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

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13

14     **ABSTRACT**

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

25 the agroforestry and the agricultural plots were  $248 \pm 31 \text{ kg C ha}^{-1} \text{ yr}^{-1}$  for an equivalent soil  
26 mass (ESM) of  $4000 \text{ Mg ha}^{-1}$  (to 26-29 cm depth) and  $350 \pm 41 \text{ kg C ha}^{-1} \text{ yr}^{-1}$  for an ESM of  
27  $15700 \text{ Mg ha}^{-1}$  (to 93-98 cm depth). SOC stocks were higher in the tree rows where  
28 herbaceous vegetation grew and where the soil was not tilled, but no effect of the distance to  
29 the trees (0 to 10 m) on SOC stocks was observed. Most of additional SOC storage was found  
30 in coarse organic fractions (50-200 and 200-2000  $\mu\text{m}$ ), which may be rather labile fractions.  
31 All together our study demonstrated the potential of alley cropping agroforestry systems  
32 under Mediterranean conditions to store SOC, and questioned the stability of this storage.

33

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

37

## 38 1. Introduction

39 Agroforestry systems are defined as agroecosystems associating trees with farming practices  
40 (Somarriba, 1992; Torquebiau, 2000). Several types of agroforestry systems can be  
41 distinguished depending on the different associations of trees, crops and animals (Torquebiau,  
42 2000). In temperate regions, an important part of recently established agroforestry systems are  
43 alley cropping systems, where parallel tree rows are planted in crop lands, and designed to  
44 allow mechanization of annual crops. Agroforestry systems are of particular interest as they  
45 combine the potential to provide a variety of non-marketed ecosystem services, defined as the  
46 benefits people obtain from ecosystems (Millennium Ecosystem Assessment, 2005; Power,  
47 2010) whilst maintaining a high agricultural production (Clough et al., 2011). For instance,  
48 agroforestry systems can contribute to water quality improvement (Bergeron et al., 2011;

49 Tully et al., 2012), biodiversity enhancement (Schroth et al., 2004; Varah et al., 2013), and  
50 erosion control (Young, 1997). But agroforestry systems are also increasingly recognized as a  
51 useful tool to help mitigate global warming (Pandey, 2002; Stavi and Lal, 2013; Verchot et  
52 al., 2007). Trees associated to annual crops store the carbon (C) assimilated through  
53 photosynthesis into their aboveground and belowground biomass. The residence time of C in  
54 the harvested biomass will depend on the fate of woody products, and can reach many  
55 decades especially for timber wood (Bauhus et al., 2010; Profft et al., 2009). Agroforestry  
56 trees also produce organic matter (OM) inputs to the soil (Jordan, 2004; Peichl et al., 2006),  
57 and could thus enhance soil organic carbon (SOC) stocks. Leaf litter and pruning residues are  
58 left on the soil, whereas OM originating from root mortality and root exudates can be  
59 incorporated much deeper into the soil as agroforestry trees may have a very deep rooting to  
60 minimize the competition with the annual crop (Cardinael et al., 2015; Mulia and Dupraz,  
61 2006). Moreover, several studies showed that root-derived C was preferentially stabilized in  
62 soil compared to above ground derived C (Balesdent and Balabane, 1996; Rasse et al., 2005),  
63 mainly due to physical protection of root hairs within soil aggregates (Gale et al., 2000), to  
64 chemical recalcitrance of root components (Bird and Torn, 2006), or to adsorption of root  
65 exudates or decomposition products on clay particles (Chenu and Plante, 2006; Oades, 1995).  
66 Compared to an agricultural field, additional inputs of C from tree roots could therefore be  
67 stored deep into the soil, but could also enhance decomposition of SOM, i.e., due to the  
68 priming effect (Fontaine et al., 2007).

69 Although it is generally assumed that agroforestry system have the potential to increase SOC  
70 stocks (Lorenz and Lal, 2014), quantitative estimates are scarce, especially for temperate  
71 (Nair et al., 2010; Peichl et al., 2006; Pellerin et al., 2013; Upson and Burgess, 2013) or  
72 Mediterranean (Howlett et al., 2011) agroforestry systems combining crops and tree rows.

73 Most studies concern tropical regions where agroforestry is a more widespread agricultural  
74 practice (Albrecht and Kandji, 2003; Somarriba et al., 2013).

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

87 In alley cropping systems, spaces between trees in tree rows are usually covered by natural or  
88 sowed herbaceous vegetation, and the soil under tree rows is usually not tilled, which may  
89 favor SOC storage in soil (Virto et al., 2011). Moreover, while trees strongly affects the depth  
90 and spatial distribution of OM inputs to soils (Rhoades, 1997), distribution of SOC stocks  
91 close and away from trees was seldom considered. Some authors reported higher SOC stocks  
92 under the tree canopy than 5 m from the tree to 1 m soil depth (Howlett et al., 2011), others  
93 found that spatial distribution of SOC stocks could vary with the age of the trees (Bambrick et  
94 al., 2010). Some authors reported that spatial distribution of SOC stocks to 20 cm depth was  
95 not explained by the distance to the trees but by the design of the agroforestry system, tree  
96 rows having higher SOC stocks than inter-rows whatever the distance to the trees (Peichl et  
97 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.

122        **2. Materials and methods**

123            *2.1. Site description*

124            The experimental site was located in Prades-le-Lez, 15 km North of Montpellier,  
125 France (Longitude 04°01' E, Latitude 43°43' N, elevation 54 m a.s.l.). The climate is sub-  
126 humid Mediterranean with an average temperature of 14.5°C and an average annual rainfall of  
127 951 mm (years 1996–2003). The soil is a silty and carbonated deep alluvial Fluvisol (IUSS  
128 Working Group WRB, 2007). From 1950 to 1960, the site was a vineyard (*Vitis vinifera* L.),  
129 and from 1960 to 1985 the field was occupied by an apple (*Malus* Mill.) orchard. The apple  
130 tree stumps were removed in 1985. Then, durum wheat (*Triticum turgidum* L. subsp. *durum*  
131 (Desf.) Husn.) was cultivated. In February 1995, a 4.6 hectare agroforestry alley-cropping  
132 plot was established after the soil was ploughed to 20 cm depth, with the planting of hybrid  
133 walnuts (*Juglans regia* × *nigra* cv. NG23) at 13 × 4 m spacing, with East–West tree rows  
134 (Fig. 1). The remaining part of the plot (1.4 ha) was kept as a control agricultural plot. The  
135 walnut trees were planted at an initial density of 200 trees ha<sup>-1</sup>. They were thinned in 2004  
136 down to 110 trees ha<sup>-1</sup>. In the tree rows, the soil was not tilled and spontaneous herbaceous  
137 vegetation grew. The cultivated inter-row was 11 m wide. Since the tree planting, the  
138 agroforestry inter-row and the control plot were managed in the same way. The annual crop  
139 was most of the time durum wheat, except in 1998, 2001 and 2006, when rapeseed (*Brassica*  
140 *napus* L.) was cultivated, and in 2010 and 2013, when pea (*Pisum sativum* L.) was cultivated.  
141 The durum wheat crop was fertilized as a conventional crop (120 kg N ha<sup>-1</sup> yr<sup>-1</sup>), and the soil  
142 was ploughed annually to 20 cm depth, before durum wheat was sown.

143

144

145



146

147 Figure 1. Hybrid walnut-durum wheat agroforestry system. Left panel: in November 2013;  
148 Right panel: in June 2014.

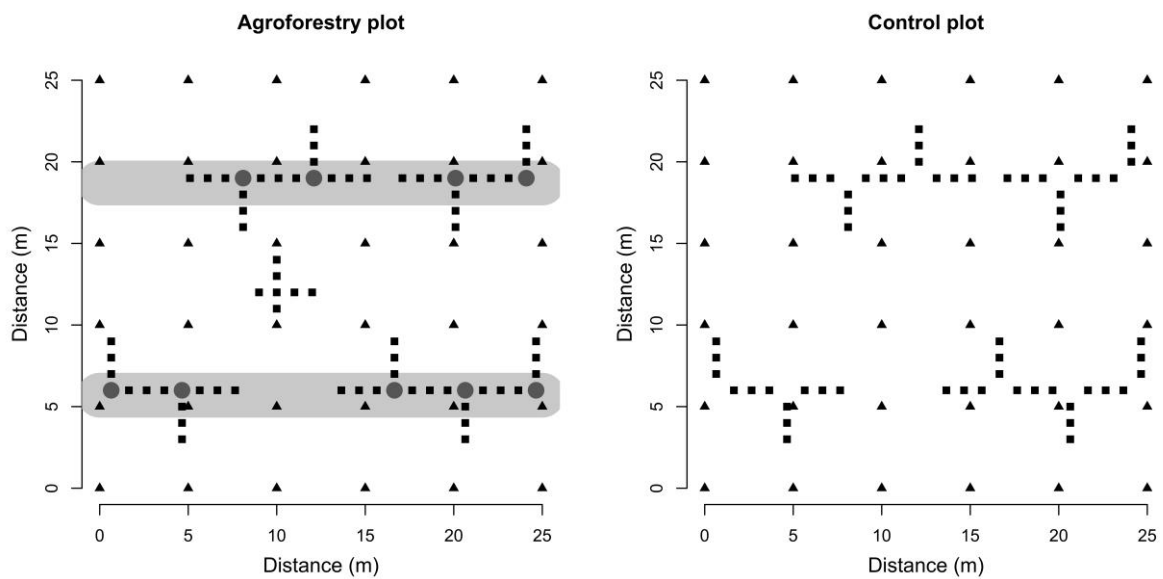
149

## 150 *2.2. Soil core sampling*

151 The experimental site was not designed as traditional agronomical experiments with blocks  
152 and replicates, but with two large adjacent plots. First, soil texture was analyzed for 24  
153 profiles down to 2 m soil depth, following a random sampling design within the two plots. In  
154 May 2013, a sub-plot of 625 m<sup>2</sup> was sampled in both plots, following an intensive sampling  
155 scheme (Fig. 2). In the agroforestry plot, this sub-plot included two tree rows, two inter-rows  
156 and nine walnut trees. Walnut trees had a mean height of  $11.21 \pm 0.65$  m, a mean height of  
157 merchantable timber of  $4.49 \pm 0.39$  m and a mean diameter at breast height of  $25.54 \pm 1.36$   
158 cm. Soil cores (n=36) were sampled on a regular grid, every 5 m (Fig. 2). Around each tree, a  
159 soil core was collected at 1 m, 2 m and 3 m distance from the tree (n=57), in the tree row and  
160 perpendicular to the tree row. Seven soil cores were sampled additionally in the middle of the  
161 inter-row to study short scale (1 m distance) spatial heterogeneity of SOC stocks far from the  
162 trees (Fig. 2). The same sampling scheme was followed in the control plot without these seven  
163 additional soil cores. Thus, 100 soil cores were sampled in the agroforestry sub-plot (40 in



164 tree rows, 60 in inter-rows) and 93 in the agricultural sub-plot (Fig. 2). All cores were  
 165 sampled down to 2 m depth using a motor-driven micro caterpillar driller (8.5-cm diameter  
 166 and 1-m long soil probe). The soil probe was successively pushed two times into the soil, to  
 167 get 0-1 m and 1-2 m cores at each sampling point. Each soil core was then cut into ten  
 168 segments, corresponding to the following depth increments: 0-10, 10-30, 30-50, 50-70, 70-  
 169 100, 100-120, 120-140, 140-160, 160-180, and 180-200 cm.



170  
 171 Figure 2. Description of the intensive sampling scheme in the agroforestry and in the control  
 172 sub-plots. Circles represent hybrid walnuts, the grey strips represents the tree rows,  
 173 triangles are for soil cores on the regular grid (every 5 m), squares are for soil cores  
 174 on transects (every 1 m).

175  
 176 *2.3. Use of field visible and near infrared spectroscopy to predict SOC*

177 As core surface had been smoothed by the soil probe, each segment was refreshed with  
 178 a knife before being scanned, in order to provide a plane but un-smoothed surface. Then, four  
 179 VNIR spectra (from 350 to 2500 nm at 1 nm increment) were acquired in the field at different

180 places of each segment, using a portable spectrophotometer ASD LabSpec 2500 (Analytical  
181 Spectral Devices, Boulder, CO, USA), and were then averaged. Reflectance spectra were  
182 recorded as absorbance, which is the logarithm of the inverse of reflectance. The whole  
183 spectrum population was composed of 1908 mean spectra (i.e. 193 cores with 10 sub-cores  
184 per core but a few samples were lost due to mechanical problems). In topsoil (0-30 cm), the  
185 soil was dry and crumbled whereas in deeper soil horizons, it was moister and had higher  
186 cohesion. Thus, two different predictive models were built: one for topsoil samples, the other  
187 for subsoil (30-200 cm) samples. The “topsoil model” for predicting SOC was built using the  
188 116 most representative topsoil samples, out of 380 samples, and the “subsoil model”, using  
189 the 142 most representative subsoil samples, out of 1488 samples. The procedure to select the  
190 most representative samples is presented below. The 0-10 cm soil layer from the tree rows (40  
191 samples) was not used for the topsoil model as it contained abundant plant debris < 2 mm  
192 (roots, leaves, etc.) and a PCA revealed that these VNIR spectra were different from the  
193 whole spectra population. SOC concentration of these samples was therefore determined with  
194 a CHN elemental analyzer, and, thus, not predicted by VNIR. The SOC concentration of the  
195 258 samples selected for building the VNIR prediction models was also analyzed using a  
196 CHN elemental analyzer.

197

#### 198 *2.4. VNIR spectra analysis and construction of predictive models*

199 VNIR spectra analysis was conducted on topsoil and subsoil samples separately, using  
200 the WinISI 4 software (Foss NIRSystems/ Tecator Infracore International, LLC, Silver Spring,  
201 MD, USA) and R software version 3.1.1 (R Development Core Team, 2013). The most  
202 representative samples, from a spectral viewpoint, were selected using the Kennard-Stone  
203 algorithm, which is based on distance calculation between sample spectra in the principal

204 component space (Kennard and Stone, 1969). For the topsoil model, the calibration subset  
205 included 104 samples (90%) selected as the most representative spectrally, and the validation  
206 subset 12 samples (10%). For the subsoil model, the calibration subset included 128 samples  
207 (90%), and the validation subset 14 samples. Fitting the spectra to the SOC concentrations  
208 determined with a CHN elemental analyzer was performed using partial least squares  
209 regression (PLSR; Martens and Naes, 1989). We tested common spectrum preprocessing  
210 techniques including first and second derivatives, de-trending, standard normal variate  
211 transformation and multiplicative scatter correction, but the best models were obtained when  
212 no pre-treatment was applied on the spectra (data not shown). Then cross-validation was  
213 performed within the calibration subset, using groups that were randomly selected (10  
214 groups), in order to build the model used for making predictions on the samples not analyzed  
215 in the laboratory. No outlier was removed. The number of components (latent variables) that  
216 minimized the standard error of cross-validation (SECV) was retained for the PLSR. The  
217 performance of the models was assessed on the validation subsets using the coefficient of  
218 determination ( $R^2$ ) and the standard error of prediction (SEP) between predicted and measured  
219 values, and also the ratio of standard deviation to SEP, denoted RPD, and the RPIQ, which is  
220 the ratio of performance to IQ (interquartile distance), i.e.  $IQ/SEP = (Q3 - Q1)/SEP$ , where  
221  $Q1$  is the 25<sup>th</sup> percentile and  $Q3$  is the 75<sup>th</sup> percentile (Bellon-Maurel et al., 2010). Then all  
222 sub-set samples (i.e., calibration and validation samples) were used to build models that were  
223 applied on the samples not analyzed in the laboratory. The performance of these models was  
224 also assessed according to  $R^2$ , SECV, RPD and RPIQ.

225

226

227

228 Table 1. External validation and prediction model results for soil organic carbon. N: numbers of samples; SD: standard deviation (mean and  
 229 standard deviation of the conventional determinations); R<sup>2</sup>: coefficient of determination; RPD is the ratio of performance to deviation,  
 230 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 -  
 231 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 <sup>-1</sup>	SD mg g <sup>-1</sup>	SEP mg g <sup>-1</sup>	Bias mg g <sup>-1</sup>	R <sup>2</sup>	RPD	RPIQ	N	Mean mg g <sup>-1</sup>	SD mg g <sup>-1</sup>	SECV mg g <sup>-1</sup>	R <sup>2</sup>	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 <sup>-1</sup>	SD mg g <sup>-1</sup>	SEP mg g <sup>-1</sup>	Bias mg g <sup>-1</sup>	R <sup>2</sup>	RPD	RPIQ	N	Mean mg g <sup>-1</sup>	SD mg g <sup>-1</sup>	SECV mg g <sup>-1</sup>	R <sup>2</sup>	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

232

233

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

242

#### 243 *2.5. Bulk densities determination*

244 Each segment was weighed in the field to determine its humid mass. Following this  
245 step, each segment was crumbled and homogenized, and a representative sub-sample of about  
246 300 g was sampled. Sub-samples were sieved at 2 mm to separate coarse fragments such as  
247 stones and living roots. Coarse fragments represented less than 1% of each soil mass and were  
248 considered as negligible. Moisture contents were determined for 23 soil cores (i.e. 230  
249 samples) after 48 h drying at 105°C, and were used to calculate the dry mass of all samples.  
250 Bulk density (BD) was determined for each sample by dividing the dry mass of soil by its  
251 volume in the soil corer tube.

252

#### 253 *2.6. Reference analysis measurements*

254 After air drying, soil samples were oven dried at 40°C for 48 hours, sieved at 2 mm,  
255 and ball milled until they passed a 200 µm mesh sieve. Carbonates were removed by acid  
256 fumigation, following Harris et al., (2001). For this, 30 mg of soil was placed in open Ag-foil

257 capsules. The capsules were then placed in the wells of a microtiter plate and 50  $\mu\text{L}$  of  
258 demineralized water was added in each capsule. The microtiter plate was then placed in a  
259 vacuum desiccator with a beaker filled with 100 mL of concentrated HCl (37%). The samples  
260 were exposed to HCl vapors for 8 hours, and were then dried at 60°C for 48 hours. Capsules  
261 were then closed in a bigger tin capsule. Decarbonated samples were analyzed for organic  
262 carbon concentration with a CHN elemental analyzer (Carlo Erba NA 2000, Milan, Italy).  
263 Isotopic measurements were performed on a few samples to check that decarbonation was  
264 well performed ( $\delta^{13}\text{C OM} = -25 \text{ ‰}$ ).

265

## 266 *2.7. Soil organic carbon stock calculation*

267 In most studies comparing SOC stocks between treatments or over time periods, SOC  
268 stocks have been quantified to a fixed depth as the product of soil bulk density, depth and  
269 SOC concentration. However, if soil bulk density differs between the treatments being  
270 compared, the fixed-depth method has been shown to introduce errors (Ellert et al., 2002). A  
271 more accurate method is to use an equivalent soil mass (ESM) (Ellert and Bettany, 1995). We  
272 defined a reference soil mass profile that was used as the basis for comparison, based on the  
273 lowest soil mass observed at each sampling depth and location. For this reference, soil mass  
274 layers (0-1000, 1000-4000, 4000-7300, 7300-10700, 10700-15700, 15700-18700, 18700-  
275 21900, 21900-25100, 25100-28300, 28300-31500  $\text{Mg ha}^{-1}$ ) corresponded roughly to soil  
276 depth layers (0–10, 10–30, 30–50, 50-70, 70-100, 100-120, 120-140, 140-160, 160-180, 180-  
277 200 cm, respectively). For the different treatments (control, tree row, inter-row), SOC stocks  
278 were calculated on this basis, soil mass was the same, whereas depth layer varied (Table 2).  
279 The effect of the ESM correction can be seen in Table S1. SOC stocks in the agroforestry plot  
280 were calculated with tree rows representing 16% of the plot surface area and inter-rows 84%:

281 
$$\text{SOC stock}_{\text{Agroforestry}} = 0.16 \times \text{SOC stock}_{\text{Tree row}} + 0.84 \times \text{SOC stock}_{\text{Inter row}} \quad (1)$$

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

284 
$$\Delta \text{SOC stock} = \text{SOC stock}_{\text{Agroforestry}} - \text{SOC stock}_{\text{Control}} \quad (2)$$

285 All SOC stocks were expressed in Mg C ha<sup>-1</sup>. SOC accumulation rates (kg C ha<sup>-1</sup> yr<sup>-1</sup>) were  
286 calculated by dividing delta stocks by the number of years since the tree planting (18 years):

287 
$$\text{SOC accumulation rate} = \frac{\Delta \text{SOC stock}}{18} \times 1000 \quad (3)$$

## 288 *2.8. Particle-size fractionation*

289 Particle-size fractionation was performed for five soil cores from the inter-rows, five from the  
290 tree rows and six from the control plot, and at four depths: 0-10, 10-30, 70-100 and 160-  
291 180 cm. Thus, 64 soil samples were fractionated, as described in Balesdent et al. (1998) and  
292 Gavinelli et al. (1995). Briefly, 20 g of 2-mm sieved samples were soaked overnight at 4°C in  
293 300 mL of deionized water, with 10 mL of sodium metaphosphate (HMP, 50 g L<sup>-1</sup>). Samples  
294 were then shaken 2 h with 10 glass balls in a rotary shaker, at 43 rpm. The soil suspension  
295 was wet-sieved through 200-µm and 50-µm sieves, successively. The fractions remaining on  
296 the sieves were density-separated into organic fractions, floating in water, and remaining  
297 mineral fractions. The 0-50 µm suspension was ultrasonicated during 10 min with a probe-  
298 type ultrasound generating unit (Fisher Bioblock Scientific, Illkirch, France) having a power  
299 output of 600 watts and working in 0.7:0.3 operating/interruption intervals. This 0-50 µm  
300 suspension was then sieved through a 20-µm sieve. The resulting 0-20 µm suspension was  
301 transferred to 1-L glass cylinders, which were then shaken by hand and 50 mL of the  
302 suspension were withdrawn immediately after. They constituted an aliquot of the entire 0-20  
303 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  $\mu\text{m}$  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  $\mu\text{m}$   
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  $\mu\text{m}$ ) 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<sup>-1</sup>. 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):

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

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

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:

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



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

330 These calculations were performed for the four depths where particle-size fractionation was  
 331 done (0-10, 10-30, 70-100 and 160-180 cm). But as the equation proposed by (Hassink, 1997)  
 332 was calibrated for topsoil layers, calculations for deep soil layers are only indicative.

333

#### 334 2.10. Statistical analyses

335 The observed variability in a soil property  $Z$  such as SOC concentration results from complex  
 336 processes operating over various spatial scales. A simple but useful statistical model for  $Z$  at a  
 337 set of observations that could be spatially located,  $\mathbf{s}_i = \{s_1, s_2, \dots, s_q\}$  is

$$338 \quad Z(\mathbf{s}_i) = \mu(\mathbf{s}_i) + \varepsilon(\mathbf{s}_i) \quad (6)$$

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

$$344 \quad \mu(\mathbf{s}_i) = \beta_0 + \sum_{j=1}^q \beta_j x_j(\mathbf{s}_i) \quad (7)$$

345 where  $x_j$  ( $j = 1, 2, \dots, q$ ) are  $q$  auxiliary variables and  $\beta_0, \dots, \beta_q$  are the associated fixed  
 346 effects. This model is referred as a Mixed Effects Model which offers a flexible framework by  
 347 which to model the sources of variation and correlation that arise from grouped data (Lark et

348 al., 2006; Pinheiro and Bates, 2000). In this work, we fitted two different linear mixed models  
349 (LMM).

350 We first fitted a LMM using the whole set of the bulk densities, SOC concentrations, and  
351 SOC stocks observations at the different depths. We used the *nlme* package (Pinheiro et al.,  
352 2013). Soil core ID was considered as a random effect to take into account a sample effect.  
353 These soil properties were then compared by depth and per location (control, tree row, inter-  
354 row). An ANOVA was performed on these models. We then used the *multcomp* package  
355 (Hothorn et al., 2008) to perform a post hoc analysis and determine which means differed  
356 significantly between the control, tree rows and inter-rows, using the Tukey-Kramer test,  
357 designed for unbalanced data. To study spatial influence on SOC stocks, “distance to the  
358 closest tree” was added to the LMM model, and an ANOVA was performed.

359 Secondly, we fitted a LMM in a geostatistical framework using the cumulated SOC stock  
360 observations for 3 depths (0-30 cm, 0-100 cm and 0-200 cm). In a spatial context, the random  
361 effects of the LMM describe spatially-correlated random variation. The LMM model is then  
362 parameterized by a global vector, called  $\Theta$ , of model parameters which include the parameters  
363 of the covariance function and the fixed effects coefficients. These can be fitted to the data by  
364 a likelihood method. Lark et al. (2006) described how the maximum likelihood estimator is  
365 biased in the presence of fixed effects and suggested that the restricted maximum likelihood  
366 estimator (REML) should be applied. Following Villanneau et al., (2011) we have tested the  
367 assumption that the random effects are spatially correlated by comparing the quality of the  
368 model-fit for spatially correlated and spatially independent models (usually called pure nugget  
369 model). Webster and McBratney, (1989) suggested that the Akaike information criterion  
370 (AIC, Akaike, 1974) should be used to compare different spatially correlated models. Once  
371 the parameters of the LMM have been fitted, they may be plugged into the best linear  
372 unbiased predictor to form the empirical best linear unbiased predictor (E-BLUP) of the

373 property at unsampled sites (Lark et al., 2006). The error variance of the E-BLUP can also be  
374 computed at any unsampled site. For this, the value of fixed effects covariates must be known  
375 at each prediction site. We therefore calculated several grids of the fixed effects with a 25 cm  
376 cell size. The use of any model of spatial variation implies that assumptions have been made  
377 about the type of variation the data exhibit. Once the model has been fitted, cross-validation  
378 can be used to confirm that these assumptions are reasonable and that the spatial model  
379 appropriately describes the variation. We therefore computed a ‘leave-one-out cross-  
380 validation’. For each sampling location,  $\mathbf{s}_i$  ( $i = 1, 2, \dots, q$ ), the value of the property at  $\mathbf{s}_i$  was  
381 predicted by the E-BLUP upon the vector of observations excluding  $Z(\mathbf{s}_i)$ , in order to  
382 compute the standardized squared prediction error (SSPE: the squared difference between the  
383 E-BLUP and the observed value divided by the computed prediction error variance (PEV)).  
384 Under an assumption of normal prediction errors, the expected mean SSPE is 1.0 if the PEVs  
385 are reliable (which requires an appropriate variogram model), and the expected median SSPE  
386 is 0.455. The spatial analysis package *GeoR* (Ribeiro and Diggle, 2001) was used for REML  
387 fitting and kriging.

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

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

394

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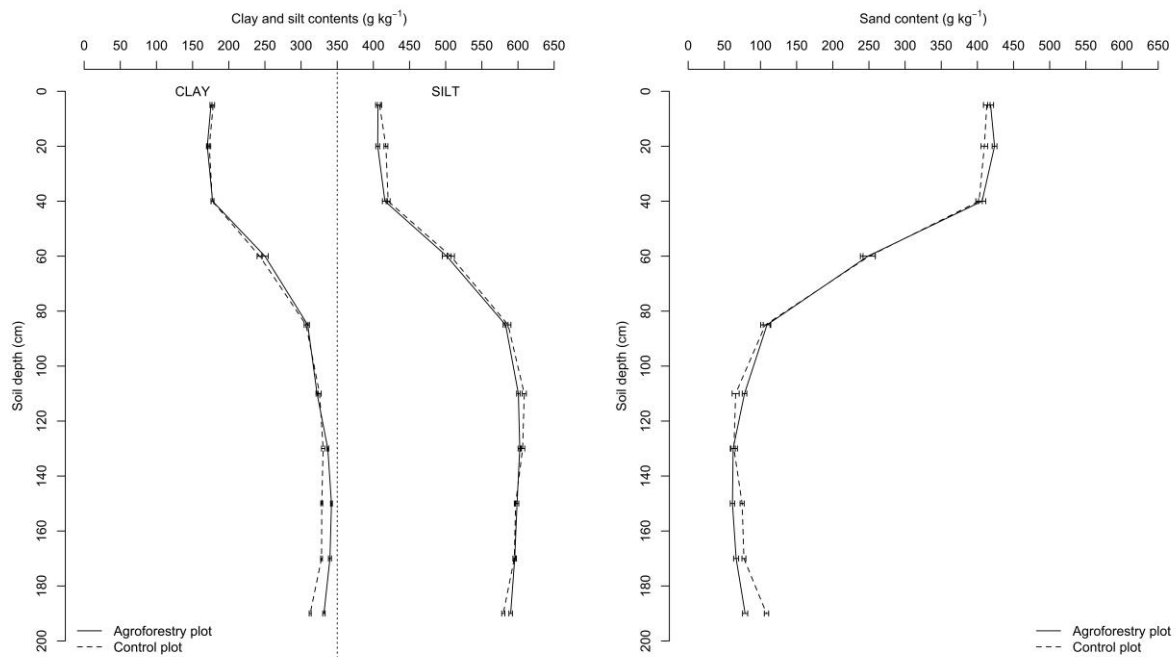
397 Table 2. Soil organic carbon stocks (Mg C ha<sup>-1</sup>) and SOC accumulation rates (kg C ha<sup>-1</sup> yr<sup>-1</sup>). Associated errors are standard errors (40 replicates  
 398 for the tree-row, 60 replicates for the inter-row, and 93 replicates for the control plot). ESM = Equivalent Soil Mass. Significantly (p-  
 399 value<0.05) different SOC stocks are followed by different letters.

Cumulated ESM (Mg ha <sup>-1</sup> )	Cumulated calculated depth to ESM (cm)			Cumulated SOC stocks (Mg C ha <sup>-1</sup> )				Δ SOC stocks (Mg C ha <sup>-1</sup> )	SOC accumulation rates (kg C ha <sup>-1</sup> yr <sup>-1</sup> )	
	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

400 **3. Results**

401 *3.1. Changes in soil texture with depth*

402 Clay, silt and sand profiles were very similar at both plots (Fig. 3). Soil texture was  
403 homogeneous in the first 50 cm. Clay and silt contents linearly increased till 100 cm soil  
404 depth to reach about 325 g kg<sup>-1</sup> and 575 g kg<sup>-1</sup> respectively, while sand content decreased. Soil  
405 texture did not change between 100 and 200 cm soil depth. Below 140 cm depth, clay and  
406 sand content were significantly different (F=71.31, P<0.001) in both plots, but the maximum  
407 difference was less than 20 g kg<sup>-1</sup>.



408

409 Figure 3. Changes in soil texture with depth in the control plot and in the agroforestry plot.

410 Error bars represent standard errors (n=100 in the agroforestry, n=93 in the control).

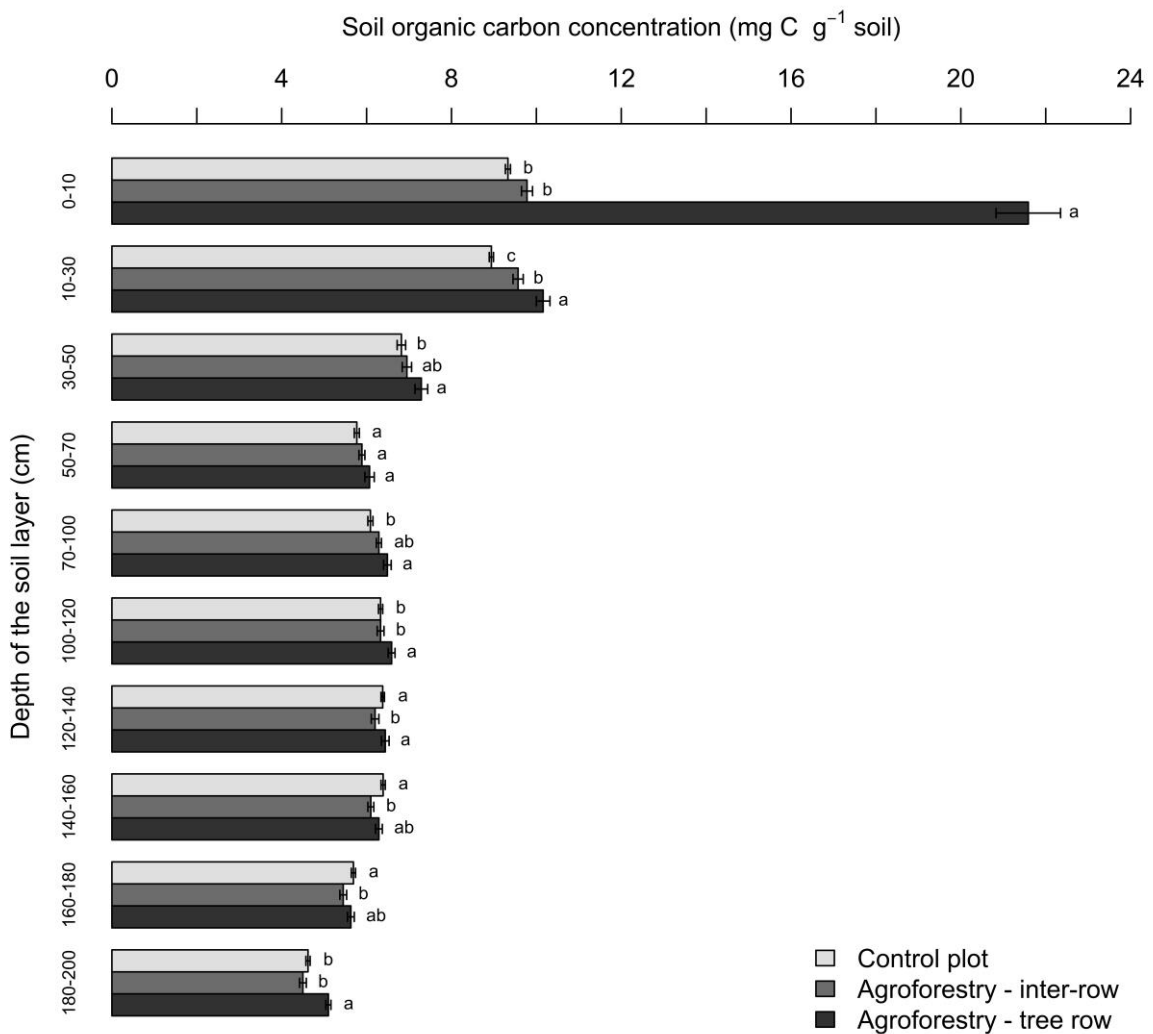
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413

414 3.2. Soil bulk densities

415 Soil bulk densities were significantly higher in the control plot than in the tree row at all  
 416 depths except for 30-50 and 140-160 cm, and higher than in the inter-row, except for 10-30  
 417 and below 140 cm depth (Table 3). In the agroforestry system, soil bulk densities were higher  
 418 in the inter-row than in the tree row for 0-10 and 10-30 cm.



419  
 420 Figure 4. Soil organic carbon concentration (mg C g<sup>-1</sup> soil) of soil layers to 2-m depth in the  
 421 control plot and in the agroforestry plot. Error bars represent standard errors (n=100  
 422 in the agroforestry, n=93 in the control). Significantly (p-value<0.05) different SOC  
 423 concentrations per depth are followed by different letters.

424 Table 3. Mean soil bulk densities ( $\text{g cm}^{-3}$ ). For a given depth, means followed by the same letters do not differ significantly at  $p = 0.05$ .

425 Associated errors are standard errors (40 replicates for the tree-row, 60 replicates for the inter-row, and 93 replicates for the control plot).

426

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

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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 ( $21.6 \pm 0.8 \text{ mg C g}^{-1}$ ) compared to the inter-row ( $9.8 \pm 0.1 \text{ mg C g}^{-1}$ ) and to the  
441    control ( $9.3 \pm 0.1 \text{ mg C g}^{-1}$ ), 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  $4000 \text{ Mg ha}^{-1}$  (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  $31500 \text{ Mg ha}^{-1}$  (to 189-196 cm depth), SOC stocks were about  $20 \text{ Mg C ha}^{-1}$  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<sup>-1</sup> (to  
460 112-115 cm depth), except for an ESM of 1000 Mg ha<sup>-1</sup> 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<sup>-1</sup> (to 26-29 cm depth),  
464 SOC stocks were 40.3 ± 0.5 Mg C ha<sup>-1</sup> and 35.8 ± 0.2 Mg C ha<sup>-1</sup> in the agroforestry and in the  
465 control, respectively. For a soil mass of 15700 Mg ha<sup>-1</sup> (to 93-98 cm depth), Δ SOC stock  
466 between the agroforestry and the control was 6.3 ± 0.7 Mg C ha<sup>-1</sup>. This difference was much  
467 lower without the ESM correction (Table S1).

468

### 469 *3.5. Soil organic carbon accumulation rates*

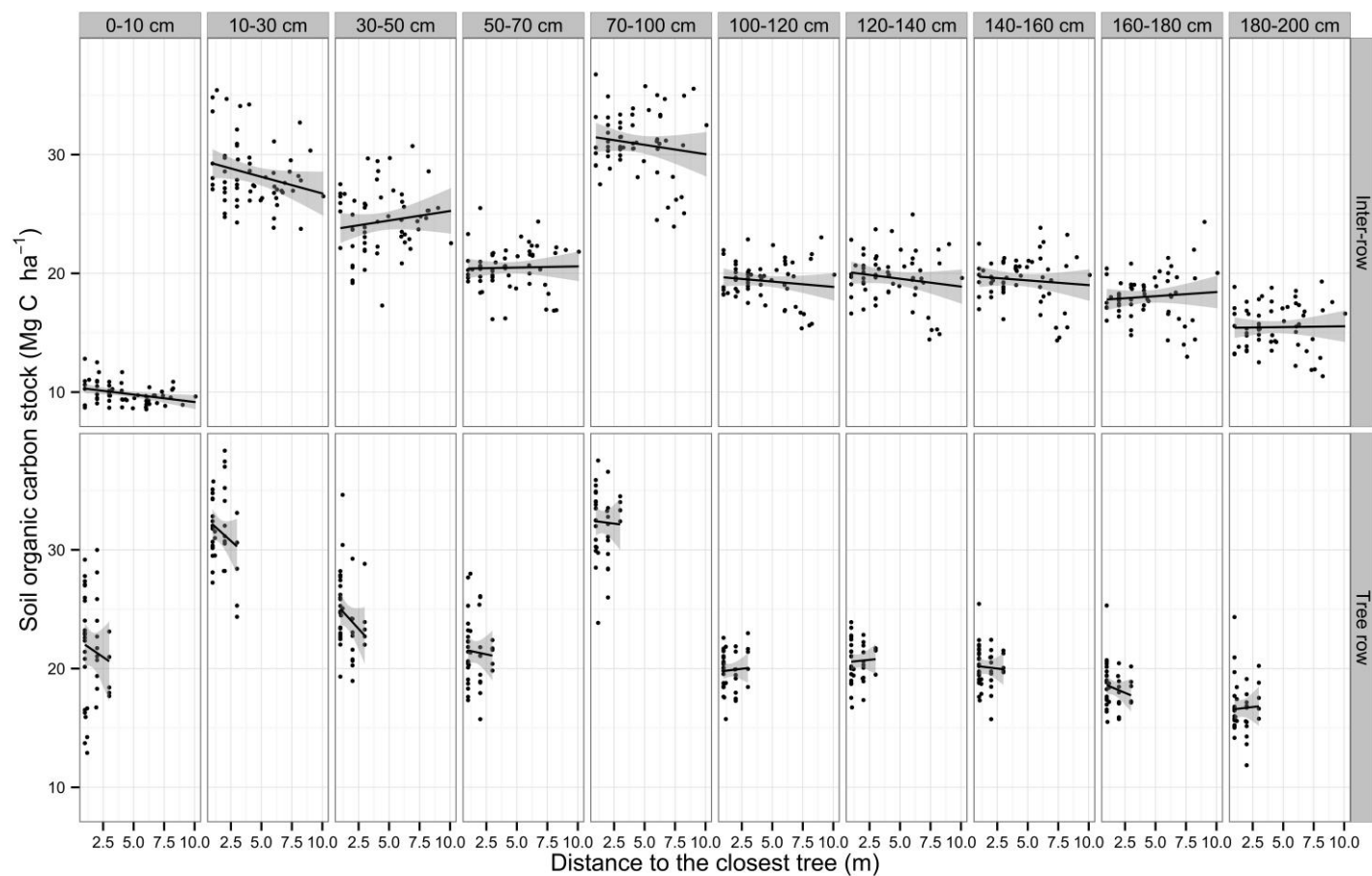
470 Compared to the control, inter-rows accumulated 115 ± 33 kg C ha<sup>-1</sup> yr<sup>-1</sup> for an ESM of 4000  
471 Mg ha<sup>-1</sup> (26-29 cm) (Table 2), and 202 ± 45 kg C ha<sup>-1</sup> yr<sup>-1</sup> for an ESM of 15700 Mg ha<sup>-1</sup> (93-  
472 98 cm). SOC accumulation rates in the agroforestry plot compared to the control were 248 ±  
473 31 kg C ha<sup>-1</sup> yr<sup>-1</sup> for an ESM of 4000 Mg ha<sup>-1</sup>, 350 ± 41 kg C ha<sup>-1</sup> yr<sup>-1</sup> an ESM of 15700 Mg  
474 ha<sup>-1</sup>, and 183 ± 48 kg C ha<sup>-1</sup> yr<sup>-1</sup> an ESM of 31500 Mg ha<sup>-1</sup> (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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482 Figure 5. Soil organic carbon stocks (Mg C ha<sup>-1</sup>) in the agroforestry plot as a function of depth, location (tree row vs. inter-row) and  
 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<sup>-1</sup>); RMSQE, root mean  
 487 squared error (Mg C ha<sup>-1</sup>); AIC, AIC of the spatially correlated model; AIC.ns, AIC of the non-spatially correlated model;  $\beta_0$  and  $\beta_1$  the  
 488 fixed effects (Mg C ha<sup>-1</sup>). 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	$\beta_0$	$\beta_1$	Nugget	Sill	Range	Nugget to Sill ratio
Agroforestry	0-30	0.99	0.36	-0.004	20.7	585	<b>583</b>	38.1	14.8	19.7	1.3	15.2	0.94
	0-100	0.99	0.45	-0.010	43.3	<b>662</b>	665	114.1	16.4	36.0	16.3	12.8	0.69
	0-200	0.98	0.39	0.055	123.1	<b>769</b>	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	<b>357</b>	35.9	-	2.4	0.2	19.4	0.93
	0-100	1.01	0.50	0.061	25.7	<b>578</b>	579	111.2	-	20.2	11.0	12.6	0.65
	0-200	0.98	0.40	0.519	57.5	<b>665</b>	681	208.9	-	16.4	85.8	6.3	0.16

490

### 3.6. Spatial distribution of SOC stocks

491 The AIC (Table 4) of the spatially correlated model were less than that of the spatially  
492 uncorrelated model for 2 depths (0-100 cm and 0-200 cm for the agroforestry and the control  
493 plots), indicating that spatial correlation should be included in the model of variation. We  
494 tested several models of spatial variation and retained the spherical model (Webster and  
495 Oliver, 2007). For top soil depth of the two plots (0-30 cm), the AIC of the spatially  
496 uncorrelated model was slightly the smallest indicating that the residual variation could be  
497 independent once fixed effects had been included in the model. But the difference was very  
498 small so we considered the spatially correlated model for the rest of the study. The cross-  
499 validation results confirmed the validity of the fitted LMM. The nugget to sill ratio measures  
500 the unexplained part of the observed variability. The smallest value was observed for the 0-  
501 200 cm depth in the control plot and the higher was observed for the 0-30 cm depth in both  
502 plots. When mapping the SOC stocks for three fixed depths with the BLUP in the two plots, a  
503 clear pattern can be observed in the agroforestry plot, with high SOC stocks in the tree rows  
504 (Fig. 6). The fitted fixed effects indicate that, in average, the SOC stocks were 15 to 20 Mg C  
505 ha<sup>-1</sup> higher in the tree rows to 30 to 200 cm depth (Table 4). At the opposite, the control plot  
506 did not exhibit any spatial pattern.

507

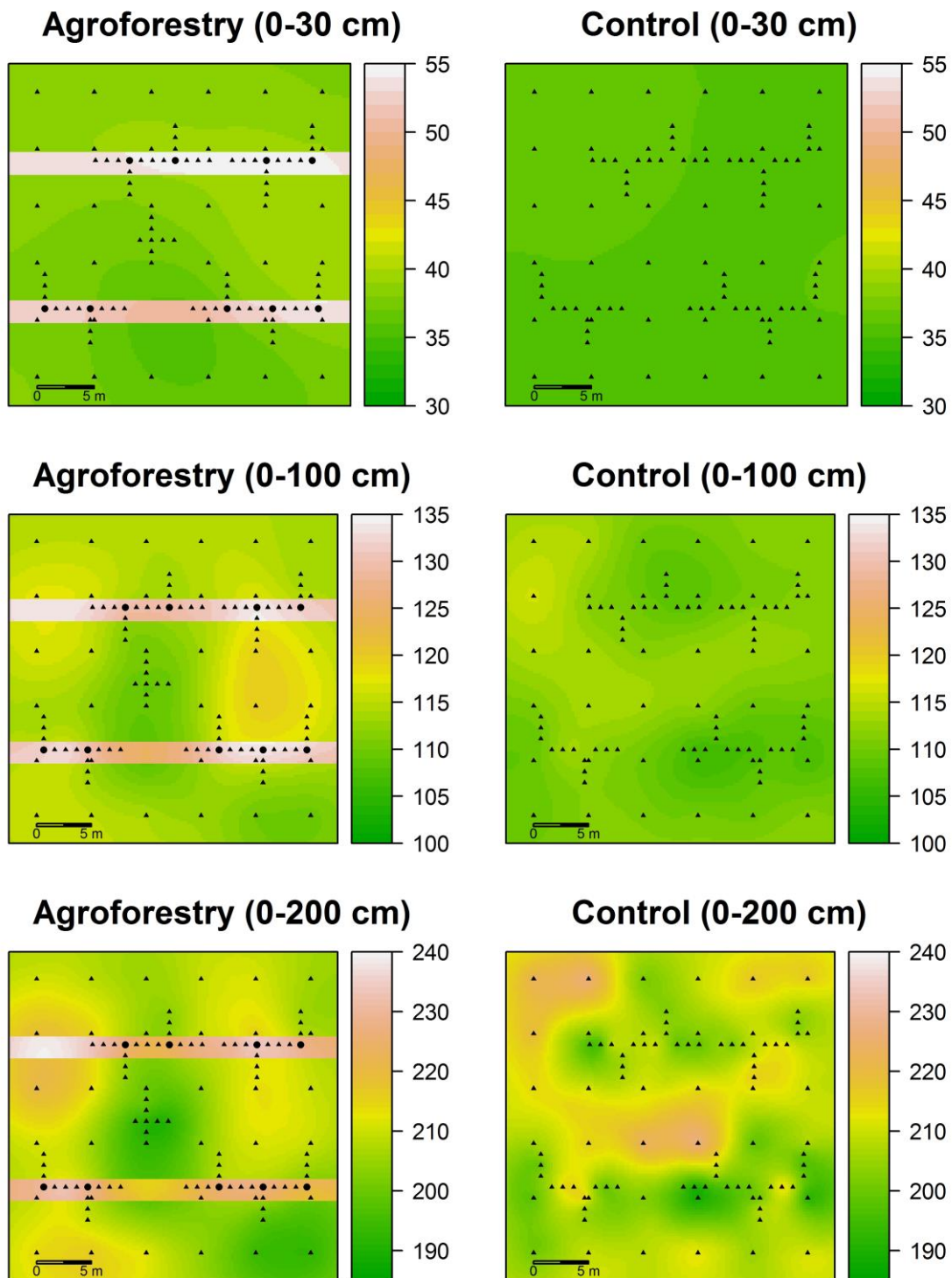
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514 Figure 6. Kriged maps of cumulated soil organic carbon stocks ( $\text{Mg C ha}^{-1}$ ) in the  
 515 agroforestry and in the control plot.

516

517 *3.7. Organic carbon distribution in soil fractions*

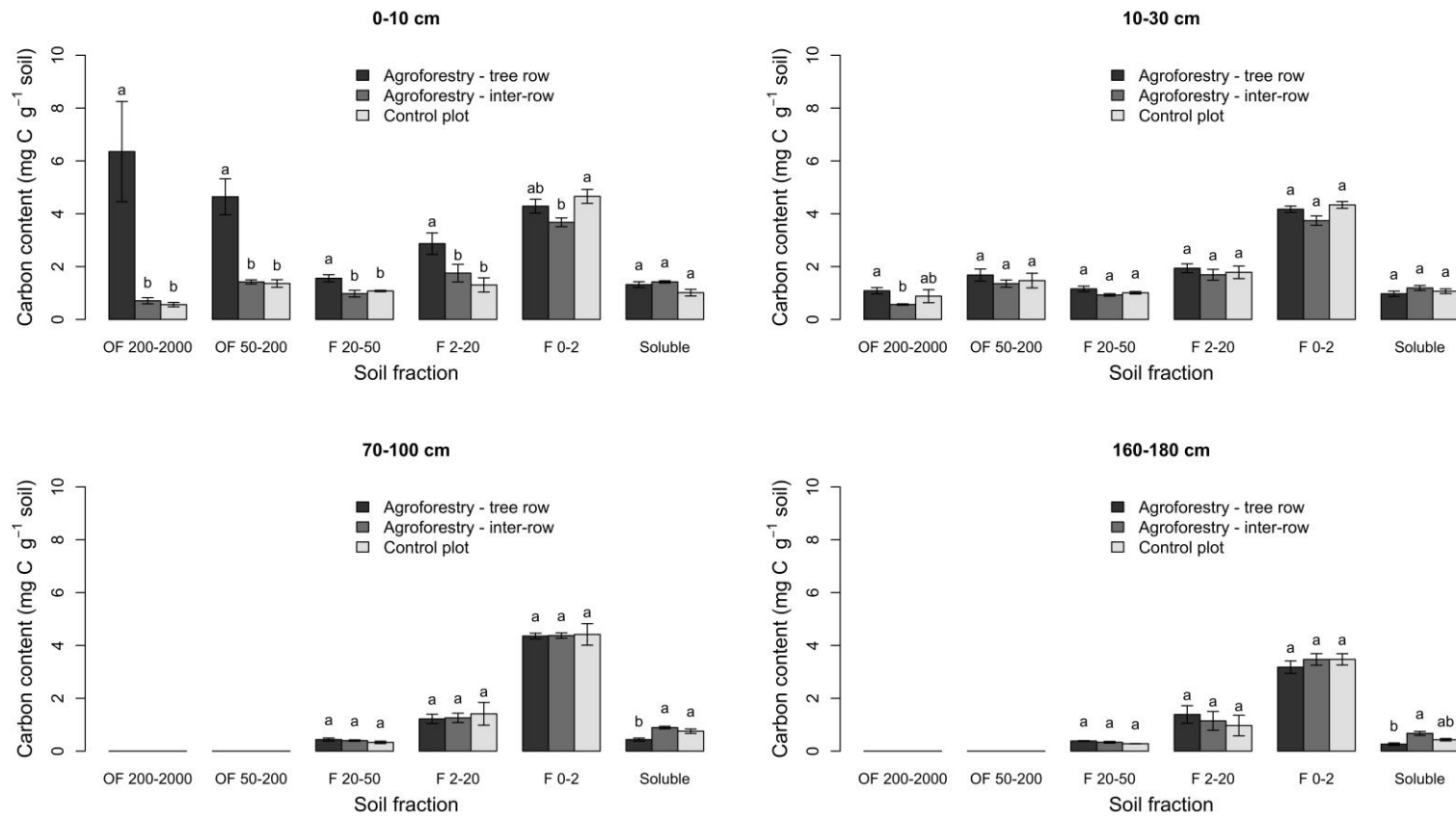
518 An average mass yield of 98% and an average carbon yield of 96% were obtained, showing  
519 the quality of the particle size fractionation. Furthermore, the variation between soil texture  
520 and soil fractionation was only 5-6% (data not shown). Soil segments used for soil  
521 fractionation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

525 For 0-10 cm depth, the distribution of OC in particle size fractions was strongly modified in  
526 the tree rows, with an important increase of C in particulate organic matter (POM) fractions  
527 (50-200  $\mu\text{m}$  and 200-2000  $\mu\text{m}$ ) compared to the inter-row and to the control (Fig. 7). An  
528 increase of C in silt size fractions (2-20  $\mu\text{m}$  and 20-50  $\mu\text{m}$ ) of the tree rows compared to the  
529 inter row and to the control was also observed. Significantly higher C concentrations in the  
530 clay fraction (0-2  $\mu\text{m}$ ) were observed in the tree row than in the inter-row (Fig. S3), but it was  
531 not the case for the amount of C in the clay fraction per gram of soil (Fig. 7).

532 Similar trends in C distribution in fractions were observed at 10-30 cm depth compared to 0-  
533 10 cm, although with much smaller differences (Figs. 7, S3). At deeper depths (70-100 and  
534 160-180 cm) there were no differences between the three locations (tree row, inter-row and  
535 control) except a lower amount of C in the soluble fraction in the tree row. The potential SOC  
536 saturation of particles  $<20 \mu\text{m}$  was not reached at any depths (Table 5), and the SOC deficit  
537 was high. The saturation capacity was far from being reached, as it amounted 17 to 40% of  
538 saturation capacity in the tree rows.

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540



541

542 Figure 7. Organic carbon contents in each soil fraction ( $\text{mg C g}^{-1}$  soil). Error bars represent standard errors ( $n=6$  in the control,  $n=5$  in the inter-  
 543 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  
 544 size ( $\mu\text{m}$ ). Means followed by the same letters do not differ significantly at  $p=0.017$  (Dunn's test with Bonferroni correction).

545 Table 5. Soil organic carbon saturation of the fractionated soil samples in the agroforestry plot.  $SOC_{sat-pot}$ , potential SOC saturation ( $mg\ C\ g^{-1}$ );  
 546  $SOC_{cur}$ , current mean SOC concentration of the fine fraction  $<20\ \mu m$  ( $mg\ C\ g^{-1}$ );  $SOC_{sat-def}$ , SOC saturation deficit ( $mg\ C\ g^{-1}$ );  $SOC_{stor-pot}$ ,  
 547 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  
 548 layers are only indicative.

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



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<sup>-1</sup>, were comparable to reported values for the Mediterranean  
564    region, i.e., 25 to 50 Mg C ha<sup>-1</sup> (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<sup>-1</sup> 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<sup>-1</sup> between 1.2 and 2.0 m soil depth in 18 years, i.e.,  
591 about 180 kg C ha<sup>-1</sup> yr<sup>-1</sup>. 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<sup>-1</sup>) in the agroforestry treatment than in the cropped alleys (17 mg C g<sup>-1</sup>), or the  
604 arable control (17.1 mg C g<sup>-1</sup>). 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<sup>-1</sup> yr<sup>-1</sup> 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<sup>-1</sup> yr<sup>-1</sup> 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

622 of tree density in Canada, Bambrick et al., (2010) and Peichl et al., (2006) also found no  
623 effect of the distance to the trees on SOC stocks to 20 cm depth. They also suggested that the  
624 18 m high poplar trees distributed litterfall equally in the crop alleys. Close to the tree rows (1  
625 to 2 m distance), the intercrop had a lower yield (15% less in 2012) compared to the middle of  
626 the inter-row at the study site (Dufour et al., 2013). On the contrary, tree fine root density was  
627 higher close to the tree rows (2.79 t DM ha<sup>-1</sup> between 0 and 1.5 m from the tree row in the  
628 inter row, and to 4-m soil depth) than in the middle of the inter-rows (1.32 t DM ha<sup>-1</sup> between  
629 3 and 4.5 m from the tree row in the inter row, and to 4-m soil depth) (Cardinael et al., 2015).  
630 Thus, lower carbon inputs from crop residues close to the tree rows may be counterbalanced  
631 with higher inputs from tree fine root mortality, explaining homogeneous distribution of SOC  
632 stocks within the inter-row (Bambrick et al., 2010; Peichl et al., 2006). In the tree row,  
633 homogeneous distribution of SOC stocks may be explained by the short distance between  
634 trees and by the presence of abundant herbaceous vegetation.

635

#### 636 *4.4. Agroforestry systems: an efficient land use to improve SOC stocks*

637 Compared to other agroforestry systems having about the same tree density, a lower SOC  
638 accumulation rate in 0-30 cm (0.25 Mg C ha<sup>-1</sup> yr<sup>-1</sup>) was observed at our site. Peichl et al.  
639 (2006) reported a SOC accumulation rate of 1.04 Mg C ha<sup>-1</sup> yr<sup>-1</sup> (0-20 cm) in a 13-year old  
640 temperate barley (*Hordeum vulgare* L.)-poplar intercropping system (111 trees ha<sup>-1</sup>). In a 21-  
641 year old agroforestry system in Canada where poplars were intercropped with a rotation of  
642 wheat (*Triticum aestivum* L.), soybean (*Glycine max* (L.) Merr.) and corn (*Zea mays* L.),  
643 Bambrick et al. (2010) estimated a SOC accumulation rate of 0.30 Mg C ha<sup>-1</sup> yr<sup>-1</sup> (0-20 cm).  
644 Our lower accumulation rate may be explained by warmer climate, higher temperatures  
645 enhancing OM decomposition (Hamdi et al., 2013). Moreover, valuable hardwood species

646 like walnut trees have a slower growing rate than fast growing species like poplar (Teck and  
647 Hilt, 1991), and therefore for a same tree age, the amount of OC inputs (leaf litter, fine roots)  
648 to the soil is lower for slow growing species.

649 Together with other climate-smart farming practices (Lipper et al., 2014), alley-cropping  
650 agroforestry systems have the potential to enhance SOC stocks and to contribute to climate  
651 change mitigation (Nair et al., 2010; Pellerin et al., 2013). No-till farming is a commonly  
652 cited agricultural practice supposed to have a positive impact on SOC stocks. But recent meta-  
653 analyses showed this practice had no effect on SOC stocks to 40 cm depth (Luo et al., 2010)  
654 or a smaller one ( $0.23 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  to 30 cm depth) than previously estimated (Virto et al.,  
655 2011). A meta-analysis also revealed that the inclusion of cover crops in cropping systems  
656 could accumulate SOC at a rate of  $0.32 \pm 0.08 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  to a depth of 22 cm (Poeplau  
657 and Don, 2015). At our site, we found a mean SOC accumulation rate of 0.13 in 0-30 cm in  
658 the inter-rows compared to the control. This rate reached  $0.25 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  for the whole  
659 agroforestry system. A companion study at this site estimated that the tree aboveground C  
660 stock was  $117 \pm 21 \text{ kg C tree}^{-1}$  (unpublished data). With 110 trees  $\text{ha}^{-1}$ , total organic carbon  
661 (SOC to 1 m soil depth + aboveground tree C) accumulation rate was  $1.11 \pm 0.13 \text{ Mg C ha}^{-1}$   
662  $\text{yr}^{-1}$ , making agroforestry systems a possible land use to help mitigating climate change (Lal,  
663 2004; Lorenz and Lal, 2014).

664

#### 665 *4.5. A long-term SOC storage?*

666 Most of additional SOC in the agroforestry plot compared to the control plot was located in  
667 coarse soil fractions (50-200  $\mu\text{m}$  and 200-2000  $\mu\text{m}$ ). These soil fractions are assumed to  
668 contain labile fractions (Balesdent et al., 1998), that are not stabilized by interaction with  
669 clays and thus prone to be decomposed by soil microorganisms. Our site might not be old

670 enough to observe a difference in the fine soil fractions as changes in the clay fractions are  
671 often long-term processes (Balesdent, 1996; Balesdent et al., 1988). For example, Takimoto et  
672 al., (2008) found in a 35-year-old *Faidherbia albida* parkland in Mali, that the silt + clay soil  
673 fraction ( $< 53 \mu\text{m}$ ) was enriched in C at depth compared with treeless systems. But on the  
674 other hand, Howlett et al., (2011) did not observe any difference for the same soil fraction in a  
675 80 year-old Dehesa cork oak (*Quercus suber* L.) silvopasture, but they found that C storage in  
676 the macroaggregate fraction (250–2000  $\mu\text{m}$ ) was 68% greater underneath *versus* away from  
677 the tree canopy (in 0-25 cm). Several studies have demonstrated that protection of C within  
678 the macroaggregate size class was affected by afforestation (Del Galdo et al., 2003; Deneff et  
679 al., 2013) and cessation of tillage (Tan et al., 2007). The fractionation method that was used in  
680 this study disrupted macroaggregates, and part of this labile fractions could be located within  
681 them and therefore be physically protected from decomposition by soil microorganisms (Six  
682 et al., 2000). Further work will focus on this aspect in order to estimate the amount of  
683 particulate organic matter located in soil aggregates. Calculation of SOC saturation revealed a  
684 high deficit of SOC of this soil compared to the theoretical value, suggesting that  
685 accumulation of SOC due to the agroforestry system could continue for decades before  
686 reaching saturation.

687

## 688 **5. Conclusion**

689 This study showed the potential of agroforestry systems to increase SOC stocks. However,  
690 despite a deep tree rooting system, additional SOC was mainly located in topsoil layers, and  
691 in labile organic fractions, making this C storage vulnerable. Tree rows were shown to be a  
692 key factor for SOC storage in alley cropping systems. Combining agroforestry systems with  
693 no-till or permanent cover systems could be a very efficient way to increase SOC stocks, but  
694 more research is needed on this aspect. To fully estimate the impact of agroforestry systems

695 on SOC sequestration, other aspects should be taken into account. For instance, higher SOC  
696 stocks in the inter-rows could increase soil fertility and reduce the need for chemical fertilizer,  
697 contributing indirectly to a reduction of greenhouse gases emissions; further work should  
698 therefore focus on nutrient cycling in these systems.

699

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991 **Supplementary materials**

992 Table S1. Soil organic carbon stocks (Mg C ha<sup>-1</sup>) and SOC accumulation rates (kg C ha<sup>-1</sup> yr<sup>-1</sup>)  
 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).

Soil depth (cm)	Cumulated SOC stocks (Mg C ha <sup>-1</sup> )		Δ SOC stocks (Mg C ha <sup>-1</sup> )	SOC accumulation rates (kg C ha <sup>-1</sup> yr <sup>-1</sup> )
	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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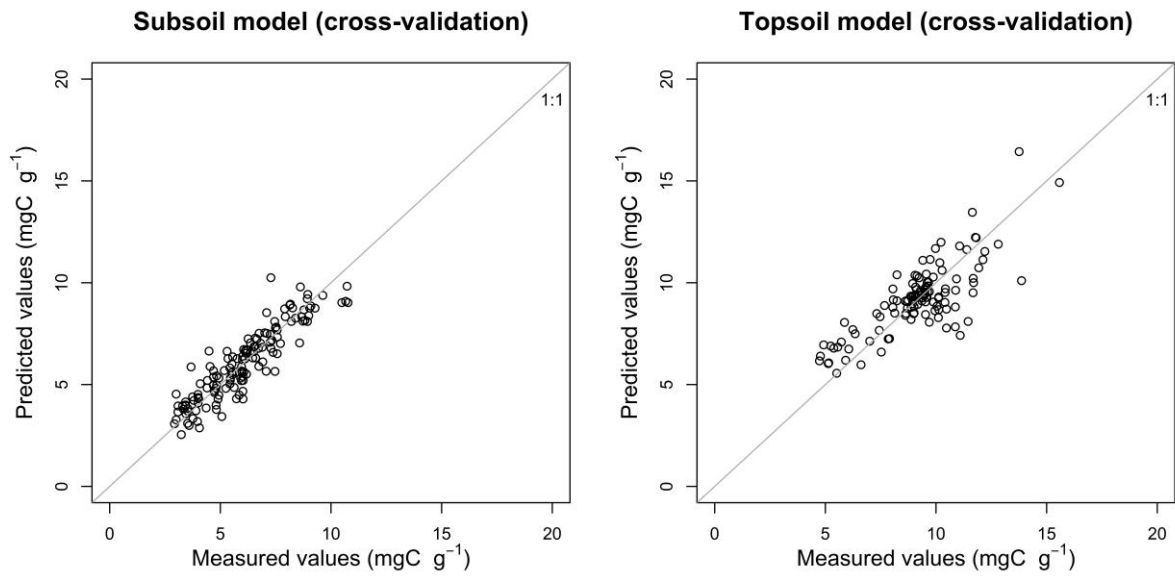
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1005 Figure S1. Measured and cross-validation predicted values of soil organic carbon  
1006 concentrations for the topsoil and subsoil models.

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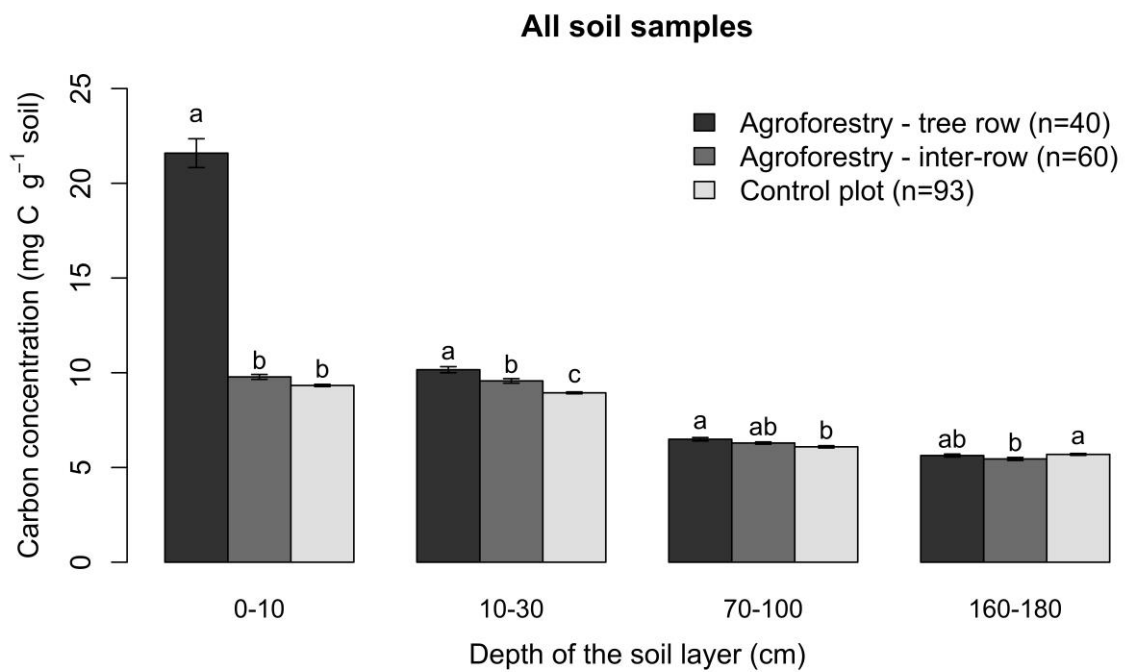
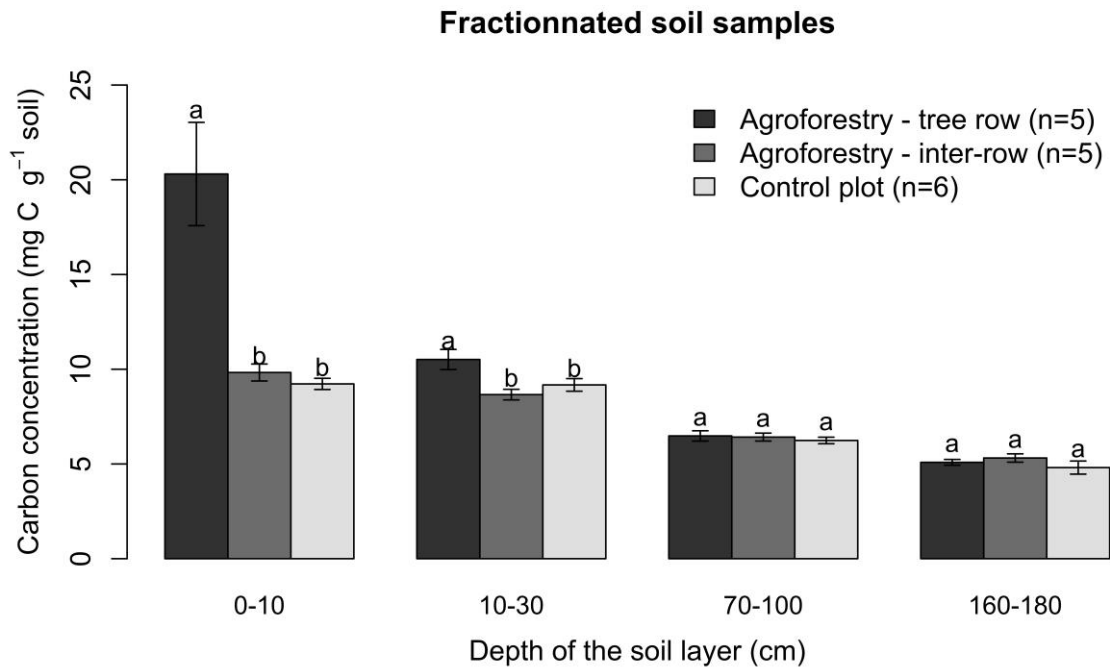
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1018 Figure S2. Carbon concentration of bulk fractionated samples. Error bars represent standard

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

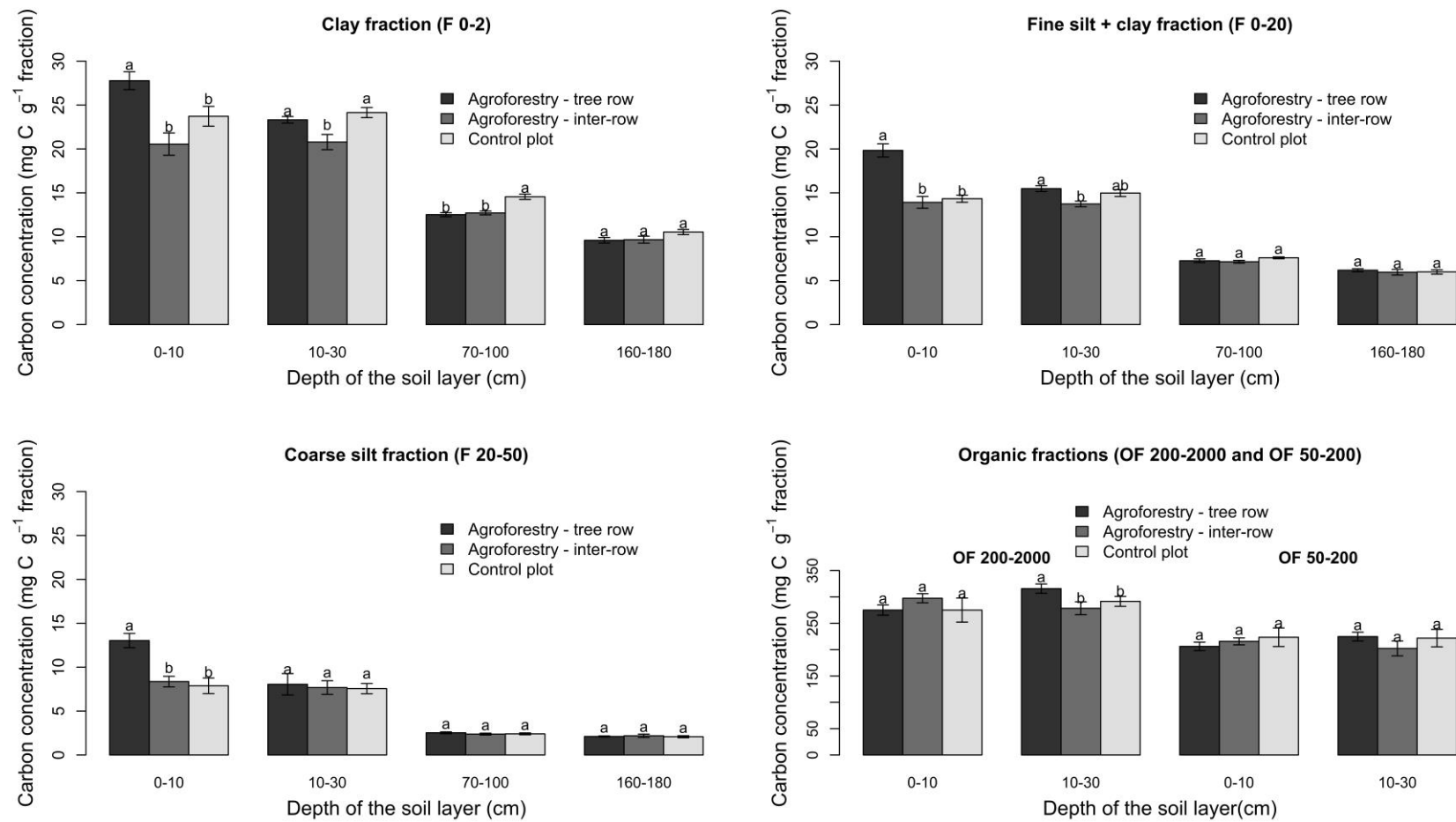


Figure S3. Carbon concentration of each soil fraction. Error bars represent standard errors (n=6 in the control, n=5 in the inter-row and in the tree row). OF = Organic fraction, F = organo-mineral fraction. Means followed by the same letters do not differ significantly at p=0.017 (Dunn's test with Bonferroni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