

MAINTENANCE OF FUNCTIONAL ANT DIVERSITY IN A COFFEE
AGROECOSYSTEM: DISTURBANCE, HABITAT COMPLEXITY, AND
IMPLICATIONS FOR SPATIAL PATTERN

by

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Abstract

Numerous mechanisms promoting assembly and maintenance of diversity have been identified, from disturbance to habitat heterogeneity to structure of species interaction networks. Understanding which mechanisms are operating and how they are interacting in the field is crucial for conserving functional diversity and important ecosystem services. This is particularly true in managed ecosystems, such as agroecosystems, where effective biocontrol often relies on associated biodiversity such as insect predators and parasitoids. Here we examined the impact of habitat complexity on the response of an arboreal foraging ant community to disturbance in a coffee agroecosystem in Chiapas, Mexico. The primary disturbance in this system is driven by an entomopathogenic fungus (*Lecanicillium lecanii*) infecting the green coffee scale (*Coccus viridis*), an important food resource for its mutualist partner, *Azteca instabilis*, a dominant ant species. We hypothesize the disruption of this mutualism forces a shift in foraging of the dominant competitor and thus has cascading effects on the arboreal foraging ant community. Furthermore, we hypothesize that increasing habitat complexity, in this case shade tree density, provides refugia and alternative foraging resources for the keystone dominant thereby encouraging a resource shift, which in turn facilitates transmission of the disturbance to the arboreal ant community. To test these hypotheses, we induced an artificial fungal epizootic in four experimental sites by spraying a suspended *L. lecanii* spore mixture on coffee bushes surrounding *A. instabilis* nest-sites in which the ant/scale mutualism was strong. Surveys of activity of all arboreal foraging ant species present were undertaken before and after the epizootic. These surveys were undertaken in both coffee bushes and shade trees within the experimental plots and were compared to

identical analyses undertaken in a control site.

We found a significant shift in foraging activity of *A. instabilis* in two of four experimental sites after the artificial epizootic. This response was correlated with shade tree density; at high tree density, we found a significant decrease in *A. instabilis* foraging activity in coffee bushes and corresponding increase in foraging activity in shade trees. Additionally, we found an increase in foraging activity of other species of ants correlated with the shift in *A. instabilis* foraging. These results suggest that increasing habitat complexity allowed *A. instabilis* to respond to disturbance and the resulting change in foraging location of the dominant competitor opened niche space for other arboreal foraging ants, promoting maintenance of functional ant diversity. These results provide insight into how complex interactions can drive spatial patterns of species distribution, and have implications for shade management as a means of promoting predatory ant diversity and thus biocontrol of coffee pests.

Keywords: *Lecanicillium lecanii*; epizootic; *Azteca instabilis*; self-organization; coffee agroecosystem; biological control

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Introduction

Functional biodiversity in both terrestrial and aquatic systems provides for and promotes important ecosystem services, from nutrient cycling to pest control (Altieri 1999, Stachowicz et al. 2002, Tylianakis et al. 2008, Duffy 2009, Simon et al. 2009, Woodward 2009). This is particularly true of associated biodiversity in agroecosystems, which provides pollination services, biological control through autonomous ecosystem function, and other beneficial services (Altieri 1999, Perfecto et al. 2004, Bianchi et al. 2006, Perfecto and Vandermeer 2006, Philpott and Armbrrecht 2006, Brosi et al. 2008, Livingston et al. 2008, Vandermeer et al. 2010). For example, Philpott and Armbrrecht (2006) discuss how protection of associated biodiversity, particularly ant diversity, promotes biological control of pests in tropical agroforestry systems. Ants can aid in the management and prevention of pests in agroforestry systems by acting as predators, chemically deterring herbivores, or causing pests to drop from plants in which they are foraging (Way and Khoo 1992, Perfecto and Castiñeiras 1998).

Ants in tropical systems can be predators, scavengers, and generalist foragers (Hölldobler and Wilson 1990). They also display a diverse array of foraging and nesting strategies from predominantly arboreal to predominantly below ground. This diversity and spatial distribution, from below ground to the canopy, allows ants to be potential biocontrol agents of a diversity of pests with different life histories and at different life stages. For instance, the coffee berry borer (*Hypothenemus hampei*), which drills into the berry and oviposits in the seed, where it is largely unreachable by conventional pesticides, is preyed upon by hemipteran-tending ants when drilling and by twig-nesting ants that can follow the drilled canal into the berry (Perfecto and Vandermeer 2006,

Larsen and Philpott 2009). Once the berry falls to the ground, a variety of ground-foraging ants prey on them as well (Gallego Ropero and Armbrrecht 2005). Given these and other important services provided by the ant community in agroecosystems, it is important to understand the mechanisms and processes by which a diverse ant community assembles and is maintained.

Assembly and maintenance of diversity result from numerous processes and underlying biotic and abiotic conditions. Examples include disturbance (Connell 1978), trophic complexity (Paine 1969), spatial pattern and self-organization (Chesson 2000, Perfecto and Vandermeer 2008), structure of species-interaction networks (Paine 1966, Hastings 1988, Bascompte and Jordano 2007), and habitat complexity (Caley and St John 1996, Hansen 2000), among others. While many of these factors have been posited in isolation, the reality in the field is likely a much more complex suite of interacting mechanisms. Here we report on the demonstrated importance of the interaction between disturbance and habitat complexity, as well as the potential broader implications for pattern and spatial self-organization, in promoting the assembly and maintenance of functional biodiversity in a coffee agroecosystem.

Connell's (1978) classic work illustrated the importance of disturbance as a mechanism for maintaining diversity in both tropical forests and coral reefs. In the absence of such intermediate disturbances, communities will tend toward equilibrium and lower species diversity due to interspecific competition and competitive exclusion (Connell 1978). However, to prevent loss of species from the community as a result of disturbance, there often must exist some mechanism or abiotic factor allowing for the community to respond and adapt to the disturbance. For example, landscape structure

and habitat complexity promote maintenance of diversity in systems ranging from tropical coral reefs (Caley and St John 1996) to temperate forests (Verschuyl et al. 2008). Structurally complex landscapes can provide more niche space for differentiation and specialization (MacArthur 1972, Ziv 1998, Ribas and Schoereder 2002). Furthermore, and perhaps more importantly in considering disturbance, complex landscapes can provide refugia from predation, parasitism (Murdoch et al. 1989), epidemic disease, and other disturbances.

The local interaction of these two factors, disturbance and habitat complexity, has potential implications for observed, landscape level spatial pattern in our study system (Perfecto and Vandermeer 2008, Vandermeer et al. 2008). In particular, our localized experimental results may be a signal that spatial self-organization is operating in this system to promote species diversity and community stability (Vandermeer et al. 2008).

While these three mechanisms -- disturbance, habitat complexity, and spatial self-organization -- are typically studied separately, in our study system these mechanisms may act in concert to promote diversity. In tropical coffee agroecosystems, an arboreal-nesting ant, *Azteca instabilis*, forms a mutualism with the green coffee scale, *Coccus viridis*, in which the ant protects the scale from predators and parasites, and the scale provides a nutrient rich secretion in return (Vandermeer et al. 2002). The spatial distribution of the *A. instabilis* ant nests (Fig. 1) is thought to arise through a process of self-organization characterized by local expansion and density-dependent mortality (Perfecto and Vandermeer 2008, Vandermeer et al. 2008). A fungal pathogen, *Lecanicillium lecanii*, attacks the local concentrations of scale insects that are associated with the clusters of ant nests, greatly reducing the scale population and possibly causing

density-dependent control of the *A. instabilis* colonies (Jackson et al. 2009), although other factors have been proposed (Vandermeer et al. 2008). We hypothesize that seasonal epizootics of *L. lecanii* create periodic disturbances, causing dynamic shifts in the foraging ranges of *A. instabilis*, thereby opening niche space to other arboreal ants and promoting diversity of the arboreal foraging ant community. We further hypothesize that the magnitudes of the shifts in the *A. instabilis* foraging range depend on the availability of alternate resources located in neighboring shade trees, i.e. habitat complexity. Additionally, if between-site differences are observed in the magnitude of foraging shifts in response to the disturbance, we hypothesize this heterogeneity could be contributing to the self-organization process.

To explore these hypotheses, we examined the impact of *L. lecanii* epizootics on the foraging behavior of *A. instabilis* and the resultant effect of this disturbance on the activity of the arboreal foraging ant community. We paid particular attention to *A. instabilis* foraging response with respect to shade tree density (a proxy for habitat complexity). In consideration of particular mechanisms (i.e. disturbance, habitat complexity, spatial self-organization) promoting assembly and maintenance of diversity, we asked three specific questions: 1) How does *A. instabilis* respond to *L. lecanii* epizootics and concomitant decrease in the food resource provided by the scale insects? 2) How does habitat complexity, in this case density and proximity of other shade trees to *A. instabilis* nest-sites, influence the response of *A. instabilis* to a local epizootic? and 3) How is the arboreal foraging ant community affected by the response of *A. instabilis* to a local epizootic? We predicted that *A. instabilis* would abandon coffee bushes with large populations of infected scale insects and respond either by expanding their foraging

activity to coffee bushes in the periphery of previously tended bushes, or by switching their foraging activity to shade trees in close proximity to nests. Furthermore, we predicted that this shift in *A. instabilis* foraging activity would impact the arboreal foraging ant community by promoting expansion of sub-dominant ant foraging activity into coffee bushes previously tended by *A. instabilis*, thereby promoting and maintaining diversity in the arboreal foraging ant community.

Materials and Methods

The study site is located at Finca Irlanda, a 300 hectare, organic coffee farm in the Soconusco region of Chiapas, Mexico (15° 11' N, 92° 20' W). The farm is a commercial polyculture (Moguel and Toledo 1999), with coffee bushes growing beneath shade trees that have been planted in an approximately uniform distribution. The dominant shade trees are comprised of several *Inga* species, *Alchornea latifolia*, and *Trema micrantha* (Martinez and Peters 1996), some of which have extrafloral nectaries and many contain various species of scale insects and aphids (Livingston et al. 2008).

Site selection and data collection

We selected experimental sites based on the following criteria. First, each site had to have one or more *A. instabilis* nest, with each nest cluster being independent from nests outside the study plot. That is, potential sites containing colonies with foragers traveling to and from nests outside of the proposed study site were rejected. This criterion was imposed to ensure that colonies could not simply respond to *L. lecanii* epizootics within treatment sites by increasing foraging on coffee bushes outside of the sites. Second, each site had to have a large number of healthy scales so that the epizootic-induced death of these scales would entail a significant reduction in the food resources available to the *A.*

instabilis colonies. Although we initially searched for sites without *L. lecanii*, this criterion proved to be too stringent, as most sites with large scale populations had at least some *L. lecanii*-infected scales. Third, we avoided sites in which the *A. instabilis* colonies were primarily foraging in shade trees, either tending other scale insects or foraging at extrafloral nectaries, as we wanted to focus on colonies whose primary carbohydrate source was *C. viridis*. Using these criteria, we selected four treatment sites and one control site ranging in size from 100 m² to 375 m². The locations of all *A. instabilis* nests, shade trees, and coffee bushes in each site were recorded using a Cartesian coordinate system.

We used an aqueous suspension of *L. lecanii* conidia cultured from an infected *C. viridis* obtained from within the farm to inoculate scale insects, thereby creating an artificial epizootic (Easwaramoorthy 1978). Following isolation from the scale insect and culturing of conidia, conidia were mass produced via solid-state fermentation using cooked rice and sorghum as substrates. We then suspended the resultant conidia in water, added Tween 80 surfactant to the suspension, and applied it directly to the scale insects using a handheld, manual pump sprayer. Each coffee plant was sprayed until the surfaces of all leaves were thoroughly wet to the point of dripping; an average of 0.25 L of suspension was applied to each plant. Each site was sprayed twice to maximize *L. lecanii*-induced scale mortality: once on the morning of July 4, 2009 and again the morning of July 18, 2009. The spore concentrations, approximately 1.9×10^5 spores/mL for the first spraying, and 2.2×10^6 spores/mL for the second application, were determined using a hemacytometer.

We censused the scale insects at each site prior to and following inoculation using the

following protocol, adapted from Vandermeer and Perfecto (2006) (Vandermeer and Perfecto 2006). Each plant was rapidly surveyed to examine scale insect abundance. If plants had fewer than 20 scales, we categorized plants as “zero scales”, as virtually every coffee plant in the farm has at least a few scale insects. For plants with between 20 and 50 scales, we categorized them as “50 scales.” For plants with more than 50 scales, a four-category protocol was applied to each branch of the plant. Branches with 0-6, 7-30, 31-70, and in excess of 70 scales were placed in categories 1, 2, 3, and 4, respectively. We then calculated an estimated total scale count for the entire plant as $(0 \times \text{branches in category 1}) + (15 \times \text{branches in category 2}) + (46 \times \text{branches in category 3}) + (150 \times \text{branches in category 4})$.

We estimated the prevalence of *L. lecanii* while performing the scale censuses. For plants with between 20 and 50 scales, we visually estimated the percentage of scales infected with *L. lecanii*. Based on this estimate, the plant was placed in one of four fungal prevalence categories: 1-10% = category 1, 11-20% = category 2, 21-50% = category 3, and > 50% = category 4. For plants assessed using the four-category scale counting protocol, the same four fungal prevalence categories were applied to each branch individually. The number of infected scales was estimated as 0.05, 0.15, 0.35, or 0.75 times the total number of scales for fungal categories 1, 2, 3, and 4, respectively.

The abundance of *A. instabilis* and the identity of the numerically dominant arboreal foraging ant species were noted for each coffee plant. Abundance was assessed using a four category protocol: < 10 foragers = category 1, 10-25 = category 2, 26-50 = category 3, and > 50 = category 4. The ants were censused twice prior to and 3 times following the experimental inoculation.

Data analysis

We employed a resampling approach to determine if the increase in the percentage of scales infected at each treatment site was significantly greater than at the control site; that is, to confirm that the *L. lecanii* inoculation significantly increased prevalence. For each treatment site, we created synthetic control and treatment groups by combining the newly infected and healthy scales from the treatment site and the control site into a single pool and resampling randomly from this pool to assemble synthetic populations of the same sizes as the post-inoculation populations. The difference between the percentage increase at the simulated control site and the percentage increase at the simulated treatment site was then compared to the observed difference in percentage increases between the control and treatment sites. This procedure was repeated 10,000 times, with the p-value being the fraction of times the difference between the simulated treatment and control sites was as great, or greater than, the observed difference. This resampling approach determines the probability that we would see, by chance alone, as large of a difference in the change in the two sites as was observed.

We also employed a resampling approach to determine if the changes in the abundances of *A. instabilis* foragers in the treatment sites were significantly different from the change observed in the control site, using the percent change in the number of *A. instabilis* foragers on an entire-site basis.

The ant forager-abundance resampling analyses were performed as follows. First, for both the treatment and control sites, we calculated the average number of foragers on each coffee plant prior to inoculation and summed these to determine the average number of foragers in the entire site. Using this average and the total number of foragers in each

site present in the final ant census, we calculated the percentage change in foragers. From these values we obtained the difference in the percentage change at the control and treatment sites. We then combined the coffee plants from both the treatment and control sites into a single pool for resampling.

For each resampling, coffee plants were randomly assigned to either the control or treatment site, resulting in simulated control and treatment groups with the same numbers of plants as the actual control and treatment sites. The difference in the percentage change in foragers at these two simulated sites was then compared to the actual, observed difference. This procedure was repeated 10,000 times. The p-value was then calculated as the fraction of resamples resulting in a difference in percentage change that was as extreme or more extreme than the observed difference.

To estimate the food resources in shade trees (extrafloral nectaries, other scale insects, etc.) available in each site, we defined a shade tree resource index. We made the simplifying assumption that the accessibility of shade tree resources to an ant nest would fall off linearly with distance from the nest. Therefore, the total shade tree resources available to a nest are:

$$\text{Shade tree resource index} = \sum_{i=1}^n \frac{1}{d_i}$$

where n is the number of shade trees in the neighborhood of the nest and d_i is the distance between the nest and the shade tree.

Results

The increase in *L. lecanii* prevalence was 20.6, 12.6, 28.3, 12.8, and 4.6 percent for Sites 1-4 and the control site, respectively. The increase was highly significant at all treatment sites compared to the control site ($p < 0.0001$).

There was a substantial contraction in the *A. instabilis* foraging range at Sites 1 and 2 following the *L. lecanii* inoculation (Fig. 2). The number of coffee bushes occupied by non-*A. instabilis* arboreal-foraging ants also increased markedly in both sites between the first and last censuses: from 13 to 23 occupied bushes in Site 1 and from 6 to 15 in Site 2. The trends were less apparent in Sites 3 and 4. In Site 3, there was an increase in the number of coffee bushes tended by *A. instabilis*, from 19 to 28, while the number of bushes occupied by other ant species was relatively unchanged, decreasing from 19 to 18. However, three bushes in this site were destroyed by a falling tree trunk just before the final census. The portion of the tree trunk that fell contained part of an *A. instabilis* nest. The foragers found on the two newly-colonized bushes located at the left edge of the site came from this fallen nest. In site 4, there was a slight increase in the number of coffee bushes tended by *A. instabilis*, from 29 to 31. However, the spatial extent of the *A. instabilis* foraging range appears to have decreased slightly (Fig. 2). The number of bushes occupied by other ant species increased markedly, from 19 to 28.

In the control site, there was a slight decrease in the number of *A. instabilis*-tended bushes, from 13 to 11, as well as a slight decrease in the spatial extent of foraging. The number of bushes occupied by other ant species increased from 2 to 6 (Fig. 3).

The change in the actual numbers of *A. instabilis* foragers at the various sites corresponds with the qualitative picture shown in Figures 2 and 3. Sites 1 and 2 exhibited decreases in *A. instabilis* foraging that were significantly greater than the decrease that occurred at the control site. Sites 3 and 4 experienced a slight increase and a slight decrease, respectively, that were not significantly different from the control (Fig. 4).

The relationship between the change in *A. instabilis* foraging and the availability of alternate resources provided by shade trees was not significant ($p=0.31$), but did exhibit a qualitative positive association between the magnitude of the decrease in the number of *A. instabilis* foragers and the shade tree resource index (Fig. 5). Thus, a higher availability of alternative, shade tree resources was associated with a larger decrease in *A. instabilis* foraging.

The change in the number of coffee plants occupied by other arboreal-foraging ant species was negatively correlated, although not significantly so ($p=0.19$), with the change in the number of *A. instabilis* foragers (Fig. 6). That is, sites with a larger decrease in the number of *A. instabilis* foragers tended to have a larger increase in the number of coffee plants tended by another ant species.

Discussion

Our results suggest that *A. instabilis* colonies are able to adapt to *L. lecanii* epizootics by shifting their foraging ranges, but only if sufficient alternative resources are available. In the absence of alternative resources, or foraging refugia, as illustrated in the shade tree resource index in Figures 5 and 6, colonies are forced to continue tending the original, decimated scale populations. As a result, colonies without foraging refugia almost certainly experience a substantial reduction in food intake. In the long term, this could lead to the forced migration or mortality of these colonies, thereby contributing to the spatial self-organization of *A. instabilis* nests in this system.

The local contraction of the foraging range of *A. instabilis*, caused by the *L. lecanii* epizootic and mediated by the availability of foraging refugia, also promotes a local increase in the functional diversity of other arboreal-foraging ant species, including twig-

nesting and ground-nesting ants. Thus, *L. lecanii* epizootics, in concert with the variable availability of foraging refugia, may contribute to both the generation of spatial structure through self-organization and the maintenance of ant biodiversity in this system.

Despite a significant increase in fungal infection at all experimental sites, *A. instabilis* did not respond equally at all sites to the disturbance and loss of resource. With increasing shade tree density, and thus more readily available alternative resources including extra-floral nectaries and other species of scale insect, foraging contracted in coffee bushes and increased in shade trees. In contrast, *A. instabilis* foraging shifted much less in response to the artificial epizootic at sites with low shade tree density (Fig. 5). Though the regression comparing percent change in number of *A. instabilis* foragers and shade tree proximity to *A. instabilis* nest-sites was not significant, this is likely due to an underestimate of alternative resource availability at experimental site 2 (Fig. 5). All shade trees at this particular site, except for the tree in which the nest is located, are citrus trees, which contained substantial, uninfected populations of *C. viridis* throughout the duration of the study. Thus, despite lower shade tree density and proximity to nest-sites, the abundance of alternative resources at site 2 is still quite high, producing an underestimate of resource availability as calculated in the shade tree resource index. Sufficiently high densities of shade trees surrounding *A. instabilis* nest-sites appear to provide abundant alternative resources, as well as potential nest-site locations, allowing adaptation to the loss of scale insect resources in coffee bushes. This finding highlights the possible importance of local habitat complexity in facilitating pattern formation.

Furthermore, in the absence of readily available alternative resources provided by adjacent shade trees (sites 3 and 4), *A. instabilis* appeared to increase the intensity of

scale-tending activities in coffee bushes affected by the fungal epizootic. Based on observations made at these two particular sites, the number of foragers tending the few healthy scales remaining drastically increased after inducing the artificial epizootic. This suggests that a localized epizootic could cause persistent stress due to deficient resource availability for colonies located in areas lacking sufficient densities of shade trees.

Additionally, our results suggest that foraging activity of other arboreal foraging ants may increase in response to the contraction of *A. instabilis* foraging in coffee as a result of *L. lecanii* epizootics. With access to alternative resources, *A. instabilis* abandons coffee bushes affected by the epizootic, retreating to shade trees as a foraging refuge and likely remaining there until the epizootic diminishes and a new scale population begins to rebuild. Thus, given structurally complex habitat, *L. lecanii* acts as a disturbance in the arboreal foraging ant community, disrupting foraging of the competitive dominant and periodically resetting niche space availability for less dominant species of ants. This biotic disturbance is not only important to the maintenance of ant diversity, but also to the maintenance of biocontrol services provided by the diversity of foraging strategies, morphologies, and life histories of the different ant species. For instance, twig-nesting ants whose occupancy is negatively correlated with Azteca presence have been shown to be important predators of the coffee berry borer (*Hypothenemus hampei*), one of the most economically important, and one of the most difficult to control, pests of coffee (Vandermeer et al. 2002, Perfecto and Vandermeer 2006, Perfecto and Vandermeer 2008, Larsen and Philpott 2009). More generally, maintenance of arboreal foraging ants in coffee agroecosystems is essential for controlling herbivores and other pests (Philpott and Armbrrecht 2006).

Nest-to-nest variability in the number and accessibility of neighboring shade trees, i.e., habitat complexity, could then lead to variability in the magnitude of *A. instabilis* range shifts, thereby promoting diversity at the landscape scale. This habitat complexity may also feed back to the self-organization process: in the absence of alternate resources, *A. instabilis* colonies are more negatively impacted by *L. lecanii* epizootics, implying that the density-dependent mortality of nests is modulated by habitat complexity.

Spatial pattern in ecology has emerged as an important variable in the study of the distribution of biodiversity at numerous scales. The spatial distribution of a population or community throughout a landscape has often been assumed to result from underlying habitat structure (Forman and Gordon 1987, Turner et al. 1990). However, there may be other factors contributing to observed spatial patterns, particularly when considering relatively uniform landscapes (Skarpe 1991, Rohani et al. 1997, Bascompte and Solé 1998, Alados et al. 2007, Scanlon et al. 2007, Solé 2007). For example, observed patterns and distributions in uniform ecosystems, such as managed ecosystems, may be the result of self-organization caused by species interactions rather than underlying habitat variables (Pascual et al. 2002, Solé and Bascompte 2006, Perfecto and Vandermeer 2008, Rietkerk and van de Koppel 2008, Vandermeer et al. 2008).

In our system, we posit that the self-organization of *A. instabilis* nest clusters may be driven by entomopathogenic fungal epizootics in dense aggregations of *C. viridis* in mutualistic association with *A. instabilis*. Such epizootics were initially expected to cause an expansion of *A. instabilis* foraging activity. With the local loss of tended coffee scale resources, we expected *A. instabilis* to expand foraging activity to coffee bushes in the periphery of previously tended coffee bushes. Had *A. instabilis* responded by

expanding foraging activity to the small populations of uninfected scales in peripheral coffee bushes, the implications for the maintenance of the colony would likely be minimal. Taking advantage of these small aggregations of unaffected scale insects would allow *A. instabilis* to begin to build up and tend sufficient populations of *C. viridis* within range of the colony, possibly leading to the formation of new satellite nests in shade trees or large coffee bushes adjacent to the expanded foraging radius.

However, in contrast with these expectations, we observed a contraction of foraging activity and resource switching in sites with the highest shade tree resources and increased intensity of foraging on a reduced number of healthy scales in sites with lower shade tree resources (Fig. 2). This indicates that *L. lecanii* may be acting to promote nest mortality in the absence of arboreal refugia, or promote nest relocation from areas with low tree density to areas with higher tree density, either way driving the observed clumped distribution of *A. instabilis* nest-sites within the farm (Fig.1). The decline in *A. instabilis* foraging activity in coffee plants in areas with abundant alternate resources, suggests that there is a significant reduction in the quantity of scale resources obtained by *A. instabilis* colonies impacted by *L. lecanii*. In the absence of sufficient alternative resources, this reduction may lead to a weakening of colonies and eventual nest movement in order to escape the fungal pathogen, or nest mortality, both supporting the hypothesis that *L. lecanii* promotes the spatial pattern of *A. instabilis* nest clusters observed at the landscape scale (Jackson et al. 2009).

In summary, we suggest that the spatial self-organization process creates clusters of *A. instabilis* nests that provide the nuclei for *L. lecanii* epizootics; these epizootics, in turn, contribute to the self-organization process and cause disturbances that promote the

diversity of the arboreal foraging ant community; and habitat complexity, in terms of accessibility of shade trees and resources therein, alters the magnitude of the disturbances, increasing diversity at the landscape scale, and influencing the mortality component of the spatial self-organization process.

Furthermore, the clumped distribution of *A. instabilis* nest clusters at the landscape scale has been demonstrated to promote biological control of important coffee pests. For example, *A. instabilis* tended patches provide an enemy free space for the development of the coccinellid beetle larvae of *Azya orbiger*a (Coleoptera: Coccinellidae), which are immune to *A. instabilis* attack (Liere and Perfecto 2008). In tending the green coffee scale, *A. instabilis* also inadvertently protects the *A. orbiger*a larvae from predators and parasitoids (Liere and Perfecto 2008). This protection allows *A. orbiger*a, an important predator of the green coffee scale, to develop into a mobile adult coccinellid and provide biological control of the scale in areas of the farm that are unprotected by *A. instabilis*. The patchy distribution of *A. instabilis* nest clusters thus provides not only an enemy free space for larval development but also untended areas in which *A. orbiger*a acts as an effective control agent for the green coffee scale (Liere and Perfecto 2008, Perfecto and Vandermeer 2008, Vandermeer et al. 2008, Jackson et al. 2009).

Our results suggest that the dynamic interaction between a biotic disturbance (*L. lecanii* epizootics) affecting the arboreal foraging ant community and local habitat complexity (density of shade trees) promotes the maintenance of functional diversity. This interaction has important implications for the spatial distribution of nest clusters of the keystone species, *A. instabilis*, and suggests the self-organization of nests may be the result of *L. lecanii* epizootics. Furthermore, maintenance of functional diversity of

arboreal foraging ants is key to biological control in coffee provided by autonomous ecosystem function (Philpott and Armbrecht 2006, Larsen and Philpott 2009). Our results suggest that self-organization of *A. instabilis* nests may be promoting maintenance of this diversity. Additionally, the spatial pattern and distribution of *A. instabilis* nests resulting from this dynamic interaction has been shown to promote biological control of coffee and ecosystem function in numerous other ways (Liere and Perfecto 2008, Perfecto and Vandermeer 2008, Vandermeer et al. 2008, Jackson et al. 2009).

These findings have potentially significant implications for coffee management. This study has demonstrated that local habitat complexity can act to dampen the effect of *L. lecanii* epizootics on *A. instabilis* colonies, maintaining *A. instabilis*, and its important biocontrol services, in the system. Habitat complexity was also demonstrated to facilitate disturbance within the arboreal foraging ant community, thus promoting diversity of arboreal ants. Management of shade tree densities in coffee agroecosystems could potentially be used to promote landscape-level pattern formation and local-level maintenance of diversity in order to maximize ant-derived biological control of coffee pests.

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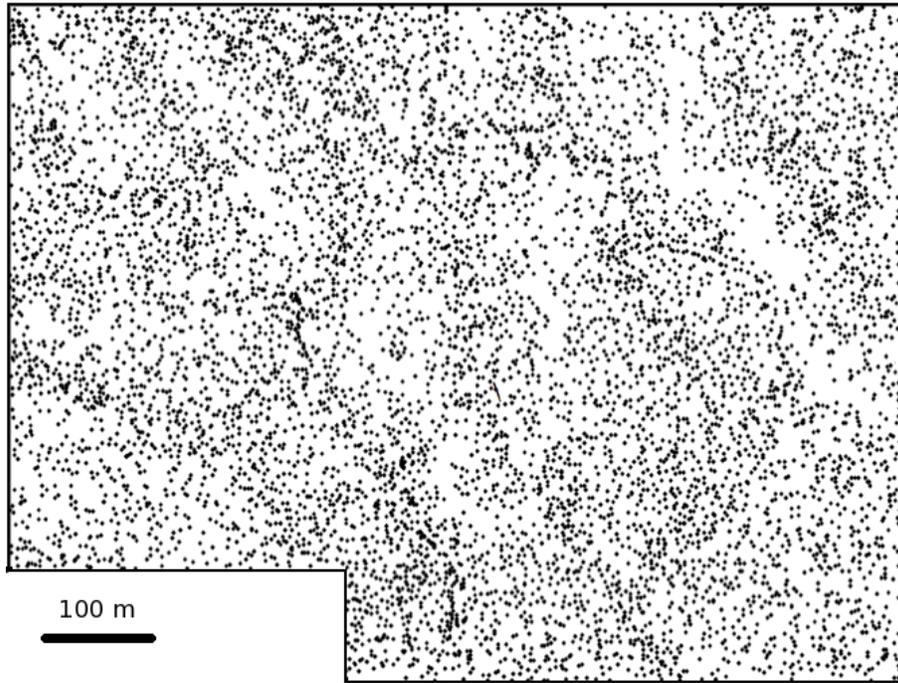
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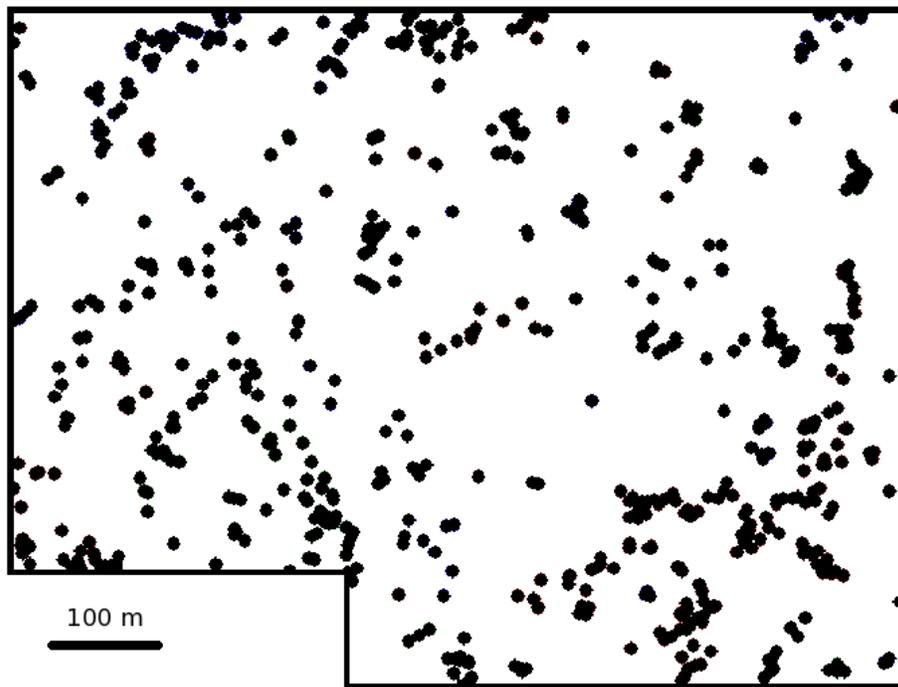
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Figures



a)



b)

Figure 1. Spatial distributions of a) shade trees and b) nests of *A. instabilis* in the 45 ha study plot.

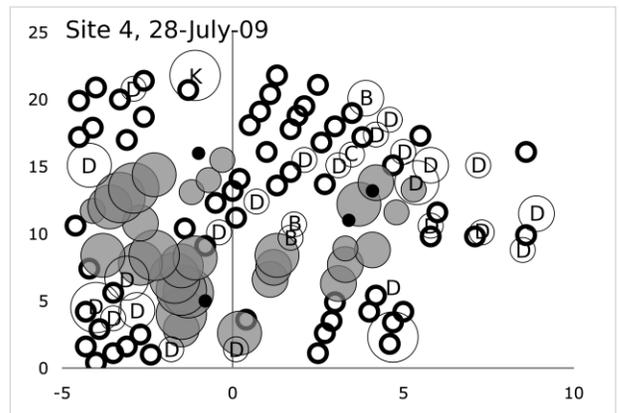
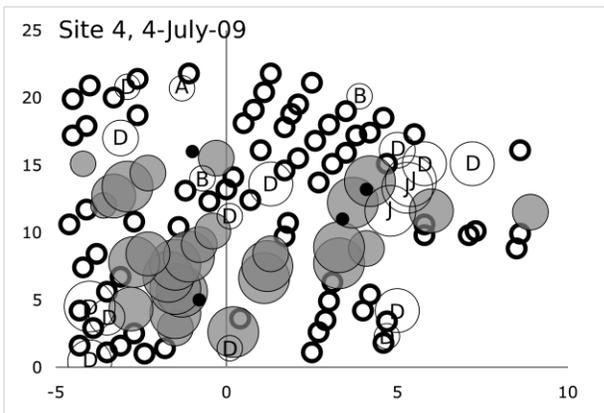
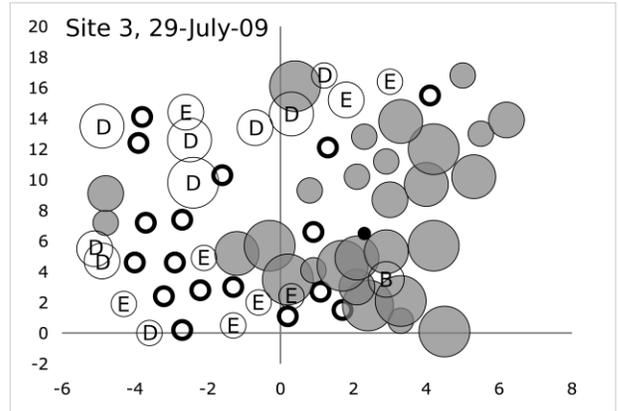
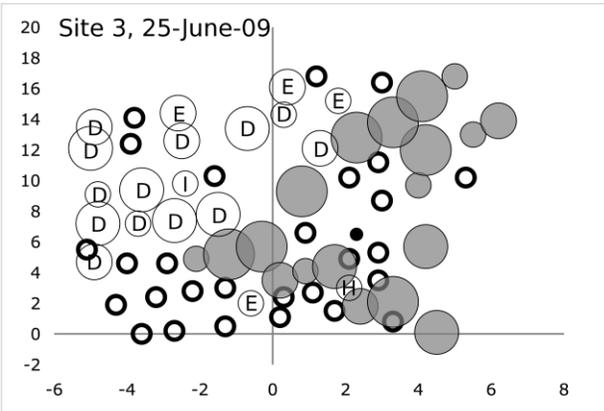
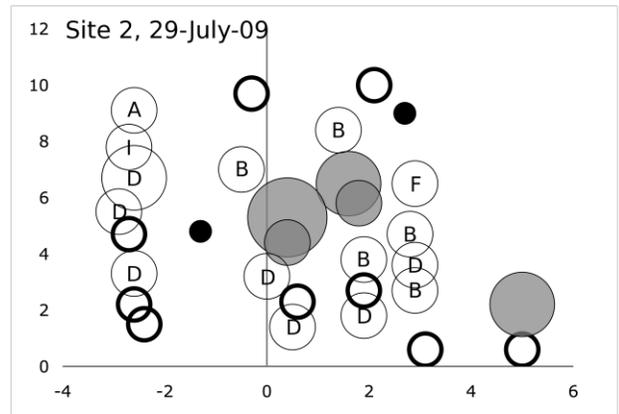
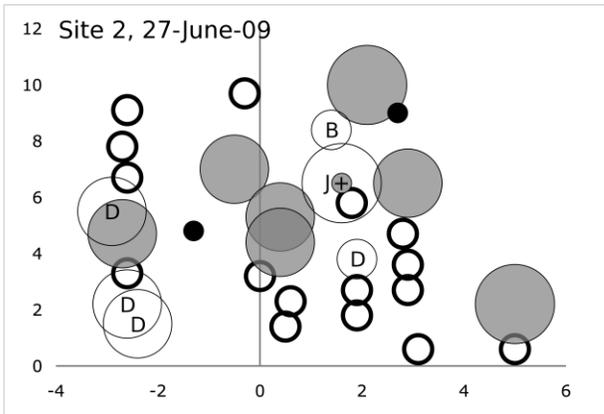
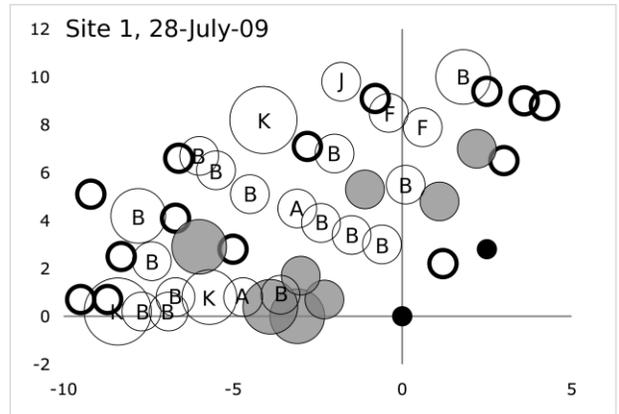
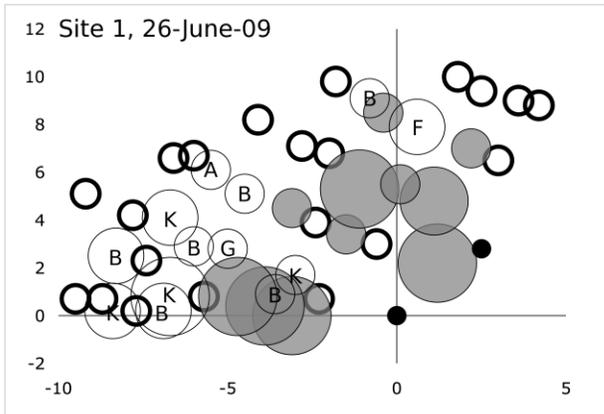


Figure 2. Spatial distributions of arboreal foraging ants in coffee bushes at four experimental sites before and after *L. lecanii* inoculation. Gray circles represent numbers of *A. instabilis* foragers. Labeled white circles represent numbers of foragers of other ant species, as follows: A = *Brachymyrmex* sp. 1, B = *Brachymyrmex* sp. 2, C = *Crematogaster carinata*, D = *Pheidole* sp. 1, E = *Pheidole* sp. 2, F = *Procrystocerus hylaeus*, G = *Pseudomyrmex ejectus*, H = *Pseudomyrmex gracilis*, I = *Pseudomyrmex simplex*, J = *Solenopsis geminata*, and K = *Technomyrmex* sp. 1. The sizes of the circles in each site correspond to one of four different abundance classes, from smallest to largest circles: < 10, 10-25, 26-50, and > 50 foragers. Unlabeled white circles represent coffee bushes without ants. Solid black circles indicate nests of *A. instabilis* in shade trees. The gray circle with a cross in site B indicates a nest of the ground-nesting ant *Solenopsis geminata*. Dimensions are in meters. Note that the axes are scaled differently in order to maximize separation between data points for visual clarity.

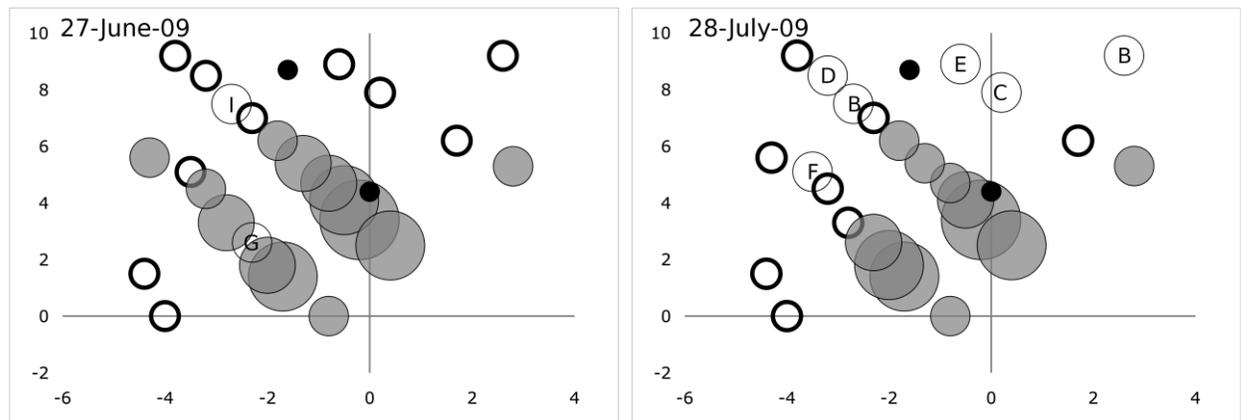


Figure 3. Spatial distributions of arboreal foraging ants in coffee bushes at the control site at the beginning and the end of the experiment. Gray circles represent numbers of *A. instabilis* foragers. Labeled white circles represent numbers of foragers of other ant species, as follows: B = *Brachymyrmex* sp. 2, C = *Crematogaster carinata*, D = *Pheidole* sp. 1, E = *Pheidole* sp. 2, F = *Procrystocerus hylaeus*, G = *Pseudomyrmex ejectus*, and I = *Pseudomyrmex simplex*. The sizes of the circles correspond to one of four different abundance classes, from smallest to largest circles: < 10, 10-25, 26-50, and > 50 foragers. Unlabeled white circles represent coffee bushes without ants. Solid black circles indicate nests of *A. instabilis* in shade trees. Dimensions are in meters. Note that the axes are scaled differently in order to maximize separation between data points for visual clarity.

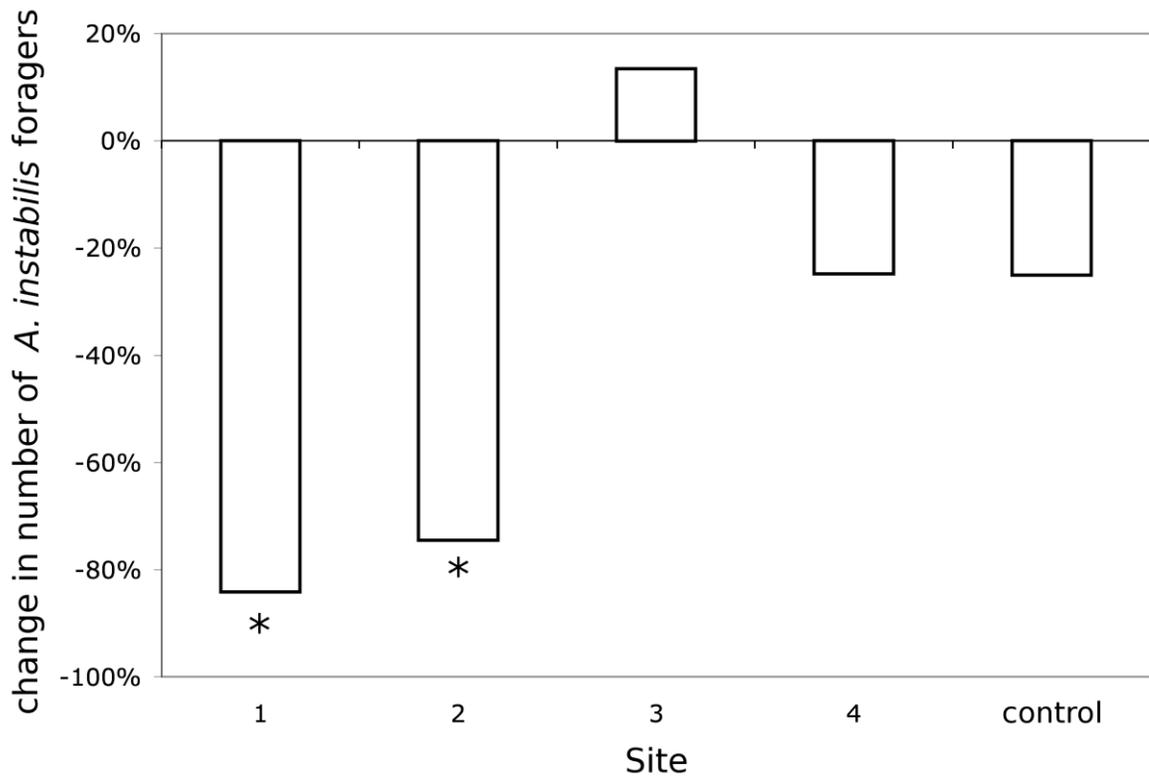


Figure 4. Change in the total number of *A. instabilis* foragers on coffee bushes at each site. Bars labeled with an asterisk are significantly different from the control ($p < 0.05$) using a resampling approach, as described in the text. Note that significance depends on both the relative difference in the change in foragers and the number of bushes at each site ($n = 18, 13, 33, 42,$ and 15 for sites 1, 2, 3, 4, and the control site, respectively).

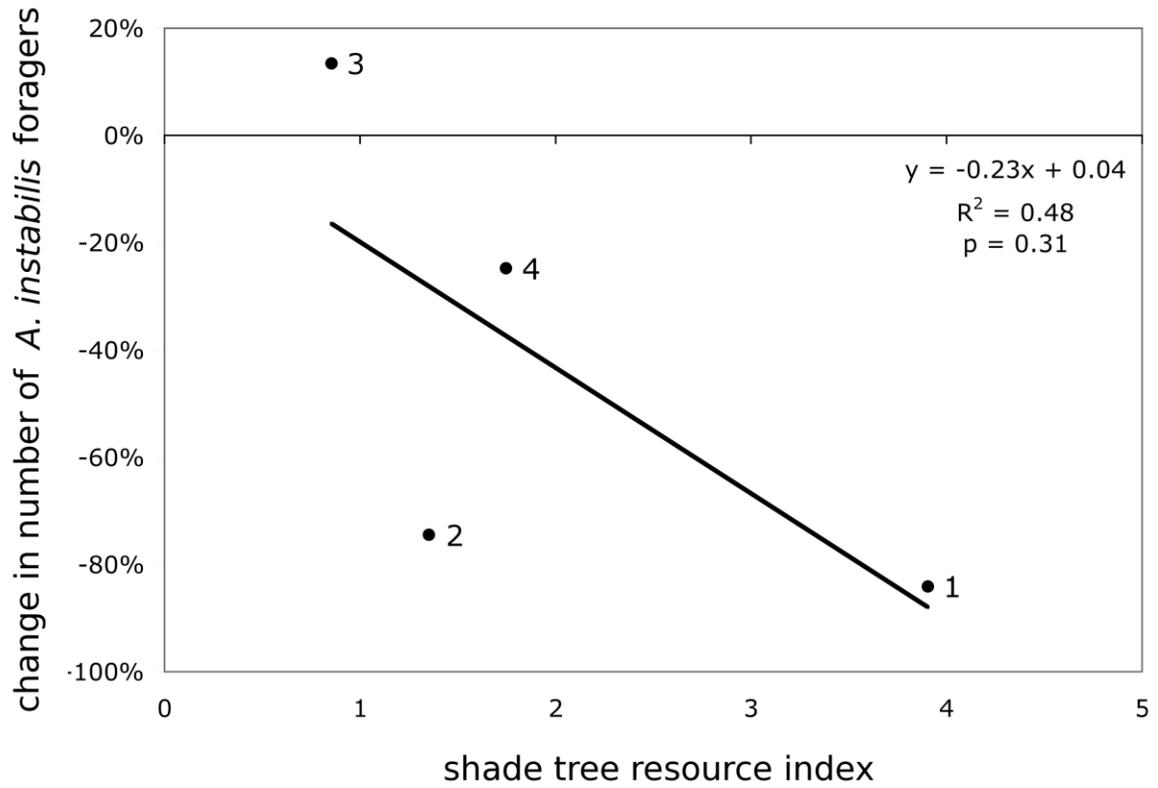


Figure 5. Change in the number of *A. instabilis* foragers on coffee bushes. Points are labeled with site numbers. The shade tree resource index is the sum over all shade trees of one divided by the distance from the ant nest to the shade tree. In sites with multiple ant nests, the shade tree resource index is an average of the indices of the individual ant nests.

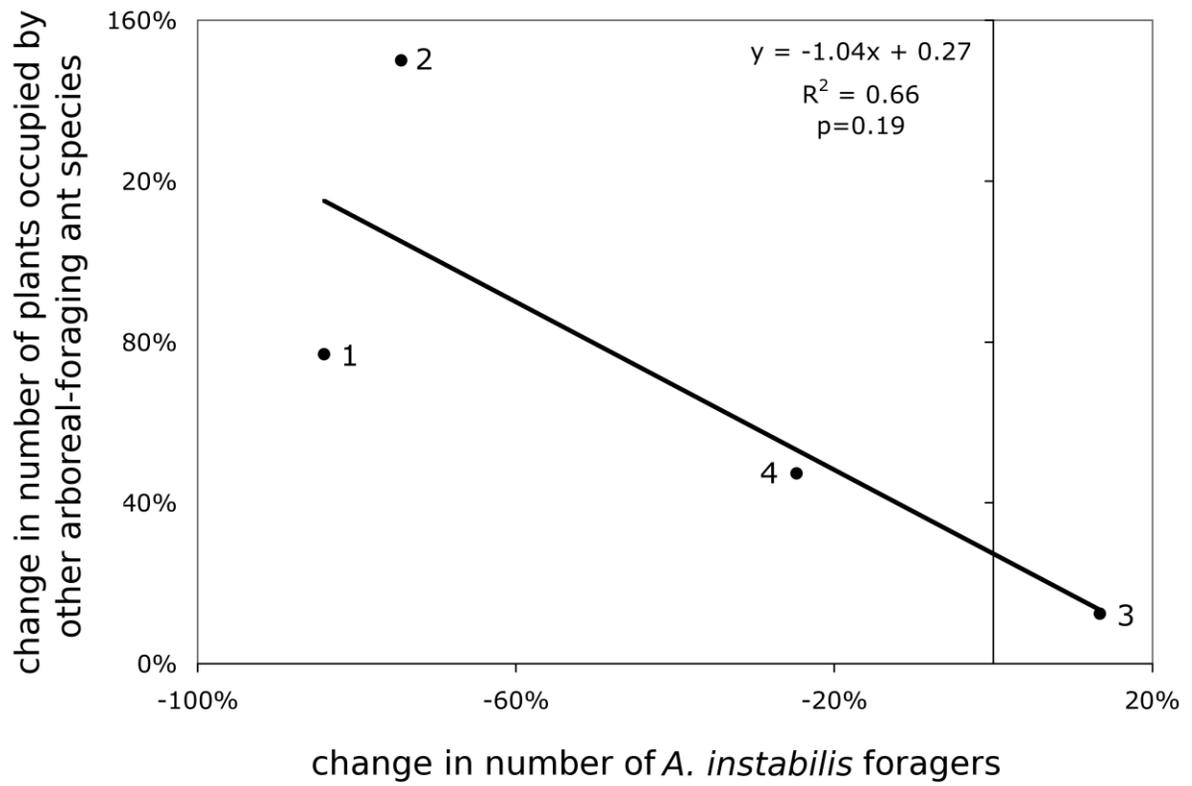


Figure 6. Change in the number of plants occupied by other arboreal-foraging ant species versus change in number of *A. instabilis* foragers. Points are labeled with site numbers.