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.

Keywords: Tree-based intercropping system, Soil mapping, Soil organic carbon storage, Soil organic carbon saturation, Deep soil organic carbon stocks, Visible and near infrared spectroscopy, Particle-size fractionation

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; Stavi and Lal, 2013; Verchot et al., 2007). 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 (Bauhus et al., 2010; Profft et al., 2009). 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 (Cardinael et al., 2015; Mulia and Dupraz, 2006). 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 (Chenu and Plante, 2006; 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 (Nair et al., 2010; Peichl et al., 2006; 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 of less than 0.5 m (Bambrick et al., 2010; Oelbermann and Voroney, 2007; Oelbermann et al., 2004; Peichl et al., 2006; Sharrow and Ismail, 2004). 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., 2011). 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

98 Oliver, 2007) have never been used to describe the spatial distribution of SOC stocks in alley
99 cropping agroforestry system although they have been recognized to be very powerful to map
100 and understand spatial heterogeneity at the plot scale (Philippot et al., 2009) especially when
101 dealing with more diverse and heterogeneous systems.

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

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

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

2. Materials and methods

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 14.5°C and an average annual rainfall of 951 mm (years 1996–2003). 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. 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 1. Hybrid walnut-durum wheat agroforestry system. Left panel: in November 2013; Right panel: in June 2014.

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. 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. 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. 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. 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.

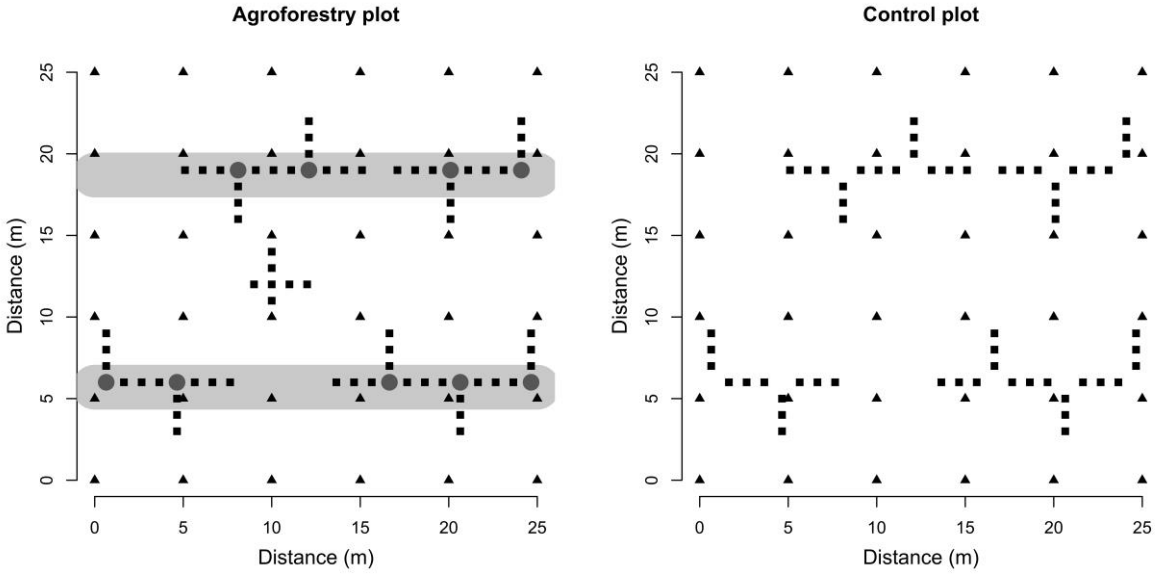

Figure 2. Description of the intensive sampling scheme in the agroforestry and in the control sub-plots. Circles represent hybrid walnuts, the grey strips represents the tree rows, triangles are for soil cores on the regular grid (every 5 m), squares are for soil cores on transects (every 1 m).

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 sub-cores 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 < 2 mm (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.

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 25^{th} percentile and Q3 is the 75^{th} 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.

Table 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).

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

Subsoil models performed better than topsoil models (Table 1, Fig. 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.

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.

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 (δ^{13}C OM = -25 ‰).

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 (Table 2). The effect of the ESM correction can be seen in Table 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):

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

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

304 removed by siphoning the upper 10 cm of the suspension left after the first sampling. This
305 represented an aliquot of the 0-2 µm fraction. A third aliquot was also collected in the upper
306 10 cm, and centrifuged two times 35 min, at 4000 rpm. This aliquot was then filtered at 2 µm
307 to get the hydrosoluble fraction. Fractions were then dried at 40°C, finely ground,
308 decarbonated and analyzed with a CHN elemental analyzer. A binocular microscope was used
309 to check if separation of coarse mineral fractions and of light organic coarse fractions (200-
310 2000 and 50-200 µm) was well done. No pyrogenic particles were observed. Organic carbon
311 contents of coarse mineral fractions were then assumed to be 0 mg C g^{-1}. A sub-sample of
312 each of the 64 selected samples was used to perform a classical textural analysis after
313 destruction of organic matter. These texture analyses were used to evaluate the quality of the
314 dispersion for soil particle size fractionation.

315

316 2.9. Calculation of SOC saturation

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

319 $$SOC_{sat-pot} = 4.09 + 0.37 \times \text{particles} < 20\ \mu\text{m} \quad (4)$$

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

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

325 $$SOC_{sat-def} = SOC_{sat-pot} - SOC_{cur} \quad (5)$$

where $SOC_{sat\text{-}def}$ 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 ($SOC_{stor\text{-}pot}$, Mg C ha^{-1}) was calculated multiplying $SOC_{sat\text{-}def}$ 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). But as the equation proposed by (Hassink, 1997) was calibrated for topsoil layers, calculations for deep soil layers are only indicative.

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, $\boldsymbol{s}_i = \{\boldsymbol{s}_1, \boldsymbol{s}_2, \cdots, \boldsymbol{s}_q\}$ 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 (Lark et

al., 2006; Pinheiro and Bates, 2000). 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 $\boldsymbol{\Theta}$, 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.

Table 2. 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.

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

3. Results

3.1. Changes in soil texture with depth

Clay, silt and sand profiles were very similar at both plots (Fig. 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}.

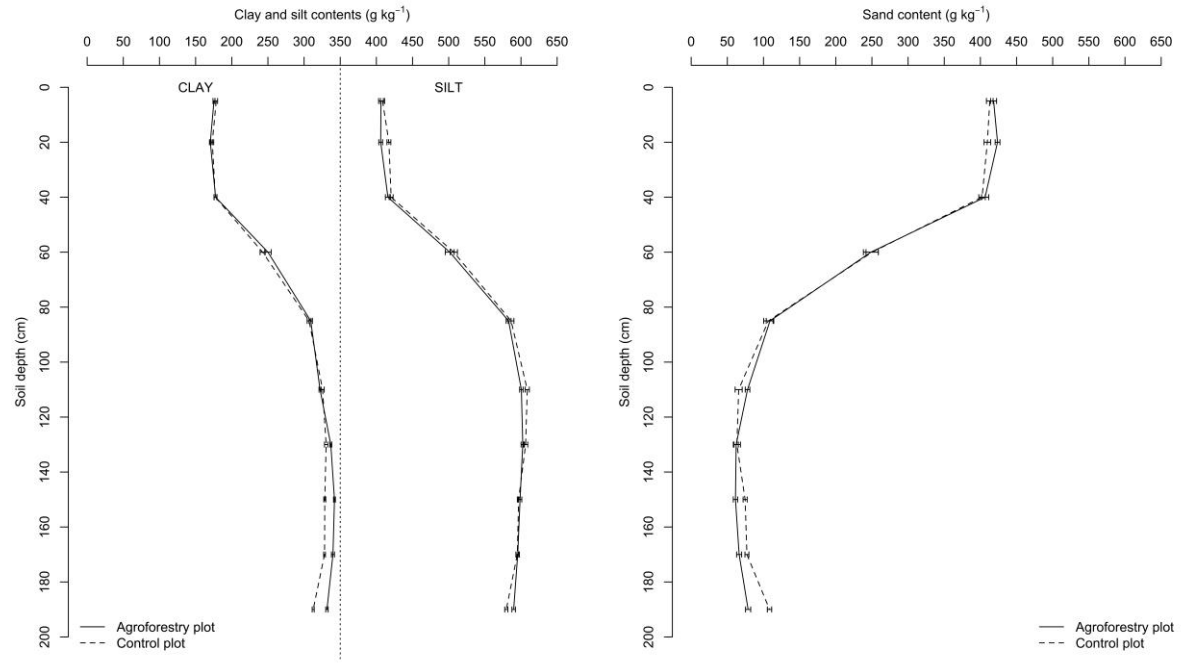

Figure 3. Changes in soil texture with depth in the control plot and in the agroforestry plot. Error bars represent standard errors (n=100 in the agroforestry, n=93 in the control).

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 3). 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.

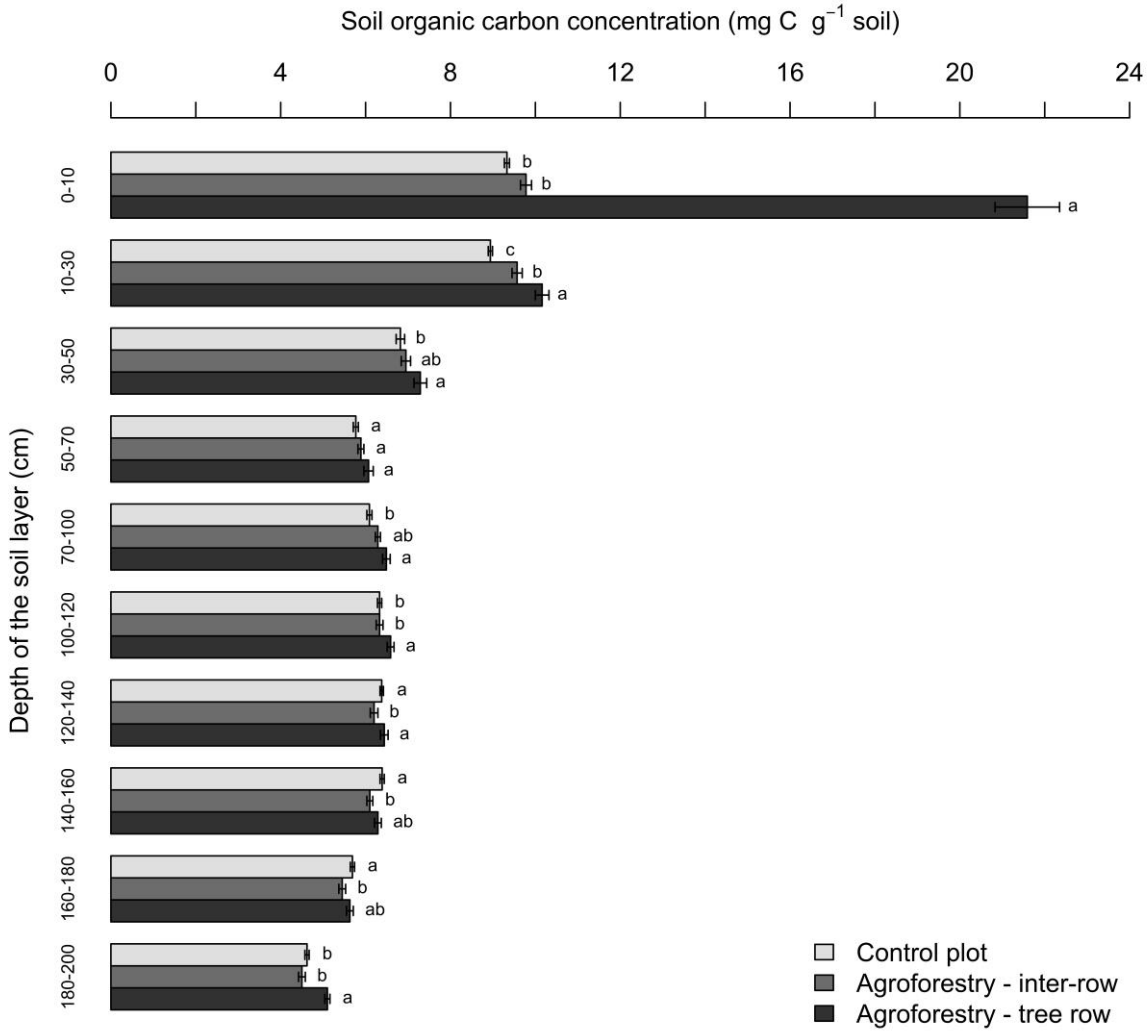

Figure 4. Soil organic carbon concentration (mg C g^{-1} soil) of soil layers to 2-m depth in the control plot and in the agroforestry plot. Error bars represent standard errors (n=100 in the agroforestry, n=93 in the control). Significantly (p-value<0.05) different SOC concentrations per depth are followed by different letters.

Table 3. 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).

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

435 *3.3. Soil organic carbon concentrations*

436 An ANOVA performed on the LMM model revealed that soil depth (F-value=270, $P<0.0001$)
437 and location, i.e., tree row vs. inter-row (F-value=171, $P<0.0001$), were the only variables
438 affecting significantly SOC concentrations. Distance to the closest tree had no significant
439 effect (F-value=1.3, $P=0.28$). As shown in Fig. 4, for 0-10 cm, SOC concentration doubled in
440 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
441 control (9.3 ± 0.1 mg C g^{-1}), whereas the latter two were not significantly different. SOC
442 concentration was significantly higher in the tree row than in the control plot to 120 cm soil
443 depth, except in the 50-70 cm soil layer where no difference was observed. SOC
444 concentration was significantly higher in the tree row than in the inter-row to 30 cm soil
445 depth.
446

447 *3.4. Soil organic carbon stocks*

448 Fig. 5 represents SOC stocks in the agroforestry plot as a function of soil depth, location and
449 distance to the closest tree. For a given depth and distance to the closest tree, variability of
450 SOC stocks was high, and there was no effect of the distance to the closest tree on SOC stocks
451 (Fig. 5). An ANOVA performed on the LMM model confirmed that SOC stocks were
452 significantly influenced by soil depth (F-value=483, $P<0.0001$) and location, i.e., tree row vs.
453 inter-row (F-value=66, $P<0.0001$), but not by the distance to the closest tree (F-value=1.5,
454 $P=0.22$).

455 For an equivalent soil mass (ESM) of 4000 Mg ha^{-1} (to 26-29 cm depth), SOC stocks were
456 significantly higher in the tree row than in the inter row and in the control (Table 2). For an
457 ESM of 31500 Mg ha^{-1} (to 189-196 cm depth), SOC stocks were about 20 Mg C ha^{-1} higher in
458 the tree rows compared to the inter-rows or to the control. Cumulated SOC stocks were

459 significantly higher in the inter-row than in the control plot to an ESM of 18700 Mg ha^{-1} (to
460 112-115 cm depth), except for an ESM of 1000 Mg ha^{-1} where not difference was found
461 (Table 2).

462 At the plot scale, cumulated SOC stocks in the agroforestry plot were significantly higher than
463 in the control plot at all depths (Table 2). For an ESM of 4000 Mg ha^{-1} (to 26-29 cm depth),
464 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
465 control, respectively. For a soil mass of 15700 Mg ha^{-1} (to 93-98 cm depth), Δ SOC stock
466 between the agroforestry and the control was 6.3 ± 0.7 Mg C ha^{-1}. This difference was much
467 lower without the ESM correction (Table S1).

468

469 *3.5. Soil organic carbon accumulation rates*

470 Compared to the control, inter-rows accumulated 115 ± 33 kg C ha^{-1} yr^{-1} for an ESM of 4000
471 Mg ha^{-1} (26-29 cm) (Table 2), and 202 ± 45 kg C ha^{-1} yr^{-1} for an ESM of 15700 Mg ha^{-1} (93-
472 98 cm). SOC accumulation rates in the agroforestry plot compared to the control were 248 ±
473 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
474 ha^{-1}, and 183 ± 48 kg C ha^{-1} yr^{-1} an ESM of 31500 Mg ha^{-1} (Table 2). The additional SOC
475 storage rates for 0-10 cm and 10-30 cm were respectively explained at 80% and 60% by the
476 tree rows.

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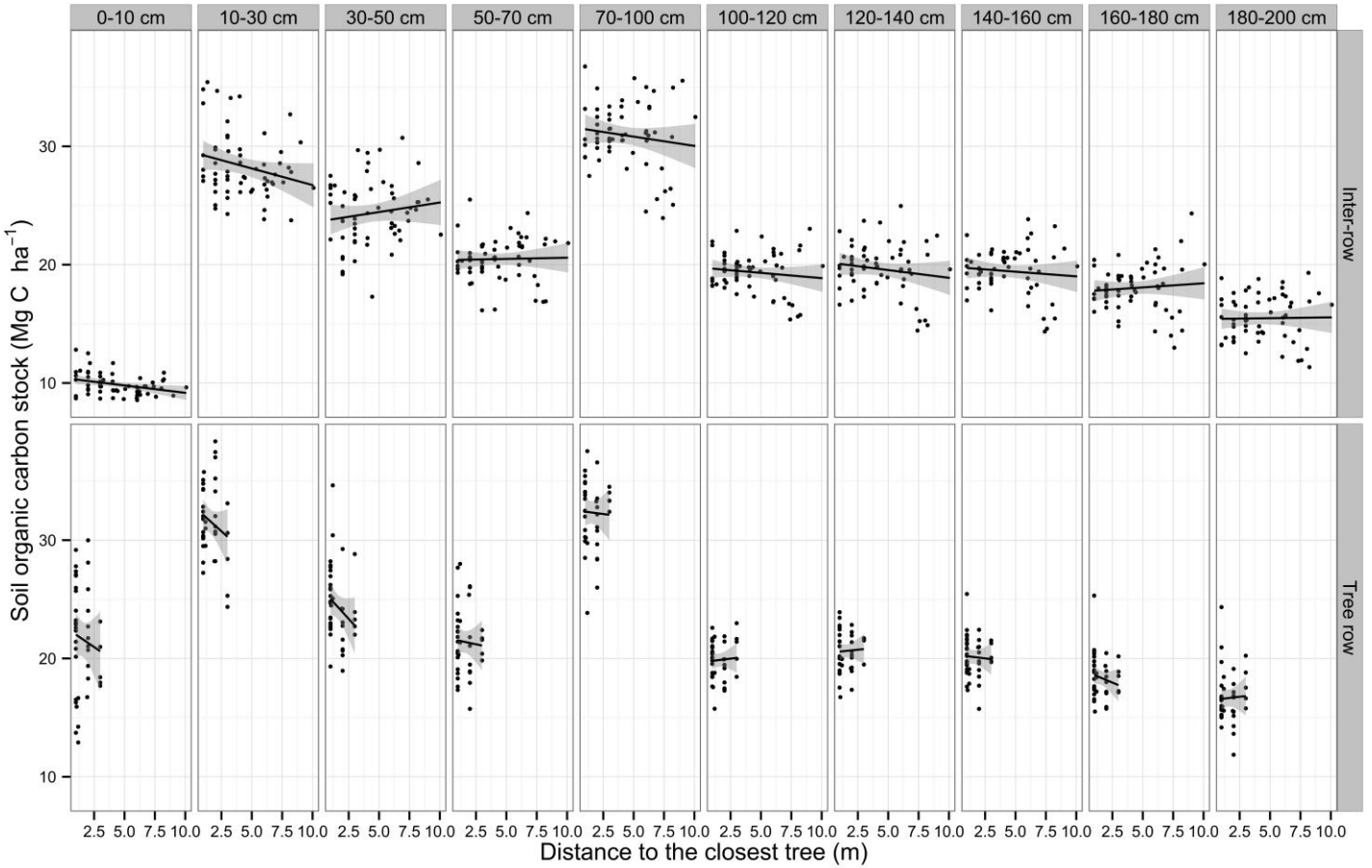


481

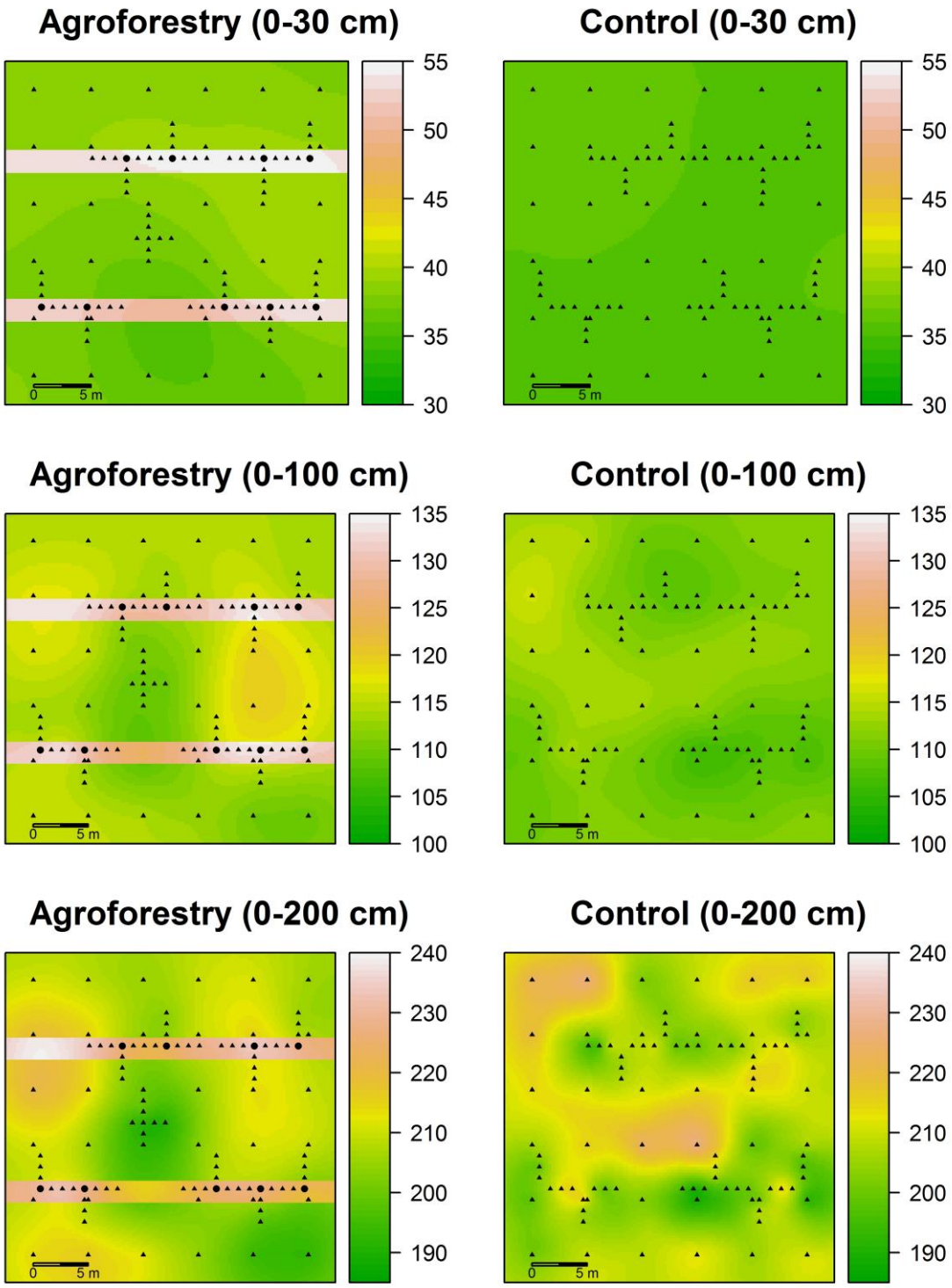
482 Figure 5. Soil organic carbon stocks (Mg C ha^{-1}) in the agroforestry plot as a function of depth, location (tree row vs. inter-row) and distance to
483 the closest tree. The lines represent the regression lines fitted using soil samples per investigated depth. The gray shades display the
484 prediction confidence interval at the 0.95 level.

485 Table 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)
486 for the 2 plots, and cross validation. SSPE, standardized squared prediction errors; ME, mean error (Mg C ha^{-1}); RMSQE, root mean
487 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
488 fixed effects (Mg C ha^{-1}). Bold characters represent the smallest AIC for each depth. The medians and the mean of the cross validation
489 statistics are within the 95% confidence interval.

	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

3.6. Spatial distribution of SOC stocks

The AIC (Table 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. 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 4). At the opposite, the control plot did not exhibit any spatial pattern.



513

514 Figure 6. Krieged maps of cumulated soil organic carbon stocks (Mg C ha^{-1}) in the
515 agroforestry and in the control plot.

516

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. 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. 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 (0-2 µm) were observed in the tree row than in the inter-row (Fig. S3), but it was not the case for the amount of C in the clay fraction per gram of soil (Fig. 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. 7, 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 5), and the SOC deficit was high. The saturation capacity was far from being reached, as it amounted 17 to 40% of saturation capacity in the tree rows.

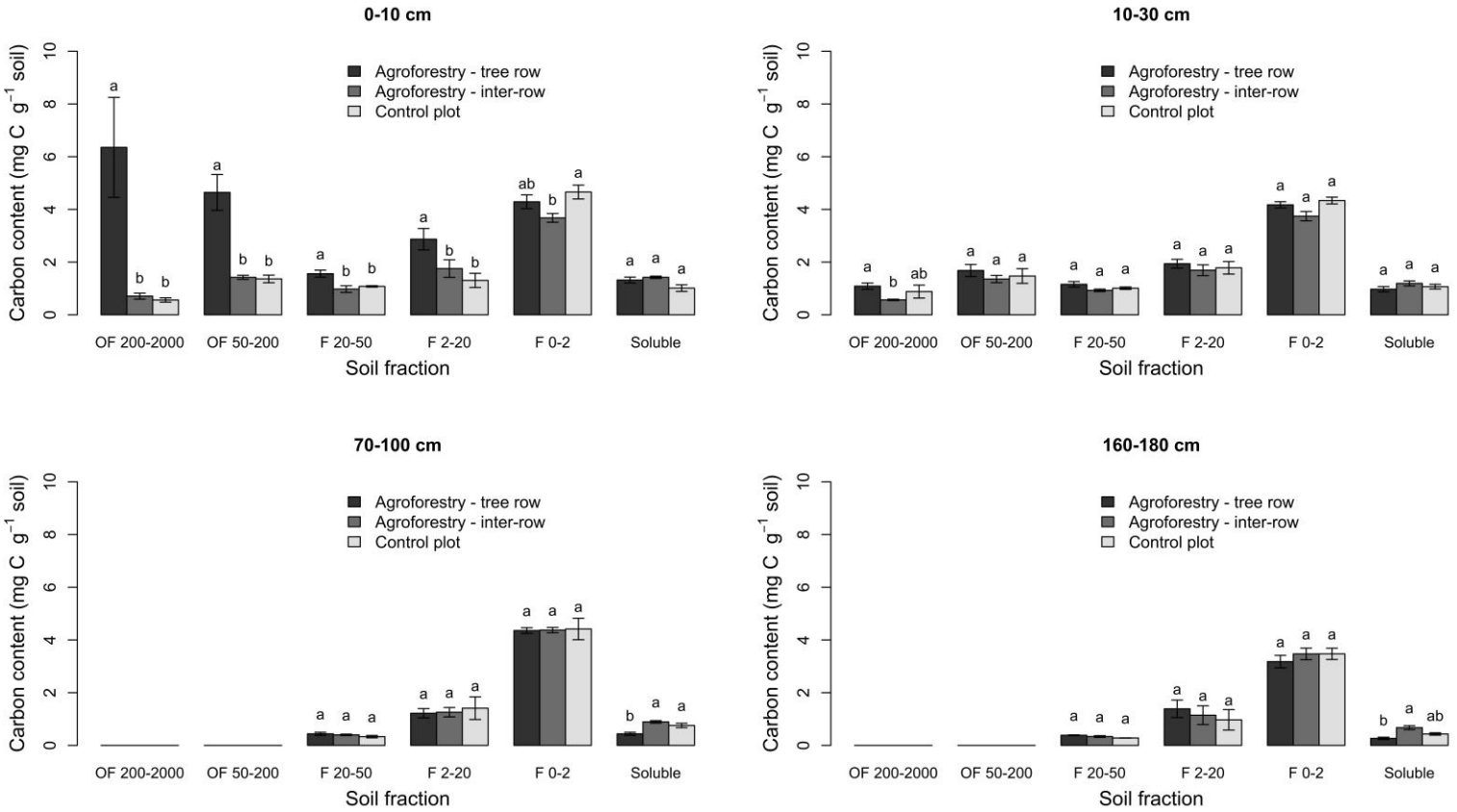


Figure 7. Organic carbon contents in each soil fraction (mg C g^{-1} soil). Error bars represent standard errors (n=6 in the control, n=5 in the inter-row and in the tree row). OF = Organic fraction, F = organo-mineral fraction. 0-2, 2-20, 20-50, 50-200 and 200-2000 represent particle size (µm). Means followed by the same letters do not differ significantly at p=0.017 (Dunn's test with Bonferroni correction).

Table 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.

	$SOC_{sat\text{-}pot}$ (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

549 *3.8. Distribution of additional OC in soil fractions*

550 For 0-10 cm depth, the additional OC stored between the tree row and the inter-row was
551 explained at 80% by POM fractions, at 15% by silt size fractions, and at 5% by clay fraction,
552 whereas the additional OC stored between the tree row and the control was explained at 80%
553 by POM and at 20% by silt size fractions (Fig. 7). For 10-30 cm, the additional SOC storage
554 between the tree row and the inter-row was explained at 50% by POM fractions, at 25% by
555 coarse and fine silt fractions, and at 25% by clay fraction (Fig. 7), whereas when comparing
556 the tree row and the control these numbers were of 50% (POM) and 50% (silt).

557

558 **4. Discussion**

559 4.1. *A shallow additional SOC storage*

560 Sampling to 2-m soil depth indicated that the 0-30 cm soil layer contained less than 20% of
561 total SOC stocks to 2-m depth, demonstrating the importance of deeper soil layers for storing
562 SOC (Harper and Tibbett, 2013; Jobbagy and Jackson, 2000). SOC stocks observed in 0-30
563 cm, from 36 to 41 Mg C ha^{-1}, were comparable to reported values for the Mediterranean
564 region, i.e., 25 to 50 Mg C ha^{-1} (Martin et al., 2011; Muñoz-Rojas et al., 2012). Additional
565 SOC storage in the agroforestry system compared to the agricultural system was mainly
566 observed up to 30 cm soil depth in the inter-row and up to 50 cm in the tree row. A
567 companion study at the same site indicated that 60% of additional OM inputs (leaf litter,
568 aboveground and belowground biomass of the natural vegetation in the tree row, tree fine
569 roots) to 2 m depth in the agroforestry plot compared to the control plot were located in the
570 first 50 cm (unpublished data). Even if 50% of tree fine root density was found between 1 and
571 4 m soil depth (Cardinael et al., 2015), it was also proven at this site (Germon et al.,
572 submitted) and at other sites (Hendrick and Pregitzer, 1996) that the turnover rate of fine roots

573 decreased with increasing depth, resulting in low OM inputs in deep soil layers. Time since
574 the tree planting (18 years) is probably not long enough to detect changes in SOC stocks at
575 deeper soil depths considering low organic inputs below 1 m depth. For 2012, organic C input
576 due to tree fine root mortality was estimated to be less than 150 kg C ha^{-1} for 100-200 cm soil
577 depth. Below 1.2 m soil depth, delta of cumulated SOC stocks between the agroforestry and
578 the control plot decreased, due to higher SOC concentrations and stocks in the control at these
579 depths. These higher SOC concentrations were linked to higher SOC concentrations in the
580 clay fraction. This difference may be due to pre-experimental soil heterogeneity, the soil in
581 the agroforestry plot may have had a lower level of SOC below 1.2 m depth before tree
582 planting. An initial heterogeneity was also proposed by Upson and Burgess (2013) who found
583 higher SOC stocks at depth in a control plot compared to an agroforestry plot in an
584 experimental site in England . This shows the limit of paired comparisons - or synchronic
585 studies - to evaluate SOC changes after land use change (Junior et al., 2013; Olson et al.,
586 2014), and pleads for long-term diachronic studies in agroforestry systems. An alternative
587 explanation could be a positive priming effect, i.e., the acceleration of native SOC
588 decomposition by the supply of fresh organic carbon (Fontaine et al., 2007, 2004) from the
589 trees. However, this seems highly unlikely since positive priming effect could not explain
590 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.,
591 about 180 kg C ha^{-1} yr^{-1}. Another hypothesis to explain higher SOC stocks below 1.2 m depth
592 in the control plot is a different belowground water regime between the two plots. Water table
593 depth at this site is known to be very variable (between 5 to 7 m). A shallower water table in
594 the agroforestry plot compared to the control plot may promote capillary action, and therefore
595 cause wetting-drying cycles that could enhance SOM decomposition in deep soil layers
596 (Borken and Matzner, 2009).

597

598 *4.2. Tree rows and SOC storage in agroforestry systems*

599 The high SOC stocks observed in tree rows accounted for an important part of SOC stocks of
600 the agroforestry plot even though tree rows only represented 16% of the surface area. In a
601 poplar (*Populus* L.) silvoarable agroforestry experiment in England, Upson and Burgess,
602 (2013) also found that the SOC concentration was greater in the top 40 cm under the tree row
603 (19.6 mg C g^{-1}) in the agroforestry treatment than in the cropped alleys (17 mg C g^{-1}), or the
604 arable control (17.1 mg C g^{-1}). Tree rows are comparable to a natural permanent pasture with
605 trees, given that spontaneous herbaceous vegetation grows and that the soil is not tilled.
606 Conversion of arable lands to permanent grasslands is recognized as an efficient land use for
607 climate change mitigation (Soussana et al., 2004). Grasslands can accumulate SOC at a very
608 high rate. For instance, it was estimated on about 20 years old field experiments that
609 conversion from crop cultivation to pasture stored SOC at a rate of 1.01 Mg C ha^{-1} yr^{-1} in 0-30
610 cm (Conant et al., 2001). In our case, SOC accumulation rate in the tree rows was 0.94 ± 0.09
611 Mg C ha^{-1} yr^{-1} in 0-30 cm. Management of tree rows could therefore have an important role in
612 improving agroforestry systems in terms of SOC storage. Improved grass species could be
613 sown in the tree rows, as well as shrubs between trees. Further research should focus on this
614 aspect to evaluate benefits in terms of SOC storage and biodiversity for instance.

615

616 *4.3. Homogeneous distribution of SOC stocks in the cropped alley*

617 There was no significant effect of the distance to the trees on SOC stocks at all depths, either
618 in the tree row or in the inter-row. This was also indicated by the maps of the SOC stocks.
619 Tree density was high at this site, and walnuts were about 13 m in height, which is also the
620 distance between two tree rows. This could explain the homogeneous distribution of leaf
621 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 crop 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., 2015). 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 distribution of SOC stocks within the inter-row (Bambrick et al., 2010; Peichl et al., 2006). 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.

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 (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., 2011). 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 0.13 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, 2004; Lorenz and Lal, 2014).

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, 1996; Balesdent et al., 1988). 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, and part of this labile fractions could be located within them and therefore be physically protected from decomposition by soil microorganisms (Six et al., 2000). 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, suggesting that accumulation of SOC due to the agroforestry system could continue for decades before reaching saturation.

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.

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991 **Supplementary materials**

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

	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

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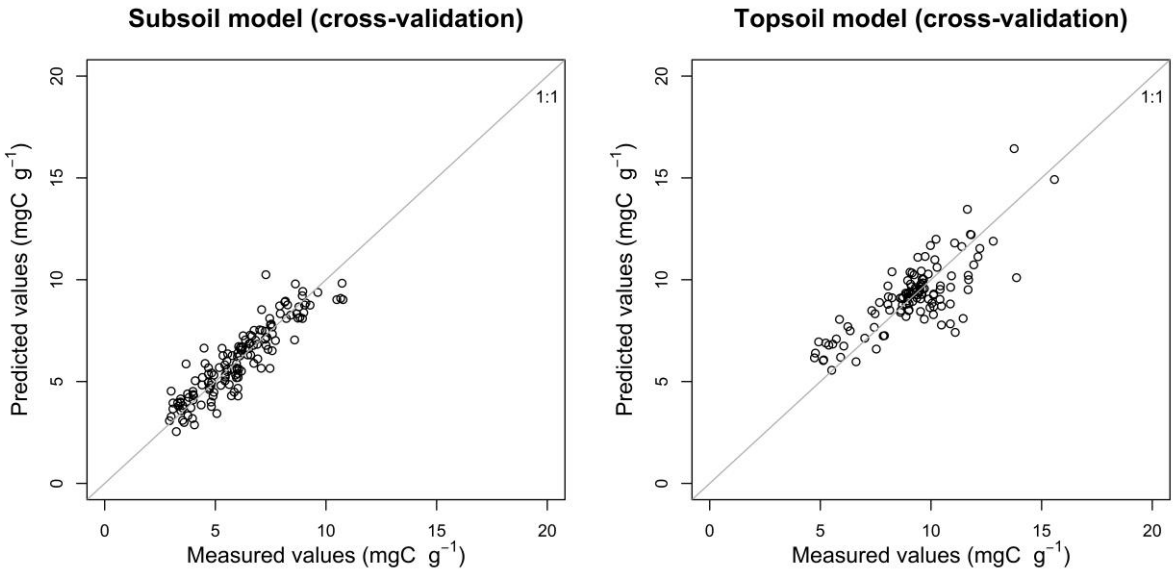
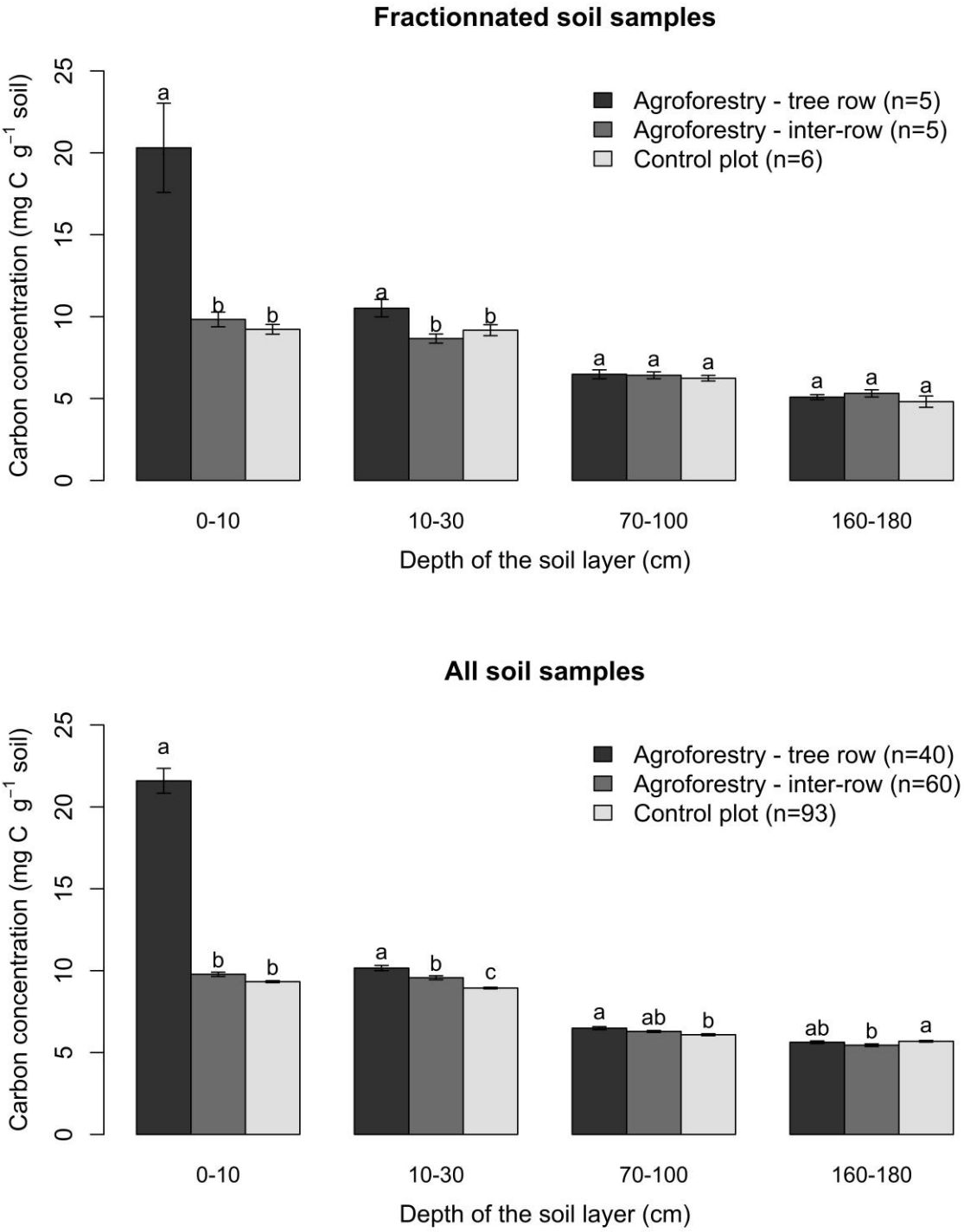


Figure S1. Measured and cross-validation predicted values of soil organic carbon concentrations for the topsoil and subsoil models.



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1018 Figure S2. Carbon concentration of bulk fractionated samples. Error bars represent standard

1019 errors (n=6 in the control, n=5 in the inter-row and in the tree row).

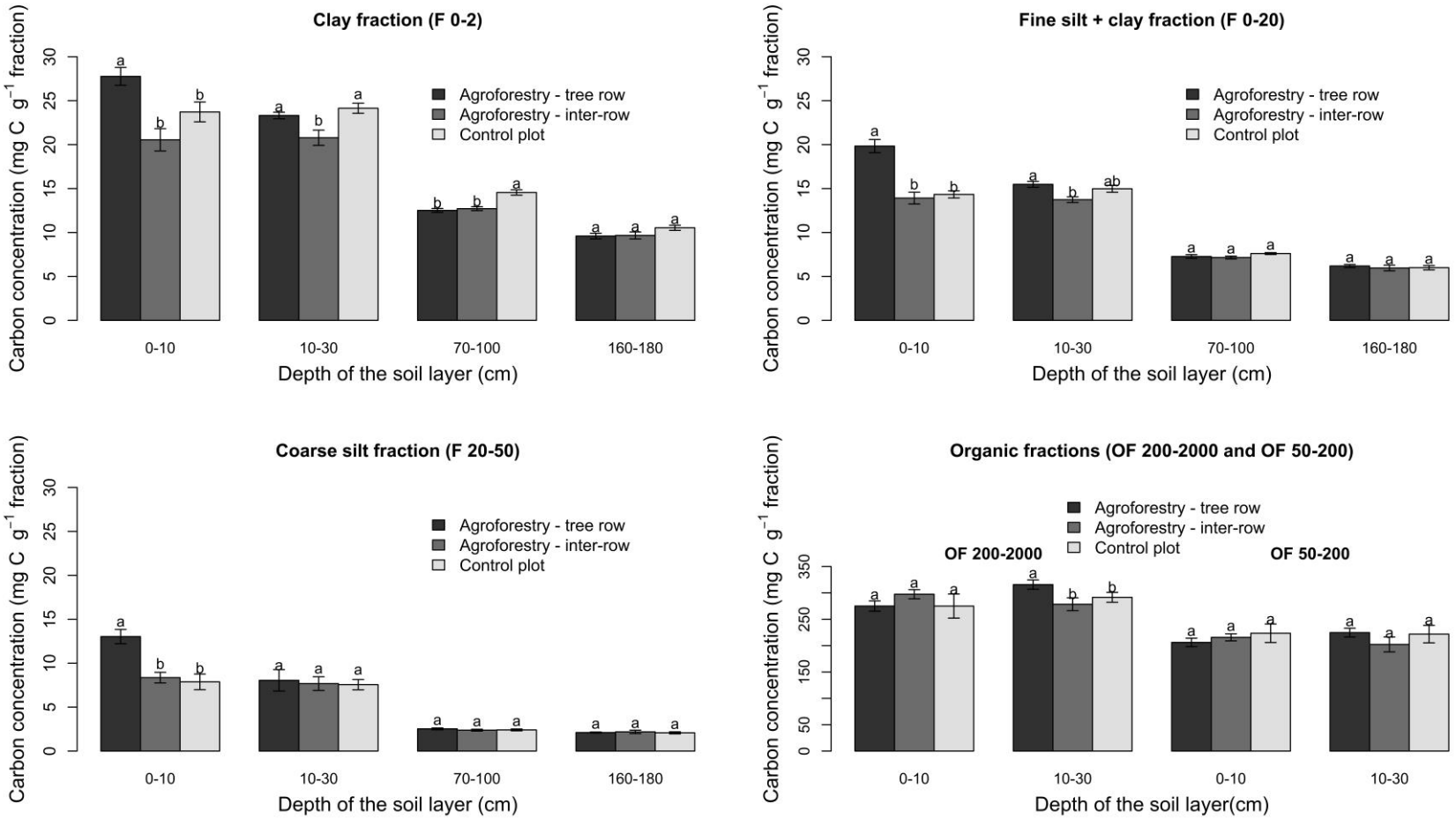

Figure S3. Carbon concentration of each soil fraction. Error bars represent standard errors (n=6 in the control, n=5 in the inter-row and in the tree row). OF = Organic fraction, F = organo-mineral fraction. Means followed by the same letters do not differ significantly at p=0.017 (Dunn's test with Bonferroni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