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Research article

# The impact of bloodmeal and geographic region on the richness, diversity, and function of internal microbial community in *Haemaphysalis qinghaiensis* from the Qinghai province, China

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ABSTRACT

*Background:* Ticks are ectoparasites that feed on blood and pose a threat to both the livestock industry and public health due to their ability to transmit pathogens through biting. However, the impact of factors such as bloodmeal and geographic regions on the bacterial microbiota of *Haemaphysalis qinghaiensis* remains poorly understood.

*Methods:* In this study, we used the v3-v4 region of the 16S rRNA gene to sequence the microbiota of *Haemaphysalis qinghaiensis* from eight groups (HY_M, YS_M, XH_M, LD_M, BM_M, LD_F_F, LD_F, and BM_F_F) in Qinghai Province.

*Results:* Significant differences in bacterial richness were observed between LD_F_F, BM_F_F, and LD_F ($P < 0.01$), and among the five groups (HY_M, YS_M, XH_M, BM_M, and LD_M) ($P < 0.05$). The bacterial diversity also differed significantly between LD_F_F, LD_F, and BM_F_F ($P < 0.01$), as well as among the five groups (HY_M, YS_M, XH_M, LD_M, and BM_M) ($P < 0.01$). The group with the highest number of operational taxonomic units (OTUs) was LD_F, accounting for 23.93 % (419/1751), while BM_F_F accounted for at least 0.80 % (14/1751). At the phylum level, *Firmicutes* was the most abundant, with relative abundance ranging from 7.44 % to 96.62 %. At the genus level, *Staphylococcus* had the highest abundance, ranging from 1.67 % to 97.53 %. The

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endosymbiotic bacteria *Coxiella* and *Rickettsia* were predominantly enriched in LD_F_F. Additionally, the 16S gene of *Coxiella* showed the highest identity of 99.07 % with *Coxiella* sp. isolated from Xinxiang hl9 (MG9066 71.1), while the 16S gene of *Rickettsia* had 100 % identity with *Candidatus Rickettsia hongyuanensis* strains (OK 662395.1). Functional predictions for the prokaryotic microbial community indicated that the main functional categories were Metabolic, Genetic information processing, and Environmental information processing across the eight groups.

*Conclusion:* This study provides a theoretical basis for the prevention and treatment of tick-borne diseases, which is of great significance for public health.

## 1. Introduction

Ticks possess the ability to transmit tick-borne pathogens (TBPs) as well as various other microorganisms, including bacteria, viruses, protozoa, and so forth. Recent research has indicated that the presence of these microorganisms, particularly bacteria, may contribute to the dissemination of TBPs and potentially affect human health [1]. Therefore, investigating the microbial community in ticks can assist in the identification of specific TBPs that cause human diseases [1,2]. This knowledge will significantly enhance our ability to anticipate and mitigate public health risks associated with tick-borne diseases (TBDs), while offering an innovative approach to controlling and preventing such diseases [1,3].

Ticks carry microorganisms that are vital to their physiology, including *Coxiella* and *Rickettsia*. *Rickettsia*, a symbiotic bacterium, is primarily transmitted by various types of ticks. For instance, *Rickettsia parkeri* has been detected in *Amblyomma maculatum* ticks [4,5]. *Rickettsia buchneri* is considered an obligate symbiont that enhances the overall health of *Ixodes scapularis* ticks by providing essential nutrients such as vitamins and amino acids [6]. However, there is currently no strong evidence supporting an obligatory relationship between bacteria and ticks. Further investigation into the presence of *Rickettsia* is necessary to examine the vectors and reservoir hosts involved. Additionally, *Rickettsia* endosymbionts have the potential to alter the microbial composition of ticks and the transmission of other *Rickettsia* pathogens. This is demonstrated by the negative correlation between the infection rates of *Rickettsia* (a pathogen) and *Rickettsia peacock* (a spotted fever group (SFG) *Rickettsia* found in *Dermacentor andersoni* [7]. *Coxiella* is an obligate intracellular bacterium that can be found in ticks worldwide, including Colombia, Brazil, Kenya, and China [8–10]. It plays a crucial role in reproduction and development. Various methods for sequencing and phylogenetic analysis of *Coxiella* have been documented in recent decades. For example, the bacterial symbionts of *Coxiella burnetii* in *Rhipicephalus sanguineus*, *Haemaphysalis longicornis*, and *Ornithodoros moubata* have been identified based on 16S rDNA sequences [11]. Pyrosequencing with barcoded eubacterial primers targeting the variable 16S rRNA gene has also revealed the presence of *Rickettsia* and *Coxiella* microorganisms in *Amblyomma americanum* ticks [12]. In China, studies on ticks such as *Haemaphysalis longicornis* (Hebei), *Dermacentor silvarum* (Hebei), and *Rhipicephalus microplus* have identified the presence of *Coxiella* and *Rickettsia* microorganisms [10,13,14]. These microorganisms were identified using 16S rRNA sequencing technology to obtain information about their composition. However, there is limited data on *Coxiella* and *Rickettsia* in *H. qinghaiensis* in China, necessitating further research to fully understand the symbiotic bacteria and their functions in *H. qinghaiensis*. Additionally, ticks have been found to commonly host bacteria such as *Staphylococcus*, *Pseudomonas*, *Enterobacter*, *Acinetobacter*, and *Stenotrophomonas*, which are also prevalent in their surrounding environments, suggesting that ticks may acquire these environmental bacteria [7].

However, the composition of the tick microbiome is not static and can vary due to factors such as geographical location, bloodmeal type, and tick gender. These factors contribute to the diversification of tick microbiome composition [15], indicating that a diverse microbial community may provide competitive advantages for ticks in their environment [16]. The presence of ticks in different geographical locations can significantly impact their microbial composition, particularly on a large scale [17,18]. Previous studies in this field have produced inconsistent findings, leading to uncertain conclusions. For example, investigations into the microbiome of *D. andersoni* ticks have shown that the environment in which tick populations are found plays a crucial role in determining the complexity of their bacterial microbiome composition [3]. A study conducted by Van Treuren et al. [17] analyzed the microbial communities of *Ixodes scapularis* and *Ixodes affinis* ticks collected from 19 different regions. The study observed that the variations in tick microbiota increased as the geographical distance between the locations increased. However, it is important to note that these alterations may be influenced by factors such as soil composition and other environmental factors, which require further investigation. The consumption of a blood meal is essential for ticks to complete their life cycle and also plays a crucial role in the transmission of pathogens [19]. How does the blood meal affect the microbial composition within ticks? A study conducted by Zhang et al. [20] demonstrated that prolonged feeding significantly altered the bacterial composition of *Ixodes persulcatus* while maintaining bacterial composition diversity. Another study revealed that the blood meal effectively influenced both the richness and diversity of the tick microbiome. The decrease in microbial diversity caused by bloodfeeding could potentially impact the vector competence of *Ixodes pacificus* and subsequently affect pathogen transmission in natural systems [21]. Furthermore, there is a complex interaction between endosymbionts and pathogenic microorganisms within tick hosts. Factors associated with blood feeding resulted in significant changes in the composition of symbiotic bacteria, which in turn influenced pathogen colonization and transmission [22]. These findings suggest that the consumption of a blood meal can indeed influence the microbial composition within ticks.

The objective of our research was to analyze the bacterial community composition of *H. qinghaiensis* collected from Qinghai, China, using high-throughput sequencing. We aimed to assess how the microbial composition of ticks in the natural environment of

*H. qinghaiensis* was influenced by sampling regions and blood meal. *H. qinghaiensis* is a unique species discovered in China that poses significant threats to animals and humans in the northwest plateau [14]. Previous studies on other ticks have demonstrated that interactions between microorganisms and coexisting bacteria could impact the development of pathogen lineages, particularly when pathogens rely on vectors or hosts for transmission. Thus, investigating the microbial diversity of *H. qinghaiensis* could enhance our understanding of its potential as a disease carrier for vertebrate hosts.

## 2. Materials and methods

### 2.1. Sample collection

A total of 186 tick samples were collected for analysis. Some of these ticks were engorged ticks found on the surface of Tibetan sheep, while the remaining ticks were collected from vegetation by dragging a 1 $m^2$ white blanket in various areas of Qinghai Province, China (Table 1). All collected ticks were then brought back to the laboratory.

### 2.2. High-throughput sequencing

Ticks were first washed in 75 % ethanol and deionized water for 5 min each to remove any environmental contaminants. Following DNA extraction, which was conducted using a E.Z.N.A.® Soil DNA Kit (Omega BioTek, USA), PCR amplification was performed targeting the 16S rRNA V3 and V4 hypervariable regions. A selection of tick samples was then sent to Majorbio Bio-pharm Technology for high-throughput 16S rRNA sequencing.

The tick samples were divided into eight groups: LD_F (Ledu Females), LD_M (Ledu Males), LD_F_F (Ledu Fed Females), HY_M (Huangyuan Males), BM_M (Banma Males), YS_M (Yushu Males), XH_M (Xunhua Males), and BM_F_F (Banma Fed Females). To amplify bacterial 16S rRNA, barcode-indexed primers (338F: 5′-ACTCCTACGGGAGGCAGCAG-3′ and 806R: 5′-GGACTACHVGGGTWTCTAAT-3′) were used, along with TransStart Fastpfu DNA polymerase (TransGen, Beijing, China). PCR was conducted on the ABI GeneAmp® 9700 PCR instrument (Applied Biosystems, California, USA). A negative control containing sterile water was included in each reaction. This primer set generated fragment lengths ranging from 401 to 440 bP. All samples were subjected to formal experimental conditions, with each sample being repeated three times.

PCR products were detected by 2 % agarose gel electrophoresis and recovered by cutting the gel using an AxyPrepDNA gel extraction kit (AXYGEN, USA). Preliminary quantitative electrophoresis results indicated that PCR products were detected and quantified using the QuantiFluor™-ST blue fluorescence quantitative system (Promega, USA). Finally, paired-end sequencing was performed on the Illumina MiSeq PE300 using a standard protocol platform (Majorbio Bio-pharm Technology, Shanghai, China).

### 2.3. Data analysis

The data was analyzed using the free online platform Majorbio I-Sanger Cloud (https://cloud.majorbio.com). The forward and reverse sequences obtained through double-ended sequencing were paired and assembled using Flash (1.2.11). To facilitate the analysis, Uparse (version 7.0.1090) was employed to cluster 97 % of OTU representative sequences with similar levels. Additionally, Mothur (1.30.2) (http://www.mothur.org/wiki/Calculators) was utilized to analyze alpha diversity, providing information on community species richness, coverage, and diversity. The Kruskal-Wallis H test was used to test for intergroup differences and to determine the significance of differences in the alpha index between groups ($p < 0.05$). Venn diagrams were employed to calculate the number of shared and unique species OTUs in each group, allowing for a more intuitive understanding of the similarity and overlap of species OTU composition in the environmental groups. Dominant species and their relative abundance were analyzed at the phylum and genus classification levels. Beta diversity was assessed using Bray Curtis analysis, and Principal Coordinate Analysis (PCoA) was conducted to compare and visualize differences between groups [23]. Furthermore, similarity analysis (ANOSIM) based on unweighted and weighted UniFrac was performed using 999 permutations to determine the variability rate (%) of bacterial composition explained by

**Table 1**
Sampling location and sample data information.

| County/Altitude (m) | Longitude and latitude | Number of ticks (n) | | | |
|---|---|---|---|---|---|
| | | Female | male | Fed Female | Total |
| Xunhua/2793 | E 102°31′16″<br>N 35°45′23″ | | 30 | | 30 |
| Huangyuan/2755 | E 101°21′35″<br>N 36°40′50″ | | 30 | | 30 |
| Ledu/2276 | E 102°46′14″<br>N 36°29′46″ | 30 | 30 | 3 | 63 |
| Yushu/4016 | E 96°51′40″<br>N 32°45′23″ | | 30 | | 30 |
| Banma/3751 | E 100°36′54″<br>N 33°4′31″ | | 30 | 3 | 33 |
| Total | | 30 | 150 | 6 | 186 |

sample type [24]. Finally, Tax4fun (0.3.1) was employed to predict the KEGG functions of the prokaryotic microbial community in the eight groups.

## 3. Results

### 3.1. Morphological characterization of H. qinghaiensis

By morphological examination, all the tick samples were identified as Haemaphysalis and on the basis of the research on *H. qinghaiensis* in the laboratory, on the basis of the above appraisal, a part of tick samples were identified as *H. qinghaiensis* was found [14,25]. Select some samples from *H. qinghaiensis* for the next experiment (Fig. 1A–D).

### 3.2. MiSeq sequencing data

No PCR bands were detected in the negative control, and a total of 24 samples were effectively sequenced (Table 1), yielding 1886987 raw reads and 1814677 optimized sequences. The coverage index of all samples exceeded 99.9 %, this outcome was further corroborated by an adequate coverage estimate for each sample, and the sequencing depth was sufficient to display microbial diversity and reflected the actual situation of the group.

### 3.3. OTU and alpha diversity analyses

Based on a similarity level of 97 %, alpha diversity analysis was performed on the 16S rRNA sequencing results. The Shannon and Chao indexes reflected microbial diversity and richness. LD_F (4.14) and HY_M (0.171) had the highest and lowest bacterial diversity, respectively, and the difference between LD_F_F (1.848) and LD_F (4.14) was significant; the bacterial diversity in the non-bloodsucking group was remarkably higher than in the bloodsucking group ($P < 0.05$). The bacterial diversity of BM_F_F was lower than LD_F_F, but there was not a significant difference between BM_F_F and LD_F_F ($P > 0.1$), The bacterial diversity of BM_F_F was lower than LD_F_F, but there was not a significant difference between BM_F_F and LD_F_F ($P > 0.1$) (Fig. 2A), it was possible that the decrease in bacterial diversity caused by *Rickettsia* resulted in no significant difference in bacterial diversity between the LD_F_F and BM_F_F. The bacterial diversity of five groups (HY_M, YS_M, XH_M, BM_M, and LD_M) was different, and there were differences among five regions ($P < 0.01$) (Fig. 2A and Table 2). The differences in bacterial diversity between XH_M and LD_M were significant ($P < 0.05$), between YS_M and LD_M were highly significant ($P < 0.01$), between BM_M and LD_M were significant ($P < 0.05$), and between HY_M and LD_M were highly significant ($P < 0.01$) (Fig. 2A).

The Chao index was used to evaluate the bacterial richness in these groups. LD_F (730.4) had the highest bacterial richness, followed by LD_M (617.8). There was a striking differences between LD_F (730.4) and LD_F_F (150.7); The bacterial richness of the non-bloodsucking group was clinically higher than that of the bloodsucking group ($P < 0.01$), so bloodsucking affected the bacterial richness. The bacterial richness of BM_F_F was lower than LD_F_F, but there was not a remarkable difference between BM_F_F and

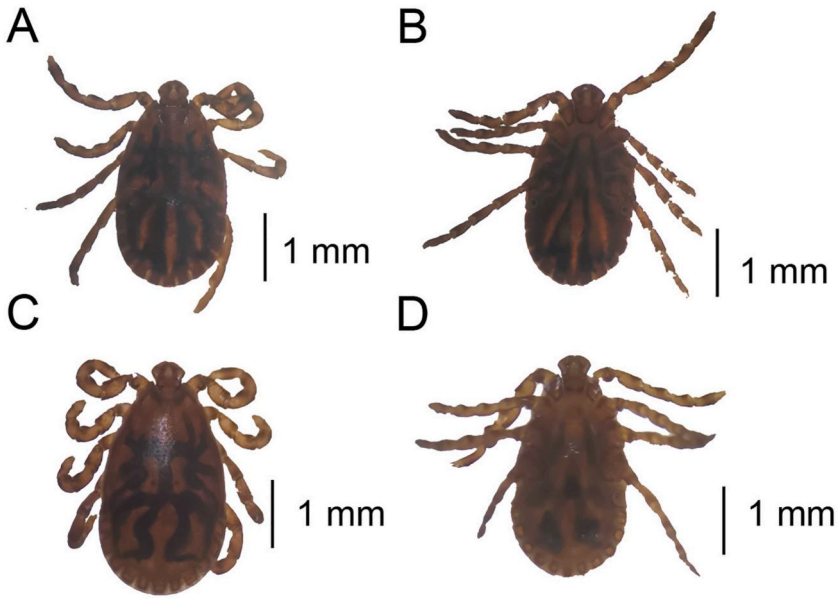


**Fig. 1.** Morphological characterization of *H. qinghaiensis*. The dorsal view and ventral view of *Haemaphysalis qinghaiensis*, All figures used the same ruler (1 mm). (A) Dorsal view of female. (B) Ventral view of female. (C) Dorsal view of male. (D) Ventral view of male.

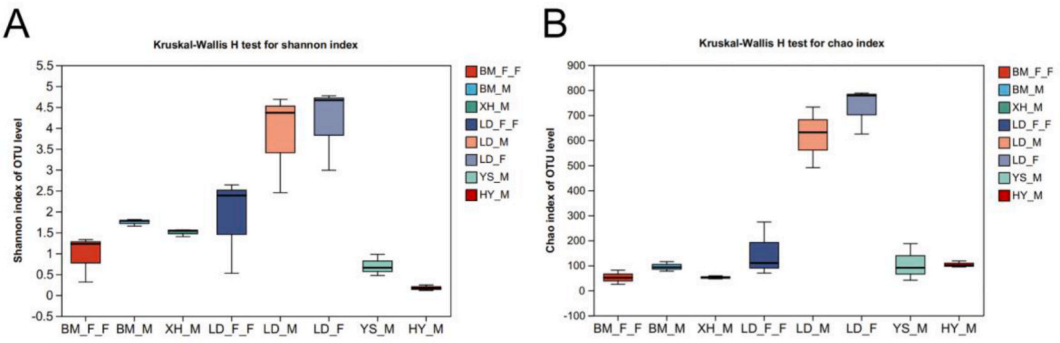


**Fig. 2.** Alpha diversity of the microflora in 8 groups. (A) Kruskal-Wallis H test for shannon index. (B) Kruskal-Wallis H test for chao index. Mean indices of samples within 8 groups of independent groups were significantly different (Wilcoxon rank-sum test: $P < 0.05$).

**Table 2**
Mean (±SD) alpha diversity indices of observed operational axonomic units (OTUs) in the bacterial communities of tick samples.

| Sample name | Shannon index | Chao index |
|---|---|---|
| BM_F_F | 0.9559 (±0.5591) | 52.04 (±28.28) |
| BM_M | 1.745 (±0.08293) | 94.96 (±19.07) |
| HY_M | 0.171 (±0.06641) | 103.8 (±12.5) |
| LD_F | 4.14 (±1.001) | 730.4 (±91.68) |
| LD_F_F | 1.848 (±1.154) | 150.7 (±108.1) |
| LD_M | 3.832 (±1.207) | 617.8 (±121.6) |
| XH_M | 1.5 (±0.08732) | 51.83 (±6.292) |
| YS_M | 0.7013 (±0.2559) | 106.1 (±74.3) |

LD_F_F ($P > 0.1$), it was possible that the decrease in bacterial richness caused by *Rickettsia* resulted in no significant difference in bacterial richness between the LD_F_F and BM_F_F. The bacterial richness of five groups (HY_M, YS_M, XH_M, BM_M, and LD_M) differed ($P < 0.05$). The differences in bacterial richness between XH_M and LD_M were significant ($P < 0.01$), between YS_M and LD_M were significant ($P < 0.01$), between BM_M and LD_M were significant ($P < 0.01$), and between HY_M and LD_M were significant ($P < 0.01$). This confirmed the impact of sampling locations and bloodmeal on bacterial richness, possibly due to different tick populations having different habitats and host bloodmeal. This also indicated that the growth and reproduction of ticks in different environments had a significant impact on the richness of bacteria (Fig. 2B and Table 2). We discovered that different sampling areas might lead to significant changes in the diversity and richness of bacteria within ticks, which might affect the transmission of pathogens to humans and animals after tick bites. Therefore, the impact of sampling areas should be considered when conducting disease monitoring and control.

An OTU was defined as reads with 97 % sequence similarity. Excluding differences between different sample sizes that affect group differences, the samples were balanced based on the lowest volume size among all samples, and a total of 1751 distinct OTUs were observed in these groups. Based on the balanced OTU data, the number of shared and unique OTUs in five groups (BM_M, XH_M, LD_M,

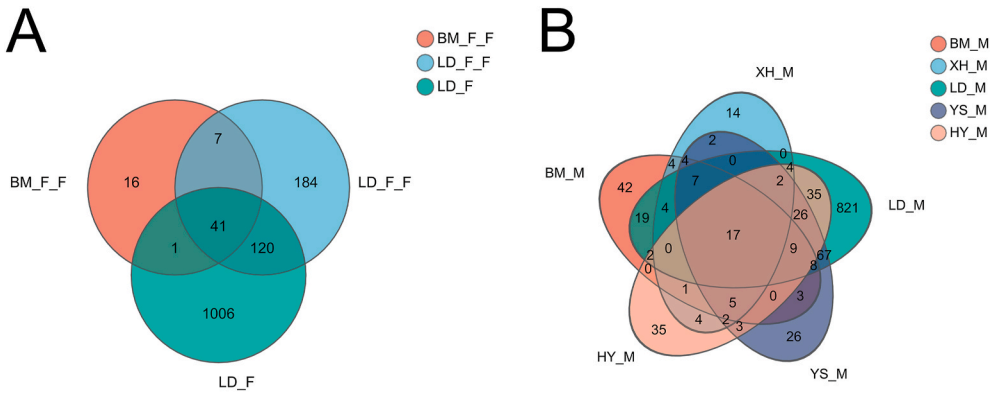


**Fig. 3.** Venn diagram of unique and shared operational taxonomic units (OTUs) between different groups for the bacterial communities. (A) BM_F_F, LD_F_F, and LD_F. (B) BM_M, XH_M, LD_M, YS_M, and HY_M.

YS_M, and HY_M) was calculated using Venn diagram, and the shared and unique OTUs of BM_F_F, LD_F_F, and LD_F were displayed using Venn diagram (Fig. 3A and B). The most significant number of OTUs was LD_F, accounting for 1168 OTUs, followed by LD_F_F, accounting for 352 OTUs. BM_F_F was the lowest, accounting for 65 OTUs. A total of 161 OTUs were shared by LD_F_F and LD_F, indicating that ticks could acquire bacteria from blood meal. Compared to LD_F_F, LD_F had more unique OTUs, indicating that bloodsucking could affect microbial species composition (such as OTUs) (Fig. 3A).

The largest number of OTUs was LD_M, accounting for 1021 OTUs, followed by XH_M, accounting for 70 OTUs; There were a total of 17 OTUs in five groups (BM_M, XH_M, LD_M, YS_M, and HY_M), and shared OTUs indicated that ticks could obtain bacteria from different regions (Fig. 3B). There were differences in the unique OTU numbers of *H. qinghaiensis* in different regions, indicating that the region affected the composition of microbial species (such as OTU). The diversity of OTUs of *H. qinghaiensis* varied in different regions, and the microbial community was influenced by the environment, adapting and changing, which might affect the ecological balance and stability.

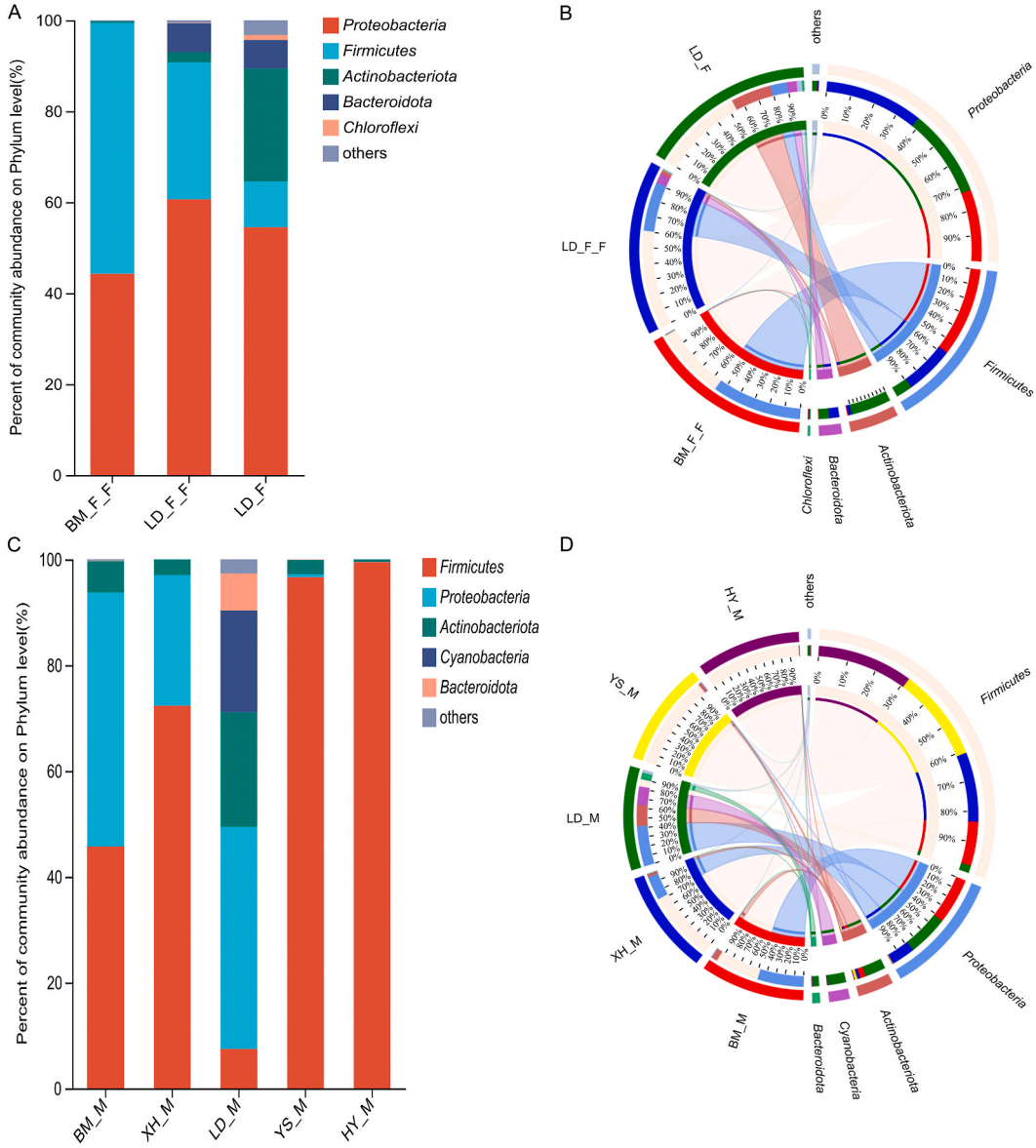


**Fig. 4.** Relative abundance (%) of bacteria communities composition at the phylum level. (A) BM_F_F, LD_F_F, and LD_F. Community Circos Plot of the community composition. (B) BM_F_F, LD_F_F, and LD_F. Relative abundance (%) of bacteria communities composition at the phylum level. (C) BM_M, XH_M, LD_M, YS_M, and HY_M. Community Circos Plot of the community composition. (D) BM_M, XH_M, LD_M, YS_M, and HY_M.

### 3.4. Bacterial microbiota composition

The bacterial communities were further assigned to 1 domain, 1 kingdom, 30 phyla, 75 classes, 191 orders, 320 families, 654 genera, 1047 species and 1751 OTU. At the phylum level, 6 phyla (*Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Cyanobacteria*, *Bacteroidetes* and *Chloroflexi*) had high relative affinity among all groups (Fig. 4A–D). The Circos sample and species relationship diagram was commonly used to display the distribution of microbial species present in different microbial samples. On one side of the circular diagram were the samples and their respective groups, while on the other side were the main dominant species. The connections through inner ribbons showed the abundance distribution of different species within the samples.The top 5 species at the phylum level included: *Firmicutes*, *Proteobacteria*, *Actinobacteriota*, *Cyanobacteria*, *Bacteroidota* (Fig. 4B–D). The relative abundance of *Firmicutes* increased with bloodsucking, while the relative abundance of *Chloroflexi* and *Cyanobacteria* showed geographical differences between groups (Fig. 4A–C). *Firmicutes* was dominant in all locations except one location where *Firmicutes* had a high relative abundance as compared to *Proteobacteria* (Fig. 4A–D).

As shown in the figure, *Firmicutes* has the highest relative abundance and existed in all groups, with percentages ranging from 7.44 % to 96.62 % for different groups, which was an absolute advantage for all groups. *Proteobacteria* followed closely, with percentage values ranging from 0.54 % to 60.63 % for different groups (Fig. 4A). The proportion of LD_F_F was the highest, reaching 60.63 %, while HY_M did not exist. The percentage values of different groups in *Actinobacteria* ranged from 0.33 % to 24.84 %, with the highest

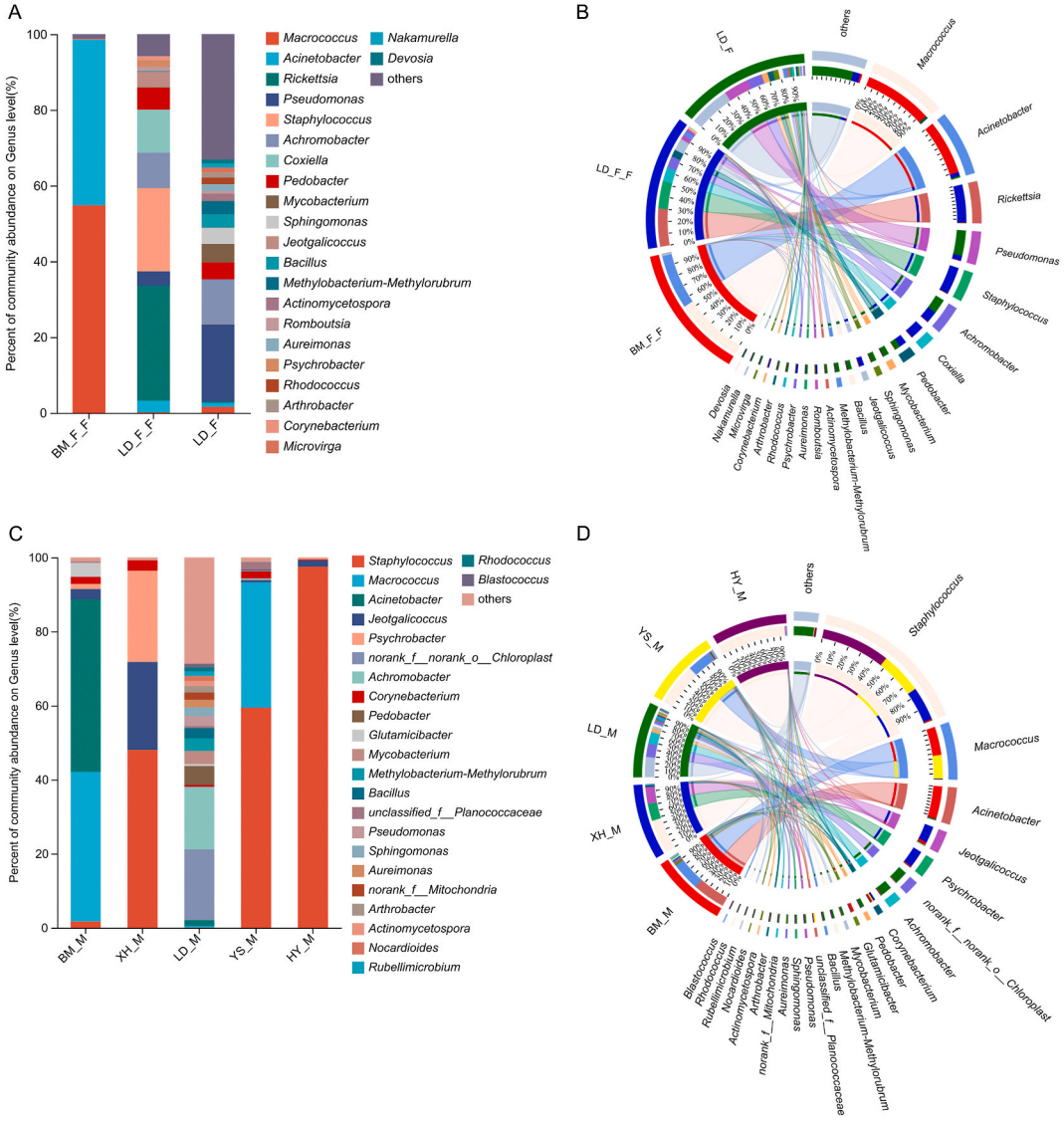


**Fig. 5.** Relative abundance (%) of bacteria communities composition at the genus level. (A) BM_F_F, LD_F_F, and LD_F. Community Circos Plot of the community composition. (B) BM_F_F, LD_F_F, and LD_F. Relative abundance (%) of bacteria communities composition at the genus level. (C) BM_M, XH_M, LD_M, YS_M, and HY_M. Community Circos Plot of the community composition. (D) BM_M, XH_M, LD_M, YS_M, and HY_M.

proportion of LD_F reaching 24.84 % and the lowest proportion of BM_F_F reaching 0.33 %. In addition, *Cyanobacteria*, *Bacteroidetes*, and *Chloroflexi* were mainly distributed in LD_M (19.25 %, 7.01 %, and 0.89 %), LD_F (0.53 %, 6.27 %, and 1.13 %), and LD_F_F (0.11 %, 6.33 %, and 0.11 %) (Fig. 4A–C). There were differences between different microorganisms in different regions.

At the genus level, a total of 654 genera were found in all groups, of which five genera (*Staphylococcus*, *Macrococcus*, *Acinetobacter*, *Achromobacter*, and *Rickettsia*) had higher relative abundance (Fig. 5A–D). The relative abundance of *Staphylococcus*, *Rickettsia*, and *Coxiella* increased with bloodsucking, while the relative abundance of *Macrococcus* and *Pseudomonas* decreased with bloodsucking. It is highly probable that ticks exhibit selectivity in their bloodsucking behavior, opting for specific bacteria. This suggested that the act of bloodsucking might exert varying impacts on different bacteria, consequently influencing the growth and propagation of ticks. The species belonging to the top 5 in terms of horizontal gene transfer included: *Staphylococcus*, *Macrococcus*, *Acinetobacter*, *Achromobacter*, and *Jeotgallicoccus* (Fig. 5B and D).

The abundance of 30 genera exceeded 1 %. *Macrococus* (54.79 %) was the most abundant genus in BM_F_F, followed by *Acinetobacter* (43.66 %). *Acinebacterium* (46.58 %) was the largest in BM_M, followed by *Macrococus* (40.43 %). *Staphylococcus* (48.06 %) was the most abundant genus in XH_M, followed by *Psychobacter* (24.65 %). *Rickettsia* (30.34 %) was the largest in LD_F_F, followed by *Staphylococcus* (21.98 %). *Chloroplasts* (19.12 %) were the most abundant genus in LD_M, followed by *Achromorphs* (16.87 %). *Acrhromobacter* (11.88 %) was the largest in LD_F, followed by *Mycobacterium* (4.84 %). *Staphylococcus* (59.44 %) was the most abundant genus in YS_M, followed by *Macrococcus* (33.83 %). *Staphylococcus* (97.53 %) was the most abundant genus in HY_M, followed by *Jeotgallicoccus* (1.59 %) (Fig. 5A–C). In summary, the majority of the dominant bacteria in *H. qinghaiensis* were environmental symbiotic bacteria, with a few being pathogens, such as *Rickettsia*.

Based on the symbionts *Rickettsia* and *Coxiella* mainly existed in LD_F_F, the species distribution and proportion of LD_F_F at various taxonomic levels were intuitively displayed from inside to outside by using the multi-level species Sunburst plot through multiple concentric circles. Selecting the classification level subordinate to OTU, it was found that OTU996 was annotated at the OTU level of *Rickettsia* of LD_F_F, accounting for 33.5 %. Their 16S genes had the highest identities of 100 % to *Candidatus Rickettsia hongyuanensis* strains (OK662395.1), 100 % to uncultured *Rickettsia* sp. Hja 192 (LC379493.1) from Ha. japonica in Japan, and 99.75 % to Uncultured *Rickettsia* sp. clone Hme-HirooL009R (MT378436.1) from *Haemaphysalis megaspinosa*, respectively (Fig. 6A). Selecting the classification level subordinate to OTU, it was found that OTU456 was annotated at the OTU level of *Coxiella* of LD_F_F, accounting for 12.5 %. A *Coxiella* strain closely related to *Coxiella* sp. isolate XinXian-HL9 (MG906671.1) identified from *H. longicornis* in China (with a sequence identity of 99.07 % for the 16S gene) was detected in LD_F_F (Fig. 6B).

### 3.5. Sample difference analysis

Then, the unweighted distance between groups was calculated according to the species abundance table of the microbiome of *H. qinghaiensis*. The variation among 8 groups was further evaluated using PCoA and ANOSIM analyses. The unweighted UniFrac PCoA (which does not account for abundance data) explained 38.45 % (PC1) and 20.23 % (PC2) of the BM_F_F, LD_F_F, and LD_F (Fig. 7A), The unweighted UniFrac PCoA explained 34.95 % (PC1) and 16.03 % (PC2) of the (BM_M, XH_M, LD_M, YS_M, and HY_M) (Fig. 7B), and LD_F_F clustered together and were distinctly separated from LD_F and BM_F_F; and five other groups (BM_M, XH_M, LD_M, YS_M, and HY_M) were separated (Fig. 7A). In addition, the ANOSIM result ($R = 0.7860$, $P = 0.001$) also showed significant differences in bacterial composition between BM_F_F, LD_F_F, and LD_F (Fig. 7A), the ANOSIM result ($R = 0.8904$, $P = 0.001$) also showed significant differences in bacterial composition between BM_M, XH_M, LD_M, YS_M, and HY_M (Fig. 7B). The weighted UniFrac PCoA and ANOSIM ($R = 0.4733$, $P = 0.001$) obtained a similar result considering the abundance of bacteria in BM_F_F, LD_F_F, and LD_F (Fig. 7C). The weighted UniFrac PCoA and ANOSIM ($R = 0.7033$, $P = 0.001$) obtained a similar result considering the abundance of bacteria in BM_M, XH_M, LD_M, YS_M, and HY_M (Fig. 7D).

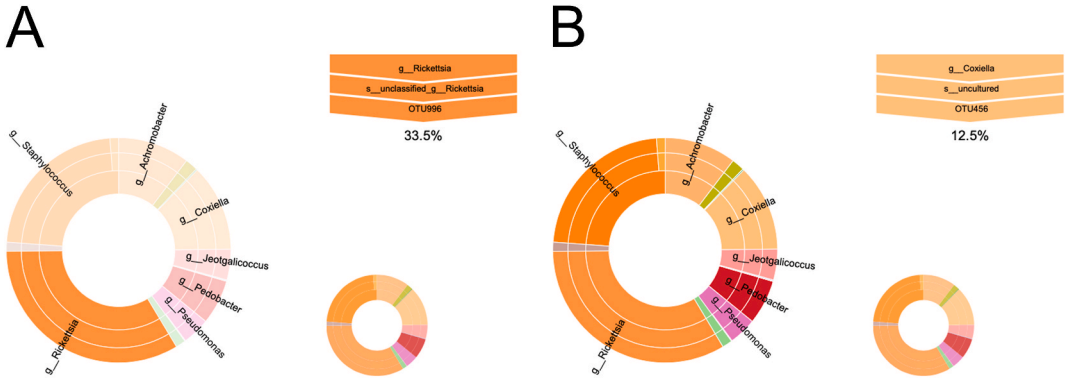


**Fig. 6.** Sunburst diagram of multilevel species in LD_F_F. The diagram displayed the species composition and proportion from the inner circle to the outer circle, representing the Genus, Species, and Operational Taxonomic Unit (OTU) levels. (A) The composition and proportion at the Genus, Species, and OTU levels for *Rickettsia*. (B) The composition and proportion at the Genus, Species, and OTU levels for *Coxiella*.

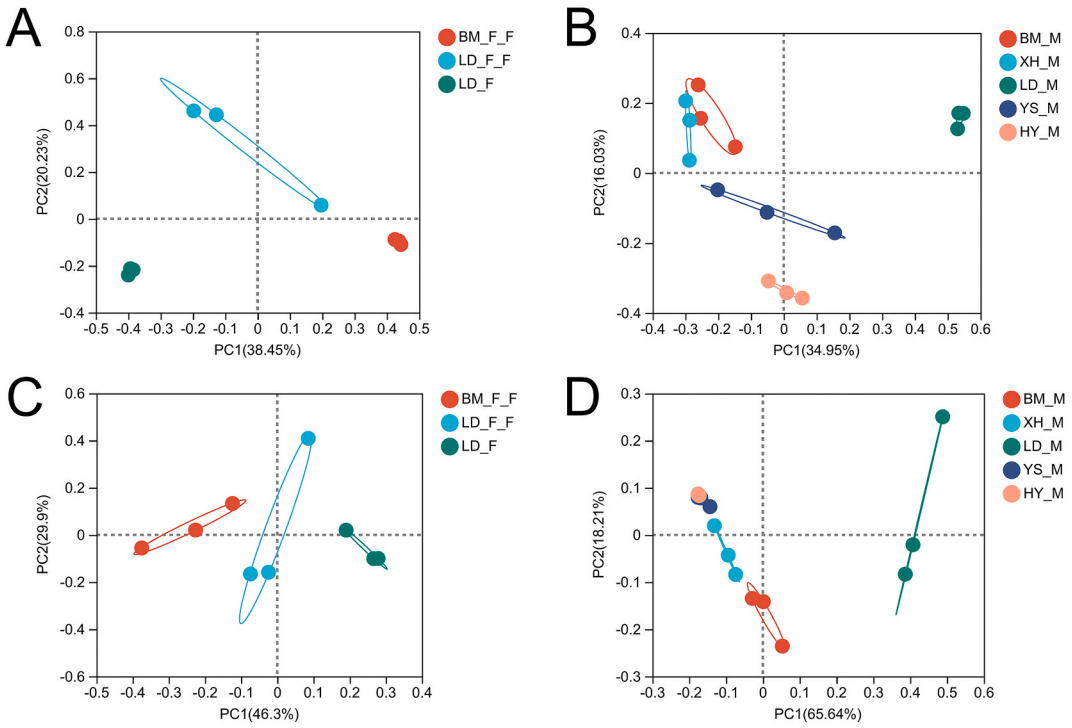


**Fig. 7.** The difference in microbial communities between different groups based on unweighted and weighted UniFrac distances. Principal coordinate analysis (PCoA) for the bacterial communities. (A,B) unweighted UniFrac distances. (C,D) weighted UniFrac distances. The points with different colors indicated different groups.

### *3.6. Function annotation analysis*

We utilized the Tax4Fun algorithm to conduct functional prediction analysis on the microbiota of *H. qinghaiensis* in each group. Differential alterations in gene pathways caused by variations in microbial community were investigated using different gene modifications from the Silva database, and gene function prediction was performed (Fig. 8). Based on findings derived from the KEGG database, alterations were observed across all groups. As depicted in Fig. 8A, six metabolic pathways underwent modifications among all signaling pathways (Metabolism, Environmental information processing, Genetic information processing, Cellular processes, Human diseases, and Organizational systems), with Metabolism, Environmental information processing, and Genetic information processing exhibiting the most significant changes while other pathways were also influenced to varying extents. The following analysis showed that bloodsucking could cause significant changes in metabolic pathways. As shown in Fig. 8B, compared with the LD_F_F, the metabolic pathways of LD_F in Membrane transport, Carbohydrate metabolism, Amino acid metabolism, Signal transduction, Xenobiotics biodegradation and metabolism, Metabolism of terpenoids and polyketides, Metabolism of other amino acids and Cell mobility were enhanced. However, in other metabolic pathways, such as Translation and Energy metabolism were lower, it indicated that the metabolism of the non-bloodsucking was decreased in terms of providing capacity. On the other hand, compared with the LD_F_F, the LD_F showed a decrease in Nucleotide metabolism, Metabolism of cofactors and vitamins, as well as a decrease in Glycan biosynthesis and metabolism, and an increase in Lipid metabolism. The above results further indicated that changes in the microbiota of *H. qinghaiensis* after bloodsucking caused changes in the signaling pathway.

## 4. Discussion

This study presents an intriguing discovery in *H. qinghaiensis*. The alpha and beta diversity results indicate that the microbial community of *H. qinghaiensis* exhibits regional and blood meal variations, aligning with previous studies conducted on *A. americanum* [26]. Interestingly, the predominant phylum found in *H. qinghaiensis* is *Firmicutes*, which differed from the top phylum observed in other tick species, such as *Ixodes ricinus*. This suggests the potential existence of tick species and geographical variations, as well as the selective effects of blood consumption on microbial types [27]. A diverse microbial community equips ticks with a competitive advantage in adapting to changing environmental conditions by accommodating variable bacterial components, as demonstrated in previous studies [28].

In our study, we also identified *Coxiella* sp. and *Rickettsia* sp. as the two main symbiotic bacteria in *H. qinghaiensis*, consistent with previous research conducted on *A. americanum* [12]. However, what sets our findings apart is the identification of *Rickettsia* as the most abundant genus, with *Coxiella* accounting for only a small portion. Further investigation should be conducted to explore the potential


**Fig. 8.** Overview of the function distribution heatmap of 16S rRNA gene-predicted functional profiles obtained with Tax4Fun. (A) level 1. (B) level 2.

competition between *Coxiella* and *Rickettsia*.

Bloodmeal is essential for ticks to complete their life cycle and is an important material for pathogen transmission [29,30]. During the process of bloodsucking, ticks experience a significant increase in weight and a variation in their liquid storage capacity. This process can also regulate the internal cellular and molecular pathways of ticks, as well as the microbial composition within them, including the quantity and location of bacteria [31].

Studies on various tick species, such as *Ixodes pacificus*, *Ixodes angustus*, *Dermacentor variabilis*, *Dermacentor occidentalis*, *Dermacentor albipictus*, and *Haemaphysalis leporispalustris*, have demonstrated that bloodmeals can affect the microbial community of ticks and potentially impact pathogen transmission [32]. Further research has shown that compared to unfed males and nymphs, fed female ticks have lower microbial abundance, suggesting that blood meals can influence the diversity of tick microbial communities [33].

In the case of *H. qinghaiensis*, the effect of bloodmeal on microbial composition was investigated through 16S rRNA sequencing analysis. It was found that the bacterial diversity of LD_F_F (1.848) was significantly lower than that of LD_F (4.14) ($P < 0.05$), and the bacterial richness of LD_F_F (617.8) was significantly lower than that of LD_F (730.4) ($P < 0.01$). These results are consistent with findings from other tick species [32]. It is speculated that bloodsucking can reduce the microbial richness of *H. qinghaiensis*, potentially modulating the tick's immune response and influencing the composition of its microbiome. Further investigation is required to comprehend the mechanisms of undefined factors in order to gain a better understanding of the interaction between ticks and the microbiome.

The diversity of the microbial community plays a crucial role in the survival and reproduction of ticks. Their adaptability and competitive advantage stem from the presence of a diverse microbial environment [34]. At the genus level species composition, it was observed that *Proteobacteria* (60.63 %) was the most prevalent phylum in LD_F_F, followed by *Proteobacteria* (54.49 %) in LD_F. In

LD_F_F, *Firmicutes* (30.14 %) was the most abundant phylum, followed by *Firmicutes* (10.06 %) in *H. qinghaiensis*. The abundance of *Proteobacteria* and *Firmicutes* in *H. qinghaiensis* was easily influenced by bloodsucking behavior and increased as the feeding progressed. Conversely, *Actinobacteriota* (24.84 %) was the most abundant phylum in LD_F, followed by *Actinobacteriota* (2.20 %) in LD_F_F. *Actinobacteriota* showed susceptibility to bloodsucking behavior in *H. qinghaiensis* and decreased as the feeding progressed.

Ticks can directly acquire bacteria from the blood meal, which can stimulate the growth of certain bacteria while also affecting the diversity and stability of the host's bacterial population. However, most acquired bacteria may be eliminated during tick development, resulting in their clearance. The loss of acquired bacteria could be attributed to their stimulation of the tick's immune system or their inability to survive in the constantly changing environment [15]. The regulation of bacterial abundance by ticks during the bloodsucking process reflects the complex and intricate relationship between them, which warrants further research and discussion.

Several studies have compared the microbiota of ticks collected from different geographic regions, revealing variations in bacterial community or structure based on the collection location [3,17,32–35]. Analyzing ticks sampled under natural conditions and their microbial composition is crucial for understanding patterns of host-microbe association and the factors influencing the tick microbiome. In this study, we analyzed adult ticks from five distinct geographic regions to maximize the likelihood of detecting microbial composition, which can vary spatially in tick populations due to complex ecological factors [36].

As is well known, ticks primarily reside in the fallen leaves of their hosts, allowing the environment to shape the microbial community indirectly (through non-biological parameter changes) or directly (through microbial interactions) [1]. It is plausible to hypothesize that variations in the tick microbiome across different sampling locations may be attributed to the acquisition of microorganisms from the soil, including soil-associated bacteria present in the microbial community of *Ixodes scapularis* [37]. Additionally, some researchers have documented the presence of a group of Lactobacillus bacteria from the soil in *Dermacentor variabilis* and *Ixodes scapularis* [22].

In addition, *Rickettsia* has been identified as nutritional endosymbionts capable of synthesizing folate, a compound that animals are unable to synthesize independently. Therefore, animals require it either through diet or mutual contact with microorganisms [38,39]. We hypothesized that engorged female ticks require a significant amount of nutritional symbiosis for reproduction, leading to the enrichment of the nutritional endosymbiont *Rickettsia* in their bodies. However, *Rickettsia* was not detected in other sampling areas, such as BM_F_F, possibly due to unique environmental conditions or host associations. These factors may allow *H. qinghaiensis* to survive and thrive without the presence of *Rickettsia* endosymbionts. Similar observations have been made for *Ixodes scapularis* in the past [24].

Although the *Rickettsia buchneri* endosymbiont was reported to be 100 % maternally transmitted in *Ixodes pacificus*, the prevalence of this symbiont in tick populations varied between 46 and 82 % across different geographical locations. This suggested that geographical location has an influence on symbiotic relationships [7,13]. Furthermore, it indicated that the relationship between *Rickettsia* and ticks is facultative rather than obligate, as the presence of *Rickettsia* is not necessary for the survival of the host [7,13].

However, under our test conditions, it is unclear whether *Rickettsia* sp. is an obligate endosymbiont of *H. qinghaiensis*, as the role of *Rickettsia* in the biology and ecology of *H. qinghaiensis* is not fully understood, and the mechanisms driving this situation remain unknown. Therefore, further research is warranted.

In our study, the phylogenetic resolution of the v3-v4 region of the 16S rRNA gene was insufficient, preventing further differentiation of *Rickettsia*. We downloaded the nucleotide sequence corresponding to OTU996 in the 16S gene and conducted a comparison on NCBI, which revealed that *Rickettsia* in LD_F_F was similar to *Candidatus Rickettsia hongyuanensis* (OK 662395.1), a species of SFG *Rickettsia* found in *H. qinghaiensis*. *H. qinghaiensis* is a three-host tick that can easily transmit pathogens among different hosts during its life cycle [14]. Therefore, the role of *H. qinghaiensis* in the spread of SFG *Rickettsia* should be further investigated. Additionally, the pathogenicity of *Rickettsia* sp. in humans requires further investigation.

*Coxiella* has also been detected in various other ticks, similar to Rickettsial bacteria, indicating that they are widely distributed symbionts in biology [40–42]. *Coxiella* rapidly proliferates to meet the reproductive needs of ticks during the life cycle of *H. longicornis* [43]. *Coxiella* was previously identified as an endosymbiont in *A. americanum*, as it was found with a frequency of 100 % in many studies of *A. americanum* in different locations [42]. In our study, the discovered *Coxiella* might also be an endosymbiont. Interestingly, it was predominantly found in LD_F_F, suggesting that bloodsucking affects the composition of symbionts in ticks. Although *Coxiella* relies on bloodsucking to obtain nutrients from the tick host, the tick host may not depend on *Coxiella* (i.e., it is not an obligatory symbiont). In this study, we demonstrated that the *Coxiella* load in the body of *H. qinghaiensis* significantly increased with bloodsucking. Furthermore, our analysis showed that bloodsucking leads to significant changes in metabolic pathways, as predicted by the Tax4Fun algorithm. Compared to LD_F_F, LD_F showed significant enhancements in metabolic pathways related to nutrient provision and membrane transport for nutrient acquisition.

Symbionts, which are microorganisms that have closely interacted with tick hosts for an extended period, are primarily studied in terms of their relationship with pathogens. The *Coxiella* genus encompasses two recognized species (*Coxiella burnetii* and *Coxiella cheraxi*), a Candidatus species (*Candidatus Coxiella mudrowiae*), and several unclassified *Coxiella* symbionts [44,45]. Notably, these endosymbionts are closely related to *Coxiella burnetii*, a highly pathogenic pathogen causing Q fever [46]. For instance, *A. americanum* may harbor *Coxiella* spp. endosymbionts that share a close relationship with the highly pathogenic *C. burnetii*, the causative agent of Q fever. It has been demonstrated that *C. burnetii* originated from non-pathogenic *Coxiella* endophytes through horizontal gene transfer and convergence [46]. This study, primarily focusing on *Coxiella* in LD_F_F, might be one of the few recent studies to identify *Coxiella* species infecting mammals. *Coxiella* DNA was found in the blood-sucking tick *H. qinghaiensis*, suggesting that the host (sheep) could be infected with this *Coxiella* strain. The 16S rRNA gene closely related to *Coxiella* sp. isolate XinXian-HL9 (MG906671.1), identified from *H. longicornis* in China (with a sequence identity of 99.07 % for the 16S gene), was detected in LD_F_F. This *Coxiella* strain may represent a *Coxiella*-like endosymbiont coexisting with the host [47]. Each possibility is intriguing and warrants further research.

Bloodsucking is a vital biological process in the life cycle and reproduction of *H. qinghaiensis*. Consequently, the diversity and species composition of its microbiome undergo changes during the bloodsucking phase, resulting in an enrichment of various bacteria such as *Rickettsia* and *Coxiella* (Fig. 6). We hypothesized that *H. qinghaiensis* may influence these functional differences by altering the microbial composition following blood feeding. To explore this further, we employed the Tax4Fun algorithm to predict the functions of microorganisms carried by *H. qinghaiensis*. Our findings indicated that bloodsucking leaded to alterations in metabolic pathways. Specifically, when comparing LD_F_F with LD_F, we observed a significant enhancement in nutrient provision and membrane transport pathways in LD_F, suggesting an increased capacity for nutrient acquisition. Moreover, the enriched pathways primarily involved the synthesis of large biomolecules, including nucleotides and folate. Although the comprehensiveness of these gene prediction algorithms remains uncertain, these results underscore the need for further investigation into the factors influencing the composition and function of tick microbiomes, as well as the potential impact of tick microbiome activity on tick adaptability and pathogen transmission. Therefore, conducting additional research and experiments is imperative to gain a comprehensive understanding of the complexity and significance of tick microbiomes. Ultimately, these efforts will contribute to the development of more effective solutions for tick-borne diseases and related issues.

## 5. Conclusions

This study investigated the structure of the microbial community in a substantial number of ticks (n = 186) collected from five different regions in Qinghai province. Our analysis revealed the influence of geographic origin and blood meal on the composition of the microbial community. Furthermore, we observed the presence of *Coxiella* and *Rickettsia* endosymbiotic bacteria in LD_F_F. Several human pathogens, including *Staphylococcus*, *Pseudomonas*, *Corynebacterium*, *Cloacibacterium*, and *Acinetobacter*, were also identified. It is important to note that different species within these genera may have distinct roles as pathogens or symbionts. This study holds significant implications for understanding the relationship between ticks and microbial communities, advancing our knowledge of the interaction between tick-borne pathogens (TBPs) and tick-borne symbionts, and providing a solid theoretical foundation for the prevention and control of ticks and tick-borne diseases at the microbial level.

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## Data availability statement

The raw data are available on the NCBI Sequence Read Archive (SRA) archive under the accession number PRJNA1052414.

## CRediT authorship contribution statement

**Shuo Jiang:** Writing – review & editing, Writing – original draft, Validation, Data curation. **Ming Kang:** Writing – original draft, Visualization, Supervision. **Zengkui Li:** Software, Methodology, Conceptualization. **Xiaoling Han:** Software, Methodology, Investigation. **Changjiang Chen:** Validation, Software, Formal analysis. **Shunfu He:** Writing – review & editing, Methodology, Conceptualization. **Xiaoyu Hu:** Writing – original draft, Visualization, Software. **Yongcai He:** Validation, Methodology, Investigation. **Yuezhong Wang:** Investigation, Conceptualization. **Zhongyu Li:** Visualization, Validation. **Jiyong Chen:** Writing – original draft, Data curation. **Pengcheng Geng:** Methodology, Data curation. **Qiang Chen:** Methodology. **Jinghua Ma:** Formal analysis. **Xiao Zhang:** Software. **Ximei Tai:** Methodology. **Ying Li:** Writing – review & editing, Visualization, Validation, Supervision.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## References

[1] S. Narasimhan, E. Fikrig, Tick microbiome: the force within, Trends Parasitol. 31 (7) (2015) 315–323, https://doi.org/10.1016/j.pt.2015.03.010.
[2] A. Wu-Chuang, A. Hodžić, L. Mateos-Hernández, et al., Current debates and advances in tick microbiome research, Curr. Res. Parasitol. Vector Borne Dis. 1 (2021) 100036–100049, https://doi.org/10.1016/j.crpvbd.2021.100036.
[3] C.A. Gall, G.A. Scoles, K. Magori, et al., Laboratory colonization stabilizes the naturally dynamic microbiome composition of field collected *Dermacentor andersoni* ticks, Microbiome 5 (1) (2017) 133–142, https://doi.org/10.1186/s40168-017-0352-9.
[4] M.G. Guizzo, K. Budachetri, A. Adegoke, et al., *Rickettsia parkeri* infection modulates the sialome and ovariome of the Gulf coast tick, *Amblyomma maculatum*, Front. Microbiol. 13 (2022) 1023980–1023992, https://doi.org/10.3389/fmicb.2022.1023980.
[5] J.R. Ramírez-Garofalo, S.R. Curley, C.E. Field, et al., Established populations of *Rickettsia parkeri*-infected *Amblyomma maculatum* ticks in New York city, New York, USA, Vector Borne Zoonotic Dis. 22 (3) (2022) 184–187, https://doi.org/10.1089/vbz.2021.0085.

[6] B. Cull, N.Y. Burkhardt, X.R. Wang, et al., The *Ixodes scapularis* symbiont *Rickettsia buchneri* inhibits growth of pathogenic rickettsiaceae in tick cells: implications for vector competence, Front. Vet. Sci. 8 (2022) 748427–748446, https://doi.org/10.3389/fvets.2021.748427.

[7] A. Ahantarig, W. Trinachartvanit, V. Baimai, L. Grubhoffer, Hard ticks and their bacterial endosymbionts (or would be pathogens), Fol. Microbiol 58 (5) (2013) 419–428, https://doi.org/10.1007/s12223-013-0222-1.

[8] E. Machado-Ferreira, V.F. Vizzoni, E. Balsemão-Pires, et al., *Coxiella* symbionts are widespread into hard ticks, Parasitol. Res. 115 (12) (2016) 4691–4699, https://doi.org/10.1007/s00436-016-5230-z.

[9] S.I. Bonnet, F. Binetruy, A.M. Hernández-Jarguín, et al., The tick microbiome: why non-pathogenic microorganisms matter in tick biology and pathogen transmission, Front. Cell. Infect. Microbiol. 7 (2017) 236–249, https://doi.org/10.3389/fcimb.2017.00236.

[10] X.L. Xu, T.Y. Cheng, H. Yang, et al., Identification of intestinal bacterial flora in *Rhipicephalus microplus* ticks by conventional methods and PCR-DGGE analysis, Exp. Appl. Acarol. 66 (2) (2015) 257–268, https://doi.org/10.1007/s10493-015-9896-1.

[11] H. Noda, U.G. Munderloh, T.J. Kurtti, Endosymbionts of ticks and their relationship to *Wolbachia* spp. and tick-borne pathogens of humans and animals, Appl. Environ. Microbiol. 63 (10) (1997) 3926–3932, https://doi.org/10.1128/aem.63.10.3926-3932.1997.

[12] L.P. Maldonado-Ruiz, S. Neupane, Y. Park, et al., The bacterial community of the lone star tick (*Amblyomma americanum*), Parasit Vectors 14 (1) (2021) 49–58, https://doi.org/10.1186/s13071-020-04550-z.

[13] L.M. Liu, J.N. Liu, Z. Liu, et al., Microbial communities and symbionts in the hard tick *Haemaphysalis longicornis* (Acari: Ixodidae) from north China, Parasite vectors 6 (1) (2013) 310–317, https://doi.org/10.1186/1756-3305-6-310.

[14] X. Liu, Z. Chen, Q. Ren, et al., Genetic diversity of *Haemaphysalis qinghaiensis* (Acari: Ixodidae) in western China, Exp. Appl. Acarol. 74 (4) (2018) 427–441, https://doi.org/10.1007/s10493-018-0242-2.

[15] S. Narasimhan, A. Swei, S. Abouneameh, et al., Grappling with the tick microbiome, Trends Parasitol. 37 (8) (2021) 722–733, https://doi.org/10.1016/j.pt.2021.04.004.

[16] D. Obregón, E. Bard, D. Abrial, et al., Sex-specific linkages between taxonomic and functional profiles of tick gut microbiomes, Front. Cell. Infect. Microbiol. 9 (2019) 298–313, https://doi.org/10.3389/fcimb.2019.00298.

[17] W. Van Treuren, L. Ponnusamy, R.J. Brinkerhoff, et al., Variation in the microbiota of Ixodes ticks with regard to geography, species, and sex, Appl. Environ. Microbiol. 81 (18) (2015) 6200–6209, https://doi.org/10.1128/AEM.01562-15.

[18] E.F. Che Lah, M. Ahamad, A. Dmitry, et al., Metagenomic profile of the bacterial communities associated with *Ixodes granulatus* (Acari: ixodidae): a potential vector of tick-borne diseases, J. Med. Entomol. 60 (4) (2023) 753–768, https://doi.org/10.1093/jme/tjad044.

[19] A.I. Krawczyk, S. Röttjers, M.J. Coimbra-Dores, et al., Tick microbial associations at the crossroad of horizontal and vertical transmission pathways, Parasite vectors 15 (1) (2022) 380–389, https://doi.org/10.1186/s13071-022-05519-w.

[20] X.C. Zhang, Z.N. Yang, B. Lu, et al., The composition and transmission of microbiome in hard tick, *Ixodes persulcatus*, during blood meal, Ticks Tick Borne Dis 5 (6) (2014) 864–870, https://doi.org/10.1016/j.ttbdis.2014.07.009.

[21] K. Ring, L.I. Couper, A.L. Sapiro, et al., Host blood meal identity modifies vector gene expression and competency, Mol. Ecol. 31 (9) (2022) 2698–2711, https://doi.org/10.1111/mec.16413.

[22] E.C. Rynkiewicz, C. Hemmerich, D.B. Rusch, et al., Concordance of bacterial communities of two tick species and blood of their shared rodent host, Mol. Ecol. 24 (10) (2015) 2566–2579, https://doi.org/10.1111/mec.13187.

[23] L. Ponnusamy, A. Gonzalez, W. Van Treuren, et al., Diversity of Rickettsiales in the microbiome of the lone star tick, *Amblyomma americanum*, Appl. Environ. Microbiol. 80 (1) (2014) 354–359, https://doi.org/10.1128/AEM.02987-13.

[24] A.I. Krawczyk, S. Röttjers, M.J. Coimbra-Dores, et al., Tick microbial associations at the crossroad of horizontal and vertical transmission pathways, Parasite vectors 15 (1) (2022) 380–389, https://doi.org/10.1186/s13071-022-05519-w.

[25] Y.C. He, J.X. Li, Y.L. Sun, et al., *Spotted fever group Rickettsia* infecting ticks (Acari: Ixodidae), yak (*Bos grunniens*), and Tibetan sheep (*Ovis aries*) in the Qinghai-Tibetan plateau area, China, Front. Vet. Sci. 8 (2022) 779387–779397, https://doi.org/10.3389/fvets.2021.779387.

[26] A.C. Menchaca, D.K. Visi, O.F. Strey, et al., Preliminary assessment of microbiome changes following blood-feeding and survivorship in the *Amblyomma americanum* nymph-to-adult transition using semiconductor sequencing, PLoS One 8 (6) (2013) 67129–67138, https://doi.org/10.1371/journal.pone.0067129.

[27] R. Rousseau, S.O. Vanwambeke, C. Boland, et al., The isolation of culturable bacteria in *Ixodes ricinus* ticks of a Belgian peri-urban forest uncovers opportunistic bacteria potentially important for public health, Int. J. Env. Res Public Health 18 (22) (2021) 12134–121477, https://doi.org/10.3390/ijerph182212134.

[28] D. Obregón, E. Bard, D. Abrial, et al., Sex-specific linkages between taxonomic and functional profiles of tick gut microbiomes, Front. Cell. Infect. Microbiol. 9 (2019) 298–313, https://doi.org/10.3389/fcimb.2019.00298.

[29] R.C. Edgar, UPARSE: highly accurate OTU sequences from microbial amplicon reads, Nat. Methods 10 (10) (2013) 996–998, https://doi.org/10.1038/nmeth.2604.

[30] K.A. Padgett, R.S. Lane, Life cycle of *Ixodes pacificus* (Acari: Ixodidae): timing of developmental processes under field and laboratory conditions, J. Med. Entomol. 38 (5) (2001) 684–693, https://doi.org/10.1603/0022-2585-38.5.684.

[31] A. Swei, J.Y. Kwan, Tick microbiome and pathogen acquisition altered by host blood meal, Ism. J. 11 (3) (2017) 813–816, https://doi.org/10.1038/ismej.2016.152.

[32] B. Chicana, L.I. Couper, J.Y. Kwan, et al., Comparative microbiome profiles of sympatric tick species from the far-western United States, Insects 10 (10) (2019) 353–364, https://doi.org/10.3390/insects10100353.

[33] R.J. Brinkerhoff, C. Clark, K. Ocasio, et al., Factors affecting the microbiome of *Ixodes scapularis* and *Amblyomma americanum*, PLoS One 15 (5) (2020) 232398–232417, https://doi.org/10.1371/journal.pone.0232398.

[34] L.H. Li, Y. Zhang, D. Zhu, Effects of antibiotic treatment on the fecundity of *Rhipicephalus haemaphysaloides* ticks, Parasit Vectors 11 (1) (2018) 242–248, https://doi.org/10.1186/s13071-018-2807-7.

[35] R.T. Trout Fryxell, J.M. DeBruyn, The microbiome of *Ehrlichia*-infected and uninfected lone star ticks (*Amblyomma americanum*), PLoS One 11 (1) (2016) e0146651–e0146669, https://doi.org/10.1371/journal.pone.0146651.

[36] E.F. Lambin, A. Tran, S.O. Vanwambeke, et al., Pathogenic landscapes: interactions between land, people, disease vectors, and their animal hosts, Int. J. Health Geogr. 9 (2010) 54–66, https://doi.org/10.1186/1476-072X-9-54.

[37] A. Hernández-Jarguín, S. Díaz-Sánchez, M. Villar, et al., Integrated metatranscriptomics and metaproteomics for the characterization of bacterial microbiota in unfed *Ixodes ricinus*, Ticks Tick Borne Dis 9 (5) (2018) 1241–1251, https://doi.org/10.1016/j.ttbdis.2018.04.020.

[38] J.L. Bodnar, S. Fitch, A. Rosati, et al., The folA gene from the *Rickettsia* endosymbiont of *Ixodes pacificus* encodes a functional dihydrofolate reductase enzyme, Ticks Tick Borne Dis 9 (3) (2018) 443–449, https://doi.org/10.1016/j.ttbdis.2017.12.013.

[39] B. Hill, B. Schafer, N. Vargas, et al., Functional analysis of *Rickettsia monacensis* strain humboldt folA dihydrofolate reductase gene via complementation assay, Ticks Tick Borne Dis 14 (6) (2023) 102217–102226, https://doi.org/10.1016/j.ttbdis.2023.102217.

[40] A. Jasinskas, J. Zhong, A.G. Barbour, Highly prevalent *Coxiella* sp. bacterium in the tick vector *Amblyomma americanum*, Appl. Environ. Microbiol. 73 (1) (2007) 334–336, https://doi.org/10.1128/AEM.02009-06.

[41] K. Clay, O. Klyachko, N. Grindle, et al., Microbial communities and interactions in the lone star tick, *Amblyomma americanum*, Mol. Ecol. 17 (19) (2008) 4371–4381, https://doi.org/10.1111/j.1365-294x.2008.03914.x.

[42] T.L. Greay, A.W. Gofton, A. Paparini, et al., Recent insights into the tick microbiome gained through next-generation sequencing, Parasit Vectors 11 (1) (2018) 12–25, https://doi.org/10.1186/s13071-017-2550-5.

[43] X.Y. Zhang, S.S. Li, K.L. Chen, et al., Growth dynamics and tissue localization of a *Coxiella*-like endosymbiont in the tick *Haemaphysalis longicornis*, Ticks Tick Borne Dis 13 (5) (2022) 102005, https://doi.org/10.1016/j.ttbdis.2022.102005.

[44] Y. Gottlieb, I. Lalzar, L. Klasson, Distinctive genome reduction rates revealed by genomic analyses of two *Coxiella*-like endosymbionts in ticks, Genome Biol. Evol. 7 (6) (2015) 1779–1796, https://doi.org/10.1093/gbe/evv108.

[45] C.K. Tan, L. Owens, Infectivity, transmission and 16S rRNA sequencing of a *rickettsia, Coxiella cheraxi* sp. nov., from the freshwater crayfish *Cherax quadricarinatus*, Dis. Aquat. Organ. 41 (2) (2000) 115–122, https://doi.org/10.3354/dao041115.
[46] O. Duron, V. Noël, K.D. McCoy, et al., The recent evolution of a maternally-inherited endosymbiont of ticks led to the emergence of the Q fever pathogen, *Coxiella burnetii*, PLoS Pathog. 11 (5) (2015) 1004892–1004914, https://doi.org/10.1371/journal.ppat.1004892.
[47] M. Lu, J. Tian, H. Zhao, et al., Molecular survey of vector-borne pathogens in ticks, sheep keds, and domestic animals from ngawa, southwest China, Pathogens 11 (5) (2022) 606–616, https://doi.org/10.3390/pathogens11050606.