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A whole-ecosystem method for experimentally suppressing ants on a small scale

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Abstract

1. Ant suppression experiments have emerged as a powerful method for assessing the role of ants in ecosystems. However, traditional methods have been limited to canopy ants, and not assessed the role of ants on and below ground. Recent advances have enabled whole-ecosystem ant suppression in large plots, but large-scale experiments are not always feasible. Here, we develop a small-scale, whole-ecosystem suppression method. We compare techniques for monitoring suppression experiments, and assess whether habitat complexity in oil palm influences our method’s effectiveness.

2. We conducted ant suppression experiments in oil palm agroforestry in Sumatra, Indonesia. We used targeted poison baits, a physical barrier and canopy isolation to suppress ants in 4 m radius arenas around single palms. We sequentially tested three suppression methods that increased in intensity over 18 months. We sampled ant abundance before and after suppression by fogging, using pitfall traps and extracting soil monoliths. We also monitored ants throughout the experiment by baiting. We tested the soil for residual poison and monitored other invertebrates (Araneae, Coleoptera, Orthoptera and Chilopoda) to test for cross-contamination. Plots were established under four oil palm management treatments that varied in their habitat complexity: reduced, intermediate and high understory complexity treatments in mature plantation, and a recently replanted plantation.

3. Post-treatment ant abundance was 92% lower in suppression than control plots. Only the most intensive suppression method, which ran for the final 9 months, worked. Baiting rarely reflected the other monitoring methods. The treatment negatively affected Orthoptera, but not other taxa. There was no residual poison in the soil. Coleoptera abundance increased in suppression plots post-treatment, potentially due to reduced competition with ants. Our findings were consistent across management treatments.
4. We developed a whole-ecosystem method for suppressing ants on a small scale in oil palm plantations. Our method represents a significant advance; previous reductions in ant abundance have not exceeded 38%. We provide the first example of ants being experimentally suppressed belowground. Baiting alone is not adequate for assessing suppression effectiveness, and testing a range of taxa for confounding impacts is important. Our study can act as a blueprint for developing suppression methods for other taxa, which offer unique insights into community ecology.

**KEYWORDS**

competition ecology, ecological redundancy, ecological resilience, exclusion experiment, Formicidae, invasive species control, whole-ecosystem experimental manipulation, Yellow Crazy Ant (*Anoplolepis gracilipes*)

## 1 | INTRODUCTION

Experimental manipulations, such as exclusions and suppressions, are the best way to separate correlation from causation when investigating the ecological role of any taxon (Fayle et al., 2015). In addition, exclusion and suppression experiments (henceforth referred to as suppression experiments) enable the ecological redundancy of a group to be tested (Gitay et al., 1996). For example, field observations showed that one ant species (*Messor barbarus*) was dispersing the majority of seeds in montado forest, but experimental suppressions of *M. barbarus* did not reduce seed dispersal rates as other species widened their diet breadth and increased dispersal activities (Timóteo et al., 2016). In this example, other ant species provided ecological redundancy for the role of *M. barbarus*. Studies that identify ecological redundancy (also termed ecological equivalence) have practical merit in addition to their theoretical value. They highlight areas of vulnerability in ecological networks and test ecological resilience (i.e. the capacity of an ecosystem to recover from disturbance; Pimm et al., 2019). Therefore, they can be used to inform management decisions and direct conservation efforts towards at-risk habitats and processes (Laliberté et al., 2010; Mori et al., 2013; Palmer et al., 1997). The taxonomic level at which suppression studies are conducted varies, with some studies comparing the effect of single versus multi-species ant suppressions (e.g. Mestre et al., 2016; Rosumek et al., 2009). Henceforth, we discuss multi-species suppression experiments, which investigate the role of ants as a whole.

Ants are a popular focus for suppression experiments (Rosumek et al., 2009; Schmitz et al., 2000) as they are highly abundant in most terrestrial systems and mediate a wide range of ecosystem functions (Elizalde et al., 2020). For example, they can increase soil health (Evans et al., 2011), redistribute nutrients and seeds (Griffiths et al., 2018), and indirectly regulate decomposition and herbivory through predation (Parr et al., 2016). Their importance is partly driven by their high abundance (Parr et al., 2016), a feature that makes suppressing ants over large areas difficult (Klimes et al., 2011). Because of this, the majority of ant suppression experiments have been small-scale canopy suppressions that use sticky barriers around trunks or branches, sometimes in combination with targeted poison baits, to suppress ant abundance (Rosumek et al., 2009; Schmitz et al., 2000). These studies have yielded important insights into the role of ants in the canopy. For example, a meta-analysis investigating canopy ant suppressions found that suppressing ants resulted in, on average, 50% more leaf-herbivores, twice as much herbivory damage, and 25% fewer fruits and seeds, compared with trees with ants present (Rosumek et al., 2009). Canopy suppressions have enabled researchers to quantify the ecosystem services provided by canopy ants in agricultural landscapes, and have found that suppression in cacao agroforestry resulted in yield reductions of 27% (Wielgoss et al., 2014), and even 50% in some cases (Gras et al., 2016). Conversely, yield was not affected by ant suppressions in citrus plantations (Piñol et al., 2012). Though these insights are valuable, they only give a partial picture; they do not assess the importance of ants on or below ground.

Whole-ecosystem suppressions of ants are rare (Griffiths et al., 2018; Klimes et al., 2011; Parr et al., 2016; Schmitz et al., 2000), and often confounded by potential impacts on other taxa. For example, they include the use of broad-spectrum insecticides (e.g. Evans et al., 2011) or the establishment of small-scale plots that exclude all non-flying invertebrates (e.g. Wardle et al., 2011). In these studies, the authors conclude that the observed effects of their treatments are the result of suppressing the most abundant invertebrates (ants, or ants and termites in the case of Evans et al., 2011). However, the nature of these experimental designs means that the role of ants cannot be conclusively separated from that of the other excluded invertebrates.

Recent methodological advances have enabled targeted whole-ecosystem suppressions of ants on large scales. Many of these advances have been driven by the need to suppress invasive ants. For example, five invasive ant species were suppressed in 41-ha plots in Australia by distributing poison baits from helicopters (Lach, 2013). Large-scale suppressions for ecological experiments are less common. Klimes et al. (2011) used targeted baits and physical barriers to suppress ant abundance by 80%-90% in 0.06 ha tropical rainforest plots. The confounding impact of having a physical barrier is reduced in large plots because they can support existing populations of non-flying invertebrates. More recently, large-scale suppressions have been conducted using
targeted granular baits that can be dispersed easily on the ground over large areas (0.64–1 ha in these cases), in a similar vein to invasive suppressions (Griffiths et al., 2018; Parr et al., 2016). With easy coverage over larger areas, the laborious physical barrier is replaced by large buffer zones.

These methodological advances are significant, but large-scale experiments are not always possible. For example, sites may be limited if the habitat is patchy, and widespread poison application may not be possible in protected areas or some agricultural landscapes. Small-scale suppressions can be more easily replicated than large-scale ones, which means that habitat variability can be accounted for by testing across multiple sites. However, whole-ecosystem ant suppressions are challenging at small scales. Denmead et al. (2017), using methods similar to those of Klimes et al. (2011), recently attempted this in 0.025 ha plots in oil palm plantations. However, the treatment did not reduce ant species richness, and ant abundance was only reduced by 38%.

In this study, we develop an effective ant suppression method in oil palm plantations and test whether it reduces ant abundance both above and below ground; the latter of which has not been assessed in previous studies. Using four habitat types that varied in their structural complexity within oil palm agroforestry, we also assess whether the effectiveness of suppression techniques varies with the physical characteristics of the ecosystem. We hypothesise that it may be harder to suppress ants in more structurally complex habitats due to increased surface area, or inaccessibility caused by vertical stratification.

This project aims to:

1. Develop a method of whole-ecosystem ant suppression that is effective at a small scale.
2. Test whether targeted ant suppression is possible belowground.
4. Test whether ants are harder to suppress in more structurally complex oil palm plantations.

2 | MATERIALS AND METHODS

2.1 | Study site

This study was conducted in oil palm plantations in Riau, Sumatra, Indonesia (NO 55.559, E101 11.619) as part of the Biodiversity and Ecosystem Function in Tropical Agriculture (BEFTA) Programme (Luke, Advento, et al., 2019). The Ministry of Research and Technology of the Republic of Indonesia (RISTEK) granted us research permission (permit numbers 426/SIP/FRP/SM/XI/2012, 72/EXT/SIP/FRP/SM/IX/2013, 44/EXT/SIP/FRP/SM/IX/2014, 52/SIP/FRP/ES/Dit.KI/II/2017, 09/EXT/SIP/FRP/ES/Dit.KI/II/2018). The estates used in this study are owned and managed by PT Ivo Mas Tunggal, a subsidiary of Golden Agri Resources (GAR), with technical advice from Sinar Mas Agro Resources and Technology Research Institute (SMARTRI, the research and development centre of GAR).

The elevation of our study plots is 10–30 m a.s.l., the average annual rainfall in the study region is 2,400 mm and the mean temperature is 26.8°C.

Oil palm yields palm oil, which is the world’s most produced vegetable oil (United States Department of Agriculture (USDA), 2019). Oil palm agroforestry is a good model system to trial ant suppression methods because ants are abundant and the plantation is structurally complex; the canopy reaches 14 m at maturity (Fayle et al., 2010; Luskin & Potts, 2011). The results of this study are relevant to similarly complex or less complex natural and agricultural habitats, such as other types of agroforestry. However, the suppression method may be less feasible or effective in habitats which are more structurally complex than oil palm, such as rainforest (Luskin & Potts, 2011).

This study was conducted across four oil palm management treatments, with three in mature first-generation plantations (planted 1988–1993), and one in a second-generation (replanted) plantation (planted 2012–2013). The palms were planted in staggered rows with 136–143 palms/ha. The mature sites were based at the BEFTA Understorey Vegetation Project (BEFTA UVP), which is a large-scale experiment within the BEFTA Programme that has experimentally altered understorey vegetation complexity since 2014. The BEFTA UVP Project includes three treatments that represent real management strategies used by oil palm growers. The treatments were applied in a randomised complete block design with six replicates separated by at least 1 km. Each plot was 150 × 150 m in size, with a central 50 × 50 m area from which data were collected. Experimental plots within a block were separated by 150 m of plantation, and ~5 m of road. For full details of the BEFTA UVP Project, see Luke, Advento, et al. (2019). The replanted sites were in a neighbouring estate ~9 km from the mature sites. The majority of this estate was second-generation oil palm planted 2012–2013. We chose six replicate plots, divided into two blocks of three. As within the BEFTA UVP sites, plots within the blocks were separated by 150 m of plantation and 5 m of road. The two blocks were separated by 1 km. See Figure S1 for a site map.

The four habitat types were as follows:

1. Reduced complexity (Reduced): Mature first-generation plantation with all understorey vegetation removed by spraying herbicide.
2. Normal complexity (Normal): Mature first-generation plantation with understorey vegetation removed from the harvesting paths and harvesting circle (~1.5 m radius area around the base of each palm) using herbicide, and large woody vegetation removed manually. Other vegetation was allowed to grow. This is standard industry practice in these estates.
3. Enhanced complexity (Enhanced): Mature first-generation plantation with the same understorey management as the normal complexity, except harvesting paths and circles were cleared by strimming rather than herbicide.
4. Replanted (Replanted): Young second-generation plantation with the same understorey vegetation management as normal complexity plots.
These four habitat types differ structurally and biologically. The normal and enhanced plots have ~10 times taller understory vegetation, ~8 times greater understory plant biomass, ~2.3 times more plant species, and cooler afternoon soil temperatures than the reduced plots (Luke, Advento, et al., 2019; Luke, Purnomo, et al., 2019). The normal and enhanced plots are more similar to each other, and the primary structural difference is in the percentage bare ground cover, which differs between all treatments (mean reduced: 56%, normal: 23%, enhanced: 6%; Luke, Advento, et al., 2019). Compared to the mature sites, the replanted sites are less vertically stratified (canopy height ~14 m vs. ~3 m), have a more open canopy, and the ground vegetation is dominated by a leguminous cover crop (Mucuna brachteata; Figure 1, pers. obs.) rather than ferns and herbaceous plants. In other oil palm plantations, an open canopy is associated with increased temperatures, lower humidity and greater wind exposure (Luskin & Potts, 2011). In the mature sites, ant abundance is lower in the reduced plots than the enhanced and normal plots, but species richness and community composition do not differ between treatments (Hood et al., 2020). For other differences between the mature sites, see the publications in Luke, Advento, et al. (2019). A study from another site has shown that ant community composition differs between younger and mature oil palm plantations (Wang & Foster, 2016), but this has not been tested at these sites.

2.2 Installation of ant suppression

We established one suppression and one control plot around single palms in each of the six replicate blocks in each management treatment in May–June 2016 (Figure 1). We randomly selected two corners of the central 50 × 50 m management area in which to place the plots, with plots 50–70 m apart.

We installed a plastic barrier in a 4 m radius circle around each palm to prevent ground and belowground ants from entering the plots. We chose this plot size (0.005 ha) as we expected it to be large enough to support most existing populations of non-flying invertebrates and, therefore, for there to be little confounding impact of having a physical barrier. In the suppression plots, the barrier was buried 0.5 m belowground and it extended 0.5 m aboveground; in the control plots, it was 0.5 m belowground only. The control barrier was installed to ensure root damage to the study palms and other aspects of soil disturbance were comparable between the treatments. By limiting dispersal, the barrier may have suppressed belowground ant abundance in the control plots, which would make our estimates of belowground ant suppression in the treatment plots conservative. In total, we installed 1.2 km of barrier over 1,350 individual work hours. We applied a thick layer of motor grease to the upper part of the barrier to prevent ants from climbing into the plots.

To prevent canopy ants entering the suppression plots, we pruned any neighbouring-palm fronds that touched suppression-palm fronds. As this could reduce the movement of other invertebrates, and because frond pruning increased canopy openness (canopy openness, measured with a spherical densiometer, increased significantly after frond pruning; linear model: 6.4 ± 1.5, df = 35, t = 4.3, p < 0.0001), we also isolated the canopy in the control plots. See Figure S2 for photographs and further details about the experimental setup.

FIGURE 1 A scheme showing the experimental setup. We established one suppression and one control plot around single palms in each of the six replicate blocks in each management treatment in May–June 2016 (Figure 1). We randomly selected two corners of the central 50 × 50 m management area in which to place the plots, with plots 50–70 m apart.
2.3 | Poisoning and maintenance of ant suppression

We maintained the plots by mending holes in the barrier, reapplying grease and poison, and isolating the canopy by pruning and manually removing encroaching vegetation. This was carried out in three phases with increasing effort. We increased the effort because the ant baiting indicated that ants were not suppressed in the earlier phases (suppression effectiveness is described in the results). The phases were as follows:

1. Phase 1 (June–November 2016): We conducted plot maintenance once every 6 weeks, with the exception of isolating the canopy, which was done fortnightly. We applied 20 g of targeted ant poison baits (Synergy Pro®: active ingredients: hydramethylnon and pyriproxyfen) to the ground inside the plots, following the manufacturer’s recommended application rate. We applied 15 g of bait to the canopy, split into four small plastic cups that were pulled into the canopy on string (Figure S2). This was 5 days of work per month.

2. Phase 2 (November 2016–March 2017): The same as Phase 1, but we doubled the frequency of visits, where we conducted plot maintenance and poisoning, to once every 3 weeks. This was 10 days of work per month.

3. Phase 3 (March–November 2017): We increased the visiting frequency to once every 2 weeks. We applied a mix of poison baits: 50% was Synergy Pro®, 30% was AMDRO® (active ingredient: hydramethylnon) and 20% was Maxforce Complete® (active ingredient: hydramethylnon). We applied 30 g to the ground inside the plots, 40 g to the ground in a 2 m radius around the outside the plots and 30 g to the canopy. We also conducted targeted searches for ground ant nests, which we poisoned when found. This was 25 days of work per month. The majority of the time was spent repairing the barrier.

We did not poison belowground directly in any of the phases, which is likely to have limited impacts on ants living in this strata. However, ant abundance belowground could have been suppressed if ground-foraging ants foraged belowground or belowground ants foraged aboveground. Furthermore, if either case occurred, whole or part baits could be transferred belowground and, therefore, poison ants that did not move between the strata.

2.4 | Thorough ant sampling pre- and post-treatment

We sampled ants pre- (March–June 2016) and post-treatment (September–November 2017) in the canopy by fogging, on the ground using pitfall traps and belowground by extracting soil monoliths. Fogging our study palms pre-treatment would have been highly destructive, so we randomly selected a palm to fog that was 30–50 m from our study palms and within the central 50 × 50 m habitat management area. To collect invertebrates, we laid six circular 110 cm diameter trays around each palm, with three touching the trunk (to sample the epiphytes) and three 2 m from the trunk (to sample the canopy). We fogged the canopy for 90 s between 8:00 a.m. and 09:15 a.m. using a synthetic pyrethroid insecticide. We collected the samples after 1.5 hr.

We used a before-after-control-impact (BACI) design for sampling ground-foraging ants with pitfall traps. We set three pitfall traps per plot at 3 m intervals. Traps were 9 cm diameter plastic cups with 75% ethanol inside. This shape and size of trap is highly effective for sampling ants, and the killing agent does not attract ant species differentially (Ahmed & Petrovskii, 2019; Sheikh et al., 2018). We covered the traps with polystyrene plates to protect them from rain. One trap was set in the harvesting circle (the ~1.5 m radius area around the base of each palm), one bordered the harvesting circle and the windrow (the understorey/area between the harvesting paths and circles), and the third was in the windrow. We chose this method as invertebrates in oil palm plantations can differ between the harvesting circle and the windrow (Ashton-Butt et al., 2018; Carron et al., 2015). Pre-treatment traps from one palm in the replanted sites were damaged and therefore discounted. Traps were open for 3 days; we sampled 855 trap-nights in total. Though using Winkler traps in combination with pitfall traps can increase the number of ground-foraging ant species recorded (Agosti & Alonso, 2000), we only used pitfall traps because Winkler traps sample few additional species in habitats with little litter (Lopes & Vasconcelos, 2008), such as oil palm (Philip et al., 2018).

We collected one soil monolith from the harvesting circle and one from the windrow in each plot to sample belowground ants. Sampling was conducted between 07:00 a.m. and 11:00 a.m. Using a standard method, we hand-searched a quadrat of soil that was 25 × 25 cm in diameter and 20 cm deep. We may have missed cryptic, microscopic species with this method. The sites were primarily sampled using a BACI design. However, belowground pre-treatment sampling was conducted as part of a different project (published in Ashton-Butt et al., 2018; Ashton-Butt et al., 2019), and the palms did not always directly overlap with the control/suppression palms in this study. In six mature sites and all of the replanted sites, the palms were different, but within 50 m of our palms. Furthermore, we sampled 9 and 11 palms (instead of 12) in the replanted and normal sites pre-treatment, respectively. Therefore, we sampled 63 palms in total. We did not fog or collect monoliths in the rain, and did not set pitfall traps in periods of heavy rain.

2.5 | Rapid ant sampling throughout the experiment

We monitored ant abundance before and throughout the experiment by baiting. We chose this method as it is faster and, in the case of fogging, less destructive than the thorough sampling methods described above. This makes it more feasible for long-term monitoring. We baited four plates in each plot every 6 weeks, with
one sugar and tuna plate in the canopy and again on the ground (Figure S2). We set baits in the morning (8 a.m.–12 p.m.) and returned approximately 6 hr later to visually estimate ant abundance on each plate. Preliminary baiting demonstrated that this time period was long enough for the ants to find the baits, but not so long that the baits were fully consumed; baits were fully consumed in <2% of cases. We identified two distinctive and large species by eye: the Yellow Crazy Ant (Anoplolepis gracilipes) and the Weaver Ant (Oecophylla smaragdina). Both species can be important predators of herbivore pests and A. gracilipes is a widespread and destructive invasive species (Bos et al., 2008; Thurman et al., 2019). Other ants were not identified to species. We did not bait in the ground, and Chilopoda belowground (n = 2,813) on = 1,688) in = 1,205). We sampled the most abundant predacious/omnivorous non-hymenopteran orders pre- and post-treatment using the thorough sampling methods described above; this was Araneae (n = 2,059) and Coleoptera (n = 1,688) in the canopy, Coleoptera (n = 17,013) and Orthoptera (n = 2,813) on the ground, and Chilopoda belowground (n = 264). We sampled predators/omnivores as they were more likely to consume the baits than herbivores. Chilopoda were identified to class rather than order to increase the sample size of belowground invertebrates.

Vertebrates could also have been impeded by the barrier. The majority of flightless vertebrates in these sites are rats and Leopard Cats (Hood et al., 2019). Leopard Cats are partially arboreal, and so are unlikely to have been impeded by the barrier. We found that rats were able to cross the barriers, as we regularly observed their claw marks in the grease of the plots (Figure S2).

2.6 | Impacts of the method on non-target taxa

Non-ant invertebrates could also have been impeded by the barrier, killed by residual poison in the soil, or killed either directly by consuming the baits or secondarily by consuming poisoned carcasses. We therefore ran several tests to quantify non-target effects. In November 2017, we collected soil at a depth of 0–10 cm from three locations (which were subsequently combined) at each of six palms (three from the suppression plots and three from control plots). We tested the soil for hydramethylnon and pyriproxyfen, using high-performance liquid chromatography (HPLC) with a Triple Quadrupole Tandem Mass Spectrometry detector. We tested whether invertebrates consumed the poison baits by baiting with it on the ground during the first round of ant baiting and monitoring which invertebrates were on the plates upon collection. We also sampled the most abundant predacious/omnivorous non-hymenopteran orders pre- and post-treatment using the thorough sampling methods described above; this was Araneae (n = 2,059) and Coleoptera (n = 1,688) in the canopy, Coleoptera (n = 17,013) and Orthoptera (n = 2,813) on the ground, and Chilopoda belowground (n = 264). We sampled predators/omnivores as they were more likely to consume the baits than herbivores. Chilopoda were identified to class rather than order to increase the sample size of belowground invertebrates.

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2.7 | Statistical analysis

All data analysis was performed in software package R version 4.0.0 (R Core Team, 2020) with R Studio version 1.2.1535 (Rs Team, 2019). We used packages tidyverse (Wickham, 2017), cowplot (Wilke, 2019) and lattice (Sarkar, 2008) for plotting and data wrangling.

We ran eight sets of generalised linear mixed effects models (GLMMs) to investigate the impact of the suppressions on the abundance of ants and selected invertebrate groups pre- and post-treatment. We modelled ant abundance (a) in the canopy, (b) on the ground and (c) belowground; (d) Araneae and (e) Coleoptera abundance in the canopy; (f) Coleoptera and (g) Orthoptera abundance on the ground and (h) Chilopoda abundance belowground. We calculated the total abundance per palm by summing the abundance in the six fogging trays (for the canopy), the three pitfall traps (for the ground) and the two soil monoliths (for belowground).

For the canopy models (n = 72), we included the interaction between habitat management (HM; categorical: four levels) and suppression treatment/time (STT; categorical: three levels—pre-suppression treatment, post-treatment control and post-treatment suppression), with the random effects plot (categorical: 24 levels) nested within block (categorical: eight levels) to account for spatial dependency (canopy abundance = HM × STT + (1/block/plot)). For the ground models (n = 95), we included the interaction between habitat management, time (T; categorical: two levels—pre-treatment and post-treatment) and suppression treatment (ST; categorical: two levels). Time and suppression treatment were not combined into one variable as in the canopy model because we sampled the same palms pre-suppression treatment and post-treatment. In addition to the random effect of plot nested in block, we included palm (categorical: 48 levels) to account for temporal dependency (ground abundance = HM × T × ST + (1/block/plot) + (1/palm)). For the belowground models (n = 92 for ants, 91 for Chilopoda), where 29 palms were paired through time but others were not, we included the interaction between habitat management and suppression treatment/time, with the random effects plot nested in block, and palm (categorical: 63 levels for ants and 62 for Chilopoda; belowground abundance = HM × STT + (1/block/plot) + (1/palm)). Outliers were removed from two models: belowground Chilopoda and ground ant abundance (see SI.1). Ant abundance belowground was patchy, and we occasionally encountered nests which resulted in high outliers. We have plotted these data, but for the models we used ant occurrence (i.e. the proportion of monoliths that had ants in them). We used a binomial distribution when modelling ant occurrence pre- and post-treatment. We modelled ant abundance (a) in the canopy, (b) on the ground and (c) belowground; (d) Araneae and (e) Coleoptera abundance on the ground and (f) Coleoptera and (g) Orthoptera abundance on the ground and (h) Chilopoda abundance belowground. We calculated the total abundance per palm by summing the abundance in the six fogging trays (for the canopy), the three pitfall traps (for the ground) and the two soil monoliths (for belowground).

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We ran GLMMs using Bayesian Markov chain Monte Carlo (MCMC) estimation in Stan using packages brms (Bürkner, 2018) and rstan (Stan Development Team, 2019). For each model, we ran four chains with 10,000 iterations and saved every 10th iteration after the initial 1,500. We used diffuse priors, with half-cauchy distributions for the random effects and normal distributions for the intercepts and betas (Zuur et al., 2015). We assessed mixing and model fit using the package bayesplot (Gabry & Mahr, 2019; Gabry et al., 2019). For model selection, we simplified the models in a stepwise manner using the package loo (Vehtari et al., 2019) to calculate the approximate leave-one-out-cross-validation information criterion (LOOIC)
for each model (Vehtari et al., 2017). We chose the model with the lowest LOOIC, unless there was a simpler, nested model (Richards et al., 2011) for which the standard error of difference in expected log predictive density overlapped with the model with the lowest LOOIC. In that instance, we chose the simpler model. To assess the difference between factor levels for terms in these simplest models, we used the package `emmeans` (Lenth, 2019) to conduct pairwise contrasts between the posterior distribution of the estimated marginal means for each factor level. Where the 95% credible intervals of these contrasts overlapped with zero, we concluded that there was little evidence to support differences between those groups.

3 | RESULTS

3.1 | Effectiveness of the suppression in reducing ant abundance

Mean ant abundance per palm in the canopy (fogging), on the ground (pitfall traps), and belowground (soil monoliths) was respectively (mean abundance ± standard error of the mean): 90 ± 19, 144 ± 33 and 66 ± 21 pre-treatment; 213 ± 58, 118 ± 11, & 99 ± 71 in the control plots post-treatment; and 21 ± 6, 12 ± 3 and 0 ± 0 in the suppression plots post-treatment (Table S1). Summing these, this amounted to a 43% increase in ant abundance in the control plots and an 89% reduction in the suppression plots, or 92% fewer ants in the suppression plots (mean 33 per plot) compared with the controls (mean 430 per plot) post-treatment. The results of the GLMMs indicated that ant abundance was reduced in the canopy and on the ground in the suppression plots post-treatment, with post-hoc comparisons showing that abundance in suppression treatments was lower than in control and pre-treatment plots, which did not differ from each other (Figure 2A,B). Habitat management had little effect on ant abundance in the canopy or on the ground, and it was not included in the final models. Belowground, ant abundance was also reduced in suppression plots post-treatment, with ants only occurring in 3 of 24 suppression plots post-treatment (Figure 2C). Results of the GLMMs showed that ant occurrence was highest pre-treatment, with slightly lower occurrence in control plots post-treatment and much lower occurrence in suppression plots post-treatment (Figure 2D). This trend was consistent across habitat types, though its magnitude varied, and habitat management was included in the final model. The interaction with suppression treatment/time was removed. See Figure S3 for model selection and Table S2 for model outputs.

Visual inspection of the baiting data showed that the suppression treatments were probably not effective for the entire period that they were implemented. In phase one, the LOESS smoothers showed that there was little evidence of reductions in ant abundance in the suppression plots in any of the habitat types, though there was a slight reduction in the proportion of plates with ants in the reduced plots (Figure 3). This was similar in Phase two. In Phase three, there was noticeable divergence between the control and suppression treatments. This divergence was not consistent between habitat types or strata (canopy vs. ground). In particular, enhanced plots did not show such a marked reduction of ants in phase three suppression plots, compared with the other habitat types (Figure 3). Visual inspection of the baiting data also showed that the abundance of the Yellow Crazy Ant A. gracilipes was reduced in the suppression plots in phase three in all habitat types (Figure S4). The Weaver Ant O. smaragdina showed a reduction in the enhanced sites, but in the other sites abundance was too low in control and suppression sites to identify a trend (Figure S4).

3.2 | Impacts of the method on non-target taxa

We found no traces of hydramethylnon or pyriproxyfen in the soil. We found no animals other than ants on the 48 plates with poison baits on them, and at no point in the project did we see anything else consume the baits. The suppression treatment impacted two taxa other than ants, with increases in Coleoptera abundance in the canopy (mean abundance ± standard error: 25 ± 5 pre-treatment, 13 ± 3 post-treatment control and 32 ± 11 post-treatment suppression) and decreases in Orthoptera abundance on the ground (36 ± 5 pre-treatment, 44 ± 3 post-treatment control and 3 ± 1 post-treatment suppression; Figure 4B,D). Araneae in the canopy, Coleoptera on the ground and belowground Chilopoda were not affected by the suppression treatment (Figure 4A,C,E). Habitat management had little impact on the abundance of any taxa, and was removed from all final models with the exception of ground Orthoptera. See Table S1 for mean values, Figure S5 for model selection and Table S3 for model outputs.

4 | DISCUSSION

4.1 | Effectiveness of the suppression

In this study, we successfully developed a new whole-ecosystem method for suppressing ants on a single-tree scale; there were few ants in the suppression plots post-treatment, with the thorough sampling showing an average of 92% fewer ants in the control plots compared with the suppression plots post-treatment (430 vs 33 per plot). We also provided the first example of a targeted ant suppression that reduced ant abundance belowground, with a mean of 92 per control plot compared with 0 per suppression plot post-treatment. However, we did not target this strata directly with our baiting methods, and so may have under-sampled it. Future experiments aiming to suppress belowground ants could use baiting methods that target them directly, both for the poison baiting and for monitoring ant abundance (Wong & Guénard, 2017). The baiting showed that the Yellow Crazy Ant A. gracilipes was suppressed, indicating that this method could be adapted to control this invasive and destructive species (Lach, 2013).

Ant abundance measured through baiting showed that the suppression was only effective during Phase three, which used
the most intensive suppression method. As we increased the types and amount of poison used in Phase three concurrently, we cannot determine which change caused the increase in suppression rates. Though the methods used in Phase three were highly effective in suppressing ants, they were also labour intensive. A large part of this labour cost was incurred by the need to regularly repair the barrier, and we recommend using a more robust barrier (e.g., a metal one), if conducting suppression experiments for more than 6 months.

Ground Orthoptera abundance was reduced in the suppression plots post-treatment, indicating that they may have been adversely affected by the treatment. As the majority of the Orthoptera we found were winged (pers. obs.), we think it is more likely that this was due to poisoning than being deterred by the barrier. Alternatively, the reduction may have been caused by cascading interactions resulting from ant suppression, rather than the suppression method itself. Although we did not observe Orthoptera consuming the baits, they have been found to do so in previous experiments (Parr

**FIGURE 2** (A–C) Boxplots showing the median and interquartile (IQR) ranges of ant abundance (A) in the canopy (fogging) (B) on the ground (pitfall traps) and (C) belowground (soil monoliths) in each plot. Boxplot whiskers incorporate data that are $1.5 \times$ IQR. Data beyond the whiskers are plotted individually with each point representing a single palm. Two outliers were removed from (C) to aid visual representation (normal control value 1,645 and enhanced pre-treatment value 798). (D) Scatterplot showing ant occurrence belowground with point area sized by the number of plots with that value of ant occurrence: maximum number of plots is 12 pre-treatment and 6 post-treatment. All plots are faceted by habitat management and coloured by suppression treatment and time. Pre- and post-treatment palms are paired for (B), but not (A, C, D), due to the experimental setup. Black asterisks in (A, B, D) show the median of the expected values for each group according to the simplest model of best-fit. Different letters show differences between groups determined by post-hoc comparisons. These letters only show differences within habitat management groups (i.e., in (D) the differences between habitat management treatments are not shown).
indicating that direct poisoning is likely to be the reason for reduced numbers in this case. Future studies should investigate the non-target impacts of these baits in more detail, as any effects of reducing ants may be confounded by a reduction in Orthoptera. However, previous studies that have used these baits have applied lower application levels (2.5 kg/ha) than we did in Phase three (8.85 kg/ha) and as such they may not have impacted Orthoptera in the same way (Griffiths et al., 2018; Parr et al., 2016). We did not detect a negative impact of the treatment on any other taxa sampled, which demonstrates the importance of sampling a range of taxa when testing for unintended impacts of suppression experiments. Habitat management did not influence the effectiveness of the suppression or any unintended impacts, indicating that this method can be used across a range of habitats. However, the structural complexity gradient in this study does not cover the full spectrum of structural complexity, and the suppression method may be less feasible or effective in more complex habitats, such as rainforest (Luskin & Potts, 2011).
FIGURE 4  Boxplots showing the abundance of (A) Araneae in the canopy, (B) Coleoptera in the canopy, (C) Coleoptera on the ground, (D) Orthoptera on the ground and (E) Chilopoda belowground. Boxplots are specified as in Figure 2. Plots are faceted by habitat management and coloured by suppression treatment. Pre- and post-suppression-treatment palms are paired for (B, D), but not (A, B, E) due to the experimental setup. Black asterisks show the median of the expected values for each group according to the simplest model of best-fit. Different letters show differences between groups determined by (A, B, D, E) post-hoc comparisons or (C) the model simplification process (time was the only predictor included in the final model). These letters only show differences within habitat management groups (i.e. in (D) the differences between habitat management treatments are not shown).
4.2 | Monitoring ant abundance

The thorough sampling showed large reductions in ant abundance that were not clearly detected through baiting. This result supports previous findings that baiting can be a less comprehensive method for sampling ant communities than pitfall trapping, as baits can be monopolised by one or a few dominant species (Bestelmeyer et al., 2000; Folgarait, 1998; Lopes & Vasconcelos, 2008). However, comparing between sampling methods can be problematic if some methods are active and others are passive and this affects the species sampled. In terms of suppression experiments, it is important that ant abundance is monitored regularly to test whether the treatment is working, and using non-destructive and rapid methods such as baiting are useful for this. The proportion of plates found by ants was lower in the suppression plots post-treatment, and this may therefore be a more reliable measure of suppression rates than abundance per plate as it reflected the reductions seen with the more thorough sampling methods. We therefore advise that future studies bait with multiple plates, and calculate the proportion found by ants instead of the abundance of ants on individual plates, as this overcomes the issue of ant recruitment behaviour differing by species and colony size (Planqué et al., 2010). Even so, more thorough sampling methods should be used in addition to baiting. This may be particularly important in structurally complex habitats, as we found that baiting was more successful at detecting reductions in management treatments with lower habitat complexity: i.e. the reduced and replanted sites. This could be due to differences in ant species recruitment behaviour and existing differences in community composition between the sites (Hood et al., 2020; Planqué et al., 2010). In conclusion, baiting is not an accurate measure of ant abundance, and past suppression experiments that have solely used this method may have underestimated suppression effectiveness.

4.3 | Choosing small-scale or large-scale suppression

Researchers should consider the advantages and disadvantages of small-scale and large-scale suppressions before conducting their studies. Large-scale suppressions that use buffer zones (as in Griffiths et al., 2018; Parr et al., 2016) reduce the need for constructing barriers, which can be costly to install and maintain. Furthermore, treatment effects on wide-ranging invertebrates, such as winged species, are more likely to be detected at larger scales. In the case of ant suppressions, lower poison rates are needed, and non-target taxa (including Orthoptera) do not seem to be adversely affected at these levels (Griffiths et al., 2018; Parr et al., 2016). However, a major advantage of small-scale suppressions is their versatility; they can be conducted in places where large-scale experiments are not feasible. For example, in this study, we conducted suppressions within an existing experimental manipulation, and future studies could stratify suppressions along gradients, such as elevation, or conduct suppressions in protected habitats or agricultural landscapes where widespread poison application is not possible. Another advantage of small-scale suppressions is that they are easily replicated, which means that habitat variability can be accounted for by testing multiple sites. Conducting small-scale suppressions on a single-tree level is particularly useful in the context of perennial agriculture, as the impact of suppression on crop yield can be measured. In conclusion, the scale of suppression experiments should be decided in the context of site availability, habitat variability, the ecology of the taxa being suppressed (e.g. their range sizes), the targeted outcomes, and the resources available to install and maintain the experiment.

4.4 | Suppression experiment workflow

Here we outline a workflow to guide future suppression experiments.

1. Select sites: Consider the scale of the suppression using the guidance above.
2. Option for pre-treatment sampling: Sample the taxa being suppressed and a range of non-target taxa.
3. Option to physically isolate the suppression plots: Consider partially isolating control plots if there are non-target effects in the context of targeted outcomes (e.g. installing barriers can damage tree roots).
4. Test and then apply targeted poison baits: Conduct baiting experiments to test whether non-target taxa are attracted to the baits prior to widespread application.
5. Maintain plots and monitor suppression effectiveness: Maintain the plots and monitor the taxa being suppressed and a range of non-target taxa to test for cross-contamination. Option to test for residual poison in the ecosystem.

5 | CONCLUSIONS

This study makes an important methodological advancement in ant suppression techniques. It provides the first example of a targeted suppression that is effective on belowground ants, allowing for their impact on biodiversity and ecosystem functions to be experimentally determined. We suggest that techniques should be developed to suppress other key invertebrate taxa using similar methods to those described here, as this will facilitate research into the ecological role of these taxa. With increasing ecological uncertainty caused by anthropogenic change, it is important to experimentally test ecological networks to identify areas of vulnerability and direct future conservation efforts accordingly.

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CONFLICT OF INTEREST
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