



## Dump size and distance from the nest define the investment in waste management in *Sericomyrmex mayri* (Formicidae: Myrmicinae: Attini)

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**ABSTRACT.** The farming system practiced by non-leaf-cutting ants *Sericomyrmex mayri* (Formicidae: Myrmicinae: Attini) involves a mutualistic interaction with symbiotic fungi. Their colonies' success depends on several factors, such as the fungus garden protection against pathogens. Garden contamination may depend on the aspects involved in waste management. However, specific local conditions, including nest and dump sizes, nest-dump distances and abiotic conditions are relevant and overlooked, especially for non-leaf-cutting ants. We aimed to explore the natural history involved in *Sericomyrmex mayri* waste management. Hence, we documented the relationships between 1) the foraging rate and waste removal activities with dump size, 2) the size of refuse dumps and their distance from nest entrances, and 3) the foraging activity and the rate of waste disposal with climatic conditions. The study was developed in the Atlantic Forest, Southeastern Brazil, wherein 33 colonies' activities were monitored for three consecutive days, during different day-periods. More prominent dumps received less waste and were located at further distances from the nest. Additionally, daily temperature and humidity positively influenced waste disposal and foraging activities, respectively. These findings provide information on how dump size and distance from the nest may define the investment of waste management of non-leaf-cutting ants.

[Keywords: fungus garden; mutualism; fungus-growing ants]

**RESUMEN.** El tamaño del basurero y la distancia del nido definen cuánto invierte *Sericomyrmex mayri* (Formicidae: Myrmicinae: Attini) en el manejo de los residuos. El sistema de cultivo que practican las hormigas no cortadoras de hojas *Sericomyrmex mayri* (Formicidae: Myrmicinae: Attini) incluye una interacción mutualística con hongos simbióticos. El éxito de sus colonias depende de varios factores, como, por ejemplo, la protección del jardín del hongo contra los microorganismos patógenos, lo cual es clave debido a la naturaleza obligatoria de esta asociación. Se sabe que la contaminación del jardín del hongo se relaciona con el manejo de los residuos por parte de las obreras. Por lo tanto, nuestro objetivo fue investigar la historia natural de la gestión de residuos en *Sericomyrmex mayri*. Para esto, documentamos las relaciones entre 1) las actividades de recolección de residuos y forrajeo con el tamaño del basureros, 2) el tamaño de los basureros y su distancia de las entradas de los nidos, y 3) la actividad de forrajeo y la tasa de eliminación de residuos con las condiciones climáticas. El estudio se desarrolló en el Bosque Atlántico, al sudeste de Brasil, donde se monitoreó la actividad de 33 colonias durante tres días consecutivos, en diferentes momentos del día. Los basureros más grandes recibieron menos residuos y se encontraron a distancias mayores del nido. Además, la temperatura y la humedad diarias influyeron de manera positiva en la eliminación de residuos y las actividades de forrajeo, respectivamente. Estos hallazgos aportan información sobre cómo el tamaño del basurero y la distancia desde el nido pueden definir la inversión en el manejo de desechos de hormigas no-cortadoras de hojas.

[Palabras clave: jardín de hongos; mutualismo; hormigas cultivadoras de hongos]

### INTRODUCTION

The farming system practiced by Attini ants involves a mutualistic interaction with symbiotic fungi (Chapela et al. 1994). These ants have been traditionally considered to maintain their fungal gardens as pure cultures in almost total isolation from other organisms (Müeller et al. 2001). Hence, garden protection against pathogens is an important strategy to achieve fungus cultivation success. Garden contamination relies on distinct aspects

of waste management. For example, the location of waste (in underground chambers or on external refuse piles) may affect the probability of fungal infections, as well as the quantity of organic waste deposited (Farji-Brener et al. 2016). Since refuse dumps are known as a source of fungal pathogens, some authors suggest that waste disposal should be located as far as possible from the nest foraging entrances to avoid contamination (Weber 1972; Bot et al. 2001; Farji-Brener et

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al. 2016). Additionally, it is expected that bigger colonies are more active, producing more substantial amounts of waste and refuse piles, and that their activities will be positively influenced by temperature and humidity (Ribeiro and Navas 2007; Gibb et al. 2015).

Over 250 species of fungus-growing ants (Formicidae: Myrmicinae: Attini: Attina) occur in the New World, mostly in the Neotropical Region (Brandão and Mayhé-Nunes 2008; Ješovnik et al. 2018). The majority of Attini genera (17 out of 19) cultivate fungus on dead organic material (e.g., dried leaves, flowers, insect frass), while the evolutionarily derived genera, *Atta* and *Acromyrmex*, so-called leaf-cutting ants, use freshly cut vegetation as substrate (Schultz and Brady 2008). Mycophagy has evolved multiple times (Hammond and Lawrence 1989), as an essential form of nourishment (Wheeler 2015). Most studies on fungus-growing ants are focused on leaf-cutting species (*Atta* and *Acromyrmex*), particularly involving its control and agricultural importance (Cherrett 1986; Leal et al. 2014). Although non-leaf-cutting species represent the majority of Attini diversity (Mehdiabadi and Schultz 2010), their natural history still is overlooked.

To our knowledge, there are little field observations about the waste management of non-leaf-cutting ants. *Sericomyrmex* (Mayr 1865) is a Neotropical widespread non-leaf-cutting genus that practices generalized higher agriculture (i.e., so-called non-leaf-cutting "higher Attini"), in which they cultivate a symbiotic Leucoprineae tribe fungi (Schultz and Brady 2008; Mehdiabadi and Schultz 2010). In this obligate association, the ant provides to the fungus nutrients and protection against parasites and competitors, disperse their hyphae, and feed on them (Weber 1972; Hölldobler and Wilson 1990). *Sericomyrmex* comprises 11 species (10 recorded for Brazil) that nest in the soil, especially in forest clearings and ravines (Baccaro et al. 2015; Ješovnik and Schultz 2017). Their nest can reach 79 cm in depth, hold up to 6000 workers, and sustain up to 18 chambers (Ješovnik et al. 2018). Nests entrances are featured by a hole on the ground (Ješovnik and Schultz 2017) and their refuse dumps are external.

Here we explored some aspects of the natural history involved in *Sericomyrmex mayri* waste management. Its colonies vary in size (with ca. 800 to 5000 individuals), are located near the

soil surface, and have external refuse dumps (Weber 1969; Ješovnik et al. 2018). *S. mayri* workers are small, cryptic, move slowly and mostly forage alone or eventually forming short columns in nest entrance immediate area (Ješovnik 2018; Ješovnik and Schultz 2017). However, some aspects involved in *S. mayri* waste management, such as dump size and nest-dump distance, are practically unexplored (only a single record of 50 cm (Ješovnik et al. 2018)). In this study, we documented the relationships between 1) the foraging rate and waste removal activities with dump size, 2) the size of refuse dumps and their distance from nest entrances, and 3) the foraging activity and the rate of waste disposal with climatic conditions.

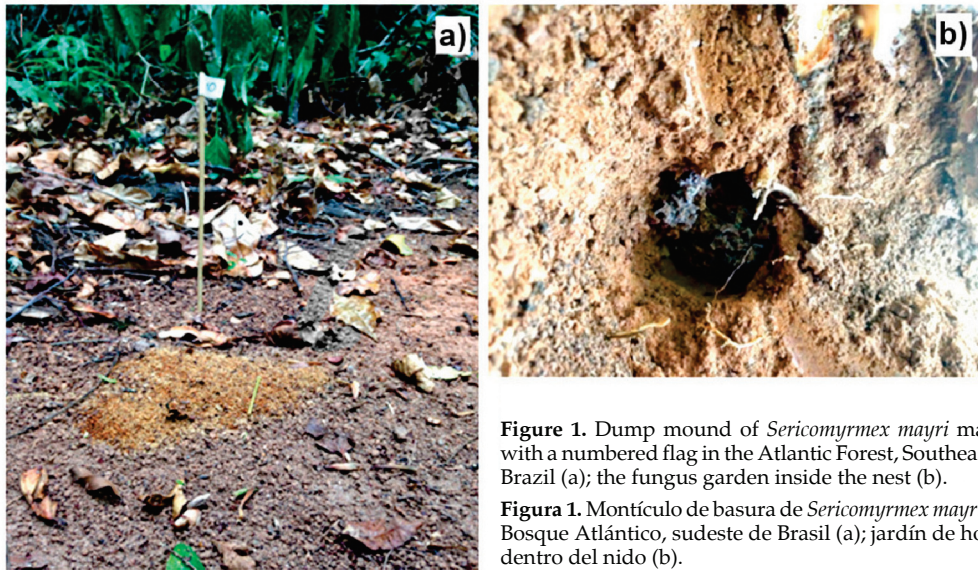
## MATERIALS AND METHODS

### *Study site*

The study was conducted in Rio Doce State Park, Minas Gerais, Brazil. The soil in the region is acid with the predominance of clayey content (Souza et al. 2012). The climate is Aw, according to Köppen's classification, with markedly rainy periods from November to February and well-defined droughts from March to October (Alvares et al. 2013). The average annual rainfall varies from 1350 to 1900 mm and relative air humidity and temperature are around 79% and 23 °C, respectively (CBHRD 2013). The field experiment was performed along "Vinhático" trail (19°42'23" S - 42°34'33" W), a secondary forest path of three meters wide and 1.04 km long (Lopes et al. 2002).

### *Sampling design*

We selected all *Sericomyrmex mayri* colonies found along the trail that were spaced by at least 10 meters. Their colonies in the studied site are easy to locate due to the dark yellow coloration of their external refuse dumps, which are quite distinct from forest ground (Figure 1a). Thus, a total of 33 colonies and its corresponding dumps (recognized by previous observations of midden workers along nest-dump paths) were tagged and enumerated with flags (Figure 1a). We sampled at least one specimen from each colony to validate species identification, and few ones were open to characterize the fungus garden (Figure 1b, evidencing fungus garden). Ant identification was determined with the key available in Ješovnik and Schultz (2017).



**Figure 1.** Dump mound of *Sericomyrmex mayri* marked with a numbered flag in the Atlantic Forest, Southeastern, Brazil (a); the fungus garden inside the nest (b).

**Figura 1.** Montículo de basura de *Sericomyrmex mayri* en el Bosque Atlántico, sudeste de Brasil (a); jardín de hongos dentro del nido (b).

Observations were performed during the end of the dry season (October of 2017), in the mornings (8:00 to 12:00 h) and afternoons (12:00 to 17:00 h). Throughout the sampling days, there was no rain in the region, which assures that dump size and its distance from the nest remain similar throughout the days. Each colony was monitored four times for approximately seven minutes at different periods of the day, to encompass a broader heterogeneity of local day-period conditions. Hence, 33 colonies were monitored per 28 minutes, during three consecutive days, in order to cover the lack of a broader temporal sampling. Observations consisted of registering the number of workers performing two main activities: waste management (i.e., midden workers depositing waste on the external refuse dumps or transporting it along nest-dump paths), and foraging (i.e., workers carrying food sources, such as pieces of flowers and leaves, and patrolling nest territories). Eventual patrolling events were grouped with foraging due to the difficulty of distinguish them in certain situations; for instance, when the food source is liquid. Distances between dumps and nest entrances were measured, and dumps size were estimated according to the circle area ( $\pi.r^2$ ). Air temperature and relative humidity were recorded for each observation using a thermo-hygrometer.

#### *Statistical analyses*

The first and third objectives were tested with generalized linear mixed-effects models (GLMMs). In GLMMs, we fitted fixed and random effects that accounted for our repeated

measurements (Crawley 2013). We included the sampling period as a random effect on the intercept for all models (1 | period), to cope with our repeated temporal sampling in each nest (Bates et al. 2014). First, to verify the relationship of workers' disposing waste and foraging activities with dump size, we fitted dump size as response variable (with Gaussian distribution) and both workers' activities as predictor variables of fixed effects. To test the second objective (the relationship between the size of refuse dumps and their distance from the nest entrance), we built a generalized linear model (GLM). In this model, the nest-dump distance was the response variable (Gaussian distribution) and the dump size the predictor variable. Lastly, to test the relationship of temperature and humidity (predictors of fixed effects fitted in simple and quadratic terms) with the number of workers disposing of waste in dumps (Poisson distribution) and the number of foraging workers (Poisson distribution), we built two distinct GLMM models.

Models' residuals were analyzed, as well as the suitability of error distribution chosen. Complete models were simplified until the minimum suitable model by backward selection removing non-significant variables ( $P < 0.05$ ). We performed all analyses in R (R Development Team 2017).

## RESULTS

We registered a maximum of 15 workers foraging ( $1.15 \pm 0.19$ ; mean  $\pm$  SE) and 15 workers performing waste disposal ( $1 \pm 0.2$ )

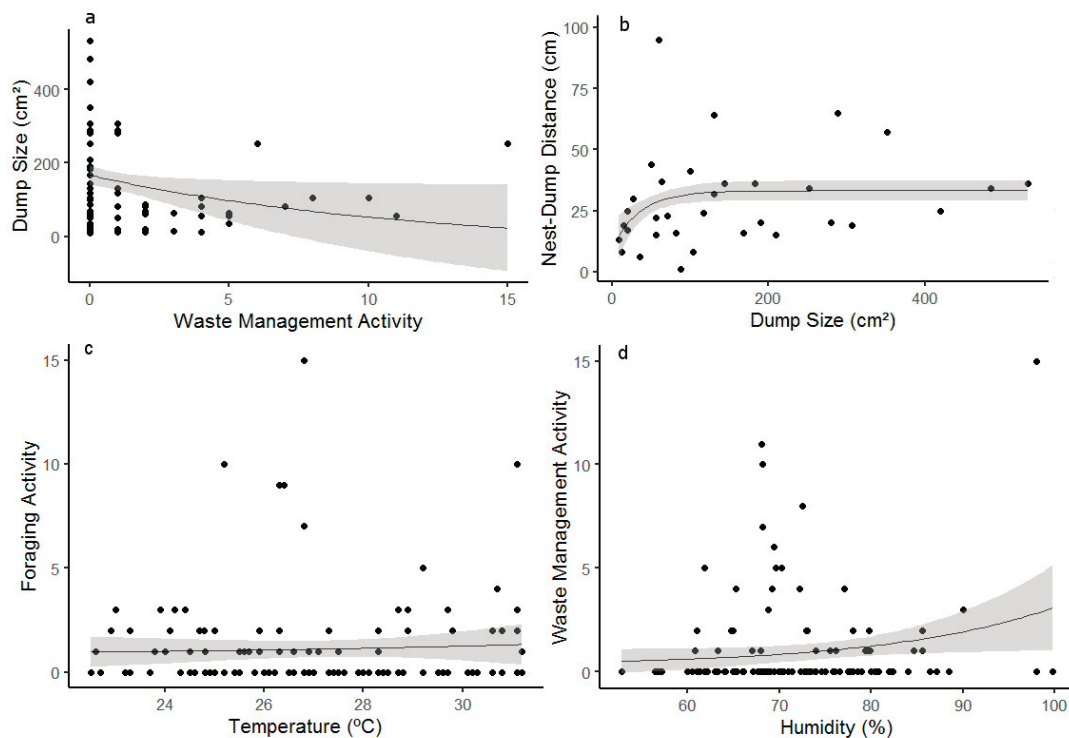
per observation. The size of the studied dumps ranged from eight to 532 cm<sup>2</sup> (153±12), while nest-dump distances varied from one to 95 cm (28.9±1.67). The temperature during the study varied in 8.5 degrees (from 22.7 °C to 31.2 °C; 26.8±0.19), whereas the humidity ranged from 52.8% to 99.8% (76.8±0.75).

We observed reduced waste disposal activities (including zero activity) in larger dumps (GLMM, estimate: -0.0846, z-value: -19.734,  $P < 0.001$ ) (Figure 2a), while foraging activity was not related with dump size (GLMM, estimate: -0.0014, z-value: -0.454,  $P = 0.650$ ). Moreover, bigger dumps were more distant from its respective nest (GLM, deviance: 2004.4,  $F: 5.6134, P = 0.01932$ ) (Figure 2b). Additionally, temperature during the day positively affected foraging activity (GLMM, estimate: 1.12, z-value: 2.33,  $P = 0.0197$ ) (Figure 2c), but did not affect waste disposal (GLMM, estimate: 2.764, z-value: 1.318,  $P = 0.1876$ ). Humidity positively affected the activity of midden workers (GLMM, estimate: 3.92, z-

value: 6.1,  $P < 0.001$ ) (Figure 2d), but did not affect foraging (GLMM, estimate: 0.00032, z-value: 0.023,  $P = 0.9819$ ).

## DISCUSSION

Our study revealed that larger dumps receive less waste disposal and are located at further distances from the nest. We also observed that waste disposal and foraging are higher during wetter and warmer periods of the day, respectively. These results reveal some interesting aspects involved in waste management of *Sericomyrmex mayri*. We observed that larger dumps tend to receive less waste and are further from their nests. It seems that shorter distances may allow the same individual to perform the same path multiple times in a shorter time. Some findings indicate that the fungus-growing ant *Acromyrmex lobicornis* tends to dispose waste in closer dumps, as this material can be recycled in periods of lower resource availability (Farji-Brener and Tadey 2012). Conversely, short



**Figure 2.** Smaller waste management activities (including zeros) were observed in bigger dumps (a), and larger dumps are further from its respective nest (b). Foraging activity is greater during warmer periods (c), while waste management is higher during wetter periods (d). Gray shadows represent the confidence intervals of dependent variables. Curves were fitted with parameters from GLM (a) and GLMM (b, c, d) models.

**Figura 2.** Las menores actividades de manejo de residuos (incluyendo ceros) se observaron en los basureros más grandes (a). Los basureros más grandes se ubicaron más lejos de sus respectivos nidos (b). La actividad de forrajeo es mayor durante periodos más cálidos (c), mientras que el manejo de residuos es mayor durante periodos más húmedos (d). Las sombras grises son los intervalos de confianza de las variables dependientes. Las curvas fueron modeladas con los parámetros de modelos GLM (a) y GLMM (b, c, d).

nest-dump distances can restrict foraging territories, as foraging workers often avoid paths that cross waste piles and contact with midden workers (Farji-Brener et al. 2016). Actually, larger (>350 cm<sup>2</sup>) and more distant (>50 cm) dumps had almost no activity (Figure 2b), which might indicate its desertion. Dump desertion is related to the risk of contamination (Curtis et al. 1999), as infected parts likely are discarded in external mounds (Bot et al. 2001). This behavior might be related to dump size, as it usually reflects colony size and age (Hart et al. 2002). Therefore, larger dumps generally are older, have more infectious agents, and hence, might be abandoned when its dimension assumes the infection risk. These relationships may suggest a trade-off between the risk of keeping a waste pile that is close enough to assure an effective waste management (i.e., allowing several disposal rounds in a shorter time), without limiting foraging activity (i.e., preventing midden and foraging workers meetings).

Waste disposal and foraging activities increased in wetter and warmer periods of the day, respectively. Several studies show the positive effects of abiotic conditions - especially temperature - on ant activity (e.g., Nielsen 1986; Diamond et al. 2012). Nevertheless, very humid environments generally favor pathogen proliferation in waste (Farji-Brener and Tadey 2012). Indeed, we observed peaks of waste and foraging activities in intermediate values of temperature (ca. 27 °C) and humidity

(ca. 65%). These findings suggest that colony activity may be restricted both by abiotic conditions on foragers, as well as by the risk of contamination of midden workers.

In summary, our study brings insights into how dump size and distance from the nest may define the investment of waste management by *Sericomyrmex mayri*. It is plausible that to reduce the infection risk, larger refuse dumps receive less waste deposition when reaching a specific volume and are located far from the nest entrance. These associations suggest a trade-off among refuse size, refuse location, and foraging rate. However, since our data come from a short-sampling period, studies encompassing larger periods and some experimental protocol are needed to confirm whether the location of refuse dumps and their abandon at certain size/age are behaviors that effectively reduce the risk of infection via refuse contamination.

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