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Experimental suppression of ants foraging on rainforest vegetation in New Guinea: testing methods for a whole-forest manipulation of insect communities

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Abstract. 1. Ants are extremely abundant in lowland tropical forests where they are important predators, plant mutualists, and herbivores. Their complex role in tropical plant–insect food webs can be best assessed by experimental manipulation of their abundance. Historically, ant exclusion experiments have had a small-scale focus, such as single trees. Here, we test a new ‘whole-forest’ method of ant exclusion, using treated canopy bait stations, in a diverse tropical rainforest in New Guinea.

2. We conducted a 10-month manipulative experiment in primary and secondary rainforests. In each forest type, a 625 m^2 treatment plot was isolated from the surrounding forest and 135 bait stations treated with fipronil, S-methoprene, and hydramethylnon were placed in trees to suppress ants. Ant activity was monitored in the forest canopy and understorey with an additional 65 stations in treatment and control plots.

3. We achieved a dramatic decline in ant abundance in treatment plots compared with controls in both forest types, with an average decrease in ant numbers per station of 82.4% in primary and 91.2% in secondary forest. In particular, native dominant species *Oecophylla smaragdina*, *Anonychomyrma* cf. *scrutator* in primary forest, and invasive *Anoplolepis gracilipes* in secondary forest were greatly affected. In contrast, *Tapinoma melanocephalum* flourished in treatment plots, perhaps benefiting from reduced competition from other ant species.

4. Our study demonstrates that it is possible to selectively eradicate most of the foraging ants in a structurally complex tropical forest. We propose whole-forest manipulation as a novel tool for studying the role of ants in shaping plant–insect food webs.

Key words. Ant exclusion, bait traps, canopy, food webs, Formicidae, tropical forests.

Introduction

Ants are extraordinarily abundant insects in lowland tropical forest canopies, where they can represent 20–40% of all arthropod biomass (Floren & Linsenmair, 1997; Davidson *et al*., 2003). They are therefore likely to have a major influence on the structure of forest plant–insect food webs at both evolutionary and ecological scales (Hölldobler & Wilson, 1990; Heil & McKey, 2003; Moreau *et al*., 2006).

Ants are considered a keystone group of invertebrate predators in tropical forests, exerting high predation pressure on insect herbivores (e.g. Olson, 1992; Novotny *et al*., 1999; Floren *et al*., 2002). However, many ant species also act as mutualists of plants or of insect herbivores (Davidson *et al*., 2003; Blüthgen *et al*., 2004). These mutualisms may provide the major resources needed to maintain the exceptionally high biomass of ants in tree canopies via ‘cryptic herbivory’ on exudates produced by the symbionts (Hunt, 2003).

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Plant–insect food webs of tropical forests are so complex that manipulative experiments are one of the few feasible approaches to their study (Morris *et al*., 2004). This is particularly true for ants, with their multiple and often conflicting roles in the same ecosystem. Although numerous ant exclusion experiments have been conducted in tropical environments, they were usually limited to small spatial scales, typically individual trees or tree branches, and focused on specialised interactions, such as ants on myrmecophytic plants or extrafloral nectaries (e.g. Koptur, 1984; Fiala *et al*., 1994; Dejean *et al*., 2006; Agarwal & Rastogi, 2008). Such small-scale manipulations generally resulted in higher abundance of herbivores (Whalen & Mackay, 1988; Fiala *et al*., 1994) and/or greater herbivore damage of plant leaves in the absence of ants (Koptur, 1984; De La Fuente & Marquis, 1999; Dejean *et al*., 2006). The removal of ants on larger spatial and temporal scales has not been attempted in the tropics, with the exception of a few specific studies that aimed to remove a single dominant species (see Majer, 1976; Andersen & Patel, 1994; Adams & Tschinkel, 2001; Gibb & Hochuli, 2004; Abbott & Green, 2007; Hoffmann, 2010).

Small-scale exclusion experiments in a forest are unduly influenced by the surrounding non-manipulated matrix. For example, the results of ant eradication from a single tree would still depend on the colonisation of the tree by other arthropods from the neighbouring trees. Herbivore assemblages on tropical trees comprise many rare species and are heavily dependent on such immigration (Novotny & Basset, 2000). Large, 'whole-forest' experiments that manipulate components of local food webs over relatively large areas of diverse vegetation are therefore required in the tropics (Dyer & Letourneau, 2003; Morris *et al*., 2004). However, such studies on ants have not been attempted, probably due to practical and logistical difficulties.

Here, we tested the feasibility of broad-scale and selective exclusion of ants from the entire above-ground vegetation within a contiguous patch of diverse lowland rainforest, using a novel method of bait stations treated with insecticides and placed directly in the forest trees. We focused primarily on ant species foraging on vegetation, i.e. those most likely interacting with insect herbivores (Novotny *et al*., 1999; Floren *et al*., 2002), homopteran symbionts, and plant nectaries (Davidson *et al*., 2003; Blüthgen *et al*., 2004).

Materials and methods

Study area

The study was conducted between June 2007 and April 2008 in a tropical lowland forest near Wanang village, Madang province, Papua New Guinea (100–200 m a.s.l., 5° 14′ S 145° 11′ E). The site has a mosaic of primary and secondary vegetation and is part of an extensive region of mixed evergreen forest on latosols in the basin of the Ramu river (Paijmans, 1976; Wood, 1982). The climate in the region is humid with mean annual rainfall of 3500 mm, a mild dry season from July to September, and mean monthly temperature of 26 °C. (McAlpine *et al*., 1983).

Local primary forest vegetation was characterised by 1348 stems per ha with diameter at breast height (DBH) > 5 cm, consisting of 215 species, having a basal area of 33.2 m^2, and canopy up to 45 m; secondary forest vegetation had 1213 stems per ha, representing 90 species and a basal area 15.0 m^2, and canopy height about 20 m (Damas, 2009). The secondary forest was approximately 10-year-old vegetation regrowth on the site of an abandoned food garden, surrounded by primary vegetation. The primary forest chosen for experiments was part of a 1-ha area marked for traditional slash and burn subsistence farming by indigenous landowners.

Study design

Our experiment followed a before–after-control-impact (BACI) design (Green, 1979) with two treatment levels (ant treatment and control), and 12 levels of time (one before the start of the treatment, 11 during the treatment). Paired treatment and control plots, 25 × 25 m each, were established, one pair in primary and one pair in secondary forest (i.e. one replicate for each forest type). We replicated surveys after treatment but not before treatment as our main focus was to test the methods for a long-term ant suppression. We regard this set up as sufficient for a pilot concept of the study to test the feasibility of such methodologically ambitious experiment. As such, our experiment represents the largest manipulative treatment of ants in tropical forest to date.

The combined effect of two methods was used as a treatment to suppress ants; the isolation of treatment plots and distribution of treated baits. The two treatment plots were isolated from the surrounding forest by felling a narrow strip of vegetation along the perimeter and, where necessary, cutting branches and lianas overlapping into the plot. The plots were also fenced using a 0.5 m high plastic sheet, buried 10 cm in the soil, and coated with insect glue (Tanglefoot®, Contech Enterprises Inc., Victoria, British Columbia) to prevent the movement of epigeic ants to and from the plot. The glue was regularly checked and replaced whenever needed. The two control plots were not isolated from the surrounding forest. The distance between treatment and control plots was 50–100 m.

We used 40 canopy and 25 understorey bait stations per plot to monitor ant activity. The number of stations was higher in the canopy because this stratum comprises a larger area and our focus was to distribute stations evenly across the tallest trees. An additional 135 canopy bait stations per treatment plot were used to eliminate ants on vegetation. The distribution of bait stations was adjusted to the structure of the vegetation. In particular, most trees with DBH > 10 cm had at least one monitoring (untreated) and one treated bait station, either within the crown or mid-trunk in contact with branches, epiphytes, or lianas. Occasionally, one treated canopy station was also placed at the trunk base if intense foraging of arboreal ants was noted there. The understorey bait stations were placed in a 5 × 5 m grid in each plot on vegetation between 1 and 2 m above the ground.

Ant activity was surveyed 12 times in each plot, i.e. approximately once every 3 weeks for 9 months. The treated bait was applied nine times during that period and stations were left suspended for 2 days on every treatment date and then removed. The survey of ant activity followed approximately 1 week after each treatment, with the exception of a 6-week period after the third treatment date, where ants were surveyed repeatedly without treatment. Surveys of the control plots followed 1–3 days after the survey of the respective treatment plots. All experiments were performed in dry weather and each survey of ant activity in one plot (or one treated bait application) was accomplished in 1 day.

Bait stations and ant sampling

Two types of bait stations were used to attract ants; one in the canopy and one in the understorey. The canopy type was constructed for both the survey of ant activity (i.e. ant trapping) and the delivery of treated bait. The understorey type was used only for monitoring of ant activity. The canopy station was cylindrical semi-enclosed trap of a novel design. It was made from a plastic pipe (25 cm long, 6 cm in diameter, wall 3 mm thick) and closed at both ends with plastic removable caps so that bait could be easily inserted inside. Entrance holes (12 holes 15 mm in diameter and 20 holes 8 mm in diameter) for ants were drilled into the wall of the pipe. Two sizes of holes were used to allow the access of more species of different sizes to the station at the same time. Four flexible nylon strips (150 mm long and 20 mm wide) were attached to the cap to improve its contact with surrounding vegetation and maximise ant access (Figure S1). We adapted a method of Kaspari (2000) to place canopy stations on trees. A fishing-line with a lead weight was first shot over a suitable canopy branch using a wrist-rocket-style catapult and then replaced by a nylon string attached to the bait station. Each canopy station was finally suspended in a tree on the nylon string, which made possible to lower and raise station as needed. The understorey bait station was a square piece of gauze 5 × 5 cm, smeared with untreated bait (referred as attractant hereafter) and pinned on plant stems or leaves between 1 and 2 m above the ground (Figure S2).

Canned tuna (Diana®, tuna in oil, RD Tuna Canners Inc., Madang, PNG) and a concentrated sugar solution (Golden Crush®, cordial, Coca-Cola Amatil Ltd., Lae, PNG) were mixed in at a ratio 5 : 1 and used as an attractant. Preliminary experiments confirmed the mixture to be attractive to a broad range of arboreal ants unlike to other arthropods (P. Klimes, pers. obs.) Approximately 20 g of the attractant per station (two tablespoons) were spread and pressed onto the inner sides of each canopy station, or spread over each understorey station.

To assess ant activity, canopy stations were put in place for 5 h between 10.00 and 15.00 hours and understorey stations for 3 h between 11.00 and 14.00 hours. After this time, the stations were checked for ants. The number of workers for each observed morphospecies was recorded according to the scale 0; 1–10; 11–100; and >100 individuals and several specimens of each morphospecies were collected in 95% ethanol for identification. The shorter positioning time of understorey bait stations was chosen to minimise labour cost and temporal variation in ant activity for each survey. In this way, both strata within individual plots could be surveyed within the same day.

Three insecticides (treatment products) were applied as treatment in the additional canopy bait stations. The products were fipronil (REGENT®, 800 g kg^{-1}, BASF AG Co., Ludwigshafen, Germany), S-methoprene (Pre-strike® 1.5%, Wellmark International Inc., Dallas, TX) and hydramethylnon (MAXFORCE®, Bayer CropScience Co., Research Triangle Park, North Carolina, 1%). All the three treatment products are commonly used in baits for ant eradications (see for more Tingle *et al*., 2000; Lee *et al*., 2003; Abbott & Green, 2007; Hoffmann, 2010). Both fipronil and S-methoprene were prediluted in water and mixed with the attractant to concentrations of 0.01% and 0.0015% respectively, and 0.5 g of hydramethylnon granules were spread on the bottom of each bait station. The three products were combined in every treated bait station because the aim of the study was to eradicate as many ants as possible, not test the efficacy of individual treatment products. The treated bait was enclosed inside the pipe so that only insects actively crawling into the bait station could come into the contact with it.

The initial phase of the experiment (i.e. isolation of the plots and positioning of bait stations) took approximately 90 person-days in each forest type, with an average time of 1.5 person-hours to set up one canopy station in a tree. To prepare one bait station with treated bait, including subsequent retrieval and cleaning after 2 days of exposure, took 12 person-days in each forest plot. One survey took 6 person-days per plot. Setting up of the whole experiment was therefore considerably time-consuming and labour intensive with over 680 person-days required altogether. Nevertheless, the scope of the experiment was manageable for a team of six people.

Ant identification

All individuals were sorted to morphospecies and identified to genus using Bolton (1994). The morphospecies were further identified using reference collections at the Biology Centre of the Czech Academy of Science and the Harvard Museum of Comparative Zoology, online image databases (http://www.antweb.org; Janda *et al*., 2010; Pffeifer, 2010) and with specialist assistance. Morphological identifications of 48 species in this study were confirmed by COI sequences and compared with approximately 1500 COI sequences in the New Guinea ant species database (in collaboration with Consortium for Barcoding Life, http://www.formicidaebol.org). Vouchers are deposited at the Biology Centre of the Czech Academy of Science.

Data and statistical analyses

It was only possible to establish one treatment and one control replicate in each forest type because of the large scale at which the manipulations were applied, which made establishing plots a time and labour-intensive process. Any statistical test of treatment effects therefore faces the problem of pseudoreplication (Hurlbert, 1984) as samples (bait stations) were pseudoreplicated on both spatial and temporal scales. This is a common problem for whole-ecosystem studies, where treatments are often impossible to replicate (e.g. unpredictable, large-scale, or spatially restricted natural phenomena) or logistically difficult to replicate (Oksanen, 2001; Hoffmann, 2010). Contrasting suggestions have been made about the use of inferential statistics in such situations. Either their use should be avoided (Hurlbert, 1984), or, if large scale has priority over replication, then their cautious use may be considered acceptable (Oksanen, 2001). Considering the limitations of the study design, we decided to report the treatment impact on ant activity without statistical analysis. For analysis of effects on species richness and composition, we used more conservative tests to minimise temporal pseudoreplication and the probability of type I errors (paired and split-plot design).

The number of ants per bait station was used to assess the effect of ant suppression treatment on ant activity. Because our abundance estimates were semi-quantitative, we used the lower bound of each category, i.e. 0, 1, 11, and 101 ants, as a conservative estimate of ant abundance for calculations. The changes in ant abundance during the experiment were compared with the abundance in the control plots, using the average abundance of individuals per station for every survey and leaving out the initial survey from the calculation. Data from the canopy and understorey bait station were calculated separately and temporal changes were reported graphically.

The number of ant species was estimated for the combined samples from all 65 monitoring stations per plot obtained during each survey. Species numbers were compared between treatment and control plots by paired t-tests, leaving out the initial surveys from the analysis (i.e. 11 replicates). Statistica ver. 9.1 (StatSoft Inc., 2010) was used for the calculation.

The response of ant species composition to the TREATMENT (i.e. two levels) and TIME (12 levels) variables was tested by the redundancy analysis (RDA), a direct ordination method (Leps & Smilauer, 2003). The presence/absence of species in samples was used as dependent variable (i.e. number of species in a particular bait station on a particular day). Rare species found in <3 samples were excluded from the analysis. The RDA was calculated in Canoco for Windows Version 4.5 (ter Braak & Smilauer, 1998) and significances of all canonical axes were tested by the Monte Carlo permutation test with 999 permutations (Leps & Smilauer, 2003). Because data from bait stations were not independent, permutations were constrained by split-plot design according to the matrices and entered environmental variables (Leps & Smilauer, 2003). All effects were tested independently for the primary and secondary forest plot and for the canopy and understorey (four combinations). First, the combined effect of treatment level and time of the survey (i.e. interaction of TREATMENT and TIME variables) was tested. Second, we tested the difference in species composition between the initial survey prior to the suppression experiment (i.e. T0) and the subsequent surveys, separately in the treatment plots and in the control plots.

Results

Ant fauna

Ants were highly abundant and species-rich in the studied plots, with approximately 60–100% of bait stations occupied by ants prior to treatments and generally higher ant activity-density in secondary forest plots (Fig. 1, Figure S3). Ant abundance in the primary forest was higher in the canopy than in the understorey, but no such difference was apparent in the secondary forest plots (Fig. 1).

In total, we found 72 species of ants, including 22 rare species with only one or two occurrences (Table S1). The two forest types supported ant communities that were different in species richness as well as composition. Bait stations in the primary forest recorded 63 species, with the three most abundant species, *Oecophylla smaragdina* (Fabricius, 1775), *Anonychomyrma* cf. *scrutator* (Smith F., 1859) and *Technomyrmex albipes* (Smith F., 1861) ($>10\%$ of occurrences in the control plot, Table 1). Secondary forest bait stations recorded 26 species, but were dominated by one invasive species *Anoplolepis gracilipes* (Smith F., 1857) ($>70\%$ of occurrences in the control plot, Table 1). Only 17 species were recorded in both forest types.

Impact on ant activity

The ant suppression treatments produced a significant decline in the activity of ants in both forest types and in both strata (Fig. 1). There was a decline to almost zero individuals per bait station after the first application of treatment in both forest types and strata. Although ant activity rose slightly later, it remained remarkably lower than in control plots following subsequent treatments (Fig. 1). The average abundance of individuals per station in the canopy declined after treatment by 82.4% ± 2.7% of the abundance in control (mean ± SE) in the primary forest treated plot and by 91.2% ± 4.1% in the secondary forest plot. Ant abundance in the understorey decreased even more, by 90.3% ± 3.3% and 94.2% ± 2.4%, respectively. Analogous trends after treatment were also observed in the proportion of stations discovered by ants (Figure S3).

Ants were more abundant in the secondary forest plot, and also declined more in response to the ant suppression treatment, in comparison with the primary forest plot (Fig. 1). The estimation of the average number of ant workers per bait station after treatment was 2.5 ± 1.4 individuals in the treatment plot versus 15.5 ± 3.9 individuals in the control plot in primary forest and 1.6 ± 0.6 versus 39.1 ± 4.7 individuals in secondary forest, respectively (mean ± SE).

Impact on ant species diversity and composition

The treatments significantly reduced the number of species per survey in the primary forest to 8.6 ± 2.4 in the treatment plot versus 20.1 ± 2.8 in the control (paired t-test: $t_{10} = -8.93$, $P < 0.001$), but not in the secondary forest (6.4 ± 2.8 species in the treatment plot vs 4.8 ± 2.2 species in the control plot; $t_{10} = 1.35$, $P = 0.2$; Fig. 2).

Species composition was significantly affected by the treatment in both strata and forest types as documented by the RDA permutation tests (Table 2). The ant community in the initial survey prior to experiment (T0) was significantly

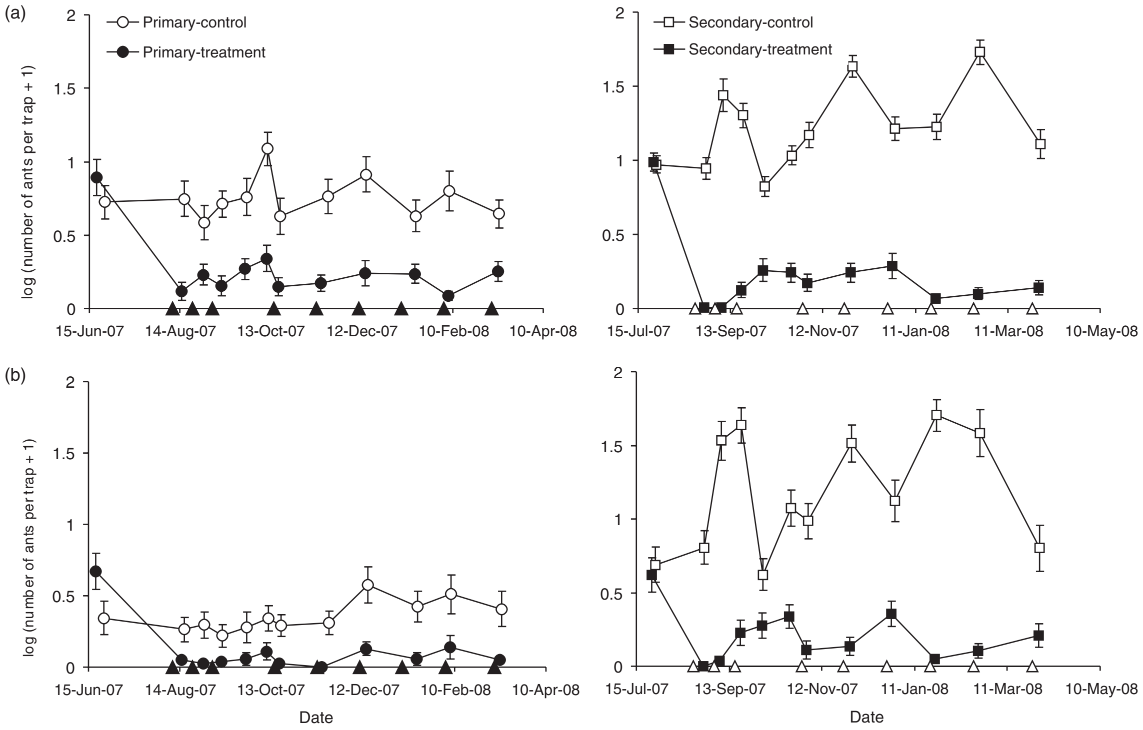

Fig. 1. Activity of ants in the canopy (A) and understorey (B) of primary and secondary forest plots. The average (± SE) of $\log_{10}$ (number of individuals per bait station +1) was used as an index of ant activity in the treatment and control plots. The first point in each time series corresponds to the natural situation before the start of the ant suppression experiment. Treatment dates are marked by triangles on the axes.

Table 1. Overview of the most frequent species in the ant communities of primary and secondary forest plots.

	Primary forest				Secondary forest			
	Canopy		Understorey		Canopy		Understorey	
Species name	T	C	T	C	T	C	T	C
Anonychomyrma cf. *scrutator**	4.0	11.0	0.7	—	1.0	—	—	—
*Anoplolepis gracilipes**	—	—	—	—	5.4	93.1	6.7	79.0
*Camponotus vitreus**	0.4	3.8	—	1.7	1.0	0.2	—	—
Crematogaster flavitarsis	2.1	4.4	3.3	1.3	1.0	0.6	—	5.0
Crematogaster cf. *emeryi**	0.4	8.5	—	0.7	—	—	—	—
*Diacamma rugosum**	0.2	3.1	0.3	5.3	—	—	—	—
Monomorium floricola	2.5	2.7	0.3	—	3.3	4.0	4.0	1.0
Monomorium sp. 003	—	—	1.3	4.7	—	—	—	—
*Oecophylla smaragdina**	1.5	13.8	2.7	1.3	3.3	—	0.3	—
Nylanderia vaga	—	0.4	—	5.0	—	—	—	—
Pheidole sp. 003	—	0.6	—	4.0	—	—	—	—
Pheidole sp. 004	—	0.8	—	3.3	—	—	—	—
Philidris cf. *cordata*	0.6	0.4	0.7	—	—	—	6.7	—
*Tapinoma melanocephalum**	8.3	—	3.7	—	10.4	0.2	9.3	1.3
*Technomyrmex albipes**	3.8	10.4	1.0	4.7	1.0	—	4.0	—

The values show the percentage of bait stations visited in each forest strata (canopy, understorey) and plot (T, treatment; C, control) averaged for all surveys. Only species that visited more than 3% of stations are included. Species marked by an asterisk had the best fit in the redundancy analysis (RDA) for canopy data ($\geq$1% in species variability explained by the ordination, Fig. 3). Full ant species list is in Table S1.

different from the subsequent surveys in the treatment plots, both in the canopy and understorey of primary and secondary forest. In contrast, species composition at T0 did not differ significantly from subsequent surveys in the control plots, except for a marginal difference of canopy fauna in the primary forest plot (Table 2).

The impact of ant suppression on canopy ants at the species level is documented by ordination diagrams for

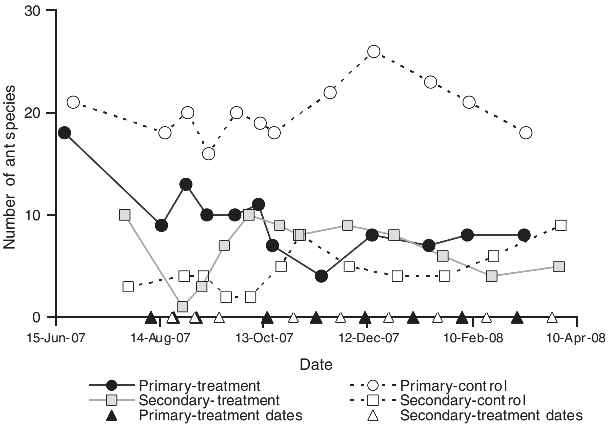

Fig. 2. Species richness of ants in the primary and secondary forest plots. The total number of ant species obtained from 65 bait stations for each sampling date is reported (for both forest strata together). Treatment dates are marked by triangles on the X axis.

both forest plots (Fig. 3). The entire ant community was affected by the treatment as most of the species had their optimum occurrence in the control plots and in the initial survey of treatment plots (Fig. 3). Similar ordination results were obtained for understorey stratum (figure not shown). *Oecophylla smaragdina*, *A. gracilipes*, and *A*. cf. *scrutator*, which were the most common species initially, were virtually excluded by the end of ant suppression experiment (Fig. 4). In contrast, *Tapinoma melanocephalum* (Fabricius, 1793) was the only abundant species in the treatment plots with activity gradually increasing during the experiment, in the canopy as well as understorey of primary and secondary forest (Figs 3 and 4, Table 1). Only a few other species were positively correlated with treatment (for example *Monomorium floricola* (Jerdon, 1851) and *Monomorium* sp. 002), but their increase in activity was only temporal and limited to one forest stratum.

Discussion

Concerted attempts at broad-scale suppression of ants have been attempted many times over the past century (Williams *et al*., 2001; Hoffmann *et al*., 2010). However, the aim of such studies has been the extirpation of targeted invasive species, never entire ant assemblages. In the tropical forests, total ant exclusion has only been attempted for small areas (e.g. Fiala *et al*., 1994; Dejean *et al*., 2006; but see Abbott & Green, 2007). Our results clearly demonstrate that entire ant assemblages can be successfully suppressed within a highly complex rainforest system containing abundant and diverse ant fauna. Moreover, this study shows that such manipulative experiments can be maintained for a 9-month period with ant activity decreased to 10% of its original value. This was achieved using relatively simple methods, i.e. isolation of forest plots combined with a novel approach of setting stations with poisoned bait in the tree canopies.

Based on the continuous observations during the experiment, we assume that our exclusion method was selectively targeting ants. We did not observe any other animals accessing the treated baits and we did not notice any dead insects around the stations, except ants. The short exposure of the treated bait in enclosed stations and the relatively small concentration of toxic compounds used made it unlikely that other animals came into contact with it. There might have been some potential for secondary effects of treatment products (e.g. fipronil) in the food chain, via arthropods feeding on dead ants, however, no such

Table 2. Redundancy analysis (RDA) of ant species composition (presence–absence of species) based on inter-sample distances (see Materials and methods for a fuller explanation of variables).

Explanatory variables	Canopy						Understorey					
Primary forest	Matrix*	Eigall†	%‡	%all§	*F*¶	*P*	Matrix	Eigall	%	% all	*F*	*P*
TREATMENT × TIME††	960 × 31	0.05	3.3	5.2	2.23	***	600 × 21	0.07	3.3	7	1.89	***
SUPPRESSION: T0‡‡	480 × 15	0.02	2.4	2.4	11.9	***	300 × 5	0.08	7.9	7.9	25.7	***
CONTROL: T0‡‡	480 × 24	0.004	0.4	0.4	2.01	**	300 × 18	0.002	0.2	0.2	0.45	0.92
Secondary forest												
TREATMENT × TIME	960 × 11	0.54	53.1	54.3	48.4	***	600 × 12	0.42	40.5	42.2	18.3	***
SUPPRESSION: T0	480 × 10	0.14	13.5	13.5	74.5	***	300 × 9	0.11	10.9	10.9	36.4	***
CONTROL: T0	480 × 4	0.003	0.3	0.3	1.52	0.18	300 × 6	0.001	0.1	0.1	0.4	0.72

*Number of samples time species.

†Sum of all canonical eigenvalues.

‡The per cent variation in species data explained by the first two ordination axes.

§The per cent variation in species data explained by all canonical axes.

¶F-values and significances of all canonical axes assessed via Monte-Carlo permutation (F-ratio value, $\alpha = 0.05$, 999 runs per analysis), ***$P = 0.001$, **$P < 0.01$.

††Test of the effect of treatment–time interaction. Split-plot design, 80 whole plots (canopy) and 50 whole plots (understorey) permuted completely at random, 12 split-plots not permuted. See Fig. 3 for ordination diagrams for canopy ant fauna.

‡‡Test of the difference between T0 (the initial survey prior to the suppression experiment) and the remaining surveys. Data for SUPPRESSION (treatment plot) and CONTROL (control plot) tested separately. Split-plot design, 40 whole plots (canopy) and 25 whole plots (understorey) not permuted, 12 split-plots permuted completely at random.

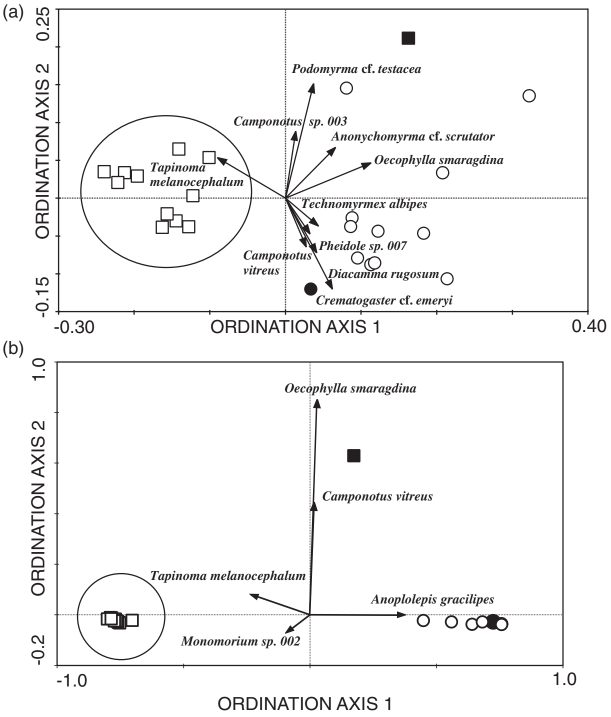

Fig. 3. Species - environment diagram for redundancy analysis (RDA) of ant species composition in the canopy of primary (A) and secondary (B) forest. Variation of the community composition (presence-absence of ant species) is explained by interaction of the ant reduction treatment (TREATMENT variable: ☐ treatment and ○ control) and the date of sampling (TIME variable: 12 surveys). Markers refer to centroids of explanatory variables, arrows to species. Rare species (unique and duplicates) were excluded from the analysis. Solid black symbols refer to the natural state prior to ant suppression, empty symbols to the surveys carried out thereafter. The samples from treated plots after suppression form a cluster marked by a circle. Only the species with the best fit to the ordination model ($\geq$ 1 % in species variability explained by the ordination) are shown. See Table 2 for the percentage of variation explained by the ordination axes.

effects have been reported following large-scale application of poisoned baits (Marr *et al*., 2003).

We found the ant attractant used in this study (tuna in oil and sugar) was very palatable to ants in the study area. These foods are known to attract a wide range of ant species of various diet preferences and are commonly used as bait for ant surveys in the tropical habitats (Greenslade, 1971; Majer, 1976; Addison & Samways, 2000). Nevertheless, it is also known that bait trapping is a selective method as some ant species might not be attracted to the bait. We acknowledge that especially for some species, such as specialist feeders or cryptic species that live in the aerial soil or under bark or in hollow tree branches (e.g. *Hypoponera*, *Solenopsis*, *Strumigenys*) this kind of manipulation would be less effective. However, this study was focused on targeting the species actively foraging on trees that are most likely to affect insect herbivores as predators and/or mutualists (Novotny *et al*., 1999; Addison & Samways, 2000; Blüthgen *et al*., 2004), so the cryptic and uncommon species were not the primary focus of this experiment.

Behaviourally dominant or abundant species are known to usurp and actively exclude other species from clumped resources such as baits (Andersen 1992; Gibb & Hochuli, 2004; von Aesch & Cherix, 2005). Thus, it could be expected that treatments would have affected only a few highly abundant and dominant species, not the entire ant community. However, this was not the case as the observed change in species composition between treatment and control plots shows that the ant community as a whole was affected.

Previous sampling of ant species by a variety of other methods in the study area indicates that approximately two-thirds of the local arboreal species pool were present at our bait stations (Janda, 2007; Klimes *et al*., 2009). Most of the species caught at baits were also found nesting on trees (54 species from 72, Table S1). This implies that only a small proportion of species attending bait stations were ground nesting species, which is in contrast with other rainforests sites (Oliveira-Santos *et al*., 2009; Tanaka *et al*., 2010). We also did not find any evidence for increased activity of ground nesting ant foragers in trees after the abundance of arboreal ants has been reduced by the treatment. This observation corresponds with the generally low activity of ground-nesting ants on trees in the area (Janda, 2007). It is known that some ant species have nocturnal activity in tropical forests (von Aesch & Cherix, 2005; Tanaka *et al*., 2010) but we assume these species were affected by our treatment because bait stations were also operated overnight.

Because of the lack of replication, the observed differences in ant activity between forest types and strata might be perceived rather as contrasts between the studied plots than reflecting general patterns for the regional rainforest. However, the lower activity of ants in the understorey stratum compared with the canopy of the primary forest plots agrees with observations from other tropical forests (Olson, 1992; Basset *et al*., 2003). The higher abundance of ants in secondary forest with similar levels of activity in both strata was probably due to the high numbers of *A. gracilipes* workers at majority of baits (>100 individuals per bait station) compared with any other species in this study. Although, this species is usually a ground nester it is also very active in trees and shows a remarkably effective monopolisation of food resources in disturbed habitats (Abbott & Green, 2007; Drescher *et al*., 2010).

Although the treatment effect was successful in both forest plots, it appeared to be somewhat more effective in the secondary forest plot. Several factors could contribute to this pattern. First, the same number of bait stations was set in both forest plots. Thus, since the secondary plot had fewer trees and a lower canopy (i.e. more baits per leaf area), the effect on ant community there could have been stronger. Second, the treatment might have a more dramatic effect on ant activity where only one species (*A. gracilipes*) was dominant (see also Abbott & Green, 2007). Third, the more inter-connected lower strata of the secondary forest may facilitate more rapid colonisation of baits.

Secondary forest plots had a much lower diversity of ants than primary forest plots. This corresponds with many earlier studies that have demonstrated that ant diversity in tropical rainforests usually declines with the human disturbance (e.g. Floren *et al*., 2001; Dunn, 2004). The initial ant diversity

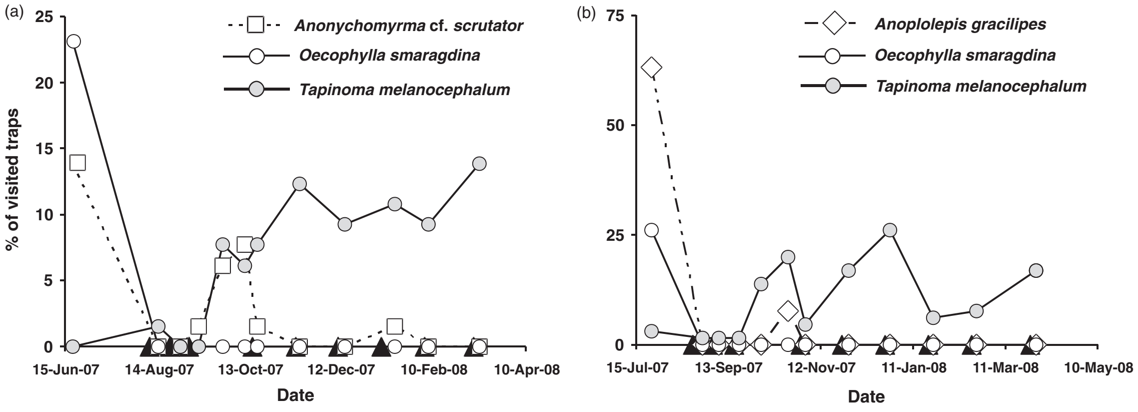

Fig. 4. Trends in activity for the most common ant species in primary forest (A) and secondary forest (B) treatment plots. The percentage of bait stations, from the total of 65, visited by at least one individual from a particular species, is reported as an index of ant activity. The first point in each time series refers to the natural state before ant suppression, followed by samples obtained thereafter. Treatment dates are marked by triangles on the X axis.

could also be negatively influenced by the presence of *A. gracilipes* as has been shown recently elsewhere (Bos *et al.*, 2008; Drescher *et al.*, 2010). The species richness of ants was therefore reduced significantly in the primary forest by the experiment but not in the secondary forest, where richness was already low.

The dramatic decline to nearly zero abundance of ants after the first application of treated baits demonstrates the efficacy of the suppression method. However, the subsequent increase in ant activity indicated that the treatment has to be applied repeatedly (approximately once per month) to prevent the colonisation of suddenly vacant niches by both immigrants and species previously less abundant in the plot, as well as from subsequent recovery of nests where reproductive individuals persisted. Such species might benefit from lower interspecific competition that resulted from the exclusion of the more active or ecologically similar species (Andersen & Patel, 1994; Gibb & Hochuli, 2004; Chong & Lee, 2009). Indeed, initially the most abundant ant species were the first affected by the experiment because of their tendency to monopolise the bait stations (e.g. *O. smaragdina*, *A. gracilipes*). Later, subdominant species benefited from the exclusion of numerically and/or behaviourally dominant species.

The species responding most positively to the suppression treatment, *T. melanocephalum*, is small, generalistic species (von Aesch & Cherix, 2005). Its workers are unlikely to attack large insect herbivores, however, they can predate on eggs (Way *et al.*, 1989) and deter pollinators (Agarwal & Rastogi, 2008). Although *T. melanocephalum* is well known tramp species of global distribution (Wetterer, 2009), it was not common in our studied area (P. Klimes, unpublished). The proliferation of *T. melanocephalum* during manipulation in both studied plots thus suggests that native species might prevent it from colonising new areas under normal circumstances.

Implementation of the method we describe for the suppression of rainforest ants is logistically taxing as it requires a large number of treated bait stations (∼2000 per ha) that have to be deployed frequently. Nevertheless, we are confident that such 'whole-forest' manipulative experiments in tropical rainforests could be adequately replicated if sufficient resources are available. For instance, based on our calculations, suppression of ants in one forest type with five randomly assigned replicates of paired plots (∼0.1 ha each) would require approximately 1000 treated and 500 monitoring canopy stations. Such an experiment would demand a roughly threefold increase in effort compared with this pilot study (i.e. 600 person-days to set up the whole experiment and 36 person-days to apply one treatment and one survey to one pair of plots), however it should be feasible for around nine people working in the field.

In conclusion, we have successfully tested a new method for the suppression of ant assemblages in complex tropical vegetation. Despite not excluding ants from the forest entirely, and with differential effects across species, we believe the overall effects were strong enough and benign to other arthropods. As such, this method could facilitate the realisation of future broad-scale manipulative experiments of food webs (Dyer & Letourneau, 2003; Morris *et al.*, 2004), thus advancing knowledge of ecosystem dynamics and clarifying the complicated role of ants in tropical communities, including their impact as predators, mutualists, and competitors of parasitoids and other predators.

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Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/j.1365-2311.2010.01250.x

Figure S1. Canopy bait station in the primary forest visited by *Oecophylla smaragdina* (photo P. Klimes).

Figure S2. Understorey bait station occupied by the yellow crazy ant *Anoplolepis gracilipes* on a leaf in the understorey of secondary forest (photo P. Klimes).

Figure S3. The percentage of baits visited by ants in the forest canopy (A, 40 bait stations) and in the understorey (B, 25 bait stations). The first point in each time series refers to the natural state prior to ant suppression, followed by samples obtained thereafter.

Table S1. List of ant species caught during the study. Columns in the table show sum of occurrences at bait stations for the whole study: P, primary forest; S, secondary forest; Σ, total. Species marked with an asterisk refer to species of probable ground-nesting habit (not found nesting on trees in the studied area).

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