nature portfolio

Peer Review File

Differential responses of termite gut bacterial and fungal community to tropical forest conversion

Corresponding Author: Dr Shengjie Liu

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Major Comments
1. Abstract

Line 28: Provide the full form of RDA (Redundancy Analysis) at first appearance.

Line 31: When referring to bacteria, please provide the genus name.

Line 34: Provide the species name mentioned in this sentence.

The abstract states “whereas use more time…” — please clarify and rewrite for better readability.

In the keywords, authors include the termite genera Ancistrotermes and Odontotermes, but these genera are not mentioned anywhere in the abstract. Either mention them in the abstract or revise the keywords accordingly.

2. Introduction

Lines 56–57: The phrase “health and ecological functions … among other” is unclear. Please clarify what “among other” refers to.

Citation Pattern: Citation formatting is inconsistent throughout the Introduction. Please check and correct citations in lines 54, 62, 63, 83, 85, 88, and subsequent similar issues.

Line 90: Sentence is unclear or grammatically incorrect; please revise.

Line 107: Sentence structure needs correction for clarity.

3. Methodology

Authors collected more samples from forest area than from urban plantation, but fewer colonies were collected from natural forest than rubber plantation. This is contradictory—please justify the sampling design and provide a clear explanation.

Line 125: Mentions “3–16 colonies,” whereas elsewhere it states “30 termites per sample.” Please resolve this discrepancy.

Line 127: States “frozen at –80°C”; please provide the make and model of the freezer or instrument used.

Missing Citations: No reference is provided for the methodology. Please clarify whether

this is a newly developed protocol, or

adopted/adapted from published literature.

In the latter case, proper citations must be added.

Abbreviations without full forms appear throughout the section (e.g., lines 127, 135, 145, 172, 241, 256, 265, and others). Please ensure that each abbreviation is written in full at its first occurrence.

4. Results

Line 238: Please provide the species name discussed in this statement.

Line 239: Mention the level of statistical significance (e.g., $p < 0.05$, $p < 0.01$).

Line 246: Remove the apostrophe from angustignathus.

Citation formatting issues similar to the Introduction also appear in the Results—please revise accordingly.

5. Figures

Figures 1 & 2: Abbreviations are used, but full forms are not provided in the figure captions, especially for species names.

Figures 1–4: Figures are not clear; high-resolution images should be provided.

Figures 5 & 6: The manuscript text discusses Levels 1, 2, and 3, but the figures show only Level 3. Please clarify this inconsistency or revise the figures accordingly.

Minor Comments

Check spelling, grammar, and consistency throughout the manuscript.

Species names must be in italics and correctly formatted.

Ensure uniform terminology for habitat types (e.g., natural forest vs rubber plantation vs urban plantation).

Reviewer #2

(Remarks to the Author)
The manuscript investigates how tropical forest conversion to rubber plantations affects the gut bacterial and fungal communities of four termite species in Xishuangbanna, China. The authors combine high-throughput sequencing, community composition analyses, functional analyses, and co-occurrence network approaches to evaluate the relative effects of host identity and habitat type on termite gut microbiomes. The topic is timely and relevant, as understanding microbiome responses to land-use change is central to predicting ecosystem resilience under anthropogenic pressure.
The main finding is that termite host identity is a stronger determinant of bacterial community composition, whereas both host species and habitat type influence fungal communities. While the study presents valuable data and a solid experimental design, its conceptual and mechanistic advances are limited. The termite species selected represent feeding guilds that do not directly consume plant substrates, three are fungus-growing termites and one is a facultative inquiline. In this context, it is possible that the gut microbiota did not fully capture the effects of forest type. Analyzing the fungal comb microbiota, rather than the termite gut community, might have been a more suitable approach to assess the influence of forest conversion on termite-associated microbial assemblages.
Methodologically, the study is competently executed, but several aspects of the statistical analyses need clarification or improvement, especially when simultaneous effects are evaluated.
Minor comments
Introduction
• Line 65: Diet changes have distinct effects on termite gut microbiota, particularly within the family Termitidae. Please see https://doi.org/10.3389/fevo.2025.1625443 for further discussion on dietary influence in termitids.
• Line 96: It is not clear why these specific termite species were selected. Are they the most common or ecologically dominant species in the studied habitats? Please clarify the rationale for species selection.
Methodology
• Line 182: PERMANOVA was used to evaluate compositional differences in the microbiome. However, the dispersion of data appears to vary among termite species and forest types. Because PERMANOVA is sensitive to differences in dispersion from the centroid, which can lead to false significance, it would be useful to test data dispersion using the permdisp function.
• Line 197: A two-way ANOVA or a LMM (with colony as random factor) might be more appropriate to evaluate the interaction between termite species and forest type, since some species responded differently to the rubber plantation impact.
• Line 210: It is unclear whether the authors conducted a standard RDA or a tb-RDA analysis. Please clarify which constrained ordination method was used.
Results
• Lines 241–252: Please verify data dispersion before running the PERMANOVA analyses. Because PERMANOVA is sensitive to heterogeneity of dispersion, unequal group variances could lead to false significance. Testing this assumption (with the permdisp function) would strengthen the validity of the results.

• Line 254: Scores for termite species and forest types were not provided. Please include these, along with the adjusted R^2 values for each analysis, to facilitate a more comprehensive interpretation of the ordination results.
• Line 281: Functional analyses should emphasize CAZyme activity, as the majority of the termite gut microbiota are involved in lignocellulose degradation in termitids. Shifts in CAZyme functional profiles could be directly associated with changes in food quality and availability following forest conversion.
• Line 293: Consider using heatmaps to visualize differences in the predicted functional profiles of the gut microbiota. This graphical approach would allow clearer comparisons across termite species and forest types.
• Line 294: Since microbiota characterization represents the main focus of the study, this part of the results should be placed at the beginning of the Results section. In addition, please provide taxonomic bar plots (taxaplots) showing both phylum- and genus-level classifications to better visualize shifts in microbial community composition.
• Line 551: The RDA triplots indicate potential variable collinearity. It would be advisable to retain only the environmental variables that most strongly contribute to the observed patterns to improve model interpretability and reduce redundancy.
Discussion
The discussion should be revised to better contextualize the biology of the termite species investigated. Since most of the species analyzed belong to the subfamily Macrotermitinae, which cultivate the symbiotic fungus Termitomyces, their gut microbiota primarily reflects the digestion of fungal material rather than direct decomposition of plant substrates. This distinction is critical because the influence of forest conversion on gut microbial composition may be indirect, mediated through changes in fungal comb microbiota. In non-fungus-growing Termitidae, the gut microbiota directly processes lignocellulosic material derived from soil organic matter or plant litter, making it more sensitive to vegetation composition and forest type. In contrast, fungus-growing termites rely on their fungal symbiont for pre-digestion of plant biomass, leading to a more stable and specialized gut microbiota largely decoupled from external food variability.
Therefore, the current dataset likely did not show the direct effects of forest conversion on termite-associated microbiomes. To capture these effects, the study should have included soil-feeding, wood-feeding or litter-feeding termites whose diet is directly influenced by forest vegetation structure. Alternatively, analyzing the fungal comb microbiota in addition to the termite gut community would provide a more complete understanding of how environmental changes cascade through the termite–fungus–microbiome system.
Furthermore, this section should temper generalizations about the ecological implications of forest conversion and emphasize that responses of termite microbiota to habitat change are likely to be feeding guild-specific. Termites with soil-based diets or wood-based diets are expected to show very different microbial shifts compared to fungus-growing taxa. Clarifying this distinction will strengthen the ecological interpretation and make the conclusions more biologically meaningful.

Reviewer #3

(Remarks to the Author)
This manuscript investigates the effect of converting natural tropical forests into monoculture rubber plantations on the gut microbiota of four termite species in Xishuangbanna, China. Using amplicon sequencing, functional predictions, and co-occurrence network analysis, the authors conclude that termite host identity is the dominant driver of gut bacterial diversity and function, whereas fungal composition and functional guilds respond strongly to land-use change.
The topic is timely, and the study system is ecologically important. However, there are several issues that must be addressed before the manuscript can be considered for publication.

Major comments:

1. The sampling design is unbalanced, with four natural forests compared to two rubber plantations, and highly uneven numbers of colonies per species across habitat types. For some species, the sample size per habitat is minimal (3–5 replicates), which increases the risk of both false negatives and false positives, thereby weakening the robustness of the conclusions drawn from the analyses. The Discussion section should explicitly acknowledge these design constraints and their implications for interpreting habitat-related differences in gut microbiome structure and function.
2. The manuscript suggests that termite gut fungal communities closely reflect environmental fungal communities because all fungal genera detected in the gut were also present in the soil. However, this apparent complete overlap is likely driven by the coarse taxonomic resolution of ITS amplicon sequencing, which often lacks the ability to distinguish closely related or functionally distinct fungal lineages at finer scales. In addition, the absence of negative controls raises the possibility that some taxa may represent low-level environmental or laboratory contamination rather than true gut residents. Furthermore, the soil fungal data appear to have been collected at the site level rather than paired with each individual colony, limiting the ability to draw robust conclusions about direct environment–gut correspondence. These methodological constraints should be acknowledged, and interpretations regarding environmental filtering or gut–environment similarity should be presented with appropriate caution. Also clarify how many soil samples were sequenced and how they map to termite samples.
3. The authors explicitly state that no negative controls were included during DNA extraction or library preparation. This represents a significant limitation, particularly for the fungal ITS dataset, as ITS markers are highly susceptible to reagent- and handling-related contamination, and also gut fungal communities in termites often have low biomass. Hence, without controls, it is impossible to distinguish true gut residents from contaminants introduced during dissections, DNA extraction, or reagents. This uncertainty should be acknowledged in the manuscript, and the authors may wish to apply more conservative filtering criteria for fungal ASVs or discuss how potential reagent contaminants could influence the observed patterns of the fungal community.

4. The interpretation of functional predictions appears overstated given the methodological constraints of the tools used. For

PICRUSt2, the reliability of inferred bacterial functions is uncertain because many termite gut bacterial lineages lack closely related reference genomes. Functional inference for fungi using FUNGuild is even more limited, as ecological guild assignments are inherently coarse and become particularly unreliable when based on genus-level classifications. The authors should discuss the limitations of applying PICRUSt2 to termite gut microbiomes and clearly acknowledge that FUNGuild-based functional assignments are putative and should be interpreted with caution rather than as definitive indicators of functional change.

5. The Discussion section includes several mechanistic interpretations suggesting that observed microbiome differences arise from dietary shifts in termites or from environmental filtering driven by soil conditions. However, the study did not directly measure termite diets, nor did it quantify feeding substrates or resource availability across sites. As a result, any statements linking gut microbial changes to diet, such as “nutrient limitation may increase microbial network complexity,” remain speculative.

6. The manuscript draws attention to differences in gut microbial patterns between the three Odontotermes species and Ancistrotermes dimorphus, but it provides limited ecological context regarding their feeding guilds, nest characteristics, or foraging behaviors. These traits are known to strongly influence termite gut microbiomes through differences in diet composition, substrate specialization, and exposure to environmental microbes. Including more information on each species’ dietary breadth, trophic group, nest substrate, or foraging depth would substantially strengthen the biological interpretation of the observed microbiome differences.

Minor Comments
1. Ensure consistent use of scientific names (italicization).
2. Use “natural forest” vs “rubber plantation” consistently; avoid mixing with “primary forest”
3. Clarify whether “three Odontotermes species had similar bacterial compositions” (L299) is based on statistical tests or visually from bar plots.
4. Provide GPS coordinates or site descriptions either in the main text or Supplementary file
5. Cite more recent work on microbiome responses to land-use change in invertebrates.
6. Ensure that all supplementary tables referenced in-text (S1–S9) actually match descriptions (L250- you refer to “Table S1” for PERMANOVA whereas it should be Table S2)
7. The Abstract and Discussion sections would benefit from concise wording, as both contain some repetition of the key findings. Streamlining these sections would improve clarity and readability.
8. L30 change to “....bacterial communities…”
9. L34-35: what do you mean by “community function”?
10. Figure 1 shows only the bacterial and fungal richness. What about other alpha diversity indices (Shannon index and Faith’s phylogenetic diversity)?
11. Figure 2, NMDS plots should display stress values.
12. Figure 4 is visually overwhelming. It is better to give a table with information about the number of edges, etc., instead of bars, and increase the image size of the network
13. Provide rarefaction curves

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
The manuscript has been improved according to the reviewer’s comments. However, there are still some issues related to the statistical analyses that should be addressed before acceptance. When comparing gut bacterial composition, the authors used PERMANOVA with both unweighted UniFrac and Bray–Curtis distances. The results indicated a stronger “separation” when using unweighted UniFrac distances, including a possible interaction between termite species and forest type for fungi. Nevertheless, the PERMDISP analyses for bacteria revealed a significant heterogeneity among termite species, indicating that groups differ in dispersion rather than (or not necessarily in) centroid location. In this case, an alternative statistical approach should be used (see Wang, Y., U. Naumann, S. T. Wright & D. I. Warton, 2012. mvabund: an R package for model-based analysis of multivariate abundance data. Methods in Ecology and Evolution 3: 471–474).

It would be useful to add a heatmap highlighting the most important CAZymes across termite species and forest types. The heatmaps currently shown in the manuscript (Figure 5) are difficult to read.

Reviewer #3

(Remarks to the Author)
I have reviewed the revised manuscript and the authors’ point-by-point responses to the reviewers’ comments. The authors have satisfactorily addressed all the issues raised and have implemented the suggested revisions appropriately.

In my opinion, the manuscript has been significantly improved and now meets the standards required for publication. I

therefore recommend that the manuscript be accepted for publication.

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
The manuscript has been revised and improved following the suggestions. I consider it appropriate for publication.

Reviewer #1 (Remarks to the Author):

Major Comments

1. Abstract

Line 28: Provide the full form of RDA (Redundancy Analysis) at first appearance.

Response: The suggested change was made.

Line 31: When referring to bacteria, please provide the genus name.

Response: The suggested change was made.

Line 34: Provide the species name mentioned in this sentence.

Response: The suggested change was made.

The abstract states “whereas use more time…” — please clarify and rewrite for better readability.

Response: Thank you for highlighting this issue. We have revised the sentence for better readability by replacing "whereas" with "while" in the first sentence (line 26) of the abstract.

In the keywords, authors include the termite genera Ancistrotermes and Odontotermes, but these genera are not mentioned anywhere in the abstract. Either mention them in the abstract or revise the keywords accordingly.

Response: Thank you for pointing this out. As suggested, we have added the termite genera *Ancistrotermes* and *Odontotermes* to the abstract (line 32) to align with the keywords.

2. Introduction

Lines 56–57: The phrase “health and ecological functions … among other” is unclear. Please clarify what “among other” refers to.

Response: We have revised the sentence as suggested to clarify the listed functions. The updated text (lines 53-54) now states: “Subsequently, gut microbes play critical roles in termite health and ecological processes, including nitrogen fixation, hydrogen metabolism, and pH maintenance.”

Citation Pattern: Citation formatting is inconsistent throughout the Introduction. Please check and correct citations in lines 54, 62, 63, 83, 85, 88, and subsequent similar issues.

Response: The citation formatting has been checked and standardized throughout the text. All in-text citations and the reference list now follow a consistent style.

Line 90: Sentence is unclear or grammatically incorrect; please revise.

Response: We have revised the sentence for better grammatical structure and clarity. The text now is (lines 89-90): “Thus, accounting for host species identity is essential to accurately assess the effects of land-use change on gut microbial communities.”

Line 107: Sentence structure needs correction for clarity.

Response: We have revised the sentence to improve its structure and clarity. The text now is (lines 108-110): “compared to natural forests, rubber plantations are expected to host termite gut microbial communities with reduced network complexity and functional guilds. This reduction is attributed to the distinctive termite food resources and environmental conditions in plantations.”

3. Methodology

Authors collected more samples from forest area than from urban plantation, but fewer colonies were collected from natural forest than rubber plantation. This is contradictory—please justify the sampling design and provide a clear explanation.

Response: We thank the reviewer for this observation, which allows us to clarify the sampling design. The lower success rate in actually locating termite colonies within natural forest resulted in fewer colonies collected (24 in natural forests vs. 34 in rubber plantations). This difference is primarily attributed to the practical difficulty of finding nests amidst the dense understory vegetation of natural forests, as explained in the revised text on lines 140-142.

Line 125: Mentions “3–16 colonies,” whereas elsewhere it states “30 termites per sample.” Please resolve this discrepancy.

Response: There is no discrepancy: 3-16 colonies refer to the number of independent biological replicates (nests) per termite species. 30 termites refer to the standardized number of individuals

pooled to form a single composite sample from each of those colonies. We have revised the text on lines 143-145 to make this hierarchical relationship explicit.

Line 127: States “frozen at –80°C”; please provide the make and model of the freezer or instrument used.

Response: The suggested change was made.

Missing Citations: No reference is provided for the methodology. Please clarify whether this is a newly developed protocol, or adopted/adapted from published literature. In the latter case, proper citations must be added.

Response: The necessary citations for the methodology have been incorporated into the text as suggested.

Abbreviations without full forms appear throughout the section (e.g., lines 127, 135, 145, 172, 241, 256, 265, and others). Please ensure that each abbreviation is written in full at its first occurrence.

Response: We have carefully reviewed the manuscript and ensured that every abbreviation is now spelled out in full at its first occurrence.

4. Results

Line 238: Please provide the species name discussed in this statement.

Response: The suggested change was made.

Line 239: Mention the level of statistical significance (e.g., $p < 0.05$, $p < 0.01$).

Response: The suggested change was made.

Line 246: Remove the apostrophe from angustignathus.

Response: The suggested change was made.

Citation formatting issues similar to the Introduction also appear in the Results—please revise accordingly.

Response: We have checked and corrected the formatting throughout the Results section to ensure consistency.

5. Figures

Figures 1 & 2: Abbreviations are used, but full forms are not provided in the figure captions, especially for species names.

Response: The suggested change was made.

Figures 1–4: Figures are not clear; high-resolution images should be provided.

Response: The suggested change was made.

Figures 5 & 6: The manuscript text discusses Levels 1, 2, and 3, but the figures show only Level 3. Please clarify this inconsistency or revise the figures accordingly.

Response: We have revised the manuscript text to ensure that the description of Figures 5 accurately matches the presented data (KO level 3).

Minor Comments

Check spelling, grammar, and consistency throughout the manuscript.

Response: We have carefully checked the manuscript for spelling, grammar, and consistency. Additionally, our co-author, Jocelyn Behm (a native speaker), has thoroughly reviewed the text to ensure language quality and formatting consistency.

Species names must be in italics and correctly formatted.

Response: The suggested change was made.

Ensure uniform terminology for habitat types (e.g., natural forest vs rubber plantation vs urban plantation).

Response: The suggested change was made.

Reviewer #2 (Remarks to the Author):

The manuscript investigates how tropical forest conversion to rubber plantations affects the gut bacterial and fungal communities of four termite species in Xishuangbanna, China. The authors combine high-throughput sequencing, community composition analyses, functional analyses, and co-occurrence network approaches to evaluate the relative effects of host identity and habitat type on termite gut microbiomes. The topic is timely and relevant, as understanding microbiome responses to land-use change is central to predicting ecosystem resilience under anthropogenic pressure.

The main finding is that termite host identity is a stronger determinant of bacterial community composition, whereas both host species and habitat type influence fungal communities. While the study presents valuable data and a solid experimental design, its conceptual and mechanistic advances are limited. The termite species selected represent feeding guilds that do not directly consume plant substrates, three are fungus-growing termites and one is a facultative inquiline. In this context, it is possible that the gut microbiota did not fully capture the effects of forest type. Analyzing the fungal comb microbiota, rather than the termite gut community, might have been a more suitable approach to assess the influence of forest conversion on termite-associated microbial assemblages.

Response: We thank the reviewer for this insightful observation regarding the critical importance of termite feeding biology for interpreting gut microbiota responses to forest conversion. We fully agree that the dominance of fungus-growing termites in our dataset necessitates careful contextualization. As the reviewer astutely points out, their gut microbiota processes pre-digested fungal material derived from their cultivated *Termitomyces* symbionts, rather than directly decomposing plant substrates. This reliance on an internalized food source likely buffers these termites' gut communities from direct impacts of changing external vegetation, contrasting sharply with non-fungus-growing guilds e.g., soil−feeders, wood−feeders whose gut microbiota directly processes externally derived organic matter and are thus predicted to be more sensitive to forest conversion.

We have revised the Discussion significantly to address this crucial point, see Limitation and Future Perspectives in Discussion section (lines 459−492) of the revised manuscript.

Methodologically, the study is competently executed, but several aspects of the statistical analyses need clarification or improvement, especially when simultaneous effects are evaluated.

Response: We thank the reviewer for the positive assessment of our methodological execution and for the constructive suggestions regarding statistical analyses. We have thoroughly revised the statistical analysis section to address these points. These modifications are detailed in our point-by-point responses below and have been incorporated into the revised manuscript.

Minor comments

Introduction

• Line 65: Diet changes have distinct effects on termite gut microbiota, particularly within the family Termitidae. Please see https://doi.org/10.3389/fevo.2025.1625443 for further discussion on dietary influence in termitids.

Response: We have added the following sentence to the manuscript and cited the recommended article (line 63-64): "In addition, the litter-feeding termite *Cornitermes cumulans* showed shifts in bacterial abundance and a pronounced decline in enzymatic function in response to dietary changes (Menezes et al., 2025)"

• Line 96: It is not clear why these specific termite species were selected. Are they the most common or ecologically dominant species in the studied habitats? Please clarify the rationale for species selection.

Response: To clarify, we have revised the text (lines 96-99) to read: "We selected four termite species (*Ancistrotermes dimorphus, Odontotermes angustignathus, Odontotermes gravelyi,* and *Odontotermes yunnanensis*) for this study based on their dominance and critical roles in local nutrient cycling and soil engineering processes such as litter decomposition and bioturbation"

Methodology

• Line 182: PERMANOVA was used to evaluate compositional differences in the microbiome. However, the dispersion of data appears to vary among termite species and forest types. Because PERMANOVA is sensitive to differences in dispersion from the centroid, which can lead to false significance, it would be useful to test data dispersion using the permdisp function.

Response: We have now conducted homogeneity of multivariate dispersions tests using the permdisp function as recommended. The analyses were performed separately for both grouping factors: termite species and forest type (line 205-211).

• Line 197: A two-way ANOVA or a LMM (with colony as random factor) might be more appropriate to evaluate the interaction between termite species and forest type, since some species responded differently to the rubber plantation impact.

Response: We agree that this is an appropriate analytical method. In fact, this is the exact method we employed: a two-way ANOVA with colony as a random factor. We have revised the relevant text to state this more clearly (line 234-236).

• Line 210: It is unclear whether the authors conducted a standard RDA or a tb-RDA analysis. Please clarify which constrained ordination method was used.

Response: Thank you for raising this point for clarification. We confirm that we performed a standard Redundancy Analysis (RDA) with linear constrained ordination and we have revised the relevant text to state this more clearly (line 247).

Results

• Lines 241–252: Please verify data dispersion before running the PERMANOVA analyses. Because PERMANOVA is sensitive to heterogeneity of dispersion, unequal group variances could lead to false significance. Testing this assumption (with the permdisp function) would strengthen the validity of the results.

Response: We have now conducted homogeneity of multivariate dispersions tests as recommended. The analyses were performed separately for both grouping factors: termite species and forest type (line 312-317 and line 323-326).

Key findings: For bacteria, the tests revealed significant heterogeneity among termite species ($F = 15.06$, $p < 0.001$), indicating that differential group variances contribute to the observed compositional differences. In contrast, dispersion did not differ significantly between forest types. For fungi, no significant heterogeneity in multivariate dispersion was detected for either termite species or forest types.

• Line 254: Scores for termite species and forest types were not provided. Please include these, along with the adjusted R^2 values for each analysis, to facilitate a more comprehensive interpretation of the ordination results.

Response: We have extracted and provided the RDA axes 1 and 2 scores for both termite species and forest types with the adjusted R^2 values for both bacteria and fungi (line 333-343). The complete scores have been added to the supplementary Table S4.

• Line 281: Functional analyses should emphasize CAZyme activity, as the majority of the termite gut microbiota are involved in lignocellulose degradation in termitids. Shifts in CAZyme functional profiles could be directly associated with changes in food quality and availability following forest conversion.

Response: Thank you for your valuable suggestion. We agree that focusing on carbohydrate-active enzyme (CAZyme) activities is important for understanding lignocellulose degradation by termite gut microbiota, especially in the context of food quality changes following forest conversion. In response to your comment, we have added detailed analyses of CAZyme profiles in both the Methods and Results sections. In Methods, we have added the description to specify how CAZyme families were derived from predicted EC numbers (line 216-222). In Results, we now include the analysis of CAZyme functional profiles (line 367-369).

• Line 293: Consider using heatmaps to visualize differences in the predicted functional profiles of the gut microbiota. This graphical approach would allow clearer comparisons across termite species and forest types.

Response: We have added a heatmap to illustrate the relative abundance of key predicted functional categories across the experimental groups. This heatmap is now included as Figure 5 (see revised manuscript).

• Line 294: Since microbiota characterization represents the main focus of the study, this part of the results should be placed at the beginning of the Results section. In addition, please provide taxonomic bar plots (taxaplots) showing both phylum- and genus-level classifications to better

visualize shifts in microbial community composition.

Response: The section "Bacterial and fungal community composition" is now placed as the first subsection of the Results (lines 263–286). As suggested, we have generated taxonomic bar plots at both phylum and genus levels (supplementary Fig. S2, S3) to illustrate compositional shifts.

• Line 551: The RDA triplots indicate potential variable collinearity. It would be advisable to retain only the environmental variables that most strongly contribute to the observed patterns to improve model interpretability and reduce redundancy.

Response: We sincerely thank the reviewer for raising this important point regarding variable collinearity in the RDA analysis. We agree that collinearity can complicate the interpretation of constrained ordination results, and we fully appreciate the suggestion identify most strongly contribute of environmental predictors.

During our revision, we carefully considered implementing forward selection. We also conducted a correlation analysis among all environmental variables to quantitatively assess the degree of collinearity. The analysis revealed that most variable pairs showed only moderate to weak correlations. Notably, only three pairs exhibited correlations with $|r| > 0.7$: TC and TN ($r = 0.89$), TC and HyN ($r = 0.71$), and BG and NAG ($r = 0.74$). A correlation heatmap visualizing these relationships has been added as Supplementary Fig. S6.

After extensive deliberation and model comparison, we decided to retain the full set of environmental variables in our final model for the following reasons:

1, Ecological Interpretability and Biological Significance: The variables identified as correlated (e.g., TC, TN, HyN) are not merely statistical covariates. They represent interconnected components of the soil nutrient environment that jointly and logically influence termite ecology and their gut microbiota. Removing a biologically core variable like Total Nitrogen (TN) solely because it correlates with Total Carbon (TC) would render the model ecologically incomplete and misleading for inference.

2, Model Performance: We compared the full model with several reduced models obtained through forward selection. While new models showed marginally lower collinearity, the overall explanatory power (adjusted R^2) did not improve meaningfully. More importantly, the ecological narrative became less coherent when statistically selected but biologically peripheral variables

were retained over core, correlated ones.

To address the reviewer's concern transparently, we have now:

1, Added a statement in the Methods (lines 251-252) acknowledging the collinearity among some predictors and explaining our decision to retain them for ecological reasons.

2, Ensured that the Results explicitly note that correlated variables should be interpreted as part of a broader environmental gradient rather than as independent effects.

We believe this approach maintains the ecological integrity of our analysis while openly addressing the statistical issue raised. Thank you again for this thoughtful comment, which has strengthened the transparency of our methodology.

Discussion

The discussion should be revised to better contextualize the biology of the termite species investigated. Since most of the species analyzed belong to the subfamily Macrotermitinae, which cultivate the symbiotic fungus Termitomyces, their gut microbiota primarily reflects the digestion of fungal material rather than direct decomposition of plant substrates. This distinction is critical because the influence of forest conversion on gut microbial composition may be indirect, mediated through changes in fungal comb microbiota. In non-fungus-growing Termitidae, the gut microbiota directly processes lignocellulosic material derived from soil organic matter or plant litter, making it more sensitive to vegetation composition and forest type. In contrast, fungus-growing termites rely on their fungal symbiont for pre-digestion of plant biomass, leading to a more stable and specialized gut microbiota largely decoupled from external food variability. Therefore, the current dataset likely did not show the direct effects of forest conversion on termite-associated microbiomes. To capture these effects, the study should have included soil-feeding, wood-feeding or litter-feeding termites whose diet is directly influenced by forest vegetation structure. Alternatively, analyzing the fungal comb microbiota in addition to the termite gut community would provide a more complete understanding of how environmental changes cascade through the termite–fungus–microbiome system.

Furthermore, this section should temper generalizations about the ecological implications of forest conversion and emphasize that responses of termite microbiota to habitat change are likely to be feeding guild-specific. Termites with soil-based diets or wood-based diets are expected to show

very different microbial shifts compared to fungus-growing taxa. Clarifying this distinction will strengthen the ecological interpretation and make the conclusions more biologically meaningful.

Response: We sincerely thank the reviewer for these exceptionally insightful and constructive comments regarding the critical biology of the termite species studied. The reviewer is absolutely correct in highlighting the fundamental distinction between fungus-growing and non-fungus-growing termites, and how this shapes the interpretation of our results in the context of forest conversion.

Our response and revisions:

1. We have fully incorporated this perspective into a new subsection titled "Limitations and Future Perspectives" at the end of the Discussion section.
2. In this new paragraph, we explicitly acknowledge that our dataset, dominated by *Macrotermitinae*, likely reflects indirect effects mediated via the fungal comb, and therefore may not capture the direct effects of forest conversion on termite gut microbiomes involved in primary decomposition.
3. We have tempered our generalizations, emphasizing that responses are feeding guild-specific. We now clearly state that future work should include soil- or wood-feeding termites for more direct assessment, and/or analyze the fungal comb microbiota to understand system-level cascades.

These revisions have significantly strengthened the biological grounding and ecological interpretation of our discussion. We are grateful for the reviewer's expertise, which has greatly improved the manuscript.

Reviewer #3 (Remarks to the Author):

This manuscript investigates the effect of converting natural tropical forests into monoculture rubber plantations on the gut microbiota of four termite species in Xishuangbanna, China. Using amplicon sequencing, functional predictions, and co-occurrence network analysis, the authors conclude that termite host identity is the dominant driver of gut bacterial diversity and function, whereas fungal composition and functional guilds respond strongly to land-use change.

The topic is timely, and the study system is ecologically important. However, there are several issues that must be addressed before the manuscript can be considered for publication.

Major comments:

1. The sampling design is unbalanced, with four natural forests compared to two rubber plantations, and highly uneven numbers of colonies per species across habitat types. For some species, the sample size per habitat is minimal (3–5 replicates), which increases the risk of both false negatives and false positives, thereby weakening the robustness of the conclusions drawn from the analyses. The Discussion section should explicitly acknowledge these design constraints and their implications for interpreting habitat-related differences in gut microbiome structure and function.

Response: We sincerely thank the reviewer for highlighting sampling design limitations. We acknowledge the imbalance in habitat representation 4 forests vs. 2 plantations and uneven replication per species-habitat combination 3–5 replicates for some species, which could affect statistical power in direct habitat comparisons. To address this:

1. We have explicitly discussed this limitation in the revised Discussion (Limitations and Future Perspectives section, line 459-492), noting its potential implications for interpreting species-specific habitat responses.
2. Crucially, inter-replicate consistency strengthens core findings: Despite low replication for some species, our analysis revealed minimal variation in microbiome composition among colonies from the same habitat and species (supplementary Fig. S2, S3). This high intra-group stability—particularly for dominant taxa and functional pathways—suggests that key habitat-driven patterns are robust and not artifacts of sampling imbalance.
3. We contextualized habitat claims: Conclusions about habitat effects are focused on

generalizable patterns supported by multi-species trends, with caution applied to interpretations of under-replicated species.

We agree that expanded sampling would refine species-resolution insights, but the replicate consistency supports the overall validity of habitat-associated microbiome shifts reported.

2. The manuscript suggests that termite gut fungal communities closely reflect environmental fungal communities because all fungal genera detected in the gut were also present in the soil. However, this apparent complete overlap is likely driven by the coarse taxonomic resolution of ITS amplicon sequencing, which often lacks the ability to distinguish closely related or functionally distinct fungal lineages at finer scales. In addition, the absence of negative controls raises the possibility that some taxa may represent low-level environmental or laboratory contamination rather than true gut residents. Furthermore, the soil fungal data appear to have been collected at the site level rather than paired with each individual colony, limiting the ability to draw robust conclusions about direct environment–gut correspondence. These methodological constraints should be acknowledged, and interpretations regarding environmental filtering or gut–environment similarity should be presented with appropriate caution. Also clarify how many soil samples were sequenced and how they map to termite samples.

Response: We sincerely thank the reviewer for these insightful methodological comments, which have helped us improve the rigor and clarity of our manuscript.

1. Clarification on soil sampling and mapping:

We have revised the Methods section to explicitly state (line 189-192): “In total, 20 soil samples in natural forest and 10 soil samples in rubber plantation were sequenced. This site-level design provided a robust profile of the environmental microbial community available to termites within each habitat, though it does not enable pairwise matching with individual termite colonies.” This clarifies the sample count and the logical link (site-level representation) between soil and termite samples.

2. Integration of methodological constraints into the Discussion:

As suggested, we have added a dedicated paragraph in the Limitations and Future Perspectives section of the Discussion (line 479-486). It now acknowledges: (i) the taxonomic resolution limits of ITS sequencing, (ii) the absence of negative controls, and (iii) the site-level compositing of soil

samples, explaining how each factor warrants caution in interpreting the gut–environment fungal overlap. We explicitly state that "Thus, interpretations of fungal community assembly and environmental filtering remain provisional."

These revisions directly address the reviewer's concerns by providing the requested clarifications and incorporating the necessary methodological caveats into our interpretation.

3. The authors explicitly state that no negative controls were included during DNA extraction or library preparation. This represents a significant limitation, particularly for the fungal ITS dataset, as ITS markers are highly susceptible to reagent- and handling-related contamination, and also gut fungal communities in termites often have low biomass. Hence, without controls, it is impossible to distinguish true gut residents from contaminants introduced during dissections, DNA extraction, or reagents. This uncertainty should be acknowledged in the manuscript, and the authors may wish to apply more conservative filtering criteria for fungal ASVs or discuss how potential reagent contaminants could influence the observed patterns of the fungal community.

Response: We thank the reviewer for this crucial point regarding the absence of negative controls and the specific vulnerability of fungal ITS data from low-biomass termite gut samples to contamination. We fully agree that this represents a significant methodological limitation. We have substantially revised the corresponding section in the Limitations part of the Discussion to explicitly acknowledge this concern, incorporate the reviewer's specific reasoning regarding ITS contamination risk and termite gut fungal biomass, and describe the implications for interpreting our fungal community data (line 479-486).

4. The interpretation of functional predictions appears overstated given the methodological constraints of the tools used. For PICRUSt2, the reliability of inferred bacterial functions is uncertain because many termite gut bacterial lineages lack closely related reference genomes. Functional inference for fungi using FUNGuild is even more limited, as ecological guild assignments are inherently coarse and become particularly unreliable when based on genus-level classifications. The authors should discuss the limitations of applying PICRUSt2 to termite gut microbiomes and clearly acknowledge that FUNGuild-based functional assignments are putative and should be interpreted with caution rather than as definitive indicators of functional change.

Response: We thank the reviewer for this critical insight regarding the limitations of functional prediction tools. We fully agree that the interpretation of PICRUSt2 and FUNGuild outputs requires careful contextualization, especially given the unique composition of termite gut microbiomes and the coarse resolution of genus-based guild assignments. To address this, we have added a dedicated paragraph to the Limitations and Future Perspectives section of the Discussion (lines 487−492):

PICRUSt2 Uncertainty: We explicitly acknowledge that the accuracy of bacterial functional predictions is compromised by the scarcity of reference genomes for termite-specific bacterial clades, which increases uncertainty in inferred metabolic pathways.

FUNGuild Provisionality: We emphasize that fungal guild assignments are inherently speculative at the genus level, as many genera exhibit ecological plasticity, and FUNGuild's categorical designations cannot capture functional nuance.

We have also revised the Results and Discussion sections to replace assertions of functional "changes" with more cautious phrasing e.g., "suggested shifts," "putative alterations" and clarified that FUNGuild assignments are not definitive ecological labels. These edits ensure our conclusions align with the predictive tools' limitations and address the reviewer's valid concerns.

5. The Discussion section includes several mechanistic interpretations suggesting that observed microbiome differences arise from dietary shifts in termites or from environmental filtering driven by soil conditions. However, the study did not directly measure termite diets, nor did it quantify feeding substrates or resource availability across sites. As a result, any statements linking gut microbial changes to diet, such as "nutrient limitation may increase microbial network complexity," remain speculative.

Response: We thank the reviewer for this critical and fair assessment regarding the speculative nature of our dietary and environmental filtering interpretations. We agree entirely that our study lacked direct measurements of termite diet and site-specific resource availability, which limits the strength of causal inferences.

Our response and revisions:

We have revised the Discussion throughout to temper mechanistic language. All statements linking

gut microbial changes directly to diet or environmental filtering have been rephrased to present these ideas as plausible hypotheses or potential explanations consistent with broader ecological theory, rather than as established conclusions.

We have added explicit caveats where such interpretations are offered. For example, the sentence regarding nutrient limitation and network complexity now reads (line 397-400): "The observed shifts in gut bacterial diversity in *O. angustignathus* following forest conversion are consistent with a potential dietary modification driven by altered food resource availability (Huang et al., 2013). This interpretation, however, remains speculative in the absence of direct dietary data."

6. The manuscript draws attention to differences in gut microbial patterns between the three *Odontotermes* species and *Ancistrotermes dimorphus*, but it provides limited ecological context regarding their feeding guilds, nest characteristics, or foraging behaviors. These traits are known to strongly influence termite gut microbiomes through differences in diet composition, substrate specialization, and exposure to environmental microbes. Including more information on each species' dietary breadth, trophic group, nest substrate, or foraging depth would substantially strengthen the biological interpretation of the observed microbiome differences.

Response: We thank the reviewer for highlighting the importance of ecological context in interpreting gut microbiome differences. We agree that functional traits are critical drivers of termite microbiota assembly. As suggested, we have now included a detailed ecological characterization of the study species in the revised manuscript. This information has been added to lines 126–138.

Minor Comments

1. Ensure consistent use of scientific names (italicization).

Response: We have carefully reviewed the manuscript and ensured that all scientific names are now consistently italicized throughout the text.

2. Use "natural forest" vs "rubber plantation" consistently; avoid mixing with "primary forest"

Response: The suggested changes have been made.

3. Clarify whether “three Odontotermes species had similar bacterial compositions” (L299) is based on statistical tests or visually from bar plots.

Response: We have clarified the text as suggested. The observation of similar compositions among *Odontotermes* species was indeed made from visual inspection of Fig. S2. The revised sentence (line 268-271) now reads: “Visual comparison of the bacterial family composition bar plots indicated that the three *Odontotermes* species shared the same dominant families in comparable proportions, whereas *A. dimorphus* differed, exhibiting a lower relative abundance of Bacteroidetes (36%) and a higher abundance of Spirochaetes (38%).”

4. Provide GPS coordinates or site descriptions either in the main text or Supplementary file

Response: We have now provided the GPS coordinates for all study sites in the Supplementary Fig. S1.

5. Cite more recent work on microbiome responses to land-use change in invertebrates.

Response: We have added five recent studies (2021-2025) that specifically investigate invertebrate-associated microbiome responses to land-use change.

6. Ensure that all supplementary tables referenced in-text (S1–S9) actually match descriptions (L250- you refer to “Table S1” for PERMANOVA whereas it should be Table S2)

Response: We have carefully checked all in-text references to Supplementary Tables and Figs and corrected them to match their actual content and descriptions.

7. The Abstract and Discussion sections would benefit from concise wording, as both contain some repetition of the key findings. Streamlining these sections would improve clarity and readability.

Response: Thank you for this suggestion. We have revised both the Abstract and Discussion sections to eliminate redundant phrasing and improve overall conciseness, clarity, and readability.

8. L30 change to “....bacterial communities…”

Response: The suggested change has been made.

9. L34-35: what do you mean by “community function”?

Response: We change to “fungal functional potential”.

10. Figure 1 shows only the bacterial and fungal richness. What about other alpha diversity indices (Shannon index and Faith’s phylogenetic diversity)?

Response: The Shannon index and Faith's phylogenetic diversity results are indeed available and have been included as Supplementary Figures S5 and S6, respectively. Following a suggestion from another reviewer to reduce redundancy in the main text (as these indices showed broadly similar trends to richness), we placed these supporting analyses in the supplement. The patterns observed are consistent with and reinforce the conclusions drawn from the richness data presented in Figure 1.

11. Figure 2, NMDS plots should display stress values.

Response: The suggested changes have been made.

12. Figure 4 is visually overwhelming. It is better to give a table with information about the number of edges, etc., instead of bars, and increase the image size of the network

Response: The suggested changes have been made.

13. Provide rarefaction curves

Response: The suggested changes have been made.

Reviewer #2 (Remarks to the Author):

The manuscript has been improved according to the reviewer's comments. However, there are still some issues related to the statistical analyses that should be addressed before acceptance. When comparing gut bacterial composition, the authors used PERMANOVA with both unweighted UniFrac and Bray–Curtis distances. The results indicated a stronger "separation" when using unweighted UniFrac distances, including a possible interaction between termite species and forest type for fungi. Nevertheless, the PERMDISP analyses for bacteria revealed a significant heterogeneity among termite species, indicating that groups differ in dispersion rather than (or not necessarily in) centroid location. In this case, an alternative statistical approach should be used (see Wang, Y., U. Naumann, S. T. Wright & D. I. Warton, 2012. mvabund: an R package for model-based analysis of multivariate abundance data. Methods in Ecology and Evolution 3: 471–474).

Response: Thank you for your valuable suggestions regarding the statistical analyses in our manuscript. We have carefully considered your concerns and followed the recommended approach to re-analyze the data accordingly.

Accordingly, we have updated the "Data analysis for termite gut microbiomes" section to include a detailed description of this approach (line 209-216), and we have revised the "Results" section to reflect the outcomes of the mvabund analysis (line 322-325).

It would be useful to add a heatmap highlighting the most important CAZymes across termite species and forest types. The heatmaps currently shown in the manuscript (Figure 5) are difficult to read.

Response: We have thoroughly revised Figure 5 and added new supplementary figure S8 to address the readability issues you identified. The revised Figure 5 now provides a more accessible and informative overview of the key CAZyme patterns driving differences among termite species and forest types.