

Effects of two locust control methods on wood-eating termites in arid Australia

Kimberly Maute¹  · Kristine French¹ · Paul Story² · C. M. Bull³ · Grant C. Hose⁴

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Abstract Termites are ubiquitous detritivores and are a key influence on soil function and nutrient cycles, particularly in arid and semi-arid ecosystems. Locust control presents a unique hazard to termites and the effective functioning of ecosystems as a consequence of the overlap between pesticide applications and termite populations in grassland and desert landscapes. We monitored the effects of locust control methods using ultra-low-volume (ULV) barrier application of a chemical pesticide, fipronil, and a blanket application of a fungal biopesticide, *Metarhizium acridum*, on wood-eating termites in arid western New South Wales, Australia. We tested the hypothesis that spray

applications decrease termite activity at wood baits using a BACI designed field experiment over 2 years. Our replicated control and treatment sites represented the spatial scale of Australian locust control activities. There was no detectable impact of either locust control treatment on termite activity, bait mass loss or termite community composition measures. Non-significant differences in termite survey measures among sites suggested that climate and environmental conditions were stronger drivers of our termite measures than the single, localized and unreplicated application of pesticides more commonly used in locust control operations in arid Australia. A lack of evidence for an impact of our fipronil or *Metarhizium* application methods supports their use as low hazard locust control options with minimal large scale and longer-term effects on termites in Australian arid rangelands. Future research would be necessary to determine the probable short-term impacts of treatments on individual termite colonies and the possible impacts on non-wood eating termite species in the arid-zone.

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✉ Kimberly Maute
kmaute@uow.edu.au

Kristine French
kris@uow.edu.au

Paul Story
Paul.Story@agriculture.gov.au

C. M. Bull
michael.bull@flinders.edu.au

Grant C. Hose
grant.hose@mq.edu.au

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Introduction

Though rainfall and soil type have the greatest influence on primary productivity in arid and semi-arid ecosystems, detritivores and herbivores have indirect impacts on productivity through their roles in nutrient cycling (Culliney 2013; Huntly 1991; Morton et al. 2011). Termites are abundant detritivores which influence levels of soil porosity, aeration, organic matter, and water infiltration and storage (Bignell 2006). By increasing soil productivity,

¹ School of Biological Sciences, University of Wollongong, Wollongong, NSW 2522, Australia

² Australian Plague Locust Commission, GPO Box 858, Canberra, ACT 2601, Australia

³ School of Biological Sciences, Flinders University, GPO Box 2100, Adelaide, SA 5001, Australia

⁴ Department of Biological Sciences, Faculty of Science, Macquarie University, Sydney, NSW 2109, Australia

termites can influence the growth of plants and are also a key prey item for invertebrate and vertebrate predators (Craig et al. 2006; Greenville and Dickman 2005; Morton et al. 2011). Therefore termites are considered to be a key influence on soil function, nutrient cycles and food webs in arid systems (Jouquet et al. 2011). Nutrient cycling in more mesic environments is largely facilitated by not only termites, but also earthworms, mites, springtails and litter invertebrates (Lee and Foster 1991; Petra and Hendrix 2007). Termites are also considered key arid ecosystem engineers due to the lack of high densities of large herbivores which may substantially influence nutrient cycling in arid systems (Milchunas and Lauenroth 1993; Morton et al. 2011). A decline of termite activity is often correlated with rangeland degradation in wet tropical savannas, supporting the view that termites provide important ecosystem services (Dawes-Gromadzki 2005; Whitford et al. 1992). However, the diversity and activity of termites in the Australian arid-zone is much lower than the diversity and activity in savannahs and temperate regions (Abensperg-Traun and Steven 1997; Watson and Abbey 1993). Worldwide, the role of arid-zone termites is also relatively unknown.

Termites are sensitive to several application of pesticides commonly used in agriculture and locust control, suggesting potential flow-on effects on the nutrient cycling within ecosystems (Mamadou and Sarr 2009; Peveling et al. 2003; Rouland-Lefèvre 2011; Su and Scheffrahn 1990). Locust control presents an important possible hazard to termites because of the large overlap between areas of locust control operations and the natural landscapes where termites are prevalent (Peveling 2001; Story et al. 2005). However, few field studies have attempted to measure the effect of chemical locust control on termites (Peveling et al. 2003; Mamadou and Sarr 2009; Steinbauer and Peveling 2011). In contrast to chemical pesticides, non-chemical locust control, such as by orthopteran-targeted fungal biocontrol, may have little effect on termites. Moreover, 50 years of research and repeated attempts to develop an effective fungal biocontrol for wood-eating termites has failed to result in a biopesticide which can control termite colonies in field situations (Chouvenc et al. 2011; Milner 2003). This resilience of termites to biocontrol efforts suggests that fungal pesticides used in locust control should have little detectable effect on Australian termite colonies in the field.

Locust control in Australia is characterized by regular surveillance and rapid treatment of emerging locust populations often in remote, arid parts of eastern Australia to prevent larger populations migrating to cropping regions (Hunter 2004). Increasing constraints on the use of chemical pesticides for locust control necessitated the development of a fungal biopesticide to enable spraying over

organic pastoral leases and ecosystems or species deemed sensitive to chemical pesticides. In Australia, full cover blanket spraying of *Metarhizium acridum* (hereafter referred to as *Metarhizium*) is used in these circumstances and has been shown to have little effect on non-orthopteran arthropods and vertebrates compared to chemical pesticides (Arthurs et al. 2003; Zimmermann 2007). Concomitantly, improvements in application techniques for existing chemical insecticides have reduced the amount of chemical applied, thereby minimising the environmental impacts of locust control programs more broadly (Story et al. 2005). The use of barrier treatments for the ultra-low-volume (ULV) formulation of the phenyl pyrazole insecticide, fipronil, is one such example. Barrier treatments use aircraft to deposit strips of pesticide at an angle of 90° to the prevailing wind direction, leaving 300–500 m wide strips of untreated vegetation between each barrier (Story et al. 2005). Using this technique, locusts that are not directly over-sprayed will move into treated areas, while the pesticide is still active thus acquiring a lethal contact and oral dose (Story et al. 2005). Additionally, spray applications are seldom repeated in the same location, and predominantly represent single-dose applications (Story et al. 2005), which are likely to have a lower impact on non-target fauna than repeated treatments commonly used for pest control in more highly managed agro-ecosystems.

We monitored the effects of the two current locust control methods (outlined above) on wood-eating termite activity at baits by quantifying termite activity before and after a single ULV reduced area treatment application of fipronil and a single blanket application of a fungal biopesticide formulation of *Metarhizium* (Driver and Milner, isolate FI-985, marketed as Green Guard®). Monitoring termites in Australian arid lands is challenging due to the lack of above-ground mounds, cryptic behaviour, variable seasonal activity and low population densities compared to termites in tropical or higher rainfall areas (Abensperg-Traun 1993; Abensperg-Traun and Steven 1997; Dawes-Gromadzki 2003). While studies in higher rainfall tropical and temperate areas in Australia have recorded between 29 and 55 species, there are often <10 species recorded in arid zones (Abensperg-Traun and Steven 1997). However, baiting has been shown to provide a particularly efficient, relatively accurate and repeatable technique for following the activity of termites through time in arid landscapes (Davies et al. 2013; Taylor et al. 1998). This is particularly true for wood-eating termites, which play an important role in arid ecosystems in which woody shrubs predominate during drought, and grass, dung and leaf litter availability is irregular (Abensperg-Traun 1991, 1993). Despite their ecological significance and sensitivity to chemical pesticides, subterranean wood-eating termites have rarely been studied in locust

control research and only harvester termite groups have been monitored during control spray trials in the past (Mamadou and Sarr 2009; Peveling et al. 2003; Steinbauer and Peveling 2011).

Our large scale field experiment tested the hypothesis that locust spray applications would decrease termite activity at wood baits within arid grasslands in western NSW, Australia. We designed our field experiment to approximate the scale of locust control activities in Australia by using replicated 70 ha control and sprayed treatment sites. Because fipronil is already recognised as an effective termiticide at high dosages (Vargo and Parman 2012), we predicted that the application of fipronil would have a larger impact on termites than *Metarhizium* treatments or untreated controls. The use of fipronil barrier treatments or *Metarhizium* blanket treatments was also predicted to allow rapid recovery of affected termite colonies because of the relatively small areas treated and ultra-low dosages used. The use of an experimental design which approximates methods relevant to the management of locust control will help determine the relative hazards of these practices to termite communities in Australia and elsewhere.

Materials and methods

Study area

Research was conducted at Fowlers Gap Arid Zone Research Station, near Broken Hill, NSW Australia (S31.087034, E141.792201; Fig. 1). Although there were no locust outbreaks at the time of the study, this site is within the geographical region of western New South Wales where destructive locust outbreaks and spray operations have historically occurred. This property is a working sheep station also managed for biodiversity conservation. It has cool winters and hot summers (average maximum summer temp for Jan: 36 °C, winter average minimum 6 °C for July) with an annual average rainfall of 292 mm (Meteorology 2014). Sites in the current study were located in arid habitat with no trees, irregular large areas of bare soil and a sparse ground layer dominated by woody shrubs and perennial grasses. The shrub layer was dominated by Chenopodiaceae species (*Atriplex*, *Bassia* and *Maireana* sp.), while grasses were dominated by the genera *Astrebla*, *Dichanthium*, *Panicum* and *Eragrostis*.

Study design and setup

We used a BACI (before, after, control, impact) experimental design (Green 1979) to test the effects of two pesticide treatments on termites. At all sites, termite-

attracting baits were placed on-site and monitored for a year before treatment and a year after treatment. Nine sites, each approximately 1 km in diameter, were spaced at least 2 km apart. Sites were placed haphazardly within a 20 × 15 km area and randomly allocated to each of three treatments: control, fipronil treatment and *Metarhizium* treatment (Fig. 1). Each site contained six monitoring arrays with five arrays placed in a circular pattern around a centre array. Placement was determined by random number generation determining an angle within each of five sections of a circle and a location between 200 and 500 m from the centre array. All arrays were at least 200 m apart. At each array, we placed 24 termite baits (20 × 4 × 2 cm wooden blocks of kiln dried and untreated *Eucalyptus regnans*) in a cross formation at 8, 24 and 40 m from a central stake (total n = 1296 baits). Former studies in Australia have shown that common wood-eating termite species across the continent are able to feed on the wood of *E. regnans* (Dawes-Gromadzki 2003; Evans and Gleeson 2001). Although this tree is not native to our site, the wood represented a readily available bait choice likely to repeatedly attract wood-eating termites. At each of the three distances (8, 24, and 40 m) along each of the four arms of each array, we secured one bait to a stainless steel tent peg on the soil surface, and second bait 5 cm below the ground (Fig. 1). Baits were weighed to the nearest 0.05 g and then left in the field for a year, either during the pre-treatment year or the year post-treatment. Pre-treatment baits were set out in February 2012 and collected in February 2013. The treatment sprays were applied in late February 2013, after pre-treatment baits were collected. To ensure that our baiting also measured the time termites took to discover baits after spray, the post-treatment baits were not set in the same locations within the monitoring arrays as the pre-treatment baits, but were each placed 2 m to the right of the pre-treatment bait locations in late February 2013 and collected in February 2014. We inspected each bait for termite activity before treatment in June, September, December 2012 and February 2013, and after treatment in June, September, December 2013 and February 2014. During inspection, observers turned over above-ground baits and dug up below ground baits so that the presence of termites, tunnels or evidence of termite feeding could be recorded during each visit. Baits were quickly returned to their original location and below ground baits were re-buried after inspections. Though it is likely that the disturbance caused by checking baits may have acted as a deterrent to termites or an attractant for other fauna, these effects would have been consistent among treatments and years.

Termite activity was recorded as presence due to wood-feeding, presence due to tunneling near baits or absence of any activity. When presence due to feeding was observed,

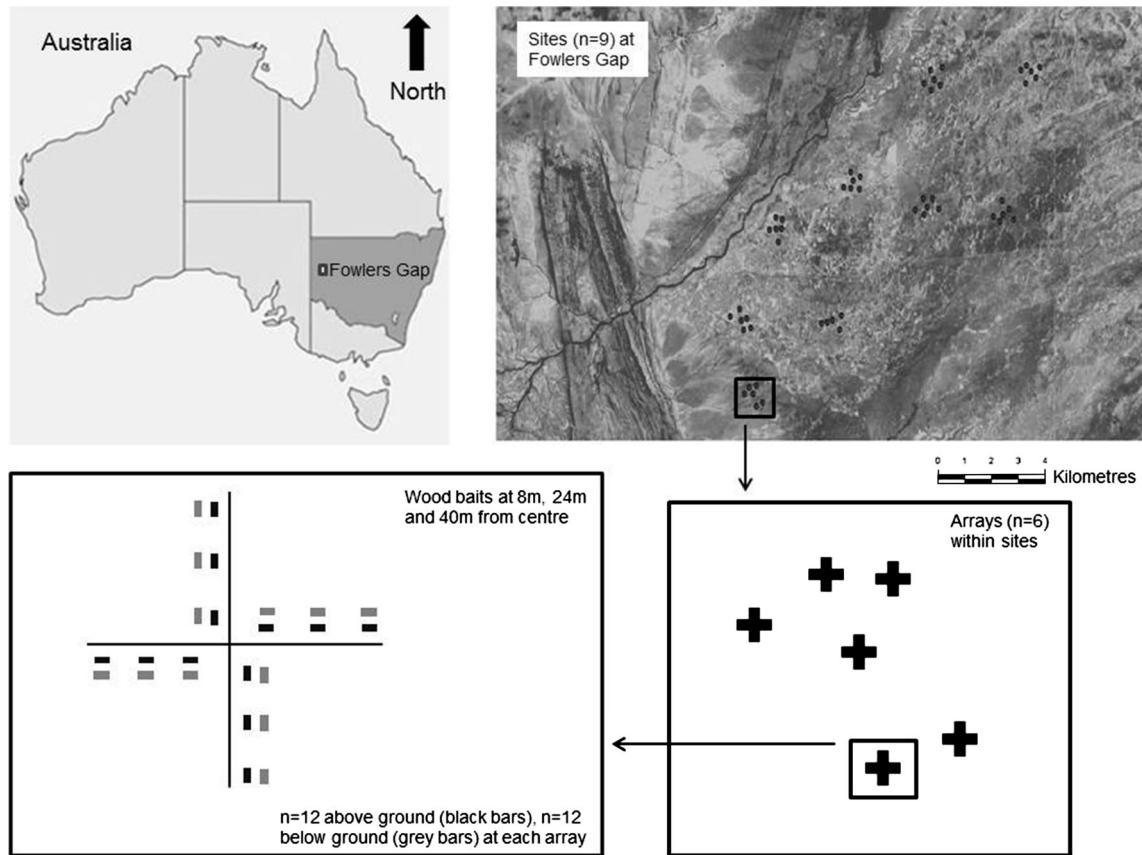


Fig. 1 Diagram representing location of study sites at Fowler's Gap Arid Zone Research Station and placement of 24 baits within each of six arrays, within each of nine sites

the amount of bait consumed was recorded on a scale of 1–5, with 1–4 corresponding to each fourth of the bait consumed, and five corresponding to complete consumption of the bait. Baits were scored according to the percentage of bait consumed in total; therefore, if a bait was scored as 1 in June, and if, by September, further consumption had not removed more than 1/4 of the bait, the score remained as 1. Also, if termite tunneling activity (but not bait consumption) was recorded in June, this bait was not re-scored for termite activity if no new tunnels were found later in the year, but nor would it gain a score if more tunnels were found by February because it was difficult to confidently identify new tunnels. The result was a maximum score of 1 for non-wood eating termite activity. Even after 1 year, less than 3 % of all baits were recorded with a score higher than 1. Therefore in subsequent analyses, we used only presence of wood-eating termites, presence of termite tunnels or absence of any termites. For each array we then calculated, separately for above and below-ground baits, the percent of the 12 baits with termites present. We recognise that lower activity scores in 1 year may simply reflect a reduction in termite activity under altered climatic conditions, but our main hypothesis was that there would

be relatively less evidence of termites finding baits in sprayed than unsprayed sites that were surveyed at the same time and under similar climatic conditions.

In February of each year, collected baits were cleaned of debris or termite tunnels, re-dried in an oven at 80 °C for 10 days and then weighed to the nearest 0.05 g. Worker and soldier termites were collected for identification when encountered on or in the baits during all months. We attempted to identify all termite specimens to morphospecies; however, five of the nine recorded morphospecies were only identified when soldiers were present. This required us to sometimes lump the five large Termitinae species into a general grouping named 'large Termitinae sp.' Because of time and funding constraints, morphospecies were not further identified to species. Both wood-eating and grass, litter, and detrital-eating termites were regularly found to use the baits as cover for tunnels. Less than 3 % of litter decomposition bags (mesh size 0.28 or 5 mm) filled with 40 g of air dried native grass (*Astrelba pectinata*), and also pegged to the soil surface at all sites in both years, attracted termites, and therefore these were not used to monitor harvester termite activity or diversity.

Spray treatments

The experimental spraying was conducted at a time when there was no locust threat, and when no other spraying was conducted in the region. Our experimental sprays were applied in late summer, a time when historical locust control records indicate that spray operations have occurred more frequently than at other times of the year in this region (Australian Plague Locust Commission, APLC, unpublished data). This is also a season when warmer and more humid conditions are likely to promote termite activity near the soil surface.

Pesticide treatments were applied cross-wind from a Piper Brave (PA36) fixed-wing aircraft equipped with two Micronair AU5000 rotary atomizers (Micron Sprayers) in the same manner as operations for locust control. The spray plane was equipped with a Satloc differential global positioning system (Hemisphere GPS) for spray guidance using a constant flow rate. Within each treated site, three arrays were directly sprayed and three were not. Oil sensitive cards (Spray Systems Co. IL, USA) placed at all arrays confirmed that spray was confined to the three targeted arrays within each treatment site, and not within the three untargeted arrays within each site.

Fipronil (Adonis 3UL formulated at 3 g a. i./L) was applied as a ULV formulation using barrier treatments, which involve the spray plane applying a swath of pesticide (two swaths spaced at 300 m per site, covering three arrays) allowing the cross-wind to drift pesticide across each array corresponding to a dose per unit area of 0.25–1.25 g a. i./ha). Green Guard[®] ULV (*Metarhizium* conidia suspended in corn oil) was applied as a blanket treatment using cross-wind spraying with slightly overlapping tracks (50 m spacing) resulting in a continuous area or ‘block’ of treatment over half of each site (three arrays). While fipronil action occurs through both contact and ingestion, *Metarhizium* action occurs mainly through contact (Gunasekara et al. 2007; Zimmermann 2007). Several grasshoppers (*Chortoicetes terminifera*) showing pink coloration indicative of *Metarhizium* infection were found near the sites during the week after spray confirming that viable conidia were used in our application of this biological insecticide. Spray parameters for each of the treatments are as in Maute et al. (2015). A large scale experiment at these sites also confirmed that the application of both treatments had a short-term impact on terrestrial insects (unpublished data), but no detectable impacts on reptiles (Maute et al. 2015), soil microbes or grass litter decomposition (unpublished data).

Statistical analysis

Percent termite activity at baits was calculated by averaging activity scores per array (percent of baits with evidence

of termites present) separately for above-ground ($n = 12$) and below-ground ($n = 12$) baits at each array ($n = 18$ arrays per treatment, 54 arrays in total) and for each month of observation (June, September, December, and February) in each year of observation (pre-spray, post-spray). Because activity levels were low early in each year, only the cumulative measure of all activity recorded over each year in February was used in analysis. Therefore, each array was given one value ($x/12\%$) for both above and below ground baits during pre-treatment and post-treatment. The effect of treatment (control, fipronil or *Metarhizium*), depth (above or below-ground baits), time (pre or post-treatment) and site nested in treatment (random factor) on arcsine transformed percent termite activity at baits was analysed using a Restricted Maximum Likelihood (REML) random effects model (JMP Pro 11.0.0, SAS Institute Inc. 2013). Treatment, depth and time were included as fixed effects, as were all possible fixed interaction terms, but not interaction terms for the random effect of site. This analysis was performed separately for activity recorded from wood-feeding, and also for the combined activity recorded from wood-feeding and tunneling. Because baits were set in different locations within arrays each year, repeated measures analysis was not appropriate. Because the data were nonparametric, we used the Wilcoxon test for post hoc analysis to explore the direction of significant effects for all analyses of termite activity.

Bait mass loss was first calculated by subtracting the final mass of each individual bait from its initial mass for pre and post-treatment years. Percent of original mass lost was then calculated to control for differences in initial mass among baits. The effect of bait depth (above or below-ground), treatment (control, fipronil or *Metarhizium*) and time (pre or post-treatment) and site nested in treatment (random factor) on percent bait mass lost was analysed using REML random effects models (JMP Pro 11.0.0, SAS Institute Inc. 2013).

We also compared the difference between mean bait mass loss for baits with no termite activity to mass loss for baits with termite activity using Welch’s unequal variances t-tests. Because data were continuous, we used Tukey–Kramer HSD post hoc analysis to explore the direction of significant effects for all bait mass loss analyses.

We also separately analysed the effects of fipronil or *Metarhizium* (sprayed or unsprayed arrays), depth, year and array nested in site (random factor) on termite activity and on mean bait mass loss within the three sites for each pesticide using REML models. A separate analysis of within site effects of spray (sprayed and unsprayed arrays), depth and time was calculated using REML random effects models for fipronil and *Metarhizium* sites. We used retrospective power analysis based on the sample size and

variance of termite activity or bait mass loss data to estimate the probability that our sampling procedure would detect differences among treatments (JMP Pro 11.0.0, SAS Institute Inc. 2013). Site nested in treatment was set as a random factor in our analyses. This removed much of the variation resulting from array placement within the three sites allocated for each treatment, and our power analysis therefore used the variation among the 18 arrays per treatment.

The termite morphospecies that were identified were pooled for each array to reduce the number of non-detections (zero values) analysed. The effects of treatment, site and time on untransformed termite community composition data were analysed using PERMANOVA (Bray Curtis used to create resemblance matrix) and pairwise comparisons to suggest which treatments or times were dissimilar. We used the similarity percentages module in PRIMER (SIMPER) to identify which species accounted for more of the dissimilarities between times and visualised the data using a nonmetric MDS (PRIMER 6.1.11 & PERMANOVA+ 1.0.1, PRIMER-E Ltd, 2008). The effects of spray within treatments and time on untransformed termite community composition data were also analysed separately for fipronil and *Metarhizium* using PERMANOVA.

Results

Even after 12 months in the field, we recorded wood-feeding and other termite activity at, on average, only 27 % of below ground baits and 20 % of above ground baits per array (Fig. 2). Mean termite activity levels increased over time within each year for both above and below ground baits, and the increase from December to February was significantly greater in below-ground baits compared to above-ground baits (Fig. 2). In the BACI design, an impact of the pesticide spray treatment should be detected by a significant treatment \times time (pre- vs post-spray) interaction. Analysis of cumulative annual termite activity at baits using a REML random effect model revealed no significant interactions and no significant main effect of treatment or time (Table 1; Fig. 3); depth was the only significant factor for both wood-eating activity and all for all termite activities combined (Table 1). Termite activity in baits was greater in those that were buried compared to those on the surface (Fig. 2). There were no significant effects on termite activity of sprayed vs unsprayed arrays within sites either for fipronil or for *Metarhizium* sites (interaction terms treatment \times time $F_{1, 60} = 0.28$, $P = 0.60$ fipronil, $F_{1, 60} = 0.06$, $P = 0.81$ *Metarhizium*; online supplementary material Table A). Because non-wood eating termites were only detected at 0–8 % of all baits, depending on site, these non-normal data were not analysed separately.

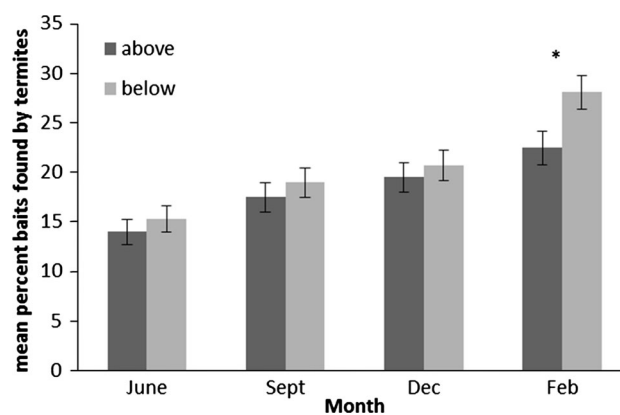


Fig. 2 Activity for both wood-eating and other termites observed at baits (bait depth is above or below-ground) inspected in each month (times pooled). Bars represent mean percent of baits found by termites within each array (\pm SE). Significant difference (*asterisk*) between depths determined by Wilcoxon test, $P = 0.01$, all other comparisons non-significant ($P < 0.05$)

There were no significant effects of any factor, nor were there significant interactions between factors in analyses of the percent bait mass loss using data either from arrays across all sites (Table 2; Fig. 3), or from arrays within each of the two types of spray treatment (online supplementary material Table B). The mean bait mass loss was $7.03 \% \pm 0.02$ SE for baits without evidence of termite activity. When those baits were excluded from the analyses, still no factors were significant, although bait depth was nearly significant, with higher percent mass loss for below ground baits ($P = 0.06$, online supplementary material Table C; above ground mean $11.16 \% \pm 1.59$ SE, below ground mean $15.85 \% \pm 1.59$ SE). Analysis of differences in mean bait mass loss between baits with and without termite activity suggested that termites removed significantly more mass than other decomposition processes alone for both above ground and below ground baits (above, $F_{1, 363} = 10.72$, $P < 0.001$, $t = 3.27$; below, $F_{1, 443} = 33.24$, $P < 0.0001$, $t = 5.77$). Mass loss of baits with no evidence of termite activity averaged 7 % over the course of the study (above $7.3 \pm 0.9 \%$ SD, below $6.8 \pm 0.9 \%$ SD), and based on percent mass loss at baits with termite activity (above $8.9 \pm 9.6 \%$ SD, below $10.8 \pm 14.9 \%$ SD), we can assume that termites were contributing a third or more of all wood bait mass loss, for the proportion of baits that were attacked (up to 35 % attacked at each array), over 2 years.

Power analysis of overall termite activity sampling suggested our design had an 88 % chance of detecting a 30 % difference among treatments, or a 35 % chance of detecting a 15 % difference between treatments ($n = 18$ arrays per treatment, $std = 0.19$) and that, in relation to bait mass loss, our design had a 91 % chance of detecting a

Table 1 Analysis of the effects of treatment (control, fipronil and *Metarhizium*), time (pre or post-treatment, n = 108 per time) and bait depth (above or below-ground) on either wood-eating termite or all

termite activity at baits using REML random effect models with site nested in treatment designated as a random factor

Factor	Wood-eating termite activity degrees of freedom		F value	P value	All termite activity degrees of freedom		F value	P value
	Numerator	Denominator			Numerator	Denominator		
Treatment	2	6	0.24	0.80	2	6	0.17	0.85
Time	1	200	0.18	0.67	1	200	0.10	0.75
Depth	1	200	6.77	0.01	1	200	6.51	0.01
Treatment × time	2	200	1.16	0.33	2	200	0.84	0.43
Treatment × depth	2	200	0.03	0.97	2	200	0.13	0.88
Time × depth	1	200	0.12	0.73	1	200	0.66	0.42
Treatment × time × depth	2	200	0.26	0.77	2	200	0.24	0.79

Significant P-values are shown in bold

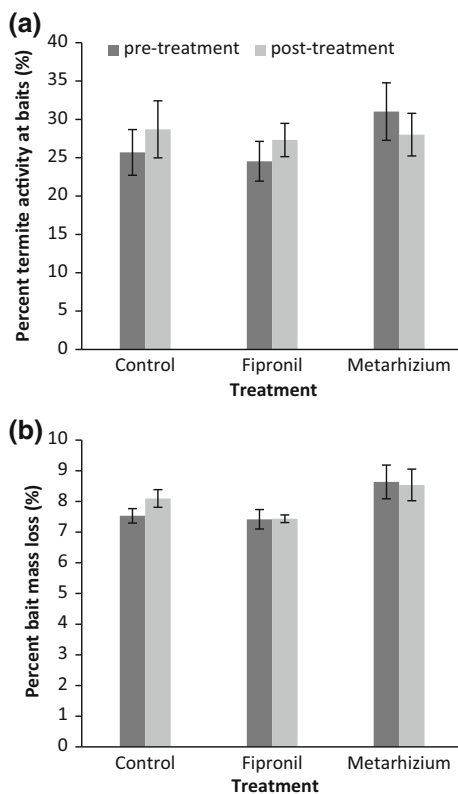


Fig. 3 Percent of baits with both wood-eating and other termite activity **a** and percent bait mass loss **b** for fipronil, *Metarhizium* and control treatments during pre and post-treatment years. Bars represent mean ± SE

10 % difference in percent mass loss between treatments (n = 18 arrays per treatment, std = 0.06).

Termite community analysis using PERMANOVA indicated that composition differed among sites nested within treatments (site (treatment) $F_{6, 107} = 1.69$, $P = 0.03$) but that differences were consistent between pre

and post-treatment sampling times (time × site (treatment) interaction $F_{6, 107} = 0.98$, $P = 0.50$; treatment × time $F_{2, 107} = 0.37$, $P = 0.85$; time $F_{1, 107} = 0.91$, $P = 0.46$, treatment $F_{1, 107} = 2.28$, $P = 0.09$). This suggested that there was no treatment effect on termite community composition. Pairwise tests and MDS visualization of dissimilarities showed that *Metarhizium* and fipronil communities were very similar ($T = 0.88$, $P = 0.46$), while control site communities were less similar to both *Metarhizium* and fipronil treatments (*Metarhizium* vs control, $T = 1.72$, $P = 0.19$; control vs fipronil, $T = 1.91$, $P = 0.10$), though not significantly different (Fig. 4). SIMPER analysis of dissimilarities suggested that trends were largely driven by the presence/absence of a small and common wood-eating *Microcerotermes* sp. and the lumped grouping of the five similar larger species, many of which were likely to be litter, grass and bark-eating *Drepanotermes* sp. (Table 3). Analysis of termite community composition in sprayed and unsprayed arrays within treatments using PERMANOVA showed no significant effects of spray, time, or of any interactions (online supplementary material Table D).

Discussion

The application of the locust specific biocontrol, *Metarhizium*, and the chemical pesticide, fipronil had no detectable effect on wood-eating termite activity, bait mass loss or community composition at wooden baits at our sites. Previous studies investigating the effect of fipronil on termites revealed negative impacts on mound-building termites in Madagascar and northern Australia (Peveling et al. 2003; Steinbauer and Peveling 2011). However, both previous studies used either blanket treatment application of fipronil and/or fipronil at much higher application rates than in the current study. These previous studies focused on

Table 2 Analysis of the effects of treatment (control, fipronil and *Metarhizium*), time (pre or post-treatment) and bait depth (above or below-ground) on percent termite baits mass loss at all baits using REML random effects models

Factor	Degrees of freedom Numerator	F value Denominator	F value	<i>P</i> value
For all sites (n = 288)				
Treatment	2	6	0.56	0.60
Time	1	6	0.11	0.75
Depth	1	6	1.01	0.35
Time × treatment	2	6	0.17	0.84
Depth × time	1	6	0.19	0.68
Depth × treatment	2	6	1.01	0.42
Time × treatment × depth	2	6	0.26	0.78

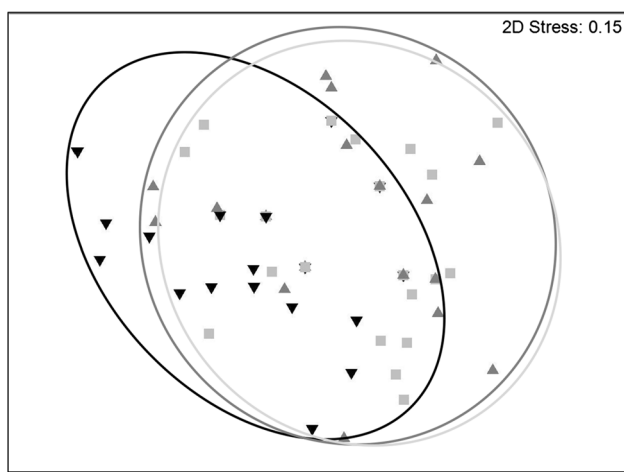


Fig. 4 Visualization of the effect of treatment on community composition using MDS, based on Bray–Curtis similarity (Primer 11.1). The location of symbols represent the relative dissimilarity of community composition among arrays within sites for both before and after sampling times (n = 36 per treatment). Treatment abbreviations: *Metarhizium* = grey triangles, Control = black triangles, fipronil = squares

mound-building termites, which represent grass and litter feeding species and differs from this current study which focused on the detection of impacts on wood-eating termites. Furthermore, the previous Australian study lacked significant pre-treatment monitoring, reducing the rigour of their analysis (Steinbauer and Peveling 2011). Although at odds with these previous studies, our research provides a statistically rigorous, long-term study at realistic spatial scales using relevant control agents and application regimes. Our results support the proposition that these locust control methods, now commonly used in Australia, are unlikely to impact arid wood-eating termite communities over the relatively long timeframes we have considered. From our long-term sampling approach, we cannot rule out possible short-term decreases in individual abundance within termite colonies, or longer-term impacts on less common and difficult to survey grass and litter feeding termites. Those species were not as well represented by our baiting experiment. These generalisations may not be

transferrable to other habitat types, other termite communities, or other application procedures and different fipronil application methods could have significant and long-lasting impacts on other termites in different environments (Peveling et al. 2003). However, the current method of applying these insecticides in Australia does appear to be safe for the termite species we surveyed.

There are four possible explanations why the locust control treatments we used did not impact wood-eating termites in our study. First, it is highly likely that exposure to pesticides did not cause high mortality over the long-term of our observations, as the dose of pesticide administered was insufficient to elicit a population-level response. As mentioned earlier, the ultra-low volume fipronil dosage was used and the pesticide was applied in barriers, which were hypothesized to have a lower hazard to fauna than higher dose blanket treatments which have impacted mound termites in Madagascar (Peveling et al. 2003). Our results agree with this hypothesis. Second, it is possible that most individual termites were not exposed to the spray treatments because they were underground when the sprays were applied. Australian wood feeding termite species rarely access the surface, and all termites visit the surface less during dry conditions (Abensperg-Traun 1993; Evans and Gleeson 2001). Fipronil control of wood-eating termites is effective in field and laboratory conditions due to the high toxicity of fipronil to many termite species (Vargo and Parman 2012). However, termite control is reliant on a large proportion of workers being exposed through high dose treatments of underground soil or fumigation which both result in substantial contact and ingestion poisoning (Chen et al. 2015; Vargo and Parman 2012). Locust control spraying, as in our study, uses neither underground soil nor fumigation applications. Thus it is possible that full colony collapse did not occur during our study, as an insufficient proportion of workers were exposed to fipronil during the dry conditions at our sites. Third, it is possible that there was rapid recovery of colonies from any short-term mortality that did occur, reducing our ability to detect immediate short-term impacts

Table 3 Community analysis using SIMPER shows species which determined 90 % of dissimilarities among treatments

Pairwise difference Termite morphospecies	Feeding preference ^a	Between <i>Metarhizium</i> (M) and control (C)			Between fipronil (F) and control (C) treatments		
		M Average presence	C Average presence	Contribution of morphospecies (%)	F Average presence	C Average presence	Contribution of morphospecies (%)
<i>Microcerotermes</i> 1	Wood	0.33	1.25	48.32	0.39	1.25	42.22
<i>Termitinae</i> sp.	Wood or other	0.25	0.14	14.58	0.42	0.14	19.33
<i>Drepanotermes</i> 4	Litter, bark	0.03	0.22	9.17	0.17	0.22	11.74
<i>Drepanotermes</i> 1	Litter, bark	0.19	0.08	8.33			
<i>Amitermes</i> 1	Grass, detrital	0.06	0.06	4.32	0.11	0.06	5.76
<i>Drepanotermes</i> 3	Litter, bark	0.14	0.00	4.29			
<i>Heterotermes</i> 1	Wood	0.11	0.03	4.16			
<i>Drepanotermes</i> 2	Litter, bark				0.08	0.08	4.77
<i>Microcerotermes</i> 2	Wood				0.14	0.03	6.56

Average presence represents mean number of times the species was encountered per array (n = 9 per treatment), pooled across both times

^a Feeding preference for each genus based on Australian literature (Dawes-Gromadzki 2003; Park et al. 1993; Watson and Perry 1981)

on termites. In Niger, aerial application of chlorpyrifos ethyl and fenitrothion to termites lowered colony activity in the field, however, activity levels returned to pre-spray conditions within 75 days (Mamadou and Sarr 2009). Our sampling did not look at short-term changes in termite activity, but took a longer-term view and began monitoring termites 4 months post-treatment. Consequently, our experiment would not have detected any immediate effects of fipronil or *Metarhizium* on termite activity. Fourth, it is conceivable that termite colonies were impacted by the spray, but dispersal from unaffected colonies in nearby unsprayed areas allowed rapid local recovery. This scenario is unlikely, as most Australian termite species take longer than 4 months to construct a new colony, and it is also unlikely that individuals were able to re-colonize abandoned sections of a polycalic nest or forage in new areas within ten metres of their surviving nest within that time (Abe et al. 2000).

Only 20–35 % of the baits we provided were found by termites and few baits were fully consumed each year. In contrast, baiting studies from more mesic habitats in Australia have observed 90–100 % of baits found or consumed by termites in as little as 6 months (Abensperg-Traun 1993). The low level of activity we recorded supports the hypothesis that termite abundance and diversity is lower in the arid zone than the northern savannas and rainforest or south western woodlands of Australia (Abensperg-Traun and Steven 1997). Our finding of low activity at baits also matches reports of lower termite abundance in low rainfall areas in Africa and North America (Davies et al. 2013; Taylor et al. 1998). If termite abundance is low in our study area, this may result in

slower nutrient cycling in these arid temperate grasslands compared to mesic tropical and temperate regions where a higher abundance of termites and other decomposer fauna such as earthworms drive faster soil nutrient cycles (Culliney 2013; Petersen and Luxton 1982). However, our measure of activity at baits does not measure termite abundance, and could be an artefact of the unknown feeding preferences of the local termite species. Further study including other sampling methods would be needed to determine if termites are less abundant and diverse in arid Australia.

Our power analysis suggests there was sufficient replication using 1296 bait stations averaged across 18 replicate per treatment in our experiment to detect small changes in termite activity and bait mass loss. Other studies using fewer than our 18 sample sites (or 432 baits) per treatment have succeeded in detecting termite responses to disturbance. For example, multiple studies using a minimum of four replicate sample sites (and ten baits) per treatment determined a significant reduction in termite species diversity and activity in response to grazing and to other agricultural practices (Abensperg-Traun et al. 1996; Holt et al. 1993, 1996; Mugerwa et al. 2011). Despite the low levels of bait discovery and mass loss in our study compared to other studies, our level of sampling was robust, supporting the interpretation that our lack of observed treatment differences was indeed due to there being no effect of the pesticide applications on the target wood-eating species. For these reasons, we feel that it is unlikely that a larger number of replicates would have resulted in finding a significant impact of spray treatment on wood eating termites in this habitat.

Our finding that the effect of bait depth on percent mass loss was similar among treatments and between years suggests that wood-eating termite abundance was relatively uniform across our study area. It is unsurprising that termites did not consume higher amounts of wood placed above-ground, as wood-eating termites spend considerable time underground (Abensperg-Traun 1993; Evans and Gleeson 2001). It is possible that in this arid environment, where dead wood on the soil surface is rare due to a lack of trees, wood eating termites commonly find woody material partially buried, in the form of dead chenopod shrub and perennial grass roots. In another study conducted at our site, only 2–4 % of litter bags filled with native *Astrebla* sp. grass attracted termites, suggesting that surface feeding termites are extremely difficult to monitor using baiting in this habitat (unpublished data). Termite activity measures also suggested that above-ground baits were not found by termites as often as below-ground baits. Despite these differences between bait locations (above and below ground), we found consistently more bait mass loss in baits with evidence of termite activity than in those apparently unvisited by termites. This suggested that termite activity resulted in significant mass loss at both above and below ground baits. For instance, in below ground baits, those with signs of termite activity lost an additional 3 % of initial mass above the mean of 7 % mass loss for those without termite activity. These measures clearly show that termites were responsible for a significant proportion of wood decomposition over the course of our study, and support the hypothesis that they are important components of the decomposition process in the Australian arid zone, albeit at a much slower rate than recorded in many mesic environments. This finding partially agrees with previous findings that above ground decomposition in arid environments is largely controlled by photodegradation, and less by bacteria, fungi and invertebrates such as termites (Austin 2011).

Grass and litter foraging termites (such as species of *Drepanotermes* and *Amitermes*) may not have been detected as often as wood eating species because they are less attracted to wood baits (Dawes-Gromadzki 2003). However, wood baits should have created shelter from high temperatures and a moister microenvironment, which would attract non-wood eating species of termites. Past research on *Drepanotermes* species has suggested that these termites are attracted to trees and woody grass roots, as they often harvest leaves and bark, as well as grass and dung (Noble et al. 2009; Park et al. 1993). Though these species made up a small proportion of the activity at baits, the lack of a statistically significant impact of treatments on all termite activity and community composition suggests that treatments did not have enough of an impact to result in the collapse of harvester termite colonies, as was seen

with higher concentrations of fipronil in previous studies in Madagascar and Australia (Peveling et al. 2003; Steinbauer and Peveling 2011). The determination of harvester termite species responses to locust control treatments at our sites would require the trial of additional baiting methods using paper or dung-based baits, or soil sampling, and was beyond the scope of this study.

Although termite activity and bait mass loss levels were similar among treatments, there were minor differences in termite community composition among treatments. There was no significant interaction between year and treatment effects, suggesting that differences in termite assemblages or activity were not due to pesticide treatments. Instead the among-treatment effects suggest an underlying difference among habitats at different sites may be driving minor differences in species compositions. Subterranean wood-eating termites (*Microcerotermes* sp.) made up a large proportion of the communities at control sites, and both subterranean (*Microcerotermes*, *Amitermes* sp.) and above-ground foraging termites (*Drepanotermes* sp.) were more common at treatment sites. This suggested that we should find a higher rate of bait mass loss at control sites, because wood-eating termites made up a larger proportion of the community in these areas. However, we found no significant differences in bait mass loss across sites. One explanation is that decrease in the mass of baits measures how active a colony is at a bait once it is found, and not necessarily how frequently the baits are found by a certain species of termite. The relatively small decreases in the mass of baits (mean of 11–16 % per array) due to termite activity and the lack of a difference in the percent termite activity measure among treatments supports the hypothesis that termite abundance was similar among sites. Therefore the differences in community composition seen among treatments are not clearly correlated with the decrease in bait mass, and are likely due to small scale differences in environmental conditions among sites. Since our treatments were allocated to sites randomly, and were well spread across the study area, any apparent differences in termite communities among treatment and control sites are probably a stochastic pattern and not a consequence of a compromised experimental design.

Conclusions and future research

This study found no detectable impact of locust control using current application methods for fipronil or *Metarhizium* treatments, on termite activity or bait mass loss in arid Australian rangeland. Few other field studies have attempted to measure the effect of chemical locust control on termites, and all previous research has focused in areas where harvester termite abundance and diversity is high

(Peveling et al. 2003; Mamadou and Sarr 2009; Steinbauer and Peveling 2011). We believe that this is the first study attempting to determine the impact of locust control on arid zone wood-eating termites. Non-significant trends in activity levels and variation in measures among sites suggest that climate and local environmental conditions may be driving differences in termite activity and species distributions, a result that mirrors our similar research on reptile communities at this site (Maute et al. 2015). Though termites have previously been shown to be sensitive to chemical control, many studies have suggested that termites are resilient to some agricultural practices such as grazing and biocontrol (Abensperg-Traun 1992; Abensperg-Traun and Milewski 1995; Chouvinc et al. 2011; Dawes-Gromadzki 2005; Vargo and Parman 2012). It is not surprising that termites did not respond to the orthopteran specific *Metarhizium* treatment, and a lack of evidence for an impact of fipronil treatment is encouraging; supporting the use of ultra-low volume barrier treatments as a low hazard locust control option in Australian arid rangelands. However, important information on the immediate or sublethal effects of locust control on termites is still missing, and impacts could differ in other environments, climatic conditions and under different spray application procedures. Future research focusing on the effects of fipronil on a smaller temporal and spatial scale, in different habitats, in higher rainfall regions, and using full blanket spray treatments, where these are relevant, would provide a better understanding of the short term, comparative effects of different pesticide applications on arid zone termites. Grass and litter foraging termites common in higher rainfall regions are likely to be at greater risk during times of increased activity on the soil surface and short-term impacts on these important detritivores could have important local effects within arid ecosystems.

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