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4 **1 Effects of nest building by the dominant hunting ant, *Ectatomma* sp. 2 (*E.***
5 **2 *ruidum* complex), on Andean coffee plantations**

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24 **Keywords:** ant bioturbation, *Ectatomma ruidum* sp. 2, shaded vs. sun coffee, ecological
25 function, plant growth.

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29 **Abstract**

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32 Soil removal by ants when building or adapting their nests generates ecological changes
33 within the soil and on its surface, impacting both soil physical properties and plant growth.
34 *Ectatomma* sp. 2 (*E. ruidum* complex) is a dominant hunting ant in numerous neotropical
35 agricultural systems; however, its bioturbation impact on agroecosystems of economic
36 importance, such as coffee, has not been studied. We evaluated it on coffee seedlings in
37 Andean plantations of Colombia. Sixty colonies of this ant (30 in sun coffee, 30 in shaded
38 coffee) were added within enclosed experimental units (EUs) containing coffee seedlings,
39 and compared with their respective controls without ants. On average, dry soil removal by
40 colony was 104.4 g during the two months experimental period (1.6 g day⁻¹ colony⁻¹), 13
41 times higher than in controls. A conservative estimate of their global bioturbation impact
42 was calculated at between 59.3 and 203.1 kg dry soil ha⁻¹, considering only two months of
43 digging activity year⁻¹ colony⁻¹, a likely underestimation of the actual digging activity of
44 this species. Positive effects of the presence of ants were found on several plant growth
45 variables and on soil microporosity in shaded coffee plantations. Taking into consideration
46 only the 14 colonies that were fully active until the end of the experiment, dry soil removal
47 was on average 228.8 g (3.5 g day⁻¹ colony⁻¹) during the two months experimental period

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32 and coffee seedling growth in these EUs was significantly higher than in controls,
33 indicating that, at the global scale, our bioturbation estimate could be higher under less
34 restricted natural conditions and higher nest densities as found in other regions. *Ectatomma*
35 *ruidum* sp. 2 appears as a functional agent in agroecosystems due to its ubiquity and its
36 high bioturbation impact during the nest excavation period of activity, providing support
37 services in agroecosystems and neotropical natural systems.

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1 | INTRODUCTION

Ants are the most diverse group of social insects (Bolton et al., 2007; Ward, 2009). However, of the more than 16,000 valid species of ants currently recognized (AntWeb, 2019), very few species have been thoroughly studied with regard to their nest structure and their impact on their environment. Most ant nests are hypogeous and their building can result in the removal of considerable volumes of soil (Hölldobler & Wilson, 1990). Due to their activity and abundance in agroecosystems and natural systems, some species may impact deeply ecological processes (Folgarait, 1998), but our understanding of the principal ecosystemic services supplied by ants is still very limited (Del Toro et al., 2012). Two of these ecosystem services are particularly important: bioturbation resulting in soil turnover, both at the superficial and subsurface levels, where ants act as ecosystem engineers (Lobry de Bruyn & Conacher, 1994; Lavelle et al., 2006; Tschinkel, 2015; Tschinkel & Seal, 2016), and changes in soil physical properties, which, in turn, may provide benefits for neighbouring plants (Lobry de Bruyn & Conacher, 1990; Eldridge et al., 2009).

Bioturbation by ants is evidenced through soil mounds deposited while subterranean nest construction or enlargement is carried out by workers. This process of soil excavation for nest construction results in the vertical movement and redistribution of organic and inorganic materials through the soil profile (Folgarait, 1998). For example, the fungus-growing ant *Atta laevigata* builds between nearly 1,150 and 7,900 chambers, probably moving more than one million cm³ soil per mature nest (Moreira et al., 2004). Similarly, in two different coastal ecosystems of eastern and southeastern Sweden, ant bioturbation essentially performed by *Lasius niger* and *Formica exsecta* reached between 300-400 kg ha⁻¹ y⁻¹ (Persson et al., 2007), while in New Mexico (USA), the average quantity of soil moved to the surface by a whole ant assemblage reached approximately 842 kg ha⁻¹ for the two month period (August-September) of surface activity only (Whitford et al., 1986). In arid soils of Australia, nests of *Aphaenogaster barbigula* have an average density of entrances of 88,000 ha⁻¹, and exert a global bioturbation effect of 3,360 kg ha⁻¹ y⁻¹, demonstrating the importance of just one of the ant species in this region in terms of soil removal (Eldridge & Pickard, 1994). Another species of the same genus, *A. longiceps*,

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4 70 exerts a bioturbation effect of 8,410 kg ha⁻¹ y⁻¹ which can reach the extraordinary high
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6 71 values of 68,380 kg ha⁻¹ y⁻¹ in sandy soil (Richards, 2009).
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10 73 Apart from changing the physical and chemical properties of the soil, bioturbation
11 74 generates a higher infiltration of water in the soil, both through nest entrances at the
12 75 surface, and as a result of increased macroporosity. For example, in Australian zones where
13 76 nests of *A. barbigula* were present in high densities, water infiltration averaged 23.3 mm
14 77 min⁻¹, i.e. about four times more than in zones without nests of this species (Eldridge,
15 78 1993). In Australia, Evans and coauthors found no effect of ant nesting in agricultural
16 79 areas; however, two years later they found three times as much water infiltration in
17 80 agricultural areas where ants nested in than in areas without ant nests (Evans et al., 2011).
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26 82 Physical changes to the soil, associated with the additional aggregation of organic
27 83 material gathered by ants and deposited inside or outside their nests (both of plant and
28 84 animal origins), impact on its water-holding capacity and soil fertility (Farji-Brener &
29 85 Werenkraut, 2017). Soil changes in the vicinity of the nests or mounds of numerous ant
30 86 species also have a positive influence on the growth of neighbouring plants (Shukla et al.,
31 87 2018). Even the nests of some invasive ant species, such as *Solenopsis invicta* can exert
32 88 positive effects on plant growth (Lafleur et al., 2005) and in some dominant ants with long-
33 89 lasting mounds, such as *Lasius flavus* in the Czech Republic, positive effects on mound
34 90 vegetation can last several years after the disappearance or the substitution of the original
35 91 ant occupant (Kovář et al., 2001).
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46 93 In the Neotropics, *Ectatomma ruidum* sp. 2 (*E. ruidum* complex) is a dominant
47 94 ground-dwelling predatory ant species of great interest due to its functional importance in
48 95 relation to biological systems altered by humans (agroecosystems) (Fontalvo-Rodríguez &
49 96 Domínguez-Haydar, 2009) and for its potential as a biological control agent (Lachaud,
50 97 1990). It is commonly found in coffee, cocoa and corn plantations, presenting densities
51 98 which vary from 500 to 6,000 nests ha⁻¹ (Levings & Franks, 1982; Santamaría et al., 2009),
52 99 but can occasionally exceed 10,000 nests ha⁻¹ (Schatz & Lachaud, 2008).
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101 Although *E. ruidum* sp. 2 nest density tends to be high, the role of this species on
102 bioturbation, soil porosity and water infiltration through the surface nest entrances has not
103 been documented. It is to be expected that nest galleries that connect nest chambers with
104 the surface could improve water movement through the soil, as has been documented with
105 termites and other ant species (Lobry de Bruyn & Conacher, 1990). Furthermore, *E. ruidum*
106 sp. 2 can exhibit high predation rates (several million prey ha⁻¹ y⁻¹, see Lachaud et al.
107 (1996)) thus adding large amounts of organic material to their nests that enhances soil
108 fertility around the nest area. Such effects can occur during the colony life cycle and after
109 nest abandonment. Due to the characteristics exhibited by *E. ruidum* sp. 2 in
110 agroecosystems, such as high nest density ha⁻¹, high predation rates, and high
111 myrmecochorous activity (Lachaud et al., 1996; Gutierrez-Rapalino & Domínguez-Haydar,
112 2017), this species has been considered as an important functional agent in neotropical
113 agroecosystems and natural systems (Schatz & Lachaud, 2008; Domínguez-Haydar &
114 Armbrrecht, 2011). In order to examine whether field data support the significant ecological
115 function played by this ant species, we evaluated, for the first time in coffee
116 agroecosystems, the role of *E. ruidum* sp. 2 in bioturbation through measuring soil removal
117 activity and the effect of this removal on both water infiltration and growth of neighbouring
118 coffee seedlings over a relatively short time span. We hypothesized that the presence of
119 excavating colonies of *E. ruidum* sp. 2 causes significant changes both in soil and
120 neighboring plants. Therefore, our objective was to test whether the presence of *E. ruidum*
121 sp. 2 results in higher bioturbation, more changes in soil physical properties, and faster
122 growth of coffee seedlings than equivalent areas without colonies of this ant.

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2 | MATERIALS AND METHODS

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2.1 | Study area

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128 The study was carried out in the Southwestern region of the Colombian Andes, in the
129 municipality of Caldono, Department of Cauca (2°49' N, 76°32' W). The mean annual
130 temperature is 21.5 °C, with a total annual rainfall of 2,191 mm distributed in a bimodal
131 pattern with two high peaks in April-May and October-November. Altitude varies between
132 1,000 and 2,000 m.a.s.l. The area is predominantly agricultural and coffee production has
133 increased over the past years, generally in the form of 1-20 ha small farms. The Department
134 of Cauca is the sixth coffee producer in Colombia covering 74,000 hectares (FNC, 2010).
135 In 2009, shaded coffee plantations dominated the area, covering about 72% of the
136 cultivated area, the remaining 20,500 ha being covered by coffee plantations without shade
137 (FNC, 2010). We define “sun” coffee plantation as an uncovered or “shadeless” coffee
138 crop, in which there are no trees surrounding the coffee bushes; a “shaded” coffee
139 plantation consists of coffee bushes intermingled with 10-25 m height trees such as *Inga*
140 spp., *Erithrina* spp., *Cordia alliodora*, *Persea americana*, and *Citrus sinensis*, among
141 others (Fig. 1).



142 **Fig. 1.** Aerial image showing an example of both, the sun coffee and shaded coffee
143 management plots ("farm") where experimental units with and without colonies of the ant
144 *Ectatomma ruidum* complex sp. 2 were installed. The farms were located in the town of El
145 Rosal, municipality of Caldono, Cauca - Colombia.

147 2.2 | Experimental design

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149 Between August 2016 and May 2017, 20 experimental coffee plots (which will be referred
150 to as “farms”) were randomly chosen, 10 under shade and 10 under sun, all of them at

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6 152 1) neighbouring plots around each farm differed in management: for example, farms were
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8 153 separated by a tomato crop, a family orchard or a pasture; 2) if there was no other different
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10 154 management, farms were separated by at least 100 m; 3) sun and shaded coffee should form
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12 155 a mosaic in the landscape, that is, sun and shaded farms should be intermingled over the
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14 156 landscape and not grouped.

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17 158 In each farm, six bottomless polymer mesh baskets, or experimental units (which
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19 159 will be referred to as "EUs"), 50 cm in diameter and 30 cm in height (Fig. 2), were
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21 160 installed. Each EU was separated by a distance of 1 metre. The first 15 cm of each EU was
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23 161 buried within the soil and the remaining 15 cm was left exposed above ground and isolated
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25 162 from walking insects by applying Tanglefoot® insulator around the top edges. Two coffee
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27 163 seedlings (*Coffea arabica*-variety *caturra*) in a state of “chapola” with the bare root
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29 164 (“chapola” are sprouted seedlings, approximately 5 cm high, that have produced the first
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31 165 pair of primary leaves) were planted inside each EU (Fig. 2). Three weeks after the
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33 166 chapolas were planted, three EUs were randomly assigned as “treatment” and three as
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35 167 “control” for each farm.



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51 168 **Fig. 2.** Photo showing an experimental unit (EU) consisting of an exclusion basket, with or
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53 169 without an *E. ruidum* sp. 2 colony, used both in shaded and sun coffee plantations.

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56 171 Colonies of *E. ruidum* sp. 2, previously extracted from depths between 50 and 90
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58 172 cm and kept in the laboratory for three weeks, were added to each of the 60 treatment EUs.
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60 173 Each colony contained 70 workers on average, together with their respective queen and

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174 between 10-20 larvae and five pupae. No ants were added to the control EUs. Ant colonies
175 were fed with *Acheta domesticus* crickets or tuna-in-oil (1 cm³) every two days, and with
176 honey and water two times a week. Control EUs were supplied with the same food as
177 treatment EUs, and any food remains were removed from both EUs every other day.

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179 **2.3 | Soil removal**

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181 Once ant colonies had been added in each treatment EU, the soil removed by *E. ruidum* sp.
182 2 workers (Fig. 3) during the process of building their nest was collected every two days
183 over a period of 66 days. The soil was removed by gentle brushing with the aid of a spatula.
184 Each collection action took one whole day between 7:00AM and 5:00PM. The procedure
185 was always conducted in the same order, alternating treatment and control EUs, and also
186 shaded and sun coffee farms. The soil collected in the control EUs allowed an assessment
187 of the action of ant species other than *E. ruidum* as well as additional excavating
188 invertebrates present in the EUs (mostly earthworms, Gryllidae, Gryllotalpidae, spiders,
189 and Scarabaeidae larvae). Soil material was introduced in letter-sized paper envelopes and
190 the samples were immediately oven dried at 45 °C for 24 hours and weighed on a high-
191 resolution precision balance (Adventure™ Ohaus GLP/GMP) using a 50 mL beaker.

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199 **Fig. 3.** Example of soil particles removed during nest excavation by *E. ruidum* sp. 2
200 workers in a treatment EU.

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202 **2.4 | Plant growth variables**

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204 Plant height was measured from the base of the ground to the top of the plant (apical
205 meristem without including the leaf, which will be referred to as “length”). The diameter of
206 the stem was measured 1 cm above ground level using a Mitutoyo® 0.01 mm precision
207 digital calibrator. All measurements were taken every two weeks (six measurements in
208 total), for three months, the first measurement being recorded on the day the “chapola” was
209 planted. All measurements were taken on the same day and, for logistical reasons, always
210 started at the same farm. The length of the seedling from the main root tip toward the apical
211 meristem, plant dry weight and root dry weight were recorded at the end of the experiment.

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213 **2.5 | Soil infiltration**

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215 Water infiltration was measured twice for each EU: on day one, when ant colonies were
216 introduced to treatment EUs (T1) and two months later (T2) at the end of the experiment
217 (T1: zero day elapsed, and T2: 66 days elapsed). Measurements were taken using disk
218 infiltrometers (Mini Disk Infiltrometer, Decagon Devices, Inc.) placed simultaneously in
219 the control and treatment EUs; these measurements were taken in all of the 120 EUs of the
220 study. The infiltrometer was placed vertically on the soil surface, 10 cm from the nest
221 entrance in the treatment, and in the center of the basket in the control EUs. The upper and
222 lower chambers of the infiltrometers were filled with water. The upper chamber serves to
223 control suction and the lower chamber stores the water for infiltration. The water was
224 passed through a porous stainless-steel disk 0.3 cm high and 4.5 cm in diameter, located at
225 the end of the lower chamber, and using a suction rate of 2.0 cm. The initial volume of the
226 water was 85 cm³ and, in accordance with the type of soil in the EU, the time interval for
227 manual readings was adjusted to 30 min. The initial volume of water and the volume of
228 filtered water were tabulated (in mL), recording volumes at regular interval times of 30 s
229 for a total of 30 min per EU. Cumulative infiltration over time for each EU was calculated
230 using Equation 1 (Philip, 1957):

$$I = C_1 t + C_2 \sqrt{t} \tag{1}$$

232 Where I is the cumulative infiltration, C₁ is the curve of the infiltration accumulation
233 vs. time, C₂ is the relation to the absorption of the soil and t is the time.

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235 **2.6 | Physical properties of the soil**

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237 Various physical parameters of the soil were measured at the end of the experiment:
238 apparent density, macropore drainable porosity, micropore field capacity, total porous
239 space, and humidity of the sampling. To achieve this, samples were taken from the first 10
240 cm top soil layer of each EU, 10 cm from the nest entrance in the treatment EUs and in the
241 centre of the control EUs, filling a metallic cylinder with 50 g of soil extracted from each
242 EU. Analyses were carried out at the Environmental Physics Laboratory at the Universidad
243 del Valle (ILAMA Research Group). The NTC 5167 gravimetric method was used for all
244 of the fore-mentioned parameters.

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246 **2.7 | Statistical analysis**

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248 For all analyses, both land use (sun vs. shaded coffee) and ant presence (with vs. without *E.*
249 *ruidum* sp. 2) were considered a fixed factor, while “farm” was a random factor. In all cases
250 we examined the possible interaction between the factors in relation to each of the response
251 variables measured in the experiment. A deviance analysis was carried out associated with
252 a generalized linear model (GLM) with a Poisson distribution error term, and adding the
253 “farm” block factor to control for possible variability between one farm and another. One
254 model was run with homogeneous variances and one model with heterogeneous variances.
255 The second model (heterogenous variances) was selected according to the AIC and BIC
256 information criteria.

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258 In order to determine whether measurement time influenced plant growth variables,
259 we considered it in the model, together with land use and ants presence, as fixed factors. In
260 this case, the possible interaction among the three factors (time, land use, and ant presence),
261 and their possible effect on plant growth variables (response variables: stem diameter and
262 seedling length) were examined. For this, a deviance analysis was carried out associated
263 with a mixed linear model (LMM) with heterogeneous variances according to the AIC and
264 BIC information criteria, taking for this case the "basket" as the random factor and the
265 "farm" factor as a block within the model.

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4 266 Statistical analyses were carried out using the R distribution program, version 3.4.0
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6 267 (R Development Core Team, 2017), determining statistical differences to a 5% significant
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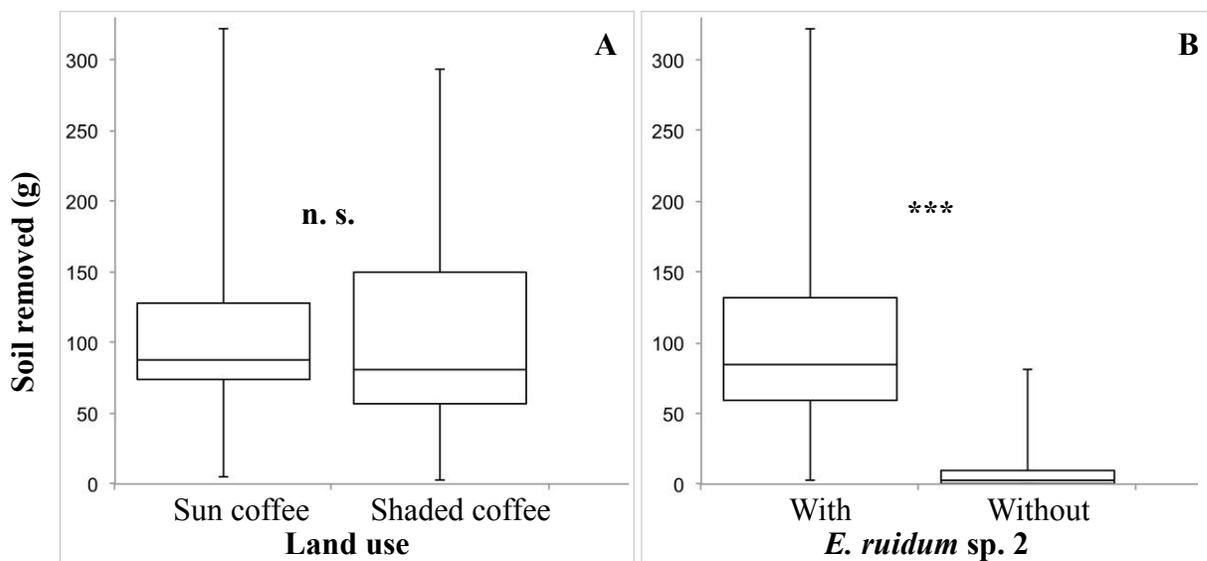
10 269 11 270 **3 | RESULTS** 12 13 271

14 272 **3.1 | Soil removal** 15 16 273

17 274 The digging activity of *E. ruidum* sp. 2 resulted in a statistically significant higher amount
18 275 of soil removal in the treatment EUs when compared with the control EUs over the
19 276 complete experiment. On average, over the 66 days of recording, the cumulative soil
20 277 removal in each EU containing an *E. ruidum* sp. 2 colony was 112.6 ± 66.3 g dry soil vs.
21 278 8.2 ± 14.4 g in each control EU (Fig. 4B). On average, each *E. ruidum* sp. 2 colony of
22 279 approximately 70 workers removed 104.4 g dry soil during the two month period of the
23 280 experiment ($1.6 \text{ g day}^{-1} \text{ colony}^{-1}$), almost 13 times more than the global soil removal
24 281 performed by other invertebrates (arthropods, annelids or other ant species) present on an
25 282 equivalent surface during the same period of time. On average, soil removal in sun coffee
26 283 was 114.7 ± 65.7 g per EU vs. 106.3 ± 68.4 g in shaded coffee, and neither land use nor
27 284 farm variables had any statistically significant effect on soil removal ($\chi^2 = 0.436$ and 0.093 ,
28 285 respectively; N.S.) (Fig. 4A). However, because soil removal gradually decreased as the
29 286 experiment continued (Fig. 5) due, in part, to the death of various colonies and the low soil
30 287 removal activity of numerous other colonies, these results underestimate the real
31 288 bioturbation impact of *E. ruidum* sp. 2 in coffee plantations. Only 14 *E. ruidum* sp. 2 (six in
32 289 sun coffee, eight in shaded coffee) were still fully active at the end of the experiment;
33 290 however, given that the EUs could not be disturbed during data collection, the final absence
34 291 of a colony could only be established at the end of the experiment. Out of the 60 colonies
35 292 which have been added at the beginning of the experiment, 23 demonstrated very limited
36 293 activity. Twenty three other colonies almost completely disappeared, probably due to either
37 294 interference competition with *Wasmannia auropunctata* which, in some cases, was already
38 295 occupying the territory, or due to stress factors during the translation and establishment of
39 296 the colonies in the EUs. The cumulative soil removal during the 66 days experimental
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297 period for the 14 EUs that were fully active until the end of the experiment, reached 237.0
298 ± 44.7 g of dry soil per colony (267.3 ± 29.6 g in sun coffee vs. 214.3 ± 41.3 g in shaded
299 coffee). In fact, taking into account soil removal by other invertebrates (8.2 g per EU), each
300 one of these 14 *E. ruidum* sp. 2 colonies removed on average 228.8 g dry soil (Fig. 5A),
301 that is $3.5 \text{ g day}^{-1} \text{ colony}^{-1}$, more than twice the previous assessment.

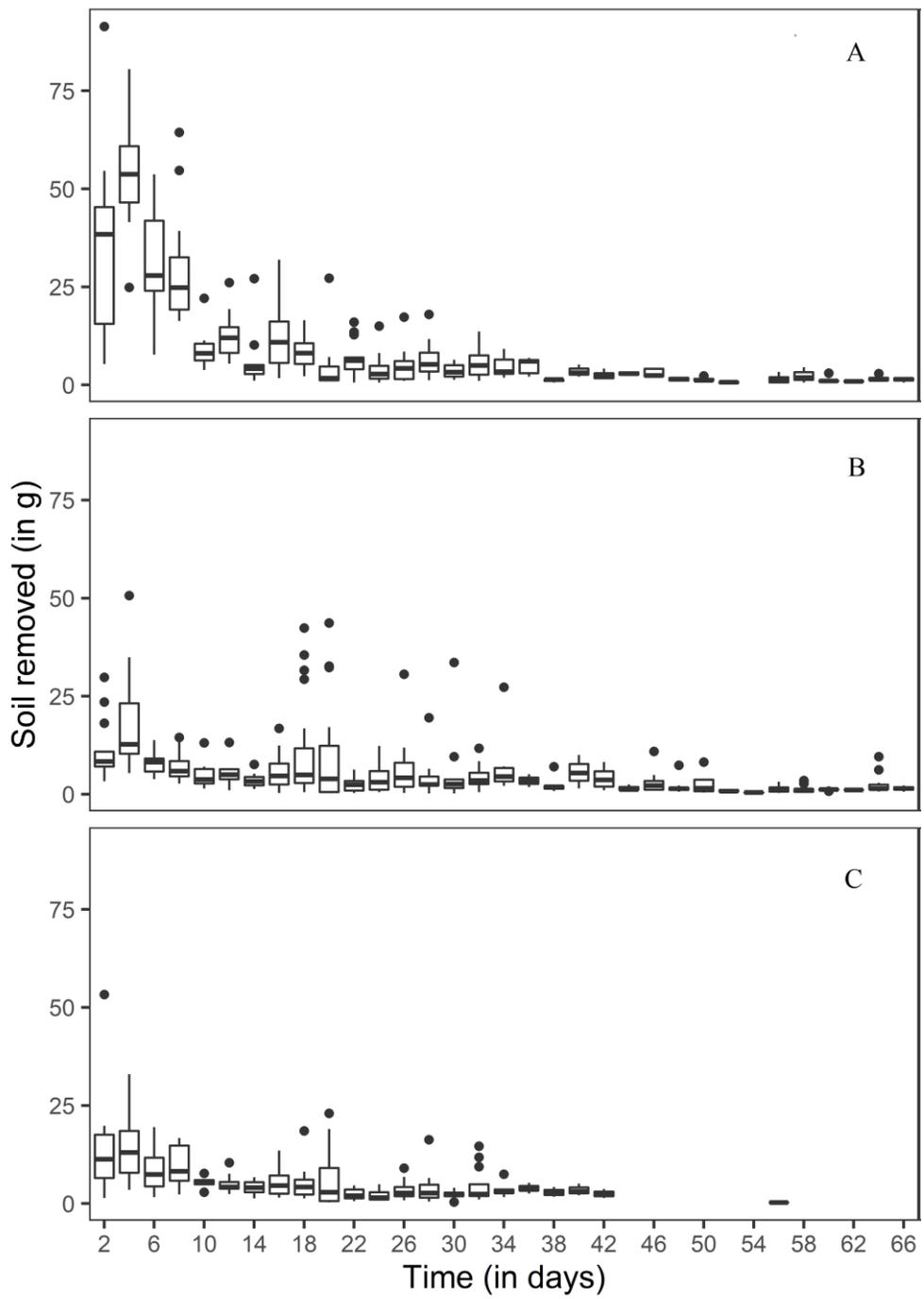


313 **Fig. 4.** Box diagrams of cumulative soil removal during the experiment: A) in sun and
314 shaded coffee with *E. ruidum* sp. 2 (n = 30 for each land use), B) in all the EUs with or
315 without *E. ruidum* sp. 2 (n = 60 for each treatment) (n.s.: not significant; ***: $P = 2e-16$).
316 Vertical bars show minimum and maximum values.

318 The GLM analysis to evaluate differences in the weight of soil removed at each
319 collection event (every two days) in the EUs with or without *E. ruidum* sp. 2 revealed that
320 the time of collection played a significant role. In all nests, soil removal was higher at the
321 start of the experiment and decreased significantly as the experimental period progressed.
322 Such effect was particularly evident for the 14 colonies which were active until the end of
323 the experiment ($\chi^2 = 670.093$; $p \ll 2e-16$) (Table 1; Fig. 5A), but was also discernible in
324 the remaining 23 colonies which showed very discrete soil removal activity ($\chi^2 = 165.395$;
325 $p \ll 2.2e-16$) (Fig. 5B) and even in the 23 colonies which disappeared during the course of
326 the experiment ($\chi^2 = 126.446$; $2e-16$) (Fig. 5C). The average quantity of soil removed was
327 always significantly higher during the first weeks of the experimental period, regardless of

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328 whether the colonies were active until the end of the experiment (Table 1), remaining at a
329 very reduced level after five weeks for all colonies. There was no significant effect related
330 to the factor "land use" (that is, between sun and shaded coffee plantations) ($\chi^2 = 0.037$;
331 N.S.), contrary to the "farm" factor which was significant ($\chi^2 = 47.995$; $p \ll 0.05$).
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335 **Fig. 5.** Box diagrams of soil removal by *E. ruidum* sp. 2 in all coffee plots during the two
 336 month experimental period. A) Soil removal in the 14 treatment EUs where colonies
 337 remained active until the end of the experiment; B) Soil removal in the 23 treatment EUs
 338 where colonies showed low activity until the end of the experiment; C) Soil removal in the
 339 23 treatment EUs where colonies did not remain active until the end of the experiment. •:
 340 outliers. Vertical bars show minimum and maximum values.

341

342 **Table 1.** Average and standard error of soil (in g) removed weekly by *E. ruidum* sp. 2
 343 colonies during the first four weeks of the experimental period, according to the activity
 344 level of the colonies.

345

	1st week	2nd week	3rd week	4th week
Colonies which were active until the end of the experiment (n = 14)	151.2 ± 36.7	30.5 ± 15.6	20.1 ± 11.2	17.9 ± 6.8
Colonies which presented very discrete soil removal activity (n = 23)	28.9 ± 27.7	11.2 ± 6.2	24.3 ± 26.3	14.3 ± 14.0
Colonies which disappeared over the course of the experiment (n = 23)	27.5 ± 23.9	7.7 ± 6.9	9.0 ± 9.5	9.0 ± 9.1

346

347 **3.2 | Seedling growth and physical properties of the soil**

348

349 Taking into account all the experimental units, the statistical analyses showed significant
 350 differences in the majority of the measured plant growth variables (seedling length,
 351 seedling length from the main root tip, root dry weight) when land use was considered, but
 352 none related to the presence or absence of *E. ruidum* sp. 2, or the factor 'farm' (Table 2).
 353 Water infiltration (T2) at the end of the experiment and macropore drainable porosity were
 354 higher in sun coffee than in shaded coffee, while microporous field capacity was lower
 355 (Table 2). However, presence or absence of *E. ruidum* sp. 2 colonies had no significant
 356 effect on the physical properties of the soil.

357

358 **Table 2.** Statistical results (A) and description of plant growth response variables (B).

359 Table 2A: Deviance analysis for all the variables measured at the end (except T1

360 infiltration) of the experiment within the EUs located in sun and shaded coffee plantations.
 361 (*): Significant difference at the 0.05 level threshold. Table 2B: Average and standard
 362 deviation of plant growth variables and soil physical properties; n = 60 for all cases.
 363 (A)

Variable	Land use (shaded/sun coffee)		Presence/absence of <i>E. ruidum</i> sp. 2		Interaction Land use and Presence/absence of <i>E. ruidum</i> sp. 2		Farm (block)	
	Statistics	p-value	Statistics	p-value	Statistics	p-value	Statistics	p-value
Length of the seedling (cm)	$\chi^2_{(df=1)} = 5.695$	0.017*	$\chi^2_{(df=1)} = 2.268$	0.132	$\chi^2_{(df=1)} = 0.365$	0.546	$\chi^2_{(df=19)} = 0.709$	0.399
Length of the seedling from main root tip (cm)	$\chi^2_{(df=1)} = 5.016$	0.025*	$\chi^2_{(df=1)} = 2.478$	0.115	$\chi^2_{(df=1)} = 0.527$	0.468	$\chi^2_{(df=19)} = 1.729$	0.189
Plant dry weight (g)	$\chi^2_{(df=1)} = 2.249$	0.134	$\chi^2_{(df=1)} = 0.461$	0.497	$\chi^2_{(df=1)} = 0.829$	0.362	$\chi^2_{(df=19)} = 0.398$	0.528
Root dry weight (g)	$\chi^2_{(df=1)} = 8.474$	0.004*	$\chi^2_{(df=1)} = 1.198$	0.274	$\chi^2_{(df=1)} = 0.257$	0.612	$\chi^2_{(df=19)} = 0.042$	0.838
Stem diameter (mm)	$\chi^2_{(df=1)} = 2.884$	0.089	$\chi^2_{(df=1)} = 0.081$	0.776	$\chi^2_{(df=1)} = 0.148$	0.701	$\chi^2_{(df=19)} = 2.336$	0.126
T1 infiltration (ml)	$\chi^2_{(df=1)} = 0.247$	0.619	$\chi^2_{(df=1)} = 0.163$	0.687	$\chi^2_{(df=1)} = 0.213$	0.644	$\chi^2_{(df=19)} = 0.475$	0.491
T2 infiltration (ml)	$\chi^2_{(df=1)} = 8.212$	0.004*	$\chi^2_{(df=1)} = 0.268$	0.604	$\chi^2 = 0.136$	0.713	$\chi^2_{(df=19)} = 0.406$	0.524
Apparent density (g.cc ⁻¹)	$\chi^2_{(df=1)} = 1.495$	0.221	$\chi^2_{(df=1)} = 0.010$	0.919	$\chi^2 = 0.021$	0.886	$\chi^2_{(df=19)} = 0.048$	0.827
Total porous space (%)	$\chi^2_{(df=1)} = 1.449$	0.229	$\chi^2_{(df=1)} = 0.005$	0.942	$\chi^2_{(df=1)} = 0.282$	0.596	$\chi^2_{(df=19)} = 4.025$	0.045*
Sample humidity (%)	$\chi^2_{(df=1)} = 7.270$	0.07	$\chi^2_{(df=1)} = 0.092$	0.762	$\chi^2_{(df=1)} = 1.664$	0.197	$\chi^2_{(df=19)} = 2.724$	0.099
Macropore drainable porosity (%)	$\chi^2_{(df=1)} = 6.632$	0.010*	$\chi^2_{(df=1)} = 0.060$	0.806	$\chi^2_{(df=1)} = 0.282$	0.595	$\chi^2_{(df=19)} = 1.742$	0.187
Micropore field capacity (%)	$\chi^2_{(df=1)} = 4.879$	0.027*	$\chi^2_{(df=1)} = 0.000$	0.997	$\chi^2_{(df=1)} = 1.498$	0.221	$\chi^2_{(df=19)} = 0.547$	0.459

364

365 (B)

Variable	Shaded coffee	Sun Coffee	With ants	Without ants
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Length of the seedling (cm)	33.1 ± 8.5	36.9 ± 6.9 *	33.9 ± 7.2	36.1 ± 8.4
Length of the seedling from main root tip (cm)	19.2 ± 6.8	21.7 ± 5.9 *	19.3 ± 5.3	21.5 ± 7.4
Plant dry weight (g)	1.1 ± 1.1	1.5 ± 1.3	1.1 ± 0.9	1.5 ± 1.4
Root dry weight (g)	0.8 ± 0.4	1.0 ± 0.4 *	0.9 ± 0.4	1.0 ± 0.4
Stem diameter (mm)	1.2 ± 0.7	1.6 ± 1.1	1.3 ± 0.8	1.4 ± 1.0
T1 infiltration (ml)	73.2 ± 12.2	71.3 ± 14.8	71.8 ± 13.4	72.6 ± 13.7
T2 infiltration (ml)	15.0 ± 12.4	23.1 ± 18.5 *	19.6 ± 16.6	18.6 ± 15.9
Apparent density (g.cc ⁻¹)	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.1	0.9 ± 0.1
Total porous space (%)	61.0 ± 4.0	61.7 ± 6.9	61.3 ± 5.6	61.5 ± 5.7
Sample humidity (%)	41.6 ± 5.7	38.9 ± 6.6	40.4 ± 6.8	40.1 ± 5.7
Macropore drainable porosity (%)	18.0 ± 5.8	20.9 ± 7.9 *	19.4 ± 7.3	19.6 ± 6.8
Micropore field capacity (%)	43.0 ± 5.1	40.8 ± 5.7 *	41.9 ± 6.1	41.9 ± 5.0

366

367 In the LMM the deviance analysis to determine which of the variables were affected
 368 by land use and time as the experiment progressed (six measurements in total in three
 369 months) confirmed that ant presence did not influence stem diameter but, together with the
 370 factor 'farm', had a positive effect on seedling length ($p < 0.05$) (Table 3).

371

372 **Table 3.** LMM deviance analysis of plant growth variables as the experiment progressed
 373 (six measurements) (*): Significant difference at the 0.05 level threshold.

Factor	Stem diameter		Seedling length of the plant	
	Statistics	p-value	Statistics	p-value
Land use	$\chi^2_{(df=1)} = 2.290$	0.130	$\chi^2_{(df=1)} = 0.739$	0.0390
Presence of <i>E. ruidum</i> sp. 2	$\chi^2_{(df=1)} = 0.971$	0.324	$\chi^2_{(df=1)} = 4.212$	0.040*
Time	$\chi^2_{(df=5)} = 740.603$	$< 2e-16 *$	$\chi^2_{(df=5)} = 3024.426$	$< 2e-16 *$
Farm	$\chi^2_{(df=19)} = 15.73$	0.227	$\chi^2_{(df=19)} = 27.458$	0.007 *
Land use*Presence of <i>E. ruidum</i> sp. 2	$\chi^2_{(df=1)} = 3.19$	0.073	$\chi^2_{(df=1)} = 0.936$	0.333
Land use*Time	$\chi^2_{(df=5)} = 9.079$	0.106	$\chi^2_{(df=5)} = 21.307$	0.0007 *
Presence of <i>E. ruidum</i> sp. 2 *Time	$\chi^2_{(df=5)} = 0.881$	0.972	$\chi^2_{(df=5)} = 3.132$	0.679
Land use*Time*Presence of <i>E. ruidum</i> sp. 2	$\chi^2_{(df=5)} = 1.811$	0.875	$\chi^2_{(df=5)} = 1.515$	0.911

374

Seedling measurements were influenced by the activity exhibited by the *E. ruidum* sp. 2 colonies present in the treatment EUs. In the eight treatment EUs that remained active until the end of the experiment in shaded coffee plantations, the presence of *E. ruidum* sp. 2 had a significant positive effect both on the total length of the plant (mean \pm SD: 20.8 \pm 3.9 cm vs. 15.3 \pm 2.5 cm) and on the length from the main root tip (mean \pm SD: 35.8 \pm 4.6 cm vs. 28.2 \pm 4.4 cm) (Table 4 and Fig. 6). A similar significant positive effect due to the presence of *E. ruidum* sp. 2 was observed on the stem diameter (mean \pm SD: 2.1 \pm 1.0 cm vs. 0.7 \pm 0.6 cm) in the six treatment EUs still active at the end of the experiment in sun coffee plots (Table 4). Moreover, micropore field capacity was significantly higher in presence of *E. ruidum* sp. 2 colonies in shaded coffee (mean \pm SD: 43.4 \pm 3.1 vs. 39.7 \pm 3.1) (Table 4).

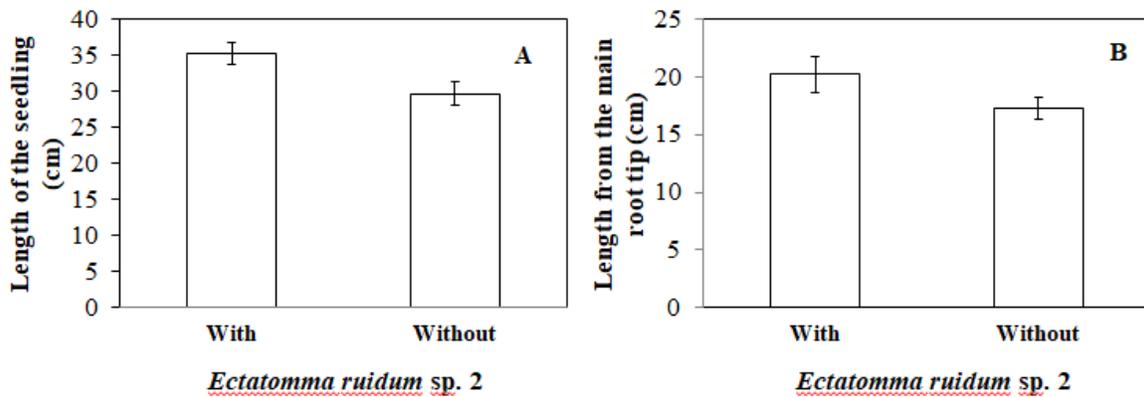


Fig. 6. Comparison of two plant growth variables in shaded coffee plots. The bar on the left represents the eight EUs with *E. ruidum* sp. 2 colonies that were active until the end of the experiment and the bar on the right the 30 EUs without *E. ruidum* sp. 2 colonies: A) Length of the seedling, B) Length of the seedling from the main root tip. Vertical bars show error values.

Table 4. Comparison (Student-*t* test) between theoretical (null hypothesis) and observed data obtained for four variables recorded in the 14 EUs with *E. ruidum* sp. 2 colonies presenting activity until the end of the experiment (6 in sun coffee, 8 in shaded coffee).

Variable	Land use	Theoretical <i>t</i>	Observed <i>t</i>	<i>p</i>
Length from the main root tip	Shaded	2.14	3.38	0.0045

Seedling length	Shaded	2.14	3.40	0.0043
Micropores field capacity	Shaded	2.14	2.37	0.0325
Stem diameter	Sun	2.23	2.95	0.0144

396

397 **4 | DISCUSSION**

398

399 To our knowledge, this is the first controlled field experiment that quantifies the
 400 contribution of a dominant hunting Ectatomminae species to the ecosystem function of
 401 bioturbation, soil physical properties and coffee plant development in Andean
 402 agroecosystems. Although multiple factors may affect the performance of artificially added
 403 colonies in a field experiment, the evidence provided in this study supports that ants
 404 actually play a prominent role in vertical movement of soil in agroecosystems. Even though
 405 it was a field experiment carried out in real agroecosystems, it aimed to control several
 406 factors such as the size (demography) of the added colonies, food and water supply, area of
 407 the enclosures, location of the treatment and control EUs, and standardized conditions of
 408 coffee seedlings planted in the EUs, for which we consider our results rather conservative.

409

410 **4.1 | Bioturbation and physical properties of the soil**

411

412 As expected, our data confirmed that colonies of *E. ruidum* sp. 2 removed high quantities of
 413 soil during the first steps of nest excavation, suggesting that this species can actually be
 414 considered as a functional agent providing ecosystem support service to soils in Andean
 415 tropical agroecosystems, especially when considering its natural history. In the coffee
 416 plantations where our experiments were performed, each of the 14 persistently active
 417 colonies of *E. ruidum* sp. 2 removed an average of 228.8 g dry soil during the 66 days of
 418 the experiment. However, for the full complement of the 60 colonies used in this study, the
 419 average soil removal fell to slightly less than half this value (104.4 g, that is, about 1.6 g
 420 day⁻¹ colony⁻¹) providing perhaps a more realistic estimate of the global bioturbation impact
 421 of this species at the population level as it takes into consideration both the variability in
 422 activity among the colonies and the potential disappearance of several of them, two
 423 parameters which resulted relatively high in our control field conditions. The highest soil

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424 removal occurred at the beginning of the experiment, that is, when new nests were
425 excavated by *E. ruidum* sp. 2. Adding ant colonies to the soil could artificially stimulate the
426 digging activity of the ants, which was higher during the first week, as expected, and very
427 limited after the fifth week (Table 1; Fig. 5). The density of the *E. ruidum* sp. 2 colonies
428 found in paddocks adjacent to our study coffee zone has previously been estimated between
429 568 and 1,945 nests ha⁻¹ in shaded and sun cattle pastures, respectively (Santamaría et al.,
430 2009). Both using the same nest density estimate for coffee plantations and considering the
431 excavation of one nest per colony per year, as known to occur in *Pogonomyrmex badius* (see
432 Tschinkel 2015), a conservative estimate of the cumulative bioturbation impact of *E.*
433 *ruidum* sp. 2 in Andean coffee plantations during the two months of digging activity can be
434 calculated at between 59.3 and 203.1 kg dry soil ha⁻¹ (between 0.9 and 3.1 kg dry soil ha⁻¹
435 day⁻¹ over 66 days), a quite important impact despite both the limited period of activity and
436 the very restrictive natural conditions.

437
438 However, such figures are very likely underestimated and three main factors have to
439 be considered. First, extremely high nest densities (> 5,000 nests ha⁻¹) can be found in
440 various other regions (Schatz & Lachaud, 2008), suggesting that the global bioturbation
441 impact of *E. ruidum* sp. 2 could be much higher and could deeply impact its habitat. In
442 southeastern Mexican coffee plantations, for example, where densities of 5,900 to 11,500
443 nests ha⁻¹ have been reported (Schatz & Lachaud, 2008), the global bioturbation impact
444 could be conservatively estimated at around 9.3 to 18.2 kg ha⁻¹ day⁻¹ during the period of
445 nest excavation activity, that is, an impact roughly equivalent to that of much more
446 populous species such as *A. barbigula* in arid soils of Australia (approximately 9.2 kg ha⁻¹
447 day⁻¹) (Eldridge & Pickard, 1994). Second, the assumption of only one nest excavated by
448 colony is probably also underestimated, at least in the study zone. Additional studies on this
449 ant species in coffee plantations in the Andean region (Quevedo Vega, 2015; Berrod, 2017;
450 Santamaría, 2017; CS, unpubl. data) suggest that *E. ruidum* sp. 2 presents both a high nest
451 turnover and a local polydomy (several nests for one ant colony), two factors promoting the
452 construction of numerous new nests during each climatic season (annual bimodal
453 precipitation). Specifically, we acknowledge that bioturbation caused by *E. ruidum* sp. 2 is
454 not constant throughout the year, as was also observed by Whitford et al. (1986) for an ant

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455 assemblage in New Mexico. However, the above mentioned local polydomic nesting
456 behaviour, together with the high nesting turnover and the annual wet-dry switching
457 periods in tropical areas, lead us to suggest that this species can enhance vertical soil
458 movement in agroecosystems during a more substantial part of the year than the two
459 months considered previously for our bioturbation estimate. Third, finally, as repetitively
460 noted, the average weight of soil removed could be almost duplicated if considering
461 colonies in more optimal conditions as were the 14 persistently active colonies of our
462 experiment. Better conditions could thus allow to expect a cumulative bioturbation impact
463 of *E. ruidum* sp. 2 during the two months of digging activity between 130 and 445 kg dry
464 soil ha⁻¹ (between 2.0 and 6.7 kg dry soil ha⁻¹ day⁻¹ over 66 days), an impact equivalent to
465 that obtained in Sweden for the combined bioturbation of both *L. niger* and *F. exsecta* over
466 a full-year (300-400 kg ha⁻¹ y⁻¹; see Persson et al., 2007).

467
468 However, in terms of soil physical properties, no differences were detected in any
469 EUs due to the presence of the ants (Table 2), with the exception of total porous space. This
470 could be due to the fact that, over a short time span, nest construction activity is not
471 affecting these properties through the first 10 cm profile level. The architecture of *E.*
472 *ruidum* sp 2. nests (Santamaría Velasco & Salas Quinchucua, 2014; Poteaux et al., 2015)
473 could partially explain these results as the entrance is a simple hole (3-5 mm in diameter)
474 followed by a vertical 10-20 cm long narrow tunnel which leads to chambers (from 3 to 12)
475 distributed over 80 cm depth in the study area and up to 120 cm in some regions, depending
476 on the soil type. Although most of the physical properties did not show differences due to
477 the presence of ants, micropore field capacity was five percent higher in the 14 nests that
478 remained active until the end of the experiment compared to EUs without ants (Table 4).
479 Micropores help to retain water in soil, and may release it when there is a potential
480 difference, such as that generated around the roots which can provide a benefit for the
481 growth of the plant (INTAGRI, 2017), particularly during the dry season in the tropical
482 Americas. This benefit provided by *E. ruidum* sp. 2 is similar to that found in Martinique
483 (Blanchart et al., 1999), where the activity of earthworms resulted in soils with more than
484 40% field capacity which decreased when earthworms were removed. This change in field
485 capacity, together with bioturbation by ants, will contribute to the understanding of the role

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486 of *E. ruidum* sp. 2 in Andean agroecosystems. Furthermore, even though no evidence was
487 found that nest construction activity by this ant species profoundly impacts the physical
488 properties of soil (except micropore field capacity) within the first 10 cm from the surface
489 during a short period of two months, any impact at deeper soil levels could eventually
490 affect upper levels over a longer time span.

491

492 **4.2 | Seedling growth**

493

494 Overall, considering all colonies involved in our experiment, we did not find evidence that
495 the presence of *E. ruidum* sp. 2 affected the growth of coffee seedlings over the short term.
496 The differences were detected only for land use (Table 2) and not for the presence of ants.
497 Most of the seedlings grew faster in sun than in shaded coffee plantations, a reasonable
498 result that could be explained because the “*caturra*” coffee variety used here is adapted to
499 grow under sunny conditions and possesses less nodes per branch and less floral buds on
500 the existing nodes when planted in the shade (Arcila et al., 2007; DaMatta & Rodríguez,
501 2007). Additionally, when seedlings were planted, adult coffee plants were always present
502 in the immediate vicinity of the EUs, providing a degree of protection from the harsh
503 conditions in the open agroecosystems.

504

505 As the experiment progressed, there were highly significant effects of ant presence
506 on seedling length (but not on stem diameter) (Table 3), which demonstrates that this
507 variable is highly dependent on local conditions, such as farm condition, previous history
508 (grasslands, or cassava or other crops prior to coffee), management and the presence of ant
509 activity. This is supported by the significant “farm” effect and land use-time interaction
510 effect. The variable of stem diameter did not present significantly different results, with the
511 exception of time, maybe because it is a more stable variable which also needed more time
512 to exhibit any treatment effects. The unexpected global lack of a positive effect due to the
513 presence of *E. ruidum* sp. 2 appeared to be associated with difficulties in the establishment
514 of some colonies and, above all, to the very unequal level of activity among them, which is
515 why the positive effects were found only when considering the colonies that remained
516 active throughout the entire experimental period. Therefore, when considering only

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517 colonies that were always active (Table 4), their presence in the EUs did result in a
518 significant positive effect on various plant growth variables, *i.e.* total length of the plant and
519 length from the main root tip toward the apical meristem in shaded coffee and stem
520 diameter in sun coffee seedlings.

521

522 It should be pointed out that the greatest movement of soil by removal occurred
523 during the first stage of nest establishment whereby nutrients are added to the soil through
524 nutrient cycling. Newly added nutrients likely come from sources such as the prey brought
525 to *E. ruidum* sp. 2 nests (this hunting species may collect up to 260 million prey ha⁻¹ day⁻¹
526 in cacao plantations in Mexico; see Lachaud et al. (1996)) and from corpses of dead
527 nestmates. In our study, limited amounts of prey were provided in order to control the
528 nutrients added (both in treatment and the control EUs). These nutrients could eventually
529 contribute to the seedling growth during the final stage of the experiment, where plants in
530 shaded coffee plots added with *E. ruidum* sp. 2 that were active throughout the entire
531 experimental period were approximately 4 cm higher when compared to plants from
532 control EUs in shaded coffee (Fig. 6). This result is consistent with that reported by
533 Wagner & Nicklen (2010) where *Acacia constricta* plants benefited from soil nutrients
534 derived from ant nests. Additionally, the result is also consistent with observations in other
535 ants such as *Pheidole* spp., where C, N, P and NO₃-N amounts were higher in soil with
536 ants than in those without ants (Shukla et al., 2013). In India, Seema (2016) found
537 evidence that soils with different ant species present the highest K and P concentrations,
538 when compared to soils without ants. The results of a previous pilot experiment, conducted
539 under identical conditions as the present study, but in the experimental station of the
540 Universidad del Valle (Cali, Colombia) over an area of about 60 m² (Rondón, 2017),
541 demonstrated that coffee seedlings grew significantly faster in length and diameter in the
542 presence of *E. ruidum* sp. 2 colonies. However, control EUs at each of the sites did not
543 allow detection of any differences, suggesting that over a short time scale, ant excavation
544 activity does not affect neighbouring coffee seedlings, especially if colonies are weak and
545 almost do not perform excavation activity. This is supported by the fact that more than two
546 thirds of the colonies (46 from the 60 ant colonies) liberated in treatment EUs had to
547 survive harsh conditions such as being enclosed in a very small area, competing with

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548 already established aggressive ants (namely *Wasmannia auropunctata*), and depending on
549 resources provided by the researchers. Considering that, even under these unfavorable
550 conditions our data clearly showed that *E. ruidum* sp. 2 contributes to supporting
551 ecosystem services in tropical agroecosystems through bioturbation exhibited during nest
552 construction, it is highly probable that their impact, both on soil physical characteristics
553 and plant growth, would be higher under less restrictive, natural conditions.

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555 Finally, from our data we conclude that excavating colonies of *E. ruidum* sp. 2 do cause
556 significant changes in bioturbation (through soil particle removal), and in only one of the
557 soil physical properties measured. Additionally, we conclude that coffee seedlings grew
558 faster when they were close to burrowing colonies of this ant species. These results
559 support the idea that *E. ruidum* sp.2 is a functionally important soil agent in Neotropical
560 agroecosystems.

561

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563

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573

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