Ecological Entomology

Ecological Entomology (2018), **43**, 621–628 DOI: 10.1111/een.12637

Nutrient transfer supports a beneficial relationship between the canopy ant, *Azteca trigona*, and its host tree

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Abstract. 1. Energy fluxes between ants and plants have been a focal point for documenting mutualistic behaviour. Plants can provide resources to ants through the production of extrafloral nectaries. In exchange, ants can fertilise plants through their nutrient- and microbe-rich refuse.

2. Here, we test a potential facultative mutualism between the carton-nesting canopy ant, *Azteca trigona*, and their host trees. Through observational and experimental approaches, this study documents how nutrient transfer provides a basis for this beneficial ant–plant relationship.

3. In a greenhouse experiment, fertilisation with sterilised refuse (i.e. nutrients only) increased seedling growth three-fold, while the refuse with its natural microbial community increased growth 11-fold.

4. Total root density was doubled in refuse piles compared with the surrounding area *in situ*. On average, refuse provides host trees and the surrounding plant community with access to a > 800% increase in N, P and K relative to leaf litter.

5. *Azteca trigona* preferentially nests in trees with extrafloral nectaries and on large, longer-lived tree species.

6. Given the nutrient-poor nature of the Neotropics, host trees probably experience significant benefits from refuse fertilisation. Conversely, *A. trigona* benefit from long-term stable structural support for nests and access to nutrient-rich extrafloral nectaries. Without clear costs to either *A. trigona* or host trees, it is proposed that these positive interactions are preliminary evidence of a facultative mutualism.

Key words. Ant–plant, *Azteca trigona*, fertilisation, nutrient transfer, species interaction.

Introduction

Mutualisms are interspecific interactions that result in reciprocal net benefits (Keeler, 1985; Bronstein, 1994, 2001, 2015). They are widespread and can increase ecosystem diversity and stability (Jander, 2015). Documenting mutualisms requires a detailed analysis of the costs and benefits to both interacting parties (Herre & West, 1997). This cost–benefit approach has helped to inform the mechanisms of numerous mutualisms such as the exchange of carbon for phosphorus between plants and mycorrhizae (Herre *et al.*, 1999), or the pollination and nutrient exchange between moths and yucca plants (Addicott, 1986). However, the strength of mutualistic relationships can vary dramatically across ecological space and time (Bronstein, 2015), and mutualisms are highly susceptible to exploitation (Bronstein, 2001; Sachs & Simms, 2006). The threat of exploitation encourages interacting partners to maintain fitness benefits while minimising costs (Herre & West, 1997; Herre *et al.*, 1999). By-product benefits, such as plants benefiting from ant foraging and waste products, are one way in which mutualisms can arise and persist with little cost to participants (Sachs *et al.*, 2004; Jones *et al.*, 2015; Hernandez *et al.*, 2017).

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Ant–plant relationships are useful model systems to explore mutualisms. Ant–plant interactions are common – up to one-third of all woody plants in a forest support ant associations (Schupp & Feener, 1991). They exhibit a diversity of ecological relationships (Beattie, 1985; Heil & McKey, 2003), ranging from obligate mutualism (e.g. *Cecropia peltata* trees and their *Azteca* ants; Janzen, 1969) to loose facultative relationships (e.g. extrafloral nectaries increasing plant visits by aggressive ants; Bronstein *et al.*, 2006). Ant–plant mutualisms are often seen as plants paying aggressive ants to protect the plant from herbivores and competing plants (Janzen, 1966; Heil & McKey, 2003; Chamberlain & Holland, 2009). The payment is frequently in the form of carbon-rich extrafloral nectar. By-products of ants can also provide nutrients to plants through the accumulation and deposition of colony waste (i.e. excreta and food waste; Sagers *et al.*, 2000). Such myrmecotrophy is of low cost to the ants and of considerable benefit to the nutrient budget of the recipient plants, especially in nutrient-poor environments (Beattie, 1989; Dejean *et al.*, 2012; Pringle *et al.*, 2013). Myrmecotrophy occurs in a variety of forms. Where plants provide internal chambers that house ant colonies (domatia), nutrient-rich ant waste piles solely benefit the host plant, leading to a close relationship (Sagers *et al.*, 2000; Mayer *et al.*, 2014). Similarly, ants nesting in canopies can facilitate development of concentrated epiphyte communities deemed 'ant gardens'. The concentration of nutrients in ant waste provides essential resources to the epiphyte community, leading to an increase in epiphyte establishment and growth (Blüthgen *et al.*, 2001; Orivel & Leroy, 2011). Ants, in turn, benefit from the epiphyte roots that strengthen the structure of their nest, and occasionally are fed through epiphyte extrafloral nectaries (Orivel & Leroy, 2011).

A looser myrmecotrophic relationship exists between soil-nesting ants and nearby plants that use their roots to 'forage' in the waste piles of ant colonies (Farji-Brener & Medina, 2000; Wagner & Nicklen, 2010). Wagner and Nicklen (2010) demonstrated that extrafloral nectaries on the desert shrub, *Acacia constricta*, encourage the soil-nesting ant, *Formica perpilosa*, to nest nearby and hence fertilise the shrub with their concentrated activity. Studies of a dominant carton-nesting canopy ant, *Azteca trigona,* suggest a similar mutualism. *Azteca trigona* feeds on extrafloral nectaries and hemipteran honeydew. They build nests of chewed plant material on the outside of various tree species in the Neotropics and defend their territories against diverse biotic intruders (Wheeler, 1942; Adams, 1994). *Azteca trigona* refuse accumulates below the nest, and these piles of refuse are three-fold richer in nitrogen (N), seven-fold richer in phosphorus (P), and 23-fold richer in potassium (K) than the surrounding leaf litter (Clay *et al.*, 2013). The refuse piles contain a diverse microbial community (*Pseudomonas* spp., *Rhizobiales* spp., and *Enterobacter* spp.), with bacteria known to promote plant growth (Lucas *et al.*, 2017).

Here we explore the relationship between *A. trigona* and the trees they nest on, focusing on the exchange of resources via refuse and extrafloral nectar. We examine the degree of host tree specificity in *A. trigona*, and test if tree species with extrafloral nectaries are more likely to attract the nests of *A. trigona*. To examine the potential for ant refuse to promote tree growth, we test whether the nutritious and microbe-rich refuse from *A. trigona* increases seedling growth in a greenhouse environment. Finally, to explore whether trees respond to refuse in natural conditions, we test for higher incidence of root foraging in refuse piles throughout the forest. Together, our results demonstrate that *A. trigona* preferentially nests on trees providing food sources and may benefit their host tree through long-term fertilisation.

Material and methods

Study site

This study was conducted throughout the Barro Colorado National Monument (BCNM) in Panama [Barro Colorado Island (BCI): 9′10′N, 79′51′W]. The BCNM is a seasonally moist tropical forest that receives an average of 2600 mm year^{-1} of rain . There is a pronounced dry season with < 10% of the rainfall occurring from mid-December to late April (Leigh, 1999). Additional information regarding this field site, including a detailed map of the trail system within the BCNM, is available through the Smithsonian Tropical Research Institute.

Azteca trigona *nest survey: testing for extrafloral nectary preference and host tree specificity*

To determine if *A. trigona* has host tree specificity and concentrates nests near extrafloral nectary sources, we performed two surveys 5 years apart. In 2011, we surveyed the westernmost third of the 50-ha forest dynamics plot located on BCI, Panama [for details on the 50-ha plot, see Hubbell & Foster (1983)]. Using the 5-m grid of the 50-ha plot, we carried out a transect census of *A. trigona* colonies. The census area included the 5 m on each side of the post to ensure the entire plot area was examined. When a nest was located, we recorded its length, height from the ground and the tag of the tree it was located on. From this tree tag number we were able to determine tree species, diameter at breast height (dbh), and location within the plot. Nests within 10 m of each other were determined to be the same colony (Clay *et al.*, 2013). The plot area was re-censused in 2016, by the same individuals, using the same methodology as 2011. We were able to determine whether nests found in 2016 were the same as those found in 2011 by comparing tree tag numbers as well as nest height and size records. We were also able to use 50-ha plot census data to determine whether trees hosting *A. trigona* nests experienced higher levels of mortality compared with background mortality rates, by comparing the number of trees hosting *A. trigona* in 2011 that died by 2016 with whole-plot mortality rates in that same time period. Additionally, we tested whether *A. trigona* non-randomly associated with trees with specific characteristics (i.e. extrafloral nectaries, large dbh). Species hosting extrafloral nectaries were classified in accordance with the database provided by Muehleisen (2013).

To determine the relative size of host trees and potential to use refuse resources below nests, we extrapolated dbh of host trees to determine average crown area. From our survey we determined 20 and 30 cm as our most common host tree dbh.

Using these values we calculated the average host tree crown area as 41.33 m^2 using values provided by O'Brien *et al.* (1995). We also calculated daily refuse production under a crown as 7.66 g (Clay *et al.*, 2013) and average daily leaf litter fall as 2.75 g m^{-1} day^{-1} (Sayer *et al.*, 2012). Then, we used the leaf litter and refuse nutrient level data provided by Clay *et al.* (2013) to calculate the average amount of each element (by weight) available to a host tree from refuse as compared with leaf litter inputs. Values for these calculations are included in the Supplementary Methods and Table S1 in File S1.

Examining the influence of refuse fertilisation on seedling growth

In 2015, ant refuse was collected from beneath 10 separate colony nests of *A. trigona* located along the Thomas Barbour trail on BCI. Buckets raised up on stilts and covered in fine mesh were placed below nests to catch the refuse before it could be colonised by soil microbial communities (Clay *et al.*, 2013). Refuse was collected every 3 days. Soil located 10 m away from refuse buckets was collected at each point for soil addition treatments. Half of the refuse and soil collected was sterilised by heating it to 250 °C for 1.5 h, while the other portion was added as live microbial input on seedlings.

To test the prediction that refuse and associated microbes facilitate plant growth we set up four fertilisation treatments: (i) refuse addition; (ii) sterilised refuse addition; (iii) soil addition; and (iv) sterilised soil addition. For each fertilisation treatment, we filled 12 tree pots (height 24 cm, width 10 cm, volume 1.65 litres) with a 50:50 mixture of sterilised local soil and rock. Our focal species, *Ochroma pyramidale* (balsa wood), is a common pioneer tree on BCI whose seeds are wind-dispersed and have physical dormancy. It has small seeds that require light gaps to establish and germinate (Croat, 1978). *Ochroma pyramidale* is a commonly used species in greenhouse experiments (Dalling *et al.*, 2013; Zalamea *et al.*, 2014), allowing us to compare our results with previous studies.

Seeds were collected from the soil seed bank below the crowns of three reproductive *O. pyramidale* adults. Seeds were then surface-sterilised in a bath of 10% sodium hypochlorite (bleach) solution for 10 min. Immediately following surface sterilisation, seeds were placed in 100 °C water for 30 s to break their physical dormancy (Zalamea *et al.*, 2014). They were then allowed to germinate in containers with fresh potting soil for 2 weeks (Fosforo Soil, Panama City, Panama). We transplanted into each pot three 2-week-old *O. pyramidale* seedlings (96 total), as described by Dalling *et al.* (2013). The initial dry mass of five randomly selected seedlings was determined at the time of the transplant. Seedlings were grown in full sun, on racks open on both sides and with a clear plastic roof to eliminate rainfall from watering plants. Seedlings were watered every other day and pot locations were randomised every 5 days. Seedlings received 5 g (roughly the average amount of refuse deposited each week by an *A. trigona* nest) of sterilised or unsterilised refuse or soil once a week and were grown for a total of 42 days.

At the time of harvest, seedlings were extracted and gently washed, then separated into root, leaf and stem fractions to examine above- and below-ground biomass. Specific leaf area (SLA; leaf area per unit leaf mass; cm^{-2} g^{-1}) of wet leaves was measured using an automated leaf area meter (LI-3000A, Li-Cor, Lincoln, Nebraska). The final biomass was measured after drying for 72 h at 60 °C.

Quantifying root foraging in refuse piles

To assess whether trees were preferentially foraging in *A. trigona* refuse piles, we located 15 *A. trigona* nests along the Edwin Willis trail on the Gigante peninsula of BCNM. We measured the distance from the trunk of the tree to directly underneath the nest at each location. Soil cores were taken in refuse piles directly below nests (0 m), then in a randomised direction at 0.5, 1 and 10 m away using a 5-cm-diameter split-sleeve core sampler (AMS, American Falls, Idaho). Cores were taken to a depth of 10 cm. A 0.5-mm sieve was used to rinse roots and separate them from soil particles. Once cleaned, the roots were sorted into two categories [< 1 mm diameter (fine) and > 1 mm (coarse)], as suggested by Cheng *et al.* (2009). The roots were dried to a constant mass at 60 °C, and then weighed.

Statistical analyses

All analyses were performed in the statistical environment R (R Development Core Team, 2013). All variables were tested for normality via the Wilks–Shapiro test (Sokal & Rohlf, 1981). For plant growth analysis, we tested for treatment effects on seedling growth and SLA using linear models, followed by a Tukey honest significant difference (HSD) using the *multcomp* package (Hothorn *et al.*, 2008). We ran a two-way ANOVA with root size (fine or coarse), categorical distance from the refuse pile, and their interaction to compare root densities in and near refuse piles. We tested for differences among distances from the refuse pile using a Tukey HSD. A *G* test with the Yates correlation correction (McDonald, 2009) was used to determine if *A. trigona* nests were associated with common tree species, tree size classes, trees with increased mortality and trees with extrafloral nectaries more than expected by chance.

Results

Azteca trigona *host tree demography*

Our initial survey in 2011 found 97 colonies on 194 trees (223 total nests) in the 16.67-ha area examined. The same survey in 2016 found 123 colonies on 142 trees (164 total nests). Of the 194 trees hosting nests in 2011, 48 (34%) were hosting the same nests in 2016. Average colony density increased from 5.82 to 7.69 colonies ha^{-1}, although total nests decreased from 13.38 to 9.84 nests ha^{-1}.

Azteca trigona nests were positively associated with trees with beneficial traits. Nests were often aggregated on trees with extrafloral nectaries (2011, $G = 57.91$, d.f. = 1, $P < 0.001$; 2016, $G = 34.14$, d.f. = 1, $P < 0.001$; Table 1 in File S1). Furthermore, nests were frequently found on larger trees, creating an aggregation of nests on a larger size class distribution of trees as compared with that of all the trees on the 50-ha plot (Table 2; Table S2 in File S1).

Azteca trigona was associated with specific host tree species. In 2011, 14.5% (44 of 303) of the tree and shrub species found on the plot had *A. trigona* nests in them. Similarly, in 2016, 13.9% (42 of 303) of the tree and shrub species found on the plot had *A. trigona* nests in them. This is far fewer species than would be expected if *A. trigona* nested in trees at random (2011, $G = 303.43$, d.f. = 1, $P < 0.001$; 2016, $G = 285.32$, d.f. = 1, $P < 0.001$). *Azteca trigona* nests were most frequently found in the tree species *Trichilia tuberculate* ($N = 42$ in 2011, $N = 19$ in 2016) an over-canopy tree with an average density of 259 stems ha^{-1} (Belk *et al.,* 1989). This species was used as a host tree more frequently than would be expected based on its abundance within the plot (Table 1). Multiple additional species had a high association with nests and are detailed in Table 1. Host trees did not experience different rates of mortality compared with background rates ($G = 0.654$, d.f. = 1, $P = 0.42$).

Plant growth is enhanced by refuse due to nutrient and microbial inputs

Trees supporting *A. trigona* nests receive a highly concentrated point-source fertilisation of macro- and micronutrients. While refuse is only equal to 6.7% of the weight of leaf litter underneath an average tree crown (Sayer *et al.*, 2012), it contains a potent level of nutrients equal to 20% of the total available N, 41% of total P, and 136% of total K found in the leaf litter under a tree crown (Table S1 in File S1). More specifically, in the area where refuse accumulates under a tree (*c.* 1 m^2), refuse input comprises 833% of N, 1739% of P, and 5747% of K provided by leaf litter.

In our greenhouse experiment, refuse fertilisation increased seedling growth and altered biomass allocation relative to soil fertilisation. However, these effects were minimal without the microbial community of the refuse (Fig. 1). Unsterilised refuse (i.e. refuse with its unaltered microbial flora) caused an 11-fold increase in total biomass relative to soil treatments, whereas sterilised refuse only caused a three-fold increase ($F_{3,44} = 80.81$, $P < 0.0001$; Fig. 1). Unsterilised refuse-treated plants allocated 45% of their total biomass to roots, whereas sterilised refuse and both soil treatments allocated only 37% of their total biomass to the roots. Neither soil nor refuse fertilisation treatments had significant effects on SLA ($F_{3,44} = 1.77$, $P = 0.17$).

Plants concentrate root foraging in Azteca trigona *refuse*

Refuse piles increased root foraging ($F_{4,114} = 12.41$, $P < 0.001$). The dry mass of roots in refuse piles was double the amount of roots in locations 1 and 10 m away ($F_{3,114} = 4.01$, $P = 0.009$; Fig. 2). The effects of distance from the refuse pile were particularly strong for fine roots (Fig. 2). However, the mass of coarse roots was greater than fine roots in each soil core ($F_{1,114} = 37.38$, $P < 0.001$), and both root sizes exhibited the same general pattern (root size × distance interaction, $F_{3,111} = 0.34$, $P = 0.80$). There was no difference in root density across each nest, ruling out intercolonial differences ($F_{13,39} = 6.84$, $P = 0.64$).

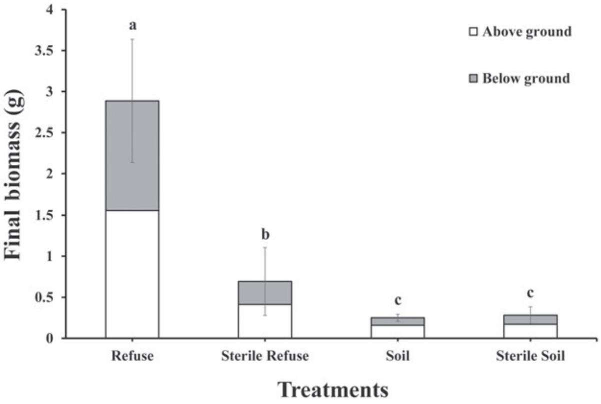

Fig. 1. Average total biomass of seedlings after 6 weeks of fertilisation. Total biomass bars are split into above- (white) and below-ground (grey) values. Error bars represent standard deviations of the total biomass values and letters indicate significant differences among treatment types: live refuse (RL), sterile refuse (RS), live soil (SL) and sterile soil (SS). Live refuse treatments have significantly higher biomass.

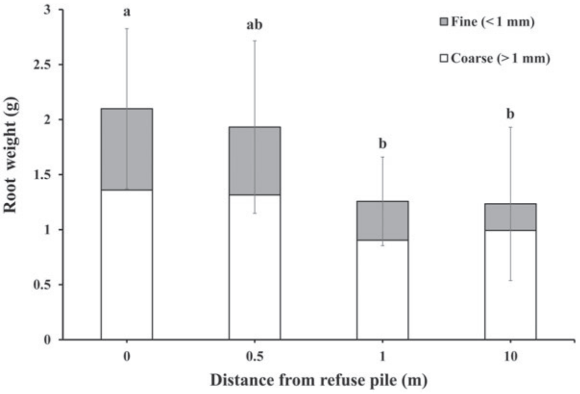

Fig. 2. Average biomass of coarse (> 1 mm, white) and fine (< 1 mm, grey) roots at distances from underneath nests (in m). Error bars represent standard deviations for total root biomass values, and letters indicate significant differences between distances. Root densities were highest in refuse piles and this effect was seen up to 0.5 m away.

Discussion

Ant–plant relationships range from obligate symbioses to facultative, conditional associations that are maintained when the benefits to ant and plant exceed the costs. Here we describe an association similar to that exhibited between the ant *Formica perpilosa* and the desert shrub, *Acacia constricta* (Wagner & Nicklen, 2010). Like the *F. perpilosa*, which nests below *A. constricta*, *A. trigona* colonies congregated on trees that provide extrafloral nectaries. The concentration of ants provides a nutrient-rich reward for plants in the form of refuse, which is actively foraged in by surrounding roots, and may confer additional benefits to surrounding seedlings. Moreover, many of the

Table 1. Results of the *G*-tests with the Yates correlation correction (McDonald, 2014) for tree species exhibiting high association with *Azteca trigona* nests. Trees with extrafloral nectaries (EFNs) are also noted.

Tree species	Year	*G*-test	d.f.	*P*	EFNs
Alchornea costaricensis	2016	3.551 64	1	0.059	Yes
Alchornea costaricensis	2011	13.541 91	1	< 0.001	Yes
Apeiba membranacea	2011	7.410 328	1	0.006	No
Astronium graveolens	2016	7.037 124	1	0.008	No
Chimarrhis parviflora	2016	3.922 504	1	0.048	No
Drypetes standleyi	2016	19.678 92	1	< 0.001	No
Drypetes standleyi	2011	21.098 55	1	< 0.001	No
Guarea Guidonia	2016	7.048 582	1	0.008	Yes
Gustavia superba	2016	10.557 21	1	0.001	Yes
Gustavia superba	2011	17.346 83	1	< 0.001	Yes
Heisteria concinna	2016	9.775 206	1	0.002	No
Heisteria concinna	2011	12.2281	1	< 0.001	No
Hirtella triandra	2016	34.809 45	1	< 0.001	Yes
Hirtella triandra	2011	33.304 12	1	< 0.001	Yes
Inga marginata	2011	9.905 782	1	0.002	Yes
Pouteria reticulata	2016	15.8004	1	< 0.001	No
Sloanea terniflora	2011	3.658 433	1	0.056	Yes
Trichilia tuberculate	2016	7.738 496	1	0.005	No
Trichilia tuberculate	2011	33.169 32	1	< 0.001	No

Table 2. Size class distributions of all the trees on the 50-ha plot as well as the proportion of those trees hosting *Azteca trigona* colonies.

Size class	Range (mm)	2011 whole plot (%)	2016 whole plot (%)	2011 *A. trigona* host trees (%)	2016 *A. trigona* host trees (%)
1	10–50	77.2	76.5	5.56	1.6
2	51–100	12.8	13.7	11.67	15.9
3	101–150	4.3	4.5	15.56	19.8
4	151–200	1.8	1.7	12.78	11.1
5	201–250	1.1	1	13.88	15.1
6	251–300	0.7	0.7	12.22	11.1
7	≥ 301	2.0	1.9	28.33	25.4

nutrients in the refuse probably originated from *A. trigona* prey, including herbivores, inhabiting the canopy of the host tree. In the nutrient-limited Neotropics (Dalling *et al.*, 2016), the relationship between *A. trigona* and its host tree may play an important role in shaping the local community through the exchange of key nutrients.

Seedlings fertilised with live microbial refuse saw an 11-fold increase in growth. While we acknowledge that in natural settings multiple trees may use refuse piles, we stress that host trees need only exploit a portion of the nutrients to gain large nutritional benefits. For example, if host trees only have access to 20% of the refuse pile, the nutritional benefit is equivalent to a substantial portion of the total P (8% of leaf litter) and K (27%) contained by all of the litterfall under its crown. This effect is particularly important because P and K are limiting nutrients for plants at our study site (Kaspari *et al.*, 2008; Wright *et al.*, 2011).

The potentially beneficial effects of *A. trigona* refuse extend beyond its nutrient content. Previous studies highlight the importance of beneficial microbial flora in supporting plant growth and defending against pathogens (Compant *et al.*, 2005; Van der Heijden *et al.*, 2008). Our greenhouse experiment demonstrated that the refuse microbiome amplified the effects of refuse on plant productivity (Fig. 1). This amplified growth could be due to the multiple plant growth-promoting bacterial taxa found in *A. trigona* refuse (Lucas *et al.*, 2017). Similarly, refuse contains an abundance of antimicrobial-producing *Actinomycetes*. All seedlings fertilised with sterilised (non-living) microbial refuse had large amounts of visible detrimental fungal colonisation (J.M. Lucas, pers. obs.), whereas live refuse additions had no visible fungal growth. This is anecdotal evidence that the rich microbial community found in refuse can provide protection against potential fungal pathogens.

Because *A. trigona* colonises trees long after the seedling stage (> 10 mm dbh), it is difficult to determine the extent to which *A. trigona* refuse benefits host trees during nest residency. We found no evidence that host trees are hindered by the presence of *A. trigona*; results from the 50-ha plot survey demonstrate that, over a 5-year period, trees hosting colonies do not experience increased mortality. Apart from enhanced growth and no obvious fitness cost, the benefits received by *A. trigona* post-seedling fertilisation could include increased investment in reproductive structures (Willson & Price, 1980). If host trees benefit from increased access to limiting nutrients, then we could expect greater investment in reproductive structures by host trees than by conspecific trees without *A. trigona* nests. The impact of refuse fertilisation on the reproductive success of mature trees requires further investigation in order to determine the degree to which host plants may benefit from *A. trigona* presence.

Azteca trigona ants were non-randomly associated with tree species that produce extrafloral nectaries. As these ants are major consumers of simple carbohydrates, nesting near extrafloral nectaries probably provides an important food source (Wheeler, 1942; Clay *et al.*, 2013). In a similar study exploring the contribution of ant waste to plants, Pinkalski *et al.* (2015) demonstrated that ants given access to sugar produced more faeces than those without access to sugar. Increased nutrient availability for plants can increase the production of ant rewards (Folgarait & Davidson, 1994; Heil *et al.*, 2000; however, see de Sibio & Rossi, 2016). Furthermore, ant aggression increases with increased carbohydrate availability (Grover *et al.*, 2007; Ness *et al.*, 2009; González-Teuber *et al.*, 2012). Thus, through a positive feedback loop, trees that provide extrafloral nectaries may benefit from increased nitrogen and macronutrient deposition, as well as increased ant-mediated defence (González-Teuber *et al.*, 2012; Pinkalski *et al.*, 2016). In return, ants may be rewarded with additional nutritional resources. However, we emphasise that trees providing extrafloral nectaries were not an obligate requirement for host tree selection. Additional studies that examine the distance and/or connectivity between non-extrafloral nectary host trees and a neighboring tree with extrafloral nectaries might provide insight into the importance of access to extrafloral nectaries in nest site selection.

In summary, we provide initial support for a facultative mutualism between *A. trigona* and their host trees. However, three avenues require further attention. We predict that, similar to the aggressive behavior documented in *Azteca chartifex* (Dejean *et al.*, 2009), *A. trigona* defend their host tree against herbivores. Although it is not resolved, early results provide preliminary support for this prediction (J.M. Lucas, unpublished data). Second, we must explore where *A. trigona* colonies forage for nutrients, and whether they are strongly reliant on host tree extrafloral nectaries. Finally, dendro-ecological measurements and monitoring of the reproductive structure production of host trees will determine whether *A. trigona* fertilisation increases host tree growth and fitness.

Acknowledgements

First, we thank our anonymous referees and the associate editor for their input. Their comments have greatly improved the quality of this manuscript. We thank Carolyn Gigot, Kate Henderson, Annika Salzberg, and Andy Van Alst for their help in the field. We thank Carolina Sarmiento, Camilo Zalamea, and Scott Mangan for their help in the design of our greenhouse study. We thank Jesse Czekanski-Moir and Evan Gora for help with the analysis. We also thank Oris Acevedo, Belkys Jimenez, and STRI staff on Barro Colorado Island, Panama, and ANAM for permits. We thank the Yanoviak laboratory for comments throughout the project design and manuscript writing process. This work was supported by funding from the Biology Department (OU), Graduate Student Senate (OU), NSF Graduate Fellowship Program ID 2014170874 (awarded to JL), NSF DEB 0842038 (principal investigators MK and A. Kay), NSF Macrosystems EF-1065844 (principal investigator MK), and the Research Experience for Undergraduates program run by the Smithsonian Tropical Research Institute. The authors declare they have no conflict of interest.

JL and NC conceived the initial study and carried out the 50-ha plot survey experiment. JL conducted the greenhouse and root density experiments. JL wrote the manuscript with support from NC and MK. JL and MK provided funding for the project. MK helped to supervise the project.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

File S1. Supplementary material including calculations and values for chemistry of refuse and leaf litter, and statistical results for size class values of trees hosting *A. trigona*.

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Accepted 19 April 2018
First published online 17 May 2018
Associate Editor: Sophie Evison