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

L. P. Saad, D. R. Souza-Campana, O. C. Bueno, and M. S. C. Morini

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

Materials and Methods

Study Site

The study was conducted in rural areas of the state of São Paulo, southeast Brazil, in seven municipalities: Análândia (22° 06’ 15.3’’S, 43° 16’ 48.8’’W), Itu (22° 41’ 41.6’’S, 44° 15’ 31.5’’W), Mogi das Cruzes (22° 52’ 41.0’’S, 45° 59’ 40.3’’W), Mogi Mirim (22° 59’ 38.3’’S, 46° 11’ 16.9’’W), São José dos Campos (22° 59’ 14.0’’S, 46° 12’ 17.7’’W), São Carlos (22° 54’ 40.8’’S, 45° 41’ 15.4’’W), and Sertãozinho (22° 48’ 49.9’’S, 44° 58’ 16.0’’W). The study area is part of the São Paulo State Atlantic Forest Reserve and is highly diversified by altitude, topography, and altitude variation. Seven sugarcane fields (Hole 1981, Lavelle et al. 1997, Johnson 2001).
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–Tullgren funnels, where it remained for 120 h (Rodrigues et al. 2008). All ants were sorted and separated...
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).

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. 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 ($P < 0.05$).

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.
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

---

**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.
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 that in forests.
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 D. brunnifer (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 Negrissoli 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. eggeeri, which feed on springtails (Mahanarva spp.) and enter in the soil (Brandão et al. 2009). These microarthropods are particularly abundant in vinasse-fertilized sugarcane soils (Pasquali et al. 2012), which have a higher pH (Laime et al. 2012). In both forest remnants and sugarcane crops, 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 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**

The authors would like to thank FAPESP (grant Nos 2012/50197-2 and 2014/50280-2) and CAPES for the financial support, and Universidade Federal de São Carlos (Araras Campus) and Fazenda da Aeronáutica de Pirassununga for giving us permission to collect samples in their properties.

**References Cited**

Andersen, A. N. 1997. Using ants as bioindicators: multiscale issues in ant community ecology. Conserv. Ecol. 1: 8.

Andersen, A. N., and A. Brault. 2010. Exploring a new biodiversity frontier: subtropical ants in northern Australia. Biodivers. Conserv. 19: 2741–2750.

Baccaro, F. B., R. M. Feitosa, F. Fernández, I. O. Fernandes, T. J. P. Izoz, and R. R. C. Solar. 2015. Guia para os gêneros de formigas do Brasil. Editora Inpa, Manaus, AM.

Barros, E., A. Neves, E. Blanchart, E. C. M. Fernandes, E. Wandelli, and P. Lavelle. 2003. Development of the soil macrofauna community under silvopastoral and agrosilvicultural systems in Amazonia. Pedobiologia 47: 273–280.

Bradly, S. G., B. L. Fisher, T. R. Schultz, and P. S. Ward. 2014. The rise of army ants and their relatives: diversification of specialized predatory Doryline ants. Evol. Biol. 14: 93.

Braga, D., J. N. Louzada, R. Zanetti, and J. H. C. Delabie. 2010. Avaliação rápida da diversidade de formigas em sistemas de uso do solo no sul da Bahia. Neotrop. Entomol. 39: 464–469.

Brandão, C. R. F., R. R. Silva, and J. H. C. Delabie. 2009. Formigas (Hymenoptera). In A. R., Pazzini and J. R. P. Parra (eds.), Biocologia e nutrição de insetos: Base para manejo integrado de pragas. Embrapa Informação Tecnológica, Brasília, DF.

Buss, D. F., D. F. Baptista, and J. L. Nessimian. 2003. Bases conceituais para a aplicação de biomonitoramento em programas de avaliação da qualidade da água de rios. Cad. Saúde Pública. 19: 465–473.

Cardoso-Leite, E., T. B. Corve, R. G. Ometto, D. C. Cavalcanti, and M. I. Paganini. 2004. Fitosociologia e caracterização sucessional de um fragmento de mata ciliar, em Rio Claros/SP, como subsídio à recuperação da área. Rev. Inst. Flor. 16: 31–41.

Cabello, P. E., F. P. Scognamiglio, and F. J. C. Terán. 2009. Tratamento de vinhaça em reator anaeróbio de leito fluidizado. Eng. Ambiental. 6: 321–338.

Canellas, L. P., A. C. X. Velloso, C. R. Marciano, J. F. G. P. Ramalho, V. M. Rumpianek, C. E. Rezende, and G. D. A. Santos. 2003. Propriedades químicas de um camissolo cultivado com cana-de-açúcar, com preservação do palhão e adição de vinhaça por longo tempo. Rev. Bras. Ciênc. Solo. 27: 935–944.

Clarke, K. R. 1993. Non-parametric multivariate analysis of changes in community structure. Aust. J. Ecol. 18: 117–143.

Colwell, R. K. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9.1 User’s Guide and application.

Cuezzo, F., and R. J. Guererro. 2012. The ant genus Dorymyrmex Mayr (Hymenoptera: Formicidae: Dolichoderinae) in Colombia. Psyche J. Entom. 2012: 1–24.

Decaens, T. 2010. Macroecological patterns in soil communities. Global Ecol. Biogeogr. 19: 287–302.

Duffren, M., and P. Legendre. 1997. Species assemblages and indicator species: the need for flexible asymmetrical approach. Ecol. Monogr. 67: 345–366.

Ekroos, J., J. Helioila, and M. Kuuasari. 2010. Homogenization of lepidopteran communities in intensively cultivated agricultural landscapes. J. Appl. Ecol. 47: 439–467.

Folgarait, P. J. 1998. Ant biodiversity and its relationship to ecosystem functioning: a review. Biodivers. Conserv. 7: 1221–1244.

Fowler, H. G., L. C. Forti, C. R. F. Brandão, J. H. C. Delabie, and H. L. Vasconcelos. 1991. Ecologia nutricional de formigas. In A. R. Panizzi and J.R.P. Parra (eds.), Ecologia nutricional de insetos e suas implicacões no manejo de pragas. São Paulo, SP.

Fowler, H. G., J. H. C. Delabie, and P. R. S. Moutinho. 2000. Hypogaec and epigaec ant (Hymenoptera: Formicidae) assemblages of Atlantic coastal...
