

# Medium to long-term patterns of soil properties in forest restoration models in seasonally dry Atlantic Forest biome

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## Research Article

**Keywords:** Forest restoration, land uses, ecosystem services, soil fertility, soil organic carbon

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26 **HIGHLIGHTS**

27

- 28 • NF regenerated after coffee was the most depleted soil fertility and SOC storage
- 29 • Grass pasture and legume plantations showed smaller soil acidity and larger base  
30 concentration than NF areas
- 31 • SOC was large in NF regenerated after selective cutting and grass pasture
- 32 • Legume trees decreased SOC storage by 35% after planting on grass pasture

33

34 **ABSTRACT**

35 The medium- to long-term impacts of forest restoration models on soil properties are not  
36 well documented in the Atlantic Forest biome, especially in a seasonally dry climate. We  
37 assessed patterns of topsoil fertility, soil organic carbon (SOC) storage, and isotopic  
38 signatures of  $^{13}\text{C}$  in gradient with three areas with natural regeneration of native forest  
39 (NF) with 50 years of old, two areas with monocultures of  $\text{N}_2$ -fixer and non- $\text{N}_2$ -fixer tree  
40 species, six years after planting on grass pasture, and one area with grass pasture in the  
41 last 40 years of age. In each area, three to nine composite soil samples were collected at  
42 0-20 cm soil depth during the rainy season for analysis. The topsoil of the NF regenerated  
43 after coffee was the most depleted soil fertility and SOC storage in our vegetation  
44 gradient. The grass pasture and forest plantations showed smaller soil acidity and a larger  
45 base concentrations than the NF areas. The SOC storage was larger in NF after grass  
46 pasture and selective cutting, followed by grass pasture, with 80, 76, and 69  $\text{Mg ha}^{-1}$ ,  
47 respectively. The planting of legume trees decreased SOC storage by approximately 35%  
48 during SOC storage, independent of  $\text{N}_2$ -fixing capacity. The SOC derived from trees  
49 increased rapidly in the first six years of tree plantation ( $\approx 3 \text{ Mg SOC ha}^{-1} \text{ year}^{-1}$ ). The

50 recovery of soil fertility and SOC storage by long-term natural regeneration differs  
51 according to historical land use. Differences in N<sub>2</sub>-fixation capacity had little effect on  
52 soil properties in the medium term.

53

54 **Keywords:** Forest restoration, land uses, ecosystem services, soil fertility, soil organic  
55 carbon.

56

## 57 INTRODUCTION

58 The promotion of forest restoration and ecosystem services has been extensively  
59 debated in the Atlantic Forest biome of Brazil (Latawiec et al., 2015; Chazdon et al.,  
60 2017). This biome has the last 12 million hectares of abandoned land to be restored, and  
61 the other 15 million hectares of pasture (Chazdon et al., 2016). The planting of trees and  
62 natural regeneration are the two most used restoration interventions in the Atlantic Forest  
63 biome. The improvement of soil fertility and soil organic carbon (SOC) storage is one of  
64 the ecosystem services expected from the medium to long term after the restoration of  
65 agricultural lands. However, there are few field studies showing soil data for restorations  
66 with monocultures of native trees that have been established in the Atlantic Forest biome  
67 in the last decade (Mendes et al., 2019). In tropical regions, these concerns have mainly  
68 been evaluated for some tree genera, mainly *Eucalyptus* and *Pinus*, and N<sub>2</sub>-fixing trees  
69 such as *Acacia*, *Albizia*, *Leucaena*, and *Casuarina* genera (Marín-Spiotta and Sharma,  
70 2013; Shi et al., 2016).

71 Soil carbon loss due to land-use change is the second major cause of the increasing  
72 CO<sub>2</sub> in atmosphere after fossil fuels, and is mainly responsible for the increase in  
73 greenhouse gas emissions in tropical countries (Assad et al., 2013; Locatelli et al., 2015).  
74 Moreover, the conversion of land use affects several soil properties besides SOC (Guo

75 and Gifford, 2002; Don et al., 2011), such as acidity, nitrogen, phosphorus, and cation  
76 exchange (Shi et al., 2016). In general, the pools of C, N, and other nutrients in natural  
77 forest soil (NF) are significantly reduced due to the conversion of NF to traditional  
78 agriculture, mainly with non-sustainable practices (Don et al., 2011; Lal, 2018). The  
79 conversion of NF to grass pasture has a controversial impact in the short to medium term  
80 (Guo and Gifford, 2002; Don et al., 2011). However, the recovery of soil properties often  
81 occurs when agriculture and grass pastures are restored by afforestation over the long  
82 term (Don et al., 2011). At the same time, there is an equal lack of soil data in natural  
83 forests regenerated after agriculture and grass pasture in the last half century, when it  
84 intensified the protection of native forests in Brazil (from 1964).

85         The effect of tree planting on soil fertility and SOC storage varies with previous  
86 use and management intensity (Laganière et al., 2010), and functional traits of the species  
87 (De Deyn et al., 2008). Positive effects on soil fertility and C status are often found when  
88 bare land and cropland are converted in tree plantations with stand age higher than 10-30  
89 years (Guo and Gifford, 2002; Shi et al., 2016). However, the same patterns were less  
90 frequent when grass pastures were converted to tree plantations (Shi et al., 2016).  
91 Moreover, plantations using management practices with minimum soil interventions  
92 show higher SOC storage than plantations with high management intensity (Laganière et  
93 al., 2010). The N<sub>2</sub>-fixing legume tree species are an exception because several studies  
94 have shown a faster positive effect of these functional groups on soil properties when  
95 established in agricultural and grass pasture areas (Shi et al., 2016; Marín-Spiotta and  
96 Sharma 2013). However, few studies have been conducted to compare the effects of  
97 legume trees with different capacities to N<sub>2</sub>-fixation on soil properties after afforestation  
98 of grass pastures in the Atlantic Forest biome (Barroso et al., 2018).

99           We carried out a field investigation to assess patterns of soil fertility (*i.e.*, pH, Al,  
100 P, K, Ca, Mg, base exchange), C and N concentrations, SOC storage, and isotopic  
101 signatures of <sup>13</sup>C in a gradient of vegetation localized in the seasonally dry Atlantic Forest  
102 biome in Southeast Brazil. The gradient of vegetation was composed of NF with at least  
103 50 years of natural regeneration after selective logging, and after coffee agriculture or  
104 grass pasture, one grass pasture area with at least 40 years of age, and two monocultures  
105 with N<sub>2</sub>-fixing *Anadenanthera peregrina* var. *peregrina* (L.) Speg. and non-N<sub>2</sub>-fixing  
106 *Schizolobium parahyba* var. *amazonicum* (Huber & Ducke) Barneby, six years after  
107 planting on grass pastures. Our hypotheses were as follows: 1) NF with at least 50 years  
108 of natural regeneration would show similar soil property patterns, and 2) the N<sub>2</sub>-fixing  
109 legume trees would improve soil properties by a major magnitude compared to non-N<sub>2</sub>-  
110 fixing legume trees.

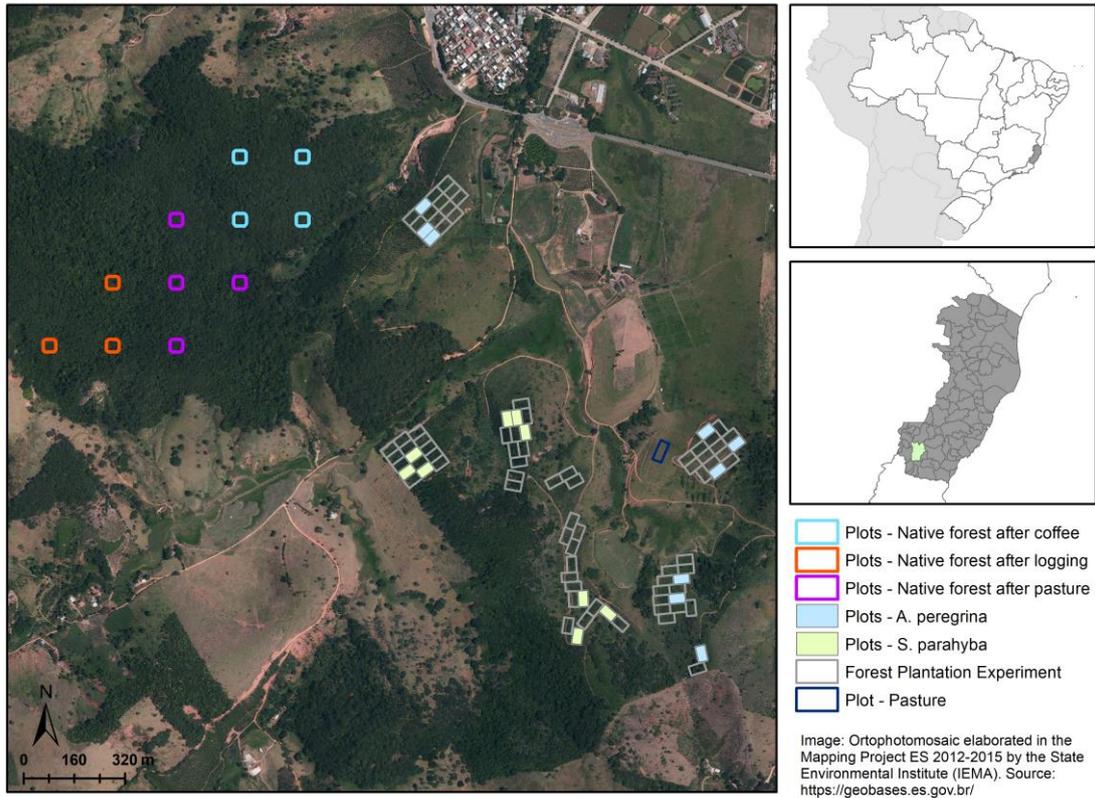
111

## 112 **MATERIAL AND METHODS**

### 113 **Study area**

114           The study was carried out at the campus of *the Instituto Federal do Espírito Santo*  
115 (IFES), municipality of Alegre, in southern Brazil (Fig. 1). The vegetation was spread  
116 over an area that describes a vegetation gradient ordinary to the Atlantic Forest biome of  
117 southeast Brazil. It involves the regeneration of NF, grass pastures, and tree plantations.  
118 The climate is classified as Aw, with rainy summers and dry winters. The mean annual  
119 rainfall is approximately 1200 mm with a dry period (< 50 mm) from May to September,  
120 and the mean annual temperature is 23 °C (Alvares et al., 2013). The relief region varies  
121 from wavy to mountainous, with the occurrence of mountains and culvert chains and an  
122 average altitude of 150 m (IBGE, 1987). The slope ranged between 15% and 45% in most

123 sampling areas, and the main soil type was Ferralsols (Lorenzoni-Paschoa et al., 2019;  
124 Bigi et al., 2021).  
125



126  
127 **Fig. 1** Localization of plots in the vegetation gradient

128  
129 The natural forest is a forest fragment of seasonally dry forest with a total area of  
130 109.6 hectares. The fragment was previously subjected to three different disturbances:  
131 coffee plantations, African grass pasture, and selective tree cutting (Lorenzoni-Paschoa  
132 et al., 2019). This previous land use and management occurred at least 50 years ago. The  
133 fragments contained a minimum of 153 identified tree species. However, the main tree  
134 species were *Pseudopiptadenia contorta*, *Parapiptadenia pterosperm*, and *Apuleia*  
135 *leiocarpa*. The mean value of the basal area in the fragment was 29 m<sup>2</sup> ha<sup>-1</sup>, and there was

136 no statistically significant difference between previous land use and management  
137 (Lorenzoni-Paschoa et al., 2019).

138 The plantation of *A. peregrina* trees and *S. parahyba* trees was established in areas  
139 managed with grass pastures for more than 40 years with *Brachiaria* sp. dominant species.  
140 We used data from nine of these experimental plots per species planted in 2011 with a 3  
141 m × 3 m tree spacing (stand density of 1111 trees ha<sup>-1</sup>) (Bighi et al., 2021). The seedlings  
142 were produced from seeds originating from stock plants located in Dom Elizeu, state of  
143 Pará, Brazil, in Reserva Natural Vale in Linhares, state of Espírito Santo (Instituto  
144 Ambiental Vale). The seedlings of *A. peregrina* were not inoculated with rhizobial  
145 strains. However, natural nodules were observed in the study area (Mendes et al., 2021).  
146 The cattle were removed before planting, and the pasture was desiccated by applying  
147 glyphosate. The seedlings were planted in pits with dimensions of 30 cm × 30 cm × 30  
148 cm. Each seedling received 220 g of commercial fertilizer (NPK 06-30-06) more  
149 micronutrients (0.4 g of B, Cu; and Zn) per hole at the time of planting. Maintenance  
150 occurred in the first 12 months after planting, with replanting, crowning, and the control  
151 of leaf cutter ants.

152

### 153 **Soil sampling**

154 For the regenerated NF, the soil data determined by Lorenzoni-Paschoa (2016) and  
155 Neves (2018) at the 0-20 cm soil layer in 11 permanent plots were used. Each plot had an  
156 area of 400 m<sup>2</sup> (20 m × 20 m). Four plots were localized in the zones with NF regenerated  
157 after grass pasture and agriculture (coffee), and three plots were localized in the zone with  
158 the selective cutting of trees (Fig. 1). During the rainy season of 2015, ten soil samples  
159 per plot were collected and mixed in one soil sample. Soil fertility and texture were  
160 assessed in this study (Lorenzoni-Paschoa, 2016). Soil N and C concentrations and SOC

161 storage were determined in the rainy season of 2018 from five simple soil samples  
162 collected per plot and mixed (Neves, 2018). Soil density was also measured at two points  
163 per plot, using a metal ring with a volume of 106.7 cm<sup>3</sup> (Neves, 2018). Finally, the same  
164 soil samples collected by Neves (2018) were used to determine the natural abundance of  
165 <sup>13</sup>C in each plot.

166 For the grass pasture area, soil samples collected in the 0-20 cm layer on two  
167 occasions were used. Soil fertility and soil density were determined in soil samples  
168 collected from grass pasture areas during the rainy season of 2010, 12 months before tree  
169 plantation (Campanharo, 2017). On this occasion, 360 simple samples distributed  
170 systematically in all areas with tree plantations were collected (Fig. 1). The samples were  
171 mixed into six composite samples for the fertility analysis. Soil density was measured in  
172 60 samples distributed systematically in all areas used for planting tree legumes. For N  
173 and C concentrations, natural abundance of <sup>13</sup>C, and texture, one area with grass pasture  
174 adjacent to the forest plantation was used for soil samples collected (Fig. 1) during the  
175 rainy season of 2017. In this area, 18 soil samples were mixed into three composite soil  
176 samples for analysis.

177 For the tree plantation area, soil fertility and soil density in the 0-20 cm layer  
178 determined by Campanharo (2017) in the rain season of 2017, six years after planting,  
179 were used. In each plot with tree plantation, the 0-20 cm soil layer was collected from six  
180 different horizontal points from six different trees (Mendes et al. 2021). These six simple  
181 soil samples were mixed in one composite sample per plot and used for fertility analysis.  
182 The soil density was determined using a 5 cm diameter metal ring in a single sample  
183 collected from the central region of each plot. Furthermore, the N and C concentrations,

184 SOC storage, isotopic composition of  $^{13}\text{C}$ , and soil texture were determined using soil  
185 samples collected by Campanharo (2017).

186

### 187 **Laboratory analysis**

188 All analyses of soil fertility, soil density, and soil texture were carried out  
189 according to Embrapa (1997). The soil samples for soil density assessment were weighed  
190 on a precision balance after drying at 105 °C for 72 h. The proportions of clay, silt, and  
191 sand were determined using a pipetting method. The soil samples used for chemical  
192 analysis were air-dried, macerated, and sieved (0.5 mm).  $\text{pH}_{\text{H}_2\text{O}}$  was determined to be  
193 1:2.5. Phosphorus (P) and potassium (K) were extracted using Melich 1. Exchangeable  
194 Al, Ca, and Mg were extracted using KCl (1 mol L<sup>-1</sup>).

195 Soil N concentration was determined according to the Kjeldahl method  
196 (Mendonça and Matos, 2005). Soil C concentration and  $\delta^{13}\text{C}$  were determined using a  
197 20–20 Hydra mass spectrometer (ANCA-GSL, SERCON Co., Crewe, Uk) (Boutton,  
198 1991). The  $\delta^{13}\text{C}_{\text{VPDB}}$  signature (i.e., using the Vienna Pee Dee Belemnite standard) was  
199 calculated.

200

### 201 **Data analysis**

202 The SOC storage (Mg ha<sup>-1</sup>) at 0-20 cm was estimated using the soil C  
203 concentration (%) multiplied by the soil mass measured in each vegetation.

204 The amounts of SOC derived from trees and grass were calculated by multiplying  
205 the SOC storage by the proportion of C derived from each vegetation. The proportion of  
206 SOC derived from trees and grass was estimated using Equations 1 and 2, respectively:

$$207 \quad \% C \text{ trees} = \left( \frac{\delta \text{ soil tree} - \delta \text{ soil pasture}}{\delta \text{ soil ref} - \delta \text{ soil pasture}} \right) * 100 \quad (1)$$

208 
$$\% C_{grass} = 100 - \% C_{trees} \quad (2)$$

209 Where: % C trees or % C grass is the proportion of SOC derived from trees and grass;  $\delta$   
210 is the  $\delta^{13}C$  (‰) value determined in each plot by tree plantation (N=9), and in each plot  
211 with NF regenerated after pasture (N=4);  $\delta$  soil pasture is the mean value of  $\delta^{13}C$  (‰) in  
212 soil sampled in pasture area; and  $\delta$  soil ref is the mean value of  $\delta^{13}C$  (‰) in soil sampled  
213 in NF regenerated after selective cutting (N=3).

214 The mean values of the chemical and physical properties of the soil were  
215 compared among areas using one-way ANOVA for normal data or the Kruskal-Wallis  
216 test for non-normal data. The mean values of soil properties in each of the five areas were  
217 compared with the control group NF after selective cutting using Dunnett's method for  
218 normal data, or Dunn's method for non-normal data. Normality was tested using the  
219 Shapiro-Wilk test. Statistical analysis was performed with SigmaPlot 13 (Systat, 2014),  
220 using a statistical threshold value of  $P = 0.05$ .

221

## 222 **RESULTS**

223 There were different patterns of soil fertility among the areas in the studied  
224 vegetation gradient (Tab. 1). Soil acidity and Al concentration were higher mainly in the  
225 regenerated coffee than in the grass pasture or legume plantations. The mean P  
226 concentrations were generally larger in legume tree plantations, but the difference was  
227 not statistically significant. The base concentrations were often smaller in the NF-coffee  
228 culture than in the other areas. In addition, the base concentrations were often larger in  
229 grass pasture and legume tree plantations than in NF areas. However, statistical  
230 differences among the areas were found only for Mg concentrations.

231           The mean N concentration values ranging from 0.19 to 0.25 % were statistically  
232 equal among the areas (Tab. 1). In contrast, the C concentration was larger in NF-SC and  
233 NF-grass, followed by grass pastures. At the same time, the C concentrations were similar  
234 in legume trees and NF-coffee plantations. The C concentration was 1.6 time higher in  
235 the reference area NF-SC than in the tree plantation and NF-coffee. The  $\delta^{13}\text{C}$  values were  
236 statistically higher in grass pasture and tree plantation areas than in the reference NF-SC.  
237  $\delta^{13}\text{C}$  assumed intermediate values between NF and grass pastures.

238           Soil density was higher in grass pasture areas than in forest areas (Tab. 1). The  
239 soil texture was statistically equal among areas, with clay concentrations ranging from  
240 50% to 56%, and sand concentrations from 35% to 42%.

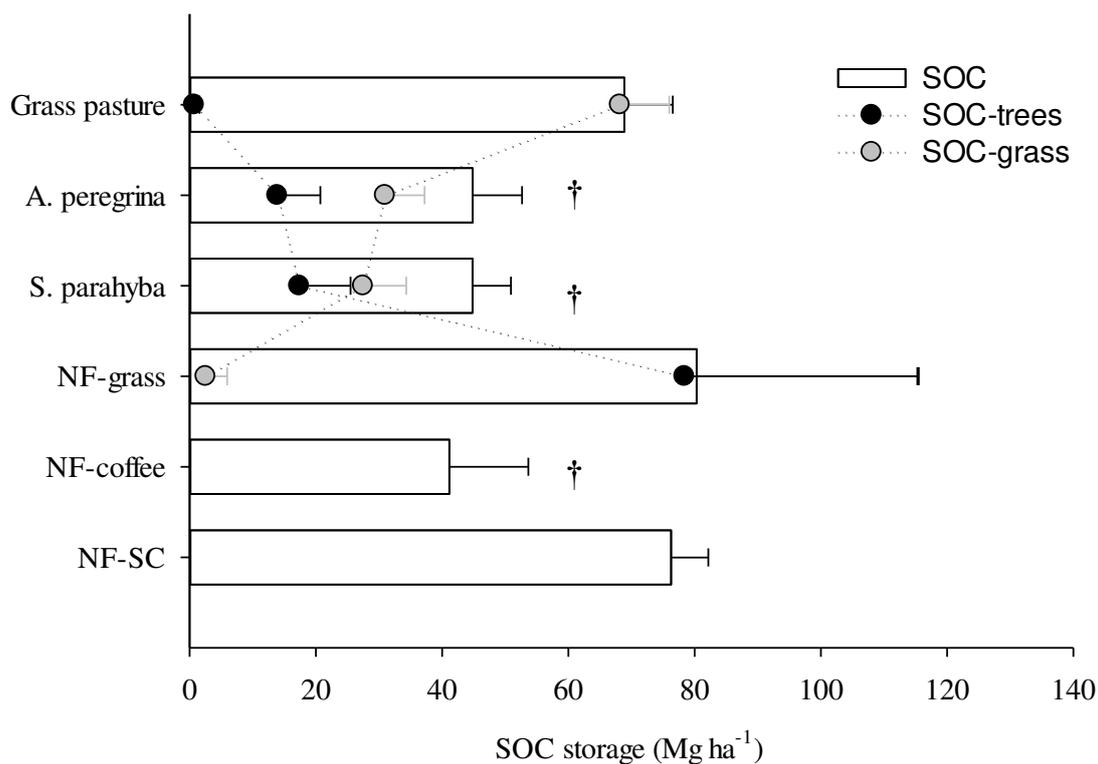
241           The SOC storage values were 1.7 times higher in NF-SC, NF-grass, and grass  
242 pasture ( $\approx 75 \text{ Mg ha}^{-1}$ ) than in tree plantation and NF-coffee areas (Fig. 2). At the same  
243 time, the SOC storage was similar among the NF-coffee and legume tree plantations, with  
244 mean values ranging from 41 to 44  $\text{Mg ha}^{-1}$ .

245           The SOC derived from the tree increased from 0.7 in grass pasture to 78  $\text{Mg ha}^{-1}$   
246 in NF-grass. In addition, the SOC derived from the tree was slightly larger in *S. parahyba*  
247 than in *A. peregrina*, with 19 and 15  $\text{Mg ha}^{-1}$ , respectively. Furthermore, the SOC derived  
248 from grass decreased from 68  $\text{Mg ha}^{-1}$  in grass pasture to  $\sim 29 \text{ Mg ha}^{-1}$  in legume tree  
249 plantations and 2  $\text{Mg ha}^{-1}$  in NF-grass.

250 **Tab. 1** Soil proprieties in vegetational gradient composed of native forest (NF) regenerate after the selective cutting of trees (SC), grass  
 251 pasture (grass), agriculture (coffee), grass pasture, and legume tree species plantations. \* Significant differences among areas. † Significant  
 252 difference between each area and the NF-SC control

Variables	Vegetational gradient					
	NF - SC	NF - grass	NF - coffee	Grass pasture	<i>S. parayba</i>	<i>A. peregrina</i>
pH (water) *	5.15 ± 0.70	5.18 ± 0.63	4.46 ± 0.13	5.74 ± 0.33	5.45 ± 0.53	5.33 ± 0.19
Al (cmol <sub>c</sub> dm <sup>-3</sup> ) *	0.29 ± 0.44	0.30 ± 0.22	0.78 ± 0.28†	0.09 ± 0.12	0.09 ± 0.12	0.06 ± 0.04
P (cmol <sub>c</sub> dm <sup>-3</sup> )	1.55 ± 0.47	2.23 ± 0.31	1.80 ± 0.41	2.00 ± 0.40	2.71 ± 0.78	2.79 ± 2.41
K (cmol <sub>c</sub> dm <sup>-3</sup> )	92.77 ± 41.71	101.03 ± 33.04	44.83 ± 13.82	72.46 ± 18.65	69.67 ± 38.40	53.33 ± 24.27
Ca (cmol <sub>c</sub> dm <sup>-3</sup> )	1.72 ± 1.09	1.95 ± 1.46	0.47 ± 0.47	2.44 ± 1.37	2.52 ± 2.03	3.07 ± 2.04
Mg (cmol <sub>c</sub> dm <sup>-3</sup> ) *	1.16 ± 0.35	1.21 ± 0.38	0.66 ± 0.24	1.51 ± 0.66	1.33 ± 0.81	2.21 ± 1.11
CEC (cmol <sub>c</sub> dm <sup>-3</sup> )	3.43 ± 1.15	3.73 ± 1.69	2.04 ± 0.47	4.23 ± 1.91	4.14 ± 2.68	5.49 ± 3.14
C (%) *	2.74 ± 0.26	2.88 ± 1.04	1.70 ± 0.53†	2.28 ± 0.25	1.75 ± 0.30†	1.80 ± 0.31†
N (%)	0.21 ± 0.07	0.20 ± 0.05	0.25 ± 0.07	0.21 ± 0.04	0.19 ± 0.05	0.20 ± 0.03
δ <sup>13</sup> C (δ ‰) *	-26.16 ± 0.45	-25.88 ± 0.46	-26.43 ± 0.38	-16.75 ± 0.27†	-20.33 ± 1.40†	-19.59 ± 1.30†
Density (g cm <sup>-3</sup> ) *	1.39 ± 0.03	1.37 ± 0.14	1.21 ± 0.04	1.51 ± 0.04	1.28 ± 0.11	1.24 ± 0.06
Clay (%)	54.85 ± 2.77	55.13 ± 5.54	49.76 ± 3.89	52.88 ± 20.09	51.46 ± 8.33	56.46 ± 4.45
Sand (%)	37.10 ± 3.78	35.99 ± 2.62	42.18 ± 4.98	40.98 ± 12.62	39.73 ± 4.69	35.15 ± 6.31

253



254

255 **Fig. 2** Mean values of SOC storage ( $\pm$  standard deviation) at 0-20 soil layer in  
 256 vegetational gradient compose by native forest (NF) regenerate after the selective cutting  
 257 of trees (SC), grass pasture (grass), agriculture (coffee), and in grass pasture, and legume  
 258 tree species plantations. The amounts of SOC derived from trees (black) and grass (grey)  
 259 plants area showed by scattering lines. † Difference significant between each area and  
 260 control NF-SC

261

262 **DISCUSSION**

263 The topsoil of NF regenerated after coffee culture was depleted in most soil  
 264 properties compared to other NF after selective cutting and grass pasture. In fact, the soil  
 265 acidity, Al, and base concentrations in the NF-coffee area were the lowest soil fertility  
 266 class of soil fertility for the Espírito Santo state (Prezotti et al., 2007). In contrast, NF  
 267 regenerated after grass pasture showed similar patterns of soil fertility and C pools

268 compared to the NF after selective cutting. Guo and Gifford (2002) showed that the  
269 replacement of natural forests by agriculture was responsible for the reduction of C stocks  
270 by up to 60% in the first 30 cm of depth. However, the authors found that the conversion  
271 of natural forests to grass pastures does not result in a reduction in the C stock in regions  
272 with annual rainfall ranging from to 1000-3000 mm.

273 A set of factors could explain the differences observed in soil properties between  
274 NF regenerated after coffee and grass pasture areas, in addition to natural soil variability.  
275 The conversion of land use from NF to agriculture and livestock is achieved by harvesting  
276 all valuable wood species followed by the burning of forest slash. The traditional  
277 management of coffee culture, where the plants are alone, favors the loss of SOC and soil  
278 nutrients (Sales et al., 2013), especially in the early ages due to little ground cover. In  
279 addition, coffee plants were likely unfertilized in the 1960s. Grass pastures are widely  
280 known for their ability to store organic matter in soil Abdalla et al. (2018). The lower  
281 values of SOC storage in grass pasture area than in NF after selective cutting could be  
282 caused by occasional burning in the past of the seasonally dry Atlantic Forest. Although  
283 specific information about the historical burning in these areas is unknown, burning is a  
284 common practice used in agriculture and grass pastures in the region of study (Gobbo et  
285 al., 2016). Finally, we observed great stand deviation in SOC storage in the NF after the  
286 grass area. This was caused by one plot that showed high SOC storage ( $119 \text{ Mg ha}^{-1}$ ) and  
287 CEC concentration ( $6.24 \text{ cmol}_c \text{ dm}^{-3}$ ) in the gradient. This plot had the lowest altitude and  
288 the slope of the NF area. The CEC concentration found in this plot was the highest among  
289 soil fertility classes in the Espírito Santo state (Prezotti et al., 2007). Thus, we noticed  
290 the removal of this plot of the calculations, and the mean SOC storage values for NF after  
291 grass were reduced to  $67 \pm 13 \text{ Mg ha}^{-1}$ . This value is very similar to that observed for

292 grass pasture areas. The CEC value in the other three plots of NF after grass area was 2.89  
293  $\text{cmol}_c \text{dm}^{-3}$ .

294 The soil impact of coffee culture could be observed in the long term in the  
295 seasonally dry Atlantic Forest region. Although natural regeneration faced a more  
296 stressful soil environment in the coffee area than in the grass pasture area, the basal area  
297 of the stand was statistically equal between NF areas (Lorenzoni-Paschoa et al., 2019).  
298 Moreover, litterfall production and N, P, and C inputs were very similar among the NF  
299 areas (Neves, 2018, unpublished data). Thus, the availability of nutrients in the soil likely  
300 has less effect on the long-term accumulation of biomass after 50 years. However,  
301 differences in the structure and phytosociology of vegetation between NFs have been  
302 observed (Lorenzoni-Paschoa et al., 2019). These authors found similar species richness  
303 and diversity between NFs regenerated after selective cutting of the tree and after grass.  
304 At the same time, the pattern difference in NF after coffee showed slower advancement  
305 of the successional stage of vegetation in this area than in NF after grass (Lorenzoni-  
306 Paschoa et al., 2019).

307 Further studies could assess more details of soil type and parental material to  
308 confirm the tendencies in NF areas. Paz et al. (2016) showed that clay-rich soils have  
309 higher SOC storage than sandy-rich soils. However, in sandy soils, there was an increase  
310 in SOC storage over the time of forest succession. On the other hand, the slow recovery  
311 of SOC storage in NF regenerated after coffee may be explained by the high clay content  
312 in the topsoil layer. Moreover, the area with NF regenerated after grass pasture still  
313 presents recalcitrant forms of grass-derived C, possibly protected in macroaggregates (Six  
314 et al., 2002).

315           The planting of both legume trees decreased in about 35% the SOC storage in 0-  
316 20 cm layer in relation to pasture area at six years after planting. This difference in SOC  
317 storage is between the highest observed in reviews and meta-analyses (Guo and Gifford,  
318 2002; Don et al., 2011; Shi et al., 2016). Mass correction (Don et al., 2011) was not used  
319 to minimize the effects of land use on topsoil density because of small differences in  
320 patterns observed for SOC storage. Using the correction of soil mass with native forest  
321 after selective logging as a reference por example, the estimated decrease in SOC by  
322 legume tree planting on grass pasture was 22%. However, we showed that the patterns  
323 are different for N<sub>2</sub>-fixing tree species because, in general, there is an increase in SOC  
324 storage after the establishment of this species (Guo and Gifford, 2002; Shi et al., 2016).  
325 Moreover, the change in the isotopic composition of <sup>13</sup>C suggested a faster loss of labile  
326 soil C derived from grass in the first six years after planting. At the same time, the input  
327 of SOC derived from trees was slightly higher in *S. parahyba* than in *A. peregrina* trees  
328 up to six years after (2.8 versus 2.3 Mg SOC ha<sup>-1</sup> year<sup>-1</sup>). This indicates a more rapid  
329 turnover of SOC after the introduction of non-N<sub>2</sub>-fixing legumes that have a higher  
330 growth rate in stem diameter and total height than the N<sub>2</sub>-fixing *A. peregrina* Bigli et al.  
331 (2021).

332           The δ<sup>13</sup>C values of the soil were slightly lower in the plantations of *S. parahyba*  
333 than in *A. peregrina*. Plots with *S. parahyba* were completely occupied by *Brachiaria*  
334 sp., while the presence of grasses in the understory was drastically reduced in plots with  
335 *A. peregrina* (Campanharo, 2017). The presence of grasses should lead to higher δ<sup>13</sup>C  
336 values in the soil with *S. parahyba* than in *A. peregrina* trees. This pattern may be  
337 explained by the functional traits of trees that influence the litter decomposition process  
338 De Deyn et al. (2008), as well as the presence of understory that may prevent loss of litter

339 and SOC in *S. parahyba*. For example, the  $\delta^{13}\text{C}$  value was smaller in the leaf litter of *S.*  
340 *parahyba* than in *A. peregrina* (Paula et al., unpublished data). Furthermore, it is likely  
341 that SOC derived from both legume trees occurred in the sand fraction because of the  
342 significant negative correlation by Spearman correlation between clay content and SOC  
343 derived from trees (-0.523; P=0.0255; N=18). Further studies should assess more details  
344 about the litter quality of this legume species, as well as the correlations between texture  
345 and SOC derived from species over the long term in this planting.

346         Soil fertility generally improved after the introduction of tree legumes into grass  
347 pasture areas, although the differences were not statistically significant. These results may  
348 also be explained by the effect of fertilizer in the planting, which has been cycled by trees  
349 over the years. However, the differences in chemical properties, although subtle, indicate  
350 that soil fertility (*e.g.*, CEC) was likely affected by the N<sub>2</sub>-fixer trees compared to *S.*  
351 *parahyba*, but not by N and C pools. Faster improvement in soil fertility occurs with the  
352 introduction of fast-growing N<sub>2</sub>-fixing trees, mainly on severely degraded land in the  
353 Atlantic Forest biome Macedo et al. (2008). However, this does not appear to have been  
354 the case for our grass pasture, since soil fertility parameters were similar or greater in the  
355 grass pasture than in the NF.

356

## 357 **CONCLUSION**

358         Our results identified different patterns of topsoil properties among restoration  
359 models with long-term natural regeneration areas and medium-term legume tree  
360 plantations. Fifty years after traditional coffee culture, the 0-20 cm soil depth still showed  
361 higher soil acidity and lower soil fertility parameters, as well as SOC storage, while soil  
362 properties were more similar between NF after grass pasture and selective cutting. Thus,  
363 the patterns of topsoil properties suggest that long-term natural regeneration may be

364 insufficient to recover the original topsoil in previous coffee areas of seasonally dry  
365 tropical climates. However, further studies are needed to assess more soil and land  
366 historical details to confirm our results. Legume tree plantations established on grass  
367 pasture showed similar trends of decrease in approximately 35% of the SOC storage  
368 compared to grass pasture area. However, the SOC derived from trees increased rapidly  
369 in the first six years of tree plantation ( $\approx 3 \text{ Mg SOC ha}^{-1} \text{ year}^{-1}$ ). Furthermore, the  
370 differences in soil fertility suggested a more positive effect of *N*<sub>2</sub>-fixer *A. peregrina* than  
371 non-*N*<sub>2</sub>-fixer *S. parahyba*, although statistically significant differences were not found up  
372 to six years after planting.

373

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