# PROCEEDINGS B

## Research

CrossMark
click for updates

**Author for correspondence:**
Kirsten M. Prior
e-mail: priorkm@gmail.com

†Present address: Department of Biology, University of Florida, Gainesville, FL 32611, USA.

**THE ROYAL SOCIETY**
PUBLISHING

# Mutualism between co-introduced species facilitates invasion and alters plant community structure

Kirsten M. Prior[1,2,†], Jennifer M. Robinson[1,2], Shannon A. Meadley Dunphy[1,2] and Megan E. Frederickson[1,2]

[1]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario, Canada M5S 3G5
[2]Koffler Scientific Reserve, University of Toronto, King City, Ontario, Canada L7B 1K5

Generalized mutualisms are often predicted to be resilient to changes in partner identity. Variation in mutualism-related traits between native and invasive species however, can exacerbate the spread of invasive species ('invasional meltdown') if invasive partners strongly interact. Here we show how invasion by a seed-dispersing ant (*Myrmica rubra*) promotes recruitment of a co-introduced invasive over native ant-dispersed (myrmecochorous) plants. We created experimental communities of invasive (*M. rubra*) or native ants (*Aphaenogaster rudis*) and invasive and native plants and measured seed dispersal and plant recruitment. In our mesocosms, and in laboratory and field trials, *M. rubra* acted as a superior seed disperser relative to the native ant. By contrast, previous studies have found that invasive ants are often poor seed dispersers compared with native ants. Despite belonging to the same behavioural guild, seed-dispersing ants were not functionally redundant. Instead, native and invasive ants had strongly divergent effects on plant communities: the invasive plant dominated in the presence of the invasive ant and the native plants dominated in the presence of the native ant. Community changes were not due to preferences for coevolved partners: variation in functional traits of linked partners drove differences. Here, we show that strongly interacting introduced mutualists can be major drivers of ecological change.

## 1. Introduction

Mutualisms affect community structure and dynamics by linking the fates of interacting species and generating cascading indirect effects (e.g. [1–4]). Most mutualisms involve interacting guilds of potential partner species that often share little or only a very diffuse coevolutionary history [5]. If species within a guild of mutualists (e.g. pollinators, seed dispersers) vary greatly in the quantity or quality of goods or services that they provide to partners, then gains or losses of mutualist species may have large effects on communities [6,7]. By contrast, if species in a guild are essentially interchangeable, then mutualistic networks should be resilient to changes in species identities ([8–10]; but see [11,12]). Many models predict network resilience because they assume that interaction frequency is a good surrogate for partner quality [13], but even in generalized mutualisms changes in partner identities can have complex effects on communities when interacting partners vary in functional traits [4,6].

Many ecological communities have recently gained numerous introduced species that interact with native species and one another [12,14,15]. There is interest in the role that mutualisms between co-introduced species (i.e. species introduced from the same region) play in facilitating invasions (e.g. [14,16–18])—or to what extent do species 'not take over the globe by combat, but by networking' [19]? In population dynamical models, positive feedback between mutualists tends to generate runaway population growth [20]. Simberloff & Von Holle [21] proposed that such an autocatalytic process might commonly occur between mutualistic invasive species, accelerating their spread and amplifying their impacts on ecosystems; they coined this idea 'invasional meltdown'. Invasional meltdown

occurs when there are stronger positive feedbacks between introduced mutualists than between introduced and native mutualists. Variation in partner quality (e.g. resulting from differences in functional traits) between introduced and native species within mutualistic guilds may generate such feedbacks.

Here, we examine how an invasive ant impacts plant communities by dispersing seeds of native and invasive plants. The approximately 11 000 myrmecochorous plant species worldwide [22] have seeds with lipid-rich appendages (elaiosomes) that attract ants. Foraging ants pick up seeds and carry them back to their nests (primary dispersal), where they remove elaiosomes and feed them to ant larvae [23]. Intact seeds are deposited in waste middens inside or outside nests (secondary dispersal). Plants benefit by being moved away from parent or sibling plants [24], ending up in favourable microsites [25] or avoiding predation [26]. Ant colonies can benefit from the nutrition gained by their larvae [27,28]. Myrmecochory is considered a generalized mutualism [29,30], but recent studies suggest that there is large variation in the quality of dispersal services provided by different ant species. Certain ant species belonging to a guild of scavenging, omnivorous ants act as high-quality dispersers, and often play a disproportionately large role in seed dispersal [30–33].

This dependency on a few high-quality dispersers means that myrmecochorous communities are probably sensitive to changes in ant communities. In fact, myrmecochorous communities are vulnerable to disruption by invasive ants that often locally extirpate or severely reduce the abundance of native ants and that, as seed dispersers, are typically poor substitutes for the native species they replace [34–37]. Compared to native ants, invasive ants often disperse fewer or smaller seeds, remove elaiosomes *in situ*, or move seeds shorter distances or to less optimal sites ([34,36–38]; but see [39]). Most studies have focused on only a few invasive ant species, particularly *Linepithema humile* and *Solenopsis invicta* [34,36,37]. Neither of these species originates from regions where myrmecochory is common, nor have they been described as seed dispersers in their native ranges (i.e. they are probably low-quality dispersers) [22,36].

Native to Eurasia, *Myrmica rubra* L. (Myrmicinae) has invaded North America following its introduction to the eastern United States in the early twentieth century [40]. Like other invasive ants it forms large colonies with numerous interconnected nests (polydomy) and multiple queens (polygyny) [34,40,41]. Unlike *L. humile* and *S. invicta*, *M. rubra* is an important seed disperser in its native range [23,42]; thus, it may be adapted to consuming elaiosomes as a part of its diet and may have coevolved with Eurasian myrmecochores. Several plant species that *M. rubra* disperses in its native range have also been introduced to North America, meaning that old and potentially co-adapted partnerships between mutualists are now occurring in a new location where they overlap with native myrmecochores and native seed-dispersing ants.

Here, we take advantage of the introduction of *M. rubra* to ask how: (i) variation in mutualist quality between native and invasive ants belonging to the same behavioural guild, and (ii) the co-introduction of mutualistic ant and plant partners influence plant communities. We created mesocosms of ant and plant communities in a North American forest habitat that were stocked with either the invasive ant or a native 'keystone disperser', *Aphaenogaster rudis* s.l. (Myrmicinae) and a plant community comprising several native myrmecochores and one co-introduced, invasive myrmecochore (figure 1). In this context, our mesocosm approach is unique; most previous
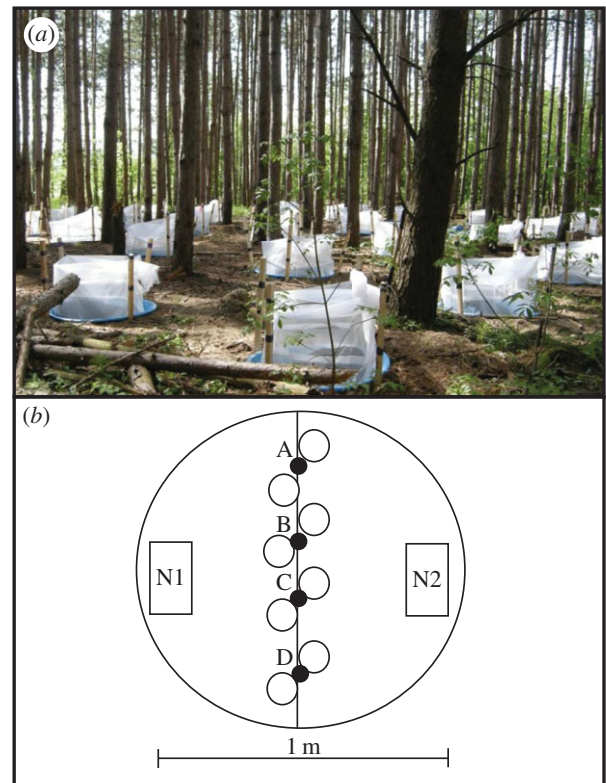


**Figure 1.** Experimental design. (a) Mesocosms were set up in a 6 × 7 array in a red pine plantation at KSR. Mesh enclosures prevented ants and seeds from entering or escaping the mesocosms. (b) Each mesocosm contained two adult plants of four myrmecochore species planted along a central line in the middle of the mesocosm: *A. acutiloba* (position A), *A. canadense* (position B), *S. canadensis* (position C) and *C. majus* (position D). Each individual plant was planted offset from these positions in different directions along the central line (see circles in figure). We placed wire rings (10 cm in diameter) around the base of each adult plant to estimate dispersal. Two pieces of rotting red pine wood (approx. 10 × 20 cm) were placed at either end of the mesocosms to act as ant nests (N1 and N2). (Online version in colour.)

research has compared seed removal between invaded and uninvaded sites (e.g. [6,43]). Results in these previous studies may be confounded by pre-existing differences between invaded and uninvaded sites or a higher overall density of ants in invaded sites [34,36]. Furthermore, we were able to measure not only the rates of seed removal, but also plant recruitment and performance. This provides a significant advantage over most studies that assume that seed removal frequency is a sufficient measure of dispersal quality ([39,43]; except see [6,24]). We coupled our mesocosm experiment with laboratory assays and laboratory and field behavioural trials to confirm and explain patterns in our experimental communities.

## 2. Material and methods

### (a) Natural history of myrmecochorous plants and seed-dispersing ants

Our study was conducted at the Koffler Scientific Reserve (KSR) (44°02′ N, 79°32′ W) which covers approx. 350 ha of the Oak Ridge's Moraine in southern Ontario, Canada and comprises deciduous and mixed forest. *Aphaenogaster rudis* is the dominant ant in forests at KSR and acts as a keystone disperser for myrmecochorous plants in North American deciduous forests [32]. The taxonomy of *A. rudis* is under consideration; our study species

belongs to the 'A. rudis group' which may consist of several species that are difficult to separate based on morphology ([44]; electronic supplementary material). Colonies of A. rudis are monogynous, contain several hundred medium-sized (approx. 4 mm) workers and may nest in soil but prefer rotten wood [44]. Aphaenogaster rudis reproduces annually, when queens start new colonies independently after nuptial flights [44].

Myrmica rubra is a medium-sized ant (approx. 4 mm) that disperses seeds of myrmecochores, as well as being insectivorous and tending homopterans [40,42]. Colonies are polygynous and polydomous, with queen numbers ranging from 1 to approximately 200, and workers from approximately 300 to over 10 000 [40,41]. Queens start new colonies independently or by budding off from larger colonies with some workers [40,41]. Myrmica rubra occurs in various habitats, including forests, nesting at the roots of plants, in soil and within rotten wood [40]. Although M. rubra is considered invasive because of its dense populations and painful sting [34,40], little is known about its ecological effects in its introduced ranges (except see [45]).

The native ant A. rudis disperses seeds of many native myrmecochores, including Asarum canadense L. (Aristolochiaceae), Anenome acutiloba L. (Ranunculaceae) and Sanguinaria canadensis L. (Papaveraceae) [28,46]. These plants are shade-tolerant perennials commonly found in woodlands at KSR and surrounding areas. Native to Eurasia, Chelidonium majus L. (Papaveraceae), has been widely distributed throughout North America [47] and occurs at KSR. This species is biennial or perennial and usually occurs in forest edges and disturbed forests [48]. Myrmica rubra disperses C. majus seeds in its native range [23]. Both introduced species co-occur with native myrmecochores and A. rudis in their introduced ranges [49].

## (b) Mesocosm experiment

We set up mesocosms in the early spring of 2012 in a red pine plantation at KSR that provided even shading. Each mesocosm consisted of a plastic pool (1.2 m diameter, 45 cm deep) with drainage holes that were covered with fine mesh. This is an appropriate scale to study myrmecochory as dispersal in this system happens within 1 m of the parent plant [49]. We filled pools with a layer of sand (approx. 15 cm) and then soil (native top soil, compost, sand and peat (Gro-max (Arnts, Whitby, Ontario))). Each pool was dug into the forest floor to mediate soil temperature. Two adult nursery plants of native plants A. acutiloba, A. canadense, S. canadensis and the invasive plant C. majus were planted down the middle of each pool (figure 1). We sewed fine mesh (625 holes per square inch, no-see-um netting (Skeeta, Bradenton, FL, USA)) to thick plastic sheeting (8 mil, vinyl) that was sunk into the soil and sealed to the bottom of the pool. Mesh was sealed at the top to form a dome, and the plastic was painted with fluon (Insect-a-Slip (Bioquip, Rancho Dominguez, CA, USA)) to keep ants in and to prevent access by granivores, large herbivores and competitors.

We placed mesocosms 2 m apart in a $6 \times 7$ array with 42 mesocosms in total (figure 1; 35 were used in this study). Five treatments were assigned in a stratified random design, each with seven replicates: (i) one A. rudis colony, (ii) two A. rudis colonies, (iii) one M. rubra colony, (iv) two M. rubra colonies, and (v) no ants (control). The full design included an interspecific interaction treatment (results not reported here) for use in a subsequent study. Throughout, we call one ant colony the 'low-density' treatment and two ant colonies the 'high-density' treatment. Density treatments reflected the natural density of focal ant species; both can have one to multiple colonies within 1 m² [40,44].

We collected A. rudis colonies from multiple locations at KSR and M. rubra colonies from nearby forests (electronic supplementary material). Colonies were standardized so that they each contained a single queen, 350 workers and up to 100 brood

(larvae and pupae). Colonies were transferred to mesocosms on 13 June 2012 under rotten logs that were placed perpendicular to the line of adult plants (figure 1). We fed each colony one medium-sized cricket and cotton soaked in honey-water every 2–3 days until 30 August 2012.

We harvested seeds from naturally occurring plants at KSR between 29 May and 22 June 2012 (electronic supplementary material, table S1). Seeds were stored at 4°C until the beginning of the experiment. On 14 June 2012, we scattered 30 A. acutiloba seeds within a 10 cm wire ring around the base of each adult A. acutiloba plant (60 seeds per mesocosm). On 22 June, we similarly added 30 A. canadense seeds, 30 S. canadensis seeds and 150 C. majus seeds to the base of each adult plant of the same species. The number of seeds and the timing of seed additions were chosen to reflect traits of plant species (electronic supplementary material, table S1). Chelidonium majus seeds are significantly smaller than native species and are difficult to see on the soil surface. Thus, to measure dispersal for this species, we placed 30 of the 150 C. majus seeds on a piece of white filter paper under each adult C. majus plant and estimated dispersal from these 30 seeds.

We monitored dispersal for 3 days by counting the number of seeds remaining at the base of the adult plants. Dispersal happens quickly in this system; most seeds were dispersed within 24 h (electronic supplementary material, figure S1). Native myrmecochores are slow-growing plants with seeds requiring a period of cold dormancy before emergence, and plants taking several years to become reproductive [51]. Chelidonium majus seedlings emerge quickly, just a few weeks after seeds dehisce from plants, and become reproductive the following year [48]. Chelidonium majus seedlings started to emerge on 30 July 2012 and were counted once a week until 19 September 2012. The following spring, we measured plant recruitment by counting all native plant seedlings three times until we stopped observing new emergence on 5 June 2013. We also counted C. majus plants on the same three dates in 2013, including both newly emerged seedlings and established, reproductive plants that had germinated in 2012 (about 70% of C. majus plants present in the mesocosms in 2013 had germinated in 2012; see the electronic supplementary material, figure S2). Established C. majus plants started flowering on 5 June 2013 and flowers were counted once a week until 21 August 2013. Native plants did not produce flowers because they take several years after germination to become reproductive [51].

## (c) Laboratory and field ant behavioural trials and seed trait measurements

We conducted several laboratory and field trials to compare behavioural traits between A. rudis and M. rubra, and to confirm and explain results that we found in the mesocosms. For laboratory trials, colonies were collected in September 2013 and standardized to contain one queen, 250 workers and 50 brood (electronic supplementary material). Colonies of each species ($n = 10$) were allowed to enter a foraging arena ($43 \times 34 \times 14$ cm) that contained 10 seeds of each plant species on small (6 cm) Petri dishes. For the first hour, we counted the number of foraging ants in the arena, and the number of ants in contact with seeds every 10 min. We also counted the number of seeds moved from the Petri dish every hour for 6 h and then again at 24 h. From these trials, we examined differences between ant species in the rate and number of dispersed seeds, recruitment to seeds and preferences for seeds of different plant species.

We conducted field trials on three separate occasions at various sites including KSR (figure 4b, electronic supplementary material figures S3a and S3b reflect trials on three different dates). Ten colonies of each ant species were located at each site. Multiple dishes holding 10 seeds each and covered with caging material (1.3 cm mesh size, $12 \times 12 \times 7$ cm) to prevent
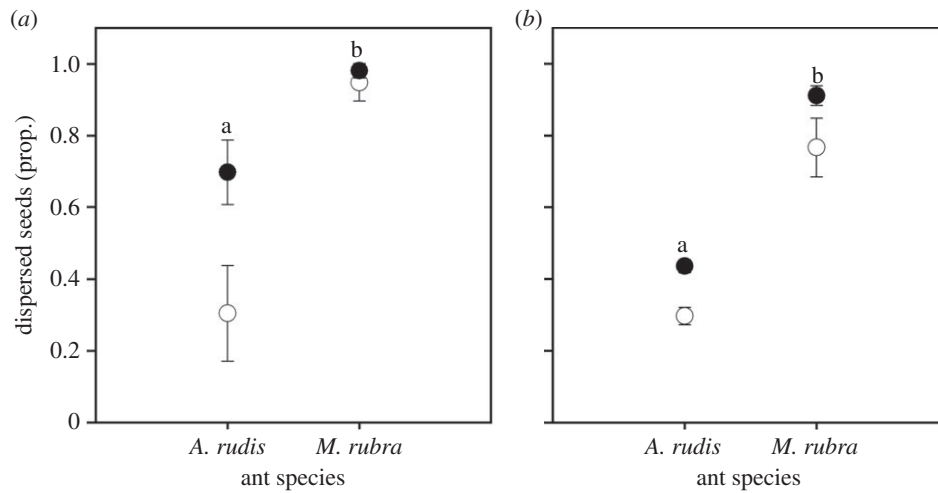
**Figure 2.** Seed dispersal. Mean ($\pm$ s.e.m.) proportion of dispersed seeds of the (a) invasive plant, *C. majus,* and the (b) native plants 3 days after seeds were added to mesocosms ($n = 7$). Open circles represent low-density ant treatments (one colony), and filled circles represent high-density ant treatments (two colonies). Different letters indicate statistically significant differences between ant species. Density treatments were also significantly different (native plants $p = 0.008$; invasive plant $p = 0.029$).

rodents from accessing seeds were placed 10 cm from the centre of the colony at multiple coordinates. Dispersal from the dishes was monitored every hour for 3–5 h and once the next morning. We also ran dispersal trials at three different time periods: morning (9.00–13.00), mid-day (14.00–18.00) and overnight (19.00–8.00). Different colonies in the field were used for each set of trials so that we were not measuring dispersal on 'satiated' colonies.

We measured several seed traits, including: diaspore mass (seed plus elaiosome), the proportion of diaspore mass that is elaiosome mass, and free oleic acid concentrations in elaiosomes of each myrmecochore species. Ten diaspores were weighed (wet weight) with and without their elaiosomes. Elaiosomes were removed from 12 previously frozen seeds of native species and 18 seeds of *C. majus*. Three elaiosomes were pooled to measure oleic acid concentrations in *C. majus*, because elaiosomes were too small to be analysed singly. Oleic acid concentration (ng mg$^{-1}$ elaiosome) was measured using combined gas chromatography-mass spectrometry (GC-MS) (electronic supplementary material).

### (d) Statistical analyses

We conducted generalized linear models (GLMs) with quasi-binomial errors to examine ant species, colony density and interaction effects on the proportion of seeds dispersed in our mesocosms on the third day for all plant species together, as well as native plants (pooled, figure 2; each species separately see the electronic supplementary material, figure S4) and the invasive plant separately. We pooled native plants because the effects of ant species on native versus invasive plants were planned as *a priori* comparisons. To correct for multiple tests, *p*-values were corrected using Bonferroni adjustments. Quasi-binomial errors were used to correct for overdispersion, and significance was tested with *F*-tests.

To examine the effect of ant treatments on plant recruitment, we calculated the log response ratio (ln RR), a commonly used metric in ecological studies [51]:

$$\ln \mathrm{RR}_{recruitment} = \ln \left( \frac{\text{recruitment in ant treatments}}{\text{mean recruitment in controls}} \right).$$

We calculated ln RR$_{recruitment}$ for each replicate in ant treatments as the natural log of recruitment divided by the mean recruitment in controls. Calculating effect sizes is a good way to compare the quality of mutualistic services provided by each ant species: a positive effect size represents a facilitative effect of ants on plants, a negative effect size an antagonistic effect. We conducted GLMs

with Gaussian errors to examine the effect of ant species, colony density and their interaction for all plant species pooled. We conducted similar analyses for native plants (pooled) and the invasive plant separately, using Bonferroni-adjusted *p*-values (see separate analysis for native plants in electronic supplementary material, figure S5). Significance was tested with $\chi^2$-tests. We calculated ln RR$_{flowering}$ for *C. majus* plants and analysed this effect size in a similar way. We also examined ant species effects on plant community structure by conducting a principle component analysis on recruitment of all four plant species and conducting a GLM (Gaussian errors) to test for differences in the first two principle components among ant treatments (electronic supplementary material).

Generalized linear mixed models (GLMMs) with binomial error distributions were conducted to compare the proportion of seeds dispersed by *A. rudis* and *M. rubra* in laboratory and field trials. Ant species was a fixed factor and time was a random factor. A similar analysis was conducted on the number of ants in contact with seeds in the laboratory trials, but using a Poisson distribution. To examine ant preferences for the invasive plant, a GLMM with a binomial error distribution was also conducted to compare the proportion of *C. majus* seeds dispersed out of all seeds dispersed by *A. rudis* and *M. rubra* in laboratory trials. GLMs with quasi-binomial error distributions were conducted to compare the proportion of seeds dispersed by ant species during different time periods in field trials. We conducted GLMs with Gaussian errors on log-transformations of oleic acid content, on seed size and the ratio of elaiosome to diaspore mass among plant species followed by Tukey tests to examine differences in seed traits among plant species.

All statistical tests were conducted in R v. 2.15.1 (R Core Team, Vienna, Austria 2012).

## 3. Results

The invasive ant, *M. rubra*, dispersed 87 $\pm$ 4% (s.e.m.) of seeds added to the mesocosms; the native ant, *A. rudis*, dispersed only 40 $\pm$ 4%. Dispersal was significantly higher in *M. rubra* treatments than in *A. rudis* treatments for both the invasive plant (GLM: $p < 0.001$; figure 2a) and the native plants (GLM: $p < 0.001$; figure 2b). Dispersal patterns were similar for all native plants when analysed separately (electronic supplementary material, figure S4). Seed dispersal was generally
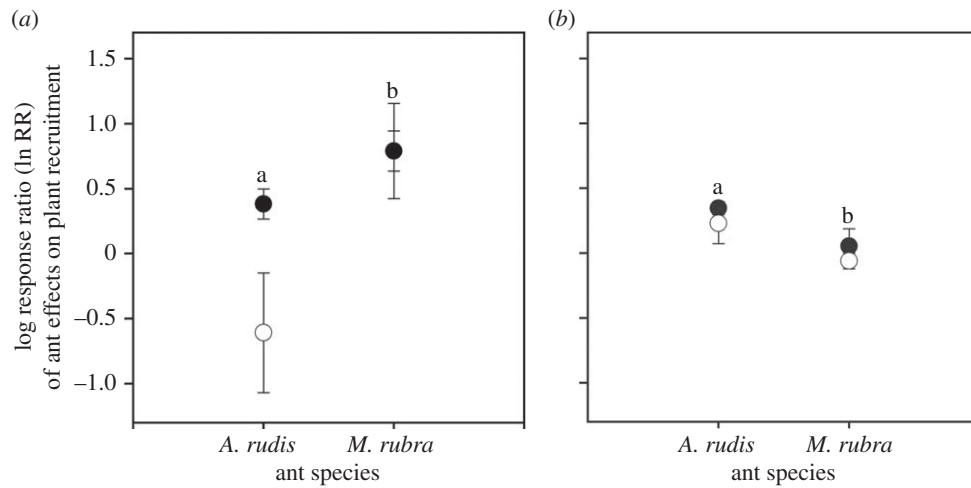
**Figure 3.** Plant recruitment. Mean ($\pm$ s.e.m.) log response ratio (ln RR) of ant effects on plant recruitment of the (a) invasive plant *C. majus* (seedlings and established plants), and the (b) native plants (seedlings) 1 year after seeds were added to mesocosms ($n = 7$). Open circles represent low-density ant treatments (one colony), and filled circles represent high-density ant treatments (two colonies). For the invasive plant, the effect sizes are similar for the low- and high-density *M. rubra* ant treatments (i.e. the symbols are overlapping). Different letters indicate statistically significant differences between ant species. A positive effect size represents a facilitative effect of ants on plants, a negative effect size an antagonistic effect.
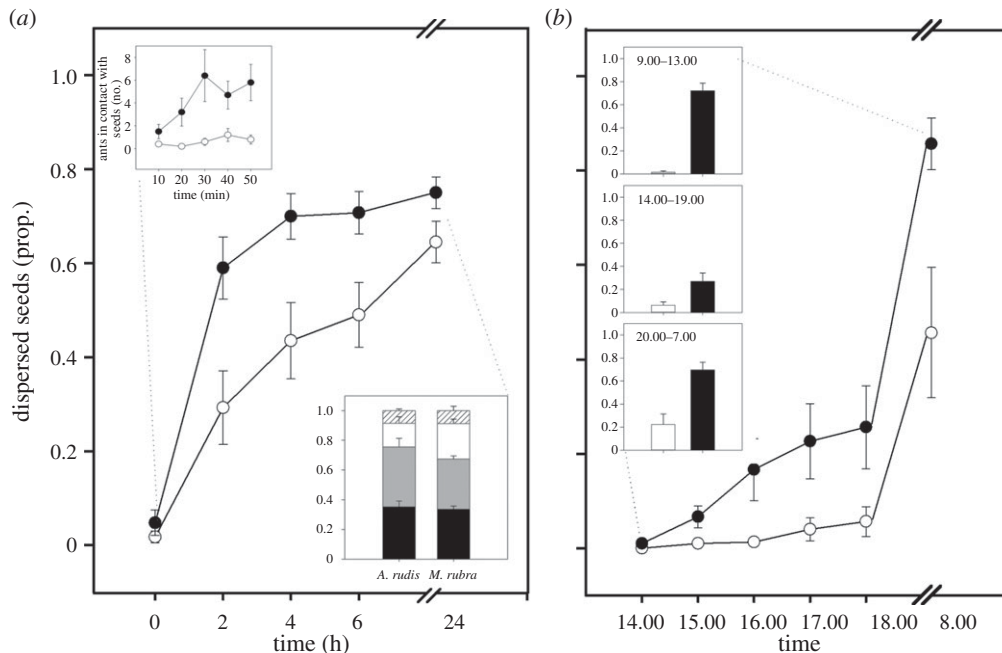


**Figure 4.** Ant behavioural traits. (a) Mean ($\pm$ s.e.m.) proportion of dispersed seeds of all plant species in the laboratory trial for *A. rudis* (open circles) and *M. rubra* (filled circles) colonies ($n = 10$). (Top inset) Mean ($\pm$ s.e.m.) number of ants in contact with seeds within the first 50 min of the laboratory trial. (Bottom inset) Mean ($\pm$ s.e.m.) proportion of dispersed seeds out of all dispersed seeds of *C. majus* (black), *A. acutiloba* (grey), *A. canadense* (white) and *S. canadensis* (hatched). (b) Mean ($\pm$ s.e.m.) proportion of dispersed seeds in a field trial conducted on August 22, 2012 ($n = 10$). (Inset) Mean ($\pm$ s.e.m.) proportion of dispersed seeds during three separate time periods in the field; filled bars, *M. rubra*; open bars, *A. rudis*.

enhanced by the presence of two ant colonies compared to only one ant colony (invasive plant: $p = 0.029$; native plants: $p = 0.007$), but there was no interaction between colony density and ant species (invasive plant: $p = 1.000$; native plants: $p = 0.724$).

*Myrmica rubra* had a strong facilitative effect on recruitment of the invasive plant compared to *A. rudis* ($p < 0.007$; figure 3a). *Aphaenogaster rudis* had a somewhat stronger facilitative effect on recruitment of the native plants compared with *M. rubra* (GLM: $p = 0.016$; figure 3b), with similar trends for all native plants (electronic supplementary material, figure S5). The invasive ant (but not the native ant) facilitated recruitment of the invasive plant compared to the no-ant controls (*M. rubra* versus no ants, $p = 0.004$; *A. rudis* versus no ants, $p = 0.841$); and the

native ant (but not the invasive ant) facilitated native plant recruitment compared to the no-ant controls (*A. rudis* versus no ants, $p = 0.035$; *M. rubra* versus no ants, $p = 0.968$; see the electronic supplementary material). There were no effects of colony density (invasive plant: $p = 0.219$; native plants: $p = 0.584$), or an interaction between ant species and colony density on plant recruitment (invasive plant: $p = 0.218$; native plants: $p = 1.000$). Ant species had divergent effects on plant community structure (electronic supplementary material, figure S6).

*Chelidonium majus* plants that recruited in the mesocosms produced more flowers in *M. rubra* than *A. rudis* treatments (GLM: $p = 0.050$; electronic supplementary material, figure S7). Colony density had a marginally significant effect on

flowering (GLM: $p = 0.064$), with no effect of the interaction between colony density and ant species ($p = 0.281$). Both ant species had facilitative effects on *C. majus* flowering compared to the no-ant controls (*A. rudis* versus no ants, $p = 0.002$; *M. rubra* versus no ants $p < 0.001$; see the electronic supplementary material).

*Myrmica rubra* recruited to and picked up seeds faster than *A. rudis* in laboratory trials (GLMM: $p < 0.001$; figure 4*a*, inset). As in our mesocosms, *M. rubra* also moved more seeds more quickly to their nests from depots than *A. rudis*. This was the case in one 24 h laboratory trial (GLMM: $p = 0.011$; figure 4*a*) and in three separate 17–20 h field trials (GLMM: $p < 0.001$; figure 4*b*; electronic supplementary material, figure S3). *Myrmica rubra* is active around the clock, whereas *A. rudis* is active mostly at night (GLM: morning $p < 0.001$; mid-day $p = 0.011$; night $p = 0.002$; figure 4*b*, inset).

In our laboratory trials, we did not find that the native and the invasive ant preferred seeds of different plant species. Specifically, ants did not prefer species with which they historically co-occurred: the native and the invasive ant did not differ in their preference for the invasive plant, *C. majus* (GLMM: $p = 0.668$). Rather, both species had similar preferences with high preferences for *C. majus* and *A. acutiloba* (native), and lower preferences for *A. canadense* and *S. canadensis*, both natives (figure 4*a*, inset). Oleic acid content differed among plant species (GLM: $p < 0.001$; electronic supplementary material, figure S8) and was particularly high in *C. majus* compared with native myrmecochores ($p < 0.050$). Seeds also differed among species in diaspore size (GLM: $p < 0.001$) and the ratio of elaiosome to seed size (GLM: $p < 0.001$). Small diaspore size and high levels of oleic acid together explained preferences by both ant species for *C. majus* and *A. acutiloba* (see the electronic supplementary material).

## 4. Discussion

Compared to the native 'keystone disperser' *A. rudis*, introduction of the invasive ant *M. rubra* shifted the plant community in our mesocosms to become dominated by the invasive plant. This response was not due to ant preferences for plants with which they historically co-occurred. Instead, community shifts resulted from variation in partner traits that created a particularly strong facilitative link between the two invaders. While both ant species preferred and dispersed seeds of the invasive plant, the invasive ant promoted the success of the invasive plant to a much higher degree than the native ant. As discussed below, this occurred because functional traits of *M. rubra* and *C. majus* enabled strong interactions between these species: the invasive ant dispersed more seeds overall than the native ant and the invasive plant responded more strongly to dispersal than the native plants. This study shows that co-introduced mutualists can facilitate invasion and highlights that changes in partner identity can have large effects in generalized mutualisms when species interactions are altered via trait-mediated pathways.

Variation in ant behavioural traits creates differences among ant species in how workers pick up and process seeds and in where they are eventually deposited [36,49]. Thus, even if ant species belong to the same behavioural guild of high-quality seed dispersers, ant species are unlikely to be functionally redundant mutualists and can have significant and complex effects on plant communities. Despite the prediction that communities of generalized mutualists may be resilient to extinctions or introductions [8–10], some generalized mutualisms may be particularly vulnerable to species introductions because new generalist species may integrate easily into networks [12,15,53] while varying in functional traits [4]. Our mesocosm approach provides an advantage over the majority of seed dispersal studies which assume that dispersal quantity (i.e. frequency of dispersed seeds) is a proxy for dispersal quality (i.e. plant recruitment). Importantly, measuring dispersal frequency only would have led us to incorrectly conclude that this mutualism is enhanced by *M. rubra* for all plant partners, while failing to reveal that ant identity alters plant communities because plant partners have different traits that cause them to differentially respond to dispersal.

*Myrmica rubra* was a superior disperser to *A. rudis* throughout the whole dispersal process (figure 4). This is probably owing to differences in ant behavioural traits. The invasive ant, *M. rubra*, recruited to and moved seeds to nests quickly, along with moving more seeds (primary dispersal). From a previous study, we also know that *M. rubra* handles seeds at a faster rate (approx. 2 d in nest) compared to *A. rudis* (up to 11 d) before secondarily dispersing seeds outside of nests [49]. *Myrmica rubra* probably disperse more seeds faster because they require a lot of resources to support their large, polydomous colonies and rapid colony growth (i.e. by colony budding) [40]. On the other hand, previous studies have found that *A. rudis* colonies become quickly satiated with myrmecochorous seeds [54]. Perhaps their monodomous, slow-growing colonies require fewer resources. In our mesocosms, *A. rudis* dispersed less than half of the seeds we offered them, while *M. rubra* dispersed almost all of the seeds provided. We found similar results in our laboratory trial, and in two out of three of our field trials (figure 4; electronic supplementary material, figure S3). This suggests that *M. rubra* colonies were not satiated in any of these cases and they would have dispersed more seeds if available. Also, since we standardized ant colony size and *M. rubra* has larger colonies than *A. rudis* in nature, our results are probably conservative with respect to differential effects of these ant species on plant communities.

*Myrmica rubra* dispersed more seeds of all plant species, yet native and invasive plants differed in their response to dispersal. Dispersal did not translate into recruitment success for all plant species equally, because plant species have different traits that influence how they respond to dispersal. The invasive plant, *C. majus,* dominated in the *M. rubra* treatments because it greatly benefitted from being dispersed away from adult plants (and *M. rubra* dispersed more seeds). In fact, few *C. majus* seedlings emerged under adult plants, and none established and became reproductive under adult plants (electronic supplementary material, figure S2). This strong positive response to dispersal is probably owing to several traits of *C. majus*, such as: the adult plants are large and bushy, they have a fast growth rate, and they are not shade tolerant [48]. These traits probably mean that competition from adult plants greatly suppresses the establishment of non-dispersed offspring. *Chelidonium majus* possesses a number of traits that are common among invasive plants: small seeds, high seed output, fast growth rates and good resource competitors [55]. Thus, while we studied only one invasive plant, other invasive myrmecochores with similar traits may also have strong positive responses to dispersal.

By contrast, recruitment of native seedlings was not greatly enhanced in the *M. rubra* treatments. Even though many seeds were dispersed by the invasive ant, native plants benefitted little from being dispersed. Native seedlings may fare better near conspecific adults because these species are shade tolerant [51] and have many fewer leaves than *C. majus*. Kalisz *et al.* [24] similarly found that dispersal did not increase the recruitment of *Trillium grandiflorum* (a common native myrmecochore), although it did decrease relatedness among neighbours. For long-lived native perennials, the benefits of dispersal may be realized primarily in the long-term, if competition becomes increasingly important as plants grow and reproduce or if dispersal increases disease resistance or mating success. Native plants did fare slightly better in the presence of the native ant, *A. rudis* than *M. rubra* (figure 3b). This could be because *M. rubra* may negatively indirectly affect native plants by facilitating the success of the fast-growing, competitive invasive plant, *C. majus*. Alternatively, *M. rubra* may have deposited some seeds in suboptimal locations. Patterns of seed deposition differed between ant species. *Aphaenogaster rudis* often deposited seeds just outside nests that largely remained under the rotten wood provided (N1 and N2 in figure 1b). *Myrmica rubra* created multiple nests and deposited seeds throughout the mesocosm. Some of these locations may be inferior sites for plant recruitment (electronic supplementary material, figure S9).

Compared to the native ant, the invasive ant increased recruitment of the invasive plant by a factor of 8.2 and flowering by a factor of 1.7. These effects could have long-term consequences for population dynamics, providing evidence for invasional meltdown (or mutualist facilitation) [17,21]. Although we do not know if the extraordinary success of the invasive plant when paired with *M. rubra* feeds back to benefit the invasive ant, feedback is likely. Elaiosomes are a lipid-rich food resource and can increase colony growth or reproduction [27,28]. *Chelidonium majus* may provide a significant nutritional advantage to ant colonies because they can produce thousands of seeds continuously from June through to September, whereas native myrmecochores produce only a handful of seeds once in the spring (electronic supplementary material, table S1). In our experiment, *C. majus* produced more flowers in *M. rubra* treatments than in *A. rudis* treatments (electronic supplementary material figure S7), suggesting that more seeds with lipid-rich elaiosomes would be available to *M. rubra* than *A. rudis* colonies. Further, *M. rubra* may take better advantage of greater seed abundance because *A. rudis* colonies satiate quickly [54].

Ant species did not have preferences for the plant species with which they historically co-occurred. This is not surprising given that myrmecochory probably did not evolve in response to specific ant species, but to a guild of omnivorous ants multiple times independently [22,30]. Certain traits, such as small diaspores, high oleic acid content and a high ratio of elaiosome to seed are generally attractive to ants [31,54,56]. *Chelidonium majus* has higher levels of oleic acid compared with native myrmecochores; this coupled with its small diaspore size may explain why both ant species preferred the invasive plant.

Mesocosms provided a powerful approach to examine community-level dynamics in our system. However, we were able to examine only some benefits of myrmecochory, such as dispersal away from adult plants and seed handling by ants. Although we could not evaluate if seeds benefitted from being deposited in resource-rich microsites, this benefit

is expected to be important largely when seed-dispersing ants have large, long-lived nests (e.g. *Formica* spp.; [24]). *Aphaenogaster rudis* has small, ephemeral nests [57], and secondarily disperses seeds into leaf litter, an environment not unlike where seeds are deposited when they dehisce from their parent plant [50]. Predator avoidance is another benefit of myrmecochory that was not directly tested in mesocosms, but since it is mediated by the rate at which seeds are retrieved, *M. rubra* may also prove a superior disperser in this regard.

Also, given that our mesocosms were simplified communities, we do not know if the plant community change we observed in the presence of invasive versus native ants occurs in natural communities. However, because both ant species prefer and readily disperse *C. majus*, it is likely that they can accelerate the spread of *C. majus*, *M. rubra* more so than *A. rudis* because it disperses more seeds in general. Also, given the fast growth rate of *C. majus*, this species probably establishes easily in communities of slow-growing forest perennials. Surveying the distribution and abundance of *M. rubra*, *A. rudis*, and native and invasive myrmecochores at sites across the region would provide an excellent avenue for future research.

Diffuse or generalized mutualisms are often thought to be resilient to species introductions and extinctions ([8–10]; except see [11,12]). This view arises from models of networking species which assume that interaction frequency is a good measure of partner quality [13]. Our results suggest that the consequences of changing partner identities in mutualistic networks will be complex because interactions are mediated by traits of networking species, which in turn may have indirect effects [4]. Even though both of the ant species in our study belong to the same guild of seed-dispersing ants [30], they were not interchangeable. As species are moved around the globe and interact with new partner species or coevolved species in new contexts, communities will be altered in complex ways, potentially having large ecological and evolutionary ramifications [58]. Given their potential for strong facilitative links and feedbacks, co-introduced mutualists could be major drivers of ecological change worldwide.

# References

1. Frederickson ME, Greene MJ, Gordon DM. 2005 'Devil's gardens' bedeviled by ants. *Nature* **437**, 495–496. (doi:10.1038/437495a)

2. Rudgers JA, Clay K. 2008 An invasive plant-fungal mutualism reduces arthropod diversity. *Ecol. Lett.* **11**, 831–840. (doi:10.1111/j.1461-0248.2008.01201.x)

3. Goheen JR, Palmer TM. 2010 Defensive plant-ants stabilize megaherbivore-driven landscape change in African savanna. *Curr. Biol.* **20**, 1768–1772. (doi:10.1016/j.cub.2010.08.015)

4. Brosi BJ, Briggs HM. 2013 Single pollinator species losses reduce floral fidelity and plant reproductive function. *Proc. Natl Acad. Sci. USA* **110**, 13 044–13 048. (doi:10.1073/pnas.1307438110)

5. Stanton ML. 2003 Interacting guilds: moving beyond the pairwise perspective on mutualism. *Am. Nat.* **162**, S10–S23. (doi:10.1086/378646)

6. Christian CE. 2001 Consequences of a biological invasion reveal the importance of mutualism for plant communities. *Nature* **413**, 635–639. (doi:10.1038/35098093)

7. Traveset A, Richardson DM. 2006 Biological invasions as disruptors of plant reproductive mutualisms. *Trends Ecol. Evol.* **4**, 208–216. (doi:10.1016/j.tree.2006.01.006)

8. Memmott J, Waser N. 2002 Integration of alien plants into a native flower-pollinator visitation web. *Proc. R. Soc. Lond. B* **269**, 2395–2399. (doi:10.1098/rspb.2002.2174)

9. Memmott J, Waser NM, Price MV. 2004 Tolerance of pollination networks to species extinctions. *Proc. R. Soc. Lond. B* **271**, 2605–2611. (doi:10.1098/rspb.2004.2909)

10. Díaz-Castelazo C, Guimarães Jr PR, Jordano P, Thompson JN, Marquis RJ, Rico-Gray V. 2010 Changes in a mutualistic network over time: reanalysis over a 10-year period. *Ecology* **91**, 793–801. (doi:10.1890/08-1883.1)

11. Aizen MA, Morales CL, Morales JM. 2008 Invasive mutualists erode native pollination webs. *PloS Biol.* **6**, e31. (doi:10.1371/journal.pbio.0060031)

12. Traveset A, Heleno R, Chamorro S, Vargas P, McMullen CK, Castro-Urgal R, Nogales M, Herrera HW, Olesen JM. 2013 Invaders of pollination networks in the Galápagos Islands: emergence of novel communities. *Proc. R. Soc. B* **280**, 20123040. (doi:10.1098/rspb.2012.3040)

13. Vázquez DP, Morris WF, Jordano P. 2005 Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecol. Lett.* **8**, 1088–1094. (doi:10.1111/j.1461-0248.2005.00810.x)

14. Grosholz ED. 2005 Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. *Proc. Natl Acad. Sci. USA* **102**, 1088–1091. (doi:10.1073/pnas.0308547102)

15. Heleno RH, Olesen JM, Nogales M, Vargas P, Traveset A. 2012 Seed dispersal networks in the Galápagos and the consequences of alien plant invasions. *Proc. R. Soc. B* **280**, 20122112. (doi:10.1098/rspb.2012.2112)

16. O'Dowd DJ, Green PT, Lake PS. 2003 Invasional 'meltdown' on an oceanic island. *Ecol. Lett.* **6**, 812–817. (doi:10.1046/j.1461-0248.2003.00512.x)

17. Simberloff D. 2006 Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? *Ecol. Lett.* **9**, 912–919. (doi:10.1111/j.1461-0248.2006.00939.x)

18. Green PT, O'Dowd DJ, Abbott KL, Jeffery M, Retallick K, Mac Nally R. 2011 Invasional meltdown: invader–invader mutualism facilitates a secondary invasion. *Ecology* **92**, 1758–1768. (doi:10.1890/11-0050.1)

19. Margulis L, Sagan D. 1986 *The origins of sex: three billion years of genetic recombination*. New Haven, CT: Yale University Press.

20. May RM. 1982 Mutualistic interactions among species. *Nature* **296**, 803–804. (doi:10.1038/296803a0)

21. Simberloff D, Von Holle B. 1999 Positive interactions of nonindigenous species: invasional meltdown? *Biol. Invasions* **1**, 21–32. (doi:10.1023/A:1010086329619)

22. Lengyel S, Gove AD, Latimer AM, Majer JD, Dunn RR. 2009 Ants sow the seeds of global diversification in flowering plants. *PLoS ONE* **4**, e5480. (doi:10.1371/journal.pone.0005480)

23. Servigne P, Detrain C. 2008 Ant-seed interactions: combined effects of ant and plant species on seed removal patterns. *Insectes Soc.* **55**, 220–230. (doi:10.1007/s00040-008-0991-8)

24. Kalisz S, Hanzawa FM, Tonsor SJ, Thiede DA, Voigt S. 1999 Ant-mediated seed dispersal alters pattern of relatedness in a population of *Trillium grandiflorum*. *Ecology* **80**, 2620–2634. (doi:10.1890/0012-9658(1999)080[2620:AMSDAP]2.0.CO;2)

25. Culver DC, Beattie AJ. 1980 The fate of viola seeds dispersed by ants. *Am. J. Bot.* **67**, 710–714. (doi:10.2307/2442664)

26. Heithaus ER. 1981 Seed predation by rodents on three ant-dispersed plants. *Ecology* **62**, 136–145. (doi:10.2307/1936677)

27. Bono JM, Heithaus ER. 2002 Sex ratios and the distribution of elaiosomes in colonies of the ant, *Aphaenogaster rudis*. *Insectes Soc.* **49**, 320–325. (doi:10.1007/PL00012655)

28. Turner KM, Frederickson ME. 2013 Signals can trump rewards in attracting seed-dispersing ants. *PLoS ONE* **8**, e71871. (doi:10.1371/journal.pone.0071871)

29. Bronstein JL, Alarcón R, Geber M. 2006 The evolution of plant-insect mutualisms. *New Phytol.* **3**, 412–428. (doi:10.1111/j.1469-8137.2006.01864.x)

30. Giladi I. 2006 Choosing benefits or partners: a review of the evidence for the evolution of myrmecochory. *Oikos* **112**, 481–492. (doi:10.1111/j.0030-1299.2006.14258.x)

31. Gove AD, Majer JD, Dunn RR. 2007 A keystone ant species promotes seed dispersal in a 'diffuse' mutualism. *Oecologia* **153**, 687–697. (doi:10.1007/s00442-007-0756-5)

32. Ness JH, Morin DF, Giladi I. 2009 Uncommon specialization in a mutualism between a temperate herbaceous plant guild and an ant: are *Aphaenogaster* ants keystone mutualists? *Oikos* **118**, 1793–1804. (doi:10.1111/j.1600-0706.2009.17430.x)

33. Manzaneda AJ, Rey PJ. 2009 Assessing ecological specialization of an ant-seed dispersal mutualism through a wide geographic range. *Ecology* **90**, 3009–3022. (doi:10.1890/08-2274.1)

34. Holway DA, Lach L, Suarez AV, Tsutsui ND, Case TJ. 2002 The causes and consequences of ant invasions. *Annu. Rev. Ecol. Syst.* **33**, 181–233. (doi:10.1146/annurev.ecolsys.33.010802.150444)

35. Sanders NJ, Gotelli NJ, Heller NE, Gordon DM. 2003 Community disassembly by an invasive species. *Proc. Natl Acad. Sci. USA* **100** 2474–2477. (doi:10.1073/pnas.0437913100)

36. Ness JH, Bronstein JL. 2004 The effects of invasive ants on prospective ant mutualists. *Biol. Invasions* **6**, 445–461. (doi:10.1023/B:BINV.0000041556.88920.dd)

37. Rodriguez-Cabal MA, Stuble KL, Nuñez MA, Sanders NJ. 2009 Quantitative analysis of the effects of the exotic Argentine ant on seed-dispersal mutualisms. *Biol. Lett.* **5**, 499–502. (doi:10.1098/rsbl.2009.0297)

38. Ness JH, Bronstein JL, Andersen AN, Holland JN. 2004 Ant body size predicts dispersal distance of ant-adapted seeds: implications of small-ant invasions. *Ecology* **85**, 1244–1250. (doi:10.1890/03-0364)

39. Stuble KL, Kirkman LK, Carroll CR. 2010 Are red imported fire ants facilitators of native seed dispersal? *Biol. Invasions* **12**, 1661–1669. (doi:10.1007/s10530-009-9579-0)

40. Groden E, Drummond FA, Garnas J, Franceou A. 2005 Distribution of an invasive ant, *Myrmica rubra* (Hymenoptera: Formicidae), in Maine. *J. Econ. Entomol.* **98**, 1774–1784. (doi:10.1603/0022-0493-98.6.1774)

41. Elmes GW. 1973 Observations of the density of queens in natural populations of *Myrmica rubra* L. (Hymenoptera, Formicidae). *J. Anim. Ecol.* **42**, 761–771. (doi:10.2307/3136)

42. Gorb SN, Gorb EV. 1999 Effects of ant species composition on seed removal in deciduous forest in eastern Europe. *Oikos* **84**, 110–118. (doi:10.2307/3546871)

43. Rodriguez-Cabal MA, Stuble KL, Guénard B, Dunn RR, Sanders NJ. 2012 Disruption of ant-seed dispersal mutualisms by the invasive Asian needle ant (*Pachycondyla chinensis*). *Biol. Invasions* **14**, 557–565. (doi:10.1007/s10530-011-0097-5)

44. Lubertazzi D. 2012 The biology and natural history of *Aphaenogaster rudis*. *Psyche* **2012**, 1–11. (doi:10.1155/2012/752815)

45. Ness JH, Morales MA, Kenison E, Leduc E, Leipzig-Scott P, Rollinson E, Swimm BJ, von Allmen DR. 2012 Reciprocally beneficial interactions between introduced plants and ants are induced by the presence of a third introduced species. *Oikos*

8

rspb.royalsocietypublishing.org  *Proc. R. Soc. B* **282**: 20142846

122, 695–704. (doi:10.1111/j.1600-0706.2012.20212.x)

46. Beattie AJ, Culver DC. 1981 The guild of myrmecochores in the herbaceous flora of West Virginia forests. *Ecology* 62, 107–115. (doi:10.2307/1936674)

47. Mack RN. 2003 Plant naturalization and invasions in the eastern United States: 1634–1860. *Ann. Mo. Bot. Gard.* 90, 77–90. (doi:10.2307/3298528)

48. Kang H, Primack RB. 1991 Temporal variation of flower and fruit site in relation to seed yield in celandine poppy (*Chelidonium majus*: Papaveraceae). *Am. J. Bot.* 78, 711–722. (doi:10.2307/2445092)

49. Prior KM, Saxena K, Frederickson ME. 2014 Seed handling behaviors of native and invasive seed-dispersing ants differentially influence seedling emergence in an introduced plant. *Ecol. Entomol.* 39, 66–74. (doi:10.1111/een.12068)

50. Canner JE, Dunn R, Giladi I, Gross K. 2012 Redispersal of seeds by a keystone ant augments the spread of common wildflowers. *Acta Oecol.* 40, 31–39. (doi:10.1016/j.actao.2012.02.004)

51. Whigham DF. 2004 Ecology of woodland herbs in temperate deciduous forests. *Annu. Rev. Ecol. Syst.* 35, 583–621. (doi:10.1146/annurev.ecolsys.35.021103.105708)

52. Hedges LV, Gurevitch J, Curtis PS. 1999 The meta-analysis of response ratios in experimental ecology. *Ecology* 80, 1150–1156. (doi:10.1890/0012-9658(1999)080[1150:TMAORR]2.0.CO;2)

53. Stouffer DB, Cirtwill AR, Bascompte J. 2014 How exotic plants integrate into pollination networks. *J. Ecol.* 102, 1442–1450.

54. Heithaus ER, Heithaus PA, Liu SY. 2005 Satiation in collection of myrmecochorous diaspores by colonies of *Aphaenogaster rudis* (Formicidae: Myrmicinae) in Central Ohio, USA. *J. Insect*

*Behav.* 18, 827–846. (doi:10.1007/s10905-005-8743-3)

55. van Kleunen M, Weber E, Fischer M. 2010 A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecol. Lett.* 13, 235–245. (doi:10.1111/j.1461-0248.2009.01418.x)

56. Fischer RC, Richter A, Hadacek F, Mayer V. 2008 Chemical differences between seeds and elaiosomes indicate an adaptation to nutritional needs of ants. *Oecologia* 155, 539–547. (doi:10.1007/s00442-007-0931-8)

57. Culver DC, Beattie AJ. 1978 Myrmecochory in viola: dynamics of seed-ant interactions in some West Virginia species. *J. Ecol.* 66, 53–72. (doi:10.2307/2259181)

58. Kiers ET, Palmer TM, Ives AR, Bruno JF, Bronstein JL. 2010 Mutualisms in a changing world: an evolutionary perspective. *Ecol. Lett.* 13, 1459–1474. (doi:10.1111/j.1461-0248.2010.01538.x)

9

rspb.royalsocietypublishing.org  *Proc. R. Soc. B* 282: 20142846