

Floral visitation by the Argentine ant reduces bee visitation and plant seed set

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Abstract. Ants often visit flowers, but have only seldom been documented to provide effective pollination services. Floral visitation by ants can also compromise plant reproduction in situations where ants interfere with more effective pollinators. Introduced ants may be especially likely to reduce plant reproductive success through floral visitation, but existing experimental studies have found little support for this hypothesis. Here, we combine experimental and observational approaches to examine the importance of floral visitation by the nonnative Argentine ant (*Linepithema humile*) on plant species native to Santa Cruz Island, California, USA. First, we determine how *L. humile* affects floral visitor diversity, bee visitation rates, and levels of pollen limitation for the common, focal plant species island morning glory (*Calystegia macrostegia* ssp. *macrostegia*). Second, we assess the broader ecological consequences of floral visitation by *L. humile* by comparing floral visitation networks between invaded and uninvaded sites. The Argentine ant and native ants both visited island morning glory flowers, but *L. humile* was much more likely to behave aggressively towards other floral visitors and to be the sole floral occupant. The presence of *L. humile* in morning glory flowers reduced floral visitor diversity, decreased rates of bee visitation, and increased levels of pollen limitation. Network comparisons between invaded and uninvaded sites revealed differences in both network structure and species-level attributes. In invaded sites, floral visitors were observed on fewer plant species, ants had a higher per-plant interaction strength relative to that of other visitors, and interaction strengths between bees and plants were weaker. These results illustrate that introduced ants can negatively affect plant reproduction and potentially disrupt pollination services at an ecosystem scale.

Key words: Argentine ant; bee visitation; biological invasion; *Calystegia macrostegia*; Halictidae; island morning glory; *Linepithema humile*; pollen limitation; pollination; visitation network.

INTRODUCTION

Over 85% of terrestrial plant species rely on pollination services provided by animals (Ollerton et al. 2011). Plants use floral displays to attract effective pollinators, but this strategy also exposes plants to visitation by ineffective pollinators (Schemske and Horvitz 1984, Waser et al. 1996, Irwin et al. 2001, Traveset and Richardson 2006). Ants, for example, visit flowers of many plant species from a variety of environments (Rico-Gray 1989, Bosch et al. 1997, Blüthgen et al. 2004, Junker et al. 2011), but usually do not provide pollination services (Hölldobler and Wilson 1990, Lach et al. 2010). Ants do pollinate some plant species (Gómez et al. 1996, Ashman and King 2005, de Vega et al. 2009), but, for most plants, the disadvantages of

floral visitation by ants are believed to outweigh the advantages (Beattie et al. 1984, Hölldobler and Wilson 1990, Lach et al. 2010). The fact that certain plants possess structural barriers and chemical repellents that restrict floral visitation by ants provides evidence that these insects can reduce plant fitness (Junker et al. 2011, Ballantyne and Willmer 2012). Given the ubiquity of floral visitation by ants, it seems somewhat surprising that relatively few studies investigate how this phenomenon affects plant reproduction or influences selection on floral traits (Ashman and King 2005, Ness 2006, Galen and Geib 2008).

Compared to interactions involving ants and plants native to the same region, interactions involving introduced ants may be especially likely to affect plant reproductive success. A lack of evolutionary history between ants and plants can increase the likelihood of floral visitation (Junker et al. 2011). In addition, certain introduced ant species have been documented to aggressively interact with other floral visitors, attain

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high levels of abundance, and exhibit strong preferences for carbohydrate resources (Lach 2003, 2007). Although the existing body of work on floral visitation by introduced ants clearly documents decreased visitation by other insects, studies to date have found little experimental evidence that these effects negatively affect plant reproduction (Blancafort and Gomez 2005, Lach 2007, 2008). Previous studies on this topic have also restricted their focus to a single plant species. Clarifying the importance of floral visitation by introduced ants within the ecosystems that they invade requires an understanding of how ants affect networks of interactions among plants and pollinators. Introduced ants, for example, could usurp links between plants and legitimate pollinators without compensating for reductions in pollination services. In general, the extent to which introduced species (especially nonnative floral visitors) alter the structure of pollination networks remains poorly understood (Tylianakis et al. 2010, Burkle and Alarcón 2011, Traveset et al. 2013).

Here we combine experimental approaches and network analyses to clarify the ecological importance of floral visitation by the Argentine ant (*Linepithema humile*) for plants native to Santa Cruz Island, California. Our study has two components. We first address how *L. humile* affects floral visitor diversity, bee visitation rates, and pollen limitation on a common focal plant species, the island morning glory (*Calystegia macrostegia* ssp. *macrostegia*). Second, we construct floral visitation networks in plots with and without *L. humile* to quantify the extent to which this invader influences the strength of interactions between bees and native plants.

METHODS

Study system

Santa Cruz Island is the largest of the eight California Channel Islands and supports over 600 species of native vascular plants (Junak et al. 1995), over 100 species of native bees (Thorpe et al. 1994), and at least 30 species of native ants (Wetterer et al. 2000). The Argentine ant has been present on Santa Cruz Island for several decades, and, as of 2010, occupied four separate areas (each located >1 km apart from one another) that total approximately 227 ha (~2% of total island area) of scrub, grassland, and riparian habitats. We focus in detail on how the Argentine ant affects the island morning glory, which is endemic to the northern Channel Islands (Junak et al. 1995). Island morning glory is a semi-deciduous perennial that produces numerous, bisexual flowers from early spring through late fall. The long blooming period of this species, coupled with its abundance and widespread distribution on Santa Cruz Island, likely make it an important source of nectar and pollen for a variety of native pollinators. At least 11 native bee species, for example, visit the flowers of island morning glory on Santa Cruz Island (Thorpe et al. 1994).

Experimental design

To examine the effects of floral visitation by the Argentine ant on island morning glory, we made comparisons based on both observational data (invaded sites vs. uninvaded sites) and experimental ant removals (invaded sites vs. removal sites). For both types of comparisons, we consider the same set of response variables: floral visitor diversity, bee visitation rate, and pollen limitation. Observational data on floral visitor diversity and bee visitation rates consisted of measurements made during four sampling periods (June 2011, September 2011, June 2012, and September 2012) at six sites (three invaded, three uninvaded). Pollen limitation was measured only in 2012. Each uninvaded site was in the general vicinity of an invaded site and was separated by >1 km from another uninvaded site. Within each site, individual focal morning glory plants were separated by at least 10 m.

In addition to comparisons based on observational data, we conducted an Argentine ant removal in 2012. We used ground baits containing Optigard Flex liquid toxicant (Syngenta Crop Protection, Greensboro, North Carolina, USA), containing the active ingredient thiamethoxam, to remove *L. humile* from a 4-ha portion of one of the invaded areas (see Appendix A). No floral visitors of island morning glory, aside from ants (nearly all *L. humile*), were observed at any of the ground baits. This treatment, which we initiated in mid-June, decreased *L. humile* abundance to the point that it was almost completely absent from the treatment area by September (Appendix A). To assess how Argentine ant removal influenced each response variable, we made pre-removal comparisons between plants in the removal area and plants in an adjacent invaded area (left untreated) in early June, and post-removal comparisons between plants in the removal area and plants in an adjacent invaded area (left untreated) in September. Individual plants are treated as experimental units in all analyses based on experimental data. All analyses on island morning glory data were conducted in R (R Development Core Team 2012).

Floral visitor diversity on island morning glory

To quantify floral visitor diversity on the island morning glory, we inspected 16 ± 2 (mean \pm SE) flowers on each plant on one day in each sampling period and recorded the proportion of flowers with each type of floral visitor (identified to the lowest taxonomic level possible in the field; see Appendix B). We conducted sampling under sunny conditions between mid-morning and mid-afternoon. These surveys tallied 10 887 arthropods belonging to 33 species (or morpho-species) visiting 3917 morning glory flowers. Using these data, we calculated the Shannon diversity index (H') for each plant in each sampling period. For comparisons based on observational data, plants were surveyed at each site in June 2011 (invaded, $n = 12, 13,$ and 5 plants; uninvaded, $n = 13, 15,$ and 17 plants), September 2011

(invaded, $n = 10, 9,$ and 7 plants; uninvaded, $n = 14, 13,$ and 6 plants), June 2012 (invaded, $n = 11, 20,$ and 11 plants; uninvaded, $n = 13, 14,$ and 11 plants), and September 2012 (invaded, $n = 5$ and 7 plants; uninvaded, $n = 8, 8,$ and 14 plants). For analysis, we averaged diversity estimates across all plants within each site and sampling period and used these means as data points in a linear mixed-effects model (Crawley 2013). In this analysis, based on observational data, invasion status (invaded vs. uninvaded) was treated as a fixed effect, and sampling period was treated as a random effect. For comparisons based on experimental data, we used Wilcoxon rank-sum tests to compare plants from invaded and treatment sites before the removal of *L. humile* (invaded, $n = 10$ plants; treatment, $n = 10$ plants) and then again after removal (invaded, $n = 7$ plants; treatment, $n = 9$ plants).

Bee visitation rates on island morning glory

To estimate bee visitation rates (visits per minute) and to quantify behavioral interactions between ants and other floral visitors, we conducted 5-min focal observations at each of 2.8 ± 0.1 flowers per plant in each sampling period. This sampling effort yielded data on 2137 floral visits across 689 focal observation periods. During each 5-min focal observation, we recorded the identity of each floral visitor (identified to the lowest taxonomic level possible in the field; see Appendix B), duration of each visit, and (if relevant) behavioral interactions between ants and other visitors. We collected data on visitation rates for all floral visitors, but we focus on bees, because of their presumed importance as pollinators of the island morning glory (Thorpe et al. 1994) and its congeners (Ushimaru and Kikuzawa 1999). Interactions between ants and other floral visitors were considered aggressive when ants chased visitors away from flowers or neutral when ants and other visitors co-occurred in a flower without apparent aggression. For comparisons based on observational data, plants were surveyed at each site in June 2011 (invaded, $n = 12, 13,$ and 5 plants; uninvaded, $n = 13, 8,$ and 7 plants), September 2011 (invaded, $n = 11, 10,$ and 7 plants; uninvaded, $n = 15, 13,$ and 6 plants), June 2012 (invaded, $n = 11, 20,$ and 11 plants; uninvaded, $n = 13, 14,$ and 11 plants), and September 2012 (invaded, $n = 5$ and 4 plants; uninvaded, $n = 8, 9,$ and 8 plants). For analysis, we averaged bee visitation rates (all bee taxa pooled) across plants (after averaging rates across flowers within each plant) within each site and sampling period and used these means in a linear mixed-effects model (Crawley 2013). For this analysis based on observational data, invasion status (invaded vs. uninvaded) was treated as a fixed effect, and sampling period was treated as a random effect. For comparisons based on experimental data, we used Wilcoxon rank-sum tests to compare invaded and treatment sites before the removal of *L. humile* (invaded, $n = 10$ plants; treatment, $n = 10$ plants) and then again

after removal (invaded, $n = 4$ plants; treatment, $n = 4$ plants).

Pollen limitation in the island morning glory

To determine if floral visitation by the Argentine ant increases pollen limitation for the island morning glory, we determined seed set for open-pollinated and hand cross-pollinated flowers. Open-pollinated flowers were unmanipulated and received visits by pollinators. For the hand cross-pollination treatment, we applied non-self pollen from ≥ 5 pollen donors collected from synchronously blooming plants located >1 km away. Before and after hand cross-pollination, flowers in this treatment were covered with fine-mesh nylon bags; bagging prevented deposition of self-pollen that could interfere with cross pollen in this partially incompatible species. Once plants set fruit, we collected and counted seeds. We estimated pollen limitation as pollen limitation index (PLI) = $1 - ([\text{mean seed set for open flowers}] / [\text{mean seed set for flowers that received cross-pollination}])$ (Larson and Barrett 2000). As PLI standardizes the reproduction of open flowers by the maximum attainable reproduction at each site, this measure is robust to among-site variation in factors other than pollen receipt. Thus, we may attribute differences among treatments in PLI to the degree to which *L. humile* disrupts pollination. For comparisons based on observational data, the numbers of plants surveyed at each site were invaded ($n = 7, 8,$ and 8 plants) and uninvaded ($n = 8, 8,$ and 7 plants). We used a split-plot ANOVA to test for differences in pollen limitation between invaded and uninvaded sites; invasion status (invaded vs. uninvaded) was treated as a fixed, whole-plot effect, and blocks (sites) were considered random effects (Crawley 2013). Individual plants were treated as experimental units in this analysis. Prior to analysis, PLI values were arcsine square root transformed. For PLI comparisons based on experimental data, we used separate Wilcoxon rank-sum tests to compare invaded and treatment sites before *L. humile* removal (invaded, $n = 4$ plants; treatment, $n = 4$ plants) and then again after removal (invaded, $n = 5$ plants; treatment, $n = 5$ plants).

Floral visitation networks

To assess the broader ecological importance of floral visitation by *L. humile*, we constructed floral visitation networks at four, 1-ha plots interspersed among our island morning glory study sites but separated by ≥ 1 km from one another. The four plots included two plots that were invaded by *L. humile*, and two plots that were not invaded. Vegetation in all network plots consisted primarily of shrub species (<2 m in height) common on Santa Cruz Island (Junak et al. 1995). We surveyed each plot monthly from May to October 2012. During each survey we recorded all floral visitors at all plant species in bloom along four, 100×2 m belt transects running the length of each plot. These surveys yielded 3914 floral visitor observations by 54 species (or

morphospecies) of arthropods on 18 native plant species (Appendix C). The floral visitation network for each plot is based on the visitation data for that plot pooled across months.

To determine the degree of similarity in network topology, we performed orthogonal, least-squares Procrustes analyses (Alarcón et al. 2008) between each pair of floral visitation networks ($n = 6$ comparisons). The Procrustes algorithm minimizes the sums-of-squares distances between corresponding observations in two visitor \times plant interaction matrices by translating, reflecting, rotating, and scaling one matrix to fit the other (Jackson 1995, Jones 2002). The approach is analogous to mapping the positions of arthropod visitor landmarks between two superimposed floral landscapes (Alarcón et al. 2008). We used the FATHOM toolbox (Jones 2002) implemented in MatLab R2013a (The Mathworks, Natick, Massachusetts, USA) to produce the m^2 goodness-of-fit statistic, which varies from 0 to 1; smaller m^2 values indicate better fit (Jackson 1995, Jones 2002). To determine the significance of m^2 values, we used permutation tests (10 000 permutations per test; Jackson 1995, Peres-Neto and Jackson 2001). Values were first standardized (mean = 0, variance = 1) by plant species so that each species would contribute equal weight. To simplify interpretation, we converted m^2 values to the congruence between networks, which can be interpreted as the similarity in network topology expressed as a percent (congruence = $[1 - m^2] \times 100$; Alarcón 2010).

Procrustes analysis also produces vector residuals for each visitor morphospecies; residuals are a measure of the deviation in the position of each morphospecies between the two superimposed networks (Jackson 1995, Peres-Neto and Jackson 2001). We also calculated other species-level network parameters for each visitor morphospecies: species degree (S , the number of plant species visited by each visitor morphospecies) and species diversity (H , Shannon diversity index for the diversity of plant species visited by each visitor morphospecies). For each plant species we calculated the interaction strength of all of its associated floral visitors (the proportion of visits to each plant species by each visitor morphospecies out of the total number of visits that a plant species received).

RESULTS

Floral visitor diversity on island morning glory

In floral visitation surveys, over 90% of individual visitors at uninvaded sites were bees, beetles, and ants, and these insects (aside from *L. humile*) were mostly absent from island morning glory flowers at invaded sites (Table 1). Comparisons based on observational data and those based on experimental data both revealed reductions in floral visitor diversity in areas with the Argentine ant. With respect to the observational surveys, floral visitor diversity on island morning glories in areas with *L. humile* was lower compared to

TABLE 1. Visitation on island morning glory flowers by ants, bees, and beetles.

Taxon and sampling period	Uninvaded	Invaded
Native ants		
June 2011	0.32 \pm 0.06	0.02 \pm 0.02
September 2011	0.56 \pm 0.03	0.07 \pm 0.07
June 2012	0.37 \pm 0.17	0.01 \pm 0.01
September 2012	0.58 \pm 0.01	0.00 \pm 0.00
Native bees		
June 2011	0.18 \pm 0.09	0.01 \pm 0.01
September 2011	0.25 \pm 0.05	0.03 \pm 0.01
June 2012	0.27 \pm 0.05	0.00 \pm 0.00
September 2012	0.30 \pm 0.05	0.01 \pm 0.01
Native beetles		
June 2011	0.47 \pm 0.06	0.02 \pm 0.01
September 2011	0.04 \pm 0.01	0.01 \pm 0.01
June 2012	0.32 \pm 0.03	0.05 \pm 0.05
September 2012	0.02 \pm 0.01	0.00 \pm 0.00

Notes: Table entries show mean (\pm SE) proportion of flowers on a plant with each type of visitor; values are averaged across plants in uninvaded sites ($n = 3$ sites) and invaded sites ($n = 3$ sites). Species (or morphospecies) that make up each of the three groups are provided in Appendix B.

that in areas without *L. humile* (Fig. 1a; linear mixed-effects model, $t_4 = 5.63$, $P < 0.01$). With respect to the experimental removal data, floral visitor diversity did not differ between plants in invaded and treatment sites prior to Argentine ant removal (Fig. 1b; Wilcoxon rank-sum test, $W = 41.5$, $P = 0.39$), whereas after removal diversity was greater in the treatment site compared to that in the invaded site (Fig. 1b; Wilcoxon rank-sum test, $W = 10.5$, $P = 0.013$).

Floral visitation surveys revealed that island morning glories in invaded and uninvaded sites did not differ from one another with respect to the proportion of their flowers with ants present (linear mixed-effects model, $t_4 = 1.15$, $P = 0.20$). Across all sampling periods at invaded sites, Argentine ant workers were present (in at least one flower) on 100% of island morning glories surveyed ($n = 110$ observations). Two native ant species were occasionally observed in island morning glory flowers near the perimeter of the invasion; *Monomorium ergatogyna* was present (in at least one flower) on 8% (9/110) of plants surveyed, and *Formica moki* was present on 4% (4/110) of plants surveyed. Across all sampling periods at uninvaded sites, seven ant species were detected in morning glory flowers. *Monomorium ergatogyna* was present on 71% (94/132) of plants surveyed (with workers in at least one flower); *Crematogaster marioni* was present on 35% (46/132); *Formica moki* was present on 26% (34/132); *Pheidole hyatti* was present on 5% (7/132); *Dorymyrmex bicolor* was present on 4% (5/132); *Tapinoma sessile* was present on 3% (4/132); and *Camponotus maritimus* was present on 2% (2/132).

Although the frequency of ant visitation to island morning glory flowers did not differ between invaded and uninvaded sites, focal flower observations revealed

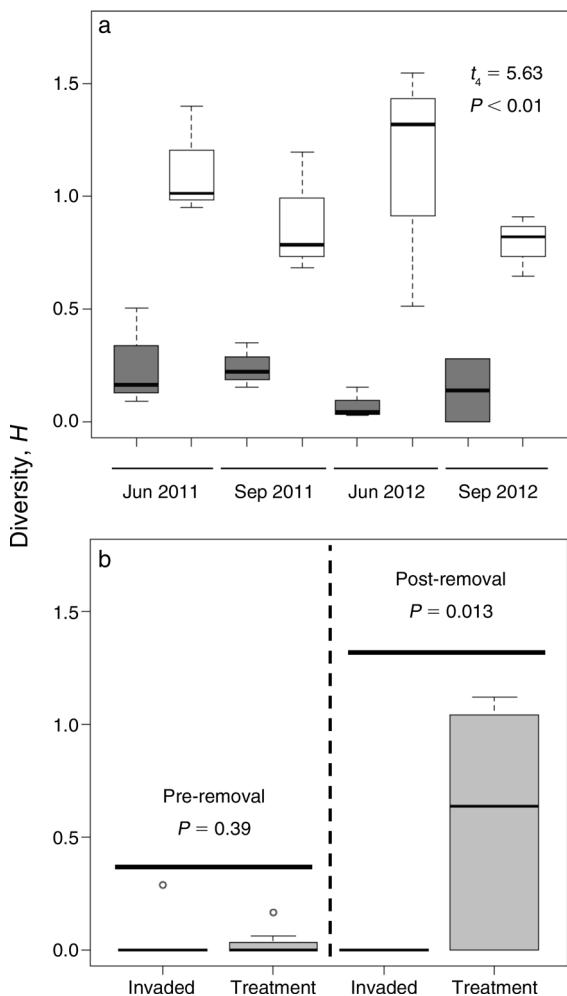


FIG. 1. (a) Shannon diversity indices (H) for floral visitors on island morning glory study sites invaded by and uninvaded by the Argentine ant, as evidenced across four sampling periods (June and September 2011, June and September 2012). T value and P value for the effect of invasion status from a linear mixed-effects model are shown. (b) Shannon diversity indices (H) for floral visitors at invaded and treatment sites before (June) and after (September) Argentine ant removal. P values from Wilcoxon rank-sum tests are shown. For (a) and (b), box plots show the median (bold line), first and third quartiles (upper and lower edge of boxes), and minimum and maximum (whiskers). The unfilled circles represent outliers. White signifies uninvaded sites, dark gray signifies invaded sites, and light gray signifies treatment sites.

differences in how ants behaved towards other visitors and in the likelihood that ants and other arthropods occurred in flowers. Across all sampling periods, Argentine ant workers usually showed aggression towards other floral visitors (48 out of 51 interactions observed), whereas native ants seldom exhibited aggression (1 out of 184 interactions observed; chi-square test, $\chi_1 = 206.23$, $P < 0.01$). A video example of Argentine ant aggression is provided in Appendix D that shows an *L. humile* worker driving off a halictid bee (*Augochlo-*

rella pomoniella) from a morning glory flower. Island morning glory flowers with at least one Argentine ant worker present were less likely to be simultaneously visited by another floral visitor (18 out of 905 flowers) compared to flowers with native ants (330 out of 668 flowers; chi-square test, $\chi_1 = 498.69$, $P < 0.01$).

Bee visitation rates on island morning glory

Comparisons based on observational data and those based on experimental data both revealed reductions in bee visitation rates on island morning glory flowers in areas occupied by the Argentine ant. Data from observational surveys indicated that rates of bee visitation on island morning glories in areas with *L. humile* were lower than those in areas without *L. humile* (Fig. 2a; linear mixed-effects model, $t_4 = 6.38$, $P < 0.01$). Moreover, prior to experimental removal of the Argentine ant, bee visitation rates did not differ between plants in invaded and treatment sites (Wilcoxon rank-sum test, $W = 37$, $P = 0.21$), whereas after removal bee visitation rates increased on plants in the treatment site relative to those in the invaded site (Fig. 2b; Wilcoxon rank-sum test, $W = 0$, $P = 0.029$).

Pollen limitation in island morning glory

In comparisons based on observational data, island morning glories experienced greater pollen limitation in areas where *L. humile* was present compared to where it was absent (Fig. 3a; split-plot ANOVA, $F_{1,2} = 28.89$, $P = 0.033$); block was not significant in this analysis ($F_{1,1} = 6.17$, $P = 0.24$). At the five sites for which we have PLI data for both June and September 2012, PLI site means were higher in September compared to those in June at all five sites, and the mean PLI value based on these site means was higher in September compared to that for June (paired t test, $t_4 = 3.48$, $P = 0.025$).

Consistent with comparisons based on observational data, experimental data showed that Argentine ant removal decreased pollen limitation relative to levels apparent in the invaded control (Fig. 3b). Prior to *L. humile* removal, pollen limitation did not differ between plants in invaded and treatment sites (Wilcoxon rank-sum tests, $W = 25$, $P = 0.89$), whereas after *L. humile* removal pollen limitation was lower for plants in the treatment site compared to those in the invaded site (Wilcoxon rank-sum tests, $W = 9$, $P < 0.01$).

Evidence that bees play a key role in the pollination of island morning glory comes from the negative relationship between the pollen limitation index and bee visitation rate (simple linear regression, $F_{1,9} = 11.89$, $P < 0.01$, $R^2 = 0.57$). The data points in this regression are based on site means from the 2012 sampling periods (observational data).

Floral visitation networks

The presence or absence of the Argentine ant significantly influenced the topology of floral visitation networks. Procrustes analyses revealed that the two

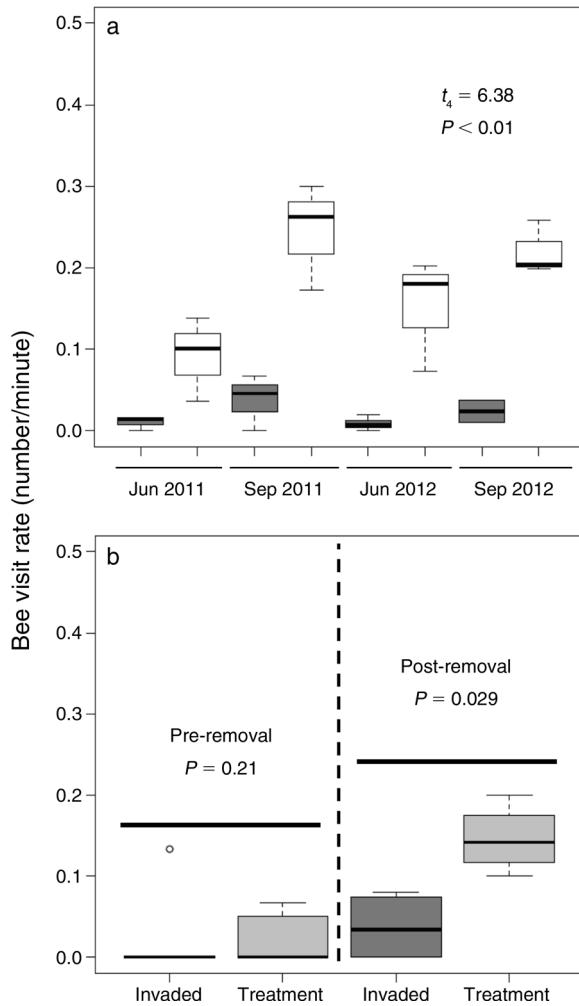


FIG. 2. (a) Bee visit rate on individual island morning glory flowers at study sites invaded by and uninvaded by the Argentine ant, as evidenced across four sampling periods (June and September 2011, June and September 2012). Values of t and P for the effect of invasion status from a linear mixed-effects model are shown. (b) Bee visit rate on individual island morning glory flowers at invaded and treatment sites before (June) and after (September) experimental Argentine ant removal. See Fig. 1 for explanation of box plot components.

invaded networks significantly resembled each other (54.52 % congruence, $m^2 = 0.45$, $P = 0.0044$), as did the two uninvaded networks (49.49% congruence, $m^2 = 0.51$, $P < 0.01$). Networks with and without *L. humile*, in contrast, were dissimilar ($6.48\% \pm 1.38\%$ [mean \pm SE] congruence [$n = 4$ pair-wise comparisons], $m^2 = 0.94 \pm 0.014$, $P > 0.05$). Consequently, the congruence value for the two invaded plots was higher than that of the mean congruence value from the invaded–uninvaded plot comparisons (one-sample t test, $t_3 = 34.82$, $P < 0.01$), and the congruence value for the two uninvaded plots was higher than that of the mean congruence value from invaded–uninvaded plot comparisons (one-sample t test, $t_3 = 31.17$, $P < 0.01$). Moreover, residual vectors

for floral visitors were larger from the invaded–uninvaded network comparisons (0.11 ± 0.016 [$n = 4$ pair-wise comparisons]) than they were from the comparison between the two invaded networks (0.066 ± 0.010 ; paired t test, $t_{53} = 2.49$, $P = 0.016$). A similar (but non-significant) pattern was also detected between the residual vectors for floral visitors from the invaded–uninvaded network comparisons vs. those from the comparison between the two uninvaded networks (0.082 ± 0.006 ; paired t test, $t_{53} = 1.65$, $P = 0.11$).

Species-level analyses revealed that across all visitor taxa, species degree, S , was greater in uninvaded networks (1.75 ± 0.17 [mean \pm SE]) compared to invaded networks (1.11 ± 0.18 ; paired t test, $t_{53} = 2.89$, $P < 0.01$). Similarly, species diversity (0.32 ± 0.049 [$H \pm$ SE]) was greater in uninvaded networks compared to invaded networks (0.16 ± 0.036 ; paired t test, $t_{53} = 3.13$, $P < 0.01$). In invaded networks, the Argentine ant had

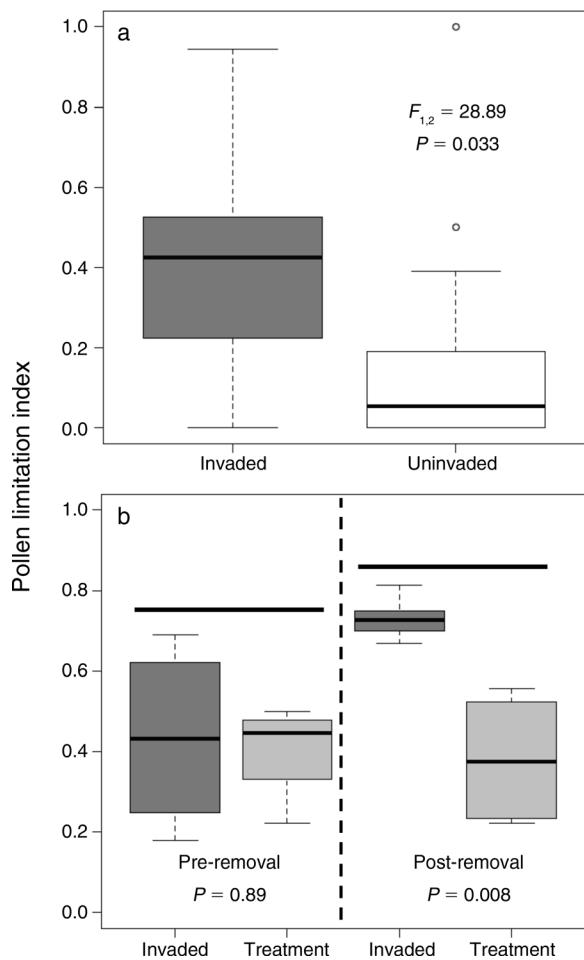


FIG. 3. (a) Pollen limitation indices (PLI) for island morning glory at sites invaded by and uninvaded by the Argentine ant in June 2012. F value and P value from a split-plot ANOVA are shown. (b) PLI at invaded and treatment sites before (June) and after (September) experimental Argentine ant removal. P values from Wilcoxon rank-sum tests are shown. See Fig. 1 for explanation of box plot components.

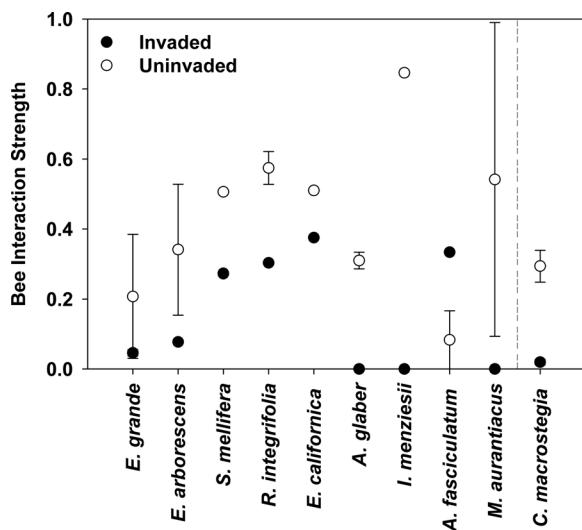


FIG. 4. Bee interaction strength (mean \pm SE) for each plant species for networks invaded by and uninvaded by the Argentine ant. Plant species included were species in bloom within both invaded and uninvaded plots. (See Appendix C for a list of all taxa included in floral visitation networks.) Plant species include *Eriogonum grande*, *Eriogonum arborescens*, *Salvia mellifera*, *Rhus integrifolia*, *Encelia californica*, *Acmispon glaber*, *Isocoma menziesii*, *Adenostoma fasciculatum*, and *Mimulus aurantiacus*. Values for *Calystegia macrostegia* are from floral visitation surveys.

higher interaction strength across surveyed plant species (0.36 ± 0.06) compared to those of bees (0.09 ± 0.03 ; all taxa combined) or beetles (0.07 ± 0.03 ; all taxa combined; Wilcoxon rank-sum tests, $P \leq 0.01$ in both cases). In networks without *L. humile*, in contrast, the interaction strength for native ants (0.26 ± 0.07 ; all taxa combined) did not differ from those of bees (0.22 ± 0.05 ; all taxa combined) or beetles (0.11 ± 0.04 ; all taxa combined; Wilcoxon rank-sum test, $P \geq 0.05$ in both cases). Moreover, in comparisons of plots with and without *L. humile*, invaded plots had fewer bee visits per plant (Wilcoxon rank-sum tests, $W = 25.5$, $P < 0.01$) and a lower bee interaction strength per plant (Fig. 4; Wilcoxon rank-sum test, $W = 26$, $P = 0.012$).

DISCUSSION

In this study, we employ large-scale, ant-removal experiments to test whether introduced ants negatively affect plant reproduction, and network analyses to gauge how visitation by introduced ants alters networks of interactions among plants and pollinators. Our results illustrate that introduced ants can negatively affect plant reproduction and potentially disrupt pollination services at an ecosystem scale. With respect to the island morning glory, floral visitation by the Argentine ant reduced floral visitor diversity, decreased rates of bee visitation, and increased levels of pollen limitation. Network analyses suggest that effects experienced by the island morning glory may extend to numerous other plant species. Networks from invaded and uninvaded

plots markedly differed; invaded plots had a lower species degree, a reduced diversity of plants visited by floral visitors, a higher per-plant interaction strength for ants relative to those of other visitors, and weaker interaction strengths between bees and plants.

To our knowledge, this study provides the first experimental evidence that floral visitation by introduced ants negatively affects plant reproduction. In past studies, removal of the Argentine ant from inflorescences did not affect seed set (Blancafort and Gomez 2005, Lach 2007, 2008). Removal of ants at the scale of individual inflorescences could obscure effects of ant visitation, because legitimate pollinators might avoid plants occupied by ants, even if ants are present on only some inflorescences (Irwin et al. 2001). More generally, the likelihood that floral visitation by introduced ants will negatively affect plant reproduction may depend on differences in floral geometry (which could affect the likelihood or outcome of interference), susceptibility to pollen limitation, variation in carbohydrate availability (which could influence ant behavior or local abundance), or differences in the taxonomic composition of the floral visitor assemblage (Lach 2003). In contrast to earlier studies (Blancafort and Gomez 2005, Lach 2007, 2008), small-bodied bees (mostly species in the Halictidae; Appendices B–C) were common in our study, and they appear sensitive to displacement from flowers (Appendix D). Although interference between *L. humile* and halictids was easily observed, our results do not allow us to isolate this behavior as the sole mechanism responsible for reduced pollinator visitation. Exploitation (Irwin et al. 2001) or modification (de Vega and Herrera 2013) of floral resources could also be responsible.

Native ants frequently visited the flowers of a variety of plant species in our study, and this observation is consistent with reports from other Mediterranean-climate ecosystems (Bosch et al. 1997, Blancafort and Gomez 2005, Lach 2007, 2008). Although no study that we are aware of compares the behavior of native ants and introduced ants with respect to how they interact with other floral visitors, the common ant species in our study strikingly differed in this respect. *Monomorium ergatogyna*, which was the most common native ant floral visitor in this study, is a tiny, slow moving species that seems unlikely to interact strongly with other floral visitors either directly (through interference) or indirectly (through exploitation of floral nectar). The Argentine ant in contrast is well known to displace other floral visitors (Blancafort and Gomez 2005, Lach 2007, 2008), perhaps due to its aggressive behavior, high levels of activity, or abundance. Like many other introduced ant species, the Argentine ant also exhibits strong preferences for carbohydrate resources, including nectar, which can elevate rates of colony growth and enhance traits related to colony performance (Kay et al. 2010, Wilder et al. 2011).

Network analyses revealed that the reductions in floral visitor diversity and bee visitation caused by *L. humile* on island morning glory could well extend more broadly to include numerous other plant species. Procrustes analysis, for example, revealed that congruence in network topology was tied to the presence or absence of *L. humile*. Moreover, residual vectors for visitor taxa, which measure the difference in interactions between networks, were largest for comparisons between invaded and uninvaded plots, suggesting that most visitor species altered their visitation patterns in response to the presence of *L. humile*. Indeed, visitor species established fewer links to plants within invaded networks. It seems likely that floral visitors may be avoiding plants on which *L. humile* is present (as appears to be the case for island morning glory). In essence, the Argentine ant has usurped interactions from other floral visitors, in particular bees (Fig. 4), and appears to be functioning as a central hub species in invaded networks. These results underscore the capacity for introduced floral visitors to restructure floral visitation networks (Aizen et al. 2008, Traveset et al. 2013) and suggest that future studies consider how plant reproduction may be compromised by such changes.

The effects of floral visitation by the Argentine ant could be exerting ecological and evolutionary pressure on a variety of plant species. Weakened interactions between bees and plants, in particular, would be expected to have profound consequences on plant reproduction (Bascompte et al. 2006), given that bees, compared to other floral visitors, transfer the majority of pollen at the community level (Alarcón 2010). Large-scale removal of the Argentine ant, however, led to an apparent and relatively rapid restoration of pollination services, at least for island morning glory. This finding may reflect the non-consumptive nature of most interactions between bees and the Argentine ant (Appendix D; Schmitz et al. 2008) or behavioral flexibility by pollinators following removal of an introduced species (see also Hanna et al. 2013). Given that removal areas were surrounded by habitat lacking *L. humile*, pollinators could readily immigrate into treatment areas. Enhancement of pollination services represents a potential conservation gain that could result from management or, if possible, eradication of introduced ants, such as *L. humile*.

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SUPPLEMENTAL MATERIAL

Ecological Archives

Appendices A–D are available online: <http://dx.doi.org/10.1890/14-0542.1.sm>