Title: Continuous soil carbon storage of old permanent pastures in Amazonia

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Abstract

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

Introduction

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

After deforestation, in livestock systems, pastures are often established and managed with little concern for sustainability: only one exotic C4 grass species (e.g. *Brachiaria sp*.) is usually planted and managed without a forage use plan. Pastures are subjected to cycles of over- or under-grazing which favour encroachment by shrubs and trees (de Faccio Carvalho, 2006). After pasture establishment, burning is a common technique to reduce tree encroachment (Kauffman et al., 1998; Navarette et al., 2016b). However, pasture burning leads to emissions of radiatively active aerosols (e.g., CH4, CO, N2O, NOx, nonmethane hydrocarbons) (Kauffman *et al.,* 1998). Furthermore, burning decreases the supply of organic C and N in soils reducing soil organic C (SOC) stock of pastures. The consequence of this SOC depletion is a rapid loss of pasture productivity (< 5 years) pushing farmers to abandon the degraded land and constantly move and clear more land to cover their forage needs. However, other management options do exist; establishing the pasture with a mixture of plant species (e.g. C4 (grass) and C3 (legumes)) combined with appropriate grazing rotation plans makes it possible to control shrubs and trees encroachment without burning (de Faccio Carvalho, 2006). With favourable agricultural practices, SOC stocks can accumulate in the surface layers of the soil (Trumbore *et al.,* 1995; de Moraes *et al.,* 1996; Neill *et al.,* 1997; Cerri *et al.,* 2003, 2004). However, SOC accumulation often ceases a few years after the pasture was established (Neill *et al.,* 1997; Cerri *et al.,* 2003, 2004) suggesting that, contrary to tropical forests, tropical pastures are unable to sequester C in the long-term. It is generally predicted that the change in SOC stocks resulting from a land use change is maximal in the first years, and decreases exponentially until the SOC pool attains a new equilibrium (e.g. Lal, 2004). This assertion arises from decades of observations of SOC dynamics in surface soil layers subject to different agricultural practices (Johnston *et al.,* 2009; Poeplau *et al.,* 2011).

However, there is increasing evidence that ecosystems such as permanent pastures and forests can continuously accumulate carbon (Syers *et al.,* 1970; Schlesinger, 1990; Knops & Tilman, 2000; Sanderman, 2003; Chen *et al.,* 2013; Yu *et al.,* 2013). Indeed, chronosequence studies have shown SOC to be accumulated over millennia without reaching equilibrium (Syers *et al.,* 1970; Schlesinger, 1990; Knops & Tilman, 2000). Global networks on ecosystem CO_2 exchanges show net CO_2 uptake by most undisturbed grasslands and forests, suggesting accumulation of carbon in these ecosystems (Sanderman, 2003; Chen *et al.,* 2013; Yu *et al.,* 2013). The long-term ecological research network (LTER) has also produced evidence of long-term accumulation of SOC, though an equilibrium state can be reached in the surface layers after more than 100 years (Smith, 2014). Long-term SOC accumulation preferentially occurs in deep soil layers where microbial mineralization is extremely low (Fontaine *et al.,* 2007; Callesen *et al.,* 2016; Guan *et al.,* 2016; Stahl *et al.,* 2016). Unfortunately, C dynamics in deep soil layers has often been neglected, explaining the lack of robust information on the long-term C sink of permanent pastures.

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

An alternative method to the direct measurement of SOC stock changes in ecosystems is to measure the net ecosystem exchange (i.e. NEE) at the system boundaries. This approach provides a high temporal resolution and changes in C stock can be detected within one year. Hence, flux measurements may be a good method to validate direct measurement of changes in soil C stocks over time as provided by chronosequence studies. To our knowledge, only two pastures sites have been instrumented on deforested pastures in Amazonia, a two flux towers in Brazil (Goncalves *et al.*, 2013) and another one in Panama (Wolf *et al.,* 2011). Moreover, a quantitative comparison of these two methods for C storage evaluation in Amazonian pastures has not yet been undertaken.

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

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

Materials and Methods

Study sites

The study was conducted along the coastline in French Guiana (~ 200 km), South America (5°16’54”N, 52°54’44”W). The average annual rainfall was 3041 mm and the air temperature was 25.7 °C (Paracou station; Gourlet-Fleury *et al.,* 2004). The study focused on a hilltop zone with clayey soils, classified as Ferralsols or Acrisols according to the IUSS Working Group WRB (2006). The study comprised 24 pastures (latitude 4°43’31.0728’’; 5°27’36.5934’’ and longitude -53°57’27.8382’’; -52°27’26.0172’’ (Fig.1)), established after deforestation of native rainforest between 1976 and 2010. They were distributed in four typical cattle farming areas along the Guyanese coast, together with one representative native forest site in each area (Fig. 1). Farmers were interviewed to determine land-use history such as the date of forest conversion or agricultural management, for more information on farmers see Stahl *et al.* (2016). The condition required for a site to be included in the study was that all chronosequence soil forming factors have remained constant since deforestation, the age of the pasture being the discriminant variable (Huggett, 1998). The following criteria were chosen to ensure the sites were comparable: (i) the parent material had to be the same, i.e. Precambrian metamorphic formation, which we checked on geological outcrops along nearby roads; (ii) the sites had to be situated in a hilltop zone with only a slight slope to avoid major transport of sediments in riverbeds; (iii) no crop rotation or land-use change should have been implemented since forest conversion, nor fertilisation prior to sampling, which was checked by identifying the land-use history in interviews with the farmer; and (iv) soils had to be comparable in terms of pH, which was checked by soil analysis (Table S1a,b,c). According to the farmers, these pastures were established by slash-and-burn but had not been burned since. After the pasture establishment the shurbs were slashed every two-four years in order to limit their propagation. The pastures were managed by eight different farmers applying all rotational grazing plans at an animal stocking density of ~1 LSU ha^{-1} (Livestock Standard Unit (Palmer & Ainslie (2005)). The pastures were grazed (not harvested) by cows, except for four, which were grazed by goats (~ 10 goats ha^{-1}). The vegetation in the pastures was dominated by C4 species (mainly the grass *Brachiaria humidicola*).

Botanical determination

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

Soil sampling

In each pasture and in the native forest plot, eight soil cores were sampled using a jackhammer equipped with a drill gauge, volume= 3,32x 10^{-3} m^3 (Cobra TT, Eijkelkamp, The Netherlands). The litter layer was removed before the soil was sampled. The sampling plan consisted in two parallel transects spaced 30 m apart. Along each transect, the distance between each of the four soil cores was 10 m. Each core was split into three layers: 0-20; 20-50; 50-100 cm, dried (48 h at 60 °C to constant mass) and sieved at 2 mm. Each layer of the eight cores was pooled proportionally to its mass to obtain a composite sample for each site. The mass of fine soil (< 2 mm), soil texture, soil organic C content and its isotopic composition ($\delta^{13}C$), soil organic N content and coarse particulate organic matter (> 200 μm) were quantified in each layer (Table S1).

Soil analysis

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

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

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

$$C4_i = C_{toti} \times \frac{(\delta \mathrm{soil}_{C4i} - \delta soil_{C3i})}{(\delta \mathrm{root}_{C4i} - \delta soil_{C3i})} \qquad \text{Eq 2}$$

where C_{toti} is the total C stock in the soil layer i, $C3_i$ is the C stock originating from the forest and C3 pasture species in the soil layer i and $C4_i$ is the C stock originating from grasses in the pasture, present in the soil layer i; $\delta soil_{C4i}$ is the $\delta^{13}C$ isotopic composition in the pasture in the soil layer i and $\delta soil_{C3i}$ is the $\delta^{13}C$ isotopic composition in the native forests and in the C3 pasture species in the soil layer i, and $\delta root_{C4i}$ is the $\delta^{13}C$ isotopic composition of the roots of C4 grass (i.e. -12.4 ‰) in the soil layer *i*.

Quantification of soil C storage

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

For the chronosequence study, the SOC stocks were quantified in three soil layers (0-20, 20-50 and 50-100 cm) and in two soil C pools: fresh C (particulate organic matter : POM) > 200 μm) and humified C (POM < 200 μm) in each pasture. The POM were separated from the soil by "washing" the soil samples on a 200 μm sieve several times. Fresh C accounts for the plant material (root and shoot litter) recently incorporated into the soil while humified C accounts for the organic matter transformed by soil microorganisms. Whether free in the soil or adsorbed on soil particles, humified C is slowly decomposed by microorganisms (mean residence time can be decades or thousands of years) due to its low accessibility and energetic value for soil microorganisms (Sutton & Sposito, 2005; Fontaine *et al.*, 2007; Dungait *et al.*, 2012). The SOC stocks were corrected to an equivalent soil mass. This correction is appropriate to standardise the different sites sampled in our chronosequence approach (Stahl *et al.*, 2016). First, we standardised the soil C stock in each plot by applying the average fine soil mass (< 2 mm) for all plots (0-100 cm, native forests and pastures, n = 28) (Ellert *et al.*, 2002; Bahr *et al.*, 2014). Second, we corrected soil C stock using the same method of equivalent soil mass for each soil layer. After correcting for fine soil, we added a correction for clay content (Zinn *et al.*, 2005) to account for the close relationship between clay and C content. We standardised C stocks using the mean clay content in each soil layer in all the plots (native forests and pastures, n = 28). These corrections were applied to reduce the variance between sites due to the soil characteristics (fine soil, clay content, see Stahl *et al.*, 2016). Finally, soil C storage is given by the slope between SOC stock and time.

Meteorological and eddy covariance measurements

To estimate the ecosystem C sink activity, net ecosystem exchange (NEE) of CO_2 was measured by two flux towers in two pastures (one 4 and one 33 years old). The NEE was also quantified in the nearby native forest using the same eddy covariance measurements (same analyser and procedure).

In more details, in March 2010, one young pasture (4 years old) and one old pasture (33 years old) were equipped with a meteorological weather station and eddy covariance towers. At each site, a meteorological station provided 30-min averaged values of global radiation, air temperature, soil temperature and water content (at depths of 10 and 30 cm) and rainfall. All meteorological data were collected at 1 min intervals and compiled as 30 min averages or sums with a CR1000 datalogger (Campbell Scientific Inc., Shepshed, UK). Each tower was composed of a fast response (20 Hz) sonic anemometer (Solent R3, Gill Instruments, Lymington, UK) and a closed path CO_2-H_2O analyser (LI-7000, LI-Cor Inc., Lincoln, USA) installed at a height of 275 cm from the ground. The net ecosystem CO_2 exchange (NEE) for each 30 min period was calculated based on the mass exchange between the ecosystem and the atmosphere according to standard methodologies (Aubinet *et al.*, 2000). NEE was computed as the sum of CO_2 eddy fluxes. In 2003, the same equipment was installed in the native forest at the top of self-supporting 55-m high metal tower (Bonal *et al.*, 2008). The distance between the forest and the old pasture site is 8 km, while the young pasture site is located at a distance of 50 km from the two other sites. Gaps and poor quality data were reconstructed using the gap-filling strategy of Reichstein *et al.* (2005).

Statistical analyses

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

Results

Soil organic C dynamic and pasture age

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

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

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

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

C storage determined using the eddy covariance method

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

Drivers of soil organic C stock

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

The analysis of isotopic composition ($\delta^{13}C$) of humified C showed that the contribution of C4 plants (i.e. *Brachiaria sp*) to the stock of humified C stock increased linearly with the age of the pasture ($R^2 = 0.66$, soil C4 stock = 1.13 * age + 15.71, $p < 0.001$) (Fig. 3). The contribution of C3 plants originating from the native forest and the C incorporated by new C3 plants such as shrubs (i.e. *Spermacoce verticillata*), and legumes (i.e. *Mimosa pudica*) decreased continuously during the first part of the chronosequence (0-24 years) (Fig. 3). After 24 years, the pattern was reversed and a significant increase in the contribution of C3 plants material in humified C was observed until the end of the chronosequence ($R^2 = 0.30$, Soil C3 stock = 0.086 * age^2 - 3.059 * age + 76.33, p = 0.02).

Pasture species composition

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

Discussion

Dynamics of SOC storage in tropical pastures

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

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

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

The C sink of old tropical pastures and native forest

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

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

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

To our knowledge, this is the first time that C storage capacity of ecosystems is measured simultaneously by eddy covariance and chronosequence in the tropics. The convergence of results from these two methods show the powerfulness of these methods when they are developed with caution and give strong credit to our findings. Nonetheless, further studies should improve our understanding of pasture effect on regional C cycle by investigating larger geographic area on the one hand and conducting a full greenhouse gas exchange (CO_2, CH_4 and N_2O) measurements on the other. Moreover, a better estimation of carbon storage could be realized by calculating the net carbon storage (NCS, Soussana *et al.,* 2010). NCS is calculated as NEE subtracting methane emission, live weight gain and C leaching of the pastures.

The localization of C storage

Our study indicates that the C sink of old tropical pastures results from an accumulation of SOC in the deep soil layers (> 20 cm). In fact, the deep soil layers contain more than half of the total carbon stocks of up to 1m soil depth (i.e. 28 and 26%, for the 20-50 cm and the 50-100 cm soil layer, respectively, Stahl *et al.,* 2016). These findings demonstrate the need to include deeper soil layer in the carbon budget assessment of ecosystems in order to avoid underestimating soil C stock and storage (Jobbagy & Jackson 2000; Stahl *et al.,* 2016). The un-accounting of deep soil layers in C budget of a number of studies may explain why they report a constant or decreasing soil C stock in pastures > 30 years (Neill *et al.,* 1997; Cerri *et al.,* 2004; Powers & Veldkamp 2005; Bahr *et al.,* 2014).

Drivers of C storage

Soil humified and fresh-C stock

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

Origin of the humified C stock in deep soil

Two process could explain the origin of the humified C stock in deep soil in our study.

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

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

Carbon derived from C3 and C4 plants

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

Link between C and N stock

The SOC storage in old pastures was significantly correlated with the accumulation of soil organic N and, depending on the site of the pasture, with an increase in the soil C:N ratio (Table 3, Fig. S1). Similar relations have been shown for soil profiles of tropical pastures (Groppo *et al.,* 2015; Stahl *et al.,* 2016) and chronosequences in the Colombian Amazon (Navarrette *et al.*, 2016b). Thus, the SOC storage in old pastures can be explained by two processes stimulated by the development of C3 plants in tropical pastures: (i) the development of C3 legumes, which fix atmospheric N_2 and incorporate organic N in the soil. The higher N availability for microorganisms favours microbial production of humified compounds from fresh N-compounds (Dijkstra *et al.,* 2004) and preservation of pre-existent humified compounds from microbial mining (Fontaine *et al.,* 2004, 2011; Ramirez *et al.,* 2012); (ii) the development of C3 plants with a high C:N ratio (like shrubs with deep roots) leading to humified compounds with a high C:N ratio (Stahl *et al.,* 2016) in deep layers. The subsequent increase in the humus C:N ratio (Table S1a, b, c), enabling sequestration of more C per unit of N present in the ecosystem throughout the soil profile.

To conclude, we shown that the old tropical pastures can restore the C storage observed in native forest. But it is clear that to preserve forest biodiversity and C stocks, efforts to curb deforestation should continue to be a priority. These efforts have enabled a major reduction in forest clearing, but progress remains fragile (Davidson *et al.,* 2012) depending on the sustainability of agricultural systems. We show that, two decades after the establishment, the tropical pastures accumulate SOC over time, suggesting that they can be exploited by farmers in the long-term without the loss of soil fertility often observed in cultivated soils (McGrath *et al.,* 2001). In order to mitigate the climate change sustainable pasture managements may increase soil C stocks, soil quality and while providing sufficient forage without extending pasture area. Moreover, the humified-C stored in the deeper soil layers are likely to provide unlimited C accumulation and conservation of C in the long term.

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

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

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Supporting information

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

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

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

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

Tables

Table 1 Soil C stocks under native forests and pastures separated into two age classes (100 cm depth, ≤ 24 years old and ≥ 24 years old). Numbers represent mean ± SE. n is the number of plots. Different letters indicate significant differences ($p < 0.05$) in mean 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 ± 7.36 ab	85.54 ± 5.57 b	117.73 ± 9.64 a

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 *

Values indicate potential C storage by the ecosystems (i.e. NEE), a positive value implies the emission of carbon from the ecosystem to the atmosphere. Carbon storage quantified by eddy covariance is the mean ± SE of annual net ecosystem exchange (NEE) for a period of four years (2011-2014) for the native forest and the two pastures. Values followed by the same lower case letter did not differ significantly among sites according to posthoc Kruskal Nemenyi test ($p < 0.05$). Carbon storage quantified by the chronosequence study corresponds to the slope of the linear regression of soil C stock with the age of the pastures (Fig.2) ± SE. ns indicates no significant relationship between the soil C stock and the age of pastures, * significant relationship ($p < 0.05$).

Table 3 Effect of pasture age, soil N stock and soil C:N ratio on soil C stocks in the chronosequence study. A linear mixed model with pasture conditions as a random effect 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	

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

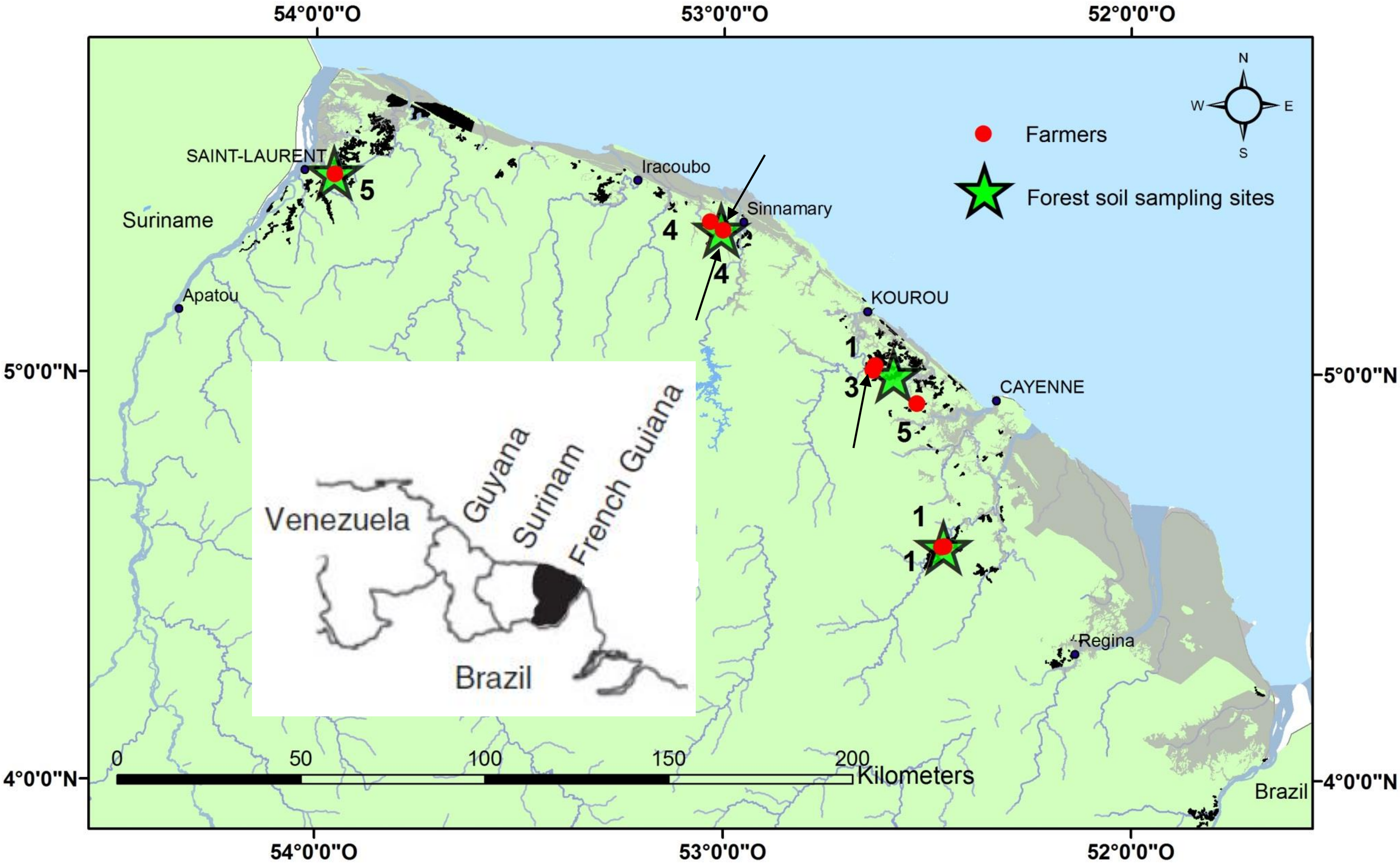
Plant type	Main species	Pastures ≤ 24 yr	Pastures ≥ 24 yr	Significant difference
C3 legumes	*Calopogonium mucunoïdes, Desmodium ovalifolium, Desmodium adscendens, Mimosa pudica*	3.0 ± 2.1	9.0 ± 3.4	*
C3 shrubs	*Spermacoce verticillata, Solanum sp*	1.3 ± 0.7	5.0 ± 1.6	**
Total C3		5.6 ± 2.6	14.0 ± 4.6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

Figure captions

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

Fig. 2 Soil carbon stocks for two soil fractions and different soil layers along the chronosequence. (a) Soil carbon stocks to a depth of one metre under pastures ≤ 24 years old and (b) pastures ≥ 24 years old. (c) Soil humified C (< 200 µm, open blue circles) and fresh C (> 200 µm, open red circles) stocks under pastures ≤ 24 year and (d) under pastures ≥ 24 years old. (e) Soil humified-C stock in the three soil layers (0-20 cm: blue squares; 20-50 cm: red circles; 50-100 cm: orange triangles) under pastures ≤ 24 years old and (f) pastures ≥ 24 years old. Dashed lines mean no significant relationship between soil C stocks and the age of the pasture. Solid lines mean a significant linear relationship was found between soil C stocks and the age of the pasture.

Fig. 3 Soil carbon stocks originating from C3 and C4 plants (1 m depth) along the chronosequence. The C4 plant signature (black circles) in soil C mostly results from a single grass (*Brachiaria sp.*) planted when the pastures were established. The C3 plant signature (white circles) have several possible origins: soil C inherited from the native forest and C incorporated by new C3 plants like shrubs (i.e. *Spermacoce verticillata*), and legumes (i.e. *Mimosa pudica*) colonizing pastures. Solid lines show a significant relationships between soil C stocks and the age of the pasture.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Forest soil sampling sites
SAINT-LAURENT
5
Iracoubo
Sinnamary
4
4
Suriname
Apatou
KOUROU
1
3
1
5
CAYENNE
1
1
Regina
Venezuela
Guyana
Surinam
French Guiana
Brazil
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50
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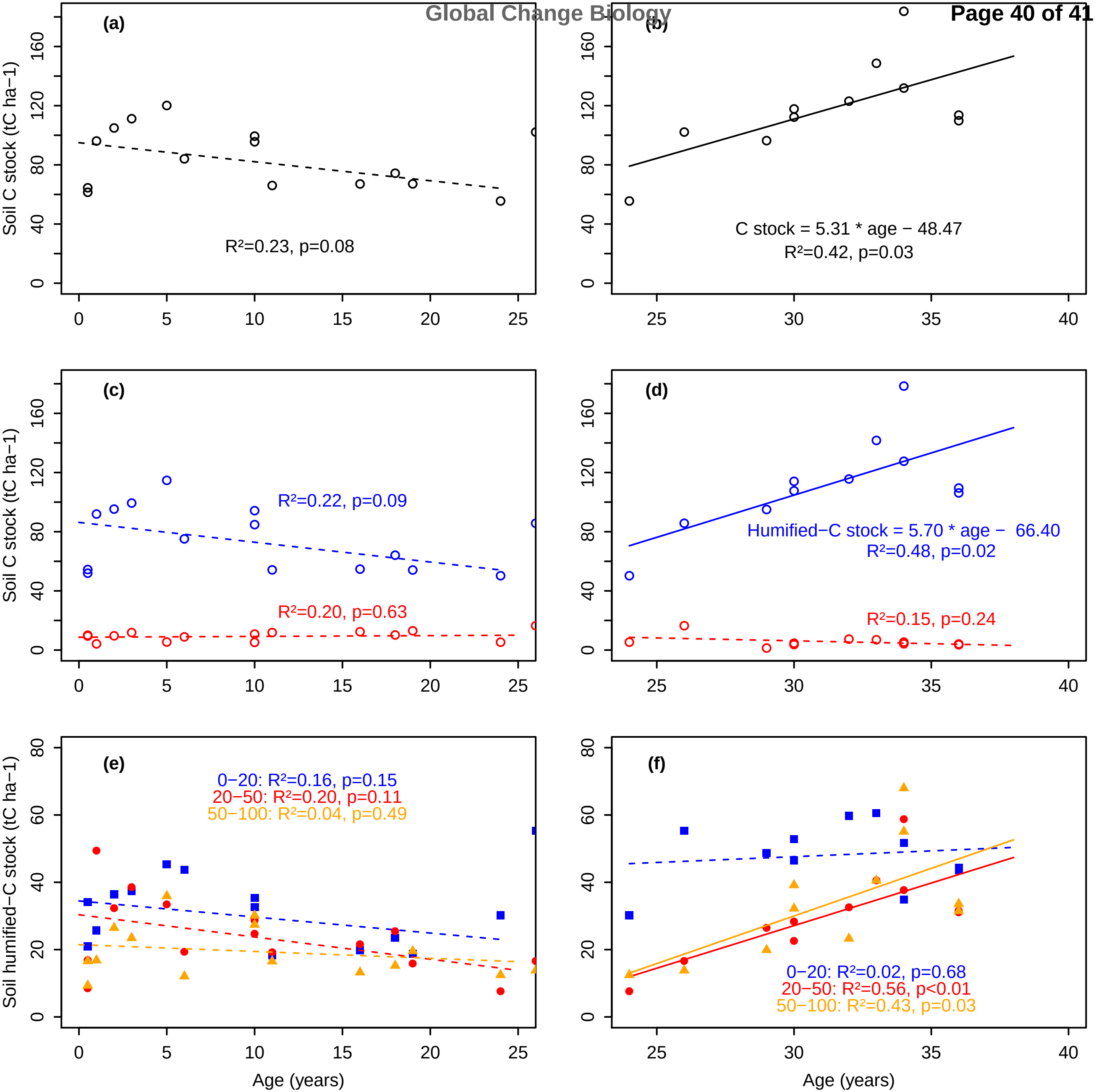
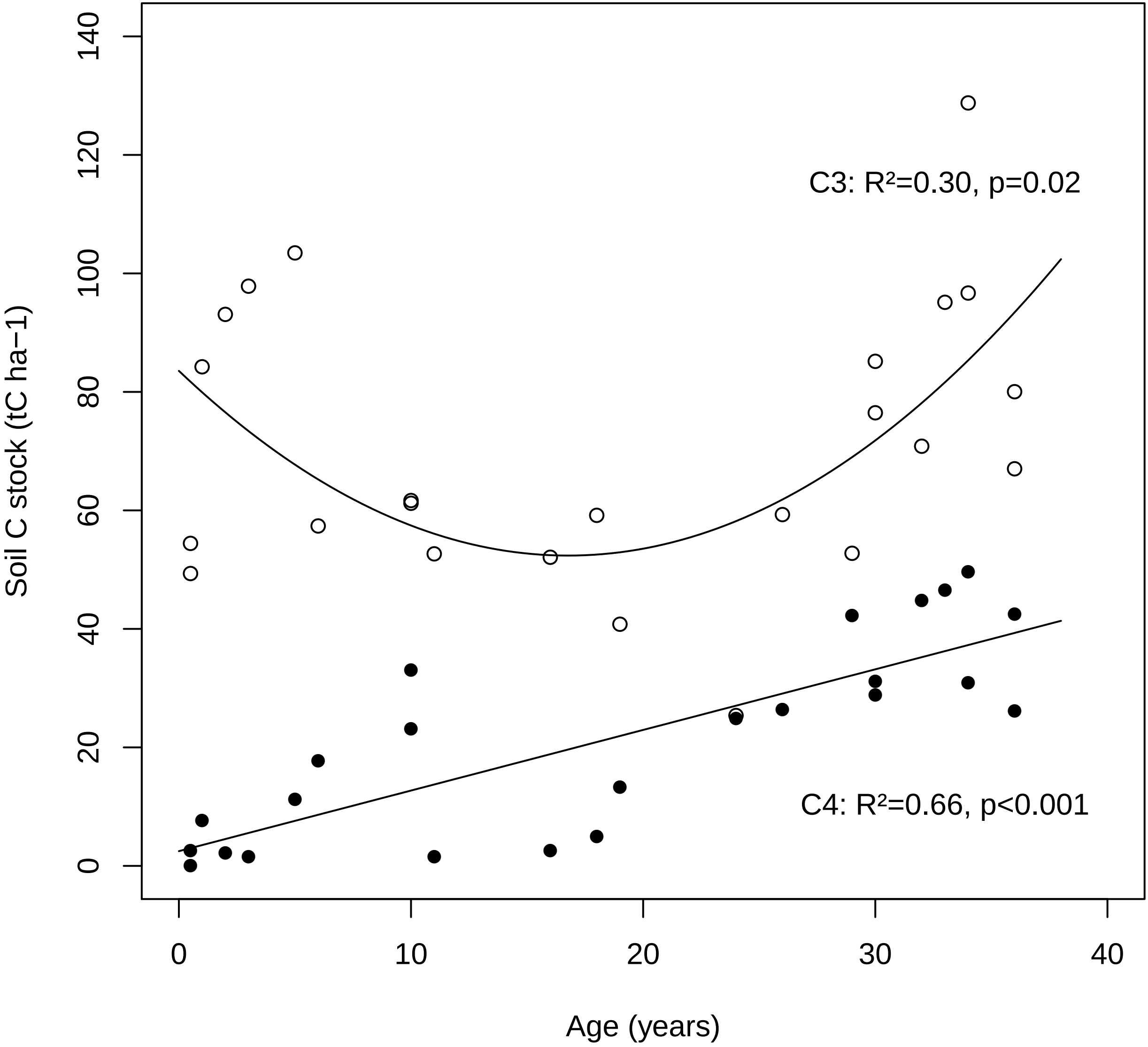
150
200
Kilometers
Brazil


(a)
R²=0.23, p=0.08
(b)
C stock = 5.31 * age − 48.47
R²=0.42, p=0.03
(c)
R²=0.22, p=0.09
R²=0.20, p=0.63
(d)
Humified−C stock = 5.70 * age − 66.40
R²=0.48, p=0.02
R²=0.15, p=0.24
(e)
0−20: R²=0.16, p=0.15
20−50: R²=0.20, p=0.11
50−100: R²=0.04, p=0.49
(f)
0−20: R²=0.02, p=0.68
20−50: R²=0.56, p<0.01
50−100: R²=0.43, p=0.03
Soil C stock (tC ha−1)
Soil humified−C stock (tC ha−1)
Age (years)
0
40
80
120
160
0
20
40
60
80
0
5
10
15
20
25
30
35
40


C3: R²=0.30, p=0.02
C4: R²=0.66, p<0.001
Soil C stock (tC ha−1)
Age (years)
0
20
40
60
80
100
120
140
0
10
20
30
40