Guo 2014) and seems to operate also in Mediterranean agroforestry systems. Soil temperature is likely to drive RER and root mortality through complex mechanisms; in

particular its influence on root metabolic activity and nutrient mineralization rates (Fornara et al. 2009). In our study, as the soil temperature never attained higher than 24°C below a depth of 0.5 m and therefore, never reached levels deleterious for root growth (McCormack and Guo 2014). Although we found a positive influence of soil temperature on RER, soil temperature is highly influenced by soil depth (Pregitzer et al. 2000) as was RER, making it difficult to differentiate edaphic and temperature factors. Thus, further experiments should be performed to disentangle the effects of soil temperature and depth.

Surprisingly, the lack of relationship with soil volumetric moisture and root growth was in contrast to previous studies in forest ecosystems in a Mediterranean climate (López et al. 1998; Misson et al. 2006). These studies found that root growth was less rapid in very dry conditions. At our site, a water table is present at a depth between 5 and 7 m and soil volumetric moisture varied little during spring and summer months throughout the entire soil profile (data not shown), except in the topsoil. However, soil volumetric moisture was only recorded between April and July 2013 and not during the driest season (August-September 2012). The availability of soil water content during the study period may explain the lack of correlation between RER and soil volumetric moisture.

2.4.5 High turnover rate

Fine root turnover estimates of walnut trees were between 1.4 and 2.8 yr^{-1}, i.e. higher than those commonly reported for forest ecosystems (Priess et al. 1999), confirming our third hypothesis. According to other studies in temperate forests, fine root turnover was about 0.7 yr^{-1} (Gill and Jackson 2000; Withington et al. 2003; Espeleta et al. 2009). However, some studies on temperate tree species estimated fine root turnover rates of similar magnitude than that found in our study. Tierney and Fahey (2002) found a range between 0.7 and 2.6 yr^{-1} for sugar maple (*Acer saccharum* Marsh.), American beech (*Fagus grandifolia* Ehrh.) and yellow birch (*Betula alleghaniensis* Britt.). McCormack and Guo (2014) found turnover rates between 0.5 and 2.5 yr^{-1} for 12 temperate tree species in USA, and Brunner et al. (2013), in their global review of several European *Fagus sylvatica* forests, found that fine root turnover ranged from 0.2 to 2.9 yr^{-1} (using a sequential coring method). The turnover rates we measured were closer to those estimated in tropical climates e.g., 1.3 and 1.8 yr^{-1} for *Eucalyptus grandis* W. in Brazil (Jourdan et al. 2008), 2.0 yr^{-1} for *Eucalyptus sp.* in Congo (Thongo M'bou et al. 2008) and more than 2.0 yr^{-1} in several cloud forest tree species in

Venezuela (Graefe et al. 2008). Turnover rates are usually faster in warm climates than cold climates, because turnover is linked to root growth, which is in turn driven largely by soil temperature.

2.4.6 Prerequisites and limits of the minirhizotron technique

The minirhizotron technique is considered as a reliable approach to estimate fine root turnover and longevity (Majdi et al. 2001; Hendricks et al. 2006). A stabilization time is usually required to ensure that soil disturbance at the installation of the minirhizotrons does not lead to a flush of fine roots which would over-estimate normal root growth. For example, minirhizotrons studies initiated after 8-10 months of stabilization in temperate forests (Hendricks et al. 2006) and after 5-6 months of stabilization in tropical forests (Graefe et al. 2008). Although the stabilization time was short in our study (two months between installation and the first scan), the highest net fine root production (Fig. II-2.3a) and RER (Fig. II-2.2) were measured during the spring, i.e., about 1 year after the installation of the minirhizotrons. A flush of fine root production, which is characteristic right after the installation of the tubes, was not observed in the first months of monitoring. The reliability of our results is demonstrated by a comparison with six other minirhizotrons set up at the soil surface in the same agroforestry plot 6 months before the installation of the tubes in the deep pit. The comparison analysis showed that median lifespans were not significantly different for the six minirhizotrons installed 6 months before and those obtained from measurements in our study pit at the same depth (0.0-0.7 m), regardless of the diameter class (Table II-2.S1). Flushes of fine root production were synchronous between the six surface tubes and the tubes in the pit at the same depth. (Figure II-2.S2). However, root production and RER were slightly lower in the tubes installed 6 months before in the plot, compared to those in the pit. Therefore, the stabilization period may have quantitatively influenced fine root production more than root phenology. Even though fine root growth at the onset of the study period might have been slightly overestimated, comparisons of root phenology and mortality between soil layers are reliable since all the tubes inside the deep pit were installed simultaneously at all depths. Measurements are still on going and in the future they should allow us to determine patterns of root dynamics over a period of several years and allow us to link together root decomposition, respiration and nutrient and water uptake.

2.4.7 Implications for agroforestry management and growth models

Hybrid walnut trees in an agroforestry system under Mediterranean conditions revealed an unexpected functioning of fine roots. The rapid fine root turnover in the topsoil and the fine root growth in deep soil layers in the winter may reflect tree plasticity in response to the heterogeneity of soil conditions. In Mediterranean agroforestry systems, competition with the intercrops induces extremely variable soil conditions throughout the year, forcing the tree roots to explore and tap deeper layers (Mulia and Dupraz 2006; Cardinael et al. 2015c). Establishing deep root systems will also help agroforestry trees to withstand long or intense summer droughts which are expected to increase in the future in the Mediterranean region (Solomon et al. 2009).

Among the ecosystem services that are expected from temperate agroforestry systems, two depend on the rooting patterns and dynamics that we have demonstrated in this study. Active fine roots in deep soil horizons in the winter may be effective at capturing N leached below the rooting zone of the annual crops by the autumn and winter rains. This process will aid the control of environmental pollution from N (Andrianarisoa et al. 2015). Regarding C sequestration in the soil, the fate of deep roots is of high significance. A lower turnover rate of fine roots in deep horizons compared to the topsoil would reduce the amount of C potentially sequestered, while the low mineralization rate in deep soil layers would increase the potential sequestration. The balance between the two processes needs a careful appraisal. Process-based models of ecosystem functioning also need to be modified to take into account these patterns of fine root phenology and turnover (Mulia et al. 2010). The Hi-sAFe model of tree-crop interactions in agroforestry (Talbot et al. 2014) includes a 3D opportunistic tree root growth module. In this model, root phenology was so far assumed to be totally synchronous with leaf expansion, and root turnover was assumed to be independent of the distance to tree trunk and the soil layer depth. Such hypotheses need to be modified to comply with the findings of this study, and may result in a very different fate of the tree-crop system as a whole.

2.5 Supplementary material

	Diameter classes (mm)		
	0.0-0.5	0.5-1.5	1.5-2.0
Median lifespan (days)	127 ± 3.05 c	134 ± 3.14 b	229 ± 4.10 a
Turnover (yr^{-1})	2.8	2.1	1.7
N (Number of roots)	265	77	52

Table II-2.S1. Median lifespan of individual roots estimated using proportional hazard regressions per diameter class at 0.0-0.7 m soil depth horizon obtained with the minirhizotrons in the plot. Standard deviations are indicated. Different letters in the same line denote significant differences ($p < 0.05$) between diameter classes and depth classes.

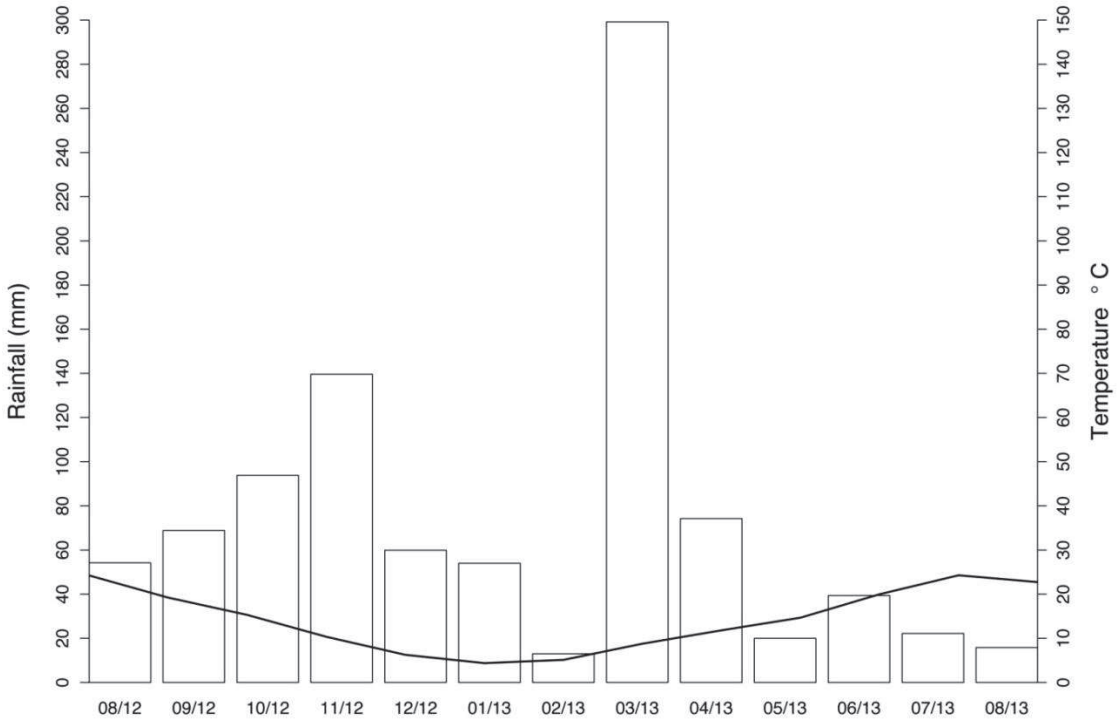


Figure II-2.S1. Ombrothermic diagram of the study period; air temperature (°C); rainfall (mm).

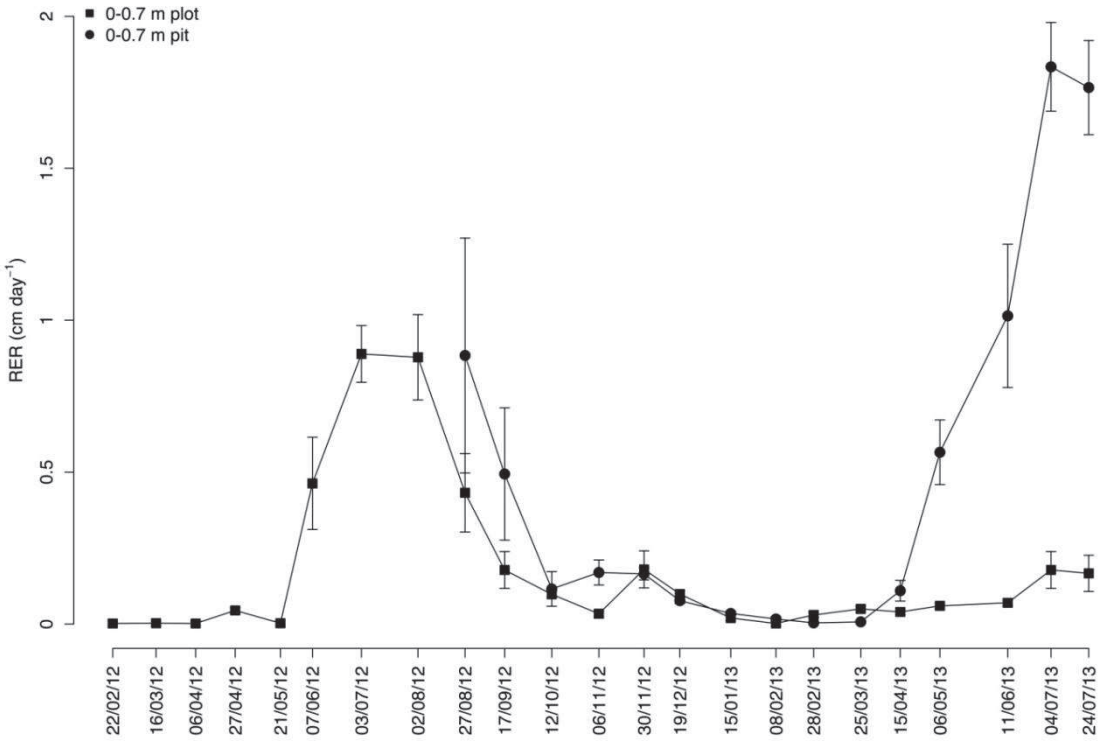


Figure II-2.S2. Mean daily root elongation rate (RER, cm day^{-1}) at a depth of 0.0-0.7 m in the pit and in the plot over time. Vertical bars represent standard deviations (not shown when smaller than the symbol size).

Chapitre 3

Bilan des entrées de carbone organique dans le sol – comparaison d'une parcelle agroforestière et agricole sous climat méditerranéen

Article en préparation (***couplé avec la Partie 3, chapitre 2***)

Can fresh OM inputs drive SOC storage in alley cropping agroforestry systems? A study case in a Mediterranean context using experimental and modeling approaches

Rémi Cardinael, Bertrand Guenet, Tiphaine Chevallier, Christian Dupraz, Claire Chenu

3.1 Materials and methods

3.1.1 Study site

The experimental site was located in Prades-le-Lez, 15 km North of Montpellier, France (Longitude 04°01' E, Latitude 43°43' N, elevation 54 m a.s.l.). The climate is sub-humid Mediterranean with an average temperature of 15.4°C and an average annual rainfall of 973 mm (years 1995–2013). The soil is a silty and carbonated deep alluvial Fluvisol (IUSS Working Group WRB 2007). In February 1995, a 4.6 hectare alley cropping agroforestry plot was established with the planting of hybrid walnut (*Juglans regia* × *nigra* cv. NG23) trees at a current density of 110 trees ha^{-1}. Trees were planted at 13 m × 4 m spacing, and tree rows were East–West oriented. The cultivated inter-rows are 11 m wide. The remaining part of the plot (1.4 ha) was kept as a control agricultural plot. Since the tree planting, the agroforestry inter-rows and the control plot were managed in the same way. The associated crop was

durum wheat (*Triticum turgidum* L. subsp. *durum*) and the soil was ploughed to a depth of 0.2 m before sowing. The wheat crop was fertilized with an average of 120 kg N ha^{-1} yr^{-1}. Crop residues (wheat straw) were also harvested, but about 25% remained on the soil. Tree rows were covered by a spontaneous and herbaceous vegetation. Two successive vegetation types occurred during the year, one in summer and one in winter. The summer vegetation was mainly composed of *Avena fatua* L., and was 1.5 m tall. In winter, the vegetation was a mix of *Achillea millefolium* L., *Galium aparine* L., *Vicia* L., *Ornithogalum umbellatum* L. and *Avena fatua* L, and was short (20 cm).

3.1.2 Carbon stock in the tree aboveground biomass and in the stump

Three hybrid walnuts were chopped down in 2012. Trunk circumference was measured every meter up to maximum height of the tree to estimate its volume. The trunk biomass was estimated multiplying the trunk volume by the wood density that was measured at 616 kg m^{-3} during a previous work at the same site (Talbot 2011). Then, branches were cut, the stump was uprooted, and they were weighted separately. Samples were brought to the laboratory to determine their moisture content, which enabled calculation of the branches and stump dry mass. Three samples of the trunk and of the branches were analyzed with a CHN elemental analyzer (Carlo Erba NA 2000, Milan, Italy) to determine their carbon concentration. Carbon concentration of the stump was supposed to be the same as the carbon concentration of the trunk. Dry biomass of the trunk, of branches and of the stump were multiplied by their carbon concentration to get a carbon stock for the tree aboveground biomass and for the stump.

3.1.3 Tree fine root density

In March 2012, a deep pit was open in the agroforestry plot, perpendicular to the tree row (Fig. II-3.1). This pit was 5 (length) × 1.5 (width) × 4 m (depth). Tree fine root (≤ 2 mm) distribution was mapped up to 4 m depth, and tree fine root biomass was quantified in the tree row and at different distances in the inter-row. A detailed description of the methods used can be found in Cardinael *et al.*, (2015b). Only results concerning the first two meters of soil will be presented here. Briefly, tree fine root impacts were counted using a grid with regular squares (10×10 cm), and a relationship was established between tree fine root impacts and tree fine root biomass, enabling the prediction of fine root biomass along the soil profiles.

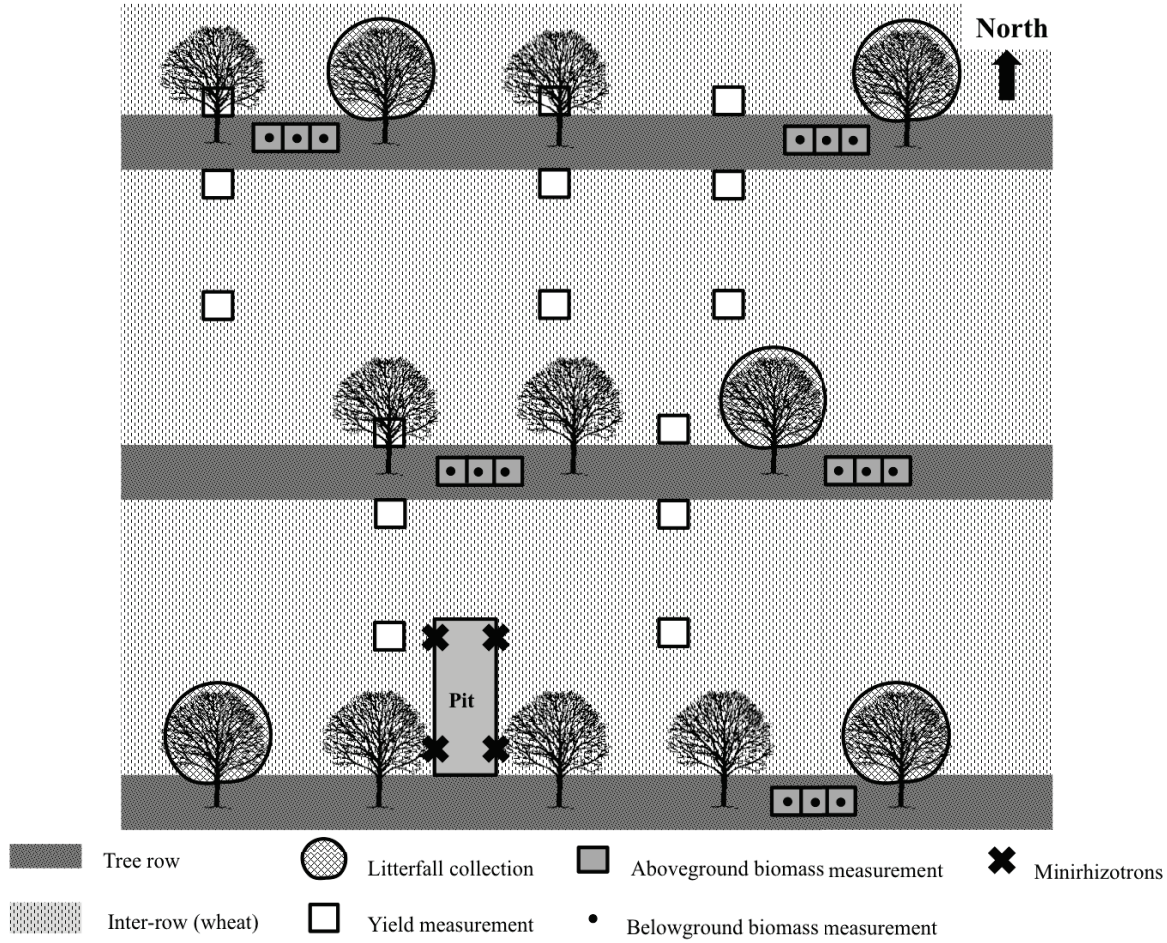


Figure II-3.1. Sampling protocol for the estimation of organic inputs in the agroforestry plot.

3.1.4 Tree fine root turnover

In July 2012, sixteen minirhizotrons were installed in the agroforestry pit, at 0, 1, 2.5 and 4 m depth, and at two distances from the tree (Fig. II-3.1). Soil temperature and moisture sensors were also installed at these depths. Tubes were 1.05 m long, had an inner diameter of 7.6 cm, and were inserted in the soil with a 4° degree angle. Tree root growth and mortality (diameter ≤ 2 mm) was monitored during one year using a scanner (CI-600 Root Growth Monitoring System, CID, USA), and analyzed using the WinRHIZO Tron software (Régent, Canada). A detailed description of the methods used can be found in Germon *et al.,*(submitted for publication).

3.1.5 Tree litterfall

In 2009, the crowns of two walnut trees were packed with a net in order to collect leaf litterfall from September to January. The same was done in 2012 with three other walnut trees (Fig. II-3.1). Leaf biomass was dried at 40°C, and 3 subsamples were analyzed with a CHN elemental analyzer (Carlo Erba NA 2000, Milan, Italy) to determine their carbon concentration. Leaf biomass was then dried at 105°C to determine total dry matter (kg DM $tree^{-1}$). The diameter at breast height (*DBH*) was also measured for each walnut. The ratio between leaf dry matter and *DBH* was calculated for the 5 replicates.

3.1.6 Aboveground and belowground input from the crop

Since the tree planting in 1995, yield and total aboveground biomass of the crop were measured in both the control and the agroforestry plot (Dufour et al. 2013). In the control plot, five replicates of 1 m^2 each were sampled. In the agroforestry plot, five transects were sampled (Fig. II-3.1). Each transect was made of three sampling plots (1 m^2 each), 2 m North from the tree, 2 m South from the tree, and 7 m from the tree (middle of the inter-row). Based on radiation measurements (hemispherical pictures) taken at different distances in the inter-row in 2012, it was estimated that yield measurements 2 m North from the trees were representative of a width of 4 m and yield measurements in the middle of the inter row were reprensentative of a width of 6 m. The carbon content of the wheat straw was analyzed on 5 sub-samples, after they were oven-dried at 40°C for 48 hours, using a CHN elemental analyzer (Carlo Erba NA 2000, Milan, Italy).

In March 2012, a 2 m deep pit was opened in the agricultural control plot (Prieto et al. 2015a), wheat fine root distribution was mapped, and root biomass was quantified to the maximum rooting depth (1.5 m). The root:shoot ratio of the durum wheat was measured in the control plot. We assessed that durum wheat grown in the agroforestry plot had the same root:shoot ratio, and wheat root biomass in the agroforestry plot was estimated using this ratio. As the same maximum rooting depth of the durum wheat was observed in the agroforestry plot than in the control plot, we assessed that wheat root distribution within the soil profile was not modified, but only its biomass.

3.1.7 Above and belowground biomass of the tree row vegetation

As two types of herbaceous vegetation grew in the tree rows during the year, samples were taken in summer and winter. In late June 2014, twelve squares of 1 m^2 each were positioned in the tree rows, around 4 walnut trees. In January 2015, six squares of 1 m^2 each were positioned in the tree rows, around 2 walnut trees. The middle of each square was located at 1 m, 2 m and 3 m, respectively, from the selected walnut tree (Fig. II-3.1). All the aboveground vegetation in each square was collected, and the humid mass was weighed. In the middle of each square (Fig. II-3.1), root biomass was sampled with a cylindrical soil corer (inner diameter of 8 cm). Soil was taken in three soil layers, 0-10, 10-30 and 30-50 cm. In the laboratory, soil was gently washed with water through a 2 mm mesh sieve, and roots were collected. Roots from the herbaceous vegetation were easily separated from walnut roots, as they were soft and yellow compared to walnuts roots that were black. After being sorted out from the soil and cleaned, humid mass of roots was weighted. For the aboveground and belowground biomass, sub-samples were oven-dried at 105°C for 48 hours to calculate the dry mass. The carbon concentration of the above and belowground biomass was analyzed using a CHN elemental analyzer.

3.2 Results

3.2.1 Carbon stock in aboveground biomass of walnut trees

The aboveground (trunk + branches) carbon stock of walnut trees reached 10.57 ± 0.97 t C ha^{-1} (Table II-3.1).

	Biomass (kg DM)	Carbon stock (kg C $tree^{-1}$)	Carbon stock (t C ha^{-1})
Trunk	123.53 ± 9.76	55.06 ± 4.35	6.06 ± 0.48
Branches	95.60 ± 17.85	40.98 ± 7.65	4.51 ± 0.84
Stump	47.59 ± 2.39	21.21 ± 1.07	2.33 ± 0.12
Total	266.72 ± 20.48	117.25 ± 8.87	12.9 ± 0.98

Table II-3.1. Carbon stocks in the aboveground biomass and in the stump of walnut trees.

3.2.2 Tree fine root input

Tree fine root biomass was higher in the tree rows, and decreased with increasing distance from the trees (Table II-3.2).

	Tree fine root biomass (t DM ha^{-1})			
Soil depth (cm)	Tree rows	Inter-rows		
		]0, 1.5] m	]1.5, 3.0] m	]3.0, 4.5] m
0-10	0.18 ± 0.02	0.19 ± 0.03	0.03 ± 0.01	0.01 ± 0.01
10-30	0.32 ± 0.05	0.54 ± 0.05	0.34 ± 0.04	0.11 ± 0.03
30-50	0.50 ± 0.05	0.36 ± 0.04	0.18 ± 0.02	0.12 ± 0.02
50-100	0.80 ± 0.09	0.31 ± 0.03	0.31 ± 0.03	0.19 ± 0.03
100-150	0.35 ± 0.04	0.22 ± 0.03	0.19 ± 0.03	0.19 ± 0.02
150-200	0.17± 0.03	0.30 ± 0.03	0.20 ± 0.03	0.15 ± 0.02
Total	2.32 ± 0.13	1.92 ± 0.09	1.25 ± 0.07	0.77 ± 0.06

Table II-3.2. Walnut tree fine root biomass as a function of depth and distance to the tree rows. DM = Dry matter. Errors stand for standard errors.

Tree fine root turnover ranged from 1.7 to 2.8 yr^{-1} depending on fine root diameter, with an average turnover of 2.2 yr^{-1} for fine roots ≤ 2 mm and to a depth of 2 m (Germon *et al.*, submitted for publication).

3.2.3 Leaf litterfall

Total leaf biomass was 8.96 ± 1.45 kg DM tree^{-1} and the carbon concentration of the walnut leaves was 44.94 ± 0.37 mg C g^{-1} (Table II-3.3). The ratio between leaf biomass and DBH was 0.0277 ± 0.0024 t C tree^{-1} m^{-1}. At the plot scale, leaf litterfall was estimated at 0.73 ± 0.06 t C ha^{-1}.

Type of OM	Organic C concentration (mg C g^{-1})	C:N	Number of replicates
Walnut trunk	445.7 ± 1.0	159.1 ± 25.2	3
Walnut branches	428.6 ± 1.7	62.2 ± 11.7	3
Wheat straw	433.2 ± 0.7	55.5 ± 2.1	5
Wheat root	351.4 ± 19	24.8 ± 2.1	8
Walnut leaf	449.4 ± 3.7	49.1 ± 0.4	3
Walnut fine root	412.2 ± 2.7	31.0 ± 3.1	8
Summer vegetation (ABG)	448.4 ± 1.9	37.8 ± 2.2	5
Summer vegetation (roots)	314.5 ± 8.3	33.8 ± 1.7	6
Winter vegetation (ABG)	447.7 ± 5.3	11.2 ± 0.4	3
Winter vegetation (roots)	397.4 ± 5.0	24.7 ± 0.7	3

Table II-3.3. Organic carbon concentrations and C:N ratio of the different types of organic matter. ABG: aboveground. Errors stand for standard errors.

3.2.4 OM inputs from the crop

In the control plot, wheat root biomass reached 6.52 ± 0.84 t DM ha^{-1} to 1.5 m depth (Table II-3.4). Root biomass decreased exponentially with depth (R^2=0.99). Organic carbon concentration of wheat roots was 351.4 ± 19 mg C g^{-1} (Table II-3.3).

Soil depth (cm)	Wheat root density (t DM ha^{-1})
0-10	1.36 ± 0.11
10-30	1.95 ± 0.22
30-50	1.25 ± 0.22
50-100	1.48 ± 0.55
100-150	0.48 ± 0.55
Total	6.52 ± 0.84

Table II-3.4. Wheat fine root biomass in the control plot in 2012. Errors stand for standard errors.

The root:shoot ratio was 0.79 ± 0.12 in the control plot. Yield and straw biomass were higher in the control plot than in the agroforestry plot (Table II-3.5). Annual variability of yield is about 10%.

Plot	Location	Yield (t ha^{-1})	Straw biomass (t DM ha^{-1})	Aboveground biomass (t DM ha^{-1})	Estimated belowground biomass (t DM ha^{-1})
Control		3.04 ± 0.29	3.69 ± 0.29	8.25 ± 0.63	6.52 ± 0.84
Agroforestry	2 m north	1.39 ± 0.39	2.34 ± 0.31	4.60 ± 0.85	3.61 ± 0.86
	7 m (middle)	2.10 ± 0.24	2.96 ± 0.16	6.26 ± 0.44	4.92 ± 0.81
	2 m south	2.32 ± 0.33	3.05 ± 0.23	6.51 ± 0.63	5.11 ± 0.91

Table II-3.5. Aboveground and belowground biomass of the wheat crop in the agroforestry and in the control plot in 2012. Yield, straw biomass and aboveground biomass were measured. Belowground biomass for the control was measured in the field. Other belowground values were estimated using the root:shoot ratio. Errors stand for standard errors.

3.2.5 OM inputs from the tree row vegetation

Distance from the trees had no effect on above and belowground biomass of the herbaceous vegetation (data not shown). The summer aboveground biomass was almost three times higher than in winter, whereas the belowground biomass was two times higher (Table II-3.6).

Biomass (t DM ha^{-1})		Summer	Winter
Aboveground		3.50 ± 0.23	1.24 ± 0.19
Belowground	0-10 cm	0.70 ± 0.10	0.42 ± 0.03
	10-30 cm	0.50 ± 0.07	0.16 ± 0.03
	30-50 cm	0.28 ± 0.04	0.10 ± 0.02
	Total	1.48 ± 0.13	0.68 ± 0.05

Table II-3.6. Aboveground and belowground biomass of the herbaceous vegetation in the tree rows. DM = Dry matter. Errors stand for standard errors.

3.2.6 OM inputs and SOC stocks: a summary

Tree rows in the agroforestry system received two times more OM inputs compared to the control plot (Fig. II-3.2), and 65% more than inter-rows. Globally, the agroforestry plot had 41% more OM inputs to the soil than the control plot to 2 m depth (3.80 t C ha^{-1} yr^{-1} compared to 2.69 t C ha^{-1} yr^{-1}). In the control plot, 85% of OM inputs are root litters. Root inputs represent 71% of OM inputs in the inter-rows, and 50% in the tree rows.

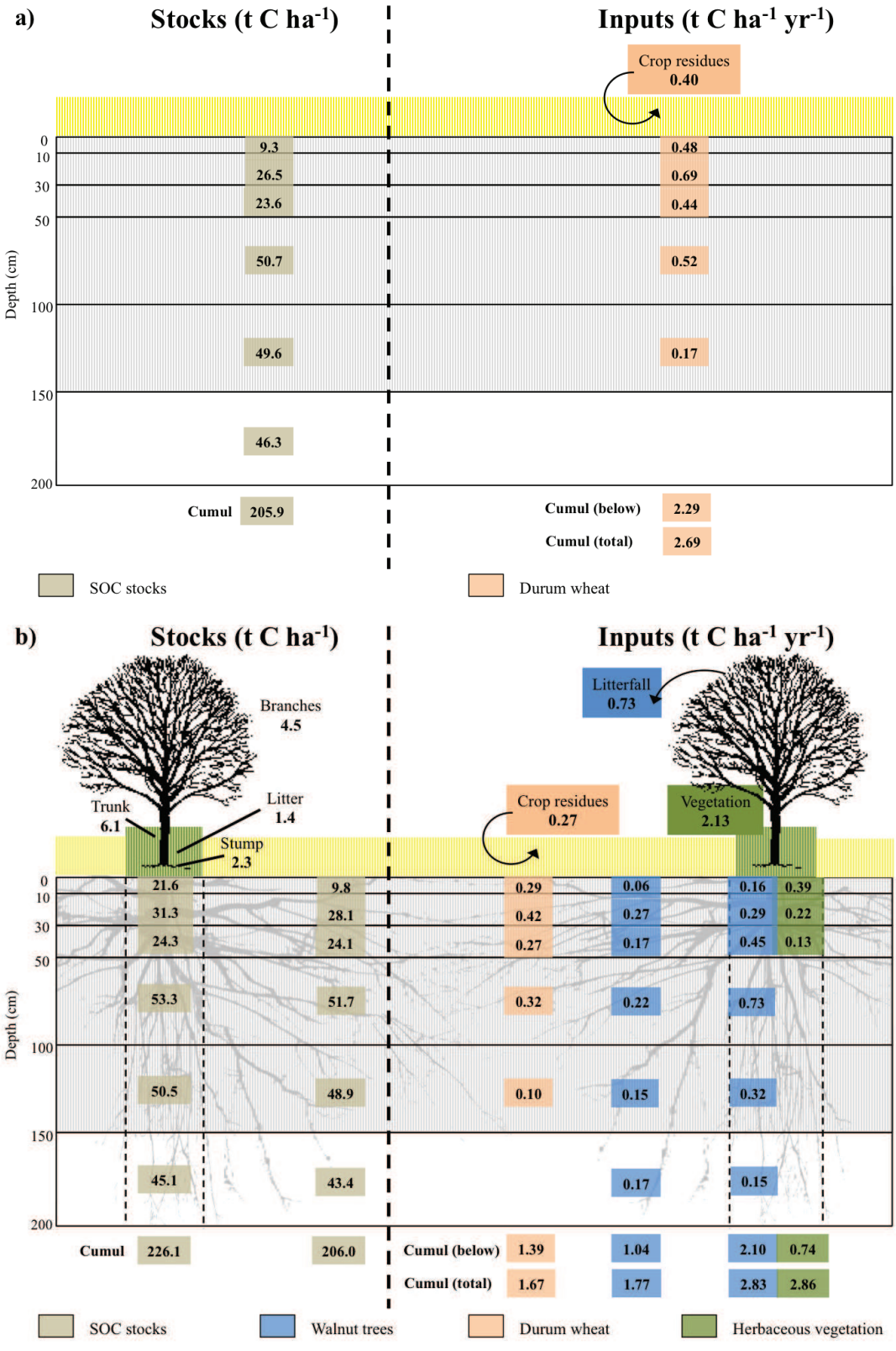


Figure II-3.2. Soil organic carbon stocks and organic carbon inputs to the soil a) in the agricultural control plot, b) in the 17 year old agroforestry plot. Below: belowground ; Total : aboveground + belowground. Values in the inter-row of the agroforestry plot represent average values north from the edge of the tree row to the middle of the inter-row. SOC stocks data are issued from Cardinael *et al.*, (2015a).

In the first 50 cm of soil, delta of cumulated OM inputs between the agroforestry plot and the control plot measured in 2012 in both plots was similar to observed delta SOC stocks (Fig. II-3.3). In 50-100 cm, OM inputs are probably underestimated in the tree row as root density of the herbaceous vegetation was only sampled in the first 50 cm of soil. Below 100 cm, delta SOC stocks are negative, while delta OM inputs are still positive. Modelling will provide us an insight on this phenomenon: is it a consequence of a pronounced priming effect in deep soil layers or a consequence of an initial heterogeneity at the site (higher SOC stocks at depth in the agroforestry plot before the establishment of the experiment)?

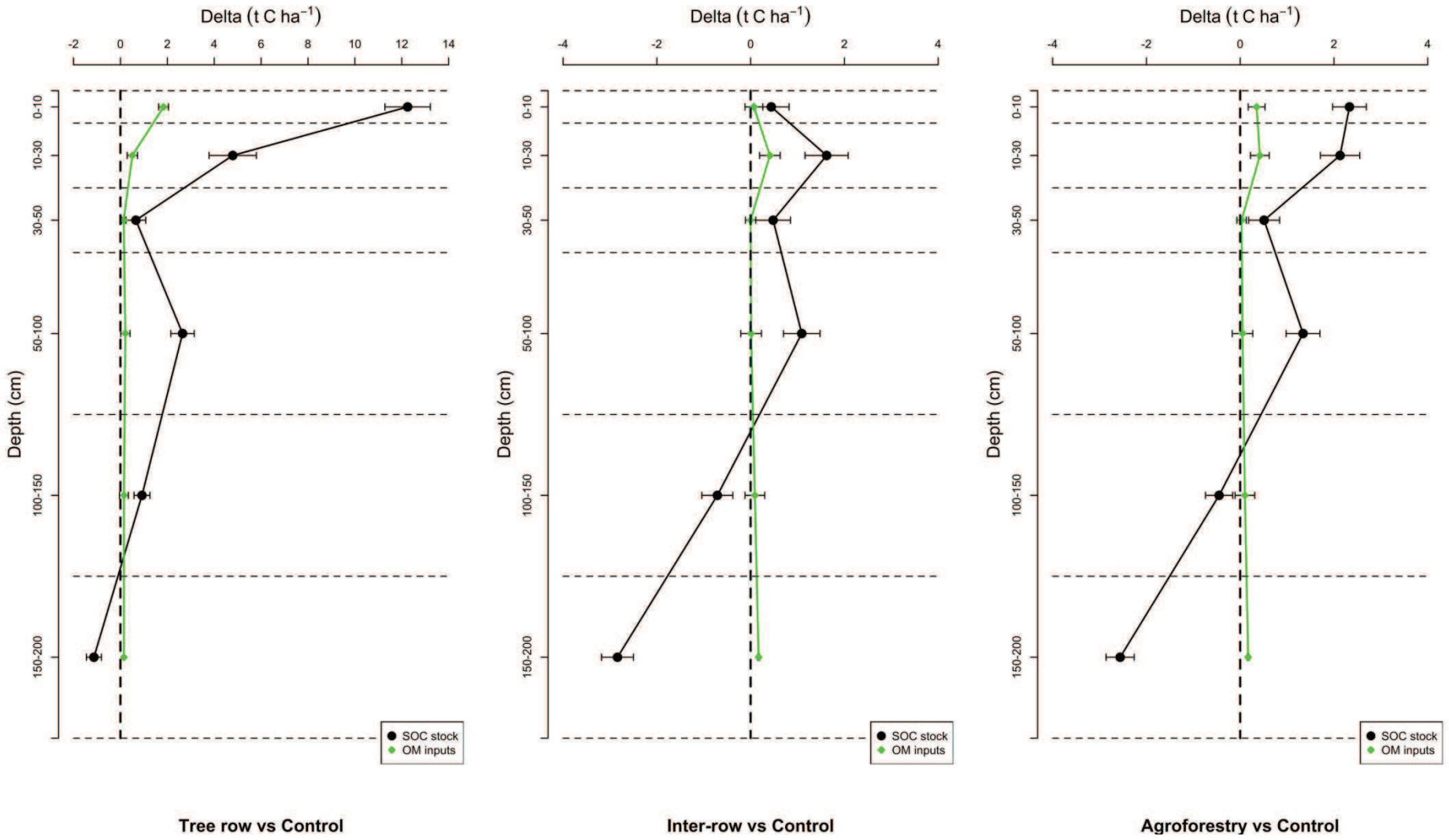


Figure II-3.3. Measured delta SOC stocks (t C ha^{-1}) and OM inputs (t C ha^{-1} yr^{-1}) between a 17-year-old agroforestry plot and an agricultural control plot. Delta SOC stocks data are issued from Cardinael *et al.*, (2015a).

Partie III :

Minéralisation et modélisation de la dynamique du carbone organique

Chapitre 1

Minéralisation du carbone organique superficiel et profond d'une parcelle agroforestière et agricole – une approche par incubation

Article en préparation

Stabilization and mineralization of shallow and deep soil organic carbon under a Mediterranean agroforestry system

Thomas Cozzi, Rémi Cardinael, Tiphaine Chevallier, Cyril Girardin, Valérie Pouteau, Claire Chenu

1.1 Introduction

Agroforestry systems are a type a agroecosystems where trees and crops or animals are associated within the same field (Nair 1993). These last decades, more interest have been accorded to these systems as they usually provide enhanced ecosystem services compared to treeless farming systems. For instance, trees can reduce pollution of water by absorbing part of leached nitrates (Tully et al. 2012), enhance biodiversity at the farm scale (Burgess 1999) and provide a variety of goods such as timber, fiber, fruits or firewood. In addition, agroforestry systems also have a positive impact on soil organic carbon (SOC) stocks (Albrecht and Kandji 2003; Lorenz and Lal 2014). Several studies showed an increase of SOC stocks following the establishment of agroforestry systems (Oelbermann et al. 2004; Bambrick et al. 2010; Cardinael et al. 2015a). Thus, these agricultural practices can be seen as a mean to mitigate greenhouse gases accumulation into the atmosphere.

SOC dynamics are still insufficiently understood, especially at depth (Salomé et al. 2010; Rumpel and Kögel-Knabner 2011). Studies showed that SOC dynamics is slower at depth because substrate quality usually decreases with depth, i.e., the organic matter at depth is less biodegradable than the organic matter in topsoil (Rumpel and Kögel-Knabner 2011). Microbial biomass is lower in deep soil layers (Taylor et al. 2002; Eilers et al. 2012) and interactions between organic matter and minerals vary with depth (Kleber et al. 2005; Mathieu et al. 2015). Moreover, decomposition of SOM at depth may be limited by a lack of energy for microorganisms (Fontaine et al. 2007). Mineralisation rates of SOC therefore tend to decrease with soil depth. Estimation of mineralisation potentials at different soil depths is essential, especially for modelling purposes (Braakhekke et al. 2011; Braakhekke et al. 2013; Guenet et al. 2013).

Little is known about the stability and dynamics of SOC in agroforestry systems. A few studies have characterized the form and location of additional SOC in order to evaluate the stability of SOC under agroforestry systems. Two studies showed that additional SOC was located in particulate organic matter (POM, 50–200 and 200–2000 μm), a rather labile fraction (Howlett et al. 2011; Cardinael et al. 2015a). On the opposite, Haile et al. (2010) found in a silvopastoral system that most of SOC in deep soil profiles was derived from tree components and was located in a fine sized fraction (< 53 μm) considered to be relatively stable because associated to mineral particles. Del Galdo et al. (2003) also showed that afforestation resulted in significant accumulation of carbon associated with microaggregates (53–250 μm) and silt-clay (< 53 μm). Agroforestry systems can also have a positive impact on water stability of soil aggregates (Udawatta et al. 2008), and therefore increase the residence time of aggregates that would physically protect SOC from decomposition.

Two main strategies have been developed in the literature to gain information on the physical protection of organic matter: (i) to separate physical fractions presumably differing in their extent of provided protection, quantify SOC in fractions and measure its turnover rate using stable isotopes (Puget et al. 2000; Six et al. 2000b) or (ii) to physically disrupt soil aggregates and measure the resulting flush of CO_2 upon mineralization, indicative of the presence within aggregates of decomposable but protected organic matter (Beare et al. 1994; Balesdent et al. 2000; Chevallier et al. 2004).

The aims of this study were twofold: (i) assess soil organic carbon mineralisation potential as a function of soil depth in an agroforestry plot and in an agricultural control plot (ii) compare

the effects of soil structure on SOC mineralisation in both fields for topsoil and subsoil samples, using different levels of disruption of aggregates.

We hypothesized that mineralisation potentials would decrease with increasing soil depth, in both the agricultural control plot and the agroforestry plot. We also hypothesized that the soil structure disruption would enhance SOC mineralisation more in the agroforestry plot than in the control plot as more OM could be located within soil aggregates and protected therein.

We performed soil incubations with soil sampled at four depths (0-10, 10-30, 70-100 and 160-180 cm) in an 18 year old agroforestry plot and in an adjacent agricultural control plot. SOC stocks at this site have been studied previously (Cardinael et al. 2015a), as well as the forms of SOC storage using particle-size fractionation.

1.2 Materials and methods

1.2.1 Site description

Soils were collected in an experimental site 15 km North of Montpellier, France (Longitude 04°01′ E, Latitude 43°43′ N, elevation 54 m a.s.l.). The climate is sub-humid Mediterranean with an average temperature of 15.4°C and an average annual rainfall of 873 mm (years 1995–2013). The soil is a silty and carbonated deep alluvial Fluvisol (IUSS Working Group WRB 2007). The site comprises an alley cropping agroforestry system, associating walnut (*Juglans regia* × *nigra* cv. NG23) trees and durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.), and an adjacent agricultural control plot where only the annual crop is cultivated. Trees were planted in 1995, in parallel tree rows (13 × 4 m spacing) and have a current density of 110 trees ha^{-1}. A spontaneous and herbaceous vegetation grows in the tree rows.

1.2.2 Soil collection and samples description

Soil samples were collected in May 2013 using a motor-driven micro caterpillar driller (Cardinael et al. 2015a). Four samples were taken in the control plot, four in the inter-row and four in the tree row, at 0-10, 10-30, 70-100 and 160-180 cm. Soil samples were air-dried before storage. Soil organic carbon (SOC) concentration was analyzed on sub-samples with a

CHN elemental analyzer (Carlo Erba NA 2000, Milan, Italy) after carbonates were removed by acid fumigation, following Harris et al., (2001). Soil texture was also analyzed.

SOC concentration decreased with increasing depth, and almost doubled in 0-10 cm in the tree row compared to the control and the inter-row (Table III-1.1). Clay and silt texture increased with increasing soils depth (Table III-1.1).

	SOC concentration (mg C g^{-1}) (% C-POM ± se)			Soil texture (g kg^{-1}) clay/silt/sand
Depth (cm)	Control	Inter-row	Tree row	
0-10	8.74 ± 0.38 (20 ± 2)	10.78 ± 0.34 (22 ± 1)	19.14 ± 1.81 (52 ± 5)	188/419/393
10-30	9.12 ± 0.39 (22 ± 3)	9.42 ± 0.33 (22 ± 1)	10.36 ± 0.73 (26 ± 2)	180/409/411
70-100	6.22 ± 0.29 (0)	6.40 ± 0.12 (0)	6.21 ± 0.40 (0)	295/545/160 (0)
160-180	6.17 ± 0.20 (0)	5.32 ± 0.31 (0)	5.68 ± 0.21 (0)	334/577/89 (0)

Table III-1.1. Soil analyses of incubated samples. C-POM: ratio between carbon amount in particulate organic matter (POM) and total SOC carbon. se: standard error. For SOC concentrations, n=4 at each depth. For C-POM, n=5 for each depth, except in the control where n=6. For soil texture, n=12. Associated errors are standard errors.

1.2.3 Laboratory incubations, soil respiration and soil microbial biomass

Two series of incubations were performed one after another. The first incubation comprised four soil replicates per location (control, tree row, inter-row) and per depth (0-10, 10-30, 70-100 and 160-180 cm). Soil samples were sieved at 5 mm (n=48). Additionally, four other sub-samples from the tree row at 10-30 and 70-100 cm, were hand milled to pass a 200 µm sieve (n=8). The second incubation comprised four replicates per depth (10-30 and 70-100 cm) from the tree row (n=8), sieved at 5 mm, and of four other replicates per depth where the soil was dispersed at < 50 µm (n=8). The soil was dispersed following the procedure described by Balesdent et al., (1998). Briefly, 40 g of soil was shaken during 16 h in 180 mL of deionized water with 10 glass balls in a rotary shaker, at 65 rpm to disrupt the aggregates. The suspension was then frozen at -20°C during 16 h and was then freeze-dried. This procedure was chosen to avoid the mineralisation that would have occurred during the drying of the suspension at 40°C in an oven. For both incubations and any treatment, 40 g of soil was placed in 500-mL glass jars with Teflon® rubber stoppers crimped on with aluminium seals.

All soil samples were moistened to reach a water potential of -0.03 MPa (pF 2.5). Immediately after adding the water, the glass jars were flushed with CO_2 free air (19% O2, 81% N2). Soils were then incubated at 20°C in the dark. The CO_2 concentration and the $\delta^{13}C$ of the CO_2 were measured after 1, 3, 7, 14, 21, 28, 35 and 44 days for the first incubation, and after 1, 3, 7, 14, 22, and 27 days for the second incubation. The CO_2 concentration was determined with a micro GC (Agilent 3000A). The isotopic composition ($\delta^{13}C$, ‰) of the CO_2–C was determined using a GC (Hewlett-Packard 5890) coupled to an Isotope Ratio Mass Spectrometer (GC-IRMS; Isochrom Optima, Micromass). Jars were aerated when necessary and flushed again to ensure that CO_2 concentrations did not exceed 10,000 ppm.

At the end of the incubations, microbial biomass was determined for each sample using 5 g of soil, and following the fumigation-extraction method described by Vance et al., (1987). The solution extracted with K_2SO_4 (5g/L) was then analysed with a TOC analyser (TOC 505A, Autosampler ASI-5000A) to quantify microbial carbon.

1.2.4 Isotopic calculations

The CO_2 concentration measured in each glass jar was a mix of CO_2 originating from the mineralisation of organic compounds and from the dissolution of carbonates:

$$CO_{2_{measured}} = CO_{2_{organic}} + CO_{2_{inorganic}} \qquad (1)$$

where CO_2 concentrations are expressed in ppm.

Carbon isotope ratio is presented in δ notation, defined as follows:

$$\delta\ ‰ = \frac{R_{sample} - R_{PDB}}{R_{PDB}} \times 1000 \qquad (2)$$

where R_{sample} is the $^{13}C/^{12}C$ isotope ratio of the sample and R_{PDB} is the $^{13}C/^{12}C$ ratio of the international pee dee belemnite (PDB) standard (Coplen 1995). The analytical precision of the $\delta^{13}C$ measurements was 0.1‰. We assumed that the carbonates in the soil solution were in isotopic equilibrium with the solid carbonates (Bertrand et al. 2007). The proportion of CO_2 evolved from inorganic carbon (f_{SIC}) was estimated using a two-end member mixing model (Balesdent et al. 1987; Ramnarine et al. 2012):

$$\delta^{13}C_{CO_2} = f_{SIC} \times \delta^{13}C_{SIC} + (1 - f_{SIC}) \times \delta^{13}C_{SOC} \qquad (3)$$

The equation can be rewritten as:

$$f_{SIC} = \frac{\delta^{13}C_{CO_2} - \delta^{13}C_{SOC}}{\delta^{13}C_{SIC} - \delta^{13}C_{SOC}} \qquad (4)$$

The $\delta^{13}C_{SOC}$ value was determined on soil samples from the tree row, inter-row and control at each depth. Carbonates were removed after soil was exposed to HCl (37%) vapors for 8 h, following the acid fumigation protocol described in Harris et al., (2001). Samples were analysed with a CHN elemental analyzer (Carlo Erba NA 2000, Milan, Italy) coupled to an Isotope Ratio Mass Spectrometer. The $\delta^{13}C_{SIC}$ was measured on total soil samples that were put in the oven at 550°C for 6 h to remove residual organic matter. Powdered carbonate (10 mg) reacted with orthophosphoric acid during 16 h at 25°C in a reaction vessel that had previously been purified of any gases with a vacuum pump and liquid nitrogen. After the reaction, the CO_2 is expanded into the evacuated system, and then condensed into an U-trap with liquid nitrogen cooling (McCrea 1950; Jones and Kaiteris 1983; Mucciarone and Williams 1990). The system is pumped to remove any residue of non-condensable gas. The carbon dioxide is measured in a manometer, and then transferred to a sample tube to be used on the Isotope Ratio Mass Spectrometer (GC-IRMS; Isochrom Optima, Micromass).

$\delta^{13}C_{SIC}$ did not vary with soil depth nor with location (tree row, inter-row, control), and equaled -3.05 ‰. $\delta^{13}C_{SOC}$ varied with depth and location. In the tree row, it was -27.55 and -26.34 ‰ at 0-10 and 10-30 cm, respectively, while it was -26.02 ‰ and -25.91 ‰ at the same depths for both the inter-row and the control. At 70-100 and 160-180 cm, it was -24.65 ‰ whatever the location.

1.2.5 Determination of SOC decomposition rates

Mineralization rates were initially rapid and stabilized after nearly two weeks (see paragraph 1.3.2). The initial rate was affected by rewetting the samples as well as by the aggregate disruption treatment. We then decided to focus on the second part of the mineralization kinetics, from day 14 to day 44. Linear equations ($y = k \times x + b$) were fitted on the curves representing the cumulated proportion of total SOC mineralised as a function of time, from day 14 to day 44, and for each depth. The *k* coefficients represent SOC decomposition rates.

1.3 Results

1.3.1 Contribution of inorganic carbon to evolved CO_2 from soil

The $\delta^{13}C_{CO_2}$ of total measured CO_2 ranged from about -19‰ to -21‰ for top soil layers from the beginning to the end of the incubation, and from -10‰ to -15‰ for deep soil layers. The contribution of inorganic carbon to the CO_2 emission from the soil increased with soil depth (Fig III-1.1). At 0-10 cm, about 20% of the CO_2 originated from the carbonates, at 10-30 cm about 30%, at 70-100 cm about 50% and at 160-180 cm about 60%. The maximum contribution of carbonates to CO_2 emission was always observed during the first day of the incubation, except for the tree row in 0-10 cm. For all depths considered, no difference was found between the origin of soil samples (tree row, inter-row or control). The contribution of soil inorganic carbon to CO_2 emissions was not impacted by soil disruption, except during the first day, where it was increased.

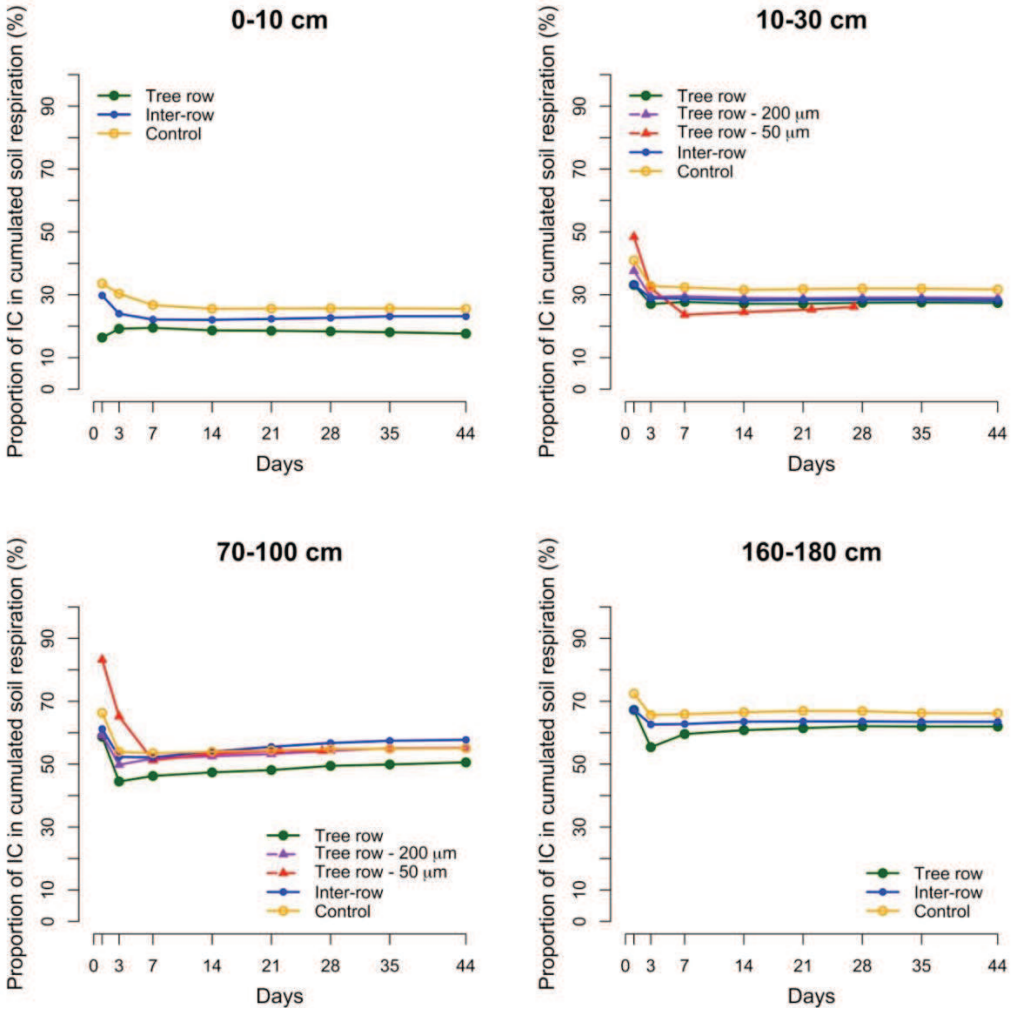


Figure III-1.1. Proportion of inorganic carbon to soil respiration for soil samples sieved at 5 mm, at 200 µm or dispersed at 50 µm. IC: inorganic carbon. Associated errors are standard errors (n=4 per day and per modality).

1.3.2 Cumulated CO_2 respiration from undisrupted samples

At 0-10 cm, cumulated CO_2 emission from organic carbon mineralisation was three times higher in the tree row than in the inter-row and in the control (Fig III-1.2). At 10-30 cm, soil respiration was lower in the control, whereas at 70-100 cm it was lower in the inter-row. At 160-180 cm, no difference was observed between modalities.

1.3.3 Proportion of SOC mineralised

In topsoil layers, between 1 and 2% of total SOC was mineralised after 44 days of incubation (Fig III-1.3). At 0-10 cm, a higher proportion of total SOC was mineralized in the tree row than in the inter-row. For subsoil layers, the amount of total SOC mineralised ranged from 0.1 to 0.3%. At 70-100, SOC from the inter-row was less mineralised, whereas at 160-180 cm, SOC from the control was less mineralised.

1.3.4 SOC decomposition rates with depth

The SOC decomposition rate reached 0.0314 day^{-1} in 0-10 cm in the tree row and was two times higher than in the inter row or in the control (Table III-1.2). No clear difference was observed between modalities below 10 cm. SOC decomposition rates decreased exponentially with increasing depth (Fig III-1.4), and reached about 0.002 day^{-1} in 160-180 cm.

Depth (cm)	Location	*k* (day^{-1})	*b* (%)	R^2
0-10	Tree row	0.0314	0.7922	0.996
	Inter-row	0.0151	0.6664	0.975
	Control	0.0189	0.8165	0.976
10-30	Tree row	0.0142	0.5459	0.983
	Inter-row	0.0168	0.5243	0.991
	Control	0.0127	0.4262	0.987
70-100	Tree row	0.0037	0.1548	0.977
	Inter-row	0.0022	0.1107	0.979
	Control	0.0037	0.1188	0.980
160-180	Tree row	0.0021	0.0569	0.986
	Inter-row	0.0027	0.0579	0.983
	Control	0.0018	0.0417	0.989

Table III-1.2. Coefficients of linear regressions performed on curves from Fig. III-1.2 from day 14 to day 44. The *k* coefficients represent SOC decomposition rates.

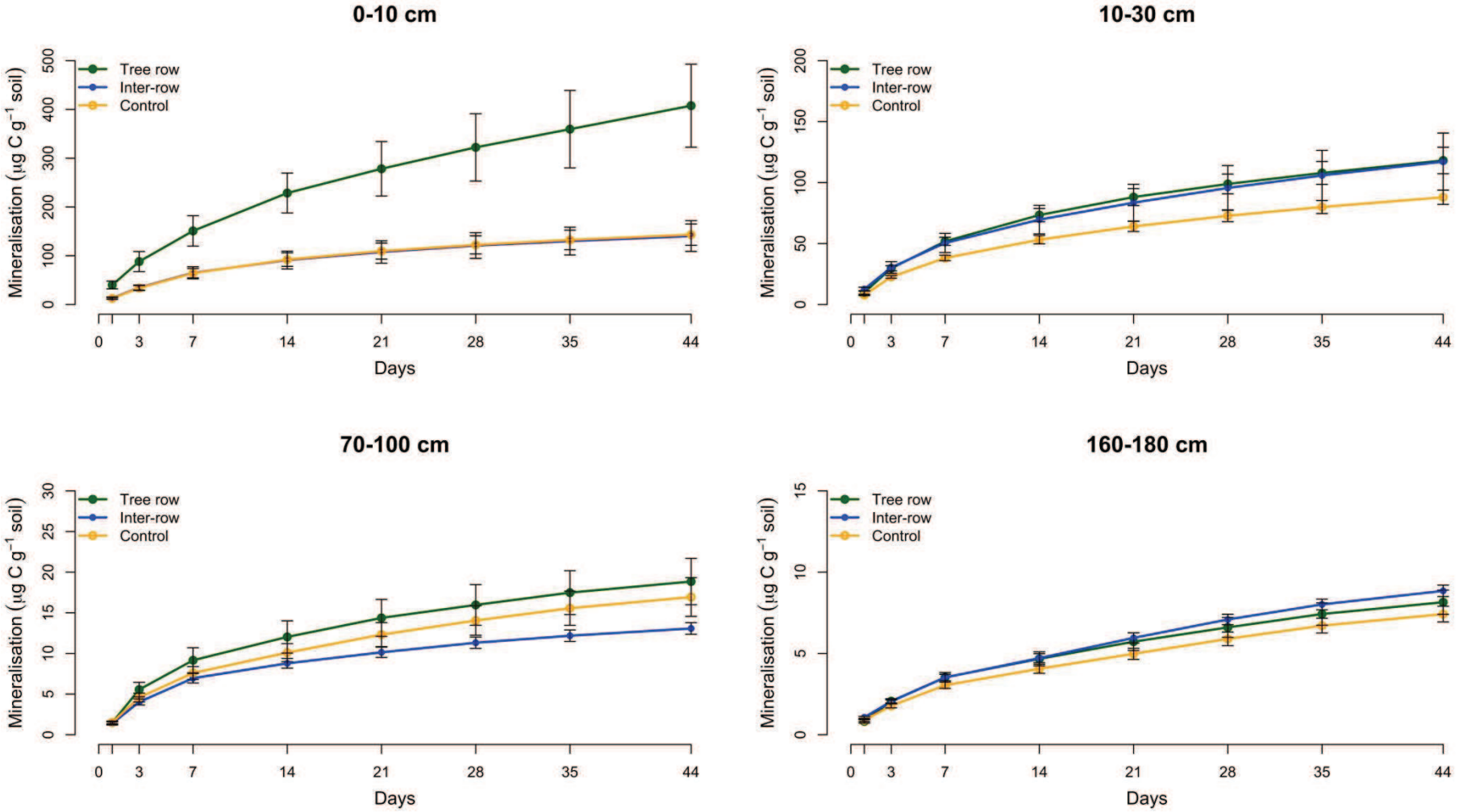


Figure III-1.2. Cumulated organic C-CO_2 respiration of incubated soil samples sieved at 5 mm. Associated errors are standard errors (n=4 per day and per modality).

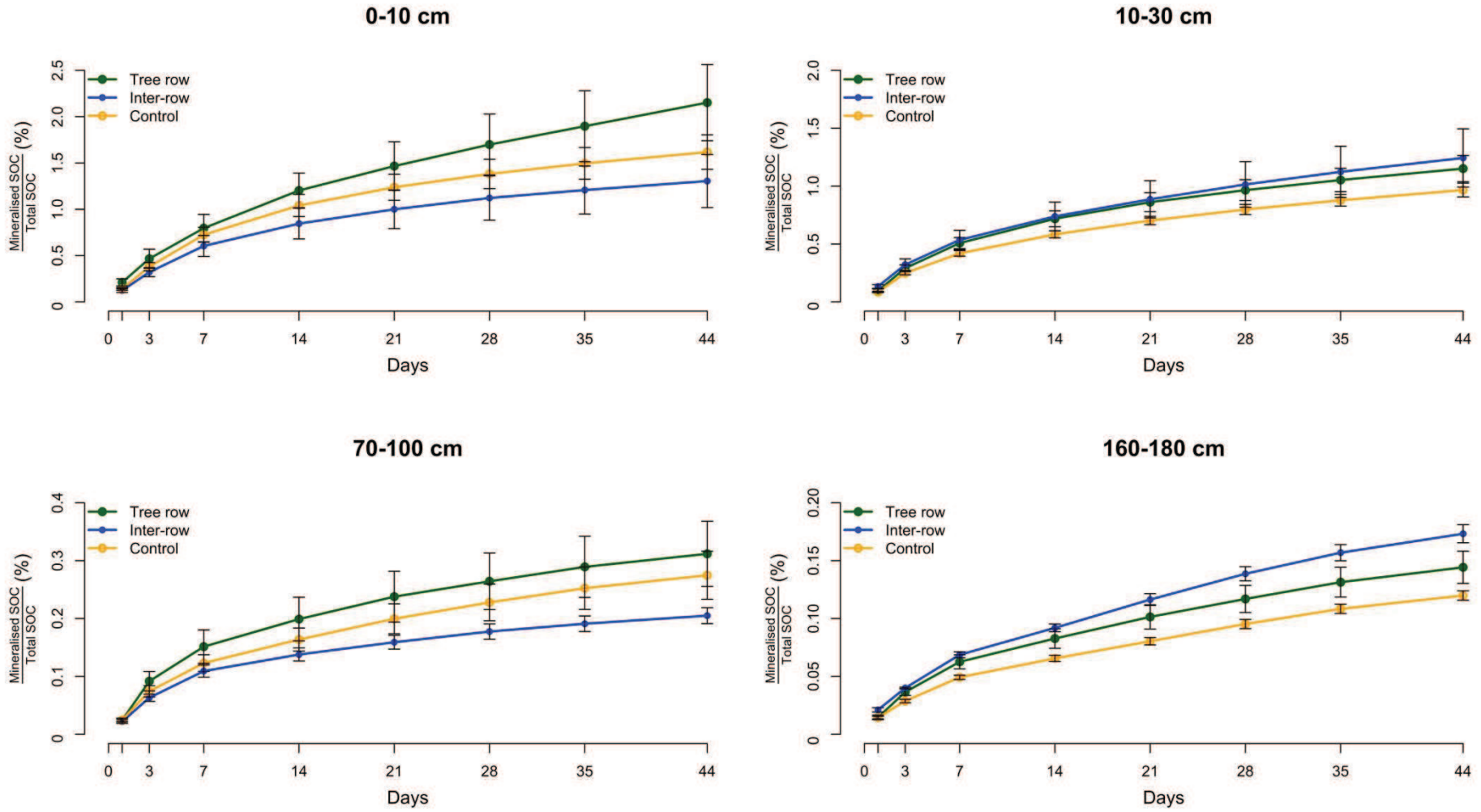


Figure III-1.3. Cumulated proportion of total SOC mineralised during an incubation of soil samples sieved at 5 mm. Associated errors are standard errors (n=4 per day and per modality).

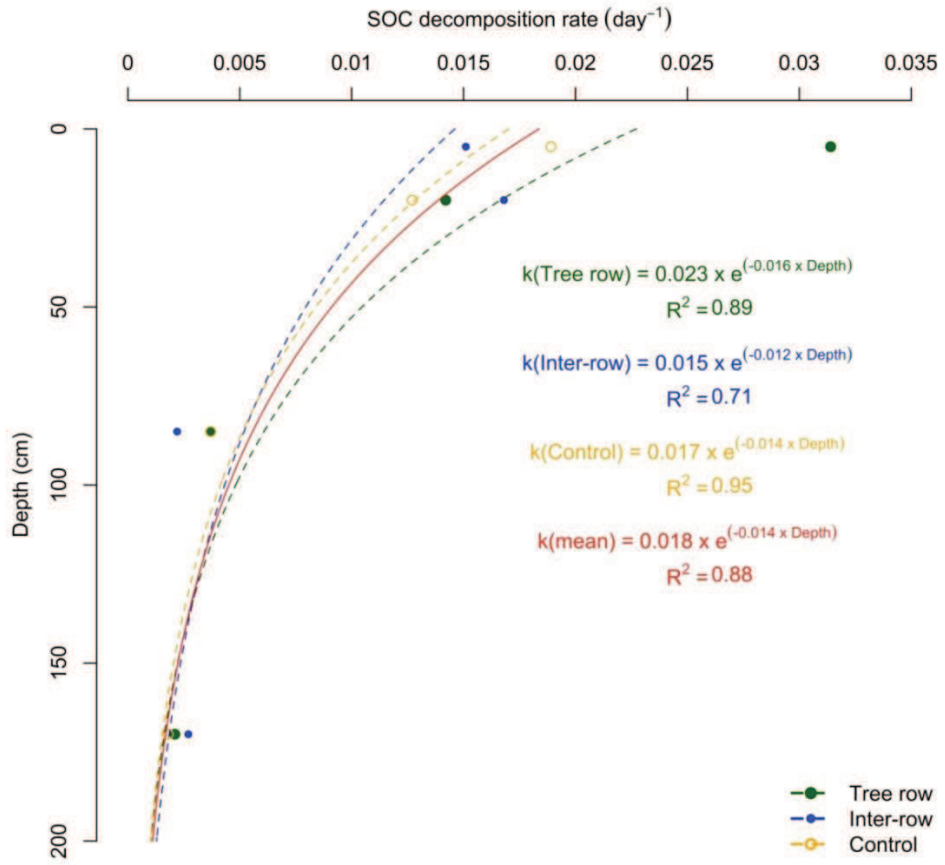


Figure III-1.4. SOC decomposition rates (k, day^{-1}) as a function of soil depth (cm).

1.3.5 Microbial biomass with depth

At 0-10 cm, microbial biomass was three times higher in the tree than in the inter-row or in the control (Fig III-1.5). No difference was observed between the inter-row and the plot at 0-10 cm and at 10-30 cm. At 70-100 and at 160-180 cm, negative values indicate that microbial biomass was below the detection threshold and therefore extremely low.

1.3.6 Effect of soil disruption on SOC mineralisation

No significant difference was observed between the two series of incubations concerning soil samples sieved at 5 mm (i.e. no disruption treatment) (Fig III-1.6). At 10-30 cm, mineralisation of soil samples sieved at 200 µm was significantly higher than for soil samples sieved at 5 mm, whereas at 70-100 cm no difference was observed. The mineralisation of soil samples dispersed and sieved at 50 µm was not significantly different either from soil samples sieved at 5 mm, or from soil samples sieved at 200 µm, at 10-30 and at 70-100 cm.

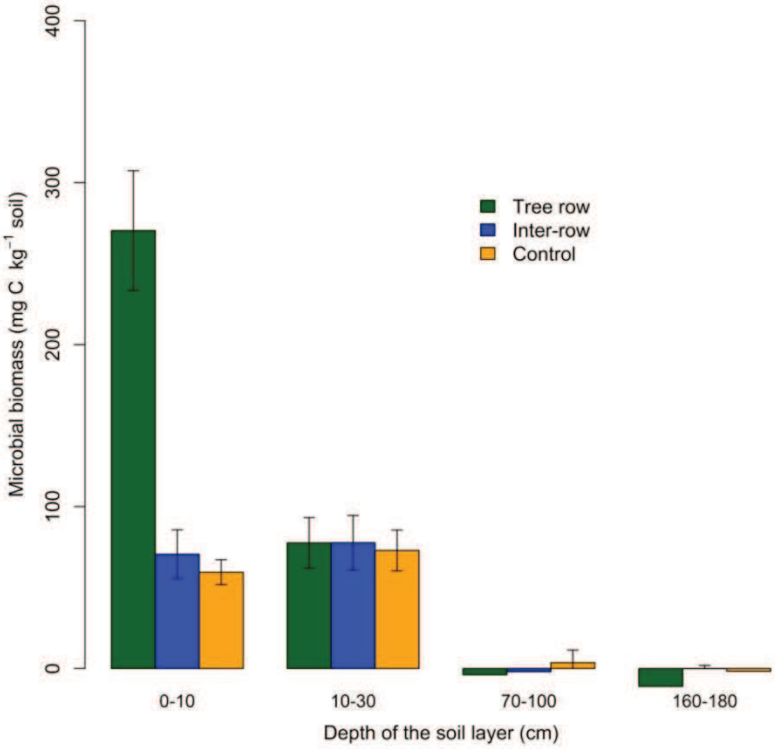


Figure III-1.5. Microbial biomass after 44 days of incubation.

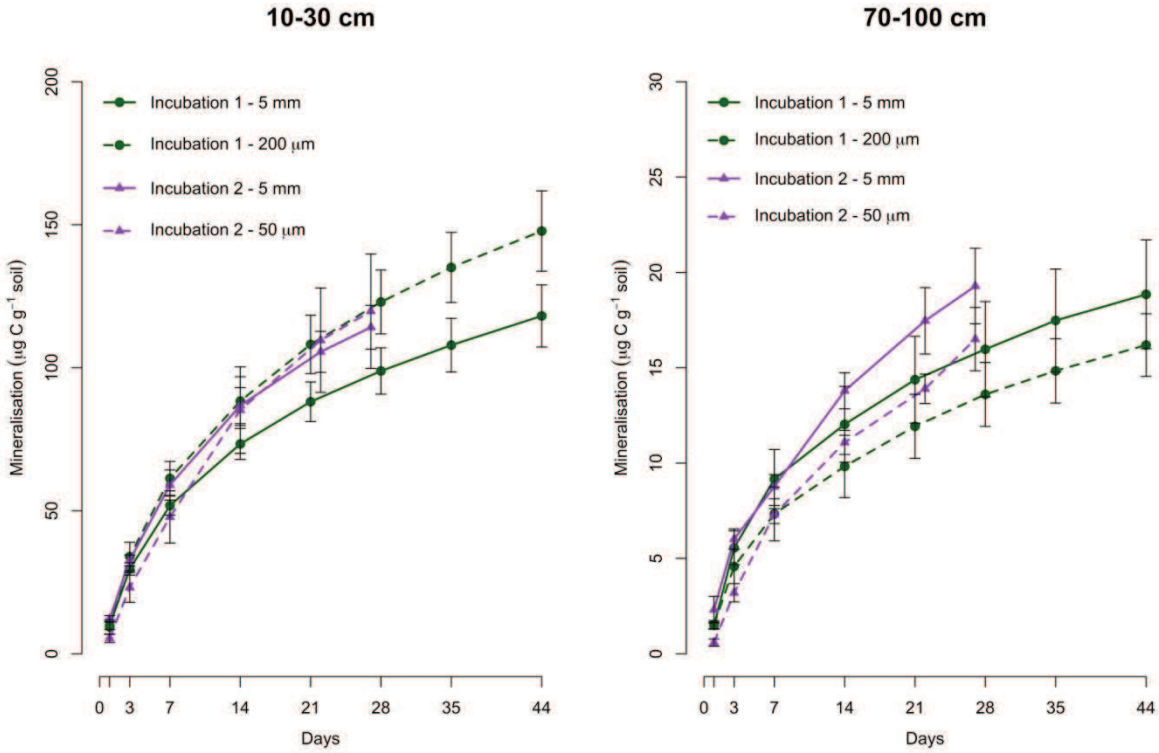


Figure III-1.6. Cumulated organic C-CO_2 respiration of incubated soil samples sieved at 5 mm, at 200 µm or dispersed at 50 µm. Associated errors are standard errors (n=4 per day and per modality).

1.4 Discussion

1.4.1 Mineralisation and soil depth

A clear distinction appeared between the 0-10 cm layer in the tree row and other soil layers and locations (inter-row and control). The highest cumulated respiration was observed in this layer, but also the highest proportion of respired SOC and the highest microbial biomass. A previous study within the same site revealed that this soil layer was rich in particulate organic matter (50-200 and 200-2000 µm) (Cardinael et al. 2015a), which are rather labile fractions. The highest proportion of SOC which is respired at 0-10 cm in the tree row was therefore probably due to the mineralisation of particulate organic matter, i.e. decomposing plant debris.

1.4.2 SOC stabilization processes

Disruption of soil structure to 200 µm had a little but significant effect at 10-30 cm, suggesting that a fraction of SOC is protected within soil macroaggregates. SOC physical protection within macroaggregates may be less important at depth as we observed no effect of soil structure disruption to 200 µm at 70-100 cm

Disruption of soil structure to 50 µm at 10-30 cm revealed no additional respiration while an effect was observed at 200 µm. This absence of effect may be a consequence of freeze-drying following soil dispersion that reduced the microbial biomass (Fig III-1.S1), or to the shortest incubation time, which may have counterbalanced the disruption of soil structure at a finer scale than in the first incubation.

At 70-100 cm, disruption of soil structure had no effect on SOC mineralisation, suggesting that physical protection is not important at these scales at depth, contrasting with the results of Salomé et al. (2010). Clay content increased with soil depth, and SOC is probably stabilized at a finer scale, i.e. clay-particle size, as it was shown by different authors in several contexts (Moni et al. 2010; Schrumpf et al. 2013).

1.4.3 Role of carbonates in CO_2 emissions

Our results confirm that ignoring the contribution of inorganic carbon to soil respiration could lead to great overestimations (Bertrand et al. 2007; Tamir et al. 2011; Ramnarine et al. 2012).

In topsoil layers, about 30% of soil respiration could be attributed to the dissolution of carbonates, while at depth this proportion attained 50 to 60%. Carbonates content was constant with depth, this higher proportion is probably due to the fact that SOC mineralisation was lower at depth, but it would be interesting to study carbonates forms with depth. During soil incubations, contribution of carbonates is maximal during the first days of incubation, following the re-humectation of soil samples, and then it stabilizes. Soil dispersion and shaking with glass balls increased this contribution of carbonates during the first days, suggesting the reactive surface of carbonates had been increased due to a destruction of small carbonates stones.

1.5 Conclusion

SOC mineralisation rate decreased exponentially with depth, suggesting that SOC is less biodegradable in deep soil layers. However, clay and silt contents increased with depth. Therefore, the lower biodegradability of SOC at depth could be explained by both a lower quality substrate and a higher SOC stabilisation on mineral particles. The absence of increased SOC mineralisation following disruption of soil suggests that physical protection of SOC in aggregates > 50 µm is not a major process of SOC stabilization at this site, and that agroforestry did not improved this protection. We conclude that additional SOC stored at this site, mainly made of particulate organic matters not occluded within soil macroaggregates, is vulnerable and not stable.

1.6 Supplementary material

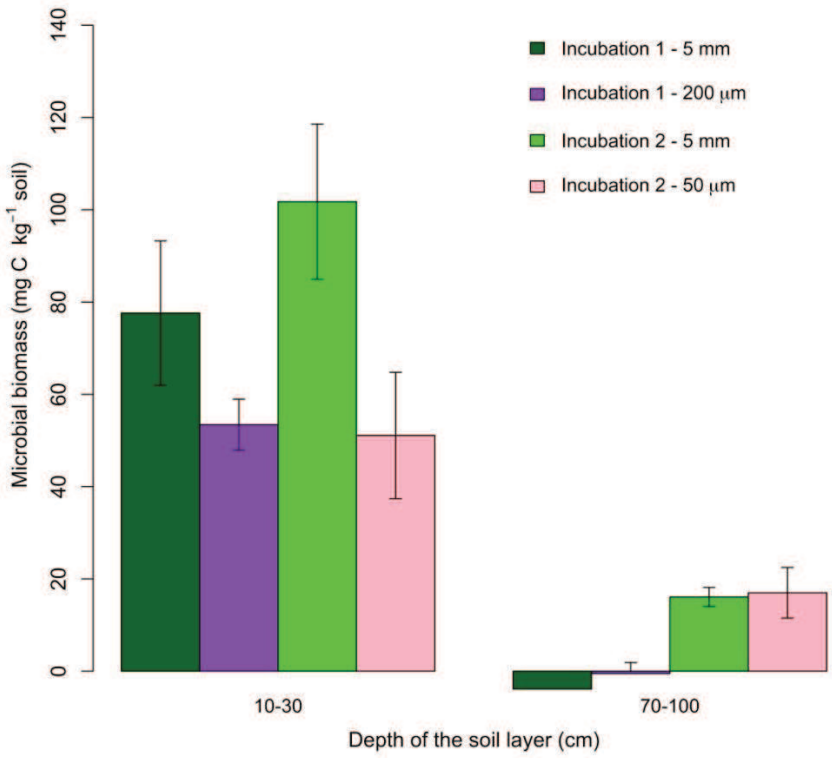


Figure III-1.S1. Microbial biomass after 44 days of incubation (incubation 1) and after 27 days of incubation (incubation 2), following different levels of soil disruption.

Chapitre 2

Modélisation des dynamiques de carbone en système agroforestier – la nécessité d'un modèle discrétisé en profondeur

Article en préparation (***couplé avec la Partie 2, chapitre 3***)

Can fresh OM inputs drive SOC storage in alley cropping agroforestry systems? A study case in a Mediterranean context using experimental and modeling approaches

Rémi Cardinael, Bertrand Guenet, Tiphaine Chevallier, Christian Dupraz, Claire Chenu

2.1 Introduction

Agroforestry systems are complex agroecosystems combining trees and crops within the same field (Nair 1985; Somarriba 1992; Nair 1993). These systems are at the interface between agricultural systems and forest systems. Alley cropping agroforestry systems are a type of agroforestry systems where parallel tree rows are intercropped with annual crops. Some studies showed that these systems can be very productive, with a land equivalent ratio (Mead and Willey 1980) reaching up to 1.3 (Graves et al. 2007; Dupraz et al. 2010). This means that agroforestry systems can produce up to 30% more biomass on the same area of land compared to crops and trees grown separately. This performance can be explained by a better use of water, nutrients and light by the agroecosystem throughout the year. Indeed, in temperate and in Mediterranean regions, farmers usually grow one crop per year, and the association of trees

can extend the growing period at the field scale, especially when winter crops are intercropped with trees having a late bud break (Burgess et al. 2004). Trees grown in agroforestry systems usually grow faster than the same trees grown in forest ecosystems, because of their lower density, and because they also benefit from the crop fertilization (Balandier and Dupraz 1999; Chaudhry et al. 2003; Chifflot et al. 2006). However, a decrease of crop yield can be observed in mature and highly dense plantations, especially close to the trees (Yin and He 1997; Burgess et al. 2004; Dufour et al. 2013).

Part of the additional biomass produced in agroforestry is used for economical purposes, such as timber or fruit production. However, leaves, tree fine roots, pruning residues and the herbaceous vegetation growing in the tree rows will return to the soil, contributing to a higher input of organic matter (OM) to the soil compared to a classical agricultural field (Peichl et al. 2006).

Higher OM inputs to the soil could therefore explain higher SOC stocks observed in agroforestry systems (Albrecht and Kandji 2003; Lorenz and Lal 2014). Indeed several studies showed increased SOC stocks in agroforestry systems. For example, Cardinael *et al.*, (2015a) showed an additional SOC stock of 6 Mg C ha^{-1} to 1 m depth in a 18-year-old alley cropping agroforestry system compared to an agricultural plot. Higher SOC stocks were also found in Canadian agroforestry systems, but were only quantified to 20 cm depth (Oelbermann et al. 2004; Peichl et al. 2006; Bambrick et al. 2010). However, studies combining SOC stocks with OM inputs in agroforestry system are scarce (Peichl et al. 2006), especially when considering deep soil layers.

Introduction of trees in an agricultural field modifies the amount, but also the distribution of fresh organic matter (FOM) input to the soil, both vertically and horizontally (Peichl et al. 2006; Bambrick et al. 2010; Howlett et al. 2011). FOM inputs from the trees (leaf and root litter, exudates) decrease with increasing distance from the trunk and with soil depth (Moreno et al. 2005). On the contrary, crop yield usually increases with increasing distance from the trees (Li et al. 2008; Dufour et al. 2013). The proportion of FOM coming from the crop residues or from the trees changes with distance from the trees, soil depth, and time.

In agroforestry systems, tree fine root distribution within the soil profile is also strongly modified due to the competition with the crop, inducing a deeper rooting compared to trees grown in forest ecosystems (Mulia and Dupraz 2006; Cardinael et al. 2015c). Deep soil layers may therefore receive significant OM inputs from root mortality and exudates. Root carbon

has a higher mean residence time in soil compared to shoot carbon (Rasse et al. 2005; Kätterer et al. 2011), because root residues are preferentially stabilized within microaggregates or adsorbed on clay particles. Moreover, environmental conditions (temperature, moisture) are more buffered in subsoil than in topsoil with reduction in oxygen concentration. The microbial biomass is also lower in deep soils compared to surface soils (Fierer et al. 2003; Eilers et al. 2012), resulting in lower decomposition rates. Deep root carbon input in the soil could therefore contribute to a more stable SOC storage. However, recent studies showed that addition of FOM – a source of energy for microorganisms - to the subsoil enhanced decomposition of ancient carbon, a process called « priming effect » (Fontaine et al. 2007). More generally, priming effect could be defined as the control of soil organic matter (SOM) mineralization by the availability of fresh organic matter. Nevertheless, considering priming effect as a major driver of carbon dynamic in deep soils is still matter of debate (Salomé et al. 2010). Priming effect is usually stronger when induced by labile molecules like root exudates than by root litter coming from decomposition of dead roots (Shahzad et al. 2015). Therefore, the net effect of deep roots on SOC stocks has to be assessed, especially in agroforestry systems.

Several models have been developed to simulate interactions for light, water and nutrients between trees and crops (van Noordwijk and Lusiana 1999; Duursma and Medlyn 2012; Charbonnier et al. 2013) or to predict growth and yield in agroforestry systems (van der Werf et al. 2007; Graves et al. 2010). These models are crucial as they allow virtual experiments to best design and understand complex processes in these systems. However, none of these models has been made to simulate SOC dynamics in agroforestry systems. Oelbermann & Voroney, (2011) evaluated the ability of the Century model (Parton et al. 1987) to predict SOC stocks in tropical and temperate agroforestry systems, but with a single-layer modeling approach (0-20 cm). The approach of modeling a single soil layer assumes that deep SOM does not play an active role in carbon cycling, even if it was shown that deep soil layers contain important amounts of SOC (Jobbagy and Jackson 2000), and that part of this deep SOC could cycle on decadal timescales due to root inputs or to dissolved organic carbon transport (Baisden and Parfitt 2007; Koarashi et al. 2012). The need to take into account deep soil layers when modeling SOC dynamics is now well recognized in the scientific community (Elzein and Balesdent 1995; Baisden et al. 2002), and several models have been proposed (Braakhekke et al. 2011; Guenet et al. 2013; Koven et al. 2013; Taghizadeh-Toosi et al. 2014). Using vertically discretized soils is particularly important when modeling the impact of

agroforestry systems on SOC stocks, but to our knowledge, vertically spatialized SOC models have not yet been tested for these systems.

The aims of this study were twofold: (i) propose a model of soil C dynamics in agroforestry systems able to account for both vertical and lateral spatial heterogeneities and (ii) test whether variations of fresh organic matter input could explain increased SOC stocks both using experimental data and model runs. We based our study on a well-documented long-term agroforestry experiment in South France.

For this, we first compiled data on FOM inputs to the soil acquired in a 17 year old agroforestry plot and in an agricultural control plot, in which SOC stocks to 2 m soil depth have been recently quantified (Cardinael et al. 2015a). FOM inputs comprised tree fine roots (Cardinael et al. 2015c), tree leaf litter, aboveground and belowground biomass of the crop and of the herbaceous vegetation in the tree rows.

We modified a model proposed by Guenet *et al.*, (2013), to create a 2D model (depth × distance), the SOCRATES model (Soil Organic CaRbon dynamic in AgroforesTry systEmS). Based on data acquired since the tree planting in 1995 (crop yield, tree growth), and on FOM inputs, we modeled SOC dynamics to 2 m soil depth in the agroforestry and the agricultural control plot. We evaluated the model against measured SOC stocks along the profile and used the opportunity to test the importance of the priming effect in deep soil C dynamic in an agroforestry system.

Predict SOC storage capacity of agroforestry systems on the long-term is not an easy task, and the rotation length can impact the amount of stored carbon. We compared here two different management scenarios using this model.

2.2 General description of the SOCRATES model

2.2.1 The OM decomposition

We adapted a model developed by Guenet et al. (2013) where total OM is split in two pools, the FOM and the soil organic matter (SOM) for each soil layer (Fig. III-2.1). Input to the FOM pool comes from plant litter and the distribution of this input within the profile is

assumed to depend upon depth from the surface (z) and upon distance from the tree (d). Equations describing inputs to the FOM pool ($I_{t,z,d}$) at a given depth, distance and time (t) are fully explained in paragraph 2.4.

FOM mineralisation (FOM_{dec}) is assumed to be governed by first order kinetics, being proportional to the FOM pool, as given by:

$$\frac{\partial FOM_{dec}}{\partial t \partial z \partial d} = -k_{FOM} \times FOM_{t,z,d} \times f_{clay,z} \times f_{moist,z} \times f_{temp,z} \qquad (1)$$

where $FOM_{t,z,d}$ is the FOM carbon pool at a given time (t, in years), depth (z, in m) and distance (d, in m), and k_{FOM} is its decomposition rate. The rate modifiers $f_{clay,z}$, $f_{moist,z}$ and $f_{temp,z}$ are functions depending respectively on clay content, soil moisture and soil temperature at a given depth z, and range between 0 and 1.

The f_{clay} function originated from the ORCHIDEE model (Krinner et al. 2005):

$$f_{clay,z} = 1 - 0.75 \times Clay_z \qquad (2)$$

where $Clay_z$ is the clay content (%) of the soil at a given depth z.

The $f_{moist,z}$ function originated from the meta analysis of Moyano *et al.*, (2012) and is affected by soil properties (clay content, SOC stocks). Briefly, the authors fitted linear models on 310 soil incubations to describe the effect of soil moisture on decomposition. Then, they normalized such linear models between 0 and 1 to apply these functions to classical first order kinetics. All details are described in Moyano *et al.*, (2012). To save computing time, we calculated $f_{moist,z}$ only once using measured SOC stocks instead of using modelled SOC stocks and repeated calculation at each time step.

The temperature sensitivity of soil respiration is expressed as Q_{10}:

$$f_{temp,z} = Q_{10}^{\frac{temp_z - temp_{opt}}{10}} \qquad (3)$$

with $temp_z$ being the soil temperature in K at each soil depth z and $temp_{opt}$ a parameter fixed to 304.15 K. The Q_{10} value was fixed to 2, a classical value used in models (Davidson and Janssens 2006).

Once the FOM is decomposed, a fraction is humified (e) and another is respired as CO_2 ($1-e$) (Fig. III-2.1) following equations 4 and 5.

$$Humified\ FOM_{t,z,d} = e \times \frac{\partial FOM_{dec}}{\partial t \partial z \partial d} \qquad (4)$$

$$Respired\ FOM_{t,z,d} = (1-e) \times \frac{\partial FOM_{dec}}{\partial t \partial z \partial d} \qquad (5)$$

Two mathematical approaches are available in the model to describe the mineralisation of SOM: a first order kinetics, as given by eq. 6 or an approach developed by Guenet *et al.*, (2013) introducing the priming effect, i.e., the mineralisation of SOM depends on FOM availability, and given by eq. 7:

$$\frac{\partial SOM_{dec}}{\partial t \partial z \partial d} = -k_{SOM,z} \times SOM_{t,z,d} \times f_{moist,z} \times f_{temp,z} \qquad (6)$$

$$\frac{\partial SOM_{dec}}{\partial t \partial z \partial d} = -k_{SOM,z} \times SOM_{t,z,d} \times \left(1 - e^{-PE \times FOM_{t,z,d)}}\right) \times f_{moist,z} \times f_{temp,z} \qquad (7)$$

where $SOM_{t,z,d}$ is the SOM carbon pool at a given time (t, in years), depth (z, in m) and distance (d, in m), $k_{SOM,z}$ is its decomposition rate at a given depth z, and PE is the priming parameter. The parameters $f_{moist,z}$ and $f_{temp,z}$ are functions depending respectively on soil moisture and soil temperature at a given depth z, and affecting the decomposition rate of SOM. They correspond to eq. 2 and 3. The decomposition rate $k_{SOM,z}$ is an exponential law depending on soil depth (z):

$$k_{SOM,z} = a \times e^{-b \times z} \qquad (8)$$

The shape of the function (i.e. the *b* parameter) was determined by incubating soils from different soil layers during 44 days at 20°C and a water potential of pF 2.5 (results not shown, Cozzi et al., in prep). We used the soil incubations to determine only the *b* parameter because with such short term incubations, a complete estimation of $k_{SOM,z}$ over the soil profile overestimates the decomposition rates because the CO_2 measured during the incubations is mainly coming from labile source of C. The *a* parameter was optimized following the procedure described in paragraph 2.6. The effect of clay on SOM decomposition was implicitly taken into account in this equation as clay content increased with soil depth.

A fraction of decomposed SOM returns to the FOM assuming that part of the SOM decomposition products is as labile as FOM (*e*) and another is respired as CO_2 (Fig. III-2.1).

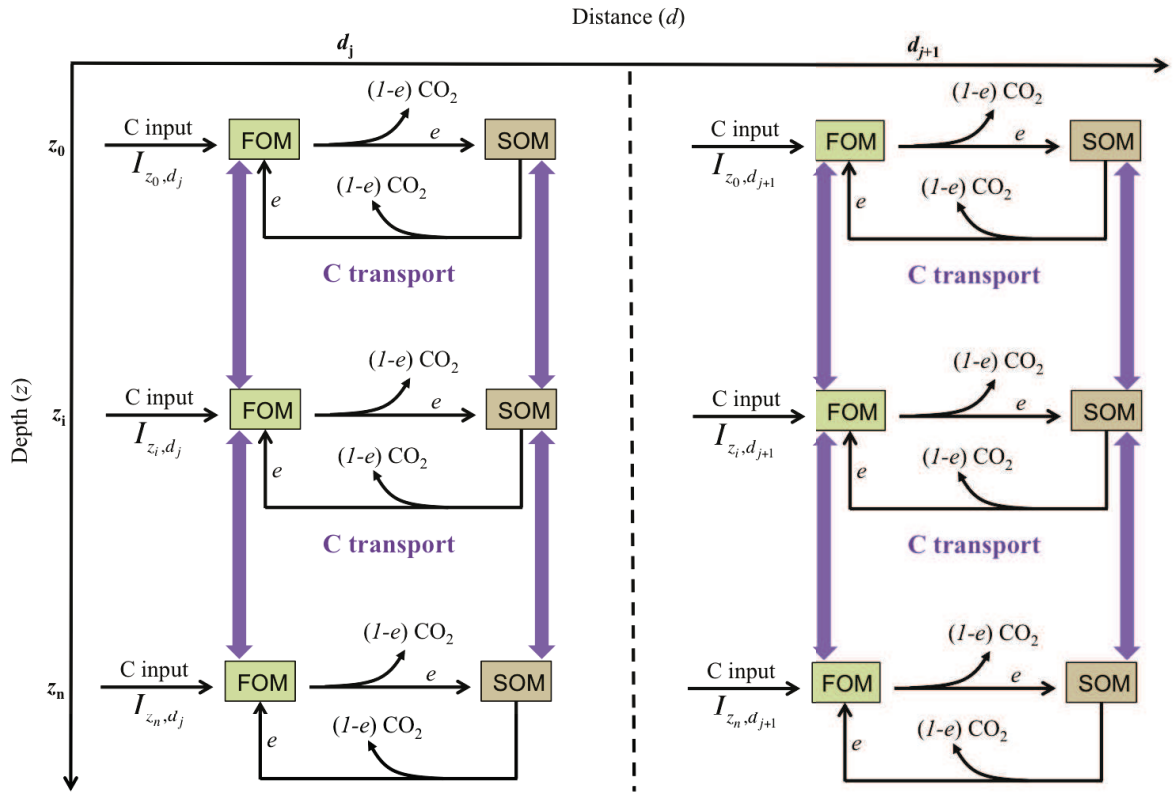


Figure III-2.1. Schematic representation of the pools and the fluxes of the model. Modified from Guenet et al. (2013).

2.2.2 Carbon transport mechanisms

Transport of C between the different soil layers was represented by both advection and diffusion mechanisms (Elzein and Balesdent 1995), which have been shown to usually better describe C transport in soils (Bruun et al. 2007; Guenet et al. 2013).

Advection is defined by:

$$F_A = A \times C \qquad (9)$$

where F_A is the flux of C transported downwards by advection, A the advection rate (mm yr^{-1}).

Diffusion is represented by the Fick's law:

$$F_D = -D \times \frac{\delta^2 C}{\delta^2 z} \qquad (10)$$

where F_D is the flux of C transported downwards by diffusion, $-D$ the diffusion coefficient ($cm^2\ yr^{-1}$) and C the amount of carbon in the pool subject to transport (FOM or SOM).

To represent the effect of soil tillage ($z \leq 0.2$ m), we added another diffusion term using the Fick's law but with infinite values of D to represent the mixing due to tillage. It must be noted that no tillage effect on decomposition was represented here because of the large unknowns on these aspects (Virto et al. 2012; Dimassi et al. 2013).

In this model, the flux of C transported downwards by advection and diffusion (F_{AD}) was represented as the sum of both mechanisms, following Elzein and Balesdent (1995):

$$F_{AD} = F_A + F_D \qquad (11)$$

Finally, the FOM and SOM pools dynamics correspond to:

$$\frac{\partial FOM}{\partial t \partial z \partial d} = I_{t,z,d} + \frac{\partial F_{AD}}{\partial z} + e \times \frac{\partial SOM_{dec}}{\partial t \partial z \partial d} - \frac{\partial FOM_{dec}}{\partial t \partial z \partial d} \qquad (12)$$

$$\frac{\partial SOM}{\partial t \partial z \partial d} = \frac{\partial F_{AD}}{\partial z} + e \times \frac{\partial FOM_{dec}}{\partial t \partial z \partial d} - \frac{\partial SOM_{dec}}{\partial t \partial z \partial d} \qquad (13)$$

The model was run at a yearly time step. The model was run from the ground (0 m) to 2 m depth, and from the tree (0 m) to 3.5 m in the inter-rows (4.5 m from the tree). The spatial resolution was 0.1 m both vertically and horizontally.

2.3 General equations describing the site

2.3.1 Soil characteristics

Soil texture and soil bulk densities of the agroforestry and the agricultural control plots were fully presented in Cardinael et al. (2015a) and were used for this work. Soil temperature and moisture sensors were installed in a pit in the agroforestry plot at 30, 130, 280 and 400 cm in April 2013, and soil temperature and moisture were measured during 11 months. We used these data to fit the following equations:

$$T = -0.89 \times z + 288.24 \qquad (14)$$

where T is the soil temperature (K) and z is the soil depth (m).

$$H = 0.05 \times z + 0.28 \qquad (15)$$

where H is the soil volumetric moisture (%) and z is the soil depth (m).

Soil pH was also measured along the soil profile:

$$pH = 0.16 \times \ln(z) + 8.36 \qquad (16)$$

Soil temperature, soil moisture and soil pH were considered to be the same in the agroforestry and in the control plot.

2.3.2 Tree growth

Several parameters such as crop yield and litter input (leaf, roots) were linked to the growth of the trees, which was measured in the field since the establishment of the experiment. We used the *DBH* as a surrogate of the tree growth preferentially to the tree height as the field measurements were more accurate. Indeed, *DBH* is easier to measure than tree height, especially when trees are getting older. To describe the temporal dynamic of *DBH* since the tree planting, we fitted a linear equation:

$$DBH \begin{array}{ll} 0.01, & y \leq 3 \\ 0.0157 \times y - 0.0391 \ (R^2 = 0.997) & 3 < y \leq 20 \end{array} \qquad (17)$$

where *DBH* is the diameter at breast height (m) and *y* represents the time since tree planting (years).

2.3.3 Crop yield

The mean yield in the control plot was 3.79 ± 0.40 t DM ha^{-1}. In the agroforestry plot, the yield was the same as in the control plot during the 8 first years. Then, mean crop yield decreased linearly with time (increasing *DBH*) and can be described using the following linear equation:

$$Rel\ Y_{AF_t} = -119.52 \times DBH_t + 106.59 \ \ (R^2 = 0.20) \qquad (18)$$

where $Rel\ Y_{AF_t}$ is the mean relative crop yield (%) in the agroforestry plot compared to the control plot at the year *t*, and DBH_t is the diameter at breast height (m) at the year *t*.

A quadratic equation gave the best fit of crop yield as a function of distance to the tree when we considered the entire inter-row (data not shown). In the model, we aim to predict SOC stocks up to 3.5 m in the inter-row. In this case, a linear increase of crop yield with increasing distance to the tree gave similar results than the quadratic equation and was more parsimonious:

$$Rel\ Y_{AF_d} = 4.11 \times d + 64.30 \qquad (19)$$

where $Rel\ Y_{AF_d}$ is the relative crop yield (%) in the agroforestry plot compared to the control plot at a distance *d* (m) from the tree.

Finally, the crop yield in the agroforestry plot was modeled as follows:

$$Y_{AF_{t,d}} = \begin{matrix} 3.79, & t \leq 8 \\ 1.2 \times Rel\ Y_{AF_d} \times Y_C \times Rel\ Y_{AF_t} \quad (R^2 = 0.24), & 9 < t \leq 20 \end{matrix} \qquad (20)$$

where $Y_{AF_{t,d}}$ is the crop yield (t DM ha^{-1}) in the agroforestry plot at the year *t* and at a distance *d* (m) from the tree, $Rel\ Y_{AF_t}$ is the mean relative crop yield (%) in the agroforestry plot compared to the control plot at the year *t*, Y_c is the crop yield (t DM ha^{-1}) in the control field, and $Rel\ Y_{AF_d}$ is the relative crop yield (%) in the agroforestry plot compared to the control plot at a distance *d* (m) from the tree. Nevertheless, because we used 3 linear equations to describe the crop yield in the agroforestry plot, we accumulated the errors and finally came up with a standard underestimation of the crop yield in the agroforestry plot that we corrected by multiplying our estimation by 1.2.

2.4 Carbon inputs to the FOM pool

2.4.1 Leaf litterfall

The ratio between leaf biomass and DBH was 0.0277 ± 0.0024 t C tree^{-1} m^{-1} (R^2=0.62). With a density of 110 trees ha^{-1}, we applied this linear relationship in the model to describe the C input to leaf litter:

$$L_t = 3.05 \times DBH_t \qquad (21)$$

where L_t is the leaf litter input (t C ha^{-1}) at the year *t*, and DBH_t the diameter at breast height the year *t*.

2.4.2 Tree fine root biomass

Tree fine root distribution and biomass within the soil profile was quantified in 2012 by Cardinael et al. (2015b). In the tree row, there was no effect of the distance from the tree (0 to 1 m) on tree fine root biomass, that was 1.01 t C ha^{-1} to 2 m depth. In the inter-row, tree fine root biomass decreased with increasing distance from the tree and was represented by an exponential function (R^2=0.9):

$$TFRB = \begin{matrix} 1.01, & 0 \leq d \leq 1 \\ 1.29 \times e^{-0.28 \times d} \quad (R^2 = 0.90), & 1 < d \leq 4.5 \end{matrix} \qquad (22)$$

where *TFRB* represents tree fine root biomass to 2 m depth (t C ha^{-1}), and *d* the distance to the tree (m).

We considered a linear increase of tree fine root biomass with increasing DBH. We performed a linear regression between *TFRB* in 2012 and *TFRB* in the first year (biomass considered as negligible):

$$TFRB_{t,d} = \begin{matrix} 3.69 \times DBH_t, & 0 \leq d \leq 1 \\ 4.70 \times DBH_t \times e^{-0.28 \times d}, & 1 < d \leq 4.5 \end{matrix} \qquad (23)$$

where $TFRB_t$ represents tree fine root biomass to 2 m depth (t C ha^{-1}) at the year *t*, DBH_t the diameter at breast height (m) at the year *t*, and *d* the distance to the tree (m).

To estimate tree fine root input due to mortality, tree fine root biomass was multiplied by the root turnover set at 2.2 yr^{-1} (Germon et al., submitted).

2.4.3 Distribution of tree fine roots within the soil profile

A changing distribution of tree fine roots within the soil profile was taken into account with increasing distance to the tree:

$$p_{TFRB,z,d} = \begin{array}{ll} 13.92 \times e^{-1.39 \times z} \quad (R^2 = 0.68), & 0 \leq d \leq 1 \\ a \times e^{-b \times z}, & 1 < d \leq 4.5 \end{array} \qquad (24)$$

and

$$a = 10.31 - 1.15 \times d \qquad R^2 = 0.69 \qquad (12)$$

$$b = -1.10 + 0.19 \times d \qquad R^2 = 0.51 \qquad (13)$$

Finally,

$$p_{TFRB,z,d} = \begin{array}{ll} 13.92 \times e^{-1.39 \times z}, & 0 \leq d \leq 1 \\ (10.31 - 1.15 \times d) \times e^{-(-1.10+0.19 \times d) \times z}, & 1 < d \leq 4.5 \end{array} \qquad (25)$$

where $p_{TFRB,z,d}$ is the proportion (%) of total tree fine root biomass (*TFRB*) at a given depth *z* (m), and at a distance *d* from the tree (m).

The distribution of the tree fine roots within the soil profile was considered to be constant with time.

2.4.4 Aboveground carbon input from the crop

The carbon input to the soil from the aboveground part of the crop was:

$$ABC_{crop,t,d} = Y_{t,d} \times \frac{straw\ biomass}{Y} \times C_{straw} \times (1 - Export) \qquad (26)$$

where $ABC_{crop,t,d}$ is the aboveground carbon input from the crop (t C ha^{-1}) at the year *t*, $Y_{t,d}$ is the crop yield (agroforestry or control) at the year *t* and at a distance *d*. The mean ratio between straw biomass and crop yield equaled 1.03. The carbon concentration of straw (C_{straw}) was 43.32 ± 0.07 mg C g^{-1}. Straw was exported out of the field after the harvest. It was estimated that only 25% of the straw biomass was left on the soil (*Export*=75%).

2.4.5 Belowground carbon input from the crop

Root biomass of the wheat crop was quantified to 2 m soil depth in the control plot, but no roots were observed below 1.5 m. Total fine root biomass in the control was 2.29 ± 0.32 t C ha^{-1}.

The distribution of crop roots within the soil profile was described as follows:

$$p_{CRBc,z} = \begin{cases} 26.44 \times e^{-2.59 \times z}, & z \leq 1.5 \\ 0, & z > 1.5 \end{cases} \quad (27)$$

where $p_{CRBc,z}$ is the proportion (%) of total crop root biomass in the control plot at a given depth z (m).

The carbon input to the soil from the belowground part of the crop was:

$$BEC_{crop,t,d} = Y_{t,d} \times \frac{AB_{crop}}{Y} \times Root{:}shoot \times C_{root} \quad (28)$$

where $BEC_{crop,t,d}$ is the belowground carbon input from the crop (t C ha^{-1}) at the year t and at a distance d, $Y_{t,d}$ is the crop yield (agroforestry or control) at the year t and at a distance d. The mean ratio between the crop aboveground biomass and the crop yield $\frac{AB_{crop}}{Y}$ equalled 2.45 ± 0.15. The *root:shoot* ratio of the wheat equaled 0.79 ± 0.12. The carbon concentration of wheat root (C_{root}) was 35.14 ± 1.90 mg C g^{-1}.

2.4.6 Aboveground and belowground carbon input from the herbaceous vegetation in the tree row

In January 2015, the aboveground biomass of the vegetation was 0.56 ± 0.09 t C ha^{-1} and the root biomass was 0.27 ± 0.02 t C ha^{-1} to 50 cm depth. In June 2014, it was 1.57 ± 0.11 t C ha^{-1} and 0.46 ± 0.04 t C ha^{-1} for the aboveground and belowground biomass respectively. Total aboveground carbon input was 2.13 ± 0.14 t C ha^{-1} yr^{-1} and total belowground carbon input was 0.74 ± 0.05 t C ha^{-1} yr^{-1} to 50 cm depth.

The belowground carbon input of the vegetation in the tree row ($BEC_{veg,z}$, t C ha^{-1}) at a given depth z (m) was described by the following equation:

$$BEC_{veg,z} = \begin{matrix} 0.44 \times e^{-3.12 \times z}, & z \leq 1 \\ 0, & z > 1 \end{matrix} \qquad (29)$$

Aboveground and belowground biomasses of the vegetation in the tree row were supposed to be constant over time.

2.5 Comparison of models

The model was developed using R 3.1.1 (R Development Core Team 2013). Model predictions with and without priming effect were compared calculating the coefficients of determination, root mean square errors (RMSE) and Bayesian information criteria (BIC).

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(x_i - x_\iota)^2} \qquad (30)$$

where i is the number of observations (1 to N), x_i is the predicted value and x_ι is the observed value.

$$BIC = N \times \ln\ \sigma_e{}^2\ + k\ \times \ln\ N \qquad (31)$$

where N is the number of observations, $\sigma_e{}^2$ is the error variance, and k is the number of model parameters.

2.6 Optimization procedure

Five parameters were optimized with a statistical approach based on a Bayesian framework (Tarantola 1987; Tarantola 2005; Santaren et al. 2007). These parameters were *A*, the advection rate, *D,* the diffusion coefficient, *e* the humification yield, *a* the coefficient of the k_{SOM} rate from equation (8), and *PE* the priming coefficient. The model was fitted to the data using a Bayesian curve fitting method described in Tarantola (1987). We aimed to find a parameter set that minimizes the distance between model outputs (SOM) and the corresponding observations (SOC), considering model and data uncertainties, and prior information on parameters. With the assumption of Gaussian errors for both the observations and the prior parameters, the optimal parameter set corresponds to the minimum of the cost function J(x):

$$J\ x\ = 0.5 \times\ (y - H\ x\ )^t \times R^{-1} \times\ y - H\ x\ + (x - x_b)^t \times {P_b}^{-1} \times (x - x_b) \quad (32)$$

that contains both the mismatch between modelled and observed SOC stock and the mismatch between a priori and optimized parameters. x is the vector of unknown parameters, x_b the vector of a priori parameter values, *H()* the model and y the vector of observations. The covariance matrices P_b and *R* describe a priori uncertainties on parameters, and observations, respectively. Both matrices are diagonal as we suppose the observation uncertainties and the parameter uncertainties to be independent. To determine an optimal set of parameters which minimizes J(x), we used the BGFS gradient-based algorithm (Tarantola 1987). We performed several optimizations starting with different parameter prior values to check that the results did not correspond to a local minimum. To optimize the parameters we only used the data coming from the control plot.

2.7 Management scenarios

Additionally to the model description, we compared here two simple scenarios in terms of SOC storage, with a maximum duration of agroforestry system of 90 years. The first scenario was 3 successive rotations of 30 years each. The second was 2 successive rotations of 45 years each. We compared here trees having the same growth rate (walnut trees), but the model could be used to compare trees having different growth rates.

2.8 Results

2.8.1 Correlation matrix and values of optimised parameters

The optimised parameters and their prior modes are presented in Table III-2.1. The most important correlations were observed between *e* and *A* which control the humification and the transport by advection, between *e* and *PE* which controls the effect of the FOM on SOM decomposition, but also between *A* and *PE* (Fig. III-2.2). Considering the method used to optimise the parameters, these important correlation factors hinder the presentation of the model output within an envelope. Therefore, we presented the model results using the optimised parameter without any envelope (Fig. III-2.3).

Model parameter	Meaning	Prior range	Posterior modes ± variance (prior modes)	
			Model without *PE*	Model with *PE*
a	coefficient from the equation of the SOM decomposition	$1e^{-8}$-0.01	$0.0001 \pm 2.42e^{-7}$ (0.000098)	$0.0001 \pm 2.42e^{-7}$ (0.000098)
D	diffusion coefficient (cm^2 yr^{-1})	$1e^{-6}$-1	$0.000964 \pm 2.33e^{-3}$ (0.000964)	$0.000584 \pm 2.64e^{-4}$ (0.000964)
A	advection rate (mm yr^{-1})	$1e^{-6}$-1	$0.002578 \pm 5.51e^{-4}$ (0.00854)	$0.001499 \pm 1.10e^{-3}$ (0.00854)
e	humification yield	0.01-1	$0.31 \pm 3.47e^{-5}$ (0.34)	$0.22 \pm 1.06e^{-4}$ (0.34)
PE	priming coefficient	0.1-160	-	13.74 ± 8.18 (31.99)

Table III-2.1. Summary of optimized model parameters. *PE*: priming effect.

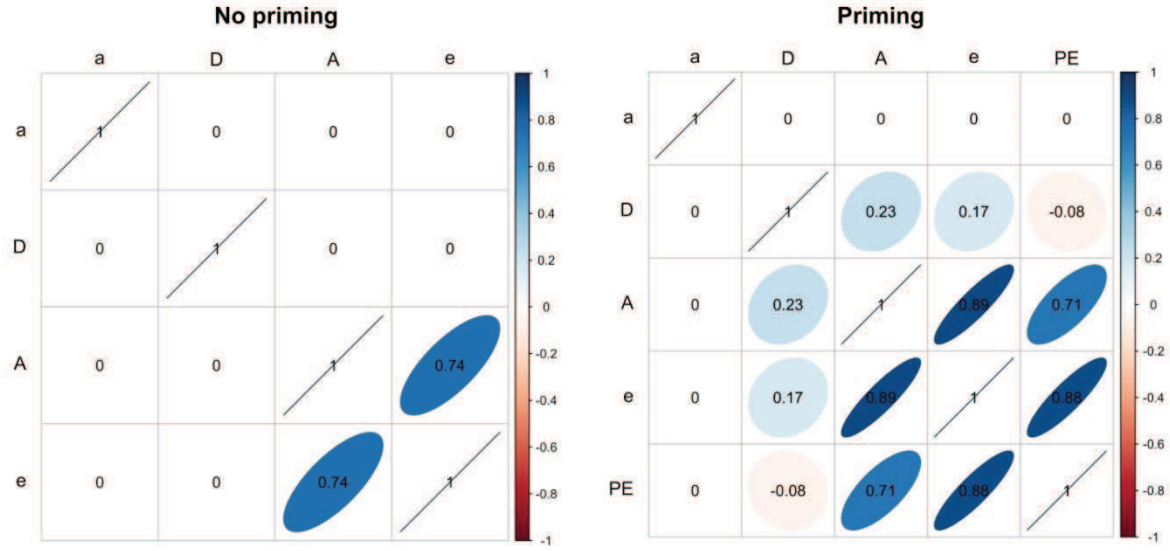
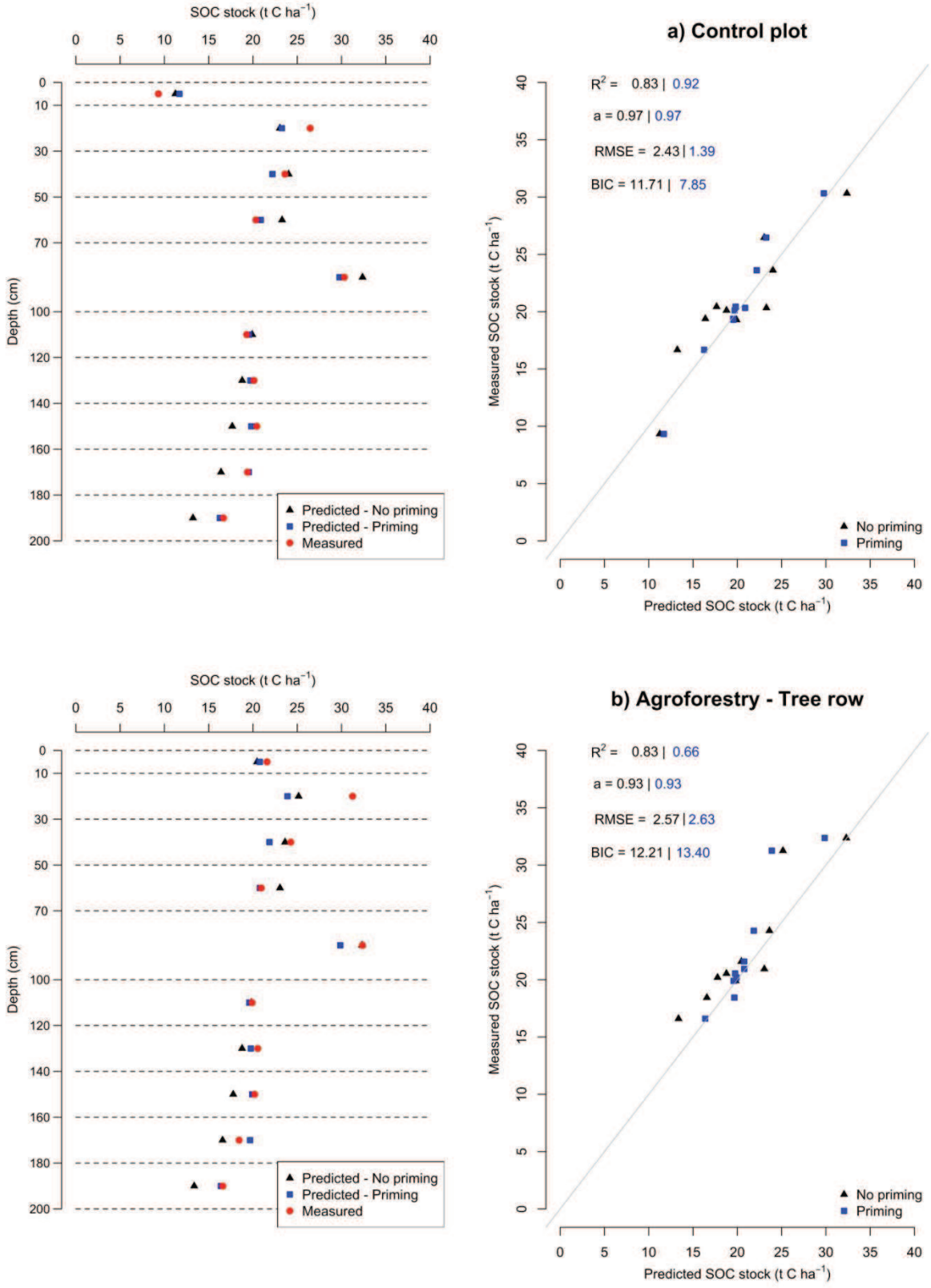


Figure III-2.2. Correlation matrix of optimized parameters. *a* is the coefficient from the equation (8) for the SOM decomposition rate, *D* is the diffusion coefficient (cm^2 yr^{-1}), *A* is the advection rate (mm yr^{-1}), *e* is the humification yield, and *PE* is the priming coefficient.

2.8.2 Model predictions and observed SOC stocks

When considering the whole soil profile, the lowest RMSEs were obtained in the inter-row (Fig. III-2.3c) but were also low in the control plot (Fig. III-2.3a), and a bit higher in the tree row (Fig. III-2.3b). The introduction of the priming effect (PE) in the model largely improved predictions in the control plot, but only slightly in the inter-row, and did not in the tree row as BICs were higher with PE than without PE (Fig. III-2.3b). However, if we calculate RMSEs and BICs for topsoil layers (0-1 m) and for subsoil layers (1-2 m), priming effect improved a lot the predictions for subsoil layers (Table III-2.2). In all cases, the major source of errors

came from the 10-30 cm layer, SOC stocks were always underestimated. Globally, predicted SOC stocks were higher with the PE than without PE (about 2 t C ha^{-1}).



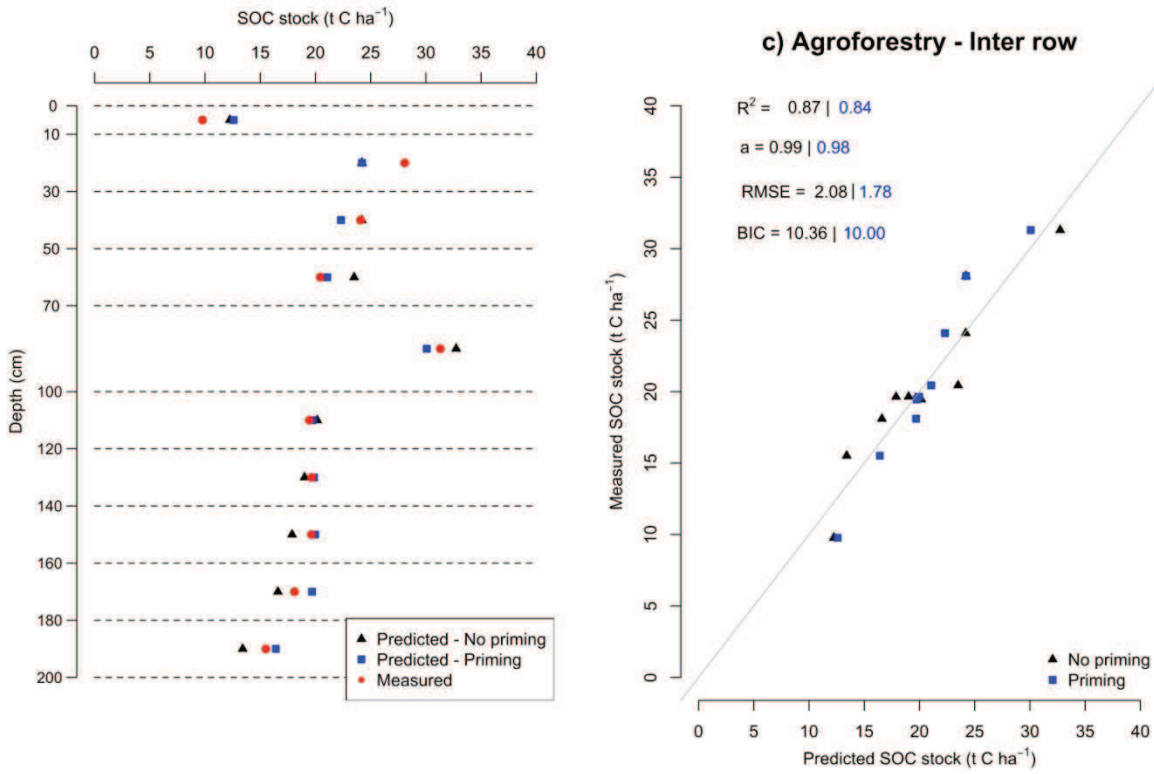


Figure III-2.3. Predicted and measured SOC stocks (t C ha^{-1}) in a) the control plot, in b) the agroforestry - tree row, and in c) the agroforestry – inter-row. Standard errors of measured values were very small (n=100) and not represented here to make the graph clearer. R^2 is the coefficient of determination, *a* is the regression coefficient (y=ax), RMSE is the root mean square error (t C ha^{-1}), and BIC is the Bayesian information criterion.

		All soil profile		Topsoil (0-1 m)		Subsoil (1-2 m)	
		without *PE*	with *PE*	without *PE*	with *PE*	without *PE*	with *PE*
Control	RMSE	2.43	1.39	2.39	1.92	2.47	0.40
	BIC	11.71	7.85	6.57	5.63	5.63	-1.15
Tree-row	RMSE	2.57	2.63	2.94	3.66	2.14	0.69
	BIC	12.21	13.40	7.49	8.42	6.10	1.16
Inter-row	RMSE	2.08	1.78	2.55	2.37	1.46	0.85
	BIC	10.36	10.00	6.87	6.54	4.43	2.08

Table III-2.2. Comparison of model predictions for the different modalities. PE: priming effect. RMSE is the root mean square error (t C ha^{-1}), and BIC is the Bayesian information criterion.

2.8.3 Predicted SOC stocks in two dimensions

Under wheat cover, the model predicted no effect of the distance to the tree on SOC stocks in an 18-year-old agroforestry plot, but a clear separation between SOC stocks under the tree row and the inter-rows is observed (Fig. III-2.4).

Additional SOC stocks in the agroforestry plot compared to the agricultural control plot were mainly located in the first 30 cm of soil.

For 0-30 cm, delta stock between the tree row and the control was 11.3 t C ha^{-1} without priming effect, and 9.7 t C ha^{-1} with priming effect, while delta stock between the inter-row and the control was 2.2 and 1.8 t C ha^{-1}, without and with priming, respectively. The highest SOC stocks and delta stocks were in the tree row, in 0-10 cm.

2.8.4 Delta SOC stocks and cumulated FOM

Delta SOC stocks between the tree row and the control, and between the inter-row and the control were well represented up to 1 m depth, and were explained by cumulated FOM stocks through the years (Fig. III-2.5). Below 1 m, observed delta SOC stocks were negative, and not predicted by the model whatever the decomposition scheme used.

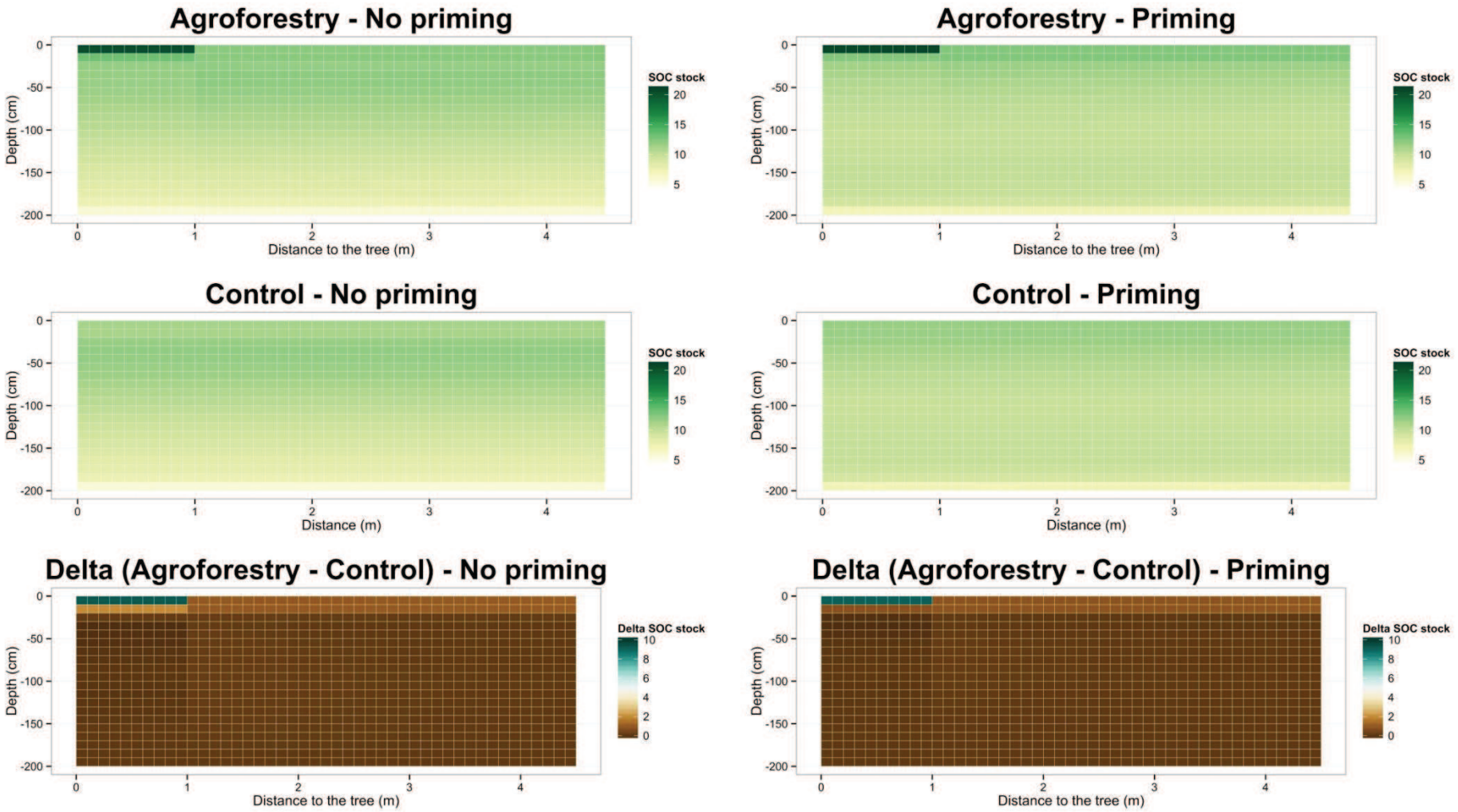


Figure III-2.4. Representation of predicted SOC stocks (t C ha^{-1}) in two dimensions, as a function of distance to the tree (m) and depth (cm), without or with priming effect. The simulated agroforestry plot is 18 years old

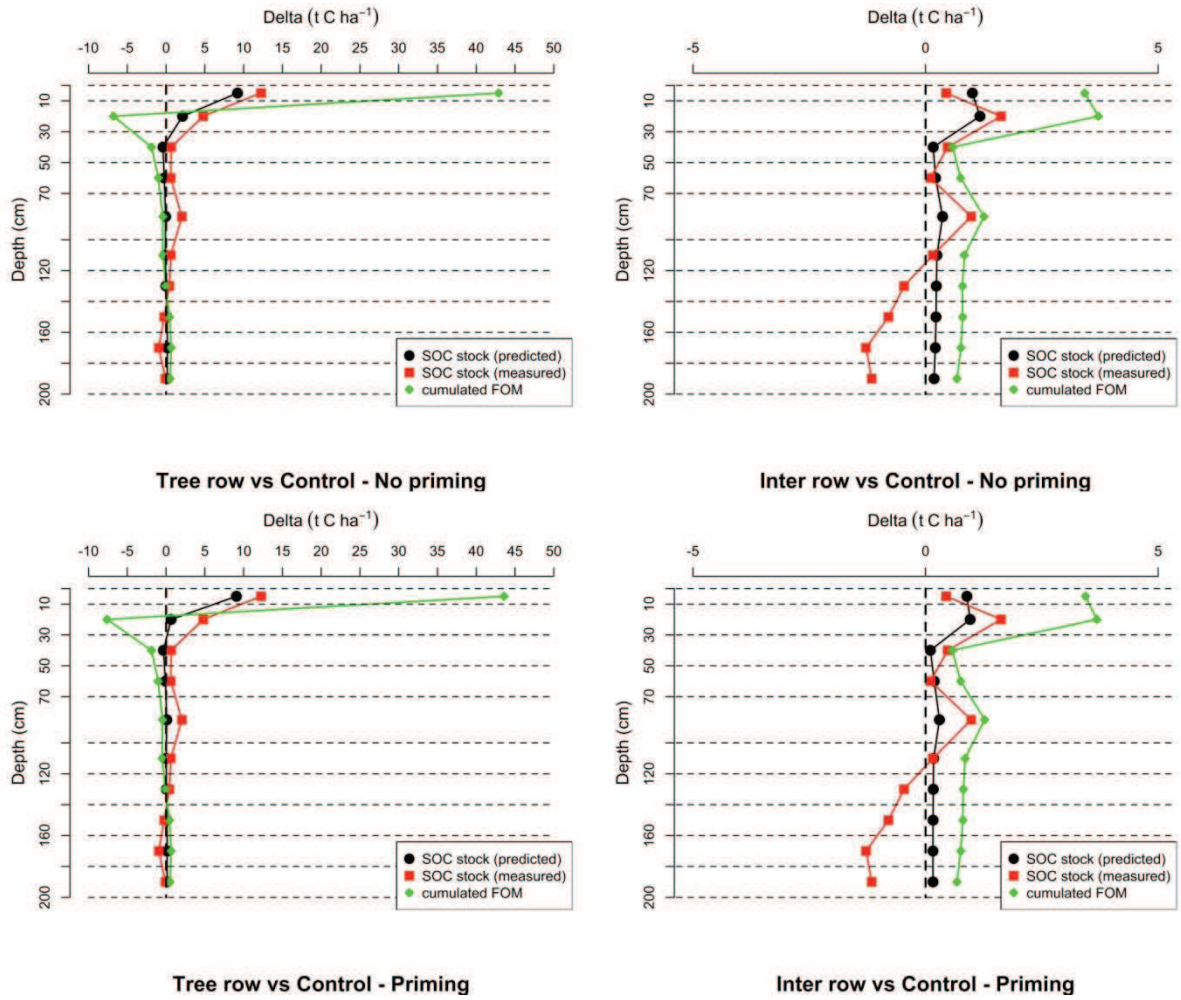


Figure III-2.5. Delta SOC stocks (t C ha^{-1}) and cumulated FOM stocks in an 18 year old agroforestry plot.

2.8.5 Comparison of different scenarios

Predictions showed that favoring older trees in a rotation is better for SOC storage (Fig. III-2.6). Indeed, two tree rotations of 45 years instead of three tree rotations of 30 years old increased the delta SOC stock between the tree row and the control of 30 t C ha^{-1} in 90 years, and of 11 t C ha^{-1} between the inter-row and the control.

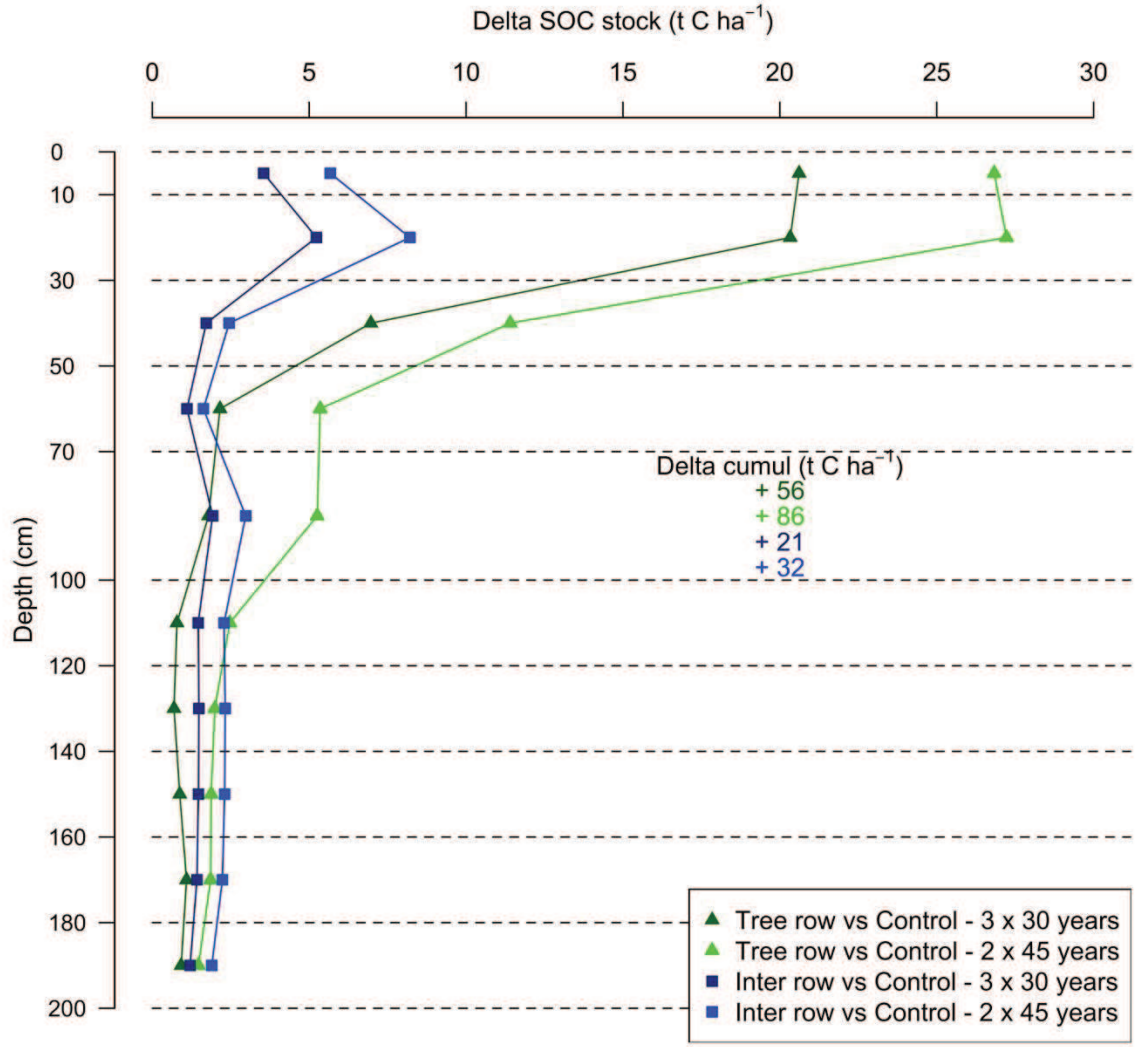


Figure III-2.6. Comparison of two different management scenarios in terms of soil organic carbon storage. The first scenario is 3 tree rotations of 30 years and the second is 2 tree rotations of 45 years.

2.9 Discussion

2.9.1 Model predictions

Model predictions were very satisfactory with or without representation of priming effect. In particular the shape of the profiles was well reproduced by the model. The model was able to represent SOC stocks in different soil layers in both the control and the agroforestry plot, and confirms the interest of vertically discretized models. Surprisingly, the predictions of SOC stocks were a bit better in the inter-rows than in the tree rows whereas organic inputs were more complex and more difficult to model. Optimized parameters were obtained on SOC stocks from the control plot. In the control plot and in the inter-rows, the soil is tilled down to 20 cm, which is not the case in the tree row. Transport parameters could be a bit different in

the tree rows compared to the inter-row, especially in top soil layers and explain this difference of prediction (the SOC stock in 10-30 cm in the tree row is the worst predicted one).

In the inter-rows, the plasticity of the tree root system due to soil tillage and competition with wheat roots was not taken into account (Cardinael et al. 2015c), but predictions were good. In this model, we were not able to represent this specific tree root pattern, and tree root profiles were fitted using a decreasing exponential, such a wheat root profiles. We were not able to implement root growth in the model due to a lack of data. Root distribution in the soil as a function of depth and distance was considered constant, only root biomass increased with tree growth. Modeling of crop yield in the agroforestry plot was also hazardous as regressions on data were not good, but it did not impact the capacity of the model to predict SOC stocks in inter-rows. This result suggests that a simple root dynamic could satisfactory represent organic inputs in the soil, at least in the first 20 years of tree growth.

However, it could be interesting to couple the SOCRATES model with a model describing root architecture and root growth (Jourdan and Rey 1997), using for instance voxel automata (Mulia et al. 2010), and compare predictions with our results.

2.9.2 SOC storage and OM inputs

With the same parameters related to mineralization and obtained after an optimization on the control plot, the model reproduced well the SOC stocks in the agroforestry plot. It suggests that the higher SOC stocks observed in the agroforestry are mainly due to an increase of OM inputs and not to a reduction of the decomposition. The difference of OM inputs was able to explain both amount and spatial distribution of additional SOC storage. However, we considered here the same soil temperature and soil moisture in both field, and it may not be the case. Reduced temperature and lower evapotranspiration is often observed under agroforestry systems (Lott et al. 2009; Clinch et al. 2009), and further work is needed to assess the impact of this microclimate on SOC decomposition.

2.9.3 Priming or not priming?

The introduction of priming effect was necessary to describe SOC dynamics in particular for subsoil, below 1 m depth, suggesting that SOC mineralisation is governed by fresh organic

inputs in deep soil layers. Indeed, when priming is incorporated, the RMSE as well as the BIC values are generally lower. BIC is a metric that takes into account the number of parameters in the model whereas RMSE doesn't. When priming is represented the model needs one more parameter and the better performance of the model with priming observed with RMSE might be only the result of an over parameterization. The reduced BIC values also observed in most of the cases suggest that priming is a necessary process to reproduce SOC stocks in our study site. However, priming is here considered as rate modifier driven by the FOM availability (eq. 7) and not simply a phenomenon accelerating the decomposition when FOM is added. It is important to note that with first order kinetics, like the one presented in eq. 6, the decomposition parameters are fitted on data where priming naturally occurs and therefore it is implicitly represented assuming a constant priming rate over time. Using eq. 7, we do not need to assume a constant priming rate over time and we consider that the SOC mineralization is driven by the availability of FOM. As a consequence, the mineralization rate calculated with priming might be sometimes higher and sometimes lower than the mineralization rate calculated without priming. This may explain why predicted SOC stocks with priming were higher than predicted SOC stocks without priming, which might appear as not intuitive.

Nevertheless, even with the priming effect, the model does not predict negative delta stock between the agroforestry and the control plot in deep soil layers (below 1 m). Thus, priming effect may not be considered as the main driver of the observed SOC stocks differences and the higher SOC stocks in the control plot in subsoil probably results from an initial heterogeneity of the alluvial soil, as suggested by Cardinael et al. (2015a).

2.9.4 Management and SOC storage

The model revealed that extending the tree rotation had a positive impact on SOC storage. Old trees produce more biomass and more organic inputs to the soil (leaves, roots) than young trees, and have therefore a more pronounced impact on SOC stocks. We compared here different rotations with trees having the same growth rate, but a more realistic scenario would be to compare short rotations with fast growing trees (e.g. poplars) and long rotations with slow growing trees (e.g. walnuts). Trade-offs between profitability and SOC storage could be assessed for instance by coupling this model with the Yield-SAFE model (Graves et al. 2007; Graves et al. 2010).

2.10 Conclusion

Agroforestry systems are generally considered as good candidates to mitigate climate change because of the high carbon storage observed in those systems in particular within the soil. Here we design a model able to predict quite well observed SOC stocks in a particular site. The model may be considered as mechanistic and consequently, it may be useful to disentangle between different mechanisms (importance of the input, priming, etc.). The model needs now to be evaluated on other sites with other trees and crops species to test its robustness but it is a promising tool to estimate the carbon storage capacity of a site. In particular, it might be a good tool to evaluate the best rotation rate or the best tree species to maximize soil carbon storage.

Discussion générale et perspectives

1. Stockage de carbone dans les systèmes agroforestiers

Cette thèse a permis une quantification du stockage de carbone organique dans le sol sous différents systèmes agroforestiers en France. En moyenne, les systèmes agroforestiers « linéaires » étudiés permettent un stockage additionnel de carbone de 0.24 (0.09 – 0.46) t C ha^{-1} an^{-1} sur 0-30 cm par rapport à une parcelle agricole. Ce chiffre est du même ordre de grandeur que celui proposé par Pellerin et al. (2013), et valide leur estimation d'environ 0.3 t C ha^{-1} an^{-1} (0.03 à 0.41 t C ha^{-1}) pour les systèmes agroforestiers en France.

Qu'indiquent ces chiffres ? Est-ce important ?

Au niveau mondial, les émissions de gaz à effet de serre (GES) provenant de l'utilisation de carbone fossile représentent 8.9 Gt C an^{-1} (Le Quéré et al. 2014), tandis que les stocks de carbone organique globaux sont estimés entre 1560 - 2300 Gt C (Jobbagy and Jackson 2000; Houghton 2007). Ainsi, les émissions annuelles de C fossile sont de l'ordre de 4 à 5‰ du stock de carbone organique. On obtient le même chiffre si l'on divise l'augmentation nette annuelle de GES dans l'atmosphère (4.3 Gt C) par les stocks de C des sols de la planète jusqu'à 30 cm de profondeur (800 Gt C). Cela signifie **qu'une variation de 4 à 5‰ des stocks de C des sols globaux peut soit doubler l'augmentation nette annuelle des GES dans l'atmosphère, soit la compenser**.

En France, les stocks de C moyens des sols cultivés sont de 50 t C ha^{-1} sur 0-30 cm (GIS sol 2011). Une augmentation des stocks de C de 4‰ par an correspond donc à un stockage de 0.2 t C ha^{-1} an^{-1} (avec une densité apparente du sol de 1.3 g cm^{-3}). Ainsi, les taux de stockage observés dans les sols sous systèmes agroforestiers permettraient de répondre localement à cet objectif des 4‰. Ce chiffre des 4‰ (voir le lancement du projet de recherche international « 4 pour 1000 » par le ministre de l'agriculture) est cependant à prendre avec précaution à l'échelle globale. En effet, il est calculé à partir des stocks de C globaux qui sont sous différents types et occupation des sols et sous différents climats. Or, les sols sous forêts ou sous prairie par exemple sont souvent saturés en carbone, leur potentiel de stockage est donc faible voire nulle. Il faudrait calculer ce taux théorique en ne prenant en compte que les stocks de C des parcelles agricoles au niveau mondial. Ce chiffre serait sans aucun doute très

largement supérieur aux 4‰. De plus, ces taux de stockage ne sont valables qu'un certain temps, jusqu'à ce que le sol atteigne un nouvel équilibre (Arrouays et al. 2002), au maximum son potentiel de saturation (Hassink 1997).

L'agroforesterie n'est pas le seul système agricole ou pratique agronomique qui permette de stocker du carbone dans les sols. En effet, l'introduction de cultures intermédiaires permet de stocker de 0.24 ± 0.11 t C ha^{-1} an^{-1} sur 0-30 cm (Pellerin et al. 2013) à 0.32 ± 0.11 t C ha^{-1} an^{-1} sur 0-22 cm (Poeplau and Don 2015). Les taux de stockage du semis direct ont été estimés à 0.15 ± 0.15 ha^{-1} an^{-1} (Pellerin et al. 2013). La conversion de parcelles cultivées en prairies permanentes permet de stocker 0.44 ± 0.24 ha^{-1} an^{-1} (Arrouays et al. 2002), du même ordre de grandeur que l'afforestation. Ainsi, l'agroforesterie fait partie d'un ensemble de solutions à la disposition des agriculteurs et des décideurs afin d'augmenter les stocks de C du sol. Par rapport à d'autres pratiques agronomiques (semis direct, cultures intermédiaires…) ; l'agroforesterie implique néanmoins une modification plus importante du système de culture. Mais si l'on prend en compte le stockage de carbone dans la biomasse aérienne et souterraine des arbres (1.08 ± 0.30 t C ha^{-1} an^{-1}), cela fait de l'agroforesterie l'une des pratiques agricoles les plus séquestrantes, ce qui pourrait justifier d'une politique dédiée d'appui à l'agroforesterie pour lutter contre l'effet de serre.

2. Répartition spatiale et en profondeur des stocks de carbone

L'analyse spatiale des stocks de carbone l'a confirmé : l'agroforesterie crée des hétérogénéités fortes au sein d'une parcelle agricole. Quelles que soient les parcelles échantillonnées, les stocks de carbone du sol ne dépendaient pas de la distance aux arbres mais étaient fonction de leur localisation, dans la ligne d'arbres ou dans l'inter-rang cultivé. La végétation herbacée sur les lignes s'apparente à une prairie permanente, le stockage de carbone y est important. Le stockage de carbone dans les jeunes parcelles agroforestières semble uniquement dû à ces « bandes enherbées ». Le fait de n'avoir observé aucun effet de la distance à l'arbre dans les inter-rangs sur les stocks de C vient probablement du fait que les parcelles âgées échantillonnées étaient toutes relativement « denses », avec une largeur maximale de l'inter-rang de 11 m, largeur moins importante que la hauteur des arbres. Les litières aériennes des arbres sont globalement réparties de façon homogène sur des inter-rangs de cette largeur, et les moindres résidus de culture dus aux pertes de rendement à proximité

des arbres sont compensés par des apports racinaires des arbres. On peut faire l'hypothèse que dans les parcelles agroforestières actuellement mises en place avec des largeurs de 30 à 40 m entre deux lignes d'arbres, un effet distance à l'arbre pourra être mesuré dans les années à venir. Nos résultats permettent de simplifier grandement les protocoles d'échantillonnage de sol futurs des parcelles agroforestières. Pour les parcelles jeunes et pour les parcelles denses, un prélèvement d'échantillons représentatifs sur la ligne d'arbre, ainsi que dans l'inter-rang permet d'avoir une bonne estimation du stock de carbone de la parcelle, sans avoir à tenir compte des distances aux arbres. La prudence s'impose cependant lorsque la parcelle agroforestière échantillonnée est une parcelle peu densément peuplée (< 50 arbres ha^{-1}, et > 20 m entre 2 lignes d'arbres), et suffisamment âgée (15-20 ans minimum), où un gradient de stocks de C en fonction des distances aux arbres peut apparaitre.

Malgré les hypothèses faites sur l'apport de carbone en profondeur, le stockage de carbone a principalement été observé dans les 30 premiers centimètres du sol. Si du carbone est bien injecté en profondeur, il l'est cependant en bien moindre quantité qu'en surface, car la densité de racines, mais aussi leur turnover est plus faible. Dans la fosse mise en place sur le domaine expérimental de Restinclières, nous avons observé qu'en profondeur les racines empruntaient assez souvent les conduits réalisés par les racines précédentes (Fig. IV-1).

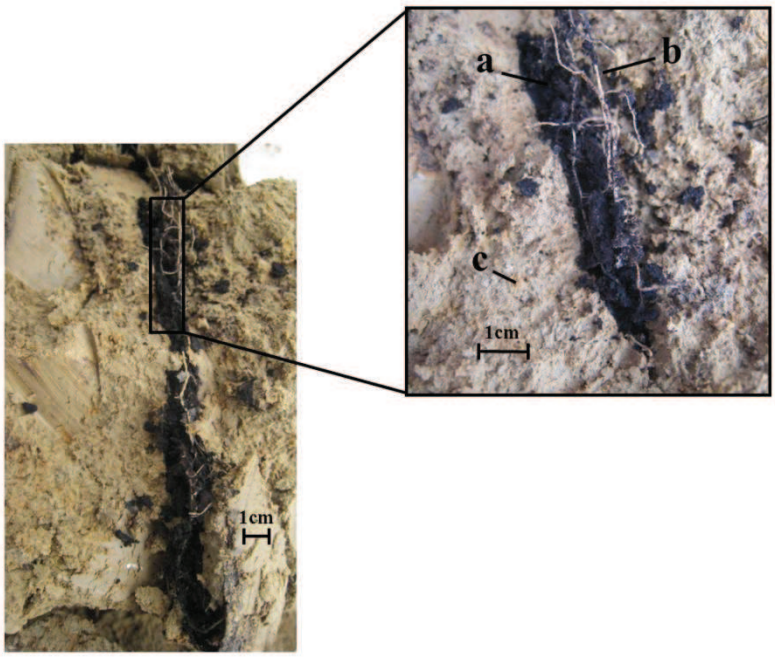


Figure IV-1. Photo de jeunes racines fines de noyer observées à 2 m de profondeur sur le site expérimental en agroforesterie de Restinclières. a) Racine morte en décomposition ; b) Nouvelle racine fine de noyer ; Sol environnant (« bulk soil »).

En profondeur, le sol se fissure moins bien qu'en surface (moins de cycles d'humectation-dessiccation), la densité apparente du sol est forte, et le sol plus pauvre en nutriments qu'en surface, les racines profitent des galeries créées par les précédentes. Nous avons observé des nouvelles racines poussant à l'intérieur d'anciennes racines en décomposition (Fig. IV-1). Elles profitent du conduit, mais aussi des nutriments libérés par la décomposition des anciennes racines. De plus, les racines présentent moins de ramifications horizontales, elles sont surtout orientées verticalement (Cardinael et al. 2015c). Ainsi, les dépôts de carbone en profondeur se font de manière très localisée. Lors d'un prélèvement, ce carbone est homogénéisé au sein du « bulk soil », et en quantité trop faible pour être détecté de façon significative lors des analyses de carbone réalisée sur du sol tamisé à 2 mm. Malheureusement, la parcelle agroforestière la plus ancienne que nous ayons échantillonné (41 ans) chez un agriculteur a été installée sur un sol superficiel, et n'a donc pas permis d'étudier l'impact sur les stocks de carbone profond du sol. A Restinclières, les stocks de carbone plus importants observés en profondeur (en dessous de 1 m 20) dans la parcelle agricole par rapport à la parcelle agroforestière sont probablement issus d'une hétérogénéité initiale de la parcelle comme le suggèrent les travaux de modélisation. Ce problème montre les limites des études synchroniques dans l'étude de l'impact de pratiques agronomiques sur le sol (Costa Junior et al. 2013). Il semble nécessaire de mettre en place des essais de longue durée en agroforesterie, avec une approche diachronique (c'est-à-dire une caractérisation de l'état initial d'une parcelle agricole qui va être plantée en agroforesterie, et non une comparaison de 2 parcelles qui apporte un biais liés aux hétérogénéités du sol) et avec la réalisation de plusieurs blocs afin d'avoir une représentativité statistique robuste.

3. Stabilité du stockage de carbone sur le long terme

Le fractionnement granulo-densimétrique de la matière organique a révélé que la majorité du carbone additionnel dans la parcelle agroforestière noyers hybrides/blé dur se trouvait sous une forme de matière organique particulaire, c'est-à-dire sous la forme de matières organiques en cours de décomposition, essentiellement des débris végétaux de la taille des sables (50-200 et 200-2000 µm) (Cardinael et al. 2015a). Cette forme de carbone a un temps de résidence de l'année à une vingtaine d'années, contrairement aux matières organiques adsorbées sur les argiles qui ont un temps de résidence moyen supérieur à 50 ans (Balesdent 1996; Six et al. 2002). De plus, les incubations de sols ayant eu leur structure détruite à différentes échelles (jusqu'à 50 µm), n'ont pas montré de sur-minéralisation de la matière organique par rapport

aux sols non déstructurés. Le carbone organique sur cette parcelle n'est donc pas non plus protégé « physiquement » au sein d'agrégats de cette taille (Besnard et al. 1996). Il pourrait être intéressant d'étudier cette protection physique à une échelle plus fine, sur d'autres parcelles, car il semble que ce processus soit surtout significatif à l'échelle du micro-agrégat de la taille des limons, et pas au niveau des macro-agrégats (Virto et al. 2008; Moni et al. 2010; Virto et al. 2010). Le fait que ce stockage de carbone soit pour le moment principalement localisé en surface le rend également vulnérable face au changement d'usage. Ces stocks seront préservés tant que l'agroforesterie sera maintenue sur le site, mais risquent d'être perdus si la parcelle redevient une parcelle agricole classique.

Dans le cadre du projet Agripsol, des comptages de vers de terre ont été réalisés par l'UMR Ecobio sur les différentes parcelles du projet. De façon inexpliquée, aucun ver de terre n'a été trouvé sur les parcelles agricoles et agroforestières du domaine de Restinclières. Or, ces ingénieurs du sol ont un fort impact sur la dynamique et la stabilisation du carbone des sols. En ingérant de la matière organique et du sol, ils la stabilisent au sein de microagrégats dans leurs turricules (Bossuyt et al. 2005; Bertrand et al. 2015). Leur absence pourrait expliquer en partie pourquoi ce carbone additionnel n'est pas stabilisé. Sur les autres sites échantillonnés, l'abondance et la biomasse des vers de terre est plus importante en agroforesterie que dans les parcelles agricoles témoins. Notamment, sur le site de Saint-Jean-d'Angély, après 41 ans d'agroforesterie, le sol rougeâtre de la « terre de groie » a pris une couleur brunâtre beaucoup plus foncée. C'est sur ce site que la plus forte augmentation des stocks de carbone a été observée (Fig. I-2.3). Il pourrait être très intéressant de faire du fractionnement granulo-densimétrique sur ce site, afin notamment de comparer les quantités de carbone associé aux argiles entre les 2 parcelles.

4. Importance des racines dans le stockage de carbone

Les racines participent au stockage de C des sols à travers la mortalité racinaire et la production d'exsudats. Nous avons montré que les apports de carbone par mortalité racinaire représentaient 85% des apports de MO dans la parcelle agricole (les pailles sont exportées, seuls les chaumes restent), et 70% dans la parcelle agroforestière (Fig. II-3.2). Ces résultats confirment d'autres études qui ont déjà montré que les racines étaient une source importante de carbone au sol (Balesdent and Balabane 1992; Rasse et al. 2005). Nous avons basé nos estimations sur une fosse profonde – ce qui est rare -, et sur des mesures de biomasses

racinaires uniquement réalisées après l'ouverture de la fosse au printemps. Or, l'on sait que la production de racines varie dans le temps (Steinaker and Wilson 2008; Abramoff and Finzi 2015). La période de mesure peut donc influencer l'estimation des biomasses racinaires. De plus, nous avons montré qu'il y avait une diminution du turnover des racines avec la profondeur du sol (Germon et al., submitted for publication), cependant l'estimation que nous en avons faite en profondeur est très probablement sur-estimée du fait de la durée de suivi relativement courte (1 an). Cette étude est maintenant poursuivie par une autre doctorante à l'UMR AMAP, qui va également comparer l'estimation faite sur des minirhizotrons avec celle faite sur des rhizotrons. De plus, dans le cadre du projet Ecosfix, une collaboration est en cours avec le Max Planck Institute de Jena afin de comparer les prédictions issues des minirhizorons et celles faites à partir de la mesure du radiocarbone (Ahrens et al. 2014; Ahrens and Reichstein 2014). Il sera très intéressant de comparer ces résultats sur une plus longue durée, avec ceux que nous avons obtenus, et voir de quelle manière ils influencent nos estimations d'entrées de carbone au sol par mortalité racinaire.

L'apport de carbone au sol par rhizodéposition (ou exsudation) n'a pu être quantifiée lors de cette thèse. Si plusieurs méthodes existent pour piéger les exsudats, les difficultés techniques pour les quantifier *in situ* sont nombreuses (Phillips et al. 2008). Cependant, plusieurs études suggèrent que ces apports de C au sol peuvent être significatifs (Kuzyakov and Domanski 2000; Balesdent et al. 2011). Les exsudats modifient les communautés microbiennes du sol (Haichar et al. 2008; de Graaff et al. 2010), mais leur rôle dans le stockage de carbone est encore ambigu. Des études suggèrent que les exsudats, molécules labiles, peuvent s'adsorber sur les particules d'argile et contribuer au stockage de carbone (Hütsch et al. 2002). D'autres montrent que la libération d'exsudats dans le sol entraîne la décomposition de la MO du sol par co-métabolisme (ou priming effect) (Bengtson et al. 2012; Shahzad et al. 2015), ou encore que l'acide oxalique, un exsudat courant, entraîne des pertes de carbone en libérant les MO adsorbées sur les minéraux (Keiluweit et al. 2015). Même si il n'y a pas de consensus sur l'intérêt de prendre en compte le rôle du priming effect sur les dynamiques de carbone à long terme (Cardinael et al. 2015b), il semble pertinent dans le futur d'étudier ce processus dans les systèmes agroforestiers. En effet, les arbres ont accès à des horizons profonds où les microorganismes sont fortement limités en énergie, et l'apport de molécules labiles peut entraîner un priming effect important (Fontaine et al. 2007). Des marquages des arbres avec du ^{13}C par « pulse-labelling », au champ ou en écotron pourraient permettre de mieux comprendre ces processus (Epron et al. 2011; Epron et al. 2012). L'allocation de carbone des

arbres agroforestiers au compartiment souterrain pourrait être différente des arbres forestiers, car ce sont des arbres de plein soleil, ils sont ne sont pas en compétition avec les arbres voisins, et sont élagués jusqu'à 4-5 m de hauteur. Il semble très pertinent d'étudier ce processus d'allocation de carbone dans ces systèmes.

5. Influence du microclimat sur la dynamique du carbone

Le microclimat en agroforesterie est modifié par la présence des arbres, mais les effets sur l'humidité du sol sont complexes. Généralement, la température du sol sous les arbres est plus basse (Clinch et al. 2009), et les arbres peuvent également servir de coupe-vent (Oteng'i et al. 2000), ce qui se traduit par une moindre évaporation du sol. En revanche, les arbres transpirent de grandes quantités d'eau (Ong and Leakey 1999), ce qui peut créer une compétition importante pour cette ressource, notamment dans les sols superficiels ou en absence de nappe accessible pour les arbres. La redistribution hydraulique ou ascenseur hydraulique est le mouvement passif de l'eau à travers différentes couches de sol via les racines des arbres, qui dépend des gradients de potentiel hydrique à l'interface sol-plante (Prieto et al. 2012). Ces mouvements d'eau peuvent modifier les profils d'humidité du sol dans les systèmes agroforestiers (Bayala et al. 2008) et ainsi affecter la décomposition de la MO (Armas et al. 2012). La décomposition de la MO est en effet affectée par la température (Hamdi et al. 2013), et par l'humidité du sol (Moyano et al. 2012; Moyano et al. 2013). Dans le cadre de la modélisation réalisée, nous avons considéré les mêmes profils de température et d'humidité dans les parcelles agricoles et agroforestières, faute de données pour les différencier. Il serait cependant intéressant de faire une analyse de sensibilité sur l'effet d'une réduction de la température du sol (notamment en surface) sur les prédictions des stocks de carbone du sol, tout comme une modification (augmentation ou diminution) de l'humidité du sol. Dans le cas d'une baisse de température et d'humidité du sol, on pourrait s'attendre à une moindre minéralisation de la MO en agroforesterie. Des études empiriques devraient être menées sur le terrain pour étayer ces hypothèses.

La température tamponnée dans les systèmes agroforestiers pourrait également avoir un intérêt dans l'adaptation au changement climatique. Par exemple, ces systèmes pourraient permettre de limiter l'échaudage thermique des cultures lors du remplissage des grains (Brisson et al. 2010; Moore and Lobell 2015), de limiter l'augmentation des teneurs en sucre des raisins qui entraînent des degrés alcooliques trop élevés (Mira de Orduña 2010), ou encore

d'allonger les périodes de production d'herbe dans les systèmes fourragers (Dupraz and Liagre 2008).

5. Modélisation du carbone en agroforesterie

Le travail réalisé sur la modélisation des dynamiques de carbone en agroforesterie a montré l'intérêt et la nécessité d'utiliser un modèle discrétisé en fonction de la profondeur, capable de prédire les profils de carbone du sol. Le modèle calibré sur le site de Restinclières a montré sa capacité à décrire ces stocks de carbone en fonction de la profondeur. La prochaine étape sera de valider le modèle sur les autres sites du réseau de parcelles échantillonnés au cours de cette thèse. Ce type de modèle pourrait être très utile pour des modèles de partage des ressources, comme Hi-sAFe (Talbot 2011), qui prédisent la croissance des arbres et des cultures, ainsi que des dynamiques racinaires, en fonction des ressources du milieu, mais qui ne sont pas encore capables de modéliser le carbone du sol. De façon plus simple, ce modèle peut être utilisé pour tester différents scénarios de gestion des parcelles, par exemple sur l'optimisation des rotations d'arbres. En faisant des hypothèses simples sur les vitesses de croissance des arbres (en modifiant par exemple la courbe DBH=f(t)), on pourrait tester l'impact de l'utilisation d'arbres à croissance rapide ou lente sur le carbone du sol.

7. Coût en nutriments du stockage de carbone

La décomposition des matières organiques fraîches et leur transformation en matière organiques stables du sol sont limitées par la disponibilité en nutriments du sol, notamment par l'azote (N), le phosphore (P) et le soufre (S) (Kirkby et al. 2013; Kirkby et al. 2014). Les ratios stœchiométriques C/nutriments des MO fraîches varient considérablement selon leur origine. En revanche, ces ratios sont relativement stables pour la MO humifiée du sol, quels que soient le type de sol, la position géographique ou encore la gestion du sol. Kirkby et al. (2011) ont montré que ce ratio était environ de 1000:90:19:14 (C:N:P:S). Ces ratios sont également relativement constants pour les bactéries du sol (1000:250:49:26) et pour les champignons du sol (1000:103:11:9) (Cleveland and Liptzin 2007; Kirkby et al. 2011; Richardson et al. 2014). Or, la décomposition de la matière organique dans le sol est contrôlée par des processus microbiens, et est limitée par leur besoins en nutriments. De ce fait, le priming effect du sol est faible quand les nutriments sont disponibles (Fontaine et al. 2011; de

Vries 2014), et important quand les micro-organismes du sol doivent décomposer la matière organique du sol pour subvenir à leur besoins en nutriments. De plus, la disponibilité en nutriments conditionne la production de biomasse et ainsi le retour de MO au sol. Ainsi, la disponibilité en nutriments contrôle le stockage de carbone dans les sols. Le stockage de carbone à long terme s'accompagne donc d'un stockage simultané de nutriments pour satisfaire les exigences stœchiométriques de la biomasse microbienne et de la matière organique du sol (Richardson et al. 2014).

L'efficience d'utilisation du carbone par les micro-organismes (c'est-à-dire la quantité de biomasse formée par unité de substrat consommée) est déterminante (Sinsabaugh et al. 2013). Une hypothèse couramment répandue est que les champignons ont une efficience supérieure aux bactéries (Holland and Coleman 1987), mais des travaux récents montrent que cette hypothèse n'est pas vérifiée et que peu de données existent sur ce sujet (Six et al. 2006; Thiet et al. 2006). En revanche, comme l'indiquent les ratios stœchiométriques, les champignons ont des besoins en nutriments plus faibles que les bactéries et pourraient avoir un potentiel important dans le stockage de carbone du sol, notamment dans les systèmes à bas intrants. Le ratio champignons:bactéries est fortement influencé par le travail du sol, la fertilisation, mais aussi par la qualité des matières organiques apportées. En général, un travail du sol réduit, une moindre fertilisation, et des matières organiques avec un rapport C/N élevé augmentent ce ratio (Six et al. 2006).

En agroforesterie, les arbres ont accès à des horizons de sol profond que les cultures ne peuvent pas atteindre (Mulia and Dupraz 2006; Cardinael et al. 2015c), et ont donc accès un pool de nutriments plus important. De plus, les arbres associés aux cultures intercalaires sont capables de capter une partie des nitrates lixiviés sous la culture (Bergeron et al. 2011; Tully et al. 2012). Une partie de ces nutriments prélevés en profondeur par les arbres est redistribuée en surface lors de la chute des feuilles et de la mortalité des racines. Grâce à cette valorisation des pertes du système agricole mais aussi grâce à l'accès à des ressources profondes, les systèmes agroforestiers pourrait nécessiter moins d'apports en fertilisants exogènes pour les cultures, mais aussi pour stocker du carbone. Comme le suggèrent Richardson et al. (2014), une piste intéressante serait d'étudier dans quelle mesure l'acquisition de nutriments naturellement présents dans le sol pourrait être découplée ou partiellement découplée de la minéralisation de la matière organique du sol. Par exemple, la majorité du phosphore du sol est inutilisable par les plantes, seule une infime partie de P inorganique se trouve dans la solution du sol, la majorité du P étant adsorbé sur des minéraux

ou complexé à des métaux (Gérard 2016). Les associations symbiotiques des plantes, notamment à travers les mycorhizes jouent un rôle crucial dans cette acquisition du P (Hinsinger 2001; Becquer et al. 2014). L'introduction d'arbres - plantes pérennes majoritairement mycorhizées – au sein d'une parcelle agricole pourrait permettre d'augmenter cette quantité de P inorganique dans le sol, alors disponible pour les cultures. Une autre piste de recherche intéressante concerne l'utilisation d'arbres fixateurs d'azote en agroforesterie (Munroe and Isaac 2013). Ces plantes ont la particularité d'être autotrophes en C, mais aussi en N, et pourraient permettre d'augmenter la fertilité des sols agricoles, tout en fournissant de l'azote organique nécessaire à la formation de matière organique stable. De manière générale, des études portant sur l'impact des arbres agroforestiers sur la fertilité des sols, mais aussi sur les couplages entre le cycle du carbone et le cycle des autres nutriments (P, N), s'avèrent nécessaire pour mieux comprendre les processus permettant de stocker du carbone dans ces systèmes.

Afin d'évaluer le bilan de carbone global d'une parcelle agroforestière, plusieurs éléments sont à prendre en considération, et nécessitent des recherches plus approfondies sur le sujet. La moindre utilisation d'engrais du fait de la présence des lignes d'arbre entraîne par exemple une moindre consommation d'énergie pour leur fabrication, mais également une moindre émission de N_2O dans les sols. L'utilisation d'arbres fixateurs d'azote pourrait également améliorer ce bilan. En revanche, la présence des lignes d'arbre entraîne des manœuvres plus importantes pour les tracteurs, et l'utilisation de machines supplémentaires pour l'élagage et la coupe des arbres. Des études futures pourraient établir ce bilan global, afin d'estimer le potentiel de séquestration nette en carbone d'une parcelle agroforestière comparativement à une parcelle agricole.

Conclusion générale

L'objectif principal de cette thèse était de quantifier le stockage de carbone organique du sol (COS) dans différents systèmes agroforestiers en France, comparativement à des parcelles agricoles de référence. De plus, un intérêt particulier a été porté à la compréhension des mécanismes sous-jacents permettant d'expliquer ce stockage de carbone additionnel, mais également à l'évaluation de sa stabilité.

Sur le site expérimental de Restinclières, après 18 ans d'agroforesterie, nous avons quantifié un stockage additionnel de COS jusqu'à 1 m de profondeur. Un taux moyen de stockage de COS a été estimé à 0.24 (0.09-0.46) t C ha^{-1} an^{-1} sur 0-30 cm sur un ensemble de cinq parcelles agrosylvicoles en France. Notre hypothèse concernant un stockage de COS accru en agroforesterie est donc validée. La majorité du carbone additionnel est fait de matières organiques particulaires (MOP), c'est-à-dire de débris végétaux en cours de décomposition, de la taille des sables (50-200 µm et 200-2000 µm), et est majoritairement localisé dans les horizons de surface. La destruction des macro-agrégats des sols incubés n'a entraîné qu'une très faible sur-minéralisation, indiquant que ces MOP ne sont pas protégées au sein de ces agrégats. Tous ces éléments semblent indiquer un stockage de carbone très vulnérable à un futur changement d'usage des sols. L'étude des formes de stockage de COS devrait être étendue aux autres sites agroforestiers étudiés, afin de pouvoir généraliser et le cas échéant, étayer des recommandations relatives à l'utilisation de l'agroforesterie pour stocker du C dans les sols dans une perspective d'atténuation de l'effet de serre.

Aucun effet de la distance à l'arbre sur les stocks de COS n'a été mis en évidence dans l'inter-rang. Les mesures des entrées des matières organiques (MO) au sol ainsi que la modélisation ont permis de montrer que cette absence de gradient était expliqué par les importantes biomasses de racines fines des arbres à proximité des lignes qui permettaient de compenser les moindres apports de MO dus à la diminution du rendement près des lignes. En revanche, les stocks de COS les plus importants ont été mesurés sur les lignes d'arbres, qui sont aussi occupées par une végétation herbacée abondante, semée ou naturelle, et où le sol n'est pas travaillé. Cette végétation peut être assimilée à une prairie permanente en bandes, et a un rôle déterminant dans le stockage de COS dans les parcelles agroforestières, notamment dans les jeunes plantations où les arbres n'ont encore aucun effet sur le COS. Notre étude sur trop peu de parcelles n'a pas permis de mettre en évidence une différence entre les couverts de ces

lignes semés ou naturels en termes de stockage de COS. La gestion des lignes d'arbres semble être un moyen d'amélioration du stockage de carbone dans les parcelles agroforestières. Des études supplémentaires devraient permettre par exemple de trouver des couverts ayant un impact positif sur le stockage de COS, mais également sur la biodiversité ou encore le contrôle des ravageurs des cultures.

Nous avons estimé que le sol de la parcelle agroforestière recevait environ 40% de matière organique en plus que la parcelle agricole (3.80 t C ha^{-1} an^{-1} par rapport à 2.69 t C ha^{-1} an^{-1}) sur une profondeur de 2 m. Les racines fines des arbres et les racines des cultures représentent chacun environ 30% des apports de MO en agroforesterie. Ce travail confirme d'autres études quant au le rôle crucial des racines dans les apports de MO au sol. En revanche, les incubations de sols ont montré que les taux de minéralisation potentiels du COS étaient identiques dans la parcelle agricole et dans l'inter-rang de l'agroforesterie. Les sorties issues de la modélisation suggèrent que le stockage de carbone en agroforesterie est le résultat d'un apport accru de MO au sol plutôt que d'une diminution de la minéralisation du COS, ce qui valide notre hypothèse initiale. Le modèle de dynamique de carbone SOCRATES que nous avons développé s'est avéré capable de bien représenter les profils de stocks de carbone en agroforesterie sur le site de Restinclières. Des travaux ultérieurs valideront le modèle sur les autres sites agroforestiers étudiés. Avec des hypothèses simples, ce modèle pourra notamment être utilisé pour tester l'impact sur les stocks de COS de différents scénarios de gestion des rotations des arbres.

De manière générale, notre étude démontre l'intérêt et le potentiel des systèmes agroforestiers pour augmenter les stocks de COS des sols agricoles. Cependant, le stockage de carbone étant limité par la disponibilité en nutriments du sol, d'autres recherches approfondies devraient être menées sur le couplage des cycles du carbone, de l'azote et du phosphore dans les systèmes agroforestiers, par exemple en s'intéressant au rôle potentiel des arbres fixateurs d'azote.

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Liste des articles publiés durant la thèse

➢ Articles liés à la thèse:

Cardinael R, Chevallier T, Barthès BG, Saby NPA, Parent T, Dupraz C, Bernoux M, Chenu C, 2015. Impact of alley cropping agroforestry on stocks, forms and spatial distribution of soil organic carbon – A case study in a Mediterranean context. *Geoderma* 259-260:288-299. doi:10.1016/j.geoderma.2015.06.015

Cardinael R, Mao Z, Prieto I, Stokes A, Dupraz C, Kim JH, Jourdan C, 2015. Competition with winter crops induces deeper rooting of walnut trees in a Mediterranean alley cropping agroforestry system. *Plant and Soil* 391:219-235. doi:10.1007/s11104-015-2422-8

Chevallier T, **Cardinael R**, Béral C, Chenu C, Bernoux M, 2015. L'agroforesterie permet-elle de concilier production agricole et atténuation du changement climatique ? *Forêt-entreprise* 225:49-54.

Germon A*, **Cardinael R***, Dupraz C, Prieto I, Mao Z, Kim JH, Stokes A, Laclau J-P, Jourdan C, 2016. Unexpected phenology and lifespan of shallow and deep fine roots of walnut trees grown in a silvoarable Mediterranean agroforestry system. *Plant and Soil* (in press). doi:10.1007/s11104-015-2753-5.**These authors contributed equally to this work*

Cambou A, **Cardinael R**, Kouakoua E, Villeneuve M, Durand C, Barthès BG, 2016. Prediction of soil organic carbon stock using visible and near infrared reflectance spectroscopy (VNIRS) in the field. *Geoderma* 261:151-159. doi:10.1016/j.geoderma.2015.07.007

➢ Autres articles:

Cardinael R, Eglin T, Guenet B, Neill C, Houot S, Chenu C, 2015. Is priming effect a significant process for long-term SOC dynamics? Analysis of a 52-years old experiment. *Biogeochemistry* 123:203-219. doi:10.1007/s10533-014-0063-2

Prieto I, Roumet C, **Cardinael R**, Kim JH, Maeght, J-L, Mao Z, Portillo N, Thammahacksa C, Dupraz C, Jourdan C, Pierret A, Roupsard O, Stokes A, 2015. Root functional parameters along a land-use gradient: evidence of a community-level economics spectrum. *Journal of Ecology* 103:361-373. doi:10.1111/1365-2745.12351

Titre : Stockage de carbone et dynamique des matières organiques des sols en agroforesterie sous climat méditerranéen et tempéré

Mots clés : agroforesterie, carbone organique du sol, matières organiques, stockage de carbone

Résumé : L'agroforesterie est un mode d'occupation des sols qui associe des arbres et des cultures et/ou des animaux au sein d'une même parcelle. Ce système agricole pourrait jouer un rôle dans l'atténuation mais aussi dans l'adaptation au changement climatique. Le but de cette thèse est d'évaluer le potentiel de stockage de carbone organique dans les sols sous agroforesterie.
Cette étude a été réalisée sur le plus ancien site expérimental en France, suivi par l'INRA depuis 1995, mais aussi chez des agriculteurs. La quantification des stocks de carbone a été réalisée par comparaison entre des parcelles agroforestières et agricoles dont la culture annuelle est gérée de façon identique, jusqu'à 2 m de profondeur. Toutes les entrées de matières organiques au sol ont été quantifiées (racines d'arbres, feuilles, résidus et racines de la culture). La stabilité de carbone additionnel stocké a été caractérisée par du fractionnement de la matière organique, et par des incubations de sol. Un modèle de dynamique du carbone a été réalisé afin de formaliser cette dynamique en agroforesterie, notamment en profondeur.
Cette étude démontre l'intérêt et le potentiel des systèmes agroforestiers dans l'augmentation des stocks de carbone du sol, avec des taux de stockage allant de 0.09 à 0.46 t C ha^{-1} an^{-1}. Elle révèle également le rôle des lignes d'arbres dans ce stockage, et l'importance des entrées de carbone par mortalité racinaire. Elle pose cependant la question de la stabilité de ce stockage.

Title : Carbon storage and soil organic matter dynamics under Mediterranean and temperate agroforestry systems

Keywords : agroforestry, soil organic carbon, organic matter, carbon storage

Abstract: Agroforestry is a land use type where trees are associated with crops and/or animals within the same field. This agroecosystem could help mitigating climate change, and also contribute to its adaptation. The goal of this thesis was to evaluate the potential of soil organic carbon storage under agroforestry systems.
This study was performed at the oldest experimental site in France, a trial supervised by INRA since 1995, but also at farmers' fields. Soil organic carbon stocks were compared between agroforestry and agricultural plots, down to 2 m soil depth. All organic inputs to the soil were quantified (tree roots, leaf litter, crop roots and residues). The stability of addtionnal stored carbon was caracterised with soil organic matter fractionation, and soil incubations. A model of soil organic carbon dynamic was described in order to better understand this dynamic in agroforestry, especially in deep soil layers.
This study revealed the interest and the potential of agroforestry systems in increasing soil organic carbon stocks, with accumulation rates of 0.09 to 0.46 t C ha^{-1} yr^{-1}. It also reveals the role of tree rows in this storage, and the importance of carbon inputs from root mortality. However, it raises concerns about the stability of this storage.

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