



Bacterial communities associated with the midgut microbiota of wild *Anopheles gambiae* complex in Burkina Faso

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Abstract

Plasmodium falciparum is transmitted by mosquitoes from the *Anopheles gambiae* sensu lato (s.l) species complex and is responsible for severe forms of malaria. The composition of the mosquitoes' microbiota plays a role in *P. falciparum* transmission, so we studied midgut bacterial communities of *An. gambiae* s.l from Burkina Faso. DNA was extracted from 17 pools of midgut of mosquitoes from the *Anopheles gambiae* complex from six localities in three climatic areas, including cotton-growing and cotton-free localities to include potential differences in insecticide selection pressure. The v3–v4 region of the 16S rRNA gene was targeted and sequenced using Illumina Miseq (2 × 250 nt). Diversity analysis was performed using QIIME and R software programs. The major bacterial phylum was *Proteobacteria* (97.2%) in all samples. The most abundant genera were *Enterobacter* (32.8%) and *Aeromonas* (29.8%), followed by *Pseudomonas* (11.8%), *Acinetobacter* (5.9%) and *Thorsellia* (2.2%). No statistical difference in operational taxonomic units (OTUs) was found (Kruskal–Wallis FDR— $p > 0.05$) among the different areas, fields or localities. Richness and diversity indexes (observed OTUs, Chao1, Simpson and Shannon indexes) showed significant differences in the cotton-growing fields and in the agroclimatic zones, mainly in the Sudano-Sahelian area. OTUs from seven bacterial species that mediate refractoriness to *Plasmodium* infection in *An. gambiae* s.l were detected. The *beta* diversity analysis did not show any significant difference. Therefore, a same control strategy of using bacterial species refractoriness to *Plasmodium* to target mosquito midgut bacterial community and affect their fitness in malaria transmission may be valuable tool for future malaria control efforts in Burkina Faso.

Keywords Midgut bacteria · *Anopheles gambiae* s.l complex · Microbiota · Malaria

Abbreviations

<i>An. gambiae</i> s.l	<i>Anopheles gambiae</i> sensu lato
DNA	Deoxyribonucleic acid
OTUs	Operational taxonomic units
PCR	Polymerase chain reaction

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Introduction

Anopheles gambiae s.l is a major vector of malaria in sub-Saharan Africa because it transmits *Plasmodium falciparum* parasites to humans, with ensuing severe forms of malaria [1]. In 2017, an estimated 219 million cases of malaria occurred worldwide, mostly in Africa (92%), followed by South-East Asia (5%) and the eastern Mediterranean region (2%) [1]. In the absence of a vaccine, malaria prevention heavily relies on vector control by insecticide-treated bed nets (LLINs) and indoor residual spraying (IRS) [2]. Nevertheless, in Africa this control is threatened by the rise of

insecticide resistance in *Anopheles* mosquitoes [3], so that new strategies for malaria control are required [4]. The gut microbiota of mosquitoes plays an essential role in their susceptibility to *Plasmodium falciparum*, as demonstrated by previous studies [5]: the presence of antibiotics in the blood of malaria-infected people enhances the susceptibility of *An. gambiae* s.l to *P. falciparum* infection by disturbing their gut microbiota [6, 7]. Therefore, the use of drugs or particular bacteria to disturb the microbiota of specific mosquitoes and affect their vector competence or fitness is a new concept that deserves further investigations [8]. Furthermore, the *An. gambiae* gut could be often a selective eco-environment (*Proteobacteria* and *Bacteroidetes*) [9]. Symbiotic control (paratransgenesis) is an alternative method for human disease vector control [10], and consists of genetic engineering of a symbiotic microorganism to alter the capacity of the mosquito to transmit malaria [11]. This control approach aims to limit or stop the development of the parasite in the mosquito vector [12] based on an understanding of the interactions between the vector, its environment, and the pathogen [13]. This approach provides anti-disease strategies inside the mosquito hosts by harnessing their symbiotic microbes [14]. Mosquitoes are indeed continuously exposed to a variety of microorganisms, mainly bacteria from their ecosystem [15]. The microbial communities -also called microbiota-hosted by mosquitoes have been characterized by conventional culture methods [16, 17], by flow cytometry analysis [18], by shotgun metagenomic sequencing [19], or by amplicon sequencing of 16S rRNA [8, 20, 21]. These methods showed that the microbiota can modulate the immune response of mosquitoes and their ability to influence human pathogen transmission [5, 15, 22]. Changes in the microbiota limit the development of the malaria parasite in *Anopheles* [23, 24]. A recent study showed that the *Enterobacter* genus directly blocked the development of the malaria parasite by producing free radicals harmful to *Plasmodium* sp. in the mosquito gut. This resulted in 99% resistance of *An. gambiae* s.l to infection by *P. falciparum* [23]. The suppression of the majority of midgut bacteria increased the susceptibility of *An. gambiae* s.l and *Aedes aegypti* to infection by *P. falciparum* and the dengue fever virus, respectively [15]. This suggests that interactions between microbes and parasites reduce *Plasmodium* sp. transmission [25]. Decreasing the vector's transmission efficacy by modifying its microbiota is a very active and novel field of research [8, 26]. For instance, some bacteria such as *Pantoea* spp. and *Asaia* spp. are currently emerging as promising candidates for paratransgenesis [8, 27, 28]. These two genera have a high vertical transmission potential through multiple mechanisms. They are capable of horizontal infection through feeding and through mating in adult mosquitoes, indicating that they could quickly spread among natural mosquito populations [14]. The investigation

of the *An. gambiae* s.l midgut microbiota may therefore be a promising way for further developing novel, safe, and effective strategies to manipulate the vectorial capacity [21, 26] and could promote new control methods [29, 30]. For example, the recent evolution of mosquito control involves mosquitoes genetically modified by gene drive [30]. This gene drive can generate a new phenotype which could modulate a midgut bacterial community to be hostile to *Plasmodium* transmission. However, any release of laboratory-modified mosquitoes in the wild will first require characterizing local mosquito populations. Burkina Faso is divided into three agroclimatic zones with different malaria epidemiological characteristics, i.e., the Sahelian area (short transmission period lasting 2 to 3 months), the Sudano-Sahelian area (seasonal transmission lasting 4 to 6 months) and the Sudanian area (perennial transmission) [31, 32]. We investigated bacterial diversity in the microbiota of the *An. gambiae* complex in different locations of the country according to different ecological settings.

Materials and methods

Mosquito collection and identification

All mosquitoes were collected from human residential areas (indoors and outdoors) using a Prokopack aspirator (craft manufacturing) between August and October 2017. Three agroclimatic areas were surveyed: Sahelian, Sudano-Sahelian and Sudanian, with two localities *per* climatic area, one in a cotton-growing field (highly exposed to insecticides) and one away from cotton fields (unexposed to insecticides). For each locality, three sites were sampled except in the Sudanian area where only two sites were sampled from the cotton fields (Table 1).

The Sudanian climatic area (yearly rainfall > 900 mm; average annual temperature 31 °C) has malaria transmission all year round. In the Sudano-Sahelian area (yearly rainfall between 600 and 900 mm; average annual temperature 33 °C), and the Sahelian area (yearly rainfall < 600 mm; average annual temperature 35 °C), malaria transmission is seasonal, with time periods of 4 to 6 months and 2 to 3 months, respectively [33]. From indoors and outdoors of houses, a total of 340 females of the *An. gambiae* complex with no blood in their midgut were selected and dissected under a light binocular microscope (Leica EZ4, magnification: × 10) and grouped in 17 pools (one pool *per* site). Each pool included 20 individual females *per* site for each locality, field and zone (Table 1).

Table 1 Location and sites surveyed for *An. gambiae* s.l mosquito samples

No.	Fields	Localities	Sites	Geo-coordinates of the localities	
Sahelian area					
1	Cotton-growing	Kongoussi	Keilla, Rollo, Toessin	13° 19' 31.063" N	− 1° 31' 32.535" W
2	Cotton-free	Ouahigouya	Bissiguin, Yipala, Namsiguima	13° 34' 0.573" N	− 2° 24' 39.566" W
Sudano-Sahelian area					
3	Cotton-growing	Kombissiri	Kosmasson, Zemtoega, Gomsi	12° 4' 27.57" N	− 1° 20' 31.571" W
4	Cotton-free	Réo	Seboun, Réo Sect. 7, Vour	12° 19' 5.32" N	− 2° 27' 55.295" W
Sudanian area					
5	Cotton-growing	Houndé	Kohofabio, Yabiro, Kohobié	11° 29' 21.926" N	− 3° 31' 23.131" W
6	Cotton-free	Bobo	Koumi, Samagan/Logofrouso	11° 9' 53.719" N	− 4° 18' 18.555" W

Mosquito dissection

All mosquitoes samples were identified morphologically as the *Anopheles gambiae* sensu lato (s.l) complex according to the identification keys of Gillies et al. [34, 35]. Living mosquitoes were anesthetized with chloroform, and dissected under sterile conditions. Three hundred and forty non-blood-engorged females were selected for dissection. The mosquitoes were surface sterilized twice prior to dissection by washing with 70% ethanol for 5 min and rinsing in a sterile saline phosphate buffer (PBS 1 ×, pH 7.8) [36]. Dissections were performed under a light binocular microscope (Leica EZ4, magnification: × 10). Each mosquito gut was pulled out into a drop of sterile PBS 1 ×, pH 7.8 on a sterilized microscope slide. The guts were pooled (20 individual guts *per* site) into a sterile 1.5 mL Eppendorf tube containing 500 µL of RNAlater (QIAGEN, USA), and stored at − 20 °C. The samples were brought to Gembloux Agro-Bio Tech, Functional & Evolutionary Entomology laboratory (Gembloux, Belgium) and stored at − 80 °C till DNA extraction.

DNA extraction and Illumina PCR amplicons

Midgut DNA was extracted with a QIAamp DNA Micro Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. Briefly, the samples were crushed individually with plastic pestles in 1.5-mL tubes containing 180 µL of ATL buffer, and 20 µL of proteinase K were added. The mixtures were vortexed thoroughly and incubated overnight in a thermomixer (56 °C, 450 rpm). After processing and washing with the adequate products provided in the kit, the final DNA product was eluted using 20 µL of AE elution buffer and stored at − 20 °C until PCR analysis. Surfaces were cleaned with 70% ethanol, and all extractions were performed under a localized aseptic microenvironment provided by flame from a Bunsen burner.

DNA concentrations were normalized at 5–6 ng/µL with a Nanodrop spectrophotometer ND1000 V3.8.1 (Nanodrop, Wilmington, DE, United States) and stored at − 20 °C before

Illumina Miseq (2 × 250 nt) library preparation. 16 S rRNA gene libraries were generated by targeting the v3–v4 universal region of the 16S rRNA gene using the Illumina Miseq metagenomic sequencing library protocols and the 16S rRNA primers: 16S Amplicon PCR Forward Primer: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 16S Amplicon PCR Reverse Primer: 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATC. The total volume of the PCR mix was 25 µL, containing 12.5 µL of 2 × KAPA HiFi HotStart ReadyMix, 5 µM of each primer and 2.5 µL of mosquito DNA templates. The PCR reaction was performed with the following temperatures: 95 °C for 3 min, 25 cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s; a final elongation step at 72 °C for 5 min, and a storage temperature of 4 °C. Five µL of PCR amplicons were loaded in 1% electrophoresis gel and visualized under UV light to check if the expected 450-bp band was obtained. The PCR products were then sent to DNAVision sequencing center (Gosselies, Belgium) for sequencing.

Bioinformatics

Demultiplexed quality-filtered reads obtained by IlluminaSeq from the sequencing center (forward and reverse) were processed using QIIME (Quantitative Insights Into Microbial Ecology) 1.9.0 version [37]. Each sample read was paired using the `multiple_join_paired_ends.py` script. Then paired fastq files were converted into fasta form with the script `convert_fastaqual_fastq.py`, and Qiime labels were added to these fasta files using the `add_qiime_labels.py` script with a valid metadata file.

Operational taxonomic units (OTUs) were picked with `pick_open_reference_otus.py` script using SILVA database (release 132) [38, 39] at 97% similarity. The OTU table was cleaned by removing sequences corresponding to cytoplasmic (mitochondrial) contamination with the `filter_taxa_from_otu_table.py` script. Then, the `compute_core_diversity.py` script was launched at 19,495 rarefaction depth for

diversity analysis and sample comparison (*beta* diversity, OTU taxonomy summary and Kruskal–Wallis test). *Alpha* diversity indexes and their comparison were computed in R, using Phyloseq package [40]. Heatmap trees of OTUs differential abundances and Wilcoxon Rank Sum were generated with the Metacoder R package [41].

Results

Analysis of bacterial diversity at the phylum level

A total of 718,556 sequences were generated from 17 samples (one site = one sample). The average number of raw sequence reads *per* sample was $42,268 \pm 64,695$ (between 19,495 and 300,529 reads). The generated sequences were assigned to 1676 OTUs. A plot of rarefaction curves was estimated for each sample. The rarefaction depth of 19,495 sequences *per* sample was sufficient to obtain good estimates of OTU richness (Supplementary Material, Figs. A1 and A2).

A total of 17 bacterial phyla was found among all samples. The most dominant one was *Proteobacteria* (97.2%), followed by *Firmicutes* (1.0%), *Bacteroidetes* (1.0%), and *Actinobacteria* (0.2%). Altogether these 4 phyla accounted for 94–99% of the total OTUs (Fig. 1), while the other 13 phyla ranged between 1 and 6%. By order of abundance, these minor phyla included *Spirochaetes*, *Chloroflexi*, *Acidobacteria*, *Patescibacteria*, *Deinococcus-Thermus*, *Cyanobacteria*, *Armatimonadetes*, *Chlamydiae*, *Epsilonbacteraeota*, *Gemmatimonadetes*, *Halanaerobiaeota*, *Planctomycetes*, and *Verrucomicrobia*. The numbers of phyla were distributed according to the climatic areas or cotton fields as follows: the Sahelian group contained 10 phyla (Kongoussi and Ouahigouya harbored 10 and 7 phyla, respectively), the Sudano-Sahelian group contained

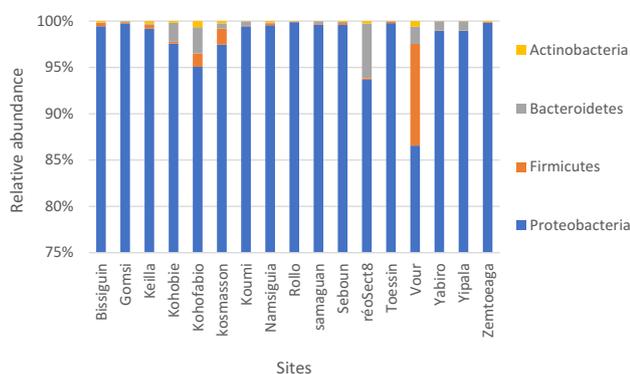


Fig. 1 Relative proportions of OTUs at the phylum level in all 17 samples (sites). Each bar represents one site, and each color stands for one phylum. Only the 4 major phyla (94–99% of the total sequences) are presented for the sake of clarity. (Color figure online)

14 phyla (Kombissiri and Réo harbored 7 and 14 phyla, respectively), the Sudanian group contained 8 phyla (Bobo and Houndé harbored 5 and 7 phyla, respectively), the cotton-growing field harbored 12 phyla, and the cotton-free field harbored 16 phyla.

Description of the core microbiome at the genus level

Out of the assigned 1676 OTUs, 14 represented 19.51% of the reads (total sequences), 22 represented 76.74% of the sequences, and 125 represented 87.65% of the sequences. These 14, 22 and 125 OTUs were found in 100%, 90% and 50% of the samples, respectively. The bacteria found in all samples belonged to 33 classes, 73 orders, 125 families and 224 genera. The most 20 bacterial genera (98.44% of total OTUs at the genus level) are presented in Fig. 2. The most dominant genus was *Enterobacter* (32.8% of OTUs), followed by *Aeromonas* (29.8%), *Pseudomonas* (11.8%), *Acinetobacter* (5.9%) and *Thorsellia* (2.2%).

A Venn diagram analysis of overlapping genera *per* area showed that 166, 155 and 128 genera were found in the Sahelian, Sudanian and Sudano-Sahelian areas, respectively, while 208 and 231 genera were detected in the cotton-growing and cotton-free fields respectively (Fig. 3). As regards climatic areas, 91 (30.4%) genera were common to all three areas, 22 (7.4%) were in common between the Sudano-Sahelian and Sahelian areas, 10 (3.3%) were in common between the Sahelian and Sudanian areas, and 26 (8.7%) were in common between the Sudanian and Sudano-Sahelian areas. In the cotton-related fields, 140 (46.8%) were in common between the cotton-growing and cotton-free fields. This suggests that 30.4% of the bacteria belonged to the three climatic zones, while 46.8% were in common between the cotton-growing and cotton-free areas.

Differential abundance of the taxonomic profiles

The Kruskal–Wallis test showed no significant difference ($FDR-p > 0.05$) in the distribution at the genus level according to the climate or cotton areas. However, the Wilcoxon Rank Sum test highlighted differential abundances in the OTUs assigned at the genus level across our samples. The site of Réo (a cotton-free field) harbored OTUs with higher abundances (Fig. 4a). A similar trend was observed when comparing the differential abundances among the climatic zones or between the cotton-growing fields in the Sudano-Sahelian area (Fig. 4b) and the cotton-free field (Fig. 4c), which displayed the highest median abundances of OTUs at the genus level.

Fig. 2 Pie chart of the predominant genera (98.44% of total OTUs) of *Anopheles gambiae* s.l. gut microbiota diversity at the genus level. Each color represents one genus, and the most abundant ones are represented from top to bottom. (Color figure online)

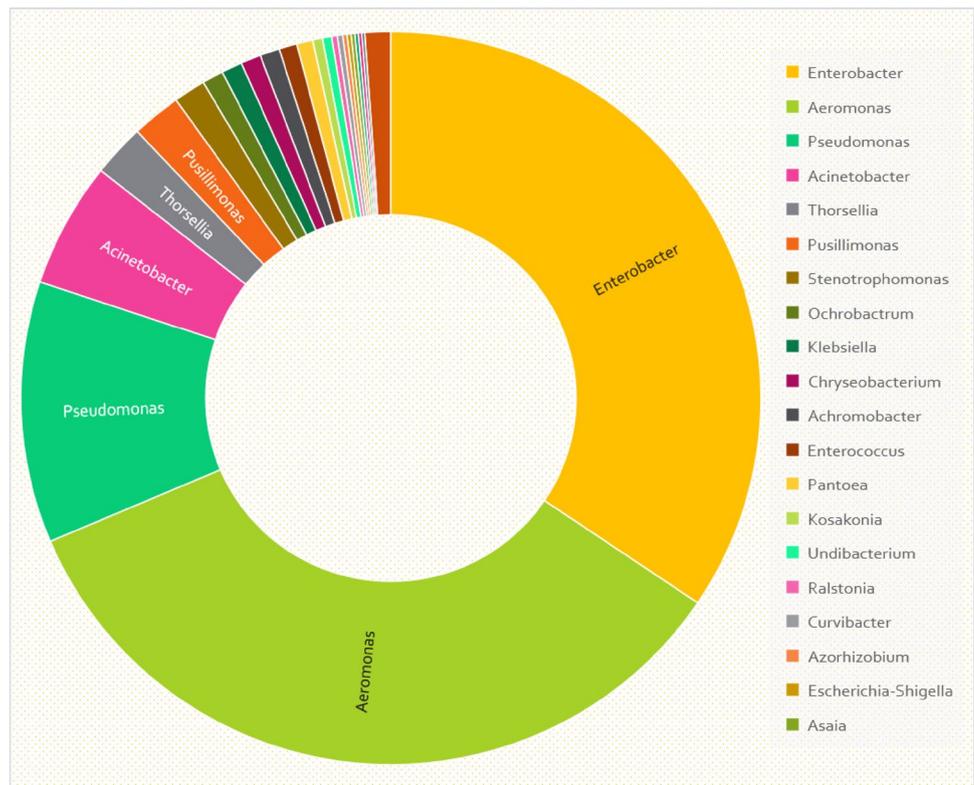
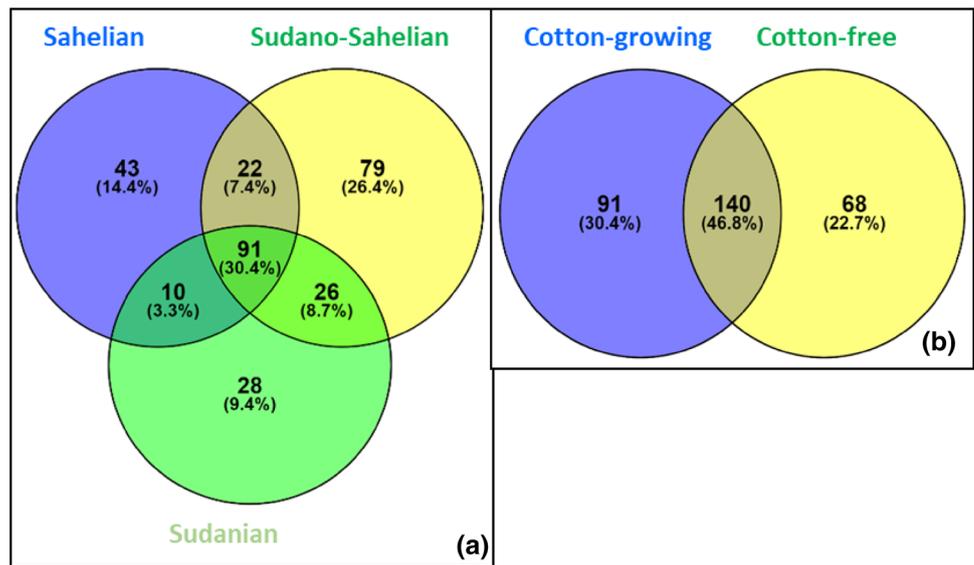


Fig. 3 Venn diagrams of the analysis of shared genera. **a** Climatic areas, **b** cotton-related fields



Bacterial community richness and diversity

Richness estimators (observed OTUs and Chao1) and diversity indexes (Shannon and Simpson) were calculated. Observed OTUs, Chao1, Simpson and Shannon indexes indicated a significant difference ($p < 0.05$) between cotton-growing and cotton-free fields *per site* in

the Sudano-Sahelian climatic area (Fig. 5). In fact, in that area, the cotton-free field of Réo was the richest and most diverse site as compared to the cotton-growing field of Kombissiri. The comparison of the *alpha* diversity indexes of the three climatic areas in each category of cotton field did not evidence any significant difference (Supplementary material A4).

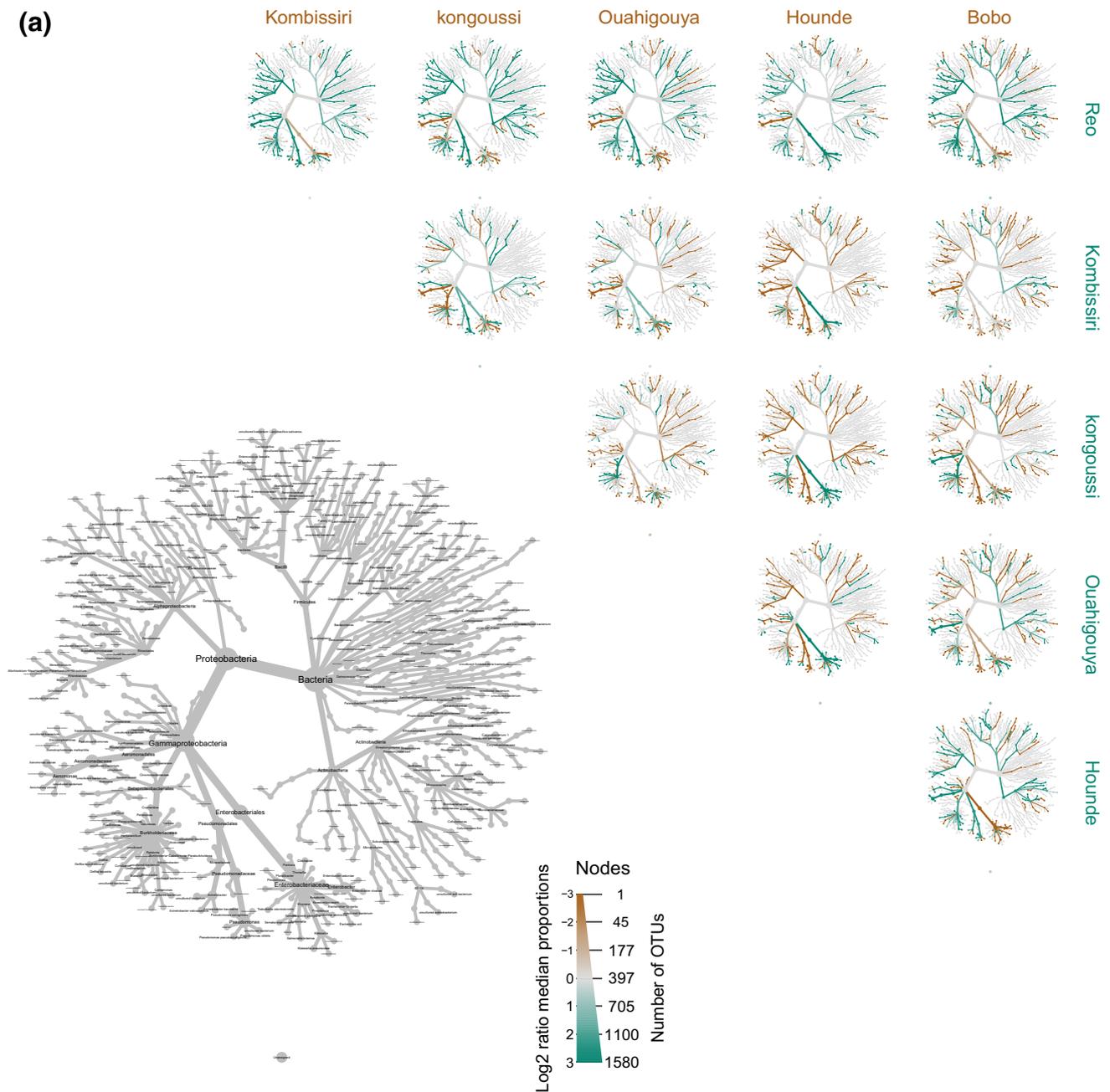


Fig. 4 a Heatmap tree of the median relative abundance tree *per site*. **b** Heatmap tree of the median relative abundance tree *per climatic area*. **c** Heatmap tree of the median relative abundance tree *per cotton-growing or cotton-free field*. Pairwise comparisons were made between the samples in the columns (brown) and the samples in the rows (blue) for all figures; each node shows one OTU, and its size is proportional to the number of reads; the node colors represent the differences in the median proportions of the log₂ ratios of the reads;

the brown color shows a greater abundance of OTUs in the samples represented in the columns, and the blue color shows a greater abundance of OTUs in the samples represented in the rows. The deeper the color, the higher the OTU abundance. In figure **c**, samples from the cotton-growing field are highlighted in blue and samples from the cotton-free field are highlighted in brown. For figures **a** and **b**, the trees in tan color are annotated trees of the shared OTUs across all samples. (Color figure online)

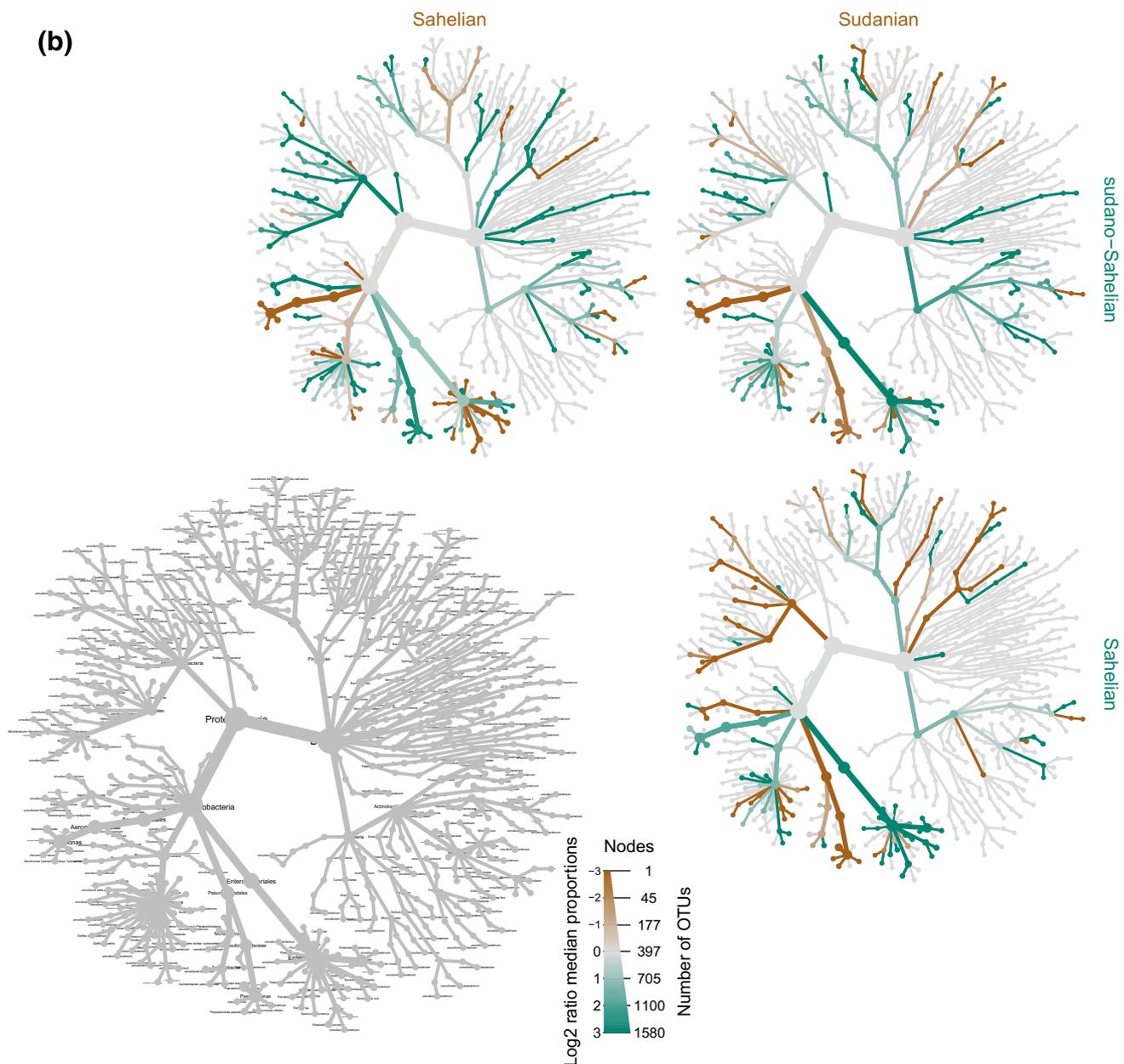


Fig. 4 (continued)

Beta-diversity analysis: microbial community structure in the midgut of mosquitoes from the *Anopheles gambiae* s.l

Our weighted UniFrac distances plotted in PCoA (Fig. 6) showed no cluster of samples, suggesting that the bacterial microbiota of the midgut of *An. gambiae* s.l was similar (Adonis PERMANOVA test, $p > 0.05$) across Burkina Faso.

Midgut microbiota of *Anopheles gambiae* s.l and interactions with *Plasmodium* infection

Based on the core microbiota description, we analyzed eight (8) genera that negatively interacted with *Plasmodium spp.* infection in *An. gambiae* s.l [8, 42]. The most abundant genus was *Aeromonas*, followed by *Enterobacter*, *Pseudomonas*, *Klebsiella*, *Pantoea*, *Asaia*, *Serratia* and *Comamonas*, whatever the climatic area or the presence or absence of cotton fields (Table 2). No significant difference was evidenced among these genera (FDR— $p > 0.05$). Nevertheless, contrasted differential abundances of some genera were observed,

(c)

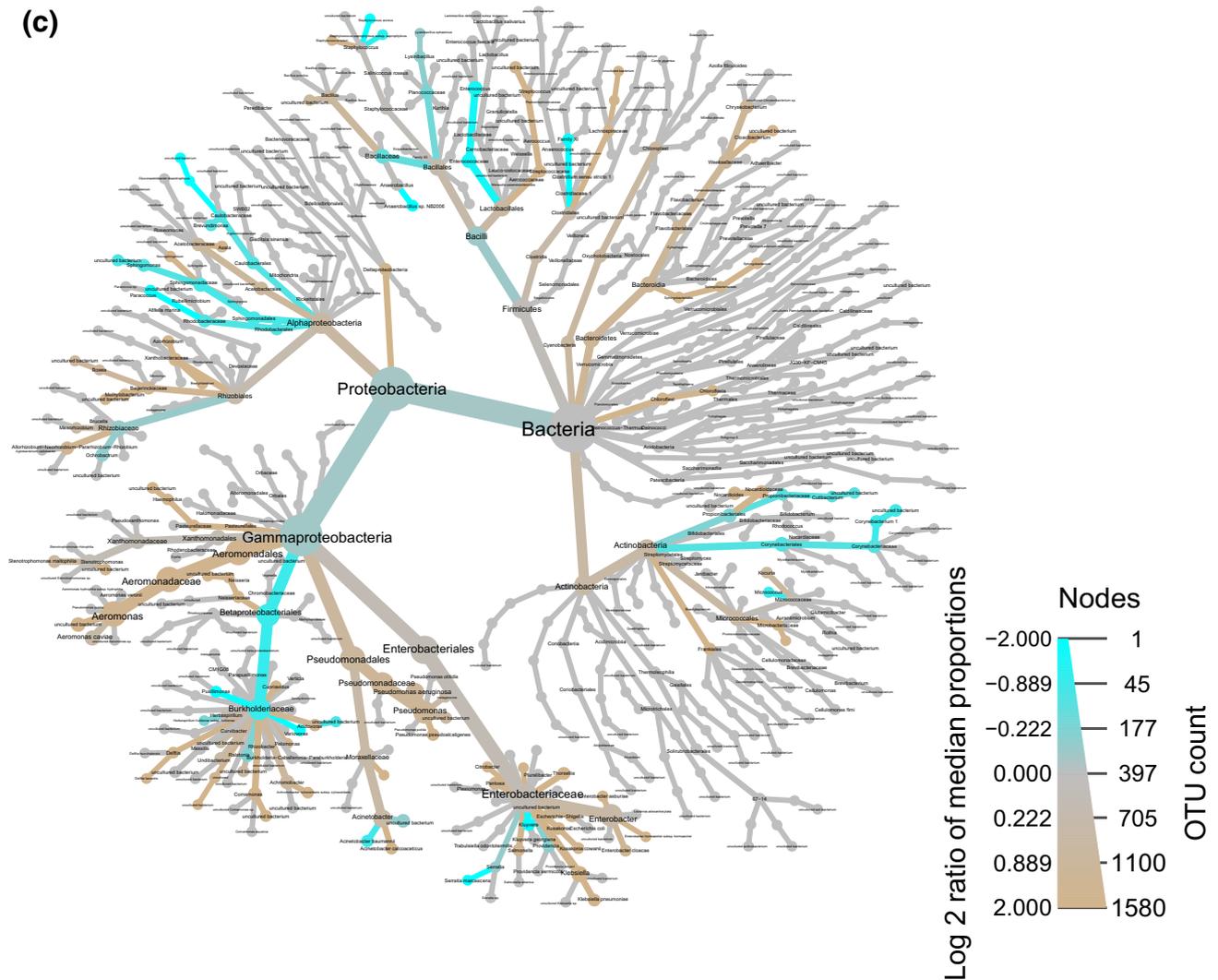


Fig. 4 (continued)

mostly for *Aeromonas*, e.g., in the Sudano-Sahelian area as compared to the Sahelian area. Rarefied abundances of these species and the situation of malaria in the areas surveyed in this study is shown in Table 2.

Discussion

Our study characterizes the microbiota of *An. gambiae* s.l females from different ecological areas that vary according to climatic conditions and to the presence or absence of cotton fields. The phylum *Proteobacteria* was predominant (97.2% of total sequences) followed by *Firmicutes*, *Bacteroidetes* and *Actinobacteria*. Other studies carried out at the phylum level found that *Proteobacteria* were often the most diverse and common among *An. gambiae* s.l [15, 21]. In Vietnam, seven phyla from the *Anopheles* microbiota

mostly belonged to *Proteobacteria*, *Firmicutes* and *Actinobacteria* [43]. In Kenya, *Proteobacteria* and *Bacteroidetes* dominated in *An. gambiae* s.l guts, with core taxa of *Enterobacteriaceae* and *Flavobacteriaceae* [9]. In our study, *Enterobacteriaceae* and *Aeromonadaceae* were the most abundant OTUs, while in another study the abundance of *Enterobacteriaceae* in the mosquito midgut significantly correlated negatively with the *Plasmodium* infection status [36]. In addition, other similar studies of other mosquito species such as *Culex quinquefasciatus*, *Aedes albopictus* and *Aedes aegypti* showed that *Proteobacteria* appeared to be the most dominant phylum [21, 30, 40, 42, 43].

Furthermore, at the genus level, *Enterobacter* was the most dominant genus in our samples, followed by *Aeromonas*, *Pseudomonas*, *Acinetobacter*, *Thorsellia*, down to *Asaia*. Among the five most common genera, *Thorsellia* was the only one found only in vector mosquitoes. This

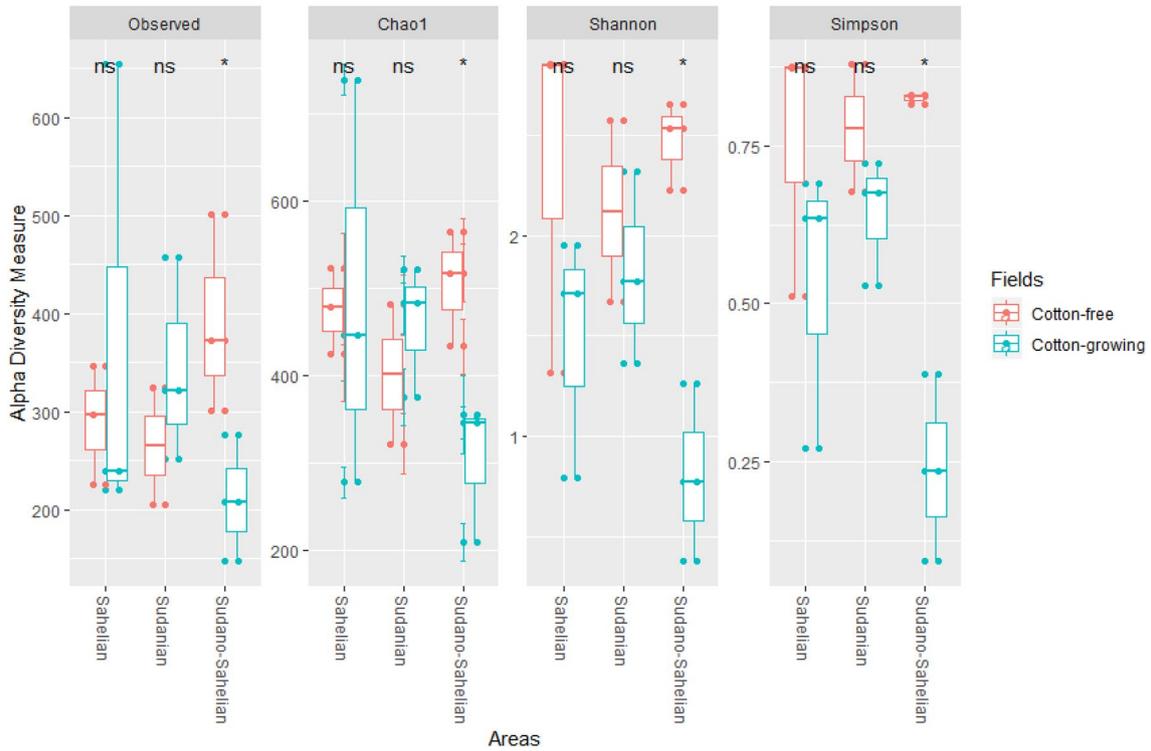


Fig. 5 Comparison of the richness and diversity of the bacterial communities harbored by *Anopheles gambiae* s.l in Burkina Faso according to climatic areas; ns: not significant; *statistically significant ($p < 0.05$)

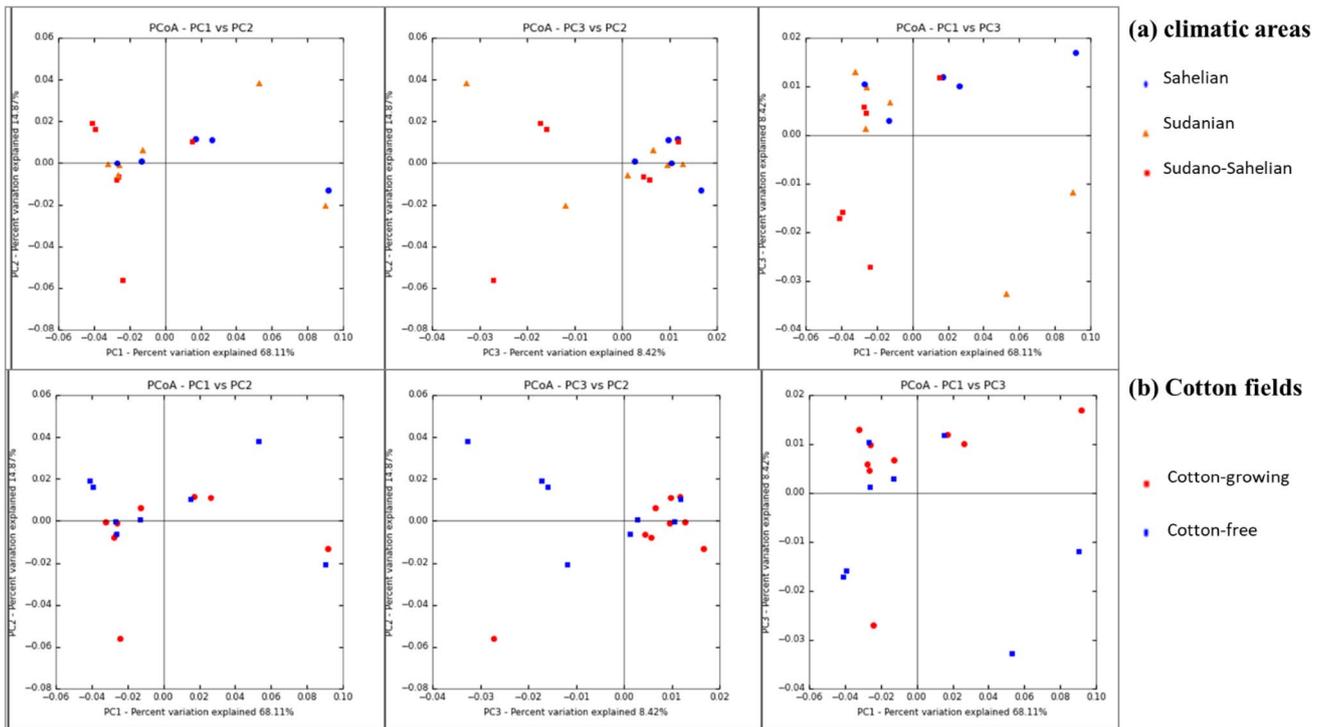


Fig. 6 2D principal coordinate analysis (PCoA) plots of the weighted UniFrac distances of *Anopheles gambiae* s.l midgut bacterial diversity according to **a** climatic areas and **b** the presence or absence of cotton fields. Each dot on the graph represents one sample. *PC* principal coordinates

Table 2 Incidence of malaria (2017) in the three climatic areas in Burkina Faso, and the bacterial OTU abundances at the genus level which could interact with *Plasmodium* spp

	Incidence rate of malaria					Relative abundance (OTUs)									
	< 1 an (%)	1–4 ans (%)	5–14 ans (%)	> 15 ans (%)	Global population (%)	<i>Serratia</i>	<i>Pseudomonas</i>	<i>Pantoea</i>	<i>Klebsiella</i>	<i>Enterobacter</i>	<i>Comamonas</i>	<i>Asaia</i>	<i>Aeromonas</i>	<i>Wolbachia</i>	<i>Thorsellia</i>
Sahelian	139	152	42	41	61	0.0002	0.0365	0.0085	0.0212	0.0981	0.0021	1E–04	0.6788	0.00,002	0.0006
Sudano-Sahelian	157	171	50	28	60	0.0008	0.1137	0.0012	0.0041	0.5341	0.0012	6E–04	0.0786	0	0.0638
Sudanian	201	204	35	30	58	0.0007	0.1892	0.0111	0.0015	0.3815	0.00002	0.004	0.2484	0	0.0034
Cotton-growing	190	201	47	33	66	0.0005	0.1196	0.0049	0.0065	0.3113	0.0013	0.003	0.3954	1.1E–06	0.0032
Cotton-free	141	150	38	33	54	0.0006	0.1067	0.0089	0.0113	0.3645	0.0009	5E–04	0.2751	0.00001	0.0421

< means less than; > means more than

bacterium was first isolated from the midgut of *An. arabiensis* in 2006 [45], and has also been reported as predominant in the midgut of adult *An. gambiae* s.l. mosquitoes captured in dwellings in central Kenya [46] in temperate climate area. Table 2 shows also that *Thorsellia* was relatively more abundant in the Sudano-Sahelian area than in the other climatic areas (Sudanian and Sahelian).

Previous studies found *Enterobacter* and *Serratia* in *Anopheles* adults collected in the field [15, 21]. The *Asaia* genus was isolated in the midgut, salivary glands and reproductive organs of different wild and laboratory colonies of *An. gambiae* s.l from Burkina Faso [47]. *Pantoea* and *Enterobacter* were the most common genera identified in field *An. gambiae* s.l from Kenya and Mali [48]. In Cameroon, the midgut microbiota of *An. gambiae* s.l was composed of members of only one phylum, i.e., *Proteobacteria*, and dominated by *Comamonas*, *Serratia*, *Pseudomonas*, *Burkoldeira* and *Brevundimonas* [49]. In Mali, the bacteria identified from *An. gambiae* s.l. were *Escherichia*, *Salmonella*, *Klebsiella*, *Pseudomonas*, *Staphylococcus*, and *Shigella* [50]. Furthermore, other studies outside Sub-Saharan Africa have shown similar results. For example, in Belgium, *Acinetobacter*, *Pseudomonas*, and *Asaia* were found in most mosquitoes, with a high prevalence of *Pseudomonas* in *Anopheles* [51], whereas in Vietnam the most prevalent genera were *Staphylococcus*, *Clostridium*, and *Bacillus* among *Firmicutes* [43]. The same study found that the *Anopheles* microbiota was greatly influenced by the local environment [43]. *An. gambiae* s.l collected in the field was also found to harbor low bacterial diversity, but significant differences were found in the core microbiota depending on the collection sites [35, 46]. Nevertheless, our results showed no significant difference in the core microbiota (abundance) among climatic areas and between cotton-growing or cotton-free fields. In contrast, richness indexes (observed, Chao1) and diversity indexes (Simpson and Shannon indexes) evidenced a significant difference in OTU richness and diversity at the genus level. The mosquitoes from the Sudano-Sahelian area hosted the highest numbers of OTUs, and so did the cotton-free fields (highest richness and diversity). This result could be explained by a greater richness of the microbiota in the Réo site than in the Kombissiri site. Moreover, Réo is a cotton-free field (Sudano-Sahelian area). Therefore, we can hypothesize a negative effect of cotton inputs such as insecticides on the bacterial richness and diversity of *An. gambiae* s.l midgut. The different environments are known to give each *An. gambiae* s.l specific bacterial profiles that provide precise and predictive information on the spatial dynamics of the population [53]. But no clustering was found from our UniFrac distance metrics plotted in the PCoA analysis, suggesting that *Anopheles* females share a similar bacterial community throughout Burkina Faso. The difference may be explained by the fact that the study by Buck et al. was

performed on single whole mosquitoes of *An. coluzzii*, while our study was performed on pooled *An. gambiae* s.l of one body part (the midgut) in the whole of Burkina Faso.

We did not find any phylogenetic structuring of the *Anopheles* midgut bacterial community according to the climatic zone or to the presence or absence of cotton fields. This result provides the basis for a more in-depth study in which a paratransgenesis application on *An. gambiae* s.l should be experienced in Burkina Faso. In addition, we found greater relative richness and diversity of the microbiota in the cotton-growing fields than in the cotton-free fields. The role of the midgut microbiota in conferring insecticide resistance in mosquitoes is unclear, while the link with insecticide resistance in agricultural pests is established [54]. A recent study reported for the first time a link between the microbiota (*Bacillus anthracis*, *B. cereus*, *B. thuringiensis*) and phenotypic resistance to the insecticide fenitrothion in *An. albimanus*, suggesting that bacterial taxonomic richness confers mosquitoes a competitive advantage in response to insecticide selection pressure [55]. We found higher relative bacterial abundance in the cotton-free fields more than in the cotton-growing field. Assuming that the microbiota diversity in *An. gambiae* l.s may have a link with the susceptibility of insecticide, this result suggests that cotton-growing field mosquitoes have a less bacterial diversity, then, they should be more resistant to insecticides. In Burkina Faso, the active ingredients of formulations for cotton crop protection belong to four insecticide classes: pyrethroids, organochlorines, organophosphates and carbamates, all used by spraying [56]. Furthermore, resistance to these molecules used to control *Anopheles* has emerged in the country [57]. In Burkina Faso, pyrethroid resistance has increased in recent years [58] as the frequencies of the 1014F *kdr* mutation and high *ace-1R* in 15 sites in 3 climatic areas [60]. Also, in rice-growing fields that use insecticide as in cotton-growing, it is noticed a high level of frequency of *kdr* L1014F mutation and *Ace-1R* mutation in *An. gambiae* s.l [61].

At the level of malaria transmission, *P. falciparum* is the most prevalent malaria parasite in 99.7% of cases in the African continent [1]. Knowing that some bacteria strongly influence malaria transmission, we hypothesized that the midgut microbiota of *An. gambiae* s.l reflected the bacterial community of the environment in which it lived and that this correlation could be one explanation for the environment-related epidemiological characteristics of malaria. Among the 224 bacterial genera detected, 8 of them genera had an *An. gambiae* s.l-*Plasmodium* interaction by reducing transmission to humans [8]. The most frequent genus was *Pseudomonas*, followed by *Aeromonas*, *Asaia*, *Comamonas*, *Enterobacter*, *Klebsiella*, *Pantoea* and *Serratia* [18, 44]. Table 2 shows greater abundance of OTUs from the genera *Pantoea*, *Klebsiella*, *Aeromonas* and *Comamonas* in the Sahelian area, of *Asaia* and *Pseudomonas* in the Sudanian

area, and of *Enterobacter* in the Sudano-Sahelian area. All these genera (OTUs) were also more abundant in cotton-growing localities. Other studies showed that the abundance of the genera *Serratia* or *Asaia* was significantly correlated negatively with *P. falciparum* infection both in the midgut and salivary glands of malaria-vector mosquitoes [44, 50, 51]. *Asaia*, *Enterobacter*, *Pantoea*, *Serratia* and *Pseudomonas* have been proposed as promising symbiotic control agents of malaria vectors such as *An. gambiae* s.l by paratransgenesis [52, 53, 62]. *Asaia* is transmitted vertically from mother to progeny, indicating that it could quickly spread [14]. This great OTU abundance of natural microbes (*Pantoea*, *Klebsiella*, *Aeromonas* and *Comamonas*) which mediate refractoriness to *Plasmodium* infection in *An. gambiae* s.l [43], has been noticed in the Sahelian area where less pluviometry is recorded in Burkina Faso and thus could explain the low transmission rate [32]. These data (low malaria transmission region) are correlated with the statistics of the 2017–2018 report about National Malaria Indicators [63]. We found *Wolbachia* in *An. gambiae* s.l. It was also recently discovered in Burkina Faso, and the authors described it as a novel *Wolbachia* strain related to but distinct from strains infecting other arthropods [64]. Infection by this recent *Wolbachia* strain was negatively correlated with the presence of *Plasmodium* parasites [55, 56]. *Wolbachia* can manipulate mosquito reproduction and immunity and thus limit the spread of arthropod human pathogens [54, 57]. *Wolbachia* are obligate in filaria and are inherited intracellular bacterial endosymbionts colonizing around half of the insect species including several *Culex* and *Aedes* mosquito species [58, 59], but rarely found in *Anopheles* species [65]. Nevertheless, *Wolbachia* has also been found in *An. gambiae* s.l and *An. moucheti* from Guinea, the Democratic Republic of Congo, Ghana, Uganda, and Madagascar [66]. This evidence of *Wolbachia* presence promotes further investigations on the possible use of natural *Wolbachia*–*Anopheles* associations to limit malaria transmission [8].

Conclusion

This study explored the midgut bacterial community of *An. gambiae* s.l, a malaria vector from Burkina Faso. No statistical difference in OTU abundances was found among the different areas, fields or localities. Therefore, the same control strategy to target mosquito midgut bacterial community and affect their fitness by bacterial refractoriness species to *Plasmodium* may be a valuable way for future malaria control efforts in Burkina Faso. Richness and diversity indexes showed significant differences in the cotton-growing fields and in the agroclimatic zones, mainly in the Sudano-Sahelian area. Potential bacterial species refractoriness to

Plasmodium were detected, with a particular great abundance in the Sahelian area where low rate of malaria has been recorded.

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Authors' contribution AAZ, AB and FF conceived and designed the study. AAZ, ZS, FY and AB supervised the data and samples collection. AAZ and ARS performed practical work, analyzed, interpreted the results and wrote the manuscript. AB, SM and FF revised the manuscript. All authors were the major contributors in writing the manuscript. All authors read and approved the final manuscript.

Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files. Fastq reads were submitted and are available in the NCBI (National Center for Biotechnology Information) Sequence Read Archive (SRA) under BioProjectPRJNA558839.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethics approval This study was approved by the National Health Ethic Committee (CERS) in Burkina Faso (reference number 2017-9-143 of 12 September 2017).

Informed consent For mosquito collection in residential areas, written informed and consent was obtained from homeowners in each location.

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