Characterization of the upper respiratory tract microbiome of turkeys

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Abstract

Background: The respiratory tracts of turkeys are the main route of infection therefore plays important roles in the overall health and performance of the birds. Understanding the poultry microbiome has the potential to offer better diagnosis and rational management of many poultry diseases. Characterization of microbial communities in the upper respiratory tract of turkeys could help better understand the role of pathogenic bacteria and other commensal or symbiotic microorganisms in the infection. The aim of this study was microbiome characterization of upper respiratory tracks of commercial turkeys using next-generation sequencing technologies.

Results: The microbiome from samples collected from commercial turkey flocks was determined using 16S rRNA metagenomic approach. Taxonomic analysis of the microbiome was done by of the V3 and V4 regions of 16S rRNA gene (MiSeq, Illumina) amplification. The phylogenetic analysis identified the 10 bacterial phyla in turkey, the most abundant were phyla *Firmicutes* and *Proteobacteria*, accounting for >99% of all the sequences. The turkey sequences represent 144 established bacterial genera. Differences between bacterial abundances were found at the family and genus level. Several defining markers of microbiome succession were identified, including the presence of *Ornithobacterium* and *Mycoplasma*.

Conclusions: Understanding the turkey's respiratory microbiome is very important. Unique informations about microbiome representing members of the four major phyla of the respiratory tract in turkeys was assembled. These results obtained in this study supply information about turkey microbiome and can be useful in controlling, diagnosing and treating commercial turkey flocks. Our study significantly broaden the knowledge of the upper respiratory tract microbiome of turkeys.

Key words: upper respiratory tract, microbiome, 16S rRNA, turkeys

Background

Next-generation sequencing has resulted in a marked increase in culture-independent studies characterizing the microbiome of humans and animals [1–6]. Much of these works have been focused on the gut microbiome of humans and other production animals [7–11]. The growing number of studies on the avian microbiome demonstrates the influence of the gastrointestinal and respiratory microbiome on the proper development and efficiency of poultry production. In recent years the studies on the bacterial microbiome of poultry have primarily focused on chickens [12–15]. Particularly focused on the composition and diversity of intestinal microbiome of chickens [11,16]. Several studies have also described the gastrointestinal bacterial community in turkeys [11,17]. Less attention has been given to turkeys and their respiratory microbiome. The stability of the avian respiratory microbiome plays a critical role in preventing the colonization of pathogens. Any disruption of microbiological composition can lead to infection. Infection with bacteria such as mycoplasmas is commonly followed by a secondary bacterial or viral infection, leading to increased morbidity and mortality [18]. Infections such as coryza or infectious laryngotracheitis may be limited to the respiratory system, at least initially [19]. The pathogens could cause chronic subclinical upper respiratory infection. Bacterial species with the potential to induce infections in the respiratory tract, such as *Escherichia coli, Ornithobacterium rhinotracheale*, are often found in association with *M. gallisepticum* or *M. synoviae* [20,21]. In some cases, respiratory infections observed in a flock may be a component of a multisystemic disease or it may be the predominant disease with lesser involvement of other organ systems [19].

The microbiome is a bacterial community including commensal, symbiotic and pathogenic microorganisms which usually colonize an area of host affecting his health status [4]. The respiratory tracts of turkeys are colonized by bacteria that play important roles in the overall health and performance of the birds. We explored the bacterial communities of the upper

respiratory tract of turkeys, which will significantly broaden the knowledge about its composition. This study was conducted to define the core microbiome colonizing the upper respiratory tract (URT) in commercial turkey flocks.

Methods

Sample collection

Samples were collected from commercial turkeys from geographically distinct farms. Respiratory samples consisted of pooled trachea swabs that were collected from 60 birds per flock. Most birds at the time of sampling did not display lesions or other respiratory signs of clinical disease, only one flock (T-URT-9) had neurological symptoms and weak respiratory signs. Swab samples were shipped to the National Veterinary Research Institute as part of a monitoring program or for diagnostic tests. Relevant flocks metadata including age, year of sampling and location of flocks are in table 1. All samples were suspended in phosphate buffered saline (PBS) (1 ml PBS per one swab) and stored at - 20 °C. Part of the suspension was centrifuged for 10 min at 10,000 rpm. The supernatant was carefully removed and the pellets were suspended in 800 µl PBS. The supernatant was used for DNA isolation.

Table 1. Flock metadata.

ID of sample	Location	Age (week)	Year of sampling
T-URT-1	lubelskie	3	2020
T-URT-2	lubelskie	3	2020
T-URT-3	warmińsko-mazurskie	30	2019
T-URT-4	warmińsko-mazurskie	52	2017
T-URT-5	warmińsko-mazurskie	3	2017
T-URT-6	wielkopolskie	22	2017
T-URT-7	warmińsko-mazurskie	3	2019
T-URT-8	kujawsko-pomorskie	8	2017
T-URT-9	kujawsko-pomorskie	6	2019

DNA extraction and 16S rRNA gene sequencing

Genomic DNA was isolated from each pooled sample with the use of Maxwell RSC PureFood Pathogen Kit (Promega, USA) according to the manufacturer's recommendations. The quantity

and quality of the DNA was determined using the Nanodrop 1000 system (Thermo Scientific). Briefly, before starting extraction 50 µl lysozyme (10mg/ml, Novazym), 6 µl mutanolysin (5KU/ml, Sigma-Aldrich), and 8 µl lysostaphin (5g/ml, Sigma-Aldrich) was added to the samples followed by incubation for 45 min at 37°C. All 16S libraries were prepared using Illumina metataxonomic protocol: "16S Metagenomic Sequencing Library Preparation". The V3-V4 regions of 16S rRNA gene were amplified using 2x KAPA HiFi Hot Start Ready Mix (Roche) and primers:

16S Amplicon PCR Forward Primer:

5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG

16S Amplicon PCR Reverse Primer:

5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC

The length of targeted region is approximately 460 bp. Primers include overhang adapter sequences, which are compatible with Illumina index and sequencing adapters. PCR was conducted according to the manufacturer's recommendations. Products were checked on Fragment Analyzer using kit: dsDNA 935 Reagent Kit. Clean up step was performed using AMPure XP beads (Beckman Coulter), according to the protocol. Index PCR step was carried out with use of 2x KAPA HiFi Hot Start Ready Mix (Roche) and dual Index adapters (Illumina) according to the manufacturer's recommendations. Clean up step was again performed using AMPure XP beads (Beckman Coulter), according to the protocol. Libraries were checked and average libraries sizes were determined on Fragment Analyzer, using kit: dsDNA 935 Reagent Kit. Quantification of the libraries was carried out with use of Qubit 3.0 fluorometer (Thermo Fisher Scientific). Normalization of the libraries was performed according to the protocol, libraries were pooled in equimolar concentration and then denatured according to the Illumina protocol and diluted to the final concentration 20 pmol. The diversity of the run was ensured

by compositing metataxonomic with WGS Illumina libraries. The sequencing run was performed on Illumina MiSeq platform and MiSeq reagent kit V3 (600 cycles) with paired reads.

16S rRNA gene taxonomy assignments

The 16S rRNA gene sequencing data was processed through the Quantitative Insights into Microbial Ecology (QIIME 2Core 2020.2) [22] and Krona [23]. The sequences were clustered into operational taxonomic units (OTUs) using dada2 denoise-paired method with parameters: --p-trim-left m which trims off the first m bases of each sequence (Illumina indexes trimming); --p-trunc-len n which truncates each sequence at position n (forward reads n=300, reverse reads n=242) allowing to remove regions of sequences below quality score 15. During this step, reads were also corrected and chimeric sequences filtered. Trained Silva 132 99% OTUs (full-length) classifier was used to assign taxonomy to sequences. Sampling depth was even to 62,338 to ensure that every sample was taken into consideration in analysis.

To compare and illustrate the overall URT microbial community structures of the turkeys flocks Krona charts that allow comparison between microbiomes based on detailed phylogenetic composition were generated. Krona charts were generated using the - krona_qiime.py from Qiime2_pipeline_IT_EMP.md(https://github.com/lokeshbio/AmpliSeq/blob/master/Qiime2_pipeline_IT_EMP.md#krona-plots). The Venn diagrams, including all OTUs generated by the OTU picking step, were calculated using the website Bioinformatics & Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn). The metadata of flocks and corresponding taxonomic classifications in Krona charts have been included as Additional files 1 and 2 respectively.

Statistical analysis of the URT microbiome of turkeys

The relative taxa abundance of the flocks is presented as a mean % value. Alpha and beta diversity plots were measured using the Shannon indexes. To assess the association between

142 the microbial community of the upper respiratory tract of turkeys ANCOM analysis
143 implemented in QIIME 2 was performed on an unweighted UniFrac distance matrix of 9
144 samples [24]. The Kruskal-Wallis test was used to detect significant differences in richness and
145 diversity between bacterial communities present in URT in turkeys. Construction of heatmaps
146 was performed using the QIIME 2 feature-table plugin. Principal coordinate analysis graphs
147 (PCoA) were constructed to visualize sample clustering by bacterial community composition.
148 A Venn diagram was constructed to reveal bacterial OTUs at the genera level that were unique
149 or shared between different flocks. The OTUs observed in any samples in a flock were counted.

150 **Results**

151 **URT microbiome of turkey**

152 Metagenomic methods have been used to describe the microbial community structure of URT
153 in turkeys. We characterized microbial communities by sequencing V3-V4 regions of 16S
154 rRNA gene. Flocks from nine different commercial farms concurrently in this study were used.
155 The bacterial diversity in the URT were generally similar but in some cases differences in
156 bacterial diversity were noted (Figure 1).

157 The sequences from turkeys represent 10 different phyla including one unclassified, 68
158 established bacterial family and 144 genera (Figure1, 2). Over 99% of the URT microbiota of
159 the turkeys flocks was comprised of *Firmicutes* (69,11% ± 20,53%), *Proteobacteria* (26,41%
160 ± 16,90%), *Bacteroidetes* (2,31% ± 2,17%), *Actinobacteria* (2,26% ± 4,94%), *Tenericutes*
161 (0,015% ± 0,03%), *Cyanobacteria* (0,087% ± 0,218%) and unclassified phylum (0,002% ±
162 0,004%). In one flock (T-URT-1) a very small number of bacteria belonging to phylum
163 *Patescibacteria* (0,003%) was found. However, in another flock - T-URT-2 was also a small
164 number of bacteria from two different phyla: *Synergistetes* (0,01%) and *Verrucomicrobia*
165 (0,01%). The microbiomes of T-URT-7, T-URT-8 and T-URT-9 have a higher abundance of
166 OTUs from *Bacteroidetes* and *Actinobacteria* phylum than others flocks. In addition, the

167 microbiome of T-URT-9 was dominated by bacteria from the *Firmicutes* phylum (Figures 1).
168 The most common bacterial OTUs in this flock were *Enterococcus* but also classified OTUs
169 *Actinobacter, Psychrobacter, Neisseria* and also to the species level: ORT and *M.*
170 *gallisepticum.*
171 In all flocks *Firmicutes* was dominated by *Bacilli* and *Clostridia, Proteobacteria* was largely
172 represented by *Gammaproteobacteria* and *Alphaproteobacteria. Bacteroidetes* consistent only
173 of the class *Bacteroidia. Actinobacteria* was the most abundant classes in the phylum
174 *Actinobacteria.* The most common bacterial OTUs found on average in URT samples were
175 *Enterococcus* (38,78%), *Lactobacillus* (12,22%), *Escherichia-Shigella* (12,04%),
176 *Pseudomonas* (1,3%) and unclassified *Enterobacteriaceae* (1,81%) (Figure 3 and Additional
177 file 1 and 2). By comparing the microbiome datasets between the flocks we identified several
178 bacterial clasess, including *Bacilli* and *Clostridia*, that were differentially expressed.
179 Upon examining the microbiome composition of turkeys flocks, we found that in each sample
180 are bacteria belonging to a small number of taxonomic classifications. The tracheal swabs
181 included OTUs classified as *Ornithobacterium, Mycoplasma, Gallibacterium, Avibacterium*,
182 *Staphylococcus* and *Streptococcus* (Table 2, Figure 3). The majority of the reads were not
183 distinguishable to the genera node.
184 Table 2. Selected OTUs in turkeys URT

ID	*Mycoplasma*	*Ornithobacterium*	*Gallibacterium*	*Avibacterium*	*Staphylococcus*	*Streptococcus*
T-URT-1	-	-	-	-	+	-
T-URT-2	-	-	-	-	+	+
T-URT-3	-	+	+	-	+	+
T-URT-4	-	-	+	+	+	+
T-URT-5	-	-	-	-	-	-
T-URT-6	+	+	-	-	-	-
T-URT-7	+	+	-	-	+	-
T-URT-8	-	+	-	-	+	+
T-URT-9	+	+	-	-	+	+

185

In order to evaluate microbiota differences between samples, we analyzed the b-diversity based on unweighted UniFrac for these groups. The UniFrac distance matrix was represented through PCoA (Figure 4). The effects of ORT and *Mycoplasmas* the occurrence and age on bacterial composition were visualized by PCoA based on the weighted UniFrac distance matrix (Figure 4). PCoA clearly distinct samples based on occurrence of these two pathogens. A Venn diagram was constructed to reveal bacterial OTUs that were unique or shared between different flocks (Figure 4).

The richness of the microbial communities in some flocks was decreasing with the age but flocks did not differ significantly in diversity. The PCoA graph showing that the age of flocks with *Mycoplasma* OTUs were at 3 to 22 weeks and birds whit ORT OTUs were at 3-30 week (Figure 4, Table 1). The Faith PD phylogenetic diversity based on the geographical localization of flocks was no significant difference between flocks (Kruskal-Wallis Test) (Figure 5).

We performed a composition analysis of microbiome (ANCOM) to identify key genera discriminating the microbiota of turkey flocks. From the all observed OTUs at the genus level, two unclassified *Enterococcus* (W = 193), (W=143) and ORT (W = 160) showed a significant difference ($p < 0.05$) in abundance between the microbiome in the URT in *Mycoplasma* positive flocks (Figure 6).

Discussion

Understanding the poultry microbiota has the potential to offer better diagnosis and rational management of many poultry diseases including the use of antibiotics. New sequencing technologies enable us to characterize the turkey's respiratory microbiome without using traditional culturing techniques. Through 16S rRNA gene variable regions analysis of the whole bacterial community from tracheal swabs samples, with possibility detection of pathogens without the need for culture. Some of the respiratory pathogens are carried in the healthy flocks and the factors that cause the switch between carriage and disease (such as inhibition by

211 commensal bacteria or intercurrent viral infections) are only partly understood. Most of the
212 current research on the commensal bacteria (microbiota) of poultry has been mainly focused on
213 the gut microbiome of chicken, and less attention has been given to turkeys and respiratory
214 microbiome.

215 In this study, we tested samples from turkey flocks of different ages and geographically
216 distinct farms. We identified bacterial profiles of URT microbiome of turkey and provided new
217 insights for further identification of novel pathogens in farm flocks.

218 Based on the results of this study, a number of avian commensals as well as pathogens
219 belonging predominantly to the phyla *Firmicutes, Proteobacteria, Bacteroidetes,*
220 *Actinobacteria,* and *Tenericutes,* have been reported. These are the main phyla in the respiratory
221 tract reported also in domestic and wild birds [25,26], and similarly like those present in the
222 respiratory tract microbiota of other animals [27,28]. At the genus level *Lactobacillus,*
223 *Enterococcus, Escherichia-Shigella, Morganella* were most abundant and represented the
224 bacterial genera in the respiratory tract of turkeys. In this study, 68 taxa at the family level were
225 observed (Figure 2, 3). One hundred and forty-four genera were found in the URT of turkeys.
226 Results of the study on the digestive system of chickens and turkeys demonstrated that age and
227 environment appear to play a key role in the initial stages of turkey bacterial microbiome
228 maturation [17,29]. In other studies, significant differences in the richness and diversity of
229 respiratory track communities were observed between age groups but in the nasal cavity of
230 chickens [15]. However, the tracheal community composition shifted very gradually as the
231 chicken aged. In this study, tracheal swabs samples were analyzed at the OTU level for specific
232 subsets of OTUs representing geographical localization of sampling and age of birds (Figure
233 5). The richness of the microbial communities in some flocks was decreasing with the age but
234 flocks did not differ significantly in diversity (AMOVA). It was clear from this analysis that
235 there were shared subsets of OTUs present across all samples, unique subsets that were defining

236 of OTUs that were dependent on flocks age. In turkeys, as in chickens, the most common
237 bacteria identified in the respiratory tract were members of the *Lactobacillales* and along with
238 members of the *Enterobacteriales* [12,30]. In this study, *Lactobacillus* and *Enterococcus* were
239 detected in all URT samples; although their relative abundance was varied in some flocks. Some
240 OTUs displayed a temporal trend and were found more prominently in later aged turkeys, such
241 as those classified as *Enterococcus* and earlier age such as *Lactobacillus*.
242 Core microbiome analysis suggests that the URT microbiome is distinct from gut and litter
243 samples, but seems to be partially reflective of both. The data from this study indicate that the
244 turkey URT microbiome is a combination of exposure to the litter environment and preferential
245 selection for microbes from that environment with the capacity to colonize. Bacteria that have
246 previously been found as members of the turkeys gut microbiota, such as *Ruminococcus,*
247 *Clostridium, Virgibacillus, Blautia* and also *Brachybacterium, Brevibacterium,*
248 *Staphylococcus, Corynebacterium* and *Weissella* were also isolated in this study [11,17]. They
249 were found in ileum and litter samples, but were absent from cecal samples. Bacteria previously
250 isolated from poultry house air, such as *Jeotgalicoccus* [31] were also found in the respiratory
251 tract in chickens [12] and now also in URT of turkeys.
252 We note that flocks T-URT-1 and T-URT-2 contained a more diverse microbiome than the
253 other flocks. The cause of this could be various factors such as housing and environmental
254 conditions, age, or performance stress. In URT of these flocks a small abundance of bacteria
255 from three phyla: *Patescibacteria, Verrucomicrobia* and *Synergistetes* were found. The bacteria
256 from the family *Akkermansiaceae* (phylum *Verrucomicrobia*) are mucin-degrading bacteria and
257 may be the most prestigious microorganisms among the next-generation probiotics [27]. The
258 bacteria from phylum *Synergistetes* inhabit a majority of anaerobic environments including
259 animal gastrointestinal tracts, soil, and wastewater treatment plants and they are also present in
260 sites of human diseases such as cysts, and areas of periodontal disease [32]. The *Patescibacteria*

phylum is the newly defined superphylum and has been found to be prevalent in groundwater, sediment, lake, different aquifer environments and also in soybean. This superphylum has small genomes and a presumed symbiotic or parasitic lifestyle [33,34]. However, we also do not know the role of bacteria that we found as unclassified at the phylum level, therefore a further study of turkeys' respiratory microbiomes is needed.

Several bacterial taxa identified in these study have been described as positively affecting chicken flock performance. These include *Bacteroides, Faecalibacterium, Parabacteroides* in the gut; and *Bifidobacterium, Corynebacterium, Dietzia, Staphylococcus* in the trachea [28]. In another study identified *Faecalibacterium* as being correlated with enhanced feed conversion rates in chickens *Bacteroides* have been reported as core components of the poultry microbiota in other studies, and perhaps deserve attention for their possible probiotic capabilities [11,28,35,36].

In the URT of turkeys, potential respiratory pathogens including *Avibacterium, Gallibacterium, Mycoplasma*, and *Ornithobacterium* were found (Table 2). Multifactorial respiratory disease in poultry is often associated with bacterial factors including *E. coli*, ORT, *Mycoplasma gallisepticum, M. synoviae* are frequently implicated. The most pathogenic bacterial species causing significant diseases of poultry are *Mycoplasma gallisepticum* (mycoplasmosis) and ORT (respiratory disease) [25]. The upper respiratory tract is a reservoir of opportunistic pathogens, which can proliferate and infect the air sacs when poultry immune system is compromised due to stress or primary viral infections. Interestingly, most birds at the time of sampling did not display respiratory signs of clinical disease. One flock T-URT-9 had neurological symptoms and weak respiratory signs. Such neurological problems have been reported in turkeys due to meningoencephalitis caused by MG neurotropic strains [37]. Presents of ORT can also induce osteomyelitis of the cranial bones in turkeys causing nervous signs, movement disturbances and recumbence [38]. This highlights the potential importance of these

pathogens in the environment, causing also subclinical disease and subsequently impacting the performance of birds. Community level similarities in the bacterial microbiome of samples were compared using principal coordinates analysis (Figure 4). Samples were stratified primarily by the age of flock. Using AMOVA based upon distance matrix, bacterial communities from all sample types were distinct ($P < 0.001$). Upon visualization of the PCoA plots, *Mycoplasma* positive and ORT positive samples did not overlap and were distinct from each other. However, all sample types shifted similarly over time on the plot, indicating that bird age has an impact on the bacterial microbiome. Venn diagram showing the number of OTUs at the genus level that were unique or shared between flocks with and without *Mycoplasma* and *Ornithobacterium.* Therefore, the interaction between turkeys and these species should be studied to understand the function of the respiratory tract microbiota of turkeys.

Our study identified bacterial profiles of turkeys respiratory microbiome and provided new insights for further identification of novel pathogens in flocks of these birds species. We determined that in the URT turkey microbiome were OTUs of different bacterial species. Most of the organisms not previously associated with the respiratory tracts of birds were present in relatively low proportions. However, it is worth noting that health problems in flocks of turkeys can be caused not only by known pathogens but also by bacteria that were traditionally not considered pathogenic. The avian respiratory tract is the common site of viral pathogen entry and disease, including Newcastle disease, infectious bronchitis, or avian influenza which are dangerous to the health and life of birds. Bacterial infections not only have a devastating effect on the poultry flocks, but they also render flocks immunosuppressed and susceptible to viral infections. Therefore, it is very important for the poultry industry to prevent poultry respiratory infections. To understand the relationship between respiratory tract microbiota of turkeys and factors influencing it, an adequate number of samples will be needed in further study.

Conclusion

It is very important that various bacteria have been identified in the microbiomes, including potentially pathogenic ones. It underlines the importance of monitoring the microbiome of healthy animals for potential emerging diseases and their treatment. Understanding associations with bacteria present in the airways and potentially pathogenic bacteria highlights the importance of this study.

Abbreviations

URT: Upper respiratory tract

QIIME: Quantitative Insights Into Microbial Ecology

OTU: Operational taxonomic unit

PCoA: Principal Coordinate Analysis

Ethics declarations

Ethics approval and consent to participate

Not applicable

Consent for Publication

Not applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Contributions

OK was involved in study design, performed sample processing, data analysis and preparation of the manuscript draft. GT assisted with the sample organization, data management. AS-D

assisted with the sample processing, and analyses. AG and MS-S performed sample processing and bioinformatics analyses and were involved in the preparation of the manuscript draft. All authors read and approved the final manuscript.

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Figure legends

Fig. 1. Taxonomic diversity plot showing the relative abundance of taxa at the phylum level in each sample.

Fig. 2 Taxonomic diversity plot showing the relative abundance of taxa at the family level in each sample

Fig. 3 Heatmap of bacterial OTUs in turkeys URT

a) Heatmap of bacterial OTUs in the upper respiratory tract of turkeys. Heatmap depicting abundance of all 73 OTUs by overall abundance across samples. Normalized heatmap on taxonomic level 5 was constructed with clustering on both samples and feature axes. b) Shannon diversity index comparing URT flocks.

Fig. 4 Shared and unique OTUs at the genus level in the URT of turkeys.

a) Venn diagram showing the number of OTUs at the genus level in flocks *Mycoplasma* positive (T-URT-6, T-URT-7, T-URT-9) and flocks *Mycoplasma* negative (T-URT-3, T-URT-4,T-URT-5). **b)** Venn diagram showing the number of OTUs at the genus level in flocks *Mycoplasma* positive (T-URT-6, T-URT-7, T-URT-9) and flocks *Ornithobacterium* positive (T-URT-3, T-URT-6,T-URT-7, T-URT-8). **c), d)** Clustering of URT of turkeys according to the occurrence of *Mycoplasma* (c) and ORT (d) in the microbiome. PCoA graph showing the significantly separate clustering by community composition of the bacterial communities in tracheal swabs from turkeys of different ages (AMOVA: $P<0.001$).

Fig. 5 Comparison of URT microbiome of turkeys

a) The Faith phylogenetic diversity (PD) rarefaction curve comparing URT microbiome.

b) The Faith PD boxplots.

463 **Fig. 6** ANCOM Volcano Plot

464 In ANCOM analysis the W value represents the number of times of the null-hypothesis (the
465 average abundance of a given species in a group is equal to that in the other group), it was
466 rejected for a given species. The clr (axe x) is central log ratio.

467

468 Additional file 1 – KRONA charts

469 Additional file 2 – ID of flocks in Krona file

Figures

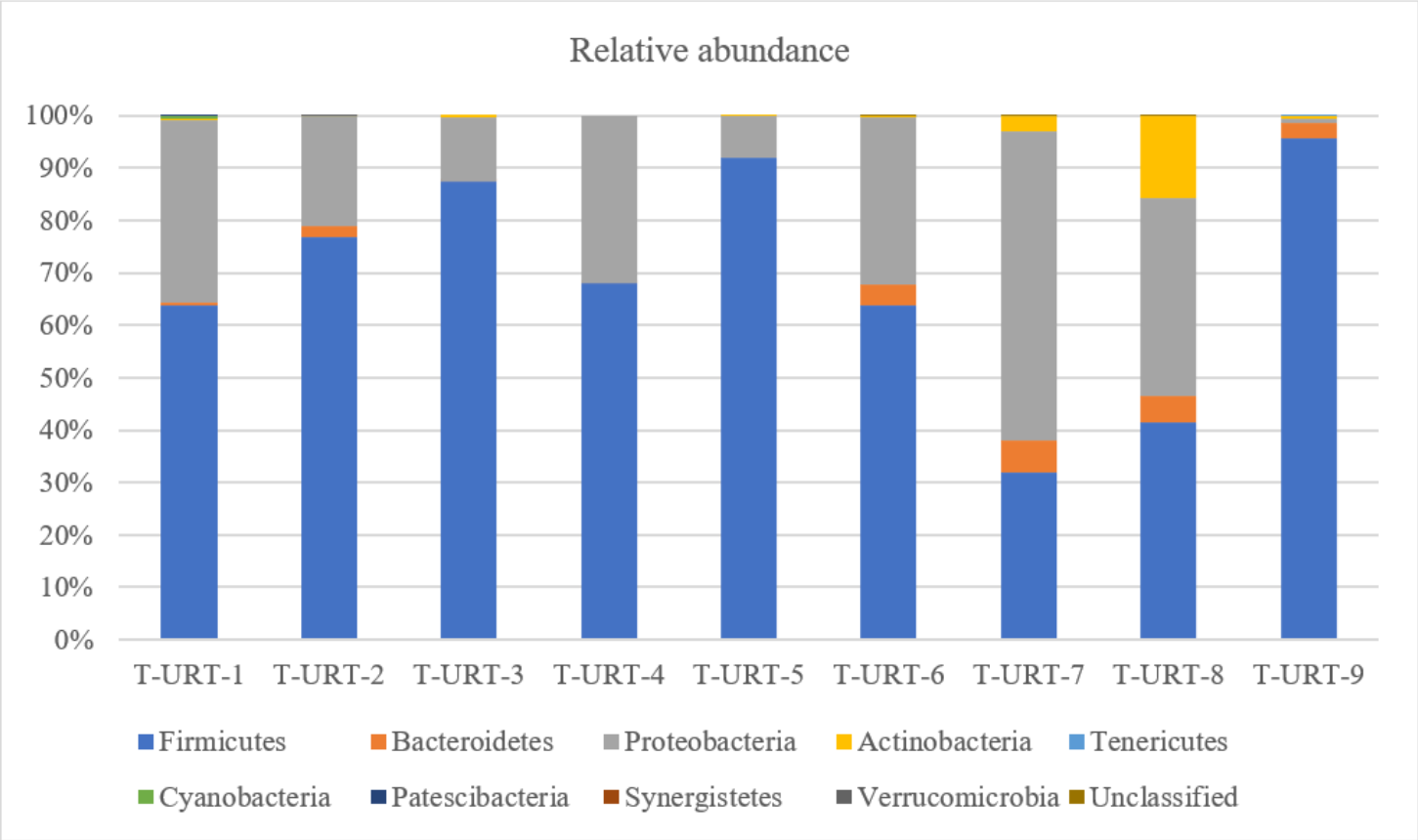

Figure 1

Taxonomic diversity plot showing the relative abundance of taxa at the phylum level in each sample.

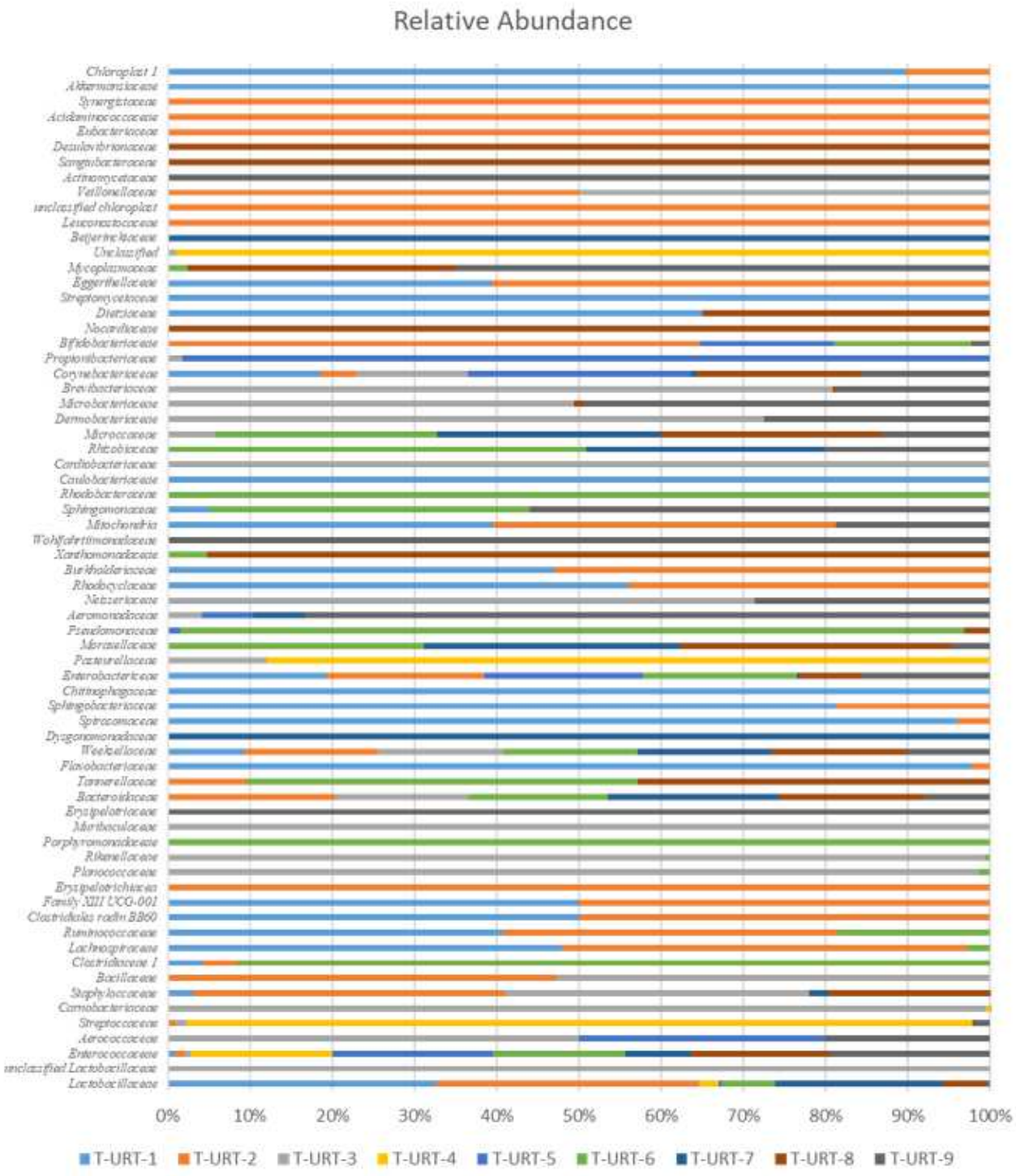

Figure 2

Taxonomic diversity plot showing the relative abundance of taxa at the family level in each sample

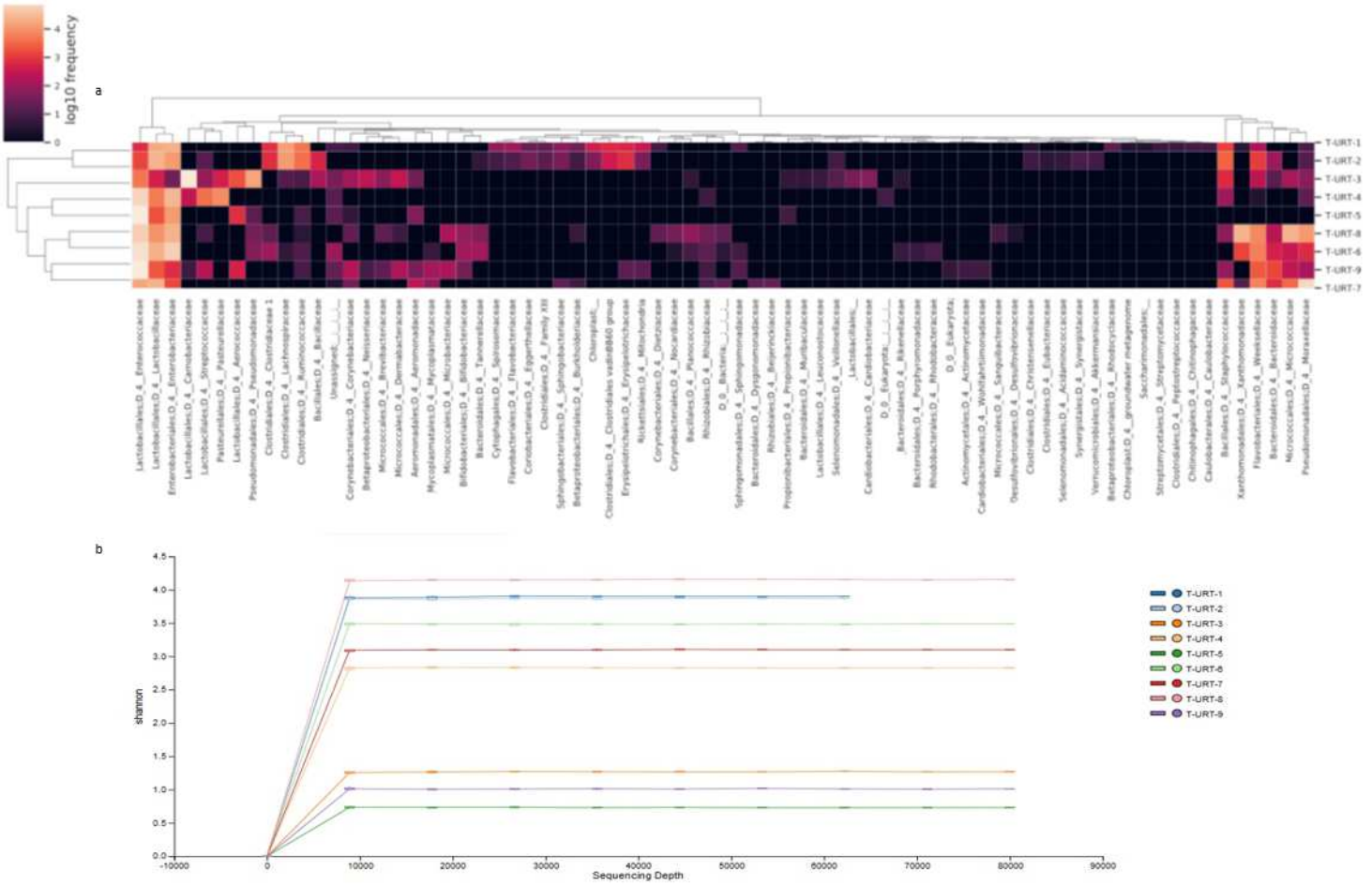

Figure 3

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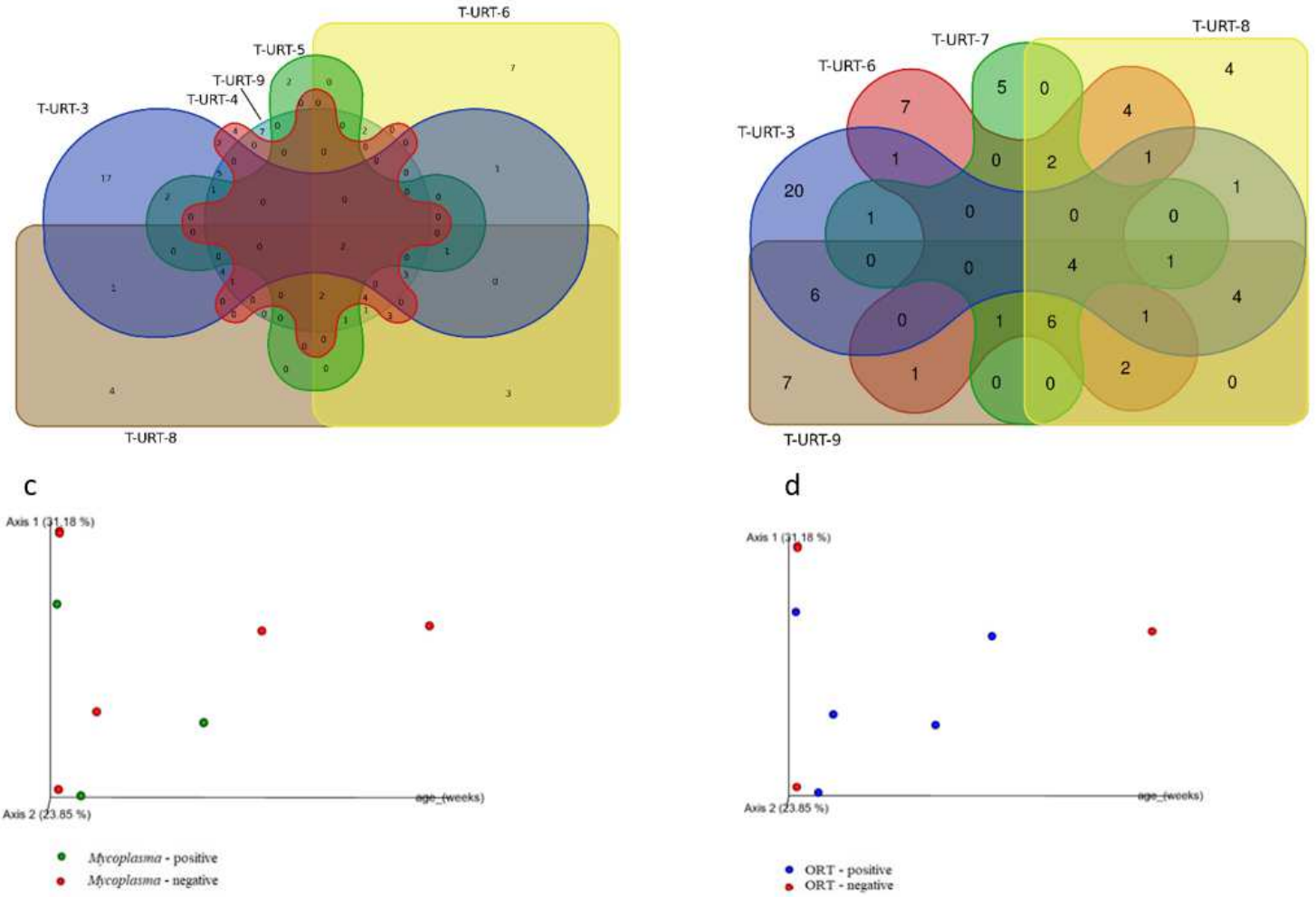

Figure 4

Shared and unique OTUs at the genus level in the URT of turkeys. a) Venn diagram showing the number of OTUs at the genus level in flocks Mycoplasma positive (T-URT-6, T-URT-7, T-URT-9) and flocks Mycoplasma negative (T-URT-3, T-URT-4,T-URT-5). b) Venn diagram showing the number of OTUs at the genus level in flocks Mycoplasma positive (T-URT-6, T-URT-7, T-URT-9) and flocks Ornithobacterium positive (T-URT-3, T-URT-6,T-URT-7, T-URT-8). c), d) Clustering of URT of turkeys according to the occurrence of Mycoplasma (c) and ORT (d) in the microbiome. PCoA graph showing the significantly separate clustering by community composition of the bacterial communities in tracheal swabs from turkeys of different ages (AMOVA: P<0.001).

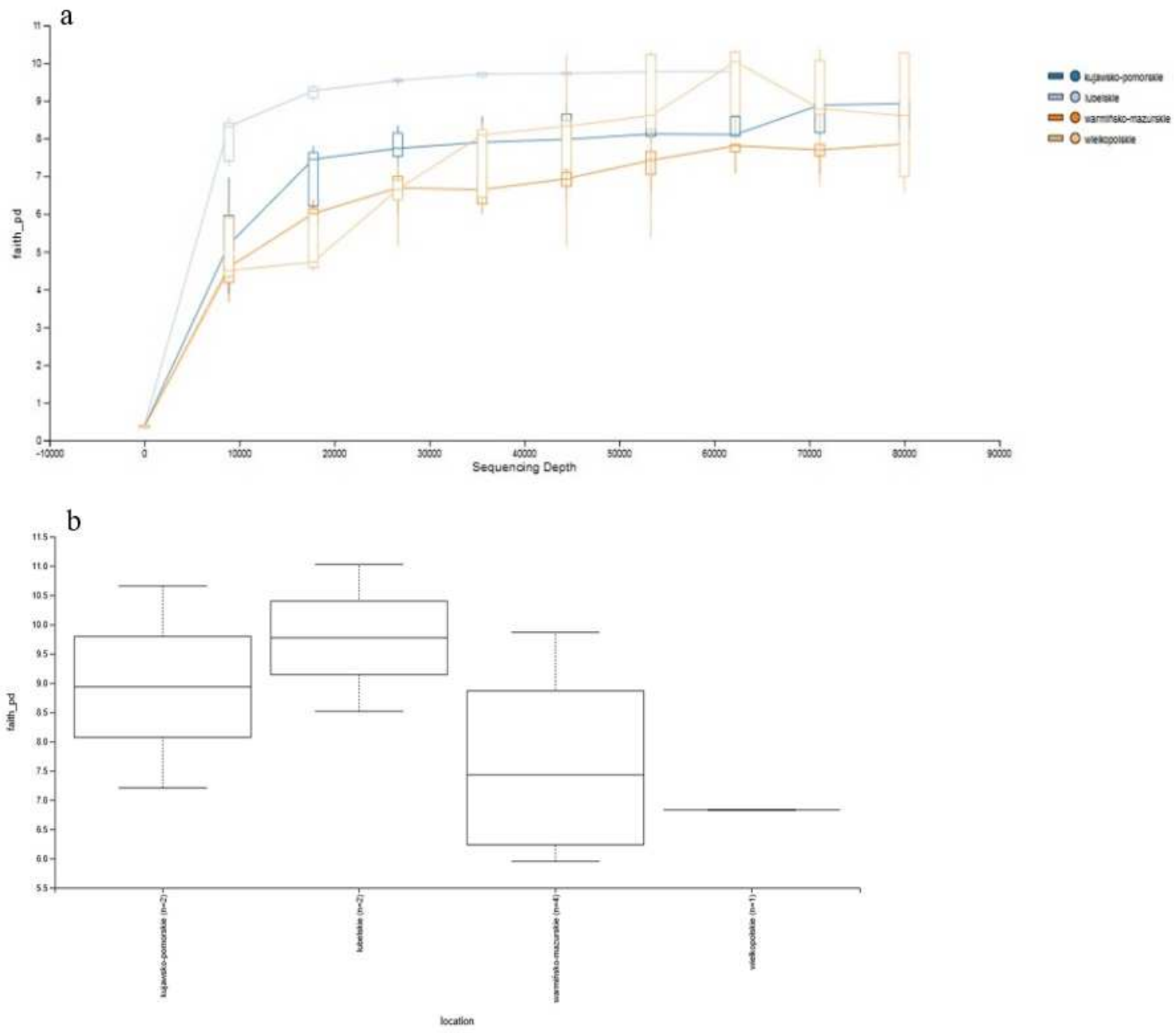

Figure 5

Comparison of URT microbiome of turkeys a) The Faith phylogenetic diversity (PD) rarefaction curve comparing URT microbiome. b) The Faith PD boxplots.

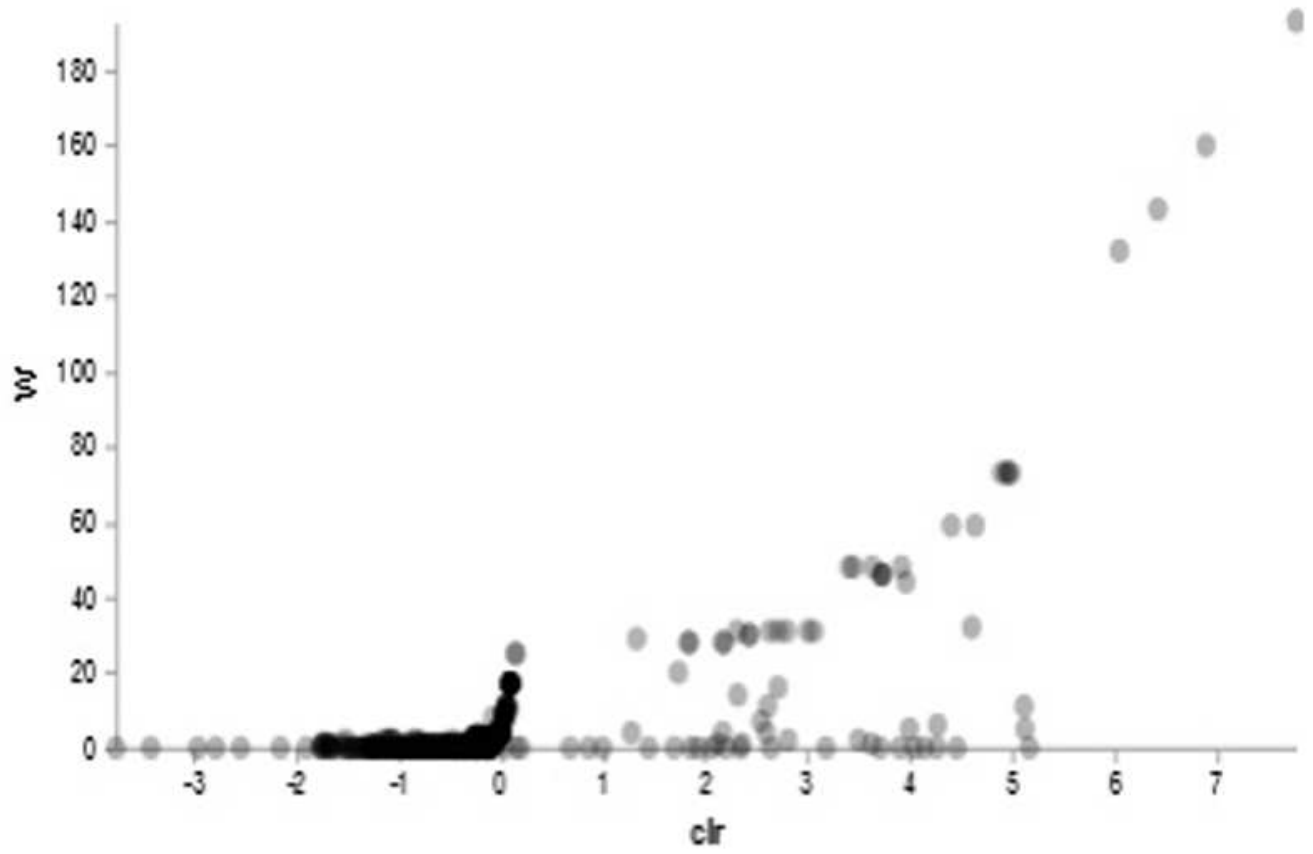

Figure 6

ANCOM Volcano Plot. In ANCOM analysis the W value represents the number of times of the null-hypothesis (the average abundance of a given species in a group is equal to that in the other group), it was rejected for a given species. The clr (axe x) is central log ratio.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile2.docx
- KRONAcharts.html