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Gut Microbial Communities of Captive and Wild Siberian Cranes and Their Associations with Soil Microbiota in Poyang Lake Wetlands, China

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Simple Summary

The gut microbiota of captive and wild Siberian cranes (*Leucogeranus leucogeranus*) and their associations with soil microbiota in the Poyang Lake wetland were analyzed and compared in this study. Captive cranes had richer and more even gut microbiota communities than their wild counterparts. While gut and soil microbiota were distinct overall, the gut communities of captive and wild cranes showed some similarity. Firmicutes was the dominant gut microbiota group. Notably, captive cranes had higher levels of certain microbiota like *Ligilactobacillus*, while wild cranes and soil samples showed more potential pathogens. Crucially, the analysis revealed a strong link between the crane's gut microbiota and the soil microbiota in their environment, suggesting soil acts as a microbial source. These findings highlight how both captivity and environmental exposure shape gut health in these endangered birds. This knowledge can help inform better management and conservation strategies for the Siberian cranes.

Abstract

Gut microbiota are integral to host health and ecological adaptation, yet their interactions with environmental microbial communities remain understudied in migratory waterbirds. Using high-throughput 16S rRNA gene sequencing, we compared gut microbiota of captive and wild Siberian cranes (*Leucogeranus leucogeranus*) and their associations with soil microbiota in the Poyang Lake wetland. Alpha diversity was significantly higher in soil than in gut microbiota, with captive cranes exhibiting greater microbial richness and evenness than wild individuals. Beta diversity analysis revealed distinct gut and soil microbiomes, with partial overlap between captive and wild crane gut microbiota. Firmicutes dominated gut communities, with *Ligilactobacillus* and *Romboutsia* enriched in captive cranes, whereas *Acidobacteria* were predominant in soil. Potential pathogens (e.g., *Escherichia-Shigella*) were more abundant in wild cranes and soil. LEfSe analysis identified 34 differentially enriched taxa, and microbial network analysis indicated stronger gut–soil microbial associations than those between captive and wild hosts, suggesting that environmental microbiota may serve as reservoirs for host colonization. These findings highlight the ecological dynamics shaping gut microbiota in response to captivity and environmental exposure, providing insights into microbial contributions to conservation strategies for Siberian cranes.

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1. Introduction

Gut microbiota play a vital role in host health and physiology, contributing to digestion, metabolism, immune homeostasis, and overall fitness [1, 2]. Their composition and diversity are shaped by intrinsic (genetics, immunity, age) and extrinsic factors (diet, habitat, environmental microbiota, age) [3-6]. Understanding these microbial communities is crucial for assessing ecological adaptations and conservation strategies for endangered species [3]. Captive breeding is essential for species conservation but imposes significant environmental shifts that can alter gut microbiota [7, 8]. Factors such as controlled diets, reduced microbial exposure, antibiotic use, and human interactions may lead to lower microbial diversity, increased opportunistic pathogens, and functional impairments in digestion and immunity [4, 9]. While some studies suggest captivity may enhance immune status, its overall impact remains debated [10]. Thus, research on gut microbiota can improve survival of animals in different living environments [11]. Evaluating gut microbiota differences between captive and wild individuals is critical for optimizing conservation management.

The Siberian crane (*Leucogeranus leucogeranus*) was classified as the critically endangered (CR) crane species by the IUCN and faces serious threats from habitat degradation and climate change [12]. With Poyang Lake Wetland as its key wintering ground, conservation efforts have focused on captive breeding and reintroduction. However, microbiota shifts in captivity may influence health and adaptability, potentially affecting reintroduction success. Despite studies on Siberian crane diet, behavior, and gut microbiota composition [13-16], the role of environmental microbiota, particularly soil microbes, remains unclear.

As a vast microbial reservoir, soil plays a fundamental role in shaping gut microbiota through feeding, foraging, and excretion [17-19]. Microbial exchange between soil and gut communities can influence host digestion, metabolism, and immunity [20, 21], yet this interaction remains understudied in avian conservation.

This study aims to: (1) characterize gut microbiota differences between captive and wild Siberian cranes, (2) analyze soil microbiota composition in their respective habitats, and (3) explore gut–soil microbial associations to assess environmental influences on gut microbiota. We hypothesize that captivity alters gut microbiota structure, habitat differences influence soil microbial composition, and wild cranes exhibit greater microbial overlap with their environment. Using 16S rRNA high-throughput sequencing, this study provides novel insights into microbiota-mediated adaptations in endangered birds, informing strategies for rewilding and conservation management.

2. Materials and Methods

2.1. Study Area and Samples Collection

Poyang Lake (28°11′–29°51′N, 115°49′–116°46′E), located at the confluence of the middle and lower reaches of the Yangtze River, in northern Jiangxi Province, is the largest freshwater lake in China. The region benefits from abundant sunlight, with a multi-year average temperature ranging from 16.5 to 17.8°C. It experiences hot, rainy summers and cold, dry winters, with an annual average precipitation of 1,450–1,550 mm. The well-developed hydrological network and rich food resources provide an optimal habitat for wintering waterfowl, particularly migratory species [22]. This study was conducted at two strategically selected sampling sites in proximity to Poyang Lake: the Siberian Crane Conservation Area (SCS) within the state-owned Nanchang Wuxing Reclamation Farm and the Poyang Lake National Wetland Park (NWP). The SCS (28°43′–28°48′N, 116°11′–116°19′E) is located in the eastern suburbs of Nanchang, adjacent to the eastern margin of Poyang Lake. This area serves as a critical conservation zone for the endangered Siberian Crane, providing a semi-natural habitat that mimics the ecological conditions of their natural wintering grounds. The NWP (28°56′–29°13′N, 116°23′–116°44′E), situated in Poyang County along the eastern shore of Poyang Lake, is a

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nationally designated wetland park that supports a diverse avian community. This site is particularly significant for its role in harboring multiple nationally protected bird species, including the Oriental White Stork (*Ciconia boyciana*), Tundra Swan (*Cygnus columbianus*), Hooded Crane (*Grus monacha*), and Red-breasted Goose (*Branta ruficollis*).

Both study sites are separated from the main body of Poyang Lake by a levee system, yet they remain in close proximity to the natural wintering habitats of Siberian cranes. These locations share similar climatic and geographical characteristics, including comparable temperature regimes, precipitation patterns, and hydrological dynamics, which facilitate comparative analyses of microbial communities across captive and wild crane populations as well as their interactions with the soil microbial community in the wetland ecosystem.

This study design enables a comprehensive investigation of the microbial ecology in both managed conservation areas and natural wetland habitats, providing valuable insights into the ecological relationships between captive and wild Siberian Cranes and their environment.

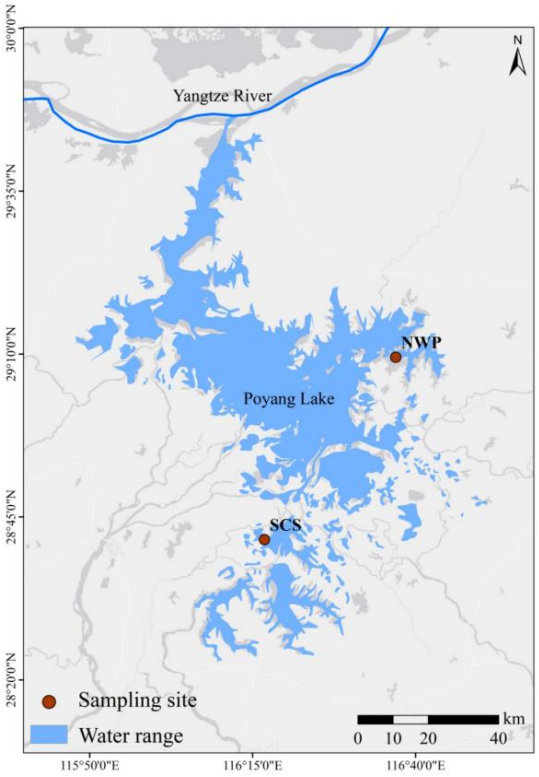

Figure 1. Map of sampling sites in the study area of Poyang Lake Wetland.

Note: Poyang Lake (28°11′–29°51′N, 115°49′–116°46′E), located at the confluence of the middle and lower reaches of the Yangtze River, in northern Jiangxi Province, is the largest freshwater lake in China. The SCS (28°43′–28°48′N, 116°11′–116°19′E) is located in the eastern suburbs of Nanchang, adjacent to the eastern margin of Poyang Lake. This area serves as a critical conservation zone for the endangered Siberian Crane, providing a semi-natural habitat that mimics the ecological conditions of their natural wintering grounds. The NWP (28°56′–29°13′N, 116°23′–116°44′E), situated in Poyang County along the eastern shore of Poyang Lake, is a nationally designated wetland park that supports a diverse avian community.

Wild Siberian cranes overwinter in Poyang Lake (28°22′–29°45′N, 115°47′–116°45′E) from late October to late March (mean residence: 150±7 days). During this time, individuals inhabiting artificial wetlands, such as rice paddies or lotus fields, remain until local food resources are exhausted. Wild Siberian cranes are primarily fed tubers such as lotus roots or rice.

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Captive Siberian Cranes are primarily fed tubers such as corn and carrots. Antibiotics are administered only under veterinary guidance to treat bacterial infections. Routine but limited human contact occurs during daily care activities such as feeding and cleaning.

Faeces and soil samples were collected from Siberian crane habitats at two locations: SCS and NWP, following their overwintering patterns. Sampling at SCS was conducted in mid-March, while at NWP, it took place in late March. At SCS, feeding hotspots were identified using binoculars, and three major crane flocks were selected for sampling. To minimize cross-species contamination, sampling was restricted to areas where no other bird species were present within a 50-meter radius during feeding. Immediately after crane departure, faeces and soil samples were collected from feeding sites, identified based on crane footprints and feeding pits. Three biological replicates of faeces samples were collected from each of the three wild crane flocks, with three adjacent soil samples obtained within a 5-meter radius per faeces deposit. This yielded a total of 18 paired samples (9 faeces + 9 soil). Due to the challenges of non-invasive sampling of wild endangered cranes, the fecal samples represent population-level snapshots from each flock, and we could not control for individual identity, sex, or age. Faeces samples were designated as the W group (biological triplicates: W1a-c, W2a-c, W3a-c; n=9), and their associated soil samples as the WT group (Paired triplicates: WT1a-c, WT2a-c, WT3-a; n=9). For captive Siberian cranes at the National Wetland Park (NWP), an identical paired sampling protocol was implemented to maintain methodological consistency. From three separate enclosures (biological replicates), triplicate faeces samples were collected per enclosure and immediately matched with adjacent enclosure soil samples (≤5 m radius, 0-10 cm depth). faeces samples from captive cranes were labeled as the Z group (Z1a-c, Z2a-c, Z3a-c; n=9 biologically independent samples), while corresponding enclosure soil samples were categorized as the ZT group (ZT1a-c, ZT2a-c, ZT3-a; n=9 spatially paired controls)

To ensure sample integrity and prevent contamination, fresh faeces and soil samples were collected using sterilized gloves and forceps. Any material that had come into contact with the ground was removed before transferring the samples into 10 mL sterile EP tubes.

The collection process was conducted with minimal disturbance to the crane populations. faeces aliquots were flash-frozen in liquid nitrogen within 15 min of collection, soils were sieved (2 mm mesh) and stored at -80°C until DNA extraction. All procedures complied with IUCN guidelines for research on endangered species (Approval No.: 20220315-001)

2.2. DNA Extraction, Amplification and High-Throughput Sequencing

Genomic DNA was extracted from faeces samples (n=18) using the faeces Microbial DNA Kit (Solarbio D2700) with bead-beating lysis (0.1 mm zirconia beads, 6 m/s for 45 s) and from soil cores (n=18) with the Foji Soil DNA Kit (DE-05514) incorporating humic acid removal steps. The extraction procedures were strictly followed according to the manufacturer's instructions for each kit. DNA integrity was verified by 1% agarose gel electrophoresis and stored at -20°C for subsequent use.

The V3-V4 hypervariable regions of bacterial 16S rRNA genes were amplified using barcoded primers 515F (5'- GTGYCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNVGGGTWTCTAAT-3') with Phusion High-Fidelity DNA Polymerase (Thermo Scientific) [23]. PCR conditions included: 95°C for 4 min (initial denaturation); 32 cycles of: 95°C/30 s, 55°C/30 s (annealing), 72°C/30 s; Final extension: 72°C/10 min. Negative controls (n=3 per batch) were processed alongside samples. Negative controls (n=3 per batch) were processed alongside samples. Paired-end sequencing was performed on Illumina MiSeq PE250 platform at Biomarker Technologies (Beijing, CAP-accredited lab).

2.3. Bioinformatics Processing and Ecological Statistical Analysis

Bioinformatics processing was performed using BMKCloud (www.biocloud.net).

1. Raw data quality control: Raw sequencing reads were quality-filtered using Trimmomatic-v0.33 (SLIDINGWINDOW:4:20, MINLEN:100). Adapter sequences were removed using cutadapt-v1.91.

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2. Amplicon Sequence Variant (ASV) Analysis: The high-resolution ASV analysis was performed to characterize microbial community composition with single-nucleotide precision. Cleaned sequencing reads were processed using the DADA2 pipeline (v1.16.0) implemented in QIIME2 (v2020.6) to generate exact biological sequence variants (ASVs) [24].

3. Diversity Analysis: Alpha diversity was assessed using QIIME2-v2020.06. Beta diversity was assessed using principal coordinates analysis (weighted UniFrac PCoA), with significance testing via PERMANOVA (999 permutations) [25]. Hierarchical clustering of samples was analyzed using Bray-Curtis distance.

4. Taxonomic Annotation and Biomarker Analysis: Taxonomic classification of amplicon sequences was performed using the SILVA database (version 138) with a confidence threshold of 85% [26]. Sequences were assigned to taxonomic ranks using the naïve Bayesian classifier implemented in QIIME2 [25]. To minimize potential misclassifications, only sequences with a confidence score above the predefined threshold were retained for downstream analyses. The community composition of each sample was statistically analyzed at various levels (phylum, class, order, family, genus, species). QIIME2 was used to generate abundance tables at different classification levels, and R was used to visualize the community structure of each sample at various taxonomic levels. Furthermore, biomarker taxa were identified using LEfSe (Kruskal-Wallis test, LDA score > 4.5, and FDR-adjusted p-values for multiple testing correction) [27].

5. Cross-Domain Correlation and Network Analysis: Inter-domain microbial correlations were assessed using SparCC methods (Sparse Correlations for Compositional Data) with thresholds of $|r| > 0.6$ and $p < 0.01$. Network visualization was performed in Gephi, employing the Fruchterman-Reingold force-directed layout [28].

3. Results

3.1. Distinct Microbial Diversity, Composition, and Host-Environment Overlap Between Gut and Soil Microbiota

High-resolution alpha diversity metrics revealed systematic variations in microbial complexity between soil and gut compartments ($p < 0.05$, Tab.1), with pronounced effects of captivity status. Soil microbiota exhibited consistently higher Ace, Chao, Simpson, and Shannon indices compared to the gut microbiota of Siberian cranes, indicating greater microbial richness and diversity in the soil environment. Among all groups, the WT (wild habitat) group demonstrated the highest values for these indices, suggesting that the soil microbiota in the habitat of wild Siberian cranes harbors the most complex and diverse microbial communities.

In contrast, the gut microbiota of wild and captive Siberian cranes displayed distinct diversity patterns. Wild Siberian cranes exhibited significantly lower gut microbial richness and diversity compared to their captive counterparts, as reflected in reduced Ace, Chao, Simpson, and Shannon indices. However, an opposite trend was observed in the soil microbiota associated with their habitats, where soil from wild crane habitats showed higher diversity than that from captive environments. These findings suggest that captivity may influence gut microbial diversity while simultaneously reducing the complexity of the surrounding soil microbial communities.

Table 1. Alpha diversity indices of gut and soil microbiota.

Sample	ASV	ACE	Chao1	Simpson	Shannon
W	220±38c	229±13c	239±13c	0.794±0.010c	3.213±0.111d
Z	415±93b	418±31b	421±30b	0.821±0.012c	3.965±0.091c
WT	734±134a	739±45a	743±44a	0.989±0.002a	8.027±0.075a
ZT	676±241a	678±80a	681±80a	0.949±0.015b	6.916±0.278b

[1] Superscript letters denote statistically homogeneous subgroups (Tukey HSD, α=0.05). W/Z: wild/captive cranes; WT/ZT: their associated soils.

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High-resolution amplicon sequence variant (ASV) analysis demonstrated distinct microbial niche specialization across host and environmental compartments (Figure 2). Only 68 ASVs (0.7% of total detected) were shared among all four groups (gut: W/Z; soil: WT/ZT).

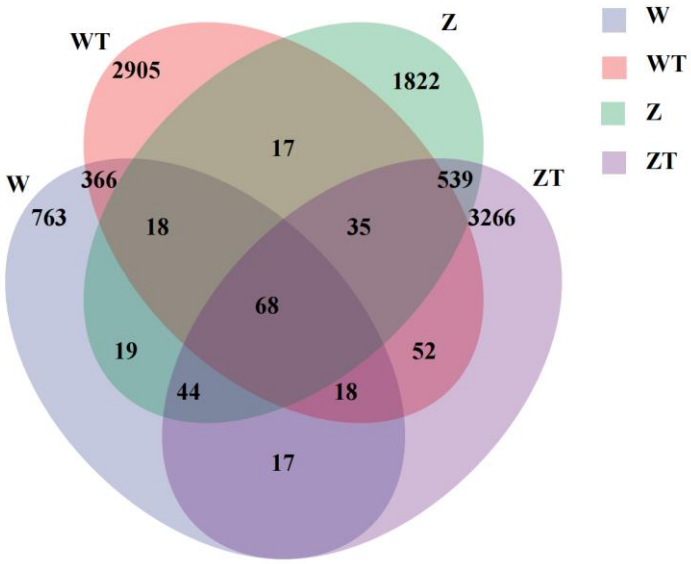

Figure 2. Venn diagram illustrating ASV distribution across Siberian crane gut and soil microbiota.

Host-environment microbial exchange varied between wild and captive cranes. Wild cranes (W) shared 470 ASVs (35.8% of their gut microbiota) with native soils (WT), while captive cranes (Z) exhibited a higher overlap with captive soils (ZT), sharing 686 ASVs (26.8%). Soil compartments (WT/ZT) accounted for 70.5% of the total unique ASVs, with captive soils (ZT) displaying the highest niche specialization (37.3% unique ASVs).

Principal Coordinate Analysis (PCoA) based on weighted UniFrac distances demonstrated a clear separation between gut and soil microbiomes across the four groups ($R^2 = 0.86$, $p < 0.01$, PERMANOVA), with Axis 1 accounting for 12.89% of the variance (Figure 3a). Notably, the wild Siberian cranes (W group) and their corresponding habitat soil (WT group) exhibited the most pronounced separation, highlighting the greatest disparity in microbial community structures between these two groups. Furthermore, hierarchical clustering analysis based on Bray-Curtis dissimilarity further supported this distinction, delineating four well-defined branches corresponding to the four groups (Figure 3b).

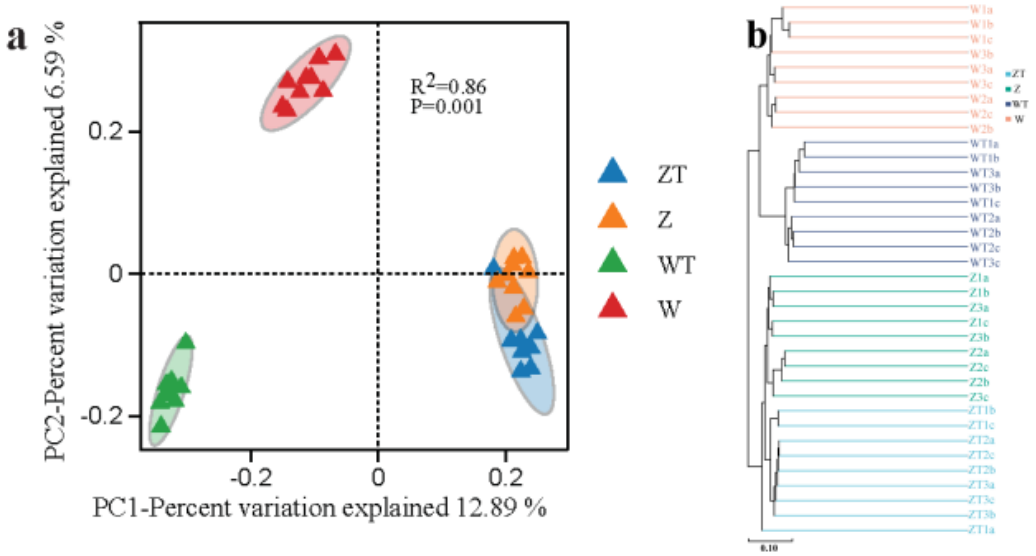

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Figure 3. Hierarchical cluster analysis of gut microbial community structure and soil microbial community structure of Siberian cranes. (a)PCoA clustering analysis (weighted UniFrac: $R^2 = 0.86$, $p < 0.01$). (b) UPGMA clustering of Bray-Curtis.

3.2. Phylum and Genus-Level Divergence in Gut and Soil Microbiota of Captive and Wild Siberian Cranes

Taxonomic profiling at the phylum level revealed a distinct host-environment dichotomy in microbial community composition (Figure 4a). Firmicutes, Proteobacteria, and Actinobacteriota were the three dominant phyla with the highest relative abundances in groups W, Z, and ZT, with their relative abundances ranging from 38.97% to 86.08%, 3.68% to 33.34%, and 0.56% to 15.14%, respectively. In the WT group, Proteobacteria, Firmicutes, and Verrucomicrobiota were the top three phyla in terms of relative abundance. Notably, the relative abundance of Firmicutes in groups W and Z was higher than in groups WT and ZT, while the relative abundance of Acidobacteriota showed an opposite trend. Compared with Z group , the relative abundance of Proteobacteria in W group increased significantly, while the relative abundances of Firmicutes, Actinobacteriota, and Acidobacteriota decreased.

At the genus level, distinct microbial signatures were observed between captive and wild Siberian cranes (Figure 4b). *Ligilactobacillus*, *Romboutsia*, and *Escherichia-Shigella* are the three most abundant genera, with relative abundances ranging from 4.62% to 60.71%, 0.48% to 15.15%, and 0.17% to 21.59%, respectively. The relative abundances of these genera vary significantly across the four sample groups. Specifically, the relative abundances of *Ligilactobacillus* and *Romboutsia* are higher in Z group compared to the other three groups, while in W group , the relative abundance of *Escherichia-Shigella* is significantly higher than in the other groups. Additionally, in groups WT and ZT, the relative abundances of other unidentified genera are 63.83% and 59.63%, respectively.

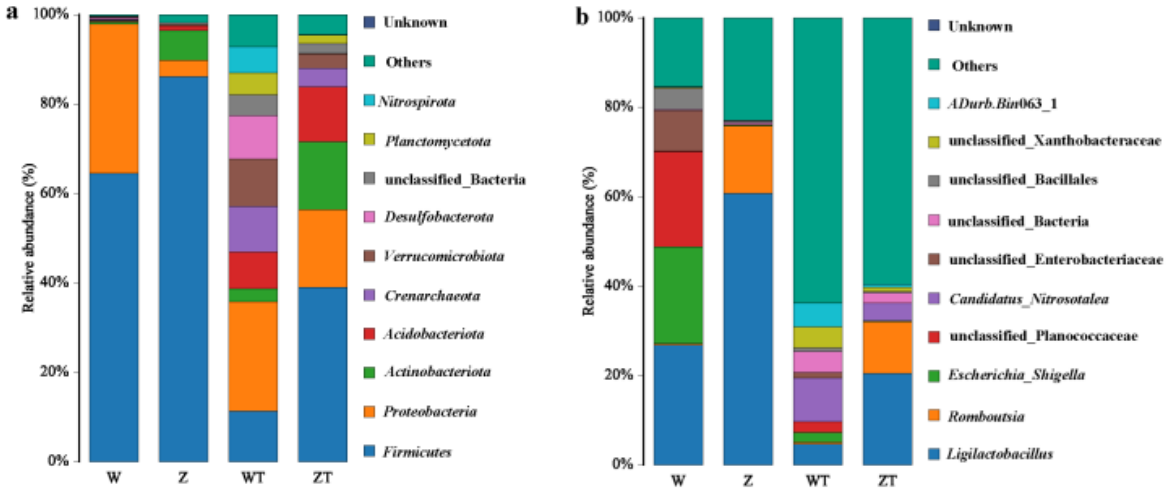

Figure 4. Microbial composition of gut and soil microbiota associated with captive and wild Siberian Cranes at the phylum (a) and genus levels (b). Stacked columns for the means of the individual samples from the four groups, indicating the relative abundance as a percentage of the total bacterial sequences per group. The taxa that have relative abundance of less than 1% were combined and are referred to as "others".

3.3. Differential Microbial Abundance in Gut and Soil Microbiomes Revealed by LEfSe Analysis

LEfSe analysis (LDA score > 4.5, $p < 0.01$) revealed 34 phylogenetically conserved biomarker taxa across five taxonomic levels, distinguishing microbial communities in the gut and soil environments (Figure 5). At the phylum level, Firmicutes predominated in the gut microbiota, while Proteobacteria was the dominant phylum in the soil microbiome, highlighting ecosystem-specific microbial composition differences.

Comparative analysis of the gut microbiomes of wild and captive Siberian cranes demonstrated significant variation in their microbiota profiles. Wild cranes (W) exhibited 10 unique biomarkers,

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while captive cranes (Z) showed 8. The gut microbiota of wild cranes was characterized by higher relative abundances of Bacilli (LDA = 5.4) and Escherichia-Shigella (LDA = 5.0), whereas captive cranes were enriched in Lactobacillus (LDA = 5.4) and Romboutsia (LDA = 4.9).

In the soil, wild crane-associated habitats (WT) were characterized by Candidatus Nitrosotalea (LDA = 4.7), while captive crane-associated soils (ZT) showed enrichment in Actinobacteriota (LDA = 4.6).

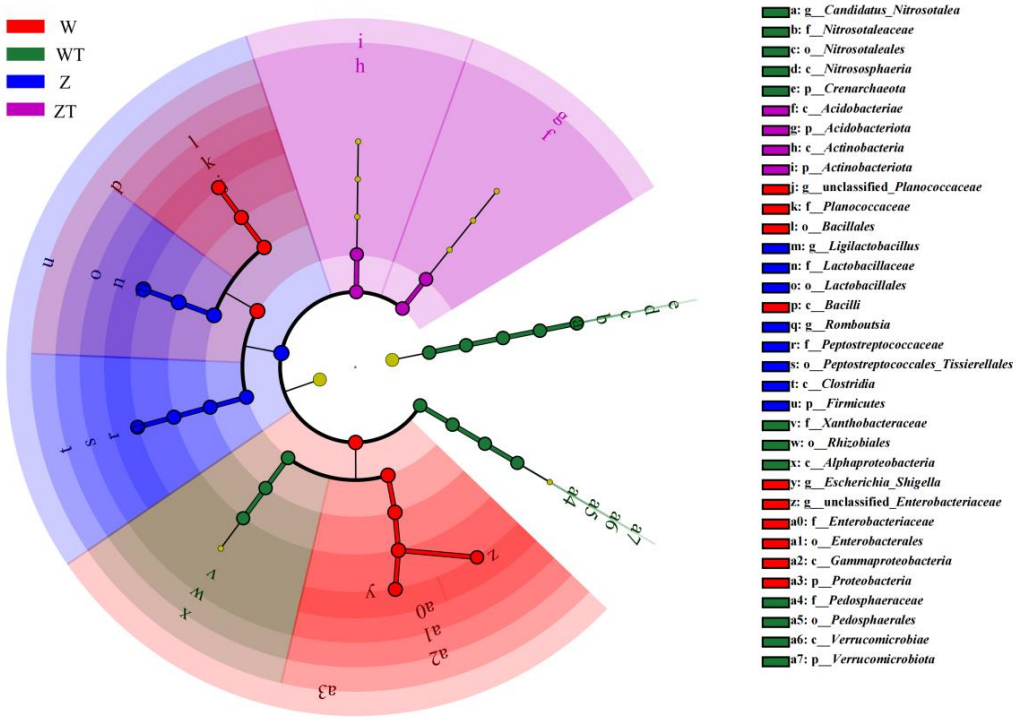

Figure 5. Differentially abundant taxa in gut and soil microbiota of Siberian Cranes identified by LEfSe analysis.

Note: The diagram displays taxonomic classification from the innermost to the outermost level: phylum, class, order, family, and genus. The size of the small circles corresponds to the relative abundance of species at each taxonomic level. Species with no significant differences are marked in yellow, while those showing significant differences are highlighted with color.

3.4. Network-Based Insights into Gut and Soil Microbiota Interactions in Siberian Cranes

From the microbial network relationship graph between captive Siberian crane and the soil of their habitat, it is observable that all the bacterial genera exhibit positive correlations (Figure 6a). Among them, the genera that have the most intimate interaction with other genera are unclassified_*Bacillales*, *Acidothermus*, unclassified_*Acidobacteriales*, unclassified_*Pedosphaeraceae*, *Subgroup_1*, *Bacillus*, *Turicibacter*, *Candidatus_Solibacter* and *Romboutsia*. These genera are concurrently significantly and positively correlated with 9 other genera ($p < 0.01$).

From the microbial network relationship graph between wild Siberian crane and the soil in the same area, it can be noted that all other genera are positively correlated, except for *Ligilactobacillus* and its associated genera, which present negative correlations (Figure 6b). Among them, the genus with the most intimate interaction with other genera is *Candidatus_Udaeobacter*, which is significantly and positively correlated with 13 other genera ($p < 0.01$). Additionally, unclassified_*Xanthobacteraceae* also has a close interaction with other genera and is significantly correlated with 12 other genera ($p < 0.01$). Furthermore, unclassified_*Pedosphaeraceae14* is significantly correlated with 11 other genera ($p < 0.01$).

From the microbial network relationship graph between captive and wild Siberian crane, it can be discerned that there are both positive and negative correlations among the bacterial genera (Figure 6c). Among them, the genera with the most intimate interaction with other genera are *Paeniclostridium*

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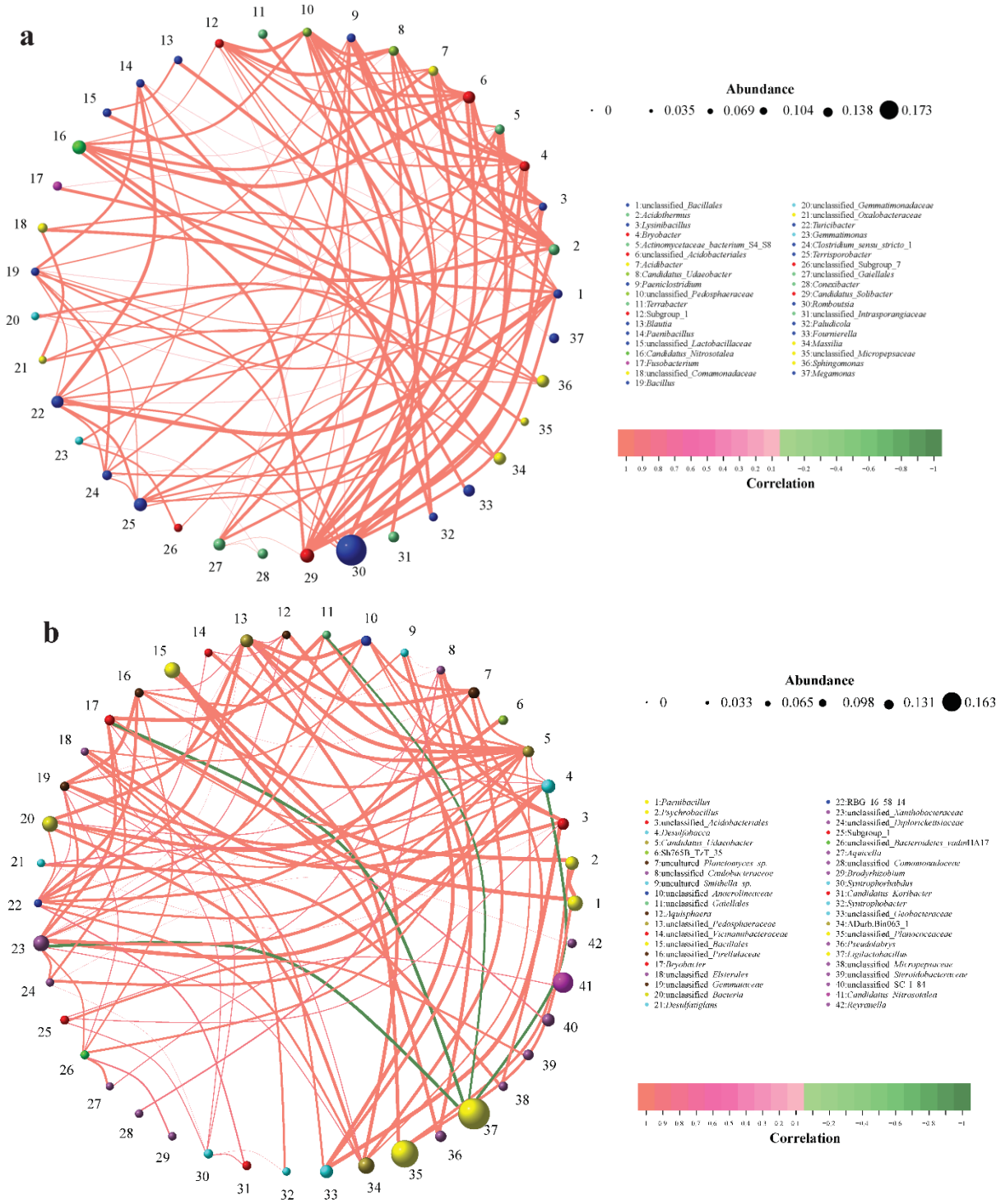
and unclassified_*Micrococcaceae*. *Paeniclostridium* is concurrently significantly and positively correlated with 12 other genera ($p < 0.01$), while unclassified_*Micrococcaceae* is significantly and positively correlated with 11 other genera ($p < 0.01$) and significantly and negatively correlated with 1 other genus ($p < 0.01$). Moreover, *Escherichia_Shigella* is solely significantly and negatively correlated with other genera ($p < 0.01$).



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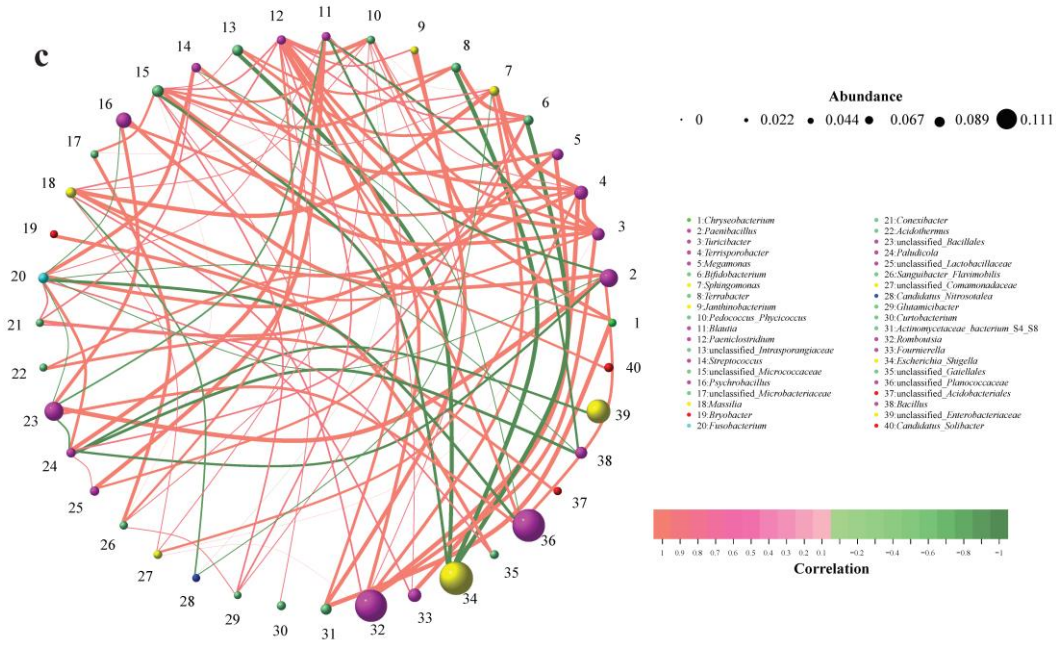

Figure 6. Correlation between the gut and soil microbiota in Siberian cranes. (a) All microbiota genera from the captive Siberian cranes faeces samples and the soil samples show positive correlations. (b) All microbiota genera from the wild Siberian cranes faeces samples and the soil samples show positive correlations, with the exception of Ligilactobacillus and its associated genera. (c) Both positive and negative correlations among microbiota genera are observed between captive and wild Siberian cranes faeces samples.

Note: Circles represent species, with the size of each circle indicating the average abundance of the species. Lines represent correlations between species, with the line thickness reflecting the strength of the correlation. Line colors denote the type of correlation: red for positive correlations and green for negative correlations.

4. Discussion

The significantly higher microbial diversity and richness observed in the soil of Siberian crane habitats compared to their intestinal microbiota underscore the distinct environmental conditions that govern microbial community assembly. The soil environment, characterized by aerobic and weakly alkaline conditions, contrasts sharply with the anaerobic and weakly acidic intestinal environment of the cranes [20]. These stark physicochemical differences impose strong selective pressures on microbial colonization, leading to distinct community structures in each habitat. Oxygen availability, pH gradients, and nutrient composition likely drive these ecological divergences, shaping microbial interactions and functional adaptations. A key finding of this study is the higher gut microbial richness and diversity in captive Siberian cranes relative to their wild counterparts. This aligns with previous studies on avian gut microbiota, which suggest that captivity alters microbial composition due to variations in diet, habitat conditions, and management practices [29]. One potential explanation is the Dietary Filtering Hypothesis, which posits that standardized captive diets may relax niche competition [30, 31], thereby permitting greater microbial richness compared to the more specialized foraging strategies of wild cranes. Captive diets, often nutritionally enriched and homogenized, may support a broader range of microbial taxa that would otherwise be constrained in a wild setting. Conversely, the Environmental Bottleneck Hypothesis suggests that anthropogenic simplification of captive soils—such as reduced plant diversity and altered microbial reservoirs—could limit microbial dispersal pathways to host guts, despite the observed increase in baseline diversity within captivity [32]. Unlike wild environments, where cranes are exposed to a dynamic and heterogeneous microbial landscape, captive settings may impose artificial constraints on microbial acquisition and transmission.

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The degree of similarity between the gut microbiota of Siberian cranes and their habitat soil underscores potential microbial exchange. Specifically, 26.77% of total gut ASVs in captive cranes and 35.80% in wild cranes overlapped with their respective soil microbiota. In contrast, only 16.08% of gut ASVs in adult black-billed gulls (*Larus saundersi*) were shared with soil [33], indicating stronger microbial associations between Siberian cranes and their habitat. This disparity may be attributed to foraging behavior—Siberian cranes feed by excavating plant roots and stems in shallow waters, increasing direct soil contact, whereas black-billed gulls primarily consume aquatic prey, limiting soil interaction. These findings align with the habitat filtering hypothesis, which posits that environmental constraints shape microbial community assembly [34]. The minimal ASV overlap (68) between wild and captive cranes suggests strong host-environment physiological filters, such as gut pH, bile acids, and immune responses, which regulate microbial colonization and create a barrier effect [35]. However, the greater ASV sharing between captive cranes and their habitat soil (Z-ZT = 686) compared to wild cranes and their soil (W-WT = 470) suggests that captivity may weaken microbial transmission barriers. This could result from altered diets and reduced exposure to diverse environmental microbial sources, leading to a captivity gradient [36]. The high number of unique ASVs in captive soil (ZT = 3,266) likely reflects anthropogenic selection pressures, including fertilizer use and reduced plant diversity, which reshape soil microbial composition. These changes may indirectly influence gut microbiota diversity in captive birds by modifying environmental microbial pools [37].

This study identified Firmicutes, Proteobacteria, and Actinobacteria as the dominant bacterial phyla in the intestinal microbiota of both captive and wild Siberian cranes, consistent with findings from wintering Siberian cranes in Poyang Lake [16]. Among these, Firmicutes was the most prevalent, which aligns with studies on the gut microbiota of other crane species, such as the white-naped crane (*Grus vipio*) [38] and the red-crowned crane (*Grus japonensis*) [39]. Firmicutes play a crucial role in the breakdown of complex carbohydrates, fatty acids, and polysaccharides in the gut, facilitating host energy metabolism [40]. Notably, the relative abundance of Firmicutes in wild Siberian cranes (W group) was 86.08%, significantly higher than in captive Siberian cranes (Z group) at 64.51%. This difference may be attributed to the higher sugar content in the natural diet of wild cranes, which could promote Firmicutes proliferation. Additionally, captive conditions may lead to a relaxation of environmental microbial filters, reducing the number of discriminatory taxa by approximately 67% (LEfSe LDA >3.0) compared to wild-type soil microbiota. This reduction could weaken microbial transmission barriers and homogenize gut microbial niches in captivity [41]. At the genus level, *Lactobacillus* and *Romboutsia* exhibited the highest relative abundances in captive Siberian cranes, whereas Escherichia-Shigella was most abundant in wild individuals. LEfSe analysis further confirmed that Firmicutes, *Lactobacillus*, and *Romboutsia* were significantly enriched in captive Siberian cranes. *Lactobacillus*, an early-known beneficial microorganism, facilitates sugar fermentation and lactic acid production, contributing to host health and immune function [42]. Similarly, *Romboutsia* plays a key role in host well-being, with its higher abundance potentially enhancing survival [43]. In contrast, *Escherichia-Shigella*, a potential pathogenic bacterium, was found in higher abundance in wild Siberian cranes, which may increase the risk of disease transmission [44]. The differences observed in microbial composition between captive and wild cranes may be partially explained by diet-mediated convergence. Standardized feeding regimens in captivity likely homogenize gut microbial niches, as evidenced by reduced β-diversity dispersion (Bray-Curtis dispersion: Z = 0.19 vs. W = 0.31; $p = 0.021$). This reduced microbial diversity may have implications for host adaptability, immune responses, and overall health [45]. Collectively, these findings suggest that while captive Siberian cranes may benefit from controlled diets and medical care, they also experience a greater variety of microbiome species related to health, potentially influencing long-term gut health and resilience to environmental challenges. Conservation strategies should consider these microbiome shifts to optimize captive breeding programs and mitigate potential health risks in reintroduction efforts.

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5. Conclusions

This study provides a novel perspective on the ecological interplay between host-associated and environmental microbiomes by integrating co-occurrence network analysis to elucidate the intricate relationships between the gut microbiota of Siberian cranes and the soil microbial communities in their wetland habitat at Poyang Lake, China. Unlike previous studies that primarily compared compositional differences between captive and wild avian microbiomes, our research reveals how microbial associations differ across habitat conditions, highlighting fundamental ecological mechanisms governing host-microbe interactions.

Notably, we uncovered a distinct microbial network structure in wild Siberian cranes, characterized by selective and potentially competitive microbial interactions, in contrast to the more homogenized associations observed in captivity. A key finding is the significant negative correlation between the genus *Lactobacillus* and the gut microbiota of wild cranes—an interaction absent in captive environments—suggesting a previously unrecognized regulatory role of *Lactobacillus* in maintaining microbial homeostasis via competitive exclusion. Additionally, the unexpected pattern of exclusively positive correlations between captive cranes and their soil microbiota challenges the prevailing assumption that captivity universally disrupts host-microbiome interactions. Instead, our results suggest that captivity induces a homogenization effect, diminishing microbial antagonism and potentially reshaping gut microbiota functions..

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Data Availability Statement: Data will be made available on request.

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