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Research article

Vinasse and Its Influence on Ant (Hymenoptera: Formicidae) Communities in Sugarcane Crops

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Abstract

Sugarcane is an important crop within the Brazilian socioeconomic landscape. There is a constant need for approaches to increase sustainability at all steps of the production chain. Irrigating sugarcane crops with vinasse is one of these approaches, because vinasse is a residue of sugarcane processing that can be used to fertilize these same crops. However, due to its chemical properties, vinasse may be harmful to soil fauna. Analyzing the structure and functional organization of ant communities is a fast and practical way to monitor sites affected by the addition of chemicals. This study compared the structure of soil ant communities in vinasse-irrigated sugarcane crops to those in secondary forests adjacent to the crops. In total, 32 genera and 107 species of ants were observed; of these, 30 species foraged in crop fields and 102 foraged in forests. Twenty-five percent of the species were present in both crops and forests. Ant communities in crop soil had poorer taxonomic composition and lower richness in each functional group compared to communities in forest remnants. However, regardless of vegetation type, epigeic ants were more diverse, and *Dorymyrmex brunneus* (crop) and *Pachycondyla striata* (forest) were very frequent. Vinasse did not increase the diversity of epigeic and hypogeic ants, but it may affect the community composition.

Key words: agriculture, beneficial arthropod, biodiversity, community ecology

The environment is a leading source of concern around the world, with an increasing demand for alternative energy sources. One of these options is ethanol from sugarcane (*Saccharum* spp.), a renewable source of energy. However, biomass distillation generates by-products, among those vinasse. In Brazil, approximately 280 billion liters of vinasse were generated in 2014, and 97% of this amount was used for land cultivation. This practice, when overused, has a negative environmental impact, because vinasse is an organic substance rich in potassium, calcium, and magnesium. However, vinasse is a low-cost fertilization alternative because it replaces fertilizers and promotes crop productivity by improving the soil structure (Silva et al. 2014).

In general, conventional farming practices cause numerous changes in the composition and diversity of soil fauna. Farming often reduces the number of species and changes the population structure of some groups (Barros et al. 2003), particularly ants. Ants are part of the soil macrofauna, and are the main contributors to soil invertebrate communities (Risch and Jurgensen 2008, Decaëns 2010), with a positive effect on soil ecosystem structure. As ecosystem engineers (Folgarait 1998, Sanders and Van Veen 2011), ants change the structural and chemical properties of the soil by building galleries that help incorporate organic matter and maintain soil porosity, drainage, and aeration and volume of organic matter (Hole 1981, Lavelle et al. 1997, Johnson 2001).

The addition of chemical substances to the soil may affect soil fauna. These impacts can be assessed through analyses of biochemical, physiological, behavioral, and metabolic changes in individuals and/or their lifecycle (bottom-up effects). Alternatively, the effects may be evaluated through changes in the structural and functional organization of communities or ecosystems (top-down effects). Top-down effects are a faster and more practical way to monitor ecosystems (Buss et al. 2003). This study compares the richness and composition of ant species in the soil of vinasse-fertilized sugarcane crops to communities in secondary forests adjacent to the crops. Given the fertilizing attributes of vinasse, we expected that communities of epigeic and hypogeic ants would be similar in the soils of crop fields and adjacent forests, because vinasse application creates the conditions for richer soil communities (Pasqualin et al. 2012).

Materials and Methods

Study Site

The study was conducted in rural areas of the state of São Paulo, southeast Brazil, in seven municipalities: Analândia (22° 06′ 15.3″

S, 47° 45′ 42″ W; elevation 804 m), Araras (22° 19′ 44.7″ S, 47° 24′ 09.8″ W, 700 m), Corumbataí (22° 14′ 34.1″ S, 47° 41′ 07.9″ W, 815 m), Ipeúna (22° 27′ 31.1″ S, 47° 41′ 10.3″ W, 611 m), Leme (22° 11′ 21″ S, 47° 23′ 53″ W, 208 m), Pirassununga (21° 56′ 42.5″ S, 47° 19′ 49.6″ W, 598 m), and Rio Claro (22° 24′ 28″ S, 47° 34′ 11″ W, 592 m).

The region's climate is classified as Cwa by Köppen's system, with hot and wet summers and cold and dry winters (Cardoso-Leite et al. 2004). Samples were collected three times, in October 2013, June 2014, and October 2014, for a total of 18 sites. Of these, nine plots were in sugarcane crops, where vinasse had routinely been used for more than 20 years to prepare the soil, and the other nine were in fragments of seasonal semi-deciduous forest. This type of forest occupies transition areas between the wet coastal ecosystem and the semiarid region (Leitão Filho 1987).

Sampling was conducted in sugarcane fields 5 months after the sprouting phase, in areas where straw covered the soil surface. All sites (n = 18; 9 in secondary semi-deciduous Atlantic forest and 9 in vinasse-fertilized sugarcane crops) had been treated with agrochemical products and herbicides recommended for traditional sugarcane cultivation (Townsend 2000). Because vinasse composition depends on the manufacturing process (Cabello et al. 2009), we selected sugarcane crops that had been fertilized with vinasse manufactured in the same plant. Therefore, the chemical composition of the vinasse was the same in all crops.

Sampling Design

Ants were collected along a 200-m linear transect in each studied site, using 2 techniques. Species foraging on the soil surface (epigeic) were collected using pitfall traps (n = 10), which were left in the field for 48 h. Ants foraging below the soil surface (hypogeic) were collected from 1 kg of soil removed with a spade to a depth of 20 cm (n = 10) along a transect parallel to the first one. This material was placed in modified Berlese–Tüllgren funnels, where it remained for 120 h (Rodrigues et al. 2008). All ants were sorted and separated

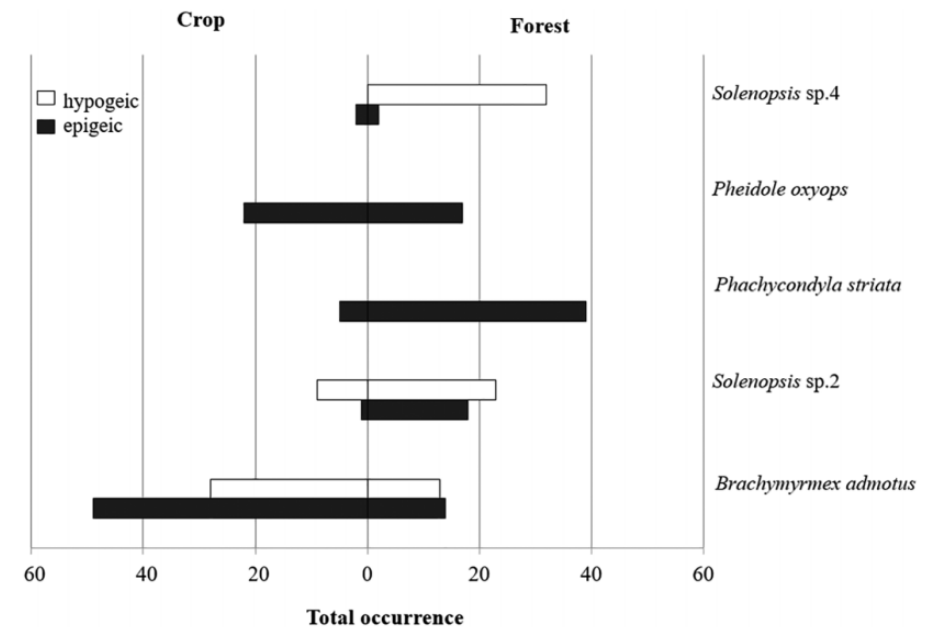

Fig. 1. Most frequent species shared between sugarcane crops and forest remnants, separated by foraging stratum.

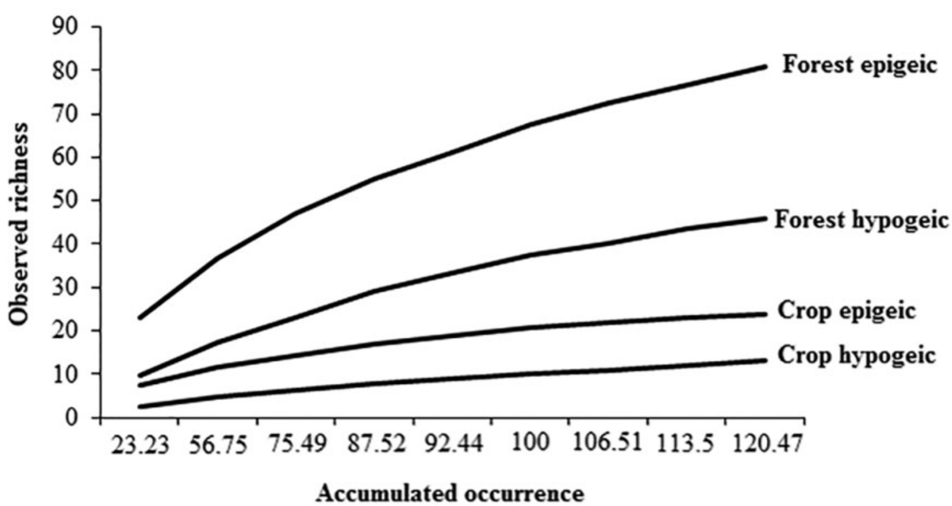

Fig. 2. Species accumulation curves showing the number of epigeic and hypogeic species collected from vinasse-fertilized sugarcane crops and forest remnants.

into subfamilies (Brady et al. 2014) and identified to the genus level (Baccaro et al. 2015). Species and morphospecies were identified according to Suguituru et al. (2015). Voucher specimens were deposited at Universidade de Mogi das Cruzes, in São Paulo, Brazil.

Data Analysis

Accumulation curves were generated based on the occurrence of species (presence–absence data). The EstimateS 9.1 (Colwell 2013) software application was used to calculate Chao 2 estimators. A Mann–Whitney test was performed to detect differences between epigeic and hypogeic communities in the number of species, as well as differences in richness among feeding/foraging types. Patterns of species composition and community structure were compared using ordination analysis (non-metric multidimensional scaling, NMDS) based on a Bray–Curtis dissimilarity matrix derived from presence–absence data (Legendre and Legendre 1998). To check the difference in this composition was performed ANOSIM similarity test (Clarke, 1993).

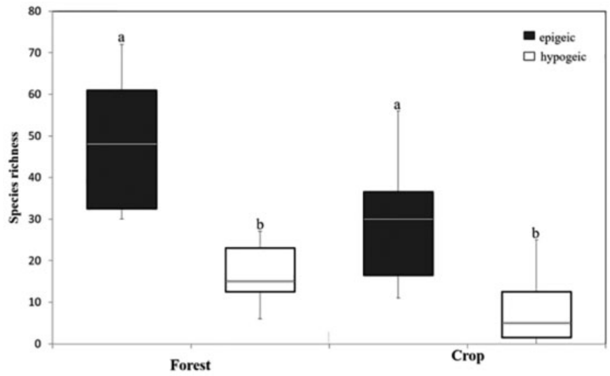

Fig. 3. Differences between epigeic and hypogeic ant species richness from forest remnants and vinasse-fertilized sugarcane crops. Lines inside boxes represent the median, lower quartile, and upper quartile; different letters indicate significant differences according to a Mann–Whitney test ($P < 0.05$).

The association of each species to each combination of foraging stratum and vegetation type (cluster) was determined using the indicator value (IndVal) method of Duffren and Legendre (1997), which expresses the fact that an indicator species is present in many sites of the cluster it indicates, in contrast to rare but exclusive species. This value is calculated for each species by multiplying a measure of specificity (A_{ij}) by a measure of fidelity (B_{ij}) to a given cluster, that is, $\text{IndVal}_{ij} = A_{ij} \times B_{ij} \times 100$, where i corresponds to a given species and j to a cluster. The value of A_{ij} is based on the average species abundance in habitat j, divided by the sum of the mean number of individuals in each cluster. The fidelity value (B_{ij}) is given by the ratio between the number of clusters in which the species occurs and the number of sites representing a cluster (Leivas and Carneiro 2012, González et al. 2013).

Indicator values ranged from 0 to 100% and were classified as followed: (1) between 50% and 70%, detector species; (2) above 70%, indicator species; and (3) 100%, species that indicates habitat exclusivity (Verdú et al. 2011). The significance of IndVal for

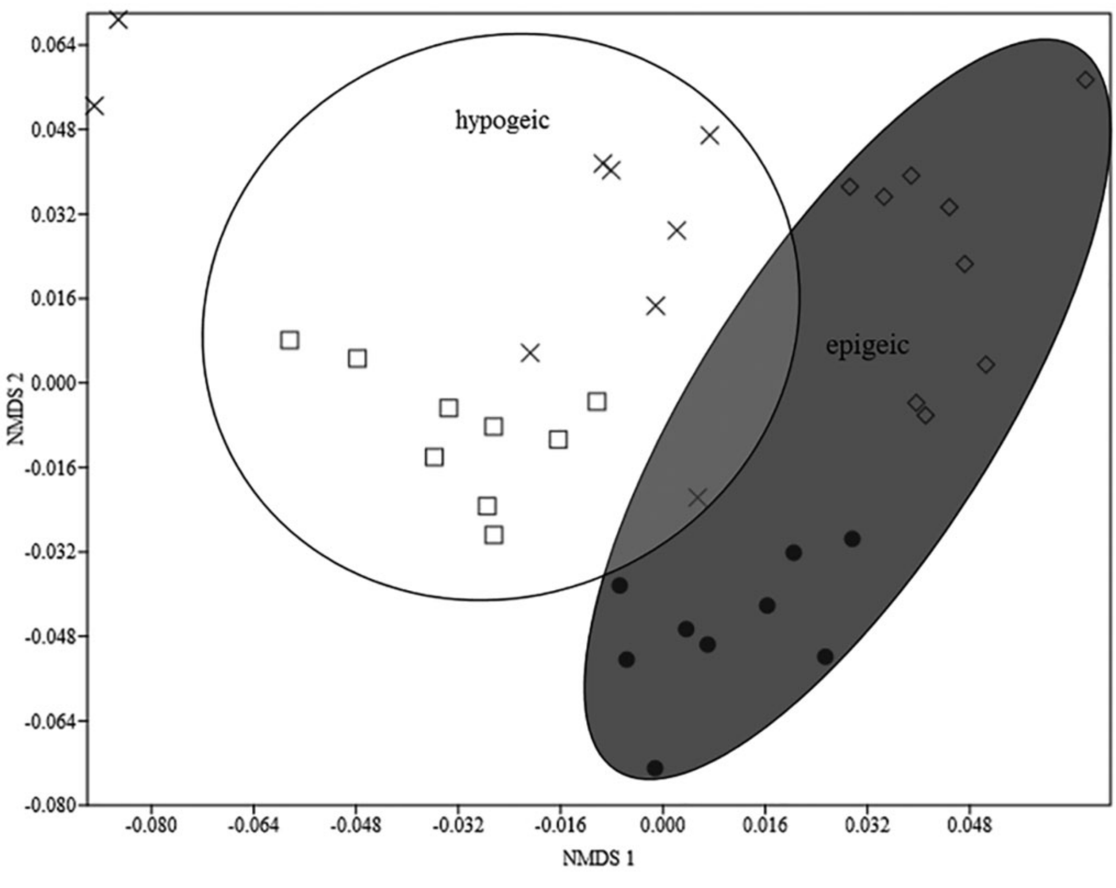

Fig. 4. Non-metric multidimensional scaling analysis (NMDS) of the structure of ant communities associated with the soil of forest remnants and sugarcane crops. Forest hypogeic Forest epigeic vinasse-fertilized sugarcane crops - hypogeic vinasse-fertilized sugarcane crops - epigeic; Stress: 0.3141.

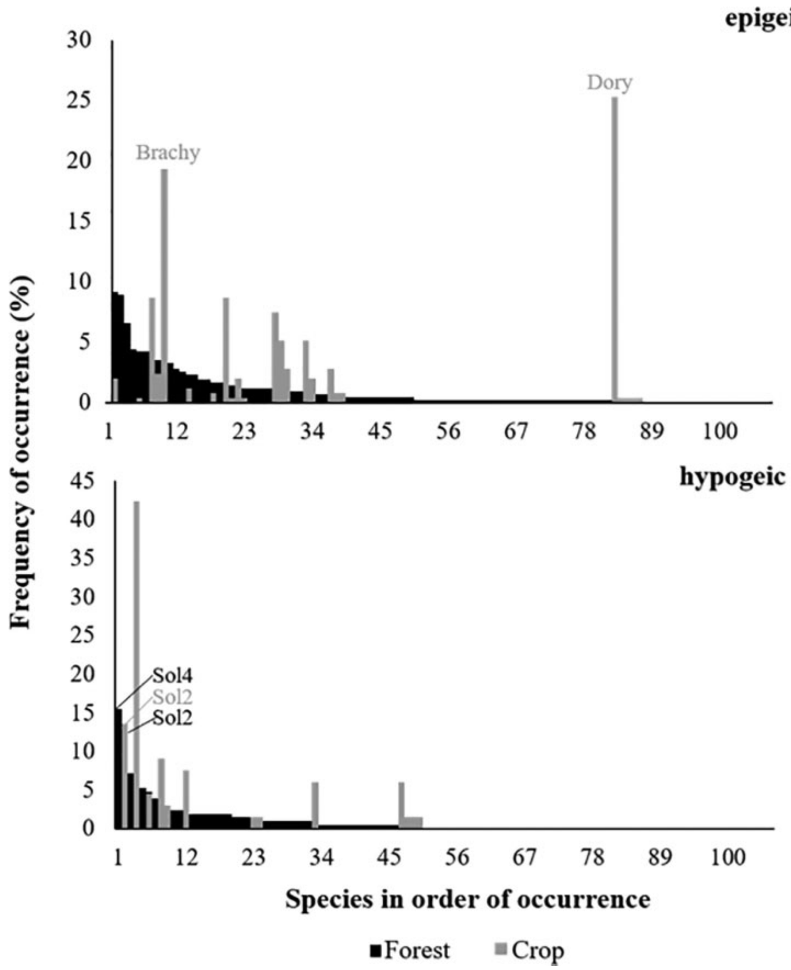

Fig. 5. Frequency of epigeic (A) and hypogeic (B) ants. Each bar represents a different species. The most frequent species in each foraging stratum are indicated in the figure. Brachy = *Brachymyrmex admotus*, Pach = *Pachycondyla striata*, Ca5 = *Camponotus* sp.5, Dory = *Dorymyrmex brunneus*, Sol4 = *Solenopsis* sp.4, Sol2 = *Solenopsis* sp.2. Note the different scales in the y axes.

each species was evaluated using Monte Carlo randomization tests (9 and 99 interactions and significance level = 0.01). Classification into feeding and foraging types followed Weiser and Kaspari (2006).

Results

In total, we found 32 genera and 107 species of ants (Supplementary material); 102 species (range 16–43) foraged in the forests, and 30 species (range 2–21) foraged in vinasse-fertilized sugarcane crops. Forest remnants had 25% ant species in common with crops. Of these, *Solenopsis* sp. 4, *Pheidole oxyops* Forel, 1908, *Pachycondyla striata* Smith, 1858, *Solenopis* sp. 2, and *Brachymyrmex admotus* Mayr, 1887 were the most frequent (Fig. 1).

Seventy-seven species were recorded exclusively in the forest; *Camponotus* sp. 5, *Wasmannia auropunctata* (Roger, 1863), *Pheidole* sp. 20, *Pheidole* sp. 45, and *Hypoponera* sp. 5 were the most frequent species. Among vinasse-irrigated sugarcane crops, only five exclusive species were recorded: *Anochetus neglectus* Emery, 1894; *Dorymyrmex brunneus* Forel, 1908; *P. marginata* (Roger, 1861); *P. subarmata* Mayr, 1884; and *Pheidole* sp. 43.

Eighty-one epigeic and 46 hypogeic species were found in forest remnants, whereas in crops there were 24 epigeic and 13 hypogeic species (Fig. 2). Based on Chao 2, however, we estimated that there were 120, 66, 27, and 21 species, respectively (Fig. 2). Species richness was higher in forest remnants compared with crops for both epigeic ($U = 14.5$, $Z = 2.2959$, $P < 0.05$) and hypogeic species ($U = 14.0$, $Z = 2.34$, $P < 0.05$; Fig. 3).

Species composition was vertically stratified between epigeic and hypogeic species in both forests and crop fields. Similarity was greater between communities in the same foraging stratum, regardless of vegetation type (ANOSIM: $R = 0.677$, $P = 0.001$) (Fig. 4). The surface of the soil was richer, and the most frequent species in the epigeic stratum were *P. striata* (forest) and *D. brunneus* (crop) (Fig. 5). In the hypogeic stratum, the most frequent species in forests and crops were *Solenopsis* sp. 4 and *B. admotus* (Fig. 5), respectively. All of these species were strongly associated with either forests or crops; *P. striata* was an indicator species of forests and *D. brunneus* was exclusive to and an indicator species of sugarcane crops (Table 1).

There were seven feeding/foraging types in forests and crop fields. The richness of omnivore/unknown, unknown/litter, and unknown/surface species did not differ between forest remnants and vinasse-fertilized sugarcane crops. However, richness differed between epigeic [fungivore/surface ($U = 19$, $Z = 1.8985$, $P < 0.05$), omnivore/surface ($U = 4$, $Z = 3.2230$, $P < 0.05$), and predator/litter ($U = 8$, $Z = 2.8698$, $P < 0.05$)] and hypogeic communities

Table 1. Species indicator values (IndVal) for each combination of vegetation type and foraging stratum.

Species	IndVal (%)	Forest		Crop		*P*	Type of indicator
		Epigeic	Hypogeic	Epigeic	Hypogeic		
Dorymyrmex brunneus	100	0/0	0/0	**64/9**	0/0	0.001	Exclusive
Pachycondyla striata	98.6	**39/9**	0/0	5/2	0/0	0.001	Indicator
Camponotus sp. 5	88.9	**38/8**	3/1	0/0	0/0	0.001	Indicator
Solenopsis sp. 3	88.9	**14/8**	10/3	0/0	3/1	0.001	Indicator
Solenopsis sp. 2	87.1/56.6	**19/8**	23/7	1/1	**9/2**	0.001	Indicator/Detector[a]
Brachymyrmex admotus	80.1/71.6	**63/7**	13/3	49/9	**28/7**	0.018	Indicator
Odontomachus chelifer	77.8	**10/7**	0/0	0/0	0/0	0.005	Indicator
Pheidole sp. 45	77.8	**19/7**	3/1	0/0	0/0	0.002	Indicator
Solenopsis sp. 4	77.8	4/2	**9/2**	2/2	0/0	0.004	Indicator
Ectatomma edentatum	67.2	**21/7**	0/0	5/2	0/0	0.026	Detector
Pheidole pr. *aper*	66.7	**8/6**	0/0	0/0	0/0	0.010	Detector
Pheidole sp. 20	66.7	**28/6**	0/0	0/0	0/0	0.010	Detector
Cyphomyrmex gr. *strigatus*	55.6	**5/5**	0/0	0/0	0/0	0.030	Detector

Numbers in forest and sugarcane columns correspond to total sample size and number of sites where the species was recorded, respectively. Values in bold indicate the vegetation types with which the species is significantly associated. *P* values show the significance of the indicator values.

[a]Indicator species for epigeic forest and detector for hypogeic in vinasse-fertilized sugarcane crops.

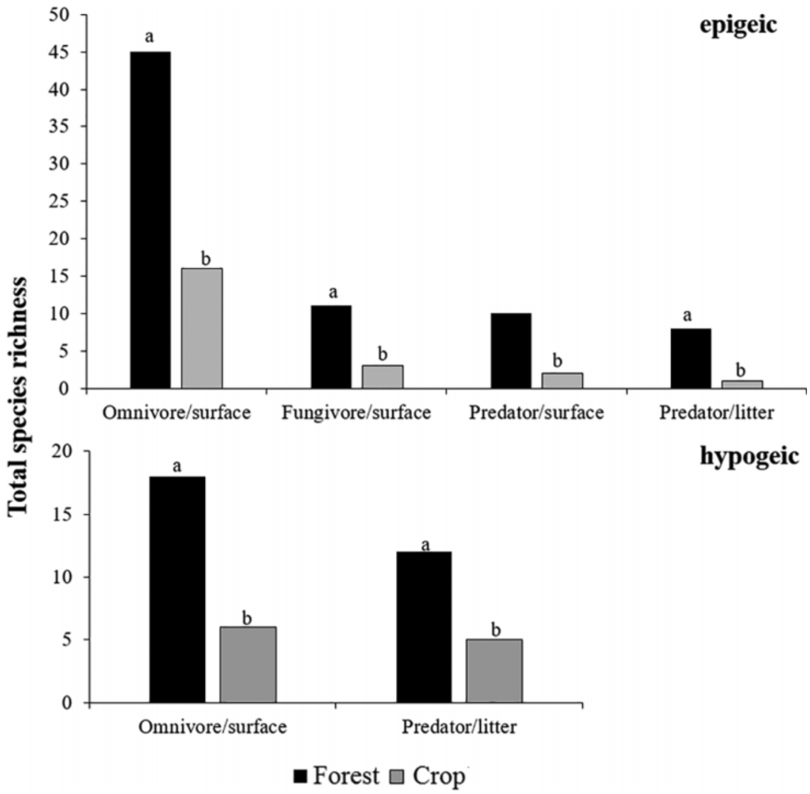

Fig. 6. Total species richness according to feeding/foraging type, vegetation type (forest or Crop) and foraging stratum (epigeic or hypogeic). Groups with the same letters are not significantly different according to the Mann–Whitney test ($P < 0.05$).

[omnivore/surface ($U = 13$, $Z = 2.4283$, $P < 0.05$) and predator/litter ($U = 13.5$, $Z = 2.3842$, $P < 0.05$)]. Omnivore/surface ants were dominant in both forests and crops (Fig. 6). *Solenopsis* spp. and *Pheidole* spp. contributed to dominance in forests, and *B. admotus* and *D. brunneus* contributed to dominance in crop fields. There were more predatory ant species in forest sites, but *Ectatomma edentatum* (Olivier 1792), *Strumigenys denticulata* Mayr 1887, *S. subedentata* Mayr 1887, and *S. eggersi* (Emery 1890) had never been recorded in crop fields before this study.

Discussion

Vinasse increases soil fertility (Canellas et al. 2003, Silva et al. 2006) and benefits the soil fauna (Pasqualin et al. 2012) and microbial communities that contribute to decomposition (Ishizaki et al. 2014). In this study, however, the soil of vinasse-fertilized sugarcane crops had poorer taxonomic composition and lower richness of ant species in each functional group than the soil of forest remnants in adjacent areas. Despite that, ant richness in crops was comparable to other rural landscapes, which are open and have sparse vegetation (Braga et al. 2010, Pacheco et al. 2013). Ant community richness increases with heterogeneity of vegetation cover and soil litter (Silva et al. 2011). Heterogeneous areas provide different resources for foraging and nesting, both essential for ant diversity (Fowler et al. 1991, Vasconcelos 2008, Silva et al. 2011).

Aside from the homogeneous landscape of farming ecosystems, the use of agrochemicals and herbicides contributes to the loss of specialist species (Ekroos et al. 2010) while increasing the abundance of generalist species, such as *D. brunneus* and *B. admotus*. *Dorymyrmex* species are very competitive in disturbed (Hölldobler and Wilson 1990, Andersen 1997, Majer and Nichols 1998) and open (Cuezzo and Guerrero 2012) environments, which explains why they were more frequent in crop fields than in forest remnants.

There are no published reports on *B. admotus* in sugarcane crops, but this species is frequently found in urban areas (Suguituru et al. 2015). In contrast, *D. brunneus* is abundant in sugarcane fields (Souza et al. 2010). This species is a predator of the sugarcane borer *Diatraea saccharalis* Fabr., 1794, a pest that is difficult to control in cultivated fields (Rossi and Fowler 2004). Frequently observed ant genera (*e.g.*, *Solenopsis* and *Pheidole*) also include species that are important control agents of sugarcane pests, such as spittlebugs (*Mahanarva* spp.) and stem borers (Guzzo and Negrisoli 2012).

Irrigating in the soil with vinasse is a sustainable and low-cost management solution. However, vinasse application did not increase the diversity of ant species that control pests, since the same *Solenopsis* and *Pheidole* species were observed in the forest. In addition, these species were more strongly associated with the forest than with the crop fields. Not even the high frequency of *D. brunneus* can be explained by vinasse fertilization, because this species is very common in sugarcane soil (Souza et al. 2010) and other types of crops (Pacheco et al. 2013). Nevertheless, other predatory species may benefit from vinasse irrigation. These species include *S. denticulata*, *S. subedentata*, and *S. eggeersi*, which feed on springtails and are primarily found in soil litter (Brandão et al. 2009). These microarthropods are particularly abundant in vinasse-fertilized sugarcane soils (Pasqualin et al. 2012), which have a higher pH (Laime et al. 2011) that favors some species of springtails (Sautter and Santos 1994, Ponge et al. 2003).

In both forest remnants and sugarcane crops, the ant communities on the soil surface (epigeic) are not the same as those in the soil (hypogeic). In tropical forests, a strong vertical stratification is observed between these types of fauna (Fowler et al. 2000; Andersen and Brault 2010), and epigeic communities are richer (Vasconcelos 2008). These data are supported by the observations of this study in crop fields.

Overall, sugarcane crops had a negative impact on the soil ant fauna, as reported for other crops (Pacheco et al. 2013), and the use of vinasse in the soil did not increase species richness. However, given the observed similarities between the soils of forests and crops, vinasse irrigation may influence ant faunal composition in each foraging stratum.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

Acknowledgments

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