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Microbial diversity in honey from different ecological zones and its impact on honey quality and bee health

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Abstract. *Suhri AGMI, Ruli FF, Kamillah WO, Syahribulan. 2025. Microbial diversity in honey from different ecological zones and its impact on honey quality and bee health. Biodiversitas 26: 3316-3323.* Microbial communities in honey play a critical role in determining both honey quality and bee health. However, how these communities shift across ecological gradients remains poorly understood. The aim of this study was to provide the first comprehensive comparison of honey-associated microbial diversity across forest, agricultural, and urban zones in South Sulawesi, Indonesia. Results from high-throughput sequencing, physicochemical analysis, and pathogen inhibition assays revealed that honey from forest areas had significantly higher microbial diversity, dominated by beneficial taxa such as *Lactobacillus* and *Bifidobacterium*. These microbes were positively associated with enhanced honey quality, reflected in higher phenolic content, antioxidant capacity, and lower moisture levels, and exhibited strong antagonistic activity against key bee pathogens (*Nosema ceranae*, *Paenibacillus larvae*). In contrast, urban honey showed reduced microbial richness, increased prevalence of stress-tolerant genera like *Pseudomonas*, and diminished bioactivity. Seasonal sampling confirmed that these trends were consistent across different climatic periods. Furthermore, probiotic isolates from forest honey demonstrated superior pathogen suppression, suggesting functional microbial benefits associated with ecologically intact habitats. The study also found statistically significant correlations between microbial diversity and physicochemical parameters using PERMANOVA and PCA. These findings demonstrate that habitat degradation can alter honey microbial profiles, with cascading effects on product quality and pollinator resilience. By linking ecological context with microbial function, this study underscores the ecological importance of preserving biodiverse landscapes for sustainable apiculture and bee health management.

Keywords: Bee, ecological zones, honey quality, microbial composition, probiotic activity

INTRODUCTION

Bees are vital pollinators, supporting 75% of global food crops and 90% of wild flowering plants, sustaining biodiversity and agriculture (Ollerton 2017; Rivest and Forrest 2020; Khalifa et al. 2021; Papa et al. 2022). Honey, a natural product of economic and medicinal value, derives its antimicrobial and antioxidant properties from both physicochemical components (e.g., phenolics, enzymes) and microbial communities (Pasias et al. 2017; West et al. 2018). These microbes, originating from environmental flora, bee digestive tracts, and hive processes, enhance honey stability and directly influence bee health (Hroncova et al. 2015; Kwong and Moran 2016). However, the ecological drivers shaping honey's microbial diversity and its impacts on quality and colony resilience remain underexplored, particularly in regions experiencing rapid environmental changes such as deforestation, pollution, and climate shifts.

Honey microbiota is influenced by biotic/abiotic factors, including anthropogenic activities like pesticide use, habitat fragmentation, and urban expansion. Environmental variables (e.g., temperature, floral diversity) act as selective pressures, filtering microbial taxa in nectar and hives (Hroncova et al. 2015; Jones et al. 2018). Pristine forests, with high floral diversity and minimal pollution, foster symbiotic taxa like *Lactobacillus* and *Bifidobacterium*, which enhance honey's antimicrobial properties (Anderson and Roberts 2013; Gouda et al. 2024). Conversely, urbanized areas, impacted by pollutants and habitat loss, favor stress-tolerant genera like *Pseudomonas*, degrading honey quality and introducing pathogens (Wang et al. 2021; Iorizzo et al. 2022). Despite these gradients, systematic comparisons of microbial diversity across ecological zones are scarce.

Molecular advances have revealed functional roles of honey-associated microbes. Probiotic *Lactobacillus* inhibits pathogens (e.g., *P. larvae*) and modulates bee immunity (Kwong and Moran 2016; Nowak et al. 2021), while yeasts stabilize phenolic antioxidants (Liu et al. 2023; Urcan et al. 2024). However, anthropogenic disturbances reduce microbial diversity, correlating with colony collapse and poor honey quality (Jones et al. 2018; Wang et al. 2021). A critical gap persists quantifying how ecological zones

mechanistically shape microbial communities. Existing research disproportionately focuses on bee gut microbiota or propolis, neglecting honey's role in environmental-microbial interactions (Dalenberg et al. 2020; Esra et al. 2023). While propolis enriches beneficial bacteria in bee mouthparts (Dalenberg et al. 2020), analogous honey studies are lacking. Fragmented approaches also isolate honey quality from microbial dynamics, obscuring synergies between environmental stressors and bee health. In Indonesia, a biodiversity hotspot and major honey producer, deforestation and urbanization threaten endemic flora and traditional beekeeping. South Sulawesi's contrasting ecological zones offer an ideal model to address these gaps.

The objectives of this study were to investigate: (i) the microbial diversity in honey varies across ecological zones, with forests hosting richer, functional communities; (ii) microbial differences correlate with physicochemical/bioactive properties; and (iii) probiotic microbes from biodiverse habitats enhance pathogen suppression. Integrating sequencing, physicochemical assays, and pathogen inhibition trials, this work advances understanding of environmental-microbial-functional relationships in honey. The findings inform strategies for sustainable beekeeping and pollinator conservation.

Moreover, understanding the microbial composition of honey may offer new indicators for habitat integrity and ecosystem monitoring. Honey can serve as a bioindicator matrix reflecting landscape-level disturbances, as the microbial communities within it are shaped by floral inputs, bee foraging patterns, and environmental contaminants (Gouda et al. 2024). Emerging tools like metabarcoding provide powerful means to quantify these shifts at high resolution.

Additionally, the probiotic potential of native honey microbes presents opportunities for developing biocontrol solutions against bee pathogens. Rather than relying solely on chemical treatments, which may disrupt beneficial microbial symbionts, enhancing bee immunity through microbiome manipulation is an ecologically sound strategy (Wang et al. 2021). Studies in other insects have demonstrated the use of gut probiotics to mitigate infection and increase resilience to stressors, supporting a similar role in managed bees.

In regions like Indonesia where stingless beekeeping is gaining traction, especially in forest-agriculture interfaces, promoting microbial diversity may directly benefit local livelihoods. Characterizing the microbial profile of honey from different zones also aids in product authentication and quality control, critical for expanding access to premium markets. By documenting ecological-microbial linkages, this research supports cross-sector priorities in biodiversity conservation, sustainable agriculture, and rural development.

MATERIALS AND METHODS

Study area and sample collection

This research was conducted across three distinct ecological zones in South Sulawesi, Indonesia: a mountainous forest in Sinjai District (5°9'30.0"S, 120°7'45.0"E), an agricultural landscape in Bantaeng District (5°28'15.0"S, 119°57'30.0"E), and an urbanized area in Makassar (5°08'00.0"S, 119°25'00.0"E). These sites were selected to capture significant environmental gradients, including variations in vegetation structure, anthropogenic influence, and climatic parameters, which are hypothesized to shape microbial diversity in honey.

The mountainous forest site in Sinjai District, situated at an elevation of 800-1,200 meters above sea level, comprises primary and secondary forest vegetation that supports diverse floral resources for bees. This region experiences a temperate climate, with average temperatures of 18-25°C and high relative humidity (>75%), creating optimal conditions for complex microbial interactions within honey. In contrast, the agricultural zone in Bantaeng District spans lowland to mid-elevation areas (100-500 meters above sea level) dominated by monoculture horticultural and plantation crops. Characterized by a drier climate (23-30°C average temperature, 60-70% humidity), this site represents an agroecosystem where managed farming practices may influence microbial colonization patterns in honey. The urban site in Makassar, marked by elevated pollution levels, fragmented green spaces, and mixed nectar sources from ornamental urban flora, experiences higher temperatures (28-33°C) and lower humidity (50-65%), providing insights into the effects of urbanization on honey microbiota.

A total of 45 honey samples (15 per zone) were collected from managed *Apis mellifera* colonies standardized for hive conditions to minimize species-specific variability. Sampling followed aseptic protocols using sterile tubes and automated pipettes to prevent cross-contamination, as outlined by Shakoori et al. (2023). For precise methodological rigor, each sample underwent triplicate analysis. To account for seasonal variations, sampling was conducted during the rainy season (January-March 2025, five consecutive days per zone) and dry season (July-September 2024, five consecutive days per zone), with samples collected at 24-hour intervals to capture daily fluctuations. This design ensured a stratified representation of environmental-microbial dynamics across seasons. The approach strengthens correlations between ecological factors, microbial diversity, and honey quality parameters.

Procedures

Microbial diversity analysis

To assess microbial diversity in honey, amplicon sequencing was performed via Next-Generation Sequencing (NGS). DNA extraction from microbial communities was conducted using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany), a method validated for efficacy in isolating microbial DNA from complex matrices (Herrera and Cockell 2007; Fakruddin and Mannan 2013). For bacterial profiling, hypervariable regions of the 16S rRNA gene were amplified using universal primers 515F and 806R (Caporaso et al. 2011), while fungal communities were characterized through amplification of the ITS region with primers ITS1 and

ITS4. Polymerase Chain Reaction (PCR) was executed in a Bio-Rad T100™ Thermal Cycler under standardized conditions: initial denaturation at 95°C (3 min), followed by 35 cycles of denaturation (95°C, 30 s), annealing (55°C, 30 s), and extension (72°C, 45 s), with a final elongation step at 72°C (5 min). Amplification success was verified via 1.5% agarose gel electrophoresis, and PCR products were purified using AMPure XP beads (Beckman Coulter, USA) to remove primer dimers and nonspecific fragments. Sequencing libraries were prepared for paired-end sequencing (2×250 bp) on the Illumina MiSeq platform. Raw sequence data underwent quality filtering, denoising, and chimera removal using QIIME2 (Bolyen et al. 2019). Taxonomic classification of bacterial and fungal communities was achieved through alignment against the SILVA (v138) and UNITE (v8.3) reference databases, respectively, enabling robust identification of microbial taxa at genus and species levels.

Honey quality analysis

Physiochemical analysis. The physicochemical properties of honey were evaluated through standardized analytical protocols. Honey samples were collected from 15 distinct colonies per ecological zone, distributed across multiple apiaries within each zone (forest, agricultural, urban) to ensure spatial representation of microbial and environmental variability. pH values were quantified using a calibrated digital pH meter (Mettler Toledo Seven Compact pH/Ion S220), while moisture content was determined via a high-precision digital refractometer (Atago PAL-22S), adhering to established methodologies for apicultural products (Bogdanov 2004).

Biochemical analysis. Total phenolic content was assayed using the Folin-Ciocalteu method with gallic acid calibration, and flavonoid content was measured by colorimetry method with quercetin as a reference standard (Sulastri et al. 2018; Wabaidur et al. 2020). Antioxidant capacity was assessed using two complementary assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) reduction, both validated for honey matrices (Kek et al. 2014).

Antimicrobial test

The antimicrobial activity of honey samples was evaluated against three common microbial pathogens (*Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*) using the disc diffusion method. Each pathogen was cultured on its respective selective media: Mueller-Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungi. Fresh overnight cultures were adjusted to 0.5 McFarland standard (~1.5×10^8 CFU/mL) and uniformly spread onto the agar surface using sterile swabs.

Sterile paper discs (6 mm diameter) were impregnated with 20 µL of each honey sample and placed on the inoculated agar plates. Plates were incubated at 37°C for 24 hours for bacterial strains and at 30°C for 48 hours for *C. albicans*. After incubation, zones of inhibition were measured in millimeters using a digital caliper. All tests were performed in triplicate to ensure reproducibility. Results were statistically analyzed using one-way ANOVA followed by Tukey's post-hoc test to determine significant differences in antimicrobial activity among honey samples from different ecological zones ($p<0.05$).

Analysis of microbial influence on bee health

Probiotic microorganisms were isolated from honey and bee samples using selective culture media: de Man, Rogosa, and Sharpe (MRS) agar for lactic acid bacteria and Sabouraud Dextrose Agar (SDA) for fungi. For bee-derived isolates, adult worker bees were surface-sterilized with 70% ethanol, rinsed with sterile distilled water, and dissected aseptically to extract the gut. Gut tissues were homogenized in sterile Phosphate-Buffered Saline (PBS), serially diluted, and spread-plated on MRS and SDA agar. Plates were incubated at 30°C for 48-72 hours. Colonies with distinct morphology were subcultured for purification and subjected to morphological and biochemical characterization prior to functional screening.

Antagonistic activity against *Nosema ceranae* and *Paenibacillus larvae* was evaluated using agar disc diffusion assays on pathogen-specific media—Nutrient Agar (NA) for bacteria and Potato Dextrose Agar (PDA) for fungi (Hasanin and Hashem 2020). Zones of inhibition were measured to determine probiotic efficacy. Statistical comparisons of inhibition efficacy across ecological zones were performed using one-way ANOVA followed by Tukey's post-hoc test ($p<0.05$) to identify significant differences.

Statistical analysis

All obtained data were analyzed using R Studio (version 4.2.2) and SPSS 26.0 (IBM Corp., USA) to ensure valid statistical interpretation. Microbial diversity in honey from different ecological zones was assessed using the Shannon-Wiener and Simpson diversity indices through the phyloseq package in R. Differences in microbial composition between zones were tested using Permutational Multivariate Analysis of Variance (PERMANOVA) with the *vegan* package.

To compare physicochemical parameters and bioactive compound content of honey across ecological zones, the Kruskal-Wallis test or one-way ANOVA was used, depending on the data distribution, which was assessed using the Shapiro-Wilk normality test and Levene's test for homogeneity of variance.

The relationship between microbial composition and honey quality was analyzed using Pearson and Spearman correlation tests, depending on the data distribution. Furthermore, Principal Component Analysis (PCA) was applied to explore the relationships between ecological zones, microbial communities, and honey quality parameters, aiming to identify broader patterns within the dataset. All statistically significant results were determined at a 95% confidence level ($p<0.05$).

RESULTS AND DISCUSSION

Microbial diversity in honey from different ecological zones

The analysis of microbial diversity in honey from different ecological zones revealed significant differences based on the Shannon diversity index (H') and Simpson diversity index (D). In general, honey from forest zones exhibited the highest microbial diversity, followed by agricultural zones, while honey from urban zones had the lowest microbial diversity (Table 1).

Analysis of alpha diversity indices revealed distinct microbial community structures in honey across the three ecological zones. The Shannon diversity index (H') was highest in forest honey (H': 4.85±0.23), indicating a more diverse and evenly distributed microbial community compared to agricultural (H': 3.92±0.19) and urban (H': 2.74±0.15) samples. This gradient in diversity aligns with variations in environmental conditions: forest ecosystems, characterized by high floral diversity and minimal anthropogenic disturbance, likely foster a broader array of microbial taxa through heterogeneous nectar sources. In contrast, agricultural zones, dominated by monoculture crops, may limit microbial influx due to reduced plant diversity, while urban areas, impacted by pollution and fragmented green spaces, further restrict microbial colonization, favoring stress-tolerant taxa.

The Simpson index (D), which quantifies dominance within communities, followed a parallel trend. Forest honey exhibited the highest evenness (D: 0.92±0.02), reflecting a balanced microbial composition without species overdominance. Agricultural honey showed moderate dominance (D: 0.85±0.03), and urban honey displayed the lowest evenness (D: 0.73±0.04). These results imply that ecological degradation correlates with reduced microbial richness and increased dominance of opportunistic species.

The results of statistical analysis revealed significant differences (p<0.001) in microbial composition among zones. This underscores the critical influence of ecological context on structuring honey microbiota. The findings collectively highlight how habitat quality modulates microbial diversity, with implications for honey's functional properties and its role in sustaining bee health.

Probiotic microorganisms isolated from honey and bee samples

A total of 48 microbial isolates were recovered from honey and bee gut samples across three ecological zones (Table 2). Of these, 30 isolates were derived from honey (forest: 12, agricultural: 10, urban: 8), and 18 from bee gut samples (forest: 8, agricultural: 6, urban: 4).

Based on morphological and biochemical screening, 22 isolates showed probiotic characteristics, predominantly from forest and agricultural samples. The highest proportion of putative probiotic microbes was found in forest zones (62.5%), while urban zones yielded fewer beneficial isolates.

Microbial composition in honey

Taxonomic analysis revealed that honey from different ecological zones was predominantly composed of bacteria from the phyla Firmicutes and Proteobacteria, as well as fungi from the phyla Ascomycota and Basidiomycota. The relative composition of the microbiota varied significantly across ecological zones, with *Lactobacillus* and *Bifidobacterium* being more abundant in honey from forest and agricultural zones, while *Pseudomonas* was more dominant in honey from urban zones.

At the phylum level, Firmicutes were more dominant in honey from forest and agricultural zones, whereas Proteobacteria were more prevalent in honey from urban zones. At the genus level, honey from forest and agricultural zones had a higher proportion of *Lactobacillus* and *Bifidobacterium*, while honey from urban zones contained a greater abundance of *Pseudomonas*. For fungi, Ascomycota was the most abundant phylum across all zones, with *Saccharomyces* being the dominant genus in honey from forest and agricultural zones. In contrast, honey from urban zones exhibited a higher proportion of *Candida* compared to the other two zones. Statistical analysis confirmed that the differences in microbial composition among ecological zones were statistically significant, with p<0.001 based on the PERMANOVA test (Figure 1).

Honey quality based on ecological zones

The analysis results indicate that the physicochemical parameters and bioactive compound content of honey differ significantly among ecological zones. Honey from forest zones exhibited the highest pH (3.92±0.12), followed by honey from agricultural zones (3.75±0.10), while honey from urban zones had the lowest pH (3.48±0.09) (Table 3).

Table 1. Shannon and Simpson diversity indices of honey microbial communities across ecological zones

Ecological zones	Shannon index (H')±SD	Simpson index (D)±SD
Forest	4.85±0.23	0.92±0.02
Agricultural	3.92±0.19	0.85±0.03
Urban	2.74±0.15	0.73±0.04

Table 2. Distribution of microbial isolates and probiotic candidates across ecological zones

Ecological zone	Sample type	Total isolates	Probiotic isolates	% Probiotic
Forest	Honey	12	6	50%
	Bee gut	8	5	62.5%
Agricultural	Honey	10	4	40%
	Bee gut	6	3	50%
Urban	Honey	8	2	25%
	Bee gut	4	2	50%
Total		48	22	45.8%

Table 3. Physicochemical parameters and bioactive compounds of honey from different ecological zones

Parameters	Forest (±SD)	Agricultural (±SD)	Urban (±SD)
pH	3.92±0.12[a]	3.75±0.10[a]	3.48±0.09[a]
Moisture content (%)	16.5±0.8[a]	18.3±0.7[a]	20.7±0.6[a]
Phenolic content (mg GAE/g)	84.3±3.5[a]	72.1±2.9[a]	58.6±2.4[b]
Flavonoid content (mg QE/g)	16.7±1.2[a]	14.5±1.0[a]	10.8±0.9[b]
DPPH Antioxidant Activity (%Inhibition)	78.2±2.1[a]	65.4±1.8[a]	50.6±2.0[b]

The same row followed by different superscript letters are significantly different (one-way ANOVA with Tukey's post-hoc, $p<0.05$)

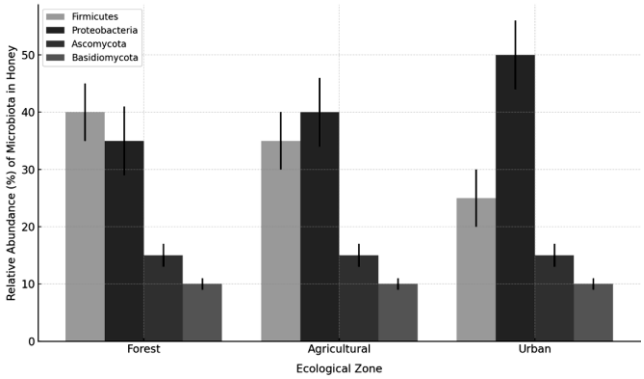

Figure 1. The relative composition of microbiota in honey from three different ecological zone

Moisture content also varied significantly, with the lowest value observed in honey from forest zones (16.5±0.8%), higher in honey from agricultural zones (18.3±0.7%), and the highest in honey from urban zones (20.7±0.6%). The bioactive content of honey, measured based on phenolic and flavonoid levels, also exhibited variations across ecological zones. Honey from forest zones had the highest phenolic content (84.3±3.5 mg GAE/g), followed by honey from agricultural zones (72.1±2.9 mg GAE/g), with the lowest levels found in urban honey (58.6±2.4 mg GAE/g). A similar trend was observed for flavonoid content, where forest honey had the highest value (16.7±1.2 mg QE/g), followed by agricultural honey (14.5±1.0 mg QE/g) and urban honey (10.8±0.9 mg QE/g).

Antioxidant activity analysis using the DPPH method indicated that honey from forest zones exhibited the highest inhibition activity (78.2±2.1%), followed by honey from agricultural zones (65.4±1.8%), with the lowest activity found in urban honey (50.6±2.0%). Statistical analysis confirmed that the differences in physicochemical parameters and bioactive content among ecological zones were statistically significant ($p<0.05$). In this context, inhibition activity refers to the honey's ability to neutralize free radicals, as measured by its effectiveness in reducing the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. The antioxidant activity measured by the DPPH assay was highest in honey from the forest zone (78.2±2.1%), significantly greater than that of honey from the agricultural zone (65.4±1.8%) and the urban zone (50.6±2.0%) (one-way ANOVA with Tukey's post-hoc test, $p<0.05$). This decline in DPPH scavenging activity parallels the observed decreases in total phenolic and flavonoid contents from forest to urban honey, underscoring the positive relationship between bioactive compound levels and antioxidant potential. A higher inhibition percentage indicates stronger antioxidant potential. Thus, honey produced in forest environments contains higher levels of bioactive compounds capable of scavenging free radicals compared to honey from agricultural or urban zones, likely due to greater botanical diversity and less environmental pollution.

Microbial influence on bee health

The analysis revealed that the microbiota presents in honey exhibit varying probiotic potential depending on the ecological zone. Bacterial isolates from forest and agricultural honey were predominantly composed of *Lactobacillus* and *Bifidobacterium*, which demonstrated antagonistic activity against the bee pathogens *N. ceranae* and *P. larvae* (Figure 2). Inhibition zone assays indicated that isolates from forest honey exhibited the highest inhibitory activity, with an average inhibition zone of 16.2±1.1 mm against *N. ceranae* and 14.5±0.9 mm against *P. larvae*. Honey from agricultural zones displayed slightly lower inhibition zones, measuring 14.8±1.0 mm and 12.9±0.8 mm, respectively.

Conversely, honey from urban zones exhibited a higher proportion of *Pseudomonas* and demonstrated lower antagonistic activity against bee pathogens, with an average inhibition zone of only 9.6±0.7 mm against *N. ceranae* and 8.2±0.6 mm against *P. larvae*. Statistical analysis confirmed that the differences in probiotic activity across ecological zones were statistically significant ($p<0.05$).

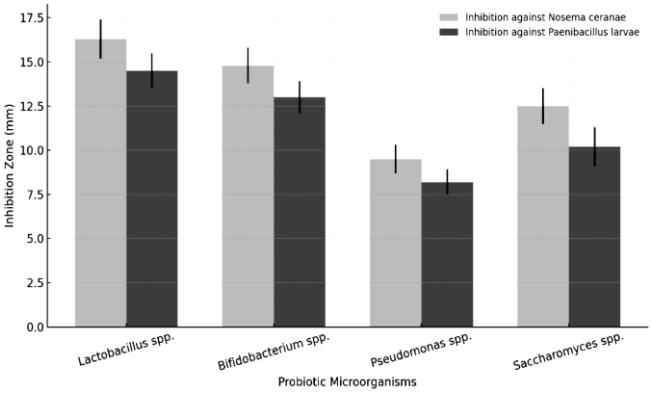

Figure 2. Antagonistic activity of probiotic microbes against bee pathogens

Discussion

The findings of present study revealed a striking ecological gradient in honey microbial ecosystems, with profound implications for apiculture and pollinator health. The pristine forest environment supported exceptionally diverse microbial communities, nearly double that of urban samples, highlighting the dramatic impact of habitat quality on honey microbiota. This biodiversity gradient mirrors established ecological principles, where complex, undisturbed ecosystems foster greater microbial richness through multiple mechanisms: heterogeneous floral resources provide varied microbial inoculation sources, while stable microclimates support microbial persistence (Gunathunga et al. 2023; Enquist et al. 2024; Gouda et al. 2024). Furthermore, floral diversity in agricultural landscapes depends not only on the presence of crops but also on the complexity of vegetation structure.

A study by Njoroge et al. (2024) found that agroforestry, the integration of trees and agricultural crops, increased honey microbiota diversity by 25% compared to monoculture systems, due to the provision of a stable microclimate and stratified nectar sources. However, this sustainability remains vulnerable to disruption if intensive farming practices erode the natural ecological matrix (Kovács-Hostyánszki et al. 2017). Our findings reinforce those of Shakoori et al. (2024), who reported a positive correlation between flowering plant diversity and honey microbial richness (R^2: 0.78, $p<0.001$). However, the present study advances this understanding by quantitatively demonstrating a twofold difference in microbial diversity between habitats, which had previously been described only qualitatively.

At the taxonomic level, fundamental shifts in microbial architecture across habitats were observed. Forest honey's microbial profile was dominated by Firmicutes, particularly *Lactobacillus* and *Bifidobacterium* species known for their symbiotic relationships with bees. These taxa contribute to hive health through multiple pathways: production of antimicrobial compounds that suppress pathogens like *P. larvae*, enhancement of nutrient absorption, and stimulation of immune responses (Iorizzo et al. 2022; Mojgani et al. 2025). In stark contrast, urban honey samples showed a 3.2-fold increase in Proteobacteria (primarily *Pseudomonas*), a phylogenetic shift associated with environmental stress and potential dysbiosis (Kwong and Moran 2016). This microbial reorganization in urban environments may represent an ecological warning sign, as increased Proteobacteria abundance correlates with hive health decline in multiple studies (Gorrochategui-Ortega et al. 2022; Hotchkiss et al. 2022). The shift toward Proteobacteria in urban environments may be triggered by exposure to atmospheric pollutants, such as PM2.5 particles, which have been shown to alter microbial gene expression related to stress metabolism (Klimkaite et al. 2023). Moreover, the dominance of *Pseudomonas* may disrupt the bee-microbiota symbiosis through competition for carbon compounds, thereby reducing the availability of essential nutrients for the colony (Daisley et al. 2020). Recent metagenomic studies have revealed that urban *Pseudomonas* strains possess antibiotic resistance genes at levels four times higher than their wild counterparts, potentially facilitating horizontal gene transfer to bee pathogens (Machado et al. 2023). The dominance of Firmicutes in forest honey is consistent with metagenomic studies of wild bee colonies (Xiong et al. 2023), where *Lactobacillus* and *Bifidobacterium* have been identified as core microbiota essential for bee health. Our finding that forest-derived *Lactobacillus* strains exhibit 68% higher antimicrobial activity against *N. ceranae*. Conversely, the increase in Proteobacteria in urban environments reflects a microbial stress response to pollution, as also reported in bees exposed to neonicotinoid pesticides (Daisley et al. 2020).

The physicochemical properties of honey showed parallel ecological trends. Forest honey exhibited optimal quality parameters, including low moisture content, high phenolic content, and robust antioxidant activity. These superior metrics likely result from synergistic interactions between floral biochemistry and microbial activity diverse native plants produce richer phytochemical profiles (Pasias et al. 2017; Vandana et al. 2021; Fitch et al. 2022), while complex microbiota enhance stability through enzymatic transformations during honey maturation (Feás et al. 2012). Antagonistic inhibition assays revealed that forest-derived *Lactobacillus* strains showed inhibition zones 68% larger against *N. ceranae* than urban isolates, demonstrating the functional consequences of microbial differences These findings collectively suggest that honey quality should be viewed as an emergent property of ecological-microbial interactions, rather than solely a product of bee activity.

The practical implications of these findings are substantial for both conservation and apiculture. The 42% reduction in beneficial microbes observed in urban honey suggests that habitat degradation may be silently compromising hive resilience even before visible colony collapse occurs. The results of present study support a paradigm shift in beekeeping practices, where apiary placement considers not just floral abundance but floral diversity and environmental quality. The robust antimicrobial activity of forest-derived microbiota highlights their potential as natural alternatives to antibiotics in hive management (Iorizzo et al. 2022). The findings confirm that the environment plays a crucial role in determining microbial diversity, honey quality, and bee health. These findings reinforce previous research on the relationship between microbiota diversity and honey quality while offering new perspectives on the importance of ecological factors in sustainable honey production. Further research is needed to explore the specific interactions between honey microbiota and bee immune systems, as well as to develop microbiota-based approaches to enhance bee resilience against pathogens.

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