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„Functional composition of ground-dwelling ant communities along a succession gradient in reforestation areas of Golfo Dulce in Costa Rica “

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Abstract

Due to agriculture and logging Costa Rica has lost many primary forests. Tropical forests are harbouring half of the Earth's terrestrial species and provide crucial ecosystem services of global relevance, making reforestation an important task. A fundamental aspect determining the success of reforestation lies in understanding the intricate web of interactions among organisms within the restored habitats. The COBIGA project near La Gamba, in the Golfo Dulce region of Costa Rica, is a response to deforestation crisis, aiming at reforestation with native tree species. Ants, as ubiquitous and highly abundant terrestrial organisms, have a substantial influence on tropical ecosystems. Their multiple roles include scavenging, predation, herbivory, and mutualistic interactions. Remarkably, they serve as vital ecosystem engineers, integral to nutrient cycling and overall ecosystem health. Given their wide distribution and ecological importance, ants are used as effective bioindicators, reflecting ecosystem conditions and functioning. This present research examines ant community responses across various stages of forest succession, aiming to reveal the status of ecosystem regeneration and functional integrity. By analysing the composition and diversity of ant assemblages at reforestation sites of different ages (two, eight and twelve years old) I wanted to reflect the trajectory of reforestation success. These sites were compared with an old-growth forest as a reference site. Habitats were described by simple vegetation data (tree circumference, leaf-litter depth, herb-layer coverage and abundance of lianas and epiphytes). Ground-dwelling ants were collected for two months during a rainy season using traps filled with canned tuna bait. Results revealed old-growth forests to harbour species which were not present in any other site, although the oldest reforestation site showed a similar species number. There were significant community differences between all sampling sites, but also small-scale variation within sites. Certain life traits were more prevalent at younger reforestation sites (i.e., feeding on nectar) which are characteristic for ants of lower trophic levels, and some at older reforestation sites (i.e., large head size) characteristic for predacious ants from higher trophic levels. Due to the chosen sampling method most of the observed ant species were omnivorous but old-growth forest harbours a substantial number of other functional groups (generalized predators, arboreal predators, arboreal omnivores) whereas the youngest reforestation site covered mostly generalized species from lower trophic levels.

Zusammenfassung

Tropische Wälder beherbergen die Hälfte der terrestrischen Arten der Erde und sind zugleich für die Ökosystemleistungen von entscheidender globaler Bedeutung. Daher ist die Rehabilitation gestörter Waldflächen eine bedeutende Aufgabe. Ein grundlegender Aspekt, der über den Erfolg einer Wiederaufforstung entscheidet, ist das Verständnis des komplizierten Netzes von Wechselwirkungen zwischen den Organismen in den wiederhergestellten Lebensräumen. Das COBIGA-Projekt in der Region La Gamba, in der Golfo Dulce Region in Costa Rica, ist eine Antwort auf die Entwaldungskrise und versucht, die Wiederaufforstung mit einheimischen Baumarten durchzuführen. Ameisen haben als allgegenwärtige und weit verbreitete terrestrische Organismen einen erheblichen Einfluss auf tropische Ökosysteme. Zu ihren vielfältigen Funktionstypen gehören Aasfresser, Räuber, Pflanzenfresser sowie Mutualisten von Pflanzen wie auch Tieren. Ameisen sind oft wichtige Ökosystemingenieure, die für den Nährstoffkreislauf und die Gesundheit des gesamten Ökosystems unerlässlich sind. Aufgrund ihrer weiten Verbreitung und ökologischen Bedeutung werden Ameisen als wirksame Bioindikatoren verwendet, die den Zustand und das Funktionieren von Ökosystemen widerspiegeln. Im Rahmen der vorliegenden Arbeit wurden die Reaktionen der Ameisengemeinschaften in verschiedenen Stadien der Waldsukzession untersucht, um die Regeneration des Ökosystems und die funktionelle Dynamik aufzuzeigen. Durch die Analyse der Zusammensetzung und Vielfalt von Ameisengemeinschaften an Aufforstungsstandorten unterschiedlichen Alters (zwei-, acht- und zwölfjährig) wollte ich den Verlauf des Aufforstungserfolgs aufzeigen. Diese Standorte wurden mit einem alten Wald als Referenzstandort verglichen. Die Lebensräume wurden anhand einfacher Vegetationsdaten beschrieben (Baumumfang, Tiefe der Laubstreu, Krautschichtdeckung und Häufigkeit von Lianen und Epiphyten). Epigäische Ameisen wurden zwei Monate lang, während einer Regenzeit, mit Fallen gesammelt, die mit Dosen-Thunfisch beködert waren. Die Ergebnisse zeigten, dass alte Wälder Arten beherbergen, die an keinem anderen Standort zu finden waren, obwohl der älteste Aufforstungsstandort eine ähnliche Artenzahl aufwies. Es gab signifikante Unterschiede in der Zusammensetzung der Lebensgemeinschaften zwischen allen Wäldern, aber auch kleinräumig innerhalb der Wiederbewaldungsflächen. Bestimmte Lebensmerkmale waren in jüngeren Sekundärwäldern stärker ausgeprägt (z. B. Nektarivorie), die für Ameisen niedrigerer Trophiestufen charakteristisch sind. Andere Eigenschaften dominierten in älteren Sekundärwäldern (z. B. große Kopfgröße), die für räuberische Ameisen höherer Trophiestufen charakteristisch sind. Aufgrund der Art der Probenahme waren die meisten der untersuchten Arbeiterinnen Allesfresser, aber alte Wälder beherbergten eine beträchtliche Anzahl weiterer funktioneller Gruppen (generalisierte Räuber, baumbewohnende Räuber, baumbewohnende Allesfresser), während an den jüngsten Aufforstungsstandorten hauptsächlich generalisierte Arten aus niedrigeren trophischen Ebenen vorkamen.

1. Introduction

There is an increasing interest in the potential for reforestation of former tropical rainforests destroyed due to agricultural or other human impacts (Chazdon 2019). Many projects worldwide attempt rebuilding these habitats with an aim to create conditions the closest to their original state. An important issue in the success of the reforestation process is to investigate the interactions between organisms in the restored habitats. Tropical forests are globally very important habitats. They are responsible for half of the Earth's terrestrial species (Dirzo & Raven, 2003) and store huge amounts of carbon (Berenguer et al., 2014). Conversion and degradation of habitats are one of the major factors influencing biodiversity decline (Newbold et al. 2015). Ants are one of the most abundant terrestrial organism on Earth (they are considered to make up to 25% of animal biomass: Fayle and Klimens 2022) and play a very important role in tropical ecosystems. Due to their high abundance in tropical ecosystems, they are considered major contributors to maintaining ecosystem services (Grimaldi & Engel, 2005). They have many different feeding strategies including large numbers of omnivores and scavengers, but many ant species also consume honeydew or extrafloral nectars, seeds, and fungi (Hölldobler & Wilson, 1990; Lach et al., 2010). In many habitats they are the most important predators on other insects and small invertebrates (Hölldobler and Wilson, 1990). For these reasons they are often used as bioindicators in land management and the status of ant assemblages is used as a proxy for conditions of tropical ecosystems. This line of research was pioneered by the classification of ants into functional groups, initially established for Australia (Andersen 1995). Several studies have shown that colonization of ant communities during ecosystem recovery reflect general patterns of the diversity of other invertebrates (Andersen and Majer 2004). Using them as bioindicators is supported by many studies on ant community responses to habitat disturbance in Australia, with certain groups being more and some less abundant in response to habitat disturbance (Hoffmann and Andersen 2003). Globally, dominant ant taxa are those prevalent in the most complex environments and those often belong to the subfamily Dolichoderinae (Andersen 2000). They are not abundant in colder climates and widely absent in Holarctic region (Andersen 1997). Another big ant group are generalized Myrmicinae which are considered subdominant, have high food exploitation capacity, but their worker body size is smaller, they occupy smaller foraging territories and show broader environmental tolerance compared to dolichoderines (Andersen 1995). Generalized Myrmicinae are prevalent in habitats of moderate stress and disturbance. The last major group is formed by opportunists which occupy wider environmental ranges but have poor competitive abilities and accordingly are more prevalent in stressed, dynamic, and disturbed habitats (Andersen and Majer 2004). The influence of environmental disturbance on ant communities is usually indirect and caused by stress due to changes in habitat structure, microclimate, and food availability (Andersen 1995). In anthropogenically altered systems, groups like specialized predators or cryptic leaf-litter species are known to decrease in richness and numbers because of their sensitivity to habitat disturbance. This impoverishment is also fostered by breaking competitive hierarchies among ant species due to changes in abundance of dominant ants to which generalized and opportunistic species are sensitive (Andersen and Majer 2004).

The COBIGA project was started as a response to the dramatic deforestation in Costa Rica happening until the 1990ies as a consequence of rapid demand of monocultures for cash crop production. While earlier the demand for bananas played a major role in deforestation (Hernandez and Witter 1996), in recent years the expansion of oil palm cultivation is a major threat (Höbinger et al. 2012). To counteract such damage, former pastures and farms are nowadays reforested with a selection of multiple native tree species based on historical documentations of primary forest inventories. Primary forests harbour higher ant species richness than secondary forests, but assemblage richness and complexity increase with time since disturbance (Silva et al. 2007). The response of community structure to the changing environment depends on the existing local to regional species pool and the species' ability to colonize new habitats in changing conditions (Gibb et al. 2015) as well as on elevation. Estimated time needed for complete faunal recovery is considered between 25-40 years (Dunn, 2004).

This present study aims at comparing ant assemblages in different succession stages of re-planted rainforests in the Golfo Dulce region in SW Costa Rica. The Peninsula de Osa and the Esquinas forests are located in the Puntarenas Province and represent, as legally protected national parks, the last remaining lowland rainforests in this part of the country. The area is one of the most humid in Costa Rica, with a very intense rainy season from August to November and a not that distinct dry period, compared to other regions, in February and March. The mean annual temperature is 27.4 °C and annual precipitation is 6241 mm (at the La Gamba field station) (Weissenhofer et al. 2019). The two national parks are surrounded by a cultivated landscape that comprises a mix of cattle pastures, oil palm plantations and various types of secondary growth. The Esquinas forests near the Pacific coast used to be connected with the Fila Cruces mountain range, which is a part of the Mesoamerican Biological Corridor, that stretches from Mexico south to Panama (Holland 2012). Today, however, connection between lowland and mountain forests is interrupted by farmland and pastures. COBIGA aims to maintain biodiversity by connecting the remaining patches of forests and simultaneously contribute to sequestration of carbon dioxide. Given the global increase in area of secondary and otherwise regenerating forests in the tropics it is important to extend conservation research into such habitats because of their ever-increasing importance for biodiversity preservation as well as for ecosystem services and functions (Chazdon et al. 2014).

Thus far, the vegetation development on the reforested areas has been investigated and documented to some detail. The COBIGA reforestation project in La Gamba shows promising results in terms of biomass accumulation and carbon dioxide sequestration by reforested trees (Hietz et al. 2019). Also, a couple of initial studies has addressed the utilization of these newly created habitats by various groups of animals. A study of Schulze et al. (2019) revealed importance of secondary forests for forest birds by offering a link to old-growth forests and therefore supporting their biodiversity (Schulze et al. 2019). However, no case study has so far been conducted on ants as essential ecosystem engineers. The only local ant study available demonstrated that spontaneously regenerating secondary forests harbour a rather rich, but nevertheless impoverished ant assemblage compared to old-growth forest, whereas ant communities in oil palm plantations are severely depauperate (Falk et al. 2019). Elsewhere in the tropics, several studies did “cafeteria experiments” with multiple baits to check for ant food preferences. Ants exist on multiple trophic levels and therefore have a broad variety of food preferences (Kaspari and Yanoviak 2001, Hashimoto et al. 2010, Rosumek et al 2018). Tuna fish traps, or in general protein-rich baits, are considered to attract mostly omnivorous ground-dwelling species, but they are the most prevalent fraction of any ant community and therefore usage of this type of baits and traps is a good method for quantifying the influence of these ants on the ecosystem.

My hypotheses focused on checking how ground-dwelling ant communities differ along a successional gradient of secondary forests and if there is a difference in predation pressure exerted by ants between primary and secondary forests.

1. Old-growth forest harbours the highest species richness with the highest number of omnivores, but also including functional specialists i.e., specialized predators or cryptic litter species.
2. Species assemblage composition differs between all four habitats, with the most homogenized community at the youngest reforestation site while the most heterogenous communities are expected in old-growth forest.
3. Old-growth forest is significantly different from the youngest reforestation site regarding species number, community composition and functional groups coverage.
4. Certain life traits are characteristic for young reforestation sites (i.e., invasiveness or feeding on nectars) and for old-growth forest (i.e., large head size characteristic for predacious ants), respectively.

2. Material and methods

2.1 Sampling sites

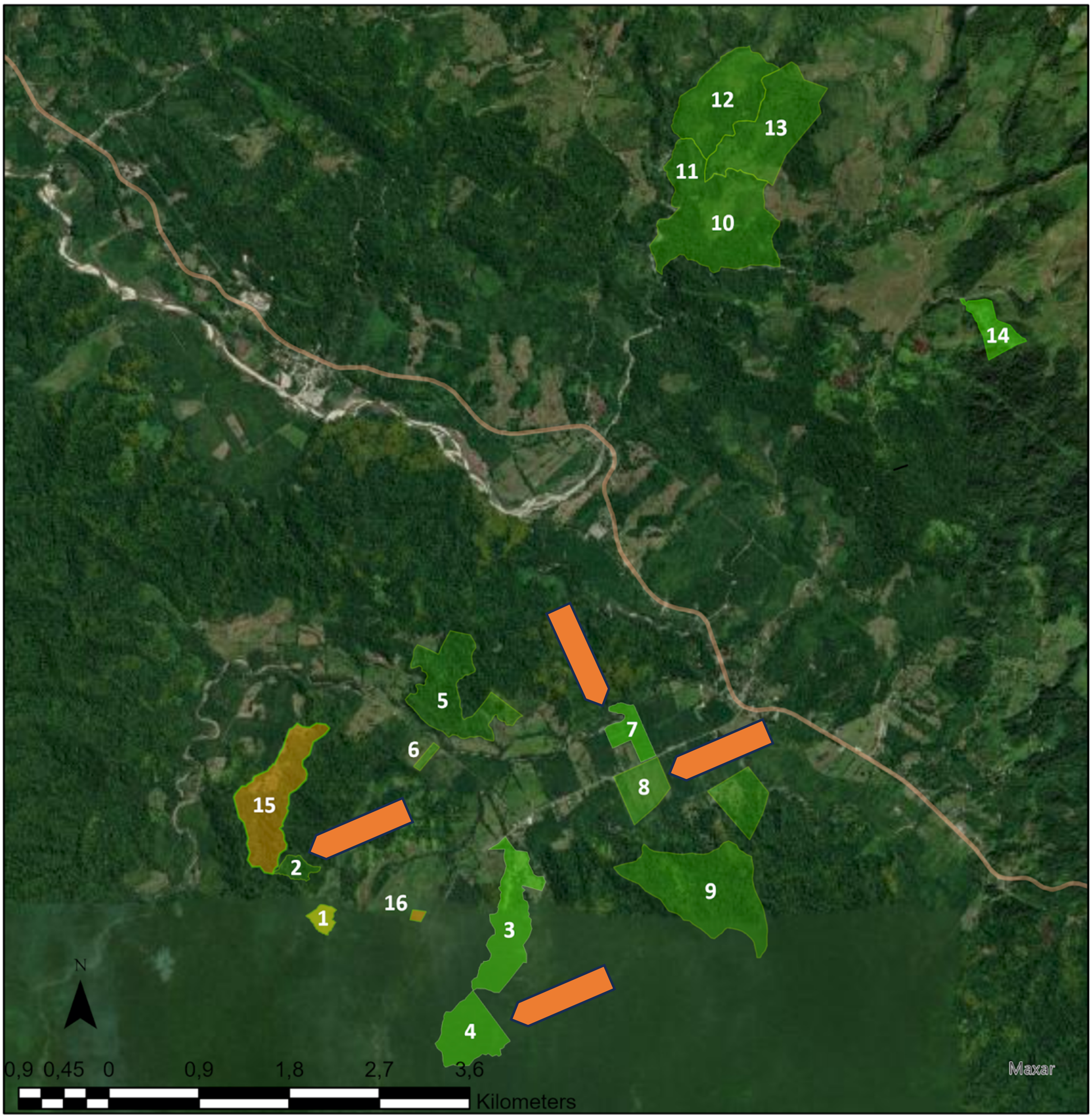


Figure 1 Map of the study sites near La Gamba in the Golfo Dulce region in SW Costa Rica. Numbers indicate properties of La Gamba station, including locations of sampling sites. My sampling sites are indicated with orange arrows. The map was modified from the La Gamba station website (https://www.lagamba.at/en/tropical-field-station/properties-of-the-tropical-field-station-la-gamba/, last accessed 22.02.2023) and originally created by A. Weissenhofer, W. Huber, T. Koukal and M. Immitzer M. in August 2022.

Ant sampling took place in the Golfo Dulce region of south-western Costa Rica near the village La Gamba. Sampling sites comprised of one primary forest stand and three reforestation sites at different stages of succession (Fig. 1). These latter sites were all abandoned former farms (fincas), which have been purchased by the Estación Tropical La Gamba S.A. during a reforestation project that aims to create a 'biological corridor' between remaining stretches of old-growth forest in the coastal lowlands and the Fila Cal mountain chain (Weissenhofer et al. 2019). Torre is a stretch of land situated directly on the border of Piedras Blancas National Park and is an area combining old-growth ravine, ridge, and slope forest (terminology according to Binz et al. 2015). Its elevation spreads between 80 to 130 m above sea level and the site covers 7 ha. My sampling took place in the area of slope forest to maintain similar conditions to other sampling sites. This site had never been cut and serves as a kind of reference to the reforestation areas in the present study. Finca La Bolsa is a mixture of primary and secondary forest as well as abandoned pasture and cropland, covers 16.55 ha and ranges from 80 to 170 m above sea level. Here, my sampling took place in the abandoned, formerly agricultural area that was reforested between 2010 and 2012 and is now completely covered by dense secondary forest of average 20 m high. Reforestation was done with 6065 tree individuals of 113 species. Finca Amable formerly was a pasture of the cultivated Russell River grass *Paspalum paniculatum* L. and surrounded by oil palm plantations (*Elaeis guineensis* Jacq.) from two sides. It also borders the Río Bonito River and the old-growth forests of the National Park Piedras Blancas to the north and the Finca Eduardo. The Río Bonito inundates its shores several times a year during the rainy season, thereby a great area of Finca Amable is flooded during the rainy season. Between 2013 and 2016 the site was planted with 10700 trees of 205 species. Finca Eduardo comprises 18 ha of former pasture, drainage ditches and 2.2 ha of oil palm plantation. The reforestation project started only in 2020 and it is planned to replant 9 ha of pasture and leave another 5.5 ha to natural succession. It is surrounded by palm oil plantations from two sites and connects the forests of the Fila Gamba with the eastern foothills of the National Park Piedras Blancas. Despite different characteristics of sampling sites, I was trying to choose similar sampling conditions, i.e., among reforested trees under closed canopy on a relatively flat area. In old-growth forest I chose an area of slope forest as the conditions were closest to reforestation sites. Vegetation at the sampling sites is depicted in Figure 2.

Figure 2 Representative pictures of sampling sites (from the top to the bottom: Eduardo, Amable, La Bolsa, Torre).

2.2 Vegetation data

Basic vegetation data was collected to describe habitat characteristics of my sampling sites. In an area of 50x50 meter around the sampling station I measured circumference (at breast height) of the ten thickest trees and calculated a mean for each sampling site. To estimate the extent of herbal layer, I took 4 photographs of understory at each sampling site and used the programme ImageJ (Schneider et al. 2014), with which I calculated a percentage of the coverage from each picture. Again, I calculated a mean of those measurements for each site. To measure leaf litter coverage, I randomly chose 8 spots close to the sampling station and measured leaf litter height from the bottom with a ruler. Again, calculated a mean of all measurements per site. Two more categorical variables were created: abundance of epiphytes and lianas. This variable has 3 levels (0- not present, 1- present but not highly abundant, 2- highly abundant). Table 1 presents all the collected measurements collected during sampling.

Table 1 Description of sampling sites including their geographical coordinates and vegetation information.

Sampling site	Geographical coordinates	Mean tree circumference [cm]	Mean herb layer coverage	Mean leaf litter depth [cm]	Epiphyte coverage	Liana coverage
Torre	8° 42' 16.5708" N 83° 12' 21.8448" W	217.0	40%	2.00	3	3
La Bolsa	8° 41' 25.8648" N 83° 11' 15.2808" W	198.9	50%	0.92	2	0
Amable	8° 42' 56.2176" N 83° 10' 32.6352" W	114.4	60%	0.58	1	0
Eduardo	8° 42' 52.4736" N 83° 10' 20.0136" W	77.9	90%	0.28	0	0

2.3 Study design

Ant sampling took place from 1st July to 9th September 2022 between 06:00 and 12:00 h. At each sampling site I chose 3 transects each consisting of 4 traps. As the conditions of each sampling site should be similar transects were placed in approximately the middle of each site to keep the distance from the edge similar. Every trap was set 10 m apart from its nearest neighbour and transects within a site were located 50 m apart from each other. Sampling was conducted only in the morning and only on rain-free days. If during the sampling rain started, ant baiting was stopped and repeated on the next day. At each site sampling was repeated for 10 days giving around 150 sampling points at each site. Sites were visited without any order meaning that each site was not sampled 10 days in a row. As a bait I used canned tuna fish in oil (soy oil) always of the same brand. It was proven to be a good bait for tropical ants (Gotelli et al. 2011, Yanoviak and Kaspari 2000). One tablespoon of tuna fish was placed on a plastic transparent plate (diameter 10 cm) and dug into the litter or topsoil so that ants foraging on the ground could easily get on it. Traps were exposed for approximately 2 hours and each hour I collected the samples into a vial filed with 70% ethanol for further analysis.

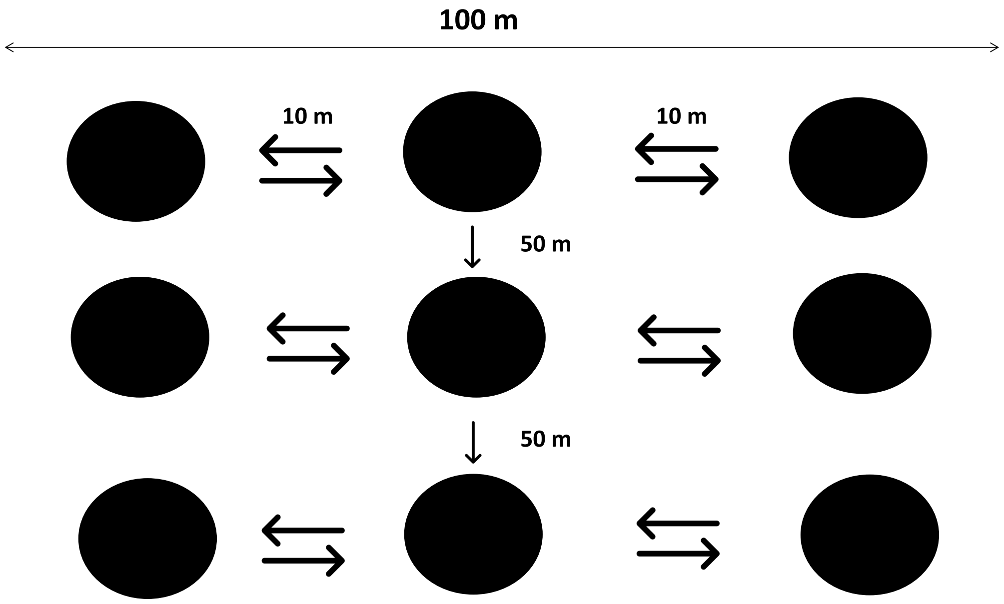


Figure 3 Schematic design of the positioning of baited traps in the sites. Three transects per site were chosen to be 100 m long, with traps randomly placed and spaced 10 m from another.

Figure 4 A trap provided with tuna fish bait, visited by 15000 ants.

2.4 Ant species identification

Specimens collected from the traps were identified on the grounds of morphological traits using the internet sources Antwiki, Antweb and resources provided by John T. Longino in 2010 from his project in Costa Rica. All samples were identified to species level if possible. Nomenclature of ant species follows Antwiki (Antwiki 2022). If Antwiki was not sufficient for identification I used resources provided by John T. Longino (Longino 2022). 50 samples which I did not manage to identify were brought to Vienna for further analysis. 12 of these samples were chosen for DNA barcoding because of difficulties with morphology-based identification. DNA was extracted from the samples using the Analytik Jena Kit innuPREP DNA Micro Kit and processed for Sanger sequencing on an AB 3730 DNA Analyzer from Applied Biosystems. As primers I used standard LepF and LepR primers. Obtained COI sequences (length 658 bp) were entered into BOLD system (http://www.boldsystems.org) and subjected to a BLAST search. Most of the results gave a match to samples affiliated with a valid scientific name, but four samples resulted only in a working name for the respective species.

2.5 Statistical analysis

2.5.1 Species richness

Observed species richness was analysed by mixed model ANOVA in the R environment (R Development Core Team, 2022) with habitat (i.e. the 4 sites) used as fixed factor. I used both data aggregated at transect level or each individual trap as sampling units for comparison in the analyses. Species richness was calculated using *iNext* package in R (Chao et al. 2014, Hsieh et al. 2022). Data frame was conducted based on indices of each species at each transect and the species accumulation curves were created for every sampling site and visualized with *ggplot2* (Wickham 2016).

2.5.2 Functional diversity

Using Antweb, Antwiki and resources provided by John T. Longino on individual species I collected information on 12 species traits on worker ant morphology, foraging stratum, invasiveness, trophic level, colony size, distributional range and food preference. Based on a synthesis chapter for the Neotropics (Groc et al. 2013) I classified all observed ant species into functional groups (Table 2) and also analysed the ant assemblages of the four study sites at this level, instead of taxonomic species.

Table 2 Classification of Neotropical ant functional groups (at genus level) according to Groc et al. (2013).

Classification A	**Classification B**		
Predators	**Arboreal predators**	**Raid-hunting predators**	**Ground-dwelling generalized predators**
	Pseudomyrmex	*Nomamyrmex*	*Anochetus* *Ectatomma* *Gnamptogenys* *Leptogenys* *Mayoponera* *Neoponera* *Odontomachus* *Pachycondyla*
Omnivores	**Arboreal omnivores**	**Generalist omnivores**	
	Azteca *Camponotus* *Dolichoderus* *Monomorium* *Tapinoma*	*Cardiocondyla* *Crematogaster* *Ectatomma* *Nylanderia* *Paratrechina* *Pheidole* *Solenopsis* *Tetramorium* *Wasmannia*	
Fungus-growers	**Leaf-cutters**		
	Acromyrmex		

2.5.3 Community composition

Community composition was first analysed using non-metric multidimensional scaling (NMDS) as a means of unconstrained ordination. As data frame I used abundance data of species at each sampling site. As abundance was considered every incidence of a species in a trap. Samples taken after 60 and 90 minutes were treated separately, meaning that if a certain ant species occurred twice (or remained after both 60 and 90 minutes) it was considered twice as occurring in this trap. The analysis was conducted using the *metaMDS* function in the *vegan* R package (Oksanen et al. 2020). Bray Curtis distances were used to perform this computation. A PERMANOVA was run afterwards to check for the significance of habitat differences in species composition. Additionally, I ran the *betadisper* function from the *vegan* package to check for homogeneity of variances between the groups. A Tukey post-hoc test was used to localize differences between habitats. Another method I used was a distance-based RDA, which is a constrained ordination method used to check for the possible impact of environmental variables (leaf litter thickness, tree circumference, herb layer coverage, lianas, and epiphyte abundance) on the ant community. This analysis was conducted using the *capscale* function in the *vegan* package. Environmental data was standardized to a mean of 0 and a standard deviation of 1 before analysis. A PERMANOVA was run to check for significance of the model.

3. Results

3.1 Identified species and species richness

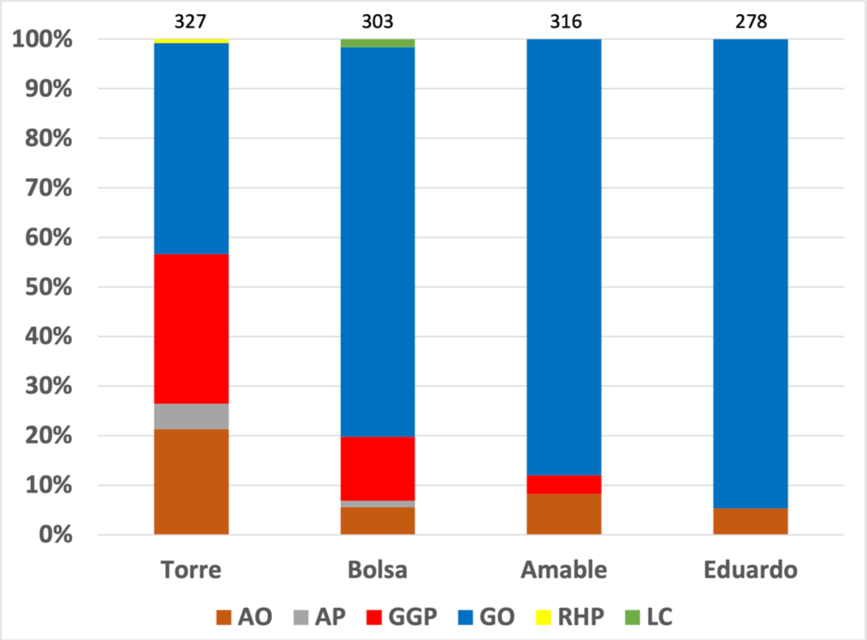


Figure 5 Bar plot of functional ant groups identified at each reforestation site, with the percentage contribution of each group (AO - arboreal omnivore, AP - arboreal predator, GGP - generalized ground-dwelling predator, GO - generalized omnivore, RHP - raid hunting predator, LC - leaf cutter). Numbers on top relate to the total number of ant observation units at each site.

Altogether I identified 43 ant species from approximately 15000 workers that occurred in the traps. Based on Groc et al. (2013) classification they were assigned to six functional groups. The percentage distribution of functional groups among sampling sites is shown on Figure 5. The broadest representation of different functional groups was observed at Finca Torre, with the highest percentage of generalized omnivores, but with generalized ground dwelling predators, arboreal omnivores and arboreal predators making up a very high fraction of all species. Finca La Bolsa shows a significantly higher percentage of generalized omnivores compared to other groups, but still covering almost all of the functional groups. Finca Amable covers only three functional groups with 80% of identified species being generalized omnivores. Finally, Finca Eduardo harboured only two functional groups with generalized omnivores covering more than 90% of identified species.

Figure 6 shows the distribution of identified species among four sampling sites. As expected, the highest number of species present only at one sampling site occurred at Finca Torre with twelve species characteristic for only that site. Finca La Bolsa had seven unique species, Finca Amable just two and Finca Eduardo had none. The diagram also shows that there were only three species present at all sampling sites and that Finca Torre and Finca La Bolsa shared six species which is a high number of species; younger reforestation sites did not show a similar trend.

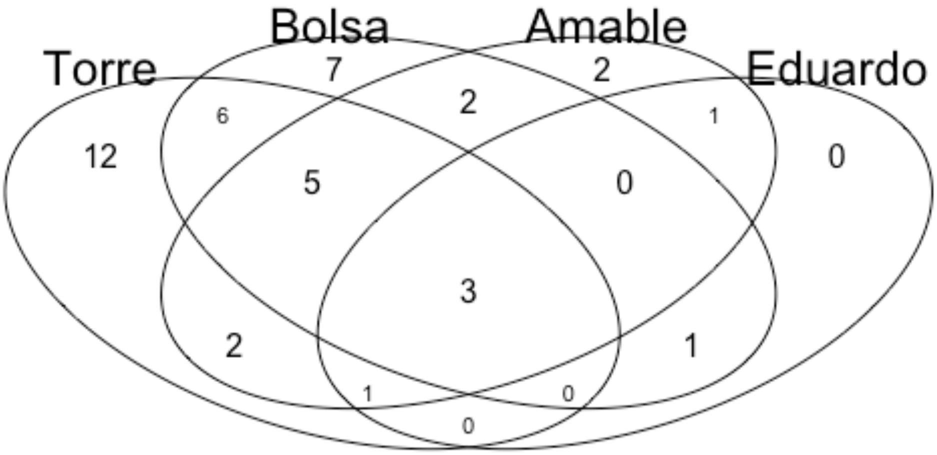


Figure 6 Venn diagram showing the numbers of observed ant species along the four reforestation sites.

Surprisingly, when using each transect as a sampling unit, the number of observed species differed significantly only between Finca Eduardo (the youngest reforestation site) and all three other habitats (8-year-old, 12-year-old and old-growth forest) (Fig.7). *Solenopsis geminata, Nylanderia steinheili* and *Monomorium floricola* are the species that occurred at every sampling site. *Dolichoderus curvilobus* was a very abundant species that occurred only in old-growth forest and the oldest reforestation site, viz. Finca La Bolsa. *Monomorium pharaonis* was very abundant at Finca Torre but did not occur at any other sampling site (Fig.8).

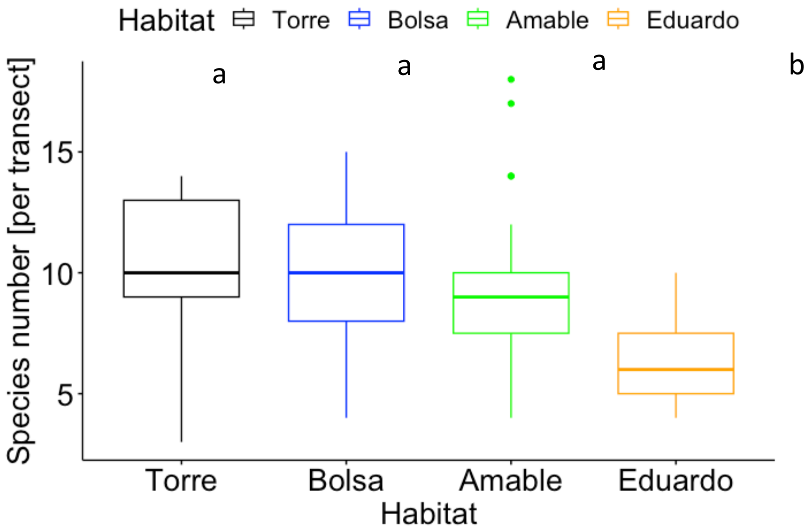


Figure 7 Box plot of observed ant species richness per transect, across the 4 reforestation sites. Differences were highly significant (ANOVA: $F_{3;123}$ = 15.08, p<0.0001). A Tukey post-hoc comparison revealed that richness per transect at Eduardo was significantly lower (p<0.0001) than at all 3 other sites (indicated by letters).

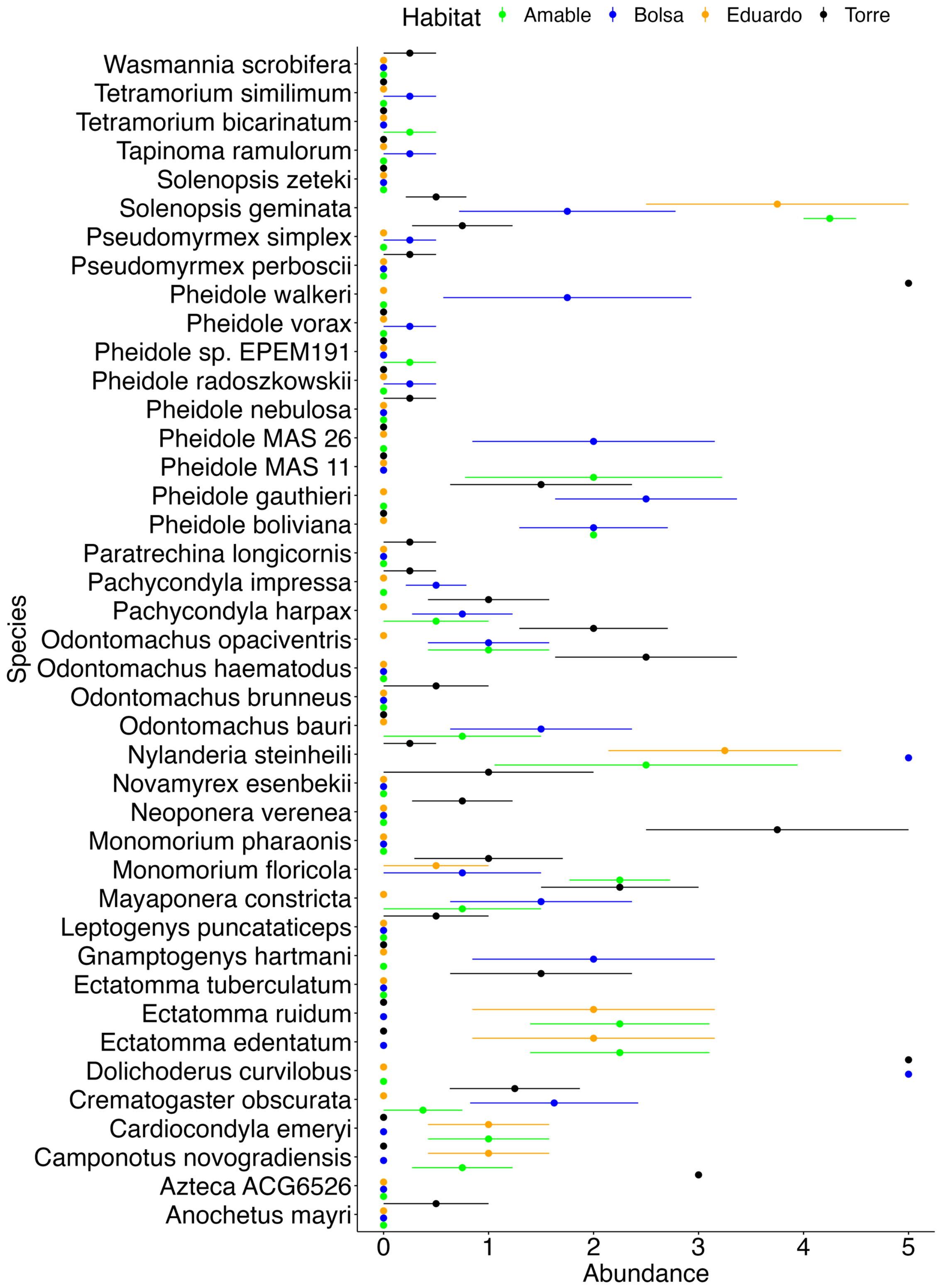
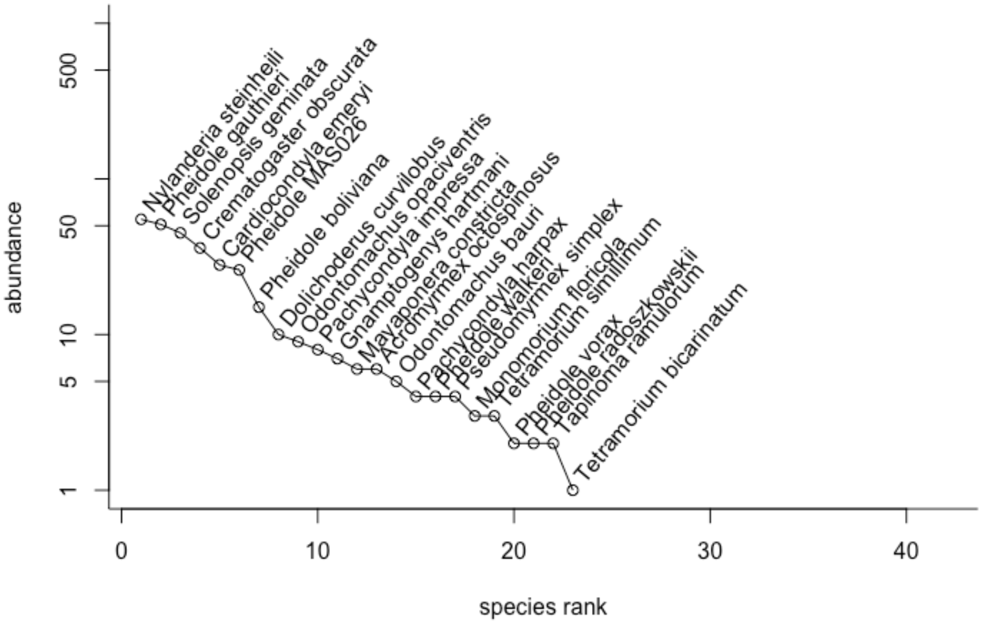
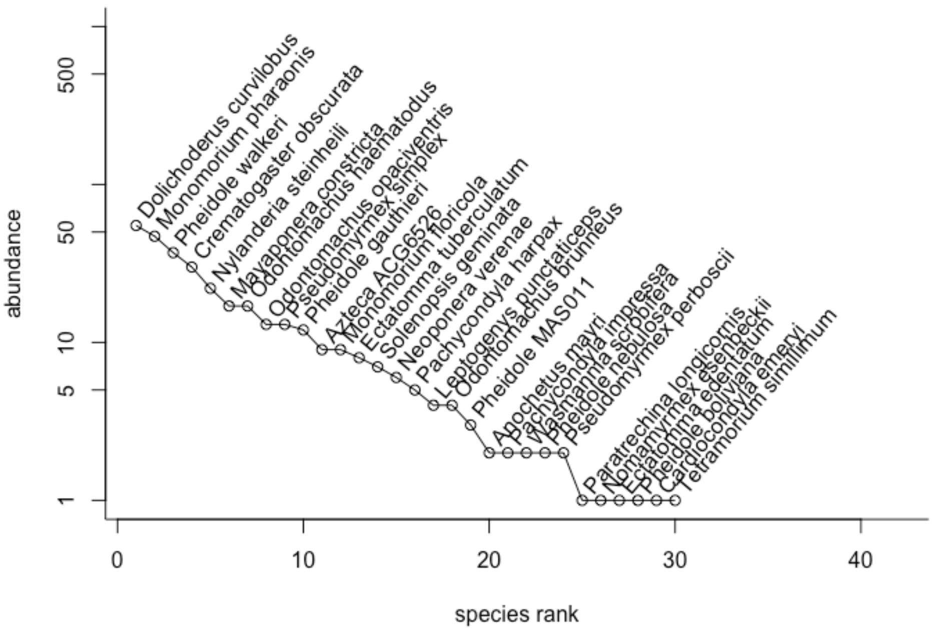
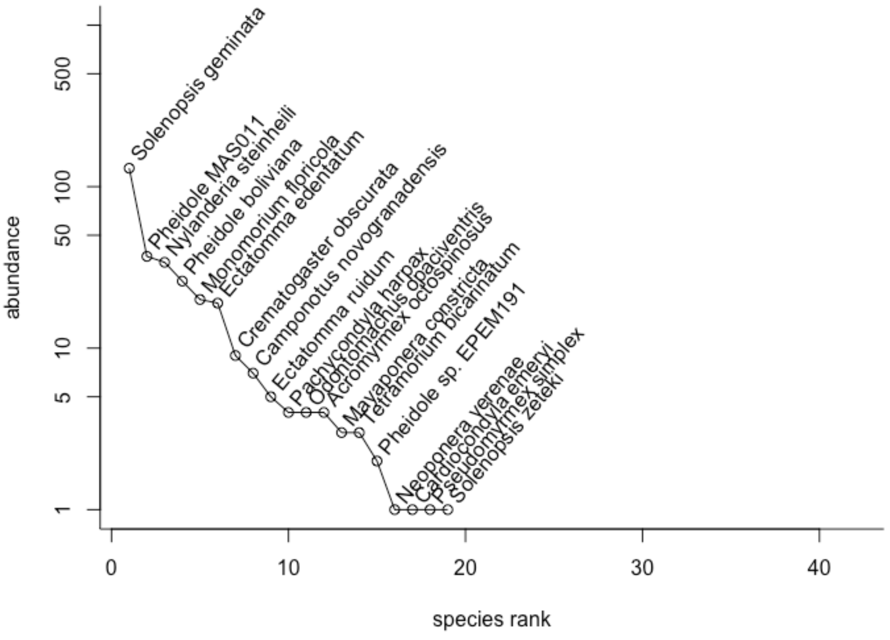


Figure 8 Mean pseudo-abundances of all observed ant species per site with 95% confidence intervals. The maximum abundance each species could get was 5 (meaning it was present in each trap throughout 2 hours of sampling every time the trap was checked).

Using rank abundance curves (Fig. 9), I illustrated how different species richness and evenness were among the four habitats. Finca Torre shows the highest number of species and has the highest species evenness. The curve looks almost identical in shape at Finca La Bolsa, showing some species with a low rank (meaning occurring often), a big group of species with a medium rank and a few which occurred but sporadically. Finca Amable shows quite a different pattern with a relatively smaller pool of species and with only one species – *Solenopsis geminata* – occupying the highest rank. There is again a pool of species that occurred more than a few times and just a few that occurred sporadically. Finca Eduardo shows again a completely different pattern with *Solenopsis geminata* having the highest rank and showing a huge distance in abundance from the species at the next rank. Additionally, Finca Eduardo shows the steepest gradient of the curve suggesting low species evenness.




abundance
1
5
10
50
500
species rank
0
10
20
30
40
Dolichoderus curvilobus
Monomorium pharaonis
Pheidole walkeri
Crematogaster obscurata
Nylanderia steinheili
Mayaponera constricta
Odontomachus haematodus
Odontomachus opaciventris
Pseudomyrmex simplex
Pheidole gauthieri
Azteca ACG6526
Monomorium floricola
Ectatomma tuberculatum
Solenopsis geminata
Neoponera verenae
Pachycondyla harpax
Leptogenys punctaticeps
Odontomachus brunneus
Pheidole MAS011
Anochetus mayri
Pachycondyla impressa
Wasmannia scrobifera
Pheidole nebulosa
Pseudomyrmex perboscii
Paratrechina longicornis
Nomamyrmex esenbeckii
Ectatomma edentatum
Pheidole boliviana
Cardiocondyla emeryi
Tetramorium simillimum


abundance
1
5
10
50
100
500
species rank
0
10
20
30
40
Solenopsis geminata
Pheidole MAS011
Nylanderia steinheili
Pheidole boliviana
Monomorium floricola
Ectatomma edentatum
Crematogaster obscurata
Camponotus novogranadensis
Ectatomma ruidum
Pachycondyla harpax
Odontomachus opaciventris
Acromyrmex octospinosus
Mayaponera constricta
Tetramorium bicarinatum
Pheidole sp. EPEM191
Neoponera verenae
Cardiocondyla emeryi
Pseudomyrmex simplex
Solenopsis zeteki

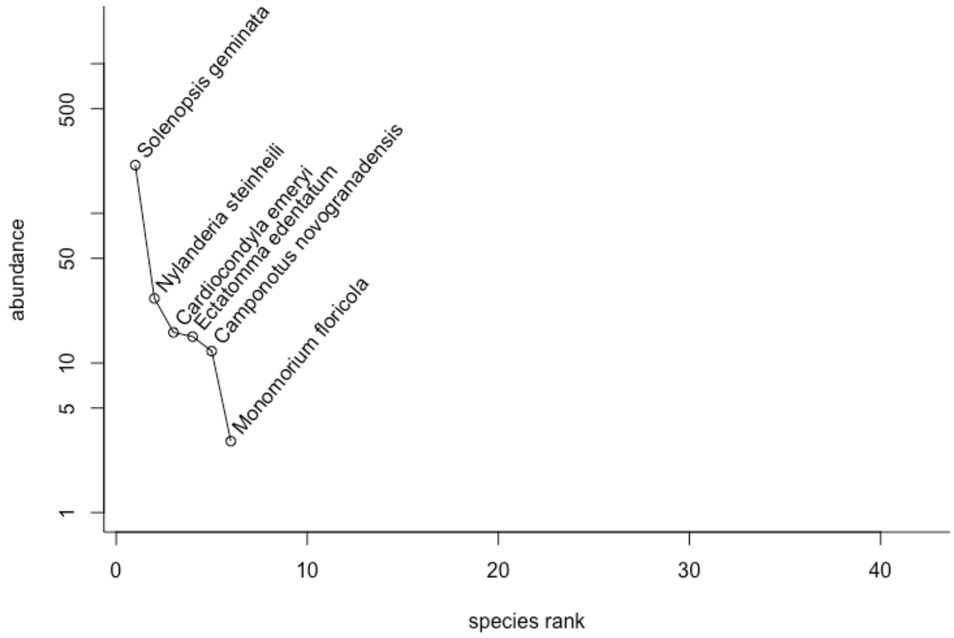


Figure 9 Rank abundance curves based on species abundances taking transects per site as a sampling unit. Note the log-scale of the y-axis. From the top: Torre, La Bolsa, Amable, and Eduardo.

According to the results (Fig. 10) we can see that Finca Eduardo is fully saturated meaning that extending sampling effort would likely not increase the recorded number of species. In three other habitats increased sampling effort might add a few species. Additionally, ant species richness in Finca Bolsa is very close to Finca Amable. At a standardized number of 200 sampling units, the four reforestation sites fall into three groups with regard to their overall ant species richness: Torre (the oldest forest site); Amable + Bolsa (mid-aged reforestations); and Eduardo (the youngest reforestation).

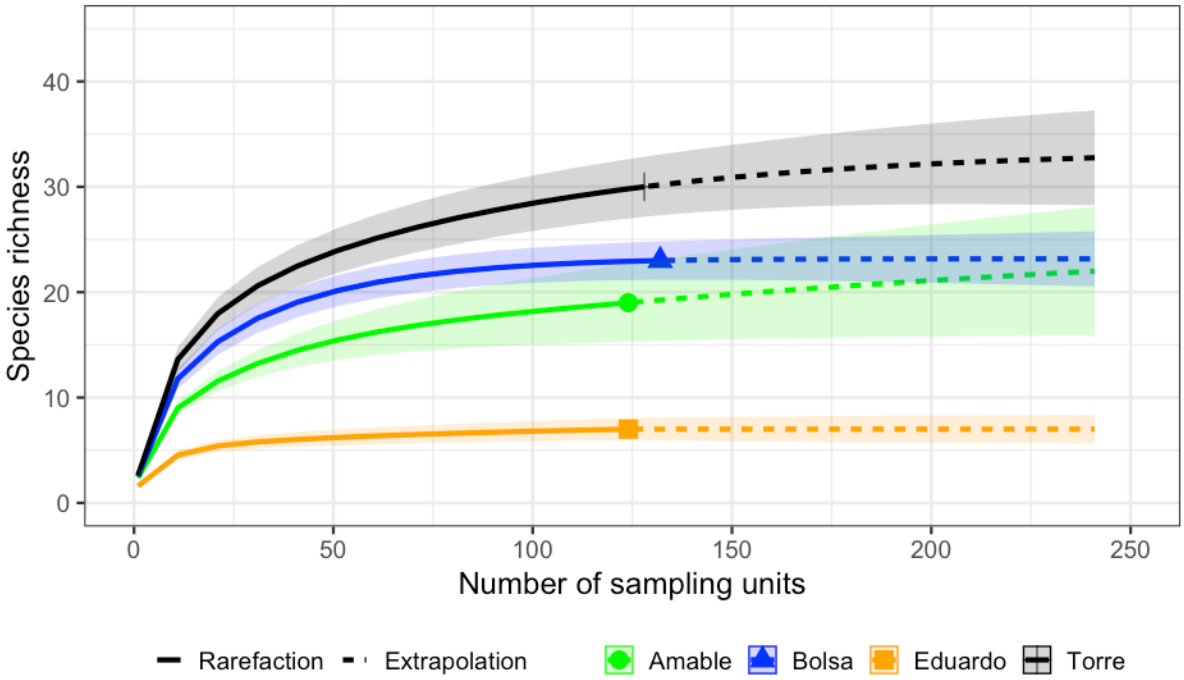


Figure 10 Species richness accumulation curves with 95% confidence limits (shaded areas) based on species incidences at each site. Sampling units were transects and the number of sampling units was the sum of abundances at each site.

3.2 Community composition analysis

Species abundance was used for computing a distance-based analysis of ant community composition among all four reforestation sites. First an unconstrained ordination (non-metric multidimensional scaling, NMDS) was computed to check for compositional differences between sites (Fig. 11). Afterwards a constrained ordination was computed to check what ecological factors might cause those differences (Fig. 16). As expected, ant communities differed significantly among the sampling sites with Finca Torre showing the highest diversity in ant composition and with the youngest reforestation site – Finca Eduardo – showing the lowest compositional diversity. A PERMANOVA test showed all these differences to be highly significant. Additionally, a multivariate test for within group dispersion was computed. The results (Fig. 12) showed a significant compositional difference within groups with Finca Torre having the highest compositional diversity and Finca Eduardo the lowest. Distances of individual bait trap sites from the centroids of each reforestation site reflect the compositional complexity of the ant assemblages at each reforestation site.

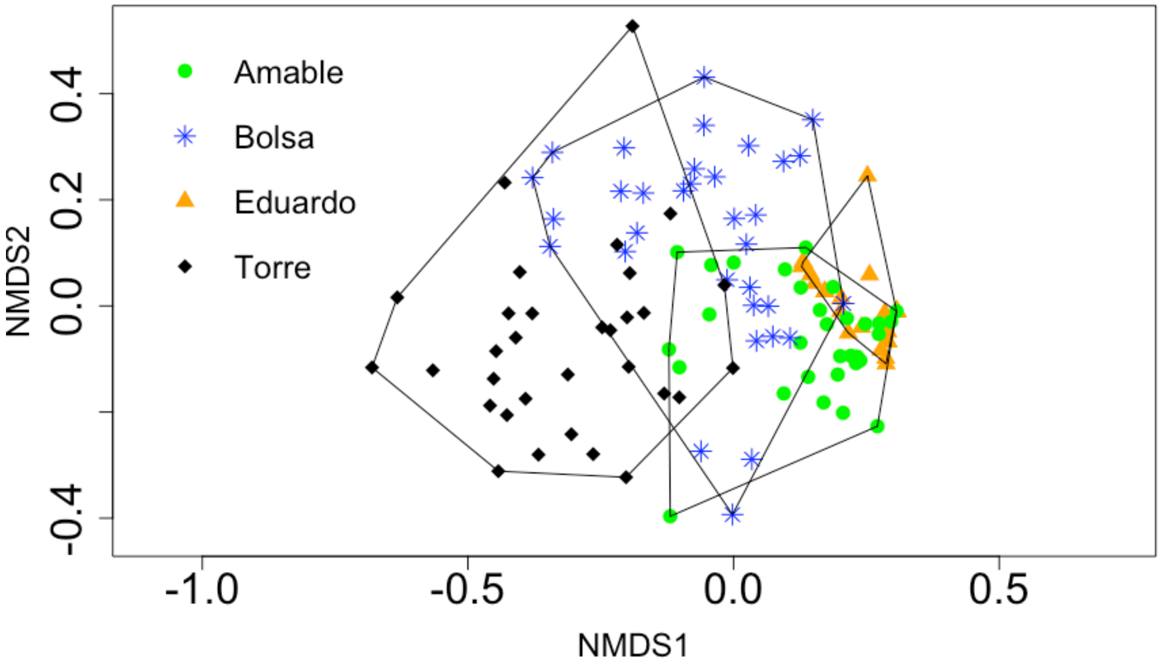


Figure 11 NMDS ordination of ant assemblages based on Bray-Curtis distances. A gradient in species composition and dispersion is visible, starting from the left (old-growth forest: Torre), across La Bosa and Amable to Eduardo (youngest reforestation site). Stress: 0.142. Data used is abundance data with individual transects per site as sampling units. PERMANOVA: F = 18.127, df=3, p<0.001.

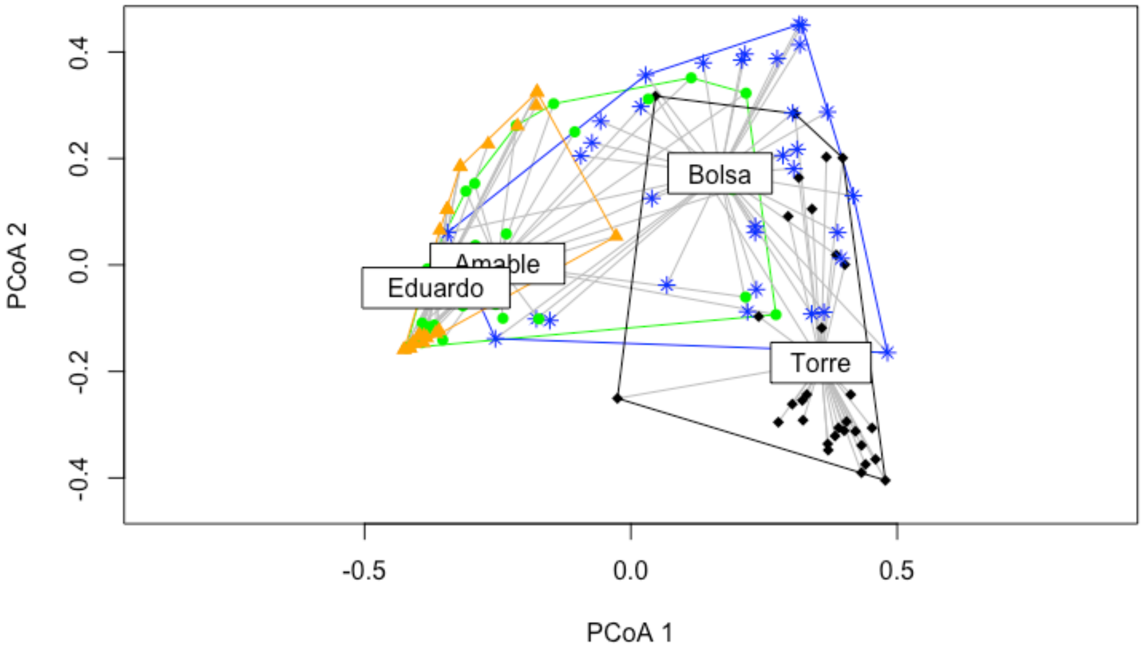


Figure 12 Principal coordinate analysis plot showing the compositional differences within habitats. Groups with points of a higher distance from the center show a greater compositional difference. Analysis of variance: F=33.769, df=3, p<0.001.

To further analyse ant community composition, I used a constrained analysis to check for an influence of environmental variables on ant community composition. After performing a PERMANOVA test I chose only environmental variables that contributed significantly to explaining ant community composition. A sequential matrix regression revealed that mean leaf litter thickness (F=29.66, p<0.001), herb layer cover (F=7.0603, p<0.001) and mean tree circumference (F=6.847, p<0.001) all contributed significantly to explaining variation in ant-assemblage composition at the individual transects.

Collectively, these three variables captured 26.2% of local variation in ant species composition. Those variables that created the best fit for the model were chosen for computing an NMDS ordination based on the results from the constrained analysis (Fig. 13). Tree circumference and leaf litter height seem to influence the composition of the communities characteristic for Finca Torre and the oldest reforestation site – La Bolsa. Herb layer explains the composition of younger reforestation sites – Finca Amable and Eduardo.

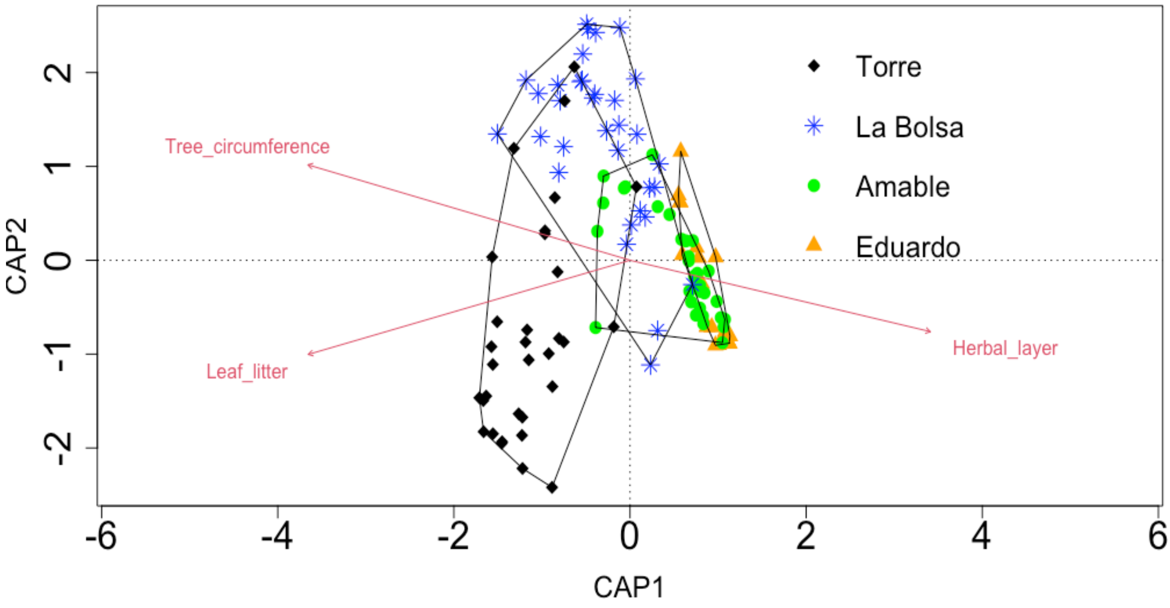


Figure 13 Ordination plot of a capscale matrix regression (based on Bray-Curtis similarities of ant assemblages) showing the influence of three explanatory variables on ant community composition among reforestation sites. Ant abundance (per transect) and vegetation data (leaf litter(mean), herb layer, tree circumference(mean), presence of lianas and epiphyte coverage) were initially included in the model. The three variables that contributed significantly to explaining variance in ant species composition (PERMANOVA) are illustrated as vectors.

3.3 Trait correlation and community weighted means of traits

I computed a Pearson correlation to look at correlation between traits on species level. As expected certain traits exclude each other and others co-occur. The highest correlation turned out to be between trait “Trophobiosis/nectarivory” and “ Polydomous” suggesting that ant species that are flexible on food preferences often have more than one nest per colony. At the same time species that are polydomous are not expected to have polymorphic workers as those traits appear to exclude each other (Fig. 14).

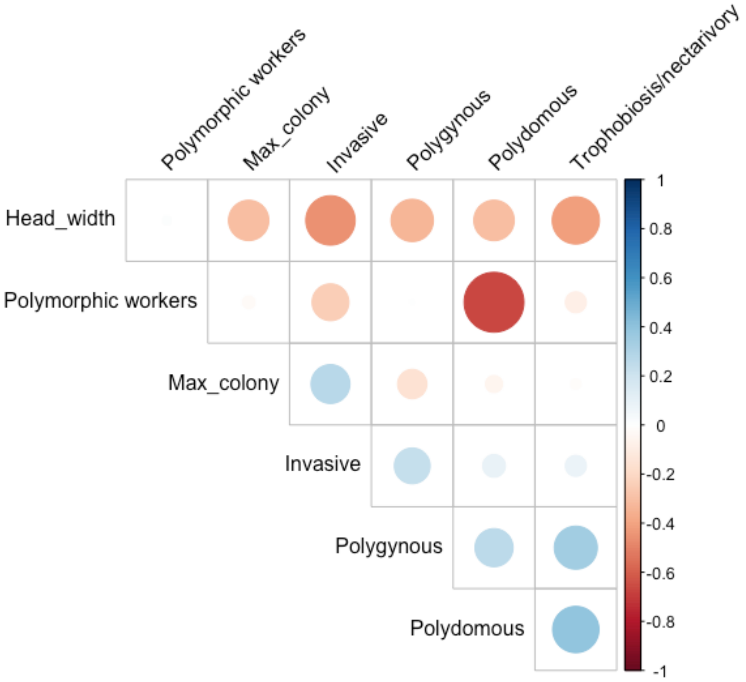
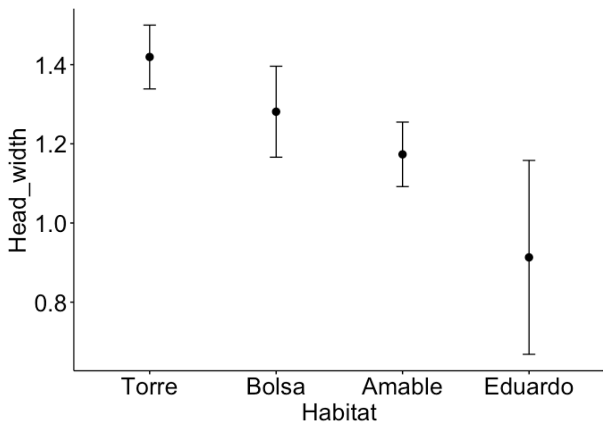
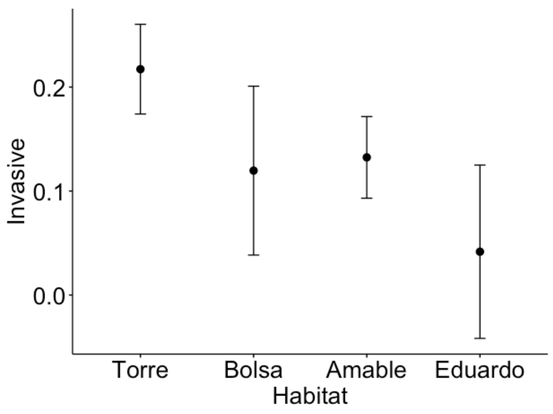
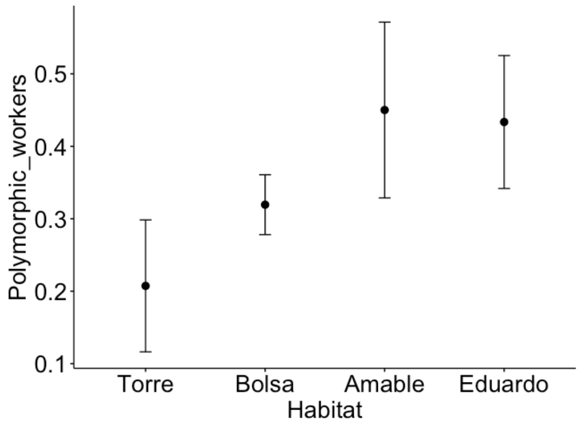


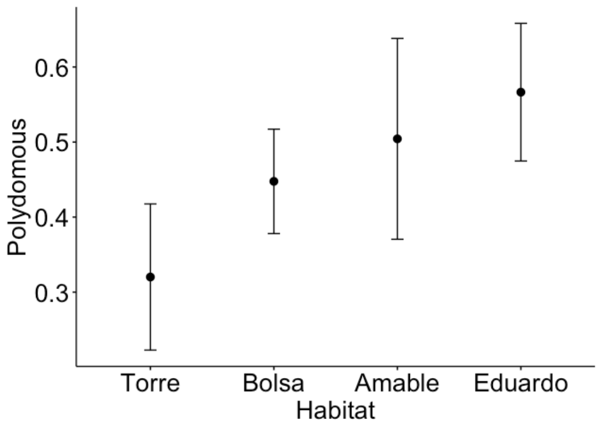
Figure 14 Pearson correlation matrix based on species traits data showing which traits tend to co-occur or rather exclude each other on the species level.

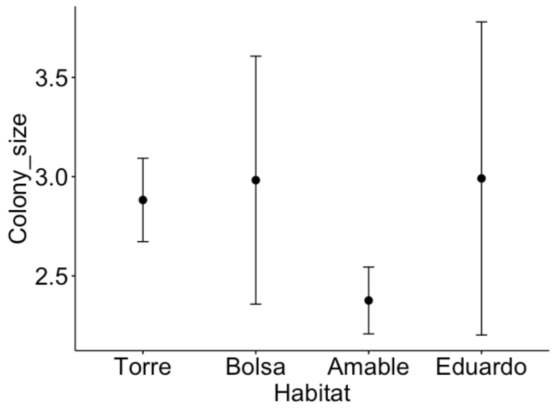
Using pseudo-abundances of species, I calculated community weighted means (CWM) at a level of a sampling site, of all the traits that I collected to describe identified species. They were used to illustrate the distribution of certain traits among the whole pool of species collected during the study (Fig. 15). Not every trait turned out to be informative in my data set (i.e., colony size or invasiveness) but some results show an explanation of certain traits being more abundant among certain habitats. High head width value is very prevalent at Finca Torre and Finca Bolsa while being significantly lower at Finca Eduardo. Obviously, species which are more dominant have a bigger head to achieve advantage over less dominant species. On the other hand, traits like feeding through trophobiosis or the occurrence of a polymorphic worker caste are more prevalent at Finca Amable and Eduardo and characterize omnivorous species which were more prevalent at those sampling sites.

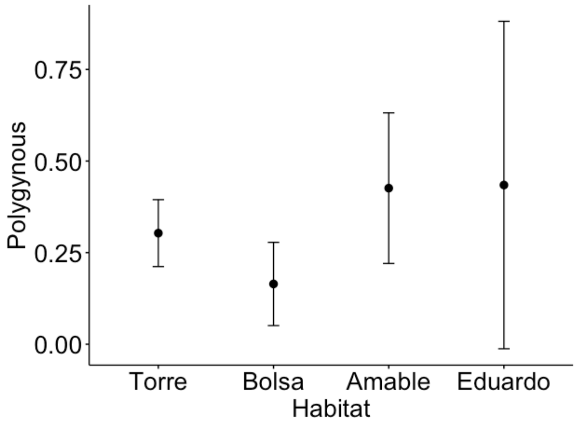

Head_width
1.4
1.2
1.0
0.8
Torre
Bolsa
Amable
Eduardo
Habitat


Invasive
0.2
0.1
0.0
Torre
Bolsa
Amable
Eduardo
Habitat


Polymorphic_workers
0.5
0.4
0.3
0.2
0.1
Torre
Bolsa
Amable
Eduardo
Habitat


Polydomous
0.6
0.5
0.4
0.3
Torre
Bolsa
Amable
Eduardo
Habitat


Colony_size
3.5
3.0
2.5
Torre
Bolsa
Amable
Eduardo
Habitat


Polygynous
0.75
0.50
0.25
0.00
Torre
Bolsa
Amable
Eduardo
Habitat

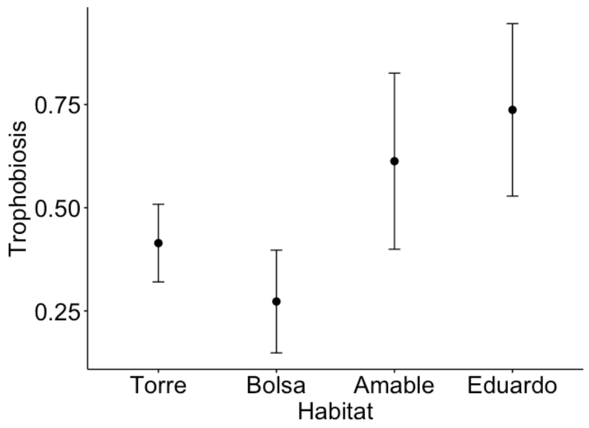

Trophobiosis
0.75
0.50
0.25
Torre
Bolsa
Amable
Eduardo
Habitat

Figure 15 Error plots with 95% confidence levels of community-weighted means for seven traits collected for the sampled ant species.

Based on the community weighted means I computed a principal component analysis (PCA) to illustrate which variables best describe the ant communities. Four variables: “Head width”, “Trophobiosis”, “Polydomous” and “Polymorphic workers” together explained 80% of variance suggesting that those traits explain the composition of community the best. The resulting ordination plot (Fig. 16) shows that there is a definite separation between old-growth forest together with the oldest reforestation site versus the two younger reforestation sites. Larger head width defined the ant community of advanced succession habitats (Finca Torre, Finca La Bolsa), whereas ant species with polymorphic workers, polydomy and showing intense trophobiotic feeding are characzteristic for the less developed succession habitats (Finca Amable, Eduardo).

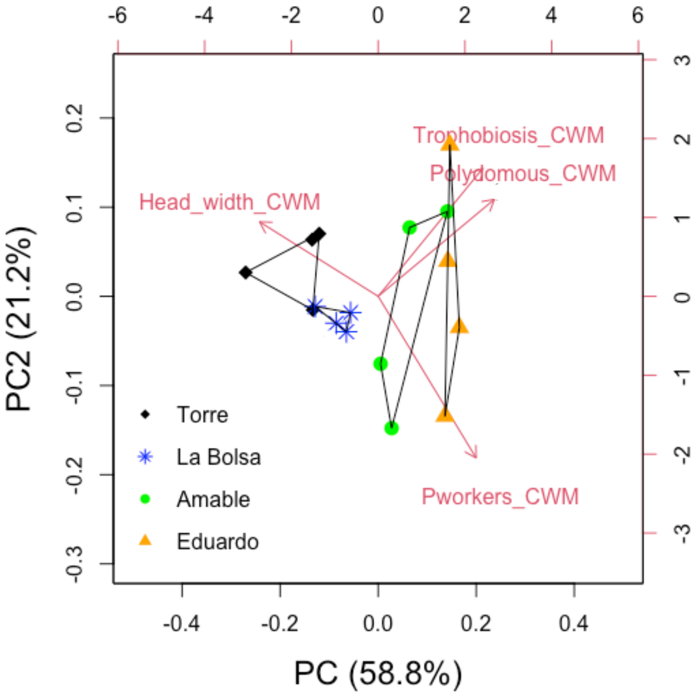


Figure 16 Ordination plot of a principal component analysis on community-weighted means of species traits, using pseudo-abundances, showing (as vectors) which species traits explain most of the variance on the community level.

4. Discussion

4.1 Species richness

Overall, I collected and identified 43 species, from 24 genera and seven subfamilies. Due to bureaucratic issues export of all the samples was unfortunately impossible and therefore I assume the realistic number of species was distinctly higher. *Pheidole* genus was probably the one for which the actual species number was underestimated due to export permit issue. DNA barcoding results revealed multiple still undescribed *Pheidole* species, and it only came from a few specimen samples. Another genus which I expect to show a higher species number was *Odontomachus* due to several situations of ants of that genus running away from the trap when sampled. Six species are considered invasive in that region. All the identified species considered invasive in the Neotropical region have a broader range of distribution. Primary forest, as expected, harbored most identified species, but the oldest secondary forest had a species number close to the one in old-growth forest. As mentioned before these numbers are only an approximation of the actual species richness of my sites due to identification difficulties at the station and export permit regulations. Nevertheless, these numbers show a trend that can be used to estimate the succession status of sampled reforestation sites. The study done by Falk et al. in the same region covered multiple sampling methods (hand sampling, pitfall traps and Winkler method) (Falk et al.2019). The species number is substantially higher, but the number of workers in my study was way higher because of the form my study had (Ant workers accumulated in large numbers in the traps). Comparing to research done in tropical secondary forest the number of species is significantly smaller due to only one sampling method. Usually studies cover multiple sampling methods, for example leaf litter samples and pitfall traps (Rocha-Ortega et al. 2013). A study conducted in Amazon with only pitfall traps and for a shorter period resulted in a very similar species number to my study (Santos et al. 2021). Bait samplings has many limitations as it only captures ground-dwelling omnivorous ants whose diet includes protein-based food products, foraging during the day. This means that groups like leaf-cutting ants (captured by Winkler method), highly specialized predators (i.e., army ants), arboreal ants, and ants inhabiting myrmecophytes shouldn't be caught by this sampling method. In practice some of arboreal ants were captured by my traps but they were a minority in identified samples. Pitfall traps are capturing more species as they are left overnight compared to baited traps being left for maximum few hours. My results showed that the oldest reforestation site starts to remind the community of Finca Torre twelve years after re-planting but remains still somewhere intermediate between Finca Amable and old-growth forest. There were only three species that occurred at all sampling sites (Figure 2). Their bionomics confirm that they all can survive in disturbed habitats and have very generalized habitat requirements (Achury et al. 2018). Those species included *Nylanderia steinheili* a species native to the Neotropics which is common in open areas (Williams et al. 2020). The spread of the species can be increased by temperature increase and transporting of goods (Williams and Lucky 2020). Another species found at all sampling sites was a fire ant, *Solenopsis geminata* whose native range includes Central and South America and whose abundance in disturbed areas tends to increase (Gotzek et al 2015). Today, *Monomorium floricola* is a worldwide distributed tramp ant considered widely spread in tropical countries (Wetterer 2010). Their occurrence in reforestation sites seems to be understandable, whereas the fact they were present in Finca Torre might be caused by human impact on primary forest. Another tramp species, *Monomorium pharaonis* was surprisingly present only at Finca Torre and showed a very high abundance there. *M. pharaonis* initially came from the

West Indies but is today very highly spread worldwide (Wetterer 2010) and its occurrence at Finca Torre can be again connected to human activity there. These observations indicate that Finca Torre, even though characterized as old-growth forest by its vegetation, suffers from faunal change induced by human activities nearby. Studies proved that due to habitat fragmentation invasive ants tend to colonize edges of forests due to more extreme conditions that are not favorable for native species (Crist,2009).

Looking at functional groups, as expected, Finca Torre covered all the functional groups identified at all sampling sites (Figure 5). In contrast, Finca Eduardo covered only two functional groups, with generalized omnivores representing more than 90% of identified species. This was expected as Finca Eduardo has very young trees, its vegetation is dominated by herbs and the whole was still mostly an open area at the time of my ant sampling. Finca Amable covered an additional functional group, viz. generalized predators. This sampling site has significantly more canopy coverage than Finca Eduardo but is still not as dense as an old-growth forest. Additionally, it is prone to floodings due to its proximity to a river, which can influence ant species richness especially in the rainy seasons as well. According to Baccaro et al. number of specialized ant species decrease in the face of increasing water table level, while number of generalized species remains the same (Baccaro et al. 2013). Additionally, activity of leaf-cutting ants and number of their nests is decreasing in the proximity of high-water levels (Sendoya et al. 2014). Finca Amable is a relatively young reforestation site, and it was not expected to see many specialized species there, but flooding could influence the ratio of more specialized-generalized species during the rainy season. Three species were “characteristic” for this site as their abundance was highest there or they were even exclusive to that site. Two *Pheidole* species (*P. boliviana* and *P. MAS011*) showed a very high abundance at Finca Amable, with an undescribed species *P. MAS011* occurring only there. *Pheidole* is one of the most speciose animal genera consisting of 900 taxonomically defined species with many being still undescribed. It is very diverse and abundant around the world but particularly prevalent in tropical regions (Wilson 2003). *Pheidole* species identified in my study were all generalized omnivores. *Dolichoderus curvilobus* was very abundant at Finca Torre and Finca La Bolsa (and present only there). It is an arboreal species whose main food source is nectar. They build big colonies and have an aggressive behavior when defending their nest. They were very abundant in that region as it was present almost daily in my traps. Another surprising although rather coincidental were two species from *Pseudomyrmex* genus. It’s a genus inhabiting plants (*Acacia* in Central America) species. They are feeding on plant-derived food bodies and extrafloral nectars (Kautz 2009).

4.2 Ant community composition

Community composition of ants is influenced by both biotic and abiotic factors (Boet et al. 2020). The major biotic factor is considered to be competition between ants (Cerda et al. 2013). It is very difficult to “capture” competition, but resource limitation is a key in understanding competition and co-occurrence of species (Law and Parr. 2020). Several studies proved that arboreal ant species co-occur less frequently than expected by chance. Dominant ant species do not co-occur due to competitive exclusion (Sanders et al.2007). According to Law and Parr (2020), canopy ant assemblages show a higher nitrogen limitation and dominant species monopolize resources high in protein. This could explain some arboreal species occurring in my protein bait traps offered on the ground. Similar study was made for ground-dwelling ants by Carval et al. and their results showed that some species in a very simple banana agroecosystem have a higher probability of co-occurring and the other ones excluding each other, but at the same time not being able to maintain numerical dominance in a trap not always led to a benefit of another species suggesting that another species needs to have a similar niche to replace another species (Carval et al. 2016). As mentioned before, competition is difficult to quantify and therefore biotic factors are often used as templates because of their structure and function (Conceição-Neto et al. 2020). Leaf litter is providing food and shelter for ground-dwelling ants and depending on tree species and density litter coverage varies substantially across forest types (Blüthgen and Feldhaar, 2010). Moreover, canopy characteristic like crown size and leaf density can create small-scaled differences in solar radiation and soil humidity which can also influence ant communities (Mejía-Domínguez et al. 2011). Based on unconstrained NMDS ordination I observed significant differences in community composition between and within study sites (Figure 14). Dispersion analysis revealed that Finca Torre showed the greatest within-site variation in species composition, due to the highest differences between individual traps and the highest species turnover after one hour of sampling. Ant samples in bait traps in Finca Eduardo, in contrast, showed very limited variation. The number of identified species there was significantly smaller compared to all other sampling sites and there were very little differences between traps (Figure 11). This indicates that on the youngest reforestation site a very homogeneous ant assemblage, structured by a very small number of locally dominant species. Constrained analysis showed that community composition differs along the environmental variables (Figure 13). The shift in ant species composition observed in old-growth Forest and at Finca La Bolsa was clearly related to the higher leaf litter coverage and larger mean tree circumference at these sites. As mentioned before leaf litter has a significant impact on ant communities and with increasing density of trees the leaf litter coverage increases respectively. Mean tree circumference is a proxy for the complexity of canopy and higher tree circumference is also characteristic for more advanced ecosystems. “Ant-Biomass Paradox” is a theory created because of a very high ant abundance in comparison to food sources. Studies revealed that arboreal ant nutrition is a spectrum with a very few true specialists and very pronounced omnivores (Davidson and Patrell 1996). Several studies used stable isotope ^{15}N to check for food partitioning among arboreal ant species. Results reveled that only very few ant groups (mostly army ants and *Leptogenys* are strictly predacious, whereas ponerine ants that are considered predators or scavengers were also found on extrafloral nectar (Blüthgen et al. 2003). The ant assemblages at the younger reforestation sites were related to the higher herb layer coverage on these young afforestations.

4.3 Community weighted means and trait co-occurrence

Complementary to the community analysis based on taxonomic identity of ants, I also explored how ecologically relevant species traits were distributed across the reforestation sites. Species traits were analyzed on three levels. Pearson correlation was used to check for correlation between traits on species level (Figure 14). This revealed that certain traits exclude each other. Traits that exclude each other the strongest in the set of species encountered were the occurrence of polymorphic workers and polydomy. Traits like polydomy and polygyny were positively correlated as was also mentioned in literature (Ellis et al.2014). Invasiveness tend to exclude the trait “head width” as large head size is rather associated with predacious ant species. Next, community weighted means of local assemblages were calculated to check for the possible prevalence of certain traits on community level at each sampling site (Figure 15). A high value of head width was significantly more prevalent in Finca Torre and La Bolsa compared to Finca Amable and Eduardo. Most of the species identified at younger sites were very small and therefore their head width was consistent. Dominant species who feed mostly on arthropods need to have bigger heads as compared to generalized omnivores. This trait is problematic due to worker polymorphism in some species. Considering that by polymorphic species workers of different sizes play different roles in a colony, larger species tend to be present in food retrieving (Wills et al. 2018) and comparison of sampled workers size might not be fully correct. Workers of species identified at older reforestation site or old-growth forest that monopolized the traps as first were bigger, meaning that those who appear at first are more advanced at locating resources. In the study of Gibb et al. 2015 it was proven that in general larger body size is correlated to more closed habitats with dense canopy but a large head size was rather characteristic for species favoring open habitats (Gibb et al. 2015). Surprisingly, invasiveness as a trait was predominant in all sites with Finca Torre showing a high score due to the abundance of *M. pharaonis.* A study done by Calcaterra et al. checked for habitat preference of several invasive ants sampled in Natural Reserve consisting of multiple habitat types. Their results revealed that almost every invasive species had a habitat preference, including wetland, low grasslands prone to floodings, or forest (Calcaterra et al.2016). This could explain why *M. pharaonis* was so abundant in Finca Torre and not present at any other sampling site. Another trait that seems to contribute to community composition is feeding on nectar (trophobiosis/nectarivory). This trait was used to distinguish species having more specialized diet and those who are more generalist about their food resources. Dominant species are considered to monopolize protein resources and more rare species are often more flexible with their food source preferences and choosing carbohydrate sources (Law and Parr, 2019). Therefore, “Trophobiosis/nectarivory” was a trait explaining the community of younger reforestation sites more significantly as baits in older reforestation site or old-growth forest were monopolized by dominant species. A study conducted by Floren et al. 2002 compared ratio of predacious and non-predacious ants on trees in a tropical lowland forest. Additionally, due to resource availability honeydew and nectar are very important components of ants in tropical forest ecosystems and ants from lower trophic levels are more abundant than pure predators (Blüthgen et al. 2003). The results showed a drastic change in that ratio suggesting disturbed habitats have a significantly higher number of non-predacious ants (Floren et al. 2002). When computing a PCA ordination based on the CWMs of the ant species traits I observed that ant assemblages segregated into those in old-growth forest and Finca La Bolsa on the one hand, and Finca Amable and Eduardo on the other, suggesting that variation in species traits explains the differences in ant community composition of older versus younger sites (Figure 16).

5. Conclusions

My results have showed that based on four sampling sites we can already observe species number differences between younger reforestation sites and the oldest reforestation site. 12-year-old reforestation site had a species number almost as high as the primary forest. Nevertheless, the composition of communities differs among sites and species turnover in traps occurred only in old-growth forest and the oldest secondary forest. Only primary forest covered all functional groups and it decreased very significantly with younger secondary forests leading to the youngest one having species from only two functional groups. Based on information gathered on ant species traits we could see certain traits being more prevalent at younger sites and others at older ones. A high number of species was characteristic only for primary forest whereas the youngest reforestation site had no unique species. Based on these results we can assume recovery of ant community takes more than 12 years as we cannot say the oldest reforestation site was fully recovered judging from compositional differences from primary forest. Abundance of invasive species in primary forest might rise an important issue that might arise with reforestation projects. They should not cause danger to native fauna, but it should be closely observed during possible conservation projects. It should be important to promote recovery of ant communities due to their important role in tropical ecosystems. Homogenous ant community allows only species of very low trophic levels and therefore excludes higher trophic level ants who play multiple important roles in the community. Ants live in a symbiosis with myrmecophile plants and perform trophobiosis with honey-dew producers and extrafloral nectar producing plants. On top of that they are the main redistributors of nutrients, biological pest control and seed dispersers. Their abundance is crucial for functional ecosystems and therefore further research on ant recovery during the reforestation process should take place.

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Appendix

Table A All identified species with functional group and sampling site abundance.

Species	**Functional group**	**Torre**	**Bolsa**	**Amable**	**Eduardo**	**Number of sites**
Camponotus novogranadensis	AO	0/19	0/19	7/19	12/19	**2**
Nylanderia steinheili	GO	21/138	55/138	36/138	26/138	**4**
Paratrechina longicornis	GO	1/1	0/1	0/1	0/1	**1**
Azteca ACG6526	AO	9/9	0/9	0/9	0/9	**1**
Tapinoma ramulorum	AO	0/2	0/2	0/2	0/2	**1**
Dolichoderus curvilobus	AO	55/68	13/68	0/68	0/68	**2**
Nomamyrmex esenbeckii	RHP	1/1	0/1	0/1	0/1	**1**
Ectatomma edentatum	GO	1/37	0/37	21/37	15/37	**3**
Ectatomma ruidum	GO	0/5	0/5	5/5	0/5	**1**
Ectatomma tuberculatum	GGP	8/8	0/8	0/8	0/8	**1**
Gnamptogenys hartmani	GGP	0/5	5/5	0/5	0/5	**1**
Anochetus mayri	GGP	2/2	0/2	0/2	0/2	**1**
Leptogenys punctaticeps	GGP	4/4	0/4	0/4	0/4	**1**
Odontomachus bauri	GGP	0/6	6/6	0/6	0/6	**1**
Odontomachus brunneus	GGP	4/4	0/4	0/4	0/4	**1**
Odontomachus haematodus	GGP	18/18	0/18	0/18	0/18	**1**
Odontomachus opaciventris	GGP	15/28	9/28	4/28	0/28	**3**
Pachycondyla harpax	GGP	6/13	4/13	4/13	0/13	**3**
Pachycondyla impressa	GGP	2/10	8/10	0/10	0/10	**2**
Neoponera verenae	GGP	6/7	0/7	1/7	0/7	**2**
Mayaponera constricta	GGP	17/26	6/26	3/26	0/26	**3**
Acromyrmex octospinosus	LC	0/5	5/5	0/5	0/5	**1**
Pheidole MAS026	GO	0/27	27/27	0/27	0/27	**1**
Pheidole boliviana	GO	1/45	15/45	29/45	0/45	**3**
Pheidole vorax	GO	0/2	2/2	0/2	0/2	**1**
Pheidole radoszkowskii	GO	0/2	2/2	0/2	0/2	**1**
Pheidole nebulosa	GO	2/2	0/2	0/2	0/2	**1**
Pheidole sp. EPEM191	GO	0/2	0/2	2/2	0/2	**1**
Pheidole MAS011	GO	3/41	0/41	38/41	0/41	**1**
Pheidole gauthieri	GO	12/65	53/65	0/65	0/65	**2**
Pheidole walkeri	GO	38/42	4/42	0/42	0/42	**2**
Wasmannia scrobifera	GO	2/2	0/2	0/2	0/2	**1**
Cardiocondyla emeryi	GO	3/50	30/50	1/50	16/50	**2**
Crematogaster obscurata	GO	29/73	35/73	9/73	0/73	**3**
Tetramorium bicarinatum	GO	0/4	1/4	3/4	0/4	**2**
Tetramorium simillimum	GO	1/4	3/4	0/4	0/4	**2**
Monomorium floricola	AO	7/32	3/32	19/32	3/32	**4**
Monomorium pharaonis	AO	43/43	0/43	0/43	0/43	**1**
Solenopsis geminata	GO	7/386	38/386	132/386	209/386	**4**
Solenopsis zeteki	GO	0/10	7/10	3/10	0/10	**3**
Pseudomyrmex perboscii	AP	2/2	0/2	0/2	0/2	**1**
Pseudomyrmex simplex	AP	12/16	4/16	0/16	0/16	**2**