

Sampling across space and time to validate natural experiments: an example with ant invasions in Hawaii

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Abstract Measuring the impacts of invasive species on recipient biotic communities can be challenging because standard ecological field experiments may be impossible, undesirable or inaccurate due to limitations of scale. Many studies therefore rely on a comparative observational approach, in which invaded areas are compared to similar nearby uninvaded areas, but this approach suffers from reduced inferential power because of the inability to randomly assign treatments. When possible, the best solution may be to incorporate comparative sampling over time, from before and after invasion, as a means of validating comparisons across space. We used this approach to assess the impacts of Argentine and big-headed ants on arthropod communities at two natural area sites in the Hawaiian Islands. Two sampling events, separated by 2 years, indicated that invaded plots at each site were significantly different from uninvaded plots in terms of overall community composition and distributions of certain indicator species. This compositional change

associated with ant invasion was accompanied by a significant decrease in arthropod species richness at one site, but not at the second site. Changes in arthropod communities from before invasion to after invasion in separate before–after plots at each site were qualitatively consistent with the aforementioned changes measured across the invasion boundaries, but were smaller in magnitude because the invasion processes were still in early stages. Together, these findings support the inference that carefully planned studies of biological invasions that use a comparative observational approach can provide an accurate picture of resultant community level changes, and illustrate an effective method for validating such studies.

Keywords Biological invasions · Invasive ants · Experimental methods · Natural snapshot experiments · Before and after sampling · Hawaii

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Introduction

Obtaining accurate assessments of the ecological impacts of species invasions is an important, yet often challenging, objective. Ecological studies aiming to measure the effects of a particular species on its biotic community or ecosystem typically employ an approach in which the species is either added to or

removed from experimental plots (e.g. Connell 1961; Fagan and Hurd 1994; Schoener and Spiller 1996; Schmitz 1997; Denno et al. 2002; Taylor et al. 2006). When studying invasive species, however, this approach is frequently unavailable. Certain invasive species can be very difficult to remove from study plots, and the method of removal may unintentionally affect other species in the communities under study. Likewise, it is commonly unethical to introduce invasive species into randomly assigned plots, from which they were previously absent, as an experimental treatment. Studies examining the ecological effects of biological invasions therefore often rely on a comparative observational approach, so-called ‘natural snapshot experiments’ (Diamond 1986), in which invaded communities are compared to uninvaded communities in nearby or similar habitats, and the differences are inferred to be caused by the invasive species (Parker et al. 1999).

The inferential power of this comparative observational approach is necessarily lower than that of true, randomized treatment experiments (Diamond 1986), and this is a real weakness. However, the experimental approach often has its own shortcomings, perhaps the most important being that of questionable realism (Diamond 1986). In the case of the experimental introduction or removal of a species, if plots of differing treatments are too close together, migration of species between plots can obscure or weaken the dynamics of interest (Spiller and Schoener 1998) and lead to inaccurate or incorrect conclusions. Similarly, if plots are too small, migration of species into the plots can mask a true reduction induced by the treatment, while the failure of other species to colonize the small plots can result in a false interpretation that they do not benefit from the treatment. Finally, the effects of a species addition or deletion may take a fair amount of time to manifest themselves, especially those involving complicated indirect pathways, so that experiments typically running over relatively short time frames (Schoener 1983) may fail to assess the effects with accuracy.

Studying the ecological effects of invasive ants exemplifies many of the above difficulties. Eradicating invasive ants is extremely difficult once they become established (Tschinkel 1993), so introducing them for the purpose of an experimental treatment can be problematic. Removing existing ants from experimental plots without affecting other species is also difficult, although recent efforts have had notable

success in removing the majority of target ants with minimal nontarget effects (King and Tschinkel 2006; LeBrun et al. 2007). These latter studies, however, were necessarily limited by small spatial and temporal scales, and were therefore designed to investigate a relatively few interactions between ant species, not the full range of potential responses within arthropod communities. Because of these various constraints, by far the most commonly used method for examining broad-ranging community impacts of invasive ants has been to compare arthropod communities in invaded areas with those in adjacent or nearby uninvaded areas (e.g. Lubin 1984; Porter and Savignano 1990; Cole et al. 1992; Heterick 1997; Human and Gordon 1997; Holway 1998; Hoffmann et al. 1999; Bolger et al. 2000). Differences other than invasive ant presence between the invaded and uninvaded sampling locations, however, have the potential to confound results in these types of studies. This may be of particular concern in cases where ants have invaded from habitat edges, which is not uncommon (e.g. Heterick 1997; Human and Gordon 1997; Bolger et al. 2000).

Perhaps the best available approach, then, is to combine comparisons of communities from across invasion boundaries with comparisons of communities across time—samples collected in the same locations before and after invasion. This has been done to assess the effects of invasive ants on native ants at two sites in California (Sanders et al. 2003; Tillberg et al. 2007). Another study used this approach to measure the impacts of an invasive ant on the wider arthropod community at an Australian site (Hoffmann and Parr 2008), although conclusions for non-ant arthropod groups were limited somewhat by low taxonomic resolution and varying sampling methods over time. In studies related to the present one (Krushelnycky 2007; Krushelnycky and Gillespie 2008), we examined the impacts of invasive ants on arthropod species and communities at five sites in the Hawaiian Islands using a comparative observational approach. In the present study, we focused on two of these sites, where we conducted repeated sampling over time that allowed us to (1) evaluate the results of comparisons made across invasion boundaries in relation to those conducted before and after invasion, and (2) gain insight into the temporal dynamics of the invasion process and its ecological effects. This method of cross-validation may be more widely applicable in situations where invading organisms form a distinct invasion front.

Methods

Study sites

The system used in this and related studies to examine population and community level effects of ant invasion on resident arthropods was comprised of five middle to high elevation mesic shrubland or savannah sites on the islands of Maui and Hawaii. They are all described in detail elsewhere (Krushelnycky and Gillespie 2008). Here, we focused on only two of these sites, both on Hawaii Island, to conduct additional sampling in both space and time. The first site, Ahumoa, is a relatively open shrubland with dense grass and herb groundcover on top of a 14,000- to 65,000-year-old substrate (Wolfe and Morris 1996), situated at 1,880 m on the southwestern slope of Mauna Kea. The second site, Huluhulu, is located at 2,040 m elevation in a 1,500- to 3,000-year-old pahoehoe lava flow (Wolfe and Morris 1996) in the saddle area between Mauna Kea and Mauna Loa, and is characterized by relatively early successional open shrubland within mostly bare rock groundcover. Both sites are located in natural areas supporting predominantly native vegetation, although the plant communities differ between them.

Each site represented a broadly homogeneous habitat undergoing active invasion by an expanding unicolonial population of invasive ants. The ant population boundaries at our sites therefore mark the extents to which ants have invaded to date, and do not represent abiotic limits that might also affect other arthropods. Furthermore, at neither site were ants invading from a habitat edge. The Ahumoa site was being invaded by the big-headed ant (*Pheidole megacephala*), while the Huluhulu site was being invaded by the Argentine ant (*Linepithema humile*). These two species are among the most dominant invasive ants worldwide, and are believed to be primarily generalist predators and scavengers, but can also engage in extensive tending of honeydew-producing Hemiptera (Holway et al. 2002). Pre-existing data on ant population spread over time were available for the Ahumoa site (C. Farmer, unpublished data), and we closely monitored spread of the ant population boundaries at both sites for over a year prior to initial sampling. Habitat homogeneity within each site was judged according to consistency of vegetative community type and species composition, as well as the lack

of apparent changes in substrate type or levels of disturbance. Surrounding areas, while sometimes harboring colonies of several relatively inconspicuous ant species (*Cardiocondyla kagutsuchi* and *Hypoponera opaciceps*), were uninvaded by any dominant, unicolonial invasive ants (i.e. species that form large, continuous expanses of cooperating nests).

Sampling design

We established three sets of replicate plots at each site that would allow us to estimate the effects of ants on the recipient arthropod communities in two ways: by comparing community compositions in invaded plots with those of adjacent uninvaded plots, and by comparing community compositions before and after invasion in a third set of plots. Hereafter, we refer to plots behind the ant population boundaries that were always invaded as ‘invaded plots’, plots ahead of the ant boundaries that were never invaded as ‘uninvaded plots’, and plots installed immediately in front of ant boundaries that were expected to be invaded during the course of the study as ‘before–after plots’. We use terminology such as community composition ‘change’ as shorthand for compositional differences measured between different plot types at a single point in time, or between two points in time in before–after plots.

At each site, we mapped the ant population boundary at points spaced at haphazard intervals of roughly 40–100 m. From each of five such points, we measured behind the ant boundary a distance of 100 m at the Ahumoa site and 175 m at the Huluhulu site to establish the invaded plots; distances were further at the Huluhulu site due to faster rates of prior ant spread. At each of these five points at each site, we randomly selected a compass bearing, and walked to the nearest habitat patch that contained all of the dominant shrub species at the site (defined as the two or three most common shrub species). In each of these patches, we installed a 5 m by 5 m sampling plot that contained all of the dominant shrub species as well as some of the intervening grassland or bare ground habitat. Each of these five invaded plots was then matched with an uninvaded plot and a before–after plot, for a total of 15 plots per site. Uninvaded plots were located 120–175 m ahead of the expanding population boundary, while before–after plots were located 25–40 m ahead of the boundary. Before–after plots were therefore roughly equidistant

from invaded and uninvaded plots, and each set of three plots formed an approximate line perpendicular to the ant population boundary. Habitat patches for uninvaded and before–after plots were chosen such that the vegetative covers matched as closely as possible those of the invaded plots situated across the ant boundary; percent covers of dominant shrub species in uninvaded and before–after plots deviated from those in matched invaded plots by <15%.

We employed three standardized sampling techniques to quantify arthropod densities in each plot. First, we placed three pitfall traps (300 ml, 80 mm mouth diameter plastic cups half-filled with a 50:50 propylene glycol:water solution), separated by at least 2 m, in each plot, with one randomly chosen trap baited around the rim with blended fish and the other two unbaited. These traps were left open for 2 weeks. Second, in each plot we collected leaf litter from three different areas, mixed it together and removed 1 l of litter, and placed this in a Berlese funnel extractor for 24 h. Third, in each plot we beat the two dominant shrub species that supported the highest arthropod richnesses at that site (Krushelnycky 2007). These plant species were *Dubautia linearis* and *Dodonea viscosa* at the Ahumoa site, and *Leptecophylla tameiameia* and *Vaccinium reticulatum* at the Huluhulu site. Each plant species received five beats, spread among multiple individual plants in the plot if possible, and all arthropods were collected onto a 1 m by 1 m beating sheet and aspirated.

Because examining species-level variables and determining arthropod species identities were priorities of this study, the laborious specimen identification process made seasonal sampling at the sites unfeasible. Instead, we sampled on two occasions at each site, both during summer when arthropod abundances and diversities should have been highest, with each sampling event separated by 2 years: August 22 to September 7, 2002, and August 6 to August 20, 2004 for the Ahumoa site; and August 15 to August 30, 2003, and August 8 to August 22, 2005 for the Huluhulu site.

Arthropod identification

A total of 97,746 arthropods were collected during the two sampling events at the two study sites. With the exception of Acari (mites), Pseudococcidae (mealybugs), parasitic Hymenoptera, and immature

individuals of a number of taxa, all individual arthropods were identified as one of 248 species or morphospecies. The vast majority of morphospecies were identified to genus. These taxonomic identifications were made with the help of many systematists, taxonomists and other specialists (see “Acknowledgments”), allowing confidence that nearly all represent unique species-level taxa; in a very few cases of uncertainty, a conservative approach was taken and taxa were grouped. All taxa are referred to hereafter as species. The species were assigned as either endemic, introduced to the Hawaiian Islands, or of unknown provenance according to Nishida (2002), other literature and the knowledge of specialists. Representative specimens are deposited at the Bernice P. Bishop Museum, the Essig Museum of Entomology, the University of Hawaii Insect Museum and the Haleakala National Park Insect Collection.

For the purpose of characterizing community composition in each plot, immatures of many taxa could be matched to adults, though for others, only determination of genus, family, or sometimes order could be made definitively. In most of the latter cases, immatures within a known taxon were assigned to species according to the relative abundances of adults within that taxon. For example, if three species of *Nysius* seed bugs (Hemiptera: Lygaeidae) occurred in a plot, numbers of immature *Nysius* in that plot were allocated to these three species according to the proportional representation of the adults in that plot. In the few cases where immatures could be identified only to an order or to a family with many species (e.g. some Lepidoptera, Coleoptera and Araneae), these individuals were excluded from analyses. The unidentified Acari, Pseudococcidae and parasitic Hymenoptera were each treated as a single species of unknown provenance.

Compositional similarity and change

All samples collected in each plot during a given sampling event (i.e. for each year) were pooled to produce a single multivariate dataset of species abundances for the plot. Overall compositional differences between plots (excluding the invading ants themselves) were assessed using Bray–Curtis distances calculated with species abundance data that were log transformed and standardized by plot

abundance totals. This measure considers the cumulative total of proportional differences in species abundances and identities. For each site and sampling event, we used hierarchical agglomerative cluster analysis to group plots according to overall compositional affinity. We used the above-described Bray–Curtis distances as the distance measure, and a flexible beta linkage method with β set to -0.25 , which yields results similar to Ward’s linkage method (McCune and Grace 2002). We also used multi-response permutation procedures (MRPP) to test whether community compositions were significantly different among plot types (invaded, uninvaded, before–after) at each site, and within each sampling event, based on inter-plot Bray–Curtis distances. MRPP is a non-parametric permutation method that tests the probability of a difference between groups of multivariate data against the null hypothesis of no difference between the groups (Mielke and Berry 2001). In addition to calculating the test statistic (T), MRPP provides an estimate of the effect size (A), the “chance-corrected within-group agreement”. A scores equal 1 when all species identities and abundances are identical within groups, equal 0 when within-group agreement is random, and are negative when within-group agreement is less than that expected by chance. The combined cluster and MRPP analyses therefore indicated the degree to which before–after plots became more similar to invaded plots and less similar to uninvaded plots after they were invaded.

We used two-sample t -tests to evaluate differences in endemic, introduced and overall species richness between plot types at each site. For each year, we compared richness between the five replicate invaded and five replicate uninvaded plots. In addition, we compared before samples with after samples in the five before–after plots at each site, adjusting for temporal changes unrelated to ant invasion. This adjustment was performed by subtracting the mean richness in uninvaded plots from the richness of each of the temporally corresponding before and after samples at the site (e.g. the adjusted richness for a before sample at Ahumoa site was calculated as the richness in that particular 2002 before–after plot minus the mean richness in 2002 uninvaded plots, while the adjusted richness after invasion was calculated as the 2004 richness in the same before–after plot minus the mean richness in 2004 uninvaded plots).

Indicator species analysis

Indicator species analysis is a method for identifying species that are tightly associated with a particular habitat, environmental condition, or group of samples (McCune and Grace 2002). It identifies taxa that maximize two qualities: the species should occur frequently in a particular group of samples and should occur nearly exclusively in that group of samples. An observed indicator value (from 0 to 100) is calculated for each species, with a perfect score of 100 indicating that a species occurred in every sample of one group and no samples of the alternate group. The probability of obtaining the observed value under the null hypothesis of no association is then calculated with a randomization procedure. We used indicator species analysis to find species that occurred commonly and predominantly in either uninvaded or invaded plots at each site, and that therefore appeared to be strongly affected by the invasion process. We made several comparisons. First, for each sampling event at each site, we compared compositions in uninvaded plots with those in invaded plots to identify species that were strongly associated with either group of plots. Second, for each sampling event, we compared compositions in uninvaded plots with those in before–after plots, and identified those species that were tightly associated with either group of plots in the second sampling event but not in the first. This singled out species that shifted their distributions over time such that they were similarly abundant in both groups of plots before invasion, but became either much less or much more abundant in before–after plots, relative to uninvaded plots, after invasion. This approach is preferable to simply comparing compositions in before–after plots between the two sampling events because of strong inter-year variation in species abundances that was unrelated to ant invasion. We used 5,000 permutations in the Monte Carlo randomization procedure to test for statistical significance of indicator species status for each comparison.

For all of the analyses in this study, sample size was limited to five replicate plots within a given group, and thus statistical power was low. We therefore did not adjust α for groups of multiple tests because we felt such a correction would be overly conservative. Instead, we focused on general trends and assumed that up to 5% of comparisons within groups of related tests may falsely reject the null hypothesis (however, P values are provided for all statistical tests). All

univariate statistical analyses were conducted in Minitab (Ryan et al. 2005), and all multivariate analyses were conducted in PC-ORD (McCune and Mefford 2006).

Results

Patterns of invasion

As observed in the pre-sampling monitoring period, ants invaded faster at the Huluhulu site than at the Ahumoa site over the course of the study (Table 1). One year after establishing the plots and completing the first sampling event, Argentine ants had invaded and spread beyond all five before–after plots at the Huluhulu site. Big-headed ants reached four of five before–after plots at the Ahumoa site after 1 year, but appeared to be very scarce in most of these and could not be found far beyond the plots. Two years after establishing the plots, at the time of the second sampling event, all five before–after plots were invaded at both sites, but ant densities were proportionately higher at the Huluhulu site. Ant densities in before–after plots had reached 25.6% of those in invaded plots by the second sampling event at the Huluhulu site, whereas they had only reached 10.7% of those in invaded plots by the second sampling event at the Ahumoa site (Table 1).

Compositional similarity and change

The compositional similarities between individual plots can be portrayed with dendrograms that display

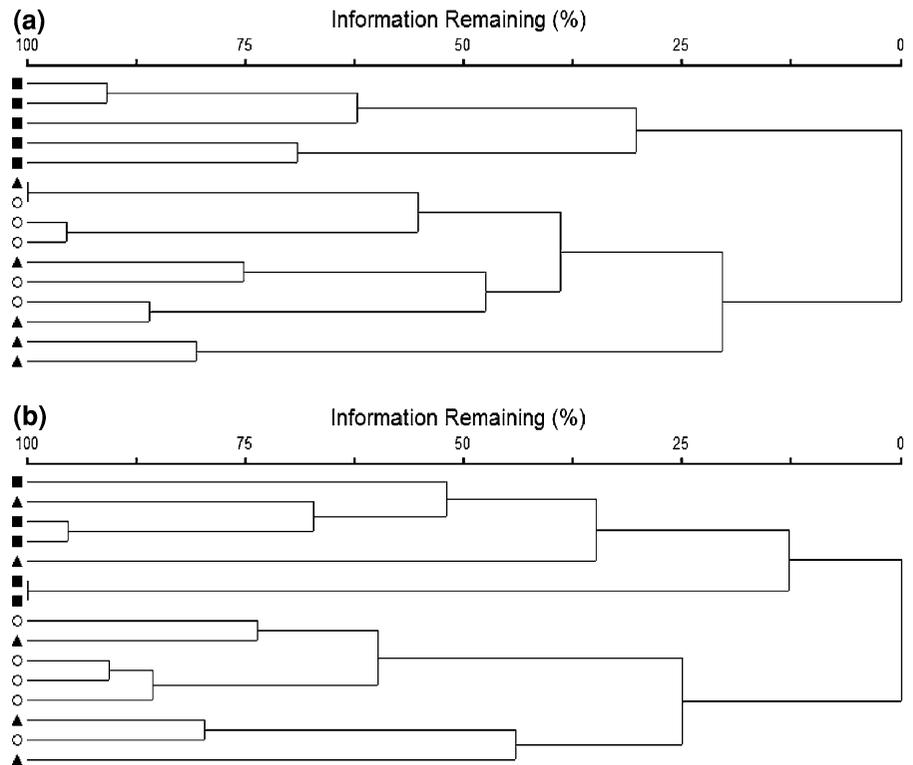
the grouping schemes produced by hierarchical agglomerative cluster analysis (Figs. 1, 2). Cluster analysis suggests that at the Huluhulu site in 2003, invaded plots had community compositions that were similar to each other, grouping together into one main cluster, while uninvaded plots and before–after plots grouped together into a second main cluster (Fig. 1a). Individual uninvaded and before–after plots were interspersed within this second cluster, indicating that compositions in before–after plots prior to invasion were similar to those of uninvaded plots. Two years later, after ants invaded the before–after plots, all invaded plots still clustered separately from all uninvaded plots, but before–after plots were now split between the two clusters (Fig. 1b). This suggests that by the time of the second sampling event, before–after plots at the Huluhulu site had begun the transition from communities characteristic of uninvaded areas to those characteristic of invaded areas. Evidence of this transition was less apparent in the dendrograms for the Ahumoa site. In 2002, all invaded plots clustered together, but an uninvaded plot and a before–after plot also grouped with this invaded cluster (Fig. 2a). The remaining uninvaded and before–after plots were interspersed in a second cluster, as at the Huluhulu site. Two years later, invaded and uninvaded plots were completely segregated, but none of the before–after plots (now invaded) clustered with the invaded plots (Fig. 2b). However, the before–after plots were now less thoroughly interspersed with uninvaded plots, with three before–after plots forming a minor cluster (Fig. 2b), providing some evidence that these before–after plots were starting to differentiate from uninvaded plots.

Table 1 Changes in ant densities, and progression of ant invasion in before–after plots, at each site over the course of the study

Site/year	Mean ant density (\pm SE)		Before–after plots	
	Invaded plots	Before–after plots	Plots invaded (%)	Invaded plot density (%) ^a
Huluhulu				
2003	346.6 (\pm 90.6)	0	0	0
2004	–	–	100	–
2005	268.2 (\pm 38.3)	68.6 (\pm 10.2)	100	25.6
Ahumoa				
2002	817.6 (\pm 142.2)	0	0	0
2003	–	–	80	–
2004	860.2 (\pm 175.3)	92.2 (\pm 43.3)	100	10.7

^a The density of ants in before–after plots, as a percent of ant density in invaded plots at the site, during a given year

Fig. 1 Dendrogram of hierarchical agglomerative cluster analysis of plot similarity among the 15 plots at Huluhulu site for the **a** 2003 sampling event, and **b** 2005 sampling event. Symbols indicate plot types: “■” invaded plot, “○” uninvaded plot, “▲” before–after plot



The MRPP analyses yielded similar results. At both sites, community compositions in invaded plots, as a group, were always significantly different from those of uninvaded plots (Table 2). Prior to invasion, communities in before–after plots at both sites were significantly different from those of invaded plots but were not significantly different from those of uninvaded plots. After invasion, before–after plots at the Huluhulu site became significantly different from uninvaded plots, and not significantly different from invaded plots. Concurrently, A , which is a measure of the degree of differentiation between two groups, decreased from the first to the second sampling event for comparisons of before–after plots with invaded plots, and increased over the same period for comparisons of before–after plots with uninvaded plots (Table 2). These trends indicate partial transition of communities in the Huluhulu before–after plots from an uninvaded character to an invaded character. At the Ahumoa site, however, before–after plots failed to become significantly different from uninvaded plots, and remained significantly different from invaded plots, after they were invaded (Table 2). As in the cluster analysis, though, there

was some evidence of community transition associated with ant invasion: while A did not decrease as expected from the first to second sampling event for comparisons of before–after plots with invaded plots, it did increase substantially for comparisons of before–after plots with uninvaded plots. This again suggests that before–after plots had begun to differentiate from uninvaded plots.

Ant invasion was always associated with declines in mean plot richness at the Huluhulu site (Fig. 3). Changes in richness calculated by comparing invaded plots with uninvaded plots were very similar for 2003 and 2005, with an average decrease of 4–5 endemic species, 5–6 introduced species, and 11–12 species overall per plot. These differences between invaded and uninvaded plots were all statistically significant. The changes in richness from before invasion to after invasion in before–after plots were qualitatively identical to those measured between invaded and uninvaded plots in 2003 and 2005, but in magnitude they were smaller by roughly half and were not statistically significant. At the Ahumoa site, ant invasion was associated with small and statistically non-significant changes, across both space and time,

Fig. 2 Dendrogram of hierarchical agglomerative cluster analysis of plot similarity among the 15 plots at Ahumoa site for the **a** 2002 sampling event, and **b** 2004 sampling event. Symbols as in Fig. 1

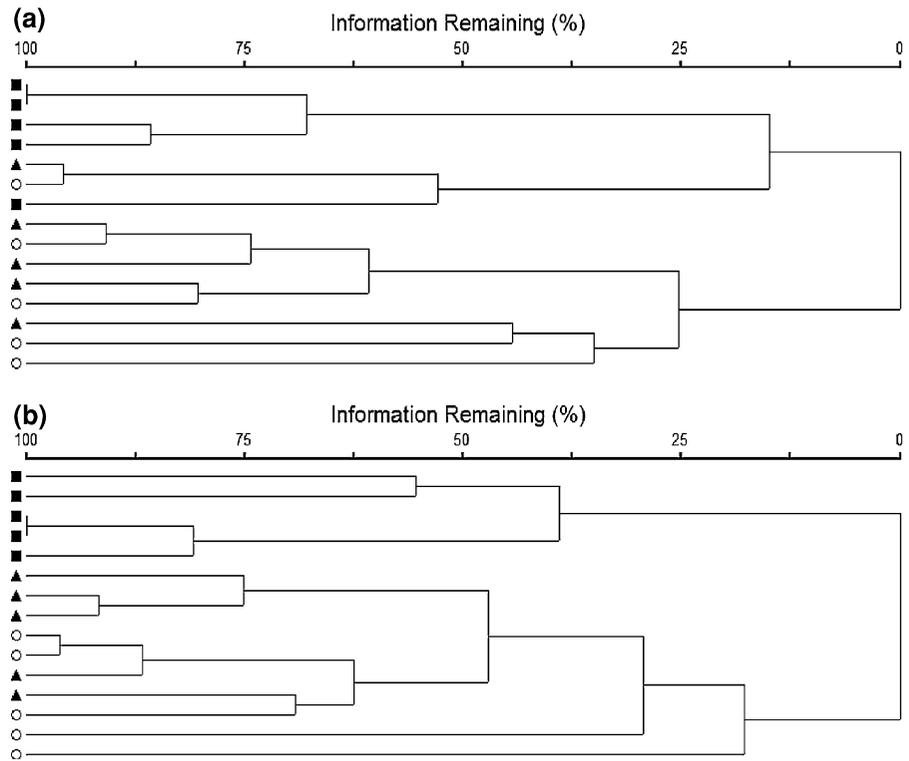


Table 2 Results of MRPP analyses comparing community composition between the different plot types at each site and for each sampling event

Comparison	Huluhulu 2003			Huluhulu 2005			Ahumoa 2002			Ahumoa 2004		
	A	T	P	A	T	P	A	T	P	A	T	P
Inv versus unin	0.113	-4.46	0.002	0.124	-5.16	0.001	0.073	-4.10	0.002	0.113	-5.08	0.002
Inv versus before-after	0.128	-4.84	0.002	0.021	-1.41	0.087	0.092	-4.27	0.003	0.121	-5.23	0.001
Uninv versus before-after	0.013	-0.78	0.210	0.026	-1.85	0.040	0.004	-0.31	0.356	0.013	-1.04	0.150

Presented for each comparison are the chance-corrected within-group agreement (*A*, a measure of effect size), the test statistic for the analysis (*T*), and *P*, the probability of incorrectly rejecting the null hypothesis that there was no difference in community composition between plots of different types. Plot types: *inv* invaded plots, *uninv* uninvaded plots, *before-after* before-after plots

in mean plot richness (Fig. 3). Changes in richness calculated by comparing invaded plots with uninvaded plots varied slightly between 2002 and 2004, but in both cases all changes were on the order of less than one to slightly greater than two species per plot. Changes in richness from before invasion to after invasion in before-after plots were similar in magnitude.

Indicator species analysis

At the Huluhulu site, eight species in six orders were tightly associated with uninvaded plots when

compared to invaded plots in either 2003 or 2005 (Table 3a), suggesting that they were highly vulnerable to invasive ants. Two of these species were identified in both years. An additional three species in two orders were strongly associated with invaded plots in either the first or second sampling event, suggesting that while they were normally relatively uncommon at this site, they became more widespread and usually more abundant in the presence of ants. None of the species at the Huluhulu site were strongly associated with invaded plots in both years. When uninvaded plots were compared with before-after plots in the second sampling event (2005, after invasion), two

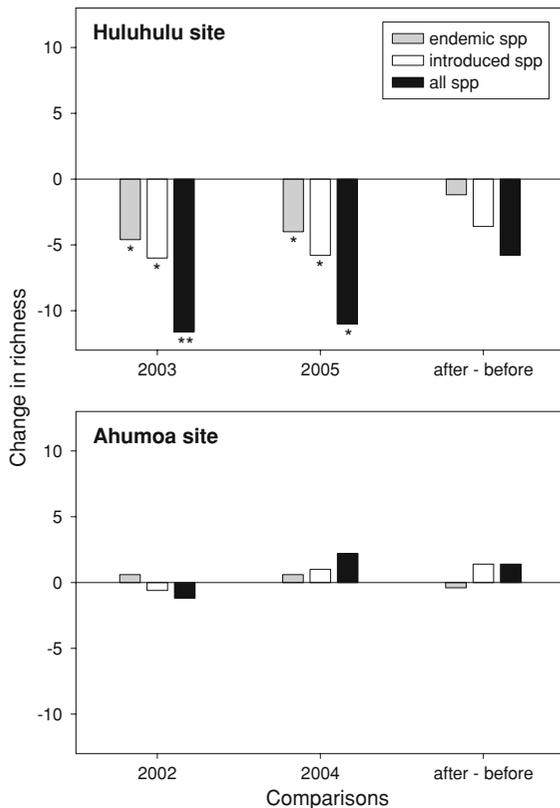


Fig. 3 Changes in mean plot richness resulting from ant invasion at the two sites. The first two groups of bars in each panel show changes in endemic, introduced and overall richness between invaded and uninvaded plots for each year indicated, and were calculated as mean richness in invaded plots minus mean richness in uninvaded plots. The third group of bars shows changes in richness from before invasion to after invasion in before–after plots, and were calculated as mean richness after invasion minus mean richness before invasion, adjusted for temporal changes in richness unrelated to invasion (see text). Significant differences in richness between groups of plots used to calculate each change are indicated (t -test, $n = 5$ for each, * $P < 0.05$, ** $P < 0.01$)

species were found to be associated with uninvaded plots while no species were strongly associated with the newly invaded before–after plots (Table 3a). Both of these species were also identified as indicators in the first set of comparisons between invaded and uninvaded plots, and neither exhibited this same skewed distribution between uninvaded and before–after plots in the first sampling event (2003, prior to invasion). This temporally changing distributional pattern strongly suggests that these two species declined in frequency and abundance in before–after plots in response to ant invasion.

At the Ahumoa site, nine species, most of which were introduced, in six orders were tightly associated with uninvaded plots when compared to invaded plots in either 2002 or 2004 (Table 3b), suggesting that they were highly vulnerable to invasive ants. Two species were again identified in both years. One of these species, the theridiid spider *Achaearanea* cf. *riparia*, appeared to respond to ant invasion in an opposite fashion at the two sites; inconsistent responses to ant invasion was fairly common for species in this study system in general (Krushelnycky 2007). In contrast to the Huluhulu site, six species in four orders, including two endemic species, were strongly associated with invaded plots at the Ahumoa site, suggesting that more species benefited from ant invasion there. One species, *Ectopsocus vachoni* (Psocoptera: Ectopsocidae), exhibited this pattern in both years. When uninvaded plots were compared with before–after plots for the second sampling event (2004, after invasion), two species were found to be associated with uninvaded plots and one species was associated with the newly invaded before–after plots. All three of these species were identified as indicators in the first set of comparisons, and none of them exhibited this same skewed distribution between uninvaded and before–after plots in the first sampling event (2002, prior to invasion). This strongly suggests that these three species shifted abundances in before–after plots in response to ant invasion.

Discussion

Repeat sampling at two sites, each being invaded by a different ant species, confirmed that invasive ants can cause significant changes in arthropod community composition in the Hawaiian Islands. Comparisons of communities from before and after invasion at these two sites were qualitatively consistent with multiple comparisons across the invasion boundaries, but were smaller in magnitude because the invasion processes were still in early stages. The sampling methods and analyses employed also revealed the rate at which these changes occurred.

Arthropod communities at both sites experienced shifts in species incidences and abundances between sampling years that were unrelated to ant invasion. For example, indicator species that were both relatively common and tightly associated with either

Table 3 Indicator species associated with ant invasion at (a) Huluhulu site, and (b) Ahumoa site

Indicator species for uninvaded plots		Indicator species for invaded plots	
Species ^a	Sig. ^b	Species	Sig.
<i>(a) Huluhulu</i>			
1. Uninvaded versus invaded plots (2003 and/or 2005)			
Araneae: ⁺ <i>Havaika</i> sp. 1	*	Araneae: <i>Achaearanea</i> cf. <i>riparia</i>	*
Collembola: ⁺ <i>Bourlettiela insula</i>	* *	Collembola: <i>Entomobrya multifasciata</i>	*
Diptera: <i>Helicobia morionella</i>	**	[‡] <i>Entomobrya</i> sp. 2	*
⁺ <i>Megaselia</i> sp.	***, **		
<i>Pollenia rudis</i>	*		
Hemiptera: <i>Aphis gossipyi</i>	**		
Lepidoptera: [‡] Noctuidae immatures ^c	*		
Thysanoptera: ⁺ <i>Haplothrips davisi</i>	**		
2. 2005 uninvaded versus 2005 before–after plots (and not identified in 2003 comparison)			
Diptera: <i>Helicobia morionella</i>	*	None	
⁺ <i>Megaselia</i> sp.	*		
<i>(b) Ahumoa</i>			
1. Uninvaded versus invaded plots (2002 and/or 2004)			
Araneae: <i>Achaearanea</i> cf. <i>riparia</i>	***, **	Araneae: <i>Meriola arcifera</i>	*
Collembola: <i>Folsomides angularis</i>	*	<i>Steotoda grossa</i>	**
Diptera: <i>Bradysia</i> sp. nr. <i>impatiens</i>	*	Coleoptera: <i>Pantomerus cervinus</i>	*
⁺ <i>Megaselia</i> sp.	*	Hemiptera: ⁺ <i>Emoloana eragrosticola</i>	* *
Hemiptera: <i>Nysius palor</i>	**	Psocoptera: <i>Ectopsocus vachoni</i>	***, **
Hymenoptera: <i>Cardiocondyla kagutsuchi</i>	**	⁺ <i>Ptycta distinguenda</i>	* *
Psocoptera: <i>Cerobasis guestfalica</i>	*		
<i>Lepinotus reticulatus</i>	*		
[‡] <i>Liposcelis</i> sp.	* *		
2. 2004 uninvaded versus 2004 before–after plots (and not identified in 2002 comparison)			
Diptera: <i>Bradysia</i> sp. nr. <i>impatiens</i>	*	Psocoptera: <i>Ectopsocus vachoni</i>	*
⁺ <i>Megaselia</i> sp.	*		

Species associated with uninvaded plots in each comparison were vulnerable to ant invasion, while species associated with invaded plots benefited from invasion. Two types of comparisons were made: (1) identifies species associated with uninvaded or invaded plots in either the first, second or both sampling events; (2) species associated with uninvaded plots or before–after plots in the second sampling event but not in the first sampling event (see text for explanation). Only species that were statistically significant indicators in at least one comparison are listed

^a Endemic species indicated with (+), introduced species have no symbol, species of unknown provenance indicated with (‡)

^b Significance of indicator species at * $P < 0.05$ or ** $P < 0.01$. For species found to be significant indicators in invaded versus uninvaded plot comparisons in both years, level of significance is indicated for both, separated by a comma. The remaining species were found to be significant indicators in only one of the 2 years

^c Most likely caterpillars of an endemic *Agrotis* sp. or spp.

uninvaded or invaded areas at each site showed only partial overlap between sampling years (Table 3). Despite these natural species shifts, overall composition was highly significantly different between invaded and uninvaded plots for both sampling years at both sites (Table 2). Likewise, differences in richness between invaded and uninvaded plots were

similar between sampling years at both sites (Fig. 3). At the Huluhulu site, invaded by Argentine ants, these changes in richness signalled declines among both endemic and introduced species, which supports previous assessments of the impacts of invasive ants on Hawaiian arthropods (e.g. Cole et al. 1992; Gillespie and Reimer 1993; LaPolla et al. 2000). In

contrast, the compositional changes at the Ahumoa site, invaded by big-headed ants, translated into only very small and statistically insignificant changes in richness in both 2002 and 2004. Although each site was invaded by a different ant species, all evidence suggests that this discrepancy in impact on richness resulted from differences in community composition between the sites, rather than from differences between the species of ant. For example, the Argentine ant and big-headed ant demonstrated very similar behavior towards common endemic Hemiptera species in laboratory trials, but the net effects on these species in the field depended on the composition of the rest of the arthropod community (Krushelnycky 2007). Moreover, the magnitude of impact on endemic richness in this study system was most strongly related to the degree to which endemic species in each community had already been lost prior to ant invasion (Krushelnycky and Gillespie 2008). Even though certain species shifted abundances (Table 3) and overall composition changed due to big-headed ant invasion, the most vulnerable species that are likely to completely disappear when ants invade (and hence drive changes in richness) were already missing at the Ahumoa site (Krushelnycky and Gillespie 2008).

Over the 2 year sampling period, compositional change in the newly invaded before–after plots progressed somewhat differently at the two sites, reflecting the differences in rate of invasion. At the Huluhulu site, Argentine ants completely invaded all five before–after plots within 1 year, and reached densities that were 25% of those in invaded plots after 2 years. Prior to invasion, community compositions in before–after plots were not significantly different from those in uninvaded plots, but were significantly different from those in invaded plots (Table 2). Two years later, after invasion, these affinities were reversed as communities in before–after plots began to change. This reversal was weak, undoubtedly because ant densities were still relatively low. In comparison, big-headed ants at the Ahumoa site had gained only a weak foothold in four of the five before–after plots after 1 year, and attained densities that were only 10% of those in invaded plots after 2 years. Consequently, the transition of community composition from an uninvaded character to an invaded character in before–after plots had barely begun 2 years after the initial sampling event.

The partial transitions of communities in the before–after plots should be attended by comparable

changes in aggregate measures, such as species richness, as well as by changes in abundances of individual species. As stated above, species richness was not very different between invaded and uninvaded plots in either 2002 or 2004 at the Ahumoa site, so changes in richness from before to after invasion in before–after plots were also small (Fig. 3). At the Huluhulu site, where richness was significantly lower in invaded plots relative to uninvaded plots in both sampling years, declines in richness over time in before–after plots were qualitatively identical but roughly half as large in magnitude (Fig. 3), and therefore exactly matched expected patterns under a scenario of partial transition.

Other analyses have shown that rare species in these communities, especially endemic ones, are particularly sensitive as a group to ant invasion (Krushelnycky 2007). It follows that rare species likely accounted for a substantial portion of the compositional changes in before–after plots. But it is difficult to demonstrate a statistically significant difference in distribution, and therefore an effect of invasion, for any given rare species. Instead, distributional patterns of certain more common species, such as those identified through indicator species analysis, can fill out part of the picture of invasion-related compositional change. At both sites, a wide taxonomic range of arthropod species was found to be tightly associated with either uninvaded plots, suggesting vulnerability to ants, or invaded plots, suggesting direct or indirect benefit from ants (Table 3). For example, shrub inhabiting endemic species at the Ahumoa site, such as the psocid *Ptycta distinguenda*, probably benefited because invading ants severely reduced numbers of introduced spiders, like *A. cf. riparia*, on shrubs (Krushelnycky 2007). When compositions in uninvaded plots were compared with those in newly invaded before–after plots at each site, much smaller subsets of indicator species were identified (Table 3). This suggests that of the species that appeared to respond either positively or negatively to ant invasion, a select few did so faster and at lower ant densities than the others.

Three fly species exhibited this early decline at the two sites, with one, an endemic phorid fly (*Megaselia* sp.), singled out at both sites. In fact, *Megaselia* sp. was strongly associated with uninvaded plots in nearly every comparison performed (Table 3). Flies might be expected to be less prone to show such

skewed distributions, particularly strong fliers like the sarcophagid *Helicobia morionella* that could easily fly into invaded areas from uninvaded areas. Ants probably heavily impact larval stages of many flies, though, and these patterns suggest that the adults of at least these three species are restricted to relatively small territories. Only one species, the introduced ground and litter inhabiting psocid *E. vachoni* at the Ahumoa site, demonstrated a fast positive response in newly invaded before–after plots. Of course, the indicator species identified in Table 3 represent only a fraction of the species affected by ant invasion. Many others could not be singled out statistically because of their rarity, and others were affected less dramatically than those identified through indicator species analysis. Even so, the observed patterns suggest that ant invasion and its attendant impacts proceeded relatively slowly at these sites. At two sites invaded by the Argentine ant in California, significant changes in the native ant fauna were observed within 1 year (Sanders et al. 2003; Tillberg et al. 2007), perhaps indicating that interactions among competing ants are more intense than those between invasive ants and many other arthropod species.

In conclusion, the comparisons of communities from before and after invasion at these two sites were consistent with the multiple comparisons of communities across the invasion boundaries. They yielded results that provided qualitatively similar assessments of changes in overall community composition, species richness, and abundances of individual species, due to ant invasion. The changes measured with the two types of comparisons, however, differed substantially in magnitude. This is to be expected, since the invasion process was still in its early stages in the before–after plots. Overall, these results support the inference that natural experiment studies that use a comparative observational approach can provide an accurate picture of community level changes caused by an invading species, provided that they are carefully planned to avoid or control for gradients in biotic and abiotic factors and other edge effects. This study also illustrates an effective method for cross-validating such comparisons. Finally, this study highlights the importance of allowing sufficient time to elapse when using this approach. The combined results suggest that after an interval of between one and 2 years of ant presence, arthropod communities had only undergone partial changes at these two sites.

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