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

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Abstract

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

Keywords

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

1. Introduction

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

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

In addition to specific morphological characteristics, the presence of crops in alleys may contribute to changing the tree fine root distribution along the soil profile (Cardinael et al., 2015b) and during different seasons (Huo et al., 2020) in AC. In shallow soils, as it is impossible for trees to go deeper, they inevitably develop root systems located in the same soil layer as the crop. In the presence of a fluctuating water table, the soil water saturation

may change the spatial tree roots distribution due to a lack of oxygen and an increase in carbon dioxide and ethylene concentrations (Armstrong et al., 1994). Climatic parameters such as wind also influence tree root development. In response to frequent movements of the aerial parts by the wind, the tree root growth is stimulated to gain a better anchorage to the ground (Coutts et al., 1999; Stokes et al., 1995; Tamasi et al., 2005). Finally, tree fine roots distribution can be modulated by active management practices of crops and trees. Successive rotation of winter crops in alleys during the first years of trees establishment would reduce the colonization of upper soil layer by tree roots. At tree bud burst, as the topsoil is already explored by crops, tree roots are less competitive in this layer for water and nutrient uptake (Dupraz and Liagre, 2008). A deep rooting is then established by trees, in addition to those already developed during winter (Cardinael et al., 2015a). Other studies have shown that tree root pruning close to the rows promotes the tree rooting under the crop roots zone (Gilman and Yeager, 1988). Ploughing before crops sowing may limit tree root development in the upper soil layer and thus protect crops for a while from root competition with trees (Korwar and Radder, 1994; Schroth, 1995). Besides, autumn ploughing destroys shallow roots but not deep roots, which have developed during the winter and continue to grow over time.
Similarly, periodical agronomic disking minimizes competition and maximizes niche separation (Jose et al., 2004). Conversely, irrigation and fertilization promote superficial root growth because they make the topsoil more attractive and stimulate the formation of tree shallow root systems (Bakker et al., 2008; Coleman, 2007; Gilman, 1990b). Finally, tree pruning was also shown to increase the depth at which trees in AC acquire nutrients (Rowe et al., 2006).
Lateral and vertical tree roots development and turnover in AC may provide an additional source of organic matter and may contribute to carbon sequestration in soil. Cardinael et al. (2017) showed higher soil organic carbon (SOC) contents in AC with ages varying from 6 to

41 years after tree planting by comparison with control crop plot. In their young stands, high SOC was observed along tree rows at 30 cm depth, possibly due to the presence of understory vegetation strips. They also observed a high amount of SOC below 30 cm depth in two 18-year-old silvoarable plots and explained it by a high density of tree fine roots measured at this depth. In most cases, the additional SOC observed in AC plot was measured at 0-30 cm depth (Cardinael et al., 2018, 2015a; Pardon et al., 2017; Peichl et al., 2006; Upson and Burgess, 2013).

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

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

2. Materials and methods

2.1. Study territory description

The study was carried out in Hauts-de-France region in northern France (Appendix 1A). The climate is oceanic with an average (between 2010 and 2020) annual temperature and rainfall of 11.5 °C and 726 mm respectively at Lille-Lesquin station (https://www.infoclimat.fr/climatologie/globale/lille-lesquin/07015.html) and a maximum elevation of 295 m. The soil type throughout the region is dominated by cambisol, punctuated with luvisol and calcaric calcisol in some areas (Appendix 1A; FAO, 2015). The soil texture is mainly silt loam but zones with sand or clay loam with flint and limestone are also noted. The region is strongly dominated by agriculture with 2 131 503 ha of usable agricultural area, *i.e.* 67 % of the territory, including 57 % of arable land, and 26 093 farms (Agreste Hauts-de-France, 2020). Afforestation represents only 16 % of the territory. At the national scale, the region is the leading producer of wheat, sugar beet, potatoes, peas, carrots and witloof chicory. In this territory, agroforestry systems are mostly silvopastoral with apple orchards for poultry, cows or sheep, riparian wood land, shelterbelts, hedgerows or alley-cropping (Nair, 1985). Alley-cropping systems are rarely developed due to farmers' fears that trees within plot will be incompatible with the agro-pedoclimatic conditions (Andrianarisoa and Delbende, 2016).

2.2. Description of studied plots

Lists from the regional council and from the chamber of agriculture were used to identify and contact agroforestry farmers in the Hauts-de-France region. The selected farmers had alley-cropping plots between 3 and 12 years old mostly with deep (> 2 m depth) loamy soil (Appendix 1B). Twenty-six farmers were selected, corresponding to these criteria, and were contacted to participate in the study. Only 11 farmers with a total of 17 plots (Table 1 and Appendix 1A) positively responded. These plots were divided into 4 age groups to create a chronosequence of AC stands: <4 years old (n=4), [4-6] years old (n=5), [7-9] years old (n=4) and [10-12] years old (n=4). The average of tree density, alley width and distance between trees within rows for all plots were 104 ± 57 trees ha^{-1}, 47 ± 41 m and 3.6 ± 2.2 m respectively (Table 1). For the whole farm, the type of farming was partly or exclusively arable land (Kempen et al., 2011) and the cultivated crops were mainly wheat, barley, rapeseed, corn, beet, potato. In all plots, the mean tillage frequency was 0.4 ± 0.3 y^{-1} (*i.e.* twice every 5 years), the mean crop rotation duration was 4 years and the fertilization was mainly carried out with synthetic fertilizers (Table 1). The soil texture of selected plots was mainly silt loam in layer 1 (0-30 cm) and layer 2 (30-100 cm) and clay loam or silt clay loam in layer 3 (> 100 cm) (Appendix 1B). The average soil pH and soil organic matter was 7.8 ± 0.4 and 21.6 ± 3.01 g kg^{-1}, respectively in layer 1. Tree rows were composed of local tall standard mixed species within which spontaneous or sowed herbaceous vegetation (hereafter referred to as "understory vegetation strips") grew on 1 m width on average on both sides. Weeds in alley were controlled chemically on the cropping area in plots from conventional farms and were scarce in plots from organic and sustainable farms. For all farmers, the understory vegetation strips were mowed, but the frequency of the cut varied according to weeds development. All information about the chosen alley-cropping plot (trees and crops):

plot design, crop rotation, tillage, tree pruning, kind of N fertilization and irrigation were collected (Table 1).

2.3. Fine roots measurement

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

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

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

2.4. Soil sampling and analyses

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

2.5. Statistical analyses

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

Equation 1: $y = ax_1 + bx_2 + c + \beta$

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

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

Equation 2:

For $l \in [1, 3]$ and for $d \in [0, 3] \not\subset 2$: $sTFRA_{l,d} = \Sigma_{s=1}^{n} TFRA_{s}$

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

Equation 3

For $l \in [1, 3]$: $sTFRA_{l} = \Sigma_{d=1}^{3} \Sigma_{s=1}^{n} TFRA_{d,s}$

Equation 4:

For $d \in [0, 3] \not\subset 2$: $sTFRA_{d} = \Sigma_{l=1}^{3} \Sigma_{s=1}^{n} TFRA_{l,s}$

Equation 5:

$sTFRA_{t} = \Sigma_{d=0}^{3} \Sigma_{l=1}^{3} \Sigma_{s=1}^{n} TFRA_{d,l,s}$

In the results section, $sTFRA_{l,d}$, $sTFRA_{l}$, $sTFRA_{d}$ and $sTFRA_{t}$ were all called sTFRA but the concerned soil layer or distance is always specified. For sTFRA calculation, data at 10 m distance from rows were also excluded because no tree fine roots were observed. Simple regression analyses were performed between sTFRA and crop management or soil variables. The Spearman's rank correlation rho or the adjusted R-squared were calculated for quantitative or qualitative variable respectively. Stepwise regression models were also tested to select the variables allowing a better explanation of the variability of each sTFRA. The selected models are those with the first three significant explanatory variables but not those with the best AIC to limit the number of explanatory variables. The *p*-value of each model and the sign of coefficient retained for each explanatory variable are presented in Table 3. For statistical analyses of SWC and SMN, only data collected before September 21th, 2020 was used because after this date, the soil was moistened by heavy and almost permanent rainfall. Multivariate regressions were used to explain SWC, SOM and SMN with site and distance from tree rows or soil layer as explanatory variables in the same way as the TFRA in equation 1. Regression analyses were followed by Tukey's tests for post hoc pairwise comparisons. One-way ANOVAs were performed to analyze the variability of SWC, SOM and SMN with stand age group as explanatory variable. The reduction of analyzed data due to samples collected after September rainfall limited the validity of some ANOVA analysis for SWC and SMN due to insufficient number of levels for some modalities (eg: stand age group, soil layer). Correlation analysis was performed to highlight the relationship between SWC, SOM and SMN and other soil and plant parameters including TFRA. Stepwise analysis was run to select the 2 or 3 best variables explaining the variability of SWC and SOM. Before running models, collinearity analysis was performed between quantitative variables using principal component analysis (PCA). Autocorrelated variables were graphically diagnosed and removed for stepwise regression analyses. For all regressions, a symbol *** was used to indicate a significant determination coefficient at $p < 0.001$ level, a symbol ** for $p < 0.01$ level and a symbol * for $p < 0.05$ level. The variable age of the plantation was used either as its numerical values (referred to as: "stand age") or as a group of stand age (referred to as:

"stand age group"). In result section, all mean values for a given variable are followed by the standard deviation.

All statistical analyses were performed with R software version 4.0.4.

3. Results

3.1. Spatial fine roots distribution

The stand age group, the soil layer and the distance from tree rows accounted for 44 % of the tree fine roots abundance variability along the chronosequence of our AC stands ($p < 0,05$). Tree species accounted for only 4 %. During the first four years of plantation, trees developed abundant fine roots at 0-30 cm depth along tree rows and at 1 m distance (Figure 2A and 3). However, at 3 m distance we also found more tree fine roots in topsoil than in L2 and L3 (Figure 2A and 3A). From 4-6 years, trees significantly expanded deep fine roots (L3) in tree rows (Figure 2B, 3B, 3E and 3F) compared to other distances, evidencing a vertical exploration of soil profile. At the same time, the amount of tree fine roots in topsoil decreased at 1 m distance compared to young stands (Figure 3B and 3G). From 7-9 years, trees continued to develop vertical deep fine roots on rows, but they started to colonize deep soil layers at 1 m distance (Figure 2C and 3G), demonstrating both vertical and lateral fine roots expansion. No significant difference of TFRA was observed between stand age in deep soil layer at 3 m distance (Figure 3G). Beyond 9 years old, we observed horizontal tree fine roots distribution at 1 m distance in layers 2 and 3 (Figure 2D and 3G). In topsoil, we rediscovered high TFRA although they were rare between 4 and 9 years old as mentioned above.

Regardless the age of the plantation, no tree fine roots were observed at 10 m from the rows.

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

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

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

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

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

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

3.3. Relationship between root distribution and soil water content

Soil water content increased with depth in all stands ($R^2 = 0.29$***) with an average of 11 ±3 %, 13 ±3 % and 17 ±4 % in layers 1, 2 and 3, respectively. Multivariate regression analysis showed that the stand age group, the distance from tree row and the soil depth explained 49 % of the variation of soil water content. In young stands, soil moisture was significantly higher in deep soil layer (L3) compared to topsoil (L1) (Figure 4A and 4F) regardless of the distance from the tree row. Soil water content significantly varied with the distance from the tree row in topsoil, whereas no effect of distance was observed in L2 and L3 (Figure 4E).
In 4-6-year-old stands, SWC also increased with soil depth regardless of distance from tree row. It varied significantly with distance in topsoil whereas no effect was observed in layer 2. In deep soil layer (L3), the soil water content was lower in tree rows compared to other distances (Figure 4B, 4E and 4F). In 7-9-year-old stands, soil water content was significantly higher in layer 3, but the two layers above were not significantly different (Figure 4C and 4F). Finally, for old stands, SWC also increased significantly with depth (Figure 4F) and with distance from tree row regardless of soil layer (Figure 4E). Stepwise regression showed that the soil organic matter, clay and limestone content explained 22 % ($p < 0.001$) of the SWC variability.
Using a bivariate linear regression, our results showed that the site and the TFRA explained 40 % ($p < 0.001$) of the soil water content variability. The same model's determination

coefficient was 0.65, 0.54 and 0.9 in topsoil, layer 2 and layer 3, respectively ($p < 0.001$). When the regression was run per distance from tree rows, the determination coefficient was highest at 1 m ($R^2 = 0.51$***), intermediate in tree rows ($R^2 = 0.36$***) and lowest at 3 m distance ($R^2 = 0.30$***). Finally, the determination coefficient was highest in the oldest stands ($R^2 = 0.50$***), intermediate in the youngest ($R^2 = 0.20$***), lowest in 4-6 years old stands ($R^2 = 0.09$*) and was not significant for 7-9-year-old stands.

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

3.4. Relationship between root distribution and soil organic matter content

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

3.5. Relationship between root distribution and soil mineral nitrogen content

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

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

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

4. Discussion

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

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

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

Despite our caution and rigor on tree fine roots recognition, it may be possible that we also counted some weed fine roots particularly in the tree rows and at 1 m distance as mentioned by Battie-Laclau et al. (2020). Very few weeds were noted in the alleys thanks to chemical or physical control by farmers. In the rows, the mechanical maintenance for young stands or trees development for old stands limited weeds growth thus only few were noticed, and their roots were easily recognizable by their color and softness. Finally, despite the different roots development patterns of our studied tree species in forest ecosystems (taproot system for walnut (Borden et al., 2017), fasciculate roots for maple (Köstler et al., 1968) and shallow horizontal roots for hornbeam (Abdi et al., 2009)), we observed that the association with crops modifies the tree fine roots distribution from their early age and the tree species explained only 4 % of the variability of the tree fine roots abundance. This demonstrated the fine root plasticity of these species shaped by agricultural work and/or crop interaction and may contribute to limiting nutrient loss outside the crop rooting zone (Bergeron et al., 2011). Our results also showed that no tree roots reached distances farther than 10 m from the rows. As some of our old stands were up to 10 m height, we expected that tree fine roots could potentially explore an equivalent distance to their height (Danjon et al., 2020). Based on the Mulia and Dupraz (2006) study with 10 years old trees, poplar roots were found beyond 8 m from the tree row, whereas no walnut roots were found beyond 3 m from the tree row. A species and/or soil effect would explain the absence of tree roots at 10 m from the row in cropping area.

4.2. Tree fine roots distribution and soil water content.

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

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

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

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

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

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

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

4.4. Tree fine roots distribution and soil mineral N content

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

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

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

Conclusion

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

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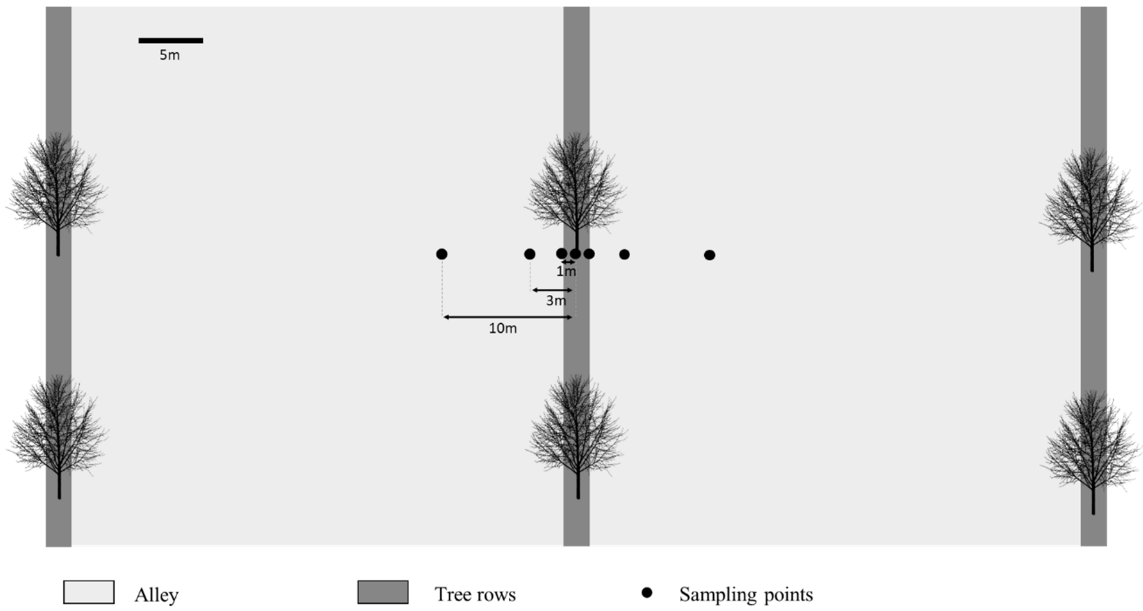


Figure 1: Description of soil cores sampling within each site.

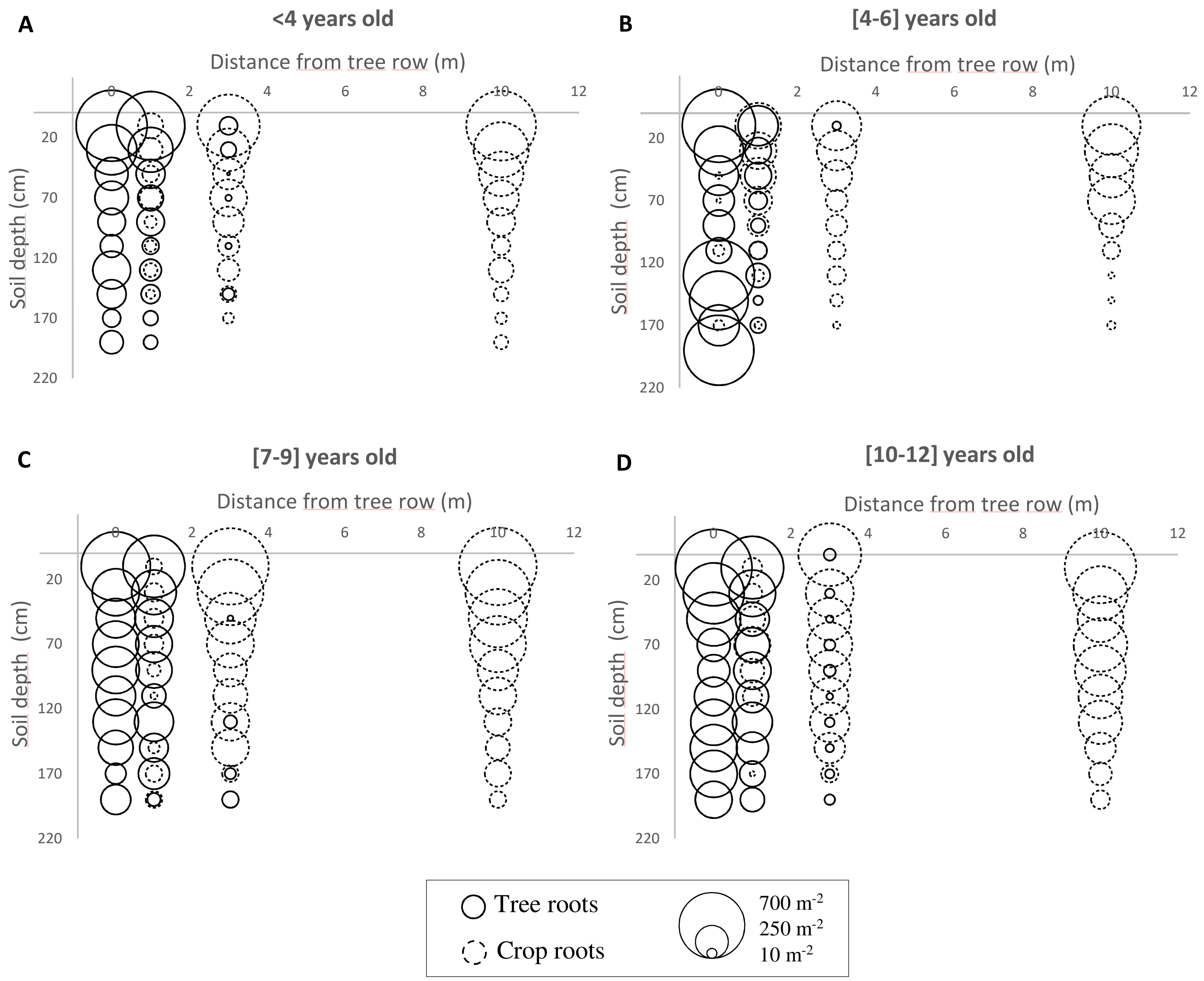
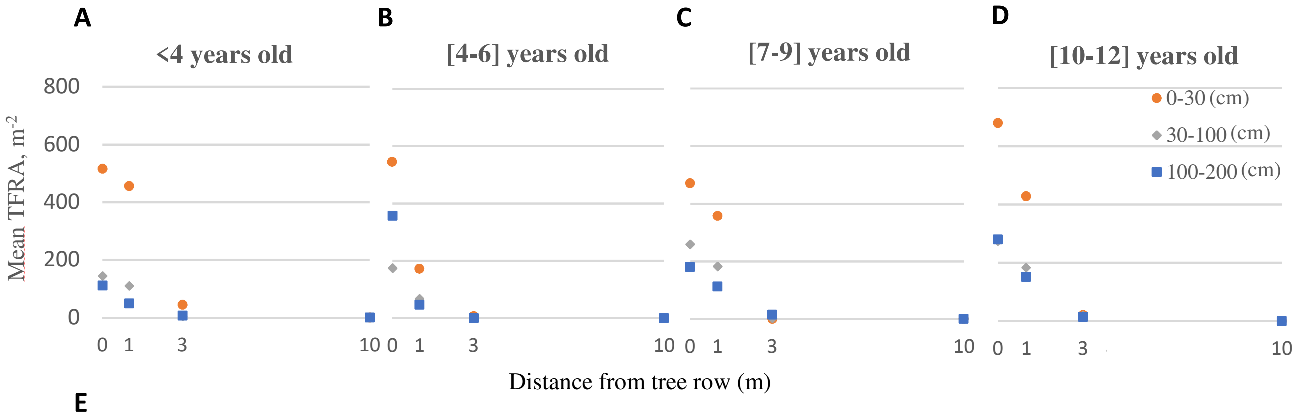


Figure 2: Lateral and vertical distribution of tree fine roots abundance (m^{-2}) down to 2 m depth at different distances from tree rows along a chronosequence of young agroforestry plots. Continuous and dotted circles represent tree and crop roots respectively. The size of circles is proportional to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



E

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

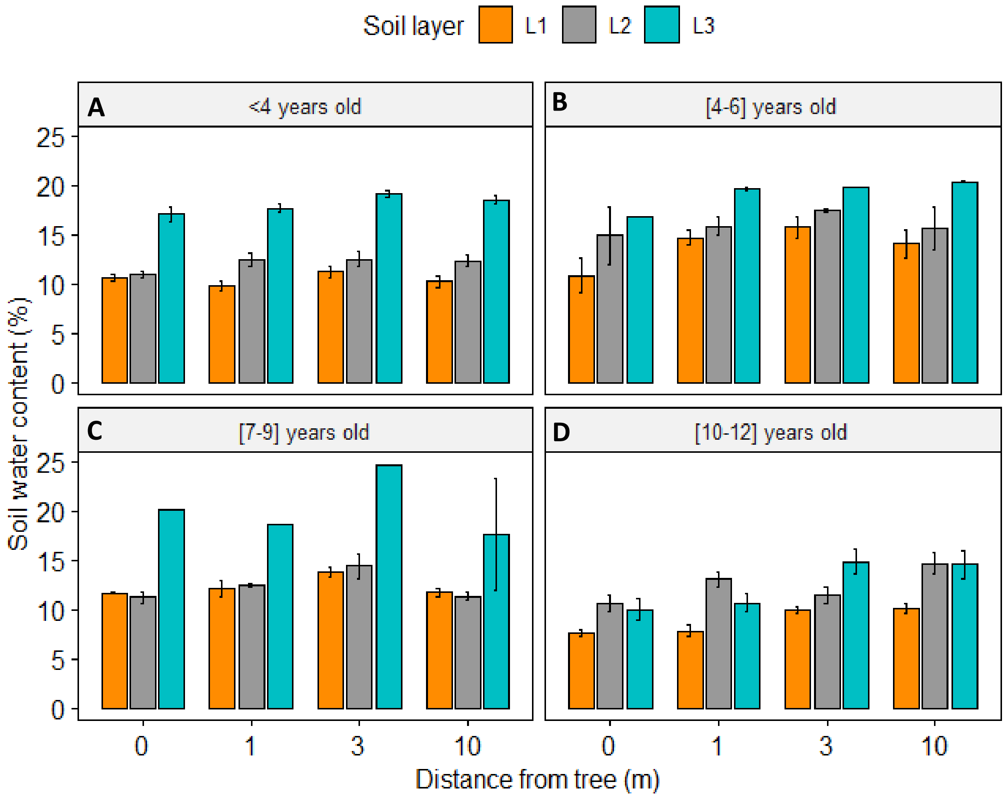
F

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

G

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

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



E

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

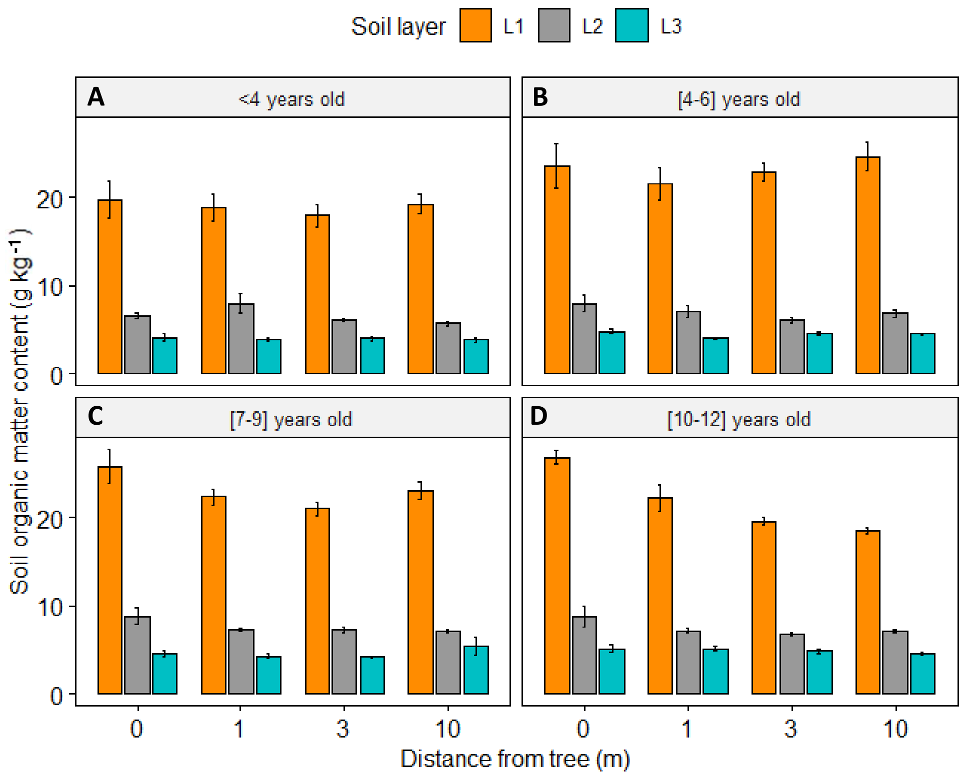
F

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

G

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

Figure 4: Variation of the soil water content (%) at 0, 1, 3 and 10 m distance from tree rows and in different soil layers along a chronosequence of agroforestry stands (A, B, C, D) and results of Tukey's test ($p < 0.05$) to compare (E) distances from tree rows for a given soil layer and a given stand age; (F) soil layer for a given distance and a given stand age; (G) stand age for a given distance from tree row and a given soil layer. Histograms are means and bars are standard errors. Letters indicate homogeneous groups: means with same letters are not significantly different. "nd" means no determined, "nv" means not validated. Vertical or horizontal arrows show the direction of reading for statistical means comparisons. Bold letters are used to facilitate table reading.



E

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

F

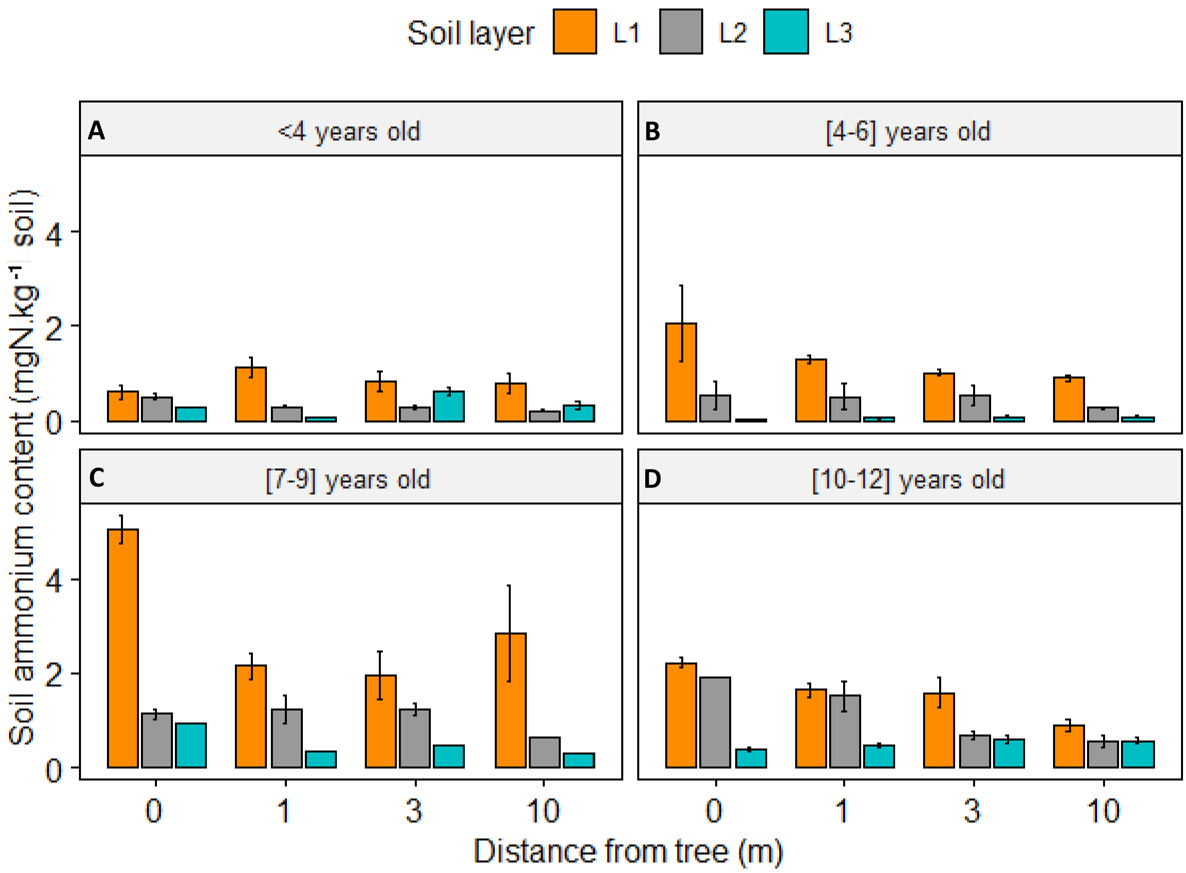
Soil layer (cm)	<4 years				4-6 years				7-9 years				10-12 years			
	Distance from tree row (m)															
	0 ↓	1↓	3 ↓	10↓	0	1	3	10	0	1	3	10	0	1	3	10
0-30	B	C	B	C	B	B	B	nv	C	C	C	nv	C	B	C	C
30-100	A	B	A	B	A	A	A	nv	B	B	B	nv	B	A	B	B
100-200	A	A	A	A	A	A	A	nv	A	A	A	nv	A	A	A	A

G

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

Figure 5: Variation of the soil organic matter content (g kg^{-1}) at 0, 1, 3 and 10 m distance from tree rows and in different soil layers along a chronosequence of agroforestry stands (A, B, C,

D) and results of Tukey's test ($p < 0.05$) to compare (E) distances from tree rows for a given soil layer and a given stand age; (F) soil layer for a given distance and a given stand age; (G) stand age for a given distance from tree row and a given soil layer. Histograms are means and bars are standard errors. Letters indicate homogeneous groups: means with same letters are not significantly different. "nv" means not validated. Vertical or horizontal arrows show the direction of reading for statistical means comparisons. Bold letters are used to facilitate table reading.



E

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

F

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

G

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

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

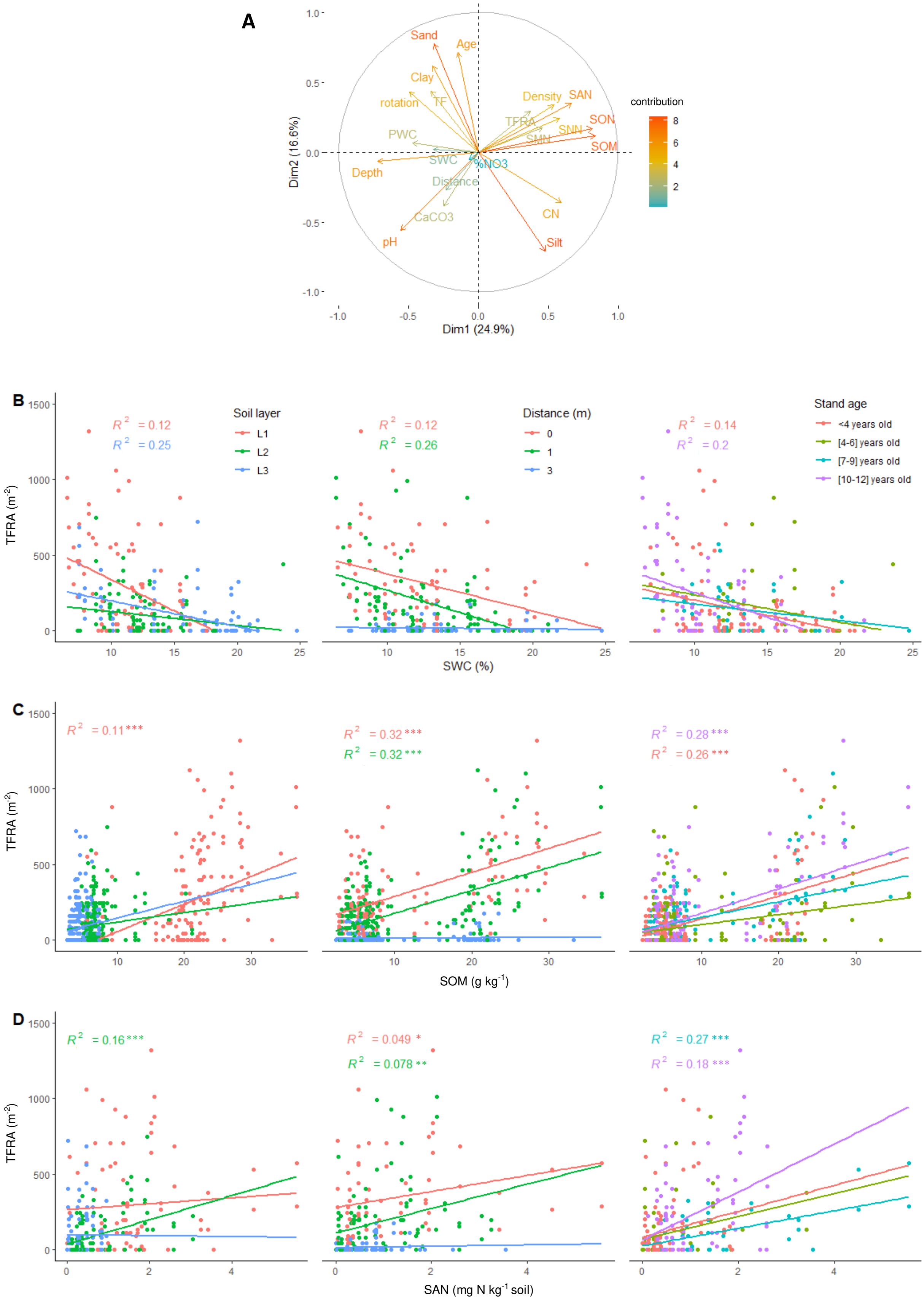


Figure 7: Correlation analysis between main studied variables: principal component analysis (A), simple linear regression between tree fine roots abundance (TFRA) and soil water content or SWC (%) (B), soil organic matter or SOM (g kg^{-1}) and (C) and soil ammonium content or

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

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

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

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

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

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

[2]Tree row/alley width indicates the distance between trees intra and inter rows respectively. For instance, 3/35 means: the distance between two trees within each row is equal to 3 m and the distance between two tree rows is equal to 35 m
[3]Crop species meaning: B: barley, beet: sugar beet, buck.: buckwheat, C: corn, einkorn: einkorn wheat, F: faba bean, lent.: lentils, P: potato, rap.: rapeseed, temp. meadow: temporary meadow, W: wheat
[4]Percentage of winter crop in rotation: for instance W, rap., W, beet = 3 winter crops on the rotation mean ¾=75%
[5]tree species studied on the site from which the distance of soil cores position was calculated
[6]Crop management meaning: Conv.: conventional, Org.: organic, RT: reduced tillage, Sus.: sustainable
[7]Temp. and Precip. mean: temperature and precipitation
[8]Fertilization meaning: M: manure, S: synthetic fertilizer
[9]According to EU classification (Kempen et al., 2011)

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

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

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

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