The gut microbiota in the common kestrel (*Falco tinnunculus*): a report from the Beijing Raptor Rescue Center

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As a complex microecological system, the gut microbiota plays crucial roles in many aspects, including immunology, physiology and development. The specific function and mechanism of the gut microbiota in birds are distinct due to their extremely special body structure, physiological attributes and life history. Data on the gut microbiota of the common kestrel, a second-class protected animal species in China, are currently scarce. With high-throughput sequencing technology, we characterized the bacterial composition and structure of the gut microbiota from 9 fecal samples from a wounded common kestrel by sequencing the V3-V4 region of the 16S ribosomal RNA gene in this study. Our results showed that *Proteobacteria* (41.078%), *Firmicutes* (40.923%) and *Actinobacteria* (11.191%) were the most predominant phyla. *Lactobacillus* (20.563%) was the most dominant genus, followed by *Escherichia-Shigella* (17.588%) and *Acinetobacter* (5.956%). A total of 28 phyla, 70 classes, 183 orders, 329 families and 681 genera were detected among the gastrointestinal bacterial communities. Further research on *Enterococcus*, *Patescibacteria* and *Deinococcus-Thermus* should be conducted in the future, with additional samples. In addition, comparison of the bacterial composition and structure between healthy and sick individuals may provide another research direction. Our results could also offer fundamental data and novel strategies for the protection of wild animals.

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Abstract

As a complex microecological system, the gut microbiota plays crucial roles in many aspects, including immunology, physiology and development. The specific function and mechanism of the gut microbiota in birds are distinct due to their extremely special body structure, physiological attributes and life history. Data on the gut microbiota of the common kestrel, a second-class protected animal species in China, are currently scarce. With high-throughput sequencing technology, we characterized the bacterial composition and structure of the gut microbiota from 9 fecal samples from a wounded common kestrel by sequencing the V3-V4 region of the 16S ribosomal RNA gene in this study. Our results showed that *Proteobacteria* (41.078%), *Firmicutes* (40.923%) and *Actinobacteria* (11.191%) were the most predominant phyla. *Lactobacillus* (20.563%) was the most dominant genus, followed by *Escherichia-Shigella* (17.588%) and *Acinetobacter* (5.956%). A total of 28 phyla, 70 classes, 183 orders, 329 families and 681 genera were detected among the gastrointestinal bacterial communities. Further research on *Enterococcus*, *Patescibacteria* and *Deinococcus-Thermus* should be conducted in the future, with additional samples. In addition, comparison of the bacterial composition and structure between healthy and sick individuals may provide another research direction. Our results could also offer fundamental data and novel strategies for the protection of wild animals.

Keywords: Common kestrel (*Falco tinnunculus*), Gut microbiota, 16S rRNA gene, High-throughput sequencing, Noninvasive

Introduction

The common kestrel (*Falco tinnunculus*) is a small raptor that belongs to Falconidae. A total of 12 subspecies are distributed widely from the Palearctic to Oriental regions (Cramp & Brooks

1992). Although listed in the least concern (LC) class by the International Union for Conservation of Nature (IUCN), the common kestrel was listed as a second-class protected animal species in China. The common kestrel is a typical opportunistic forager that catches small and medium-sized animals, including small mammals, birds, reptiles and some invertebrates (Anthony 1993; Aparicio 2000; Village 2010). Insects such as grasshoppers and dragonflies were also identified in the diet of the common kestrel (Geng et al. 2009). Common kestrels choose distinct predatory strategies when wintering and breeding to minimize the expenditure of energy, as generalist predators (Costantini et al. 2005; Masman et al. 1988). Moreover, the density, reproductive success, choice of nest sites and hunting success are different between urban and rural common kestrels (Fargallo et al. 2001; Riegert et al. 2010; Salvati et al. 1999), as well as polymorphisms at the genetic level (Rutkowski et al. 2006). Previous studies on common kestrels were comparatively comprehensive, such as those on diet and prey selection (Geng et al. 2009; Kirkwood 1980; Korpimäki 1985; Lihu et al. 2007; Souttou et al. 2007; Van Zyl 1994), behavior and diseases (Aschwanden et al. 2005; Bustamante 1994; Hille et al. 2007), and genetic variation and diversity (Nesje et al. 2000; Padilla et al. 2009; Riegert et al. 2010; Zhang et al. 2008). However, as a research hotspot, data on the gut microbiota of the common kestrel are currently very scarce. The gut microbiota has been reported to play important roles in many aspects, such as immunology, physiology and development, with the rapid progress of sequencing techniques (Guarner & Malagelada 2003; Nicholson et al. 2005). Reports concerning the gut microbiota of other avian species, such as black-legged kittiwakes (*Rissa tridactyla*) (van Dongen et al. 2013), Cooper's hawks (*Accipiter cooperii*) (Taylor et al. 2019), bar-headed geese (*Anser indicus*) (Wang et al. 2017) and hooded cranes (*Grus monacha*) (Zhao et al. 2017), have increased rapidly over the last decade. The specific function and mechanism of the gut microbiota in birds are distinct due to their extremely special body structure, physiological attributes and life history. Additionally, the ability to fly

brings additional unique differences for birds compared to other animals, as well as for their intestinal microbiota to some extent.

The aim of this study was to characterize the elementary bacterial composition and structure of the gut microbiota by sequencing the V3-V4 region of the 16S rRNA gene from a wounded common kestrel rescued by the Beijing Raptor Rescue Center (BRRC). The data we obtained could provide basic information for further protection and rescue of wild common kestrels.

Materials & Methods

Sample collection

All fecal samples were collected from an injured common kestrel in the Beijing Raptor Rescue Center (BRRC). The injured common kestrel that could not fly was found first in the Fengtai district by a rescuer on June 22nd, 2019 and then taken to the BRRC for professional rescue. The wounded common kestrel was carefully treated with several surgeries and drug therapies. Nine fecal samples (E1-E9) that may reflect the real health condition were collected from the common kestrel after relevant treatments on different days. All samples were transported immediately into the laboratory in an ice box and ultimately stored at -80°C for further experiments.

DNA extraction and PCR amplification

Microbial DNA was extracted from fresh fecal samples using an E.Z.N.A.® Stool DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's protocols. The V3-V4 region of the bacterial 16S ribosomal RNA gene was amplified by PCR (95 °C for 3 min; followed by 25 cycles at 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s; and a final extension at 72 °C for 5 min) using the primers 338F (5'-barcode-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), where the barcode is an eight-base sequence unique to each sample. PCRs were performed in triplicate in a 20 μL mixture

containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of FastPfu Polymerase, and 10 ng of template DNA.

Illumina MiSeq sequencing

Amplicons were extracted from 2% agarose gels and purified using an AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor™ -ST (Promega, U.S.). Purified amplicons were pooled in equimolar amounts and paired-end sequenced (2 × 250) on an Illumina MiSeq platform according to standard protocols.

Processing of sequencing data

Raw fastq files were demultiplexed and quality-filtered using QIIME (version 1.17) (Caporaso et al. 2010) with the following criteria. (i) The 300 bp reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window, discarding the truncated reads that were shorter than 50 bp. (ii) Exact barcode matching, 2 nucleotide mismatches in primer matching, and reads containing ambiguous characters were removed. (iii) Only sequences that overlapped longer than 10 bp were assembled according to their overlap sequence. Reads that could not be assembled were discarded.

Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed using UCHIME (Edgar et al. 2011). The taxonomy of each 16S rRNA gene sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the SILVA (SSU115)16S rRNA database using a confidence threshold of 70% (Amato et al. 2013).

Data analysis

All the indices of alpha diversity, including Chao, ACE, Shannon, Simpson, and coverage, and the analysis of beta diversity were calculated with QIIME. The rarefaction curves, rank abundance curves, and stacked histogram of relative abundance were displayed with R (version 2.15.3) (Team 2013).

The hierarchical clustering trees were built using UPGMA (unweighted pair-group method with arithmetic mean) based on weighted and unweighted distance matrices at different levels. Principal component analysis (PCA), principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS) as well as hierarchical clustering trees were calculated and displayed using QIIME and R.

The study was approved by the Animal Ethics Review Committee at Beijing Normal University. The approval number is CLS-EAW-2019-026.

Results

Overall sequencing data

A total of 389,474 reads were obtained and classified into 1673 OTUs at the 0.97 sequence identity cut-off in 9 fecal samples from a common kestrel.

Alpha diversity indices (including Sobs, Shannon, Simpson, ACE, Chao and coverage) of each sample are shown in *Table* 1. Additionally, the rarefaction curves (A) and the rank abundance curves (B) are shown in *Fig.* 1, which indicated that the number of OTUs for further analysis was reasonable, as well as the abundance of species in common kestrel feces.

Bacterial composition and relative abundance

We ultimately detected 28 phyla, 70 classes, 183 orders, 329 families and 681 genera among the 9 fecal samples from this common kestrel.

At the phylum level of the gut microbiota in the common kestrel, the most predominant phylum was *Proteobacteria* (41.078%), followed by *Firmicutes* (40.923%), *Actinobacteria* (11.191%)

154 and *Bacteroidetes* (3.821%). It is remarkable that in addition to *Tenericutes* (0.178%) and
155 *Verrucomicrobia* (0.162%), *Patescibacteria* (0.543%) and *Deinococcus-Thermus* (0.504%) were
156 also ranked in the top 10 species in the common kestrel fecal microbiota (*Table* 2).
157 The top 5 families in the gut microbiota were Lactobacillaceae (20.563%), Enterobacteriaceae
158 (18.346%), Moraxellaceae (6.733%), Bifidobacteriaceae (5.624%) and Burkholderiaceae
159 (4.752%).
160 At the genus level, *Lactobacillus* (20.563%), *Escherichia-Shigella* (17.588%) and *Acinetobacter*
161 (5.956%) were the most dominant genera. These were followed by *Bifidobacterium* (5.624%)
162 and *Enterococcus* (4.024%) (*Table* 3). These five genera in the total gut microbiota of several
163 samples accounted for a small proportion, such as for E5 (28.755%) and E6 (10.905%) and
164 especially for E4 (2.861%).
165 The stacked histogram of relative abundance for species is also demonstrated in *Fig.* 2 at the
166 phylum (A) and genus (B) levels, which could intuitively represent the basic bacterial
167 composition and relative abundance. The community structures of E1 and E9 were more similar
168 than those of the other feces samples at both levels.
169 The hierarchical clustering trees that showed the similarity of community structure among
170 different samples, which were generated by UPGMA (unweighted pair-group method with
171 arithmetic mean) with the unweighted UniFrac (*Fig.* 3A) and weighted UniFrac (*Fig.* 3B)
172 distance matrixes. Although the fecal samples were collected from the common kestrel in
173 chronological order (E1-E9) of therapy treatments, no distinct or obvious clustering relationships
174 are discernable in *Fig.* 3.
175
176 Discrepancy of community composition
177 To further demonstrate the differences in community composition among the nine samples,
178 principal component analysis (PCA), principal coordinates analysis (PCoA) and nonmetric
179 multidimensional scaling analysis (NMDS) were applied and are depicted in *Fig.* 4. For PCoA,

we chose the same two distance matrices (unweighted UniFrac in *Fig.* 4A and weighted UniFrac in *Fig.* 4B) as above to analyze the discrepancies. The NMDS plot in *Fig.* 4 was generated with the unweighted UniFrac distance matrix. The results in *Fig.* 4 were similar to those in *Fig.* 3, in which all samples scattered dispersedly, suggesting that the variation in the composition of the gut microbiota of the common kestrel was not obvious in this case over time.

Discussion

Knowledge and comprehension concerning the gut microbiota have continued to progressively develop with relevant techniques over the past decade. The application of analysis for intestinal microecology was also a research hotspot in the field of wild animal protection.

The common kestrel (*Falco tinnunculus*) is listed as a second-class protected animal species in China. Although research concerning avian species, including the common kestrel, has been increasing gradually, the available data on the gut microbiota in the common kestrel are currently limited.

We characterized the basic composition and structure of the gut microbiota from a wounded common kestrel in this study, which was rescued by the Beijing Raptor Rescue Center (BRRC). In general, the overall community structure of the gut microbiota in this common kestrel was in accordance with previous relevant characterizations in avian species, such as Cooper's hawks (Taylor et al. 2019), bar-headed geese (Wang et al. 2017), hooded cranes (Zhao et al. 2017) and swan geese (Wang et al. 2016), which included *Proteobacteria*, *Firmicutes*, *Actinobacteria* and *Bacteroidetes*.

The most predominant phylum in the fecal gut microbiota of the common kestrel was *Proteobacteria* (41.078%), which ranked after *Firmicutes* in other birds, such as cockatiels (Alcaraz et al. 2016) and black-legged kittiwakes (van Dongen et al. 2013). This crucial phylum plays many valuable roles. For instance, *Proteobacteria* is beneficial for the giant panda, which can degrade lignin in its major food resource (Fang et al. 2012). Additionally, it has been

reported that *Proteobacteria* is also the most dominant phylum in obese dogs (Park et al. 2015). The specific function of this phylum could be distinct in birds due to their unique physiological traits, as well as their developmental strategies (Kohl 2012). However, the high relative abundance of *Proteobacteria* in the total bacterial community was observed mainly in several samples that were collected during surgeries or drug treatments, such as E1 and E4. Environmental influential factors, as well as dietary changes, should also be considered an important index that could result in variations in the relative abundance of species in the gut microbiota (De Filippo et al. 2010; Scott et al. 2013).

Furthermore, the dominant genera within *Proteobacteria* in our study were *Escherichia-Shigella* (17.588%), *Acinetobacter* (5.956%), *Paracoccus* (2.904%) and *Burkholderia-Caballeronia-Paraburkholderia* (2.408%). *Escherichia-Shigella* is a common pathogenic bacterium that can cause diarrhea in humans (Hermes et al. 2009). The main cause for the high relative abundance of *Escherichia-Shigella* was the E1 (88.610%) sample, which suggested indirectly that the physical condition of the common kestrel was not normal when it was rescued by staff from the BRRC. This result was also consistent with the actual state of this wounded common kestrel that we observed.

Although *Firmicutes* (40.923%) ranked after *Proteobacteria*, its actual relative abundance was slightly lower than that in the common kestrel. As a common phylum of the gut microbiota, *Firmicutes* exists widely in both mammals and birds, which might be because of the common ancestor of amniotes (Costello et al. 2010; Kohl 2012). *Firmicutes* can provide certain energy for the host through catabolizing complex carbohydrates and sugar and even digesting fiber by some species (Costa et al. 2012; Flint et al. 2008; Guan et al. 2017).

The dominant genera in *Firmicutes* were *Lactobacillus* (20.563%), *Enterococcus* (4.024%) and *Clostridium_sensu_stricto_1* (3.586%). The relative abundance of *Enterococcus* in E5 (15.026%) contributed to the highest ranking of this genus. *Enterococcus* is not regarded as a special pathogenic bacterium due to its harmlessness and can even be used as a normal food

232 additive in related industries (Fisher & Phillips 2009; Moreno et al. 2006). *Enterococcus* species
233 are also considered common nosocomial pathogens that can cause a high death rate (Lopes et al.
234 2005). The BRRC might be a specific location similar to hospitals for raptors to some extent,
235 which could explain the high proportion of *Enterococcus* in the fecal samples of this common
236 kestrel. This genus should be given sufficient attention in subsequent studies with additional
237 samples from different individuals. Moreover, the abundance of *Clostridium* increases as more
238 protein is digested (Lubbs et al. 2009). The high relative abundance of this genus also resulted
239 primarily from certain samples (E8, 28.177%), similar to the *Enterococcus* mentioned above.
240 The third dominant phylum in the gut microbiota in our study was *Actinobacteria* (11.191%),
241 which was also detected in other species, such as turkeys (Wilkinson et al. 2017) and Leach's
242 storm petrel (Pearce et al. 2017). The abundance of *Actinobacteria* varied in different species,
243 such as house cats (7.30%) and dogs (1.8%) (Handl et al. 2011), but only accounted for 0.53% in
244 wolves (Wu et al. 2017). Within this phylum, *Bifidobacterium* (5.624%) and *Glutamicibacter*
245 (1.840%) were the primary genera. *Bifidobacterium* is closely related to the utilization of glycans
246 produced by the host, as well as oligosaccharides in human milk (Sela et al. 2008; Turroni et al.
247 2010). Noticeably, *Bifidobacterium thermophilum* was reported to be used through oral
248 administration for chickens to resist *E. coli* infection (Kobayashi et al. 2002). The detection and
249 application of *Bifidobacterium*, especially for the rescue of many rare avian species, would be
250 worth considering for curing various diseases in the future.
251 Additionally, the relative abundance of *Bacteroidetes* was 3.821% in this study, which consisted
252 mainly of *Sphingobacterium*. *Bacteroidetes* is another important component of the gut
253 microbiota that can degrade relevant carbohydrates from secretions of the gut, as well as high
254 molecular weight substances (Thoetkiattikul et al. 2013). The proportion of *Bacteroidetes*, which
255 was stable in most samples we collected except E5, would increase correspondingly with weight
256 loss for mice or changes in fiber content in rural children's daily diet (De Filippo et al. 2010; Ley
257 et al. 2006; Turnbaugh et al. 2008). To characterize the basic composition and structure of the

gut microbiota for the common kestrel more accurately, additional fresh fecal samples from healthy individuals should be collected in follow-up studies.

Furthermore, additional attention should be paid to the high ranking of *Patescibacteria* (0.543%) and *Deinococcus-Thermus* (0.504%) at the phylum level. *Patescibacteria* might be related to basic biosynthesis of amino acids, nucleotides and so on (Lemos et al. 2019). Members of *Deinococcus-Thermus* are known mainly for their capability to resist extreme radiation, including ultraviolet radiation, as well as oxidizing agents (Cox & Battista 2005; Griffiths & Gupta 2007). The specific function of certain species in these phyla for the common kestrel should be studied by controlled experiments, detailed observations or more advanced approaches, as molecular biological techniques are developed.

In addition to the quantity of samples, living environment, age, sex and individual differentiation should also be considered as influencing factors, which would cause a degree of discrepancies at all levels in the gut microbiota. In addition, A comparison of wounded and healthy samples for the bacterial composition in the intestinal microbiota is another essential research direction that may provide additional information for wild animal rescue, such as important biomarkers that indirectly indicate potential diseases.

Conclusion

In summary, using high-throughput sequencing technology in this study, we first characterized the elementary bacterial composition and structure of the gut microbiota for a wounded common kestrel in the BRRC, which could provide valuable basic data for future studies. Further research on *Enterococcus*, *Patescibacteria* and *Deinococcus-Thermus* should be conducted in the future with additional samples. The integration of other auxiliary techniques or disciplines, such as metagenomics and transcriptomics, could offer a deeper understanding of the function and mechanism of the gut microbiota, as well as the protection of wild animals.

Acknowledgments

We sincerely thank the Beijing Raptor Rescue Center (BRRC) and Professor Limin Feng for sample collection of from the common kestrel.

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Figure 1

Rarefaction Curves (A) and Rank Abundance Curves (B)

Rarefaction Curves (A) reflect the rationality of the size for sequencing data, also the bacterial diversity of each sample. Rank Abundance Curves (B) reflect the species richness by the span of curves onthehorizontal axis. While the evenness of the bacterial communities was depicted by the shape of curves.

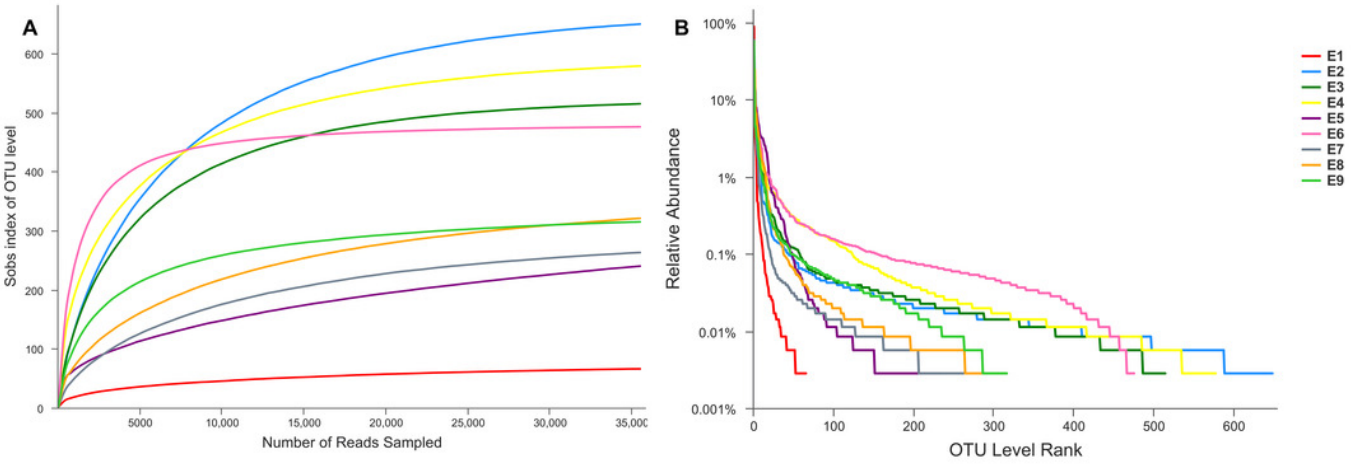

Figure 2

The histogram of relative abundance for species in Common Kestrel at phylum (A) and genus (B) level.

The histogram of relative abundance for species in Common Kestrel at phylum (A) and genus (B) level.

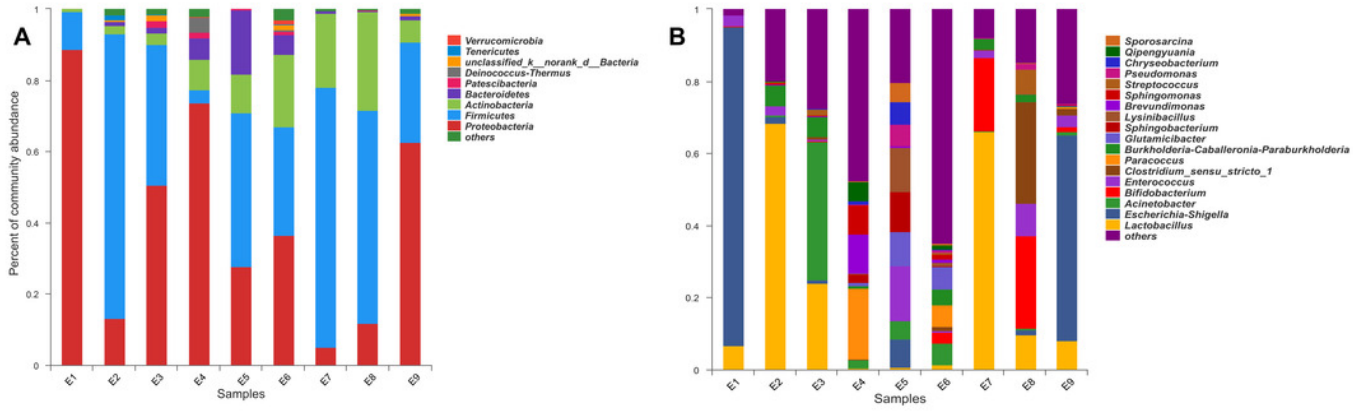

Figure 3

The hierarchical clustering trees

(A) and (B) were generated based on unweighted and weighted distance matrix at phylum level respectively.

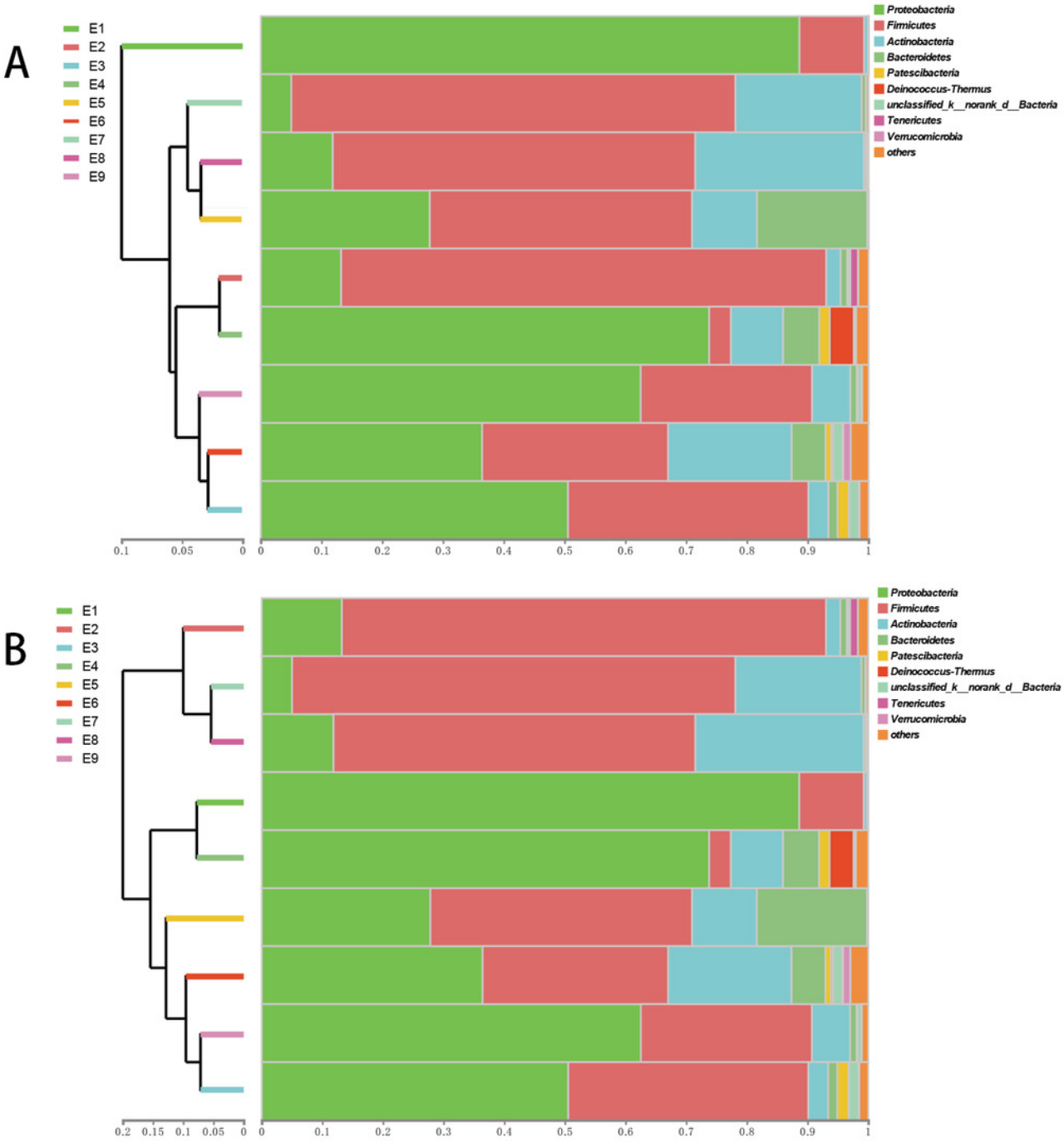
A
E1
E2
E3
E4
E5
E6
E7
E8
E9
Proteobacteria
Firmicutes
Actinobacteria
Bacteroidetes
Patescibacteria
Deinococcus-Thermus
unclassified_k__norank_d__Bacteria
Tenericutes
Verrucomicrobia
others
0.1 0.05 0
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1
B
E1
E2
E3
E4
E5
E6
E7
E8
E9
Proteobacteria
Firmicutes
Actinobacteria
Bacteroidetes
Patescibacteria
Deinococcus-Thermus
unclassified_k__norank_d__Bacteria
Tenericutes
Verrucomicrobia
others
0.2 0.15 0.1 0.05 0
0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

Figure 4

NMDS, PCA and PCoA of the bacterial population structures.

The different shape with colors represented all samples of Common Kestrel respectively. For PCoA, (A) was generated with unweighted Unifrac distance while (B) used weighted Unifrac distance.

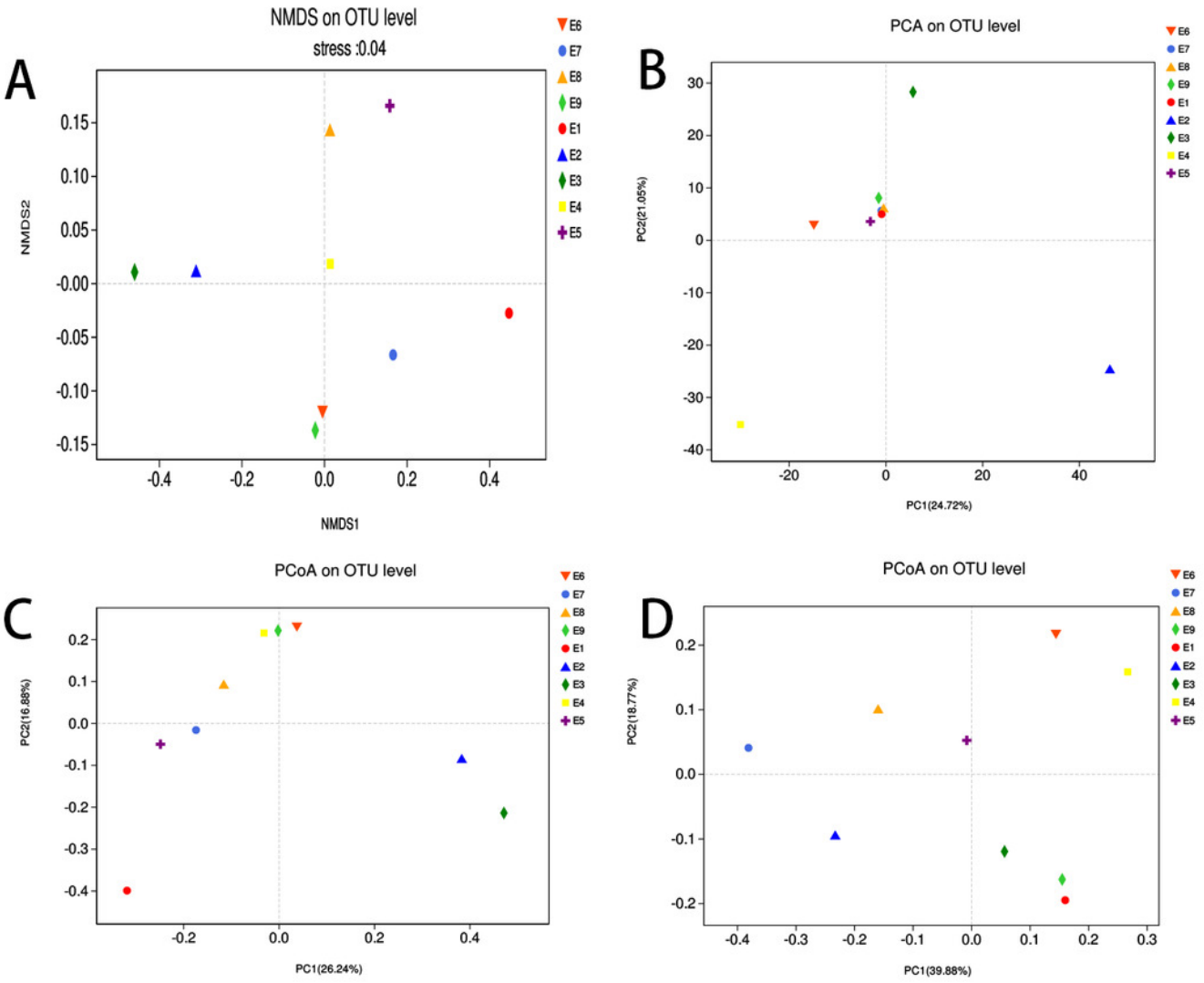

Table 1(on next page)

Alpha diversity of gut microbiota in Common Kestrel feces

1

Sample	Sobs	Shannon	Simpson	Ace	Chao	Coverage
E1	66	0.596	0.788	78.114	73.583	1.000
E2	650	2.780	0.204	674.412	672.193	0.998
E3	515	2.965	0.184	524.452	522.519	0.999
E4	579	4.233	0.053	594.498	594.050	0.999
E5	240	3.285	0.057	448.368	378.103	0.997
E6	476	4.802	0.020	479.110	480.091	1.000
E7	263	1.604	0.399	292.553	281.800	0.999
E8	321	2.706	0.143	364.651	359.519	0.998
E9	315	2.374	0.335	330.906	331.607	0.999

2

Table 2(on next page)

The relative abundance of species in gut microbiota of Common Kestrel at phylum level

The names of phyla in Table 2 represented *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Bacteroidetes*, *Patescibacteria*, *Deinococcus-Thermus*, *unclassified_K_norank_d_Bacteria*, *Tenericutes*, *Verrucomicrobia* respectively.

1

Sample	*Pro*	*Fir*	*Act*	*Bac*	*Pat*	*Dei*	*unc*	*Ten*	*Ver*	others
E1	88.630%	10.634%	0.623%	0.006%	0.003%	0.000%	0.096%	0.000%	0.006%	0.003%
E2	13.211%	79.816%	2.376%	1.085%	0.085%	0.065%	0.361%	1.291%	0.017%	1.694%
E3	50.540%	39.567%	3.286%	1.502%	1.857%	0.087%	1.553%	0.121%	0.011%	1.474%
E4	73.770%	3.574%	8.602%	5.950%	1.719%	3.960%	0.220%	0.008%	0.158%	2.038%
E5	27.797%	43.152%	10.694%	18.166%	0.104%	0.042%	0.006%	0.000%	0.000%	0.039%
E6	36.410%	30.610%	20.330%	5.572%	0.944%	0.324%	1.511%	0.135%	1.223%	2.940%
E7	5.000%	73.097%	20.770%	0.676%	0.003%	0.000%	0.076%	0.000%	0.006%	0.372%
E8	11.832%	59.652%	27.752%	0.369%	0.073%	0.003%	0.031%	0.000%	0.031%	0.256%
E9	62.507%	28.205%	6.285%	1.065%	0.096%	0.056%	0.671%	0.045%	0.006%	1.063%
Mean	41.078%	40.923%	11.191%	3.821%	0.543%	0.504%	0.503%	0.178%	0.162%	1.098%

2

Table 3(on next page)

The relative abundance of species in gut microbiota of Common Kestrel at genus level

The names of phyla in Table 3 represented *Lactobacillus*, *Escherichia-Shigella*, *Acinetobacter*, *Bifidobacterium*, *Enterococcus*, *Clostridium_sensu_stricto_1*, *Paracoccus*, *Burkholderia-Caballeronia-Paraburkholderia*, *Glutamicibacter* respectively.

Sample	*Lac*	*Esc*	*Aci*	*Bif*	*Ent*	*Clo*	*Par*	*Bur*	*Glu*	others
E1	6.618%	88.610%	0.011%	0.037%	3.140%	0.023%	0.000%	0.000%	0.000%	1.561%
E2	68.336%	1.787%	0.581%	0.034%	2.528%	0.042%	0.017%	5.567%	0.042%	21.066%
E3	24.037%	0.862%	38.448%	0.093%	0.448%	0.707%	0.101%	5.544%	0.023%	29.736%
E4	0.392%	0.166%	2.153%	0.031%	0.118%	0.214%	19.488%	0.854%	0.860%	75.724%
E5	0.693%	7.962%	5.040%	0.034%	15.026%	0.011%	0.149%	0.014%	9.411%	61.659%
E6	1.356%	0.130%	5.823%	3.086%	0.510%	1.043%	6.023%	4.445%	6.113%	71.470%
E7	66.056%	0.054%	0.211%	20.356%	2.120%	0.192%	0.031%	2.841%	0.011%	8.129%
E8	9.589%	1.536%	0.536%	25.502%	8.988%	28.177%	0.054%	2.060%	0.099%	23.459%
E9	7.988%	57.183%	0.798%	1.446%	3.340%	1.866%	0.273%	0.347%	0.000%	26.759%
Mean	20.563%	17.588%	5.956%	5.624%	4.024%	3.586%	2.904%	2.408%	1.840%	35.507%

1