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

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Abstract

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

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4 32 and coffee seedling growth in these EUs was significantly higher than in controls,
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6 33 indicating that, at the global scale, our bioturbation estimate could be higher under less
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8 34 restricted natural conditions and higher nest densities as found in other regions. *Ectatomma*
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10 35 *ruidum* sp. 2 appears as a functional agent in agroecosystems due to its ubiquity and its
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12 36 high bioturbation impact during the nest excavation period of activity, providing support
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14 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*,

exerts a bioturbation effect of 8,410 kg ha⁻¹ y⁻¹ which can reach the extraordinary high values of 68,380 kg ha⁻¹ y⁻¹ in sandy soil (Richards, 2009).

Apart from changing the physical and chemical properties of the soil, bioturbation generates a higher infiltration of water in the soil, both through nest entrances at the surface, and as a result of increased macroporosity. For example, in Australian zones where nests of *A. barbigula* were present in high densities, water infiltration averaged 23.3 mm min⁻¹, i.e. about four times more than in zones without nests of this species (Eldridge, 1993). In Australia, Evans and coauthors found no effect of ant nesting in agricultural areas; however, two years later they found three times as much water infiltration in agricultural areas where ants nested in than in areas without ant nests (Evans et al., 2011).

Physical changes to the soil, associated with the additional aggregation of organic material gathered by ants and deposited inside or outside their nests (both of plant and animal origins), impact on its water-holding capacity and soil fertility (Farji-Brener & Werenkraut, 2017). Soil changes in the vicinity of the nests or mounds of numerous ant species also have a positive influence on the growth of neighbouring plants (Shukla et al., 2018). Even the nests of some invasive ant species, such as *Solenopsis invicta* can exert positive effects on plant growth (Lafleur et al., 2005) and in some dominant ants with long-lasting mounds, such as *Lasius flavus* in the Czech Republic, positive effects on mound vegetation can last several years after the disappearance or the substitution of the original ant occupant (Kovář et al., 2001).

In the Neotropics, *Ectatomma ruidum* sp. 2 (*E. ruidum* complex) is a dominant ground-dwelling predatory ant species of great interest due to its functional importance in relation to biological systems altered by humans (agroecosystems) (Fontalvo-Rodríguez & Domínguez-Haydar, 2009) and for its potential as a biological control agent (Lachaud, 1990). It is commonly found in coffee, cocoa and corn plantations, presenting densities which vary from 500 to 6,000 nests ha⁻¹ (Levings & Franks, 1982; Santamaría et al., 2009), but can occasionally exceed 10,000 nests ha⁻¹ (Schatz & Lachaud, 2008).

Although *E. ruidum* sp. 2 nest density tends to be high, the role of this species on bioturbation, soil porosity and water infiltration through the surface nest entrances has not been documented. It is to be expected that nest galleries that connect nest chambers with the surface could improve water movement through the soil, as has been documented with termites and other ant species (Lobry de Bruyn & Conacher, 1990). Furthermore, *E. ruidum* sp. 2 can exhibit high predation rates (several million prey ha⁻¹ y⁻¹, see Lachaud et al. (1996)) thus adding large amounts of organic material to their nests that enhances soil fertility around the nest area. Such effects can occur during the colony life cycle and after nest abandonment. Due to the characteristics exhibited by *E. ruidum* sp. 2 in agroecosystems, such as high nest density ha⁻¹, high predation rates, and high myrmecochorous activity (Lachaud et al., 1996; Gutierrez-Rapalino & Domínguez-Haydar, 2017), this species has been considered as an important functional agent in neotropical agroecosystems and natural systems (Schatz & Lachaud, 2008; Domínguez-Haydar & Armbrrecht, 2011). In order to examine whether field data support the significant ecological function played by this ant species, we evaluated, for the first time in coffee agroecosystems, the role of *E. ruidum* sp. 2 in bioturbation through measuring soil removal activity and the effect of this removal on both water infiltration and growth of neighbouring coffee seedlings over a relatively short time span. We hypothesized that the presence of excavating colonies of *E. ruidum* sp. 2 causes significant changes both in soil and neighboring plants. Therefore, our objective was to test whether the presence of *E. ruidum* sp. 2 results in higher bioturbation, more changes in soil physical properties, and faster growth of coffee seedlings than equivalent areas without colonies of this ant.

2 | MATERIALS AND METHODS

2.1 | Study area

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

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

2.2 | Experimental design

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

1,450 m.a.s.l. The independence of the plots was assumed using the following arguments:
1) neighbouring plots around each farm differed in management: for example, farms were separated by a tomato crop, a family orchard or a pasture; 2) if there was no other different management, farms were separated by at least 100 m; 3) sun and shaded coffee should form a mosaic in the landscape, that is, sun and shaded farms should be intermingled over the landscape and not grouped.

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

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

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

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

2.3 | Soil removal

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

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

2.4 | Plant growth variables

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

2.5 | Soil infiltration

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

$$I = C_1 t + C_2 \sqrt{t} \quad (1)$$

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

2.6 | Physical properties of the soil

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

2.7 | Statistical analysis

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

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

Statistical analyses were carried out using the R distribution program, version 3.4.0 (R Development Core Team, 2017), determining statistical differences to a 5% significant level.

3 | RESULTS

3.1 | Soil removal

The digging activity of *E. ruidum* sp. 2 resulted in a statistically significant higher amount of soil removal in the treatment EUs when compared with the control EUs over the complete experiment. On average, over the 66 days of recording, the cumulative soil removal in each EU containing an *E. ruidum* sp. 2 colony was 112.6 ± 66.3 g dry soil vs. 8.2 ± 14.4 g in each control EU (Fig. 4B). On average, each *E. ruidum* sp. 2 colony of approximately 70 workers removed 104.4 g dry soil during the two month period of the experiment ($1.6 \text{ g day}^{-1} \text{ colony}^{-1}$), almost 13 times more than the global soil removal performed by other invertebrates (arthropods, annelids or other ant species) present on an equivalent surface during the same period of time. On average, soil removal in sun coffee was 114.7 ± 65.7 g per EU vs. 106.3 ± 68.4 g in shaded coffee, and neither land use nor farm variables had any statistically significant effect on soil removal ($\chi^2 = 0.436$ and 0.093 , respectively; N.S.) (Fig. 4A). However, because soil removal gradually decreased as the experiment continued (Fig. 5) due, in part, to the death of various colonies and the low soil removal activity of numerous other colonies, these results underestimate the real bioturbation impact of *E. ruidum* sp. 2 in coffee plantations. Only 14 *E. ruidum* sp. 2 (six in sun coffee, eight in shaded coffee) were still fully active at the end of the experiment; however, given that the EUs could not be disturbed during data collection, the final absence of a colony could only be established at the end of the experiment. Out of the 60 colonies which have been added at the beginning of the experiment, 23 demonstrated very limited activity. Twenty three other colonies almost completely disappeared, probably due to either interference competition with *Wasmannia auropunctata* which, in some cases, was already occupying the territory, or due to stress factors during the translation and establishment of the colonies in the EUs. The cumulative soil removal during the 66 days experimental

period for the 14 EUs that were fully active until the end of the experiment, reached 237.0 ± 44.7 g of dry soil per colony (267.3 ± 29.6 g in sun coffee vs. 214.3 ± 41.3 g in shaded coffee). In fact, taking into account soil removal by other invertebrates (8.2 g per EU), each one of these 14 *E. ruidum* sp. 2 colonies removed on average 228.8 g dry soil (Fig. 5A), that is $3.5 \text{ g day}^{-1} \text{ colony}^{-1}$, more than twice the previous assessment.

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

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

whether the colonies were active until the end of the experiment (Table 1), remaining at a very reduced level after five weeks for all colonies. There was no significant effect related to the factor "land use" (that is, between sun and shaded coffee plantations) ($\chi^2 = 0.037$; N.S.), contrary to the "farm" factor which was significant ($\chi^2 = 47.995$; $p < 0.05$).

Fig. 5. Box diagrams of soil removal by *E. ruidum* sp. 2 in all coffee plots during the two month experimental period. A) Soil removal in the 14 treatment EUs where colonies remained active until the end of the experiment; B) Soil removal in the 23 treatment EUs where colonies showed low activity until the end of the experiment; C) Soil removal in the 23 treatment EUs where colonies did not remain active until the end of the experiment. •: outliers. Vertical bars show minimum and maximum values.

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

	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

3.2 | Seedling growth and physical properties of the soil

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

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

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

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

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

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

Table 3. LMM deviance analysis of plant growth variables as the experiment progressed (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

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 ± 3.9 cm vs. 15.3 ± 2.5 cm) and on the length from the main root tip (mean $\pm$ SD: 35.8 ± 4.6 cm vs. 28.2 ± 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 ± 1.0 cm vs. 0.7 ± 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 ± 3.1 vs. 39.7 ± 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

4 | DISCUSSION

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

4.1 | Bioturbation and physical properties of the soil

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

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

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

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

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

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

4.2 | Seedling growth

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

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

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

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

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4 548 already established aggressive ants (namely *Wasmannia auropunctata*), and depending on
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6 549 resources provided by the researchers. Considering that, even under these unfavorable
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8 550 conditions our data clearly showed that *E. ruidum* sp. 2 contributes to supporting
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10 551 ecosystem services in tropical agroecosystems through bioturbation exhibited during nest
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12 552 construction, it is highly probable that their impact, both on soil physical characteristics
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14 553 and plant growth, would be higher under less restrictive, natural conditions.
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18 555 Finally, from our data we conclude that excavating colonies of *E. ruidum* sp. 2 do cause
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20 556 significant changes in bioturbation (through soil particle removal), and in only one of the
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22 557 soil physical properties measured. Additionally, we conclude that coffee seedlings grew
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24 558 faster when they were close to burrowing colonies of this ant species. These results
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26 559 support the idea that *E. ruidum* sp.2 is a functionally important soil agent in Neotropical
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28 560 agroecosystems.
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