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ORIGINAL PAPER

Invasive ants take and squander native seeds: implications for native plant communities

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Abstract Seed dispersal is a fundamental process in the lifecycle of all flowering plants. Many plant species have evolved specialist associations with biotic vectors to facilitate dispersal. Such specialised interactions mean that these associations are potentially highly sensitive to disruption, e.g. from invasive species. However, despite this threat we still understand remarkably little about how such perturbations affect the dynamics and efficiency of the seed-dispersal process. In this study we quantify the impacts of an invasive ant across three key phases of the seed dispersal process: seed removal, distribution and placement, in order to determine the stages of seed dispersal most vulnerable to disruption by invaders. Using the Argentine ant (*Linepithema humile*) as a model, we show that invaded sites exhibited a significant decrease in seed dispersal services across all three phases of the dispersal process, relative to non-invaded sites. Seeds dispersed in invaded sites were: (a) less likely to be transported; (b) potentially distributed over a smaller spatial area, and (c) less likely to be placed at soil depths favourable for germination and establishment compared to those dispersed in non-invaded sites. These results reveal that ant-mediated seed dispersal services are significantly reduced by an invasive species at multiple stages in the dispersal process. Reductions in the efficacy of seed dispersal, combined with shifts in the ecological and geographical patterns of dispersal, may lead to cascading impacts on plant species composition and community structure. This study shows how an invasive ant can affect seed dispersal at several stages in the dispersal process.

Keywords Ant-plant mutualism · Argentine ant · *Linepithema humile* · Myrmecochory · *Pheidole pallidula* · Seed dispersal · Iberian Peninsula

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Introduction

Seed dispersal is a vital process in the life cycle of all flowering plants (O'Dowd and Hay 1980; Howe and Smallwood 1982; Hanzawa et al. 1988). Because plants are sessile, they rely on abiotic (e.g. wind and water) and biotic (e.g. insect, bird, and mammal) vectors to disperse their seeds. Invasions by non-native species threaten the efficacy of biotic seed-dispersers, with potentially serious knock-on effects to the natural plant community structure (Ricklefs and Renner 1994; Webb and Peart 2001; Christian 2001; Ozinga et al. 2009; Ruxton and Schaefer 2012). Despite this threat, we often lack comprehensive data on how specific biological invasions alter important ecosystem processes like seed dispersal. This is because assessments are often based on studies that focus on only one aspect of the seed dispersal process (e.g. seed choice), without consideration of the entire process (e.g. whether seeds ultimately end up in favourable germination sites). This latter example can be used as a proxy for understanding the ultimate impact on plant community composition.

Ants are one of the major seed dispersal agents for angiosperms in Mediterranean climates (Lengyel et al. 2010). Ant-mediated seed dispersal (myrmecochory) is geographically widespread, and observed in at least 11 000 (4.5% of all) angiosperm plant species, across 77 (12% of all) families (Bronstein et al. 2006; Lengyel et al. 2010). Plant species that rely on this mode of dispersal use an oily seed appendage (called an elaiosome) to attract ants which then remove the seed back to their nest (Beattie 1985). In doing so, ants place the seed out of reach of danger from seed predators and of destruction by fire and waterlogging (Bond and Stock 1989; Fenner and Thompson 2005; Cuautle et al. 2005). Ants in turn benefit from the nutritious elaiosome (Gammans et al. 2005), which they feed to their larvae (Beattie 1985).

Ant behavioural and biological traits influence the efficacy of myrmecochory and thus the seed-dispersal process. These traits include: *Seed removal*: an ant must locate a seed and remove it from where it fell; *Nest distribution*: an ant must deliver the seed to its nest in an area away from the parent plant; *Seed placement*: an ant must remove the elaiosome on arrival at the nest, and discard the intact seed in a suitable place for germination and establishment (e.g. on refuse piles in or around the ant nest) (Culver and

Beattie 1980; Oliveras et al. 2005a). A mismatch between ants and their plant partners at any point in the seed dispersal process will likely modify the nature of this mutualism, reducing both seed dispersal and the survival of myrmecochorous flora. For example, not all ant species handle seeds in a way that will result in successful germination and seedling establishment (Gómez and Espadaler 1998a, b). The need for successful processing of seeds to occur at all steps makes many ant-plant interactions far more specialised than they might initially appear to be, and therefore this process is much more vulnerable to disruption than might be expected. Even small changes to ant communities may alter the composition of plant communities (Andersen and Morrison 1998; Christian 2001; Ruxton and Schaefer 2012; Warren and Bradford 2014), which can result in shorter seed dispersal distances, reduced transportation rates of seeds to ant nests, and reduced seedling germination and establishment (Bond and Slingsby 1984; Christian 2001; Gómez and Oliveras 2003; Rodriguez-Cabal et al. 2009). In addition, the effects will not necessarily be distributed equally across the native flora, which means there can be significant shifts in plant community structure, together with shifts in functional and taxonomic diversity (Bond and Slingsby 1984; Christian 2001).

Invasions by non-native ant species can significantly alter the ecological distribution and phylogenetic structure of native ant communities (Holway and Case 2000; Lessard et al. 2009). These invaded ant communities show reduced genetic and ecological diversity (Holway and Suarez 2006), resulting in a loss of both keystone ant species (Christian 2001) and ecosystem function (Andersen 1997). For example, Lessard et al. (2009) showed that across several studies, ant invaders act as a form of environmental filter, resulting in a loss of native species in a nonrandom manner. This disassembly of the native ant community structure often results in the loss of specialist ant groups, such as seed dispersers (Suarez et al. 1998; Christian 2001).

Linepithema humile Mayr, the Argentine ant (Hymenoptera: Formicidae) is one of the most invasive ant species in the world (Holway et al. 2002). Human activities have caused its rapid global spread since the 1800s, such that established populations have been found across six continents, in at least 55 countries, and on several oceanic islands (Tsutsui et al. 2001;

Wetterer et al. 2009; Suarez et al. 2001). In regions with a Mediterranean climate and/or mild winters, the first recorded introductions were in 1858 (Holway 1998; Wetterer et al. 2009). *Linepithema humile* invades both disturbed and undisturbed habitats, resulting in changes to the native ant community structure (Bond and Slingsby 1984; Holway 1998; Christian 2001; Holway et al. 2002).

Observations of invasive *L. humile* populations in native ecosystems have shown that they can alter the patterns of myrmecochory. For example, a quantitative analysis on the effects of *L. humile* on native ant community structure by Rodriguez-Cabal et al. (2009) found an average 92% reduction in the number of native ant seed dispersers within invaded regions. This loss in native seed dispersers has been demonstrated to have a detrimental impact on seed dispersal processes, with a reduction in both the distance seeds are transported and their likelihood of reaching ant nests (Gómez and Oliveras 2003; Gómez et al. 2003). While this effect has been detected in a wide variety of habitats (Christian 2001; Gómez and Oliveras 2003; Rowles and O'Dowd 2009), the degree of effect can vary tremendously between studies, either because of differences in habitat types or sampling method used.

Much of the work on the impacts of *L. humile* over the last few decades has focused on assessing their effects on seed dispersal distance, often using a single plant species (Bond and Slingsby 1984; Quilichini and Debussche 2000; Carney et al. 2003; Oliveras et al. 2005a). However, other aspects of ant behaviour and ecology, such as seed preference (variation in removal efficiency relative to different plant species), nesting ecology (distribution and location of nests across a landscape), and seed placement (post-dispersal burial depth) are often omitted or overlooked, even though they are likely to affect seed dispersal and survival (Bas et al. 2007; Renard et al. 2010). We therefore lack a single study that examines the impact of *L. humile* on seed dispersal across the full process, from seed removal, to seed placement in the nest. Such information is essential if we are to make accurate assessments of the impacts of an invasive ant species on this important ecosystem service.

In this study we evaluate how seed dispersal efficiency in invaded and non-invaded areas is affected by differences in the ants' seed-handling behaviour at three key phases in the seed dispersal process. Specifically, across four sites in Spain, we compared the seed-handling behaviour of both *L. humile* and a dominant native seed-dispersing ant, *Pheidole pallidula*. We test the hypotheses that compared to seeds in non-invaded sites, seeds in sites invaded by *L. humile* are: (1) less likely to be removed (*seed removal: hypothesis 1*); (2) distributed over a smaller area (*nest distribution: hypothesis 2*); and (3) placed at depths less favourable for germination and establishment (*seed placement: hypothesis 3*). Identifying how *L. humile* ants differ from sympatric native seed-dispersers, with regard to how they handle seeds at different phases of the seed dispersal process, will help achieve more accurate predictions as to the detrimental impact of ant invasions on this important ecosystem service. This research may then help inform any future efforts aimed at mitigating the consequences of invasion by ants.

Materials and methods

Study area and ant communities

The Argentine ant was first recorded in the Iberian Peninsula at the beginning of the nineteenth century (Espadaler and Gómez 2003). Since then its population has expanded considerably in Northern Spain, particularly in coastal regions at a rate of 7.94 ($\pm$ 2.99) metres per year (Roura-Pascual et al. 2010). The study was conducted across four sites in June–July 2014 and July–September 2015, when myrmecochorous seeds were naturally dehiscing. Two of the selected sites were known to be invaded with *L. humile* (Montilivi Campus [Site 1]: 41°58′59.20″N, 02°49′29.75″E and Castell d'Aro [Site 2]: 41°49′04.61″N, 03°04′00.68″E); the other two sites were areas not invaded by *L. humile* (Montilivi Campus [Site 3]: 41°58′59.20″N, 02°49′29.75″E and Santuari dels Angels [Site 4]: 41°58′31.18″N, 02°54′34.02″E).

Invasion status was confirmed, and ant community composition assessed for each site using both baiting and pitfall traps (Supplementary Materials 1). At invaded sites, only two ant species were present; the non-native invasive *L. humile* and native non-seed dispersing *Plagiolepis pygmaea* (Supplementary Materials 1). By contrast, at the non-invaded sites, 30 species of ants were present. The dominant native seed-dispersing species was *Pheidole pallidula*, which

represented 62% ($n = 3286$) of ant samples collected (Supplementary Materials 1). *Pheidole pallidula* is a socially polymorphic ant species with dimorphic castes (worker size: 2.2–4.5 mm) (Gómez and Espadaler 1994; Fournier et al. 2016). This species has an omnivorous diet and is characterised as a short distance seed disperser, with a mean seed transport distance of 0.46 m (Gómez and Espadaler 1998a, b). According to the Giladi (2006) seed collecting ant guild behavioural classification system, both the invasive *L. humile* and native *P. pallidula* would loosely fall within the 2nd guild ("high quality dispersers" or "removalists") seed dispersers.

The vegetation at all sites was a combination of open cork-oak secondary forest, dominated by *Quercus* and *Pinus* tree species, with herbaceous myrmecochorous plant species in the clearings.

Seed collection

Ten locally abundant myrmecochorous plant species were used in this study (Table 1). The selected species were selected because they exhibited a range of seed shapes and weights (Table 1). For each species, fruits near maturation were collected from at least 25 plants in and around the study plots and allowed to dehisce naturally in ambient conditions in the laboratory. Seeds were stored at room temperature and handled with forceps and gloves throughout the study. All trials

were conducted within two to three weeks of fruit collection.

Field experiments

Hypothesis 1 Seeds in sites invaded by *L. humile* are less likely to be removed and transported than seeds in non-invaded sites.

The dispersal rate of seeds in invaded and non-invaded sites was investigated using seed choice experiments. Within each site, ten seed hubs were set up at 10 m intervals, along a transect that was previously used in the ant community surveys (Supplementary Materials 1). Each seed hub consisted of a 10 cm^2 white card with a dome wire mesh placed on top. Ants were able to access the seeds, but larger arthropods and vertebrates were not.

In total 40 seed hubs (10 per site) were set up across the four sites. Out of the eight selected plant species, six seeds were placed on each hub (three seeds taken from two randomly chosen plant species). The seeds were placed on the seed hubs at 08:00 h and surveyed at 0.5, 1, 2, 3, 6, 12 and 24 h thereafter. At each of these time points, the different ant species on or within 5 cm of the hub, and the total number of seeds from each species remaining on the hub, were recorded. The seed choice experiments were run for six consecutive days, with seeds from each plant species being placed once on each hub. In total, 870 seeds were utilised in

Table 1 Plant species used in experiments and their respective seed traits, collected in Girona, Northern Spain, between June 2014 and July 2015

Plant family	Plant species	Collection date	Seed shape	Mean seed weight (mg)	Experiment
Asteraceae	*Cirsium vulgare* (Savi) Ten.	June 2014	Elliptic	2.9 ($\pm$ 0.26)	1 (removal)
Euphorbiaceae	*Euphorbia characias* L.	June 2014	Cylindrical	5.8 ($\pm$ 0.39)	1 (removal)
Euphorbiaceae	*Euphorbia nicaeensis* All.	June 2014	Cylindrical	6.1 ($\pm$ 0.20)	1 (removal)
Euphorbiaceae	*Euphorbia serrata* L.	June 2014	Cylindrical	6.2 ($\pm$ 0.15)	1 (removal)
Asteraceae	*Galactites tomentosa* Moench	June 2014	Elliptic	11.2 ($\pm$ 1.54)	1 (removal)
Fabaceae	*Genista linifolia* L.	June 2014	Ovoid	6.5 ($\pm$ 0.24)	1 (removal)
Fabaceae	*Genista monspessulana* (L.) L.A.S.Johnson	July 2015	Globular	5.8 ($\pm$ 0.14)	2 (placement)
Fabaceae	*Sarothamnus arboreus* Boiss.	July 2015	Ovoid	6.9 ($\pm$ 0.31)	2 (placement)
Asteraceae	*Sylibum marianum* (L.) Gaertn.	June 2014	Elliptic	13.2 ($\pm$ 1.29)	1 (removal)
Fabaceae	*Ulex parviflorus* Pourr.	June 2014	Ovoid	4.1 ($\pm$ 0.25)	1 (removal)

Mean seed weights based on 250 seeds [$\pm$ 1 SD]

this study, with 431 seeds placed in invaded ($n = 2$) and 439 seeds in non-invaded ($n = 2$) ant communities.

All statistical analyses were carried out in R (version 2.3.2) program (R Core Team 2017). We compared seed removal rates between and within invaded and non-invaded sites using a Cox Proportional Hazard-model (Cox 1972) and Log-rank test (Bland and Altman 2004) in the *survival* (version 2.42-3) and *coxme* (version 2.2-10) packages (Therneau and Grambsch 2000; Therneau 2018). Generalised linear mixed models (GLMM) using a Poisson error distribution in the *lme4* (version 1.1-17) package (Bates et al. 2015) were then used to analyse seed removal frequencies (portion of seeds removed from seed hubs after 12 h) across invaded and non-invaded sites (Crawley 2012). In both the survival and GLMM analyses, the fixed effects were invasion status (invaded vs. non-invaded) and plant species ID ($n = 8$; Experiment 1, Table 1); seed hub ID (nested within transect) was included as a random factor, which controls for the effects of site and repeated sampling of seed hubs. All generated *p* values were subjected to the Benjamini–Hochberg correction method (Benjamini and Hochberg 1995) to control for false discovery rates.

Hypothesis 2 *Linepithema humile* nests in invaded sites are less evenly distributed than *P. pallidula* nests in non-invaded sites.

Given that seeds are likely to be transported back to the ants' nest, seed distribution can be heavily influenced by the spatial distribution of nests. Therefore, the spatial patterns of ant nests within a site can potentially determine both the structure and dynamics of plant populations (Nathan and Muller-Landau 2000; Berg-Binder and Suarez 2012). If nests are clumped, seeds are less likely to be widely distributed than if nests are evenly dispersed.

The spatial distribution of nests of the dominant seed dispersing ant species in invaded (*L. humile)* and non-invaded *(P. pallidula)* sites was compared across the four sites. Within each site, 5 randomly positioned grids (30.25 m^2) were set up at least 20 metres apart, each consisting of 144 white 5 cm^2 cards, separated from each other by 50 cm (Fig. 1a). Each card was supplied with 5 g of ant bait consisting of a mix of tuna

and honey (5:1 ratio). Each card was observed for 4 h, between 8:00 am and 12.00 noon [peak foraging activity for both species (Adam Devenish pers. obs.)], for 10 consecutive days. The numbers of ant trails were recorded on each card, and trails were followed back to their nests. Each quartet of cards (Fig. 1b) was scored according to whether there was a nest present (≥ 1) or absent (0) (Fig. 1c), together with the number of ant trails leading into the nest (Fig. 1d). The number of ant trails leading to a nest entrance within a quartet was taken as an estimate for nest size; however, a caveat of this method is that we are assuming that these foraging numbers stay consistent overtime. Nonetheless, from these snapshot data, nest density for each grid ($n = 20$) was calculated based on the total number of quartets occupied by ant nests and the relative nest size. Ant trails that either originated from, or extended beyond the grids, were not included.

Grid scores were analysed using the PASSaGE: Pattern Analysis, Spatial Statistics and Geographic Exegesis Tool (version 2) program (Rosenberg and Anderson 2011). This generates a dispersion index value (*D*), based on variance (σ^2) to mean (μ) ratio (VMR; $D = \sigma^2/\mu$), which represents how clustered or dispersed the sample is. Mean grid scores were compared between invaded and non-invaded sites using a Mann–Whitney U test.

Hypothesis 3 Seeds in sites with *L. humile* invasions are less likely to be placed in locations favourable for germination and establishment compared with non-invaded sites.

There are a number of components (e.g. number of seeds transported) that need to be considered when assessing the quality of a seed disperser (Schupp et al. 2010). Within sclerophyllous vegetation, final seed placement is often considered to be one of the key aspects associated with high-quality seed dispersers. In particular post-dispersal seed placement within an ant nest heavily influences both seedling germination and survival (Christian and Stanton 2004; Gómez et al. 2005; Cumberland and Kirkman 2013). However, transport of a seed into the nest alone should not be considered sufficient evidence for optimal placement, as ant species may place seeds at depths that could be detrimental to seedling survival (Gómez and Espadaler 1998a, b). Seed placement depth was therefore

Fig. 1 Trails from ants attracted to tuna and honey bait cards (placed 50 cm apart in a grid of 30.25 m^2) were used to assess nest distribution (size and density) in invaded and non-invaded sites (Hypothesis 2). **a** Grid layout; **b** a "quartet" (cluster of four cards) in a grid and the assigned score (χ = number of trails leading into the grid); **c** ant trails leading back to nests in each quartet were recorded daily over a four-hour period for 10 days; **d** the number of ant trails within each quartet was used as a measure of ant density and hence nest size
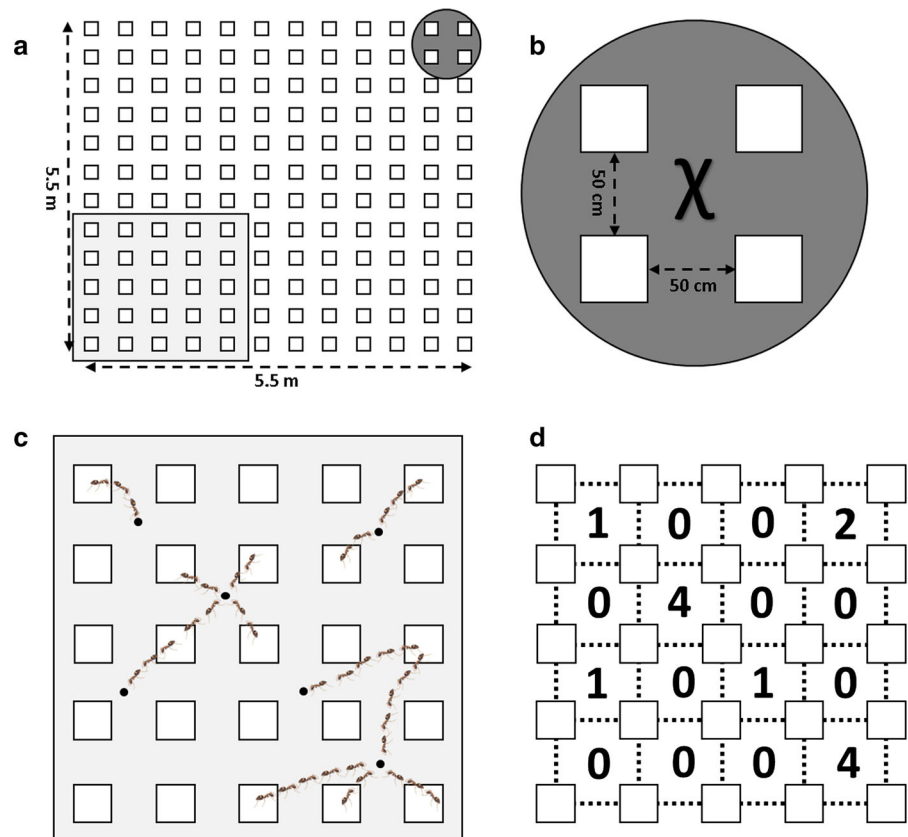
assessed in 20 nests of *L. humile* in an invaded locality (Castell d'Aro), and 20 nests of *P. pallidula* ants in a non-invaded site (Montilivi Campus). Only nests that were at least 5 m apart from each other were selected. Each nest was presented with 40 seeds, placed within 5 cm of the nest entrance: 20 of *Genista monspessulana* (French broom) and 20 of *Sarothamnus arboreus* (Black broom). These native plant species were chosen as they were not present in either locality but were naturally dispersing at the time of the trial. This means that all seeds of these species found in the ant nests would be from the experiment, rather than having been naturally dispersed.

To ensure only each target ant nest retrieved seeds, we observed them for 30 min until all the seeds were taken into the nest. If any seeds remained on the surface after this time, a Petri dish was used to cover the seeds and the nest entrance overnight. The following morning any seeds remaining on the surface

were collected. Seeds that were not collected are assumed to be have been retrieved by the ants into their nests. After 72 h, a radius of 20 cm around each nest entrance was inspected and any discarded seeds collected. This distance was selected as the capacity for an ant to transport a seed after removal of the elaiosome is limited by the morphology of the ant species (body length and mandible gap size; Gómez et al. 2005). Moreover, these seeds when discarded are often placed on refuse piles aboveground, in close proximity (< 20 cm) to the nest (Narbona et al. 2014). We are unable to rule out the potential of any post-dispersal predation from refuse piles in non-invaded regions; however, in invaded regions, no clear aboveground refuse piles for *L. humile* were reported (Adam Devenish pers. obs.). This difference in post-dispersal behaviour is likely to be due to the fact that *L. humile* (gap size: 0.6 mm; worker body length 2.6 mm) have a relatively smaller mandible gap size and body size

than *P. pallidula* (gap size: 0.64 mm; worker body length 2.2–4.5 mm) and is therefore unable to eject seeds from their nests (Crisanto Gomez, pers. obs.).

Following the above-ground inspection of the nests, each nest was excavated to a depth of 10 cm, and soil was panned using a graduated sieve (minimum wire mesh aperture: 0.5 mm) to collect any seeds. Depths below 10 cm were not excavated, as seeds deposited below this depth are unlikely to emerge (Bas et al. 2007). We thus make the assumption that any seed not found within a 20 cm radius of the nest entrance, nor within 10 cm depth in the nest classifies as an unsuccessful dispersal event.

All seeds collected were inspected for the presence of an elaiosome and for signs of seed coat damage. Seeds were classified as either: *ejected* (collected from refuse piles above ground); *buried* (collected from within the nest up to a depth of 10 cm); or *fate unknown* (not retrieved). We make the explicit assumption that these 'not retrieved' seeds were either buried > 10 cm and thus unlikely to survive; or in the instance of non-invaded regions these seeds may also have been subject to post-dispersal predation by granivorous ants from refuse piles. The numbers of seeds ejected or buried in *L. humile* and *P. pallidula* nests were compared (using an *ANOVA*), to determine whether there were differences between the placement of seeds within and outside the nests of different ant species.

Results

Hypothesis 1 Seeds in sites invaded by *L. humile* are less likely to be removed and transported than in non-invaded sites.

Seeds from all plant species were more likely to be dispersed by ants in non-invaded sites compared with invaded sites (Fig. 2a). After 12 h, 95% ($\pm$ SEM 3.6%, $n = 80$) of seeds had been removed from hubs in the non-invaded sites, compared with 49% ($\pm$ SEM 1.9%, $n = 80$) in the invaded sites (*GLMM*: $F = 65.722$, DF = 1, $p < 0.0001$). Comparison of seed removal rates revealed that seeds were ten times more likely to be removed from seed hubs in non-invaded sites compared with invaded sites (Hazard ratio, $n = 870$, 95% CI [3.8, 28.7]; Table 2).

Plant species selection was shown to have a significant effect on whether a seed was likely to removed or not in invaded sites (Log-rank test for trend: $X^2 = 65.77$, DF = 1, $p < 0.0001$). By contrast, plant species selection did not have any significant effect on seed removal in non-invaded sites (Log-rank test for trend: $X^2 = 2.432$, DF = 1, $p = 0.1188$). The fastest rates of removal in invaded sites were for the plant species *Cirsium vulgare* (52% [$\pm$ SEM 7.9%] of seeds removed after 6 h) and *Euphorbia serrata* (65% [$\pm$ SEM 6.2%] of seeds removed after 6 h); however, these removal rates were still significantly lower than seeds of the same plant species (*C. vulgare* 90% [$\pm$ SEM 4.7%] and *E. serrata* 92% [$\pm$ SEM 2.1%] dispersed (removed after 6 h) in non-invaded sites (Fig. 2b). By contrast, the lowest rates of removal of seeds in invaded sites were for *Galactites tomentosa* (22% [$\pm$ SEM 6.2%] of seeds removed after 6 h) and *Genista linifolia* (12% [$\pm$ SEM 4.1%] of seeds removed after 6 h); these rates were significantly less than the lowest rates of removal for any species in non-invaded sites (Fig. 2b).

Hypothesis 2 *L. humile* nests in invaded sites are less evenly distributed than *P. pallidula* nests in non-invaded sites.

There were significant differences in the distribution of nests of the dominant seed dispersing ant species in invaded and non-invaded sites. In the invaded sites, *L. humile* nests were clumped (mean index of dispersion score of $7.962 \pm$ SEM 0.75, $n = 10$). This was significantly different to the sympatric *P. pallidula* (*Mann–Whitney U-test*: $U = 0.0$, DF = 1, $p < 0.0001$), which exhibited a uniform distribution (mean index of dispersion score of $0.9375 \pm$ SEM 0.04, $n = 10$) in the non-invaded sites (Fig. 3).

Hypothesis 3 Seeds in sites with *L. humile* invasions are less likely to be placed in locations favourable for germination and establishment compared with non-invaded sites.

Invasive *L. humile* ants were less effective at taking seeds into their nests. *Linepithema humile* ants ($n = 20$, 63% $\pm$ SD 30.6%) retrieved significantly fewer of the 40 presented seeds into their nest compared with the native *P. pallidula* ant ($n = 20$, 100% $\pm$ SD 0%) species, which retrieved all presented seeds into their nest (*ANOVA*: $F = 89.56$,

Fig. 2 Seed removal rate differed significantly between invaded (*L. humile* present) and non-invaded (*L. humile* absent) sites. Kaplan–Meier survival curves comparing the seed removal rate of seeds placed in *L. humile* invaded (dashed) and non-invaded (solid) sites using a cox proportional hazard-model [± 95 CI]. Effect of invasion status for all plants (**a**) and for each plant species in turn (**b**) on seed removal rates shown with the *Log-rank test for trend* result [± 95% CI]
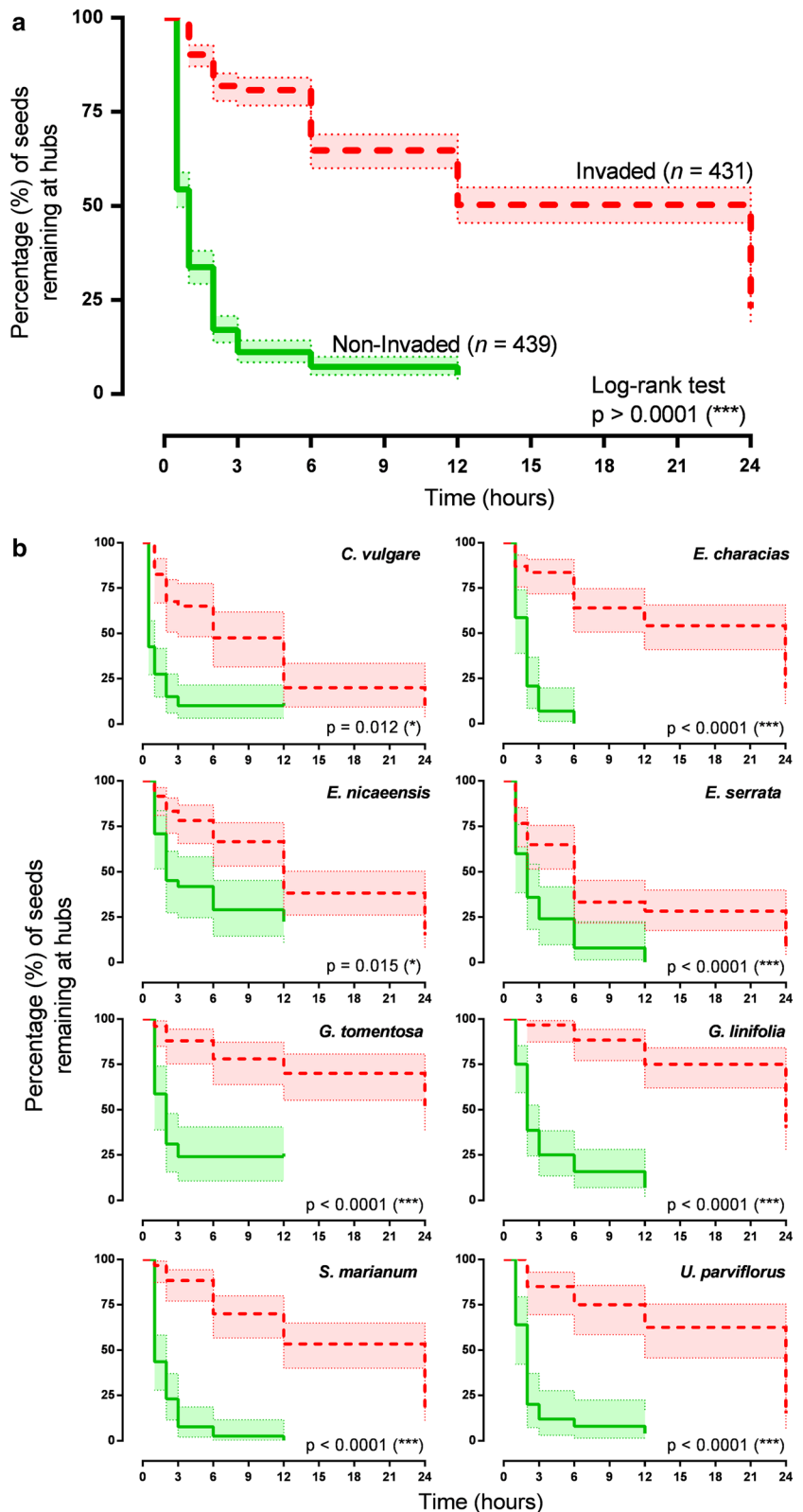
Table 2 Effects of invasion status (Model 1) and plant species (Model 2) on the removal rate of seeds from hubs using a Cox's regression analysis

Fixed effects	Coefficient	SE	Hazard ratio (HR)	95% confidence intervals for HR	p	q
Invasion status[a]	2.33	0.52	10.66	3.76–28.71	***	***
Within invaded sites[b]						
Cirsium vulgare	0.77	0.18	2.16	1.52–3.09	***	***
Euphorbia characias	0.01	0.15	1.01	0.75–1.37	0.94[NS]	0.94[NS]
Euphorbia nicaeensis	0.26	0.15	1.30	0.97–1.75	0.08[NS]	0.13[NS]
Euphorbia serrata	0.71	0.15	2.03	1.51–2.71	***	***
Galactites tomentosa	− 0.82	0.21	0.44	0.29–0.66	***	***
Genista linifolia	− 0.61	0.18	0.54	0.39–0.77	***	***
Sylibum marianum	0.07	0.15	1.07	0.79–1.45	0.64[NS]	0.85[NS]
Ulex parviflorus	− 0.03	0.18	0.97	0.68–1.38	0.86[NS]	0.94[NS]
Within non-invaded sites[b]						
Cirsium vulgare	0.28	0.18	1.33	0.94–1.87	0.11[NS]	0.22[NS]
Euphorbia characias	0.30	0.14	1.35	1.02–1.77	*	0.14[NS]
Euphorbia nicaeensis	− 0.09	0.15	0.92	0.69–1.22	0.55[NS]	0.73[NS]
Euphorbia serrata	0.26	0.14	1.30	0.99–1.71	0.06[NS]	0.17[NS]
Galactites tomentosa	− 0.16	0.15	0.85	0.64–1.14	0.29[NS]	0.46[NS]
Genista linifolia	− 0.34	0.14	0.71	0.54–0.94	*	0.13[NS]
Sylibum marianum	0.00	0.14	1.00	0.76–1.31	0.98[NS]	0.98[NS]
Ulex parviflorus	− 0.01	0.17	0.99	0.71–1.38	0.96[NS]	0.98[NS]

Random effects	Variance	SD	Log likelihood	Chi^2	p (> Chi^2)
Invasion status[a]					
Transect/seed hub ID	0.31	0.56	− 9444.3	301.02	***
Transect	0.25	0.50	− 9594.9	173.97	***
Plant species (invaded)[b]					
Transect/seed hub ID	0.13	0.36	− 4081.6	44.742	***
Transect	0.00	0.02	− 4081.6	44.739	***
Plant species (non-invaded)[b]					
Transect/seed hub ID	0.42	0.65	− 4868.4	212.05	***
Transect	0.70	0.84	− 4944.1	363.47	***

Hazard ratio (HR) is determined by the difference between the slopes of the corresponding treatments and represents the likelihood of a seed dispersal event occurring. Hubs within transects are included as random factors, to control for the non-independence of replicating hubs within sites

q = Benjamini–Hochberg corrected p values

[a]Model 1 ($\sim$ Invasion status + (1|Transect/Seed hub ID)

[b]Model 2 ($\sim$ Plant species + (1|Transect/Seed hub ID)

DF = 1, $p < 0.0001$). Furthermore, *L. humile* was more selective over which plant species it took into the nest, retrieving significantly fewer seeds of the smaller seeded *Genista monspessulana* ($n = 20$, 35% ± SD 13.1%) than the larger seeded *Sarothamnus arboreus* ($n = 20$, 92% ± SD 6.3%) (*Unpaired t test*: $t = 1.204$, DF = 38, $p < 0.0001$) (Fig. 4a).

Invasive *L. humile* ants were less likely to place seeds at depths suitable for germination and seedling establishment than the native ant *P. pallidula*. After 72 h, fewer seeds were detected at < 10 cm depth (i.e. suitable for seedling emergence) in *L. humile* nests than in native *P. pallidula* nests (*ANOVA*: $F = 24.81$, DF = 1, $p < 0.0001$). In addition, no seeds were
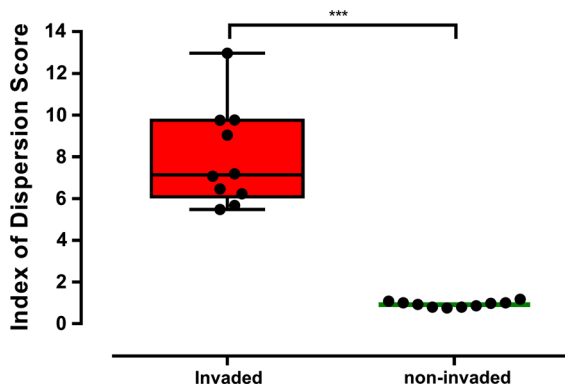
Fig. 3 Nests of the dominant seed disperser in invaded sites (*L. humile*) were more clumped than the dominant seed disperser in non-invaded sites (*P. pallidula*). Boxplot of index of dispersion scores (Y-axis) across 20 grids placed in invaded (*n* = 10) and non-invaded (*n* = 10) sites

observed to be ejected from *L. humile* nests after they had been taken into the nest (Fig. 4b), supporting our assumption that seeds not found through excavation were buried deeper than 10 cm (see Methods). By contrast, at least 15% (± SD 14.7%, *n* = 20) of *G. monspessulana* and 24% (± SD 19.6%, *n* = 20) of *S. arboreus* seeds were ejected from *P. pallidula* nests and deposited in waste dumps < 5 cm from their nest entrance. All ejected seeds lacked elaiosomes (putatively removed by the ants for nutritional needs) and a few (5%) showed signs of granivory (damaged seed coats). In *P. pallidula* nests the proportion of seeds buried and ejected did not differ significantly for either *G. monspessulana* (*Unpaired t-test*: *t* = 1.147. DF = 38, *p* = 0.2585) or *S. arboreus* (*Unpaired t-test*: *t* = 1.204. DF = 38, *p* = 0.2360) (Fig. 4b). Seeds not accounted for in non-invaded sites (classified as fate unknown; Fig. 4b) were either buried deeper within the nest or ejected and moved beyond the 20 cm search boundary.

Discussion

The invasion of ecosystems by exotic organisms is threatening long-established mutualistic relationships between their native species, including those associated with ant-plant interactions. In this study, we found evidence to suggest that across all three key phases in the seed dispersal process, the seed-handling behaviour within sites invaded by the Argentine ants
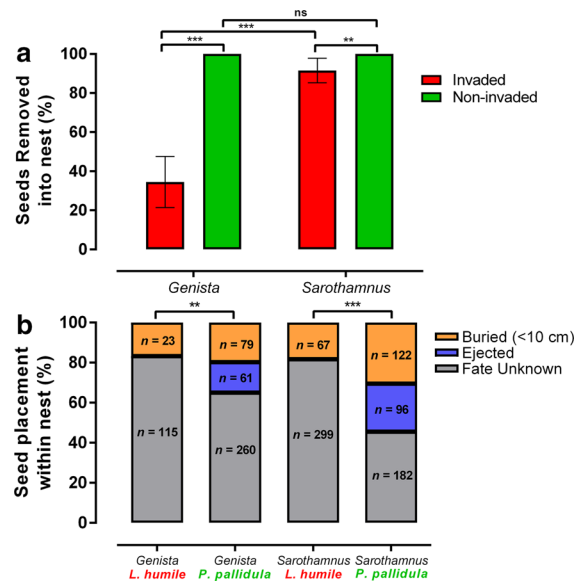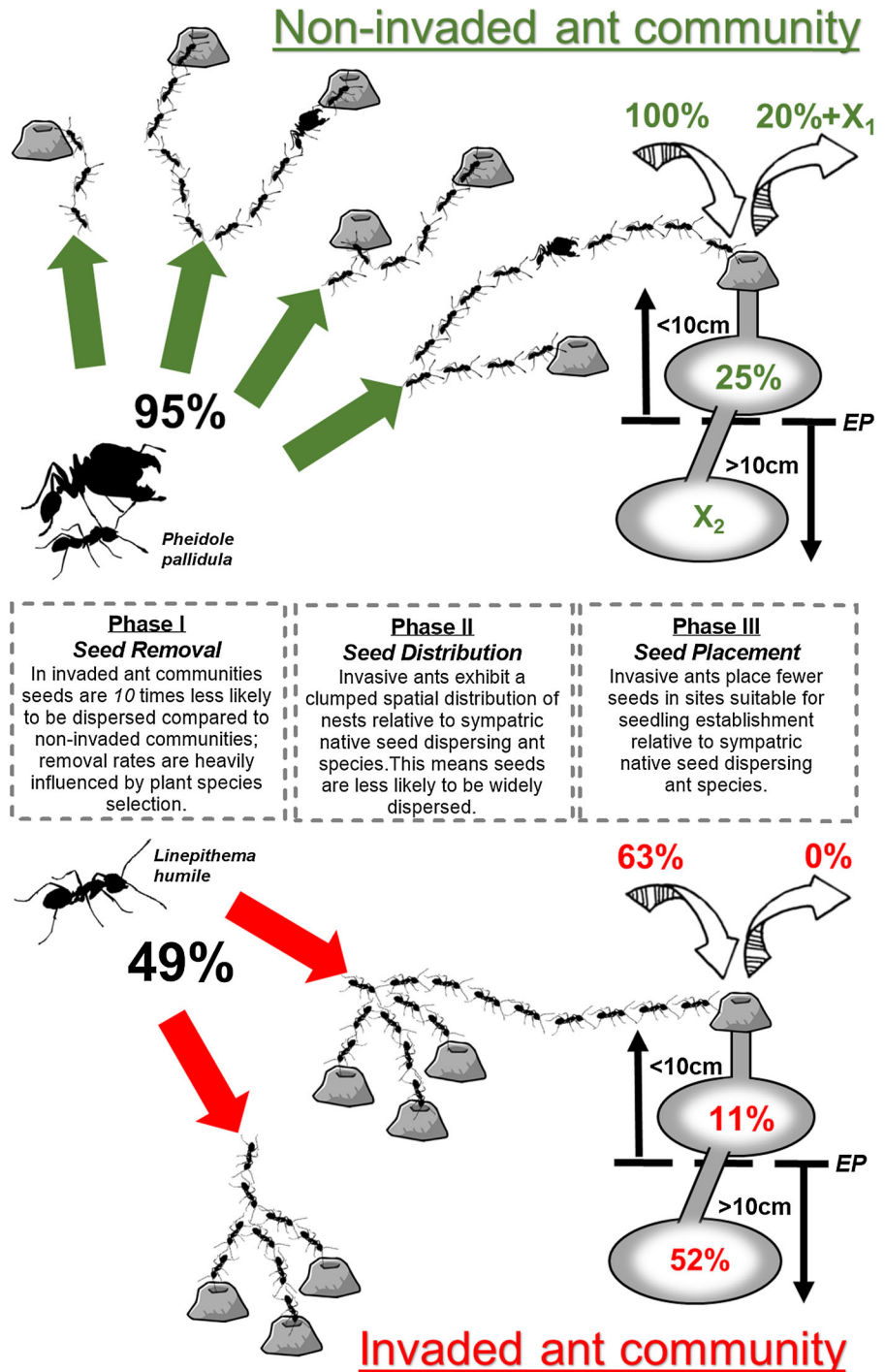

Fig. 4 Seeds were less likely to be taken into nests and placed in conditions favourable for germination and establishment in invaded (*L. humile* present) than non-invaded (*L. humile* absent) sites. **a** Percentage of seeds from two plant species (*Sarothamnus arboreus* and *Genista monspessulana*) retrieved into nests of invasive *L. humile* or native *P. pallidula* ant species [*n* = 40 nests; *n* = 40 seeds per nest (20 per species)]. **b** Fate of the seeds removed into nests (categorised as: *ejected* above ground, *buried* < 10 cm below ground and *fate unknown*) for the two plant species in invasive *L. humile* (*n* = 20; red) and native *P. pallidula* (*n* = 20; green) ant nests [± 95% CI]

differed significantly from that of the non-invaded native seed-dispersing ant community (Fig. 5). These data provide further evidence of the detrimental impacts of invasive ants on seed dispersal processes. Moreover, this study highlights the importance of quantifying seed dispersal over all stages of the dispersal process, to avoid under-estimating the impact of invasive ants on this important ecosystem service. Overall, seeds in the invaded regions (compared with seeds in non-invaded regions) were: less likely to be removed by ants; potentially distributed over a smaller area; and likely to be placed at depths less favourable for germination and establishment. These results indicate that invasive ants cause significant disruption to seed dispersal processes due to their seed-handling behaviour, and this is likely to lead to a decline in floral biodiversity and composition within and among sites. The cascade of ecological impacts resulting from these invasions is also likely to disrupt interactions among other biota which rely on these ant-plant mutualisms.

Fig. 5 Invasive Argentine ants (*L. humile*) disrupt seed dispersal across three key phases of the dispersal process. Labels: excavation point (*EP*) and seed with *fate unknown* (X_1, X_2)

Non-invaded ant community

100%   20%+X_1

<10cm
25%
EP
>10cm
X_2

95%

Pheidole pallidula

Phase I
Seed Removal
In invaded ant communities seeds are *10* times less likely to be dispersed compared to non-invaded communities; removal rates are heavily influenced by plant species selection.

Phase II
Seed Distribution
Invasive ants exhibit a clumped spatial distribution of nests relative to sympatric native seed dispersing ant species. This means seeds are less likely to be widely dispersed.

Phase III
Seed Placement
Invasive ants place fewer seeds in sites suitable for seedling establishment relative to sympatric native seed dispersing ant species.

Linepithema humile

63%   0%

<10cm
11%
EP
>10cm
52%

49%

Invaded ant community

The first experiment showed that seeds in sites invaded by *L. humile* were less likely to be removed and transported than in non-invaded sites. The loss of the primary seed disperser (*P. pallidula*) in invaded

habitats is associated with a reduction in both the rate and number of seeds being removed and dispersed. This supports previous studies (Gómez and Oliveras 2003; Bas et al. 2009) that found *L. humile* to be a poor quality seed disperser, with slower rates of uptake and removal relative to that of the native seed disperser, *P. pallidula*. As a result, seeds scattered in invaded sites will remain on the soil surface for longer, leading to either an increased risk of predation by vertebrates (Bennet and Krebs 1987; Rey et al. 2002; Carney et al. 2003), or loss by other means. Our results support the findings of other studies on ant-plant mutualisms, which identified the native sympatric species (e.g. *P. pallidula*) as a specialist seed disperser, whereas the exotic species *L. humile* is a generalist that is only pre-adapted to a narrow range of phenotypic traits (Gómez and Oliveras 2003; Witt et al. 2004). This can be further seen by the fact that within invaded regions, seed removal varied considerably between the eight selected plant species. Our study therefore showcases how seed dispersal within invaded regions may favour selected plant species.

Importantly, this experiment demonstrated that seed removal success in invaded sites varies significantly among native plant species. In sites invaded by *L. humile* the removal rate of seeds of different myrmecochorous plant species varied. By contrast, in non-invaded sites, *P. pallidula* ants removed seeds from all plant species equally. This difference is likely to be related to the morphology of both the ants (Gorb and Gorb 1995; Ness et al. 2004) and the seeds (Hughes and Westoby 1992; Mark and Olesen 1996; Garrido et al. 2002; Edwards et al. 2006; Gómez et al. 2005; Rowles and O'Dowd 2009; Boieiro et al. 2012). Since *P. pallidula* is a dimorphic ant species, with a range of worker sizes, it may be better able to handle a wide range of seed sizes and shapes, compared to the monomorphic *L. humile* (Oliveras et al. 2005b). Invasion by *L. humile* therefore is likely to lead not only to a decrease in overall seed dispersal efficiency, but also to a shift in the types of plant species being dispersed, resulting in loss of viable seeds, and a long-term change in seed bank dynamics (Bond and Slingsby 1984; Christian 2001). Such alterations of seed bank composition may limit possibilities for the regeneration of myrmecochorous plant species.

The second experiment showed that *L. humile* nests in invaded sites were less evenly distributed than *P. pallidula* nests in non-invaded sites. Both *L. humile*

and *P. pallidula* are recognised as short distance seed dispersers (Gómez and Oliveras 2003), meaning that seed dispersal is usually localised to ant nests in close proximity to the parent plant. While removal of seeds does not necessarily correlate with dispersal success or seedling establishment, spatial distribution of ant nests is likely to be a good indicator for seed dispersal patterns. Our data show that *L. humile* nests were more clumped, and thus occupied a smaller spatial area than the more evenly dispersed native *P. pallidula* nests. Clumped nest aggregations are typical of *L. humile* populations in California, as well as in their native regions (Heller and Gordon 2006; Heller et al. 2008). Such nest aggregations suggest that seeds dispersed within invaded sites are likely to be placed within a smaller spatial area than seeds removed in the non-invaded sites. This could lead to increased intra-and inter-specific competition among seeds and consequently lower seedling survival, or to a failure to colonise distant and/or newly available habitats (Quilichini and Debussche 2000; Gorb and Gorb 2003). Changes in ant nest distribution within a site may therefore alter the population genetic structure of plant species over time (Lesica and Kannowski 1998; MacMahon et al. 2000). This aspect of the seed removal process remains relatively poorly resolved within myrmecochorous systems for both non-invaded and invaded ecosystems. While we have found some evidence for this hypothesis, further work is required to account for the spatial distribution of seeds within invaded and non-invaded ecosystems.

The third experiment illustrated that seeds in sites with *L. humile* invasions were less likely to be placed in locations favourable for germination and establishment compared with non-invaded sites. Seed placement within a nest is beneficial because it decreases post-dispersal seed predation (Beattie 1985). However, placement within a nest may be detrimental if seeds are buried at a depth that increases seedling mortality (Bas et al. 2007; Narbona et al. 2007a; Renard et al. 2010). A large portion of the seeds utilised in the trial were unaccounted for; however, our results still indicate that *L. humile* removed fewer seeds into their nests relative to native *P. pallidula* ant species, and that a smaller proportion of those seeds were being placed at a depth deemed suitable for plant establishment.

Several assumptions (see Methods) have been made in interpreting out results. More research is still

required to more accurately track the placement of seeds post-dispersal and the rate of seed removal over time. This could include: improved seed tracking (Bologna and Detrain 2015), and more extensive nest excavations (using plaster) techniques, over longer timer periods (beyond the 72 h). Nonetheless, our study did highlight that, in addition to removing all presented seeds into their nest and placing more seeds at a depth suitable for plant establishment, *P. pallidula* ants also ejected as many seeds from their nests as they deposited within it. Although this behaviour has been observed before in *P. pallidula* (Gómez et al. 2003; Oliveras et al. 2005a; Bas et al. 2007), we found this behaviour to be absent in *L. humile* ants. This behaviour is likely to be driven by the morphology of the seed, and the ant ecology (Gómez et al. 2005), and could be an important and often overlooked factor in myrmecochorous seedling survival: spreading seeds across two different micro-habits (above and below ground) could maximise the chance of seedling establishment in a variable environment (Gremer and Venable 2014). For example, smaller seeds dispersed above ground in waste piles could be advantageous for survival, as seeds this size cannot withstand deep burial depths (Baskin and Baskin 1998). Deposition in waste piles above ground could also benefit seeds when there is a higher localised level of nutrients in the soil (Higashi et al. 1989) and it may further allow seeds to imbibe water more readily in low rainfall years (Merino-Martín et al. 2017). There is, however, an increased risk of predation (Bennet and Krebs 1987; Rey et al. 2002). Indeed, many *Euphorbia* seeds have non-deep physiological dormancy that is not enhanced by periods of darkness (Baskin and Baskin 1998; Narbona et al. 2007b); thus they do not require burial within an ant nest to germinate.

In conclusion, our study adds to the rich and growing literature on the impacts of invasive ants on seed dispersal processes. Specifically, we demonstrate how differences in seed-handling behaviour between invasive and native ants can be detected across several stages of the seed dispersal process. More broadly, the displacement of native species by invasive species has the potential to lead to ecological cascades of displacement across taxa. In fact, it has been shown that invasion can lead to an unravelling of important and often closely co-evolved interactions that underpin the wider structure and stability of ecosystems (Rogers et al. 2017). Future studies on assessing the impacts of invasive ant species on a key ecosystem service, such as myrmecochory, should consider the full dynamic nature of the mutualism, not just a single stage in the interaction.

Authors' contributions The project was conceived and designed by AJMD, JRB, RJN and SS; AJMD collected data, with help from CG; AJMD analysed the data; AJMD, JRB, RJN and SS led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Data availability   All data presented have been uploaded to National Environmental Research Council (NERC) Environmental Information Data Centre (https://doi.org/10.5285/375d5ea7-db23-408a-b8b3-1d4210256868). Additional data regarding the impacts of *L. humile* on ant community structure has been uploaded as Supplementary Materials 1.

Compliance with ethical standards

Conflict of interest   The authors declare that they have no competing interests.

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