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Walking through uneven terrains: Unraveling worker dynamics in foraging activity

Juiz de Fora

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Dissertação apresentada ao Programa de Pós-graduação em Biodiversidade e Conservação da Natureza da Universidade Federal de Juiz de Fora como requisito parcial à obtenção do título de Mestre em Ciências Biológicas. Área de concentração: Comportamento, Ecologia e Sistemática

Orientador: Dra. Juliane Floriano Lopes Santos

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Chapter 2 - Ant carpooling: a study on the effects of trail difficulty on hitchhiking occurrence

LIST OF ABBREVIATIONS AND ACRONYMS

F	Number of workers
q	Foraging Flow
BL	Body Length
HW	Head width
ALA	Body area considering the last pair of legs and the antennas
BA	Body area without legs and antennas
BAR	Body area ratio
LR	Multiple linear regression
LMM	Linear mixed-effects model
GLMM	Generalized Linear Mixed Model
GLM	General linear model
OW	Outbound workers
UW	Unloaded inbound workers
LW	Loaded inbound workers
HW	Loaded inbound workers with hitchhiker

LIST OF SYMBOLS

±	Plus-minus
$\bar{x}$	Mean
β	Beta
µ	Micro

SUMMARY

Presentation

This dissertation examines the impact of trail surfaces on the foraging task of leaf-cutting ants in both natural and laboratory conditions. Foraging efficiency is fundamental to the success of *Acromyrmex subterraneus* colonies, as it ensures a continuous supply of plant material for fungal cultivation. However, foraging is not only shaped by internal colony factors but also by external environmental conditions. Therefore, trail surface characteristics affect key aspects of foraging dynamics, including worker foraging flow, walking speed, and leaf transport efficiency.

Chapter 1 investigates the impact of trail surface on foraging workers by analyzing how different surfaces unevenness influences the allocation of workers, locomotion factor and leaf transport efficiency. This chapter evaluates how three trails surface with different degrees of unevenness affect movement dynamics and whether certain substrates impose greater constraints on foragers. Understanding these effects is essential for assessing the role of environmental heterogeneity in shaping foraging efficiency.

Chapter 2 builds upon this by examining how natural trail surfaces influence the occurrence of hitchhiker workers and their role in maintaining foraging efficiency. Hitchhikers, small workers that ride on leaf fragments carried by foragers, are thought to alleviate traffic congestion by reducing worker density on trails. Since terrain can impact forager mobility, it may also influence the prevalence and function of hitchhikers. This chapter explores whether hitchhiking behavior serves as an adaptive strategy to overcome the surface irregularities challenges.

Together, these chapters provide a perspective on how environmental conditions shape foraging strategies in leaf-cutting ants. By combining analyses of worker performance with behavioral adaptations like hitchhiking, this dissertation contributes to a broader understanding of how leaf-cutting ants optimize resource collection in complex and variable landscapes.

Chapter 1 - Walking through uneven terrains: Unraveling worker dynamics in foraging activity

Abstract

During foraging, leaf-cutting ant workers are faced with many challenges in the external environment, such as physical obstacles and uneven terrain, which can hinder their movement and potentially compromise foraging efficiency. The foraging efficiency must be maintained since it is crucial for the development and survival of the colonies. Under this strong selective force, we can expect that the behavioral flexibility of the workers and the mechanism of task allocation will be the tools used to allow them to overcome the difficulties. This study investigated the strategies adopted by *Acromyrmex subterraneus* workers, focusing on how trail unevenness impacts the workforce allocation, the forager worker velocity, and thus the overall foraging efficiency. We used three 3D-printed foraging trails to simulate different degrees of unevenness on which workers traveled between the colony and foraging arena. We measured the foraging flow, density of workers considering their size classes, occupancy rate (estimated area occupied by a worker), burden, worker speed, and body area ratio of workers (estimated measure of worker leg spreading). Our study demonstrates that trail unevenness significantly influenced worker density, flow, and transport efficiency. Transport efficiency decreased on more uneven trails due to reduced worker speed rather than load adjustments, suggesting that workers carried optimal loads but had to modify their stride to walk a higher unevennes surface trail. These findings highlight how physical trail conditions impact foraging coordination, emphasizing the importance of well-maintained foraging trails for optimizing worker efficiency.

Keywords: *Acromyrmex subterraneus,* trail unevenness, worker allocation, foraging efficiency, leaf-cutting ants

Introduction

The search for food is a fundamental activity for animal survival, particularly in leaf-cutting ants, whose foraging behavior relies on a sophisticated system of division of labor that involves the coordination of colony population. Forager workers collect fresh plant material to cultivate their symbiotic fungus garden, which serves as the primary food source for the colony, ensuring its survival and growth (Hölldobler & Wilson 2010).

During foraging, workers encounter diverse terrains that may hinder their locomotion and impact the leaf transport efficiency (Alma et al. 2020; Clifton et al. 2020). To mitigate these challenges, ants employ behavioral strategies that enhance their adaptability to environmental constraints. One such strategy is the construction and maintenance of physical foraging trails, which facilitate movement and improve transport efficiency.

The construction of foraging trails offers a significant advantage by reducing the number of workers required to transport the same amount of food while enabling the simultaneous use of multiple routes (Lopes et al. 2016; Bouchebti et al. 2018; Caldato et al. 2020). Movement along established trails increases worker walking speed compared to traversing uncleared surfaces, facilitating load transport and enhancing locomotion efficiency (Rockwood & Hubble 1987; Lewis et al. 2008; Sales et al. 2015). However, despite these efforts, ground surfaces remain uneven, requiring workers to adjust their locomotion and balance, particularly when carrying loads (Lewis et al. 2008; Bernadou & Fourcassié 2011; Moll et al. 2013; Norton et al. 2013; Yanoviak et al. 2017).

Beyond individual adaptations, the coordination of worker allocation in response to environmental challenges plays a crucial role in optimizing foraging efficiency (Farji-Brener et al. 2018). Task allocation operates as a self-organized mechanism driven by social interactions, determining how and when workers shift tasks to accommodate changes in environmental conditions and colony needs (Das & Gordon 2023). Given these dynamics, we hypothesize that the colony will respond to varying degrees of unevenness on foraging trails by adjusting worker allocation, while the workers will adjust their walking speed or load to maintain foraging efficiency.

In this study, we investigated how worker allocation, walking speed, and foraging efficiency vary in *Acromyrmex subterraneus* (Forel, 1893) (Formicidae: Attini) when workers are moving along foraging trails with different degrees of unevenness. By examining how leaf-cutting ants adapt to different trail surfaces, we aim to deepen our understanding of their foraging dynamics.

Material And Methods

Biological model

Four colonies of *A. subterraneus* containing 2L of symbiotic fungus garden (*Leucoagaricus gongylophorus* (Alfred Möller) Casa (1957)) maintained at MirmecoLab under controlled conditions, with a temperature of 25 ± 3°C and relative humidity of 80 ± 5%, were selected for the experimental procedure. The colonies are kept in a closed system with three compartments: the fungus chamber, the foraging arena, and the waste chamber, connected by plastic tubes. The colonies are provided daily with 10 g of fresh *Acalypha wilkesiana* Müll.Arg. leaves as a substrate for the symbiotic fungus.

Experimental design

The colonies were connected by a bridge made of three models of foraging trails 3D-printed (60 cm length, 1.2 cm width) to a foraging arena (plastic tray measuring 20 cm by 12 cm) containing 10 g of leaves of *A. wilkesiana*. Two trails were constructed with two uneven checkerboard patterns of 6 mm and 3 mm step height, and a third trail was flat, without any irregularity. The value of 6 mm is equivalent to the mean body length of Large workers ($\overline{x}$ = 5.5 mm) plus the mean deviation ($\overline{x}$ = 0.5 mm), while 3 mm is half of the former value. The large-scale surface trail exceeds the body length of workers of all size classes, simulating environmental obstacles and thus representing navigational challenges. On the other hand, a small-scale surface trail is smaller than the body length, reducing foot adhesion, friction, and contact area for walking (Clifton et al. 2020). The flat trail served as control (Fig. 1).

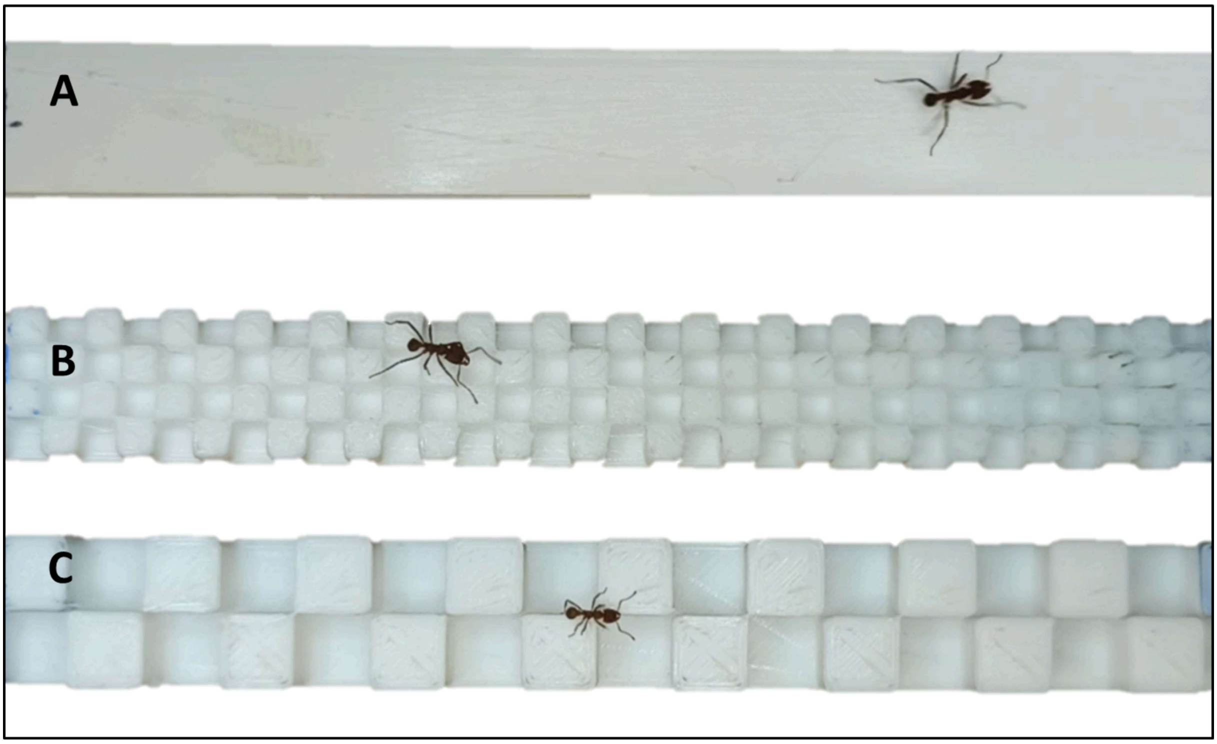

Figure 1. 3D-printed foraging trails **(A)** Flat (**B**) 3 mm (**C**) 6 mm

A camera (1080P, Full HD, 60 FPS) was positioned perpendicular over a 10 cm section in the center of the bridge to register the workers traveling during foraging. The recordings began just after the transport of the first leaf fragment and lasted 60 minutes. This procedure was made once a day and repeated five times in each colony for each trail type.

Afterward, we registered the time travel to cross a 10 cm section of the trail of six laden workers at each experimental trial (n = 234). These workers were collected and weighed as well as their respective loads using an analytical balance (0.0001 g) and later the workers were returned to the colony. The leaf loads were dried in an oven at 70°C for 48 hours to determine their dry weight. The dry weight of the workers was estimated using a linear regression between the fresh weight and dry weight ($t = 45.92$, $p < 0.0001$, $R^2 = 0.95$), using an amostral data (n = 120) of different size classes workers from the same colonies, obtained after the experimental procedure. We preferred to estimate the dry weight of the workers to avoid their removal from the colony and any interference in the foraging effort. Time travel was used to calculate the walking speed of loaded workers, while dry masses of the worker and plant load were used to calculate the burden, following the formula: **Burden** = worker mass + plant mass/worker mass (Burd 2000).

Data collection

After the transport of the first leaf fragment, we counted the number of workers (F) that were crossing a transversal line on the trail, 25 cm far from the colony entrance, during 1 minute every 5 minutes. Then we calculate the foraging flow (q) using the formula: q = F/time/trail area (s/cm^2) (Bruce & Burd 2012). During the counting, we also registered if the workers were leaving (outbound) or returning to the nest with (laden inbound) or without load (unladen inbound).

From the footage, we selected a frame (12 cm^2 of the trail) at 30 seconds after the counting of workers traveling (n = 400 frames). Using IMAGE J (Schneider et al. 2012), we took linear measurements of the body length (BL) and head width (HW) of all workers present in the frame. Also, we draw a polygon over the worker to obtain the area occupied by an individual, including the last pair of legs and the antennas (ALA) and without legs and antennas (BA) (n = 2040). With these measurements, we calculated the body area ratio (BAR) by dividing the ALA by BA (Poissonier et al. 2019). We estimated the surface area occupied by a worker using the formula: **Occupancy** = BL x HW x BAR (Poissonnier et al. 2019) and calculated the occupancy rate (sum of occupancy of all workers in the frame divided by trail area).

For these ants (n = 2040), we also determined the time spent traveling to cross a 10 cm section to obtain their walking speed, adding to the traveled distance the step height of the trail considering the number of steps they climb up and down. Besides, we counted the number of contacts (head-on, rear-end, climb) of each individual during the same trajectory. Workers that had made U-turns or presented hitchhikers over their leaves were discarded from the data analysis (n = 236).

Statistical analysis

We employed a multiple linear regression (LR) with the head width and body length of all measured workers to determine the size classes. The initial range of three size classes was that used by Calheiros et al. (2019), which was adjusted for our data until the model indicated different slopes for each size class. Different slopes indicate that the allometric coefficients are different between the size classes (LR: $F_2 = 19.27$; $p < 0.0001$; $R^2_{adjusted} = 0.67$).Therefore, the range of head width for each size class was: Small ≤ 1 mm; Media 1.1 to 1.4 mm; and Large ≥ 1.5 mm (Fig. 2).

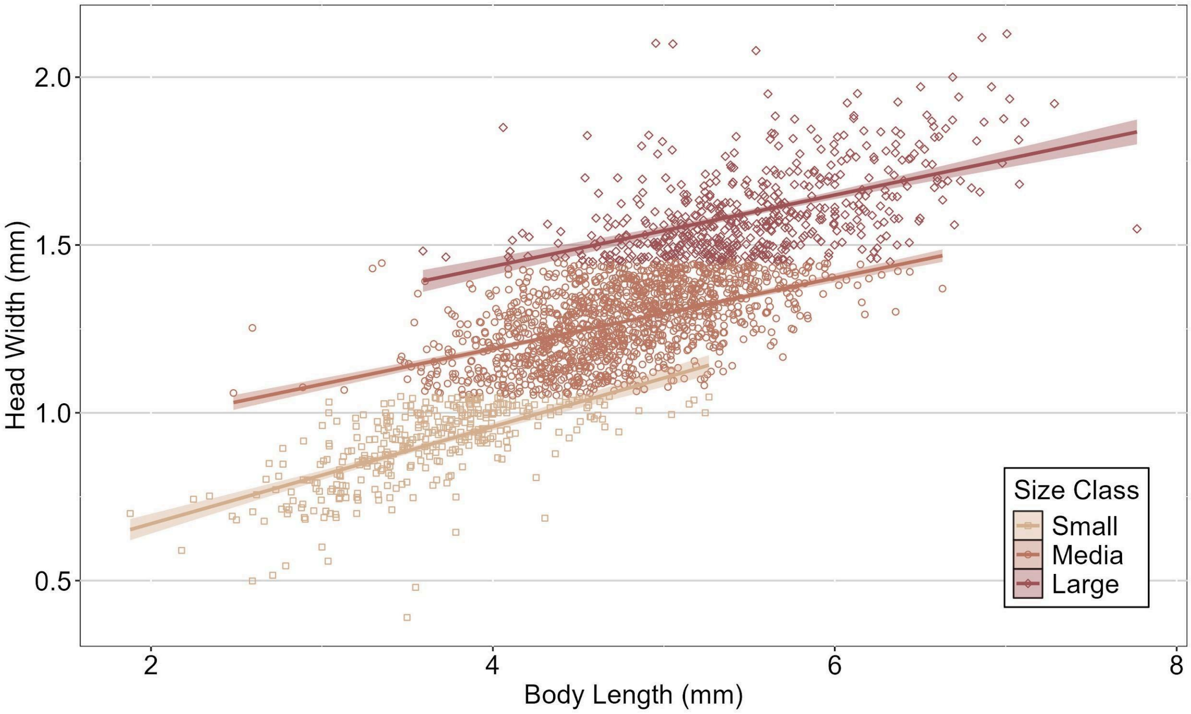

Figure 2. Linear regression between body length (mm) and head width (mm) of Small, Media, and Large workers. Symbols indicate raw data according to the worker class (Small: light brown circles, Media: medium brown triangles and Large: dark brown squares). The regression lines represent the fit through the data, with the estimated slope and intercept, and the lateral bands are confidence intervals.

To verify if the allocation of workers from different size classes alters due to trail unevenness, we used a linear mixed-effects model (LMM), with a negative binomial distribution, to analyze the variation of the number of workers considering as fixed factors the size class (categorical with three levels) and trail unevenness (categorical with three levels). The same analysis was repeated, focusing only on loaded workers, enabling us to identify which size class was most active in leaf transport.

We evaluated the foraging flow variation in function of trail occupancy rate (numerical) and trail unevenness (categorical with three levels) using an LMM. The regression slopes of each size class provided the proportional occupancy of workers from different size classes according to their size class.

To evaluate how trail unevenness affected the leaf transport efficiency [worker speed ($cm.s^{-1}$), multiplied by leaf fragment dry mass (mg)] (Rudolph & Loudon 1986; Bernadou et al. 2016), we applied an LMM considering burden (continuous) and trail unevenness (categorical with three levels) as fixed factors. Then, we analyzed how worker speed varied in function of the burden (continuous) and trail unevenness (categorical).

To verify the effect of trail unevenness on worker limb kinematics during walking, we modeled the BAR considering whether the worker was loaded or not loaded (categorical with two levels) and the trail unevenness (categorical with three levels), employing a LMM. Since BAR is an estimation of the leg stretch of a worker, this analysis can indicate how the trail unevenness interferes with the worker's tripod gait during locomotion (Moll et al. 2013).

To check the effect of the number of contacts on the worker speed (continuous), we applied a LMM considering as fixed factors the total number of contacts (numeric) and trail unevenness (categorical with three levels). The worker head width was included as a covariate in the model since the worker size has a known effect on the walking speed.

We considered colony as a random factor in all mixed models and proceeded with pairwise comparisons of the estimated means when the model revealed a significant effect of a fixed categorical factor with more than two levels. Also, the occurrence of significant interactions between the fixed factors was checked before model simplification. All analyses were performed in R software version 4.3.3 (R Core Team 2024), using the packages “multcomp” (Hothorn et al. 2008), “lme4” (Bates et al. 2015), “emmeans” (Lenth 2022), “nlme” (Pinheiro and Bates 2022), “dplyr” (Wickham et al. 2023), “DHARMa” (Hartig, 2022), and “MuMIn” (Barton 2023). The graphs were generated using “ggplot2” (Wickham 2016), “ggpubr” (Kassambara 2022), “ggh4x” (van den Brand 2024) and “ggridges” (Wilke 2024).

Results

We found that the density of workers was different on the trails (LMM: $F_2 = 8.37$, $p = 0.0002$, $R^2_{marg} = 0.44$, $R^2_{cond} = 0.54$). The Flat trail presented a lower density of workers than the 6 mm trail (Tukey's contrasts: $\beta = -0.50 \pm 0.12$, $Z = -4.07$, $p = 0.0001$) but did not differ from the 3 mm trail ($\beta = -0.27 \pm 0.12$, $Z = -2.19$, $p < 0.07$; Fig. 3A). We also found a significant difference between the density of loaded workers from the different size classes (LMM: $F_2 = 11.25$, $p < 0.0001$, $R^2_{marg} = 0.46$, $R^2_{cond} = 0.51$). In all trails, the number of Media workers was higher than Large (Tukey's contrasts: Not loaded: $\beta = 0.92 \pm 0.12$, $Z = 7.50$, $p < 0.0001$; Loaded: $\beta = 0.79 \pm 0.17$, $Z = 4.77$, $p < 0.0001$), followed by Small workers (Not loaded: $\beta = -1.27 \pm 0.12$, $Z = -10.27$, $p < 0.0001$; Loaded: $\beta = 1.63 \pm 0.19$, $Z = 8.65$, $p < 0.001$). Additionally, the number of Large workers was also higher than of Small workers among trails (not loaded: $\beta = -0.35 \pm 0.13$, $Z = -2.70$, $p = 0.0192$; Loaded: $\beta = -0.84 \pm 0.20$, $Z = -4.16$, $p = 0.0001$; Fig. 3B). These results indicate that trail unevenness could have affected the mechanism of worker allocation. On the other hand, the preferential allocation of Media workers to foraging was registered independently of trail unevenness.

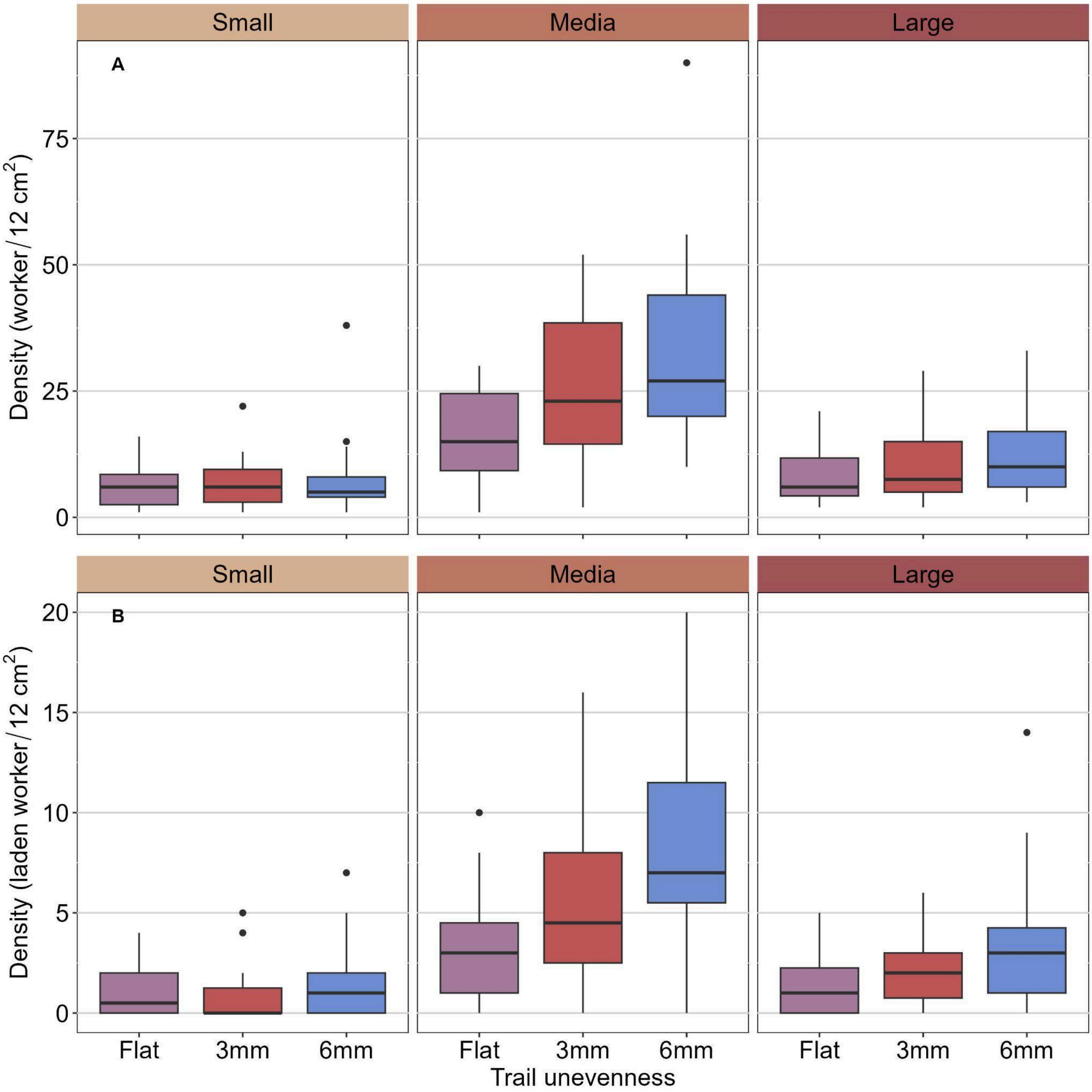

Figure 3. Density of workers (12 cm^2) of each size class on trails with different degrees of unevenness (A). Density of laden workers (12 cm^2) of each size class: Small, Media, and Large (B). Box-and-whisker plots show the median (horizontal line), interquartile range (box), and distance from the upper and lower quartiles (whiskers). The black dots represent the outliers.

The model revealed a significant interaction between trail unevenness and occupancy rate (LMM: $F_2 = 9.40$, $p < 0.0001$, $R^2_{marg} = 0.12$, $R^2_{cond} = 0.31$; Fig. 4), indicating that occupancy rates promoted different worker flow depending on the trail. The relationship between worker flow and occupancy rate was different for Flat and 3 mm trails (LMM: $\beta = 0.59 \pm 0.21$, t = 2.81, p = 0.005), while it was similar for Flat and 6 mm (LMM: $\beta = -0.03 \pm 0.21$, t = -0.15, p = 0.878). In fact, we found that the flow was 14% higher on Flat trails than on 3mm trails for the mean occupancy rate (0.2), while it becomes 12% lower when half of the trail was occupied (occupancy rate = 0.5) and was 34% lower for the maximum occupancy rate registered in this study (occupancy rate = 0.8). Regarding the comparison between Flat and 6 mm trails, the former was 23% higher for all occupancy rates.

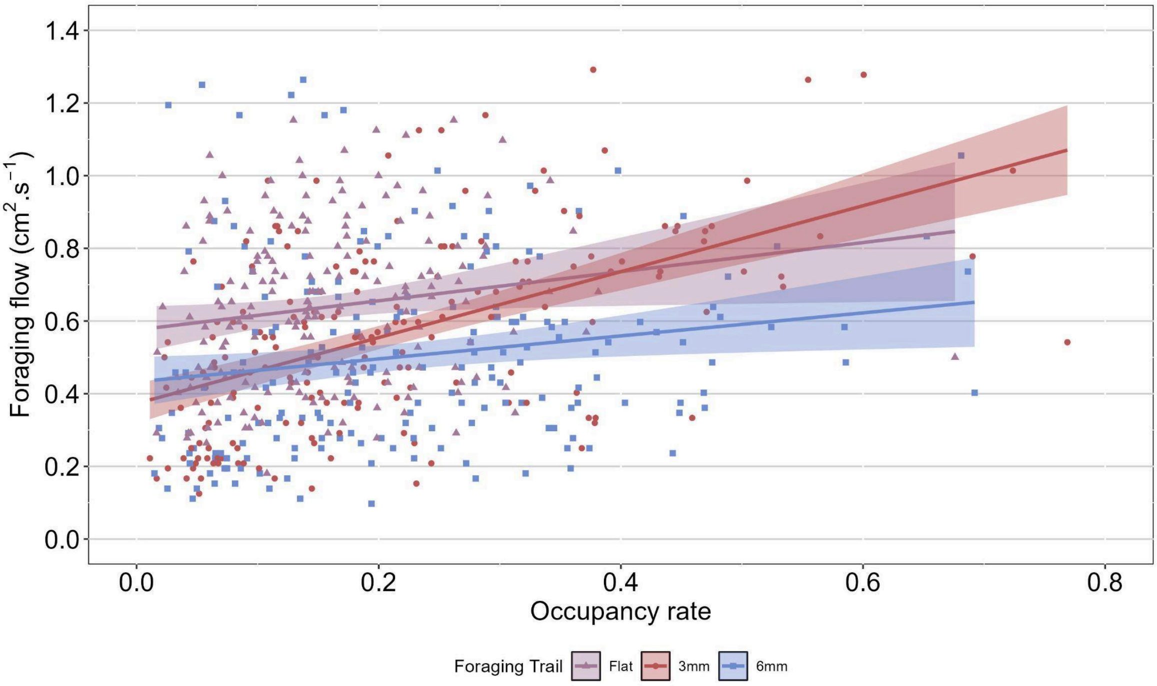

Figure 4. Foraging flow ($cm^2.s^{-1}$) variation in relation to occupancy rate at each trail (Flat, 3 mm, and 6 mm). Symbols represent the raw data, and the regression lines the fitted data, with the slope and intercept estimated by a linear mixed model. The symbols and lines are colored according to trail unevenness (Flat: purple triangles; 3 mm: red dots; 6 mm: blue squares).

As expected, we found that the transport efficiency was affected by the burden (LMM: $F_1 = 280.91$, $p < 0.0001$, $R^2_{marg} = 0.48$, $R^2_{cond} = 0.57$); the higher the burden, the higher was the efficiency. Trail unevenness also affected the efficiency ($F_2 = 92.97$, $p < 0.0001$), which was higher at Flat trails when compared to the 6 mm trail (Tukey's contrasts: $\beta = 0.44 \pm 0.08$, t = -4.56, p < 0.0001), but did not differ from the 3 mm trail ($\beta = 0.75 \pm 0.14$, t = -1.58, p < 0.25; Fig. 5a). The noted relationship between transport efficiency indicates that the unevenness of trail surfaces can affect worker locomotion or load determination.

In fact, analyzing the relationship between the walking speed of loaded workers and their burden at these trails, we found that worker speed was not affected by burden (LMM: F_1 = 3.51, p = 0.06, $R^2_{marg} = 0.41$, $R^2_{cond} = 0.45$) but was different at the trails (F_2 = 140.61, p < 0.0001). Further, worker speed was higher on Flat trails when compared to 3 mm trails (Tukey's contrasts: $\beta = 0.38 \pm 0.07$, t = 5.66, p < 0.0001) and even higher on 6 mm trails ($\beta = 1.10 \pm 0.07$, t = 16.19, p < 0.0001; Fig. 5b). These results indicate that workers were transporting an optimal load that did not affect their walking speed, and the reduction in efficiency was due to the trail characteristics.

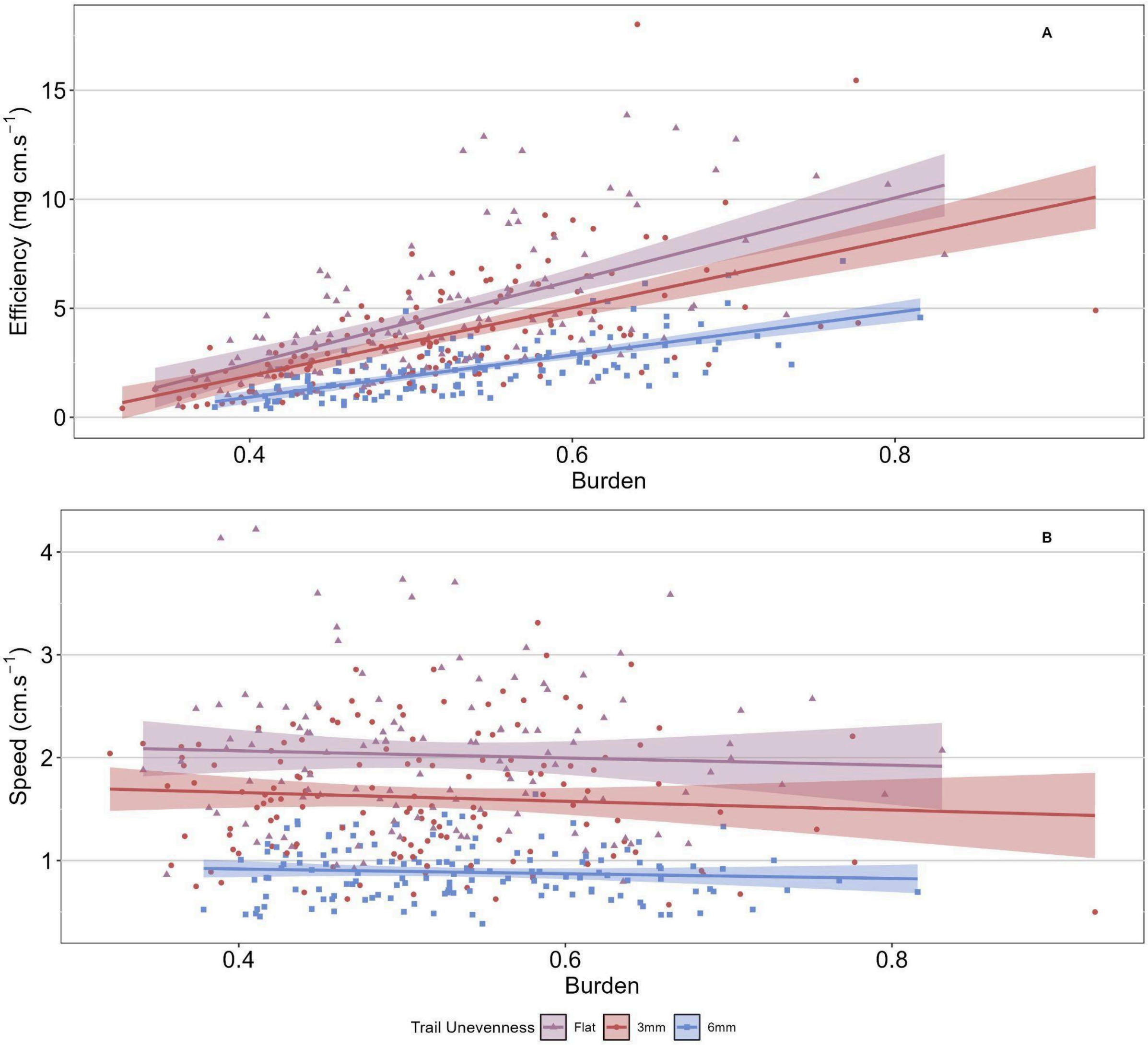

Figure 5. Relationship between (A) Efficiency (cm.s^{-1}) and Burden (B) Speed (cm.s^{-1}) and Burden of each trail with different degrees of unevenness (6 mm and 3 mm and Flat). Symbols represent the raw data, and the linear regression lines represent the fitted data. The slope and intercept are estimated by linear regression. The symbols and lines are colored according to trail unevenness (Flat: purple triangles; 3 mm: red dots; 6 mm: blue squares).

The body area ratio of workers, which estimates a measure of worker leg spreading, was higher for loaded workers (LMM: $F_1 = 37.69$, $p < 0.0001$, $R^2_{marg} = 0.06$, $R^2_{cond} = 0.10$; Fig. 6) and higher on the Flat trail when compared to the 3 mm (Tukey's contrasts: $\beta = 0.92 \pm 0.16$, $t = 5.90$, $p < 0.0001$) and even higher than the 6 mm ($\beta = 1.24 \pm 0.15$, $t = 8.33$, $p < 0.0001$), indicating that the unevenness of the trail prevented workers from spreading their legs.

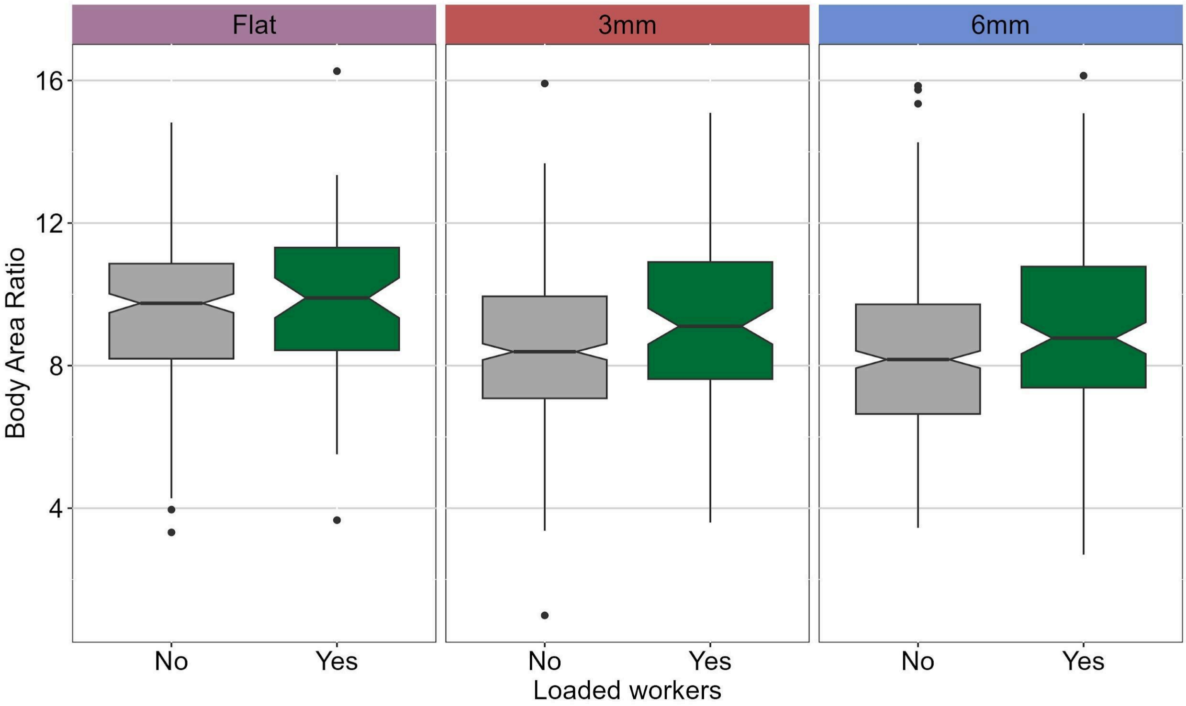

Figure 6. Loaded and unloaded body area ratio of workers by each trail unevenness. Box-and-whisker plots show the median (horizontal line), interquartile range (box), and distance from the upper and lower quartiles (whiskers). The notches give the confidence interval of the median and black dots represent the outliers.

The worker speed reduced due to the number of contacts that presented a significant interaction with trail unevenness ($F = 7.8$, $p = 0.0004$, $R^2_{marg} = 0.30$, $R^2_{cond} = 0.34$, Fig. 7). When compared to the Flat trails, the reduction on worker speed was 0.013 higher for the 3 mm trail ($\beta = 0.013 \pm 0.0006$, $t = 2.03$, $p < 0.05$) , while for the 6 mm was 0.025 higher ($\beta = 0.025 \pm 0.0006$, $t = 3.89$, $p < 0.0001$). Indicating a higher chance of traffic congestions on the 6 mm trails.

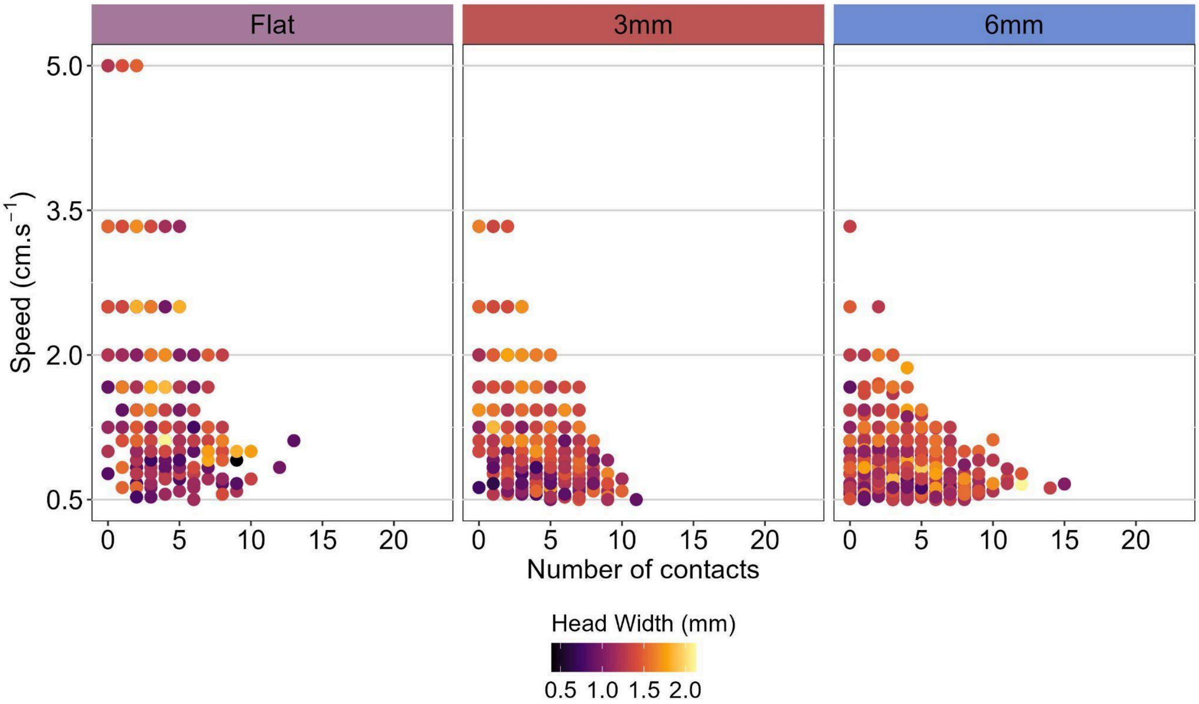

Figure 7. Relationship between worker Speed (cm.s^{-1}) and the number of contacts on each trail with different degrees of unevenness (Flat, 3 mm and 6 mm). Symbols represent the raw data and are coloured by the variation of the head width (mm) of workers.

Discussion

Our results reveal that trail unevenness promoted a significant variation of density, flow, and leaf transport efficiency of *A. subterraneus* workers. Specifically, we found that the density of workers was lower at the Flat trail when compared to the 6 mm trail. The lower density allowed a higher worker flow on Flat trails compared to both 3 mm and 6 mm trails, particularly when the occupancy rates were low (20%) and intermediate (50%). However, as at maximum occupancy registered in this study, the flow on Flat trails decreased, becoming lower than on the 3 mm trail. These results support our hypothesis that worker allocation and movement alter due to the unevenness of the trail surface. The observed differences in transport efficiency were a reflex of walking speed adjustment and not of the load. In fact, there were different values of burden that were transported at similar speed, indicating that workers were transporting their optimal load according to their size. On the other hand, the walking speed variation could be interpreted as a result of leg spreading which was more constrained at uneven trails. We suggest that in these trails, workers must adapt their stride in order to avoid the “gaps” on the trail. The walking speed was also affected in a different way by the number of contacts, which were more relevant at the uneven trails, probably due to the higher density and lower worker flow, indicating a greater chance of traffic jams at these trails.

The lower foraging flow on the 6 mm trail was due to the reduced speed of workers rather than their high frequency. This aligns with previous findings that increased substrate roughness in both natural and experimental settings can slow worker speed and increase the likelihood of dropping loads (Bernadou & Fourcassié 2007; Bernadou et al. 2011; Yanoviak et al. 2017). As previously suggested, loaded workers may reduce their speed as a strategy to minimize traffic congestion and load dropping (Farji-Brener et al. 2011). In line with our results, Poissonnier et al. (2019) demonstrated that at low densities, ant density and flow are positively correlated, while at high densities, flow stabilizes, preventing congestion despite increased worker presence.

Unexpectedly, we did not observe load adjustment in response to trail unevenness, since the burden did not vary in function of worker speed. Previous studies have shown that load adjustment is more likely when ants walks steep inclines or experience high traffic flow (Pereyra et al. 2020; Farji-Brener et al. 2011). However, while experimental constraints on trail height have been known to promote load reduction (Dussutour et al. 2009), our findings align with those of Bernadou et al. (2011) and Yanoviak et al. (2017), who reported no significant load adjustments due to trail roughness. Thus, the observed lower efficiency on the 6 mm trail resulted from slower transport speeds rather than changes in load size.

Regarding worker mobility, leg spreading was greater on the Flat and 3 mm trails than on the 6 mm trail, suggesting that greater unevenness restricted movement and required a more compact stance to maintain stability. The 61% reduction in speed of loaded workers on the 6 mm trail could be linked to stride frequency adjustments, as stride length increases linearly with speed (Wahl et al. 2015; Clifton et al. 2020). This finding is consistent with previous observations that ants slow their movements on rough terrains to maintain stability (Zollikofer 1994). Given that leaf-cutting ants prefer flat substrates for their faster and more stable locomotion (Clifton et al. 2020), our results reinforce the importance of well-maintained trails in optimizing foraging efficiency (Lopes et al. 2016; Caldato et al. 2020).

The reduction in worker speed was directly influenced by the number of contacts, which showed a significant interaction with trail unevenness. This effect was more pronounced on the more uneven trails, with the speed reduction being greater on the 6 mm trail compared to the 3 mm and Flat trails. Therefore, high uneven surfaces not only impose mechanical challenges, but also amplified worker encounters, leading to a higher risk of traffic congestion, negatively affecting resource transport efficiency.

Furthermore, the broader implications of our findings highlight how physical trail conditions influence the foraging coordination in leaf cutting ants. As shown in previous research, crowded trails can increase physical challenges and disrupt worker interactions, ultimately reducing foraging efficiency (Dussutour et al. 2007; Bouchebti et al. 2015; Poissonnier et al. 2019). Our results suggest that future studies should investigate how trail conditions influence the number of contacts and the potential for congestion, further integrating physical and social dynamics into the understanding of ant foraging behavior.

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Chapter 2 - Ant carpooling: A study on the effects of trail difficulty on hitchhiking occurrence

- Manuscript submitted to the journal Insectes sociaux

Abstract:

Hitchhiking behavior in leaf-cutting ants, where small workers ride on leaf fragments carried by foragers, has been proposed as a strategy to reduce worker density on trails and prevent traffic congestion. This study examines how different trail surfaces influence the occurrence of hitchhikers in *Acromyrmex subterraneus* and their impact on foraging efficiency. We evaluated foraging flow, burden, and hitchhiker presence on four types of trail surface: tree trunk, sidewalk, grass, and bare soil. Hitchhikers were most frequent on tree trunk trails with rough surfaces that reduce worker walking speed. Despite the additional load, foraging efficiency remained high, suggesting a trade-off between burden and walking speed. On sidewalks, efficiency was also high, but hitchhiker presence decreased at higher foraging flow, possibly due to increased worker speed reducing traffic congestion. On grass and bare soil, hitchhiker occurrence was lower, likely due to trail maintenance demands and reduced leaf load. Our results suggest that hitchhiking is context-dependent and may serve as an adaptive strategy to optimize foraging efficiency by avoiding traffic issues, particularly on trails prone to congestion.

Keywords: *Acromyrmex subterraneus*, Burden, Foraging efficiency, Leaf-cutting ants, Leaf mass, Walking speed.

Introduction

The foraging process is crucial for leaf-cutting ants (*Acromyrmex* and *Atta*), as their success depends on efficiently collecting plant material to cultivate the symbiotic fungus *Leucoagaricus gongylophorus* (Alfred Möller) Casa (1957) (Sales et al. 2021; Römer et al. 2022; Bolander et al. 2023). During foraging, forager workers move along trails, which could be constructed or only chemically marked. Therefore, trail surface can be extremely varied due to the unevenness of the terrain and the presence of many kinds of obstacles, which can make load transport more challenging.

Traffic maintenance is actually a great challenge, especially because workers who are carrying heavy loads move slower, delaying those behind, a phenomenon referred to as the 'truck-driver effect' (Farji-Brener et al. 2010). This effect can be exacerbated by the high density of workers and trail conditions, such as narrow paths, uneven terrain, and steep slopes (Sales et al. 2015; Clifton et al. 2020; Pereyra and Farji-Brener, 2020). In order to avoid disruption of the foraging flow, leaf-cutting ants must organize the traffic, exhibiting individual and collaborative strategies, like adjusting load size or even worker velocity (Dussutour et al. 2009; Bruce et al. 2017; Alma et al. 2020; Pereyra and Farji-Brener, 2020).

The hitchhiker behavior, in which small ants ride on the top of the leaf fragment carried by a forager, is supposed to be one of those strategies, as when they climb over transported leaves, they reduce the worker density along the trail (Hastenreiter et al. 2018). Hitchhikers are known to execute other activities during foraging, and their role on the trails is still controversial, with five non-exclusive hypotheses proposed to explain their occurrence.

The first hypothesis proposes that small workers ride on the leaf fragment in order to defend the forager against attacks by parasitoid phorid flies (Diptera, Phoridae) (Eibl-Eibesfeldt and Eibl-Eibesfeldt 1967; Feener and Moss 1990; Erthal and Tonhasca 2000). Indeed, a relative increase of hitchhikers in the presence of phorids has been reported (Feener and Moss 1990; Linksvayer et al. 2002; Vieira-Neto et al. 2006; Elizalde and Folgarait 2012). But even at night when phorid flies activity is expected to be reduced (Orr 1992; Feener and Brown 1993; Yackulic and Lewis 2007), hitchhikers still occur and, in some cases, are more frequent (Bragança et al. 1998; Yackulic and Lewis 2007). Thus, the single defense function is not enough to explain the hitchhiker occurrence.

The second one is known as the energy conservation hypothesis, which argues that the small workers climb over the leaf fragments to save energy otherwise spent on walking back to the nest (Feener and Moss 1990). However, hitchhikers can come down the leaf fragment before reaching the nest entrance, suggesting that the primary purpose of hitchhiking may not be energy conservation but rather performing other functions.

The third hypothesis is related to sap feeding (Stradling 1978; Vieira-Neto et al. 2006), an important nutritional source for leaf-cutting workers (Littledyke and Cherrett 1976; Stradling 1978; Bass and Cherrett 1995). Hitchhikers are observed biting the leaf where they are positioned and are also preferentially found in fresh leaf fragments that had sap than dried ones (Vieira-Neto et al. 2006). However, the retrieval of nutrients through leaf sap seems to be incidental and perhaps an opportunistic aspect of hitchhiking, since small workers can obtain it inside the nest, making improbable that they leave the nest only for sap feeding, given the associated risks of the traveling to the cutting site (Vieira-Neto et al. 2006).

The fourth hypothesis postulates that hitchhiker function is to clean and remove microbial contaminants of the leaf before entering the nest (Linksvayer et al. 2002; Vieira-Neto et al. 2006; Griffiths and Hughes 2010; Kitayama et al. 2012). However, Griffiths and Hughes (2010) reported that hitchhikers perceive contaminants only after boarding a leaf fragment, not detecting the presence of contamination beforehand. Thus, although they can perform the cleaning task when necessary, this is not the main goal of the hitchhiker behavior.

Finally, the fifth hypothesis proposes that hitchhiking is a behavioral strategy used to decrease worker density along foraging trails (Hastenreiter et al., 2018). Hitchhikers are more frequent at high foraging flows and in narrow trails, conditions in which bottlenecks, delays, and collisions between nestmates are highly expected. In fact, collision rate is recognized as a local and simple sign that allows the adjustment of the number of individuals in a task (Gordon and Mehdiabadi 1999) and is supposed to stimulate small workers to travel on the leaf fragments in order to prevent traffic jams that could compromise foraging efficiency.

Considering that the density of ants on trails and the associated risk of traffic jams can play a critical role in triggering hitchhiking behavior, we expect this could be especially true on trails whose surface characteristics can impact the forager walking dynamics. We investigate how the trail surface interferes with the occurrence of hitchhiker workers in *Acromyrmex subterraneus* (Forel, 1893) and to what extent their presence affects foraging efficiency. Thus, we assessed the relationship between four different trail surfaces in foraging flow, burden, and hitchhiking occurrence, which will provide insights about hitchhiking as an adaptive strategy of leaf-cutting ants to sustenance of foraging efficiency.

Material and methods

Experimental design and data collection

Our study was conducted between August 2021 and November 2022 on active foraging trails of *A. subterraneus* colonies located in the Campus of the Universidade Federal de Juiz de Fora (UFJF) and in the Botanical Garden, both in the municipality of Juiz de Fora, Brazil.

We selected a total of 34 foraging trails that were formed over different surfaces: Bare soil (n = 20), red-yellow latosol, a common soil type in Juiz de Fora region (Rocha, 2006); Sidewalk (n = 5), cement constructions designed for pedestrian traffic; Tree trunk (n = 4), trunks of angiosperms; and Grass (n= 5), grassy vegetation, primarily composed of plants from the *Poaceae* family.

At each trail, we established a fixed point to register the foraging flow by counting the number of outbound (OW), unloaded inbound (UW), loaded inbound (LW), and loaded inbound with hitchhiker workers (HW). We took 10 consecutive foraging flow measurements, each one lasting 1 minute at intervals of 1 minute. Also, we measured the trail width using a ruler.

Also, we established a 10 cm length section in the trail, starting from the fixed point, to register the time travel of 10 HW and 10 LW. We collected and stored these workers and their respective loads in numbered Eppendorf tubes to determine their dry weights. Foragers and hitchhikers were oven-dried for 48 h and the plant material for 24 h, both at 70 ºC, and then they were weighted on a precision analytical balance with a readability of 0.0001 grams (Sales et al. 2021).

Statistical analysis

In order to evaluate the impact of trail surfaces (categorical with four levels) on hitchhiker occurrence (binomial) probability, we submitted data to a logistic regression model including the foraging flow as an explanatory variable (continuous). The used foraging flow formula is: OW + UW + LW + HW/minute/trail width (adapted from Bruce and Burd 2012). This formula reflects the relationship among colony foraging effort, allocated workforce, and trail characteristics. Next, we calculate the probability of finding a hitchhiker on each surface, allowing us to predict the probability of hitchhiker occurrence on each surface according to the mean foraging flow at all trail surfaces.

Following, we evaluated the impact of hitchhikers on the efficiency of each forager, as they represent an additional load for the forager workers. Therefore, we calculated the burden, which is a ratio between the transported load and the forager mass (Burden = LW+HW+leaf dry mass/LW; formula adapted from Roces and Núñez 1993), and the forager efficiency, which represents the contribution of each forager to the colony's leaf input per unit of time (Efficiency = Forager walking speed x transported leaf mass; Roces and Núñez 1993). Then, we submitted the data to a general linear model (GLM) to compare the efficiency (continuous) of forager workers with or without hitchhikers (categorical with 2 levels) at each trail surface (categorical with four levels), including the burden (continuous) as a covariate.

To evaluate which components of the efficiency measure were actually impacted by hitchhiker presence, we also modeled the walking speed (continuous) and leaf mass (continuous) variation using GLMs, including burden (continuous) as a covariate (just for walking speed model), and hitchhiker occurrence (categorical with 2 levels) and trail surface (categorical with four levels) as fixed factors. Efficiency, walking speed, leaf mass, and burden were log-transformed ($\log_{10} x + 0.5$) for better fit of data. In all models, interaction terms were kept when significant. We validated all models using DHARMa package that simulates standardized residuals to visually and quantitatively check model assumptions like homoscedasticity and normality (Harting 2022).

All analyses were performed in R software version 4.3.3 (R Core Team 2024). Using the packages “fitdistrplus” (Delignette-Muller and Dutang 2015), “lattice” (Sarkar 2008), “lme4” (Bates et al. 2015), “MuMIn” (Bartoń 2023), and “emmeans” (Lenth 2024). The graphs were generated using “ggplot2” (Wickham 2016) and “ggh4x” (van den Brand 2024).

Results

What is the effect of foraging flow of different trail surfaces on hitchhiker occurrence?

The hitchhiker occurrence probability was significantly affected by the interaction between foraging flow and trail surface ($F_3 = 8.80$, $p = 0.032$). On trails whose surface was Bare soil, Grass and Tree trunk, the higher the foraging flow, the higher the hitchhiker occurrence, while on Sidewalk trails there was an inverse relationship (Fig. 1). Using the mean foraging flow ($\mu = 14.19$), the model indicated that the predicted probability of hitchhiker was 55% on Bare Soil trails. Using this trail surface as a parameter for the comparisons, we verified a significant increase of 16% for Grass, 33% for Sidewalk, and 44% for Tree trunk (Fig. 1).

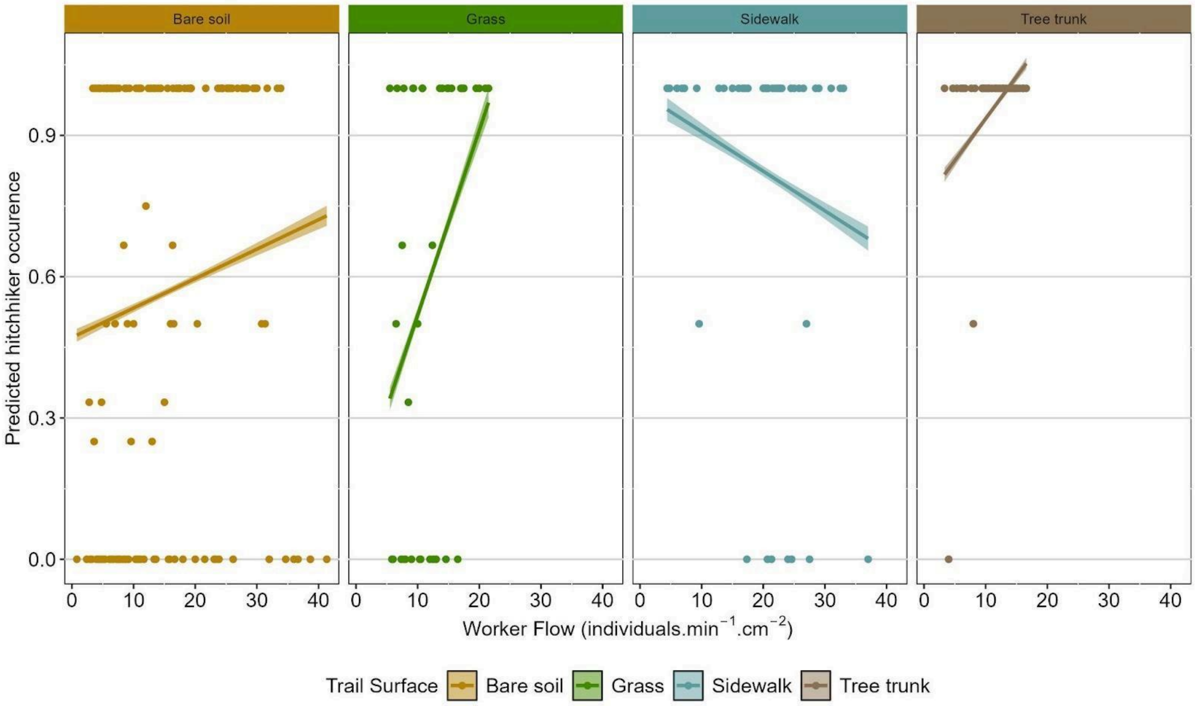

Figure 1. Predicted probability of hitchhiker occurrence according to the foraging flow (individuals.min^{-1}.cm^{-1}) and trail surfaces. Points indicate predicted data provided by a logistic regression model. The regression lines represent the hitchhiker occurrence predicted probability, with the estimated slopes and intercepts for each trail surface, and lateral bands are confidence intervals (95%).

How much do hitchhikers impact the efficiency of forager workers?

Analyzing the forager efficiency variation, we found a significant interaction between Burden and Hitchhiker occurrence (GLM: $F_1 = 5.52$, $p = 0.020$, $R^2_{marg} = 0.46$), meaning that when hitchhikers were present, the effect of burden on forager efficiency was less accentuated ($\beta = -0.42 \pm 0.18$, Fig. 2).

Trail surfaces also presented a significant effect on forager efficiency (GLM: $F_3 = 73.16$, $p < 0.0001$; Tab. 1). Foraging trails on Tree trunk and Sidewalk presented the highest efficiency. On average, the efficiency on these trails was 56% than on Bare Soil, and 31% than on Grass.

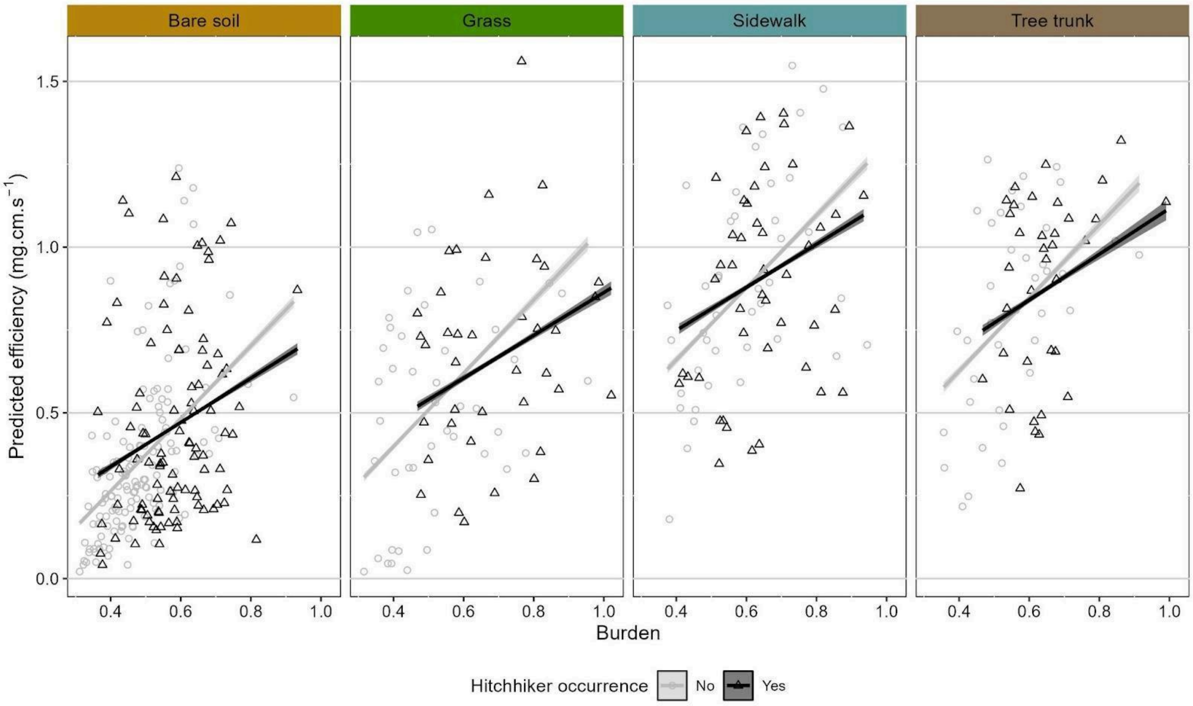

Figure 2. Forager efficiency (mg.cm.s^{-1}) of *Acromyrmex subterraneus* workers according to the burden and the presence of hitchhikers on each trail surface. Symbols indicate observed data (log 10 transformed) according to the absence (grey circles) and presence of hitchhiker (black triangles). The linear regression lines represent the predicted data, with the estimated slope and intercept, and lateral bands are confidence intervals (95%).

Table 1. Pairwise comparison of the forager efficiency (mg.cm.s^{-1}) and walking speed (cm.s^{-1}) of *Acromyrmex subterraneus* workers and their respective leaf mass (mg) by each trail surface. Values indicate the estimated difference among trail surfaces (β ± SE), the T-test calculated (t.ratio), and significant probability (p.value). Differences were considered significant when lower than 0.05.

Pairwise comparison	**Efficiency**		**Walking speed**		**Leaf mass**	
	β ± SE	**p.value**	**β ± SE**	**p.value**	**β ± SE**	**p.value**
Bare soil - Grass	-0.13 ± 0.03	0.0002	-0.05 ± 0.01	0.001	-0.18 ± 0.03	<0.0001
Bare soil - Sidewalk	-0.40 ± 0.03	<0.0001	-0.25 ± 0.01	<0.0001	-0.24 ± 0.03	<0.0001
Bare soil - Tree trunk	-0.36 ± 0.03	<0.0001	-0.12 ± 0.01	<0.0001	-0.34 ± 0.03	<0.0001
Grass - Sidewalk	-0.27 ± 0.04	<0.0001	-0.20 ± 0.02	<0.0001	-0.06 ± 0.04	0.407
Grass - Tree trunk	-0.23 ± 0.04	<0.0001	-0.07 ± 0.02	0.0006	-0.16 ± 0.04	0.0004
Sidewalk -Tree trunk	0.04 ± 0.04	0.752	0.13 ± 0.02	<0.0001	-0.10 ± 0.04	0.049

How much does hitchhiker presence affect the forager walking speed?

As expected, forager workers with higher burden were those with lower walking speed; however, the presence of hitchhikers accentuated the effect of burden by diminishing the walking speed (β = -0.18 ± 0.08), which is supported by the significant interaction between the variables burden and presence of hitchhiker (GLM: $F_1 = 5.35$, $p = 0.021$, $R^2_{marg} = 0.38$; Fig 3). We also found that the walking speed varied according to the trail surface (GLM: $F_3 = 113.57$, $p < 0.0001$; Tab. 1). When traveling on Sidewalk trails, forager workers exhibited the highest speed, reducing 24% on Tree trunk trails, 37% on Grass trails, and 44% on Bare soil trails. Therefore, high walking speed was a factor that contributed to the higher efficiency on Sidewalk but not on Tree trunk trails.

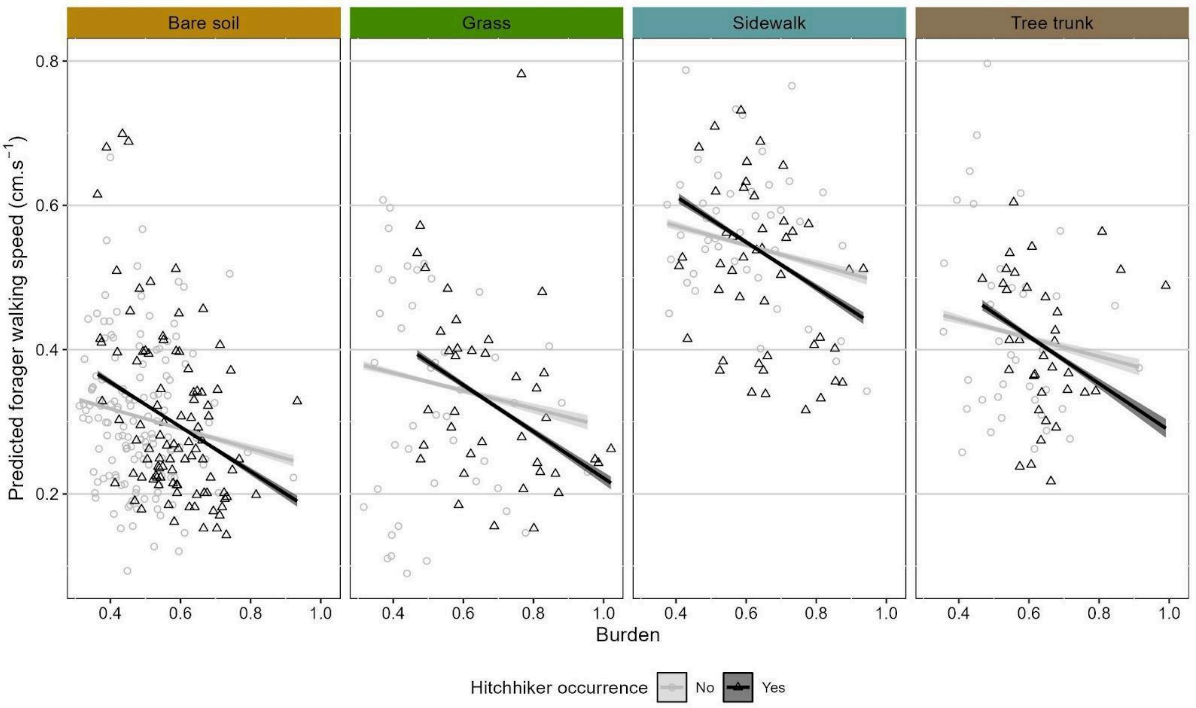

Figure 3 Walking speed (cm.s^{-1}) of *Acromyrmex subterraneus* forager workers according to their respective burden, considering the presence of hitchhikers on each trail surface. Symbols indicate observed data (log10 transformed) according to the absence (grey circles) and presence of hitchhikers (black triangles). The linear regression lines represent the predicted data with the estimated slope and intercept, and lateral bands are confidence intervals (95%).

How much leaf mass contributed to efficiency?

We found that the leaf mass varied significantly on the different surfaces (GLM: $F_3 = 48.18$, $p < 0.0001$; $R^2_{marg} = 0.26$; Fig 4), and, as expected, the hitchhiker occurred on the heaviest leaf mass fragments (GLM: $\beta = 0.14 \pm 0.02$, $F_1 = 39.39$, $p < 0.0001$). Also, the heaviest leaf fragments carried by a forager were registered at Tree trunk trails (Tab. 1), contributing to the high efficiency of these trails.

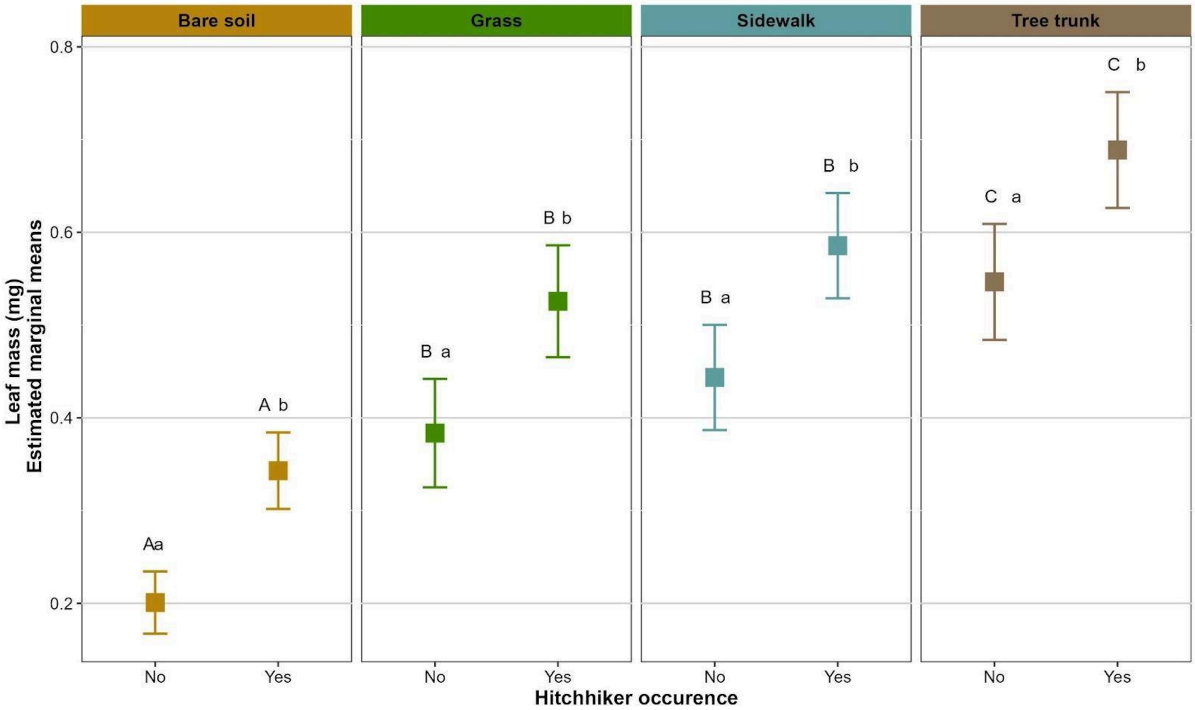

Figure 4. Data is presented as estimated marginal means of leaf dry mass (mg) obtained by data modeling (GLM) between the factors "hitchhiker" and "surface." Error bars represent 95% confidence intervals for the estimated means. Color represents trail surface types. Letters above the error bars indicate significant pairwise comparisons between levels of the factors "hitchhiker" and "surface," determined using Tukey's adjustment method. Different letters denote significant differences ($p < 0.05$). Lowercase letters indicate the difference between the hitchhiker occurrence, and uppercase letters indicate the difference between trail surfaces.

Discussion

Our results demonstrate that the occurrence of hitchhikers in the foraging trail was affected by the different surfaces. The highest likelihood of finding a hitchhiker was on the tree trunk surface, on which we also registered the higher efficiency. Thus, despite the lower foraging efficiency of foragers with hitchhikers, it seems that their occurrence is counterbalanced by the trade-off between the extra load that hitchhikers represent and efficiency. Tree trunks impose higher difficulty for workers locomotion as they are rough and often covered with epiphytic roots, moss, and lichens, reducing the worker's running speed (Yanoviak et al. 2017; Stark and Yanoviak 2020). In fact, the efficiency on tree trunk trails was improved by the transport of heavier leaf fragments, on which hitchhikers are more frequent (Hastenreiter et al. 2016). Thus, under conditions that favor the occurrence of traffic jams, the hitchhiker behavior could represent a strategy adopted by leaf-cutting ants to reduce the number of individuals along the trail (Hastenreiter et al. 2018).

On Sidewalk trails, the likelihood of finding a hitchhiker and the foraging efficiency were as high as on tree trunk trails. However, on these trails we found a kind of different scenario, as the chance of hitchhikers reduces as foraging flow increases and the walking speed was the factor responsible for raising the efficiency. We must consider that the Sidewalk surface is designed to improve human traffic (Dimaiuta et al. 2011; Kim et al. 2024), thus constituting a smoother, obstacle-free surface that probably allows a more streamlined movement of ants. Although speed generally declines as the density of moving individuals on trail increases, some ant species seem to be able to overcome this premise (*Leptogenys processionalis* (Jerdon, 1851): John et al. (2009); *Formica pratensis* Retzius, 1783: Hönicke et al. 2015), resulting in the absence of jammed traffic. An increased density on trails promotes a self-organized widening of the trail (Camazine et al. 2001) that was possible here since Sidewalk trails were not width-limited, as they were not constructed by ants as the other ones. We suggested that the likelihood of finding a hitchhiker decreases at high foraging flows because traffic jams must be rare on this surface or because foragers were so fast that the small workers were not able to climb the leaf fragments.

On Grass trails, the probability of finding a hitchhiker increased due to a small increase in worker flow, while on Bare soil surfaces, the higher probability was 75% in the higher worker flow. Grass and Bare soil trails are actively constructed by workers and consequently are subject to wear, demanding constant maintenance over time, such as trail clearing and vegetation trimming (Evison et al. 2008; Caldato et al. 2016; Alma et al. 2019; 2020). Small workers are often allocated to trail maintenance activities, and only a relatively small percentage of them hitchhike (Evison et al. 2008; Griffiths and Hughes 2010), explaining the lower hitchhiker occurrence. The foraging efficiency of these trails was lower when compared to Tree trunk and Sidewalk due to both lower walking speed and leaf mass. However, on Bare soil trails, the leaf fragment was lighter than on Grass, allowing us to suggest that the reduced leaf fragments could be one more restraint for hitchhiker occurrence (Hastenreiter et al. 2016). According to Pereyra and Farji-Brener (2020), forager workers reduce the load size in high traffic conditions; thus, another strategy to avoid traffic jams was probably already being used.

In conclusion, our findings support the hypothesis that hitchhiking is a behavioral adjustment used as a traffic signal and to manage worker density on foraging trails, thereby avoiding potential traffic congestion that could compromise foraging efficiency. The occurrence of hitchhikers varied according to trail surface characteristics, which affected both foraging flow and efficiency. On tree trunks trails, where locomotion is supposedly more compromised, leaf fragments were larger, ensuring the efficiency and allowing hitchhiker occurrence. Conversely, on Sidewalk trails, where movement was unobstructed, at high foraging flows the hitchhiker occurrence was lower, probably due to the low potential for traffic jams since foragers were very fast. On Grass and Bare soil trails, where worker-constructed trails require maintenance, hitchhiker occurrence remained low, probably because small workers would be performing maintenance tasks. Our results reinforce the idea that hitchhiking is context-dependent and may be an adaptive response to trail conditions that influence foraging dynamics in *A. subterraneus.*

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Final considerations

In the movie A Bug's Life, there is an iconic scene that captures the essence of the foraging challenges for ants: when a leaf falls from a tree and becomes a massive obstacle for ant characters. Although at first it might seem like a simple detail of nature, this scene illustrates how many challenges the forager workers face in their tiny world and how costly could be to go out for foraging. For ants, the falling leaf is not a trivial event; it represents a major threat. The leaf, falling heavily, causes the entire environment around it to change, turning it into a true battlefield and forcing the characters to push themselves to the limit to overcome the obstacle.

Here, we examined the unevenness of the trail as the main challenge to overcome in order to evaluate if the worker coordination ensured a consistent flow of leaf collection to the nest. Workers must walk through trails of varying surfaces and conditions, balancing individual locomotion with collective efforts. The maintenance of traffic flow is a major challenge, particularly due to factors such as terrain irregularity, high worker density, and the presence of obstacles. These challenges influence foraging efficiency, demanding behavioral adjustments to optimize movement and load transport.

Our study demonstrates that trail surface characteristics significantly impact foraging efficiency by altering worker mobility and traffic dynamics. Trails with rougher surfaces, such as tree trunks, impose additional locomotion difficulties but also promote adaptations that sustain efficiency, including the transport of heavier leaf fragments. In contrast, smoother, unobstructed surfaces, such as sidewalks or artificial flat trails, allow for increased walking speed, minimizing the need for other compensatory behaviors. On worker-constructed trails, such as over grass and bare soil, the demand for continuous maintenance likely influences the allocation of workers, affecting overall foraging flow. These findings emphasize how the environmental conditions were relevant in shaping the strategies used by leaf-cutting ants to maintain effective resource collection.

While this research provides valuable insights into the relationship between trail characteristics and foraging efficiency, some limitations must be acknowledged. Investigating additional variables, such as colony size, foraging distance, and seasonal influences, could provide a more comprehensive understanding of the adaptive strategies employed by these ants.

In conclusion, this study highlights the crucial role of environmental conditions in shaping foraging dynamics in *A. subterraneus*. The ability of leaf-cutting ants to adjust their behaviors in response to trail surface characteristics underscores the flexibility of their foraging strategies and the efficiency of their self-organized traffic management. These findings contribute to a broader understanding of how environmental constraints influence collective behavior in social insects, offering a foundation for future research on the mechanisms that regulate foraging efficiency under different ecological conditions.