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**CONTRIBUTION AND STABILITY OF FOREST DERIVED SOIL ORGANIC CARBON
DURING WOODY ENCROACHMENT IN A TROPICAL SAVANNA. A CASE STUDY IN
GABON**

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13 **Abstract**

14 In this study, we quantified the contribution of forest-derived carbon (FDC) to the soil organic C
15 (SOC) pool along a natural succession from savanna (S) to mixed Marantaceae forest (MMF) in the
16 Lopè National Park, Gabon. Four 1-hectare plots, corresponding to different stages along the
17 natural succession, were used to determine the SOC stock and soil C isotope composition ($\delta^{13}\text{C}$) to
18 derive the FDC contribution in different soil layers down to 1 m depth. Besides, to investigate
19 changes in SOC stability, we determined the ^{14}C concentration of SOC to 30 cm depth and derived
20 turnover time (TT). Results indicated that SOC increased only at the end of the succession in the
21 MMF stage, which stored 46% more SOC (41 Mg C ha^{-1}) in the 0-30 cm depth than the S stage
22 ($28.8 \text{ Mg C ha}^{-1}$). The FDC contribution increased along forest succession affecting mainly the top
23 layers of the initial successional stages to 15 cm depth, and reaching 70 cm depth in the MMF
24 stage. The TT suggests a small increase in stability in the 0-5 cm layer from S (146 years) to MMF
25 (157 years) stages. Below 5 cm the increase in stability was high, suggesting that FDC can remain
26 in soils for a much longer time than savanna derived C. In conclusion, the natural succession
27 towards Marantaceae forests can positively impact climate change resulting in large SOC stocks,
28 which can be removed from the atmosphere and stored for a much longer time in forest soils
29 compared to savanna soils.

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33 **Introduction**

34 Over the past century, woody plant encroachment has been a widespread phenomenon in grassland
35 and savanna ecosystems worldwide (Eldridge et al. 2011). Land use management practices such as
36 reduction of grazing and fire frequency are often the cause. In addition, ongoing climate change,
37 historic atmospheric carbon dioxide (CO₂) enrichment and the introduction of exotic plant species
38 are also potentially important drivers contributing to forest expansion into savannas (Buitenwerf et
39 al. 2012). Current trends in atmospheric CO₂ enrichment may exacerbate shifts from grass to woody
40 plant dominated ecosystems, especially where the invasive woody vegetation is capable of
41 symbiotic nitrogen fixation. Expansion of woody plants into savannas may also be favoured by
42 increases in atmospheric nitrogen (N) deposition (Boutton and Liao 2010). In addition to
43 influencing vegetation composition, woody encroachment may lead to changes in C storage and
44 dynamics. Understanding the consequences of forest expansion on ecosystem and soil organic C
45 (SOC) stocks is crucial for improving predictions of current and future effects of land use and land
46 cover changes on the global C cycle.

47 Savannas have a discontinuous tree layer which lies within a continuous herbaceous layer
48 (Veenendal et al. 2014). In tropical savannas, grasses and sedges are mainly C₄ species, i.e. use the
49 C₄ photosynthetic pathway, and they are typically enriched in ¹³C ($\delta^{13}\text{C} \approx -12\text{‰}$) compared to most
50 trees, shrubs and herbaceous plants, which utilise the C₃ photosynthetic pathway ($\delta^{13}\text{C} \approx -27\text{‰}$). In
51 fact, the C₃ metabolism is coupled with a high discrimination against ¹³C during the atmospheric
52 assimilation of CO₂ (Farquhar et al. 1989; Kohn 2010). The C isotope composition of soil organic
53 matter (SOM) in the top mineral soil is determined by inputs of organic material from the standing
54 vegetation (Boutton et al. 1998). The $\delta^{13}\text{C}$ of SOM is slightly enriched in ¹³C compared with plant
55 biomass ($\leq + 2\text{‰}$) and is stable over long-time periods (Wedin et al. 1995). Since SOM
56 accumulates over time, the $\delta^{13}\text{C}$ of deep soil layers reflects inputs of organic matter from past
57 vegetation. Thus, the $\delta^{13}\text{C}$ concentration along soil profiles can be used as an indicator of changes

58 in the abundance of C3 and C4 plants over time (Boutton et al. 1998). Taking advantage of the fact
59 that the C4-type organic matter of savanna grass is less depleted in ^{13}C than the C3-type organic
60 matter of forest trees, it is possible to partition the SOC into forest-derived C (FDC) and savanna-
61 derived C (SDC) by measuring the isotopic composition of SOC (Novara et al. 2013). This
62 information is useful for understanding SOC dynamics along the woody encroachment process and
63 the potential consequences for soil C stocks.

64 Due to the presence of a characteristic forest-savanna mosaic, Lopé National Park (LNP) in
65 central Gabon represents an ideal situation for investigating the SOC dynamics as a result of the
66 natural expansion of forests into savannas (Aubreville 1967). Anthropogenic fires have been used in
67 the area for thousands of years, and Lopé's savannas are burned annually, either as part of the Park
68 management burn plan or by fires caused by local people. Despite regular fire use, forest
69 encroachment is occurring rapidly (Jeffery et al. 2014). As a result, different stages of forest
70 colonization of soils formerly occupied by savannas can be found in the Park, with mixed
71 Marantaceae forest formations representing an intermediate successional stage between colonising
72 forests and mature forests (White 2001). Chiti et al. (2017) described the variation in SOC stocks
73 along the natural succession from savanna to mixed Marantaceae forest within the LNP, showing
74 how SOC increases as forests develop. To complement these results, we carried out another
75 sampling campaign using pseudoreplicates (Hurlbert, 1984) on the same successional stages
76 considered in the study by Chiti et al. (2017), with the aim of quantifying the contribution of FDC
77 to the observed changes in SOC. Specifically, our aims were: (a) to assess the contribution of FDC
78 versus SDC during SOC accumulation along a natural succession from savanna to mixed
79 Marantaceae forest in the LNP by using $^{13}\text{C}/^{12}\text{C}$ stable isotope ratio measurements and, (b) to
80 investigate possible changes in SOC stability along the natural succession using radiocarbon (^{14}C)
81 measurements to derive SOC turnover time (TT).

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83

84 **Materials and Methods**

85 *Area description*

86 The Lopé National Park, Gabon, (00° 10' S; 11° 36' E) covers an area of 4970 km². Mean annual
87 temperature varies between 23 °C and 31 °C while mean annual rainfall is 1474±45 mm (measured
88 from 1984 to 2004 at the Station d'Etudes des Gorilles et Chimpanzés (SEGC) in the north-east of
89 the Park). The park is part of the Congo-Ogouè Basin lowland forests and it is mainly covered by
90 closed canopy rainforest, while the north of the park is characterised by a savanna-forest mosaic,
91 with the savanna thought to be a remnant of savannas from the glacial periods (Maley et al. 2012).
92 Over twenty vegetation types have been described in the Park, including young colonising forests,
93 forests dominated by *Aucoumea klaineana* Pierre and *Sacoglottis gabonensis* (Baill.) Urb.,
94 Marantaceae forests and mature forests (White 1995). The geological substrate of the area is
95 characterised by the presence of metamorphic rocks (Schlüter 2008) and typical soils are comprised
96 within the order of Oxisols (Chiti et al. 2017).

97

98 *Vegetation types and experimental design*

99 According to the different vegetation types present within the LNP (White 2001), soil samples were
100 collected in four distinct vegetation types which represent different successional stages of the
101 natural succession from savanna to mixed Marantaceae forest: (1) savanna (S); (2) colonising forest
102 (CF); (3) monodominant forest (MF) and; (4) mixed Marantaceae forest (MMF). Compared to the
103 experimental design of the study by Chiti et al. (2017), an intermediate stage was not considered
104 due to the non-significant differences observed with the other stages. The mixed Marantaceae forest
105 is not the final stage of the succession, since it can eventually evolve into a mature forest without
106 abundant Marantaceae (White 1995). However, this climax formation does not occur in the
107 immediate vicinity, so this stage was not included in the experimental design. The S stage is
108 dominated by herbaceous species such as: *Crossopteryx febrifuga* (Afzel. ex G. Don) Benth.,
109 *Bridelia ferruginea* Benth., and *Psidium guineensis* Sw., with scarce presence of tree species (e.g.

110 no *Acacia* spp) (Cuni-Sanchez et al. 2016). The CF stage is characterised by an open canopy and
111 the presence of heliophile species such as: Okoume (*Aucoumea klaineana* Pierre), *Lophira alata*
112 Banks ex C.F. Gaertn., 1805 and *Sacoglottis gabonensis* (Baill.) Urb, which are typical of early
113 stages of forest colonisation on savannas (Cuni-Sanchez et al. 2016). The MF stage is representative
114 of areas where savanna colonisation occurred 50-100 years previously and colonising species have
115 grown up in a dense stand (White 1995), being characterised by a closed canopy of Okoume trees
116 of similar age and an open understory. The MMF stage is a mature Marantaceae forest that is
117 several hundreds of years further along the successional gradient, where remaining colonising trees
118 have matured into large trees and begun to die, allowing some recruitment of later successional
119 species. The canopy is more heterogeneous, with very large trees and an understorey dominated by
120 plant species of the *Marantaceae* and *Zingiberaceae* families (White 1995). This forest is
121 characteristic of savanna-colonising forests in central Africa with abundant megafauna (e.g. forest
122 elephants and gorillas) which greatly delay or arrest forest succession by feeding off the abundant
123 and palatable Marantaceae tree seedlings (White 1995). In all successional stages the soil type is
124 comprised within the order of Oxisols (Soil Survey Staff 2014), with a sandy clay loam texture and
125 a pH of 4-5 in the topsoil (0-30 cm depth) and a sandy clay texture and a pH of 5-5.5 in the subsoil
126 (30-100 cm depth) (Chiti et al. 2017). The soil homogeneity in the different stages excludes
127 possible bias in SOC variations due to variations in soil parameters (Chiti et al. 2017).

128

129 *Soil sampling and SOC stock determination*

130 In 2012, we delimited a one-hectare plot for each of the four vegetation types. The plots were
131 initially established for studies on vegetation dynamics (White 2001), and subsequently used for
132 studies on SOC dynamics (Chiti et al. 2017) and for aboveground biomass and C mapping studies
133 (Saatchi et al. 2011; Mitchard et al. 2012; Cuni-Sanchez et al. 2016). In each plot, a soil trench was
134 dugged for soil description down to 1 m depth. Samples from the organic horizon (litter layer) were
135 randomly collected in ten sampling points by using a 20 cm by 20 cm frame. In the same points,

136 samples from the mineral soil were collected in the topsoil at the depths of 0-5, 5-15 and 15-30 cm
137 using a cylinder of known volume (diameter = 5 cm; height = 5 cm) to determine also bulk density
138 (BD). In the case of subsoil, ten samples were collected at 30-50, 50-70 and 70-100 cm depth, using
139 an auger, while the BD was determined collecting three samples per layer in the trench using a
140 cylinder of known volume.

141 All samples were oven-dried (60 °C) to constant mass, except those for bulk density which were
142 oven-dried at 105 °C till constant mass. The litter layer was ground in a ball mill, whereas the
143 mineral soil was sieved at 2 mm and all the analyses were performed on the fine soil fraction (< 2
144 mm). In all soil samples (n=10 per layer), total C was measured on finely ground aliquots by dry
145 combustion (Thermo-Finnigan Flash EA112 CHN, Okehampton, UK), which corresponded to
146 organic C content given the absence of carbonates in these soils. The SOC stock was calculated for
147 each soil layer according to Boone et al. (1999). Changes in SOC stocks for the mineral soil were
148 discussed for two main soil compartments: 0–30 cm depth (topsoil), and 30–100 cm depth (subsoil)
149 according to the IPCC guidelines (IPCC 2006).

150

151 *Sources of SOC*

152 Besides root turnover, the sources of SOC in forests are leaf litter and small or large woody debris
153 and in savannas are litter of grasses and small trees. In the S stage, grass samples were collected
154 within the plot on three out of the ten points used for soil sampling by using a 40 cm by 40 cm
155 frame. In the forest stages CF, MF and MMF, woody debris from the main tree species contributing
156 to litter deposition (n=3 per species) were also sampled by collecting small pieces of wood directly
157 below trees. Samples were oven dried at 60 °C to constant mass, broken into small pieces using a
158 grinder in the case of wood samples, and finally milled with a ball mill.

159

160 *$\delta^{13}C$ determinations*

161 The vegetation samples collected to identify C sources to soils, and three soil samples randomly
162 selected from every layer of each stage were analysed with an isotope ratio mass spectrometer (CF-
163 IRMS, IsoPrime, GV Instruments, Cheadle Hulme, UK) connected to an elemental analyser (NA-
164 1500, Carlo Erba, Milan, Italy). Stable isotope compositions were calculated according to the
165 equation: $\delta = 1 - R_s/R_{st}$, where R_s and R_{st} are the isotope ratios of the sample and of the standard,
166 respectively. The δ -equation (in per mil; ‰) was expressed as suggested by the international
167 standard (VPDB for $\delta^{13}\text{C}$) and calculated by considering the values of the following international
168 reference materials: NBS-22 fuel oil (IAEA – International Atomic Energy Agency, Vienna,
169 Austria) and IAEA-CH6 Sucrose, for $^{13}\text{C}/^{12}\text{C}$ isotope ratio measurements. The relative precision of
170 the repeated analysis was $\pm 0.1\%$.

171 The $\delta^{13}\text{C}$ values determined for each sample were used to calculate the fraction of SOC derived
172 from the new woody vegetation (C3) and that derived from the SOC fraction of the previous
173 savanna vegetation (C4). These proportions were calculated with the mixing equation reported by
174 Gearing (1991):

175

$$176 \quad \text{Forest derived C (FDC)} = \frac{\delta^{13}\text{C}_{\text{new}} - \delta^{13}\text{C}_{\text{old}}}{\delta^{13}\text{C}_{\text{new plant}} - \delta^{13}\text{C}_{\text{old}}} \quad [1]$$

177 and

178

$$179 \quad \text{Savanna derived C (SDC)} = 1 - \text{FDC} \quad [2]$$

180

181 where FDC is the fraction (expressed as percentage) of C derived from new forest vegetation,
182 $\delta^{13}\text{C}_{\text{new}}$ is the isotope ratio of the soil sample, $\delta^{13}\text{C}_{\text{new plant}}$ is the isotope ratio of the forest vegetation
183 present in that specific stage and $\delta^{13}\text{C}_{\text{old}}$ is the isotopic ratio of the previous vegetation type, which
184 correspond to the vegetation present in the former stage of the succession.

185

186 *¹⁴C measurements*

187 An aliquot of the same samples collected at 0-5, 5-15 and 15-30 cm depth from each stage (n= 3 per
188 layer) and used for ¹³C determination was analysed for the ¹⁴C relative concentration. The samples
189 were combusted and the produced CO₂ reduced to produce Zn graphite over iron powder catalyst by
190 means of TiH₂ (at 560 °C for 8 hours), according to Marzaioli et al. (2008). Graphite was pressed
191 in Al cathodes and measured by an Accelerator Mass Spectrometer (AMS) system based on a 3MV
192 tandem accelerator at the Centre for Isotopic Research on Cultural and Environmental heritage
193 (CIRCE) of the University of Campania, Italy. Unknown samples were measured in a wheel
194 together with: i) machine (n=4) and preparation (n=3) blanks to correct for background; ii) Oxalic
195 Acid II (OXII) samples (n=4) to normalise measured ¹⁴C ratios to absolute values; iii) cellulose
196 (IAEA C3) samples (n=2) and wood (IAEA C5) standards (n=1) to check for the accuracy of the
197 entire procedure (Terrasi et al. 2008). Measured radiocarbon ratios were expressed in percent
198 Modern Carbon (pMC) according to Stuiver and Polach (1977).

199

200 *Soil organic carbon turnover time*

201 The turnover time (TT) of SOC was calculated from the ¹⁴C concentrations, using a time-dependent
202 no steady-state (TDNSS) model for S, CF and MF stages (Gaudinski et al. 2000), while for the
203 MMF stage we used a time-dependent steady-state (TDSS) model (Gaudinski et al. 2000).

204 The choice of different TT models was done because in the plots representing the first two forest
205 stages of the succession (CF and MF), aboveground biomass increased over the past 20 years
206 indicating unstable conditions, while in MMF plot no significant biomass changes were observed
207 over the same period, suggesting stable conditions for the vegetation of this stage (Cuni-Sanchez et
208 al. 2016). The S stage was also considered not stable given that it can evolve quite rapidly into CF
209 (Jeffery et al. 2014). The TDSS model relies on important assumptions: a) being at the steady state,
210 i.e. C inputs and C losses are equal; b) the ¹⁴C signature of SOC at any time depends on the ¹⁴C
211 signature of the atmosphere in previous years; c) the time-lag between the ¹⁴C value of the

212 atmosphere and new inputs to a given pool is one year for both, savanna and forest stages
213 (deciduous species); d) all C atoms in a given pool have the same probability of leaving that pool
214 (i.e., normal distribution of TT within a pool), and e) any given pool is homogenous in terms of ^{14}C
215 signature. An atmospheric radiocarbon dataset of the bomb-spike period (1950-2011) was
216 developed from Hua et al. (2013) for the southern hemisphere (SH3 zone), annually averaging all
217 available data to smooth the seasonal variability of atmospheric ^{14}C . Data gap filling and
218 extrapolation from 2011 to the year of measurements (2012) for the SH3 zone was performed using
219 the best fitting function. A pre-bomb dataset (^{14}C in the atmosphere before 1950) for the SH3 zone
220 was obtained from Levin and Hesshaimer (2000).

221 Radiocarbon concentration on the bomb-spike curve results in two possible TT's on the opposite
222 sides of the ^{14}C peak (Marín-Spiotta et al. 2008). In our case, this occurred only for ^{14}C
223 concentration higher than 104 pMC. We identified the more likely of the two solutions based on the
224 aboveground C inputs from litterfall and the SOC stock of that specific soil layer, according to
225 McFarlane et al. (2013) and Marin-Spiotta et al. (2008). For example, a ^{14}C pM value of 105.9% for
226 the 0–5 cm soil layer in the savanna corresponded to two possible TTs, 4 and 157 years. The
227 consideration that a TT of 4 years for the 0–5 cm soil layer required the same C input as the above
228 lying organic horizon, whereas a TT of 157 years required just one-third of the annual aboveground
229 litterfall, led us to assume 157 years as the most likely solution.

230

231 *Statistical analyses*

232 The statistical analyses related to the differences in C concentration, SOC stocks, $\delta^{13}\text{C}$ and ^{14}C
233 concentrations between the soil layers of the different successional stages consider the fact that this
234 study is based on simple pseudoreplication (Hurlbert 1984; Millar and Anderson 2004). We used
235 the statistical approach followed by Blanco-Canqui et al. (2006) and by Lai et al. (2014) when
236 investigating the changes in SOC and other parameters in adjacent plots. Specifically, we applied a
237 one-way ANOVA to test differences in the selected parameters (e.g. SOC stocks, $\delta^{13}\text{C}$ and ^{14}C

238 values) among the four successional stages of woody encroachment for each soil layer. The data
239 were analysed assuming a randomized experiment using the ten sampling locations within each
240 successional stage as pseudoreplicates. It is assumed that woody encroachment is mostly
241 responsible for the differences in SOC stocks, $\delta^{13}\text{C}$ and ^{14}C values among the stages because all the
242 considered plots are very close to each other. The four stages are arranged in a natural field block
243 confining the four stages on a very similar landscape position, slope, and soil features. Chiti et al.
244 (2017) reported that differences in soil texture among these stages were not significant.
245 A post hoc mean comparison was performed using Fisher's protected least significant difference
246 (LSD) method ($P < 0.001$). Pearson's correlation coefficients and associated significances were
247 calculated to assess interrelationships among the measured soil properties. The entire statistic was
248 implemented using the R software with a $p < 0.001$ (R Core Team 2016).

249

250 **Results**

251 *SOC concentration and stocks along the succession*

252 SOC concentration decreased with soil depth in all of the successional stages (Table 1). Along the
253 natural succession, SOC increased from the S to the MMF stages in most layers (Table 1). This was
254 particularly evident in the 0-5 cm layer when comparing the different stages: $12.8 \pm 0.3 \text{ g C kg}^{-1}$ (S),
255 $13.8 \pm 0.4 \text{ g C kg}^{-1}$ (CF), $17.2 \pm 0.9 \text{ g C kg}^{-1}$ (MF) and $30.7 \pm 1.9 \text{ g C kg}^{-1}$ (MMF). For the 5-15 and 15-
256 30 cm layers, no significant changes ($P > 0.001$ in all cases) were observed along the succession.
257 Below 30 cm depth the SOC increased significantly already in the first stage of the succession and
258 then remained stable along the different successional stages (Table 1).

259 In terms of C stocks, the amount of C in the litter layer (absent in the savanna plots) increased
260 along the succession: $3.6 \pm 1.5 \text{ Mg C ha}^{-1}$ in the CF, $4.8 \pm 1.9 \text{ Mg C ha}^{-1}$ in the MF, and $6.8 \pm 3.4 \text{ Mg C}$
261 ha^{-1} in the MMF (Table 1). Considering SOC stocks in the mineral soil, there were no significant
262 differences in the top 0-30 cm compartment from the savanna ($28.5 \pm 1.9 \text{ Mg C ha}^{-1}$) to the first
263 stages, CF ($31.6 \pm 2.4 \text{ Mg C ha}^{-1}$) and MF ($32.3 \pm 3.4 \text{ Mg C ha}^{-1}$), while in the MMF stage the SOC

264 stock increased significantly ($41.0 \pm 3.2 \text{ Mg C ha}^{-1}$) compared to all previous stages (Table 1). On the
265 other hand, a significant SOC increase was evident in the subsoil (30-100 cm depth) already in the
266 first stage of the succession, CF ($40.7 \pm 3.1 \text{ Mg C ha}^{-1}$), compared to savanna ($23.4 \pm 2.1 \text{ Mg C ha}^{-1}$),
267 and then remained quite stable in the following MF ($41.0 \pm 4.3 \text{ Mg C ha}^{-1}$) and MMF ($45.2 \pm 3.4 \text{ Mg}$
268 C ha^{-1}) stages (Table 1).

269

270 *Contribution from different C sources*

271 Differences in $\delta^{13}\text{C}$ values for the SOC among the stages reflect the signature of the vegetation
272 mostly contributing to C inputs (Figure 1). While in the S stage the $\delta^{13}\text{C}$ of the C source to soil was
273 -20.2‰ , when the woody species become dominant they impact greatly the $\delta^{13}\text{C}$ of SOC sources.
274 This already happened in the CF stage with values reaching -28.7‰ (Figure 1). The source of SOC
275 in the other stages clearly continue to reflect the signature of the main woody species contributing
276 to C inputs to the soil, with $\delta^{13}\text{C}$ values of the SOC source clustering at -27.4‰ and -28.8‰ for MF
277 and MMF forest stages, respectively (Figure 1). Looking at the soil, the $\delta^{13}\text{C}$ values varied in the S
278 stage from -16.7‰ at the soil surface to -22.7‰ at 70-100 cm depth (Figure 2). In the other
279 successional stages, the trend was inverted with the $\delta^{13}\text{C}$ values increasing with soil depth (Figure
280 2).

281 Within each stage, the contribution from FDC decreased normally with depth. The FDC pattern
282 between stages indicates that in the CF and MF forest stages the standing vegetation contributed to
283 the majority of the SOC in the top two layers while in the 15-30 and 30-50 cm layers less than 50%
284 of SOC derive from the standing vegetation. Below 50 cm depth no SOC deriving from the forest
285 vegetation was detected in the different layers (Figure 3). However, in the MMF forest stage all
286 SOC in the top two layers derived from forest vegetation, while in the 15-30 and 30-50 cm layers
287 the contribution was around 50%. The influence of FDC was detectable also in the 50-70 cm layer
288 although with a small contribution, about 15% of total SOC (Figure 3).

289

290 *¹⁴C concentration and SOC turnover time*

291 In the S and CF stages, the ¹⁴C concentration increased from 0-5 cm to 5-15 cm and decreased in
292 the 15-30 cm layer, while in the MF and MMF stages always decreased with depth (Figure 4). The
293 comparison of the different stages and layers indicated a decrease in the ¹⁴C concentration from the
294 S stage to the MMF stage (Figure 4). Nevertheless, even small differences in ¹⁴C concentration can
295 produce appreciable differences in the SOC turnover time. In the 0-5 cm layer the TT become
296 longer from the S stage (146±1 yr) to the CF (203±25 yr) and MF stages (204±16 yr). In the MMF
297 stage the TT is shorter than previous stages, 157±2 yr, while remaining significantly higher than the
298 S stage (Table 2). In the other two layers, the TT was significantly longer in the MMF than in the S
299 stage, with values more than double (Table 2). In the two intermediate forest stages, CF and MF,
300 the TT also increased with stand development with values significantly longer than savanna,
301 particularly in the 15-30 cm layer.

302

303 **Discussion**

304 *Effect of woody encroachment on SOC stocks*

305 In central Africa a major vegetation change occurred three thousand years ago when savannas and
306 secondary grasslands replaced mature evergreen forests (Ngomanda et al. 2009). This vegetation
307 shift has been attributed to a regional climate change (Maley et al. 2012; Neumann et al. 2012).
308 Today, the situation is reversed with savannas being encroached by forests (Delègue et al. 2001;
309 White 2001) with an expected increase in C stocks at ecosystem level, which could potentially
310 contribute to the stabilisation of the increasing atmospheric CO₂ concentration given the extent of
311 the woody encroachment process in Africa (Mitchard et al. 2013).

312 In term of SOC variations, as soon as woody species increase, SOC increases too. The increase
313 was immediately evident for the subsoil since the first stage. Differently, SOC changes in the
314 topsoil appeared only in the last stage, the mixed Marantaceae forest, although savanna C can

315 decompose faster than SOC deriving from woody vegetation (Wynn and Bird 2007). Thus, overall,
316 SOC increased with woody encroachment in this area. Precipitation has been proposed as an
317 important factor determining the trend in SOC changes upon encroachment (Jackson et al. 2002).
318 Some studies have observed a negative relationship between precipitation and SOC changes after
319 woody plant invasion on herbaceous vegetation, with SOC levels decreasing in areas of high
320 precipitation (>1200 mm) and increasing in areas with low precipitation (Jackson et al. 2002; Guo
321 and Gifford 2002). These studies suggest that high precipitation induces higher SOC losses as
322 dissolved organic C diminishing the potential SOC accumulation. Besides, under optimal moisture
323 conditions, C mineralisation is high and SOC losses via soil respiration are higher than in drier
324 sites, where low precipitation limits microbial activity favouring SOC accumulation. However,
325 even though precipitation in the Lope National Park is well above 1200 mm, we did not detect any
326 SOC loss and, in the long term, we detected a considerable SOC increases on both 0-30 and 30-100
327 cm soil compartments. The increase in SOC stock observed in this study suggests that C inputs via
328 litter and roots are higher than C losses even if precipitation is high. In particular, the change in the
329 quality of the C inputs arriving to soil has been proved to have a significant impact on the microbial
330 biomass, and as a consequence on the SOC decomposition (Shihan et al. 2017). The combined
331 effect of increasing litter inputs along the successional stages, and the presence of herbs, which still
332 dominate the understory layer of all the stages, could be responsible for the observed long-term
333 SOC increase in the topsoil. In most of the encroached sites outside the tropical area, in mature
334 forests the herbaceous layer is usually absent or greatly reduced (Gilliam 2007). The large increase
335 in SOC observed in the subsoil at the beginning of the woody encroachment process is most likely
336 the result of a different root distribution along the soil profile with deeper root systems of tree
337 species compared to savanna herbaceous species, which are concentrated in the topsoil.

338

339 *Forest contribution to soil carbon during natural succession*

340 In terms of contribution from the different vegetation types to the SOC pool, the $\delta^{13}\text{C}$ values
341 observed in top two layers of the colonising and monodominant forest stages, revealed an important
342 contribution from FDC indicating that forest vegetation can rapidly contribute to the SOC of
343 surface layers. The contribution of FDC below 30 cm depth, about 25% (colonising forest) and 20%
344 (monodominant forest) of the total SOC in the 30-50 cm layer, is probably responsible for the SOC
345 stock increase observed in the subsoil compartment of the first stages along the natural succession.
346 The $\delta^{13}\text{C}$ values observed in the 50-70 cm and 70-100 cm layers of the first two stages, where the
347 FDC contribution was absent, suggest that the intermediate forest stages developed over a soil
348 formerly occupied by savanna vegetation. On the other hand, the $\delta^{13}\text{C}$ difference between the SOC
349 source and the values observed in the lower layer of the savanna stage (+2.5‰) was lower than the
350 enrichment reported in the literature, of up to +4‰ (Sanaiotti et al. 2002; Ehleringer et al. 2000),
351 indicating that SOC inputs originate mostly from savanna vegetation. Similarly, in the forest stages
352 a difference of +8.2‰ (CF), +9.1‰ (MF) and +6.6‰ (MMF) between the signatures of the SOC
353 source and the lower soil layer revealed that SOC does not derive from forest vegetation only,
354 suggesting that all forest stages developed over a soil formerly occupied by savanna. In forest
355 ecosystems, the $\delta^{13}\text{C}$ of SOC commonly increases with soil depth by 1–3‰ relative to that of the
356 litter layer, but the reasons for this increase are not yet fully understood (Nadelhoffer and Fry 1988;
357 Wynn et al. 2005). According to Wynn et al. (2006), we quantified the uncertainty related to the
358 occurrence of a possible fractionation, which could be due to mixing of SOC from different sources
359 or ^{13}C distillation during SOC decomposing. Taking into consideration a fractionation factors of
360 0.999 (discrimination of 3‰) the measured $\delta^{13}\text{C}$ concentrations in the different layers of every
361 stage can vary of about $\pm 2.5\%$. This can results in an overestimation of the FDC contribution in
362 the different stages, which is decreasing with depth and it is varying from 16% to 18% in the 0-5
363 cm layer, and from 5% to 14% in the 30-50 cm layer. Below 50 cm of depth the FDC
364 overestimation could be possible only in the 50-70 cm layer of the last stage (about 10%), being
365 absent in the other two forest stages. Nevertheless, even taking into account the possible SOC

366 fractionation occurring along the soil depth the observed values suggest a C contribution from
367 different sources. This fact is in agreement with the findings of Oslisly et al. (2006), which dated
368 the savannas in the Lope Natural Park to more than 40,000 years ago. Given that at steady state the
369 turnover time of SOM is equivalent to the radiocarbon age, and that the SOC turnover time in the
370 mixed forest stage at 15-30 cm depth is about four centuries, the SOC from the layers below 30 cm
371 depth most likely derived from the former savanna vegetation present when the natural succession
372 took place. In the surface layers of all stages, the $\delta^{13}\text{C}$ difference between the signatures of C source
373 and that of the SOC typically resembles the ^{13}C enrichment expected during the decomposition of
374 the standing vegetation inputs (Boutton et al. 1998; Nadelhoffer and Fry 1988). These data
375 demonstrate the rapid shift in $\delta^{13}\text{C}$ signature for topsoil layers, from values characteristic of savanna
376 to values typical of forest-derived vegetation. In term of SOC stocks, the contribution from FDC in
377 the topsoil almost doubled from the colonising forest stage (21.7 Mg C ha⁻¹) to the mixed
378 Marantaceae forest stage (39.1 Mg C ha⁻¹) confirming the potential of woody vegetation to increase
379 SOC stocks. Similarly, in the subsoil, the FDC was much higher in the final stage than in previous
380 stages, despite a possible overestimation should be considered. Apart from the SOC increases
381 during the natural succession, the overall pattern of C increases at ecosystem level, as a
382 consequence of the transition from savanna to forest, is more clearly understood in the context of
383 the C stored in above ground biomass. Cuni-Sanchez et al. (2016) measured the aboveground
384 biomass in the same forest plots (savanna plot excluded), and observed an increase from colonising
385 forest (51 Mg C ha⁻¹) to monodominant forest (194 Mg C ha⁻¹) and finally to mixed Marantaceae
386 forest (247 Mg C ha⁻¹). In savannas, the aboveground C stocks can vary widely depending on tree
387 cover, from 1.8 Mg C ha⁻¹ where trees are absent, to over 30 Mg C ha⁻¹ where there is substantial
388 tree cover (Grace et al. 2006). Considering both, SOC and aboveground biomass increases during
389 the transition to forest, it become evident the huge amount of C that can be sequestered during the
390 woody encroachment process highlighting the importance of Marantaceae forest formations also for
391 climate change mitigation purposes.

392

393 *SOC stability along the natural succession*

394 The comparison of the C turnover time in soils indicated longer recycle times for the SOC in the
395 different layers within the topsoil of the forest stages than in the savanna, suggesting an effect of
396 forest vegetation in stabilising SOC. The positive effect of woody vegetation in increasing the SOC
397 stability can be explained by an increase in the recalcitrance of C inputs of woody tissues compared
398 with herbaceous vegetation (Marín-Spiotta et al. 2008). Woody plants produce compounds such as
399 waxes, suberin, cutin, and terpenoids, which are resistant to oxidation and consumption as
400 protection against parasitism and herbivory (Gleixner et al. 2001). The production of these and
401 other plant secondary compounds increase during tropical forest succession (Coley and Barone
402 1996). Compared to the 0-5 cm layer, the shorter turnover time observed in the 5-15 cm layer of the
403 savanna and colonising forest stages is probably related to a SOC leaching operated by the abundant
404 precipitations. The decrease in turnover time was particularly evident in the savanna stage, where
405 rain impacts directly the soil surface and in the colonising forest stage that still showed a not
406 uniform canopy cover. On the other hand, root depositions from herbaceous vegetation, which is
407 still abundant in the CF stage, can contribute greatly to the observed turnover time reduction, by
408 generating every year fresh C inputs, which are incorporated into the soil. Considering all the
409 stages, the trace of the ^{14}C produced with the nuclear weapon tests in the 1950's (bomb C) was
410 clearly detectable down to 30 cm depth, indicating that most of the SOC stored in the topsoil was
411 derived from the standing vegetation existing at the sites, as previously showed by the ^{13}C
412 measurements.

413 Finally, considering the two extremes of the succession, savanna and mixed Marantaceae forest,
414 the increase in FDC in the three layers included in the topsoil coincided with an increase in the time
415 of permanence of SOC in the same layers. In the final stage the time an atom of C stays in a layer
416 within the topsoil was almost double the time determined in the savanna, except in the 0-5 cm layer
417 where the increase was minimal.

418

419 **Conclusions**

420 The Marantaceae forest is an unusual successional forest for the tropics, being probably created by
421 the synergy between Marantaceae, gorillas and elephants. Because of this, such a forest type is
422 unique in respect to any other successional forests. Despite the observed results should be used with
423 caution because of the limitations caused by the use of pseudoreplicates, it is suggested that
424 Marantaceae forests have the capacity to greatly impact SOC cycling by replacing the organic
425 matter from the former savanna vegetation and increasing SOC stocks in a relatively short time.
426 Already few decades after the beginning of woody encroachment, most of the SOC in the topsoil
427 was derived from the standing woody vegetation. Furthermore, an increased stability of SOC
428 suggests that the input of carbon from Marantaceae vegetation can remain in soils for longer time
429 periods than savannah derived carbon. In conclusion, the growth of Marantaceae forest vegetation
430 into savannas had a positive impact on SOC stocks by inducing the sequestration of large amounts
431 of CO₂ from the atmosphere and by storing carbon in more stable compartments, i.e. in soils and
432 plant biomass. Therefore, woody encroachment processes, which are occurring throughout the
433 African continent and other rainforest regions across the world, may contribute significantly to the
434 terrestrial C sink by increasing stabilised forms of organic C into soil, the largest pool of the
435 terrestrial C cycle.

436

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444 Valentini R have no conflict of interest to declare.

445

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Figure legends

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Figure 1. $\delta^{13}\text{C}$ concentration (n=3) of C sources in the different stages of the natural succession.

S=savanna; CF=colonising forest; MF=Marantaceae forest; MMF=mixed Marantaceae forest.

Figure 2. $\delta^{13}\text{C}$ concentration (n=3) of SOC in the different soil layers of each stage of the natural succession. S=savanna; CF=colonising forest; MF=Marantaceae forest; MMF=mixed Marantaceae forest.

Figure 3. Contribution of forest derived C (FDC) and savanna derived C (SDC) to the total SOC stock in each layer of the colonising forest (CF), Marantaceae forest (MF) and mixed Marantaceae forest (MMF) stages.

Figure 4. ^{14}C concentration (pMC) of the SOC from the 0-5, 5-15 and 15-30 cm layers of the different stages of the natural succession. S=savanna; CF=colonising forest; MF=Marantaceae forest; MMF=mixed Marantaceae forest.

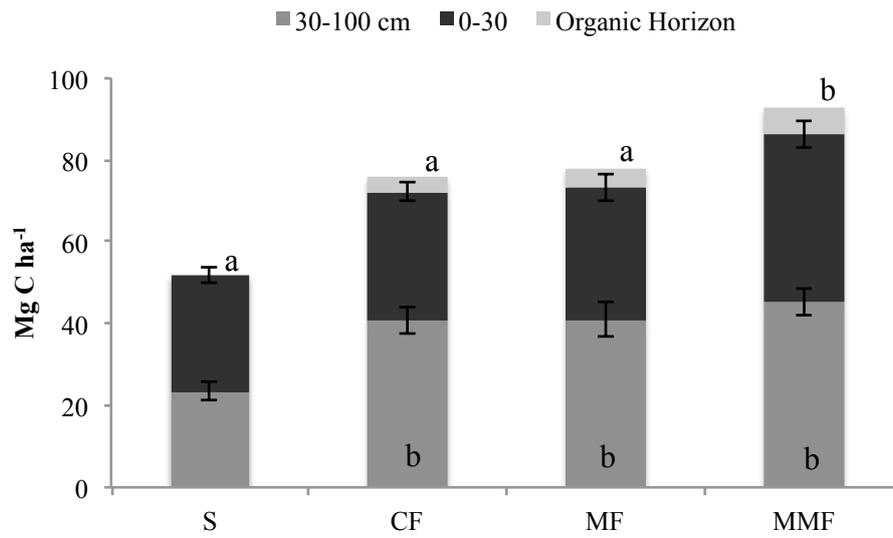


Figure 1 -

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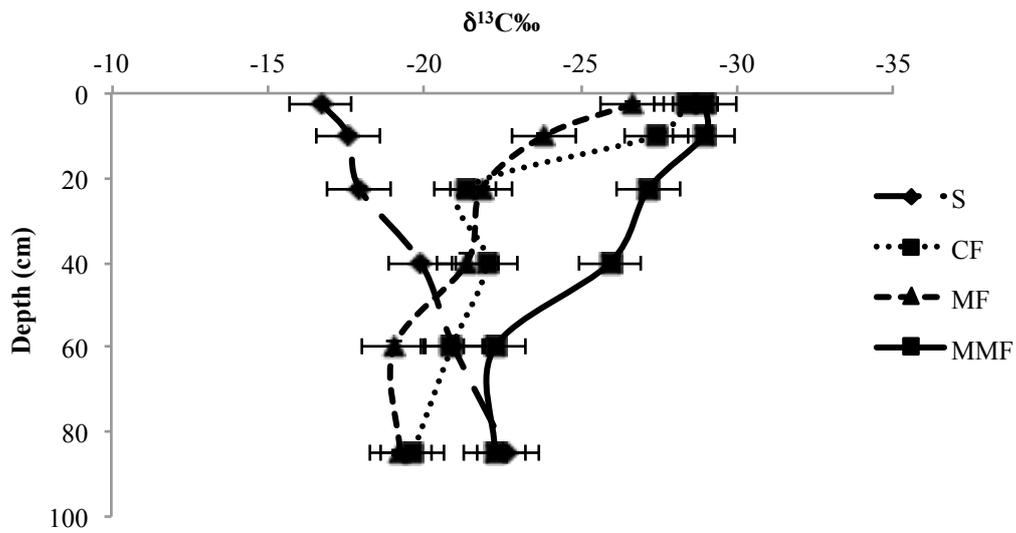


Figure 2 -

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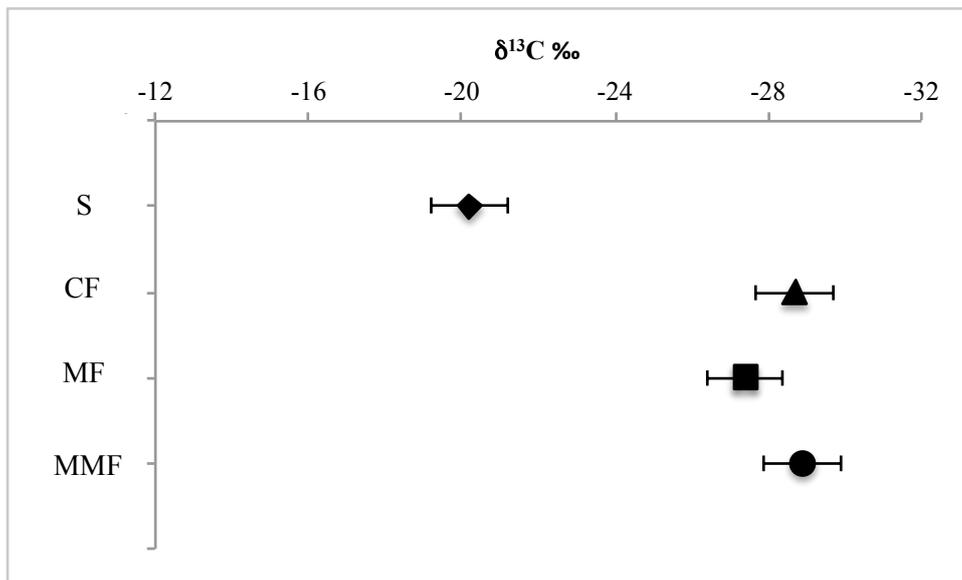
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Figure 3 -

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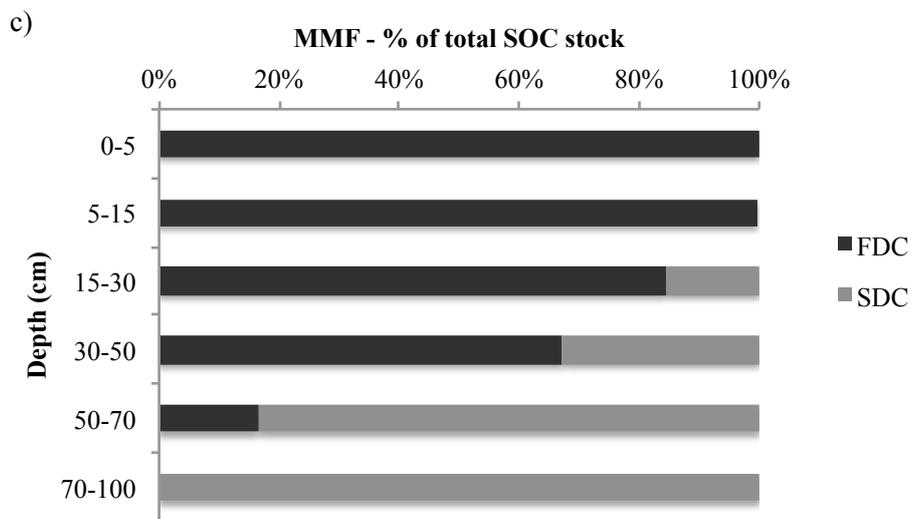
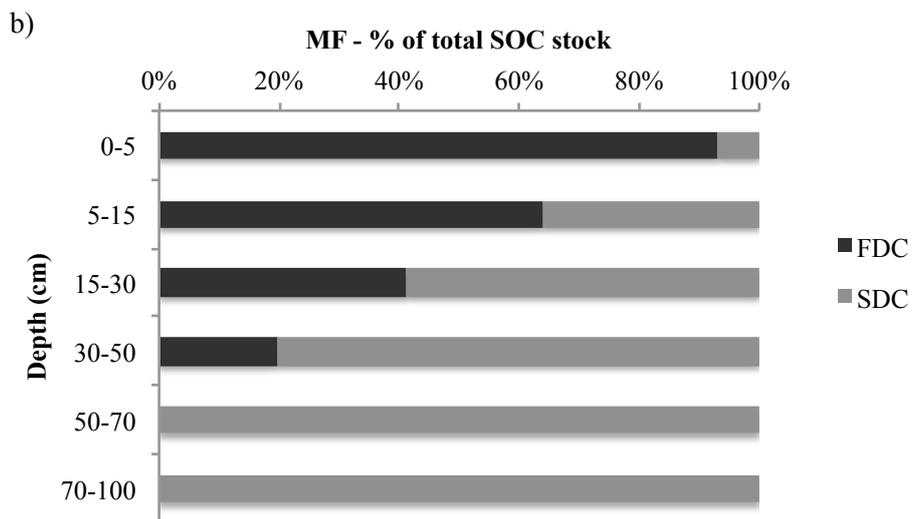
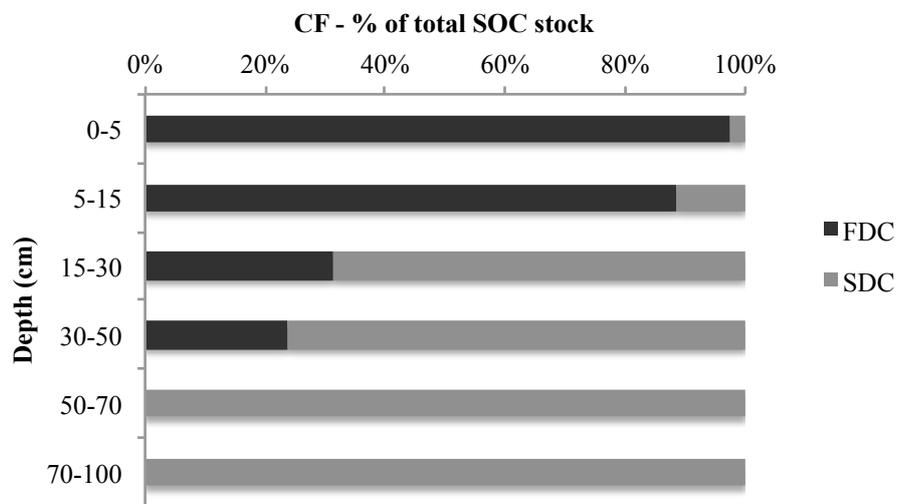
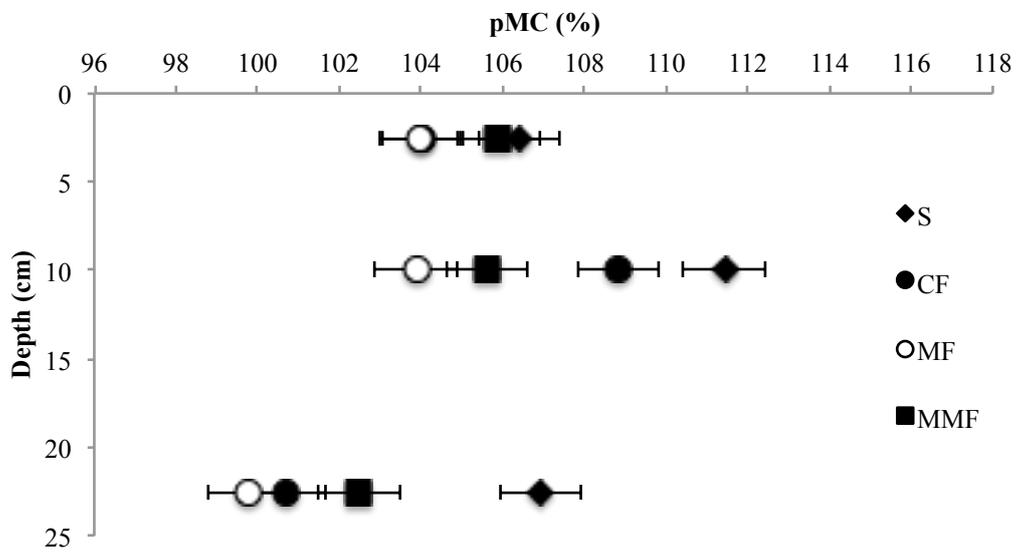


Figure 4 -

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Figure 5 -

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683 **Table 1.** Soil organic C (SOC) concentration (g C kg⁻¹) and stock (Mg C ha⁻¹) in the different soil
684 layers of each stage of the natural succession. Values are the mean of 10 samples per layer ± 1
685 standard deviation. Different letters within each line indicate differences at P<0.001 for SOC
686 concentration and stock separately (Fisher's protected LSD method).
687 S=savanna; CF=colonising forest; MF=Marantaceae forest; MMF=mixed Marantaceae forest.
688

Depth cm	S		CF		MF		MMF	
	g C kg ⁻¹	Mg C ha ⁻¹	g C kg ⁻¹	Mg C ha ⁻¹	g C kg ⁻¹	Mg C ha ⁻¹	g C kg ⁻¹	Mg C ha ⁻¹
O. Horiz.				3.6±1.5		4.8±1.9		6.8±3.4
0-5	12.8±0.3a	7.8±0.8a	13.8±0.4a	8.4±1.0a	17.2±0.9a	10.4±1.3a	30.7±1.9b	18.5±2.5c
5-15	8.7±0.4a	11.1±1.1a	9.7±0.7a	11.0±1.5a	8.5±1.2a	10.1±2.0a	8.1±0.2a	10.4±1.3a
15-30	5.9±0.5a	9.5±1.2a	8.6±0.4b	12.3±1.6b	5.9±1.0a	11.8±2.4ab	6.1±0.2a	12.0±1.4b
30-50	3.6±0.2a	7.6±0.9a	4.7±0.5a	11.6±1.7b	5.7±0.6b	13.5±2.2b	5.5±0.3b	14.6±1.8b
50-70	2.8±0.3a	6.4±0.9a	4.5±0.3b	11.4±1.5b	4.7±0.5b	11.8±1.9b	4.9±0.3b	13.7±1.6b
70-100	2.3±0.3a	9.4±1.7a	4.5±0.3b	17.7±2.1b	4.6±0.6b	15.7±3.1b	3.7±0.3b	16.9±2.4b
0-30		28.5±1.9a		31.6±2.4a		32.3±3.4a		40.9±3.2b
30-100		23.4±2.1a		40.7±3.1b		41.0±4.3b		45.2±3.4b

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706 **Table 2.** Bulk density (Mg m^{-3}) values for all the layers of mineral soil along the different stages of
 707 the natural succession toward forest. Within each column, no significant differences were observed
 708 for the same layer in the different stages (Fisher's protected LSD method). S=savanna;
 709 CF=colonising forest; MF=Marantaceae forest; MMF=mixed Marantaceae forest.
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Site	0-5 cm Mg m^{-3}	5-15 cm Mg m^{-3}	15-30 cm Mg m^{-3}	30-50 cm Mg m^{-3}	50-70 cm Mg m^{-3}	70-100 cm Mg m^{-3}
S	1.1±0.1	1.2±0.2	1.3±0.2	1.4±0.2	1.4±0.2	1.5±0.2
CF	1.1±0.2	1.2±0.2	1.3±0.1	1.3±0.2	1.4±0.2	1.6±0.3
MF	1.2±0.2	1.3±0.2	1.3±0.2	1.4±0.3	1.4±0.2	1.6±0.2
MMF	1.2±0.2	1.3±0.2	1.3±0.2	1.3±0.2	1.4±0.2	1.4±0.2

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727 **Table 3.** Soil organic C turnover time (TT) of the bulk soil samples (years) from the different stages
728 of forest succession down to 30 cm depth. Values are the mean of 3 samples per layer \pm 1 standard
729 deviation. Numbers in brackets represent the TT that was discarded. Within each column different
730 letters indicate significant differences between stages (Fisher's protected LSD method; $P < 0.001$).
731 S=savanna; CF=colonising forest; MF=Marantaceae forest; MMF=mixed Marantaceae forest.
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Site	Turnover Time		
	0-5 cm Yr	5-15 cm Yr	15-30 cm Yr
S	146 \pm 1a (5)	75 \pm 1a (13)	137 \pm 1a (6)
CF	203 \pm 25b	106 \pm 1b (9)	329 \pm 2b
MF	204 \pm 16b	208 \pm 1c	370 \pm 1c
MMF	157 \pm 2c (4)	162 \pm 1d (7)	393 \pm 25c

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