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Continuous soil carbon storage of old permanent pastures in Amazonia

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24

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27

28 **Abstract**

29 Amazonian forests continuously accumulate carbon (C) in biomass and in soil,
30 representing a carbon sink of 0.42-0.65 GtC yr⁻¹. In recent decades, more than 15% of
31 Amazonian forests have been converted into pastures, resulting in net C emissions
32 (~200 tC ha⁻¹) due to biomass burning and litter mineralization in the first years after
33 deforestation. However, little is known about the capacity of tropical pastures to restore
34 a forest C sink. Our data suggest that 24-year-old permanent tropical pastures in French
35 Amazonia can partly restore the C storage observed in native forest. A unique
36 combination of a large chronosequence study and eddy covariance measurements
37 showed that pastures stored between -1.27 ± 0.37 and -5.31 ± 2.08 tC ha⁻¹ yr⁻¹ while the
38 nearby native forest stored -3.31 ± 0.44 tC ha⁻¹ yr⁻¹. This carbon is mainly sequestered
39 in the humus of deep soil layers (20-100 cm), whereas no C storage was observed in the
40 0-20 cm layer. C storage in C4 tropical pasture is associated with the installation and
41 development of C3 species, which increase either the input of N to the ecosystem or the
42 C:N ratio of soil organic matter. Efforts to curb deforestation remain an obvious priority
43 to preserve forest C stocks and biodiversity. However, our results show that if
44 sustainable management is applied in tropical pastures coming from deforestation
45 (avoiding fires and overgrazing, using a grazing rotation plan and a mixture of C3 and
46 C4 species), they can ensure a continuous C storage, thereby adding to the current C
47 sink of Amazonian forests.

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54 **Introduction**

55 The tropical rainforest in the Amazonian basin represents about 40% of the world's
56 remaining tropical rainforest and holds one tenth of the carbon stored in terrestrial
57 ecosystems (Galford *et al.*, 2013). The Amazonian forest currently accumulates carbon
58 in biomass and in soil representing a global carbon sink of 0.42-0.65 GtC yr⁻¹ (Davidson
59 *et al.*, 2012; Brienen *et al.*, 2015). From 1960 to 2011, the Amazon basin lost
60 approximately 20% of its forests (Barona *et al.*, 2010; Karstensen *et al.*, 2013). Seventy
61 per cent of formerly forested areas have been converted into livestock pastures although
62 other land uses such as the production of soybean, maize or wood (Galford *et al.*, 2013)
63 are gaining in popularity. Deforestation causes a suppression of the forest C sink and a
64 punctual net C emission due to biomass burning and litter mineralization (aboveground
65 biomass is estimated to be around ~400 t ha⁻¹ so 200 tC ha⁻¹ in French Guiana,
66 Rutishauser *et al.*, 2010). However, the long-term effects of deforestation on the
67 regional C cycle remain uncertain and largely depend on the C dynamics of the new
68 land use.

69 After deforestation, in livestock systems, pastures are often established and
70 managed with little concern for sustainability: only one exotic C4 grass species (e.g.
71 *Brachiaria sp.*) is usually planted and managed without a forage use plan. Pastures are
72 subjected to cycles of over- or under-grazing which favour encroachment by shrubs and
73 trees (de Faccio Carvalho, 2006). After pasture establishment, burning is a common
74 technique to reduce tree encroachment (Kauffman *et al.*, 1998; Navarette *et al.*, 2016b).
75 However, pasture burning leads to emissions of radiatively active aerosols (e.g., CH₄,

76 CO, N₂O, NO_x, nonmethane hydrocarbons) (Kauffman *et al.*, 1998). Furthermore,
77 burning decreases the supply of organic C and N in soils reducing soil organic C (SOC)
78 stock of pastures. The consequence of this SOC depletion is a rapid loss of pasture
79 productivity (< 5 years) pushing farmers to abandon the degraded land and constantly
80 move and clear more land to cover their forage needs. However, other management
81 options do exist; establishing the pasture with a mixture of plant species (e.g. C₄ (grass)
82 and C₃ (legumes)) combined with appropriate grazing rotation plans makes it possible
83 to control shrubs and trees encroachment without burning (de Faccio Carvalho, 2006).
84 With favourable agricultural practices, SOC stocks can accumulate in the surface layers
85 of the soil (Trumbore *et al.*, 1995; de Moraes *et al.*, 1996; Neill *et al.*, 1997; Cerri *et al.*,
86 2003, 2004). However, SOC accumulation often ceases a few years after the pasture
87 was established (Neill *et al.*, 1997; Cerri *et al.*, 2003, 2004) suggesting that, contrary to
88 tropical forests, tropical pastures are unable to sequester C in the long-term. It is
89 generally predicted that the change in SOC stocks resulting from a land use change is
90 maximal in the first years, and decreases exponentially until the SOC pool attains a new
91 equilibrium (e.g. Lal, 2004). This assertion arises from decades of observations of SOC
92 dynamics in surface soil layers subject to different agricultural practices (Johnston *et al.*,
93 2009; Poeplau *et al.*, 2011).

94 However, there is increasing evidence that ecosystems such as permanent
95 pastures and forests can continuously accumulate carbon (Syers *et al.*, 1970;
96 Schlesinger, 1990; Knops & Tilman, 2000; Sanderman, 2003; Chen *et al.*, 2013; Yu *et*
97 *al.*, 2013). Indeed, chronosequence studies have shown SOC to be accumulated over
98 millennia without reaching equilibrium (Syers *et al.*, 1970; Schlesinger, 1990; Knops &
99 Tilman, 2000). Global networks on ecosystem CO₂ exchanges show net CO₂ uptake by
100 most undisturbed grasslands and forests, suggesting accumulation of carbon in these

101 ecosystems (Sanderman, 2003; Chen *et al.*, 2013; Yu *et al.*, 2013). The long-term
102 ecological research network (LTER) has also produced evidence of long-term
103 accumulation of SOC, though an equilibrium state can be reached in the surface layers
104 after more than 100 years (Smith, 2014). Long-term SOC accumulation preferentially
105 occurs in deep soil layers where microbial mineralization is extremely low (Fontaine *et*
106 *al.*, 2007; Callesen *et al.*, 2016; Guan *et al.*, 2016; Stahl *et al.*, 2016). Unfortunately, C
107 dynamics in deep soil layers has often been neglected, explaining the lack of robust
108 information on the long-term C sink of permanent pastures.

109 The quantification of ecosystem C sink is not easy to derive and requires specific
110 methods, sometimes in combination. The soil C sink can be directly determined by
111 measuring SOC stock change over time (e.g. with long-term ecological research, LTER)
112 (Johnston *et al.*, 2009). However, more than 5 years or even decades of monitoring are
113 required to detect significant changes in SOC stock given the high (spatial) variability
114 of soil properties. In the Amazonian region, LTER are rare and do not include grazed
115 pasture sites (Costa *et al.*, 2015). For this reason, chronosequence studies are commonly
116 used to assess SOC dynamics induced by land-use change (Neill *et al.*, 1997; Cerri *et*
117 *al.*, 2004; Carvalho *et al.*, 2010; Fujisaki *et al.*, 2015; Navarette *et al.*, 2016b; Stahl *et*
118 *al.*, 2016). This space-for-time substitution has, however, often been criticized because
119 the fields that are sampled might have had different initial soil properties (i.e. texture)
120 and be subject to different management practices. This sampling effect can be reduced
121 by a careful selection of sites based on preliminary soil descriptions and interviews with
122 farmers about their practices (Stahl *et al.*, 2016).

123 An alternative method to the direct measurement of SOC stock changes in
124 ecosystems is to measure the net ecosystem exchange (i.e. NEE) at the system
125 boundaries. This approach provides a high temporal resolution and changes in C stock

126 can be detected within one year. Hence, flux measurements may be a good method to
127 validate direct measurement of changes in soil C stocks over time as provided by
128 chronosequence studies. To our knowledge, only two pastures sites have been
129 instrumented on deforested pastures in Amazonia, a two flux towers in Brazil
130 (Goncalves *et al.*, 2013) and another one in Panama (Wolf *et al.*, 2011). Moreover, a
131 quantitative comparison of these two methods for C storage evaluation in Amazonian
132 pastures has not yet been undertaken.

133 In our study, we would like to assess whether tropical permanent pastures,
134 coming from the deforestation, maintain a long-term C sink. To this end, the C
135 dynamics of pastures and native forests was quantified in French Guiana using two
136 independent approaches: (i) a chronosequence study including the inventory of soil C
137 and N stocks to a depth of 100 cm in 24 pastures from 0.5 to 36 years old and four
138 native forests distributed across French Guiana, and (ii) measurement of NEE by eddy
139 covariance in one young (4-year-old) and one old (33-year-old) pasture included in the
140 chronosequence study, and one native forest (Bonaf *et al.*, 2008). The study only
141 included pastures managed without fires meaning that the effect of fires on long-term
142 pasture C storage cannot be determined.

143 The objectives of the present study on pastures coming from the deforestation of
144 Amazonian forest were to (1) establish the dynamics and the localization of SOC
145 storage in tropical pastures after deforestation, including deep soil layers, using two
146 independent techniques (chronosequence and eddy covariance), (2) elucidate the drivers
147 of C storage by taking into account the type (fresh versus humified organic C), the
148 origin (species composition and carbon derived from C3 and C4 plants) and the link
149 with N stock of the stored C.

150

151 **Materials and Methods**

152 *Study sites*

153 The study was conducted along the coastline in French Guiana (~ 200 km), South
154 America (5°16'54"N, 52°54'44"W). The average annual rainfall was 3041 mm and the
155 air temperature was 25.7 °C (Paracou station; Gourlet-Fleury *et al.*, 2004). The study
156 focused on a hilltop zone with clayey soils, classified as Ferralsols or Acrisols according
157 to the IUSS Working Group WRB (2006). The study comprised 24 pastures (latitude
158 4°43'31.0728''; 5°27'36.5934'' and longitude -53°57'27.8382''; -52°27'26.0172''
159 (Fig.1)), established after deforestation of native rainforest between 1976 and 2010.
160 They were distributed in four typical cattle farming areas along the Guyanese coast,
161 together with one representative native forest site in each area (Fig. 1). Farmers were
162 interviewed to determine land-use history such as the date of forest conversion or
163 agricultural management, for more information on farmers see Stahl *et al.* (2016). The
164 condition required for a site to be included in the study was that all chronosequence soil
165 forming factors have remained constant since deforestation, the age of the pasture being
166 the discriminant variable (Huggett, 1998). The following criteria were chosen to ensure
167 the sites were comparable: (i) the parent material had to be the same, i.e. Precambrian
168 metamorphic formation, which we checked on geological outcrops along nearby roads;
169 (ii) the sites had to be situated in a hilltop zone with only a slight slope to avoid major
170 transport of sediments in riverbeds; (iii) no crop rotation or land-use change should have
171 been implemented since forest conversion, nor fertilisation prior to sampling, which was
172 checked by identifying the land-use history in interviews with the farmer; and (iv) soils
173 had to be comparable in terms of pH, which was checked by soil analysis (Table
174 S1a,b,c). According to the farmers, these pastures were established by slash-and-burn
175 but had not been burned since. After the pasture establishment the shrubs were slashed

176 every two-four years in order to limit their propagation. The pastures were managed by
177 eight different farmers applying all rotational grazing plans at an animal stocking
178 density of ~ 1 LSU ha⁻¹ (Livestock Standard Unit (Palmer & Ainslie (2005))). The
179 pastures were grazed (not harvested) by cows, except for four, which were grazed by
180 goats (~ 10 goats ha⁻¹). The vegetation in the pastures was dominated by C4 species
181 (mainly the grass *Brachiaria humidicola*).

182

183 ***Botanical determination***

184 The botanical composition of pastures was determined using the Braun-Blanquet
185 method (Pott, 2011) at the start of the chronosequence survey. In each pasture, all the
186 species growing in eight quadrats (8*64 cm²) were listed and the area covered by each
187 species was estimated. Plants were also classified according to their photosynthetic
188 pathways (C3 or C4). An average of eight quadrats was calculated to determine the
189 average abundance of plant species and C3 and C4 groups per pasture. Finally, an
190 average value was calculated for the young pastures and another for the old pastures.
191 The sum of C3 and C4 is not 100% owing to presence of bare soil and the non-additivity
192 of the method. The percentages were (arcsine square root) transformed prior to analysis
193 to conform with the assumption of normality.

194

195 ***Soil sampling***

196 In each pasture and in the native forest plot, eight soil cores were sampled using a
197 jackhammer equipped with a drill gauge, volume= $3,32 \times 10^{-3}$ m³ (Cobra TT,
198 Eijkelkamp, The Netherlands). The litter layer was removed before the soil was
199 sampled. The sampling plan consisted in two parallel transects spaced 30 m apart.
200 Along each transect, the distance between each of the four soil cores was 10 m. Each

201 core was split into three layers: 0-20; 20-50; 50-100 cm, dried (48 h at 60 °C to constant
 202 mass) and sieved at 2 mm. Each layer of the eight cores was pooled proportionally to its
 203 mass to obtain a composite sample for each site. The mass of fine soil (< 2 mm), soil
 204 texture, soil organic C content and its isotopic composition ($\delta^{13}\text{C}$), soil organic N
 205 content and coarse particulate organic matter (> 200 μm) were quantified in each layer
 206 (Table S1).

207

208 ***Soil analysis***

209 Powdered soil samples were combusted and the concentration of elemental C and N in
 210 each layer was measured (IsotopeCube, Elementar, Hanau, Germany). The isotopic
 211 analyses of fine soil and roots (i.e. separation C3/ C4 species) were conducted using a
 212 Finnigan continuous flow isotope ratio mass spectrometer (Delta S, Finnigan MAT,
 213 Bremen, Germany) at the stable isotope facility at INRA, Nancy, France. Carbon
 214 isotope composition ($\delta^{13}\text{C}$, ‰) was expressed relative to the Pee Dee Belemnite
 215 standard and the analytical precision was 0.19‰ (standard deviation).

216 To determine the contribution of C derived from C3 plants (forest and pasture species)
 217 and C4 plants (pasture grasses), we applied two mass balance equations (Balesdent *et*
 218 *al.*, 1988):

$$219 \quad C_{toti} = C3_i + C4_i \quad \text{Eq 1}$$

$$220 \quad C4_i = C_{toti} \times \frac{(\delta_{soilC4i} - \delta_{soilC3i})}{(\delta_{rootC4i} - \delta_{soilC3i})} \quad \text{Eq 2}$$

221 where C_{toti} is the total C stock in the soil layer i , $C3_i$ is the C stock originating from the
 222 forest and C3 pasture species in the soil layer i and $C4_i$ is the C stock originating from
 223 grasses in the pasture, present in the soil layer i ; $\delta_{soilC4i}$ is the $\delta^{13}\text{C}$ isotopic composition
 224 in the pasture in the soil layer i and $\delta_{soilC3i}$ is the $\delta^{13}\text{C}$ isotopic composition in the native

225 forests and in the C3 pasture species in the soil layer i , and $\delta_{root_{C4i}}$ is the $\delta^{13}C$ isotopic
226 composition of the roots of C4 grass (i.e. -12.4 ‰) in the soil layer i .

227

228

229

230 ***Quantification of soil C storage***

231 The soil C storage ($tC\ ha^{-1}\ yr^{-1}$) was quantified in pastures using two independent
232 methods: (i) chronosequence, and (ii) eddy covariance measurements.

233 For the chronosequence study, the SOC stocks were quantified in three soil layers (0-20,
234 20-50 and 50-100 cm) and in two soil C pools: fresh C (particulate organic matter :
235 POM) $> 200\ \mu m$) and humified C (POM $< 200\ \mu m$) in each pasture. The POM were
236 separated from the soil by "washing" the soil samples on a $200\ \mu m$ sieve several times.

237 Fresh C accounts for the plant material (root and shoot litter) recently incorporated into
238 the soil while humified C accounts for the organic matter transformed by soil
239 microorganisms. Whether free in the soil or adsorbed on soil particles, humified C is
240 slowly decomposed by microorganisms (mean residence time can be decades or
241 thousands of years) due to its low accessibility and energetic value for soil
242 microorganisms (Sutton & Sposito, 2005; Fontaine *et al.*, 2007; Dungait *et al.*, 2012).

243 The SOC stocks were corrected to an equivalent soil mass. This correction is
244 appropriate to standardise the different sites sampled in our chronosequence approach
245 (Stahl *et al.*, 2016). First, we standardised the soil C stock in each plot by applying the
246 average fine soil mass ($< 2\ mm$) for all plots (0-100 cm, native forests and pastures, $n =$
247 28) (Ellert *et al.*, 2002; Bahr *et al.*, 2014). Second, we corrected soil C stock using the
248 same method of equivalent soil mass for each soil layer. After correcting for fine soil,
249 we added a correction for clay content (Zinn *et al.*, 2005) to account for the close

250 relationship between clay and C content. We standardised C stocks using the mean clay
251 content in each soil layer in all the plots (native forests and pastures, $n = 28$). These
252 corrections were applied to reduce the variance between sites due to the soil
253 characteristics (fine soil, clay content, see Stahl *et al.*, 2016). Finally, soil C storage is
254 given by the slope between SOC stock and time.

255

256 ***Meteorological and eddy covariance measurements***

257 To estimate the ecosystem C sink activity, net ecosystem exchange (NEE) of CO₂ was
258 measured by two flux towers in two pastures (one 4 and one 33 years old). The NEE
259 was also quantified in the nearby native forest using the same eddy covariance
260 measurements (same analyser and procedure).

261 In more details, in March 2010, one young pasture (4 years old) and one old pasture (33
262 years old) were equipped with a meteorological weather station and eddy covariance
263 towers. At each site, a meteorological station provided 30-min averaged values of global
264 radiation, air temperature, soil temperature and water content (at depths of 10 and 30
265 cm) and rainfall. All meteorological data were collected at 1 min intervals and compiled
266 as 30 min averages or sums with a CR1000 datalogger (Campbell Scientific Inc.,
267 Shepshed, UK). Each tower was composed of a fast response (20 Hz) sonic anemometer
268 (Solent R3, Gill Instruments, Lymington, UK) and a closed path CO₂-H₂O analyser (LI-
269 7000, LI-Cor Inc., Lincoln, USA) installed at a height of 275 cm from the ground. The
270 net ecosystem CO₂ exchange (NEE) for each 30 min period was calculated based on the
271 mass exchange between the ecosystem and the atmosphere according to standard
272 methodologies (Aubinet *et al.*, 2000). NEE was computed as the sum of CO₂ eddy
273 fluxes. In 2003, the same equipment was installed in the native forest at the top of self-
274 supporting 55-m high metal tower (Bonal *et al.*, 2008). The distance between the forest

275 and the old pasture site is 8 km, while the young pasture site is located at a distance of
276 50 km from the two other sites. Gaps and poor quality data were reconstructed using the
277 gap-filling strategy of Reichstein *et al.* (2005).

278

279

280

281 ***Statistical analyses***

282 The significant changes in the dynamics of pasture soil C stocks along the
283 chronosequence were analysed using the breakpoint function, *strucchange* package in R
284 software (Zeileis *et al.*, 2002). Soil C sequestration was quantified before and after the
285 breakpoint with a linear regression between pasture age and soil C stock. To determine
286 differences among the NEE of the three flux tower sites, we used a Kruskal-Wallis test
287 followed by a pairwise comparison using Tukey and Kramer (Nemenyi) test (*PMCMR*
288 package in R; Pohlert 2009). A linear mixed-effect model, *nlme* package in R (Pinheiro
289 *et al.*, 2009), was used to determine the effect of pasture age, farm, soil N stock and the
290 C:N ratio on soil C stock dynamics. In this model, the age of the pasture, soil N stocks
291 and the C:N ratio were fixed effects and farmers was a random effect. The effect of
292 pasture conditions, as a random factor, was based on model comparison using a
293 likelihood ratio test (denoted L. ratio): one model with three variables built without
294 pasture conditions (linear model) and a second model with three variables built with
295 pasture conditions as a random effect (linear mixed model). All the different
296 combinations were tested and the best model according to the AIC criterion was kept. A
297 t-test was conducted to compare the abundance of species in young and old pastures.

298

299 **Results**

300 Soil organic C dynamic and pasture age

301 Analysis of SOC stocks in the whole soil profile (0-100 cm) along the chronosequence
302 (i.e. 0 to 42 years after deforestation) indicated the presence of two distinct soil C
303 dynamics with a breaking point at 24 years of age (Fig. 2a,b).

304 Concerning the SOC stock, no significant changes were observed in pastures ≤ 24 years
305 old, whereas C stock considerably ($p = 0.03$) increased with pasture age in pastures ≥ 24
306 years old. The linear regression between pasture age and soil C stocks indicated that
307 $5.31 \pm 2.08 \text{ tC ha}^{-1} \text{ yr}^{-1}$ were accumulated in tropical pastures ≥ 24 years (Fig. 2b). Mean
308 SOC stocks in pastures ≥ 24 yr ($117.73 \pm 9.64 \text{ tC ha}^{-1}$) is higher than in forest sites
309 ($99.63 \pm 7.36 \text{ tC ha}^{-1}$) and pastures ≤ 24 yr ($85.54 \pm 5.57 \text{ tC ha}^{-1}$) ($p < 0.01$; Table 1).

310 The analysis of two soil fractions (humidified C vs. fresh C) indicated that pasture SOC
311 was dominated by humified C, which accounted for 85 to 95% of the total SOC stock
312 (Fig. 2c,d). Mirroring the dynamics of total SOC stock, humified C stock did not
313 significantly change in pastures ≤ 24 years old, whereas it increased significantly with
314 pasture age in pastures ≥ 24 years old ($R^2 = 0.48$, $p = 0.02$, Fig. 2d). Fresh C ($> 200 \mu\text{m}$)
315 did not show any pattern related to the age of the pasture.

316 The dynamics of pasture SOC stock varied considerably along the soil profile (Fig. 2f).
317 SOC stock of pastures ≥ 24 years old were significantly related to pasture age ($p < 0.01$,
318 $\text{SOC stock } 20\text{-}50 \text{ cm} = 2.52 * \text{age} - 47.5$ and $p = 0.03$, $\text{SOC stock } 50\text{-}100 \text{ cm} = 2.53 * \text{age} - 44.2$ for 20-50 cm and 50-100 cm, respectively) in the two deeper soil layers,
319 while no trend was observed in the top soil layer. The increase in SOC stock
320 corresponds to a continuous annual C storage of 2.52 and 2.53 $\text{tC ha}^{-1} \text{ yr}^{-1}$ in the 20-50
321 and 50-100 cm layers, respectively (Fig. 2f). Remarkably, no trend was observed in any
322 of the soil layers in the young pastures (Fig. 2e).

324

325 C storage determined using the eddy covariance method

326 The annual net ecosystem exchange (NEE) measured by eddy covariance of the young
327 pasture (deforested in 2008, 4 years old at the time of measurement) was C neutral (-
328 $0.31 \pm 0.48 \text{ tC ha}^{-1} \text{ yr}^{-1}$), whereas the old pasture (deforested in 1978, 33 years old at the
329 time of measurement) and the native forest site were a C sink with -1.27 ± 0.37 and -
330 $3.31 \pm 0.44 \text{ tC ha}^{-1} \text{ yr}^{-1}$, respectively (Table 2). The NEE of the three sites were different
331 (p -value = 0.012), where NEE of the native forest was markedly higher than the young
332 pasture but not for the old pasture (Table 2).

333

334 Drivers of soil organic C stock

335 The linear mixed model showed that pasture SOC stock was significantly explained by
336 a combined effect of pasture age, soil N stock and the soil C:N ratio, rather than each
337 effect individually (Table 3). The model also identified land use history (i.e. the
338 farmers) effect on SOC stocks ($p = 0.0024$).

339 The analysis of isotopic composition ($\delta^{13}\text{C}$) of humified C showed that the contribution
340 of C4 plants (i.e. *Brachiaria sp*) to the stock of humified C stock increased linearly with
341 the age of the pasture ($R^2 = 0.66$, soil C4 stock = $1.13 * \text{age} + 15.71$, $p < 0.001$) (Fig. 3).

342 The contribution of C3 plants originating from the native forest and the C incorporated
343 by new C3 plants such as shrubs (i.e. *Spermacoce verticillata*), and legumes (i.e.
344 *Mimosa pudica*) decreased continuously during the first part of the chronosequence (0-
345 24 years) (Fig. 3). After 24 years, the pattern was reversed and a significant increase in
346 the contribution of C3 plants material in humified C was observed until the end of the
347 chronosequence ($R^2 = 0.30$, Soil C3 stock = $0.086 * \text{age}^2 - 3.059 * \text{age} + 76.33$, $p =$
348 0.02).

349

350 Pasture species composition

351 The botanical composition (i.e. legumes, shrubs, C3 and C4 plant types) of the sampled
352 pastures was analysed and averaged for the two pasture age classes. Table 4 shows a
353 species shift with pasture age, with marked differences in the type of plants between \leq
354 24-year-old and \geq 24-year-old pastures. In other words, the overall abundance of C3
355 species was more than twice higher in \geq 24-year-old pastures than in \leq 24-year-old
356 pastures. More specifically, pastures \geq 24 years old had three times higher abundances
357 of C3 legumes and shrubs than young pastures (Table 4).

358

359 Discussion**360 Dynamics of SOC storage in tropical pastures**

361 Soil C dynamics in our study showed two contrasted patterns according to the age of the
362 pasture (Fig 2).

363 In pastures \leq 24 years old, SOC stocks did not significantly change over time,
364 suggesting that any sequestration or losses of C in these “young” pastures were too
365 small to be detected. In the first years after conversion ($<$ 5 year), SOC was highly
366 variable, ranging from 61 to 120 tC ha⁻¹. This variability can be due to different initial
367 SOC stocks under the native forest (Cerri *et al.*, 2004), differences in the decomposition
368 of dead wood (Navarette *et al.*, 2016a), or to differences in farmers’ deforestation
369 practices (i.e. stumping or not) (Stahl *et al.*, 2016). In the present study, the land use
370 history was shown to be an important factor controlling SOC stocks (Table 3) rather
371 than the initial SOC stocks under the native forests. Indeed, the four forest study sites
372 showed low variability compared to the young pastures (i.e. forests = 99.63 ± 7.36 and
373 young pastures = 85.54 ± 5.57 tC ha⁻¹, Table 1).

374 In contrast, pastures ≥ 24 years old converged towards a significant increase in SOC
375 stocks over time without reaching a plateau (Fig. 2b, $p = 0.03$). A large number of
376 different locations and farm management systems were included in our chronosequence
377 (five farmers for 11 pastures from 24 to 36 years old) signifying that the long-term
378 accumulation of SOC is common to many pastures of this region. This result supports
379 the idea that certain ecosystems such permanent pastures continuously accumulate soil
380 carbon in the long term (e. g. several decades). Moreover, the variability of SOC stock
381 around the regression slope (Fig. 2b) suggests that, despite our careful selection of
382 farmers and sites, there were difference in rate of SOC accumulation among pastures
383 that might be explained by different management practices.

384

385 *The C sink of old tropical pastures and native forest*

386 The chronosequence study suggests that old pastures (≥ 24 years) are a carbon sink
387 fixing $- 5.31 \pm 2.08 \text{ tC ha}^{-1} \text{ yr}^{-1}$ (Table 2). The C sink function of old pastures was
388 supported by the continuous measurement of ecosystem CO_2 exchanges by eddy
389 covariance in one old pasture. However, with $- 1.27 \pm 0.37 \text{ tC ha}^{-1} \text{ yr}^{-1}$ the C sink of this
390 specific pasture is lower than the mean C sink provided by the chronosequence study
391 for 11 old pastures. This discrepancy can be explained by the difference in rate of SOC
392 accumulation among pastures. It can also due to the uncertainty of C sink estimation
393 inherent to each method. Nevertheless, the two methods converge towards the same
394 general trend: the C balance of young pastures is nearly neutral whereas old pastures act
395 as C sink.

396 These findings indicate that the high continuous C storage by native forest ($- 3.31 \pm$
397 $0.44 \text{ tC ha}^{-1} \text{ yr}^{-1}$, Table 2) can be partly recovered in tropical pastures after 24 years.
398 Conversely, previous results for tropical pasture in Panama, showed a high C emission

399 (2.61 tC ha⁻¹ yr⁻¹) for an old pasture (50 years old) (Wolf *et al.*, 2011). This contrasted
400 result is explained by several periods with high stocking rates (2.7 LSU ha⁻¹), showing
401 that overgrazing is the major cause of carbon losses. We suggest that management with
402 low animal stocking density and no fires since establishment is necessary to ensure the
403 sustainability of pasture C sink.

404 To our knowledge, this is the first time that C storage capacity of ecosystems is
405 measured simultaneously by eddy covariance and chronosequence in the tropics. The
406 convergence of results from these two methods show the powerfulness of these methods
407 when they are developed with caution and give strong credit to our findings.
408 Nonetheless, further studies should improve our understanding of pasture effect on
409 regional C cycle by investigating larger geographic area on the one hand and conducting
410 a full greenhouse gas exchange (CO₂, CH₄ and N₂O) measurements on the other.
411 Moreover, a better estimation of carbon storage could be realized by calculating the net
412 carbon storage (NCS, Soussana *et al.*, 2010). NCS is calculated as NEE subtracting
413 methane emission, live weight gain and C leaching of the pastures.

414

415 *The localization of C storage*

416 Our study indicates that the C sink of old tropical pastures results from an accumulation
417 of SOC in the deep soil layers (> 20 cm). In fact, the deep soil layers contain more than
418 half of the total carbon stocks of up to 1m soil depth (i.e. 28 and 26%, for the 20-50 cm
419 and the 50-100 cm soil layer, respectively, Stahl *et al.*, 2016). These findings
420 demonstrate the need to include deeper soil layer in the carbon budget assessment of
421 ecosystems in order to avoid underestimating soil C stock and storage (Jobbagy &
422 Jackson 2000; Stahl *et al.*, 2016). The un-accounting of deep soil layers in C budget of a
423 number of studies may explain why they report a constant or decreasing soil C stock in

424 pastures > 30 years (Neill *et al.*, 1997; Cerri *et al.*, 2004; Powers & Veldkamp 2005;
425 Bahr *et al.*, 2014).

426

427 **Drivers of C storage**

428 *Soil humified and fresh-C stock*

429 Analysis of the two soil fractions revealed that pasture SOC stock was dominated by
430 humified C (Fig. 2c, d). In line with total SOC stock, the stock of humified C did not
431 vary significantly in young pastures while increased observed in old pastures (Fig. 2c,
432 d). In contrast, the fresh C stock remained constant throughout the chronosequence,
433 suggesting an accumulation of SOC in old pastures due to greater humification of fresh
434 C and/or preservation of humified C rather than an increase in the stock of fresh C. This
435 better preservation of humified C in old pastures show its accumulation in deep soil
436 layers where microbial mineralization activities are greatly reduced (Fontaine *et al.*,
437 2007), and where its mean residence time reaches centuries or thousands of years
438 (Freycon *et al.*, 2010).

439

440 *Origin of the humified C stock in deep soil*

441 Two process could explain the origin of the humified C stock in deep soil in our study.
442 First, the slow transfer of small organo-mineral particles compounds to deep soil layers
443 (below 20 cm) by lixiviation and heavy rains in tropics (Rumpel & Kögel-Knabner
444 2011). The lixiviation could be more important in pasture rather than in the native forest
445 where the canopy interception could limit its effect (Lloyd *et al.*, 1988). Thus, due to the
446 strong lixiviation of soil particles under wet tropical climate—the humified C is
447 transferred in deep soil layers where microbial degradation activities are extremely
448 reduced.

449 Second, the decomposition and humification of the dead deep roots of some species
450 such as grasses and legumes. In our study, roots until one meter depth were observed
451 (though at very low density), similarly to Fisher *et al.* (1994) that showed in Amazonia
452 the high contribution of deep roots grass species in carbon sequestration in deep soil
453 layers. Therefore, the slowly decomposed deep C can accumulate in the long term (at
454 least decades) leading to a continuous C storage.

455

456 *Carbon derived from C3 and C4 plants*

457 To further understand why the restoration of C storage in pastures took two decades or
458 more, we analysed the isotopic composition ($\delta^{13}\text{C}$) of humified soil organic matter. It
459 allowed to quantify the contribution to this C pool of the C4 plants (grasses and in
460 particular *Brachiaria humidicola*) and of C3 plants (residual carbon from forest and
461 new carbon from pasture). As expected, the contribution of C3 plants to humified C
462 decreases continuously during the first part of the chronosequence (0-24 years). This is
463 due to the microbial decomposition of humified C inherited of the native forest whereas,
464 the accumulation of humified C built on C4 plant compound (grasses). However, after
465 24 years, the pattern was reversed, with a significant increase in the contribution of C3
466 plants to humified C until the end of the chronosequence. To our knowledge, a
467 comparable pattern has not been reported for the Amazonia region to date. A number of
468 studies report a continuous decrease in the contribution of C3 plants residues over time,
469 whereas C4 residues increase, reaching a plateau after >20 years (Cerri *et al.*, 2004;
470 Fujisaki *et al.*, 2015; Navarrete *et al.*, 2016b). This increase in the contribution of C3
471 plants is due to the colonization of *Brachiaria humidicola* plant stand (although
472 remaining dominant) by C3 plants like legumes (e.g. *Desmodium sp.*, *Mimosa pudica*)
473 and shrubs (e.g. *Spermacoce verticillata*) (Table 4). Nevertheless, shrubs are controlled

474 by slashing every second year. These findings strongly suggests that the C storage in old
475 tropical pastures is induced by a diversification of plant species and the development of
476 C3 plants such as legumes and weeds (Table 4).

477

478 *Link between C and N stock*

479 The SOC storage in old pastures was significantly correlated with the accumulation of
480 soil organic N and, depending on the site of the pasture, with an increase in the soil C:N
481 ratio (Table 3, Fig. S1). Similar relations have been shown for soil profiles of tropical
482 pastures (Groppo *et al.*, 2015; Stahl *et al.*, 2016) and chronosequences in the Colombian
483 Amazon (Navarrette *et al.*, 2016b). Thus, the SOC storage in old pastures can be
484 explained by two processes stimulated by the development of C3 plants in tropical
485 pastures: (i) the development of C3 legumes, which fix atmospheric N₂ and incorporate
486 organic N in the soil. The higher N availability for microorganisms favours microbial
487 production of humified compounds from fresh N-compounds (Dijkstra *et al.*, 2004) and
488 preservation of pre-existent humified compounds from microbial mining (Fontaine *et*
489 *al.*, 2004, 2011; Ramirez *et al.*, 2012); (ii) the development of C3 plants with a high
490 C:N ratio (like shrubs with deep roots) leading to humified compounds with a high C:N
491 ratio (Stahl *et al.*, 2016) in deep layers. The subsequent increase in the humus C:N ratio
492 (Table S1a, b, c), enabling sequestration of more C per unit of N present in the
493 ecosystem throughout the soil profile.

494

495 To conclude, we shown that the old tropical pastures can restore the C storage observed
496 in native forest. But it is clear that to preserve forest biodiversity and C stocks, efforts to
497 curb deforestation should continue to be a priority. These efforts have enabled a major
498 reduction in forest clearing, but progress remains fragile (Davidson *et al.*, 2012)

499 depending on the sustainability of agricultural systems. We show that, two decades after
500 the establishment, the tropical pastures accumulate SOC over time, suggesting that they
501 can be exploited by farmers in the long-term without the loss of soil fertility often
502 observed in cultivated soils (McGrath *et al.*, 2001). In order to mitigate the climate
503 change sustainable pasture managements may increase soil C stocks, soil quality and
504 while providing sufficient forage without extending pasture area. Moreover, the
505 humified-C stored in the deeper soil layers are likely to provide unlimited C
506 accumulation and conservation of C in the long term.

507 The C storage in our old pastures is linked to the diversification of pasture plant
508 species and the development of C3 plants such as legumes that stimulate the production
509 of humified C. Nevertheless, the proportion of unpalatable C3 plants in studied old
510 pastures (5.0%) remained minority and did not threaten the fodder potential and thus the
511 livestock production.

512 Finally, our results are only applicable to pastures with similar sustainable
513 management practices (avoiding fires and overgrazing, using a grazing rotation plan and
514 a mixture of C3 and C4 species) and comparable pedo-climatic conditions. These
515 findings should inspire further research to generalize these management practices in
516 order to promote the C sink of tropical pastures, the soil fertility, the forage production
517 and *in fine* the preservation of rainforest.

518

519

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- 747

748 **Supporting information**

749 **Figure S1: Soil N stocks and soil C:N ratio to a depth of one metre along the**
750 **chronosequence. (a)** Soil N stocks (tN ha^{-1}) in the soil under the pasture and **(b)** soil
751 C:N ratio.

752 **Table S1a: Soil characteristics for each plot in each layer in surface soil (0-20 cm)**

753 **Table S1b: Soil characteristics for each plot in each layer in deep soil (20-50 cm)**

754 **Table S1c: Soil characteristics for each plot in each layer in deep soil (50-100 cm)**

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756 **Tables**

757 **Table 1** Soil C stocks under native forests and pastures separated into two age classes
 758 (100 cm depth, ≤ 24 years old and ≥ 24 years old). Numbers represent mean \pm SE. n is
 759 the number of plots. Different letters indicate significant differences ($p < 0.05$) in mean
 760 soil C stocks among ecosystems.

	Native forest	Pastures ≤ 24 yr	Pastures ≥ 24 yr
n	4	13	11
Soil C stock (tC ha ⁻¹)	99.63 \pm 7.36 ab	85.54 \pm 5.57 b	117.73 \pm 9.64 a

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778 **Table 2** Carbon storage or emission by native forest, young and old pastures.

	Eddy covariance (tC ha ⁻¹ yr ⁻¹)	Chronosequence study (tC ha ⁻¹ yr ⁻¹)
Native forest	- 3.31 ± 0.44 a	No data
Pasture ≤ 24 yr	- 0.31 ± 0.48 b	1.03 ± 0.85 ns
Pasture ≥ 24 yr	- 1.27 ± 0.37 ab	- 5.31 ± 2.08 *

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780 Values indicate potential C storage by the ecosystems (i.e. NEE), a positive value
781 implies the emission of carbon from the ecosystem to the atmosphere. Carbon storage
782 quantified by eddy covariance is the mean ± SE of annual net ecosystem exchange
783 (NEE) for a period of four years (2011-2014) for the native forest and the two pastures.

784 Values followed by the same lower case letter did not differ significantly among sites
785 according to posthoc Kruskal Nemenyi test ($p < 0.05$). Carbon storage quantified by the
786 chronosequence study corresponds to the slope of the linear regression of soil C stock
787 with the age of the pastures (Fig.2) ± SE. ns indicates no significant relationship
788 between the soil C stock and the age of pastures, * significant relationship ($p < 0.05$).

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798 **Table 3** Effect of pasture age, soil N stock and soil C:N ratio on soil C stocks in the
 799 chronosequence study. A linear mixed model with pasture conditions as a random effect
 800 was used to test each variable individually and in combination.

Random effect				L. ratio	p value		
Land use history				9.18	0.0024		
Fixed effect	Pasture conditions	Estimates	Std. Error	t value	p value	AIC	
Single effect							
	age	1.00	0.35	2.82	0.013	218.5	
	N stock	12.31	2.77	4.44	<0.001	207.3	
	C/N	4.29	1.57	2.73	0.015	216.6	
Combined effect						189.5	
	age	0.71	0.22	3.29	0.006		
	N stock	13.38	1.63	8.20	<0.001		
	C/N	2.14	1.05	2.03	0.063		

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811 **Table 4** Species composition and abundance (%) of C3 and C4 plants. Numbers
 812 represent the percentage of abundance (mean \pm SE). Significance levels: ns: $p > 0.1$; *:
 813 $p < 0.1$; **: $p < 0.05$ (ANOVA).

Plant type	Main species	Pastures \leq 24 yr	Pastures \geq 24 yr	Significant difference
C3 legumes	<i>Calopogonium mucunoïdes</i> , <i>Desmodium ovalifolium</i> , <i>Desmodium adscendens</i> , <i>Mimosa pudica</i>	3.0 \pm 2.1	9.0 \pm 3.4	*
C3 shrubs	<i>Spermacoce verticillata</i> , <i>Solanum sp</i>	1.3 \pm 0.7	5.0 \pm 1.6	**
Total C3		5.6 \pm 2.6	14.0 \pm 4.6	*
Total C4	<i>Brachiaria humidicola</i>	78.1 \pm 6.8	77.2 \pm 4.8	ns

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827 **Figure captions**

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829 **Fig. 1** Map of the study sites. The study included 24 pastures belonging to eight farms
830 (red circles) (Stahl *et al.*, 2016). The numbers indicate the number of pastures sampled
831 on each farm. The four reference forest sites are indicated by a green star. The area in
832 light green is the tropical forest; the grey area is swamp, and the black area is
833 agricultural land. The locations of the three flux towers (two pastures and one forest) are
834 indicated by arrows. The insert shows the location of French Guiana in South America.

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836 **Fig. 2** Soil carbon stocks for two soil fractions and different soil layers along the
837 chronosequence. (a) Soil carbon stocks to a depth of one metre under pastures ≤ 24
838 years old and (b) pastures ≥ 24 years old. (c) Soil humified C ($< 200 \mu\text{m}$, open blue
839 circles) and fresh C ($> 200 \mu\text{m}$, open red circles) stocks under pastures ≤ 24 year and (d)
840 under pastures ≥ 24 years old. (e) Soil humified-C stock in the three soil layers (0-20
841 cm: blue squares; 20-50 cm: red circles; 50-100 cm: orange triangles) under pastures \leq
842 24 years old and (f) pastures ≥ 24 years old. Dashed lines mean no significant
843 relationship between soil C stocks and the age of the pasture. Solid lines mean a
844 significant linear relationship was found between soil C stocks and the age of the
845 pasture.

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853 **Fig. 3** Soil carbon stocks originating from C3 and C4 plants (1 m depth) along the
854 chronosequence. The C4 plant signature (black circles) in soil C mostly results from a
855 single grass (*Brachiaria sp.*) planted when the pastures were established. The C3 plant
856 signature (white circles) have several possible origins: soil C inherited from the native
857 forest and C incorporated by new C3 plants like shrubs (i.e. *Spermacoce verticillata*),
858 and legumes (i.e. *Mimosa pudica*) colonizing pastures. Solid lines show a significant
859 relationships between soil C stocks and the age of the pasture.

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