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1 **Aerobic microbial activity in four tropical earthworm-soil systems. A mesocosm**
2 **experiment**

3 Running head: Microbial activity in earthworm-soil systems

4

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12

12 **Aerobic microbial activity in four tropical earthworm-soil systems. A mesocosm** 13 **experiment**

14

15 **Abstract.** Soil organic matter (SOM) quality and carbon (C) availability may be major features
 16 affecting the effect of earthworms on the aerobic processes in clayey tropical soils. In this study, we
 17 assessed the effect of an anecic (*Polypheretima elongata*), an endogeic (*Pontoscolex corethrurus*) and
 18 an epigeic (*Eudrilus eugeniae*) earthworm on the aerobic microbial activity of two tropical soils, a
 19 calcic vertisol and an acid ferralsol, with clay content >70% and very different organic C content and
 20 SOM stability. The soil-earthworm interaction was studied in a 6-month mesocosm experiment in a
 21 greenhouse using soils with and without (control soil) earthworm addition. Potential C mineralization,
 22 actual net nitrogen (N) mineralization and dehydrogenase activity (DHA), as indicators of the aerobic
 23 activity of the soils, and phosphorus (P) availability were determined during the trial. DHA was used
 24 as an indicator of the global aerobic activity. Earthworms affected little potential C mineralization but
 25 increased significantly actual net N mineralization. The increase of N mineralization in the vertisol
 26 was twice as great as, and longer (6 months vs. 3 months) than for the ferralsol. Differences between
 27 soils for N mineralization were associated with a less recalcitrant SOM in the vertisol. Available P
 28 increased 10% in the earthworm treatments. Earthworm activity improved N and P availability. DHA
 29 was 15 times higher for the vertisol than for the ferralsol, but the positive effect of earthworms on
 30 DHA was greater for the ferralsol. This effect was greater for *E. eugeniae* probably due to surface
 31 burrows generated by this epigeic earthworm, which favoured oxygen entry into the soil. Differences
 32 between the two soils were greater for DHA than for C and N mineralization, and this was observed
 33 for the control soils as well as for the earthworm treatments. This indicates that earthworm activity
 34 modified the rate of the aerobic processes but it did not affect the intrinsic biological properties of
 35 these tropical soils, which were controlled mainly by SOM quality and C availability.

36

37 **Additional keywords:** *Eudrilus eugeniae*, ferralsol, *Polypheretima elongata*, *Pontoscolex*
 38 *corethrurus*, SOM mineralization, vertisol.

39

40

41 **Introduction**

42

43 Earthworms contribute to many ecosystem services, including water regulation, nutrient cycling,
 44 carbon (C) sequestration and primary production (Blouin *et al.* 2013). Earthworm species and
 45 populations vary widely with the ecosystem management, such as differences in tillage, nutrient and
 46 pesticide inputs, and crop rotation (Lavelle 2002). The intensive cropping systems developed since
 47 about 1980 in the Caribbean region are based on heavy use of fertilizers and pesticides, which cause
 48 severe diffuse pollution of water resources and reduce soil fauna and biodiversity (Charlier *et al.*

49 2009). As an example, Clermont-Dauphin *et al.* (2004) reported that microbial activity and earthworm
50 biomass in the soils of that region decreased drastically under intensive banana monocropping.

51 Blouin *et al.* (2013) proposed a theoretical approach to assess the impact of practices including
52 earthworms on ecosystem services. These practices vary according to a gradient from passive
53 biostimulation (e.g. removal of management practices detrimental to earthworms) to active
54 biostimulation (e.g. vermicomposting, inoculation in the field). This approach may be considered as a
55 conceptual tool to identify different management techniques to preserve or to improve ecosystem
56 behaviour. Recently, it has been reported that earthworms in a tropical leptosol enhance the
57 stabilization of soil organic matter only when organic residues are applied (Fonte and Six 2010). In
58 this case, changes in management systems are probably a better way of manipulating carbon
59 sequestration in agricultural contexts than the inoculation of earthworms. Indeed, the most effective
60 use of earthworms to improve soil quality requires a detailed understanding of the interaction between
61 soils properties and earthworm activity (Lavelle and Spain 2001). McInerney and Bolger (2000)
62 assessed the effect of temperature, wetting cycles and soil texture on C and nitrogen (N) dynamics in
63 earthworm casts in temperate soils. They observed a strong protection of C and N in casts at first,
64 followed by a reduction in protection due to weakening of aggregate structure. The extent of the
65 protection phase depended on complex interactions between the three studied factors. Similar results
66 were obtained by McInerney *et al.* (2001) who observed that earthworm reworking of soil may cause
67 pulses of mineralization and reduce C stability inside clay-rich casts. Moreover, Marhan and Scheu
68 (2005) found that C mineralization in casts prevailed on C stabilisation, and this was further stimulated
69 by the addition of sand in soils. For a tropical ferralsol, Chapuis-Lardy *et al.* (2010) reported that
70 earthworm activity increased C mineralization in the short term. The same observation was made by
71 Bernier (1998) in a forest soil of the French Alps for anecic and endogeic earthworms.

72 Earthworms are currently classified in three main functional groups: epigeic living at the
73 topsoil, endogeic living near the soil surface, and the deep-burrowing anecic species (Sheehan *et al.*
74 2007). Anecic earthworms are larger than topsoil species, feeding on larger quantities of organic
75 matter on the soil surface, having the potential to incorporate this deeper in the soil profile. Another
76 classification based on feeding habits places epigeic and anecic earthworms into the group of
77 detritivores, with a regime based on plant litter and mammalian dung, and the endogeic earthworms in
78 the group of geophages, which derive their nutrition from soil organic matter (SOM) and dead roots
79 (Curry and Schmidt 2007). Indeed, contradictory results concerning the effect of earthworm activity
80 on soil C and N dynamics could be attributed to differences between earthworm species in soil
81 location and feeding habits. However, Lavelle *et al.* (2004) highlight that earthworms may not always
82 exhibit the behaviour associated to their functional group, and that a classification based more on
83 impacts on soil parameters might be more useful. This is particularly important in the Caribbean
84 region where climatic gradients on small spatial scales (i.e. 10 km) and volcanic activity have led to a
85 great diversity of clayey soils with very different soil mineralogy, C content and SOM quality, which
86 may strongly affect earthworm behaviour and functions (Clermont-Dauphin *et al.* 2004; Sierra 2006).

87 In this study, we tested the hypothesis that the effect of earthworm activity in these soils is
 88 mainly controlled by SOM quality and C availability, which depends upon the strength of SOM
 89 stabilization with the specific clay fraction of each soil group. For this, we assessed the effect of an
 90 anecic, an endogeic and an epigeic earthworm species on C and N mineralization and on the total
 91 aerobic microbial activity of two tropical soils of Guadeloupe (vertisol and ferralsol) with very
 92 different mineralogy, SOM quality and C availability. This study is part of a larger project devoted to
 93 improve soil quality in the Caribbean region using earthworm inoculation in crop (Lafont *et al.* 2007;
 94 Loranger-Merciris *et al.* 2012) and grassland (d'Alexis *et al.* 2009) systems. These studies were
 95 carried out on the same vertisol and ferralsol used in the present study. So the aim of this work was to
 96 analyse how active biostimulation by inoculation of earthworms affects some key aerobic processes in
 97 these tropical soils.

98
 99

100 **Materials and methods**

101

102 *Rationale of the experimental design*

103

104 For this study we selected an acid dark red ferralsol and a calcic vertisol (FAO Unesco 2006), with
 105 very different SOM content and SOM stability. These factors may greatly affect earthworm activity
 106 (Barot *et al.* 2007; Blouin *et al.* 2013). SOM content is lower in the ferralsol and it is very recalcitrant.
 107 Most of SOM may be considered as inert at the time scale of this study. This fraction corresponds to
 108 SOM complexed with active Fe and Al sesquioxides and has a mean residence time of 2000-5000
 109 years (Sierra *et al.* 2010). SOM in the vertisol is complexed with smectite, but the labile SOM fraction
 110 is greater than that in the ferralsol, and then C availability is also greater in that soil (Sierra *et al.*
 111 2002). These soil properties should allow to test the hypothesis relative to the effect of SOM quality
 112 and C availability on earthworm activity. Similarly, the effect of earthworm was assessed using a
 113 relatively long term mesocosm experiment (i.e. 6 months), which is required to better understand the
 114 influence of earthworms on SOM dynamic. Earthworm species used in our mesocosm experiment are
 115 already present in soils under natural conditions, at the density set in the experiment. In this way, we
 116 assume that a part of SOM characteristics of each soil are also associated to the specific earthworm
 117 activity.

118 We used three earthworm species: *Polypheretima elongata* (Perrier), *Pontoscolex corethrurus*
 119 (Muller), and *Eudrilus eugeniae* (Kinberg). Earthworm treatments were defined considering the
 120 species observed in each soil to take into account earthworm adaptation to specific soil characteristics.
 121 In fact, preliminary work indicated a high death rate of *P. elongata* when it was inoculated in the
 122 ferralsol, and the same was observed for *P. corethrurus* in the vertisol. In this way, although *E.*
 123 *eugeniae* was observed and used in both soils, *P. elongata* was observed and used only in the vertisol,
 124 and *P. corethrurus* was observed and used only in the ferralsol.

125 To assess the effect of earthworm activity, we used two indicators linked to SOM
 126 mineralization. The first indicator involved the measurement of C respiration from laboratory
 127 incubations, which reflected the potential of soil C mineralization at the moment of soil sampling. The
 128 term potential implies that C mineralization was measured under optimal environmental conditions for
 129 specific enzymes (i.e. soil temperature and moisture content). The second indicator involved the
 130 assessment of the time course of soil mineral N in the soil-earthworm systems placed under
 131 greenhouse conditions (mesocosm experiment). Because N inputs, water drainage and nitrate leaching
 132 did not occur in our soil-earthworm systems, the time course of mineral N reflected the actual
 133 cumulative net N mineralization during the experiment. Dehydrogenase activity (DHA) was also
 134 determined at each sampling date as an indicator of the average activity of the active aerobic microbial
 135 population (Tabatabai 1994; Tiquia 2005). In this way, we assessed the effect of earthworm activity on
 136 specific aerobic processes (C and N mineralization) and also on the total aerobic microbial activity
 137 (DHA). The effect of earthworm activity on exchangeable cations and available P content was also
 138 determined because they may represent constraints for microbial activity, mainly in the ferralsol
 139 (Sierra 2006).

140

141 *Soils and earthworms*

142

143 The soils are located at the experimental stations of the Institut National de la Recherche
 144 Agronomique in Guadeloupe (French West Indies). The ferralsol is located at the Duclos experimental
 145 station (16°12'N, 61°40'W, mean annual air temperature 25.5°C, mean annual rainfall 3000 mm), and
 146 the vertisol is located at the Gardel experimental station (16°17'N, 61°21'W, mean annual air
 147 temperature 27°C, mean annual rainfall 1100 mm). Both soils are under natural grassland of
 148 *Dichanthium aristatum* (Poir) C.E. Hubbard for forty years. Some characteristics of the 0-0.2 m layer
 149 of the soils are presented in Table 1. The ferralsol was developed on old volcanic ash deposits and the
 150 clay fraction is dominated by kaolinite and sesquioxides of Fe and Al. The vertisol was developed on
 151 coral reef limestone and the clay fraction is dominated by smectite.

152 *P. elongata* (anecic earthworm) and *P. corethrurus* (endogeic earthworm) are considered to be
 153 continuous breeders with high fecundity in tropical soils (Bhattacharjee and Chaudhuri 2002). Both
 154 species are frequently detected in grasslands of Guadeloupe, in vertisols for *P. elongata* and in
 155 ferralsols for *P. corethrurus*. *E. eugeniae* is a fast-growing epigeic earthworm (Lim *et al.* 2012). In
 156 Guadeloupe it was observed in ferralsols and vertisols in the topsoil layer of grasslands covered with
 157 leaf litter, and also in mounds of cattle manure. In this study, *P. elongata* and *P. corethrurus* were
 158 collected from grassland soils and *E. eugeniae* was collected from a mound of manure stored at the
 159 Duclos Experimental Station.

160

161 *Greenhouse experiment*

162

163 Soil samples were collected by removing the upper 0.2 m layer of a 1.2 m by 1.2 m quadrat within the
 164 grassland plots. The soils were ground manually to reduce aggregates to <5 mm; large grass roots
 165 were removed manually and fine roots were removed using tweezers. After this, the soils were
 166 homogenized and gently air-dried to obtain a water content of 0.25 kg/kg for the ferralsol and 0.35
 167 kg/kg for the vertisol. A 2-kg sample of each soil was separated for the chemical and biological
 168 measurements at time 0 (T0). These samples were stored at 4°C for 48 h before being used for
 169 analyses. After this, 18 kg (dry matter basis) of the soils was placed in 15-L plastic pots (0.3 m
 170 diameter, 0.3 m deep) and moistened to -30 kPa (0.31 kg/kg for the ferralsol and 0.44 kg/kg for the
 171 vertisol). Twenty earthworms were added to each pot. Earthworm biomass was about 20 g per pot for
 172 *P. corethrurus*, 22 g per pot for *E. eugeniae*, and 44 g per pot for *P. elongata*. This is equivalent to 300
 173 earthworms/m², which is the mean density observed for several extensive pastures in Guadeloupe. The
 174 pots were covered with a plastic perforated net to keep out the earthworm predators present locally
 175 such as centipedes and frogs.

176 There were 6 treatments: (i) control vertisol (without earthworm addition, called V), (ii)
 177 vertisol with addition of *P. elongata* (V+Pe), (iii) vertisol with addition of *E. eugeniae* (V+Ee), (iv)
 178 control ferralsol (F), (v) ferralsol with addition of *P. corethrurus* (F+Pc), and (vi) ferralsol with
 179 addition of *E. eugeniae* (F+Ee). There were 4 replicates of each treatment. The 24 pots were placed for
 180 6 months in a greenhouse, and arranged in a fully randomised design. Soil moisture in the pots was
 181 kept at -30 kPa throughout the trial by regular weighing and topping up. Mean daily temperature in
 182 the greenhouse varied between 26°C and 28°C.

183 The soil in the pots was sampled 3 (T3) and 6 (T6) months after the beginning of the trial. Soil
 184 cores were taken from the soil surface to the bottom of the pots (0.27 m) using a 0.1 m diameter auger.
 185 The hole made by soil sampling at T3 was filled with a 0.1 m diameter polystyrene cylinder to
 186 minimize the effect of soil disruption on microbial and earthworm activity around the sampling site.
 187 For the same reason, soil sampling at T3 and at T6 was done on opposite sides of the pots. Soil
 188 samples were homogenized and stored at 4°C for 48 h before being used for analyses. The number and
 189 the biomass of earthworms were determined in the soil remaining in the pots at the end of the
 190 experiment.

191

192 *Carbon mineralization*

193

194 Carbon mineralization was determined by short-term soil incubation using the procedure described by
 195 Sierra *et al.* (2013). For incubation, 20 g soil (dry matter basis) was placed in 50 mL glass vials. One
 196 vial containing the soil sample, one vial of distilled water, and one vial containing 1 M NaOH solution
 197 were placed in 0.5-L glass jars. The vial containing water was used to ensure water saturation of the
 198 air inside the jars and thus prevent soil drying during the incubation. Two soil incubations were carried
 199 out for each soil sample (i.e. 2 pseudo-replicates for each replicate and 48 soil incubations per
 200 sampling date). After this, all of the jars were placed in the dark at a constant temperature of 30°C for

201 21 days. Carbon mineralization was measured at 1, 2, 3, 7, 14 and 21 d from the beginning of the
 202 incubation. The CO₂ produced between two measurement dates and trapped in the NaOH solution was
 203 back-titrated with 0.4 M HCl. After this, the vial containing the NaOH solution was renewed to prevent
 204 saturation of the solution. Soil moisture was checked by weighing at each measurement date.

205

206 *Chemical and dehydrogenase activity analyses*

207

208 Approximately 200 g of each soil sample was dried at 60°C for 72 h for chemical and DHA analyses.
 209 Four sub-samples ground to <0.2 mm were used to determine total C and N, and DHA for all the
 210 treatments and sampling dates. Total C and N were determined using an element analyser (NC 2100
 211 Soil, CE Instruments, Italy). DHA was determined following the procedure described by Tabatabai
 212 (1994), and expressed as the amount of triphenyl formazan (TPF) produced with reference to the
 213 calibration curve prepared from TPF standard.

214 Four sub-samples ground to <2 mm were used to determine pH (soil : water 1 : 5 w/v),
 215 exchangeable cations and available P. Exchangeable cations were extracted using a 1 N NH₄OAc
 216 solution at pH 7, and measured by atomic absorption spectrophotometry (AAFS 240, Varian, France).
 217 Available P was determined using the Olsen-Dabin method in a mixture of 0.5 N NaHCO₃ and 0.5 N
 218 NH₄F at pH 8.5 (Morel and Fardeau 1987). Nitrate-N and NH₄-N were extracted from 20 g of moist
 219 samples by shaking for 24 h with 100 mL of 0.5 M KCl, and centrifuging, and measured with a
 220 continuous flow colorimeter (AutoAnalyzer 3, Bran Luebbe, France). Mineral N analyses were made
 221 in duplicate. Mineral N content was used to assess net N mineralization in the pots.

222

223 *Statistical analyses*

224

225 Differences between treatments and sampling dates for the chemical and biological soil properties
 226 were assessed by analysis of variance (ANOVA) followed by Tukey's test, with a two-way design (i.e.
 227 earthworm treatment x sampling date) with 4 independent replicates per earthworm treatment and date
 228 (i.e. replicates from 4 pots). Because variance was significantly different between soil treatments,
 229 ANOVA was performed separately for each soil. Differences between soils were then assessed using a
 230 *t*-test with different variance. For C mineralization, ANOVA was performed using the cumulative
 231 mineralized C on day 21 (Cmin₂₁). The analyses were performed using SAS v. 9.1 software (SAS
 232 Institute 1999). The confidence level was fixed at *P* = 0.05 for all the analyses.

233

234

235 **Results**

236

237 *Carbon and N mineralization*

238

239 The kinetics of C mineralization was similar for all the treatments, with the highest rates occurring
 240 during the first week of soil incubation (Fig. 1). The coefficient of variation (CV) of cumulated C
 241 mineralization was relatively small for all the treatments and averaged 7% throughout the soil
 242 incubations. $C_{min_{21}}$ was higher for the vertisol than for the ferralsol treatments for all the sampling
 243 dates and all the earthworm treatments (Fig. 2). $C_{min_{21}}$ decreased significantly with the sampling date
 244 in both soil treatments, and the decrease was greater for the ferralsol treatments (-80% from T0 to T6)
 245 than for the vertisol treatments (-55%). For both soils, the effect of earthworms on $C_{min_{21}}$ was only
 246 noticeable at T3, but the trend of the earthworm effect was different for each soil. For example, the
 247 treatment with *E. eugeniae* had the lowest $C_{min_{21}}$ for the vertisol (Fig. 2a), and the highest $C_{min_{21}}$ for
 248 the ferralsol (Fig. 2b). The interaction earthworm treatment \times sampling date was only significant for
 249 the vertisol treatments. Relative C mineralization, that is $C_{min_{21}}$ expressed as percentage of the total C
 250 content of each soil, was higher for the control ferralsol than for the control vertisol at T0 (Table 2). In
 251 contrast, the values for T3 and T6 were higher for the control vertisol than for the ferralsol. The values
 252 for the earthworm treatments were similar for both soils.

253 The CV of net N mineralization in the mesocosm experiment was relatively small for all the
 254 treatments and averaged 10%. For both soil treatments, actual net N mineralization was greater for the
 255 T0-T3 period than for the T3-T6 period (Fig. 3). On average, net N mineralization for the vertisol
 256 treatments was 3 times higher than for the ferralsol treatments. Although the rate of net N
 257 mineralization for the T3-T6 period decreased in both soils, it was positive for the vertisol treatments
 258 ($+14$ mg N/kg on average), whereas it was slightly negative for the ferralsol treatments (-2 mg N/kg).
 259 For the T0-T3 period, earthworm treatments had a significant positive effect on net N mineralization
 260 ($+72\%$ for the vertisol, $+76\%$ for the ferralsol). At the end of the experiment, the effect of earthworms
 261 was only noticeable for the vertisol ($+76\%$). Differences in net N mineralization between the two
 262 earthworm treatments of each soil, and the interaction earthworm treatment \times sampling date were not
 263 significant throughout the experiment. At the end of the experiment, net N mineralization expressed as
 264 percentage of the initial organic N of each soil (i.e. relative net N mineralization) was 2.4% for the
 265 vertisol and 1.5% for the ferralsol. These values were higher in the earthworm treatments: 4.2% for the
 266 vertisol and 2.5% for the ferralsol.

267

268 *Soil nutrients, pH, DHA and earthworm survival*

269

270 Initial values of total C and N, exchangeable cations, CEC and soil pH were higher for the vertisol
 271 than for the ferralsol (Table 1). For both soils, there were no effects of soil-earthworm treatments and
 272 sampling date on the level of total C and N, exchangeable cations, and pH (Table 3). Hence values of
 273 these parameters at T3 and T6 for all the treatments were close to those presented in Table 1 for the
 274 respective initial soils. Available P was also much higher in the vertisol treatments (Fig. 4a,b). For the
 275 vertisol, there was a slight but significant effect of both earthworm treatments on available P, mainly

276 at T6 (+10%). For the ferralsol, only the *E. eugeniae* treatment had a small but significant effect at T3
 277 and T6 (+11%).

278 On average, DHA in vertisol treatments was 10 times higher than in ferralsol treatments (Fig.
 279 4c,d). For the vertisol, DHA decreased from T0 to T3 and then it was rather stable. For this soil, no
 280 significant differences were observed between soil-earthworm treatments at T3 and T6 (Fig. 4c). For
 281 the ferralsol, DHA decreased between T0 and T3 and then increased until T6. For this soil, differences
 282 between treatments presented the same trend in T3 and T6: F+Ee > F+Pc > F (Fig. 4d).

283 The number of earthworms in the soil remaining in the pots at the end of the experiment
 284 averaged 15.2 ± 1.1 without significant differences between treatments. Due to soil sampling at T3 and
 285 T6, the mass of soil remaining at the end of the experiment corresponded to about 78% of the initial
 286 amount of soil put into the pots (i.e. 18 kg). Taking into account this value, the estimated recovery of
 287 earthworms was 19.5 (i.e. $15.2/0.78$), which represented 97% of the initial number put into the pots
 288 (i.e. 20). For all the earthworm treatments, no significant differences were observed between the
 289 estimated recovery and the initial number of earthworms. For earthworm biomass, the estimated
 290 recovery represented $101 \pm 4\%$ of the initial biomass, without significant differences either between
 291 treatments or in relation to the initial biomass.

292

293

294 Discussion

295

296 *Effect of the soil and earthworms on the aerobic processes*

297

298 The two major findings from this study were: (i) the effect of earthworms on the aerobic processes
 299 varied between soils as a function of SOM quality and quantity, and (ii) the potential C mineralization
 300 determined at each sampling data was not sensitive to earthworm activity and a more dynamic
 301 parameter, such as the actual net N mineralization, was better to evaluate the effect of earthworm
 302 activity.

303 Differences in absolute and relative C and N mineralization between the ferralsol and the
 304 vertisol treatments could be also due to other soil factors affecting microbial and earthworm activity.
 305 Among these factors soil pH and nutrient content differed strongly between soils. We think that these
 306 factors were less important than C availability to cause differences between soils because absolute soil
 307 respiration was relatively high at T0 in both soils when C availability was higher, and the trend of the
 308 differences in relative C respiration varied with time without change in pH or nutrient content.
 309 Similarly, although physical and mechanical properties of the ferralsol are more favourable for
 310 earthworms than those observed in the vertisol (i.e. for the ferralsol: lower bulk density, higher
 311 macroporosity, higher water availability; Sierra *et al.* 2002; Sierra 2006), earthworm activity was
 312 always much higher in the vertisol. In this way, although we cannot discard any effect of other soil
 313 properties, it appears that C availability was the major factor affecting earthworm activity in the tested

314 soils. The results of earthworm recovery indicate that earthworms adapted well to the conditions of the
 315 mesocosm experiment, and suggest that the effect of earthworm turnover was a minor factor affecting
 316 soil processes.

317 Several authors reported that relative C and N mineralization is a suitable indicator of the size
 318 of the labile fraction of SOM, and then of SOM quality and C availability for soil organisms (e.g.
 319 Fissore *et al.* 2013). To explain the temporal change of the relative mineralization we hypothesize that
 320 there were at least two C sources in the used soils: a readily mineralizable C derived from exudates
 321 and the turnover of dead roots of the original grassland, and a more stable C fraction derived from
 322 SOM. Indeed, C availability was greatest at the beginning of the experiment when labile and dissolved
 323 organic C (DOC) coming from grass root exudates and the products of root turnover were still present
 324 in both soils (Jiménez and Lal 2007). This was reflected by the highest relative C mineralization and
 325 DHA observed at T0 in both soils. At this time, the greater relative C mineralization observed in the
 326 ferralsol would indicate that labile C and DOC was relatively more important in this soil due to its less
 327 total C content (Jouquet *et al.* 2007). The reverse occurred for the absolute C mineralization and DHA,
 328 which were higher for the vertisol. This was due probably to its higher available C from SOM (Table
 329 1). Higher C availability at T0 also explains the greater rate of net N mineralization found for the T0-
 330 T3 period, and why this rate was higher for the control vertisol compared with the control ferralsol.

331 The higher net N mineralization in the earthworm treatments for the T0-T3 period could be
 332 due to three factors: (i) earthworm burrowing and mixing increased the accessibility of SOM to
 333 decomposers (Shipitalo and Le Bayon 2004; Jouquet *et al.* 2010), (ii) SOM decomposition in
 334 earthworm guts and in the fresh casts (Blouin *et al.* 2013; Majeed *et al.* 2013), and (iii) labile C
 335 secreted by earthworms induced a priming effect leading to faster SOM mineralization (Lavelle and
 336 Spain 2001). Regardless of the underlying mechanism involved in the effect of earthworm activity, the
 337 relatively high SOM mineralization in the T0-T3 period induced a strong depletion of available C,
 338 which affected the microbial activity in each soil in a different way.

339 In the ferralsol, it seems that most of labile C and DOC coming from the original grassland
 340 were exhausted within the three first months of the experiment, and then absolute and relative C
 341 mineralization decreased sharply at T3 and T6. Only *E. eugeniae* had a small positive effect on C
 342 mineralization at T3 in the ferralsol, but this was not confirmed by the net N mineralization observed
 343 in the following T3-T6 period. For that period, the negative net N mineralization observed for all the
 344 ferralsol treatments indicated N immobilization. The effect of earthworms in the ferralsol was clearer
 345 for the increase of DHA, i.e. for the global aerobic activity (Tiquia 2005). Indeed, microbial C
 346 turnover and stabilization of DOC coming from the original grassland could be two of the aerobic
 347 processes affected by earthworm activity (Jouquet *et al.* 2007), which increased DHA and N
 348 immobilization in these ferralsol treatments.

349 At T3 and T6, when C coming from the original grassland was exhausted, the relative C
 350 mineralization was two times greater for the vertisol indicating higher C availability from the SOM
 351 source in this soil. In this way, absolute C mineralization as well as net N mineralization in the vertisol

352 decreased slower and were higher than in the ferralsol. At T3, the smaller C_{min21} in the earthworm
 353 treatments of the vertisol well reflected the exhaustion of the labile C, which agrees with their higher
 354 N mineralization in the T0-T3 period. It has been reported that mixing of organic matter and fine
 355 mineral particles in the intestinal tract of earthworms can contribute to the stabilization of organic
 356 matter in stable aggregates in temperate (Bernier 1998) as well as in tropical soils (Chapuis-Lardy *et*
 357 *al.* 2010). Thus, organic matter stabilization induced by earthworms could also explain the decrease in
 358 the potential C mineralization at T3 in the earthworm treatments. As net N mineralization in the T3-T6
 359 period was higher for the earthworm treatments than for the control vertisol, it follows that earthworm
 360 activity in the T3-T6 period could compensate for a lower potential C mineralization at T3. This
 361 agrees with the results reported by McNerney *et al.* (2001) who observed that earthworm reworking of
 362 soil may induce C mineralization and reduce C stability in casts. Our results clearly indicate that
 363 potential C mineralization was less useful than net N mineralization to assess the effect of earthworms.
 364 Similar results were found by Chapuis-Lardy *et al.* (2010) to assess the effect of earthworms on N_2O
 365 fluxes by using potential denitrification in a tropical ferralsol. In contrast to the ferralsol, earthworms
 366 did not affect total aerobic activity in the vertisol, no doubt due to its relatively high current level. In
 367 fact, the level of DHA observed in vertisol treatments throughout the experiment was as high as that
 368 found currently in active composts at the end of the stabilisation period (Tiquia 2005; Sierra *et al.*
 369 2013).

370 For a tropical ferralsol, Chaudhuri *et al.* (2012) observed that earthworm activity increased
 371 available P by its effect on the mineralization of organic P and on the solubilization of mineral P.
 372 Brossard *et al.* (1996), for a tropical vertisol, and Chapuis-Lardy *et al.* (1998), for a tropical ferralsol,
 373 found that mineralization and solubilization of P may occur during gut transit and continues for a few
 374 hours after egestion of the casts. Similar results were found by Pashanasi *et al.* (1996) and Loranger-
 375 Merciris *et al.* (2012) for *P. corethrurus* in tropical soils. These results and those of N mineralization
 376 indicated that active biostimulation by earthworm inoculation had positive effects on available P and
 377 N contents, which could improve primary production on these soils.

378

379 *Soil-earthworm interactions*

380

381 Of the three earthworm species tested in this study, *E. eugeniae* had the largest effect; this was
 382 particularly noticeable for the ferralsol. In this soil, the effect of *E. eugeniae* on DHA and available P
 383 was greater than that of *P. corethrurus*. For the vertisol, differences between the two earthworm
 384 treatments were generally not significant, except for C mineralization, as discussed above. It is
 385 difficult to ascertain why differences between earthworm species were greater for the ferralsol. The
 386 larger effect of *E. eugeniae* on DHA could be due to the improvement of gaseous exchange due to the
 387 surface burrows made by this epigeic earthworm. Moreover, for tropical soils Majeed *et al.* (2013)
 388 observed that selective feeding by epigeic earthworms produced casts with high C and nutrient
 389 content, which could boost microbial activity. If this hypothesis is true for our study, the higher effect

390 of the epigeic *E. eugeniae* on available P in the ferralsol could be due to selective feeding on materials
 391 enriched in this nutrient. Chaudhuri *et al.* (2012) proposed the same hypothesis to explain the effect of
 392 *Metaphire houlleti* on the available P in a tropical ferralsol.

393 Interestingly, differences between the vertisol and the ferralsol throughout the entire
 394 experiment were much larger for DHA than for C_{min21} and net N mineralization. This different soil
 395 pattern for DHA and SOM mineralization was observed for the control soils as well as for the
 396 earthworm treatments, so it follows that earthworm activity affected the intensity of some aerobic
 397 processes in both soils, but it did not modify the intrinsic biological characteristics of each soil. As
 398 discussed above, we hypothesize that the effect of earthworms on N mineralization in the ferralsol was
 399 foreshortened because of the recalcitrant quality of SOM in this soil. That is, after depletion of the C
 400 substrate coming from the original grassland, earthworms were unable to affect organic compounds
 401 strongly complexed with active Al and Fe hydrous oxides in the ferralsol. In contrast, the effect of
 402 earthworms on N mineralization was longer and greater in vertisol because earthworms and
 403 microorganisms used a less recalcitrant SOM continuously. Indeed, further work is required to assess
 404 the C balance and the factors controlling it in nutrient-limited tropical soils affected by earthworm
 405 activity. For this, it is particularly important to consider the time scale to properly estimate the role of
 406 earthworms in terms of SOM mineralization and stabilization.

407

408

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410

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516 **Figure captions**

517

518 **Fig. 1.** Kinetics of C mineralization observed during soil incubations. T0, T3 and T6 indicate sampling
 519 dates and correspond to 0, 3 and 6 months after the beginning of the experiment. V, vertisol; F,
 520 ferralsol; Pe, *Polypheretima elongata*; Pc, *Pontoscolex corethrurus*; Ee, *Eudrilus eugeniae*. Vertical
 521 bars indicate the standard deviation (n = 4).

522

523 **Fig. 2.** Cumulative mineralized C at the end of soil incubations (day 21). Vertical bars indicate the
 524 standard deviation (n = 4). For each soil, the same letter on the histogram bar indicates no significant
 525 differences between treatments at $P < 0.05$. Differences between soils for a given sampling date were
 526 significant at $P < 0.05$. See Fig. 1 for explanation of treatment codes.

527

528 **Fig. 3.** Actual net N mineralization for the six treatments in the mesocosm experiment. Vertical bars
 529 indicate the standard deviation (n = 4). See Fig. 1 for explanation of treatment codes

530

531 **Fig. 4.** (a) and (b): available P, and (c) and (d): dehydrogenase activity (DHA), determined at each
 532 sampling date. Vertical bars indicate the standard deviation (n = 4). For each soil, the same letter on
 533 the histogram bar indicates no significant differences between treatments at $P < 0.05$. Differences
 534 between soils for a given sampling date were significant at $P < 0.05$. See Fig. 1 for explanation of
 535 treatment codes

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539**Table 1. Some characteristics of the soils used in the present study**For each parameter, values followed by different letter are statistically different at $P < 0.05$ (n = 4)

Soil	pH	Total		CEC	K	Ca	Mg	Particle size		
		C	N					<2 μm	2-50 μm	>50 μm
		(g/kg)		(cmol _c /kg)			(kg/kg)			
Vertisol	7.8a	52.4a	3.9a	52.2a	1.5a	45.4a	2.6a	0.82a	0.12b	0.06b
Ferralsol	5.5b	23.7b	2.1b	12.4b	0.1b	2.8b	0.4b	0.71b	0.18a	0.11a

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Comment citer ce document :

Sierra, J., Loranger-Merciris, G., Desfontaines, L., Boval, M. (2014). Aerobic microbial activity in a tropical acid soil system: A mesocosm experiment. *Soil Science Society of America Journal*, 78(5), 2261-2270. DOI:10.2136/sssaj2014.03.0084

541 **Table 2. Cumulative mineralized C at the end of soil incubations (day 21) expressed as % of the**
 542 **total soil C content at each sampling date**

543 Values followed by different letter are statistically different at $P < 0.05$ ($n = 4$). T0, T3 and T6
 544 correspond to 0, 3 and 6 months after the beginning of the experiment. V, vertisol; F,
 545 ferralsol; Pe, *Polypheretima elongata*; Pc, *Pontoscolex corethrurus*; Ee, *Eudrilus eugeniae*

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Sampling date	V	V + Pe	V + Ee	F	F + Pc	F + Ee
	(%)					
T0	1.0b			1.6a		
T3	0.8c	0.6d	0.5d	0.4e	0.4e	0.5d
T6	0.4e	0.4e	0.4e	0.3e	0.3e	0.3e

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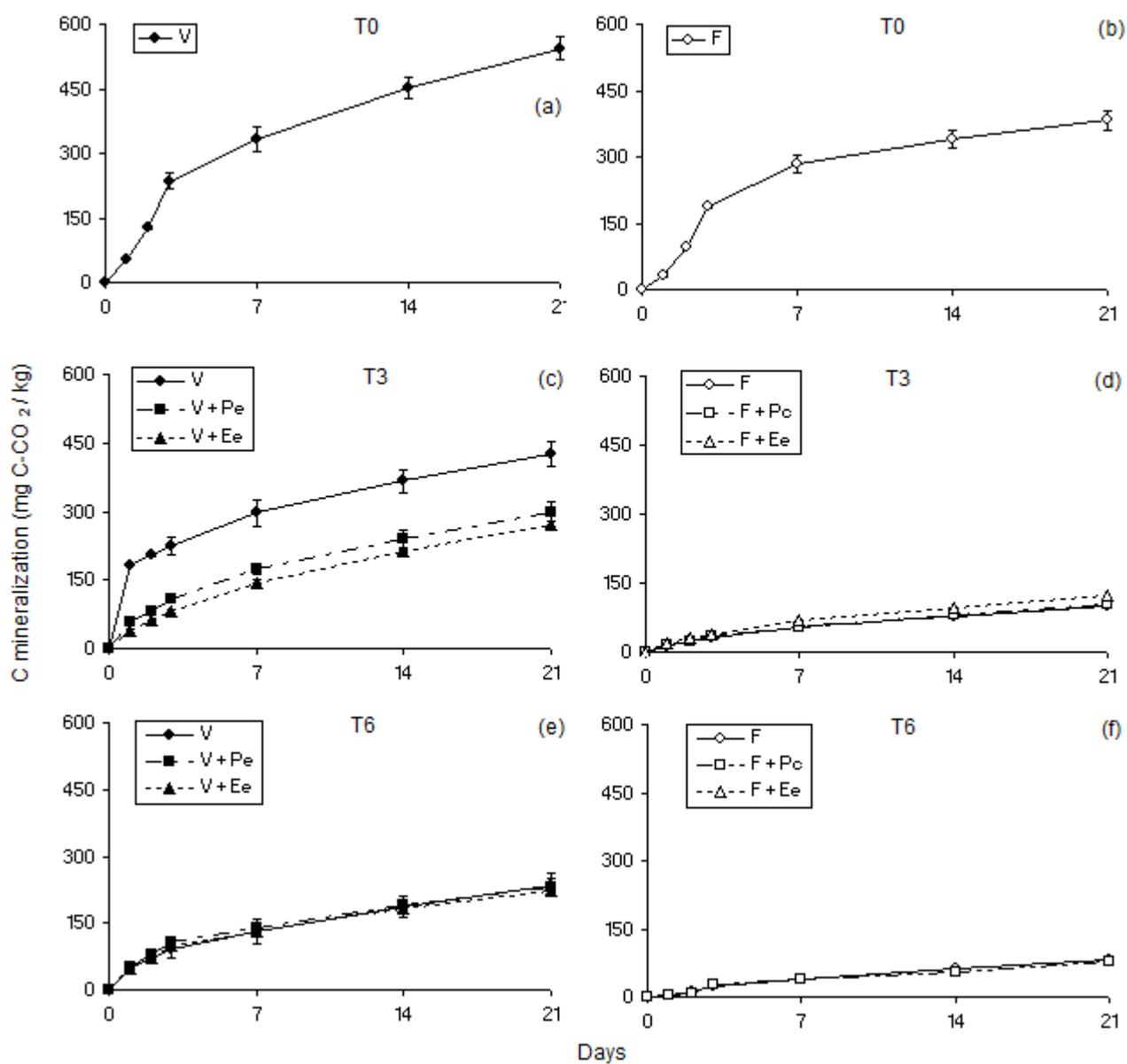
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548 **Table 3. pH, total C and N, and exchangeable cations determined three (T3) and six months (T6)**
 549 **after the beginning of the mesocosm experiment**

550 For each parameter, values followed by different letter are statistically different at $P < 0.05$ ($n = 4$). V,
 551 vertisol; F, ferralsol; Pe, *Polypheretima elongata*; Pc, *Pontoscolex corethrurus*; Ee, *Eudrilus*
 552 *eugeniae*

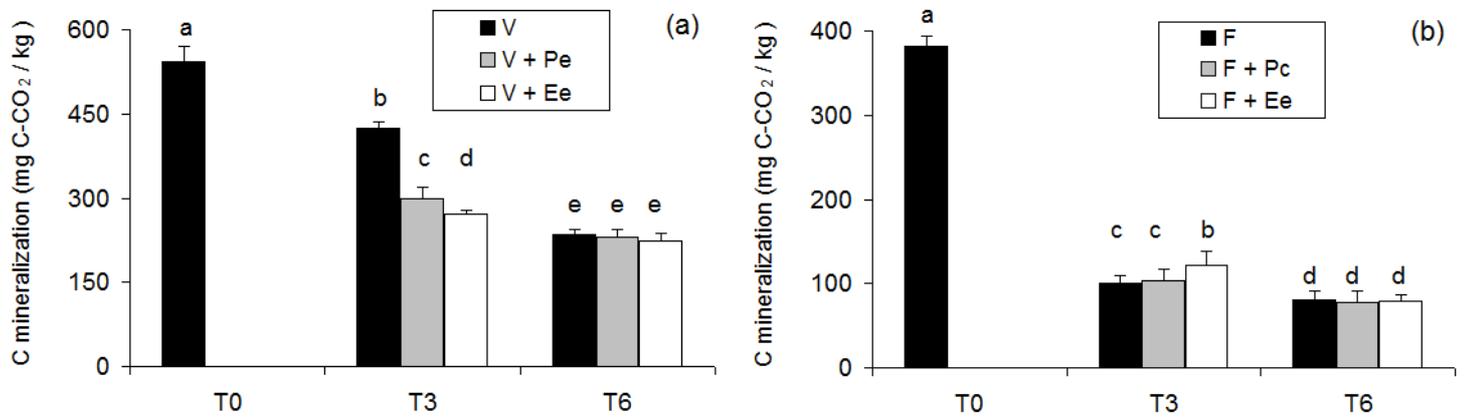
Treatment	Sampling date	pH	Total		K	Ca	Mg
			C	N			
			(g/kg)		(cmol _c /kg)		
V	T3	7.7a	51.8a	3.8a	1.5a	43.6a	2.8a
	T6	7.8a	52.0a	3.9a	1.5a	46.9a	2.9a
V + Pe	T3	7.7a	51.6a	3.7a	1.5a	44.5a	2.9a
	T6	7.9a	52.1a	3.6a	1.8a	45.1a	2.9a
V + Ee	T3	7.7a	52.3a	3.9a	1.7a	44.8a	2.7a
	T6	7.7a	52.2a	3.8a	1.6a	44.2a	2.8a
F	T3	5.4b	23.1b	2.0b	0.1b	2.8b	0.4b
	T6	5.4b	23.6b	1.9b	0.1b	2.7b	0.4b
F + Pc	T3	5.5b	22.9b	2.2b	0.2b	2.9b	0.3b
	T6	5.4b	22.4b	2.3b	0.1b	3.0b	0.4b
F + Ee	T3	5.4b	23.9b	2.0b	0.2b	3.1b	0.5b
	T6	5.4b	23.0b	2.2b	0.1b	2.9b	0.3b

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 556 **Fig. 1.**
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562 **Fig. 2.**

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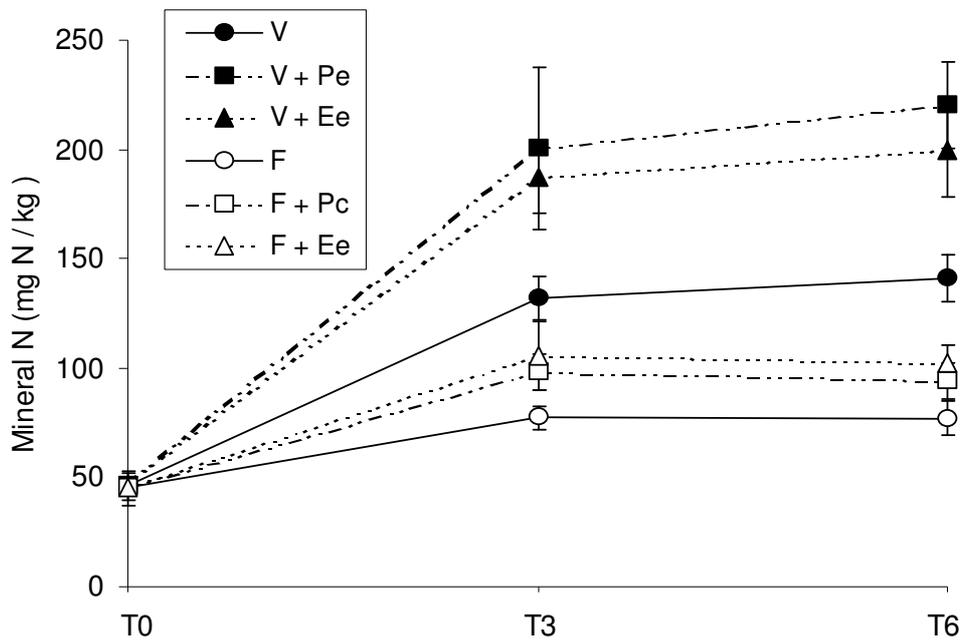
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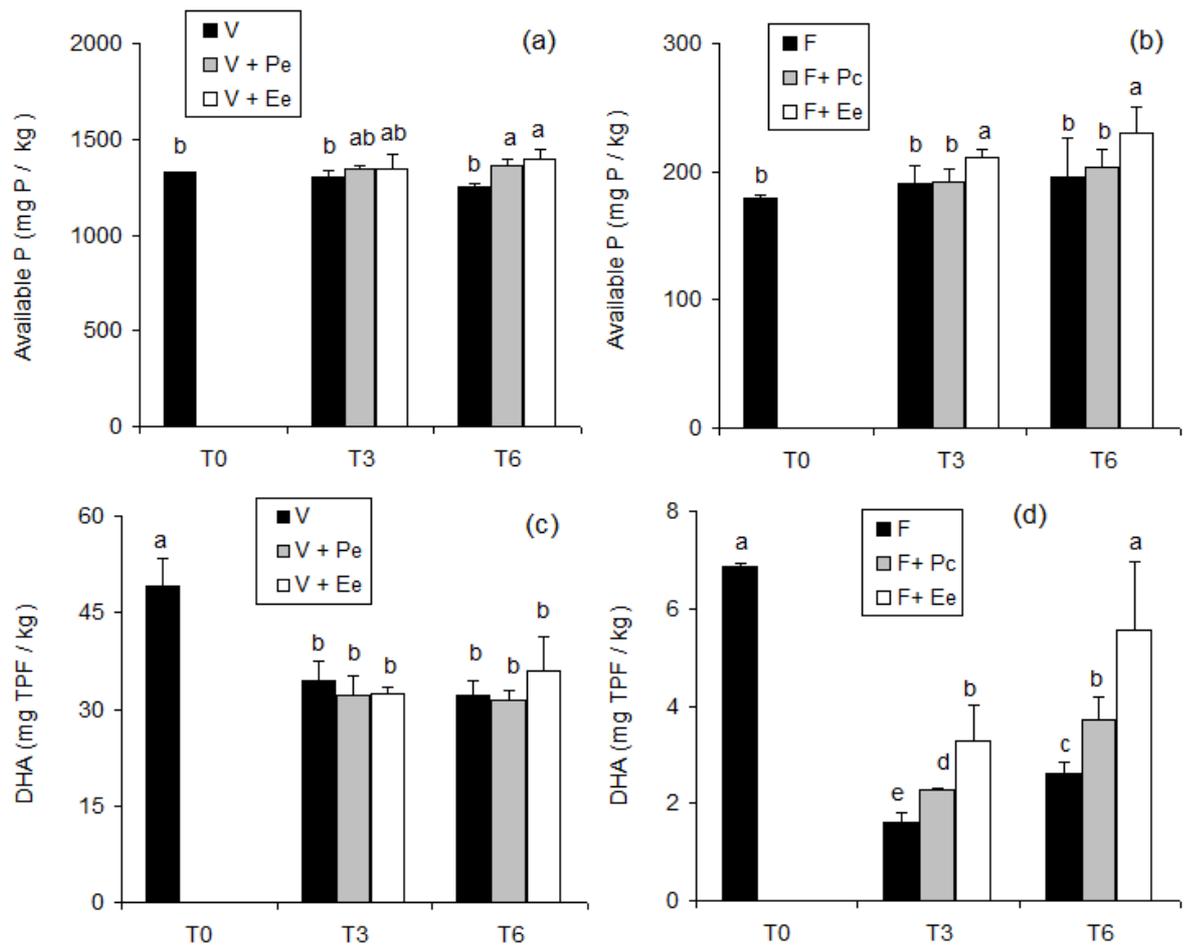
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Fig. 3.



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577 **Fig. 4.**

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