**The Relationship Between Leaf Litter and the Biodiversity of Arthropods in the Four Forest Types of the Cloud Forest**

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## 1. Introduction

A tropical forest is a complex ecosystem, full of interactions between both biotic and abiotic factors. These constant interactions support the liveliness and health of every organism. One cannot survive without the other. This perfectly describes the relationship of the trees and arthropods. Trees absorb nutrients and water from the soil with its roots, as well as add a layer of leaf litter for the insects to live in (Zheng 2017). On the other hand, many of the arthropods are decomposers, essential for recycling the energy of the system by turning dead organisms into inorganic materials available for primary producers. Additionally, arthropods provide water regulation, translocation of organic matter, and soil structure formation (Menta 2020). This symbiosis generates a positive feedback loop between the soil and arthropods, where the soil provides a habitat for the arthropods to thrive in and in return, the decomposers convert leaf litter into nutrient rich soil (Ghiglieno 2020).

Beyond being decomposers, arthropods have several other roles in the forest. Due to their prominence and ability to be easily monitored, arthropods have been utilized to gauge the biodiversity and health of ecosystems. Arthropods abundance and diversity are affected by numerous abiotic and biotic factors. Abiotic factors include climate, vegetation, and elevation while biotic factors include the number of parasites and predators around (Gonzáles-Reyes 2017). However, the main factor influencing arthropod populations is the structural complexity of the trees, where the old growth forests are the most intricate (Maleque 2006). Therefore, typically the arthropod diversity and abundance differs between forest types because each kind has a distinct structural complexity given their unique origin. Thus, it would be expected that old growth and primary forests would support more arthropods with their greater tree cover, ground vegetation, and leaf litter; and the opposite would be said young growth and planted forests. Leaf litter provides more nutrients and habitat for the insects that live in the organic layer, so theoretically, more trees would equal more leaves.

A previous researcher, Jessica Goodwin, completed a project studying the diversity of arthropods in the different forest types in the Cloudbridge Reserve. However, due to several setbacks, her experiment was quite short. A similar study was done at Cloudbridge about 15 years ago, but did not compare the diversity of arthropods to the leaf litter density (Sheenhan 2007). Not only were the collection of arthropod methods different, but it was done during the transition from wet to dry season, whereas this study was done during the rainy season. Additionally, the cloud forest has grown since then and it would be interesting to compare the results.

This research project aims to expand the previous research conducted at Cloudbridge by creating a more extensive arthropod diversity database. The results will supply a better look into the richness of the soil and allow comparisons to be made with newly forested land, which gives a way to monitor the growth of the reforested land. It is expected that there will be higher diversity in the primary forest and old growth compared to young growth and planted. A couple of sub-questions were investigated, including determining if the amount of leaf litter density is related to the diversity of arthropods and if there is a difference in abundance of arthropods in the four different forest types.

## 2. Materials and Methods

### 2.1 Study Sites and Timeline

This study was conducted at the Cloudbridge Natural Reserve, a tropical cloud forest among the Talamanca mountains in the South Pacific Foothills of Costa Rica at about an elevation of 1675m. It was orchestrated over a six-week period, from the beginning of July to the middle of August. There were four chosen areas for both the pitfall traps and leaf litter traps (Fig. 1). Each area was in one of the forest types: primary forest, old growth, young growth, and planted forest. It is important to note that planted and young forests were pasture lands in the past and the reforestation process began 20 years ago.

Collection periods were always in the morning to avoid the rain, around 8-9am. The climate was steady throughout the survey period: 18-21℃, rainfall the night before, and semi-overcast. The data collection lasted for four weeks and each week was dedicated to a forest type in this order: primary forest, young growth, old growth, and planted forest.

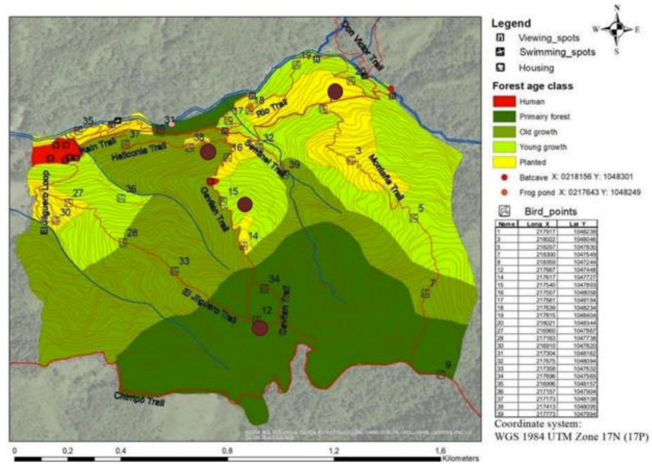


Figure 1. Leaf litter arthropods sampling locations.

### 2.2 Leaf Litter Mesh Traps

There was a total of four leaf litter traps, one placed in each of the forest types. The sites were chosen based on level ground and a small opening among trees and bushes. During the week of preparation, each of the leaf litter traps were constructed in their respective forest on a separate day. For the next six consecutive weeks, leaf litter trapped in the mesh was collected every seven days.

The leaf litter mesh traps were built using four sticks, a 1x1 meter piece of mesh, zip ties, and string. Leaf litter trapped in the mesh was collected in a Ziplock bag and brought back to the base. The leaf litter was placed on a metal tray, dried in an oven at 250°C for ten minutes, and then weighed on a kitchen scale. The density of the leaf litter was calculated by dividing the weight by its area, which was measured with a measuring tape along the width and length.

### 2.3 Arthropod Pitfall Traps

For the arthropod pitfall traps, two separate sites within each forest type were chosen. Each site consisted of three pitfall traps. A conscious effort was made to choose areas for the pitfall traps that had unique characters, such as the amount of sunlight let through the forest covering and the proximation to trees. The traps were set in the morning and the specimen were collected the next morning.

Three pitfall traps were placed apart from each other in each site within the forest type. First, holes were dug with a garden trowel about the size of a six-centimeter diameter cylindrical container. Then, the containers were placed in the holes and dirt was patted around the edges to ensure that the ground and the rim of the container were at the same level. About two centimeters of leaf litter from the surrounding area was placed into the bottom of the container, along with a piece of banana. Three tiny sticks were set into the soil around the container, allowing one centimeter to poke up from the soil. A piece of wood larger than the diameter of the container’s rim was placed on top to protect the trap from rainfall. For collection, the contents of each container were poured into a separate jar.

### 2.4 Identifying Arthropods

In the lab, the contents of each jar were dumped into a plastic container. Insects were separated from the soil, which was eventually disposed of, using tweezers and a spoon. Arthropods large enough for the eye were identified and then released back into the wild. Smaller arthropods were placed in alcohol on a petri plate to be identified under a stereoscope. The insects were identified to order using dichotomous keys for arthropods.

### 2.5 Statistical Analysis

Microsoft Excel was used to graph arthropod abundance and number of orders in each forest type. Three statistical tests were run to make comparisons among the four forest types, using the software Past 4.0. First, a chi square test was run to determine if there was a significant difference of arthropod abundance among the four forest types, then an ANOVA to test differences in leaf litter density. Finally, a value of diversity was calculated using the Shannon-Wiener Index.

## 3. Results

A total of 221 total arthropods were collected in the pitfall traps and 16 orders identified with Coleoptera and Collembola being the two most common orders (Table 1). In order of decreasing abundance, planted areas had the most insects, then young growth, old growth, and finally primary (Fig. 2). A statistically significant difference was found for comparisons between arthropod abundance within each unique forest type ($x^2=160.69$, df=54, and $p<0.05$). The young growth, old growth, and planted forest were found to have 11 arthropod orders, while the planted forest only had seven identified (Fig. 3).

Table 1. Abundance, Richness, and Diversity of individuals found in the four forest types, each with samples collected from two sites.

| | | Primary Forest | Young Growth | Old Growth | Planted Forest | |
|---|---|---|---|---|---|---|
| **Class** | **Order** | **Abundance** | **Abundance** | **Abundance** | **Abundance** | **Total** |
| Arachnida | Acari | 2 | 0 | 0 | 0 | 2 |
| | Araneae | 2 | 6 | 6 | 4 | |
| Crustacea | Isopoda | 0 | 4 | 8 | 13 | 18 |
| Chilopoda | | 0 | 1 | 1 | 1 | 3 |
| Diplopoda | | 0 | 1 | 1 | 0 | 2 |
| | Blattaria | 0 | 1 | 0 | 1 | 2 |
| | Coleoptera | 10 | 12 | 8 | 19 | 49 |
| | Collembola | 0 | 17 | 0 | 32 | 49 |
| | Dermaptera | 2 | 0 | 1 | 0 | 3 |
| | Heteroptera | 0 | 0 | 0 | 7 | 7 |
| Insecta | Homoptera | 0 | 2 | 0 | 0 | 2 |
| | Hymenoptera | 2 | 6 | 5 | 8 | 21 |
| | Isoptera | 0 | 0 | 0 | 13 | 13 |
| | Neuroptera | 0 | 0 | 1 | 0 | 1 |
| | Siphonaptera | 1 | 0 | 1 | 0 | 2 |
| | Sternorryhyncha | 0 | 1 | 2 | 0 | 3 |
| | Type 1 | 2 | 0 | 0 | 0 | 2 |
| Unidentified | Type 2 | 0 | 1 | 2 | 2 | 5 |
| | Type 4 | 0 | 0 | 0 | 2 | 2 |

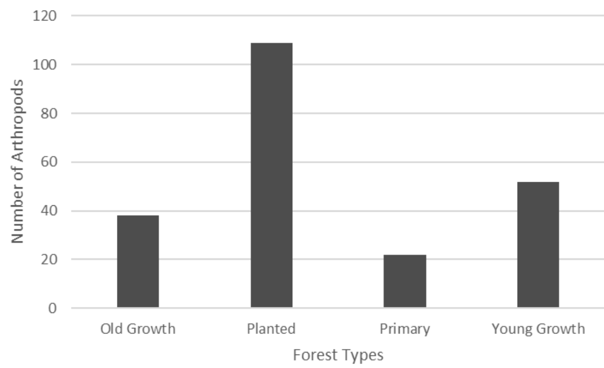


Figure 2. Arthropod abundance in the four forest types.

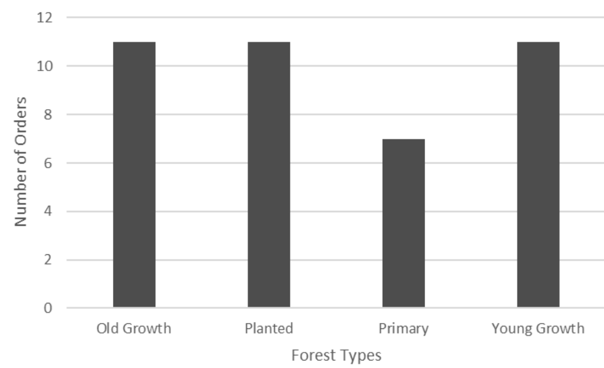


Figure 3. Arthropod richness in the four forest types.

No statistically significant difference was found when comparing the leaf litter densities between each forest type ($F (3, 8) = 2.15$, $p > 0.05$). However, the primary forest had the most leaf litter density, followed by planted forest, old growth, and young growth (Fig. 4).

Regarding diversity, it was higher in old growth forest, followed by young growth, planted forest and primary forest; however, all the forest types had a value that was less than 2.5 (Table 2).

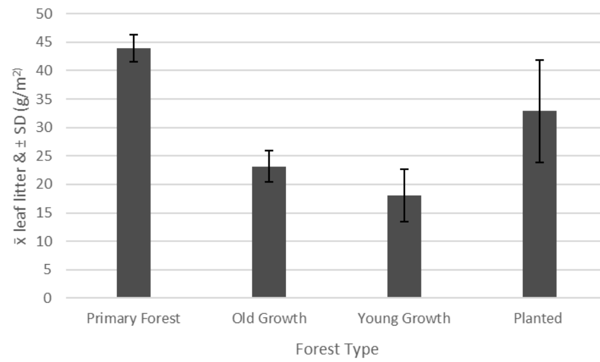


Figure 4. Leaf litter density in four forest types of Cloudbridge Nature Reserve.

Table 2. Comparison of three variables and Shannon-Wiener diversity.

| | Leaf Density (g/m2) | Arthropods (# of Individuals) | Richness (# of Orders) | Shannon-Wiener (H) |
|---|---|---|---|---|
| Primary Forest | 43.98148 | 22 | 7 | 1.779 |
| Old Growth | 23.14815 | 38 | 11 | 2.217 |
| Young Growth | 18.05556 | 52 | 11 | 2.001 |
| Planted Forest | 32.87037 | 109 | 11 | 1.996 |

## 4. Discussion & Conclusions

### 4.1 Arthropod Abundance and Richness

Seen in Figure 2a, the planted forest has a In a research project that studied arthropods among epiphyte mats in Costa Rican cloud forests, it was found that, although arthropod richness did not differ among primary and secondary forests, there was a significantly higher abundance in the latter despite the former having more complex plants (Yanoviak et al. 2006), which coincides with the results of the present study (Figure 2 and

3). The data of the study mentioned was consistent throughout the years that the research was conducted, suggesting that the age of the forest does not seem to play a significant role on fundamental arthropod parameters; according to the authors, this lack of richness may be due to the forest type spatial arrangement, considering that they were well interconnected and thus easily affecting each other.

This is unlikely the reason for this study because the chosen sites in Cloudbridge were not embedded within each other; rather they were quite isolated from each other, deep within each forest type (Figure 1). One possible cause may be the short timeline of the project and thus, the small number of surveys conducted and pitfall traps set up.

In another investigation, it was found that leaf litter arthropod abundance, diversity, and richness showed higher values in planted areas when using nucleation, a method involving planting trees in patches of different sizes, compared to more intensive tree plantation methods or natural regeneration (Cole 2016). These results can be compared to the difference between the arthropod abundance, diversity, and richness of young growth and planted in the Cloudbridge forest. Although similarly, the planted has higher abundance, it has the same amount of richness and less diversity than young growth, which does not coincide with the study mentioned above. The most obvious reasoning behind this lack of correlation is that the Cloudbridge forest did not have different methods of reforestation. There was only passive and active restoration, whereas the other study had several methods of active restoration.

**4.2 Most Abundant Orders**

In this study, the most common orders were Coleoptera and Collembola, both being 22% each of the total number of arthropods identified. The same epiphyte study from above also discovered that Coleoptera and Collembola arthropods were the most abundance order overall (Yanoviak et al. 2006). The Costa Rican International Collaborative Biodiversity Group (ICBG) extracted arthropods from Northwestern Costa Rica and found that the second most common order was Coleoptera, consisting of 15% of the total arthropods collected. The most common was Lepidoptera, butterflies, but it is not relevant to this study because insects were collected with pitfall traps, whereas the ICBG trapped arthropods with bait in bottles above the soil level; in other words, an abundance in Lepidoptera would be impossible for this study (Sittenfeld et al. 1999).

Given that Collembola and Coleoptera were yet again revealed to be most common arthropod order among Costa Rican neotropical forests in another study (Nadkarni et al. 1990), it validates the data in this study. The Collembola play a crucial role in the stability and healthiness of the surrounding environment, found in all types of temperature and extreme climates and locations (Marx 2012); whereas, Coleoptera have been found to be adaptable to extreme disturbances in the environment (Gerisch 2012). Their resilience suggests a teleological importance. When the Indonesian sugar cane plantations utilized habitat manipulation treatment to increase the diversity of arthropods and thus pest control, Collembola and Coleoptera were among the most common orders to dominate the pitfall traps (Prabowo 2021). Therefore, their abundance seems to indicate a healthy ecosystem.

**4.3 Leaf Litter Density**

The fact that the most leaf litter was found in the primary forest was expected considering it has not been tampered by humans and therefore has the oldest and most trees. For this same reason, it is interesting that the trap in planted forest had a higher amount of leaf litter density than the one placed in old growth.

A previous study inspected the amount of leaf litter in Southern Costa Rica among four different forest types: plantation (entirely planted with trees), island (trees planted in three different sized plots), control (natural regeneration), and young secondary forest. The researchers discovered that there was more leaf litter overall in plantations (Celentano et al. 2011). Naturally, this makes sense because that forest type has the largest number of trees, and thus likely the most leaf litter. It slightly coincides with the Cloudbridge study because the leaf litter density is higher in planted areas compared to natural regrowth or young growth. However, it is odd that there is less in old growth than planted, but this could be a result of a couple factors. First, the timeline of this project was half the length of theirs, which was four months. Second, the specific spots chosen for the primary and planted forest had about the same number of trees. Thus, alone, the sites do not provide an entirely accurate representation of the amount of leaf litter in each type of forest in Cloudbridge. For this study to be more accurate, more plots, traps, and times would be needed for this experiment to be significant.

### 4.4 Relationship Between Arthropod Diversity and Leaf Litter Density

Looking at Figure 4, there does not seem to be much of a pattern when comparing arthropod diversity and leaf litter density. The low diversity among all four forest types is likely due, in a least part, to the fact that the arthropods in this study were only identified to order, rather than a more specific classification. More site collection would have increased diversity as well. Focusing on the primary forest, it seems that there may be a correlation between the most leaf litter and the least diversity. The following results almost conforms to this pattern; however, young growth, with the least amount of leaf litter, does not have the most density. Plus, such a pattern does not quite make sense considering the habitat needs leaf litter provides for arthropods. More research would have to be done to delve deeper into this possible relationship.

A relevant research project conducted in Australian lowland tropical rainforest investigated the variations in diversity of arthropods and leaf litter availability throughout the year. They mainly looked at ants and beetles. It was found that when they completely removed leaf litter from a site, there were significantly fewer insects, but when they added leaf litter to the site, there was no difference (Grimbacher 2018). Noting that there was always at least some leaf litter in sites, the Australian study's results line up with this one's in concluding that there is not much of a pattern between leaf litter density and diversity. This could suggest that leaf litter arthropods only need the minimum amount of leaf litter and thus nutrients, shelter, and habitat to thrive.

More plant biomass, and thus a higher carbon-nitrogen ratio, leads to higher abundance and diversity of arthropods (Ebeling 2014). Therefore, to provide a deeper dive into the understanding of the relationship between arthropod diversity and leaf litter diversity, it would be beneficial to test and determine the leaf litter decomposition and quality.

Another study compared leaf litter herpetofauna in primary forests and abandoned cacao plantations of different ages (Heinen 1992). They found that abundance was greater in more recently disturbed areas, which had deeper leaf litter, whereas richness and diversity were greater in less recently disturbed sites. The abundance factor does line up with the current study, but not the richness and diversity. The fact that the study examining herpetofauna analyzed leaf litter straight from the ground rather than collected in traps like at Cloudbridge, where depth could not be inspected, could account for the difference in results.

### 4.5 Conclusions

Since there was no statistical difference in the arthropod diversity among the four different forest types in the Cloudbridge Reserve, this study failed to reject the null hypothesis. Although the old growth had the most diversity, the primary forest had the lowest, completely opposite of the prediction.

In the 2007 Cloudbridge project, there was significantly less arthropod abundance, richness, and diversity in the pasture compared to the old growth and secondary forest, with no significant difference between those two (Sheenhan 2007). In this study, it was found to be little difference between richness and diversity between all forest types. This serve as an indication that the soil of the planted and young growth forests has been restored successfully. The significantly higher abundance in the planted forest could be only associated with the specifically selected sites. Given these comparisons, it seems like Cloudbridge's reforesting purposes are continuing to be successful.

### 4.6 Limiting Factors

Throughout this research project, there were several limiting factors. At least five times total, coatis ate the bait of the pitfall traps, destroying them, and requiring the traps to be reset. A few pitfall traps were flooded due to unsuccessful attempts to keep out rainfall with a piece of wood. Additionally, separating insects from the soil in each jar proved to be difficult. It is likely that many smaller insects were not accounted for due to human limits.

### 4.7 Future Improvements and Suggestions

Overall, it would be helpful to do this project over a longer period of time to conduct more surveys and thus, a more accurate data set. Additionally, developing animal proof pitfall traps and a more fool proof method to separate the small insects from the soil they were collected in would help ensure more accurate results. For the future, it would be interesting to compare the biodiversity of arthropods with differing altitudes, wet and dry seasons, and bait.

## 5. Acknowledgements

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## 6. References

Celentano et al. (2011). Tropical Forest Restoration in Costa Rica: The Effect of Several Strategies on Litter Production, Accumulation, and Decomposition, *Revista de Biologia Tropical,* 59(3), 1323-1336. doi:

https://www.researchgate.net/publication/51739586

Cole et al. (2016). Leaf Litter Arthropod Responses to Tropical Forest Restoration, *Ecology and Evolution*, 6(15), 5105-5556. doi: https://doi.org/10.1002/ece3.2220

Ebeling et al. (2014). Plant Diversity Impacts Decomposition and Herbivory via Changes in Aboveground Arthropods, *PLoS ONE*, 9(9). doi: 10.1371/journal.pone.0106529

Gerisch et al. (2012). Community Resilience Following Extreme Disturbances: The Response of Ground Beetles to a Severe Summer Flood in a Central European Lowland Stream, *River Research and Applications*, 28(1), 81-92. doi: https://doi.org/10.1002/rra.1438

Ghiglieno et al. (2020). Response of the Arthropod Community to Soil Characteristics and Management in the Franciacorta Viticultural Area (Lombardy, Italy), *Agronomy*, 10(5), 740. doi: http://dx.doi.org/10.3390/agronomy10050740

Gonzáles-Reyes et al. (2017). Changes of Arthropod Diversity Across an Altitude Ecoregional Zonation in Northwestern Argentina. *Peer J*. doi: 10.7717/peerj.4117

Grimbacher et al. (2018). Temporal Variation in Abundance of Leaf Litter Beetles and Ants in an Austrailian Lowland Tropical Rainforest is Driven by Climate and Litter Fall, *Springer Nature*, 27, 2625-2640. doi: https://doi.org/10.1007/s10531-018-1558-2

Heinen et al. (1992). Comparisons of the Leaf Litter Herpetofauna in Abandoned Cacao Plantations and Primary Rain Forest in Costa Rica: Some Implications for Faunal Restoration, *Biotropica*, 24(3), 431-439. doi: https://doi.org/10.2307/2388614

Marx et al. (2012). Adaptations and Predispostions of Different Middle European Arthropod Taxa (Collembola, Araneae, Chilopoda, Diplopoda), *Animals (Basel)*, 2(4). doi: 10.3390/ani2040564

Maleque et al. (2006). The Use of Arthropods as Indicators of Ecosystem Integrity in Forest Management, *Journal of Foresty*, 104(3), 113-117. doi:

Menta et al (2020. Soil Health and Arthropods: From Complex System to Worthwhile Investiagtion, *Insects*, 11(1), 54. doi: 10.3390/insects11010054

Nadkarni et al. (1990). Invertebrates in Canopy and Ground Organic Matter in a Neotropical Montane Forest, Costa Rica, *Biotropica*, 22(3), 286-289. doi: https://doi.org/10.2307/2388539

Prabowo et al. (2021). Impact of Habitat Manipulation on the Diversity and Abundance of Benenficial and Pest Arthropods in Sugarcane Ratoon, *Biodivresitas*, 22(9), 4002-4010. doi: 10.13057/biodiv/d220948

Sittenfeld et al. (1999). Costa Rican International Cooperative Biodivesity Group: Using Insects and Other Arthropods in Biodiversity Prospecting, *Pharmaceutical Biology*, 47, 55-68. doi: 10.1076/1388-0209(200010)37:SUP;1-W;FT055

Sheenan et al. (2007). Diversity and Abundance of Subsoil and Leaf Litter Invertebrates Across Different Levels of Disturbance in a Costa Rican Cloud Forest, *Cloudbridge*.

Yanoviak et al. (2007). Arthropod Assemblages in Epiphyte Mats of Costa

Rican Cloud Forests, *Biotropica*, 39(2), 202-210. doi: 10.111/j.1744-7429.2006.00261.

Zheng et al. (2017). Tree Species Diversity and Identity Effects on Soil Properties in the Huoditang Area of the Qinling Mountains, China, *Ecosphere*, 8(3). doi: https://doi.org/10.1002/ecs2.1732