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Complexity of cacao production systems affects terrestrial ant assemblages

Chloé Durot^{a,†}, Miguel Limachi^{b,†}, Kazuya Naoki^b, Marc Cotter^a, Natacha Bodenhausen^a,
Luis Marconi^{b,c}, Laura Armengot^{a,d,*}

^a FiBL Research Institute of Organic Agriculture, Frick, Switzerland

^b Instituto de Ecología, Carrera de Biología, Universidad Mayor de San Andrés, La Paz, Bolivia

^c Herbario Nacional de Bolivia, La Paz, Bolivia

^d BETA Technological Center, Vic, Spain



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ABSTRACT

Given the rapid changes in tropical agricultural production, the evaluation of different management practices has gained interest to determine the effects of land-use change on biodiversity. The conversion of forests into agricultural land is one of the main drivers of diversity loss. Agroforestry systems have been shown as a promising option to provide suitable yields in addition to conserving biodiversity. In this study we compared species richness and community composition of terrestrial ants in six different systems in a long-term experimental site established in 2009 in Bolivia: a full-sun monoculture and an agroforestry system under conventional management, a full-sun monoculture and an agroforestry system under organic management, a highly diverse and dense agroforestry system without external inputs and a secondary forest. Using pitfall traps, we sampled ants four times during a seven-year period (2015–2021). We collected a total of 85 ant species belonging to 6 sub-families and 41 genera. More than 80% of the species were recorded in less than 10% of the traps. Species richness did not significantly differ between the systems. However, species composition mainly differed between the fallow and the production systems, and within the latter, it followed the management intensity gradient, i.e., complex agroforestry, agroforestry and monocultures. The indicator species analysis clearly showed species exclusively associated with one or more production systems, whereas others were only associated with the secondary forest. Species with specialised trophic roles were more frequent in forest and agroforestry systems. Our results showed that the disturbance generated by cultivation was the main driver differentiating ant communities, but also reinforced the importance of the complexity and management intensity of the production system. These results have strong implications for landscape management and highlights the importance of preserving natural patches of forest but also diverse and complex agroforestry systems within the agricultural matrix for ant diversity conservation.

Introduction

The conversion of forests into agricultural land is one of the main drivers of diversity loss (Rizali et al., 2020). This is especially true in Bolivia, where primary forests suffered a high conversion rate towards agricultural lands, causing further losses in their species richness and diversity (Boillat et al., 2022; Killeen et al., 2007). Cultivation of cacao (*Theobroma cacao* L.), a major global commodity in tropical areas of Latin America, West Africa and Indonesia (Akesse-Ransford et al., 2021; Kone et al., 2012), has largely contributed to deforestation (Kalischek et al., 2023). Moreover, the traditional cultivation of cacao beneath the

thinned canopy of primary or old secondary forests has been mainly replaced by full-sun monocultures or simple planted agroforestry systems to increase cacao yield at the short-term (Armengot et al., 2016), exacerbating biodiversity losses. However, agroforestry systems can widely vary in the number and diversity of tree species and combine diverse assemblages of crops and trees for food, wood or fuel (Niether et al., 2020; Haggard, 2019). Compared with monocultures, agroforestry systems can host higher diversity and abundance of wildlife, sometimes similar to forests (Nijmeijer et al., 2019; Bos et al., 2007) by acting as biodiversity reservoirs in fragmented landscapes, interconnecting forest fragments or acting as buffer zones nearby protected forest areas (Perry,

* Corresponding author.

E-mail addresses: laura.armengot@uvic.cat, laura.armengot@fibl.org (L. Armengot).

† Equal contribution

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2016; Philpott & Armbrrecht, 2006). Management intensification in cacao production due to intensive use of agrochemicals is also a threat to biodiversity (Daniel et al., 2018). Given the rapid changes in cacao plantations, the effect of different production systems has gained interest to determine the consequences of land-use change on biodiversity (Maney, 2022).

Arthropod communities can be used as indicators for assessing the effect of land disturbance or restoration (Ahuatzin, 2019; Perry et al., 2016; Solar et al., 2016). Among arthropods, ants have been widely recognized as relevant bioindicators for agricultural intensification and ecosystem disturbance (Rizali et al., 2020; Andersen, 2019; Solar et al., 2016). Ants are very diverse and abundant in most terrestrial environments of the planet (Folgarait, 1998) and play a role at all trophic levels. Ants provide provisioning (e.g., food), regulating (e.g., pollination), cultural (e.g., symbology) and supporting services (e.g., nutrient cycling) (del Toro et al., 2012), but also some disservices such as a decrease in the abundance of natural enemies (Anjos et al., 2022). The services (and disservices) that they provide are modulated by shade, i.e., pest control and plant protection are boosted in shaded crops compared to monocultures, which leads to increased yield (Anjos et al., 2022). Species richness of ant communities in managed systems has been found to be higher, lower or equal to that of forests (Narvaez-Vasquez et al., 2021; Perry et al., 2016; Kone et al., 2012). However, community composition usually differs between land uses (Martello, 2018). But up to now, most of the literature on ants compares forests with cacao plantations, either monocultures or agroforestry systems (e.g., Kone et al., 2012; Donald, 2004), but without considering the existing huge range of management intensity and complexity of the cacao production systems (Niether et al., 2020). Moreover, studies are usually limited to a very short time period. Donoso (2017) already highlighted the need for long-term studies to be able to capture changes in community structure over time.

In this sense, the long-term experimental site established in Bolivia in 2009 (<https://systems-comparison.fibl.org/project-sites/bolivia.html>) offers a unique opportunity to examine ant communities in a range of cacao production systems and a secondary forest over time, overcoming any potential confounding effect due to site-specific variations and managements that are difficult to control. The experimental site consists of six systems replicated four times: two full-sun monocultures differing in the management intensity (conventional farming vs organic farming), two agroforestry systems also under conventional and organic farming, one highly diverse and dense agroforestry system without external inputs and a secondary forest. A previous two-year study analyzing ant communities was performed in this experimental site shortly after its establishment (3–4 years), i.e., the systems compared were still young and not fully differentiated (Limachi et al., 2018). No marked differences were found in ant diversity and composition. Since age can influence ant diversity and dynamics (Kone et al., 2012), in the present study we analyse production systems entering the mature phase and over a longer time period, i.e., seven years, from 2015–2021. Ants were surveyed with pitfall traps and species richness and community composition were compared. This study aims to assess the effect of well-differentiated land-use systems on ant communities and bring some insights into the dynamics over time.

Materials and methods

Study area

The experiment was conducted at the long-term experimental site at the Sara Ana Research and Training center, located at Sara Ana (Alto Beni region), Department of La Paz, Bolivia (15°27'36" S - 67°28'17") between 2015 and 2021. The mean annual temperature of the area is 26.9 ± 0.4 °C, and the dry season extends from May to September, during which it rains less than 100 mm per month, while the average annual rainfall of 1645 ± 244 mm mostly accumulates between the

months of December and March.

The experimental site started in 2008 by clearing a twenty-year secondary forest characterized by *Guadua* spp., among other species. The experimental site consists of a complete randomized block design with 4 repetitions (Appendix A: Fig. 1). Six treatments are compared: (1) conventional monoculture (CM), (2) organic monoculture (OM), (3) conventional agroforestry (CA), (4) organic agroforestry (OA), (5) successional agroforestry system (SA) and (6) secondary forest (F). The plot size is 48 m × 48 m. Cacao trees grow in a 4 m × 4 m grid (625 trees ha⁻¹). Twelve different cacao varieties were grown in each plot, i.e., 4 international clones (TSH565, ICS1, ICS6, ICS95), 4 locally selected clones (IIa22, IIa58, III13, III6) and 4 full-sib families from the crosses of the international clones with IMC67. Due to the low yield of the full-sib families, they were grafted with local genetic material in 2017. Table 1 describes the main differences between the six systems.

Data collection

Five pitfall traps were installed, arranged equidistantly in a cross shape keeping 10 m apart in the inner 20 m × 20 m area of each plot (Limachi et al., 2018) (Appendix A: Fig. 1). For each pitfall trap, beakers were filled ¾ full with alcohol (70%) and a drop of detergent. They were buried in the ground keeping the top of the trap at the soil level for ground-dwelling insects to fall into. Samplings were conducted once a year in 2015, 2017, 2019 and 2021 in all systems in January (but in December in 2019). We always sampled during the rainy season to have comparable data between years and to maximize the potential number of species collected (Rizali et al., 2013).

The traps were left in the plots 24 h. The samples were processed in the laboratory (cleaning, separation, mounting, identification and labelling), and were all stored at the Bolivian Fauna Collection. A total of 480 traps were collected over the entire period. Identification of ants was conducted using the determination keys from Lattke (2004) and Fernández and Palacio (2006). A 50 × Wild stereo microscope was used to identify the individuals at the level of subfamilies and genera and they were assigned to morphotypes (hereafter referred as species) according to morphological parameters of each genus, which are described in Lattke (2000) and Fernandez (2003).

Statistical analysis

Analyses were carried out with the R software version 4.2.2 (R Development Core Team, 2022). We used the *vegan* package for calculating the diversity indices and the rarefaction curves ('rarecurve' function). The *glmer* function and log-likelihood ratio tests (*lme4* package) were used to calculate Chi² and *P*-values of the mixed effects models. Finally the *multicomp* and *indicspecies* packages were used for the post-hoc tests (*cld* function) and for the indicator species analysis, respectively.

Species richness

Data from the five traps of each plot were pooled and species richness was calculated as the total number of species found per plot and per year. Rarefaction curves (Coleman method) were drawn per system. The effect of the systems on ant species richness was tested using generalized linear mixed effects models with the Poisson distribution, including system, year and their interaction as fixed factors and plot nested in block as random factors. The frequency of appearance of each species was calculated by summing the total number of traps where a certain species was registered and dividing by the total number of traps (480).

Species composition

Species frequency per plot was used to compare ant species composition between systems and over the years, regardless the number of

Table 1

Description of the vegetation and the main management practices of the six systems compared.

	Shade tree and light*	Vegetation	Management
CM	0 tree ha ⁻¹ Full sun LT: 92%	Only herbaceous strata. Predominance of grass species and generalist species resistant to glyphosate (Marconi & Armengot, 2020). Bare soil.	Chemical fertilizer (Blaukorn BASF, Germany, 12–8–16-3 N-P2O5-K2O-MgO) at 112 kg ha ⁻¹ split in two doses applied in March and December to cacao trees. Weeding with brush cutters and herbicide applications, mainly Glyphosate 4-5 times per year. No other pesticides applied. Annual cacao tree pruning. Pruning residues left around the trees.
OM	0 tree ha ⁻¹ Full sun, LT: 92%	Only herbaceous strata. The perennial legume <i>Neonotonia wightii</i> was used as a cover crop and fully covered the soil during the first years. It slowly disappeared over the years while cacao tree cover increased (Marconi et al., 2022). About 10% of soil covered by the cover crop at the end of the study. No bare soil.	Locally made compost with crop and vegetation residues, hens' slurry and wood chips. 8 t ha ⁻¹ applied to cacao trees. Weeding with brush cutters. Annual cacao tree pruning. Pruning residues left around the trees.
CA	approx. 250 trees ha ⁻¹ , LT: 39%	Much less herbaceous strata compared with CM and OM due to higher litterfall and pruning residues and more shade. In the low strata coffee (<i>Coffea arabica</i>) is planted in lines. Banana trees (<i>Musa</i> spp.) were planted in the cacao tree inter-row space (4 × 4 m). Shade trees consisted mainly of legumes (8 × 8 m), such as pacay (<i>Inga ingoides</i> and <i>I. expansa</i>) and ceibo (<i>Erythrina poeppigiana</i> and <i>E. fusca</i>). With lower densities (16 × 8 m), fruit species (e.g., avocado (<i>Persea americana</i>), copoazú (<i>Theobroma grandiflorum</i>), rambutan (<i>Nephelium lappaceum</i>), and timber trees such as mara (<i>Swetenia macrophylla</i>) or Quina quina (<i>Myroxylon balsamum</i>).	Chemical fertilizer (Blaukorn BASF, Germany, 12–8–16-3 N-P2O5-K2O-MgO) at 56 kg ha ⁻¹ split in two doses applied in March and December applied to cacao trees. Weeding with brush cutters and herbicide applications, mainly Glyphosate, but less times and less quantity required compared to CM. No other pesticides applied. Annual cacao and shade tree pruning. Pruning residues left around the trees.
OA	approx. 250 trees ha ⁻¹ , LT: 39%	<i>Idem</i> as CA. In addition, the leguminous cover crop was planted at the beginning but it completely disappeared by the end of this study.	No compost applied since 2016 (before half of the dose of OM). Weeding with brush cutters. Annual cacao and shade tree pruning. Pruning residues left around the tree.
SA:	approx. 1110 trees ha ⁻¹ , LT: 26%	Highly diverse and abundant herbaceous strata, characterised by native and endemic species (Marconi &	No external inputs. Annual cacao and shade tree pruning. Pruning residues left around the trees.

Table 1 (continued)

	Shade tree and light*	Vegetation	Management
		Armengot, 2020). Additional crops in the low strata such as coffee, ginger and curcuma. Banana trees in lower density. The shade trees consist of the same design of the OA and CA, but with additional trees both coming from natural regeneration of the vegetation, planted or seeded (e.g., peach palm <i>Bactris gasipaes</i>).	
F	Not measured, but higher density and lower LT than SA	A very dense secondary forest developed after land preparation (at the same time and method than the other systems) when the land was left unmanaged. Most abundant species are <i>Cecropia</i> sp., <i>Senegalia loretensis</i> , <i>Triplaris americana</i> and <i>Guadua weberbaueri</i> .	Unmanaged

* The fraction of transmitted light (LT) was measured below the canopy. It was measured by taking four hemispherical photographs of each plot between the cacao tree lines using a Nikon CoolPix5400 equipped with an FC-E8 converter lens with a 180° angle of view. LT can vary according to the pruning activities (Niether et al., 2018)

individuals collected. A multivariate analysis based on permutations using the “adonis2” command in R was used to determine the contribution of system, year and plot to the variation in ant community composition (Anderson, 2001). The Bray-Curtis metric was used to compute the distances matrix. The partial R^2 obtained indicates the percentage of variance that is explained by each factor. The significance of the explanatory variable was obtained from an F test based on sequential sums of squares from permutations of the raw data. The permutations were restricted to each block to incorporate the experimental design. We also performed a non-metric multidimensional scaling (NMDS) to map the distance in community composition between samples in two axes.

Trophic roles and indicator species

Species were classified according to their main diet based on Agosti (2000), who established a classification according to genus. The groups were defined as: army ants, generalist foragers, seed foragers and omnivores, pollen foragers, fungi cultivators, specialized predators and generalized predators. In addition, we grouped them into specialised (i. e., army ants, pollen foragers, fungi cultivators, specialized predators) and generalist trophic groups. The percentage of species from each group per plot was compared between systems using generalized linear mixed effect models. Additionally, we used the indicator species analysis to determine if certain ant species were more associated with one system than with others (De Caceres et al., 2010).

Results

Species richness

A total of 85 ant species were recorded, belonging to 6 sub-families and 41 genera (Appendix A: Table 1). The total number of collected species per system was 60, 42, 35, 40, 31 and 33 for F, SA, OA, CA, OM and CM, respectively. The sub-families Myrmicinae and Ponerinae were

the most species-rich, with 41 and 20 species recorded, respectively (Appendix A: Table 1). The most diverse genera were *Pheidole* sp. with 13 species, *Solenopsis* sp. and *Camponotus* sp. with 6 species each and *Pachycondyla* sp. with 5 species. Between 51 and 61 species were collected per year: 55, 61, 51 and 59 species respectively for 2015, 2017, 2019 and 2021, which represents 65%, 73%, 61% and 70%, respectively, of the total species listed. A mean of 14.63 ± 1.92 species was recorded per plot, ranging from 11.25 in OM to 18.50 in F (Appendix A: Fig. 3A). Most of the species appeared at a very low frequency. Seventy species were recorded in 10% or less of the total numbers of traps sampled (480), and from those, up to 42 species were collected in 1% or less of the traps. Only two species, *Pheidole* sp. 1 and *Camponotus* sp. 1 appeared in more than 50% of the traps (57.9% and 50.8%) (Appendix A: Table 1).

Rarefaction curves for each system indicated a good completeness of the survey and showed higher total species richness in the F system (Fig. 1). The statistical analyses did not show a significant difference in species richness between the systems (Appendix A: Fig. 2A, $X^2 = 9.36$, $P = 0.09$), although there was a tendency of higher species richness in F compared to the cacao production systems. Neither year ($X^2 = 4.01$, $P = 0.26$) nor the interaction between system and year ($X^2 = 13.62$, $P = 0.55$) were significantly different (Appendix A: Fig. 2B).

Species composition

The multivariate analyses showed significant differences for the system ($F = 5.40$, $P < 0.001$) and year ($F = 6.30$, $P < 0.001$) but not for plot ($F = 1.37$, $P = 0.16$). The percentage of variation explained by the system was 20% of the total variation in species composition and year explained 14% of the variation. In the graphical representation of the community composition (NMDs), in the NMD1 axis, we observed a clear distinction between F and the production systems (Fig. 2A), but also between the different production systems, following a gradient of management intensity, i.e., SA was more similar F while CM was the most distinct system. Differences in composition between organic and conventional management within the agroforestry systems were less pronounced than within the monoculture. The NMD2 axis was related to year (Pearson R between NMD2 and year = 0.64), i.e., the samplings

performed in the last years appeared in the top part of the figure.

Ecological indicator species and trophic roles

A total of 34 species (out of 85) were associated with 1 or more systems. Fifteen species were associated only with the secondary forest whereas nineteen species were associated with 1 or more production systems (Table 2). Two species were associated only with monocultures (*Pheidole* sp. 7 and *Pogonomyrmex* sp. 1) whereas 8 species were associated with agroforestry systems exclusively. Species associated with cultivated systems (mainly monocultures and simple agroforestry systems) were found in a high percentage of traps (between 3 and 58% of total number of traps), while species associated with SA and F were less frequent (between 1% and 11%).

When looking at trophic role, 27 species were classified as foragers, 19 species were seed foragers and omnivores, 18 species were general predators, 11 species fungi cultivators, 7 species specialised predators and only 2 species and 1 species were army ants and pollen foragers, respectively (Table 2 and Appendix A: Table 1). All 7 specialised predators and the 2 army ants were species collected in less than 1% of the traps. Seed foragers and omnivores was the group that included more species with high frequency. No marked differences between systems were found in the percentage of the different trophic roles (Fig. 3A). However, we observed a higher percentage of fungi cultivators in F and SA compared with the other systems. Overall, species from specialised trophic groups (i.e., fungi cultivators, specialised predators, army ants and pollen foragers) were collected in more traps in the complex systems (81, 68, 68, 51, 46 and 48, respectively for F, SA, OA, CA, OM and CM). A total of 15 species from specialised trophic groups were recorded in F, 10 in SA and between 6 and 8 for the rest of the systems. The frequency of specialised trophic roles significantly differed between systems ($X^2 = 38.01$, $P < 0.001$; Fig. 3B). No clear differences over the years were observed in the pattern of trophic roles (data not shown).

Discussion

Our study in a long-term experimental site comparing different land-use systems showed that intensification of cacao production affects ant

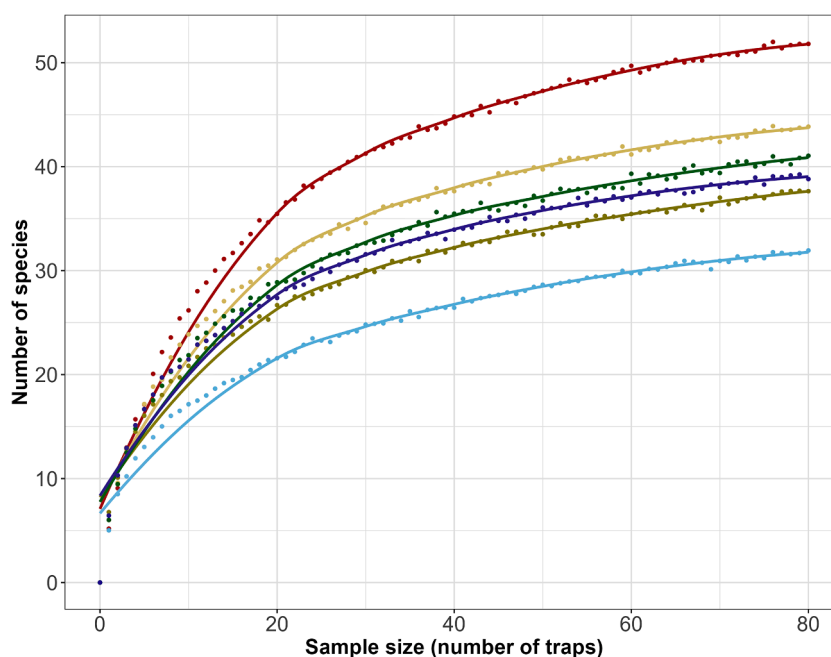


Fig. 1. Rarefied accumulation curves for the different systems.

The systems abbreviations stand for CM: Conventional monoculture, OM: Organic monoculture; CA: Conventional agroforestry, OA: Organic agroforestry, SA: Successional agroforestry, and F = Secondary Forest.

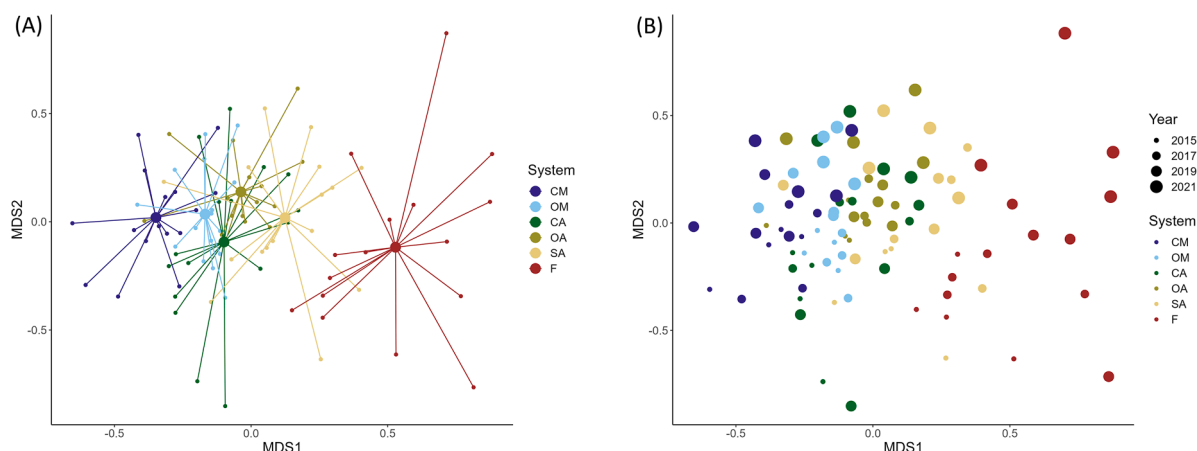


Fig. 2. Non-metric multidimensional scaling (NMDS) showing community composition of ant species for (A) different systems and (B) for the different systems and years. NMDS ordination based on Bray-Curtis dissimilarity matrix. The systems are abbreviated as follows: CM: Conventional Monoculture; OM: Organic monoculture; CA: Conventional agroforestry; OA: Organic agroforestry; SA: Successional agroforestry system; F: Secondary Forest.

Table 2

List of indicator species (34 out of 85 species). The pattern refers to the system or combination of systems where R reached its highest value.

Subfamily	Species	Pattern	R-value	P-value value	Trophic role	Frequency
Myrmicinae	<i>Pheidole</i> sp. 7	CM	0.22	0.001	SO	31%
Myrmicinae	<i>Pogonomyrmex</i> sp. 1	CM	0.58	0.001	Fo	13%
Myrmicinae	<i>Solenopsis</i> sp. 5	CM+F	0.14	0.048	SO	3%
Formicinae	<i>Camponotus</i> sp. 2	CA+CM+OM	0.16	0.013	Fo	9%
Formicinae	<i>Camponotus</i> sp. 3	CA+OA+CM+OM	0.15	0.019	Fo	5%
Myrmicinae	<i>Pheidole</i> sp. 1	CA+OA+CM+OM	0.47	0.001	SO	58%
Myrmicinae	<i>Pheidole</i> sp. 2	CA+OA+SA+CM+OM	0.24	0.001	SO	39%
Myrmicinae	<i>Pheidole</i> sp. 8	CA+OA+SA+CM+OM	0.14	0.046	SO	18%
Myrmicinae	<i>Solenopsis</i> sp. 2	CA+OA+SA+CM+OM	0.19	0.002	SO	24%
Formicinae	<i>Camponotus</i> sp. 4	CA+SA+CM	0.16	0.011	Fo	3%
Formicinae	<i>Camponotus</i> sp. 1	CA+OA+SA	0.23	0.001	Fo	51%
Ponerinae	<i>Ectatomma</i> sp. <i>permagnum</i>	OA+SA+OM	0.21	0.001	Gp	42%
Myrmicinae	<i>Pheidole</i> sp. 3	CA+OA+SA+F	0.16	0.014	SO	12%
Ponerinae	<i>Pachycondyla crassinoda</i>	CA+OA+SA+F	0.18	0.002	Gp	48%
Myrmicinae	<i>Trachymyrmex</i> sp. 1	OA+SA+F	0.20	0.001	Fu	11%
Myrmicinae	<i>Crematogaster</i> sp. 2	SA	0.29	0.001	Fo	2%
Formicinae	<i>Paratrechina</i> sp. 1	SA	0.18	0.004	Fo	5%
Formicinae	<i>Prenolepis</i> sp.	SA	0.20	0.004	Gp	1%
Myrmicinae	<i>Wasmannia</i> sp. 1	SA	0.18	0.002	Fo	6%
Myrmicinae	<i>Acromyrmex</i> sp.	F	0.17	0.027	Fu	1%
Myrmicinae	<i>Apterostigma</i> sp. 1	F	0.16	0.030	Fu	2%
Formicinae	<i>Dendromyrmex</i> sp. 1	F	0.23	0.001	Fo	1%
Ecitoninae	<i>Eciton burchellii</i>	F	0.18	0.030	A	1%
Ponerinae	<i>Ectatomma edentatum</i>	F	0.23	0.001	Gp	1%
Ponerinae	<i>Ectatomma</i> sp. 1	F	0.30	0.001	Gp	3%
Ponerinae	<i>Pachycondyla apicalis</i>	F	0.20	0.006	Gp	1%
Ponerinae	<i>Pachycondyla</i> sp. 2	F	0.29	0.001	Gp	2%
Myrmicinae	<i>Pheidole</i> sp. 10	F	0.20	0.001	SO	4%
Myrmicinae	<i>Pheidole</i> sp. 11	F	0.23	0.002	SO	1%
Myrmicinae	<i>Pheidole</i> sp. 12	F	0.25	0.001	SO	1%
Myrmicinae	<i>Pheidole</i> sp. 13	F	0.18	0.028	SO	1%
Myrmicinae	<i>Pheidole</i> sp. 4	F	0.16	0.023	SO	11%
Pseudomyrmecinae	<i>Pseudomyrmex triplanirus</i>	F	0.25	0.001	Gp	2%
Myrmicinae	<i>Sericomyrmex mayri</i>	F	0.23	0.003	Fu	1%

Trophic role according to Agosti (2000): Foragers (Fo), Fungi cultivator (Fu), Army ants (A), Pollen feeders (P), Generalized predators (Gp) and Seed foragers and omnivores (SO). Frequency: percentage of traps in which the species was found from a total of 480 traps. The system abbreviations stand for: CM: Conventional Monoculture; OM: Organic monoculture; CA: Conventional agroforestry; OA: Organic agroforestry; SA: Successional agroforestry system; F: Secondary forest.

communities. Previous studies in cacao and other tropical crops such as coffee also showed the impact of intensive management on ants (Ahuatzin, 2019; Urrutia-Escobar & Armbrrecht, 2013).

However, our study does not support the hypothesis that more complex and less intensively managed systems host higher species richness, as was found in previous studies on ant species richness (Perry, 2016; Urrutia-Escobar & Armbrrecht, 2013) but also for other taxa in the same experimental site (Naoki et al., 2017). However, some studies found no differences in ant species richness between both cultivated

(palm plantations) and non-cultivated systems (secondary forest) (Narváez-Vásquez, 2021) or along a gradient of system complexity (Donoso, 2017). Similarly, others observed a lack of effect of the removal of the understory vegetation (Hood, 2020) or the removal of shade trees (Rizali et al., 2013) on ant species richness. These results indicate that ant species richness alone might be a poor indicator of the impact of farming activities and disturbance (Casimiro et al., 2019; Solar et al., 2016). This was also the case for herbaceous species in the same experimental site, with higher number of species in both the

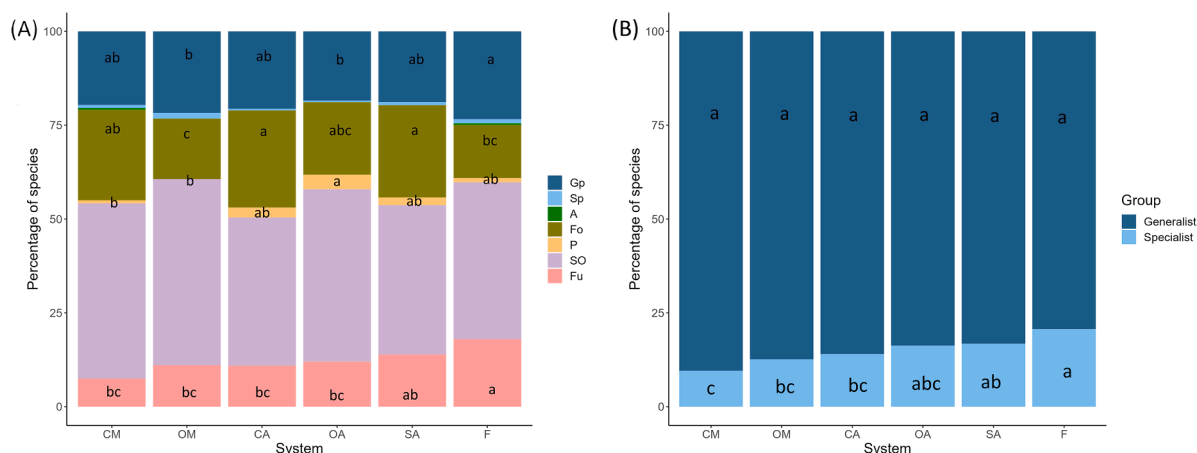


Fig. 3. Percentage of ant species in the different systems according to (A) their trophic role and (B) grouped into specialised and generalist trophic roles, averaged across all years.

Trophic role according to Agosti (2020): Generalized predators (Gp), Specialized predators (Sp), Army ants (A), Foragers (Fo), Pollen feeders (P), Seed foragers and Omnivorous (SO) and Fungi cultivator (Fu). Specialised trophic roles are: Sp, A, P, Fu. The systems abbreviations stand for CM: Conventional monoculture; OM: Organic monoculture; CA: Conventional agroforestry; OA: Organic agroforestry; SA: Successional agroforestry system; F: Secondary Forest. Different letters indicate statistically significant differences at $\alpha = 0.05$ according to pairwise comparison of the percentage of species per trophic role between the systems.

conventional monoculture and the complex agroforestry system (SA) (Marconi et al., 2020 and 2022). Ant species richness can be similar in highly different types of land-use although large differences in composition may occur (Rubiana et al., 2015), as we observed in our study.

The compositional differences were clearly shown by the NMDS representation, where the most complex and diverse agroforestry system (SA) was the most similar to the secondary forest (F), and the conventional monoculture (CM) was the most different one. In this gradient of management intensity/complexity of the systems, the differences between organic and conventional management were more marked in the monocultures compared to the agroforestry systems. This reflected well the actual differences in management, i.e., management intensity was lower and more similar in the agroforestry systems than in the monocultures (see Materials and methods). It could also reflect that the complexity and diversity of the vegetation of the systems have more influence on ant communities compared to the application of external inputs (Helms IV et al., 2021; Dos Santos et al., 2010).

The indicator species analysis identified species that helped to understand the differences in composition. For instance, species such as *Dendromyrmex* sp. 1, *Pachycondyla* sp. 2, *Pseudomyrmex triplianirus* or *Sericomyrmex mayri* were associated with the secondary forest. These species were previously identified as species living in forests and most of them have an arboreal behaviour (Weber, 1944; Fernandez, 2002; Jesovnik & Shultz, 2017). Actually, almost 50% of the species that were identified as indicator species were associated with the secondary forest. They were also species appearing mainly in very low or low frequency. On the contrary, some species were associated with all five (i.e., *Pheidole* sp. 2, 8 and *Solenopsis* sp. 2) or with four cacao production systems (i.e., *Camponotus* sp. 3 and *Pheidole* sp. 1) but not with F. These three genera, belonging to the general trophic roles of seed foragers and omnivores and generalist foragers, are considered dominant and very diverse in tropical ecosystems, probably as the result of their resilience to disturbances, large habitat plasticity and dispersal capacity (Muñoz et al., 2017; Fernández, 2003). Other studies have indicated a quick disappearance of forest specialist species with disturbance, which are replaced by species that can survive in disturbed/managed landscapes (Hagggar, 2019; Kone et al., 2012).

Although the main driver differentiating ant community composition was the disturbance caused by cultivation, i.e., the main differences were found between the secondary forest and all the other systems, we found some species, such as *Pogonomyrmex* sp. 1 and *Pheidole* sp. 7 associated only with full-sun monocultures, which reflect their

preference for open habitats. Conversely, other species only appeared in all agroforestry systems (e.g., *Pachycondyla crassinoda*, *Trachymyrmex* sp. 1) or only in the most complex one (SA, e.g., *Crematogaster* sp. 2, *Paratrechina* sp. 1, *Prenolepis* sp., *Wasmannia* sp. 1). These species have small or medium colonies (low number of individuals) (personal observation), which contrast with widespread species of genus such as *Pheidole*, *Solenopsis* and *Camponotus*. In addition, species of the genus *Prenolepis*, *Wasmannia* and *Crematogaster* are not arboreal species but they forage mainly on trees rather than on epigeous vegetation.

The trophic roles identified presented similar percentages of species across systems. The exception to this pattern was the fungi cultivators, which were more represented in the secondary forest (and partially SA) in comparison to monocultures and simple agroforestry systems. For instance, leaf-cutting ants such as *Atta cephalotes* and *Acromyrmex* sp., need a wide variety of plants to be able to offer a substrate suitable for the growth of their fungal cultures (Wirth et al., 2003). Overall, species with more generalistic trophic roles appeared in higher frequencies than the ones with specialized trophic roles, and the latter were more frequent in the more diverse systems. This indicates that the secondary forest and the complex and less intensively managed production systems can provide a suitable environment that supports the specific requirements of low-frequency species.

Our study included four samplings over a 7-year period. However, we could not observe any trend in species richness over time. We observed a turnover of species over the years (i.e., between 60%-70% of all species collected were registered each year), but this did not change the average values over the years. However, the temporal component explained some of the variability in the community composition. Some species such as *Cyphomyrmex* sp. 2, *Wasmannia* sp. 1, *Pachycondyla* sp. 1, *Pheidole* sp. 4 or *Solenopsis* sp. 1 diminished their frequency over time, while some others increased, e.g., *Pheidole* sp. 1, *Pheidole* sp. 3. *Pheidole* is a genus with highly aggressive and dominant species, which could explain the observed increase, contrary to species from other genera that diminished over time (e.g., *Cyphomyrmex*).

Conclusions

The results from this study confirm the earlier findings in this experimental site with still young and less differentiated systems, i.e., ten years older production systems did not have any effect on species richness but affected community composition. The main driver of ant communities was the disturbance created by cultivation in comparison

with natural vegetation (secondary forest), but also the different complexity/management intensity of the system. This has strong implications for landscape management and highlights the importance of preserving natural patches of forest, as well as highly diverse and complex agroforestry systems within the agricultural matrix for ant diversity conservation. These results also showed the potential of ants as indicators for assessing the impact of land-use changes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.baae.2023.10.006](https://doi.org/10.1016/j.baae.2023.10.006).

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