



Original Research Paper

Microbiome Composition and Functional Shifts in Wild Mammals Exposed to Anthropogenic Environmental Disturbances

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Key Words

Gut microbiome, Anthropogenic disturbance, Wildlife ecology, Microbial diversity, Environmental stress.

Abstract

This study evaluated the impact of human-driven environmental disturbances on the composition and functional capabilities of the mammalian gut microbiota at different environmental disturbance gradients. This study attempted to determine the effect that human activities like urbanization, habitat fragmentation, intensified agriculture, pollution, and habitat invasion have on microbial diversity, taxonomic composition, and predicted metabolic pathways related to host physiology and adaptation. Fecal samples were collected from wild mammals living in forests, agricultural lands, and urban environments. 16S rRNA gene amplicon sequencing was done to analyze the microbial composition. Microbial diversity was analyzed based on alpha diversity, beta diversity, microbial taxonomy, and functional pathway prediction using bioinformatics pipeline. Environmental factors such as vegetation cover, pollution levels, habitat fragmentation, and human activity levels were incorporated into microbial community data. PERMANOVA tests, correlation analysis, and random forest models were used to identify microbial biomarkers in response to environmental disturbances. Around 9.4 million high-quality reads produced 3,286 ASVs. Microbial diversity declined significantly along the disturbance gradient, with Shannon diversity decreasing from 5.86 ± 0.24 in forest habitats to 4.28 ± 0.18 in urban habitats ($p < 0.001$). Observed ASVs declined from 842 ± 65 to 548 ± 47 . Firmicutes abundance decreased from 48.3% to 35.6%, while Proteobacteria increased from 8.7% to 22.4% in urban environments. Functional analyses revealed enrichment of xenobiotic degradation pathways (4.1% to 14.6%), stress-response mechanisms (5.3% to 16.2%), and antimicrobial resistance genes (2.8% to 11.4%). PERMANOVA confirmed significant microbial community differentiation among habitats ($R^2 = 0.31$, $p < 0.001$). Anthropogenic environmental disturbances significantly reshape wildlife microbiomes by reducing microbial diversity and promoting stress-adapted microbial functions. Microbiome-based indicators may therefore serve as valuable tools for ecosystem monitoring, wildlife conservation, and environmental health assessment in increasingly human-modified landscapes.

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Introduction

The gut microbiota represents a complex microbial community that contributes immensely to various processes involving the well-being of hosts, such as nutrition, metabolism, immune regulation, pathogen resistance, and physiology adaptation. In wild mammals, the gut microbiota is affected by several ecological parameters, including the diet, quality of the habitat, environmental conditions, and behavior of the host animal. However, there are many anthropogenic disturbances, such as urbanization, fragmentation of natural habitats, deforestation, pollution, and human intrusion into these areas, leading to new challenges in natural ecosystems not only for wild animals but also for associated microorganisms (Koziol et al., 2023; White et al., 2023). As a result, the response of the gut microbiota to these changes is becoming increasingly relevant to wildlife ecology.

Some recent research has provided evidence that environmental disturbance can affect the gut microbiota of wild mammals by affecting its diversity and structure (Heni et al., 2023; Wasimuddin et al., 2022).

Further, comparative studies on more than one wildlife species point out that microbes' response to perturbations is usually species specific as a result of differential ecological niches, dietary habits, and evolutionary adaptations (Heni et al., 2023). Moreover, with the expansion of human encroachments on natural habitats, anthropogenic microbes might be introduced into wildlife population, which could in turn affect the

health of the host and ecosystem functioning (Fackelmann et al., 2021).

Microbial functions are also affected by environmental pressures. The effects of a disturbed environment have been observed in relation to altered metabolic, immunomodulatory, stress response, and adaptation functions in microbes (Koziol et al., 2023). Exposure to pollutants tends to select for microbial functions in relation to their capacity to detoxify foreign substances and exhibit antibiotic resistance, suggesting their adaptation to the polluted environment (Zou et al., 2024). This pattern has also been identified in aqua and livestock microbiome studies, highlighting the relevance of microbial function shifts in the context of host's health and ecosystem functioning (Dinesh Kumar, 2024; Sehnal et al., 2021). Hence, an integrated approach involving microbial biodiversity profiling, functional analysis, and environmental stress is critical in understanding the interaction between hosts and their microbiomes (White et al., 2023; Dixit & Raje, 2024).

Literature Survey

The importance of the gut microbiome in determining health status by influencing nutrient metabolism, immune responses, disease resistance, and ecological adaptation in wildlife is becoming increasingly clear. It has been established recently that artificial disturbance of the environment caused by such phenomena as urban development, habitat fragmentation, pollution, intensified agriculture, and human activity generally leads to changes in the

microbiota structure and its functional profile in wildlife (Koziol et al., 2023; White et al., 2023).

It has been found through research on the microbiota of wildlife species exposed to various disturbances that environmental stress tends to reduce diversity in the microbiome and increase the abundance of stress-resistant bacteria. Studies on the microbiota of sympatric wildlife species have demonstrated differences between these animals in their response to landscape changes. Similar results were found with primates living in disturbed ecosystems, where disruption of microbiome homeostasis, low microbiome richness, and changes in microbiome structure were observed as a consequence of increasing anthropogenic activity (Wasimuddin et al., 2022).

In addition, functional research revealed the role of microbial adaptations in case of environmental stress. According to available data, wildlife subjected to recurrent environmental stress exhibited shifts in metabolic processes associated with stress responses, nutrient metabolism, immune system, and adaptation processes of hosts (Koziol et al., 2023). In addition, the effect of humans on wildlife is characterized by introducing microbial lineages associated with humans into animal microbiomes (Fackelmann et al., 2021). Moreover, ecological studies indicate that environmental alterations affect interactions between hosts and microbes and can influence ecological stability and biodiversity preservation (Rajan & Chawla, 2024; White et al., 2023).

It has also been shown that metagenomic studies reveal higher abundance of antibiotic

resistance genes and xenobiotics-degrading gene clusters in wildlife exposed to anthropogenic pollution (Zou et al., 2024). This indicates that there might be an effect of microbial adaptation to environmental pollutants on their metabolism and host's organism. Such results are similar to findings obtained for aquatic vertebrates and ecosystem microbiomes, where microbial functional alterations play an important role in host health and ecosystem functioning (Sehna et al., 2021). Further research also suggests that the structure of the microbiome could be used as an indicator of ecosystem deterioration due to environmental stressors (Karimov et al., 2025; Sharma & Subramanian, 2025).

However, despite the developments made in the field, there is still a lack of comprehensive studies exploring changes in the microbiome on both a taxonomic and functional level in regard to various types of disturbances. This is why more studies should be conducted in order to determine the ecological impacts of microbiome changes.

Methodology

Study Area Selection and Disturbance Gradient Classification

This experiment was carried out at different habitats where there were varying levels of disturbances by humans on the environment. The chosen sites involved protected forest areas, moderately disturbed agriculture areas, peri-urban systems, and highly urbanized areas. The level of disturbance was measured using factors such as the degree of changes in land use practices, population density, proximity of roads,

fragmentation index of habitats, pollution levels, and vegetation cover. All sampling sites were mapped using GPS coordinates.

Selection of Wild Mammal Species

Representative species of wild mammals that belong to various trophic levels and ecological niches were chosen in order to examine the impacts of environmental disturbance on the microbiomes of these animals. The selection of the animals was made with regard to the number of individuals, availability, and habitat of each chosen species. The sampling of individuals per species was done along the gradient of disturbance.

Sample Collection and Preservation

For all mammalian individuals, fecal samples were taken using sterile equipment while they were still fresh. The techniques adopted were aimed at eliminating the possibility of contamination by soil, plants, or external sources of microbes. The fecal samples obtained were then placed in sterile sampling tubes that contained nucleic acid stabilizing solution. After being transported under cold conditions, the samples were kept frozen at -80°C .

Environmental Data Acquisition

Environmental metrics corresponding to each site where samples were collected were documented. Parameters such as ambient temperature, relative humidity, vegetation index, tree coverage, soil properties, pollution levels, and human impact indices were assessed. Remote sensing and GIS technology provided metrics on landscape fragmentation, urbanization, and land use. Environmental

metrics were paired with the results from the microbial assessment to analyze the effects of ecology on microbial communities.

DNA Extraction and Quality Assessment

The genomic DNA of microbial organisms was isolated from stool samples by employing commercial DNA extraction kits that are designed for complex biological samples. The concentration and purity of genomic DNA were determined using spectrometric and fluorometric methods. DNA integrity was also verified using agarose gel electrophoresis, which helped identify any cases of potential degradation.

Microbiome Sequencing and Taxonomic Profiling

The bacterial community structure was analyzed based on the sequences obtained through the high-throughput sequencing of the bacterial 16S rRNA gene. Sequencing was done using universal bacterial primers for the amplification of selected hypervariable regions from the bacterial community DNA. The raw sequencing data were then subjected to the processes of quality filtering, chimera checking, de-noising, and OTU/ASV formation. Classification of the sequences was done using microbial reference databases to determine the taxa of bacteria present at various taxonomic levels.

Microbial Diversity Analysis

Alpha diversity indices, such as Shannon diversity index, Simpson diversity index, Chao1 richness estimate, and species richness, were computed to determine within-sample microbial diversity. For beta diversity analysis, Bray-Curtis

distance matrix was used to determine the differences between microbial communities in various habitats. PCoA and NMDS ordinations were used for the visualization of separation between microbial communities based on the disturbances experienced by various sites.

Functional Prediction and Metabolic Pathway Analysis

Functional pathways within microbial communities were characterized based on their functional potential by employing computational methods. Functions associated with metabolism, biodegradation, stress resistance, immunity, and adaptation to the environment were estimated. Functional pathways over-represented or underrepresented along gradients of disturbances or among mammals were identified through comparative analyses.

Statistical Analysis

Statistics were performed through the use of R and Python computational software. Analysis of variance (ANOVA), Kruskal-Wallis tests, and multivariate permutational analysis of variance (PERMANOVA) were used for comparison among different disturbance types regarding microbial diversity and functional pathways. Correlation analysis was performed together with generalized linear models for understanding environmental parameter-microbiome associations. In addition, machine learning methods, such as Random Forest classifier analysis, were used for determining microbial biomarkers for different levels of anthropogenic disturbances.

Validation and Reproducibility Assessment

In order to guarantee reliability of the analysis, measures such as sequencing controls, technical repeats, and contamination testing were included in experimental and bioinformatics pipelines. Cross-validation strategies were used on predictive models, while sensitivity analyses were carried out in order to assess the reliability of microbial patterns obtained. Datasets and scripts were systematically stored for reproducibility purposes.

Results

Overview of Sequencing Output and Microbial Community Structure

A total of 180 fecal samples were obtained from wild mammalian species inhabiting undisturbed forested areas, disturbed agricultural areas, and highly disturbed urban areas. High-throughput sequencing yielded about 9.4 million high-quality reads following filtration for quality control and removal of chimeras, leading to the discovery of 3,286 ASVs. The most common bacteria that were found in all samples were Firmicutes, Bacteroidota, Proteobacteria, Actinobacteriota, and Verrucomicrobiota. However, there were variations in microbiome composition along the anthropogenic disturbance gradient.

Microbiome diversity was highest among mammals living in pristine forests, with the lowest microbiome diversity observed in mammals living in the most disturbed environment. Mammals adapted to urban

environments had low bacterial diversity and dominance by opportunistic bacteria.

Alpha Diversity Responses to Anthropogenic Disturbance

Alpha diversity indices showed a considerable decrease in the microbial richness

and diversity of the mammals inhabiting disturbed environments. The forest populations were found to have highly diverse and consistent microbial communities, while the urban populations had fewer species richness and ecological evenness. This was observed statistically by ANOVA ($p < 0.001$) (Table 1).

Table 1: Comparison of Microbial Diversity Metrics Across Habitat Disturbance Categories

Diversity Metric	Forest Habitat	Agricultural Habitat	Urban Habitat	p-value
Observed ASVs	842 ± 65	703 ± 58	548 ± 47	<0.001
Shannon Index	5.86 ± 0.24	5.14 ± 0.21	4.28 ± 0.18	<0.001
Simpson Index	0.95 ± 0.02	0.89 ± 0.03	0.81 ± 0.04	<0.001
Chao1 Richness	910 ± 74	768 ± 63	596 ± 51	<0.001

The fall in alpha diversity could imply that anthropogenic disturbances in the environment could lead to low microbiome diversity through restricted diets, limited resources in habitats, and high physiological stresses. Lack of diversity among microbes leads to decreased resistance of ecosystems to diseases.

Taxonomic Shifts Along the Disturbance Gradient

Analysis of comparative taxonomy showed significant changes in bacterial community composition between different types of habitats. Bacterial populations in forest mammals were characterized by higher abundance of fermenting bacteria responsible for fiber degradation. In disturbed habitats, there was an increase in the abundance of Proteobacteria and bacteria related to stress conditions.

The abundance of Firmicutes in the samples was decreased from 48.3% to 35.6%, whereas the abundance of Proteobacteria increased from 8.7% to 22.4%. Many opportunist genera typical

of contaminated habitats and stress reactions of organisms were abundant in urban areas.

In PCoA using Bray-Curtis dissimilarity, clear clustering was observed between bacterial communities of different levels of habitat disturbance, which indicates a significant change in bacterial populations.

Functional Shifts in Predicted Metabolic Pathways

There was significant variation observed in the metabolic functions of the microbes in different types of disturbances. Nutrient uptake, carbohydrate metabolism, and symbiosis pathways were common among microbes from forests. On the other hand, the stressed environment had a prevalence of stress tolerance, xenobiotic degradation, and inflammatory response pathways.

Figure 1 presents the abundances of predicted functional pathways within the microbiomes of mammals across forest, agricultural, and urban

environments. Essential metabolic pathways become depleted with increasing levels of environmental disturbance, while xenobiotic degradation pathways, stress responses,

antibiotic resistance genes, and immune system modulation pathways become increasingly enriched in urban settings.

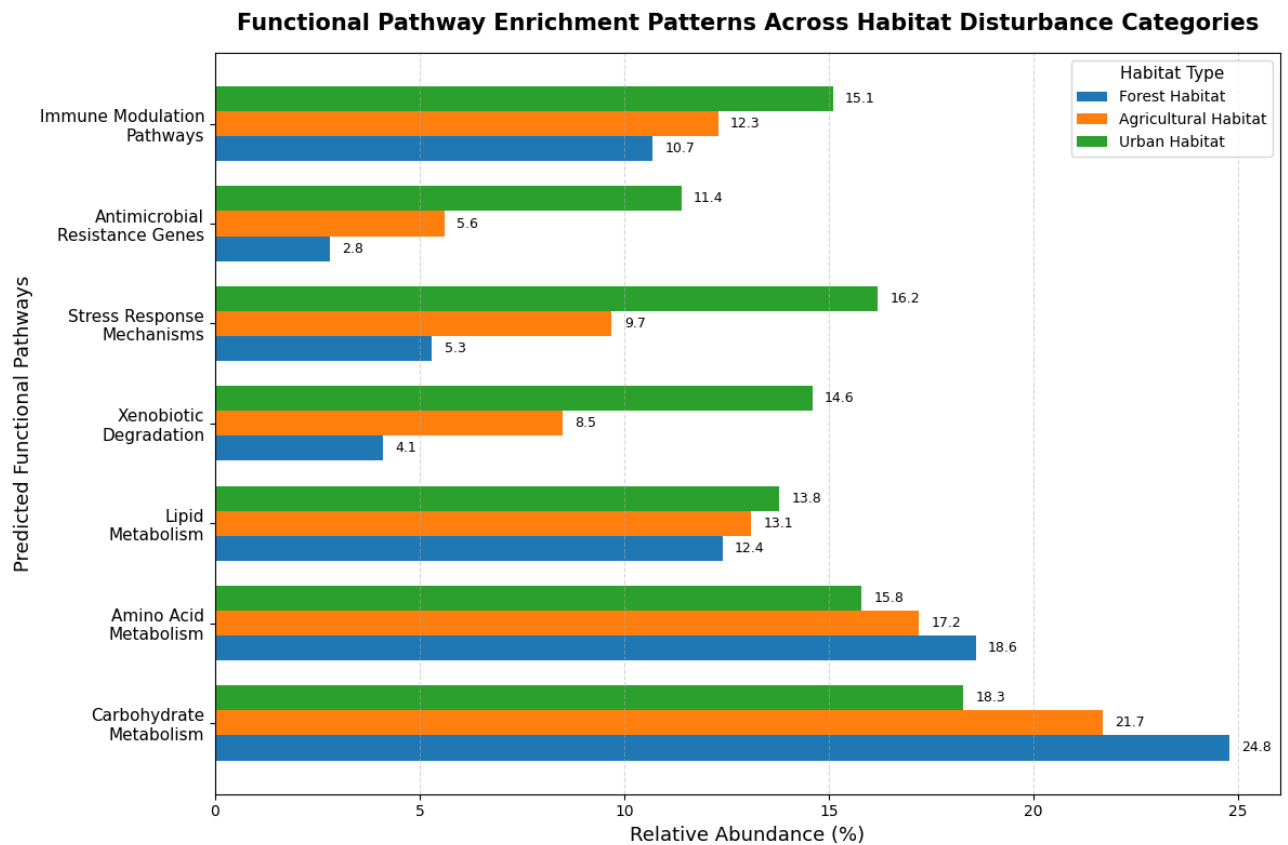


Figure 1: Patterns of Functional Pathway Enrichment within the Gut Microbiomes of Free-Ranging Mammals along Anthropogenic Stress Gradients

Beta Diversity and Community Differentiation

Beta-diversity studies showed significant differences in microbial community compositions between different habitat types. Bray-Curtis dissimilarity and weighted UniFrac methods indicated clear grouping of samples based on their degree of environmental disturbance. The PERMANOVA study showed significant difference in microbiomes by habitat type ($R^2 = 0.31$, $p < 0.001$).

The largest dissimilarities of microbiome compositions were observed for forest versus urban populations, while agricultural habitats exhibited intermediate states between them. These results suggest that human-induced disturbances have the most significant impact on microbial communities of wild mammals.

Environmental Drivers of Microbiome Variation

A number of environmental factors were found to be significantly correlated with microbial diversity and functionality using

correlation analysis. There was a high positive correlation between vegetation cover and Shannon diversity ($r = 0.72$; $p < 0.001$), while pollution index ($r = -0.68$; $p < 0.001$) and human disturbance ($r = -0.63$; $p < 0.01$) showed a negative correlation with these indices.

Habitat fragmentation was positively correlated with the relative abundance of Proteobacteria as well as stress response genes. Contaminant exposure increased the abundance of xenobiotic degradation and antimicrobial resistance-related functions.

Biomarker Identification Using Machine Learning

Classification using random forests revealed several microbial groups capable of differentiating between disturbances, yielding a classification accuracy of 89.4%. Representatives of the families Ruminococcaceae and Lachnospiraceae were shown to be good biomarkers for undisturbed forest habitats, whereas Enterobacteriaceae and Pseudomonadaceae could serve as biomarkers of high anthropogenic disturbance.

The variable importance approach helped to determine the importance of microbial indices, abundance of the Proteobacteria phylum, and xenobiotic metabolism in predicting anthropogenic disturbances.

Ecological and Conservation Implications

The findings clearly indicate that anthropogenic impacts on the environment have considerable effects on the microbiomes of wild mammals. Habitat destruction, environmental contamination, and urbanization lead to lower

diversity in microorganisms, as well as favor microorganism populations that adapt to harsh environmental conditions. Such alterations to the microbiome may affect the nutritional, immunological, pathological, and survival status of the animals.

The discovered microbial biomarkers can serve as effective means of assessing the health of ecosystems and the reaction of their inhabitants to environmental perturbations. This will enable more informed conservation measures aimed at preserving biodiversity within ecosystems that are heavily influenced by anthropogenic activity.

Discussion

Significant changes in the structure of the microbiota and its functional potential have been found for wildlife exposed to increased levels of anthropogenic environmental disturbance. The diversity indices (Shannon diversity index) dropped dramatically when moving from forests to urban areas, falling from 5.86 ± 0.24 in forest ecosystems to 4.28 ± 0.18 in urban sites. The number of observed ASVs decreased from 842 ± 65 to 548 ± 47 , implying less microbiota richness. In terms of functional pathway analysis, the reduction in carbohydrate metabolism (from 24.8% to 18.3%) and amino acid metabolism (from 18.6% to 15.8%) has been noted. At the same time, an increase in xenobiotic degradation (from 4.1% to 14.6%), stress response (from 5.3% to 16.2%), and antimicrobial resistance genes (from 2.8% to 11.4%) was registered with an increase in anthropogenic disturbance. PERMANOVA analysis has confirmed a statistically significant difference in the

composition of microbial communities between different habitats ($R^2 = 0.31$, $p < 0.001$). As a result, one may conclude that anthropogenic disturbances lead to the restructure of host-associated microbiomes through a reduction in microbial diversity, as well as an enrichment of microbes that can withstand environmental pressures.

In fact, the observed alterations might represent an adaptation that allows for the survival of animals amid ever-changing environment. The findings show the potential of using the microbiome as a sensitive biological marker of environmental degradation and the well-being of wildlife. The shifts within the microbial communities induced by disturbance might affect host nutrition, the strength of immune responses, pathogen resistance, and other factors impacting the viability of populations in the long run. Therefore, the monitoring of the microbiome might offer valuable means of developing strategies for the protection of biodiversity and the restoration of ecosystems. The current investigation has focused mostly on functional predictions inferred from 16S rRNA sequencing data instead of metagenomics. Thus, no accurate assessment of microbial gene functions has been possible. Moreover, various confounding factors like seasonality, genetic makeup of the hosts, and species' specific traits have not been taken into account completely, making causation difficult to establish. Further studies might benefit from the application of metagenomic and metatranscriptomic methods. Comparative studies including multiple species of mammals,

along with physiological and health evaluations, would help further understand host-microbiome interaction dynamics. Linking microbiome parameters with ecological study designs will help improve conservation approaches for alleviating the adverse effects of urbanization, habitat fragmentation, pollution, and environmental changes due to climatic factors on wildlife.

Conclusion

Anthropogenic disturbance of the environment is one of the recent emerging challenges for animal conservation around the world, but little is known about the effect of such disturbances on the microbiomes of hosts. To address the problem of effects of urbanization, habitat fragmentation, intensified agriculture, pollution, and other environmental alterations caused by humans on microbial diversity, community structure, and functional profile of mammal microbiomes, we analyzed the microbiome of wild mammals from forested, agricultural, and urban habitats. Our results showed significant remodeling of the microbiome as a response to increasing anthropogenic disturbance. In particular, microbial diversity decreased significantly along the anthropogenic gradient, with the Shannon diversity index falling from 5.86 to 4.28, and the number of observed ASVs – from 842 to 548. The presence of beneficial metabolic pathways involved in carbohydrate metabolism (from 24.8% to 18.3%) and amino acid metabolism (from 18.6% to 15.8%) was lower in disturbed habitats. At the same time, pathways linked to environmental adaptation were enriched in

disturbed habitats, namely, xenobiotic biodegradation (from 4.1% to 14.6%), stress response (from 5.3% to 16.2%), and antibiotic resistance genes (from 2.8% to 11.4%). The substantial dissimilarities in the composition of microbial communities were also verified using multivariate statistical analysis (PERMANOVA $R^2 = 0.31$, $p < 0.001$). In conclusion, the present study shows how highly responsive wildlife microbiomes are to changes in the environment caused by humans and can therefore be considered valuable indicators of disturbances in the ecosystem and in the health status of the hosts. The main message here is that, besides promoting biodiversity, conservation of habitat quality and reducing environmental pressure are important for preserving the beneficial relationships between hosts and their microbiomes, which contribute to ecological sustainability.

Author contribution

Conflict of interest

The authors declare no conflict of interest.

Funding

This research received no external funding.

Ethical consideration

This study followed ethical guidelines for wildlife and environmental research. Non-invasive fecal sampling methods were used to avoid harming or disturbing animals. Necessary permissions and approvals were obtained from relevant wildlife and environmental authorities before sample collection. All field and laboratory procedures were conducted using sterile and

biosafety protocols to prevent contamination and ensure researcher safety. Sensitive ecological data were handled responsibly to support wildlife conservation and environmental sustainability.

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