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RESEARCH ARTICLE

Variation in the rates of biomass removal by soil macro-fauna in different land uses at Rashad, South Kordofan, Sudan

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Abstract

One of the main implications of reducing biodiversity is the loss or decline of ecosystem function. We have previously seen in the Rashad location that agricultural practices have a lower effect on ant biodiversity. However, how they affect the environmental services, they provide is unclear. The main objective of this study was to evaluate whether the conversion of native areas into agricultural systems affects the removal of biomass carried out by ants, an important ecosystem function linked to decomposition and predation. We sampled three transects from (the Rashad district). Each sampling plot consisted of a grid of 12 pitfall traps filled with sardine baits (simulating animal organisms) and bananas (as attractive vegetable resources). In addition, grass seeds (*Sorghum bicolor*) were applied in both natural (Campo, Kubos, and forest) and agricultural settings (soy monoculture, pastures, and organic agriculture). The Results showed that ant's removal was highest in sardine with an average of 87.3g (σ ± 23.8), followed by banana (average of 70.5g, σ ± 31.5) and lowest in the seed (mean of 7.8g, σ ± 7.3) (highest $p = 0.017$). Only the soy monoculture regions showed the lowest levels of sardine removed, indicating an effect associated with the kind of land use. Because little biomass is eliminated in both natural and agricultural settings, no effect of the seeds bait has been observed. As for the banana bait, the data suggested a redundancy effect with another group of macro-fauna). Our results suggest that there is a redundancy effect with another group of macrofauna. However, macrofauna biomass (excluding ants) does not explain this biomass removal. In addition, it detected no impact of ant species composition on removed biomass. The reduction of sardine and banana biomass was correlated with ant richness, indicating that the effects on ecosystem function depend on the particularities of each evaluated role (such as resource type), the type of land use, and the ant richness in the study area.

Keywords
Biodiversity; Biomass; soil macro-fauna; land uses types; Rashad; Sudan.

Introduction

Expansion of agricultural systems affects local and global biodiversity (Lanz et al. 2018), with the extent of these impacts varying based on local characteristics, taxon, and type of agricultural system implanted (Kassa et al. 2017; Ortiz et al. 2021). However, the influence on biodiversity is just as crucial as determining what influence the growth of agricultural systems has on ecosystem function. Biodiversity-ecosystem function hypothesis, a theory debated in the early 1990s, states that a reduction in biodiversity (number of species, genetic variability, etc.) results in the decline of ecosystem processes (DeLaplante and Picasso 2011). However, this is not a universal effect (Scherer-Lorenzen et al. 2005); Reviews and meta-analyses on this subject have all come to the same conclusion that the reduction in ecological functionality is depends on the local characteristics, the assessed ecosystem's function, and the type of disturbance (Spurgeonet al. 2013; Duru et al. 2015; Boltovskoy et al. 2021).

This large number of divergent results, found in studies on ecosystem services due to the effect of anthropization, makes the predictability of these impacts complex and is depends on studies that focus on different environments, processes, and types of disturbance (Pinter-Wollman et al. 2013). However, most previous studies have focused on plant or aquatic communities' ecology (Finke and Snyder 2010; Gaertner et al. 2014; Arias-Real et al. 2021) with focusing limitedly on the ecological processes that rely on terrestrial fauna.

Among these ecological processes, we highlight the predation and decomposition exerted by terrestrial fauna, which is of fundamental importance to the ecosystem's functioning and regulation (Nichols et al. 2008). Predation allows for the control of the wide range of populations in a particular ecosystem. Few studies have examined how agricultural expansion affects biodiversity and how this manifest in the ecological processes of the Rashad, which has one of the highest, levels of endemism and degradation in the world (Underwood et al. 2009; Wilson et al. 2017). The expansion of the agricultural frontier in the Rashad is a reality with densely occupied areas mainly in the south of this biome (UNDP 2003). The effects of this occupation have been assessed using a variety of taxa, with ants being one of the most studied (reviewed by Blüthgen et al. 2003; Camarota et al. 2020), as it is one of the most abundant taxa with the highest biomass in tropical habitats (Adams et al. 2013) and interesting behaviors. In Rashad, it is estimated that ants can consume about 13 to 17% of the leaf biomass of woody plants annually (Eisawi et al. 2021). Ants can also act as predators, controlling the population of agricultural pests (Harvey and Eubanks 2004; Frizzi et al. 2020) or as seed dispersers (Aranda-Rickert and Fracchia 2012) particularly these contain some attractive seed appendage (Munguía-Rosas et al. 2009). Another important ecological service is the removal of organic biomass from the surface to the subsoil, which improves the nutritional qualities of the soil (Chen et al. 2013). Ants have different feeding habits, with the majority being omnivorous, with a few predatory, granivorous, or herbivorous species (Blüthgen et al. 2003) which contribute importantly to biodiversity-ecosystem functions. Furthermore, there is always some limiting nutritional element in the entire range of resources used by ants for their development and colonization. It had been demonstrated that ants have shown a feeding preference for those resources that contain a more significant proportion of (these limiting elements) (Feldhaar et al. 2007).

The purpose of this study was to evaluate the rates of biomass removal by ants from unique resources between native and agricultural ecosystems. For this purpose, areas of organic agriculture, soy monoculture, and pastures, in addition to native areas of the Campo, Kubos, and forest formations were selected. We attempted to test the following hypothesis: (1) there is a significant difference in the quantity of biomass removed between resources that simulate (animal organism's ants), attractive seed appendages, and grass seeds (2) changes in land use affect rates of biomass removal (3) local variables, such as ant richness, ant assembly composition, or macro-fauna biomass best explain the amount of biomass removed.

Materials and Methods

Description of the study area

This study was carried out in the Rashad district between April 2020 and June 2021, which covers an area of more than 7872 km^2 and lies in the center of the Kordofan between latitudes 10°, 13° N and longitudes 29°, 33° E. (Fig. 1). The experiment was carried out at 69 locations, Thus, in total, 14 soy monoculture, 14 pastures, 16 organic agriculture, and 22 areas of native vegetation were analyzed. It should be noted that pasture and soy monoculture, are the most common types of land use in the area, accounting for 27.6% and 11.7% of the biome area, respectively (Milazzo et al. 2013). Organic agriculture areas, on the other hand, represent an alternative way of producing vegetable food without the use of pesticides in a rapidly expanding market (Eisawi et al. 2022). (Within 22 areas of native vegetation, we selected the three phytophysiognomies that are the most common in the region, which are five localities of Campo (refers to rural formations), eight of Kubos (refers to Rashad area), and nine forests (refers to forest formations).

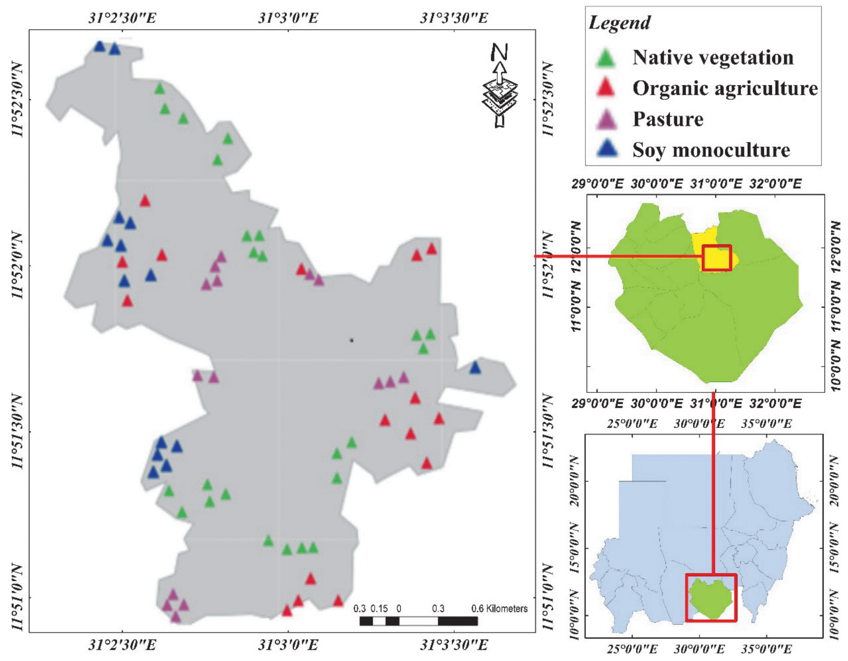

Figure 1. Map of the study area with sample distribution.

The area is in a tropical climate with an annual rainfall of between 500 and 800 mm. Most of the basement complex in the area is overlaid with Nubian sandstone. Vegetation cover in the Rashad area comprises trees and legumes dominated by the Acacia genus and annual grasses, and shrubs.

Sampling methods and experimental design

We sampled three transects 20-meter-long transects in each location (Sample plot) (Fig. 2a). Each sample plot consisted of a grid of 12 pitfall traps, which were arranged in three rows with four traps each, and with a 2 m spacing between them (Fig. 2b). The pitfall traps are 200 ml plastic cups containing sardines, bananas, and seeds (seeds of *Carica papaya* Linn). There were 12 pitfalls per plot, 36 per location, and 2,484 in all 69 sites. Biomass removal stations were set up in this experiment to sample the ant macro-fauna and quantify their rates of resource removal. These stations consisted of plastic pots of one liter sized with lids and several lateral holes in the shape of a semicircle, 1 cm high and 2 cm wide, for the macro-fauna to have access to the inside of the pots (Fig. 3a). After 24 hours, a known mass of resources (bait) was placed within each station; these stations were collected and measured once more to determine the amount of biomass removed by macro-fauna. The treatment containing seeds was dried in an oven at 50 °C for 24 hours before and after the field trial because seeds in the field absorb a lot of water. The sardine and banana baits were prepared in the laboratory, individualized and frozen, in quantities ranging from 20–30 g per biomass removal station until used in the experiment. Grass seeds were also individualized into portions of 15 grams per biomass removal station for each sample plot; 12 biomass removal stations that had been in the field for 24 hours were also installed (Fig. 3b). The sample plots were installed in each location, with the type of bait in each plot chosen at random. At the end of the experiment, we removed the stations. The ants present inside the biomass removal stations were collected and identified in the laboratory using a single key to identify subfamilies, a series of keys to identify genera, and a series of keys to identify species (Bingham 1903; Holldobler and Wilson 1990; Mathew and Tiwari 2000).

At the end of the plot, two biomass removal stations, called "control", were placed. Because the resources could lose mass due to desiccation, a control reduced these values of treatments. In addition, a three-centimeter strip of yellow adhesive trap on the inner side of the pot, which surrounded the pot preventing animals from accessing the bait, was set up (Fig. 4c). The mass lost after 24 hours was considered a loss of moisture from the resource to the environment and, therefore, was proportionally discounted from the initial mass of the other biomass removal stations. In addition, the covers of all biomass removal and control stations were covered with a layer of

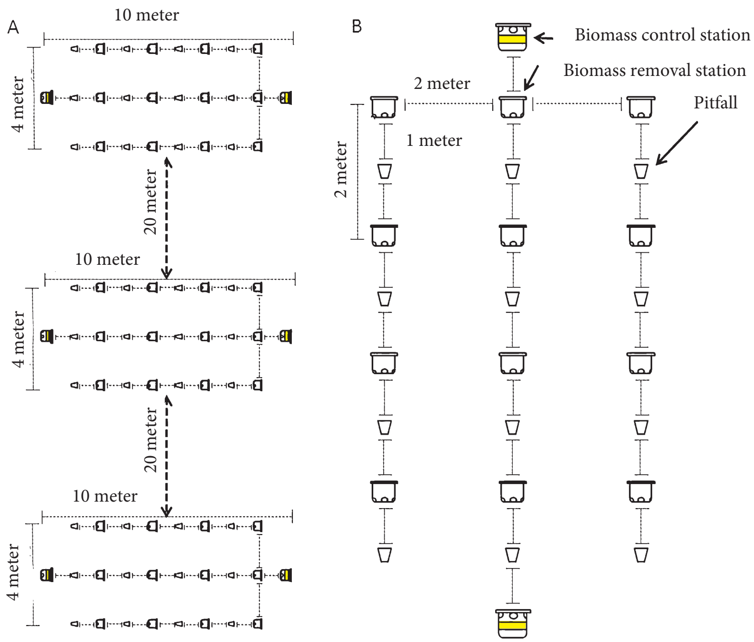

Figure 2. Methodology carried out in the Rashad District between April 2020 and June 2021. **A** As the three sample plots were distributed throughout each location, a type of bait chosen at random was used sardines, bananas and seeds in each plot. **B** Sampling plot scheme, with 12 biomass removal stations, 12 pitfalls and 2 control biomass stations at the end.

white rubberized waterproofing that was about 3 mm thick to reduce the internal temperature. The three sample plots were differentiated by the type of resource in the biomass removal stations within each location. Three types of resources were used that simulated the most common ant food available that could be obtained in large quantities and at a reasonable cost. These resources are canned sardines (simulating animal organisms), bananas of the silver variety (simulating foods such as seed or fruit aryl), and a compound of unprocessed seeds, in equal mass proportions, of rice, birdseed, and millet (simulating seeds of native grasses). In nutritional terms, sardines are very rich in sodium, protein, and total fats, bananas are rich in sugar while seeds are rich in carbohydrates to prepare the sardine (Eisawi et al. 2022), the oil was drained and then crushed with a fork; the banana was peeled and crushed.

Animals (vertebrates) attracted by the baits were kept out of the plots using an electric shock system installed alongside the experiment. Fig. 3d shows how the system was put together by attaching pieces of chicken wire to the top of the biomass removal stations and connecting them to an electric fence for livestock. Powered

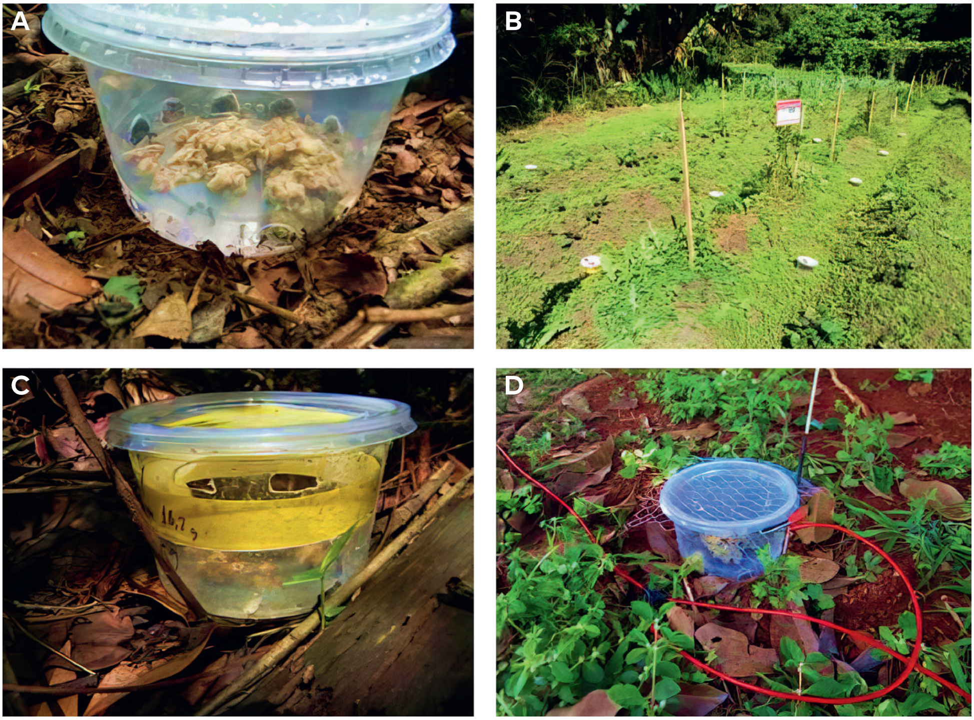

Figure 3. Photographs of the study method carried out in the Rashad District between April 2020 and June 2021. **A** Sardine bait biomass removal station; **B** The sample plot in an organic agriculture area; **C** Biomass control station; **D** Biomass removal station with a wire mesh connected to an electric shock device.

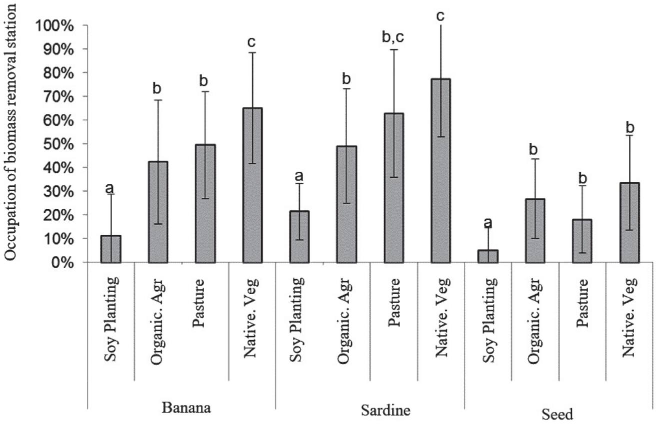

Figure 4. Proportion of biomass removal stations occupied by ants in the Rashad District between April 2020 and June 2021. Different letters mean significant differences (p <0.05) between the proportions of occupation within each type of resource, bars representing the standard deviation.

by a 12-volt battery, it was wired into each biomass removal station. Following the system's installation, no control or treatment stations were lost.

Calculation of biomass

The biomass removal rates per season were calculated, discarding the mass of potting, according to the function used by Buckley (1991):

Br = (PI x CF) - (PF)

Where:

Br is biomass removed; PI is the initial mass of food put into the station; CF is the correction factor for biomass lost by drying (formula of the calculation below); PF is the mass of the remaining food content after 24 hours.

To correct the mass of food lost due to desiccation at the biomass removal stations; (CF) was calculated with the control biomass stations, using the following Buckley (1991) formula:

CF = PF / PI

Where:

CF is the factor correction for biomass lost by desiccation; PF is the mass of the remaining food content after 24 hours; PI is the initial mass of food placed inside the control station.

Even after these calculations were, performed the sum of the food values removed per sample portion was. We accidentally lost a few biomasses removal stations (Table 1), so we estimated the total value of biomass removed per sample plot. The value was obtained using the formula by Holden & Treseder (2013):

$$B_{restimated} = (\Sigma Br_{\text{per sampling point}} / Nt) \times 12$$

Where:

ΣBr per sampling point is the sum of all biomasses removed in a sample plot; Nt is the number of biomass removal stations rescued per sample parcel and 12 is the number of stations that should exist in each sample plot.

The samples were dried at 60 ° C for 48 hours and then weighed on an analytical balance with an accuracy of 0.001g.

Table 1. Number of biomass removal stations and control stations recovered and lost in the field, between April 2020 and June 2021 in the Rashad District. The systems studied were areas of organic agriculture (Org. Agri), pasture, Soy monoculture (Soy) and areas of native vegetation (Nat.Veg). Note: The high number of lost seasons with the type of seed bait is due to the fact that, accidentally, during the field work some traps overturned and the seeds fell out of season, which were eliminated from the analysis.

Food	System	Number of biomass removal stations	Lost	Number of biomass control stations	Lost
Banana	Org. Agri	192	0	32	0
	Pasture	166	2	28	0
	Soy	168	0	28	0
	Nat.Veg	264	0	44	0
	Total	**790**	**2**	**132**	**0**
Sardine	Org. Agri	190	2	32	0
	Pasture	168	0	28	0
	Soy	166	2	28	0
	Nat.Veg	261	3	43	1
	Total	**785**	**7**	**131**	**1**
Seed	Org. Agri	183	9	29	3
	Pasture	149	19	26	2
	Soy	167	1	25	3
	Nat.Veg	255	9	41	3
	Total	**754**	**38**	**121**	**11**
Grand total		**2.329**	**47**	**384**	**12**

Data analysis

The data were divided into four habitat types: soy monoculture, organic agriculture, pasture, and native vegetation. For the native vegetation, the points that contemplate different phytophysiognomies were grouped because (1) there were no significant differences in the patterns of biomass removal; (2) the separation by phytophysiognomy to form groups with a few samples to carry out the subsequent statistical analyzes. All analyzes were performed using the R (Alvarado et al. 2020) software. The first analysis aimed to verify if the number of biomass removal stations containing ants at the end of the 24 hours in the field varied by habitat. We recorded the number of ant biomass removal in each sample plot for this analysis. Then an analysis of the generalized linear model was performed using the glm function in R.

To determine which type of bait (banana, sardine, and seed) was most removed in the system, Friedman's non-parametric test in blocks (due to the heterogeneity of variances) was performed, with an a posteriori test (Williams et al. 2001) using the Posthoc Friedman Nemenyi Test function of the PMCMR package (Pohlert and Pohlert 2018). Because the soy monoculture areas differed in terms of resource preference, the same analysis was carried out on them and used the glm function of R. We analyzed generalized linear models to see if the biomass removed from each type of bait varied according to the different habitats evaluated. A Pearson correlation was also performed between the richness and mass of ants sampled by location. Due to the non-normality of part data, heteroscedasticity problems, and the constant presence of points tending the residues; it was impossible to perform any analysis encompassing multiple factors and their interactions to explain which variables influence sardine, banana, and seed biomass removal. For this reason, Pearson correlation analyzes were performed between the variables:

Ant richness; Macro-fauna biomass; Biomass removed from sardines; Biomass removed; from bananas and biomass removed from the seed. To determine the composition of ant species that influences biomass removal rates, a canonical correspondence analysis (CCA) was performed using the CCA function of the vegan package (Oksanen et al. 2018). This analysis was based on a species matrix by location, with consideration of the frequency occurrence per plot (three plots per location). The variables were evaluated in the CCA habitats type; the number of ant species per location; macro-fauna biomass; biomass removed from sardines; bananas and seeds.

To validate the CCA, a test was performed to detect multicollinearity between the variables analyzed using the VIF. CCA function, from the vegan package (Oksanen et al. 2018) - in this test, if the VIF (variance inflation factor) values are higher than 10, indicates multicollinearity between two variables (Dormann et al. 2013). Then, the function ANOVA.CCA (vegan package), with 999 permutations, was used to determine whether the model, variables, and axes of explanation are significant. These analyzes (CCA) were repeated once again, with a matrix using only the species considered abundant (which occurred over 30 times).

List of abbreviations

NV: Native vegetation
OA: Organic agricultural
Br: Biomass removed

Results

Ant occurrence at biomass removal stations

Ants were the major insects observed within the biomass removal stations after 24 hours of collection (Table 2). Other insects such as flies, moths, and grasshoppers were found in 55 of over 2,300 biomass removal sites in the field. These indicate that the ants are responsible for most of the biomass removed, owing to earlier field observations showing that ants monopolize attractive baits by attacking any other organism that approaches. However, it is worth noting that, at the end of the experiment, the percentage of biomass removal stations occupied by ants varied significantly between habitats, being lower in the areas of soy monoculture (variation in occupation from 0 to 57%, with an average of 13%, Fig. 4).

Biomass removal rates between resource and habitat types

Ants could remove relatively large amounts of biomass per plot (maximum removed 237g, all sardines available in the VN-18 location), but this depends on the type of food (according to the Fridman block test, $X^2 = 85.544$, df = 2, $p < 0.001$) (Fig. 5a). Thus, sardine was found to be the most removed resource, with an average of 87.3g ($\sigma \pm 23.8$), followed by banana (average of 70.5g, $\sigma \pm 31.5$) and, last, seed (mean of 7.8g, $\sigma \pm 7.3$) (highest $p = 0.017$). Only for soy monoculture does the pattern differ from the others (Fig. 5b), so there was a significant difference between the biomass removed for the different resources (Fridman block test, $X^2 = 26.153$, df = 2, $p < 0.001$), with the banana being the most removed (average of 74.9g, $\sigma \pm 13.7$). The difference between biomass removed from bananas and sardines was marginally significant (p=0.06), as an average of 45.5g ($\sigma \pm 12$) of sardines was removed. At the same time, seed removal was still significantly lower (p<0.01) than the other types of resources (mean of 5.3, $\sigma \pm 7.1$).

There were no significant differences in biomass removal by habitat type (soy monoculture, pasture, organic agriculture, or native vegetation), for banana (GLM, F3.62 = 0.4565, p = 0.7136) and seed (GLM, F3.62 = 1.2561, p = 0.2972) (Fig. 5c). Sardine removal was significantly correlated with habitat type (GLM, F3.63 = 9.1317, $p < 0.001$), with only soy monoculture removing less sardine biomass than all other systems ($p < 0.001$). The values of biomass removed from sardines did not differ between organic agriculture, pasture, and native vegetation ($p > 0.05$).

Table 2. List of species captured within biomass removal stations and ordered by their occurrence in Rashad destrict between April 2020 and June 2021.

Subfamily/ Species	Banana	Sardine	Seed	Cumulative Frequency of Occurrence
Dorylinae				
Aenictus anceps Forel, 1910	74	94	31	10,7%
Anochetus traegaordhi Mayr, 1904	48	78	13	18,1%
Aenictus soudanicus Santschi, 1910	33	44	18	23,2%
Aenictus pharao Santschi, 1924	15	54	15	27,8%
Aenictus mentu Weber, 1942	29	32	8	31,5%
Formicinae				
Camponotus acvapimensis Mayr, 1862	17	41	10	35,1%
Camponotus aegyptiacus Emery, 1915	20	33	13	38,6%
Camponotus bayeri Forel, 1913	19	40	3	42,0%
Camponotus brutus Forel, 1886	14	24	10	44,6%
Camponotus carbo Emery, 1877	18	23	5	47,0%
Camponotus galla Forel, 1894	12	10	18	49,2%
Camponotus hapi Weber, 1943	4	27	7	51,2%
Camponotus kersteni Gerstäcker, 1871	5	25	8	53,2%
Cataglyphis abyssinica Forel, 1904	11	16	11	55,3%
Cataglyphis albicans Roger, 1859	12	20	3	57,2%
Cataglyphis aurata Menozzi, 1932	21	5	7	58,9%
Cataglyphis bombycina Roger, 1859	12	17	3	60,7%
Cataglyphis savignyi Dufour, 1862	16	13	3	62,4%
Cataglyphis viatica Fabricius, 1787	3	19	8	64,0%
Lepisiota canescens Emery, 1897	11	11	8	65,6%
Lepisiota capensis Mayr, 1862	3	21	5	67,1%
Lepisiota carbonaria Emery, 1892	13	11	5	68,7%
Lepisiota frauenfeldi Mayr, 1855	9	15	2	70,1%
Lepisiota gracilicornis Forel, 1892	5	16	2	71,3%
Lepisiota megacephala Weber, 1943	5	15	2	72,5%
Plagiolepis sudanica Weber, 1943	5	12	5	73,7%
Polyrhachis epinotalis Santschi, 1924	3	15	3	74,8%
Polyrhachis fissa Mayr, 1902	8	6	5	75,8%
Polyrhachis militaris Fabricius, 1782	5	10	2	76,8%
Polyrhachis schistacea Gerstäcker, 1859	6	3	8	77,7%
Polyrhachis viscosa Smith, F., 1858	9	7	1	78,6%
Myrmicinae				
Calyptomyrmex brevis Weber, 1943	5	7		80,8%
Calyptomyrmex piripilis Santschi, 1923	3	6	3	81,5%
Cardiocondyla emeryi Forel, 1881	6	4	2	82,1%
Carebara bartrumi Weber, 1943	4	6	2	82,8%
Carebara polita Santschi, 1914	1	9	1	83,4%
Carebara santschii Weber, 1943	2	5	3	83,9%
Carebara sicheli Mayr, 1862	4	5	1	84,4%
Carebara sudanensis Weber, 1943	1	6	2	84,9%
Cataulacus intrudens Smith, F., 1876	1	7	1	85,4%
Cataulacus pygmaeus André, 1890	6	1	1	85,8%
Cataulacus traegaordhi Santschi, 1914		8		86,3%
Crematogaster acaciae Forel, 1892	2	5	1	86,7%

Table 2. (continued)

Subfamily/ Species	Banana	Sardine	Seed	Cumulative Frequency of Occurrence
Crematogaster aegyptiaca Mayr, 1862	1	7		87,1%
Crematogaster chiarinii Emery, 1881	4	3	1	87,5%
Crematogaster chlorotica Emery, 1899	2	6		88,0%
Crematogaster flaviventris Santschi, 1910	2	4	1	88,4%
Crematogaster gambiensis André, 1889		6	1	88,7%
Crematogaster inermis Mayr, 1862	2	5		89,1%
Crematogaster lango Weber, 1943	2	1	3	89,4%
Crematogaster latuka Weber, 1943		5	1	89,7%
Monomorium abeillei André, 1881	3	3		90,1%
Monomorium kineti Weber, 1943	1	2	3	90,4%
Monomorium madecassum Forel, 1892	2	3	1	90,7%
Monomorium mictilis Forel, 1910	2	3	1	91,0%
Monomorium nitidiventre Emery, 1893	1	4		91,3%
Monomorium pallidipes Forel, 1910	1	3	1	91,6%
Monomorium parvinode Forel, 1894	3	2		91,8%
Monomorium pharaonis Linnaeus, 1758	3	2		92,1%
Monomorium salomonis Linnaeus, 1758	3	2		92,4%
Pheidole aeberlii Forel, 1894	1	4		92,6%
Pheidole decarinata Santschi, 1929	1	2	1	92,9%
Pheidole jordanica Saulcy, 1874	3	1		93,1%
Pheidole punctulata Mayr, 1866	3	1		93,3%
Pheidole rugaticeps Emery, 1877	3	1		93,5%
Pheidole sculpturata Mayr, 1866	2	1	1	93,7%
Pheidole sinaitica Mayr, 1862		4		93,9%
Pheidole speculifera Emery, 1877	2	2		94,1%
Pheidole termitophila Forel, 1904	3		1	94,4%
Solenopsis orbula Emery, 1875		3		94,5%
Strumigenys ludovici Forel, 1904	2	1		94,7%
Strumigenys lujae Forel, 1902	1	1	1	94,8%
Strumigenys serrula Santschi, 1910	1	2		95,0%
Tetramorium altivagans Santschi, 1914		3		95,2%
Tetramorium angulinode Santschi, 1910	1		2	95,3%
Tetramorium caldarium Roger, 1857	1	1	1	95,5%
Tetramorium cristatum Stitz, 1910		2		95,6%
Tetramorium delagoense Forel, 1894	1	1		95,7%
Tetramorium eminii Forel, 1894		2		95,8%
Tetramorium kestrum Bolton, 1980	1	1		95,9%
Tetramorium nigrum Forel, 1907		1	1	96,0%
Tetramorium nube Weber, 1943		2		96,1%
Tetramorium pullulum Santschi, 1924	2			96,2%
Tetramorium sericeiventre Emery, 1877	1	1		96,3%
Tetramorium sudanense Weber, 1943	1	1		96,5%
Tetramorium viticola Weber, 1943	2			96,6%
Tetramorium weitzeckeri Emery, 1895		1	1	96,7%
Tetramorium zonacaciae Weber, 1943		2		96,8%
Trichomyrmex abyssinicus Forel, 1894		2		96,9%
Trichomyrmex oscaris Forel, 1894		2		100%

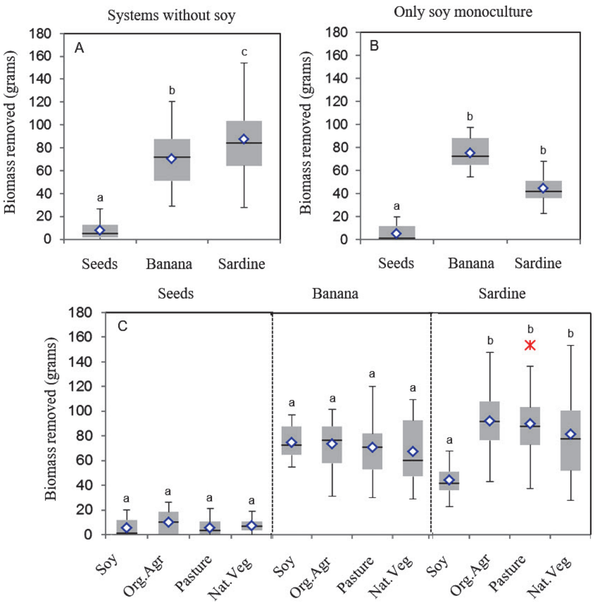

Figure 5. Biomass removed for different types of resources without counting soy monoculture (**A**), only for soy monoculture (**B**) and for different types of habitats (**C**), in the experiments carried out from in the Rashad District between April 2020 and June 2021. Different letters depict significant differences (p <0.05). Gray rectangles look from the 1st to the 3rd quartile, the horizontal line inside the rectangle represents the median, diamond in blue represents the average and an asterisk in red an outlier. Org. Agr = Organic Agriculture, Nat.Veg = Native Vegetation.

Influence of ant richness and fauna biomass

The strongest correlation found in this study was between ant richness and its biomass (r = 0.68, p <0.001) (Fig. 6). For biomasses removed from resources, we discovered that: (a) seed removal has no correlation with any of the variables presented (p> 0.05); (b) the correlations detected for the sardine and banana resources were relatively weak; and (c) sardine biomass removal correlated with the ant richness (r = 0.54, p <0.001) (Fig. 7). However, excluding soy monoculture data (Fig. 8). There was a correlation between the removal of banana biomass and ant richness (r = 0.32, p = 0.02).

Influence of species composition

As for the composition of ant species analyzed in the CCA, the models were significant (ANOVA. CCA complete F8.57 = 1.7478, p <0.001 and ANOVA. CCA only for abundant species F8.57 = 3.5721, p <0.001), as well as the first two axes that form (Fig. 9 and Fig. 10). It was also not detected multicollinearity between the variables of the models (the higher VIF was 2.25). For both models, the type of land use factor affected the species composition (for the variable land-use ANOVA. CCAs complete

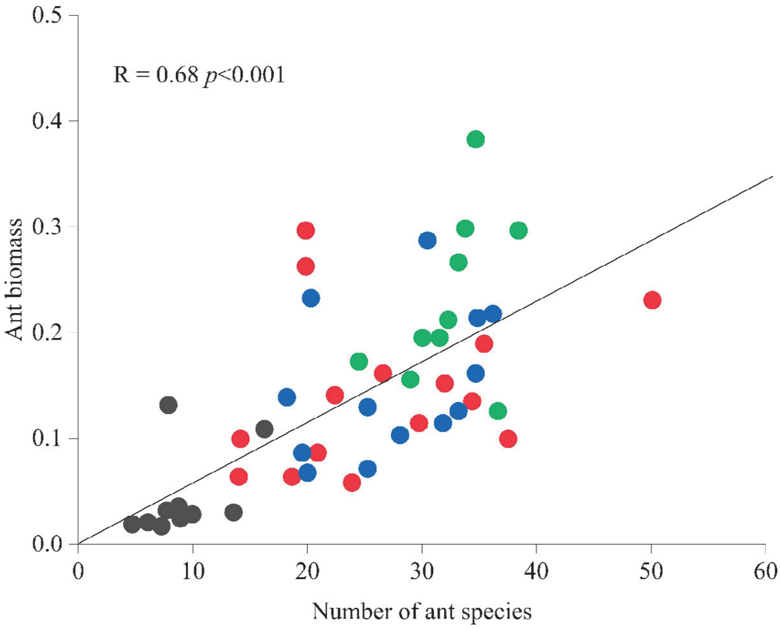

Figure 6. Pearson's correlation between number of ant species and the biomass of ants caught in the fall traps in each location. Colored dots indicate the habitat collected in the Rashad District between April 2020 and June 2021: (black) soy monoculture, (blue) pasture, (red) organic agriculture and (green) native vegetation.

F3.58 = 2.7161, p <0.001 and ANOVA. CCA only for abundant species F3.57 = 7.7573, p <0.001). The analysis of abundant species did richness significantly influence species composition (ANOVA. CCA for abundant species F8.57 = 1.5914, p = 0.044).

Discussion

Ants' occurrence at biomass removal stations

Ants are the dominant group on the surface of Rashad's soil and monopolize the resources they use. This is demonstrated by the number of biomass removal stations occupied by ants after 24 hours (Fig. 6), monopolized in the first minute of discovery. However, it detected an effect of the type of land use. In particular, soy monoculture showed a lower proportion of occupied seasons after 24 hours compared to areas of native vegetation. The existence of biomass removal stations with food without ants' presence is linked to three factors: satiety, territorialism, and the absence of ants. After 24 hours of the field experiment, some ant colonies have likely reached the satiety of food consumption, as demonstrated by Brown et al. (2001). Furthermore, ants are unlikely to come from afar to remove these baits due to their territorial behavior

(Belskaya et al. 2019). Thus, the lack of ants in the biomass removal stations shows how many ants are in their area.

Biomass removal rates between resource types

Several studies showed that ant's exhibit feeding preferences based on the most limiting nutrient in their environment (Kaspari and Yanoviak 2001; Ramalho et al. 2017). The sardine was the bait that ants consumed the most in this study, and this type of bait is widely used as an ant attractant in South Africa (Nyamukondiwa and Addison 2011), China (Wei et al. 2020) and Malaysia (Ab Majid et al. 2018). Therefore, our findings contradict those of Humle et al. (2009) from Bossou, Guinea in West Africa (using different baits), who demonstrated that soil ants choose sucrose-containing food, whereas arboreal ants choose sodium-rich resources (abundant in the sardine resource), comparable to those found by Nelson et al. (2019), using meat and sugar. According to these investigations, this occurs due to the ants' eating

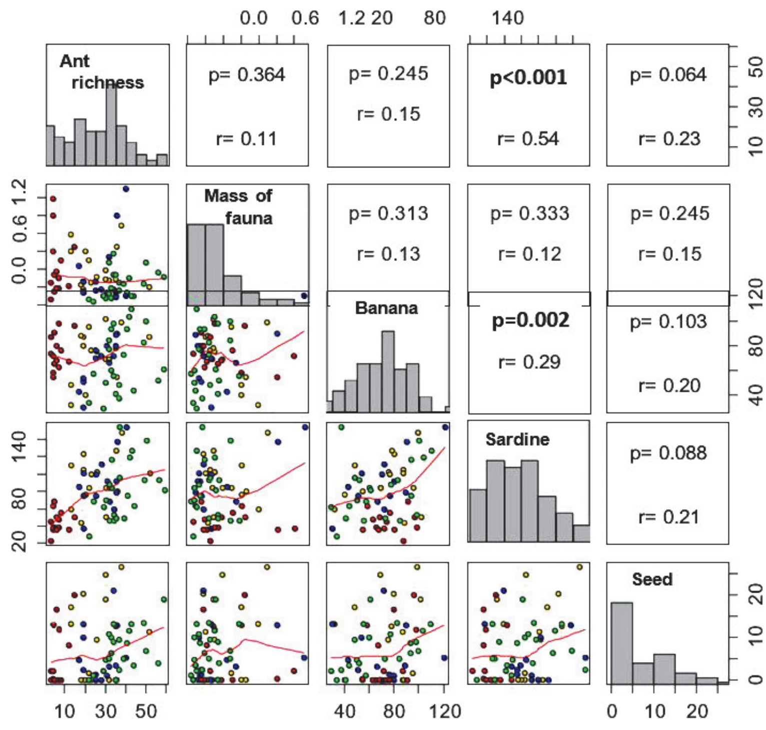

Figure 7. Pearson correlation matrix between number of ant species, macro-fauna mass (except ants) and biomass removed from banana, sardines and seeds. Significant correlation values (p <0.05) are indicated in bold. Red line represents the nonlinear trend of data dispersion. Colored dots indicate the habitat collected in the Rashad District between April 2020 and June 2021: (red) soy monoculture, (blue) pasture, (yellow) organic agriculture and (green) native vegetation.

habits, as soil-dwelling species seldom contact with sugary foods, which are more common in tree vegetation (Berndt et al. 2004). The ants removed the most food that resembled animal organisms (sardines) at the end of the 24 hours (except for soybean plantations). According to Govorushko (2019), the food resources consumed by adults and ant larvae are different. The protein and lipid resources captured by the foragers were preferably used to feed the larvae in the nest and guarantee the colony's growth. Workers use foods rich in sugars and carbohydrates for daily energy expenditure (Schabel 2006). These could be the determining factor in whether sardines and no other baits be removed further.

The values removed from sardine and banana biomass were close to each other, which shows that this can be an essential food supplement for soil microfauna, as proposed by Vasconcelos et al. (2019). Therefore, this proximity is also a clear indication that the "trophic promiscuity" in arthropods seems to be much broader than previously imagined by researchers as reported by Vepsäläinen et al. (2008). In

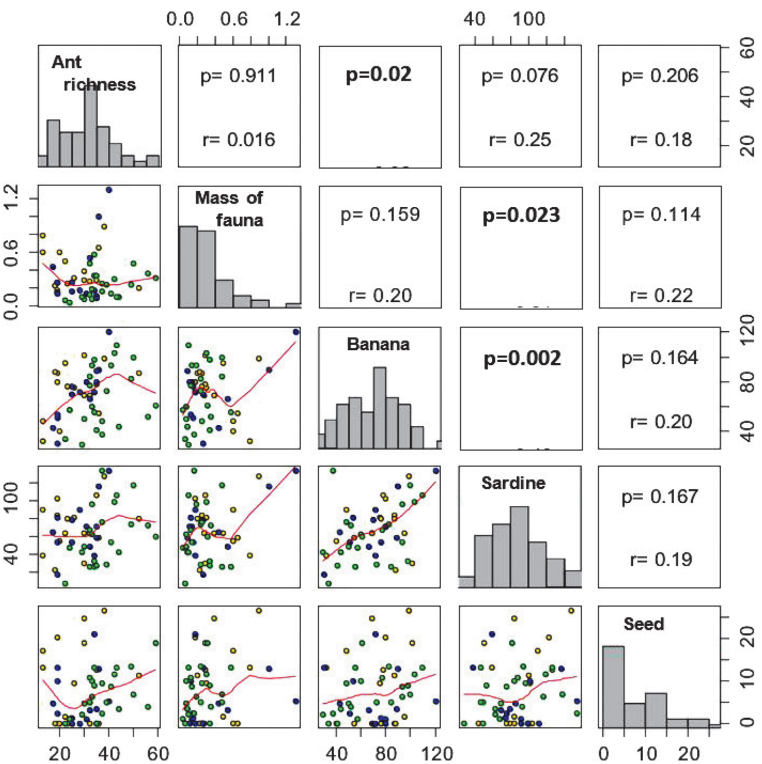

Figure 8. Pearson correlation matrix (in this case, without soy monoculture data) between number of ant species, macrofauna mass (except ants) and biomass removed from banana, sardine and seed. Significant correlation values (p <0.05) are indicated in bold. Red line represents the nonlinear trend of data dispersion. Colored dots indicate the habitat collected in the Rashad District between April 2020 and June 2021: (blue) pasture, (yellow) organic agriculture and (green) native vegetation.

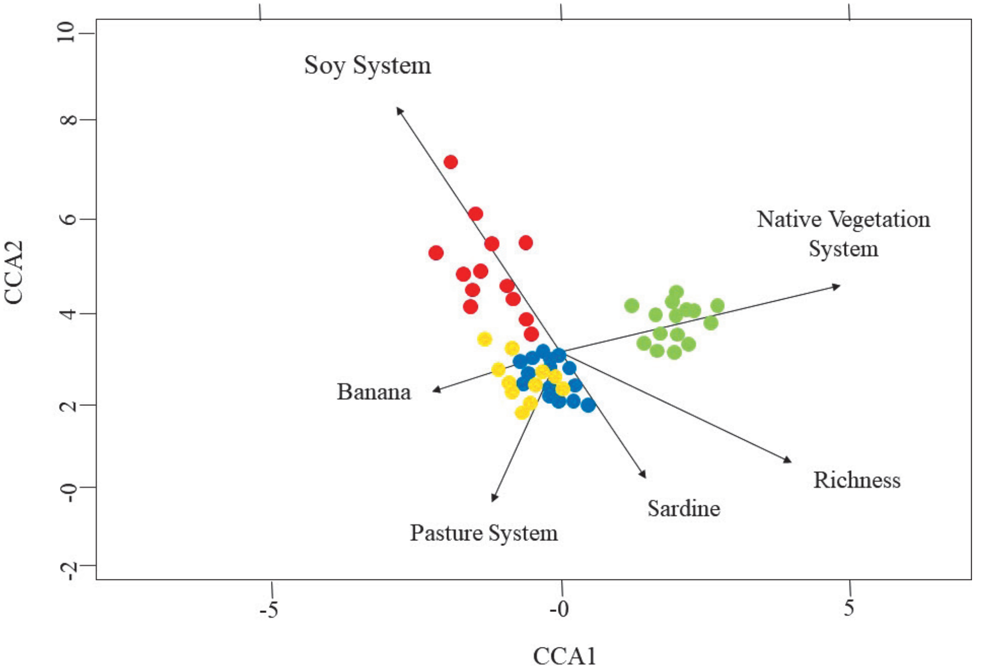

Figure 9. Canonical correspondence analysis that verifies the influence of variables on the composition of ant species, collected in the Rashad District between April 2020 and June 2021. The only significant variable in this analysis was the system in which the ants were collected ($F3.57 = 2.7151$, $p < 0.001$). (red) soy monoculture, (blue) pasture, (yellow) organic agriculture and (green) native vegetation. The position of the species in this image has been suppressed to facilitate data visualization.

addition to explaining Tobin's ant-biomass paradox (1994), he argues that the ant biomass in some ecosystems is so great that only the feeding via predation of other animals cannot explain the number of ants observed. However, it is also verified that, although studies show that ants have an important role in predation and seed dispersal (Alba-Lynn and Henk 2010), these seem to be relatively low food. These were confirmed even though it has captured species that consume seeds, such as *Leptogenys crustosa* (Campbell et al. 2014) predominantly. The behavior of biomass removal from unique resources is another factor that can influence the biomass removal rate. The sardine bait was removed by loading the pieces individually or in groups when they were huge. As for bananas and sardines, recruitment took place, usually with the monopolization of the resource, but its removal was always done individually, and the ants appeared to suck the banana rather than carry pieces between them. Seeds have neither recruitment nor monopolization, which is likely because this sort of resource in nature does not have an aggregate occurrence (and hence does not require recruitment) and is not a rare resource.

Biomass removal rates between habitats

There were no significant differences in the values removed from biomass between the different habitats for both banana and seed baits. For seed, this is probably because the consumption of this food is deficient compared to other types of food. In circumstances when the variations are minor, a method with better precision, such as counting the number of seeds, can be used to detect their biomass removals stated by Andersen and Sparling (1997). However, in our study, no factor explains the removal of seeds because, for 36 of the 67 locations analyzed, the removal of seeds was less than five grams. The values removed from bananas were independent of the type of habitat. However, considering that soy monocultures have practically no ants (in terms of individuals and species composition) compared to other habitats, it is irrational to assume that ants were responsible for this removal in the soy habitat. In addition, many of the soybean pests, especially caterpillars of the Noctuidae family, feed at night (Grinnan et al. 2013), so we suspect that there is a substitution effect for a species or group of species that perform a function similar to ants for removing banana biomass. There is also an assumption that cockroaches (Order: Blattodea)

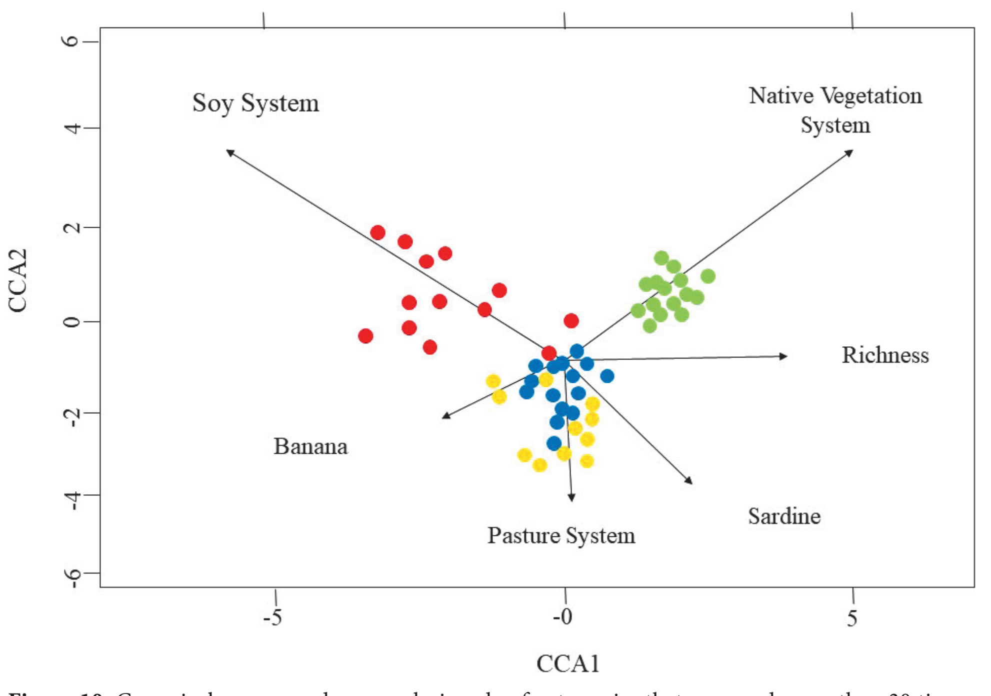

Figure 10. Canonical correspondence analysis only of ant species that occurred more than 30 times, which verifies the influence of variables on the composition of ant species collected in the Rashad District between April 2020 and June 2021. (red) soy monoculture, (blue) pasture, (yellow) organic agriculture and (green) native vegetation. The significant variables in this analysis were the system in which the ants were collected ($F3.57 = 7.7406$, $p < 0.001$) and the richness ($F8.57 = 1.5914$, $p = 0.044$).

may be a significant consumer of banana baits in soybean areas (Cheng et al. 2016). However, as no invertebrate was captured to justify this claim since few flies, moths, and grasshoppers were found at the end of the experiment within the biomass removal stations in soybeans, this assumption is only a hypothesis which is matching to the findings presented by Fischer and Larson (2019), who claim that, in the absence of a species or group of species that perform a specific ecosystem function, a second species or group of species can perform that function.

Influence of ant richness and fauna biomass

As discussed, soy is possibly a habitat where another group of invertebrates has carried out banana biomass removal. When soy is excluded from the correlation matrix (Fig. 7), it is observed that the richness of ants ($r = 0.32$) correlates with the biomass removed from the banana. The same pattern, only more robust, was also found for the biomass removed from sardines (Fig. 6). The data also show that this ant richness is positively correlated with ant biomass (Fig. 5). Three hypotheses explain why these factors affect the function of biomass removal in the ecosystem. First, about ant richness, the hypothesis of the sampling effect is suggested, which predicts that the greater the number of species, the more likely it is that any of these will play an important role in this function (Sobrinho and Schoereder 2007; Kwon 2014). There is also the hypothesis of complementarity, which implies that the quantitative role of each new species found increases the function related to them by the same proportion (Graham et al. 2009). However, Dauber and Wolters (2004) argue that both the sampling effect and the complementarity are not mutually exclusive. Therefore, these two hypotheses are likely to affect the removal of biomass at the same time. Finally, in terms of ant biomass, there is the hypothesis of more individuals (Sanders et al. 2003), which states that the more resource available, the more consumers there are (in this case, influencing the biomass removed). In similar studies, Abbott (2005) found a positive relationship between ant richness and biomass removal rate; whereas Cannicci et al. (2008) found no influence of wealth or recruitment of ants on bait removal rates but the amount of bait that the ants could transport affected the biomass removal values.

Influence of species composition

The correlations found were moderate to weak, with $r <0.7$, often values characteristic of experiments carried out in actual conditions, but which can also show the potential existence of other explanatory variables. One of these potential variables, which increases the error, is probably the ant species' identity that performs this biomass removal function. Grodsky et al. (2018); Karri et al. (2020), have addressed this issue of identity according to which the identity of the species has great relevance to determining the levels of predation. Similarly, although canonical correspondence analysis indicates that only the type of habitat or species richness is related to the composition of the ant assembly, it is undeniable that some species may play a role in removing biomass better than others remove. As an example of this, we can mention the species of the genus *Platythyrea*, all small size (with an average of 1.25 mm) that, regardless of the colony's size, could hardly remove significant quantities of the baits. However, ants of the genus *Strumigenys* (with an average of 130 mm) can remove relatively large pieces of bait at once and potentially significantly influence the total biomass removed. Thus, among these ants important to highlight the role of intermediate-sized ones, which have the behavior of recruiting, being able to remove large quantities of the baits due to the number of individuals working together, such as some species of the genus *Pheidole, Tetramorium*. The three most frequently recorded ant species in the biomass removal stations after 24 hours in the field were *Aenictus anceps*, *Anochetus traegaordhi,* and *Aenictus soudanicus* (see Supplementary File 1). In addition, these baits were found in sporadic events by ants, including *Platythyrea*, *Neivamyrmex,* and *Nesomyrmex*, which are nomadic species (without fixed colonies) that can have relatively large colonies (Sobrinho and Schoereder 2007). As a result of events, two locations NV-18 point and the NV-21 point -had to be excluded from statistical analysis - because the occurrence of correction of *Platythyrea cribrinodis* was so great that the dry mass of ants caught at each of these points was over 7.5 times greater than the third point with the most ants. This led to the total removal of sardine biomass at these specific points, around 237g and 224g.

Conclusions

The results revealed that the composition of ant species had no effect on the removed biomasses.

The ant richness is correlated with the removal of sardine and banana biomass, demonstrating that the effects on ecosystem function are dependent on the particularities of each assessed function such as resource type, the kind of different land uses, and the ant's species richness and species composition in the ecosystem. Biomass removed was highest in sardines and bananas and removed lowest in the seed. Only the soybean regions showed the lowest levels of sardine removed, indicating that there is an effect associated with the kind of land use. Further studies are needed to identify the rates of biomass removal by ants from unique resources between native and agricultural ecosystems.

Based on our findings, we suggest that future studies should examine species-specific abundance same as previously demonstrated that the abundance of *Platythyrea cribrinodis* has a significant impact on biomass removal. However, this species has also been captured in other locations. Most likely, due to a weak abundance of individuals in these locations, there has been no removal as intense as that sampled in the NV-18 and NV-21.

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