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Quantification of Ant Manure Deposition in a Tropical Agroecosystem: Implications for Host Plant Nitrogen Acquisition

Christian Pinkalski,[1]* Christian Damgaard,[1] Karl-Martin V. Jensen,[2] Renkang Peng,[3] and Joachim Offenberg[1]

[1]Department of Bioscience, Aarhus University, Vejlsøvej 25, 8600 Silkeborg, Denmark; [2]Department of Agroecology, Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark; [3]Research Institute for the Environment and Livelihoods, Charles Darwin University, Darwin, NT 0909, Australia

ABSTRACT

Ants are functionally important organisms in most terrestrial ecosystems. Being ubiquitous and abundant, ant communities can affect the availability of resources to both primary and secondary consumers. As nitrogen is a limiting nutrient for plant growth in most terrestrial ecosystems, deposition of ant manure may augment the host plants' acquisition of nitrogen. In this study, we quantified the manure deposited by colonies of the Asian weaver ant *Oecophylla smaragdina*. We developed a method to estimate the amount of manure deposited in host trees (*Mangifera indica*) based on the trail activity of *O. smaragdina*. The rate of manure deposition was on average 62.3 kg dw ha^{-1} y^{-1}, of which 0.2 kg ha^{-1} y^{-1} was deposited as urea-N, a nutrient that may be absorbed directly through the leaves, and 1.9 kg ha^{-1} y^{-1} was deposited as total nitrogen. Furthermore, ants given access to sucrose solution increased their rate of manure deposition significantly, suggesting that nectaries and/or trophobionts may play a major role in the production of ant manure. This study reveals that *O. smaragdina* can supply a significant amount of nitrogen to their host plants. In light of their remarkable abundance, the manure deposition by ants may have a hitherto unappreciated impact on the allocation of nutrients within their ecosystem.

Key words: ant–plant interactions; arthropods; food web; nitrogen cycling; nutritional ecology; *Oecophylla smaragdina*; trophobionts.

INTRODUCTION

In tropical ecosystems, ants often dominate the arthropod community in terms of numbers and biomass (Hölldobler and Wilson 1990). Due to extensive foraging, ants can consume and process large amounts of food, which may affect the availability of nutrients to other organisms within their ecosystem (Folgarait 1998). Plants hosting ants may, in addition to protection from herbivory (Bronstein 1998; Heil and McKey 2003), gain a

nutritional benefit from the refuse deposited by ants within or around the ants' nest. Myrme-cotrophy, the transfer of nutrients from ants to plants, has been investigated most thoroughly in plant species with domatia, specialized structures in which ant colonies may nest (Gegenbauer and others 2012; Solano and Dejean 2004; Treseder and others 1995). Through absorptive tissue, these myrmecophytic plants absorb nutrients from the mineralization of ant debris and excretory deposits, with up to 80% of total plant nitrogen acquisition derived from ant deposits (Solano and Dejean 2004). Correspondingly, soil nesting ants have been shown to increase the nutrient content in the soil around their nests where plants can proliferate from the increased nutrient content (Finer and others 2013; Wagner and Nicklen 2010). The majority of the studies of nutrient depositions by ants have focused on localized deposits containing both refuse and ant excreta in and around the ants nests (Clay and others 2013; Czaczkes and others 2015; Shukla and others 2013; Weiss 2006). However, many ant species, both arboreal nesting and soil nesting, forage for honeydew and insect prey in the canopy of myrmecophytic as well non-myrmecophytic trees (Campos and Camacho 2014). Here the ants may deposit ant manure as general excretion of waste (Weiss 2006) and/or in coherence with chemical communication (Höll-dobler 1983; Offenberg 2007). Therefore, the flows of nutrients in ant-plant interactions may stretch beyond depositions in the vicinity of ant nests or in closely coevolved ant plants and be relevant to plants in general. In light of the remarkable ubiquity of ants, the deposition of ant manure may thus represent a significant component in the cycling of nutrients within their ecosystem.

To determine the magnitude of manure deposition by ants across their foraging area, we assessed the ant manure deposition of the Asian weaver ant *Oecophylla smaragdina* (F.) (Hymenoptera: Formicidae). *O. smaragdina* is an arboreal predatory species that constructs nests of interwoven leaves in the canopy of trees and shrubs. The colonies can grow to a considerable size, consisting of more than a hundred nests, spanning numerous trees, and a single colony may contain hundreds of thousands of workers (Pinkalski and others 2015). *O. smaragdina* worker ants produce ant manure droplets from rectal sac fluids and deposit them throughout their territory (Beugnon and Dejean 1992; Dejean and Beugnon 1991; Offenberg and others 2004; Offenberg 2007; Peng and Christian 2013). These droplets contain various nutritious compounds such as amino acids and urea (Vidkjær and others 2015a; Vidkjær and others 2015b, unpublished data), which is commonly used as a foliar fertilizer in agriculture (Abad and others 2004; Readman and others 2002; Varga and Svecnjak 2006). The magnitude of ant manure deposition may be governed by colony growth, which in turn may be affected by various factors such as habitat conditions, temperature, and food availability (Brown and others 2004). As with other abundant ant species, *O. smaragdina* is heavily dependent of carbohydrate rich exudates from extrafloral nectaries and honeydew producing trophobionts, the availability of which may be staggered in time and space (Blüthgen and Fiedler 2002).

Being among the leading predators of other arthropods, as well as cryptic herbivores feeding on plant and homopteran exudates, *O. smaragdina* feed on several trophic levels and may play a key role in the cycling of nutrients within their ecosystem. As nitrogen is a limiting factor for plant growth in most ecosystems, (Johnson and Turner 2014; Le-Bauer and Treseder 2008), *O. smaragdina* may thus serve as conduits of nutrients for plant growth. Accordingly, it has been hypothesized that the presence of *O. smaragdina* in orchards enhances the fruit quality due to a fertilizing effect of the ant manure (Barzman and others 1996; Peng and Christian 2013).

Here we use *O. smaragdina* as a model species to assess the amount of manure deposited by ants. Models were developed to predict the yearly deposition of nutrients by *O. smaragdina*, expressed as urea-N and total nitrogen, in their host trees (*Mangifera indica*). Furthermore, we tested the prediction that access to sucrose solution, as a surrogate for plant-derived exudates, positively affects the amount of ant manure deposited and discuss how ants may affect the process of nitrogen allocation within their habitat.

METHODS

Description of Study Site

The study was performed in the wet season (Oct–Jan 2012–2013) and in the dry season (June–Aug 2013) in two mango plantations in the suburbs of Darwin, Australia (coordinates: Plantation 1: 12°23′23″S; 130°52′14″E; Plantation 2: 12°25′16″S; 130°56′19″E). Plantation 1 consisted of 76 mango trees with abundant weaver ant colonies. Some of the trees in plantation 1 had been connected by strings to facilitate the movement of ants between the trees, but otherwise the ants were not managed. Plantation 2 consisted of 110 mango trees with

abundant and unmanaged weaver ant colonies. The trees in both plantations were of similar age (15–20 years).

Darwin has a tropical climate with distinct wet and dry seasons. The wet season is characterized by high humidity and monsoonal rain with temperatures ranging from 25 to 33°C. The temperature in the dry season is similar, ranging from 22 to 32°C, but humidity is low and very little precipitation occurs (Australian Government, Bureau of Meteorology).

Estimating Ant Manure Deposition by *O. smaragdina*—Development of the Models

The conceptual framework for the following models was developed by Pinkalski and others (2015) and forms the basis for this study. The models for estimating the amount of ant manure deposited by *O. smaragdina* was constructed by combining two sub-models:

(i) Prediction of ant manure deposition based on nest volume of *O. smaragdina* (this study) and
(ii) Prediction of total nest volume in the host tree from *O. smaragdina* trail activity (Pinkalski and others 2015).

Ant Manure Deposition in Relation to Nest Volume

To estimate the amount of ant manure deposited from nests of a given volume, a total of 80 nests were sampled at three occasions: sampling 1 was performed in plantation 1 in the wet season with a total of 45 nests sampled from 4 colonies. Trees affiliated with different colonies were differentiated by following worker ant trails between the trees. If in doubt of affiliation, a few workers were transferred to the relevant tree which produced an unambiguous aggressive response among workers if the tree belonged to an alien colony (Peng and Christian 2005). Sampling 2 was performed in the same plantation in the dry season with a total of 17 nests sampled from 3 colonies. However, due to a very low abundance of ants at this time, a third sampling was performed in plantation 2 with a total of 18 nests sampled from 3 colonies.

The sampling of nests was performed during midday, as this time is the period of least ant activity, and hence, more ants are expected to remain inside the nests (Peng and others 2012). The nests were cut down, falling directly into a cardboard box that was immediately closed. On each side of the box, a hole covering almost the entire side of the box had been cut to uphold the daily variation in light. The holes were covered by white paper lining and a layer of transparent packaging tape to prevent the ants from escaping. Box sizes ranged from small (~10 l), medium (~25 l), and large (~50 l) containing small, medium, and large nests, respectively. A subset of nests was randomly selected to assess the rate of ant manure deposition (sampling 1: $n = 26$; sampling 2: $n = 14$; sampling 3: $n = 14$). The number of clearly visible, brown colored ant manure spots deposited on the white paper lining was counted every approximately two hours during the experiment. All nests were kept in the box for 24 h at ambient temperature. Subsequently, the ants were killed in a freezer, and nest dimensions were measured. Based on the cylindrical appearance of the nests, the dimensions measured were nest length (defined as the longest side of the nest) and nest circumference (measured at the center of the nest perpendicular to nest length). Nest volume was calculated as V $= 0.75 \times \pi \times \left(\frac{\text{Circumference}}{2\pi}\right)^2 \times \text{length}$, where the constant of 0.75 corrects for the bias of assuming cylindrically shaped nests (Pinkalski and others 2015). Finally, the total number of ant manure spots deposited within the 24 h in the box was counted, and the nests were dissected by hand to measure the ant biomasses (wet wt) within the nests.

Host Tree Nest Volume in Relation to Ant Trail Density

The trail density (hereafter referred to as trail score) was assessed on a total of 16 trees with 8 trees sampled in the wet season and 8 trees in the dry season. The assessments were performed in the afternoon between 16.00 and sunset, as this is the period of highest ant activity in both the wet and dry season (Peng and others 2012). Trail scores were determined on two successive days by counting the number of *O. smaragdina* trails on all major branches in the tree (Figure 1). If a trail divided into two trails at the successive division of the branch (see asterisk on Figure 1), the trail score assessments were made on the individual branches. Each trail was graded on a six-point scale divided by 6, according to the ant density on the trail. Thus, ant trails were assigned a score of 1 if there were 1–10 ants m^{-1}, 2 if there were 10–20 ants m^{-1}, 4 if there were 20–40 ants m^{-1}, and 6 if there were more than 40 ants m^{-1}. The total trail score of the tree was given as the sum of ant trails divided by 6. By a subsequent measurement of the total ant nest volume in the tree, the relation between the trail
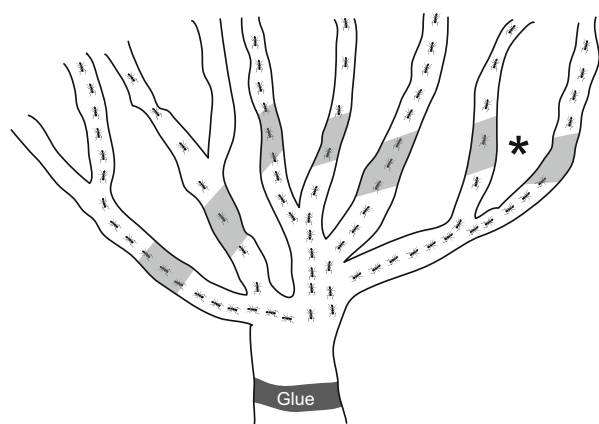
Figure 1. Schematic representation of a mango tree with ant trails. *Shaded area* represents the point for trail score determination. *Asterisk* represents a branch with successive division of ant trails (from Pinkalski and others (2015)).

score and total ant nest volume in the tree was obtained (see supplementary section S1 for an in-depth description of determining host tree nest volume in relation to trail score).

Constructing the Models for Ant Manure Deposition Assessment

By combining the relation between nest volume and ant manure deposition with the relation between trail score and total host tree nest volume, the ant manure deposition in a host tree inhabited by *O. smaragdina* could be predicted from the trail score. To assess the uncertainty of the prediction, a Bayesian latent variable model (Clark 2007; Gelman and others 2003) was fitted to the data (see supplementary section S2 for the development of the model).

As a consequence of significant differences between the sampling occasions, three model outputs were constructed: Model 1 is based on the data from sampling occasions 1–2, which had the lowest rate of manure deposition; Model 2 is based on the data from sampling occasion 3, which had the highest rate of manure deposition; and Model 3 is based on the combined data from the three sampling occasions and hence represents intermediate levels of ant manure depositions.

Application of the Models—Ant Manure Deposition Assessment in Plantation 1

In the wet season before the sampling of nests began, trail scores were determined on two successive days on all trees in plantation 1 harboring *O. smaragdina* colonies. Furthermore, the diameters of

the tree crowns were measured allowing for calculation of the projected surface area of the tree. Using the models developed in this study, the number of ant manure spots deposited in each tree was predicted. Calculation of nutrient deposition from ant manure, expressed as amount of urea-N as well as total nitrogen, was based on the data from (Vidkjær and others 2015b, unpublished data). In this study, it was found that on average, a single ant manure spot contained 133 µg dw total ant manure, 4.1 µg of total nitrogen, and 0.5 µg of urea-N.

Effect of Sucrose Solution Availability on Ant Manure Deposition

As a surrogate for nectar and/or honeydew, the effect of sucrose solution availability on ant manure deposition was tested in a separate experiment. In the dry season, 11 nests from 3 colonies in plantation 1 were cut down and placed in cardboard boxes (as above). A tube (5 mL) filled with 20% sucrose solution was placed inside the box. A cotton plug in the tube prevented the sucrose solution from leaking into the box but allowed the ants to drink. As above, the manure deposition rate throughout the experiment was assessed; the total number of ant manure spots deposited after 24 hours was counted and the nest dimensions were measured.

Statistics

To test for differences in ant manure deposition, data were normalized to ant manure spots deposited per liter of nest and ant manure spots per ant biomass. The data were log transformed to achieve normality of residuals. Comparing ant manure spot deposition between the sampling occasions 1 and 3, the effect of sampling was tested using a likelihood ratio test by comparing hierarchical linear mixed models (sampling as a fixed factor and colony as a random factor) with 2 degrees of freedom. Pairwise comparisons between sampling occasions were analyzed with linear mixed models with colony as a random factor. All Analyses were carried out using R.

RESULTS

Ant Manure Deposition in Relation to Nest Volume

The ant manure deposition in nests from sampling occasions 1–3 increased linearly as a function of nest volume (Figure 2). Although there was no
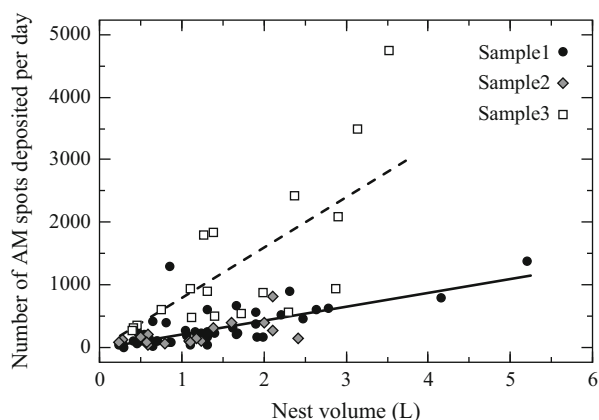
Figure 2. Relationship between nest volume and number of ant manure spots deposited per day from sample 1–3 ($n = 80$). Samples 1–2 were from Plantation 1 in the wet and dry season, respectively, and sample 3 was from plantation 2 in the dry season. The regression equation for samplings 1–2 (*thick line $R^2 = 0.47$*) and sampling 3 (*broken line $R^2 = 0.56$*) was 221 and 800 ant manure spots $day^{-1} \times$ nest volume, respectively.

difference in ant manure deposition in relation to nest volume between nests sampled from plantation 1 in the wet and dry season, respectively ($F_{1,57} = 0.3$, $P = 0.57$), nests sampled from plantation 2 in the dry season showed an almost fourfold higher ant manure deposition in relation to nest volume (Likelihood ratio $= 14.9$, df $= 2$, $P < 0.001$). Correspondingly, there was no significant difference in the number of manure spots deposited in relation to ant biomass between ants sampled in plantation 1 in the wet and dry season ($15.5 \pm$ SD 3.6 vs. $9.9 \pm$ SD 3.7 manure spots ant biomass^{-1} (g wet wt) day^{-1}, respectively; $F_{1,57} = 2.0$, $P = 0.17$), whereas ants from plantation 2 deposited significantly more manure spots in relation to ant biomass ($23.1 \pm$ SD 5.9 ant manure spots ant biomass^{-1} (g wet wt) day^{-1}; Likelihood ratio $= 6.6$, df $= 2$, $P = 0.038$). The mean ant manure deposition of the three sampling occasions combined was 17.4 (SD 3.2) manure spots ant biomass^{-1} (g wet wt) day^{-1}.

The rate of manure deposition by ants without access to sugar solution peaked during the first few hours of the experiment, which was especially pronounced by the ants from plantation 2. However, ants from plantation 1 that were provided with sucrose solution showed an increasing rate of manure deposition throughout the experiment (Figure 3). Most interestingly, this rate of manure deposition resembled a mirror image of the ants from plantation 2. Although these ants initially had a high rate of manure deposition, it declined
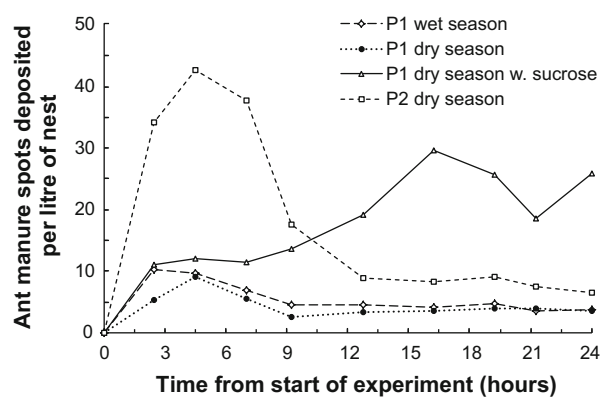

Figure 3. Rate of ant manure deposition throughout the experiment by ants from plantation 1 (P1) with access to sucrose solution (P1 dry wt. sucrose): $n = 11$) and without access (P1 wet season: $n = 26$; P1 dry season: $n = 14$ and, Plantation 2 (P2) dry season: $n = 14$). *Error bars* have been omitted for clarity.

throughout the experiment ending up with a rate similar to unfed ants from plantation 1. On the contrary, the manure deposition by ants provided with sucrose solution rose from a level similar to the ants in the same plantation without access to sucrose solution, ending up with at rate similar to the ants from plantation 2 at the beginning of the experiment.

Consequently, the number of manure spots deposited in relation to nest volume by ants with access to sucrose solution was on average 565 manure spots day$^{-1} \times$ nest volume, which was significantly different ($F_{1,24} = 35.8$; $P < 0.0001$) and almost threefold more than ants from the same plantation without access to sucrose solution (compare with regression equations in Figure 2). Furthermore, the manure deposition of ants provided with sucrose solution was not statistically different from the manure deposition of ants in plantation 2 ($F_{1,4} = 0.96$; $P = 0.38$), indicating that the amount of ant manure deposited is highly dependent on the availability of plant-derived exudates.

Ant Manure as a Function of Trail Score—The Models

The prediction models for ant manure deposition (Figure 4) showed generally a linear increase as a function of trail score. To allow for a general application, the prediction models were approximated by linear models which would allow the prediction of ant manure spot deposition in a tree directly from the trail score (Table 1). It should be noted that in the ant manure deposition experi-
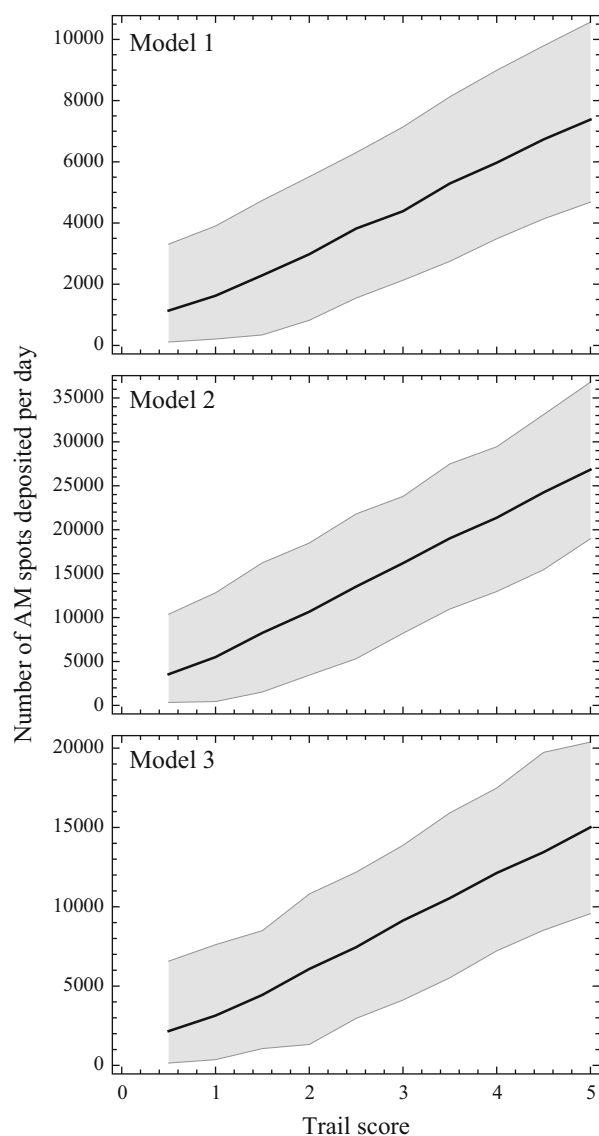
ment, on which the models are based, the ants did not have access to sucrose or water which may have negatively affected the deposition rates. Thus, it is possible that the rates have been underestimated, and the values in this study may be regarded as conservative estimates.

Ant Manure Deposition Assessment in Plantation 1

A total of 40 trees in the plantation (roughly 50% of the trees) were inhabited by *O. smaragdina* (Table 2).

Five colonies were identified with territories ranging from 2 to 14 trees. Trail scores ranged from approximately 0.5 on trees where there were only few and low density ant trails to 3.9 on the tree with the highest trail score. On such trees, most of the major branches were occupied by ant trails. The number of ant manure spots deposited, predicted by model 3 above, was on average 3556 (SD = 672) spots tree^{-1} day^{-1}, whereas the predictions from Models 1 and 2 ranged from 1830 (SD = 350) to 6108 (SD = 1134) spots tree^{-1} day^{-1}, respectively. The average tree crown area (projected surface) was 27.7 m^2 (SD = 16.0 m^2).

The number of ant manure spots was converted to average daily depositions of ant manure, urea, and total nitrogen with the calculations based on the ant manure content data from Vidkjær and others (2015b, unpublished data) (Table 3). Extrapolated to a yearly basis, the average deposition of ant manure in the plantation was 62.3 (range 32.1–107.0) kg ha^{-1} y^{-1} corresponding to 1.9 (range 1.0–3.3) kg total nitrogen and 0.2 kg (range 0.1–0.4) urea-N ha^{-1} y^{-1}.

DISCUSSION

Here we quantified the manure deposition in a tropical agroecosystem by the dominant arboreal ant *O. smaragdina*. We show that colonies of *O. smaragdina* deposit ant manure in quantities that may have hitherto unappreciated impacts on the nitrogen acquisition of host trees and that ant

Figure 4. Number of ant manure spots deposited in a tree per day as a function of trail score in each of the three model outputs. Model 1 may represent conditions under which the ant manure deposition is at a low rate, Model 2 may represent conditions that produce high ant manure deposition rates, and Model 3 is based on the data from both Models 1 and 2. *Thick lines* represent median values and *shaded areas* represent 95% credible intervals.

Table 1. Number of Ant Manure Spots Deposited per Day as a Function of Trail Score

		Model equation*	Standard deviation
#Ant manure spots tree^{-1} day^{-1}	Model 1	347 + 1407 × [trail score]	898 + 123 × [trail score]
	Model 2	705 + 5126 × [trail score]	3017 + 311 × [trail score]
	Model 3	513 + 2886 × [trail score]	1724 + 235 × [trail score]

The three model equations approximated the original models with an R^2 of at least 0.997
Model 1 may represent conditions under which the ant manure deposition is at a low rate, Model 2 may represent conditions that produce high ant manure deposition rates, and Model 3 is based on the data from both Models 1 and 2.

Table 2. Number of Colonies, Trees Occupied, Average Trail Score per Colony, and Daily Deposition of Ant Manure Spots in Plantation 1 in the Wet Season

Colony	Trees occupied	Average trail score	Ant manure spots colony^{-1} day^{-1}
1	7	0.8 [0.5–1.5]	**19,469** [10,170–33,127]
2	14	1.1 [0.7–2.3]	**50,243** [25,853–86,330]
3	13	0.6 [0.5–1.3]	**30,005** [15,888–50,597]
4	4	2.5 [1.8–3.9]	**31,399** [15,697–54,994]
5	2	1.8 [1.7–1.8]	**11,129** [5620–19,351]
Total	40	1.1 [0.5–3.9]	**142,246** [73,229–244,339]

Values in bold show the total for each colony predicted by Model 3 and numbers in square brackets represent range. For ant manure deposition, the min-max range is the predictions by Model 1 and 2, respectively.

Table 3. Average Amount of Ant Manure, Urea-N, and Total Nitrogen Deposited Daily per Tree, Area, and Ant Biomass

Average	Total ant manure (dw)	Urea-N	Total nitrogen
Per tree (mg tree^{-1} day^{-1})	**473.0 mg** [243.5–812.4 mg]	**1.66 mg** [0.9–2.9 mg]	**14.6 mg** [7.5–25.0 mg]
Per area (mg m^{-2} day^{-1})	**17.1 mg** [8.8–29.3 mg]	**0.06 mg** [0.03–0.1 mg]	**0.5 mg** [0.3–0.9 mg]
Per ant biomass (μg/g^{-1} wet wt ant biomass day^{-1})	2300 μg (SE 400)	8.1 μg (SE 3.3)	71.3 μg (SE 13.0)

Values in bold were calculated based on the predictions from Model 3, while values in square brackets represent min–max ranges calculated from the predictions of Models 1 and 2, respectively.

manure deposition seems to be highly affected by the availability of carbohydrate exudates.

The rate of ant manure deposition may be affected by the current habitat conditions, as reflected by the marked difference in manure deposition by ants from the two plantations. In plantation 1, the number of newly flushed leaves and flowers was low compared to plantation 2. Furthermore, the abundance of hemipterans was more than 10fold higher in plantation 2 (Pinkalski 2015, unpublished data). As *O. smaragdina* is heavily dependent on a continuous acquisition of carbohydrates from either nectaries or honeydew (Blüthgen and Fiedler 2002; Blüthgen and Stork 2007; Davidson 1997), the ants in plantation 1 may have suffered from carbohydrate deprivation. This is supported by the finding that the manure deposition in relation to ant biomass was higher in plantation 2. This may also explain the marked difference in the rate of manure deposition during the experiment (Figure 3). Although ants from plantation 1 initially had a very low rate of manure deposition compared to ants from plantation 2, the rates of manure deposition at the end of the experiment were similar. The high initial rate of manure deposition by ants from plantation 2 may be because these ants at the beginning of the

experiment had been well supplied with sugary exudates. Thus, concurrently with depletion of their carbohydrate supply, the rate of manure deposition decreased. Most strikingly, the ants from plantation 1 provided with sucrose solution showed a rate of manure deposition that was nearly a mirror image of the ants from plantation 2. Here, the rate initially was similar to the ants from the same plantation without sucrose solution, but during the experiment, manure deposition increased to an extent resembling the initial rate of ants from plantation 2. It is possible that with plenty of carbohydrates available, the increased rate of manure deposition is caused by deposition of excess carbohydrates rather than nitrogenous excretory compounds. However, in a laboratory study, *O. smaragdina* colonies fed solely with sugar or sugar and cockroaches in alternating 14-day cycles did not differ in ant manure urea content between the two dietary cycles (Vidkjaer and others 2015b, unpublished data). Consequently, the augmenting effect of sucrose solution on ant manure deposition may be through increased ant activity rather than deposition of excess carbohydrates. This finding also lends support to the hypothesis that carbohydrates increase colony tempo (Kay and Adler 2003; Kay and others 2012).

It cannot be excluded that the increased deposition of ant manure was caused by access to water as the experiment was performed in the dry season. However, that may be, the role of nectaries or trophobionts seems to have a critical impact of the quantity of ant manure deposition, as these, at least in the dry season, would be the only source to obtain water and carbohydrates. Stable isotope signatures have revealed that in young secondary forests, *O. smaragdina* colonies were more enriched in ^{15}N, indicating a more predacious diet, compared to adjacent mature forest. In the mature complex forest, homopteran aggregations and nectar plants were more abundant which allowed the *O. smaragdina* colonies to a larger extent to include these sources in their diet (Blüthgen and others 2003). On the other hand, the reduced diversity of suitable nectar and honeydew sources in the secondary forest, which especially may be pronounced in a monoculture agroecosystem such as a mango plantation, the *O. smaragdina* colonies were to a larger extent predatory. Consequently, the ant manure deposition may vary between different types of habitats and may be expected to be larger in habitats with abundant honeydew and nectar sources.

Oecophylla smaragdina is presently being used as bio-control agents in orchards (Offenberg 2015; Offenberg and others 2013; Van Mele 2008), and an additional benefit, besides the reduction of arthropod pests, may be the fertilizing effect of ant manure. According to Huett and Dirou (2000), the yearly nitrogen removal from a 10 t ha^{-1} production of mango crop is 11 kg ha^{-1}. Thus, the nitrogen from ant manure deposition, which on average ranged from 1.0 to 3.3 kg ha^{-1} y^{-1}, may contribute to about 9–30% of the nitrogen needed for fruit production. From a farmer's perspective, this amount seems inadequate to support full production. It may, however, augment the production of fruits. The deposition of ant manure is to a higher extent delivered where needed compared to conventional fertilizers applied on the plantation floor where nutrients can be lost due to, for example, leaching or competition from undergrowth vegetation. The activity of *O. smaragdina*, and hence the manure deposition, is highest on newly flushed leaves and fruits (Hölldobler and Wilson 1978; Offenberg 2007; Offenberg and others 2006), where the requirement for nutrients is highest. This ''on-site'' deposition of nutrients may enhance the nutrient availability for the host tree, especially if urea, a commonly applied compound for foliar fertilization, in the ant manure can be taken up directly through the leaves (Abad and others 2004;

Readman and others 2002; Varga and Svecnjak 2006; Vidkjær and others 2015a; Vidkjær and others 2015b, unpublished data). Consequently, our data may support the hypothesis of Barzman and others (1996) that manure from *O. smaragdina* positively affects the quality of fruits through fertilization, although this hypothesis remains to be tested.

The average deposition rate of *O. smaragdina* manure was 62.3 kg ha^{-1} y^{-1} corresponding to 0.2 kg urea-N and 1.9 kg total nitrogen ha^{-1} y^{-1}, respectively. In comparison, the total deposition of nitrogen from invertebrate herbivores in tropical forests (given as the sum of excreta, carcasses and unconsumed leaf fragments) has been estimated to be 18.5 kg N ha^{-1} y^{-1} (Metcalfe and others 2014). Although *O. smaragdina* was the most abundant ant species in the plantation several other ant species were also present (for example, *Odontomachus* sp., *Tetraponera* sp., *Crematogaster* sp.), by which the ant manure deposition of all ant species combined would be expected to be even higher.

The information of ant density in natural habitats is limited, although extrapolating these values to a system with a known ant biomass is possible in at least one case. In a study of the abundance of ants in temperate hardwood forests, the average biomass of ants was found to be 4.87 g dw m^{-2} (King and others 2013). Temperate climates may, however, be subject to larger variations in temperature, compared to our study where the difference between seasons was negligible, which may negatively affect the metabolic rate and hence the ant manure deposition (Brown and others 2004). Nevertheless, assuming that the amount of ant manure deposited per biomass of these ants were similar to *O. smaragdina* (using a ww:dw ratio of 3:1 to convert *O. smaragdina* biomass into dry weight, Table 3), the total nitrogen deposition from ant manure under these conditions would amount to 3.8 kg N ha^{-1} y^{-1}. In comparison, the deposition of manure by ungulates in temperate grasslands has been estimated to be about 27 kg N ha^{-1} y^{-1}. Earthworms, distinguished as important ecosystem engineers due to their effect on the cycling of nutrients (Jones and others 1994), have been estimated to excrete 41.5 kg N ha^{-1} y^{-1} in a fertilized agroecosystem (Whalen and others 2000). Finally, net nitrogen mineralization in temperate forests is on the order of 71 kg ha^{-1} y^{-1} (Perez and others 1998).

The deposition of nitrogen from ant manure in temperate climates may thus compose a smaller percentage of the system level allocation of nitrogen. Nevertheless, it may still be a significant

source of nitrogen for plants. Firstly, the manure may be deposited along the foraging trails of ants, as opposed to centralized deposits in the vicinity of ant nests. Secondly, by depositing manure in the canopy of trees, the ants may ''short circuit'' the nitrogen cycle as the mineralization step in the soil is passed over and the nitrogen is deposited directly on the trees. This process may especially be significant in tropical forests where the abundance of ants, and hence manure deposition, is recognized to be considerably higher than in temperate zones (Floren and others 2014; Hölldobler and Wilson 1990).

In addition to nitrogen, studies on the composition of insect waste have shown high concentrations of other macro nutrients. For instance, relative to leaf litter, waste from *Azteca trigona* nests were enriched sevenfold in phosphorus and 23fold in potassium (Clay and others 2013). Furthermore, bee manure from *Apis mellifera* contained phosphorous and potassium levels roughly 20 times higher than reference soil (Mishra and others 2013). Thus, ant manure may also affect the availability of other nutrients than nitrogen. In addition, due to the group recruitment behavior of ants (Hölldobler and Wilson 1990), the activity of foraging workers can be locally dense under which circumstances the deposition of ant manure may become spatially concentrated. As nitrogen is a limiting nutrient for plant growth in most terrestrial ecosystems (Johnson and Turner 2014; Le-Bauer and Treseder 2008), plants hosting large numbers of ants may gain a competitive advantage in their competition for nutrients, by which the deposition of ant manure may be a driver in the evolution of ant-plant mutualisms (Defossez and others 2011; Sagers and others 2000).

In conclusion, this study reveals that colonies of *O. smaragdina* may provide their host trees with a significant amount of nitrogen and that nutrients deposited by ants on a community scale may represent a substantial part of the nutrient cycling. Further studies on the deposition rates and composition of ant manure, as well as a more comprehensive understanding of the abundance of ant biomass, can help elucidate the evolutionary and community level significance of ant manure.

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