

Host taxonomy and temperature differentially shape gut microbiome assembly in tropical moths across an Andean-Amazonian Mountain gradient

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1 **Host taxonomy and temperature differentially shape gut microbiome assembly in tropical**
2 **moths across an Andean-Amazonian Mountain gradient**

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28

29 **Abstract**

30 **Background:** Despite the ecological relevance of microbiomes for hymenopteran pollinators,
31 the dynamics of their interaction with lepidopteran host species remain largely unexplored. Our
32 study investigates the factors influencing gut microbiota assembly in adult moths along a full
33 elevational gradient in the Peruvian Andes, spanning 3,350 meters. We chose six moth genera in
34 two families that are widely distributed along the gradient: *Eois*, *Eupithecia*, *Lomographa*,
35 *Oxydia*, *Physocleora* (Geometridae), and *Agylla* (Erebidae, Arctiinae).

36 **Results:** Our findings reveal that the composition of moth gut microbiota is predominantly
37 influenced by host taxonomy, with *Eois*, *Eupithecia*, and *Lomographa* microbiomes being highly
38 distinct from those of *Physocleora* and *Agylla*. Contrary to previous assumptions that diversity
39 decreases with elevation, we observed a significant increase in microbial diversity at higher
40 elevations, with *Oxydia* and *Physocleora* exhibiting slightly lower microbial diversity compared
41 to the other host genera. Dissimilarity of sample increased with elevational distance in particular

at higher temperatures.

Conclusions: Our results highlight that warming in tropical forests may rapidly impact host-microbial communities, further research is required to understand the ecological implications of these interactions in the context of climate change.

Keywords: Lepidoptera, gut microbiota, moths, elevation gradient, microbial diversity, Peruvian Andes, environmental gradient, community composition, temperature.

1 Introduction

Lepidoptera are one of the most diverse orders of insects, from which butterflies recognized as the most extensively studied group (Ricketts et al., 2002). In contrast, moths, which comprise the vast majority of Lepidopteran diversity, have not received a similar level of attention. Moths play crucial roles as complementary pollinators (Diniz et al., 2025) with important ecological roles as herbivores or as food for birds and bats (Li et al., 2023; Wahlberg et al., 2013). In addition, moth diversity exhibits a remarkable versatility in distribution across various habitats and temperature ranges, including elevation gradients. This broad distribution can be attributed to their ability to utilize a wide range of dietary niches as larvae and, due to their holometabolous life cycle, they have a high mobility as adults to move among habitat patches (Ricketts et al., 2002).

Lepidoptera microbiome research has primarily focused on single pest-species and their larvae (caterpillars), often only investigated under controlled laboratory conditions (Paniagua Voirol et al., 2018). This focus has overlooked the complex interactions that nectar-feeding adult

lepidopterans have with their environment, which could influence the microorganisms that reside inside the host (Shao et al., 2024). The dynamics of these microbial associations are influenced by multiple factors (Zhang et al., 2022), with the environment potentially playing a crucial role in providing a continuous influx of microorganisms and shaping interactions between the hosts and microbes (Gomes et al., 2020; Paniagua Voirol et al., 2018). The temperature of a specific habitat is expected to shape the microbial communities of host species (Holzmann et al., 2026). This is due to the varying resources and stressors associated with different temperature ranges, as seen in larvae (Gomes et al., 2020). However, in adult Lepidoptera, it remains unclear to what extent gut microbial communities are host-shaped or environmentally driven.

Given their significance in terrestrial ecosystems, moths prompt intriguing questions about bacterial associations. These microbial communities could potentially aid adaptation to diverse habitats, though direct evidence of their critical importance is still needed (Marín-Miret et al., 2022; Paniagua Voirol et al., 2018; Zhang et al., 2022). However, the variability in larval gut microbiomes raises important questions regarding the stability and functional relevance of these communities, as caterpillars harbor few or no resident bacteria compared to other herbivores (Hammer et al., 2017), leading to the perception that Lepidoptera would lack specific microbial associations, irrespective of their life stage (Shao et al., 2024). Further investigation is essential to understand how gut microbes interact with the adult stage of this important group of pollinators, especially in the context of environmental changes.

Elevation gradients in tropical mountains offer a unique system to investigate the effects of environmental factors (particularly temperature) on biodiversity, as these regions are recognized biodiversity hotspots (McCain & Grytnes, 2010). Those temperature gradients at mountains create diverse ecosystems that provide continuous variations in energetics, resources,

and stress (Wang et al., 2022). Unraveling the factors that drive diversity patterns of microorganisms alongside their host species at temperature gradients helps us in understanding ecological responses to global warming. Our study constitutes a pioneering investigation into the distribution, characteristics, and diversity of gut microbial communities in wild adult moths, comparing six different genera from two speciose families along an extended elevation gradient. As host model taxa, we chose members of two speciose families (Geometridae and Erebidae/Arctiinae). Both taxa have been studied extensively along Neotropical elevation gradients (Brehm et al., 2016, 2019), enabling us to select six genera that occur in all elevational zones. The selected taxa differ markedly in terms of their larval diet. Whilst the geometrid genera *Eois*, *Eupithecia* and *Lomographa* are regarded as specialists, members of *Oxydia* and *Physocleora* are polyphagous (Brehm, 2002). Members of *Agylla* (Erebidae), on the other hand, feed on lichens. Adults of all moths have a functional proboscis, but so far, only *Agylla* is known to play an important role in pollination networks (Diniz et al., 2025), whereas for the other species it is unknown if and which food sources are visited in the adult stage.

In contrast to existing research under controlled laboratory conditions, here we examine how host phylogeny and environmental factors, particularly temperature, influence the structure of microbial communities across species. This approach addresses a significant gap in current understanding of host-microbiome interactions in adult moths from real-world ecosystems (Marín-Miret et al., 2022; Mason et al., 2014; Mereghetti et al., 2017; Xia et al., 2017).

Temperature along these environmental gradients is a strong environmental factor significantly influencing both insects (Holzmann et al., 2026) and their gut microbiomes, and altering the composition and functionality of bacterial communities in various species, such as termites (Arango et al., 2021), honeybees (Palmer-Young et al., 2023), and mosquitoes

(Onyango et al., 2020). For instance, thermal stress can reduce microbial diversity and disrupt the balance of gut symbionts, ultimately affecting insect fitness and survival (Mason & Shikano, 2023). Moreover, temperature changes can destabilize symbiotic associations, especially with obligate symbionts that provide essential services, jeopardizing the host's reproductive success (Huus & Ley, 2021; Lemoine et al., 2020). Conversely, mutualists may adapt by modifying their metabolic activities or forming protective interactions with secondary symbionts, which could help modulate host traits and expand their viable temperature range (Brumin et al., 2011; Dallas et al., 2024; Truitt et al., 2019).

Based on previous literature, we anticipated that microbial richness would decrease with elevation or peak at intermediate elevations (Albrecht et al., 2021; Costa et al., 2023; Dickey et al., 2021; R. Li et al., 2022; Peters et al., 2016). We explored the factors underlying these potential differences in the gut microbial communities among host taxonomic groups along the elevation gradient. We hypothesized that temperature, a critical factor for ectothermic organisms in tropical mountain forests (Holzmann et al., 2026), significantly shaped the gut microbial communities of moths. Furthermore, we predicted that as elevational distance increased, microbial turnover would also rise due to community diversification, a pattern previously observed in both macroorganisms and microorganisms (Bishop et al., 2015; Wang et al., 2012).

To our knowledge, this work represents the first comprehensive examination of wild moth microbiome diversity patterns along an elevation gradient for a larger number of moth species. Our goal was to identify common patterns among gut microorganisms inhabiting the same environment, ultimately contributing to a deeper understanding of how moths and their microbiomes responded to fluctuating environmental conditions. This helps to better understand

the consequences of rising temperatures in tropical host-microbial symbiosis under the current global warming scenarios (Iltis et al., 2022).

2 Methods

2.1 Field Sampling and data collection

Study sites were described in detail by Pinos et al. (2025). The research was conducted in southeastern Peru (Figure 1), an area notable for its largely undisturbed habitats along extensive elevation gradients (Malhi et al., 2017). This region features a diverse range of ecosystems, including humid tropical lowland forests, mid-elevation cloud forests, and wet Puna vegetation above the tree line in the highlands. Along this gradient, sampling was carried out at 28 study sites from 245 to 3,588 m asl.

We aimed to collect moths observed throughout the entire elevational gradient, concentrating on six specific genera: *Eois*, *Eupithecia*, *Lomographa*, *Oxydia*, *Physocleora*, and *Agylla*. Moths were collected manually at each location using a LepiLED UV lamp (Brehm, 2017) in a “light tower” as described in Brehm (2017) in the understory of the forest. In addition, automated funnel traps (Singh et al., 2022) were operated in both the understory and the canopy. Sampling was carried out from 18:00 to 21:00 to coincide with the peak activity of moths. Nights with bright moonlight were avoided (Brehm & Axmacher, 2006). In the field, moths were identified morphologically at genus level by comparing external characters and immediately preserved in 96% ethanol within sterile collection tubes. At the research stations, samples were placed in an insulated cooler with ice packs. Moths were dissected under sterile conditions using

a magnifying lens, as described in Pinos et al. (2025). We extracted the gut region, and the samples were stored in 96-well plates with Zymo DNA/RNA shield lysis solution for nucleic acid preservation until laboratory isolation. In addition, one leg from each moth was removed for species identification confirmation through DNA barcoding.

Environmental data were measured on each plot using TMS-4 loggers (TOMST s.r.o., Prague, Czech Republic) for approximately one year (September 2022–December 2023) (Holzmann et al., 2025). Mean annual temperature (MAT) was determined by averaging all temperature readings taken during the sampling period.

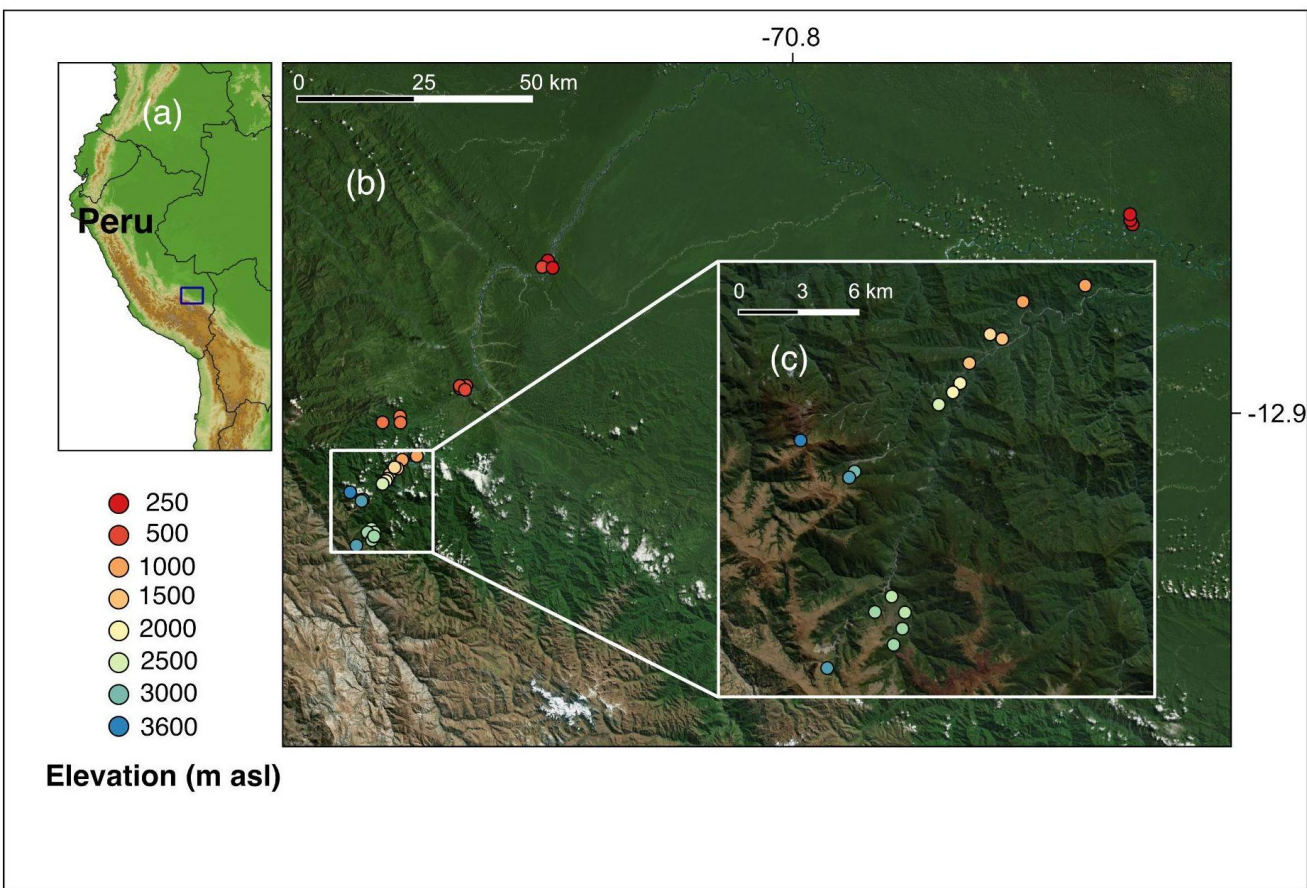


Figure 1 (a) Map of the study area along the Kosñipata valley and adjacent lowlands in Peru, (b) locations and elevations of the 28 study sites from which the moths were sampled. (c) Zoomed- in view of the high and mid elevations of the gradient. Colors of dots indicate elevation above sea level. Map created using Bing Maps. Microsoft product screen shot(s) reprinted with permission from Microsoft Corporation. For more information, visit Microsoft Print Rights.

2.2 Host identification

Sampled insects were provisionally identified in the field considering their morphological traits and confirmed through molecular characterization, using the mitochondrial cytochrome C oxidase subunit I (COI) gene sequence as a DNA barcode. DNA barcoding was carried out at the Canadian Center for DNA Barcoding (CCDB; Guelph, Ontario, Canada; <http://ccdb.ca/>). COI sequences were submitted to the Barcode of Life database (BOLD; <http://www.boldsystems.org>) for taxonomic classification. In addition, a phylogenetic analysis was conducted with the COI sequences to confirm that the target genera were correctly identified. The sequences were aligned using Muscle (Edgar, 2004) and the aligned sequences were then retrieved through a BLAST search against the GenBank database (Benson et al., 2013). Closely related taxa and outgroup sequences were selected to create a neighbor-joining tree using Mega version 11.0.13 (Hall, 2013). Individuals were included in the analysis only after morphological identification at the genus level was confirmed through barcoding. We made no attempt of identifying moths at species level because the data currently available in BOLD is still insufficient, and both Geometridae and Arctiinae contain a high proportion of undescribed species (Brehm et al., 2025).

187

188 **2.3 Microbiome profiling by 16S rRNA metabarcoding**

189 Microbiome profiling were performed according to previously published descriptions
190 (Pinos et al., 2025). Briefly, the bacterial V4 regions of the 16S rRNA gene were amplified in
191 triplicate through a dual-indexing method (Kozich et al., 2013). Negative controls in PCR were
192 included to ensure valid amplification. PCR products were gel-visualized and triplicate reactions
193 combined and quantified using a Qubit® 4.0 Fluorometer (Invitrogen, Carlsbad, CA, USA).
194 After pooling, the samples were diluted to 1.8 nM and combined with 5% Phix Control.
195 Sequencing was conducted on the Illumina MiSeq platform at the Genomics Service Unit, LMU
196 Biozentrum, using a 2 × 250 cycles reagent kit v2, adhering to the manufacturer's instructions
197 (Illumina Inc., San Diego, USA).

198 The raw sequences were processed following an established protocol (Leonhardt et al.,
199 2022). Chimeric artifacts and poorly overlapped and low-quality sequences (such as sequence
200 length <170 bp) were filtered out. Further, the sequences were clustered into Amplicon Variant
201 Sequences (ASVs) at 97% pairwise identity using VSEARCH global alignments (Rognes et al.,
202 2016). Taxonomy assignments were made through direct global alignment against the RDP_16s
203 (Edgar, 2016), GreenGenes 16s v13.5 (DeSantis et al., 2006), and SILVA 16S v123 (Quast et al.,
204 2013) databases, and chloroplast and archaeal sequences were removed.

205

206 **2.4 Data analysis**

207 All analyses were performed in R Studio (version 2025.05.1+513) using the following
208 packages: Phyloseq (McMurdie & Holmes, 2013), Vegan (Oksanen et al., 2001), iNEXT (Hsieh

et al., 2016), car (Fox et al., 2001),ggeffects (Lüdecke, 2018), tidyverse (Wickham et al., 2019),
dplyr (Wickham, 2015), patchwork (Pedersen, 2019), hillR (D. Li, 2018), lme4 (Bates, 2018),
MuMIn (Barton, 2009), and Microbiome (Lahti & Shetty, 2017).

From the ASV table, we calculated absolute counts and relative abundances (i.e. the ratio
between the number of reads belonging to the ASV in a specific sample and the total number of
reads in the sample) for each ASV.

2.4.1 Diversity and statistical analyses

Host taxon richness at each elevation was quantified as the total number of genera within
that community.

Alpha diversity

To prevent bias or confounding in empirical biodiversity (Hill's numbers of $q = 0,1$) due
to differences in abundance or sampling intensity, the bacterial sequences were filtered using a
target coverage ($\text{min SC} \geq 0.943$) (Chao & Hu, 2024). To identify the factors driving microbial
alpha diversity, we fitted generalized linear models using a Gamma error distribution with a log
link function. Microbial richness ($q = 0$) and diversity ($q = 1$, equivalent to Shannon exponential
entropy) were modeled as functions of host genus level and MAT. Explained variances were
determined using Nagelkerke's R^2 implementation in the performance package.

Beta diversity

229 We explored the variations in microbial community composition along the elevation gradient,
230 estimating the overall beta diversity using the Bray-Curtis dissimilarity index (Bray & Curtis,
231 1957) using different microbial taxonomic ranks (ASV, genus, family, order, and phylum). A
232 permutational multivariate analysis of variance model (PERMANOVA) was performed to test
233 Beta-diversity patterns. PERMANOVA was performed with the `adonis2` function using Bray-
234 Curtis distances and 999 permutations to test for differences in community composition. Then,
235 the relative contributions of host taxonomy and MAT in shaping microbial community
236 composition were estimated by variance partitioning analysis using the `varpart` function. For this
237 method, a redundancy analysis (RDA) of variables was conducted, and the respective amounts of
238 variance (i.e., individual and shared) were determined by the adjusted R^2 (Ramette & Tiedje,
239 2007). An ANOVA test was carried out to test whether there was a significant difference in the
240 individual contribution of elevation and host taxonomy to the microbial composition. We
241 visualized the differences between microbial community structures along the environmental
242 gradient with the RDA and the correlations between RDA axes and explanatory variables were
243 determined using the `'envfit'` function.

244 We tested whether bacterial community dissimilarity changes with the distance in elevation,
245 defined as the absolute difference in elevation between pairs of samples. Distance in elevation
246 represents mainly the vertical distance between sites along the elevation gradient. Pairwise
247 bacterial beta diversity was calculated using Hill numbers ($q = 0$ and $q = 1$) with the function
248 `'hill_taxa_parti_pairwise'` of the `hillR` package (effective similarity of communities: 1 identical
249 community and 2 complete dissimilarities between two samples). For each pair of samples, we
250 calculated the absolute difference in elevation. We then fitted linear mixed effects models
251 (LMMs) to assess the relationship between distance in elevation as a fixed effect and pairwise

beta diversity as the response variable. To account for the non-independence of pairwise comparisons, site identity was included as a random intercept for both samples forming each pair. Finally, we used generalized dissimilarity modeling (GDM) to predict how MAT was influencing the microbial taxonomic turnover by host genus and at the different microbial taxonomic levels (phylum, order, family, genus and ASV). GDMs were fitted on abundance-based Bray Curtis dissimilarities, using selected continuous predictors and geographic coordinates as explanatory variables (Mokany et al., 2022). Continuous predictors were fitted using monotonic I-spline transformations, which allow nonlinear relationships between environmental gradients and compositional turnover. Models were fitted by maximizing the likelihood of the observed data (Ferrier et al., 2007). Predictor importance was quantified by summing spline coefficients, and I-splines were visualized to assess the magnitude of turnover along each gradient (Fitzpatrick & Lisk, 2016).

3. Results

3.1 Host diversity

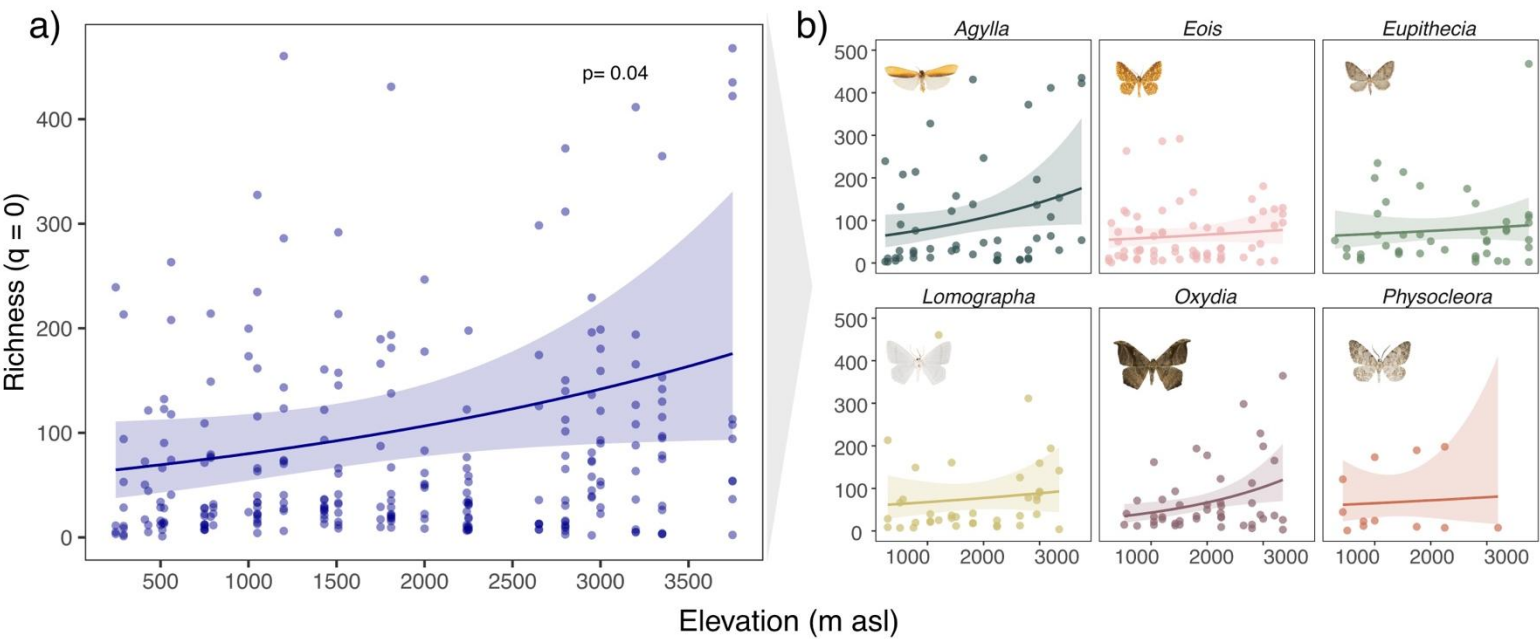
The final dataset contained a total of 255 adult moths from the six target genera within two moth families: Geometridae (n = 205) and Erebidae (n = 50). Within Geometridae: *Eois* (n = 66), occurred at elevations ranging between 245 and 3,350 m asl; *Eupithecia* (n = 43), at 290 to 3,750 m asl; *Lomographa* (n = 39), at 290 to 3,350 m asl; *Oxydia* (n = 45), at 520 to 3,350 m asl;

and *Physocleora* (n = 12), at 430 to 3,200 m asl. *Agylla* (Erebidae,) was recorded at elevations ranging from 245 to 3,750 m asl (Figure S1).

3.2 Shifts in microbial diversity along the elevation gradient

Across the complete dataset, our analyses showed significant variations in the microbial alpha diversity along the environmental gradient (Table S1). Although the elevational gradient spanned more than 3000 m, it had only a minor positive influence on bacterial richness (glm R² 0.11, p= 0.048) (Figure 2a; Table S1a), but no effect was observed in microbial diversity (Figure S2; Table S1b). Furthermore, our analysis revealed a trend toward increased gut microbial alpha diversity at the host genus level with rising elevation. However, this trend did not reach statistical significance in any case (Figure 2b; Table S1b). Overall, the models explained a modest but significant proportion of variance, i.e., 11% for richness and 19% for diversity (Table S1).

Generalized linear models (GLMs) revealed distinct patterns in how environmental temperature shapes microbial communities across different host genera. For microbial richness, MAT exerted a consistent, significant negative main effect (Table S2a), with no significant interaction terms; this indicates a uniform decline in microbial richness as temperature increases across all host groups. In contrast, microbial diversity exhibited a more complex, non-linear relationship with temperature (Table S2b). While the effects of temperature were not overall significant across the baseline reference group, a significant interaction emerged specifically for the genus *Physocleora*. Finally, microbial richness was significantly lower in *Eois* and *Oxydia*, whereas overall microbial alpha diversity was significantly lower in *Oxydia* and *Physocleora*.



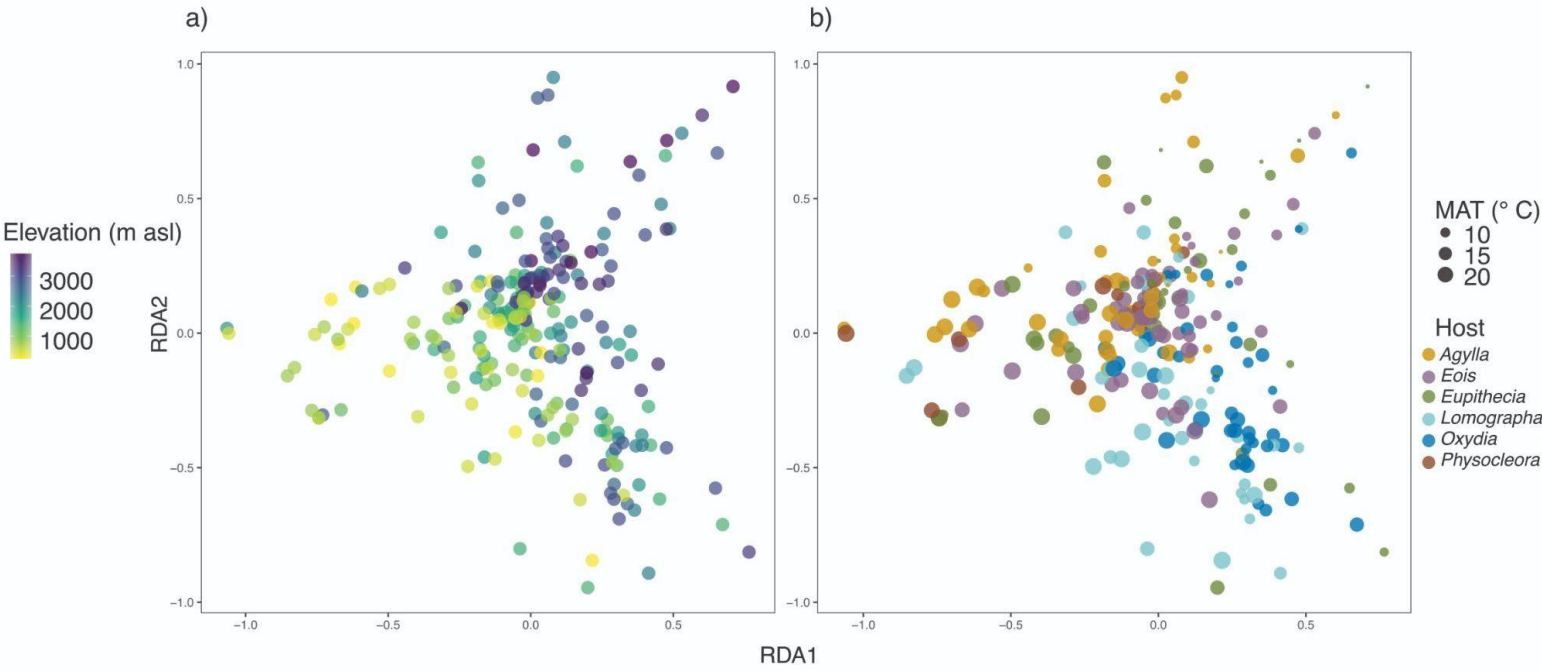
297 **Figure 2. Gut microbial richness ($q = 0$) along the elevation gradient.** a) microbial richness
298 throughout the overall dataset ($t = 1.99$, $p = 0.04$) and b) by host genus. The trend lines along
299 elevation were modeled with generalized linear models (gamma distribution, log-link function)
300 and indicate the slope estimate. The shadows are 95% confidence intervals around the estimate.

302 *Host taxonomy and MAT differentially shape microbial community composition pattern*

303 According to a PERMANOVA analysis, host taxonomy and MAT were both significant
304 factors driving gut microbial compositions across all microbial taxonomic ranks (Table S3). To
305 delve deeper into these relationships, we performed environmental fitting and variance
306 partitioning analyses (Table S4, Table S5).

Environmental fitting indicates strong influence of host taxonomy along the environmental gradient (Table S4), with persistent effect on microbial diversity across all taxonomic levels, while MAT explained variance mainly at genus and ASV level (Table S4a). Additionally, analysis of the centroid positions from the RDA reveals that *Agylla* and *Physocleora* share similar microbial communities, as indicated by their proximity across various taxonomic levels (Table S4b). In contrast, *Eois*, *Eupithecia*, and *Lomographa* exhibit more distinct microbial compositions, especially at the ASV level

Along the environmental gradient microbial communities from hosts at low elevations were separated from the ones at higher elevations (Figure 3a), and variance partitioning showed that the combination of host taxonomy and MAT explains a considerable portion of total variance, particularly evident at microbial genus level (shared contribution of 5.02%, Table S5). The unique contribution of host taxonomy alone explains up to 3.66% of variance at this level, while MAT showed only 1.46%. Additionally, temperature alone may not fully explain additional variability at higher microbial taxonomic levels, such as bacterial phylum and order level, where host taxonomy seems to be the dominant driver (Figure 3b; Figure 4).



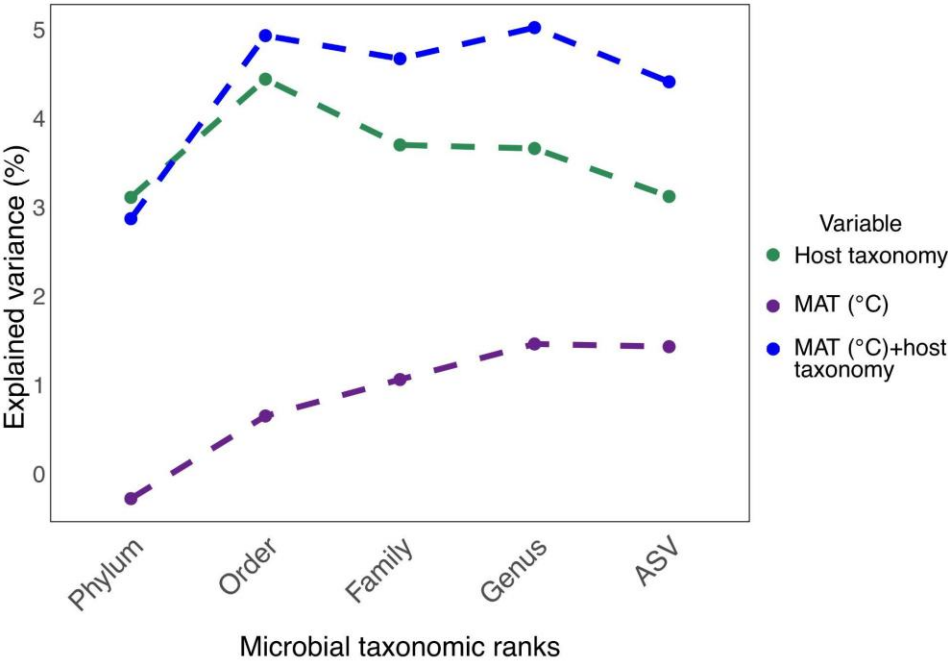
322

323 **Figure 3. Redundancy analysis (RDA) at ASV taxonomic rank of gut bacterial communities**

324 **along the elevation gradient. a) by elevation and b) by host genus and MAT represented by dot**

325 **sizes.**

326



327

328 **Figure 4. Variance partitioning results.** Scatterplot displays the percent of variance explained
329 by host taxonomy, MAT and the shared fraction between variables, across the microbial
330 taxonomic ranks (data from Table S5).

331

332 *Temperature-driven microbial turnover*

333 To evaluate how vertical distance shapes microbial communities, we modeled pairwise
334 beta diversity against absolute differences in elevation while controlling for site-specific factors.
335 Linear mixed-effects models (LMMs) revealed a significant effect of elevational distance on
336 moth microbial compositional turnover. The results indicated that both diversity (Figure 5a) and
337 richness (Figure S3) metrics increasing dissimilarity in microbial communities as the distance
338 between sampling sites increased. Distance in elevation alone explained 2% of the variance in

pairwise turnover at ASV level (Table S6). When accounting for site-specific factors, the combined model explained 53% of the variance for richness and 29% for diversity. Our findings indicate that microbial communities of moths located at similar elevations exhibit greater compositional similarity, as the distance in elevation increases, the microbial communities show more pronounced differences in composition.

Generalized Dissimilarity Modelling (GDM) showed that the microbial taxonomic turnover was higher at warmer temperatures, particularly for the host genera *Agylla* and *Eupithecia*, while the rates and magnitudes of compositional turnover remained stable along the temperature gradient for the remaining groups (Figure 5b). Thus, the rate of microbial compositional turnover, indicated by the shape of the partial ecological distance (I-spline) for MAT, was rapid and constant at low temperatures (less than ~ 10 °C) before increased sharply for *Eupithecia* (maximum partial ecological distances of ~ 0.4 , Figure 5b). For *Agylla*, turnover stabilized after ~ 14 °C, continuing to rise only slowly (maximum partial ecological distances of

0.25, Figure 5b). Additionally, GDMs showed that the highest deviance variation explained by MAT was at finer taxonomic levels (Figure 5c).

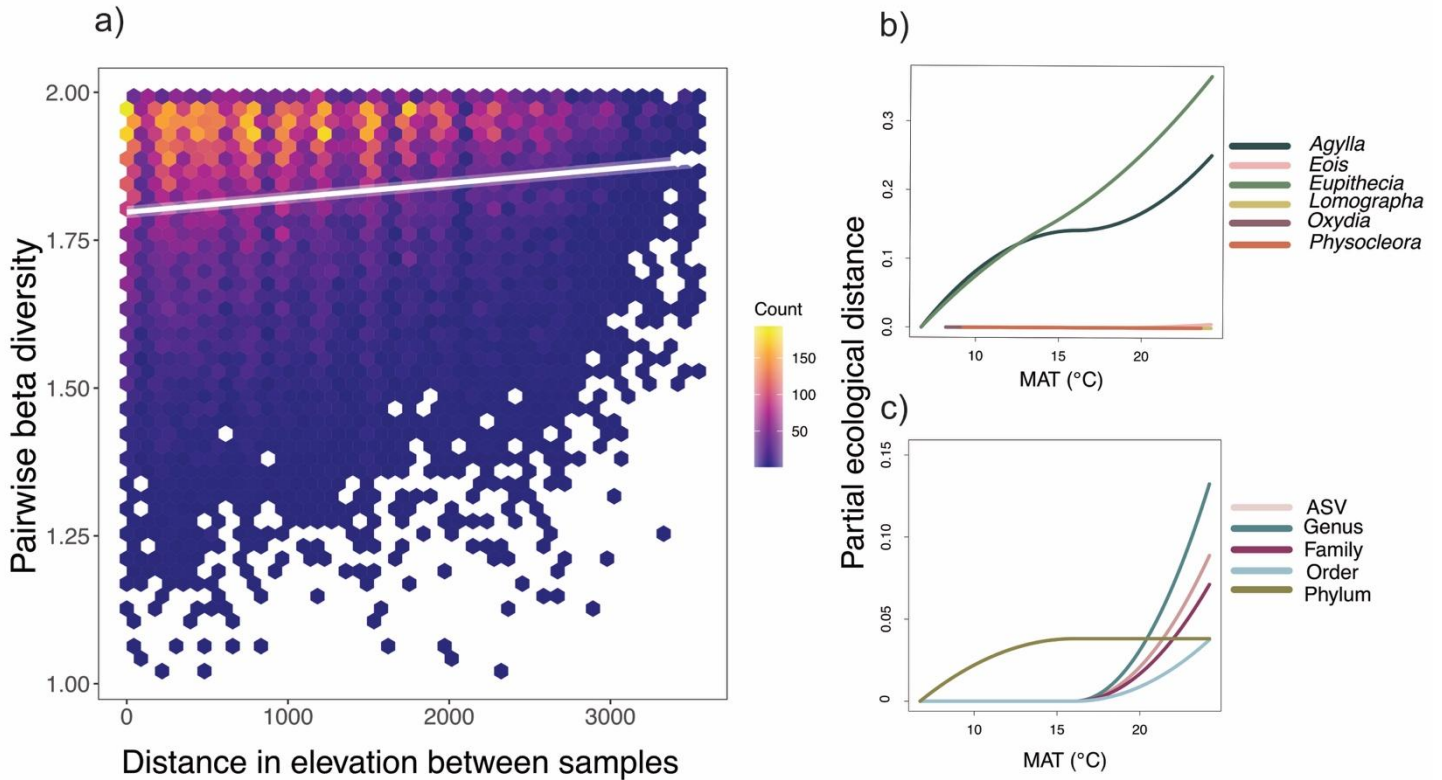


Figure 5. Microbial turnover along the elevational gradient. a) distance in elevation between samples pairs and microbial turnover (Hill order $q = 1$ at ASV taxonomic rank). Hexagonal bins represent the density of pairwise comparisons between samples, with varying colors representing different densities. The solid line demonstrates that microbial taxa turnover increases with greater vertical distance, as modeled by linear mixed effects models. b) Generalized dissimilarity model (GDM) showing the total compositional turnover with respect to MAT by host genus and c) by microbial taxonomic rank. The maximum height of each I-spline represents the total amount of compositional turnover associated with MAT, keeping all other variables constant.

4 Discussion

This study represents the first comprehensive investigation testing the effect of host-identity and environmental temperature on gut microbial communities associated with adult moths in real-world ecosystems. We compared the microbial diversity within six species rich moth genera across a complete tropical elevation gradient in Peru. Our findings reveal that host taxonomy remains the primary factor driving the structuring of gut microbial communities in a temperature gradient spanning a total of $\sim 24^{\circ}\text{C}$. However, temperature plays a crucial role in filtering microbial communities at finer taxonomic resolution along the environmental gradient.

4.1 Influence of host taxonomy in gut microbial communities

The main factor structuring the moth microbiomes along the elevation gradient is host taxonomy. Both hosts and their microbes are susceptible to environmental changes with elevation. However, gut microbiomes are protected from outside environmental conditions by the gut itself. This protection helps lessen the effects of changes in the environment, allowing the microbiomes to remain more stable even when external conditions shift (Ferguson et al., 2018; Nottingham et al., 2018). While the process of acquiring microbiomes differs significantly between solitary and social species, our findings reveal that the diversity patterns observed in moths are surprisingly similar to those in bees (Pinos et al., 2025). This suggests that, despite the solitary nature of moths, which limits microbial transmission through social interactions, there may be underlying mechanisms that promote similar diversity patterns. This phenomenon invites further exploration into how environmental factors might influence microbiome diversity in insects, paralleling the patterns seen in hymenopteran insects (Kueneman et al., 2023).

Lepidoptera are particularly adapted to navigating diverse thermal environments (Mutamiswa et al., 2023) and are good models to disentangle environmental from host effects on a temperature gradient. Each species within this order exhibits unique ecological traits that modulate thermal adaptation, ultimately influencing the composition of their associated microbial communities (Hettiarachchi et al., 2025). Our findings evidence this notion, and can be explained by the different strategies that Lepidopteran species employ for adaptation to changing environments, including microhabitat selection, basking, modifications in spatial-temporal activity cycles, and even long distance migration (Kleckova & Klecka, 2016). Moreover, morphological traits of Lepidoptera, such as wing melanism, also impact thermal regulation i.e., darker pigmentation allows for increased heat absorption, leading to microbial variation among hosts (MacLean et al., 2016). For instance, our results indicate that as MAT decreases at higher elevations, certain microbial species thrive while others decline, leading to altered community dynamics. Although interindividual variability or altered microbiome compositions have been often interpreted as a disturbed microbiome, these shifts are not necessarily negative (Williams et al., 2024). One reason for this is that many microbes have functionally redundant metabolic roles, meaning that the loss of a single taxon may not necessarily lead to the loss of a specific functional trait (Yang, 2021). Consequently, altered microbiomes could potentially increase resilience by allowing hosts to select the most beneficial microbial partners along the environmental gradient (Williams et al., 2024). In this sense, it might be that host species that are better adapted to colder conditions may harbor distinct microbial communities compared to those that thrive in warmer environments. Understanding these shifts is essential, as they might have significant ecological implications on ecosystem functioning, and how hosts could adapt to environmental warming.

4.2 Local environment influence the gut microbial communities in moths

The gut microbial communities of Lepidoptera are influenced by various aspects of their host living environments (Paniagua Voirol et al., 2018; Shao et al., 2024). Key factors include the type of ecosystem they inhabit, the distribution of vegetation, and changes in temperature (Ferguson et al., 2018; Higuera Palacio et al., 2021). Here, bacterial communities in moths respond to temperature of the host habitat. This finding agrees with previous studies, which demonstrated insect gut microbiota responses across an elevation gradient in bees (Pinos et al., 2025), and grasshoppers and crickets (Mazel et al., 2024).

Our research revealed that hosts sharing the same elevational range i.e., temperature, may harbor gut symbionts with more similar compositions compared to those from distant elevations. This finding implies that changes in temperature are likely filtering specificity of the microbial communities in particular at finer taxonomic levels, while on higher taxonomic ranks functional microbial groups are being selected by the host itself. Nevertheless, distinguishing the effects of temperature from geographical influences is challenging, as our sampling was conducted along a single elevational gradient, meaning that sites with similar temperatures are also spatially close. To better separate the impacts of temperature from geography, future studies should investigate microbiomes across multiple independent elevation gradients (Mazel et al., 2024). Although both vegetation and animal diversity tend to decline with increasing elevation in this environmental gradient, our findings reveal an opposite trend for the richness of the gut microbiome in moths, which increases at higher elevations where temperatures are lower. In comparison, microbial communities of bees along the same elevation gradient show a mixed pattern. In Pinos et al. (2025), gut microbial communities associated with honey-, stingless-, and

orchid bees exhibited a decrease in microbial richness at higher elevations. In contrast, sweat bees displayed a unimodal pattern, while bumble bees showed an increase in microbial taxa at higher elevations. Biodiversity–ecosystem function theory suggests that greater diversity enhances ecosystem functioning through complementarity (Hooper et al., 2005). Empirical studies, such as synthetic community experiments, support this, demonstrating that higher diversity boosts productivity. However, it’s still unclear if increased microbiome productivity directly benefits host health (Bell et al., 2005). While high alpha diversity has been linked to lower pathogen infections in bumblebees (Maihoff et al., 2025), some frameworks suggest it can be neutral or even harmful (Williams et al., 2024). Ecological models indicate that high diversity might destabilize microbiomes or increase competition, leading to reduced productivity (Coyte et al., 2015). For animals with few specialized symbionts, like some insects, low diversity can be optimal, as selection favors more partner-specific mutualisms (Batstone et al., 2018). Additionally, in species with mostly transient microbiomes, variations in diversity may not significantly impact host health (Hammer et al., 2019; Ravenscraft & Coon, 2026).

4.3 Temperature-driven microbial turnover in the gut communities moths

Our results provide a clear overview of how microbial taxa are organized along an elevational gradient, showing that temperature significantly influences microbial composition along with host taxonomy. Comparisons between sites indicate that bacterial communities are more uniform at similar elevations, especially at colder climates. As the distance between sites increases, microbial composition becomes more distinct, with notable turnover of taxa occurring in warmer habitats. This pattern suggests that microbial turnover accelerates beyond a certain

elevation threshold, indicating that gut communities are undergoing environmental filtering. We anticipate this classic distance decay of assemblage similarity, where communities become less similar as the distance between them increases (Bishop et al., 2015). This has been observed for various organisms and linked to community diversification (Fontana et al., 2020; Wang et al., 2012), implying that species specialize within specific elevation ranges rather than across the entire gradient. Geographical distance is well known to influence community dissimilarity (Perillo et al., 2017). However, the extent of this influence on gut symbionts remains unexplored. We can attribute observed patterns to the physiological traits of host species, such as thermal tolerances, which play a crucial role in generating species turnover (Bishop et al., 2015; Holzmann et al., 2026).

Despite these insights, the mechanisms driving this variation remain poorly understood. The interplay of multiple factors complicates the identification of causal relationships, making it challenging to draw definitive conclusions. Studies indicate that the metabolic functional potential of microbial communities is closely linked to environmental conditions, while the taxonomic variation within individual functional groups is inadequately explained by these conditions (Louca et al., 2016; Ramond et al., 2025). In this context, it is expected that similar environmental conditions will promote analogous microbial community functions, while allowing for taxonomic diversity within specific functional groups (Eisenhauer et al., 2023; Louca et al., 2018). This notion aligns with the principle of functional redundancy, wherein multiple taxonomically distinct organisms can perform equivalent metabolic functions (Louca et al., 2016). Consequently, the stability of essential functions may be upheld by diverse taxa capable of fulfilling similar ecological roles. Thus, although microbial taxonomic composition may vary significantly with increasing temperature, the underlying functional capabilities

provide a buffer that enables microbial communities to adapt and maintain ecosystem functions (Beauvais et al., 2023). This illustrates the complex interplay between environmental factors and microbial diversity, emphasizing that turnover is not merely a consequence of species loss but rather an adaptive response to environmental variability.

Nevertheless, host species exhibit varying degrees of interindividual and intraindividual (temporal) microbiome variability, and for many species, the standard level of variability remains unknown. This standard may also vary between different host species. As a result, determining the implications of microbial turnover remains unexplored. Future research should focus on elucidating the functional roles of these changing microbial communities and their potential impacts on host organisms and broader ecological networks.

5. Conclusions

This study represents the first comprehensive investigation analyzing gut microbial communities in adult moths across a temperature gradient. It highlights how the relationship between host species and temperature affects microbial diversity, showing that these microorganisms can adapt to changing environmental conditions within their ecosystems. Understanding these interactions is essential for evaluating how moth populations function ecologically and their ability to cope with climate change. This research opens the door for future studies on how non-social insects interact with their microbiomes, providing important insights for biodiversity conservation and ecosystem health.

Statements

Data availability statement

All sequence data analyzed during this study are available at NCBI
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Contributions

AP conceptualization, sample collection, molecular laboratory work, data curation,
bioinformatics and statistical analyses, interpretation of data and results, writing original draft.
PA-A, YC-C, KLH and FY sample collection, writing – review and editing. AB investigation
(sequencing). GB, IS-D, and MKP funding acquisition, writing – review and editing. AK funding
acquisition, project administration, resources. AW interpretation of data and results, writing –
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Declarations

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Supplementary Information

Below is the link to the electronic supplementary material.

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