



Cheaters and removalists: the influence of soil disturbance on ant–seed interactions in roadside vegetation

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Abstract

Myrmecochory is an important mutualistic interaction, where ants provide seed dispersal services for many plant species. We examined the influence of soil disturbances associated with roadworks activity on ant–seed interactions in roadside environments in south-eastern Australia. In the study landscape, minor rural road reserves often provide critical habitat for endangered species and woodland ecosystems. We conducted seed-depot experiments to quantify the extent of ant–seed removal, and identified the contribution of different ant species to seed removal and elaiosome consumption (cheating) interactions, in disturbed and non-disturbed roadside zones. Twenty-six ant species were recorded interacting with seeds; however, only a few species were responsible for dispersing most seeds. We found soil disturbance and roadside width influenced observed ant–seed interactions, which was largely explained by individual species habitat and behavioural traits. *Iridomyrmex purpureus* removed a higher proportion of seeds in soil disturbed zones, while *Rhytidoponera metallica* carried out more seed removals in narrow roadsides. *R. metallica* and *Melophorus bruneus* typically carried out seed removals (only), while *Monomorium* and *Pheidole spp.* almost exclusively carried out cheating behaviours. These results highlight the complex nature of ant–plant interactions is association with novel soil disturbance regimes, where ants perform a critical seed dispersal service for myrmecochorous roadside vegetation.

Keywords Ant assemblages · Grading · Myrmecochory · Road verge · Seed dispersal

Introduction

Australia is home to more than 1500 plant species that are adapted to seed dispersal by ants (a myrmecochore ‘hotspot’; Berg 1975; Lengyel et al. 2010). Myrmecochorous plants have seeds with a specialized food body attached to them, called an elaiosome, which is rich in lipids and proteins (e.g., Brew et al. 1989; Fischer et al. 2008). In this mutualism, ants gain a nutritious reward by collecting the diaspore (seed plus elaiosome), and in return, provide a critical seed dispersal service when they remove the elaiosome and deposit intact seeds in suitable sites for germination (e.g., Howe and Smallwood 1982; Giladi 2006). Previous studies have documented a strong positive correlation between the number of seed-dispersing ants and myrmecochorous plant species in an ecosystem (Berg 1975; Beattie 1985). This important

mutualism provides many benefits to myrmecochorous plants, including relocation of seeds to more favourable microsites for survival, germination and plant establishment (e.g., Giladi 2006; Rico-Gray and Oliveira 2007).

While many ant species interact with seeds, only a few may act as good seed dispersers (Giladi 2006). For example, certain ant species are more likely to remove the elaiosome in situ (cheating), without effectively dispersing the seed (Manzaneda et al. 2007; Jones et al. 2015). These ‘cheated’ seeds are less attractive to other seed-dispersing ants (Cuautle et al. 2005); therefore, the chances of seeds being dispersed into safe microsites are greatly reduced. Seeds can also be directly predated upon by ants (for example, seed harvester ants; Buckley 1982; Andersen et al. 2000) or by other predators, such as rodents and birds (Morton 1985). Recent studies have highlighted that in many ecosystem contexts, a few good seed-dispersing ants are responsible for the majority of plant dispersal services (Warren and Giladi 2014). This ‘unevenly diffuse’ mutualism (Gove et al. 2007) highlights the importance of keystone ant species in plant dispersal (Manzaneda and Rey 2009; Ness et al. 2009).

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The effectiveness of ant dispersal services to plants largely depends on the identity of the ant participant, but also the nature of the ant–seed interaction, and prevailing habitat conditions (Beattie and Hughes 2002).

Ant–seed dispersal processes can also be greatly influenced by prevailing natural and/or anthropogenic disturbance regimes (Crist 2009). Changes in myrmecochorous interactions following disturbances have been reported at various spatial and temporal scales (e.g., Garrido et al. 2002; Leal et al. 2013; Palfi et al. 2017a), where modifications to habitats conditions has altered the abundance and composition of ant communities. Disturbance effects on seed dispersal activity by ants include (a) changes in the behaviours of ants, (b) reducing or enhancing populations of specific ant species and subsequent dispersal activity, and (c) completely eliminating important seed-dispersing ant species (Gibb and Hochuli 2003; Heithaus and Humes 2003; Hoffmann and Andersen 2003; Vonshak and Gordon 2015; Andersen 2019). Invasive species and edge effects can also lead to the further disruptions of this mutualism (Christian 2001; Ness and Morin 2008; Christianini and Oliveira 2013). As a result, ant–seed interactions in relation to disturbances can be species and habitat specific (e.g., Pudlo et al. 1980; Mitchell et al. 2002; Parr et al. 2007; Leal et al. 2013; Warren and Giladi 2014).

With respect to understanding how disturbance effects ant–seed dispersal, previous studies have largely focused on the affects of fire (e.g., Majer 1982; Beaumont et al. 2011; Arnan et al. 2013). However, the effects of anthropogenic soil disturbance regimes, and its influence on important seed-dispersing species, has received less attention (but see Andersen and Morrison 1998; Palfi et al. 2017a, b). Although fire is a dominant natural disturbance process in most temperate and semi-arid ecosystems, anthropogenic soil disturbance regimes also pervade most ecosystems. For example, minor (gravel) roads are a feature of Australian agricultural landscapes, where adjacent roadside vegetation is frequently disturbed by soil grading associated with roadworks activities (Spooner et al. 2004; Bognounou et al. 2009). Roadside vegetation often provides critical refuge for remnant ecosystems and native ant species in otherwise cleared and fragmented landscapes (e.g., Bennett 1991; Major et al. 1999; Vieira-Neto et al. 2016; Kaur et al. 2019). Therefore, knowledge of the effects of soil disturbances on ant communities is important in understanding seed dispersal processes in road corridors (Suárez-Esteban et al. 2013), and for roadside conservation activities (Spooner 2015).

As part of a broader research program which investigated the effects of soil disturbances on the composition of seed-dispersing ants (Palfi et al. 2017a), and seed dispersal distances by ants (Palfi et al. 2017b), we investigated the extent to which soil disturbances may influence ant–seed behavioural interactions. Studies were conducted in linear

vegetated road corridors which experience frequent soil disturbances from roadworks and other soil grading activities. More specifically, we addressed the following questions: (1) How does soil disturbance influence seed removal rates and ant–seed behavioural interactions (seed removal vs. cheating), and (2) does soil disturbance alter the behaviour of ant species in roadside habitats? Also as Australian roadside environments are of different widths, we also investigated the effects of roadside width on ant–seed interactions.

Methods

Study area

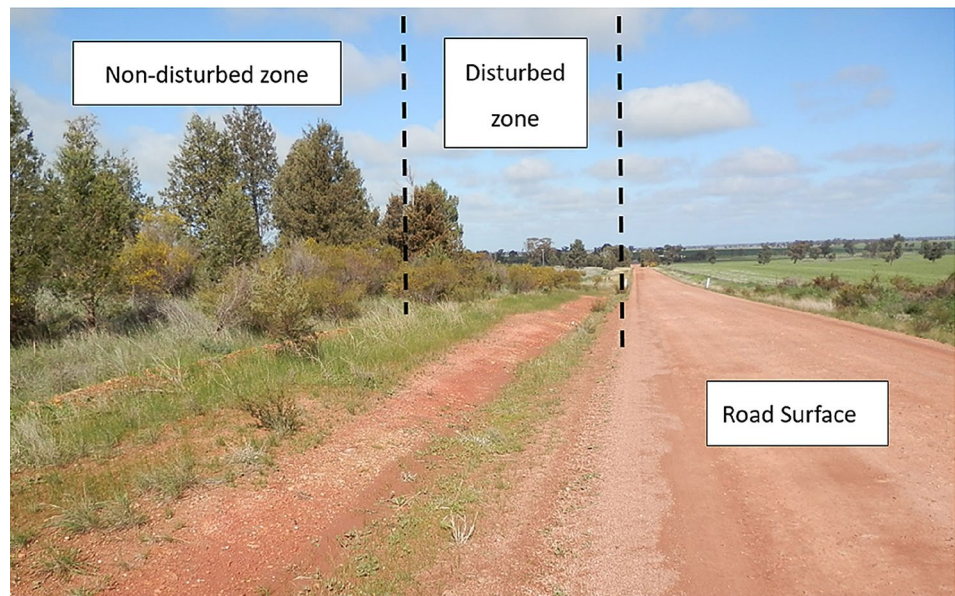
The study area (– 35.527906, 146.807167) was located in southern New South Wales, Australia. The region is characterised by low undulating hills and plains with some granite outcrops, elevation is 300–500 m asl, and where the average annual rainfall is 500–600 mm/year. This region is dominated by cropping and grazing farm systems, subdivided by a network of minor rural roads, where some are paved, but most are of gravel construction (Palfi et al. 2017a). Linear road corridors are of different widths, and possess native woodland and grassland vegetation in a range of conditions. In Australia, remnant woodland vegetation often possesses important conservation values (e.g., Yates and Hobbs 1997; Gillison 1994; Spooner and Lunt 2004).

Sampling design

Building on existing roadside vegetation datasets (Bull 1997; Spooner et al. 2004; Spooner 2005), the study was carried out in the Lockhart Council local government area (LGA), which encompasses an area of 365 km². The research was confined to one LGA to ensure a consistent approach to road management activities was applied across the study area, while sampling was undertaken. We carried out observations of ant behaviour in roadside vegetation (immediately adjacent to the road surface) which is frequently impacted by soil grading activities by heavy machinery (the ‘disturbed zone’; following Spooner et al. 2004), and the undisturbed roadside environment (the ‘non-disturbed zone’). Grading activities in the disturbed zone normally occurs 1–2 times per year, where the topsoil is graded ~0–1 cm in depth to scrape away vegetation (Fig. 1).

To conduct this study, 24 roadside sites were randomly selected, where *Acacia pycnantha* (Golden wattle) populations were present—a commonly occurring myrmecochorous shrub. *Acacia* is the largest genus in the family Mimosaceae, with more than 900 species occurring in the continent. *Acacia pycnantha* occurs in the understorey of open Eucalypt woodlands in south-eastern Australia, where

Fig. 1 Typical narrow linear road reserve in the Lockhart study region, showing roadside vegetation dominated by native *Callitris glaucophylla* and *Acacia* spp. trees. The disturbed zone is the area which is graded parallel to the gravel road edge (approx. 2–3 m width), as annotated on the image



the Lockhart LGA is situated. The species growth form is a shrub or small tree, 4–8 m tall, and it occurs on a range of soils including clays and shallow, stony loams (Tame 1992). For this study, an *Acacia* population was defined as a minimum of 30 individual plants dispersed either evenly, or forming clumped patterns, within a 60 m longitudinal section of undisturbed road verge (density approx. 30–170 stems/ha depending on roadside width). Study sites were separated by at least 500 m from each other to ensure independence of ant–plant interactions. In Australia, the area of land set aside for a road is called a road reserve, which commonly occurs in two different widths. The 24 sites were stratified into two equal groups based on road reserve width; narrow (21 m) or wide (63 m) roadsides (Fig. 1).

Observations on ant–seed interactions

The study design followed similar studies which have observed seed processes in relation to disturbance regimes (e.g., Zelikova and Breed 2008, Leal et al. 2013). At the centre of each site, two pairs of 25 m transects were placed in both the disturbed and undisturbed zones, separated by a longitudinal gap width of 10 m, using a matched pairs design. Transects ran parallel to the road edge, and positioned centrally as a function of width in their respective zones. Seed depots were then placed every 5 m along each transect, resulting in a total 24 seed depots at each site. Each seed depot represented a notional square of approximately 9×9 cm on the ground. Seeds were placed directly on the ground separated by approximately 2 cm from each other, and where necessary a palm-sized clearing of the vegetation was made to facilitate ant–seed observations (Hughes and Westoby 1990).

At each seed depot, 10 seeds of a single plant species *A. pycnantha* were offered at seed depots for each sampling period. Seeds were collected prior to the study as described in Palfi et al (2017a); *A. pycnantha* seed size is typically 3×5 mm. The research was undertaken during the summer months (December, January, February and March), when *Acacia* seed is normally released from plants. Two 4-h observational periods were performed during each day: a morning session (8:00 am to 12:00 pm) and an afternoon session (16:00 pm to 20:00 pm). Observations were curtailed during midday due to high ambient temperatures (often > 38 °C) when ants were largely inactive above ground.

Each seed depot initially contained 10 seeds, which were offered to ants at the start of each observational period. During the 4-h observational period, two observers walked along each transect, and performed observations for approximately 10 min at each seed depot to maximise the chances of observing ants interacting with seeds. The order of observation among depots was randomly selected. The following data were recorded: (1) number of seeds removed by the end of the 4-h session, or if all seeds were removed sooner, the time when all seeds were removed; and (2) the nature of ant–seed interactions ('removal' or 'cheating').

A seed removal event was determined when ants moved seeds at least 5 cm away from the edge of the seed depot, and then (usually) carried these seeds to their nests (Palfi et al. 2017b). When an ant started chewing on the elaiosome, without removing the seed itself, this behaviour was categorised as 'cheating'. The number of seeds ants cheated upon or removed, and the number of cheating individuals, was recorded at each seed depot. A seed sometimes experienced both interaction types. If a cheating behaviour was observed first, but it was subsequently removed by the same

or a different individual, this was categorised as a seed removal event.

Furthermore, two types of cheating occurred: (a) individual cheaters and (b) those that recruited other nest mates on seeds (to consume the elaiosome in situ). In the former case, this behaviour was classified as cheating if an individual belonging to the same ant species handled a seed (or multiple seeds). For the latter category, this often occurred when smaller bodied species interacted on a seed, or on multiple seeds, and their numbers usually increased over the observation period (i.e., max. 4 h). Recruiting behaviour implies that a certain number of individuals recruit to a food source, where fragments of food (in this case the elaiosome) may be consumed in situ, or can be transported back to the nest, provided the nest is situated in the vicinity (Planqué et al. 2010). In this case, the maximum number of seeds that a particular species cheated on was included in the statistical analysis (below).

Where possible, ants were identified in the field during the experiments. Otherwise, they were followed until a nest was reached, or the seed was dropped, and then captured and placed into vials filled with 70% ethanol for later identification. Collected individuals were identified in the lab to genus using Shattuck (1999) and allocated to morpho-species, while expert advice was sort for species level identification. This study was carried out in accordance with AAC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and Charles Sturt University Animal Care and Ethics Committee policy.

Data analyses

The effect of zone, width and time on the proportion of seeds removed during 4 h of exposure to ants was analysed using a generalized linear mixed model. Sites were included as random effects, and time of sampling as repeated measures within sites. Road width (narrow or wide) and zone (disturbed or undisturbed) were included as fixed factors. The proportion of seeds removed in each transect (expressed as average percentages) was cumulative across all seed depots in each transect. Assumptions for normality and homoscedasticity were confirmed using visual analysis of residual plots (Zar 2010).

Seed removal rate was calculated as the number of seeds removed (max = 10) at any given depot during the 4-h experiments/time when all seeds were removed (time units are minutes). If there were seeds left at the depot by the end of the session, the number of seeds removed was divided by the end of the observational period (i.e., 240 min). Initial analyses revealed no differences between morning and afternoon sessions; therefore, the time factor was removed from all analyses. Seed removal rate data was then averaged at

the transect level for each site, and analysed using a further GLMM.

To test for differences in the occurrence of observed ant–seed interaction behaviours between zone, time and width, a further GLMM with a binomial distribution and a logit link was fitted. Initially we conducted analyses for all ant species combined (i.e., on the proportion of all seeds removed or cheated by any ant species) following methods described above. For these analyses, the proportion of seeds (removal or cheating) was the number of seeds removed or cheated, divided by the sum of the total number of seeds that the ants interacted within each transect. Significant effects were followed up using pairwise comparisons of means and Scheffe's adjustment for multiple comparisons and interpreted using graphs of the least squares means and Wald confidence intervals (Zar 2010).

To conduct analyses for individual ant species, we repeated the same method as above for ant species with contributed to more than 5 per cent of ant–seed interactions. Due to low numbers and zero values, we combined all other ant species under an 'Others' category. For this analysis, we only included seeds that were removed or cheated. In otherwords, species relative contribution to total removal or cheating interactions was analysed. All analyses were run using SAS/STAT® (SAS 2013) within the SAS Studio (SAS 2015).

Results

Overall seed removal

A large average percentage of total seeds (85%) were removed from all seed depots combined. GLMM model results were non-significant ($p > 0.5$); however, there was a significant interaction effect between disturbance zone and roadside width on the average proportion of total seeds removed ($\chi^2 = 4.52$, $df = 1$, $p = 0.03$) (Fig. 2). Seed removal rates (i.e., measured in a 4-h period) did not significantly differ between disturbed and non-disturbed zones, narrow and wide roadsides (all tests, $p > 0.5$). For all sites combined, seeds were removed within 2 h of the experiment (mean $121 \text{ min} \pm 4.91 \text{ SE}$).

Observations on ant–seed interactions

A total of 5760 seeds were offered to ants during the observational period. For all seed depots combined, an average of 42% of seeds were observed being taken away by ants as removal events, while for 17% of seeds, the elaiosomes were consumed in situ and recorded as cheating events (Fig. 3). The GLMM models produced showed a significant interaction effect between zone and width on the average proportion

Fig. 2 Proportion of seeds removed from disturbed (D) and non-disturbed (ND) roadsides zones, for narrow and wide roadsides (shown as average per transect). Error bars represent Wald confidence intervals

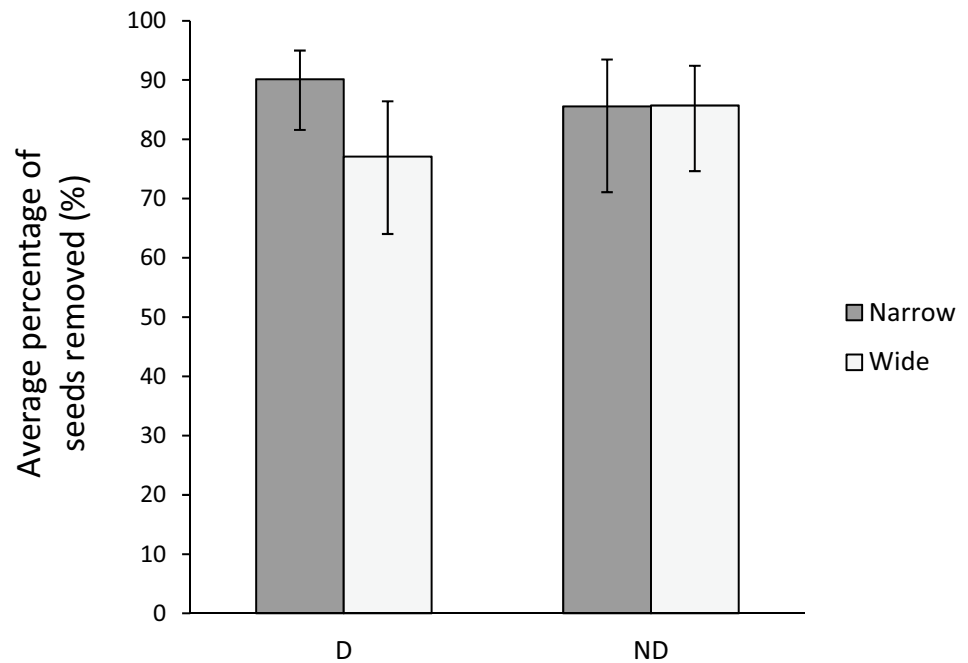
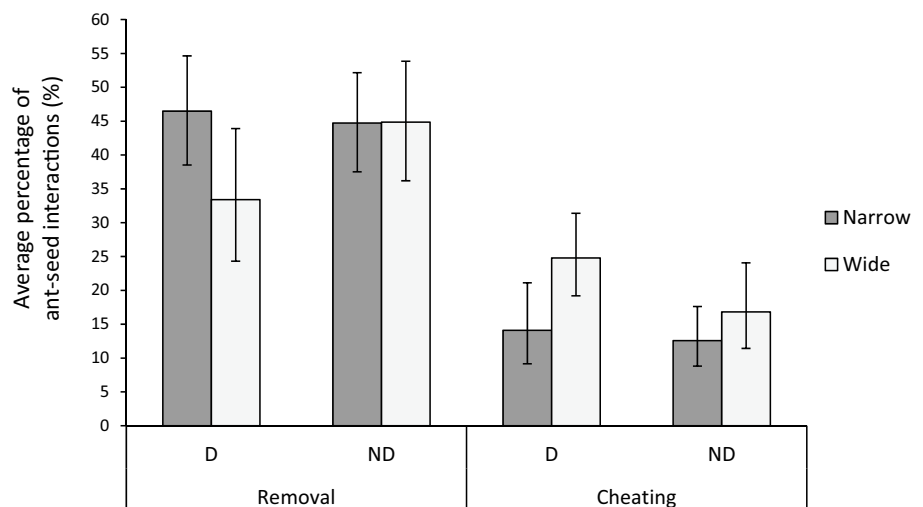


Fig. 3 Average percentage of observed ant–seed interactions, seed removal and cheating, per transect in disturbed (D) and non-disturbed (ND) zones. Error bars represent Wald confidence intervals



of seed removals ($\chi^2 = 6.03$, $df = 1$, $p = 0.01$), where there was a strong difference in seed removals in wide roadsides. There was also a marginally non-significant difference in cheating interactions between narrow and wide roadside zones ($\chi^2 = 3.66$, $df = 1$, $p = 0.055$), with a tendency for a greater proportion of seeds to be cheated at wide roadside sites (Fig. 3).

Observations of individual ant species

Twenty-six ant species were observed in engaging in ant–seed interactions, belonging to 12 genera and 5 sub-families (*Formicinae*, *Dolichoderinae*, *Ectatomminae*, *Myrmicinae* and *Ponerinae*). Of these, six ant species were

responsible for 83% of total observed ant–seed interactions (Table 1), where individual contributions to either seed removal ($n = 2442$) or cheating ($n = 983$) varied according to disturbance zone and roadside width (Fig. 4a, b). Due to low numbers, there were no statistically significant differences in seed removal or cheating behaviours found for individual species between zone and/or width, or for the interaction term (all tests, $p > 0.05$). However, descriptive statistics showed the following species patterns:

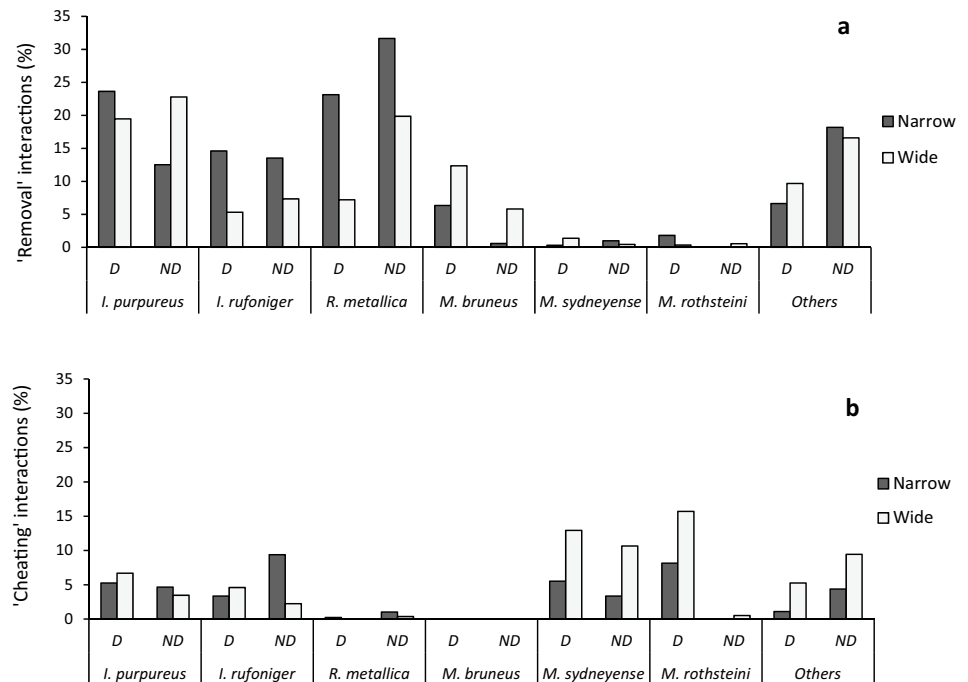
Iridomyrmex purpureus was the most abundant ant species observed at the seed depots, where a greater number of their ant–seed interactions were recorded in disturbed (mean 27.5%) than in non-disturbed zones (21.7%; Table 1). *I. purpureus* carried out both removal (average 80%) and

Table 1 Percentage contribution of six main ant species to total ant–seed interactions (wide and narrow roads combined), showing the percentage frequency of behaviour (R = seed removal; CH = cheating) for each species

Ant species	Disturbed		Non-disturbed		Total
	Ant–seed interaction (%)	Behaviour (%)	Ant–seed interaction (%)	Behaviour (%)	Ant–seed interaction (%)
<i>Iridomyrmex purpureus</i> (n = 839)	27.5	R = 80.7 Ch = 19.3	21.7	R = 80.7 Ch = 19.3	24.6
<i>Iridomyrmex rufoniger</i> (n = 514)	13.9	R = 76.4 Ch = 23.6	16.2	R = 64.7 Ch = 35.3	15.1
<i>Rhytidoponera metallica</i> (n = 731)	15.3	R = 99.6 Ch = 0.4	26.5	R = 97.3 Ch = 2.7	20.9
<i>Melophorus bruneus</i> (n = 223)	9.3	R = 100 Ch = 0	3.2	R = 100 Ch = 0.00	6.3
<i>Monomorium sydneyense</i> (n = 286)	10.1	R = 8.6 Ch = 91.4	7.7	R = 8.2 Ch = 91.8	8.9
<i>Monomorium rothseieni</i> (n = 246)	13	R = 9.8 Ch = 90.2	0.5	R = 50 Ch = 50	6.8
Others (n = 586)	10.9	R = 73.2 Ch = 26.8	24.2	R = 68.6 Ch = 31.4	17.4

All other ant species with less than 5% contribution to ant–seed interactions are combined under the ‘Others’ category

Fig. 4 Percentage contribution of six main ant species to **a** seed removal and **b** cheating interactions in disturbed and non-disturbed (ND) zones, for narrow and wide roadsides. All other ant species with less than 5% contribution to ant–seed interactions are combined under the ‘Others’ category



cheating (average 20%) behaviours (Table 1), where in wide roadsides, it was observed removing more seeds in the non-disturbed roadside zones (Fig. 4).

Rhytidoponera metallica was the second most observed species (average 21% of all interactions), where it almost exclusively participated in seed removal behaviour (Fig. 4a, b; Table 1). *R. metallica* carried out mostly seed removal activity (average 29%). Cheating activity was very low for

this species, and only average 0.4% of all of its ant–seed interactions. *R. metallica* was also more active in narrow roadside zones, where the average percentage of seed removals was almost twice that in narrow as compared to wide roadsides (Fig. 4a, b).

Almost half of the total cheating activities were performed by two *Monomorium* species (*M. rothsteini* and *M. sydneyense*), where their combined contribution to seed

removal was as low as 6%. Most *M. rothsteini* activity generally occurred in disturbed zones of narrow roadsides (Fig. 4). In contrast, *Melophorus bruneus* exclusively performed seed removal events (average 6.3% of all seed removals, Table 1), with most activity recorded in soil disturbed zones (Fig. 4). No cheating events were observed for *M. bruneus*. Combined data for all other ant species ('Others') interactions generally showed increased activity in non-disturbed roadside zones (Fig. 4; Table 1).

Discussion

Ant–seed interactions

Our observations of ant–seed interactions in the study roadside zones revealed an active assemblage of seed-dispersing (removalist) ants and mutualistic cheaters. Most seeds (85% within a 4 h period) were removed at the same rate after being placed in the disturbed and non-disturbed roadside zones. Our results follow other studies, where seed removal rates were found to be rapid in the first few hours (Beaumont et al. 2012) regardless of habitat or disturbance types (e.g., Andersen and Morrison 1998). This rapid removal of seeds from the ground can benefit plants, by minimising predation by other invertebrates and/or vertebrates (Auld and Denham 1999). Twenty-six ant species were observed in this study; however, only a few species were responsible for most ant–seed interactions: *I. purpureus*, *I. rufoniger*, *R. metallica*, *M. bruneus*, *M. sydneyense* and *M. rothsteini*. This result provides further evidence for the assertion that myrmecochory is an unevenly diffuse mutualism (Gove et al. 2007; Beaumont et al. 2011).

In our analyses, overall model results for seed removals were non-significant. However, we found a significant interaction effect between disturbance zone and roadside width on the proportion of ant–seed removals. Likewise overall model results for individual species were non-significant; however, a number of strong patterns were evident, which highlight important ecological aspects of the ant species recorded in relation to soil disturbance. For most ants, complex habitat structures make foraging more difficult, especially for larger bodied species (Gibb and Parr 2010). In this study, undisturbed roadside zones were mostly dominated by dense swards of tall (exotic) perennial grasses (Palfi et al. 2017a). Soil disturbances from roadworks, however, creates novel open habitat adjacent to the undisturbed vegetated zones, which is free of most obstacles, where some species can more easily forage. For example, *Iridomyrmex* (a large bodied species) generally carried out more seed removals within the disturbed zones of roadsides. Other studies have shown that *Iridomyrmex* prefers more open and well-sunlit conditions (Greaves 1971; Andrew et al. 2013) which occurs

in disturbed roadside zones. Disturbance mediated increases in open habitat most likely explain *Iridomyrmex* becoming behaviourally dominant in roadside vegetation (Andersen 2019).

In contrast to our findings for *Iridomyrmex*, *Rhytidoponera metallica* carried out more seed removals in narrow roadsides, where a greater percentage of interactions were recorded in the non-disturbed zones. Our findings differ to those from other studies, where *Rhytidoponera* is known to occur in a wide range of habitats (Shattuck 1999) and is recognised as a disturbance specialist (e.g., Hoffman and Andersen 2003). As narrow roads contain remnant vegetation which is often more highly modified than vegetation in wider roads (Spooner et al. 2004), may explain the patterns we recorded for this species. But we suspect more complex behavioural patterns exist. Ant community composition at any given habitat is also influenced by competitive interactions among species (Espadaler et al. 1995; Andersen 1992, 2019). *Rhytidoponera* prefers to avoid encounters with the dominant *Iridomyrmex* (e.g., Lubertazzi et al. 2010), where its subordinate behaviour allows for the co-existence of the two species in a competitive hierarchy (Gibb 2005; Mitchell et al. 2002). We observed different ant species at the same time at seed depots, where such occasions provided important insights into the competitive behaviours of these two species. For example, when a dominant *I. purpureus* individual encountered another species which was trying to remove seed from a depot, the former always robbed the seed from the latter. To counter this, *R. metallica* was always quick at removing seeds to escape from being robbed (Z. Palfi pers obs.), highlighting subordinate behaviour within the ant community.

Another key seed removalist we observed was *Melophorus bruneus*, which exclusively performed seed removal events (no cheating). *Melophorus* is a hot climate specialist (Andersen 1995) and was primarily recorded removing seeds in disturbed zones in the early afternoon period (when most other species were inactive). The conditions in the disturbance zones of roadsides during summer afternoons were normally very hot, where there is no understorey vegetation for shading (as compared to undisturbed roadside zones). As *Melophorus* spp. and *Rhytidoponera* are considered beneficial (keystone) mutualists in many Australian ecosystems (Gove et al. 2007; Majer et al. 2011). This would suggest that roadside soil disturbances can provide advantages to species specific ant–plant mutualistic processes.

In terms of elaiosome consumption by ants (cheating), we observed that this seed interaction occurred less frequently than seed removal events in roadsides, where smaller bodied *Monomorium* and *Pheidole* species (but also the dominant *Iridomyrmex* species) were mainly responsible. Other studies have also recorded a high prevalence of cheating behaviour by small bodied ant species in different habitats

(Manzaneda et al. 2007; Harris and Standish 2008; Beaumont et al. 2011). We recorded a trend (albeit marginally non-significant) for a greater level of cheating in wide compared with narrow roadsides, which reflects the activity of *Monomorium* spp. These species are widely distributed across Australia and have generalized nesting and feeding habits. Furthermore, they are able to rapidly recruit to food resources in large numbers, and defend it even against the dominant *Iridomyrmex* (Andersen 1995). Based on former studies and our data, it does appear that *Monomorium* colonies were able to obtain resources more successfully in wide, undisturbed roadside habitats.

The comparable role of *R. metallica* and *I. purpureus* as keystone seed-dispersing ants

Certain foraging, feeding and nesting strategies render an ant species as a potential keystone seed disperser: rapid food discovery, a subsequent prompt return to the nest, in conjunction with shallow depth of nests, relatively short nest life and a high nest density (e.g., Zelikova et al. 2008; Ness et al. 2009; Aranda-Rickert and Fracchia 2011). These are characteristics of *R. metallica* (Gove et al. 2007; Lubertazzi et al. 2010); a frequent removalist observed in this study. *R. metallica* individuals were regularly observed returning to the same seed depot until all seeds were individually removed (potentially by the same individual ant) and taken back to a nest. Beaumont et al. (2013) observed similar results with this species in relation to fire disturbance.

I. purpureus was the dominant species observed in the study roadsides. The large percentage of seed removals performed, coupled with occasional long-distance seed dispersal events (Whitney 2002; Pascov et al. 2015; Palfi et al. 2017a,b), suggests that this species may play a key role in facilitating plant colonization into new roadside sites. In a similar study, Gibb and Hochuli (2003) reported that a greater number of *I. purpureus* nests were recorded in cleared and well-maintained fire trails as compared to the surrounding vegetation, highlighting their affinity to open and well-sunlit habitats (Greenslade 1976)—such as the conditions experienced in the disturbed roadside zones. Further studies on the autoecology of *Iridomyrmex* (following the approaches of Lubertazzi et al. (2010) and Majer et al. (2011) would be useful to assess the relative benefits (seed removals) and costs (cheating and competitive behaviour) of *Iridomyrmex* in roadside vegetation.

Conclusions

The seed removal and cheating activity we observed by ants reveals the importance of mutualistic partners to myrmecochorous plants, where novel soil disturbances can have an

important influence on resultant seed dispersal events (Palfi et al. 2017a, 2017b). Our results show that the initial fate of seeds is largely determined by the identity and behaviour of the disperser, its response to soil disturbance regimes, and the habitat context in which the ant–seed interaction occurred. This study also highlights the importance of roadside vegetation in providing suitable habitat for native ant populations. Most ant genera we identified occur in remnant native woodland vegetation elsewhere, and generally show increased responses to disturbances (Hoffmann and Andersen 2003). *Rhytidoponera metallica* and *Melophorus bruneus* typically performed seed removal events, while *Monomorium* and *Pheidole* almost exclusively carried out cheating. *I. purpureus* removed more seeds in disturbed zones, whereas *R. metallica* was more active in non-disturbed roadside zones. Therefore, a complementary pattern in seed removal activities resulted in a similar percentage of seeds being removed from disturbed and non-disturbed roadside zones.

The interplay between the benefits (seed removal) and costs (cheating and/or seed predation; Arnan et al. 2012) help to shape the dynamics of ant–plant mutualisms. This process is often complex, where plant seeds may undergo a myriad of potential pathways while being manipulated by ant species (e.g., Hughes and Westoby 1992; Auld and Denham 1999; Bronstein 2001). Better consideration of myrmecochore activity in relation to novel anthropogenic disturbance regimes can further our ecological understanding of roadside environments, guide conservation activities, and ensure populations of myrmecochorous plants and mutualist ants persist into the future.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Ethical approval This study was carried out in accordance with Australian Code of Practice for the care and use of animals for scientific purposes and Charles Sturt University Animal Care and Ethics Committee policy. The study did not involve endangered or protected species and we did not collect ants in protected areas.

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