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 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 community of the gut from 9 fecal samples from a wounded common kestrel by sequencing the V3-V4 region of the 16S ribosomal RNA gene. 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%). Our results offer fundamental data and novel strategies for the protection of wild animals.

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30 **Abstract**

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32 including immunology, physiology and development. The specific function and mechanism of

33 the gut microbiota in birds are distinct due to their body structure, physiological attributes and

34 life history. Data on the gut microbiota of the common kestrel, a second-class protected animal

35 species in China, are currently scarce. With high-throughput sequencing technology, we

36 characterized the bacterial community of the gut from 9 fecal samples from a wounded common

37 kestrel by sequencing the V3-V4 region of the 16S ribosomal RNA gene. Our results showed

38 that *Proteobacteria* (41.078%), *Firmicutes* (40.923%) and *Actinobacteria* (11.191%) were the

39 most predominant phyla. *Lactobacillus* (20.563%) was the most dominant genus, followed by

40 *Escherichia-Shigella* (17.588%) and *Acinetobacter* (5.956%). Our results offer fundamental data

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42

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

44 throughput sequencing, Noninvasive

45

46 **Introduction**

47 Sequencing technology has developed rapidly in recent years. Meanwhile, more and more

48 research on gut microbiota have revealed its important roles in immunology, physiology and

49 development (Guarner & Malagelada 2003; Nicholson et al. 2005), as well as several basic and

50 critical process, such as nutrient absorption, vitamins synthesis and diseases in both human and

51 animals (Fukuda & Ohno 2014; Kau et al. 2011; Omahony et al. 2015) . Gut microbiota analysis

52 of wild animals is becoming a new method that may provide information for animal protection

53 and animal husbandry. Reports concerning the gut microbiota of other avian species, such as

54 Cooper's hawk (*Accipiter cooperii*) (Taylor et al. 2019), bar-headed geese (*Anser indicus*) (Wang

55 et al. 2017), hooded crane (*Grus monacha*) (Zhao et al. 2017), Western Gull (*Larus occidentalis*)

56 (Cockerham et al. 2019), herring gull (*Larus argentatus*) (Fuirst et al. 2018) and black-legged

57 kittiwake (*Rissa tridactyla*) (van Dongen et al. 2013), have increased rapidly. Although studies

58 of the microbiome of these species have increased, there has been no corresponding trend in the

59 study of each species. The specific function and mechanism of the gut microbiota in birds are

60 distinct due to their body structure, physiological attributes and life history. For example, for

61 most birds, a stable body temperature above ambient temperature ensures a high metabolic rate

62 for the birds needed for flight. Streamlined bodies, efficient breathing patterns and relatively

63 short gastrointestinal tracts are also special attributes. Meanwhile, the birds' ability to fly sets

64 them apart from other animals, altering their intestinal microbiota to some extent. However, as a

65 research focus, data on the gut microbiota of the common kestrel are currently very scarce.

66 The common kestrel (*Falco tinnunculus*) is a small raptor that belongs to *Falconidae*, which is a

67 family of diurnal birds of prey, including falcons and kestrels. A total of 12 subspecies for

68 common kestrel are distributed widely from the Palearctic to Oriental regions (Cramp & Brooks

69 1992). Although listed in the least concern (LC) class by the International Union for

70 Conservation of Nature (IUCN) (BirdLife International. 2016), the common kestrel was listed

71 as state second-class protected animals (Defined by the LAW OF THE PEOPLE'S REPUBLIC

72 OF CHINA ON THE PROTECTION OF WILDLIFE, Chapter II, Article 9) in China. The

73 common kestrel is a typical opportunistic forager that catches small and medium-sized animals,

74 including small mammals, birds, reptiles and some invertebrates (Anthony 1993; Aparicio 2000;

75 Village 2010). Insects such as grasshoppers and dragonflies were also identified in the diet of the

76 common kestrel (Geng et al. 2009). As generalist predators, common kestrels choose distinct

77 predatory strategies when non-breeding and breeding to minimize the expenditure of energy,

78   such as the strategy of the low-cost low-profit technique of perch-hunting in winter, while

79   maximized daily energy gain in summer (Costantini et al. 2005; Masman et al. 1988).

80   Previous studies on common kestrels were comprehensive, such as those on diet and prey

81   selection (Geng et al. 2009; Kirkwood 1980; Korpimäki 1985; Lihu et al. 2007; Souttou et al.

82   2007; Van Zyl 1994), behavior and diseases (Aschwanden et al. 2005; Bustamante 1994; Hille et

83   al. 2007), and genetic variation and diversity (Nesje et al. 2000; Padilla et al. 2009; Riegert et al.

84   2010; Zhang et al. 2008). As common raptors around the whole world, as well as the important

85   predators in food chains, common kestrels should be studied more deeply with the newer

86   methods and techniques.

87   The aim of this study was to characterize the bacterial community of the gut by sequencing the

88   V3-V4 region of the 16S rRNA gene of a wounded common kestrel. The data we obtained could

89   provide basic information for further protection and rescue of wild common kestrels.

90

91   **Materials & Methods**

92   Sample collection

93   All fecal samples were collected from an injured common kestrel in the Beijing Raptor Rescue

94   Center (BRRC). The injured common kestrel that could not fly was found in the Fengtai district

95   by a rescuer on June 22nd, 2019 and then taken to the BRRC for professional rescue. The

96   wounded common kestrel was carefully treated with several surgeries and drug therapies. Nine

97   fecal samples (E1-E9) that may reflect the actual state of its health were collected from the

98   common kestrel after relevant treatments on different days. The samples collection information

99   and medical records of the common kestrel were shown in *Table S1-S3* respectively. All samples

100   were transported immediately into the laboratory in an ice box and ultimately stored at -80°C for

101   further bacterial studies.

102

103   DNA extraction and PCR amplification

104   Microbial DNA was extracted from fresh fecal samples using an E.Z.N.A.® Stool DNA Kit

105   (Omega Bio-tek, Norcross, GA, U.S.) according to the manufacturer's protocols. The V3-V4

106   region of the bacterial 16S ribosomal RNA gene was amplified by PCR (95 °C for 3 min;

107   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

108   at 72 °C for 5 min) using the primers 338F (5'-barcode-ACTCCTACGGGAGGCAGCAG-3')

109   and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), where the barcode is an eight-base

110   sequence unique to each sample. PCRs were performed in triplicate in a 20 μL mixture

111   containing 4 μL of 5 × FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM),

112   0.4 μL of FastPfu Polymerase, and 10 ng of template DNA.

113

114   Illumina MiSeq sequencing

115   Amplicons were extracted from 2% agarose gels and purified using an AxyPrep DNA Gel

116   Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's

117   instructions and quantified using QuantiFluor™ -ST (Promega, U.S.). Purified amplicons were

118   pooled in equimolar amounts and paired-end sequenced (2 × 250) on an Illumina MiSeq platform

119   according to standard protocols.

120

121   Processing of sequencing data

122   Raw fastq files were demultiplexed and quality-filtered using QIIME (version 1.17) (Caporaso et

123   al. 2010) with the following criteria. (i) The 300 bp reads were truncated at any site receiving an

124   average quality score <20 over a 50 bp sliding window, discarding the truncated reads that were

125   shorter than 50 bp. (ii) Exact barcode matching, 2 nucleotide mismatches in primer matching,

126   and reads containing ambiguous characters were removed. (iii) Only sequences that overlapped

127   longer than 10 bp were assembled according to their overlap sequence. Reads that could not be

128   assembled were discarded.

129   Operational taxonomic units (OTUs) were clustered with a 97% similarity cutoff using UPARSE

130   (version 7.1 http://drive5.com/uparse/), and chimeric sequences were identified and removed

131   using UCHIME (Edgar et al. 2011). The taxonomy of each 16S rRNA gene sequence was

132   analyzed by RDP Classifier (http://rdp.cme.msu.edu/) against the SILVA (SSU115)16S rRNA

133   database using a confidence threshold of 70% (Amato et al. 2013).

134

135   Data analysis

136   All the indices of alpha diversity, including Chao, ACE, Shannon, Simpson, and coverage, and

137   the analysis of beta diversity were calculated with QIIME. The rarefaction curves, rank

138   abundance curves, and stacked histogram of relative abundance were displayed with R (version

139   2.15.3) (Dalgaard 2010).

140   The hierarchical clustering trees were built using UPGMA (unweighted pair-group method with

141   arithmetic mean) based on weighted and unweighted distance matrices at different levels.

142   Principal coordinate analysis (PCoA) was calculated and displayed using QIIME and R, as well

143   as hierarchical clustering trees.

144   This study was performed in accordance with the recommendations of the Animal Ethics Review

145   Committee of Beijing Normal University (approval reference number: CLS-EAW-2019-026).

146

147   **Results**

148   Overall sequencing data

149   A total of 28 phyla, 70 classes, 183 orders, 329 families and 681 genera were detected among the

150   gastrointestinal bacterial communities. There were altogether 389,474 reads obtained and

151   classified into 1673 OTUs at the 0.97 sequence identity cut-off in 9 fecal samples from a

152   common kestrel.

153   Alpha diversity indices (including Sobs, Shannon, Simpson, ACE, Chao and coverage) of each

154   sample are shown in *Table 1*. The Sobs and Shannon index of all samples are shown in *Fig. 1*.

155 Additionally, the rarefaction curves (A) and the rank abundance curves (B) are shown in *Fig. S1*,

156 which indicated that the number of OTUs for further analysis was reasonable, as well as the

157 abundance of species in common kestrel feces. The total sequences, total bases and OTU

158 distributions of all samples are shown in *Table S4* and Table *S5*.

159

160 Bacterial composition and relative abundance

161 At the phylum level of the gut microbiota in the common kestrel, the most predominant phylum

162 was *Proteobacteria* (41.078%), followed by *Firmicutes* (40.923%), *Actinobacteria* (11.191%)

163 and *Bacteroidetes* (3.821%). In addition to *Tenericutes* (0.178%) and *Verrucomicrobia*

164 (0.162%), *Patescibacteria* (0.543%) and *Deinococcus-Thermus* (0.504%) were also ranked in the

165 top 10 species in the common kestrel fecal microbiota (*Table* 2).

166 The top 5 families in the gut microbiota were *Lactobacillaceae* (20.563%), *Enterobacteriaceae*

167 (18.346%), *Moraxellaceae* (6.733%), *Bifidobacteriaceae* (5.624%) and *Burkholderiaceae*

168 (4.752%).

169 At the genus level, *Lactobacillus* (20.563%), *Escherichia-Shigella* (17.588%) and *Acinetobacter*

170 (5.956%) were the most dominant genera. These were followed by *Bifidobacterium* (5.624%)

171 and *Enterococcus* (4.024%) (*Table* 3). These five genera in the total gut microbiota of several

172 samples accounted for a small proportion, such as for E5 (28.755%) and E6 (10.905%) and

173 especially for E4 (2.861%), while the largest proportion was 98.416% in E1.

174 The stacked histogram of relative abundance for species is also demonstrated in *Fig. 2* at the

175 phylum (A) and genus (B) levels, which could intuitively represent the basic bacterial

176 composition and relative abundance. The community structures of E1 and E9 were more similar

177 than those of the other feces samples at both levels.

178 The hierarchical clustering trees showed the similarity of community structure among different

179 samples, which were generated by UPGMA (unweighted pair-group method with arithmetic

180 mean) with the unweighted UniFrac (*Fig. 3A*) and weighted UniFrac (*Fig. 3B*) distance matrixes.

181   Although the fecal samples were collected from the common kestrel in chronological order (E1-

182   E9) of therapy treatments, no distinct or obvious clustering relationships are discernable in *Fig.*

183   *3*.

184

185   Discrepancy of community composition

186   To further demonstrate the differences in community composition among the nine samples,

187   principal coordinates analysis (PCoA) was applied (*Fig. 4*). For PCoA, we chose the same two

188   distance matrices (unweighted UniFrac in *Fig. 4A* and weighted UniFrac in *Fig. 4B*) as above to

189   analyze the discrepancies. The results in *Fig. 4* were similar to those in *Fig. 3*, in which all

190   samples scattered dispersedly, suggesting that variation in the composition of the gut microbiota

191   of the common kestrel was not obvious over time.

192

193   **Discussion**

194   Knowledge and comprehension concerning gut microbiota have continued to progressively

195   develop with relevant techniques over the past decade (Guarner 2014; Li et al. 2014; Qin et al.

196   2010). The application of analysis for intestinal microecology continues to be also a research

197   focus in the field of wild animal protection.

198   The common kestrel (*Falco tinnunculus*) is listed as a second-class protected animal species in

199   China. Although research concerning avian species, including the common kestrel, has been

200   increasing gradually, the available data on the gut microbiota in the common kestrel were

201   currently unknown.

202   We characterized the basic composition and structure of the gut microbiota from a wounded

203   common kestrel in this study, which was rescued by the Beijing Raptor Rescue Center (BRRC).

204   In general, the overall community structure of the gut microbiota in this common kestrel was in

205   accordance with previous relevant characterizations in birds, such as Cooper's hawks (Taylor et

206   al. 2019), bar-headed geese (Wang et al. 2017), hooded cranes (Zhao et al. 2017) and swan geese

207 (Wang et al. 2016), which included *Proteobacteria*, *Firmicutes*, *Actinobacteria* and

208 *Bacteroidetes*.

209 The most predominant phylum in the fecal gut microbiota of the common kestrel was

210 *Proteobacteria* (41.078%), which ranked after *Firmicutes* in other birds, such as cockatiels

211 (*Nymphicus hollandicus*) (Alcaraz et al. 2016) and black-legged kittiwakes (van Dongen et al.

212 2013). This crucial phylum plays many valuable roles. For instance, *Proteobacteria* is beneficial

213 for the giant panda, which can degrade lignin in its major food resource (Fang et al. 2012).

214 Additionally, it has been reported that *Proteobacteria* is also the most dominant phylum in obese

215 dogs (Park et al. 2015). The specific function of this phylum could be distinct in birds due to

216 their unique physiological traits, as well as their developmental strategies (Kohl 2012). However,

217 the high relative abundance of *Proteobacteria* in the total bacterial community was observed

218 mainly in several samples that were collected during surgeries or drug treatments, such as E1 and

219 E4. Sample E1 was collected on 23rd June that the day after kestrel rescued from the wild. On

220 22nd June, the kestrel was bandaged with silver sulfadiazine cream (SSD), also subcutaneously

221 injected with 10 ml and orally administered with 4ml lactated ringer's solution (LRS)

222 respectively. The increased level of *Proteobacteria* was associated with some cardiovascular

223 events, inflammation and inflammatory bowel disease (Amar et al. 2013; Carvalho et al. 2012).

224 Although the kestrel's weight increased 34 grams when E4 was collected, it just ate a mouse's

225 head. Combined with the status when kestrel was rescued, we speculated that the increased

226 proportion of *Proteobacteria* may reflect its food consumption or gastrointestinal status to some

227 extent. Environmental influential factors, as well as dietary changes, should also be considered

228 an important index that could result in variations in the relative abundance of species in the gut

229 microbiota (De Filippo et al. 2010; Scott et al. 2013).

230 Furthermore, the dominant genera within *Proteobacteria* in our study were *Escherichia-Shigella*

231 (17.588%), *Acinetobacter* (5.956%), *Paracoccus* (2.904%) and *Burkholderia-Caballeronia-*

232 *Paraburkholderia* (2.408%). *Escherichia-Shigella* is a common pathogenic bacterium that can

233   cause diarrhea in humans (Hermes et al. 2009). The main cause for the high relative abundance

234   of *Escherichia-Shigella* was the E1 (88.610%) sample, which suggested indirectly that the

235   physical condition of the common kestrel was not normal when it was rescued by staff from the

236   BRRC. This result was also consistent with the actual state of this wounded common kestrel that

237   we observed (*Table S3*).

238   Although *Firmicutes* (40.923%) ranked after *Proteobacteria*, its actual relative abundance was

239   only slightly lower than that in the common kestrel. As a common phylum of the gut microbiota,

240   *Firmicutes* exists widely in both mammals and birds, and this ancient symbiosis may be linked to

241   the common ancestor of amniotes (Costello et al. 2010; Kohl 2012). *Firmicutes* can provide

242   certain energy for the host through catabolizing complex carbohydrates, sugar, and even by

243   digesting fiber in some species (Costa et al. 2012; Flint et al. 2008; Guan et al. 2017).

244   The dominant genera in *Firmicutes* were *Lactobacillus* (20.563%), *Enterococcus* (4.024%) and

245   *Clostridium_sensu_stricto_1* (3.586%). The relative abundance of *Enterococcus* in E5

246   (15.026%) contributed to the highest ranking of this genus. *Enterococcus* is not regarded as a

247   pathogenic bacterium due to its harmlessness and can even be used as a normal food additive in

248   related industries (Fisher & Phillips 2009; Moreno et al. 2006). *Enterococcus* species are also

249   considered common nosocomial pathogens that can cause a high death rate (Lopes et al. 2005).

250   Meanwhile, these species are also associated with certain infections, including neonatal

251   infections, intraabdominal and pelvic infections, as well as the nosocomial infections and

252   superinfections (Murray 1990). Coincidentally, prior to the collection of sample E5, kestrel was

253   anesthetized for the treatment of the right tarsometatarsus injury. The right digit tendon of kestrel

254   was exposed before managing the wound, without any function. Although ensuring the

255   sterile conditions, we inferred that the kestrel was infected by certain bacteria during the surgery.

256   The BRRC could be regarded as a specific hospital for raptor, which could explain the high

257   proportion of *Enterococcus* in the fecal samples of this common kestrel. However, this genus

258   should be given sufficient attention in subsequent studies with additional samples from different

259 individuals. The abundance of *Clostridium* increases as more protein is digested (Lubbs et al.

260 2009). *Clostridium difficile* has been reported to be associated with certain diseases, such as

261 diarrhea and severely life-threatening pseudomembranous colitis (Kuijper et al. 2006; Pepin et al.

262 2004). The high relative abundance of this genus also resulted primarily from certain samples

263 (E8, 28.177%), similar to the *Enterococcus* mentioned above. And it's remarkable that the

264 collection of sample E8 was in the same situation as E5. On 13th July, the kestrel also underwent

265 surgery under anesthesia. While E5 was collected, the kestrel's status was still normal according

266 to relevant records. These results indicated that the high relative abundance of certain pathogens

267 may not show any symptoms of illness for kestrel. In general, the abnormal situation of E5 and

268 E8 still need to be paid enough attention. Moreover, to minimize the influences due to the

269 individual differences, more samples from different individuals should be collected for further

270 study.

271 The third dominant phylum in the gut microbiota in our study was *Actinobacteria* (11.191%),

272 which was also detected in other species, such as turkeys (*Meleagris gallopavo*) (Wilkinson et al.

273 2017) and Leach's storm petrel (*Oceanodroma leucorhoa*) (Pearce et al. 2017). The relative

274 abundance of *Actinobacteria* varied in different species, such as house cats (7.30%) and dogs

275 (1.8%) (Handl et al. 2011), but only accounted for 0.53% in wolves (Wu et al. 2017). Within this

276 phylum, *Bifidobacterium* (5.624%) and *Glutamicibacter* (1.840%) were the primary genera. The

277 presence of *Bifidobacterium* is closely related to the utilization of glycans produced by the host,

278 as well as oligosaccharides in human milk (Sela et al. 2008; Turroni et al. 2010). Noticeably,

279 *Bifidobacterium thermophilum* was reported to be used through oral administration for chickens

280 to resist *E. coli* infection (Kobayashi et al. 2002). The detection and application of

281 *Bifidobacterium*, especially for the rescue of many rare avian species, would be worth

282 considering for curing various diseases in the future.

283 Additionally, the relative abundance of *Bacteroidetes* was 3.821% in this study, which consisted

284 mainly of *Sphingobacterium*. *Bacteroidetes* is another important component of the gut

285 microbiota that can degrade relevant carbohydrates from secretions of the gut, as well as high

286 molecular weight substances (Thoetkiattikul et al. 2013). The proportion of *Bacteroidetes*, which

287 was stable in most samples we collected except E5 (18.166%), would increase correspondingly

288 with weight loss for mice or changes in fiber content in rural children's daily diet (De Filippo et

289 al. 2010; Ley et al. 2006; Turnbaugh et al. 2008). However, the weight of kestrel was increasing

290 during the collection of E5 and E8. Additionally, although kestrel underwent surgery on 4th July,

291 the reason for the high proportion of *Bacteroidetes* in its fecal sample E5 were unclear. To

292 characterize the basic composition and structure of the gut microbiota for the common kestrel

293 more accurately, additional fresh fecal samples from healthy individuals should be collected in

294 follow-up studies.

295 Furthermore, additional attention should be paid to the high ranking of *Patescibacteria* (0.543%)

296 and *Deinococcus-Thermus* (0.504%) at the phylum level. *Patescibacteria* might be related to

297 basic biosynthesis of amino acids, nucleotides and so on (Lemos et al. 2019). Members of

298 *Deinococcus-Thermus* are known mainly for their capability to resist extreme radiation,

299 including ultraviolet radiation, as well as oxidizing agents (Cox & Battista 2005; Griffiths &

300 Gupta 2007). The specific function of certain species in these phyla for the common kestrel

301 should be studied by controlled experiments, detailed observations or more advanced

302 approaches, as molecular biological techniques are developed.

303 In addition to the quantity of samples, living environment, age, sex and individual differentiation

304 should also be considered as influencing factors, which would cause a degree of discrepancies at

305 all levels in the gut microbiota. In addition, A comparison of wounded and healthy samples for

306 the bacterial composition in the intestinal microbiota is another essential research direction that

307 may provide additional information for wild animal rescue, such as important biomarkers that

308 indirectly indicate potential diseases.

309

310 **Conclusion**

311   In summary, using high-throughput sequencing technology in this study, we first characterized

312   the elementary bacterial composition and structure of the gut microbiota for a wounded common

313   kestrel in the BRRC, which could provide valuable basic data for future studies. Further research

314   on *Enterococcus*, *Patescibacteria* and *Deinococcus-Thermus* should be conducted in the future

315   with additional samples. The integration of other auxiliary techniques or disciplines, such as

316   metagenomics and transcriptomics, could offer a deeper understanding of the function and

317   mechanism of the gut microbiota, as well as the protection of wild animals.

318

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322

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Figure 1

Sobs index and the Shannon index of samples

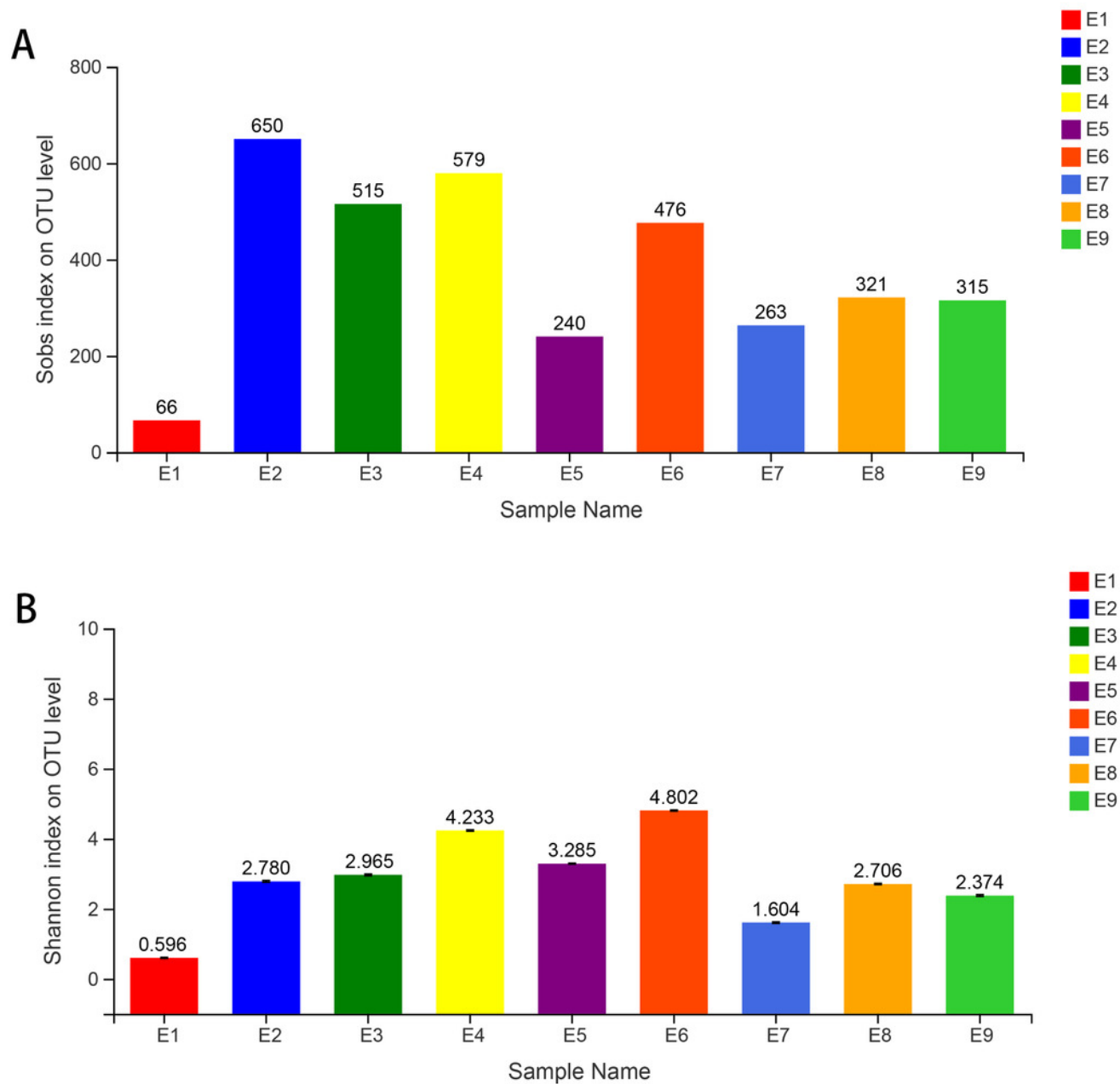



Figure 2

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

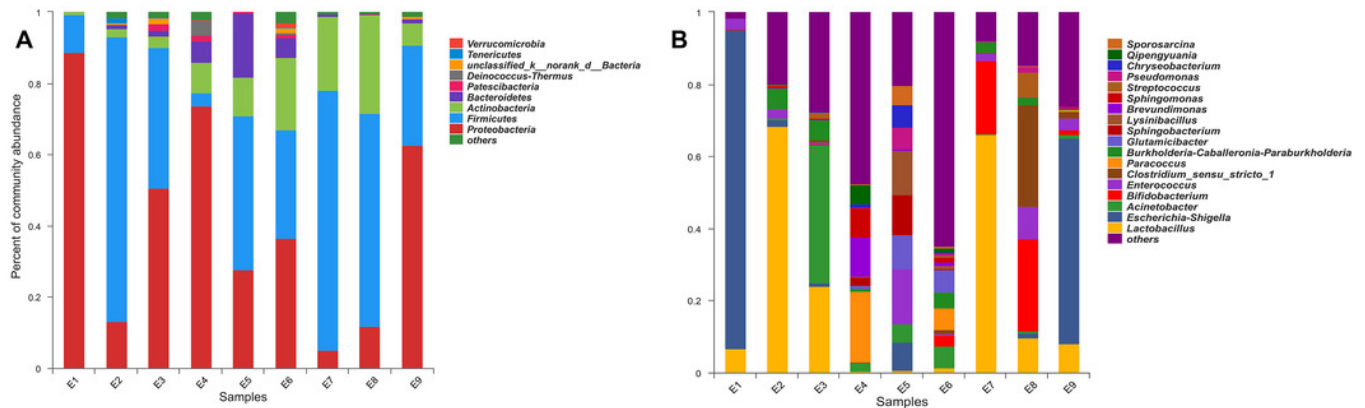



Figure 3

The hierarchical clustering trees

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

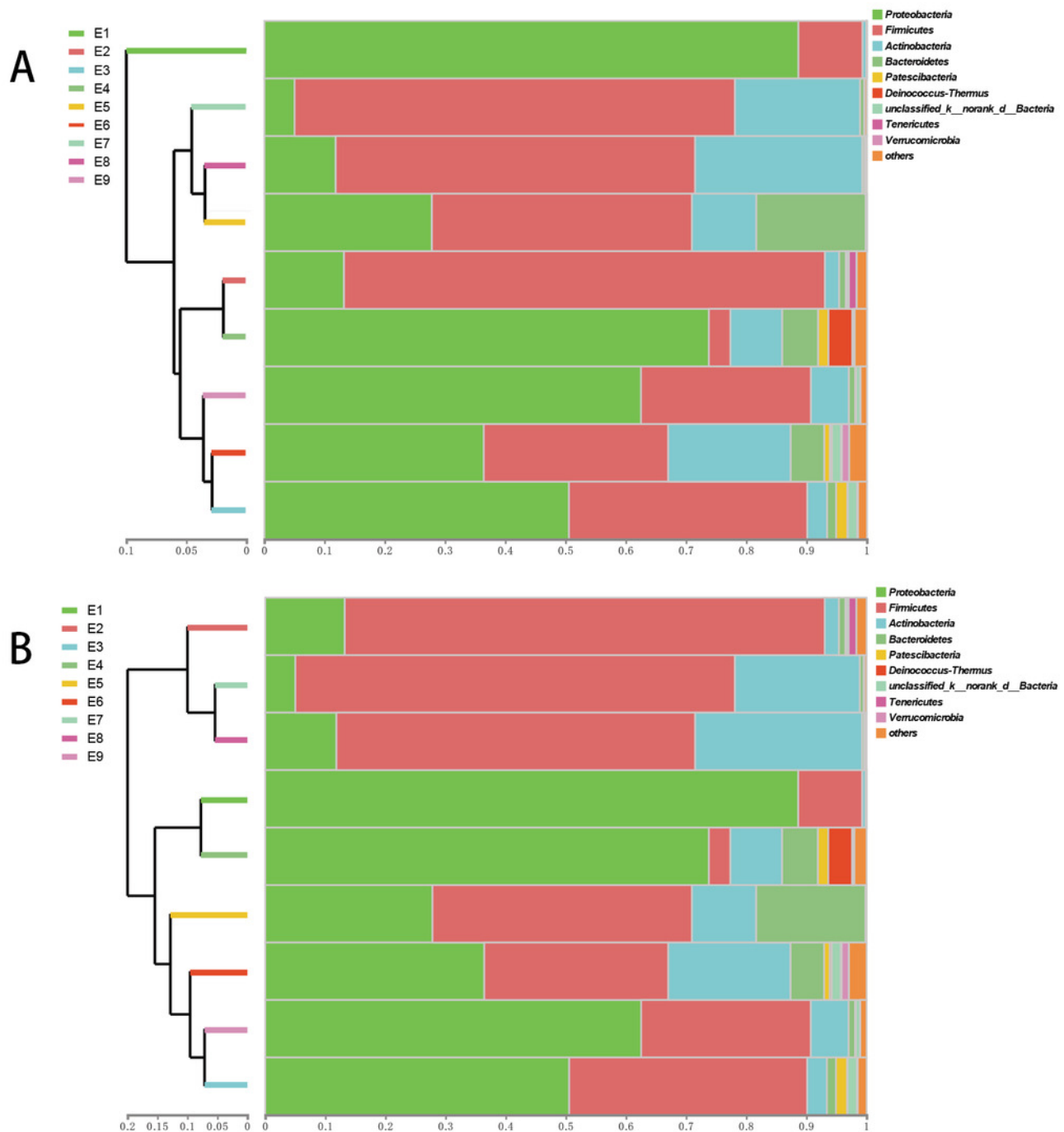
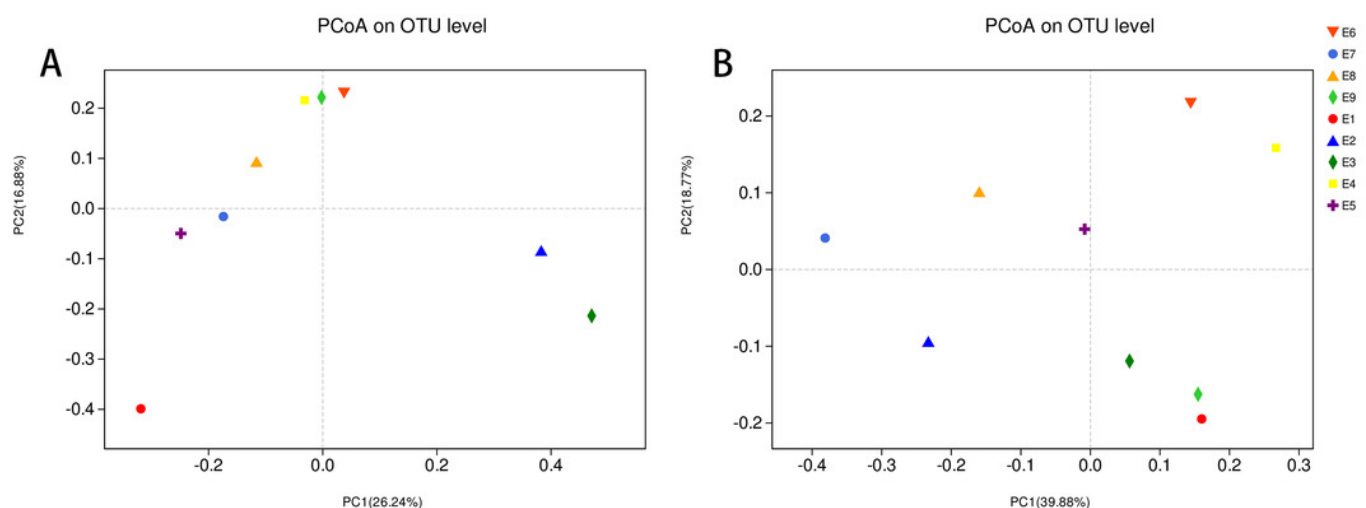



Figure 4

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.

Alpha diversity of gut microbiota in Common Kestrel feces

Sample	Sobs	Shannon	Simpson	Ace	Chao	Coverage
E1	66	0.596	0.788	78.114	73.583	1.000
E2	649	2.780	0.204	674.412	672.193	0.998
E3	515	2.965	0.184	524.452	522.519	0.999
E4	578	4.233	0.053	594.498	594.050	0.999
E5	235	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	317	2.706	0.143	364.651	359.519	0.998
E9	317	2.374	0.335	330.906	331.607	0.999

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.

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%

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%