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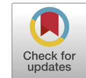

# Bacterial diversity in honey bee environment: Embu County, Kenya

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## ABSTRACT

In Kenya, small-scale farmers are increasingly venturing into honeybee keeping supplementing their income. However, cases of honeybee populations decline characterized by colony losses, hive absconding and migrating swarms have been documented, resulting in a decline in the number of colonized hives thus raising concerns about their sustainability. Honeybee and colony fitness is dependent on bacterial symbioses, and their disruption leads to disease susceptibility. To assess bacteria associated with honeybees in Kenya, we collected honeycombs, honey, adult worker bees and frame scraping samples from different agro-ecological zones within Embu County. To determine the bacterial composition we characterized the hive microbiota using the targeted metagenomic culture-independent 16S rRNA gene sequencing. Honeybee-associated bacteria community in the hive materials was dominated by *Lactobacillus, Leuconostoc, Fructobacillus, Bacillus, Gilliamella, Frischella, Enterobacter, Bombella* and *Serratia* across the sampling environment. In the sample types, adult worker bees lacked the *Lactococcus* genus but had the other phylotypes consistently similar to those in the larvae samples. Genus *Saccharibacter* however was absent in honeycomb and larvae samples. Genus *Lactococcus* was present in all the sample types except in the adult worker bee samples. The significant observation was the presence of genera *Serratia* and *Enterobacter*, opportunistic environmental bacteria. High levels of these bacteria in adult and larva samples may present a potential disruption of the microbial community and increase disease susceptibility. Honey production declines as a result of the weakened colony. Regular monitoring will be ideal in maintaining the colony health and thus respond to the growing global market and achieving the African union's Agenda 2063 on agricultural productivity and production.

*Abbreviations:* 16s rRNA, 16s ribosomal RNA; OTUs, operational taxonomic units; ACE, richness diversity; DNA, deoxyribonucleic acid; PBS, phosphate buffer saline solution; EDTA, ethylenediaminetetracetic acid; Trris—HCl, tris hydrochloride; NaCl, sodium chloride; 1% SDS, sodium dodecyl sulfate; CCS, circular consensus sequences; UCHIME, undetected chimeras; BLASTn, basic local alignment search tool; SSU, small subunit; QIIME, quantitative insights into microbial ecology; NMDS, non-metric multidimensional scaling; IFS, international foundation for scientists; Bp, base pair; DEPC, diethyl pyrocarbonate.

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## Introduction

Honeybees (*Apis mellifera*) are key players in the ecosystem and in many natural environments. Besides the production of honey and other hive products such as wax, royal jelly, venom, propolis, bee pollen and bee bread, honeybees also serve as pollinators of plants which are important in food production and ecological balance [1].

Beekeeping in Kenya plays a fundamental role not only in forested areas but also in arid and semi-arid regions, with most beekeepers relying on traditional knowledge and skills [2]. Further, the practice is divided into intensive and extensive systems, both relying on the managed and wild bees. Extensive system involves beekeeping in large areas far away from human populations, usually attracting wild swarms, while intensive system involves beekeeping on small to medium farms on agriculturally productive land [3]. Honeybees have become an increasingly popular livestock for small-scale Kenyan farmers to supplement their livelihoods in recent years through the sale of honey and hive products for consumption, medicinal value, industrial use [4] and pollination of agricultural and non-agricultural ecosystems resulting in improved yields [5]. Arid and semi-arid land areas make up the largest land area in Kenya and are also the leading honey producing sites in Kenya setting its rank among the highest honey producing countries in Eastern Africa [6]. Similarly, most beekeeping activities within Embu county are carried out in arid and semi-arid lands which have similar conditions with other arid and semi-arid lands in Kenya [7].The beekeeping industry in Kenya is estimated to produce more than 20,525 t of honey and 2504 t of beeswax per annum which is however lower than the country's demand. This could be attributed to honey supply decline, consumer nutritional awareness and the general increase in human population [6].

Despite the significant role played by the honeybees, cases of bee population decline have been reported globally [8]. Across geographies, the decline has been variable, such as in the USA, 59% colonies were lost between 1947 and 2005. In Europe, 25% were lost between 1985 and 2005 [9]. Colony losses and migrating swarms have been documented in Kenya, resulting in a decline in the number of colonized hives Absconding is a common occurrence following reproductive swarming which produces new colonies. However, abscondment associated with non-reproductive swarming has been reported as a major cause of the colony decline [10]. Furthermore, habitat loss, climate change, unregulated application of agrochemicals and malnutrition have also been linked to absconding [11,12].

At the hive level, the provision behavior and nest building in bees facilitates the contact between nestmates and subsequent transfer of microbes [13]. Distinct bacterial clusters have been observed within the hives despite the expected homogeneous and ubiquitous distribution throughout the nests. Eight distinct bacterial phylotypes have consistently been reported; *Lactobacillus* spp. Firm 4 and *Lactobacillus* spp. Firm 5, *Bifidobacterium* spp., *Snodgrassella alvi, Frischella perrara, Gilliamella apicola, Bartonella apis*, and Alpha 2.1 [14]. *Lactobacillus* and *Bifidobacteria* are involved in food production and honey bee host defense against pathogens and other microbes collected during forage [15]. *Bartonella* is actively involved in aerobic respiration [16]. *Gilliamella* and *Frischella* abundance has been associated with disruption of honey bee host health, while *Snodgrassella* abundance has been associated with increased gut bacteria composition which assists in bee nutrition and health [17]. Bacterial symbioses have been viewed as key to host fitness and their disruption thus can lead to disease susceptibility. Understanding the bacterial community structure disruption requires prior knowledge on the native homeostatic bacterial community and factors influencing its variability [18]. Majorly, these bacterial communities associated with the food stores are a combination of bacteria originating from the hive and those derived from the host gut, surface and the foraging environment [19]. This creates the need to promote good beekeeping practices by regular monitoring and routine surveillance related to constraining factors that are crucial in honeybees health improvement [20].

In Kenya, few studies on bacteria associated with honeybees have been carried out [21] targeting the gut microbiota., this prompts for further study to understand the community composition in the region. Assessing the bacterial diversity of honeybees will provide valuable information about their overall health status and productivity, ultimately benefiting bee farmers in sub-Saharan Africa as well as in increasing agricultural productivity and production security as mentioned in the African Union's Agenda 2063. Thus, we sought to evaluate the bacterial diversity found within hive materials and their distribution in hives, habitats, agro-ecological zones within Embu County, Kenya to understand the community composition in the region. Here we used targeted metagenomic culture-independent 16S rRNA gene sequencing to determine the bacterial community structure of honeybees.

## Materials and methods

### *Study site characteristics*

Honeycombs, honey, adult worker bees and frame scrapping were collected within Embu County, Kenya for this study. Embu County lies between latitude 0° 34 S and longitude 37° 37 E. The region is characterized by a typical agro-ecological profile of cold and wet upper zones to hot and dry lower zones. We classified the sampling sites into four agro-ecological zones namely, upper highlands characterized by annual rainfall ranging from 1750 to 2000 mm and temperature of 15.8 °C to 17.7 °C, upper midlands with annual rainfall ranging from 980 to 1800 mm and temperature of 17.5 °C to 20.9 °C, lower midlands with annual rainfall ranging from 700 to 1100 mm and temperature of 21 °C to 23.9 °C and the inland lowlands with annual rainfall ranging from 590 to 710 mm and temperature of 24 °C to 25.4 °C [22]. The localities were classified as either grassland, where the lands were open fields with little tree cover or forest, where the lands were dominated by trees, close proximity to a forest and whereby agroforestry was practiced.

### *Sample collection and processing*

Honeycombs, honey, adult worker bees, larvae and the scrapings from the frames were collected across the different agro-

**Table 1**
Summary of the metadata list showing samples sources, sample types, collection sites description, crops types and the location coordinates.

| Code | Sub county | Coordinates of place | Sample id | Hive type | Sample type | Habitat types | Agro-ecological Zone | Food crops | Cash crop | Fruit crops |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Mbeere south | 0°48′00.4″S 37°33′33.6″E | Makima | Top Bar | Honey | Grassland | Inland Lowland (IL) | Millet and Sorghum | Khat | mangoes |
| 2 | Embu north | 0°26′08.5″S 37°28′14.4″E | Kamama | Traditional | Honey | Forest | Upper Highlands (UH) | Beans | Macadamia nuts | Avocado |
| 3 | Embu north | 0°24′16.2″S 37°31′22.1″E | kianjokoma | Top Bar | Combs | Forest | Upper Highlands (UH) | Maize | Tea | bananas |
| 4 | Mbeere north | 0°26′39.4″S 37°47′44.6″E | Ishiara | Traditional | Frames | Grassland | Lower Midland (LM) | Maize | Khat | mangoes |
| 5 | Mbeere north | 0°35′08.5″S 37°34′03.9″E | Nthawa | Top Bar | Honey | Grassland | Lower Midland (LM) | Millet and Sorghum | Khat | mangoes |
| 6 | Embu west | 0°25′55.5″S 37°25′41.9″E | Kibugu | Traditional | Larvae | Grassland | Upper Midland (UM) | vegetables | Coffee | passion fruit |
| 7 | Embu west | 0°32′20.7″S 37°29′20.0″E | Muthatari | Top Bar | Combs | Grassland | Upper Midland (UM) | Maize | Coffee | mangoes |
| 8 | Embu north | 0°29′25.0″S 37°28′55.4″E | Kiangima | Top Bar | Frames | Forest | Upper Highlands (UH) | Maize | Coffee | Avocado |
| 9 | Embu west | 0°29′12.5″S 37°30′44.1″E | Kivwe | Top Bar | Larvae | Grassland | Upper Midland (UM) | Maize | Coffee | Avocado |
| 10 | Embu east | 0°25′11.6″S 37°23′29.1″E | Gitumbi | Traditional | Honey | Forest | Inland Lowland (IL) | Maize | Khat | mangoes |
| 11 | Embu west | 0°28′02.5″S 37°30′22.7″E | Karingari | Top Bar | Adult Bees | Grassland | Upper Midland (UM) | Maize | Coffee | bananas |
| 12 | Embu east | 0°34′09.4″S 37°32′19.0″E | Kiamuringa | Top Bar | Combs | Grassland | Upper Highlands (UH) | Maize | Khat | mangoes |
| 13 | Mbeere south | 0°48′53.0″S 37°40′16.3″E | Ivinge | Traditional | Honey | Grassland | Inland Lowland (IL) | Cassava | Khat | mangoes |
| 14 | Embu west | 0°26′26.1″S 37°30′42.7″E | Makengi | Top Bar | Combs | Grassland | Upper Midland (UM) | Maize | Coffee | bananas |
| 15 | Embu west | 0°31′13.6″S 37°30′40.0″E | Kiethiga | Traditional | Frames | Grassland | Upper Midland (UM) | Maize | Coffee | bananas |
| 16 | Embu west | 0°29′04.0″S 37°29′28.7″E | Gatunduri | Traditional | Honey | Grassland | Upper Midland (UM) | Maize | Coffee | bananas |
| 17 | Embu north | 0°43′00.9″S 37°40′47.7″E | Kivoti | Traditional | Honey | Forest | Upper Highlands (UH) | Maize | Coffee | Avocado |
| 18 | Embu west | 0°31′13.4″S 37°28′35.0″E | Karurina | Top Bar | Honey | Grassland | Upper Midland (UM) | Maize | Macadamia nuts | mangoes |
| 19 | Embu west | 0°28′45.1″S 37°30′43.3″E | Nemburi | Top Bar | Combs | Grassland | Upper Midland (UM) | Maize | Coffee | bananas |
| 20 | Embu west | 0°28′14.3″S 37°27′34.4″E | Mutunduri | Top Bar | Frames | Grassland | Upper Midland (UM) | vegetables | Coffee | Avocado |
| 21 | Embu north | 0°25′49.6″S 37°28′53.4″E | Manyatta | Top Bar | Honey | Forest | Upper Highlands (UH) | vegetables | Tea | passion fruit |
| 22 | Embu north | 0°26′40.8″S 37°28′39.2″E | Kigari | Top Bar | Combs | Forest | Upper Highlands (UH) | Maize | Tea | bananas |
| 23 | Embu north | 0°25′13.5″S 37°28′05.3″E | Kairuri | Traditional | Adult Bees | Forest | Upper Highlands (UH) | Maize | Coffee | Avocado |
| 24 | Embu west | 0°26′57.6″S 37°32′49.6″E | Gikuuri | Top Bar | Combs | Grassland | Upper Midland (UM) | Maize | Coffee | bananas |
| 25 | Embu west | 0°25′44.2″S 37°30′32.0″E | Kianjuki | Traditional | Adult Bees | Grassland | Upper Midland (UM) | Maize | Coffee | passion fruit |
| 26 | Embu west | 0°28′34.8″S 37°25′53.9″E | Kangaru | Traditional | Larvae | Grassland | Upper Midland (UM) | Maize | Macadamia nuts | passion fruit |
| 27 | Embu west | 0°24′49.9″S 37°30′38.7″E | Kavutiri | Top Bar | Combs | Grassland | Upper Midland (UM) | Maize | Coffee | Avocado |
| 28 | Embu north | 0°42′13.2″S 37°04′33.0″E | Mukangu | Traditional | Larvae | Forest | Upper Highlands (UH) | Maize | Coffee | mangoes |
| 29 | Embu north | 0°21′36.9″S 37°32′25.7″E | Kanja | Top Bar | Combs | Forest | Upper Highlands (UH) | Maize | Macadamia nuts | mangoes |
| 30 | Embu north | 0°27′55.8″S 37°35′24.8″E | Ugweri | Traditional | Combs | Forest | Upper Highlands (UH) | Maize | Coffee | Avocado |

**Table 2**
Summary of the samples and their diversity metric indices.

| sample Id | Sample type | Sequences | OTU | chao1 | ace | Simpson | Shannon | Fisher alpha | Goods Coverage |
|---|---|---|---|---|---|---|---|---|---|
| S1 | Honey | 13,008 | 16 | 37.00 | 24.93 | 0.35 | 1.09 | 2.37 | 1.00 |
| S2 | Honey | 5417 | 12 | 19.00 | 25.98 | 0.02 | 0.12 | 1.70 | 1.00 |
| S3 | Combs | 12,598 | 23 | 26.00 | 25.64 | 0.77 | 2.76 | 3.65 | 1.00 |
| S4 | Frames | 5845 | 22 | 23.50 | 26.07 | 0.64 | 2.38 | 3.46 | 1.00 |
| S5 | Honey | 9639 | 10 | 10.00 | 10.37 | 0.53 | 1.24 | 1.37 | 1.00 |
| S6 | Larvae | 12,931 | 12 | 12.00 | 12.35 | 0.30 | 1.02 | 1.70 | 1.00 |
| S7 | Combs | 10,254 | 17 | 24.50 | 24.76 | 0.33 | 1.01 | 2.55 | 1.00 |
| S8 | Frames | 3015 | 38 | 60.75 | 51.81 | 0.32 | 1.26 | 6.66 | 0.99 |
| S9 | Larvae | 21,073 | 6 | 7.00 | 9.75 | 0.01 | 0.04 | 0.76 | 1.00 |
| S10 | Honey | 36,872 | 37 | 55.20 | 56.91 | 0.53 | 2.02 | 6.45 | 0.99 |
| S11 | Adult Bees | 4701 | 24 | 31.00 | 33.42 | 0.76 | 2.69 | 3.83 | 1.00 |
| S12 | Combs | 13,447 | 14 | 20.00 | 21.75 | 0.66 | 1.94 | 2.03 | 1.00 |
| S13 | Honey | 10,547 | 31 | 40.00 | 45.02 | 0.34 | 1.38 | 5.21 | 1.00 |
| S14 | Combs | 51,807 | 11 | 14.75 | 18.92 | 0.02 | 0.09 | 1.53 | 1.00 |
| S15 | Frames | 7692 | 28 | 67.00 | 50.46 | 0.61 | 2.07 | 4.61 | 0.99 |
| S16 | Honey | 2156 | 57 | 62.50 | 67.37 | 0.86 | 3.71 | 10.93 | 0.99 |
| S17 | Honey | 7372 | 39 | 44.00 | 49.21 | 0.70 | 2.46 | 6.87 | 1.00 |
| S18 | Honey | 7850 | 19 | 26.00 | 41.45 | 0.74 | 2.36 | 2.91 | 1.00 |
| S19 | Combs | 12,326 | 5 | 6.00 | 9.09 | 0.08 | 0.29 | 0.62 | 1.00 |
| S20 | Frames | 10,881 | 31 | 40.43 | 50.52 | 0.62 | 2.11 | 5.21 | 0.99 |
| S21 | Honey | 15,272 | 45 | 55.50 | 58.67 | 0.32 | 1.39 | 8.18 | 0.99 |
| S22 | Combs | 124,766 | 7 | 8.00 | 8.88 | 0.53 | 1.19 | 0.91 | 1.00 |
| S23 | Adult Bees | 9844 | 27 | 40.75 | 52.28 | 0.59 | 2.09 | 4.41 | 0.99 |
| S24 | Combs | 20,518 | 6 | 7.00 | 7.91 | 0.02 | 0.11 | 0.76 | 1.00 |
| S25 | Adult Bees | 9548 | 14 | 15.50 | 15.97 | 0.77 | 2.34 | 2.03 | 1.00 |
| S26 | Larvae | 8565 | 11 | 14.00 | 14.63 | 0.50 | 1.46 | 1.53 | 1.00 |
| S27 | Combs | 13,402 | 6 | 6.00 | 6.00 | 0.62 | 1.51 | 0.76 | 1.00 |
| S28 | Larvae | 14,144 | 12 | 15.00 | 15.15 | 0.64 | 1.82 | 1.70 | 1.00 |
| S29 | Combs | 12,813 | 11 | 11.33 | 12.85 | 0.57 | 1.44 | 1.53 | 1.00 |
| S30 | Combs | 10,997 | 10 | 25.00 | 44.33 | 0.09 | 0.36 | 1.37 | 1.00 |

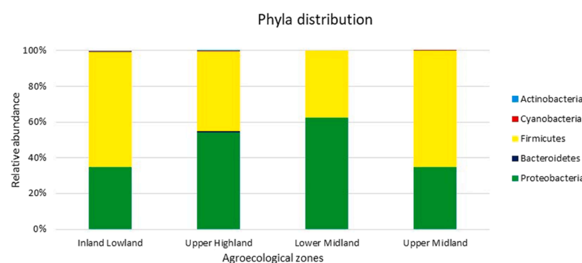


**Fig. 1.** Percentage read abundance and distribution of bacteria at the phylum level across the agro-ecological zones.

ecological zones within Embu County [Table 1]. These samples were obtained from two hive types, namely, the traditional hive which provides only an enclosure for the bee colony and top-bar hive which has movable combs and are easily inspected. Bee samples were collected in a cap tube and preserved in 70% ethanol. Honey combs and frame scrapings were cut using a sterile blade and put in sterile zip lock bags with sticker labels and placed in cooler box and transported to the laboratory where they were refrigerated at 4 °C. Samples were collected in duplicates to ensure a proper representation per site and later pooled during DNA extraction. All samples were collected during May and June 2021 honey harvesting period.

### *DNA extraction and sequencing*

One gram of the honeycomb, frame scrapings and honey samples was used as the starting materials for DNA extraction. The material samples were each separately ground using mortar and pestle and suspended in 10 ml phosphate buffer saline solution (PBS) for homogenization. In adult bee samples, three abdomens per tube were pooled and macerated, while for the larvae samples five larvae per tube were picked from combs using sterile forceps and were washed using 95% ethanol before maceration. One milliliter of the sample extract was aliquoted into sterile 2 ml tubes, centrifuged at 13,200 rpm and the supernatant discarded. The pellet containing the cells was resuspended in 100 µl solution A (400 mM Tris–HCl (pH 8.0), 100 mM EDTA (pH 8.0)), 10 µl of lysozyme solution (20 mg/ml) added and incubated at 37 °C for 30 min in a water bath. Four hundred microliters of a lysis buffer (400 mM Tris–HCl (pH 8.0), 60 mM EDTA (pH 8.0), 150 mM NaCl, 1% SDS) was added and gently mixed by inverting the tubes several times. Ten microliters of proteinase K (20 mg/ml) were added, and the mixture incubated at 65 °C in a water bath for 1 hour. An equal volume of chloroform

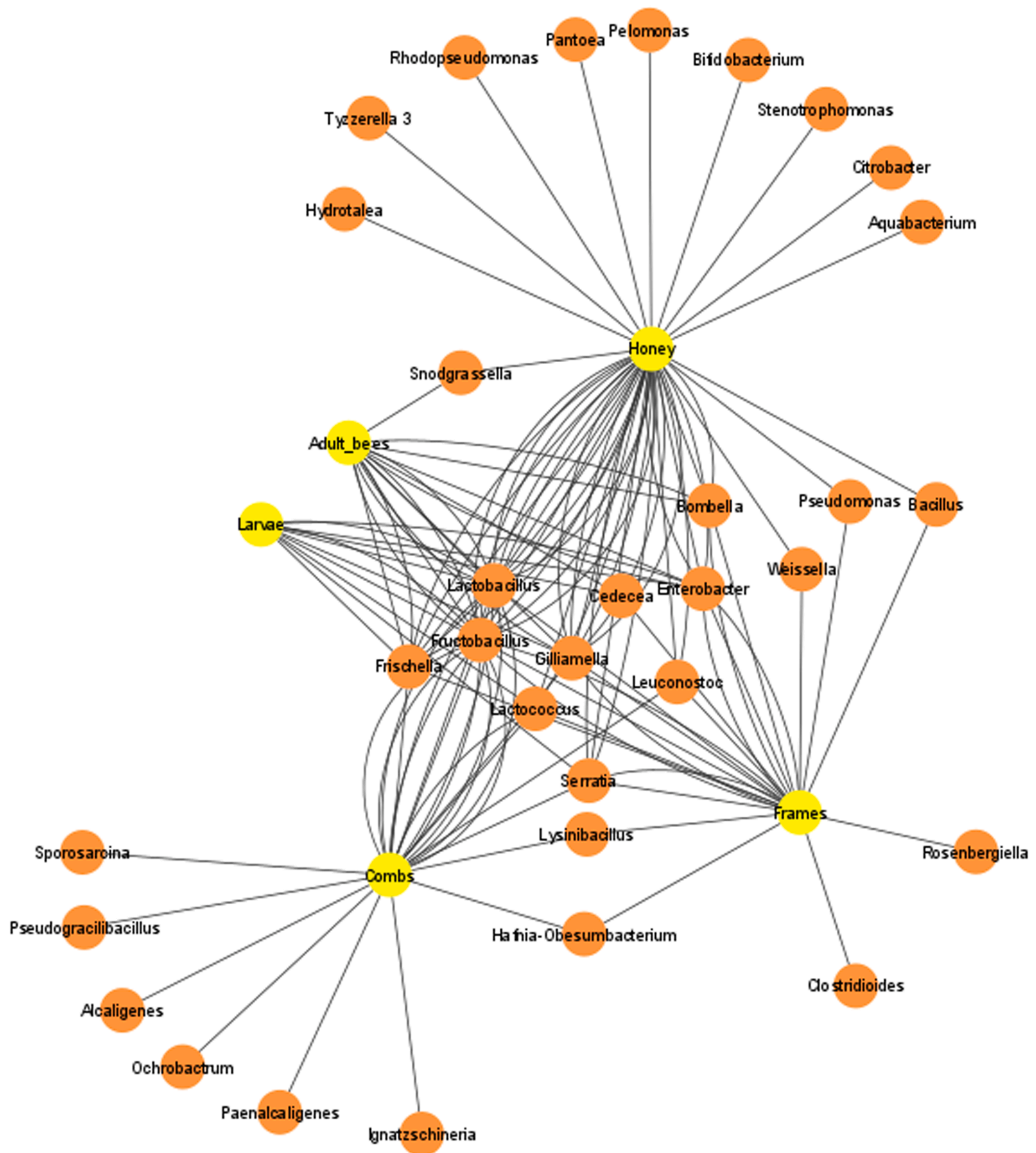


**Fig. 2.** Phylogenetic tree showing a Cytoscape analysis of the sample types and the abundant bacteria genera. Sample types represented as source nodes, and the bacteria genera as target nodes.

was added and centrifuged at 13,200 rpm for 10 min at 4 °C. The supernatant was transferred into a new tube and the volume noted. Sodium acetate (pH 5.2) was added in a volume of 150 µl to the supernatant. Equal volume of isopropyl alcohol was added to the mixture. The tubes were mixed by inverting them gently and centrifuged at 13,200 rpm for 10 min and the supernatant discarded. The DNA pellet was washed in 300 µl of 70% ethanol, centrifuged at 13,200 rpm for 1 min and the supernatant discarded. The tubes were allowed to air-dry and thereafter the DNA was resuspended in 30 µl of nuclease free DEPC treated water. Amplicon generation was then done at Inqaba Biotech, SA on a Sequel system by PacBio (www.pacb.com) using universal primers 27F and 1496R to amplify the entire 16S rRNA gene [23].

*Sequence analysis*

Raw subreads were processed through the SMRTlink (v9.0) Circular Consensus Sequences (CCS) algorithm to produce highly

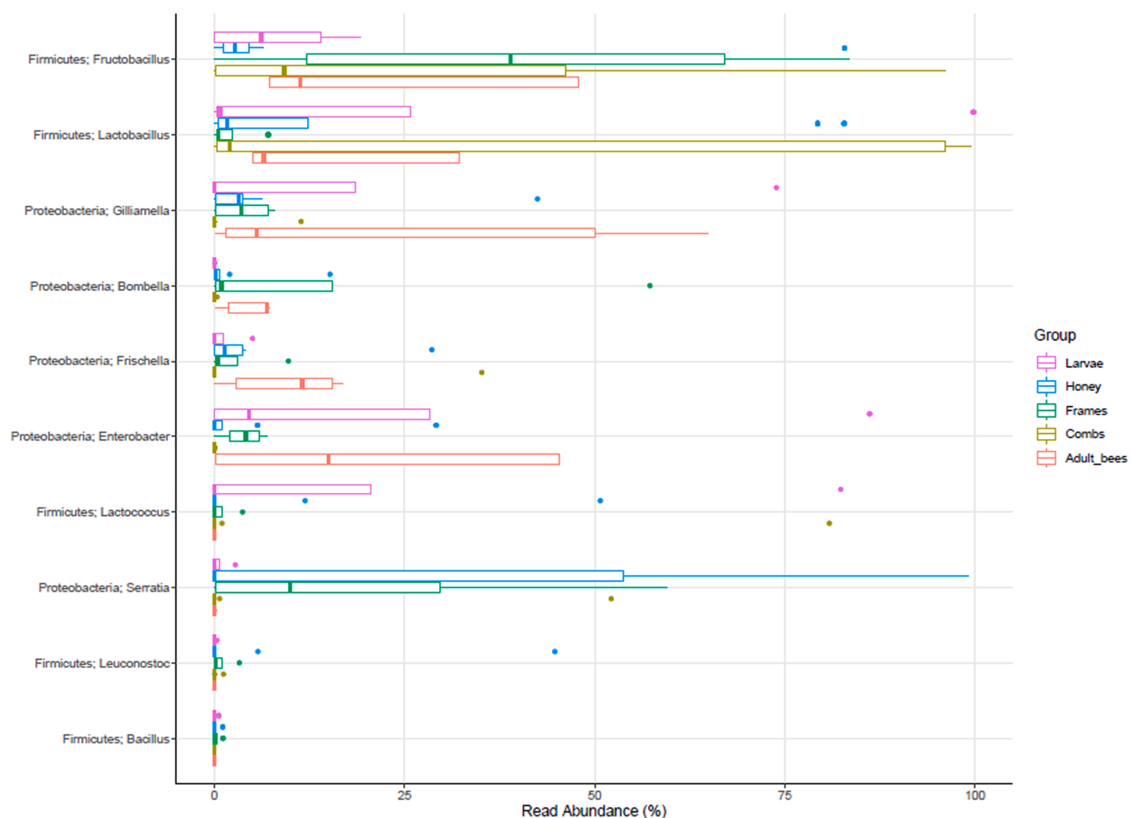


**Fig. 3.** Box plot showing the percentage read abundance of the ten most abundant genera observed in the study. Plot colors representing the sample types, that is, purple larvae, blue honey, green frames, yellow combs and red adult bee samples respectively.

accurate reads (>QV40). Dereplication and clustering was done using VSEARCH (v2.14). This included sorting and size-filtering of the reads to ≥1000 bp and dereplication of the full-length sequences. The sequences were then denoised and evaluated for potential chimeric sequences using UCHIME package v.11. [24]. A sequence identity cutoff of 97% was chosen to pick operational taxonomic units (OTUs) from the quality filtered, denoised, non-chimeric sequences. Representative OTUs were picked using VSEARCH v2.14 [25] Classification was performed by BLASTn [26] with standard settings using the non-redundant SILVA SSU reference dataset v132 as a classification reference.

*Microbial community analysis*

Eukaryotic OTUs were removed using filter_otu_table.py in QIIME. Statistical analyses were performed using the R packages Phyloseq [27], Ampvis2 [28] and Vegan [29]. Species richness was estimated using Chao1, Good’s coverage and Shannon’s diversity estimators while community evenness was evaluated using Pielou’s index. A phylogenetic tree was constructed using FastTree v2.1.7 [30] and saved in Newick format after rooting the midpoint using FigTree v1.4.4 [31]. An OTU network was generated using QIIME, filtered using an edge cut-off of 0.001 and then visualized in Cytoscape (version 3.9.1) with an “edge-weighted spring-embedded layout”. Network edges were weighted by association value [32,33] Sample types were used as source nodes and the bacteria genera as target nodes. To visualize the multivariate dispersion of community composition, a non-metric multidimensional scaling (NMDS) was performed using Bray-Curtis dissimilarities employing the “vegan” package [34]. Sequences from this study have been deposited into the SRA under the accession SRP383332.

*Microbial diversity and composition per sample type*

Differences in alpha diversity richness between the samples were visualized in plots using Phyloseq where the most abundant genera within the sample types were represented. To visualize the distribution of species across the agroecological zones, a heatmap of the top 30 species with the highest percentage read abundance was generated using Ampvis2.

## Results

We determined the diversity of bacteria within hive materials to elucidate the microbial community associated with honeybees. In

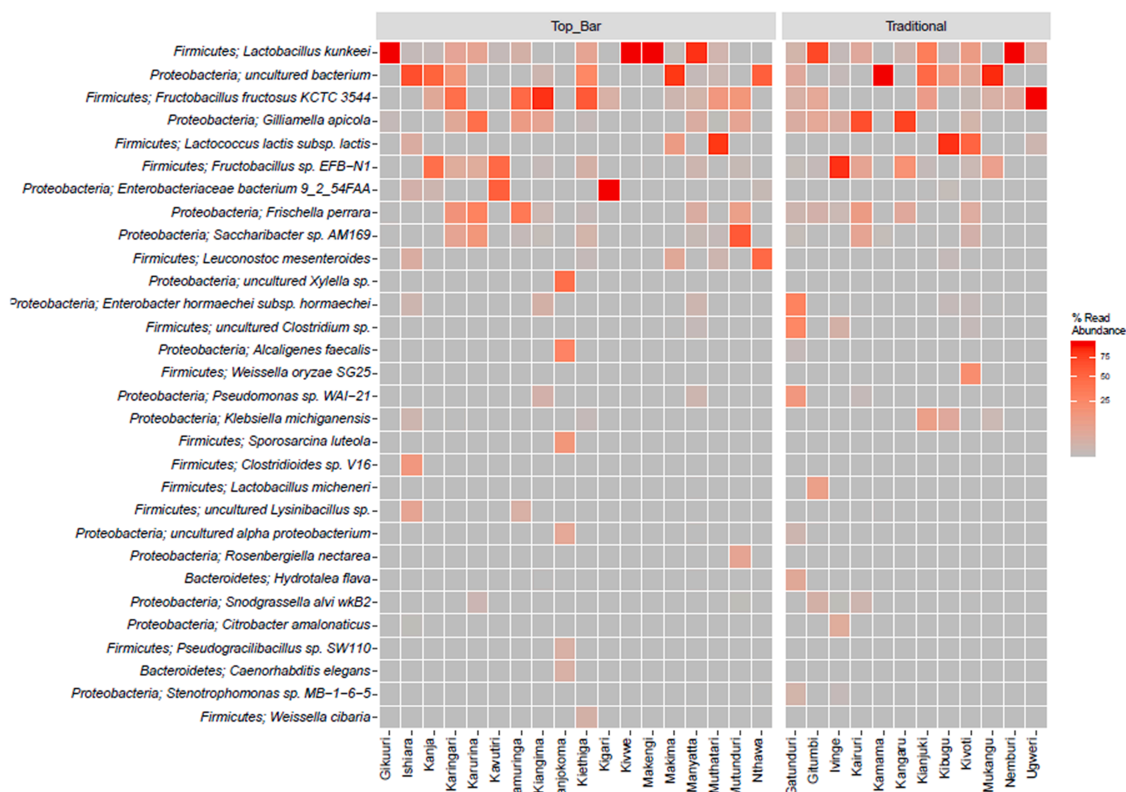


**Fig. 4.** Heatmap showing the percentage read abundance of the top 30 species across the hive types. Distribution trend of the 10 abundant genera across the hive types except for the Enterobacter and Leuconostoc in the traditional hive type.

this study we collected samples of raw honey, combs, scraps of frames, larvae and adult bees during the honey harvesting season in the region.

*Sequence read analyzed and taxa generated*

In total, 499,300 nucleotide sequence reads were obtained across the 5 sample types used in this study. The number of sequences in honey samples ranged between 2156 and 36,872 reads, and between 10 and 57 OTUs. Sequence reads in comb samples ranged from 10,254 to 124,766 reads, whereas OTUs ranged from 5 to 23. A total of 8565 to 21,073 sequence reads were found in larvae samples, while the OTUs ranged between 6 and 12 OTUs per larvae samples. Among the adult bee samples, sequence reads were between 4701 and 9844 reads, while the OTUs ranged between 14 and 27. In the frame samples, the sequence reads ranged from 3015 to 10,881 reads, while the OTUs ranged from 22 to 38 [Table 2]. Honey samples recorded the highest nucleotide observations while comb samples recorded least nucleotide observations.

*Bacterial alpha diversity*

Bacterial alpha diversity indices across the different samples were evaluated and compared using the ACE, Chao 1, Shannon, Simpson, Good's coverage and Fisher alpha diversity metrics [Table 2]. Based on Good's coverage estimator, the dominant phylotypes were represented in the data as the values ranging between 99 and 100%. Sample S16 had the highest Chao1 and ACE indices (62.50 and 67.37) respectively, indicating a greater species richness, while S27 had the least Chao1 (6.00) and ACE (6.00) indices. Sample S9 had the lowest Simpson (0.01) and Shannon (0.04) indices, indicating unequal richness of bacterial species across the samples [Table 2]. Firmicutes (55.1%), Proteobacteria (44.4%), Bacteroidetes (0.3%), Actinobacteria (0.1%), and Cyanobacteria (0.1%) phyla dominated all the agro-ecological zones [Fig. 1]. Phylum Firmicutes was predominated by the Lactobacillales (52.5%), Clostridiales (1.5%) and Bacillales (12.2%) orders. Order Lactobacillales comprised of seven genera, while Bacillales and Clostridiales had five and two genera respectively. Sequences belonging to the phylum Proteobacteria were represented mainly by the orders Acetobacterales, Rhizobiales, Sphingomonadales, Betaproteobacteriales, Cardiobacteriae, Enterobacteriales, Oceanospirillales, Orbales, Pseudomonadales and Xanthomonadales. Orders Bifidobacteria, Micrococcales and Propionibacteria predominated the Actinobacteria phylum, while orders Chitinophagales, Cytophagales, Flavobacteria and Sphingobacteria predominated the Bacteroidetes phylum. To summarize the abundant genera shared across the sample types, a phylogenetic tree was generated. Major bacteria genera shared across

**Fig. 5.** Heatmap showing the percentage read abundance of the top 30 species across the sample types. Variation in distribution observed in adult bee and larva samples whereas comb, frame and honey samples were rich in diversity.

the sample types included *Frischella, Gilliamella Lactobacillus, Serratia* and *Leuconostoc* while the less predominant bacteria genera featuring in specific sample types included *Alcaligenes* and *Sporosarcina* in comb samples, *Rosenbergiella* in frame samples and *Pantoea, Aquabacterium and Rhodopseudomonas* in honey samples [Fig. 2].

*Bacterial composition across the sampling environment*

A box plot was generated to visualize the most abundant genera based on the number of nucleotide sequence reads for each sample type [Fig. 3]. The ten abundant genera across agroecological zones, hive and habitat types were used to illustrate the disparities in bacterial diversity and composition across the environments represented in the sampling sites at varying abundances [Figs. 4, 5, 6 and 7]. The most abundant genera represented were *Lactobacillus, Fructobacillus, Gilliamella,* uncultured *Proteobacteria, Saccharibacter, Enterobacter, Leuconostoc, Frischella* and *Enterobacter*.

*Bacterial composition in hives*

Genera *Lactobacillus, Fructobacillus, Gilliamella, Lactococcus, Frischella, Saccharibacter* and uncultured *Proteobacteria* were abundant across the two hive types. Genera *Enterobacteria* and *Leuconostoc* however were only present in the top bar hive types [Fig. 4]. *Fructobacillus* genus was the second most abundant in our study. The combs and frame scrapping samples from the upper midland and upper highland agroecological zones were the richest in *Fructobacillus* composition.

*Bacterial composition in sample types*

Genera *Lactobacillus, Fructobacillus, Gilliamella*, uncultured *Proteobacteria* and *Frischella* were abundant in all the sample types. Genera *Leuconostoc* and *Enterobacter* were present in combs, honey and frame samples and only absent in adult bee and larvae samples. Genus *Saccharibacter* however was absent in comb and larvae samples while genus *Lactococcus* was present in all the sample types except the adult worker bee samples [Fig. 5].

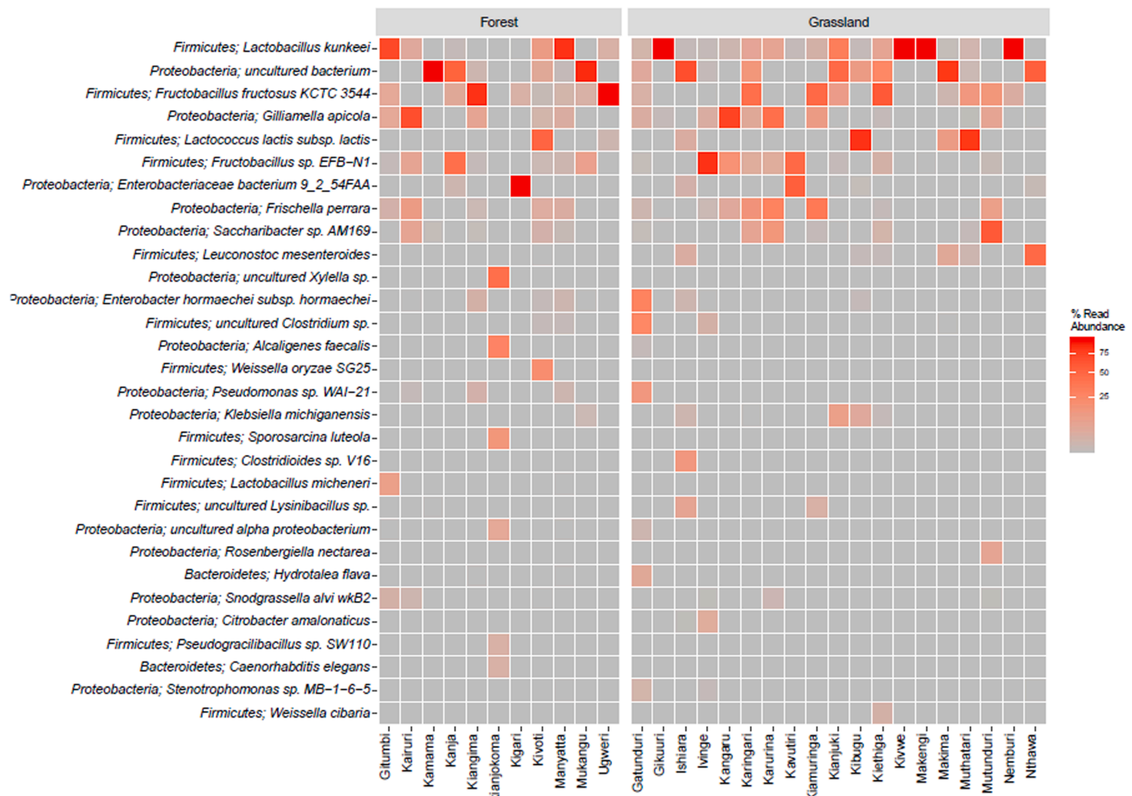


**Fig. 6.** Heatmap showing the percentage abundance of the top 30 species across the habitat types. Abundant genera were shared across the habitats, variation evident in the forest habitat which lacked the Leuconostoc genus.

*Bacterial composition in habitat types*

Genera *Lactobacillus, Fructobacillus, Gilliamella, Lactococcus, Frischella, Saccharibacter, Enterobacter* and uncultured *Proteobacteria* were abundant in forest and grassland habitats. The variation was only observed in forest habitat which lacked the *Leuconostoc* genus [Fig. 6].

*Bacterial composition in agroecological zones*

Variations were observed across the agro-ecological zones. *Lactococcus* and uncultured *Proteobacteria* were present in all agro-ecological zones. Genera *Lactobacillus, Fructobacillus, Gilliamella* and *Frischella* were present across the zones except in the lower midlands. Genus *Leuconostoc* was only present in inland lowland and lower midland agroecological zones. Genus *Saccharibacter* was present in upper highlands and upper midland agroecological zones while genus *Enterobacter* was reported in all zones except the inland lowland agroecological zone [Fig. 7]. To summarize the bacterial composition across sample types, principal coordinate analysis plot [Fig. 8] was used to illustrate the similarity and dissimilarity in the sampling sites.

## Discussion

In this study the bacterial composition was determined across the sample types in the different agro-ecological zones. The differences in bacteria composition were observed across the sample types. Adult bee samples were predominated by genera *Gilliamella, Enterobacter, Bombella, Fructobacillus* and *Lactobacillus. Lactococcus* was the most abundant genus in larvae samples. Genera *Serratia* and *Lactobacillus* were found abundant in honey samples while comb and frame samples were predominated by genera *Fructobacillus, Lactobacillus*, and *Serratia*. [35] and [36] reported similar compositions. *Fructobacillus* has been described to inhabit the flower nectar, pollination environment and honey bee sources [13], this might explain their high abundance in comb and frame scrapping samples.

*Lactobacillus* are considered to be the core gut bacteria and key in bee nutrition [37]. *Lactobacillus kunkeei* has been associated with flowers, pollen, honey, hive surfaces and the honeybee gut [38]. In our study, combs and honey samples were the most diverse in *Lactobacillus* genus composition across all the agroecological zones except in the lower midlands perhaps because it is a semi-arid region. *Lactobacillus* presence has been associated with antimicrobial potency against honey bee pathogens [39]. Genus *Serratia* has been reported to form part of non-core bacteria and is widely present at low frequencies in honeybee guts thus considered as a signifier

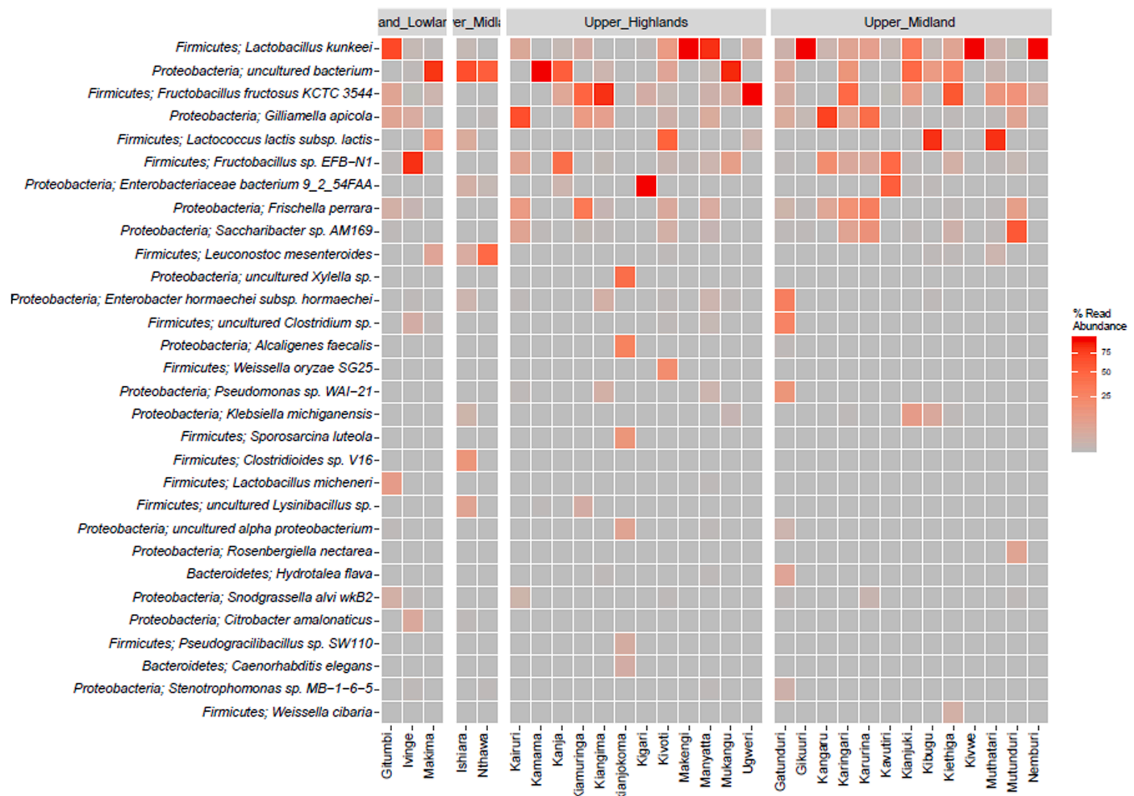


**Fig. 7.** Heatmap showing the percentage abundance of the top 30 species across the agroecological zones, with and_Lowlan denoting Inland Lowland and er_Midl denoting Lower Midlands respectively.

of characteristic bacterial composition in bees [38]. Honey samples were the richest in abundance of *Serratia* genus in our study. *Serratia* has also been reported in other insect species: *Serratia symbiotica* a symbiont of the aphids is thought to be acquired from the foraging environment [39]. *Serratia marcescens* an opportunistic bacterial pathogen has also been associated with increased lethality in honeybees following the exposure to glyophosate [40]. Similarly, genus *Enterobacter* was abundant in adult bee and larvae samples. *Enterobacter* abundance has been associated with significant disruption of the normal bee gut bacterial community composition. The disruption is occasioned by higher content of species within genera *Enterobacter, Hafnia, Serratia, Klebsiella* and *Pantoea* which are opportunistic environmental bacteria [13]. Genera *Lactococcus* was abundant in bee larvae. *Lactococcus lactic* has been reported as specific to honeybee bacteria species and generally considered as non-pathogenic and beneficial to their host [41]. Genus *Leuconostoc* has been associated with healthy apiaries [42] this might explain their occurrence in top bar hives in our study as the associated hive inspection in top bar hives are non-invasive and thus less contamination is experienced. Genus *Bombella* has been reported to inhibit fungal infection by reducing the number of spores produced per infection [43] This may explain their presence in the adult and frame samples.

In this study, *Gilliamella* was predominantly abundant in agriculturally active lands in the upper highlands and upper midlands of the county. The high abundance of *Gilliamella* in honeybees has been reported to positively correlate with chronic exposure to glyophosate, a broad spectrum herbicide, which is the most widely used herbicide worldwide in weed management [40]. According to this study, genus *Gilliamella* was the third most abundant, whereas it was reported as the most abundant in the gut microbiota of Kenyan *Apis mellifera scutellata* [21]. The variations could have resulted from agricultural practice in the areas where maize was the main food crop which requires less attention, reportedly because of use of the hybrid seeds and infrequent spraying in a season [44]. *Gilliamella* abundance has been reported to increase in honey bees that are chronically exposed to the insecticides Thiamethoxam and Fipronil, as well as to the herbicide glyphosate [21].

Alternatively, the altitude and humidity also play part in *Gilliamella* composition. [21] reported *Gilliamella* abundance as negatively correlated with altitude and positively correlated with humidity. *Gilliamella apicola* has been reported to metabolize variable carbohydrates and derive energy from pollen components and ability to resist toxic effects of the sugar components [45].

Our study provides an insight into the core bacterial community members in Embu County, Kenya. The major groups represented were *Lactobacillus, Fructobacillus, Gilliamella,* uncultured *Proteobacteria, Saccharibacter, Enterobacter, Leuconostoc, Frischella* and *Enterobacter* which resembled those found in studies of the microbiota of honeybees [14]. These bacteria likely play a significant role in nutrition and host defenses against pathogens. A significant observation was the presence of *Serratia* and *Enterobacter* genera, which are considered as opportunistic environmental bacteria suggesting the potential disruption of microbiota probably by chemical spray

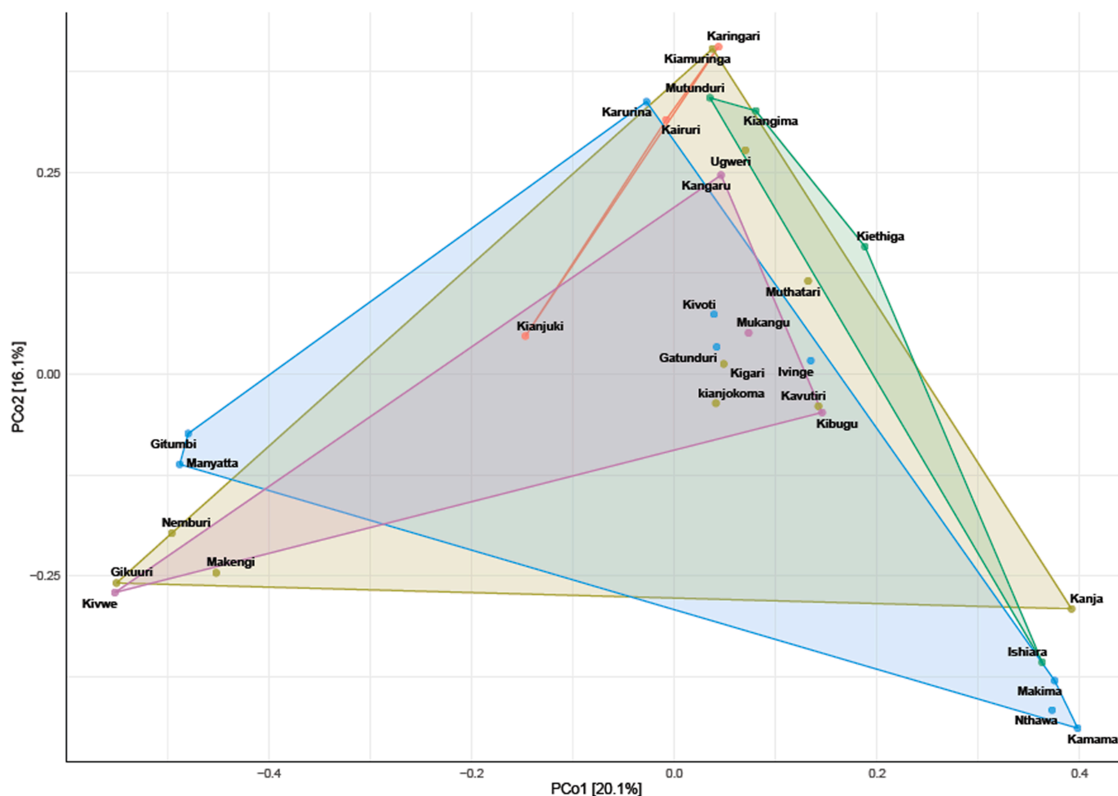


**Fig. 8.** PCoA plot showing ecological distance between sampling sites based on bacterial composition. Sample types are plotted as points in different colors. Blue indicating honey samples, green frame samples, red adult bee samples, yellow comb samples and purple indicating larvae samples respectively.

exposure from the foraging environment. We also observed disparities in phylum abundance across the agro-ecological zones which probes for further research on the roles and functions of these bacteria in the physiology and health of the honeybees. A better understanding of the central function of these bacteria and differences in phylotypes in the hive environment might help address the challenge of the honeybee stress and decline and boost the beekeeping at large.

## CRediT authorship contribution statement

**James K. Njoroge:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Moses Njire:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Julianna Maina:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Romano Mwirichia:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Franklin N. Nyabuga:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing. **Julius Mugweru:** Conceptualization, Formal analysis, Investigation, Project administration, Software, Supervision, Writing – original draft, Writing – review & editing.

## Declaration of Competing Interest

The authors of this manuscript declare that they have no competing interests to disclose. The study was funded by the IFS grants [No. 1I3_B_040304). The authors declare that they have no conflicts of interest with any person or organization that might have an interest in the results of this study. The authors have received no personal benefits or have been promised any future benefits in return for publishing this work. The authors confirm that the results reported in this study are original and have not been published elsewhere. We would like to affirm that all aspects of the study have been conducted in accordance with the ethical standards and guidelines for research. All necessary research approvals were obtained prior to conducting the study.

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