Seasonal Dynamics and Hitchhiking Behaviour in *Atta cephalotes*

A comparison of foraging and minim hitchhiking behaviour in leafcutter ants in Madre de Dios, Peru

Image credit: Menka Belgal

Flore Allessie

HAS Green Academy

5 January 2026

Seasonal Dynamics and Hitchhiking Behaviour in *Atta cephalotes*

A comparison of foraging and minim hitchhiking behaviour in leafcutter ants in Madre de Dios, Peru

RESEARCH PAPER

Flore Allessie

Student number: 540340561

Host Institution: Kawsay Biological Station, Madre de Dios, Peru

Home Institution: HAS Green Academy, 's-Hertogenbosch

Supervisor: Osama Almalik

Module: Year 3– Applied Biology

5 January 2026

Table of contents

1. Preface

This report presents the outcomes of an ecological research project conducted during a 20-week internship at Kawsay Biological Station in the Madre de Dios region of the Peruvian Amazon. The internship was carried out as part of the Applied Biology programme at HAS Green Academy.

The research focused on the foraging ecology of leafcutter ants (*Atta cephalotes*) and was based on field observations and data collection in the surrounding tropical rainforest. The study was designed as an experimental field study and conducted independently during the internship period.

I would like to thank Osama Almatik, Stephanie Riofrio, Roxana Ballon, Raul Bello, and the associated biologists and volunteers at Kawsay Biological Station for their guidance and support throughout the internship.

Flore Allessie

January 2026

2. Abstract

Leafcutter ants (*Atta cephalotes*) are dominant herbivores in Neotropical rainforests and play an important role in ecosystem functioning through their foraging and fungus-cultivation activities. Among their foraging behaviours, minim workers may hitchhike on leaf fragments carried by larger workers, yet the ecological context in which this behaviour occurs remains incompletely understood. This study examined whether minim hitchhiking behaviour and the functional composition of collected leaf material vary between seasons.

Field observations were conducted during a 20-week internship at Kawsay Biological Station in the Madre de Dios region of the Peruvian Amazon. The frequency of minim worker hitchhiking was recorded during nocturnal foraging at a single *A. cephalotes* nest. In addition, collected plant material was classified into functional leaf categories based on traits relevant to cutting, processing, and fungal cultivation. Observations were made during the dry season and the early rainy season.

Hitchhiking behaviour was observed in both seasons but occurred significantly more frequently during the dry season than during the early rainy season. In contrast, seasonal differences in the functional composition of collected leaf material were limited, with similar dominant leaf categories harvested across seasons.

This study demonstrates that minim hitchhiking behaviour in *Atta cephalotes* varies seasonally at a local scale, with a higher frequency observed during the dry season compared to the early rainy season. In contrast, no statistically significant seasonal differences were detected in the functional composition of collected leaf material. Together, these findings suggest that seasonal variation in hitchhiking behaviour may reflect changes in foraging or handling conditions along foraging trails, rather than shifts in plant selection. Further research across multiple colonies and seasons is required to assess the generality of these patterns.

Overall, this study indicates that minim hitchhiking behaviour in *A. cephalotes* can vary seasonally at a local scale. Such behavioural flexibility may reflect variation in handling demands or environmental conditions along foraging trails, rather than solely reflecting changes in plant selection. Further research across multiple colonies, sites, and seasons is required to determine the generality of these patterns.

3. Introduction

The Amazon rainforest, Earth's largest tropical forest, is an irreplaceable reservoir of life, harboring the greatest biodiversity on the planet (Guayasamin et al., 2024). Within the western Amazon lies the Madre de Dios region, characterized by high biological diversity and a wide range of species and ecological interactions; for instance, a single Amazonian tree can host more ant species than occur in the entire British Isles (Wilson, 1987). Ants are ecologically dominant in tropical ecosystems, accounting for up to 25% of the total animal biomass in the New World tropics (Hölldobler & Wilson, 1990).

Leafcutter ants (*Atta cephalotes*) are among the most dominant herbivores in the Neotropics and have practiced agriculture for millions of years, predating human agriculture (Schultz & Brady, 2008). They harvest vast amounts of vegetation to sustain their obligatory mutualistic fungus, *Leucoagaricus gongylophorus* (Hölldobler & Wilson, 2011). Foraging follows a well-defined sequence: cutting and transporting leaves, fragmenting and treating them with fecal enzymes, and inoculating the substrate with fungal mycelium, which grows into nutrient-rich hyphal swellings called gongylidia, the colony's main food source (Schultz & Brady, 2008; Aylward et al., 2012).

Through their nest-building activities, *A. cephalotes* act as ecosystem engineers, modifying soil structure, aeration, and nutrient cycling. By mixing nutrient-poor subsoil with organic-rich surface layers, they enhance carbon and nutrient heterogeneity (Swanson et al., 2019). Furthermore, the concentration of waste from their fungus gardens creates highly valuable nutrient hotspots; studies show that refuse dumps contain critical nutrients at concentrations that are, on average much larger than adjacent non-nest soils (Swanson et al., 2019; Farji-Brener & Tadey, 2009). Despite these positive effects, leafcutter ants can also damage human agriculture, causing crop losses worth billions annually (Hölldobler & Wilson, 1990; Masiulionis & Samuels, 2025).

Beyond their role as ecosystem engineers, the social organization of leafcutter ants underpins their ecological dominance. Colonies can contain millions of workers and are capable of removing up to 17% of the available leaf biomass in some ecosystems (Aylward et al., 2012; Hölldobler & Wilson, 1990). Their colonies are structured into morphologically distinct castes specialized for different tasks such as cutting, transporting, or processing vegetation (Hölldobler & Wilson, 1990). A schematic overview of the caste system of *Atta cephalotes* is provided in Appendix A (Figure A1). Among these, minim workers are noteworthy for their hitchhiking behaviour, in which they ride on leaves carried by larger workers (Linksvayer et al., 2002).

Hitchhiking behaviour in leafcutter ants is often interpreted as an adaptive defense against attacks from parasitic phorid flies (Feener & Brown, 1993; Orr, 1992). However, increasing evidence suggests that this is a multifunctional and context-dependent behaviour, with additional roles in microbial cleaning, sap feeding, and stabilization of leaf fragments during transport (Vieira-Neto et al., 2006; Linksvayer et al., 2002; De Araújo Galvão et al., 2019). The relative importance of these functions is likely to vary with foraging substrate properties and environmental conditions, yet how such variation manifests across seasons remains poorly understood.

Leafcutter ants are selective in the composition of leaf material harvested, although local availability strongly influences foraging decisions (Rockwood, 1976; Wirth et al., 2003). Foraging preferences are further shaped by the requirements of their obligate fungal symbiont; leaf material unsuitable for fungal cultivation is avoided even when it poses no direct risk to the ants themselves (Saverschek, 2010). Leaf traits such as secondary compounds, toughness, and nutrient content affect substrate selection (Howard, 1987; Nichols-Orians & Schultz, 1990).

Seasonality in the New World tropics is primarily manifested by distribution patterns of rainfall, and expressed in terms of "dry" and "rainy" seasons (Rockwood, 1975). In Madre de Dios, the rainy season lasts from November to April and a dry season from May to October. For leafcutter ants, the rainy season provides abundant foliage, whereas the dry season restricts plant availability and may constrain resource choice (Wirth et al., 1997). Leaf quality for leafcutter ants is determined by factors such as tenderness, nutrient content, and the presence or quantity of secondary plant chemicals (Hölldobler & Wilson, 1990).

Leaf toughness and lignin content affect cutting efficiency and energetic costs (Cherrett, 1972; Howard, 1987), latex and secondary compounds interfere with processing and fungal growth (Stradling, 1978), while water content influences both transport costs and pathogen risk within the fungus garden (Howard, 1987; Currie et al., 1999). This functional classification allows comparison of foraging patterns across seasons while retaining ecological relevance. Seasonal dynamics are expected to alter substrate availability and functional traits relevant to cutting, processing, and fungal cultivation.

While the ecological roles and foraging impacts of leafcutter ants are well documented, how seasonal variation in leaf availability and substrate quality influences hitchhiking behaviour and leaf selection remains poorly understood. This study therefore examined seasonal variation in minim hitchhiking behaviour and the functional composition of leaves collected by *A. cephalotes* in the Madre de Dios region of the Peruvian Amazon, to evaluate whether observed behavioural shifts were linked to changes in substrate characteristics or environmental conditions.

4. Methodology

4.1 Study Area: Kawsay Biological Station, Madre de Dios, Peru

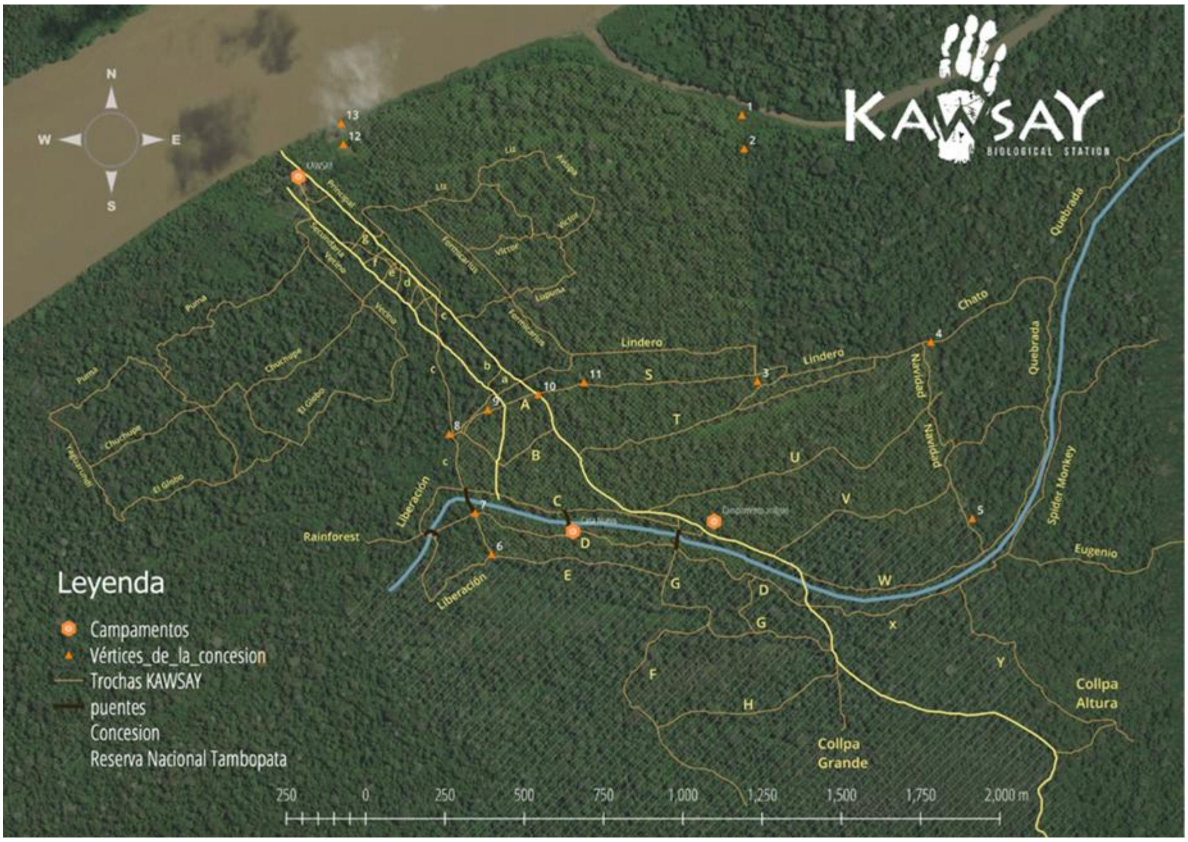

Figure 4.1: Map of the Kawsay Biological Station research grid, trail system, and surrounding concessions in the Madre de Dios region, Peru. Source: Kawsay Biological Station.

Within the western Amazon lies the Madre de Dios region, covering roughly 85,300 km², of which nearly 7.9 million hectares remain forested, representing about 12% of the Peruvian Amazon (Banco Central de Reserva del Perú, 2020; WWF).

Kawsay Biological Station is located in southeastern Peru, within the Madre de Dios region (Tambopata province). The station lies on the right bank of the Madre de Dios River, approximately one hour downstream by boat from Puerto Maldonado. The 200-hectare conservation concession forms part of the buffer zone of the Tambopata National Reserve and consists of lowland Amazonian rainforest with riparian forest and riverine terraces. The area supports high biodiversity and provides basic research infrastructure, including accommodation, trail systems, solar power, and communal facilities. Research focused on the largest *Atta cephalotes* nest within the concession, as determined by visible nest structure and foraging activity, located in an area of secondary forest.

4.2 Observation of Minim Hitchhiking Behaviour

Minim hitchhiking behaviour of *A. cephalotes* was observed at a single nest with four active foraging trails. Observations were conducted at a central section of each trail. At the start of each sampling night, a fixed reference point was selected at the location along the trail where ant traffic was visually highest at that time. All observations for that trail and night were conducted at this reference point. Inbound foraging ants passing this reference point while carrying leaf fragments were counted, defined as ants moving from the foraging source toward the nest. Minims hitchhiking on leaf fragments were recorded concurrently, with each minim counted individually. Minim hitchhiking abundance was calculated as the number of minim workers observed per leaf-carrying media worker during each observation interval.

For each trail, two consecutive two-minute observation intervals were conducted per sampling night. This procedure was repeated across all four trails. Sampling was conducted on ten evenings during the dry season and ten evenings during the rainy season, starting at approximately 20:45 h. This resulted in 80 observation intervals per season (160 in total). The same observation protocol was applied in both seasons.

4.3 Foraging Source Identification and Tree Characterisation

To assess foraging source use, four active foraging trails from a single leafcutter ant colony were selected per sampling night. Each trail was followed to the vegetation source from which leaf fragments were collected. At each source tree, geographic coordinates were recorded using Avenza Maps, tree diameter at breast height (DBH) was measured, and photographs were taken to support species identification. Tree identification to species level was completed during daytime surveys.

Sampling was conducted on ten occasions per season, resulting in 40 recorded source trees per season (80 trees in total). Individual trees could be recorded multiple times if they were repeatedly used as foraging sources.

Due to low observation numbers per individual tree species, species-level statistical analyses were not performed. Foraged tree species were assigned to functional leaf categories based on literature descriptions (Appendix A, Table A1). Classification was based on reported mechanical properties (e.g. relative toughness and lignin content), physiological characteristics (e.g. water content), and the presence of chemical defenses such as latex or secondary metabolites. No direct measurements of leaf traits were conducted in this study.

Category 1 included species described as having soft, low-lignin leaves with relatively low structural and chemical defenses. Category 2 comprised species with intermediate leaf toughness, often associated with latex production or moderate chemical defenses. Category 3 consisted of pioneer species reported to have high water content and low tissue density. Category 4 included hardwood species characterized by tough, high-lignin leaves with high structural investment and reduced suitability for fungal degradation.

4.4 Data analysis

All statistical analyses were performed in R (RStudio version 2024.04.1 + 748). Prior to hypothesis testing, the assumption of normality was assessed for all continuous response variables using Shapiro–Wilk tests and visual inspection of Q–Q plots. Because the data deviated from normality, non-parametric statistical methods were applied throughout. For all statistical tests, significance was assessed at $\alpha = 0.05$. P-values below this threshold were considered statistically significant. Effect sizes were reported where appropriate to complement significance testing.

To assess whether leafcutter ants foraged from trees of different sizes across seasons, diameter at breast height (DBH) values were compared between the dry and rainy season using a Wilcoxon rank-sum test. The same test was used to compare seasonal differences in minim hitchhiking abundance, expressed as the average number of minim workers per media worker.

For analyses of leaf material selection, observations were summarized in contingency tables of leaf material categories by season. Because several cells contained low expected counts, Fisher's Exact Test was used to test for differences in the distribution of leaf categories between the dry and rainy season. Post-hoc pairwise Fisher's Exact Tests were conducted to assess category-specific differences, with p-values adjusted for multiple comparisons using the Benjamini–Hochberg procedure. Effect sizes for contingency analyses was quantified using Cramer's V (small = 0.1, medium = 0.3, large = 0.5). In addition, a Monte Carlo–simulated chi-squared test (10,000 replicates) was performed to verify the robustness of results under low expected counts.

Descriptive statistics were calculated and data were visualized using boxplots, violin plots, and stacked bar charts.

5. Results

5.1 Minim hitchhiking behaviour

Minim hitchhiking behaviour differed between seasons (Figure 5.1), with a higher median number of minim workers hitchhiking per leaf-carrying media worker during the dry season compared to the rainy season.

Minim hitchhiking behaviour was consistently observed in both seasons, but the number of hitchhiking minim workers per leaf-carrying media worker was low.

A Wilcoxon rank-sum test indicated that this seasonal difference was statistically significant ($W = 4210.5$, $p = 0.028$).

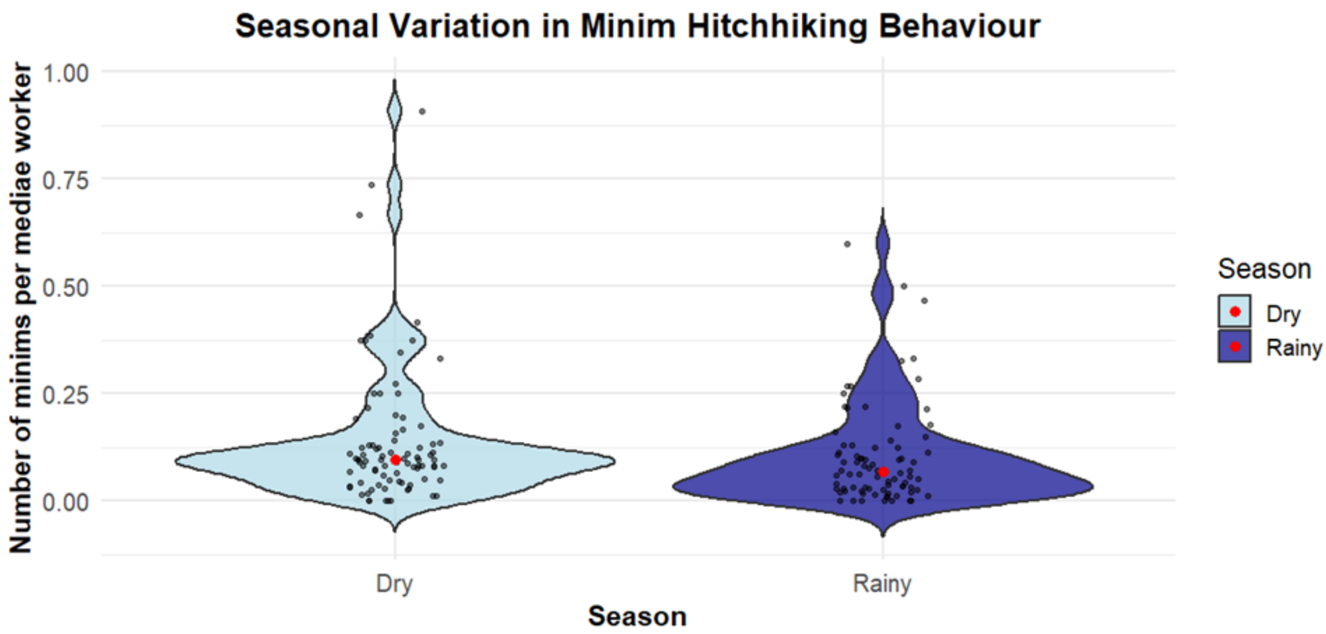

Figure 5.1. *Seasonal variation in minim hitchhiking behaviour of A. cephalotes. Values represent the number of minim workers per leaf-carrying media worker per observation interval for the dry and rainy seasons. Violin plots show the distribution of observations; red dots indicate the median.*

5.2 Foraging source identification and tree characterisation

The composition of leaf material collected by leafcutter ants differed between seasons in terms of proportional representation of functional leaf categories (Figure 5.2; Table 5.1).

Soft, low-lignin leaf categories were proportionally more represented during the dry season, whereas leaves of medium toughness and latex-associated defenses were more common during the rainy season. The remaining leaf categories showed similar proportional contributions across seasons.

A Fisher's Exact Test indicated no statistically significant difference in the overall distribution of leaf material categories between the dry and rainy season (p = 0.125). The strength of association between season and leaf category was moderate (*Cramer's V* = 0.27). A Monte Carlo–simulated chi-squared test yielded a comparable outcome (p = 0.119). The spatial distribution of harvested trees relative to the nest is shown in Figure 5.3. Tree size, characterized by DBH, did not differ significantly between seasons (Appendix B, Figure B2). Species-level frequencies underlying these functional categories are provided for descriptive purposes in Appendix B (Figure B1).

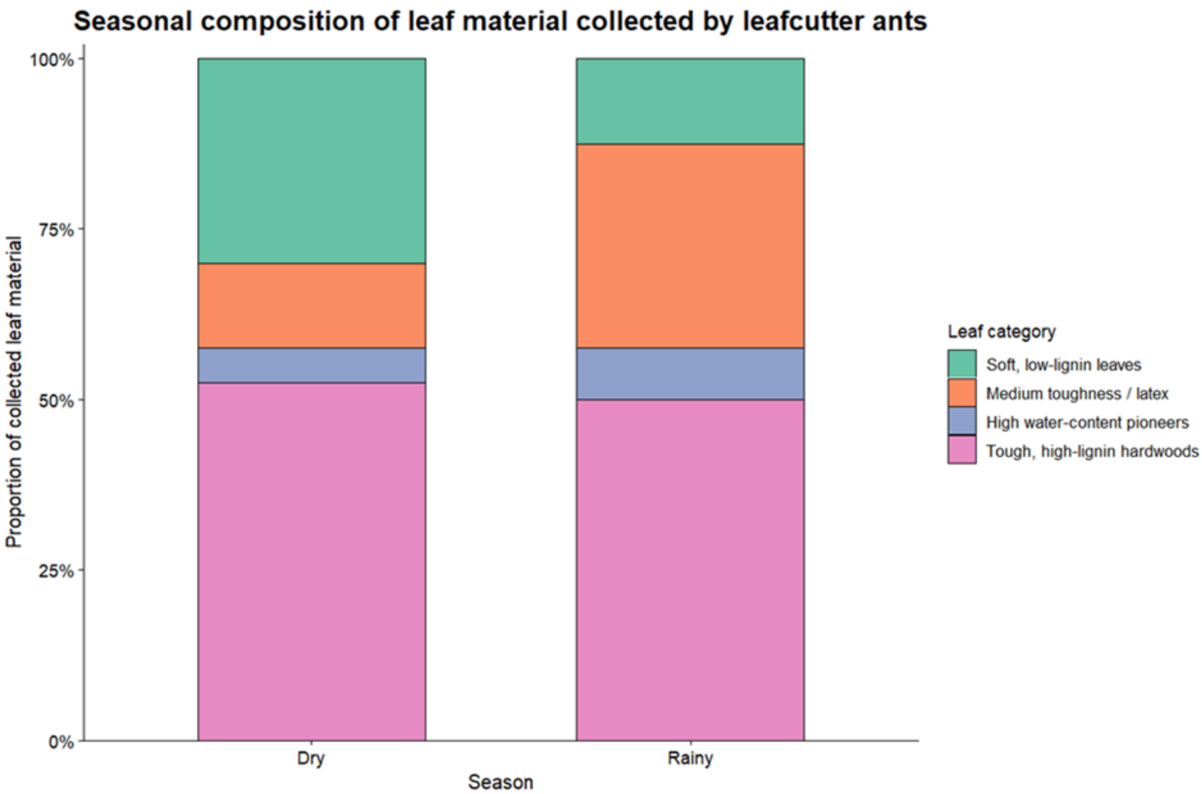

Figure 5.2. *Seasonal composition of leaf material collected by A. cephalotes. Bars represent the proportional contribution of four functional leaf categories to the total foraged material during the dry and rainy season.*

Table 5.1. *Number of foraging observations per functional leaf category recorded during the dry and rainy season.*

Leaf category	Dry season	Rainy season
1	12	5
2	5	12
3	2	3
4	21	20

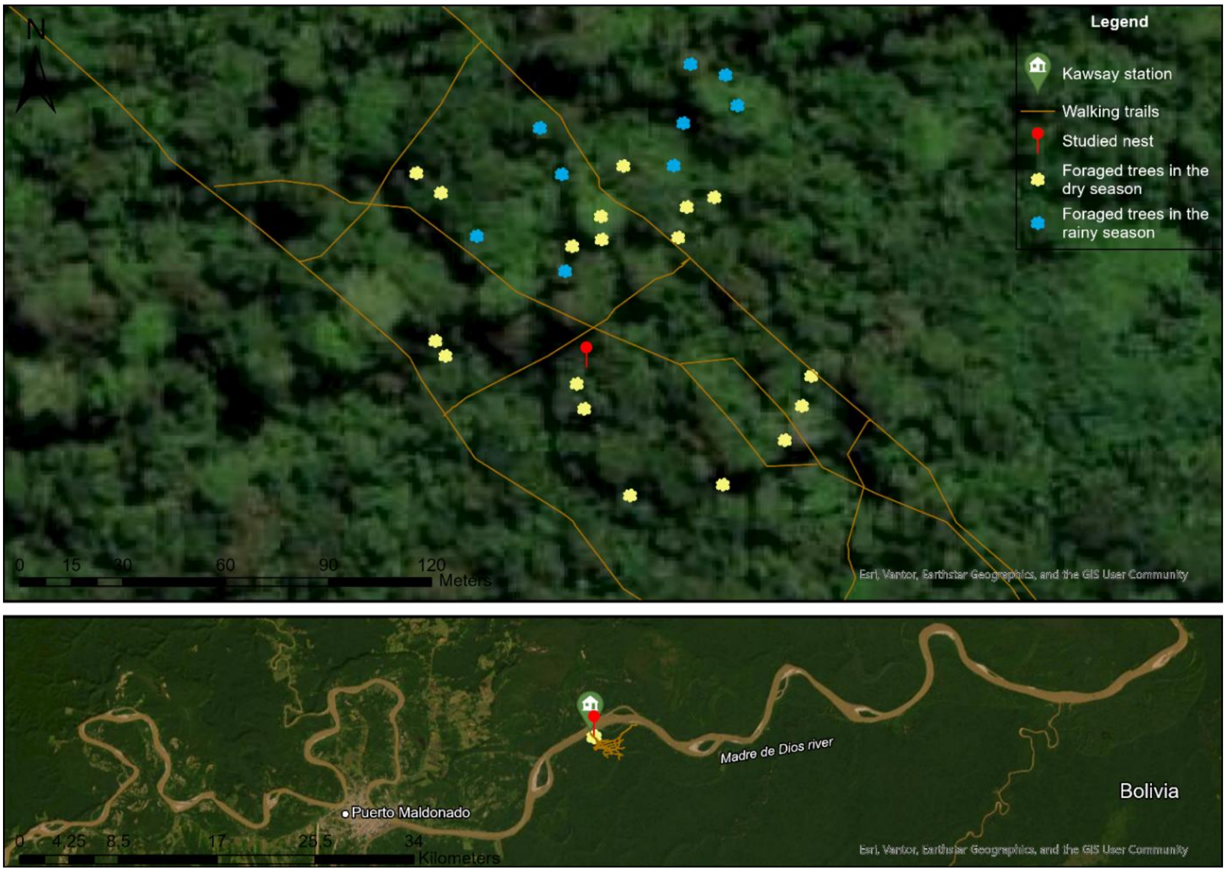

Figure 5.3. *Detailed map of the study area showing the location of the studied A. cephalotes nest and trees harvested during the dry and rainy seasons. The inset map indicates the position of the field station along the Madre de Dios River.*

6. Discussion and Conclusion

This study demonstrates that hitchhiking behaviour of minim workers in *Atta cephalotes* varies seasonally, with a higher frequency during the dry season than during the early rainy season. This indicates that minim deployment along foraging trails is not fixed, but responds to changing environmental conditions. In contrast, seasonal differences in the functional composition of collected plant material were subtle, suggesting that behavioural adjustments in hitchhiking may be more sensitive to environmental variation than changes in overall foraging choices.

All observations were conducted at night, when parasitic phorid flies are largely inactive (Orr, 1992). Hitchhiking observed in this study therefore cannot be interpreted as a direct response to parasitoid pressure, which challenges one of the most commonly proposed explanations for this behaviour (Feener & Moss, 1990). Although *A. cephalotes* forages both diurnally and nocturnally, nocturnal activity often predominates (Wetterer, 1990). If hitchhiking served exclusively as a defense against diurnal parasitoids, its frequent occurrence during nocturnal foraging would be difficult to explain (Vieira-Neto et al., 2006; Bish et al., 2020).

Hitchhiking is increasingly interpreted as a multifunctional behaviour, with microbial cleaning of leaf fragments representing one of its most strongly supported functions (Vieira-Neto et al., 2006; Linksvayer et al., 2002). Experimental studies show that the proportion of fragments carrying hitchhikers increases significantly in response to fungal contamination (Vieira-Neto et al., 2006). This hygienic role is ecologically important because collected plant material serves as the substrate for the colony's obligate fungal symbiont (*Leucoagaricus gongylophorus*), which constitutes the ants' primary food source (Hölldobler & Wilson, 1990).

Fungi in the genus *Escovopsis* are pronounced antagonists of the fungal garden and can severely reduce colony fitness (Currie et al., 1999; Currie 2001). Microbial infections are controlled through a combination of behavioural and chemical defenses, including the use of the workers' metapleural glands, which produce antifungal compounds transferred to leaf fragments through licking and associated grooming behaviours (Fernández-Marín et al., 2003; Fernández-Marín et al., 2006; Goes et al., 2020). By reducing microbial contamination prior to nest entry, hitchhiking minim workers likely enhance substrate quality and contribute to the long-term stability and productivity of the fungal garden.

Longer durations without rain can allow microbes to accumulate on leaf surfaces, and seasonal shifts can significantly alter the composition of microbial communities, with distinct bacterial and fungal taxa dominating during the dry season (Li et al., 2022; Peñuelas et al., 2012). These seasonal changes in substrate condition may increase the need for mechanical cleaning and preprocessing of leaf fragments, offering a plausible explanation for the higher hitchhiking frequencies observed during the dry season.

Seasonal differences in the functional composition of collected plant material were not statistically significant and thus more subtle, indicating that substrate selection remains broadly consistent across seasons. Tree species with tough, high-lignin leaves dominated foraging observations in both the dry and rainy season. These leaves impose higher mechanical and processing costs, including increased cutting effort and slower fungal degradation, due to lignin content (Veličković et al., 2023). Tree species within this category often contain secondary metabolites such as tannins, that may further reduce suitability for fungal cultivation (Nichols-Orians & Schultz, 1989; Howard, 1987; Barbehenn et al., 2011). Despite these constraints, *A. cephalotes* consistently harvested this functional leaf category, indicating a reliance on abundant and readily available plant material under natural forest conditions rather than active selection for low-quality substrates.

Soft, low-lignin leaves contributed proportionally more to collected material during the dry season than in the rainy season, whereas leaves of intermediate toughness and latex content were more prominent during the rainy season. Increased humidity during the rainy season can reduce mechanical resistance and brittleness of leaves, potentially lowering cutting costs and allowing exploitation of a broader range of functional leaf categories (Cherrett, 1972; Howard, 1987). These patterns should be interpreted as descriptive trends rather than evidence for seasonal shifts in substrate preference.

Despite the seasonal difference observed, hitchhiking remained a relatively infrequent behaviour overall, with most leaf-carrying workers traveling without a minim escort. This pattern suggests that hitchhiking entails energetic or logistical costs and is therefore deployed selectively rather than continuously (Wetterer, 1990; Feener & Moss, 1990). In combination with the relatively stable composition of collected plant material, this pattern suggests that seasonal changes in hitchhiking reflect shifts in handling demands rather than fundamental changes in foraging strategy.

The absence of statistically significant seasonal differences in functional leaf composition should be interpreted in light of methodological constraints. Because observation numbers per individual plant species were low, foraged material was grouped into broad functional leaf categories. Assignment to these categories was based on dominant leaf traits reported in the literature and therefore does not capture potential intraspecific or site-specific variation. This reduction in taxonomic resolution enabled ecologically relevant comparisons under limited sample sizes but may limit the generalizability of the results beyond the studied system.

Additionally, observations were restricted to a single colony and conducted during a transitional seasonal period. In the Madre de Dios region, the onset of the rainy season is characterized by a rapid increase in rainfall and humidity as part of the broader Amazon basin's seasonal rainfall cycle (Espinoza Villar et al., 2009). Sampling during this transition may limit the detectability of seasonal differences in functional leaf composition, particularly if changes in plant traits lag behind climatic shifts.

In conclusion, this study shows that *A. cephalotes* flexibly adjusts its foraging-related behaviour to seasonal environmental conditions, most notably through increased deployment of hitchhiking minim workers during the dry season. This behavioural shift is more plausibly linked to variation in substrate quality and handling demands than to parasitoid defense. Seasonal effects on leaf selection were modest, indicating that foraging choices are largely shaped by vegetation structure and resource availability rather than strong seasonal preferences. These findings support the view of hitchhiking as a flexible aspect of substrate processing that enhances fungal cultivation under variable conditions. For future research and monitoring, incorporating higher-resolution leaf trait data and multiple colonies across seasons would improve understanding of how behavioural flexibility contributes to the ecological success of leafcutter ants.

References

Aylward, F. O., Currie, C. R., & Suen, G. (2012). The evolutionary innovation of nutritional symbioses in leaf-cutter ants. *Insects* 3: 41–61.

Banco Central de Reserva del Perú (2020). *Caracterización del Departamento de Madre de Dios*. BCRP, Lima, 62 p.

Barbehenn, R. V., & Constabel, C. P. (2011). Tannins in plant–herbivore interactions. *Phytochemistry* 72: 1551–1565.

Bish, C. M., Alderdice, J., Ruchti, L., & Davis, M. S. (2020). Hitchhiking behavior of a worker subcaste in leaf-cutter ants (*Atta cephalotes*). *Georgia Journal of Science* 78: Article 48.

Cherrett, J. M. (1972). Chemical aspects of plant attack by leaf-cutting ants. In: Harborne, J. B. (Ed.), *Phytochemical ecology*. Academic Press, London: 13–24.

Currie, C. R. (2001). A community of ants, fungi, and bacteria: A multilateral approach to studying symbiosis. *Annual Review of Microbiology* 55: 357–380.

Currie, C. R., Mueller, U. G., & Malloch, D. (1999). The agricultural pathology of ant fungus gardens. *Proceedings of the National Academy of Sciences of the United States of America* 96: 7998–8002.

De Araújo Galvão, A. R., Bailez, O., Viana-Bailez, A. M., Abib, P. H., Pimentel, F. A., & Lavinscky Pereira, T. P. (2019). Parasitism by phorids on leaf cutter ants *Atta sexdens* (Linnaeus, 1758) in natural and agricultural environments. *Zoological Science* 36: 357–364.

Espinoza Villar, J. C., Ronchail, J., Guyot, J. L., Cochonneau, G., Naziano, F., Lavado, W., De Oliveira, E., Pombosa, R., & Vauchel, P. (2009). Spatio-temporal rainfall variability in the Amazon Basin countries (Brazil, Peru, Bolivia, Colombia, and Ecuador). *International Journal of Climatology* 29: 1574–1594.

Farji-Brener, A., & Tadey, M. (2009). Contributions of leaf-cutting ants to soil fertility: Causes and consequences. In: Lucero, D. P. & Boggs, J. E. (Eds.), *Soil fertility*. Nova Science Publishers, New York: 81–91.

Feener, D. H. Jr., & Brown, B. V. (1993). Oviposition behavior of an ant parasitizing fly, *Neodohrniphora curvinervis* (Diptera: Phoridae), and defense behavior by its leaf-cutting ant host *Atta cephalotes*. *Journal of Insect Behavior* 6: 675–688.

Feener, D. H. Jr., & Moss, K. (1990). Defense against parasites by hitchhikers in leaf-cutting ants: A quantitative assessment. *Behavioral Ecology and Sociobiology* 26: 17–29.

Fernández-Marín, H., Zimmerman, J. K., Rehner, S. A., & Wcislo, W. T. (2006). Active use of the metapleural glands by ants in controlling fungal infection. *Proceedings of the Royal Society B: Biological Sciences* 273: 1689–1695.

Fernández-Marín, H., Zimmerman, J. K., & Wcislo, W. T. (2003). Nest-founding in *Acromyrmex octospinosus*: Demography and putative prophylactic behaviors. *Insectes Sociaux* 50: 304–308.

Goes, A. C., Barcoto, M. O., Kooij, P. W., Bueno, O. C., & Rodrigues, A. (2020). How do leaf-cutting ants recognize antagonistic microbes in their fungal crops? *Frontiers in Ecology and Evolution* 8: Article 95.

Guayasamin, J., Ribas, C. C., Carnaval, A. C., Carrillo, J. D., Hoorn, C., Lohmann, L. G., Riff, D., Ulloa Ulloa, C., & Albert, J. S. (2024). Evolution of Amazonian biodiversity: A review. *Acta Amazonica* 54 (Especial 1): Article e54bc21360.

Hölldobler, B., & Wilson, E. O. (1990). *The ants*. Harvard University Press, Cambridge, 732 p.

Hölldobler, B., & Wilson, E. O. (2011). *The leafcutter ants: Civilization by instinct*. W. W. Norton & Co., New York, 160 p.

Howard, J. J. (1987). Leafcutting ant diet selection: The role of nutrients, water, and secondary chemistry. *Ecology* 68: 501–515.

Li, M., Hong, L., Ye, W., Wang, Z., & Shen, H. (2022). Phyllosphere bacterial and fungal communities vary with host species identity, plant traits and seasonality in a subtropical forest. *Environmental Microbiome* 17: Article 29.

Linksvayer, T. A., McCall, A. C., Jensen, R. M., Marshall, C. M., Miner, J. W., & McKone, M. J. (2002). The function of hitchhiking behavior in the leaf-cutting ant *Atta cephalotes*. *Biotropica* 34: 93–100.

Masiulionis, V. E., & Samuels, R. I. (2025). Investigating the biology of leaf-cutting ants to support the development of alternative methods for the control and management of these agricultural pests. *Agriculture* 15: Article 642.

Nichols-Orians, C. M., & Schultz, J. C. (1989). Leaf toughness affects leaf harvesting by the leaf cutter ant, *Atta cephalotes*. *Biotropica* 21: 80–83.

Orr, M. R. (1992). Parasitic flies influence foraging rhythms and caste division of labor in the leaf-cutter ant, *Atta cephalotes*. *Behavioral Ecology and Sociobiology* 30: 395–402.

Peñuelas, J., Rico, L., Ogaya, R., Jump, A. S., & Terradas, J. (2012). Summer season and long-term drought increase the richness of bacteria and fungi in the foliar phyllosphere of *Quercus ilex*. *Plant Biology* 14: 565–575.

Rockwood, L. L. (1975). The effects of seasonality on foraging in two species of leaf-cutting ants (*Atta*). *Biotropica* 7: 176–193.

Rockwood, L. L. (1976). Plant selection and foraging patterns in two species of leaf-cutting ants (*Atta*). *Ecology* 57: 48–61.

Saverschek, N., Herz, H., Wagner, M., & Roces, F. (2010). Avoiding plants unsuitable for the symbiotic fungus: learning and long-term memory in leaf-cutting ants. *Animal Behaviour* 79: 689–698.

Schultz, T. R., & Brady, S. G. (2008). Major evolutionary transitions in ant agriculture. *Proceedings of the National Academy of Sciences* 105: 5435–5440.

Stradling, D. J. (1978). The influence of size on foraging in the ant, *Atta cephalotes*, and the effect of some plant defence mechanisms. *Journal of Animal Ecology* 47: 173–188.

Suen, G., *et al.* (2011). The genome sequence of the leaf-cutter ant *Atta cephalotes* reveals insights into its obligate symbiotic lifestyle. *PLoS Genetics* 7: e1002007.

Swanson, A. C., *et al.* (2019). Welcome to the *Atta* world: A framework for understanding the effects of leaf-cutter ants on ecosystem functions. *Functional Ecology* 33: 1386–1399.

Veličković, M., *et al.* (2024). Mapping microhabitats of lignocellulose decomposition by a microbial consortium. *Nature Chemical Biology* 20: 1033–1043.

Vieira-Neto, E. H. M., Mundim, F. M., & Vasconcelos, H. L. (2006). Hitchhiking behaviour in leaf-cutter ants: An experimental evaluation of three hypotheses. *Insectes Sociaux* 53: 326–332.

Wetterer, J. K. (1990). Diel changes in forager size, activity, and load selectivity in a tropical leaf-cutting ant, *Atta cephalotes*. *Ecological Entomology* 15: 97–104.

Wilson, E. O. (1987). The arboreal ant fauna of Peruvian Amazon forests: A first assessment. *Biotropica* 19: 245–251.

Wirth, R., Beyschlag, W., Ryel, R. J., & Hölldobler, B. (1997). Annual foraging of the leaf-cutting ant *Atta colombica* in a semideciduous rain forest in Panama. *Journal of Tropical Ecology* 13: 741–757.

WWF (n.d.). *Madre de Dios: A treasure of biodiversity for the world*. https://www.wwf.org.pe/ Accessed on: 5-9-2025.

Appendices

Appendix A – Supplementary biological and classification information

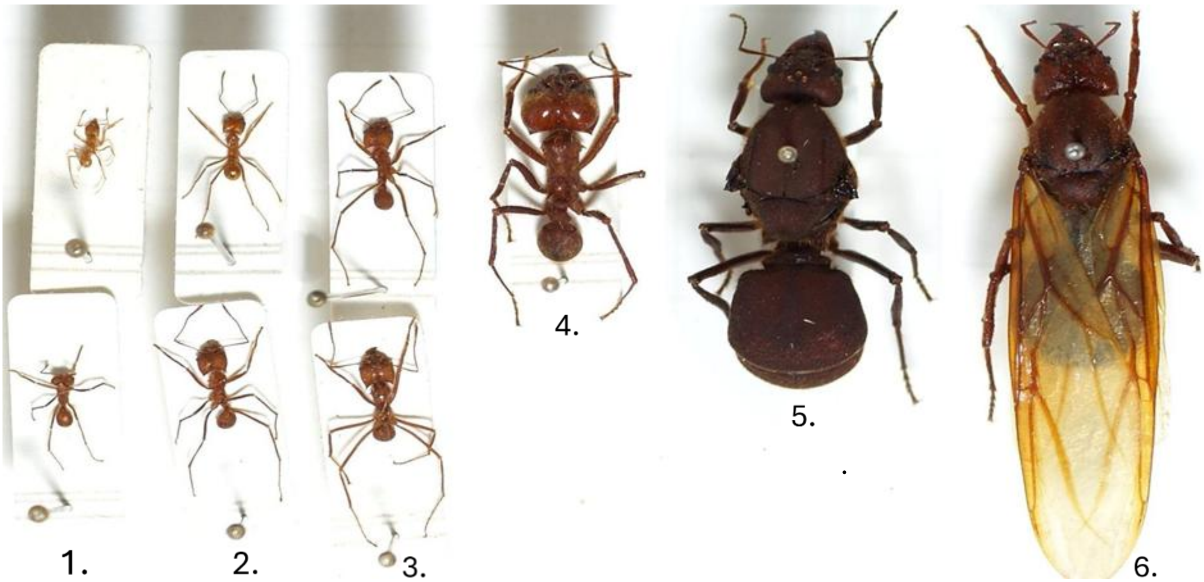

Figure A1. *Caste system of Atta cephalotes, illustrating the distinct castes: (1) minim, (2) minor, (3) media, (4) major (soldier), (5) queen, and (6) drone (male). Source: Suen et al. (2011).*

Table A1. *Functional leaf categories and associated tree species.*

Category	Category descriptions	Foraged tree species
1	Soft, low-lignin leaves with low structural and chemical defenses, as reported in the literature	*Oxandra acuminata* *Inga edulis* *Ceiba samauma* *Nectandra cissiflora* *Ocotea bofo*
2	Leaves of intermediate toughness commonly associated with latex or moderate secondary defenses	*Brosimum utile* *Margaritaria nobilis* *Clarisia biflora* *Sapium glandulosum*
3	Pioneer species characterized in the literature by high water content and low tissue density	*Spondias mombin*
4	Tough, high-lignin hardwood species with high structural investment and reduced substrate quality	*Sloanea terniflora* *Terminalia oblonga* *Apuleia leiocarpa*

Appendix B – Supplementary foraging and tree species figures

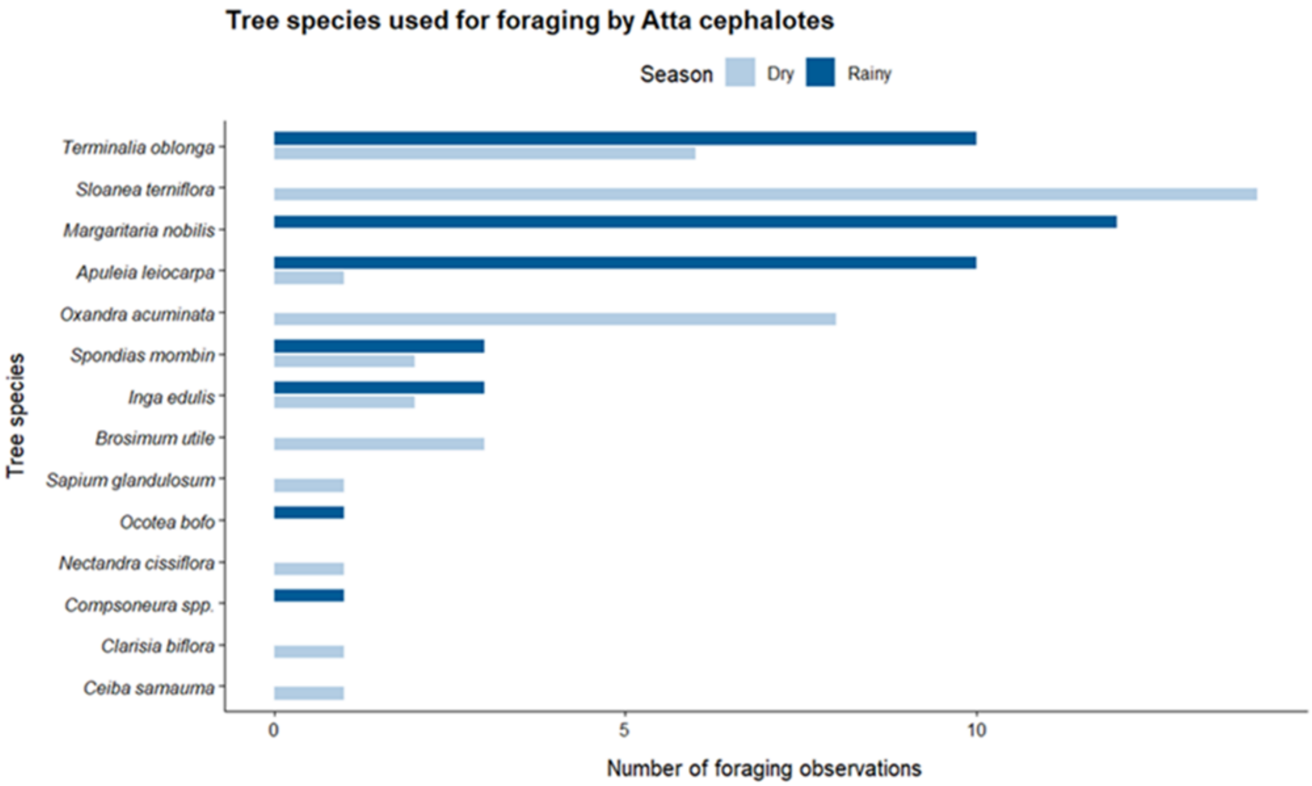

Figure B1. *Frequency of foraging observations per tree species recorded during the dry and rainy season for a single Atta cephalotes colony. Data are shown descriptively to illustrate the species composition underlying the functional leaf categories; no statistical analyses were performed at the species level.*

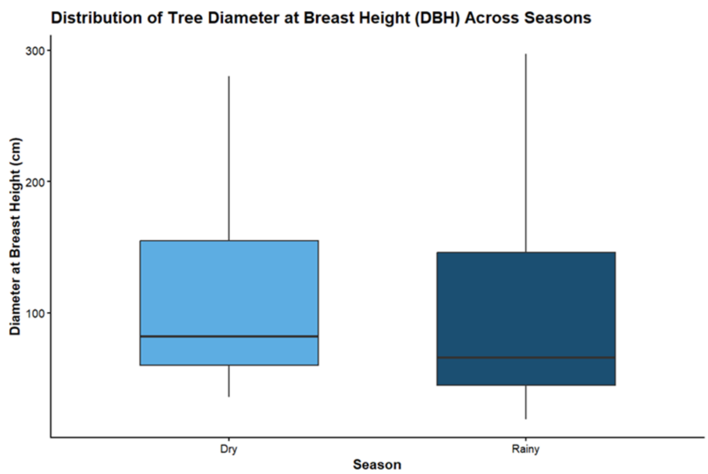

Figure B2. *Distribution of tree diameter at breast height (DBH) across the dry and rainy season.*