

**How specific habitat factors affect
Lepidoptera sub-family biodiversity at
Cloudbridge Nature Reserve**

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Abstract

Tropical montane cloud forests are important biodiversity hotspots, many have been altered for different reasons, such as agricultural land use and are undergoing or have plans to undergo restoration. Understanding how different stages of forest regeneration influence insect communities is essential for evaluating conservation success. Butterflies are widely used as ecological indicators because of how sensitive their populations are to environmental change and habitat structure. There is still a lot undiscovered when it comes to understanding the effects of forest regeneration stages on butterfly communities in cloud forests. This study investigated how butterfly (*Lepidoptera*) subfamily biodiversity varies between three habitat types at Cloudbridge Nature Reserve in the Talamanca mountain range, Costa Rica. The forest types studied included old growth, natural regrowth and planted forest. Timed transect surveys were conducted along established trails within the reserve during suitable weather conditions, and butterflies were observed and identified to subfamily level. Butterfly abundance differed between habitats with planted forest supporting the most individuals while natural regrowth showed the highest Shannon diversity level. These results provide evidence that regenerating forests can support diverse butterfly communities and highlight ecological importance of forest restoration. Despite the impacts of past land use, ecosystems show the potential to recover and develop into healthy and functioning habitats.

Introduction

Biodiversity is important for the health and functioning of ecosystems and provides numerous benefits such as environmental balance, ecosystem services, and stability (Hooper et al., 2022). Costa Rica is one of the most biodiverse areas in the world despite only occupying a small proportion of the Earth's landmass, due to its geographical position acting as a land bridge between North and South America (Rodríguez-H et al., 2002). Costa Rica is recognised for its rich diversity of species including butterflies (Chacón et al., 2025). The Cloudbridge nature reserve within Costa Rica is situated at 1550m - 2600m in the Talamanca mountain range and is specified as a tropical montane cloud forest (Powell et al., 2022). Cloud forests are specialised habitats characterised by high, constant moisture levels with coverage being approximately 1% of the world's woodland. High levels of biodiversity can often be observed here, supporting many endemic and specialised species that inhabit these remote locations (Wilson and Rhemtulla, 2018). This creates an ecosystem with high diversity but lower abundance, with the vertical stratification from the lowlands and the niche climate causes biodiversity hotspots (Álvarez et al., 2024).

Butterflies (*Lepidoptera*) play a significant role in ecosystem functioning as pollinators, transferring pollen between plants supporting the reproduction and biodiversity of these habitats. Subsequently pollination is a key part of the plant life cycle. Butterflies are an effective biodiversity indicator as changes in environmental conditions strongly influence stages in the butterfly life cycle, including reproduction, larval development and migration (Mukherjee and Hossain, 2024). These changes in climate or habitat structure can have a

direct effect on butterfly populations therefore presence, absence, abundance and diversity of population shifts can be used to inform current conservation status (Wenda et al., 2025). As a result butterflies can be used as ecological indicators to understand the overall health of an ecosystem as an indicator for the health of the environment (Niharika et al., 2024). A common method is to conduct butterfly surveys for assessing ecosystem health and evaluating success of conservation efforts (Fox et al., 2022). Butterflies are a practical option to research compared to other invertebrates, as many species are relatively easy to observe and identify, making them a viable option in ecological research (Habel et al., 2025). Their visibility also allows data to be obtained with minimal disturbance to the surrounding environment, unlike other invertebrate sampling techniques, which may involve soil disturbance or other destructive sampling methods.

Forest structure and habitat composition have a significant effect on butterfly diversity and behavior, although this is species dependent. Forest structure varies in, canopy cover, vegetation density and plant diversity, all these variables can affect food availability and light intensity. An example of forest type is old growth forest, containing complex vegetation with greater canopy cover due to larger trees (Mildrexler et al., 2020). However, newly regenerated forests are more likely to have simpler vegetation structures, potentially supporting fewer butterfly communities. Altitude can also have a significant effect on butterfly communities due to changes in temperature and oxygen availability (Santorufio et al., 2021). As altitude increases, environmental conditions become more specialized resulting in changes in diversity and distribution of certain species (Zhao et al., 2023). In tropical environments, higher elevation generally supports lower species abundance, however, it is likely to contain specialized or endemic species adapted to survive in these conditions (Pires et al., 2020).

Understanding how butterfly communities react to different forest types is particularly important in regions where forests are undergoing regeneration or restoration. Cloudbridge has undergone disturbance and destruction due to agricultural pressures, however, it has recently begun restoration with management practices. Assessing and monitoring biodiversity is also important to evaluate whether the current restoration techniques are sustainable and successful for the future.

The study aims to evaluate how different forest types influence butterfly (*Lepidoptera*) diversity at Cloudbridge Nature Reserve. Specifically, the study investigates how different stages of forest regeneration, old growth, natural regrowth and planted forest function as habitats for different butterfly subfamilies.

It was hypothesized that butterfly abundance would differ between forest types, with plantation and natural regrowth areas supporting higher numbers of individuals due to sunlight levels, open habitat and altitude levels. However old growth was predicted to have greater diversity due to a more complex structure and higher plant diversity. Based on the Intermediate disturbance Hypothesis natural regrowth forests may also support relatively high butterfly diversity because intermediate levels of disturbance can allow species associated with open and mature habitats to coexist (Gautam et al., 2025). Richness was expected to

remain similar between all areas due to connectivity and the mobility of butterflies with metapopulations. Overall, the prediction was that habitat quality would be the most significant factor affecting the butterfly communities at Cloudbridge Nature Reserve.

Methods

Study site

The study was conducted at Cloudbridge Nature Reserve located in the Talamanca mountain range of Costa Rica. The nature reserve ranges from 1550 m to 2600 m above sea level and is classed as a tropical montane cloud forest habitat. Data collection took place during January – early April 2025, which is part of the dry season. Cloudbridge contains a mixture of forest types due to previous land use and conservation efforts. The three forest types studied were old growth forest representing relatively undisturbed original vegetation, natural regrowth regenerated naturally after the land was used for agricultural reasons and planted forest the active reforest area as part of conservation efforts since 2022 (Figure 1).

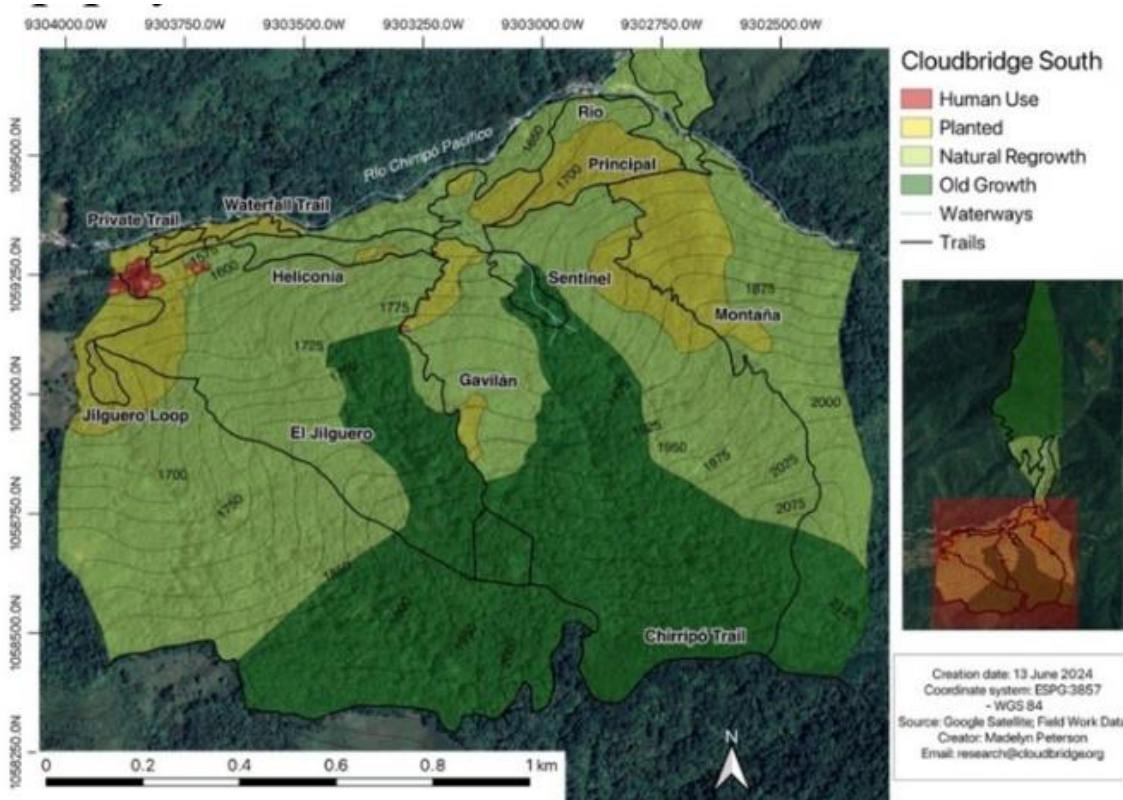


Figure 1a – Map of sampling trails in Cloudbridge Nature Reserve (El Jiguero and Montana). Key shows colours of different forest types.

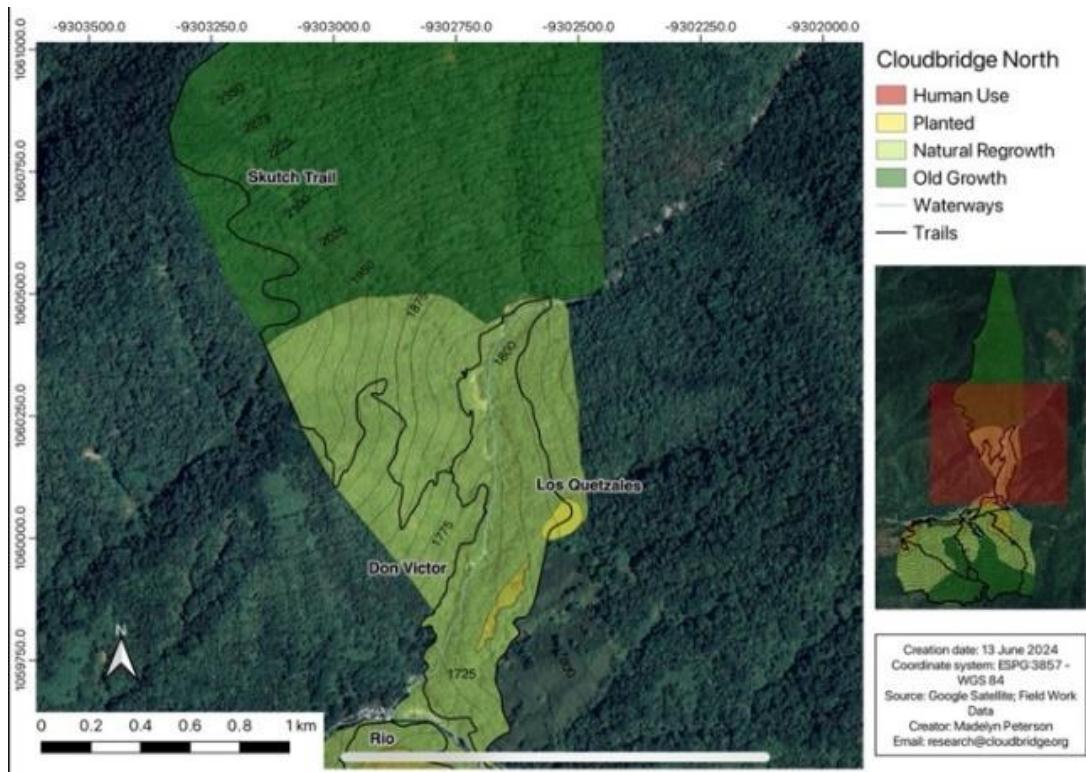


Figure 1b – Map of sampling trails in Cloudbridge Nature Reserve (Don Victor and Skutch). The key shows colours of different forest types.

Sampling method

Butterflies are generally inactive during wet conditions (Evans et al., 2019) therefore surveys were only conducted during dry weather when environmental conditions were suitable for butterfly activity. Surveys were conducted along four established trails within the reserve, as shown in Figure 1. The trails used were El Jiguero, Don Victor, Montana and Skutch, these were selected as they pass through all the forest types required.

On each trail, three transect areas were used, one in each forest type present in the reserve. A 20-minute timed transect survey through each forest type was conducted each day on a different trail. Skutch and El Jiguero trails were completed together as Skutch was needed for the old growth forest. Surveys were completed by two individuals walking slowly, transects were conducted downslope from higher to lower elevations. All butterflies observed were recorded on a digital device, which could be carried out offline. Altitude measurements were taken at the beginning and end of the transect using a handheld GPS device. Surveys were conducted over a five-week period, resulting in a total of 15 transect surveys across the three habitat types.

Identification

All butterflies were recorded and identified to a subfamily level. Identification was carried out visually in the field where possible. If unable, photographs were taken and later compared to Costa Rican identification guides or uploaded to iNaturalist species identification app. If no identification was possible then the individual was recorded as unidentified. This is

important to demonstrate the total overall number of individuals present although it cannot be used in the diversity statistics.

Statistical analysis

After data collection in the field, notes were then uploaded into Microsoft Excel and converted into a suitable format to be analysed after being uploaded to RStudio64. The data included the variables survey number, habitat type, trail, butterfly subfamily and abundance. Butterfly diversity was analysed using several analysis techniques. Species richness was calculated as the total number of butterfly subfamilies recorded in each forest type. The Shannon Diversity Index (H') was used to calculate and evaluate biodiversity, accounting for species richness and relative abundance for each species. Similarity between communities was also assessed using the Jaccard similarity coefficient, which allowed comparisons of how similar butterfly communities were between the different habitat types. Graphs were also produced to visualize the data allowing patterns to be easily interpreted.

Results

Abundance across forest types

A total of 634 individual butterflies were observed across all surveys, representing multiple different subfamilies and habitat types. Butterfly abundance varied between all habitat types with planted forest supporting the highest number of individuals, followed by natural regrowth and then old growth, supporting the lowest number of individuals Figure 2. Analysis of variance (ANOVA) revealed that the differences were significantly different ($p = 0.004$), indicating a significant effect of habitat type on butterfly abundance resulting in greater abundance in planted.

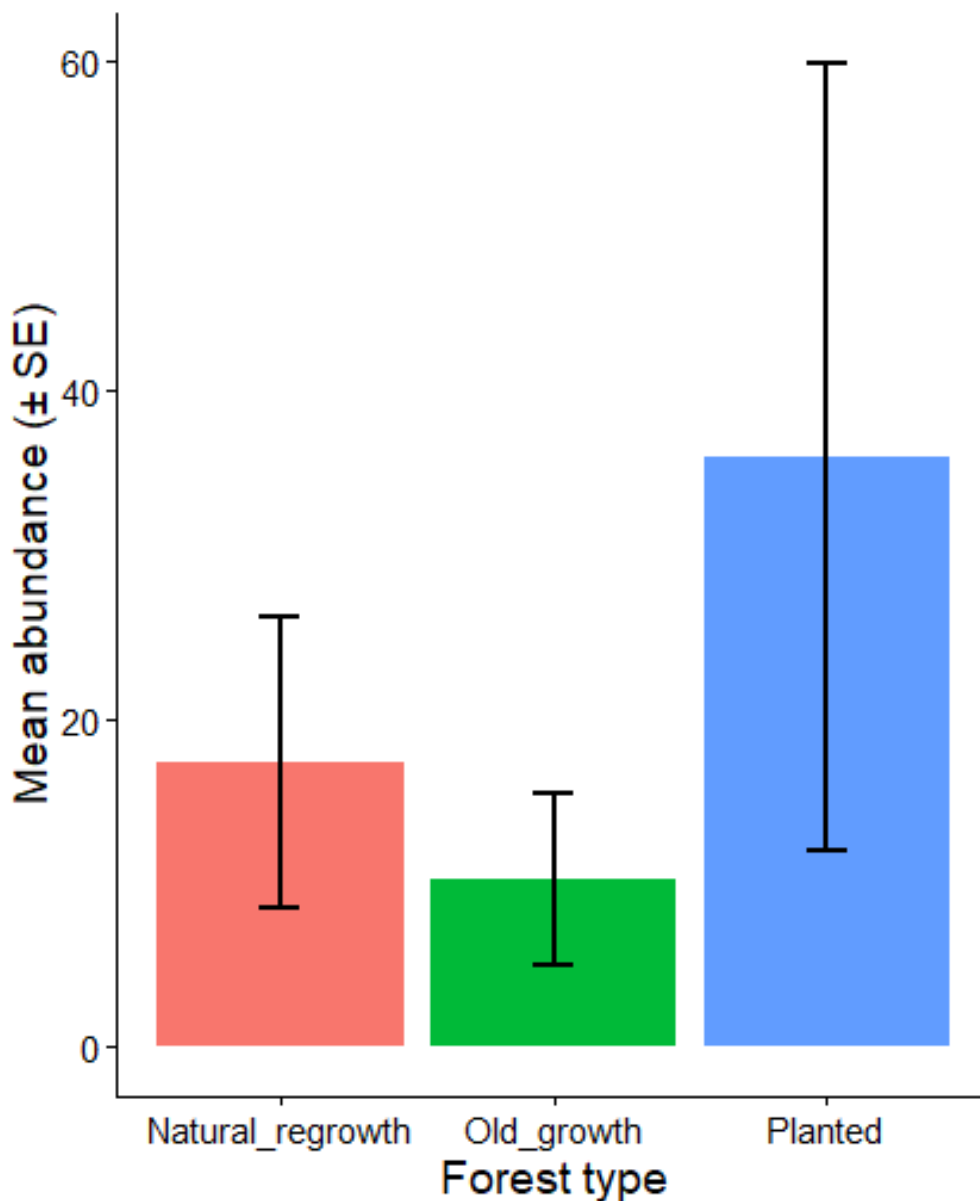


Figure 2 – Mean butterfly abundance (±SE) across forest types at Cloudbridge Nature Reserve.

Butterfly diversity across forest types

Shannon diversity index varied across habitats (Figure 3), with natural regrowth Forest exhibiting the greatest diversity and planted forest had the lowest diversity. Despite this visual trend, the values from the ANOVA test were not significant, $p=0.069$. Although the results were not statistically significant, there was a trend towards higher diversity in natural regrowth ($p<0.05$), a trend that may be interpreted as increased complexity of the habitat links to a greater diversity level.

Shannon Diversity of Butterfly Subfamilies Across Forest Types

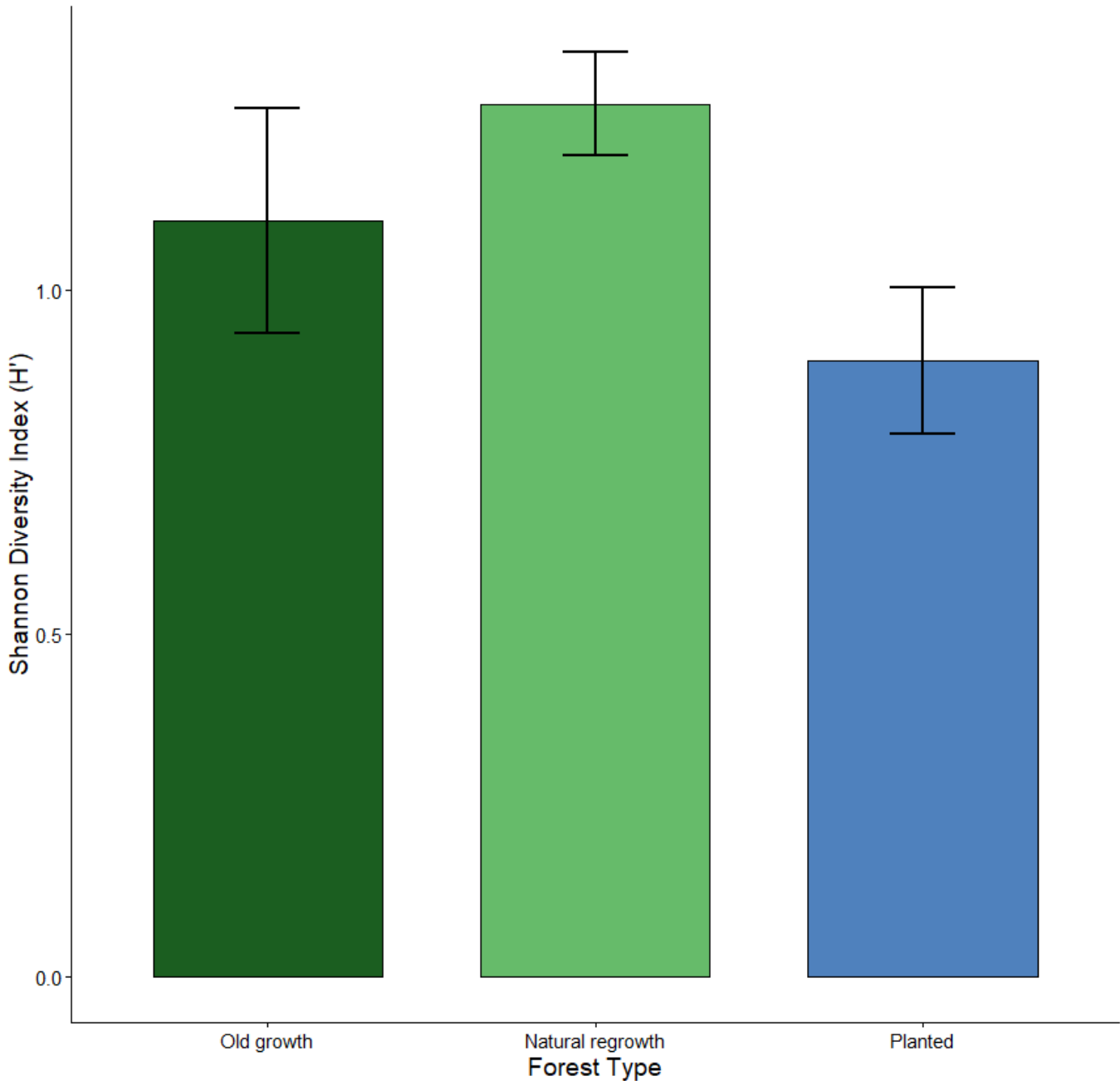


Figure 3 - Mean Shannon index (H') (\pm SE) across old growth, natural regrowth and planted forest

Butterfly community composition

Total butterfly abundance differed between forest types and subfamilies (Figure 3). Across all habitats *Ithomiinae* (289) were the most abundant subfamily, with the greatest abundance occurring in the planted forest. *Satyrinae* (217) were also commonly recorded across the reserve and showed relatively similar abundance between all habitat types. *Heliconiinae* and *dismorphiinae* (54), followed by the next greatest abundance. *Pierinae* (19) and *Riodininae* (17) were recorded in small populations throughout.

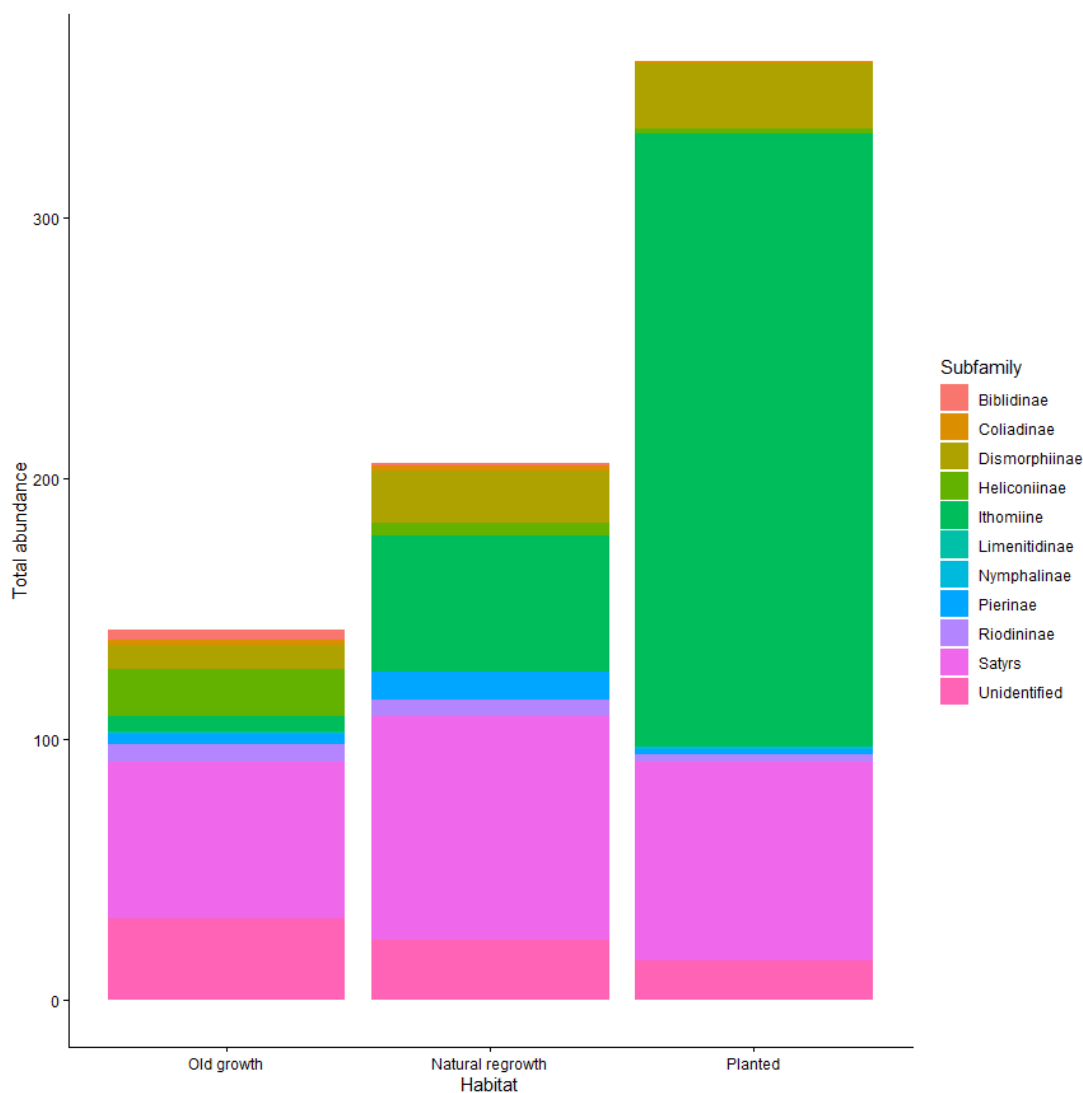


Figure 4 - total butterfly abundance by subfamily across forest types. The key beside the graph shows what colour represents each subfamily.

Altitude of transects

Mean altitude between habitats (Figure 5). Old growth was the highest elevation 1991m followed by natural regrowth 1878m and then the planted area 1838m. This pattern reflects the topographic layout of the reserve.

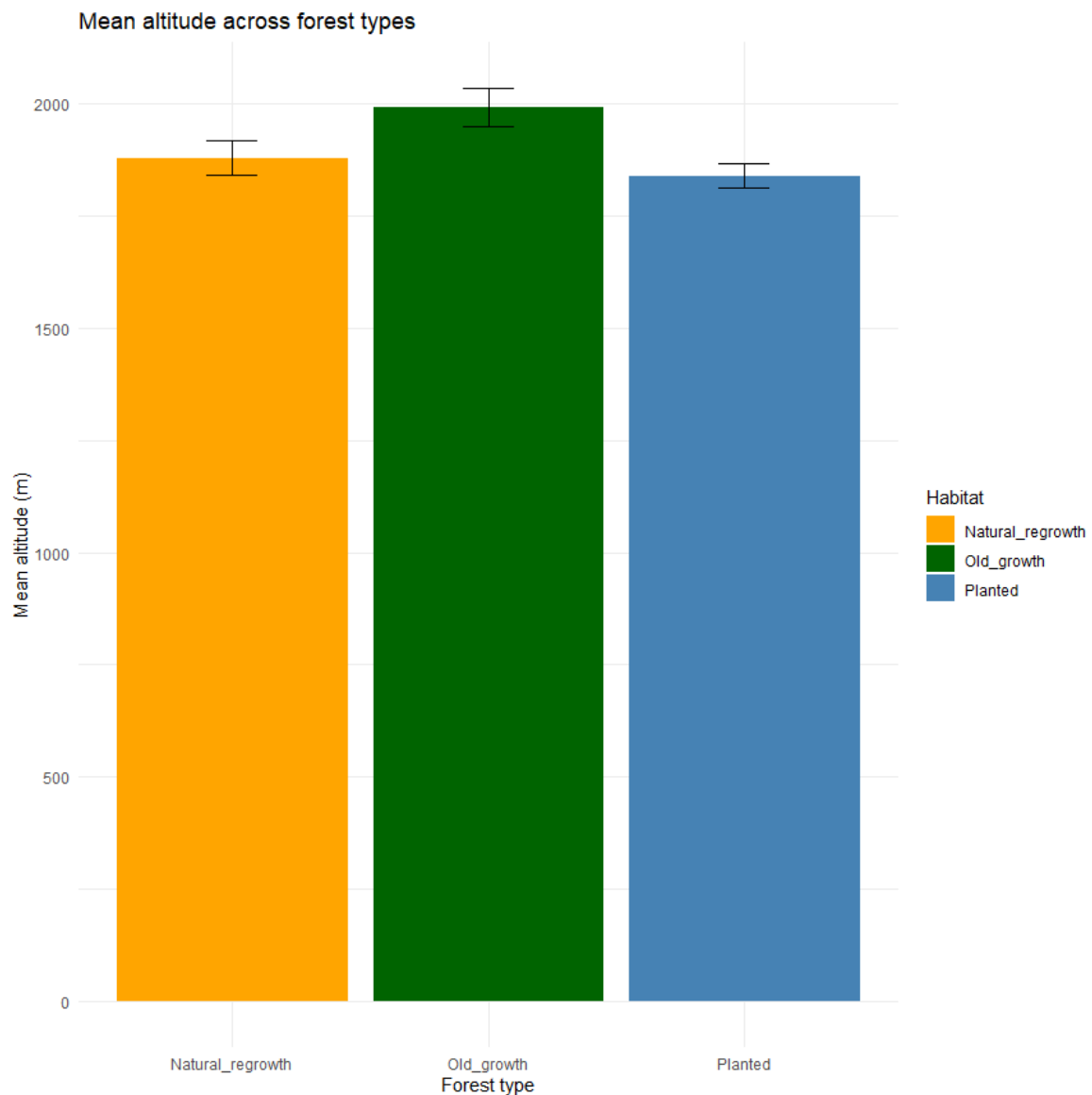


Figure 5 – mean altitude (\pm SE) of butterfly survey transects areas across natural regrowth old growth and planted forest. Bars show the mean altitude calculated from the midpoint of the maximum and minimum of each survey.

Community similarity between habitats

Jaccard similarity analysis indicated relatively high similarity in butterfly community composition across all forest types (table 1). The greatest similarity occurred between natural regrowth and old growth forest (0.90), while the value between planted and old growth was 0.73.

Table 1 – Jaccard similarity index comparing butterfly community composition between forest types. Values closer to 1 indicate greater similarity in subfamily composition.

	Natural regrowth	Old growth	Planted
Natural regrowth	1	0.9	0.8
Old growth	0.9	1	0.727272727
Planted	0.8	0.727272727	1

Species richness across forest types

Species richness measured as subfamilies recorded did not differ much at all throughout all their habitat types ($p=0.284$). Almost identical numbers of subfamilies were observed throughout all habitats (Table 2). Indicating that even if abundance and diversity vary, groups present may remain similar.

Table 2 – results of ANOVA tests examining the effects of habitat type on butterfly abundance, Shannon diversity and species richness.

Metric	Test used	Statistics	p-value	significance
Abundance	ANOVA	F (2,42) = 6.23	0.004	**
Shannon diversity	ANOVA	F (2,42) = 2.86	0.069	-
Species richness	ANOVA	F (2,42) = 1.30	0.284	Ns

Significant codes: ** $p < 0.001$, * $p < 0.05$, - $p < 0.1$, ns = not significant

Discussion

Summary of key findings

This study investigated how butterfly communities differ across forest types to assess the success of current conservation strategies at Cloudbridge Nature Reserve using butterfly diversity as the main indicator for success. The results partially supported the study hypothesis that forest type influences butterfly diversity, as butterfly abundance differed significantly throughout the habitats with planted forest hosting the most individuals, while old growth supported the fewest individuals. However, Shannon diversity was the greatest in the natural regrowth forest, although the difference was not significant. Species richness did not vary significantly between habitats. This suggests the habitats support a similar number of taxonomic groups, although their relative abundance may vary between habitats. Community similarity analysis followed the same trend, demonstrating a considerable overlap in subfamily composition between habitats. The results suggest that forest types may affect butterfly abundance and diversity differently within Cloudbridge Nature Reserve (Mihindikulasooriya et al., 2014).

Effects of forest type on butterfly abundance and diversity

The higher abundance level in planted forests is unlikely to be due to the forest type as butterfly diversity is generally greater in old growth forests compared to planted forests (Milheiras et al., 2020), (Vasconcelos et al., 2019). This result is more likely due to geographical factors such as altitude and light intensity, as planted forests offer less canopy cover than old growth forests (Ramovs and Roberts, 2003). Butterflies generally show a preference for sunlight, being heliotherms they obtain most of their heat from the sun (Clench, 1966). Butterflies generally need to warm up their muscles to achieve the necessary temperatures for flight (Heinrich, 1974). The habitat preference shown in the results may also be due to altitude. Planted forests represented the lowest altitude surveyed and as habitat altitude increased, abundance declined significantly. A lower altitude is more suited to butterflies and generally hosts a higher abundance as shown in previous studies (Oliveira et al., 2025). This could be due to increased temperatures at lower altitudes, compared to that of higher elevation (Körner, 2007). Studies have also shown links between butterfly abundance and open habitat diversity (Anirban Mahata et al., 2023), this highlighting the importance of open canopy habitats for butterfly activity. This can be linked to the planted forest at Cloudbridge having an open canopy unlike the old growth that is a denser and more complex vegetation structure due to structural, composition and ecological factors (Franklin and Robert Van Pelt, 2004). The greater number of individuals observed in the planted areas can also be linked to previous land use, as these areas were formerly grazeland, which has been associated with increased butterfly abundance (Balmer and Erhardt, 2000).

Although the planted forest supported the highest abundance, the natural regrowth forest exhibited the highest Shannon diversity however these results were not significantly different. This has also been observed in other studies as stated in the results butterfly richness and abundance were found to be lower in natural closed forests and higher in disturbed forests

(Lien Van Vu, 2008). This pattern may support the intermediate disturbance hypothesis, where it is stated local species diversity is maximized at intermediate levels of disturbance (Gautam et al., 2025). In this case that would be at the natural regrowth area. This showed that although more individuals were present in the planted area, the distribution and evenness was greater within the regenerated forest area, which partially followed the hypothesis. The next greatest Shannon value was old growth forest, these forests generally offer greater ecological complexity with greater plant diversity and richness with more diverse microhabitats (Koh and Sodhi, 2004). Increased plant diversity can also create more larval host plants, which is important for butterfly reproduction and development and provides a primary food source for caterpillars (Aprillia et al., 2024). As a result of this, an increased abundance of butterflies can coexist and reproduce, subsequently increasing overall diversity. In contrast the planted forest may provide a suitable habitat for certain species, however, more specialized species may not be able to survive in the ecosystem offered. They provide a much simpler vegetation structure and fewer plant species due to age. This may explain the results showing planted forests have a lower Shannon index value. This has also been observed in other studies in tropical rainforests (Mildrexler et al., 2020). These findings support the message of preserving natural old growth Forest, while also highlighting that regenerating forests can support diverse butterfly communities. Although it may take critical management plans over long periods to reach substantial levels of ecological complexity.

Despite clear differences in butterfly abundance and small visual differences in Shannon diversity between habitats, subfamily richness stayed relatively consistent throughout each habitat. Statistical analysis showed no difference between the number of subfamilies present between old growth, natural regrowth and planted forests. This suggests that the number of individuals varied, however many of the same taxonomic groups were present across the reserve. Other studies have shown that disturbed and regenerating forests can support similar taxonomic groups although abundance does not stay consistent (Addo-Fordjour et al., 2015). Potential reasons for this may be the distance between forest types, all forest types are situated within the same reserve with strong connectivity. Butterflies are very mobile organisms, their ability to fly makes moving between different habitats straightforward and enables them to utilize different nectar sources and host plants. This means that dispersal between forest types is very likely. Differences in abundance and diversity still help support the hypothesis of habitat quality affecting butterfly communities (van Halder et al., 2015).

Community composition and habitat connectivity

Community similarity analysis using the Jaccard similarity index showed a high level of overlap in butterfly subfamily composition between forest types. Although slight differences in richness and abundance were observed, many of the same subfamilies were shared across different forest types. The greatest similarity was seen between old growth and natural regrowth. This suggests that naturally regenerating forests may provide suitable habitat for more specialised species. This would be expected as the plants in a naturally regenerated area are more likely to be native. Butterflies are known to use a variety of different habitats throughout their lifecycle (Habel et al., 2022). This supports the data showing that individuals are likely to be active and move between habitats, it also shows positives in the restoration

efforts currently in place as communities are overlapping throughout forest types. Suggesting the regenerated areas have created a healthy environment in which butterflies can survive and create an environment with similar properties to the original forest.

Environmental factors influencing butterfly distribution

Altitude is another factor that can influence butterfly distribution in this study and potentially be the reason for certain hypotheses not being perfectly followed. The old growth forest on the reserve was located at the highest altitude (approximately 2000m), while planted forest occurred at lower elevations around 200m lower. Changes in altitude can be linked to multiple different environmental factors that can affect butterfly diversity such as temperature, humidity and oxygen availability (Dar et al., 2022). In tropical environments, other studies have shown a negative correlation between the increase in elevation and species abundance but often the presence of specialized or endemic species well adapted to the conditions as shown in this study where at a greater altitude diversity decreased but the presence of endemic species increased above 1500m (Oliveira et al., 2025). This may explain why the old growth showed a lower abundance even though in theory there should be more favourable conditions for the butterflies. Also, this may potentially show why diversity was greater, as certain specialised species may be well adapted to survive at the altitude of the old growth such as the Cloud-forest Catone *catonephele chromis* (Duong and Junger, 2015). Species such as this would struggle to survive further down within the planted forest due to adaptations.

Implications for forest restoration and conservation

The findings from the study underline the importance of forest regeneration for supporting butterfly diversity and how it can help overall ecosystem health. Although planted areas supported the greatest abundance of butterflies, natural regrowth exhibited the highest Shannon diversity. This may suggest that allowing forests to regenerate naturally, alongside active restoration efforts, may be an effective strategy for supporting insect communities. Shannon diversity is often considered a stronger indicator of ecosystem health than abundance, as it incorporates both species richness and evenness, allowing it to highlight potential dominance by particular species (Guiaşu and Guiaşu, 2003). This indicates that when restoring forests, it is important to base the new environment on the original structure as much as possible, and actions should be taken to stop deforestation primarily. The relatively high similarity between forest types indicates that restoration efforts at Cloudbridge may be successful. Planted areas are also relatively new, being planted in 2002, showing the rapid progress of the reserve. The continuation of monitoring butterflies can aid in assessing the progress in restoration and conservation techniques at the reserve, acting as a strong indicator species of the overall biodiversity of the reserve relying a lot on the surrounding environmental health (Paredes Puc et al., 2026). As butterflies are closely linked to plant communities through nectar resources and larval host plants, their presence can reflect wider ecosystem recovery and vegetation complexity (Ghazanfar et al., 2016). Increasing butterfly diversity within regenerating forests may therefore indicate broader improvements in ecosystem functioning and habitat quality.

Study limitations and future research

Several limitations should be considered when reviewing the results of this study. Data collection was only taken over a short time during the dry season. It is shown in other studies that the best time to collect data on butterflies is early dry season May – July for peak diversity and breeding (Flockhart et al., 2013). However not every species follows the same breeding calendar in Costa Rica due to climate elevation and habitat, so a year-round study can produce improved data (Matamoros-Calderón et al., 2025). Another limitation of the study was the identification only being recorded to the subfamily level, which can affect the accuracy of biodiversity data. Field identification of butterflies can also be challenging due to morphological similarities between species. If a photograph could not be obtained it was difficult to identify, especially if the individual was currently in motion when sighted. Future studies should attempt to reach a higher level of identification accuracy however, this can be challenging with the number of butterflies present in the area and lab tests must be carried out to differentiate certain species. Increased surveys and a greater range of altitudes can also improve the accuracy of the results.

Conclusion

This study examined how different forest types influence butterfly subfamily diversity at Cloudbridge Nature Reserve. Results showed that butterfly abundance differed significantly between habitats, with planted forests supporting the highest number of individuals. However, natural regrowth exhibited the greatest Shannon diversity, suggesting that naturally regenerating habitats can support more diverse butterfly communities. Species richness and composition remained stable throughout the different forest types indicating the reserve has strong connectivity between areas. These findings highlight the success of regeneration at Cloudbridge and highlight the ecological value of natural forests. Although old growth forests did not exhibit the highest diversity in this study, its protection remains essential as these habitats provide complex ecological structures and support specialised species that may not be present in younger forests. While butterflies are widely used as indicators of ecosystem health, they cannot fully represent all aspects of biodiversity and ecosystem functioning. Continued monitoring of butterflies across the reserve can help to assess the long-term success of conservation at the reserve. Collecting data throughout the year would provide a stronger understanding of butterfly populations, as many species show seasonal variation in activity and distribution.

Reference list

Artificial intelligence tools were used in this report to assist with planning of structure, grammar and help with correct packages to use on RStudio and format of the reference list. All assistance was reviewed to ensure it aligned with my intentions.

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