



# Invasive ants disperse seeds farther than native ants, affecting the spatial pattern of seedling recruitment and survival

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## Abstract

Mutualists can vary in the quantity and quality of service which they provide to their partners. Variation in seed disperser quality depends on seed-processing traits, dispersal distance, and deposition location, all of which ultimately affect plant fitness. Here, we compared these aspects of seed dispersal quality between a native and an invasive ant species, and examined how they affect competition and plant performance. Using experimental mesocosm communities, we examined how these two ant species affect the spatial pattern of recruitment and establishment for four myrmecochorous plant species, including one invasive species. We measured the locations of dispersed seedlings relative to ant nests, adult plants, and other dispersed seedlings, as well as measured the effects of location on plant performance. The invasive ant, *Myrmica rubra*, secondarily dispersed seeds farther from its nests, creating a less clumped pattern of seedling recruitment compared to the native ant, *Aphaenogaster rudis*. Plant species responded differently to dispersal. Invasive seedlings recruited farther from adult plants than native seedlings, and had higher survival the farther they were from conspecifics. In contrast, native plants had higher survival and grew taller when dispersed farther from invasive plants. We show that seed-dispersing ant partners differ in mutualist quality creating differences in dispersal distance and deposition location that affects a plant's competitive environment. Our results reveal the potential for long-term consequences on plant community structure with changing ant partner identity. We emphasize the need to examine dispersal quality in addition to quantity to uncover the importance of partner identity in structuring communities.

**Keywords** *Aphaenogaster rudis* · Biological invasions · Myrmecochory · *Myrmica rubra* · Seed dispersal · Spatial patterns

## Introduction

Many plants depend on animals to disperse their seeds. The effectiveness of an animal as a seed disperser depends on both the number of seeds it moves and on whether dispersed seeds survive, germinate, and grow into adults (Schupp 1993; Schupp et al. 2010). Where a seed ends up after dispersal establishes the abiotic and biotic environment, it will experience throughout its life. This includes local resource availability and microhabitat conditions; as well as the strength of its interactions with other plants, animals (e.g., herbivores), and soil or other microbes (Kjellson 1991; Kalisz et al. 1999; Gorb and Gorb 2003; Tanaka and Tokuda 2016). Thus, where an animal moves, a seed affects plant performance for many years following the dispersal event, and is a key component of an animal's seed dispersal effectiveness. However, because it is difficult to track seeds through space and monitor their fates through time, most studies measure only the quantity and not the

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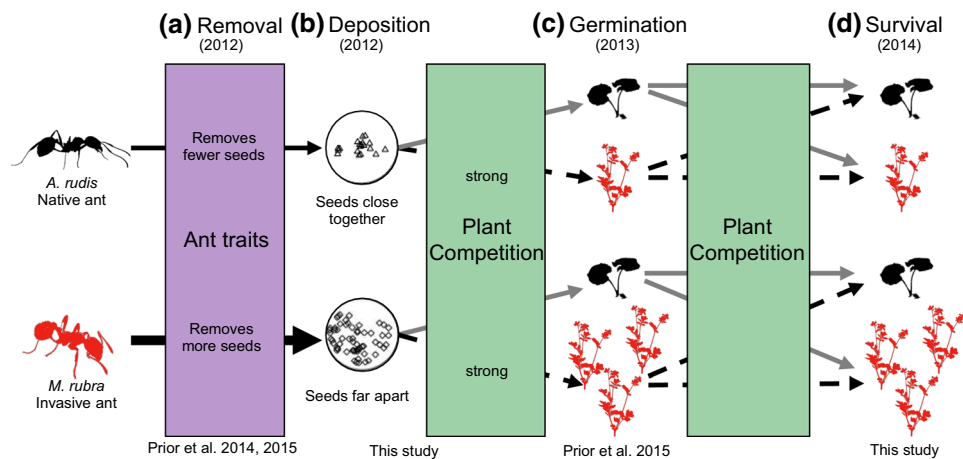
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quality of seed dispersal (including our own, Meadley Dunphy et al. 2016). To fully understand the effectiveness of different seed dispersers, we need to measure their effects on the whole dispersal process, from how many seeds they move, to where they deposit seeds, to the effect of deposition location on plant performance. Here, we investigate how two seed-dispersing species differ in their dispersal quality. In particular, we compare (1) how the ant species affect the spatial pattern of seedling recruitment and (2) the effect of deposition location on later stages of plant performance, including plant survival and growth (Fig. 1).

We focused on seed dispersal by ants (myrmecochory), which, especially in eastern North America, is a widespread mutualistic interaction (Beattie and Culver 1981; Handel et al. 1981). Generally, seed-dispersing ants pick up seeds with nutrient-rich food bodies (elaiosomes) attached, bring them to their nests (primary dispersal), remove and feed the elaiosomes to their larvae, and finally deposit seeds in a midden (secondary dispersal) (Gorb and Gorb 2003; Giladi 2006). Worldwide, the seeds of an estimated 11,000 plant species have elaiosomes (i.e., are myrmecochores) (Lengyel et al. 2009), and in the deciduous forests of northeastern North America, as many as 30% of herbaceous plants have seeds with elaiosomes (Beattie and Culver 1981; Handel et al. 1981). In eastern North America, members of this diverse plant guild have their seeds dispersed primarily by

*Aphaenogaster rudis* s.l. This ant is considered a keystone mutualist, because *A. rudis* disperses as many as 70% of the seeds it encounters and is also the most common woodland ant (Ness et al. 2009). Plants gain multiple benefits from dispersal by ants including reduced parent–offspring or seedling competition (Kalisz et al. 1999; Boyd 2001), reduced seed predation (O’Dowd and Hay 1980; Heithaus 1981), and directed dispersal to favorable microhabitats (Gibson 1993).

The outcome of myrmecochory is often sensitive to changes in the local ant assemblage, because ant species differ in how they interact with seeds (Gorb and Gorb 1999; Giladi 2006; Ness et al. 2009; Prior et al. 2015). Most myrmecochory studies measure only the rate of seed removal or quantity of dispersal from depots by different ant species, because the small size of ants and the seeds which they carry makes them difficult to track (Canner and Spence 2011). Differences in seed removal rates between ant species are often attributed to differences in ant or seed size, because small ants are unlikely to move large seeds (e.g., Christian 2001; Ness et al. 2004). However, the quality of seed dispersal also varies among ant species, including the length of time which a seed remains within an ant nest (Prior et al. 2014), the distance seeds are dispersed (Ness et al. 2004; Leal et al. 2014a), and deposition location (Gorb et al. 2000), all of which can affect plant fitness. In eastern North American forests, myrmecochorous plants have historically been



**Fig. 1** Conceptual illustration of the mechanisms affecting the seed dispersal process in our experimental mesocosms. For both ants and plants, native and invasive species are represented by black and red cartoons, respectively. Colored boxes represent mechanistic filters determining different stages in the dispersal process—seed deposition, and seedling germination and establishment. Solid black arrows show a positive effect of the mechanistic filters, while dashed arrows show a negative effect. Gray arrows indicate no significant effect. **a** Variation in ant traits such as colony size, foraging rate, and seed handling time affected how many seeds each ant species initially removed. The invasive ant, *M. rubra*, removed more seeds overall regardless of plant species (Prior et al. 2014, 2015). **b** Although we did not directly measure seed deposition location, seedling locations

suggest that *A. rudis* deposited seeds in a clumped pattern closer to their nests, while *M. rubra* spread seeds out around mesocosms. Representative *A. rudis* and *M. rubra* mesocosms show seedling locations. **c** More seedlings of the invasive plant, *C. majus*, germinated in the *M. rubra* mesocosm than any other plant–ant combination (Prior et al. 2015). The current study shows that that outcome was likely a result of a combination of strong parent–offspring competition in *C. majus* and *M. rubra* moving seeds farther away from the parent plants. **d** Here, we show that for seedlings of all species, survival was negatively affected by the distance to the nearest *C. majus* plant. Native plants also grew taller when they were dispersed farther from *C. majus*. Distance to the nearest native plant did not affect seedling survival (color figure online)

most likely to interact with *A. rudis* (Ness et al. 2009), but with the recent introduction of ant species around the globe (McGlynn 1999; Suarez et al. 2010; Wetterer and Radchenko 2011), ant-dispersed plants are increasingly likely to also interact with different ant species, including invasive ants.

There are about 200 introduced ant species globally (Holway et al. 2002), and several are known to change the outcome of myrmecochory in their introduced ranges (e.g., Ness and Bronstein 2004; Stuble et al. 2009; Rodriguez-Cabal et al. 2011). The invasive ants that have been well studied (e.g., *Linepithema humile* and *Solenopsis invicta*) are mostly tropical in origin and generally are not adapted for seed dispersal in their native ranges. Consequently, they are often ineffective seed dispersers in their introduced ranges. Invasive ants can reduce dispersal indirectly by outcompeting native ants that provide superior dispersal services (Christian 2001; Ness 2004; Stuble et al. 2009; Rodriguez-Cabal et al. 2011); or directly by picking up fewer seeds, moving seeds shorter distances, or eating elaiosomes in situ (Holway et al. 2002; Carney et al. 2003; Ness and Bronstein 2004; Ness 2004). However, recent work has shown that some invasive ants can be high-quantity seed dispersers in their introduced ranges. In particular, the European fire ant, *Myrmica rubra*, is an important seed disperser in its native range (Gorb and Gorb 1999, 2003; Fokuhl et al. 2007), and also disperses seeds in its introduced range in North America (Gammans et al. 2018), where it actually disperses more seeds faster than the keystone mutualist, *A. rudis* (Prior et al. 2015). Our previous work has shown that *M. rubra* and *A. rudis* differ in seed removal rates, seed handling time in nests, and, importantly, plant recruitment (Prior et al. 2014, 2015).

Ant introductions, or other changes to the composition of the local ant assemblage, do not affect all myrmecochorous plant species equally. Much depends on how strongly plant species respond to ant dispersal. Plant species vary in their seed dispersal requirements, yet this aspect of myrmecochory is often overlooked (except see Gorb et al. 2013; Tanaka and Tokuda 2016). Depending on the strength of parent–offspring or sibling competition, predation pressure, or the benefits of reaching a new microhabitat, plants may evolve seed or elaiosome traits that attract more appropriate ant partners. For example, species that experience higher seedling mortality near adult plants may evolve traits that promote longer dispersal distances by ants such as smaller seeds, larger elaiosomes, or more signaling compounds (Hughes and Westoby 1992; Fischer et al. 2008; Leal et al. 2014b). Comparing two closely related myrmecochorous sedges, Tanaka and Tokuda (2016) found that only one benefited from dispersal away from adult plants; this species also had seeds with larger elaiosomes that were more attractive to larger ant species that moved seeds longer distances. Thus, plants may be under selection to preferentially associate with

ant species that best meet their dispersal needs, and plant species may also be affected differently by changes in ant community composition.

In this study, we investigated how four myrmecochorous plant species with two different life-history strategies respond to dispersal by two ant species, *A. rudis* or *M. rubra*. These two species belong to the same behavioral seed-dispersing guild, but vary in traits related to seed dispersal. Three common myrmecochores native to our study region, *Anemone acutiloba* L. (Ranunculaceae), *Asarum canadense* L. (Aristolochiaceae), and *Sanguinaria canadensis* L. (Papaveraceae), are typical of most native myrmecochores in eastern North American forests (Beattie and Culver 1981; Handel et al. 1981). These plants are slow-growing, small-statured (< 25 cm tall), shade-tolerant, long-lived forbs that produce 10–30 seeds annually. We also studied the weedy, invasive plant *Chelidonium majus* L. (Papaveraceae), which is dispersed by *M. rubra* in its native range in Europe (Servigne and Detrain 2008), and also overlaps with *M. rubra* in its introduced range in North America (Prior et al. 2015). *Chelidonium majus* has a life history typical of ruderal plants that is also often associated with weedy or invasive species (Baker 1965; Sutherland 2004). This plant is faster growing, taller (up to 1 m), and shorter lived (2–5 years) than the native species in our study, and each plant produces thousands of seeds continuously throughout the summer (Kang and Primack 1991). Compared to seeds of the three native plant species, the seeds of *C. majus* are smaller with relatively large elaiosomes; their elaiosomes have more oleic acid—an important signaling compound—and they are preferred by both *A. rudis* and *M. rubra* (Prior et al. 2015). Because of *C. majus*'s larger size, seed output, and fast growth (also traits associated with other invasive plants), we expected *C. majus* seedlings to benefit more from dispersal away from adult plants: i.e., we expected stronger parent–offspring or sibling competition in *C. majus*, compared to the slow-growing native plant species. In other words, we predict that because of traits that allowed *C. majus* to become invasive, it will respond to dispersal both differently and more strongly than our native plants.

Despite belonging to the same behavioral guild of seed dispersers, we previously found that the invasive ant, *M. rubra*, disperses more seeds of all plants species more quickly than *A. rudis* (Prior et al. 2015). This is not surprising given that these ant species differ in life-history and colony traits (see “Study system and site”) that correspond with *M. rubra* being invasive (e.g., large multi-nest and multi-queen colonies), and that make them more active and likely more resource-demanding than *A. rudis*. Prior et al. (2015) also found that patterns of seedling recruitment were somewhat disconnected from the number of seeds removed by either ant species, suggesting that these ants also differ in another aspect of disperser effectiveness in addition to

quantity. Specifically, while *M. rubra* moved more seeds of all plant species, more native plants recruited when seeds were dispersed by the native ant and more invasive plants recruited when seeds were dispersed by the invasive ant (Fig. 1). This suggests that *A. rudis* and *M. rubra* differ in the spatial pattern of seed deposition, and that plant species differentially respond to dispersal. Thus, although the two ant species differ in dispersal quantity, it was likely a difference in disperser quality (placement of seeds) that determined which plant species dominated the community.

Here, using the same experimental mesocosms, we expand on Prior et al. (2015) by examining how ant placement of seeds affects plant performance, resulting in the disparate recruitment patterns found in Prior et al. (2015). We assess whether *A. rudis* and *M. rubra* differ in where they deposit seeds in relation to ant nests, adult plants, and other seed(ing)s; and assess how dispersal location affects plant survival and height 2 years after seed dispersal (Fig. 1). We predict that the divergent effects on plant communities in mesocosms dominated by either ant species are driven by both differences in the quality of dispersal services and in the responses of plant species to dispersal. More specifically, we predict that the mutualist species with invasive traits will be particularly strong interactors, such that the invasive ant provides high-quality dispersal to the invasive plant that also has a strong positive response to dispersal. Importantly, our study provides a particularly thorough investigation of the influence of seed disperser identity in seed dispersal mutualisms by measuring the whole dispersal process from removal to deposition location to plant establishment—the two later steps being understudied, yet important components of dispersal.

## Methods

### Study system and site

We conducted this study at the Koffler Scientific Reserve at Joker's Hill (KSR, 44°02'N, 79°32'W) near King City, Ontario, where we set up mesocosms in a red pine plantation. The deciduous-mixed forests of KSR are rich in native myrmecochorous species, including the long-lived spring ephemerals *A. acutiloba*, *A. canadense*, and *S. canadensis*, and the shorter lived invasive myrmecochore *C. majus*. The most abundant ant in KSR forests is *Aphaenogaster rudis*. These medium-sized (approx. 4 mm) ants are general scavengers and nest in rotting wood and occasionally in soil, forming monogynous colonies with several hundred workers (Lubertazzi 2012). *Aphaenogaster rudis* is a polyphyletic group—having phylogenetically interspersed clades with closely related *A. picea* (DeMarco and Cognato 2016)—and species in this group are difficult to distinguish

morphologically (Umphrey 1996), but may differ in their distributions and climate preferences (Warren et al. 2011). We follow the previous studies (Lubertazzi 2012; Prior et al. 2015) in referring to our study species as *A. rudis*, but recognize that the taxonomy of this group is unresolved.

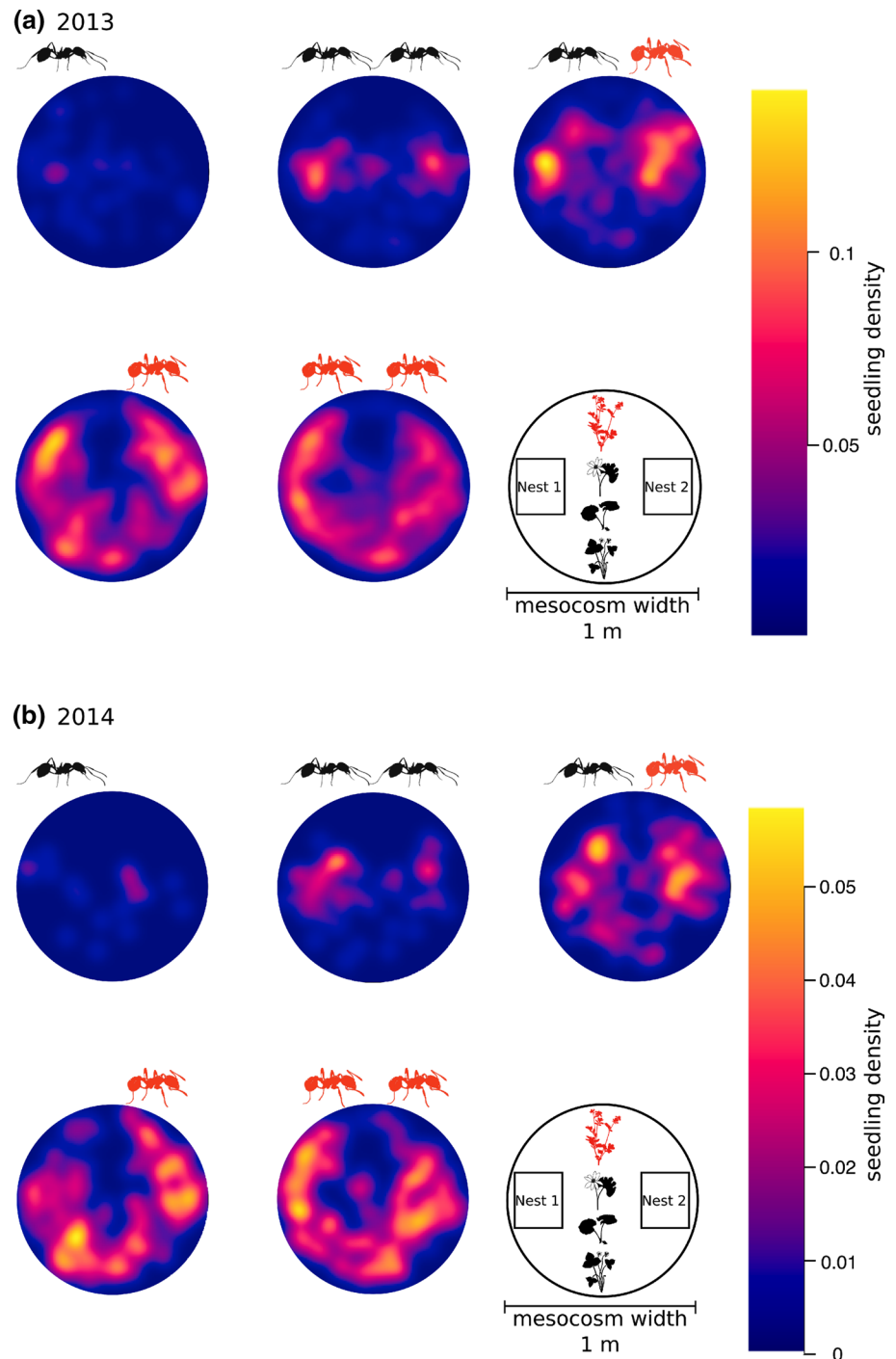
The second ant species in our study is the invasive European fire ant, *M. rubra*, which was introduced to North America over 100 years ago and has been in Ontario since at least 1975 (Grodén et al. 2005). *Myrmica rubra* lives in large colonies with multiple queens (polygyny) and multiple nests (polydomy) (Grodén et al. 2005). Its workers are approximately the same size (4 mm) as *A. rudis* workers, and are mainly insectivorous. Although *M. rubra* is uncommon in forests at KSR, it is abundant in fields at KSR and at nearby forested sites. *Myrmica rubra* disperses seeds of myrmecochorous plants in both its native (Gorb and Gorb 1999; Fokuhl et al. 2007) and introduced ranges (Prior et al. 2014, 2015).

### Mesocosm experiment

In the spring of 2012, we set up 42 experimental mesocosms in a 6 × 7 array (described in detail in Prior et al. 2015). Mesocosms were constructed out of plastic pools (1.2 m in diameter and 45 cm deep), dug into the ground, and filled with new soil that did not have a pre-existing seed bank; this size reflects the average seed dispersal distance (50 cm) of *A. rudis* (Ness et al. 2009; Canner et al. 2012). Each mesocosm was encased in fine-mesh netting to prevent colonization by other plant species, to minimize herbivory, and to keep ants and seed(ing)s in. We confirmed that the soil did not contain seeds and that the mesh netting was effective, because we observed very few seedlings of plant species other than what we added to mesocosms; we also removed any plants that were accidentally introduced. In mid-to-late May, we planted pairs of nursery-grown adult plants (either flowering or with buds) of *A. acutiloba*, *A. canadense*, *S. canadensis*, and *C. majus* (see inset in Fig. 2). Randomization of adult plant location was not possible, because it would require far more replicates per treatment than was feasible for the scope of this study. We added ant colonies to mesocosms to create six treatments with seven replicates each: (1) one *A. rudis* colony (AR), (2) two *A. rudis* colonies (ARAR), (3) one *M. rubra* colony (MR), (4) two *M. rubra* colonies (MRMR), (5) one *A. rudis* and one *M. rubra* colony (ARMR), and (6) a no ant's control. We collected ant colonies either at KSR (*A. rudis*) or in forests of nearby Toronto (*M. rubra*); we standardized each to contain 350 workers, 100 brood, and one queen. Ant colonies were added to mesocosms in two locations, with locations in one-colony treatments randomly chosen (see Fig. 2). We initially transferred colonies in plastic tubing that was loosely buried in the soil underneath a piece of rotten wood; however, both ant species made



**Fig. 2** Intensity plots of the distribution of seedlings that recruited in each ant treatment in **a** 2013 and **b** 2014. Plots are composites of all replicates within a treatment. Intensity values are counts per  $\text{cm}^2$ . Number and color of ants indicate the treatments and location of nests: single black ant, AR=one *A. rudis* colony; two black ants, ARAR=two *A. rudis* colonies; one red ant, MR=one *M. rubra* colony; two red ants, MRM=two *M. rubra* colonies; one black and one red ant, ARM=one *A. rudis* and one *M. rubra* colony. The last panels (modified from Prior et al. 2015) show the experimental design of the mesocosms. Nest 1 and Nest 2 are the locations of the original ant nests. Two adults of each plant species were planted ~10 cm on either side of the center line, in the order indicated by the icons; from top to bottom: *C. majus*, *S. canadensis*, *A. canadense*, and *A. acutiloba*. Seeds were placed at the base of each adult plant



new nests or nest entrances under the rotten wood or within other locations in the mesocosms. We collected seeds of our four focal myrmecochorous plant species between 29 May and 22 June 2012 from several nearby natural populations at KSR and stored them at 4 °C until use (up to 1 week maximum). We added 30 haphazardly chosen seeds of native plants at the base of each adult native plant on 14 June 2012 (*A. acutiloba*) or on 22 June 2012 (*A. canadense* and *S. canadensis*); these dates closely reflect the natural timing

of fruit dehiscence of these species at our site (Gordon et al. 2019). We also added 150 *C. majus* seeds underneath each of the invasive adult plants on 22 June 2012. The number of seeds added to the mesocosms reflects the approximate number of seeds produced per plant for each species, with a higher number being added for *C. majus* that produces a highly variable number of seeds depending on plant size (Kang and Primack 1991; Lobstein and Rockwood 1993; Gorb et al. 2000; see Prior et al. 2015 for details). Seedpods

of adult plants in mesocosms were removed. At the end of the first growing season, we added insecticidal baits to kill ant colonies.

Seedlings of *C. majus* started emerging about a month after seeds were added and continued into the following year. Native seedlings began emerging the following spring, 1 year after ant dispersal; fewer than 10% of native and invasive seedlings emerged 2 years after dispersal. In the spring of 2013, we recorded seedling recruitment of all four plant species, and we recorded seedling height and survival in late July, 2014. Each seedling and adult plant was individually labeled for long-term monitoring. Initial ant nests under the rotten wood were also marked, along with new nests in the soil that we located from openings in the soil where we observed ant activity.

## Data analysis

In 2013 and 2014, we photographed each mesocosm to obtain location data for all dispersed seedlings (i.e., seedlings that germinated > 10 cm from adult plant), adult plants, and ant nests. We analyzed photographs using the ImageJ software to obtain polar coordinates for each plant, which were then converted to Cartesian coordinates and plotted in R v. 3.2.5. We used the contributed R package spatstat (Baddeley et al. 2015) to calculate distances among dispersed seedlings, and from dispersed seedlings to ant nests and adult plants. We performed several analyses to compare the spatial locations of seedlings across ant treatments. We examined: (1) the overall spatial pattern of seedlings using the second-order spatial summary statistics; (2) where seedlings recruited in relation to ant nest locations; (3) the location of seedlings in relation to other plants; and (4) the effects of spatial location on the survival and growth of dispersed plants 2 years after ant dispersal. Because of low seedling recruitment in some of the mesocosms with only one ant colony, all analyses were conducted separately for mesocosms with one versus two ant colonies. Results from the single-ant colony treatments are provided in the Supporting Information, and often showed no statistically significant difference between ant species.

## Spatial pattern of seedling recruitment

To compare the spatial distributions of seedlings that recruited in each ant treatment, we estimated seedling intensity (i.e., the modeled point pattern, conditioned on the observed number of seedlings per unit area) using an isotropic Gaussian kernel with a standard deviation of 5 cm. We created summary plots of the kernel estimate of intensity for all replicates combined in each ant treatment for dispersed seedlings present in 2013 and 2014. This produced visual

representations (i.e., heat maps) of the densities of seedlings in each ant treatment (Fig. 2).

We used the second-order spatial summary statistics to determine whether the distribution of seedlings in each ant treatment was clumped or segregated at different spatial scales. For each mesocosm, we calculated Ripley's  $L$ -function and the empty-space function,  $F(r)$ . The  $L$ -function is a transformation of Ripley's  $K$ -function, which stabilizes the variance of the estimator, making the results easier to interpret visually. The empirical  $K$ -function,  $\hat{K}(r)$ , is the cumulative average number of neighboring points within a radius  $r$  from a point, standardized by the intensity and with a weighted edge correction term (Baddeley et al. 2015). Estimates of  $\hat{K}(r)$  are compared with the theoretical  $K$ -function for a Poisson process under complete spatial randomness; values of  $\hat{K}(r) > K_{\text{pois}}(r)$  indicate clustering of the point pattern, and values of  $\hat{K}(r) < K_{\text{pois}}(r)$  indicate a regular point process. The empty-space function,  $F(r)$ , describes the probability that a point will lie within the radius,  $r$ , of a reference location. Values of the empirical  $\hat{F}(r)$  are interpreted opposite to those of  $K(r)$ ; values of  $\hat{F}(r) < F_{\text{pois}}(r)$  indicate clustering of the point pattern, and values of  $\hat{F}(r) > F_{\text{pois}}(r)$  indicate a regular point process (Baddeley et al. 2015). Summary statistics were calculated for each mesocosm, and we compared spatial patterns among treatments using studentized permutation tests (Hahn 2012) with 999 random permutations on  $r$  in 0–30 cm for  $L(r)$ , and  $r$  in 0–25 cm for  $K(r)$ .

## Seedling locations in relation to ant nests

To determine whether *A. rudis* or *M. rubra* secondarily disperses seeds different distances from their nests, we first analyzed the proportion of seedlings that germinated within 20 cm of where the ant nests were originally located (Nest 1 and Nest 2 in Fig. 2; purple circles in Fig. 4c). This 20 cm radius is the furthest distance from the original nest locations to the nearest mesocosm edge (see purple circles in Fig. 4c). We fit a generalized linear model (GLM) with a gamma error distribution to the proportion of seedlings that recruited within 20 cm of the original ant nests, with ant treatment as a fixed effect. Seedlings were pooled across plant species, because there was no effect of plant species on the distance to nests (see Fig. S1); proportions of seedlings within 20 cm of ant nests were averaged within mesocosms before analysis.

Ants created new nests after we added them to mesocosms, and we tested whether ant treatment affected the number of new nests using a one-way ANOVA. We also measured the distance from each seedling to the nearest ant nest entrance—be it an original or new nest—took the average for each mesocosm, and then fit a GLM with a gamma error distribution to examine the effects of ant treatment and

number of nests on the distance from seedlings to the nearest ant nest.

### Seedling locations in relation to other plants

To explore possible competitive interactions between adult plants and seedlings, we calculated distances between seedlings and adults; we also calculated the nearest-neighbor distance for each seedling to test for competition among seedlings. For each native and *C. majus* seedling, we calculated the distance to the nearest adult native plant (of any species) and the distance to the nearest *C. majus* adult. Following Prior et al. (2015), we used invasive and natives as two plant groups, pooling the three native plants together because of their similar life histories and their similar dispersal or recruitment (they all responded similarly to the two ant species). We analyzed *C. majus* separately because of its different life-history and inherently invasive traits, and because it differed in recruitment compared to native plants in our previous study. We also calculated the distance to the conspecific adult plants for each seedling. All data were first averaged within mesocosms before analysis, to avoid pseudo-replication. We used linear mixed models to analyze distances from seedlings to adult plants, with treatment, plant type (native or invasive; or plant species), and interaction effects as fixed factors, and the individual mesocosm identity as a random factor.

We used GLMs with gamma error distributions to compare mean nearest-neighbor distances among ant treatments. Nearest-neighbor distances were calculated and analyzed separately for invasive and native plants. Ant treatment, the number of dispersed seedlings, and their interaction were included as fixed factors in the model. Because nearest-neighbor distances were strongly correlated with the number of plants in a mesocosm, we also calculated the expected nearest-neighbor distance for each mesocosm based on 1,000 random spatial arrangements of the same number of seedlings in the mesocosm. We then ran linear models to examine the effect of ant treatment on the difference between the expected and observed mean nearest-neighbor distances. This allowed us to examine the effect of ant treatment on seedling nearest-neighbor distances without the confounding influence of seedling density, which differed among ant treatments.

### Effects of spatial location on seedling survival and growth

We explored the effect of spatial location on seedling performance by modeling the relationship between spatial dispersion (i.e., nearest-neighbor distance or distance to adult plant) and seedling survival or growth. We also modeled the relationship between the distance from a seedling to the mesocosm center and seedling performance to determine

whether there were edge effects. For these analyses, the invasive and native species were analyzed separately; we pooled seedlings across all mesocosms and treatments, because there were no treatment effects independent of treatment effects on spatial dispersion (see above and Results). We modeled the probability that a *C. majus* seedling survived from 2013 to 2014 in a binomial generalized linear mixed model (GLMM) with distance to the nearest *C. majus* neighbor, distance to *C. majus* adult plant, and distance to the center of the mesocosm as fixed factors, and mesocosm identity as a random factor. We also ran a linear mixed model with the same fixed and random factors on the height of *C. majus* individuals that survived to 2014, 2 years after ants dispersed seeds. Similarly, we modeled the probability that a native seedling survived from 2013 to 2014 in a binomial GLMM with plant species, distance to the nearest dispersed *C. majus* seedling, distance to the nearest adult *C. majus*, and all possible interactions as fixed effects and mesocosm identity as a random effect, and ran a linear mixed model on native plant height in 2014 with the same fixed and random effects. Model selection was performed using AIC criteria, with the result that distance to the mesocosm center and distance to native plants were not included in models of native plant performance, because they did not improve model fit. Significance of all models was tested with type III ANOVAs.

## Results

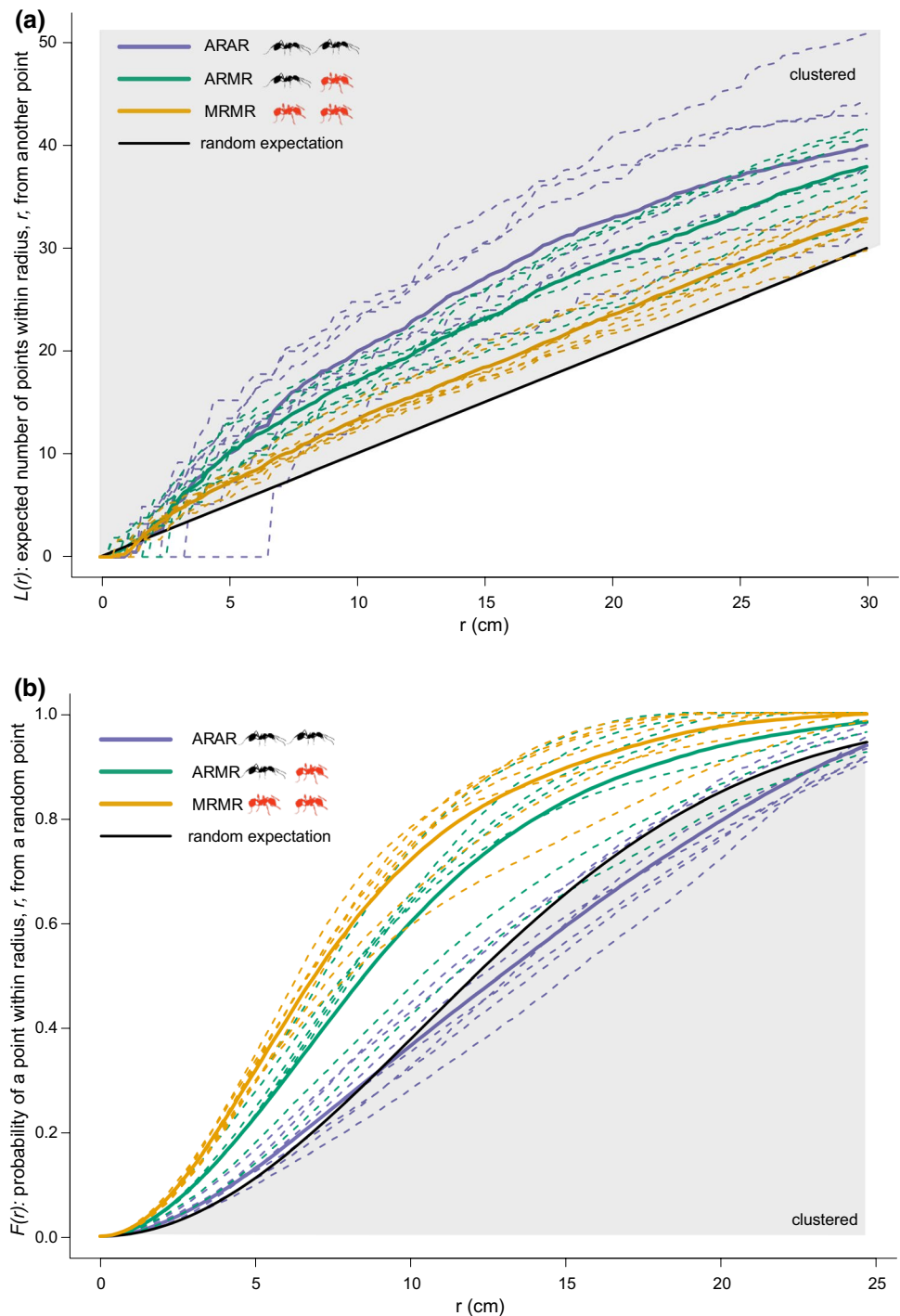
### Spatial pattern of seedling recruitment

Seed dispersal by *A. rudis* or *M. rubra* resulted in different spatial patterns of seedling recruitment. Seedlings in mesocosms with *A. rudis* recruited near the original ant nests, while seedlings in mesocosms with *M. rubra* were spread out around the entire mesocosm (Fig. 2). In all treatments, seedling densities were lowest near adult *C. majus* plants (position A in Fig. 2). High seedling mortality between 2013 and 2014 greatly reduced the overall intensity of points, explaining the different scales in Fig. 2a, b.

The Ripley's *L*-function for each treatment showed that seedlings were clustered in the mesocosms (Fig. 3a), yet the strength of clustering depended on ant treatment, with the ARAR treatment deviating farther from a random Poisson process than the MRMR treatment (studentized permutation test:  $T = 843.11$ ,  $p = 0.003$ ). At very small spatial scales ( $r < 2.5$  cm), seedling distributions more closely matched a pattern of complete spatial randomness, with some replicates even showing inhibition (Fig. 3a).

Similarly, the empty-space function,  $F(r)$ , showed differences among ant treatments (studentized permutation test:  $T = 3354.8$ ,  $p = 0.001$ ; Fig. 3b). The empirical curves for MRMR and ARMR mesocosms lie above the

**Fig. 3** **a** Ripley's  $L$ -function and **(b)** the empty-space function,  $F(r)$  at a given radius,  $r$ , for seedlings in mesocosms with two ant colonies. Dashed lines show the  $L$ - or  $F$ -functions of individual mesocosms, solid colored lines are the mean  $L$ - or  $F$ -functions for each treatment, and solid black lines are the theoretical  $L$ - or  $F$ -function for a Poisson process (complete spatial randomness). Empirical curves that fall above the theoretical  $L$ -function or below the theoretical  $F$ -function show signs of a clustering pattern (gray area), while empirical curves that fall below the theoretical  $L$ -function or above the theoretical  $F$ -function suggest a regular pattern. See Fig. 2 caption for treatment names



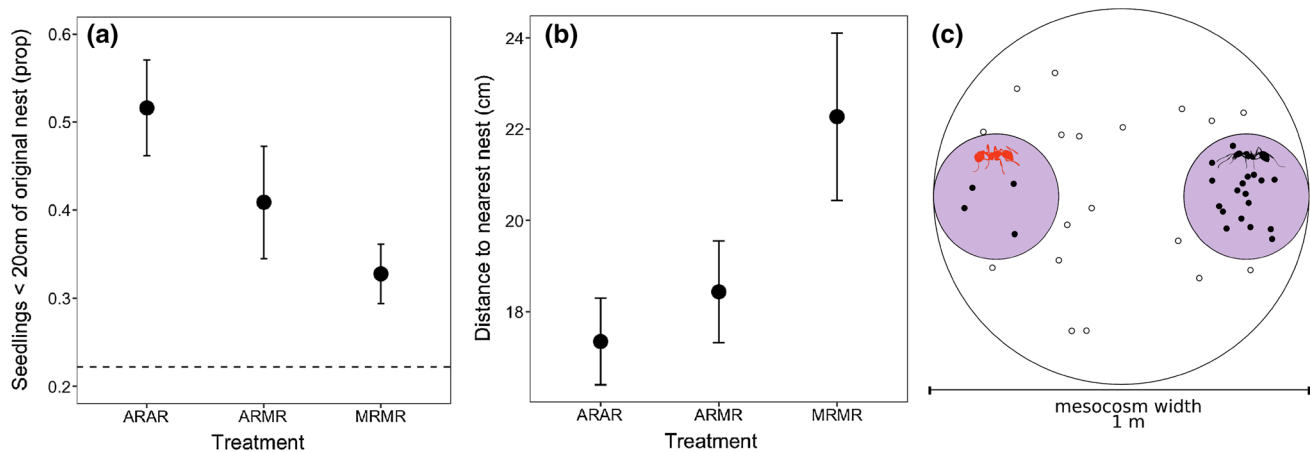
theoretical  $F$ -function ( $\hat{F}(r) > F_{\text{pois}}(r)$ ); thus, the empty-space distances are shorter than expected under a Poisson process, indicating a regular pattern. In contrast, seedlings in ARAR mesocosms showed clustering at  $r > 10$  cm. Since the values of  $F(r)$  are probabilities, for any random fixed location in a mesocosm, there is a 70% chance that a plant will be within 10 cm in a MRMR mesocosm, a

60% chance that a plant will be within 10 cm in an ARMR mesocosm, and only a ~35% chance that a plant will be within 10 cm in an ARAR mesocosm.

### Seedling locations in relation to ant nests

Seedlings recruited closer to *A. rudis* than *M. rubra* ant nests. A greater proportion of seedlings recruited within 20 cm of





**Fig. 4** Seedling locations relative to ant nests for all plant species combined. **a** Mean ( $\pm$  1SE) proportion of seedlings within 20 cm of the original ant nests; dashed line shows expected seedling density under complete spatial randomness. **b** Mean ( $\pm$  1SE) distance from a seedling to the nearest ant nest. **c** Example mesocosm showing the

20-cm radius around the two original ant nests (purple circles), and dispersed seedlings that germinated within (solid symbols) or outside (open symbols) this area. This mesocosm had one *M. rubra* (left side) and one *A. rudis* (right side) colony. See Fig. 2 caption for treatment names

the original ant nests in ARAR than MRMR mesocosms, with ARMR mesocosms intermediate, although the effect of ant treatment was only marginally significant (GLM: ant treatment:  $F_{2, 18} = 3.36$ ,  $p = 0.058$ ; Fig. 4a). Since the area within 20 cm of the two original nests is 22.2% of the total area of each mesocosm (dashed line in Fig. 4a), seedling densities around *A. rudis* nests were more than double what we would expect under complete spatial randomness.

*Myrmica rubra* made more new nests than *A. rudis* (ANOVA: ant treatment:  $F_{4, 30} = 8.08$ ;  $p < 0.001$ , Fig. S3); specifically, *M. rubra* made 1.3 new nests for every nest made by *A. rudis* in both the single and double ant colony treatments. Even so, seedlings recruited farther from ant nests in MRMR than in ARMR or ARAR mesocosms (GLM: ant treatment:  $F_{2, 18} = 8.52$ ,  $p = 0.002$ ; Fig. 4b, Fig. S1). The number of ant nests also had a significant effect on the distance from seedlings to the nearest ant nest (GLM: number of nests: coefficient =  $+0.007 \pm 0.001$  SE,  $F_{1, 17} = 23.2$ ,  $p < 0.001$ ); the distances between seedlings and the nearest ant nests were highest in the MRMR treatment, which also had the most nests.

### Seedling locations in relation to other plants

*Chelidonium majus* seedlings recruited farther from adult plants than native seedlings did (Table S1; Fig. 5a, b). There was also a marginally significant effect of ant treatment on the distance to *C. majus* adult plants (Table S1). Seedlings varied in recruitment distance from their conspecific parent plants (Fig. S5, Table S2). Adult plants (*C. majus* and *A. acutiloba*) that were planted closer to the mesocosm edge

had seedlings that recruited the farthest from their adult plants.

The mean observed nearest-neighbor distances were always smaller than the expected nearest-neighbor distances; as expected given the Ripley's *L*-functions, ant-dispersed seedlings germinated closer to one another than predicted under spatial randomness (Fig. 5c). *Chelidonium majus* seedlings were more clumped in the ARAR mesocosms than in the MRMR or in the ARMR (Fig. 5c, Table S3). Before accounting for the difference in the number of seedlings in each treatment, however, there was no treatment effect on the (raw) mean nearest-neighbor distances among *C. majus* seedlings in mesocosms, and the nearest-neighbor distances depended only on the number of dispersed *C. majus* seedlings (Table S4).

In terms of raw nearest-neighbor distances, the native plants were farther apart when they were dispersed by *A. rudis* (Table S4). However, there was no difference between the observed and expected mean nearest-neighbor distances among treatments for native plants (Fig. 5c, Table S3).

### Effects of spatial location on seedling survival and growth

Plants that were dispersed farther from the *C. majus* adult plants and from other *C. majus* seedlings had a higher chance of survival. Survival of *C. majus* to 2014 significantly depended on both an individual's nearest-neighbor distance and its distance to the *C. majus* adult plants (GLMM: distance to nearest *C. majus*:  $z_{1, 649} = 2.88$ ,  $p = 0.004$ ; distance to adult:  $z_{1, 648} = 2.15$ ,  $p = 0.03$ ), but there was no edge effect (distance to center:  $z_{1, 647} = 0.37$ ,  $p = 0.71$ ). In

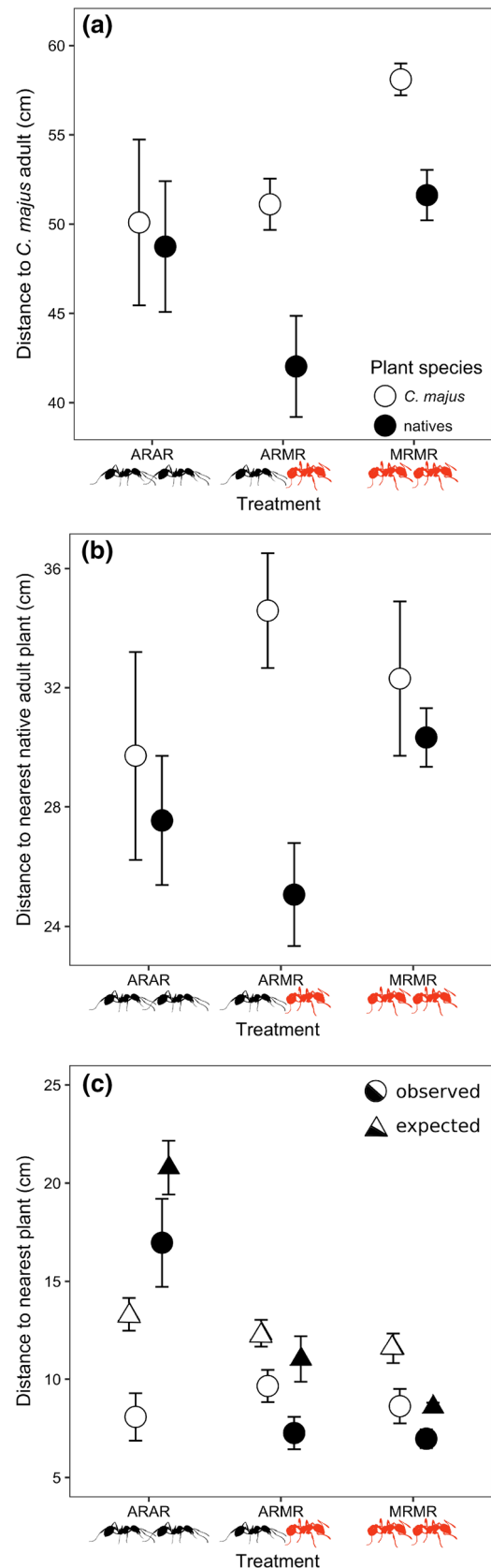
**Fig. 5** Mean ( $\pm$  1SE) distances from *C. majus* (open circles) or native (filled circles) seedlings to the nearest adult (a) *C. majus* or (b) native plant. c Mean ( $\pm$  1SE) observed (circles) and expected (triangles) nearest-neighbor distances between *C. majus* (open symbols) or native (filled symbols) plants. See Fig. 2 caption for treatment names

contrast, *C. majus* height in 2014 significantly depended on only an individual's distance from the center, and not on the nearest-neighbor distance, or distance to the *C. majus* adult plants (linear mixed model: distance to nearest *C. majus*:  $\chi^2_{1, 106} = 1.35$ ,  $p = 0.25$ ; distance to adult:  $\chi^2_{1, 106} = 2.84$ ,  $p = 0.09$ ; distance to center:  $\chi^2_{1, 106} = 17.4$ ,  $p < 0.001$ ; Figs. S5, S6b). In other words, *C. majus* plants closer to the center of a mesocosm grew taller.

There was also a negative effect of growing near a *C. majus* plant on the survival and height of native plants. There was a significant interaction effect between plant species and the distances to *C. majus* plants on native plant survival (binomial GLM: species  $\times$  distance to nearest *C. majus*:  $\chi^2_{2, 736} = 9.45$ ,  $p = 0.009$ ; species  $\times$  distance to nearest *C. majus*  $\times$  distance to *C. majus* adult:  $\chi^2_{2, 736} = 10.33$ ,  $p = 0.006$ ), but there were no other significant interaction terms. Native plants that grew closer to dispersed *C. majus* plants grew shorter (linear mixed model: distance to nearest *C. majus*:  $\chi^2_{1, 410} = 9.11$ ,  $p = 0.003$ ; Fig. S7a). The three native plant species also differed in their heights (linear mixed model: plants species:  $\chi^2_{2, 410} = 319.06$ ,  $p < 0.001$ ; Fig. S7); however, there was no effect of the distance to *C. majus* adults (linear mixed model: distance to *C. majus* adult:  $\chi^2_{1, 410} = 2.05$ ,  $p = 0.152$ ) on plant height. Thus, for the native species, germinating farther away from *C. majus* increased both their chance of survival and growth, but to different degrees for each plant species.

## Discussion

We already know that *M. rubra* and *A. rudis* differ in dispersal quantity (Prior et al. 2015); here, we suggest a mechanism by which ants differ in dispersal quality that ultimately affects plant performance and alters plant communities (Fig. 1). Figure 1 summarizes the main findings from this study and how they fit into our previous findings, showing how differences in both ant and plant traits combine to create divergent plant communities. *Myrmica rubra* not only picks up more seeds but also moves them longer distances than *A. rudis*. The two ant species deposited seeds in different locations within the mesocosms, affecting the spatial pattern of seedling recruitment and plant survival. *Myrmica rubra* secondarily dispersed seeds farther from its nests than *A. rudis*, resulting in more regular spacing among seedlings compared to the more clumped pattern in mesocosms with *A. rudis*. As a result, the invasive plant, *C. majus*, grew closer together



than expected when dispersed by *A. rudis* compared to *M. rubra*. Seedlings of *C. majus* also grew farther from all adult plants than seedlings of the three native plant species, likely because *C. majus* was more negatively affected by competition than the native plants. These spatial characteristics had fitness effects on *C. majus*; more spatially isolated *C. majus* seedlings were more likely to survive. *Chelidonium majus* also exerted a strong competitive effect on the survival and size of native plants, with native plants growing near a dispersed or adult *C. majus* plant being shorter and less likely to survive. These results explain why plant communities diverged, with the invasive plant dominating in the presence of the invasive ant and native plants in the presence of the native ants (Prior et al. 2015). Our results suggest that differences in behavior and colony organization between seed-dispersing ant species can have far-reaching consequences for plant communities by changing both the number of seeds moved and the spatial pattern of seedling recruitment, ultimately affecting plant success.

Although dispersal agents that move many seeds quickly are not necessarily effective seed dispersers, many studies measure only seed removal and extrapolate to plant fitness (e.g., Carney et al. 2003; Servigne and Detrain 2008; Meadley Dunphy et al. 2016). Other studies focus on primary dispersal (i.e., the distance from the parent plant to the ant nest) (e.g., Horvitz and Schemske 1986; Ness et al. 2004; Tanaka and Tokuda 2016); and even fewer studies examine secondary dispersal (but see Kalisz et al. 1999; Canner et al. 2012; Zhu et al. 2017) or how the whole dispersal process affects plant performance and communities (but see Christian 2001). To realistically assess their effectiveness and compare seed-dispersing species, it is critical to not only compare primary dispersal, given that secondary dispersal establishes the microhabitat and competitive environment that a seedling will experience for its lifetime. Prior et al. (2015) demonstrated that seed dispersal and seedling recruitment are not synonymous and that the differences between seed dispersal and seedling recruitment depend on both the plant and ant species. Here, we show a mechanism behind this finding, that seedling survival depends on deposition location, which differs between ant species. Only a handful of other studies have shown long-term benefits of myrmecochory that are not evident from simply measuring rates of seed removal, such as long-term survival and reproductive success (e.g., Kjellsson 1991) or reduced sibling densities (Kalisz et al. 1999).

We found that *A. rudis* secondarily dispersed seeds in a more clustered pattern than *M. rubra*. The Ripley's *L*-function showed that seedlings were spatially clustered overall and that clustering was more pronounced when seeds were dispersed by *A. rudis* than by *M. rubra* (Fig. 3a). The empty-space *F*-function, however, suggested regular spacing of seedlings in mesocosms with *M. rubra*; only mesocosms

with two colonies of *A. rudis* showed clustering (Fig. 3b). Because seedling density was higher in mesocosms with two *M. rubra* colonies (Prior et al. 2015), the same radius around a random location is more likely to include a seedling in *M. rubra* than *A. rudis* mesocosms, potentially explaining why seedlings in *M. rubra* mesocosms were regularly spaced according to the *F*-function, but clustered according to the *L*-function. Only the *L*-function analysis accounts for differences in point intensity among ant treatments (Baddeley et al. 2015).

The difference in colony structure between *A. rudis*, which has monodomous nests, and *M. rubra*, which has polydomous nests, was evident in our mesocosms. *Myrmica rubra* created more new nests and nest entrances than *A. rudis* (Fig. S3). Because of the larger number of *M. rubra* nests, if both ant species secondarily dispersed seeds similar distances, the mean distance from a seedling to the nearest ant nest would be shorter in *M. rubra* mesocosms. Instead, seedlings recruited furthest from ant nests when seeds were dispersed by *M. rubra* (Fig. 4b), suggesting that *M. rubra* workers move seeds longer distances than *A. rudis* workers during secondary dispersal. In other words, differences in the spatial pattern of seedling recruitment between *A. rudis* and *M. rubra* mesocosms may be the result of ant worker behavior more than colony structure. In other systems, ant species also vary in the distance or deposition location of secondary dispersal, affecting spatial patterns of plant recruitment (Gorb and Gorb 2003; Canner et al. 2012; Crisanto and Espadaler 2013; Bottcher et al. 2016) with certain plant species benefiting differently from spatial dispersal strategies of different ant species (Gorb et al. 2000).

In nature, *M. rubra* colonies are much larger than *A. rudis* colonies, and thus likely require more nutrition and are able to process more seeds. However, because we standardized colony size at the beginning of the experiment, and colonies were added to mesocosms only a few days before seeds, not enough time elapsed to create large differences in colony size before seed dispersal; thus, colony size likely did not contribute to differences among ant treatments in our experiment. In nature, colony size likely influences dispersal quantity, whereas differences in worker behavior between ant species may be a more important determinant of their dispersal quality.

By design, the mesocosms had a pre-existing spatial structure, with adult plants and seeds of each species planted in the same order down the centerline and ant nests always initially offset on either side (Fig. 2). While this initial spatial set-up likely influenced the final outcome of the seedling distribution patterns of all mesocosms and treatments to the same degree, the resulting spatial patterns of dispersed seedlings did not reflect where the seeds started, but rather, where the ants dispersed the seeds and how plant species germinated in response to dispersal. Since adult plants of

*A. acutiloba* and *C. majus* were planted closer to the edges of the mesocosms, the maximum distance from seedlings to these adult plants was greater than the maximum distance to *A. canadense* or *S. canadensis* adults, which were planted closer to the center. We saw this effect when comparing mean distances between seedlings and conspecific adults (Fig. S5), yet *C. majus* seedlings recruited farther from both *C. majus* adults and native adults than native seedlings. Overall, despite the pre-existing spatial set-up, we have shown that the two ant species were able to restructure the plant communities creating different patterns of spatial recruitment.

The size (120 cm in diameter) and nest density of the mesocosms was intended to reflect the average seed dispersal distance (50 cm) and nest density (> 1 colony per square meter) of *A. rudis* (Ness et al. 2009; Canner et al. 2012). The size and shape of the mesocosms constrained seed dispersal to some degree, as ants could not expand their colonies or move seeds beyond the mesocosm edges. Although we found evidence for an edge effect on *C. majus* seedling height, there was no effect on survival, suggesting that the physical constraints of the mesocosm design did not become pronounced until *C. majus* plants grew large. There was also no edge effect on the survival or height of the native species. In the MRMR and ARMR mesocosms, many seedlings grew around the mesocosm periphery, but not in the ARAR or single-ant colony mesocosms. This could result from competitive or territorial dynamics when *M. rubra* is faced with another ant colony; in Ukraine, the rate of seed removal by *M. rubra* depends on what other ant species are present (Gorb and Gorb 1999). Similar competitive dynamics may be occurring in our experiment, and may account for the high number of seedlings around the edges of mesocosms when *M. rubra* colonies share the space. Preliminary work on competition between *A. rudis* and *M. rubra* for seeds suggests that *A. rudis* is competitively dominant to *M. rubra* (Prior, unpublished data).

The mesocosm design that we used is a powerful way to test how the local assemblage of seed-dispersing animal species affects the spatial pattern of plant recruitment and ultimately plant community dynamics. We were able to experimentally compare seed dispersal quantity (Prior et al. 2014) and quality (this study) among ant assemblages in a way that would not have been possible in natural communities. However, we could not test for all possible benefits of myrmecochory in our experimental mesocosms. The mesh netting deterred rodents, so we could not assess whether seed dispersal by ants helps seeds escape predation by rodents (O'Dowd and Hay 1980; Heithaus 1981); however, other work suggests rodents very rarely consume myrmecochorous seeds at our study site (Meadley Dunphy et al. 2016). We also did not test whether *A. rudis* and *M. rubra* deposit seeds in different microhabitats (Gibson

1993); in our mesocosms, we used homogenized nursery soil that likely varied little in micronutrient content. Although there is some chance that ants may have modified local soil conditions, perhaps creating microhabitats that were more or less well suited for germination. Another limitation of our design is that we measured the spatial pattern of seedling recruitment to make inferences about the spatial pattern of seed deposition by ants. Yet, some plant mortality presumably occurred between seed deposition and seedling recruitment, and it probably did not occur entirely at random with respect to space. Although we do not report actual seed dispersal distances here, seedling recruitment and survival is more informative about plant fitness, because it measures offspring success.

Biological invasions and other global change drivers are changing the composition of many ant communities (Bertelsmeier et al. 2015), including ant communities in the deciduous forests of eastern North America where many herbaceous plants depend on ants for seed dispersal (Warren et al. 2015; Prior et al. 2015). The addition of a new ant species can change seedling recruitment and plant assemblages, whether through reduced dispersal (e.g., Christian 2001; Rodriguez-Cabal et al. 2011) or because of different dispersal characteristics between introduced and native ants (e.g., Carney et al. 2003; Ness and Bronstein 2004; Ness 2004). Unlike other well-studied invasive ants, *M. rubra* is not a poor seed disperser; in fact, it is in many ways a superior disperser compared to the native “keystone” seed-dispersing ant, *A. rudis*. *Myrmica rubra* moves more seeds more quickly (Prior et al. 2015) and secondarily disperses seeds outside its nests both faster (Prior et al. 2014) and farther (this study) (Fig. 1). Plant species respond to these different dispersal modes differently, depending on their life-history strategies and invasion history. In our experiment, this created mesocosms that were dominated by the invasive plant *C. majus* in the presence of *M. rubra*, because the traits of *C. majus* meant it benefited more from the high-quality dispersal services of the invasive ant. We found that the two seed-dispersing ant species created divergent plant communities, and that plant communities were dominated by the invasive plant in the presence of the invasive ant. This likely occurred, because the life-history strategies that confer invasiveness in these mutualist species (e.g., polygyny for the ant and fast growth for the plant) make them particularly strong interactors. Taken together with Prior et al. (2015), our results suggest the potential for long-term consequences on plant community structure with changing ant partner identity.

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