Suppression of savanna ants alters invertebrate composition and influences key ecosystem processes

Running head: Savanna ants influence key processes

(*Ecology* Notes)

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Abstract (200 words max)

In almost every ecosystem, ants (Hymenoptera: Formicidae) are the dominant terrestrial invertebrate group. Their functional value was highlighted by E.O. Wilson (1987) who famously declared that invertebrates are the "little things that run the world". However, while it is generally accepted that ants fulfil important functions, few studies have tested these assumptions and demonstrated what happens in their absence. We report on a novel large-scale field experiment in undisturbed savanna habitat where we examined how ants influence the abundance of other invertebrate taxa in the system, and affect the key processes of decomposition and herbivory. Our experiment demonstrated that ants suppressed the abundance and activity of beetles, millipedes and termites, and also influenced decomposition rates and levels of herbivory. Our study is the first to show that top-down control of termites by ants can have important ecosystem consequences. Further studies are needed to elucidate the effects ant communities have on other aspects of the ecosystem (e.g. soils, nutrient cycling, the microbial community) and how their relative importance for ecosystem function varies among ecosystem types (e.g. savanna vs. forest).

Key words: Formicidae, termites, beetles, herbivory, decomposition, manipulative experiment

Introduction

Biodiversity is integral to many ecosystem processes and services (MEA 2005), yet there is little quantification of the contribution specific taxa make and specific knowledge of cascading

interactions, especially in natural systems and at large-scales (Balvanera et al. 2006). Given the rapid rate of global biodiversity loss caused by habitat change, degradation and climate change, it is essential for us to understand the effects of species declines and local extinctions on ecosystem processes and ultimately the consequences of this loss for humanity (i.e. ecosystem services).

Ants (Hymenoptera: Formicidae) are known to be important for many ecosystem functions; with Wilson (1987) specifically highlighting ants (among other invertebrates) when he famously declared they were the "little things that run the world". They are a dominant and conspicuous terrestrial invertebrate group, particularly in tropical and sub-tropical ecosystems, with an estimated 30,000 species globally (Lach et al. 2010). They can constitute up to 75% of all ground-active macro-invertebrate individuals in tropical forests (P. Eggleton, unpublished), and up to 70% in African savannas (C.L. Parr, unpublished). Ants perform a number of roles in ecosystems as predators, omnivores, seed harvesters, and tenders of aphids and scale-insects (Hölldobler and Wilson 1990, Lach et al. 2010). Ants are also considered key ecosystem engineers involved with bioturbation of soil (del Toro et al. 2012), and, more recently, have been shown to aid soil carbon sequestration (Dom 2014).

It is generally accepted that ants fulfil important functions, yet few studies have tested these assumptions experimentally to demonstrate what happens in their absence. Consequently, the contribution ants make to ecosystem functions and processes remains to be quantified. Most studies that have experimentally manipulated ant populations and communities have done so at very small scales of only a few square metres (e.g. Andersen and Patel 1994, Gibb and Hochuli 2004, Wardle et al. 2011) or in the case of arboreal ants, at the level of branches (Fernandes et al. 2005) or individual trees (e.g. Klimes et al. 2012). Nevertheless many ecosystem processes

operate at much larger scales and so such studies are limited in the questions that can be posed and the conclusions that can be drawn.

While there have been larger-scale experiments in which the number of insects (and other invertebrates) were reduced using large quantities of broad spectrum pesticide sprays, this method prevents quantification of the individual contributions of different taxa. For example, herbivory experiments (e.g. Mauricio and Rausher 1997, Agrawal et al. 2012) that removed all herbivores from plants to look at changes in chemical defences, could not apportion the relative importance of different herbivores. Similarly, in an experiment aimed at examining the collective role of ants and termites in the soil, Evans et al. (2011) demonstrated that in the absence of soil invertebrates, soil nitrogen and soil porosity decreased (reducing soil water infiltration), leading to reduced wheat yield. Although these results were striking, the lack of specificity of the manipulations prevented quantification of individual taxon contributions (i.e. ants, termites and other taxa).

Specifically for ants, there are no experimental studies documenting the influence ants have on other invertebrate groups at large-scales. So, for example, while ants are considered to be the most important predators of termites (Deligne et al. 1981, Fayle et al. 2015), there are no community-level studies demonstrating the importance of ant predation in limiting termite populations, or that show the importance of termites as food for ants. Neither is it clear to what extent ants influence important ecosystem processes, whether directly through their own activity (e.g. soil macroporosity) or indirectly through effects on other invertebrates (e.g. decomposition and herbivory). A key issue that has plagued better understanding of these kinds of questions is that, until now, it has not been possible to manipulate specific elements of the ground-level community at a large-scale.

Here, we demonstrate, first, that it is possible experimentally to suppress the abundance of ants at large scales using a targeted baiting approach, and, second, we use this field experiment in undisturbed savanna habitat to explore how ants influence the abundance of other invertebrate taxa in the system, and thereby affect the processes of decomposition and herbivory. If ants control termite abundance we predicted that in the absence of ants, decomposition by termites would increase. We also anticipated that where ant abundance was suppressed, herbivory would be higher as a result of reduced predation on invertebrate foliar herbivores. In addition, we expect to find an increase in the number of large surface-foraging invertebrate predators due to the relaxation of interference competition from the suppressed ants (Hawes et al. 2013). Here we report on the immediate responses to the treatment by examining processes that were likely to have a relatively rapid response.

Study site

The study was conducted at the Wits Rural Facility (WRF), near Hoedspruit, Limpopo, South Africa (24°33'25S, 31°05'46E). The 350 ha site is covered with lowveld savanna at an elevation of ~580m. Dominant tree species include *Terminalia sericea*, *Combretum collinum*, *Sclerocarya birrea* and *Dichrostachys cinerea*. The grass layer is predominantly *Panicum maximum, Pogonathria squarrosa, Hyparrhenia* spp., and *Heteropogon contortus*. The area receives austral summer rainfall (mean annual rainfall is 651mm ±123, 1970–1997). The sites have nutrient-poor sandy soils. The study was carried out from October 2012 to October 2013.

Methods

A total of eight plots (four treatment and four control plots) were distributed across the WRF site. Plots were spaced a minimum of 500 m apart. Control plots were 0.25 ha in size, whereas treatment plots covered 1 ha (100 m x 100 m) with sampling confined to the central 0.25 ha (i.e.

with a 25 m encircling buffer). The buffer areas surrounding the core sampling area on treatment plots were designed to reduce the risk of ants from nests outside the suppression zone foraging into the experimental plot. We used 1 ha plots because we wanted to (a) determine whether large-scale suppression was practically feasible, (b) key processes such as decomposition can require large-scales particularly where mound-building termites are present and have an over-dispersed distribution, and (c) needed a buffer area around our central 0.25 ha sampling plot.

Chemical treatments

We used two synthetic insecticides in baits to suppress ants on treatment plots. We used the commercial product Maxforce® Ant Bait Granules (Bayer, Environmental Science), with the active ingredient Hydramethylon at 1% (w/w) mixed into a granular bait (corn grit with soybean oil). Hydramethylon is an amidinohydrazone, a metabolic inhibitor that blocks the electron transport chain in the mitochondria (Hollingshaus 1987). It is considered a slow-acting toxicant because it usually requires 24 or more hours to become effective with death occurring after several days (Hooper-Bùi & Rust 2001). We chose the Maxforce® bait because it was designed to appeal specifically to ants and has been used successfully in reducing populations (including eradication) of pest ants in natural systems (e.g. Plentovich et al. 2010). A similar product with the same active ingredients has also been used to control invasive ants in disturbed and urban habitats (e.g. Hoffmann and O'Conner 2004).

In addition, because ant species can differ in their food preference (Hölldobler and Wilson 1990) and we were uncertain whether all ants would accept Maxforce® bait, we supplemented Maxforce® baits with our own protein-carbohydate bait. We made our own bait from cat food pellets (Whiskas brand, mostly grain based carbohydrates and protein) and jelly (gelatine & suger) with the active ingredient imidacloprid (at 5 ppm w/v; Premise from Bayer

AgroSciences). Imidacloprid is a neo-nicotinoid insecticide: its mode of action is to block the acetylcholine receptors of motor neurones (Bai et al. 1991). At high doses it is fast-acting and would kill a foraging ant before it could return the bait to the nest. However, it does not kill the foraging ants at the very low dose we used (T.A. Evans, unpublished data), thus the imidicloprid bioaccumulates in the nest before reaching lethal doses (Daane et al. 2008).

In October 2012 we applied 2.5 kg of Maxforce® to each treatment plot (100 m x 100 m) by hand, with a team of six people walking in parallel lines (spaced ~5 m apart) across the plots to ensure even distribution of the bait. We applied 6 kg of the homemade baits in a similar manner. A smaller amount of Maxforce® was applied as a 'top-up' in December 2012 (675 g/ plot) and March 2013 (500 g/ plot). In March 2013, 6 kg of the homemade baits was also applied to each treatment plot (as in October 2012). No further treatments were applied after March 2013.

We planned our baiting method to minimize, if not completely negate, detrimental non-target effects. First, both of the insecticides have low toxicity to most non-target organisms such as terrestrial vertebrates and plants (Bayer Environmental Science 2003, 2006). Second, the baits were designed to appeal to ants, in the size and composition of the granules. Only a few other invertebrates were observed on the baits (e.g., flies, crickets and cockroaches) and while the baits might appeal to small omnivorous and carnivorous mammals, we applied the baits during daylight hours, when ants are at their most active and these organisms are less active. Third, once collected and returned to the ant nest, the insecticides would be sequestered below ground and thus be unavailable to surface foraging organisms. Fourth, the quantity of insecticides we applied was extremely low and below biologically relevant levels. The quantities of insecticides in the foraging territories of even the smallest and most vulnerable mammals, such as shrews and mice,

were lower than the LD50s. Therefore, the amount of insecticide was insufficient to kill these mammals, even if they were able to find and eat all of the bait spread over their foraging territories before it was discovered and removed by ants. However this is highly unlikely as the diurnal ants had the entire day to remove the bait before the nocturnal mammals became active. Both active ingredients are unlikely to persist in the ecosystem as they are rapidly degraded in soil by microbes (Abernethy and Walker 1993, Anhalt et al. 2007). We refer to the term 'toxic bait' when referring to either Maxforce or homemade 'cat food-jelly' baits.

Sampling for invertebrates and ecosystem processes

We assessed the abundance, richness and composition of ground-active invertebrates (including ants and beetles) in each sampling plot (control and treatment) using pitfall traps (nine per plot, diameter 12 cm) open for three days on two sampling occasions (Pre-treatment: October 2012 and Post-treatment: March 2013). All invertebrates collected in pitfall traps were identified to major groups, with ants identified to species and beetles (Coleoptera) sorted to subfamily, and where possible, genus.

We estimated epigeaic ant abundance on the plots with monitoring transects to assess ant abundance rapidly. This method was used to test whether we had successfully reduced the abundance of ants, and as an additional, and rapid measure, of forager ant abundance. We placed 15 squares of paper with a teaspoon of cat food (containing no toxins) in each plot in a line with each paper spaced 3m apart. We inspected the monitoring lures 40 minutes later, and scored ant abundance at each lure as follows: 0 = 0 ants; 1 = 1 ant, 2 = 2-5 ants, 3 = 6-10 ants, 4 = 11-20 ants, 5 = 21-50 ants, 6 = >50 ants (see Parr 2008). Monitoring transects were run in October 2012, December 2012, March 2013, and October 2013.

We used toilet roll consumption by termites as a proxy of decomposition as most decomposition in semi-arid African savannas is by termites (Collins 1981, Schuurman 2005). Using an established protocol, we assessed toilet roll consumption and decomposition using nine cellulose baits (unbleached, single ply, unscented toilet paper rolls) pegged to the soil surface of each plot. We scored cellulose baits for termite consumption post-treatment in December 2012, March 2013 and June 2013. Toilet rolls were replaced after the March 2013 scoring as little paper was remaining. We estimated levels of consumption using the following scale: 0 = 0 consumed; 1 = 1-25%; 2 = 26-50%; 3 = 51-75%; 4 = 76-99%; 5 = all consumed (Davies et al. 2012).

We assessed levels of invertebrate foliar herbivory post-treatment in March 2013 by collecting 20 leaves at random from the terminal branches of ten *T. sericea* trees within each plot. We scored each leaf visually for the level of invertebrate foliar herbivory: 0 = 0% consumed, 1 = <10% consumed, 2 = 10-25% consumed, 3 = 26-50% consumed, 4 = 51-75% consumed, 5 = >75% consumed. We defined damage as tissue area or mass removed by chewing invertebrate herbivores (Knepp et al. 2005). We did not conduct sampling to assess levels of herbivory at other times (e.g. in June or October) as *T. sericea* is deciduous and had no leaves during these times.

Analyses

We analysed ant abundance data using ANOVA with mean data collected from each plot. In cases where we had repeated measurements (i.e. monitoring transects) we tested each time period separately, as there were large seasonal effects. Invertebrate abundance data were analysed for each major group separately using generalized linear mixed models (poisson distribution) with plot as the random factor and treatment as the fixed factor. Community level

effects were tested using multivariate analyses. We used ordination methods that assumed linear responses as we did not expect any taxonomic groups to be completely expunged by the treatments because we explicitly targeted ants. We employed Principal Components Analysis (PCA) to explore how different genera of ants were affected by the suppression treatment. This is a descriptive rather than a probabilistic approach. Compositional differences in major taxonomic groups (non-ant groups) due to treatments (within pitfall traps) were tested using a constrained method, Redundancy Analysis (RDA), using a Monte Carlo permutation test approach to test for a treatment effect. Cellulose bait consumption was analysed using an ordered logistic regression (i.e. with score as an ordered factor) with plot as a random factor and treatment as a fixed factor. Differences in percentages of leaf herbivory were tested using Fisher's exact test.

Results

Pre-treatment (October 2012), ants made up 53% of all macrofauna in pitfall traps. Our pitfall data show that the suppression treatment successfully reduced the abundance of ants by 76% (mean reduction overall, comparing treatment plots with control plots in March), with a maximum reduction of 93%. Pre-treatment, there was no significant difference (ANOVA, $F_{1,6}$= <0.01, P>0.9) in ant abundance (or any other macrofauna group) between the control and future treatment plots, however, only five months later (March 2013) there were significantly higher abundances of ants in pitfall traps on control plots (ANOVA, $F_{1,6}$= 17.9, P< 0.001; mean (± SE) abundance of 26 (±6) and 6 (± 6) on control and treatment plots, respectively) (Fig. 1a.). In March 2013, 73% of all individuals caught in pitfall traps on control plots were ants, whereas this figure was only 27% on treatment plots. Data from the monitoring transects supported the decline in ants on treatment plots, and also indicated that substantial reductions in ant abundance were achieved on treatment plots within two months (i.e., by December 2012; Fig. 1b.).

Differences in ant abundance between sampling periods on control plots are due to seasonal effects. The toxic bait treatments affected all ant genera equally, with pitfall trap data showing that all genera declined in abundance in the treatment plots (Fig. 2) and there was no change in composition

The RDA showed that overall there was a clear treatment effect on the composition of other major taxonomic groups from the March 2013 pitfall traps (Monte Carlo test, 999 permutations, Pseudo-F= 11.6, P <0.001, Fig. 3). Beetle abundances (log +1 transformed to correct for non-normality) were significantly higher on treatment plots (GLMM, z = 2.4, p = 0.016). The beetle response was driven primarily by two large bodied beetle species: a species of *Psammodes* (Tenebrionidae) and a species of *Anachalcos* (Scarabaeidae, a dung beetle), which showed the greatest responses. Millipedes were also higher in abundance on treatment plots (GLMM, z = 2.4, p = 0.018). Other major taxa were not negatively affected by the suppression of ants (Fig. 3); indicating that there were no detrimental non-target effects of the treatment.

Consumption of the cellulose baits by termites (i.e. decomposition) did not differ among plots in December 2012, approached significance in March 2013 (Ordered logistic regression, z = 1.714, P=0.08), and was significantly higher on treatment plots in June 2013 (Ordered logistic regression, z = 2.5, P=0.012). Levels of intermediate-high foliar herbivory (i.e. >50% herbivory) were greater on treatment plots than control plots (Fisher's exact test, P=0.006) in March 2013.

Discussion

While most exclusion studies have focused on small-scales (<2 m^2), our study demonstrates that it is possible to suppress ant abundances across large areas (1 ha) of undisturbed habitat. Indeed, we were able to do this to such an extent as to influence ecosystem processes and other invertebrate groups in the system within only a few months. Furthermore, we were able to

undertake the exclusion so that unintended detrimental non-target effects on other taxa were negligible (or positive due to the ant suppression) (Fig. 3). Our study contrasts with other suppression studies where chemical treatments reduced the abundance of all invertebrates (e.g. Evans et al. 2011), and although invasive ant eradication experiments show non-target effects on invertebrates are relatively minimal (Stork et al. 2014), some taxa can be negatively affected (e.g. crickets, cockroaches, Plentovich et al. 2010). The combination of toxic bait types used in our study and the specific manner they were applied not only reduced the abundance of ants overall, but also resulted in all surface foraging ant genera being equally affected by them.

Seasonal changes in ant abundance were evident with the abundance of ants post-treatment higher on control plots than pre-treatment. Ant abundance and activity are greatest in the austral summer, wet season (Lindsey and Skinner 2001) and our pre-treatment data are from October (the transition between the wet and dry seasons in the Southern hemisphere), whereas the post-treatment comparison data are from the end of summer (March).

It was striking that the significant positive response from particular invertebrate groups was detected within only a few months after the treatment was applied (5 months). As predicted, large ground-active beetles increased in abundance on treatment plots. Particularly responsive were *Psammodes*, a large ground-dwelling tenebrionid, and *Anachalcos*, a big roller dung beetle. We suggest that this increase is likely due to competitive release of large, surface-active beetles and reduced interference by ants (e.g. Hawes et al. 2013), although release from predation may also be possible. We expected large predatory beetles (e.g. carabids and staphylinine staphyinids) to be particularly affected by the ant suppression treatments because we thought ants might out-compete these groups, but there were too few sampled in both control and treatment plots to allow a comprehensive analysis to be conducted. Millipedes also showed a significant, positive

response in the absence of ants, although the mechanism for this is unclear. Some savanna ants are specialist predators of millipedes (e.g., *Plectroctena mandibularis*, Bolton 1974), so it may be possible that the increase in millipedes was in response to predation-release from *P. mandibularis* and other predatory ant species (e.g. *Leptogenys*).

Our findings also revealed that within only a short period of time, it was possible to detect important top-down effects of ants on two major ecosystem processes: decomposition and herbivory. Ours is the first study to show effects on ecosystem functioning at the ecosystem scale through the indirect effects of ant predation. Cellulose consumption by termites, and thus decomposition rates, were higher on treatment plots. Given the specificity of the cellulose baits in attracting only termites, this suggests that the abundance and activity of termites increased in the absence of ants. While ants are considered to be the most important predators of termites (Deligne et al. 1982, Fayle et al. 2015), we have demonstrated that top-down control of termites by ants can have important ecosystem consequences. The cascading effects for ecosystem functioning remain to be further investigated.

Finally, our study found ants can influence leaf herbivory by foliar invertebrates. While it is generally understood that ants can protect host plants from herbivores (e.g. Bronstein 1998, Rosumek et al. 2009) and ant-plant-insect herbivore interactions are common in cerrado savanna (Sendoya and Oliveira 2015), this has been less well-studied in African savannas, particularly at large scales. Our results therefore confirm the important role of ants in regulating insect herbivory. It remains to be seen how the top-down role of ants may vary depending on the presence of liquid food provided by insect herbivores and the plant.

In conclusion, our study has demonstrated that it is possible to suppress ant communities at the ecosystem scale, that using this method non-target effects can be negligible, and that

ecosystems respond very rapidly to the reduction in this dominant consumer group. Further studies are needed to elucidate the effects ant communities have on other aspects of the ecosystem (e.g. soils, nutrient cycling, the microbial community) and how their relative importance for ecosystem function varies among ecosystem types (e.g. savanna vs. forest). Predicting the effects of their loss or change in abundance is especially important when considering ecosystem functioning in highly transformed habitats. Are ants always the 'little things that run the world'?

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Figure legends

Figure 1. Boxplots showing ant abundance collected (a) with pitfall traps and (b) at lures on control and treatment plots pre- and post-treatment at Wits Rural Facility, Limpopo, South Africa. T = treatment plot, C = control plot. October 2012 and March 2013 for pitfall traps, and October 2012, December 2012, March 2013 and October 2013 for lures. Shaded boxes are treatment data, clear boxes are control data.

Figure 2. PCA of the ant data collected from pitfall traps post-treatment (March 2013) on control and treatment plots. The blue (left) envelope is around treatment plot positions and the black, dashed (right) envelope is around control plot positions. Genus abbreviations: Crematog = *Crematogaster*, Camponot = *Camponotus*, Ocymyrmx = *Ocymyrmex*, Odontoma = *Odontomachus*, Monomori = *Monomorium*, Lepisiot = *Lepisiota*, Bothropn = *Bothroponera*, Tetramor = *Tetramorium*, Meranopl = *Meranoplus*, Plectro = *Plectroctena*, Polyrhac = *Polyrhachis*.

Figure 3. RDA of invertebrate communities on control and treatment plots five months post-treatment, in March 2013. Taxa showing a significant response are marked with *.
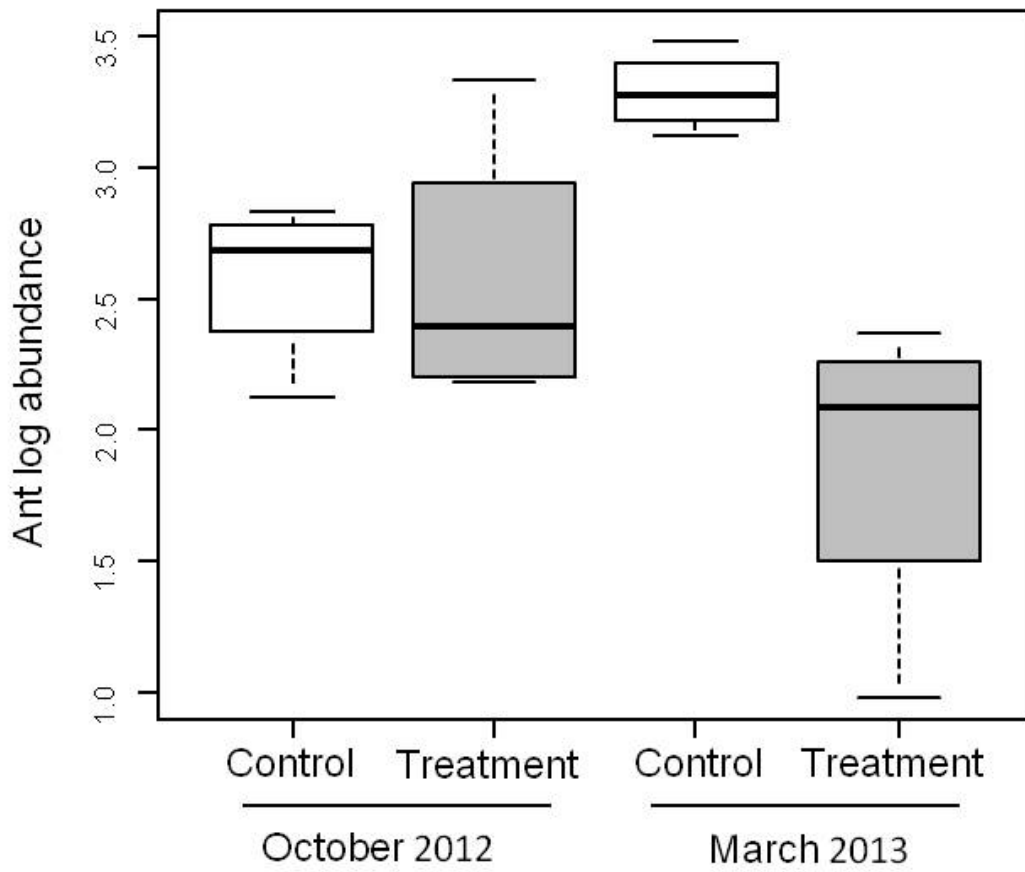
Fig. 1a

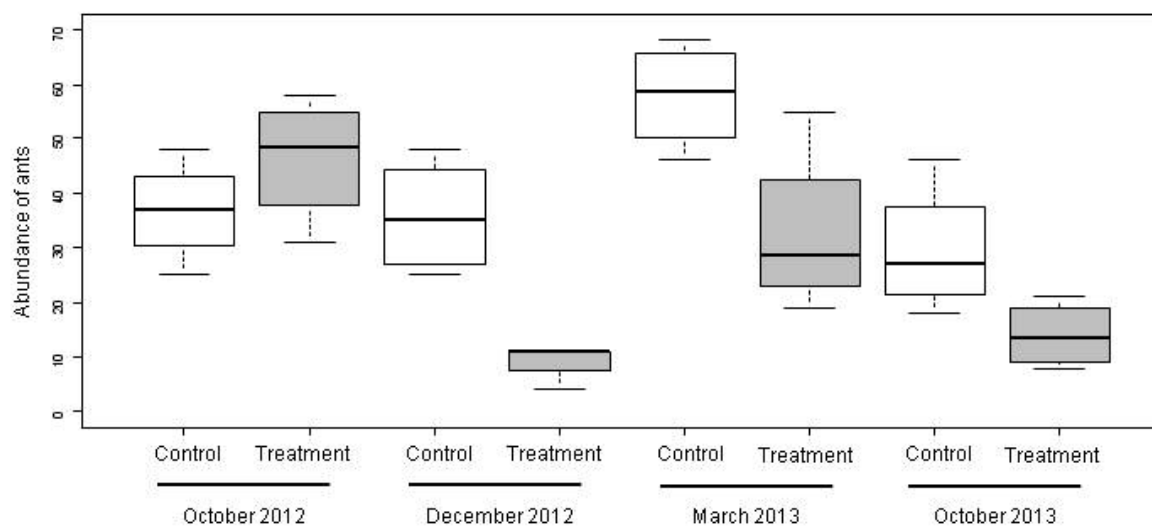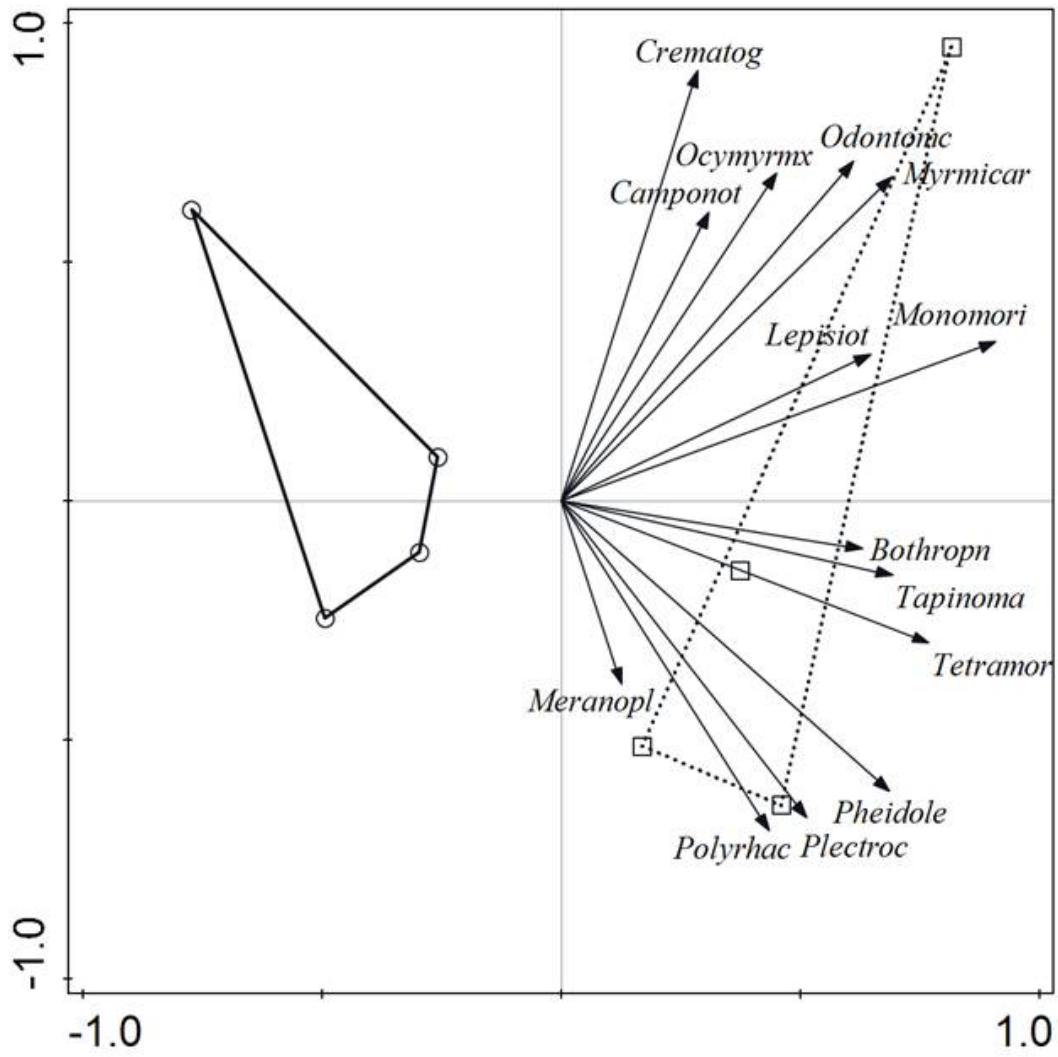Ant log abundance

| Control | Treatment | Control | Treatment |

October 2012   March 2013

Fig. 1b

Fig. 2

Fig. 3