The integrated analysis of digestive physiology and gastrointestinal microbiota structure in Changle goose

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ABSTRACT Changle goose in Fujian, China is a rare genetic resource and in urgent need to be protected. Understanding the characteristics of digestive physiology and spatial variation of gastrointestinal microbiota is crucial for developing nutritional intervention strategies to improve intestinal health and production performance of goose. Hence, histomorphological assay was used for observing development status of proventriculus, jejunum, and cecum in 70-day-old Changle geese, whereas digesta from 6 alimentary canal locations (crop, proventriculus, gizzard, jejunum, cecum, and rectum) were collected for 16S rRNA gene sequencing and short chain fatty acids (**SCFAs**) quantitative analysis. The histomorphological observation indicated that the jejunum and cecum of Changle goose were well developed. The alpha diversity analysis revealed that, except rectum, microbiota in other noncecum sections were in high diversity as cecum. The Nonmetric MultiDimensional Scaling (**NMDS**) analysis showed that microbial community of proventriculus, gizzard, and jejunum formed a cluster, which distinctly discrete with the microbiota of the other gastrointestinal locations. Additionally, the proportions of Proteobacteria, Bacteroidota, and Campilobacterota at the phylum level and *Lactobacillus, Streptococcus, Helicobacter*, and *Subdoligranulum* at the genus level exhibited tremendous alternations among different gastrointestinal locations. The characteristic bacterial composition in each section was further disclosed by analyzing the core and feature Amplicon Sequence Variants (**ASVs**) and SCFAs pattern. Importantly, 7 body-weight-associated ASVs and 2 cecum-development-related ASVs were identified via correlation analysis. In a whole, our findings provided the first insights into the specialized digestive physiology of Changle geese and distinctive regional distribution of gastrointestinal microbiota, which laid the important foundation for improving growth performance through microbiota manipulation in geese.

Key words: Changle goose, gastrointestinal microbiota, regional distribution, digestive physiology

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INTRODUCTION

In order to strengthen the conservation and usage of the rare germplasms, the third national survey of livestock and poultry genetic resources is conducting in China. Changle goose in Fuzhou city, Fujian province is a famous local breeding, which possesses excellent meet quality and unique flavor. However, due to the absences of genetic improvement and feeding standard, the growth performance is poor, further leading to the decline in feeding scale, dropping from 27,000 in 1980 to 3,500 nowadays. Hence, developing strategies to improve the growth performance is vital for saving this disappearing germplasm resource.

The 20% variations in growth performance of animals can be attributed to the diet nutrition. Nonetheless, there are few researches on goose digestive structure and physiology, which are fundamental for the understanding of nutritional requirement and ration formulation of goose. Meanwhile, goose is a well-known herbivore that can utilize 40% to 50% dietary crude fiber (Du, 2011), and gut microbiota plays central roles in this process. Although both the cecal and fecal microbiota of goose have been evaluated in the previous researches (Gao et al., 2016; Xiang et al., 2019; Liu et al., 2020; Li et al., 2022; Xi et al., 2022), the foregut microbiota is also vital

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for nutrient digestion and well-being but have attracted less attentions (Chen, 2005). More importantly, the systematic and comprehensive insights into the alternations in microbial compositions and structures in different gastrointestinal (**GI**) locations of goose can advance the understanding of the microbial regional distribution and gradual changing pattern, then paving the way for further studies related to growth performance improvement via gut microbial modulation.

In the present study, the intestinal physiological characteristics and the longitudinal distribution of gastrointestinal microbial communities of Changle goose were determined by histomorphological observation and 16S rRNA gene sequencing, respectively. The short chain fatty acids (**SCFAs**) were also detected by gas chromatography to elucidate the metabolic pattern of microbiota. Above all, our results would offer valuable information for deepening the knowledge about the microbial spatial changes in goose, and facilitate efforts for developing microbiota intervention strategies to improve growth performance.

MATERIALS AND METHODS

All management and experimental procedures followed the animal care protocols approved by the Fujian Agriculture and Forestry University Animal Care and Use Ethics Committee. All experimental protocols were approved and the methods were conducted according to the relevant guidelines and regulations.

Experiment Design and Sample Collection

A total of twelve 70-day-old male Changle geese (Adult geese) were randomly chosen from Changle Goose Breeding Farm in Fuzhou of Fujian province in China. Geese were fed with the same commercial feed from Fujian Jinzhenghe Feed Co., Ltd., and the diet was formulated according to the feeding standard DB32/T 2691-2014. The body weight (**BW**) of each goose was recorded after fasting overnight. The slaughter weight and the lengths of small intestine and cecum after slaughter were also measured. Segments of proventriculus, jejunum, and cecum in the middle part were also collected and fixed in 4% formaldehyde solution for morphological observation via HE staining. The digesta from crop, proventriculus, gizzard, jejunum, cecum, and rectum was gathered, immediately snap-frozen using liquid nitrogen, and stored at −80°C for 16S rDNA sequencing and SCFA detection. The collection sites of digesta sample are displayed in Figure 1.

Histomorphology Observation

The histomorphology analyses of proventriculus, jejunum, and cecum randomly from 6 gooses referred to the procedure in a previous study (Chen et al., 2018). The tissues, in paraffin blocks, were cut to 4 μm sections, and stained with hematoxylin and eosin. Samples were observed with a microscope (Eclipse ci, Nikon, Melville, NY) and representative photographs of the proventriculus, jejunum, and cecum were recorded with an imaging system (Digital Sight DS-Fi2, Nikon, Melville, NY). Besides, the height of villus and depth of crypt in jejunum were measured and their ratio was calculated later on. Herein, only villi and crypts that were cut longitudinally from top to bottom were considered.

16S rDNA Sequencing and Data Analysis

The procedure of 16S rDNA sequencing was described in detail in our previous study (Liu et al., 2018). The simplified workflow was presented here. The bacterial DNA of digesta were extracted by using Stool DNA Kit (D4015-01, Omega Bio-Tek, Norcross, GA), then amplified with the primers of V3-V4 region in bacterial 16S rDNA. The amplicons were purified and then sequenced via the Illumina MiSeq Platform. After quality control and assembly, the sequencing data were used for analyses of α diversity (Chao index and Shannon index), β diversity (Nonmetric MultiDimensional Scaling (**NMDS**) and Analysis of similarities) and variation between groups at the phylum and genus levels on the online platform of Majorbio Cloud Platform (www.

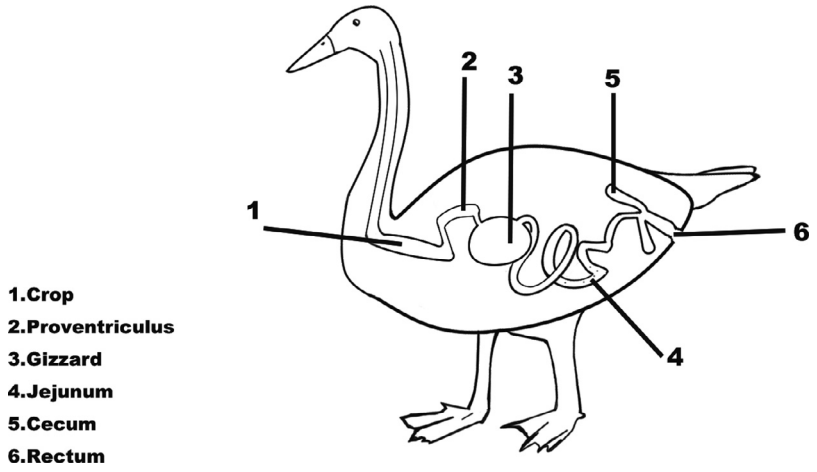

Figure 1. The schematic diagram of digesta sample collection.

majorbio.com). Common and unique Amplicon Sequence Variants (**ASVs**) among different gastrointestinal locations were visualized by the upset plot (Chen et al., 2021). Random forest analysis was used to identify the feature ASVs in each part of GI tract (Zheng et al., 2019). Spearman correlation analysis was performed between site-specific ASVs and weight (body weight and slaughter weight), jejunal-specific ASVs and jejunal development parameters (villus height, crypt depth and villus height / crypt depth (**V/C**)), and cecal-specific ASVs and cecal development parameters (cecal length and relative cecal length) (Liu et al., 2017).

All raw sequencing data have been deposited in the NCBI Sequence Read Archive under the BioProject PRJNA791802.

SCFA Detection

Concentrations of main SCFAs (including acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate) in the digesta samples were analyzed by gas chromatography (7890A, Agilent, Santa Clara, CA). Briefly, 0.7 g digesta was thawed and suspended into 1.5 mL ultrapure water, vortexed and then kept for 30 min on ice. Each sample was centrifuged at 10,000 g and 4°C for 15 min, and 1-mL supernatant was transferred into a 1.5-mL centrifuge tube to mix with 0.2-mL crotonic acid-metaphosphate acid, then kept for 30 min at 4°C, followed by centrifugation at 10,000 g and 4°C for 10 min. Later, 0.3-mL supernatant was collected and added into 0.9-mL methanol, followed by vortex and 5-min centrifugation at 8,000 g and 4°C. Afterward, the supernatant was analyzed by means of GC using a flame ionization detector, with the oven temperature from 100°C to 190°C (N_2 was used as the carrier gas at the flow rate of 1 mL/min).

Statistical Analyses

Statistical analysis was performed using GraphPad Prism 9.0 (GraphPad, San Diego, CA) if not stated otherwise in the methods section. Differences between groups were calculated using 1-way analysis of variance, whereas Wilcoxon rank-sum test was adopted to analyze the relative abundance at different taxon levels, with multiple comparisons corrected by False Discovery Rate. Statistical significance was assumed at $P < 0.05$. In figures, significances are annotated with the following markers: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; unless stated otherwise, data are reported as mean ± SE.

RESULTS

The Body Weight and GI Tract Development of Changle Goose

As shown in Table 1, the body weight and slaughter weight of 70-day-old Changle geese are 3.70 ± 0.214 kg and 3.40 ± 0.169 kg, respectively. The lengths of small intestine and cecum are 248.08 ± 16.577 cm and 51.46 ± 4.736 cm, and relative lengths are 67.19 ± 4.54 cm/kg BW and 15.15 ± 1.39 cm/kg BW. The histomorphological observation of GI tract displayed in Figure 2 showed that the mucosal ridges, papillae, and secretory adenomeres of proventriculus are clear and distinct. The tubular gland lobules are well organized. The jejunal villi are intact and regular. Goblet cells are apparent and distributed along the central axis of villi and the crypt in lamina propria are closely arranged. The villus height, crypt depth and V/C of jejunum are 1178 ± 182.7 μm, 317.0 ± 37.34 μm and 3.87 ± 0.830, respectively (Table 1). In cecum, there are numerous folds; many densely arranged straight tubular glands and more goblet cells than jejunum.

The Microbial Diversity in Goose GI Tract

The alpha diversity indexes comparison analysis results are presented in Figure 3A. There are no significant differences in Chao index, but the Shannon index in cecum is significantly higher than rectum ($P < 0.05$). Regarding the beta diversity, the NMDS analysis based on bray-curtis distance suggests that microbial structures among different locations are distinctive (stress = 0.182, R = 0.631, $P = 0.001$) (Figure 3B). Among these, structures of the stomach and small intestine microbiota are obviously separated with that of crop, cecum, and rectum.

The Microbial Composition in Goose GI Tract

At the phylum level, the proportions of Firmicutes, Proteobacteria, Campilobacterota, and Bacteroidota are notably different among different locations (Figure 4A, Table 2, $P < 0.05$). Firmicutes is the most predominant phylum in each compartment of goose GI tract, and its abundance in cecum is the highest (over 70%) and markedly richer than rectum ($P < 0.05$). The foregut possesses higher proportion of Proteobacteria, especially in crop (over 30%). Meanwhile, Bacteroidota and Campilobacterota are more abundant in the cecum and rectum, respectively ($P < 0.05$).

Table 1. The body and GI tract development of Changle goose.

Items[a]	Body weight (kg)	Slaughter weight (kg)	Villus height (μm)	Crypt depth (μm)	V/C[b]
Value	3.7 ± 0.21	3.4 ± 0.17	1178 ± 182.7	317 ± 37.34	3.87 ± 0.83
Items	Length of small intestine (cm)	Length of cecum (cm)	Relative length of small intestine (cm/kg BW)	Relative length of cecum (cm/kg BW)	/
Value	248.08 ± 16.58	51.46 ± 4.74	67.19 ± 4.54	15.15 ± 1.39	/

[a]Values are presented by mean ± SD.
[b]V/C, villus height / crypt depth in jejunum.

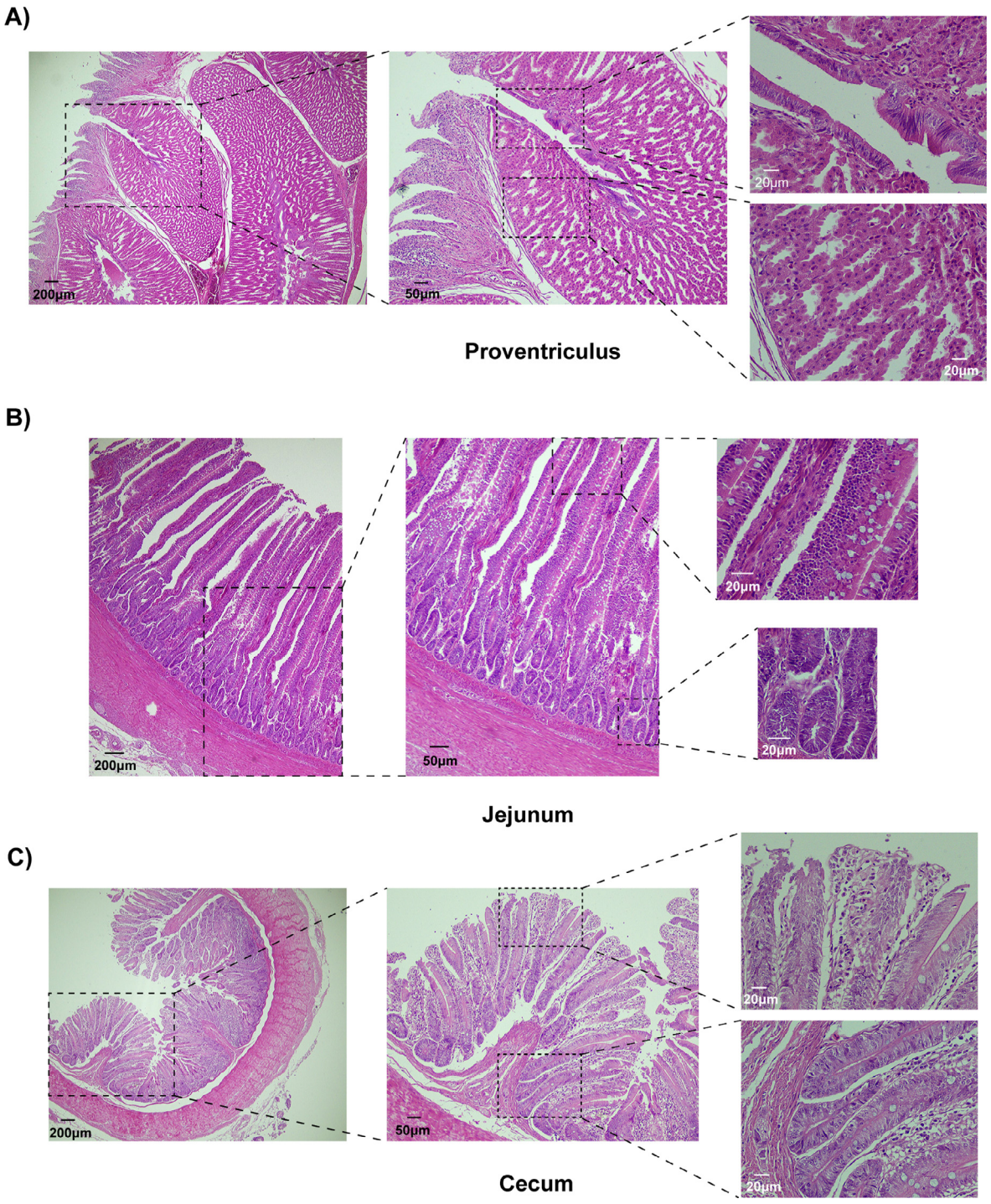

Figure 2. The histomorphology of proventriculus, jejunum, and cecum in Changle goose.

At the genus level, the remarkable differences in relative abundances among different locations are observed as well (Table 3, $P < 0.05$). The top 15 most abundant genera are displayed in Figure 4B. Among these, the relative abundances of genera of Firmicutes such as *Lactobacillus, Streptococcus, Subdoligranulum, Bacillus, Enterococcus*, and *Peptococcus* exhibited tremendous alternations in various parts of GI tract. In addition, the noticeable changes in the proportions of *Helicobacter, Gallibacterium, Campylobacter, Prevotella, Neisseria* derives from the other phyla, like Campilobacterota, Proteobacteria, or Bacteroidota are also observed.

Core and Specific ASVs in Goose GI Tract

As shown in Figure 5, average over 50% of ASVs are specific in each site of goose GI tract with 45 core ASVs existing in each part of GI tract. The top 10 differential-abundance core ASVs are presented in Figure 6 and 90% are members of Firmicutes. Among these, 4 ASVs are annotated to *Lactobacillus* species, 3 ASVs to *Subdoligranulum* species, and another 4 ASVs to *Streptococcus, Peptococcus*, and *Bacillus* species, respectively. On the other hand, a total of 80 feature ASVs are identified in the different GI parts

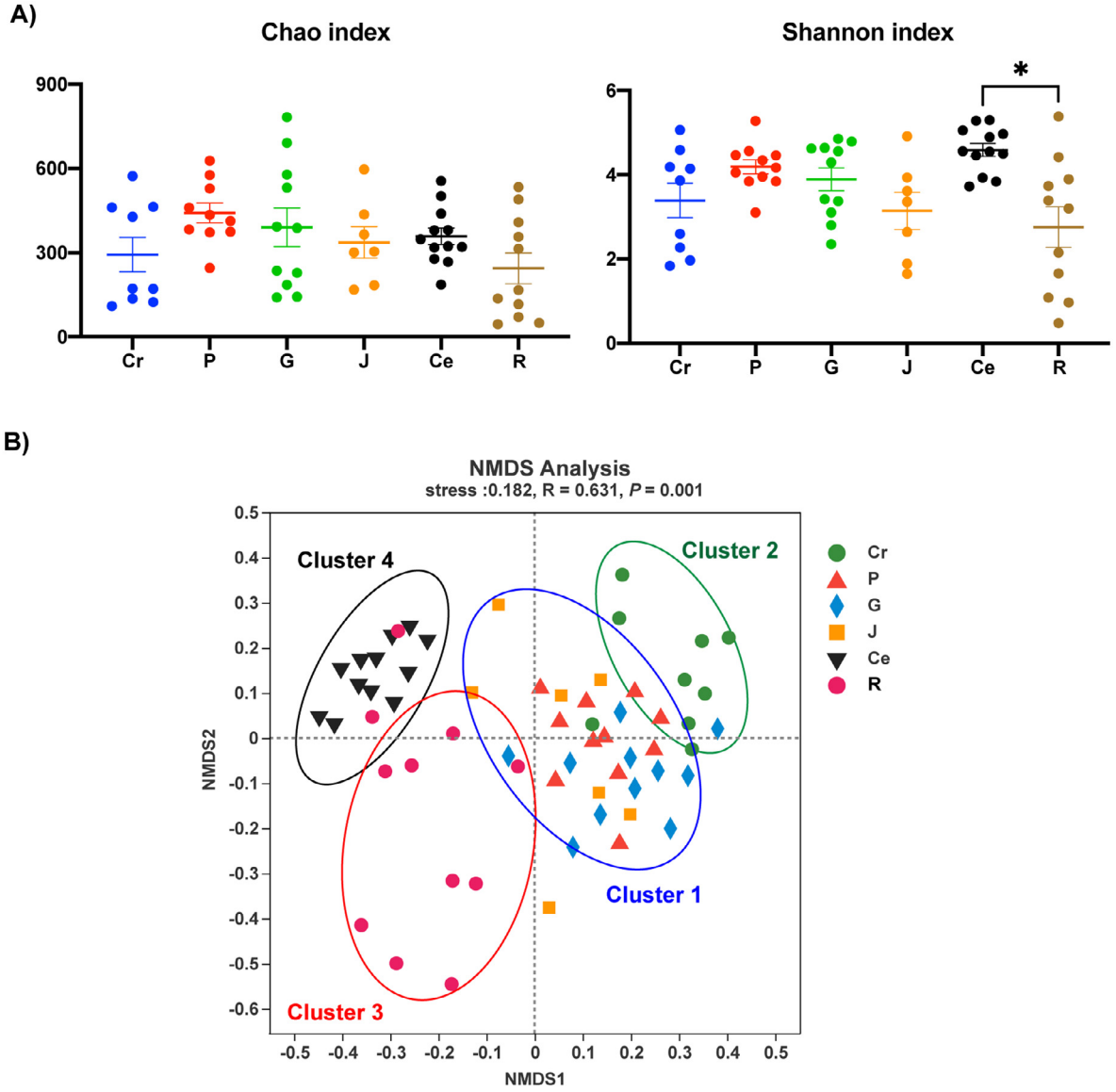

Figure 3. The α and β diversities of gastrointestinal microbiota in Changle goose. The Chao and Shannon indexes (A) and NMDS and ANOISIM analyses on ASV level (B) are showed here. ANOISIM, analysis of similarities; Ce, cecum; Cr, crop; G, gizzard; J, jejunum; NMDS, Nonmetric MultiDimensional Scaling; P, proventriculus; R, rectum.

by using random forest algorithm (Figure 7). Among them, ASV1031 aligned to *Avibacterium endocarditidis* is the most representative in crop, whereas ASV1002 mapped to *Neisseria species* and ASV20 anchored to *Lactobacillus* species is the most predominant in proventriculus and gizzard respectively. In addition, ASV136, ASV427, and ASV470 are the most representative in jejunum, cecum, and rectum, respectively.

The Gastrointestinal SCFAs Profile of Goose

The SCFAs profile in each part of GI tract is also distinctive (Figure 8). The highest SCFAs production is found in cecum, which denotes the most active microbial fermentation. The contents of acetate and propionate in cecum are notably different with crop, gizzard, and proventriculus ($P < 0.05$). Meanwhile, the content of butyrate in cecum is remarkably higher than proventriculus, gizzard, and jejunum ($P < 0.05$).

The Association Analysis Between Site-Specific ASVs and Development-Related Parameters

There is no jejunal-specific ASVs obviously associated with jejunal development parameters (villus height, crypt depth, and V/C) through Spearman association analysis. However, 7 BW-related ASVs are identified. Of which, ASV1037 (*Pasteurellaceae bacterium 199871*), ASV5 (*Rothia* spp.), and ASV466 (*Faecalitalea* spp.) are positively correlated with BW and ASV1030 (*Lysinibacillus* spp.), ASV485 (*Blautia* spp.), ASV432 (*Streptococcus* spp.), and ASV590 (*Subdoligranulum* spp.) are negatively correlated with BW (Figure 9A). Moreover, ASV552 (*Christensenellaceae R-7 group*

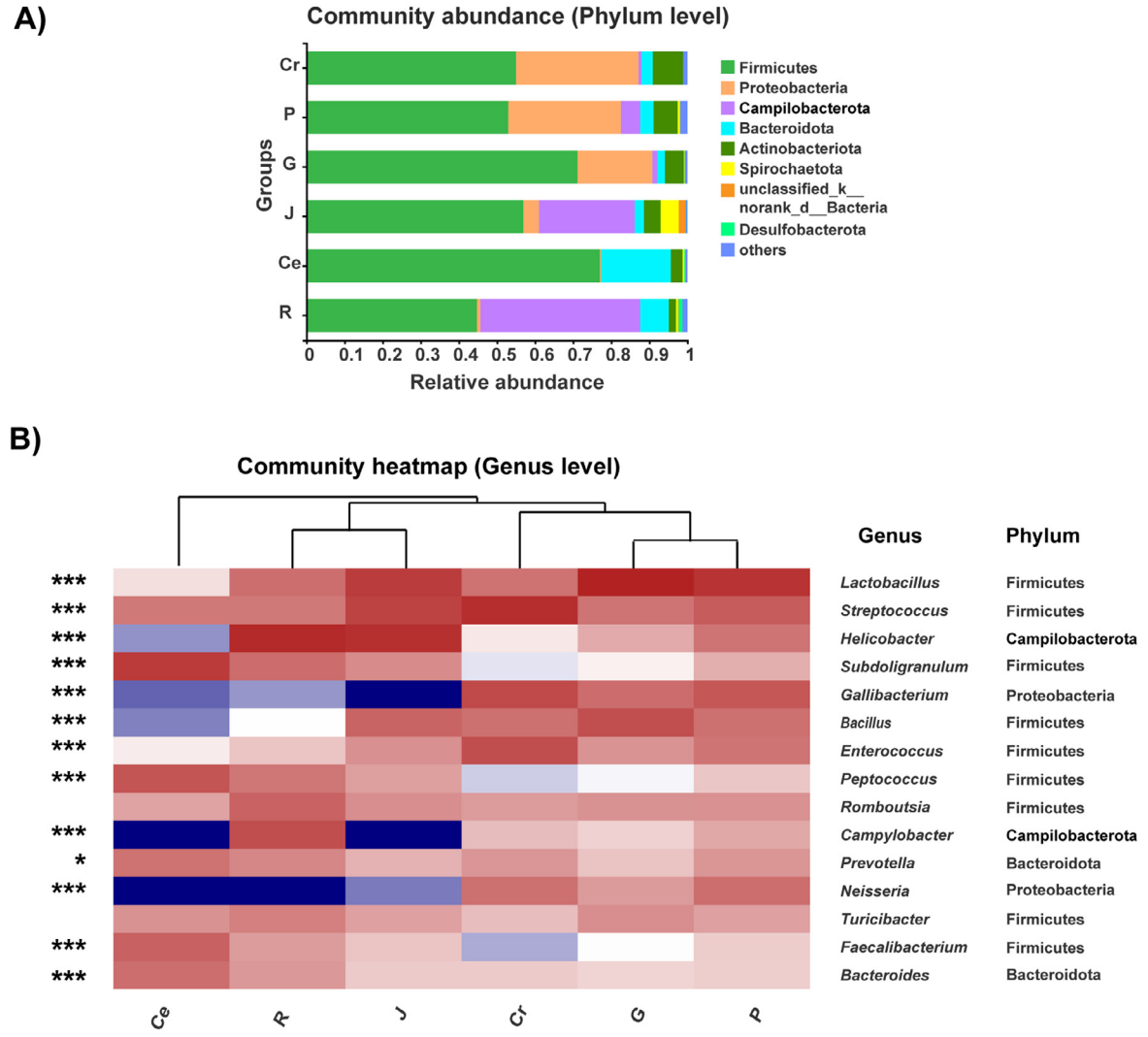

Figure 4. The gastrointestinal microbiota composition of goose at phylum (A) and the genus levels (B).

spp.) and ASV656 (*Erysipelatoclostridium* spp.) are found to be positively and negatively correlated with cecal length, respectively (Figure 9B).

DISCUSSION

Geese are herbivorous waterfowl. Their high digestibility of crude fiber provides an alternative to reduce feed cost and grain consumption, then alleviating the contradiction between people and livestock in competing for grain. And their excellent adaption to various water environment contributes to a world-wide distribution of goose raising. Hence, there are huge economic and social benefits of goose raising.

Changle geese raised in Fujian, China are small-sized breed. Benefit from the 3-generation genetic breeding by native specialist, the BW of male geese at 70 days of age can reach to 3.7 kg and both the relative lengths of small intestine and cecum of Changle geese are longer than Sichuan white geese (BW: 3.55 kg) fed with 4% crude fiber at the same age and gender (Jin et al., 2020), whereas the relative length of small intestine is shorter and the relative length of cecum is longer than Hainan geese (BW: 3.1 kg) (Li, et al., 2019). In addition, the jejunal development of Changle geese is better than

Table 2. The variance analysis of bacterial abundance among different gastrointestinal locations at the phylum level (Top 5).[1]

Taxon	Crop	Proventriculus	Gizzard	Jejunum	Cecum	Rectum	*P* value
Firmicutes	0.530 ± 0.066^{ab}	0.550 ± 0.082^{ab}	0.712 ± 0.067^{ab}	0.569 ± 0.138^{ab}	0.770 ± 0.035^{a}	0.448 ± 0.095^{b}	0.045
Proteobacteria	0.296 ± 0.052^{a}	0.321 ± 0.069^{a}	0.196 ± 0.054^{ab}	0.041 ± 0.015^{bc}	0.003 ± 0.001^{d}	0.009 ± 0.003^{cd}	<0.001
Bacteroidota	0.035 ± 0.010^{b}	0.030 ± 0.012^{b}	0.020 ± 0.009^{b}	0.024 ± 0.017^{b}	0.183 ± 0.036^{a}	0.075 ± 0.028^{b}	0.002
Campilobacterota	0.051 ± 0.030^{ab}	0.008 ± 0.002^{b}	0.013 ± 0.004^{b}	0.251 ± 0.140^{ab}	0 ± 0^{c}	0.420 ± 0.113^{a}	<0.001
Actinobacteriota	0.063 ± 0.016	0.079 ± 0.021	0.050 ± 0.019	0.044 ± 0.011	0.031 ± 0.007	0.019 ± 0.007	0.065

[1]In the same row, values with different letter superscripts (a, b, c, d) mean significant difference ($P < 0.05$), whereas no letter superscript or same letter superscipt means no significant difference ($P > 0.05$). Values are presented by mean ± SE.

Table 3. The variance analysis of bacterial abundance among different gastrointestinal locations at the genus level (Top 15).[1]

Taxon	Crop	Proventriculus	Gizzard	Jejunum	Cecum	Rectum	*P* value
Lactobacillus	0.043 ± 0.020^{bc}	0.230 ± 0.049^{a}	0.369 ± 0.091^{a}	0.182 ± 0.044^{ab}	0.002 ± 0.001^{d}	0.048 ± 0.044^{cd}	<0.001
Streptococcus	0.270 ± 0.098^{a}	0.077 ± 0.029^{ab}	0.041 ± 0.018^{b}	0.154 ± 0.085^{ab}	0.035 ± 0.021^{b}	0.036 ± 0.018^{b}	<0.001
Helicobacter	0.002 ± 0.002^{cd}	0.041 ± 0.030^{bc}	0.009 ± 0.003^{bc}	0.251 ± 0.137^{ab}	0 ± 0^{d}	0.307 ± 0.091^{a}	<0.001
Subdoligranulum	0.001 ± 0.000^{c}	0.009 ± 0.003^{b}	0.001 ± 0.000^{bc}	0.022 ± 0.016^{b}	0.188 ± 0.030^{a}	0.050 ± 0.032^{b}	<0.001
Gallibacterium	0.127 ± 0.063^{a}	0.088 ± 0.032^{a}	0.050 ± 0.036^{a}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	<0.001
Bacillus	0.046 ± 0.031^{a}	0.046 ± 0.019^{a}	0.109 ± 0.050^{a}	0.062 ± 0.058^{a}	0 ± 0^{b}	0.001 ± 0.000^{b}	<0.001
Enterococcus	0.112 ± 0.061^{a}	0.040 ± 0.016^{ab}	0.018 ± 0.009^{bc}	0.019 ± 0.010^{abc}	0.002 ± 0.000^{c}	0.005 ± 0.000^{c}	<0.001
Peptococcus	0 ± 0^{c}	0.005 ± 0.002^{b}	0.001 ± 0.000^{c}	0.013 ± 0.009^{b}	0.093 ± 0.019^{a}	0.039 ± 0.026^{b}	<0.001
Romboutsia	0.014 ± 0.011	0.019 ± 0.009	0.018 ± 0.005	0.02 ± 0.013	0.011 ± 0.002	0.065 ± 0.038	0.424
Campylobacter	0.006 ± 0.002^{ab}	0.010 ± 0.005^{ab}	0.003 ± 0.002^{bc}	0 ± 0^{cd}	0 ± 0^{d}	0.113 ± 0.055^{a}	<0.001
Prevotella	0.016 ± 0.007^{ab}	0.017 ± 0.008^{ab}	0.005 ± 0.002^{b}	0.008 ± 0.004^{b}	0.043 ± 0.010^{a}	0.025 ± 0.009^{ab}	0.017
Neisseria	0.046 ± 0.016^{a}	0.047 ± 0.021^{a}	0.014 ± 0.006^{a}	0 ± 0^{b}	0 ± 0^{b}	0 ± 0^{b}	<0.001
Turicibacter	0.006 ± 0.004^{b}	0.012 ± 0.005^{ab}	0.020 ± 0.007^{a}	0.012 ± 0.009^{ab}	0.018 ± 0.008^{ab}	0.030 ± 0.016^{ab}	0.051
Faecalibacterium	0 ± 0^{d}	0.004 ± 0.002^{bc}	0.001 ± 0.001^{cd}	0.005 ± 0.002^{bc}	0.066 ± 0.016^{a}	0.014 ± 0.006^{b}	<0.001
Bacteroides	0.004 ± 0.004^{c}	0.004 ± 0.002^{bc}	0.003 ± 0.003^{c}	0.004 ± 0.003^{bc}	0.048 ± 0.014^{a}	0.016 ± 0.006^{ab}	<0.001

[1]In the same row, values with different letter superscipts (a, b, c, d) mean significant difference ($P < 0.05$), whereas no letter superscript or same letter superscipt means no significant difference ($P > 0.05$). Values are presented by mean ± SE.

Jiangnan and Yangzhou geese based on the comparison of villus height and crypt depth (Lu et al., 2011; Yu et al., 2019). Furthermore, the morphology of the middle cecum observed in our study is similar with a report on cecal structure of White roman geese (Chen et al., 2002), with many tubular glands and goblet cells. As we all known, the physiologic functions of cecum refer to bacterial fermentation, nutrient absorption, vitamin synthesis, immunological response, and so on (Garcia, 2006). The sound cecum is indispensable for low-quality feedstuff utilization of waterfowl. So, the above-mentioned results indicated that the jejunal and cecal development in Changle geese is standing out among small-sized Chinese geese and can exploit greater advantages on food digestion.

It was mentioned earlier that the revelation of microbiota regional distribution along GI tract could be conducive to comprehensively understand the role of microbiota on nutrient utilization and intestinal health. By analyzing alpha diversity of microbiota in 6 parts of GI tract, a further verification about the vital role of cecum on bacterial fermentation is made. Besides, except cecum, other sectors of GI tract also possess strong richness and diversity of microbiota, suggesting that there may be some undisclosed effects of noncecum microbiota on nutrient digestion and gastrointestinal immunologic regulation. The NMDS analysis also shows that noncecum microbiota can be roughly divided to 3 clusters, proventriculus, gizzard, and jejunum as Cluster 1, crop as Cluster 2, and rectum as Cluster 3. The distinctive bacterial structure in crop (Cluster 2) may result from its unique physiologic structure and function. Similar results were presented in semiartificially reared bar-headed geese (*Anser indicus*), possessing

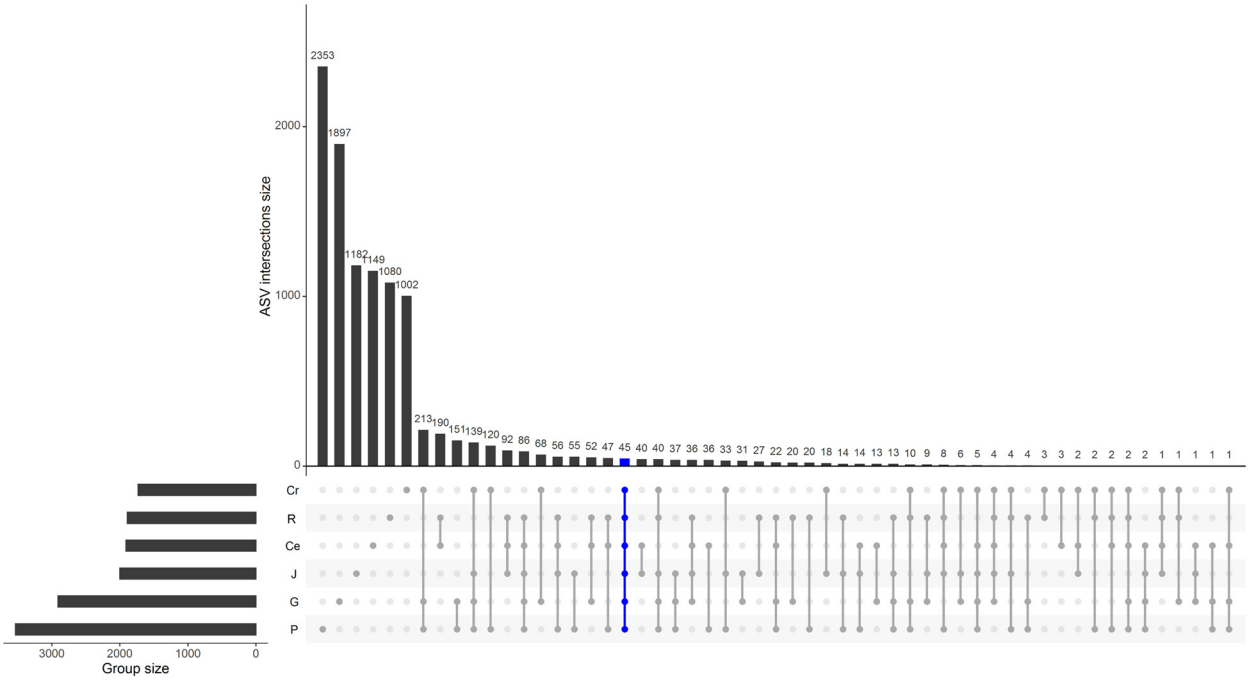

Figure 5. The ASV Upset plot of goose gastrointestinal microbiota.

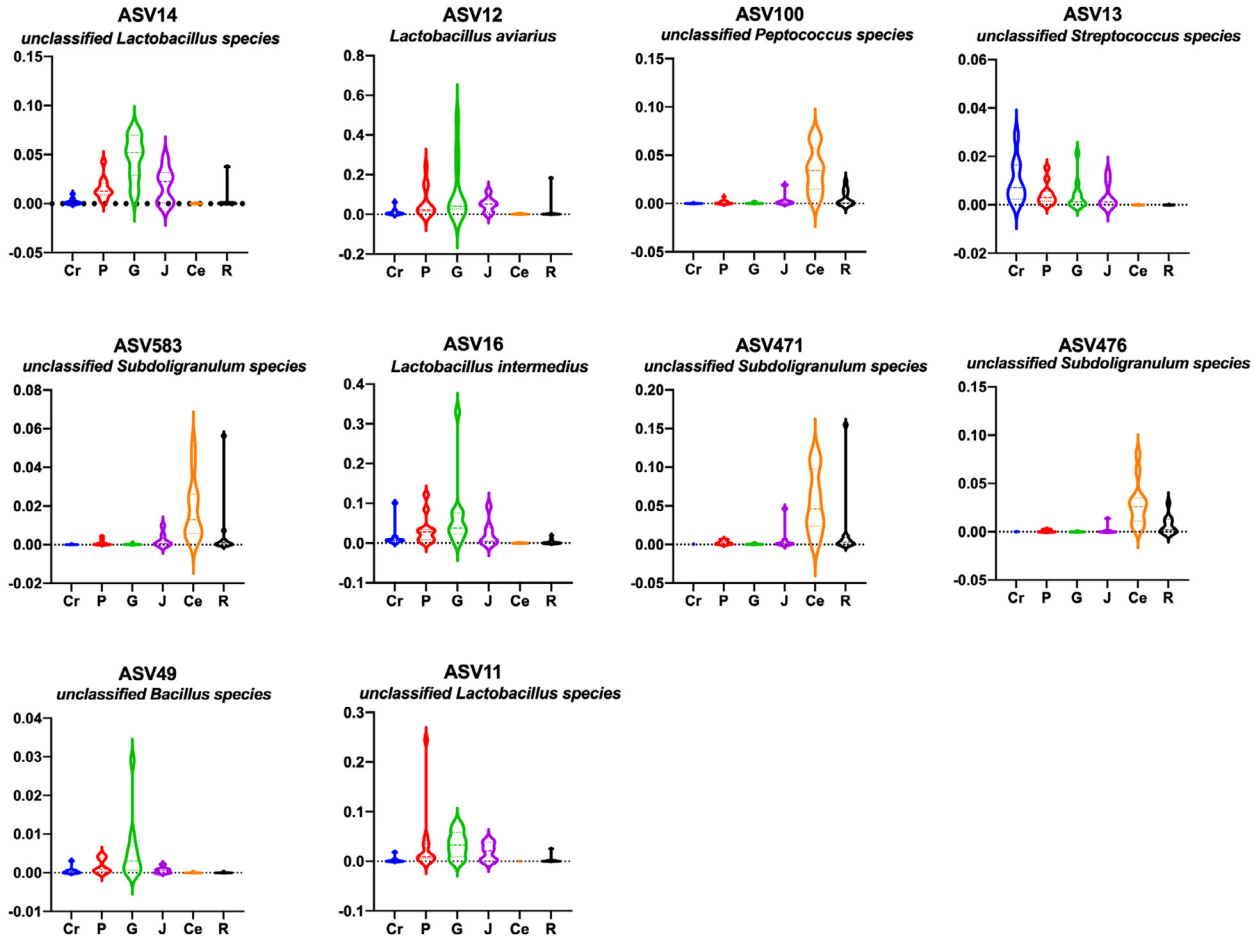

Figure 6. The top 10 different-abundance core ASVs among different GI parts.

higher bacterial richness in crop and gizzard than cecum (Wang et al., 2020). At the same time, a report that conducted in 180-day-old Taihu geese, a small-sized breed raised in Taihu Lake Basin, also suggested that proventriculus possessed the richest bacterial species comparing with gizzard, duodenum, jejunum, ileum, cecum, and rectum, and bacterial diversities in cecum and gizzard were higher than jejunum and ileum (Yang et al., 2018). In GI tract of muscovy ducks (*Cairina moschata*), the Chao 1 and Shannon indexes in proventriculus were the highest as well (Yang et al., 2020). Overwhelming evidences imply an irreplaceable role of high-richness microbiota in foregut on waterfowl health.

At the phylum level, Firmicutes is the most abundant in each location. The multiple comparison results directly demonstrate that the abundance of Firmicutes in cecum is higher than rectum. Besides, there are great discrepancies on the GI distribution of Proteobacteria, Bacteroidota, and Campilobacterota, which are enriched in foregut (especially crop), cecum, and rectum, respectively. The habitats of Proteobacteria and Bacteroidota in Changle geese are similar to other birds and such distribution characteristics may be mainly dependent on their distinct luminal environment (Wilkinson et al., 2017; Xiao et al., 2017; Yang et al., 2018; Wang et al., 2020). The higher oxygen exposure, lower degree of digestion, and anterior to GI tract lead to the enrichment of Proteobacteria species in the foregut to a large extent. And the stronger fiber digestibility of Bacteroidota species results in their predominant statue in cecum (Thomas et al., 2011). But unlike other reports, the high richness of Campilobacterota in rectum is characteristic in our study. Campilobacterota, previously called Epsilonproteobacteria, is the set of Gram-negative and spiral-shaped motile bacteria (van der Stel and Wösten, 2019). *Helicobacter* spp. and *Campylobacter* spp. are the representative genera of Campilobacterota. Generally, most members of Campilobacterota are commensal bacteria in the upper GI tract of mammals and birds. In our study, high oxygen availability similar to foregut may account for the enrichment of Campilobacterota in rectum of Changle geese.

Correspondingly, at the genus level, most high-abundance genera belong to Firmicutes. Herein, dominant genera, *Lactobacillus, Streptococcus*, and *Enterococcus*, are lactic acid bacteria, mostly settling down in the upper GI tract (Chen, 2005). Meanwhile, there are 3 *Lactobacillus* strains among top 10 different-abundance core ASVs in GI tract of Changle geese, disclosing the strong adaptability of *Lactobacillus* strains to luminal environment of GI tract and great preference of mutualistic symbiosis from host.

In addition, *Subdoligranulum* spp., similarly the core bacteria in goose GI tract, are enriched in cecum. The random forestry analysis shows ASV427 (an unclassified *Subdoligranulum* species) is the Top 1 feature ASV in

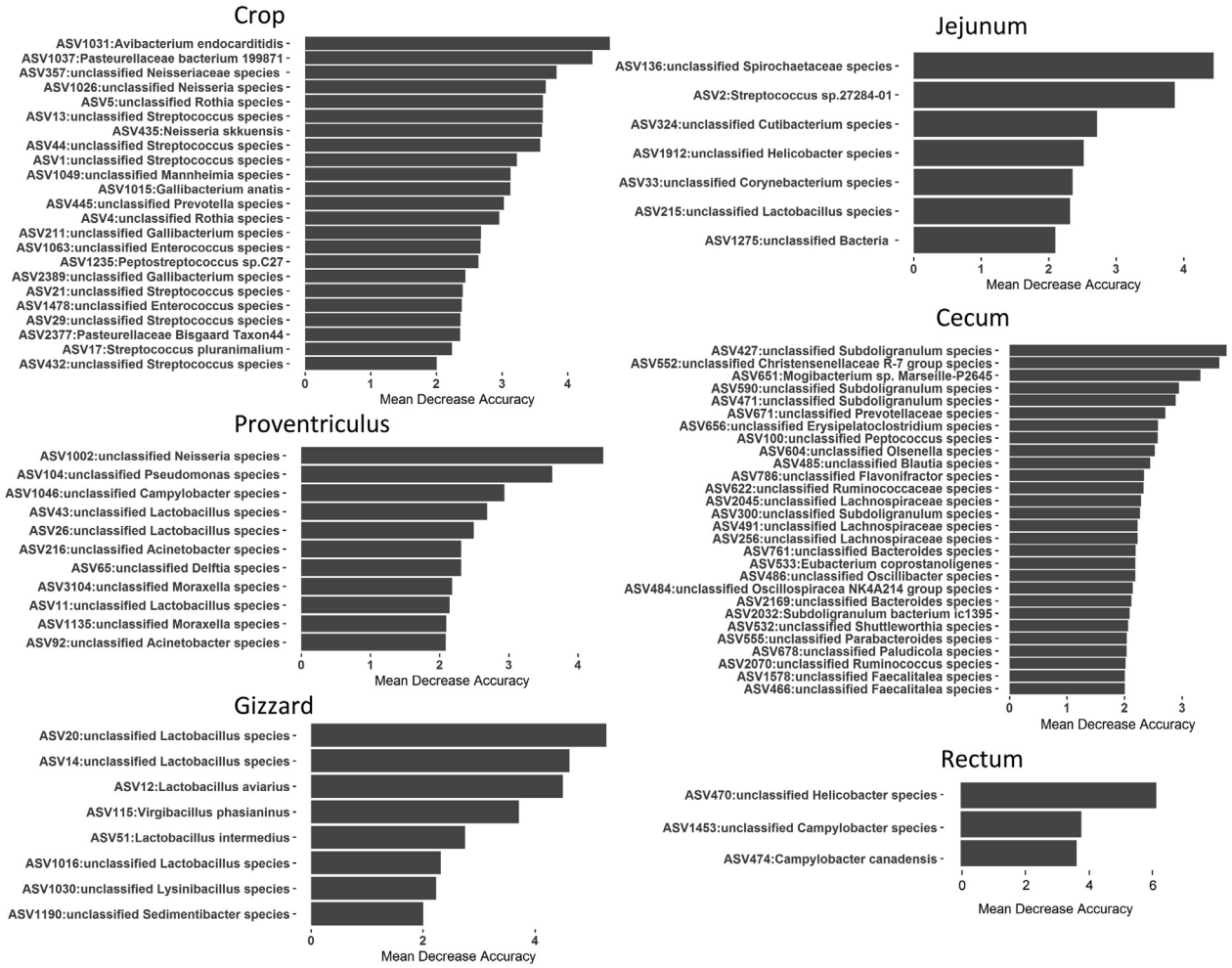

Figure 7. The feature ASVs in every GI parts based on Random Forestry Analysis.

cecum. *Subdoligranulum* spp. belong to the family Ruminococcaceae and are closely related to the genus *Faecalibacterium*. Ruminococcaceae species, which are butyrate producers and cellulose degraders, were linked with low feed-conversion ratio in chickens on multiple occasions (Torok et al., 2011; Stanley et al., 2012, 2013). Besides, it was reported that the relative abundance of an unclassified *Subdoligranulum* species decreased in diarrhea patients (Liu et al., 2022). Meanwhile, the abundance of *Subdoligranulum* spp. was strongly correlated with *Akkermansia muciniphila* and dietary fiber treatment could stimulate their growth (Van Hul and Le Roy, 2020). Hence, *Subdoligranulum* spp. were expected as probiotic candidates (Van Hul and Le Roy, 2020). So far, *Subdoligranulum variabile*, a strictly anaerobic, non-spore-forming, butyrate-producing, Gram-negative bacterium, is the only one species in this genus isolated (Holmstrøm et al., 2004) and there are some conflicting results on its application. The mono-bacterial therapy with it could prevent from food allergy, whereas 8-wk treatment of *Subdoligranulum variabile* did not improve diet-induced metabolic disorders of mice (Abdel-Gadir et al., 2019; Van Hul and Le Roy, 2020). In our study, correlation analysis results showed that the proportion of ASV59 (*Subdoligranulum* spp.) in cecum, along with ASV485 (*Blautia* spp.), is negatively related with body weight of goose. As a result, its benefit to health is still inconclusive. Additionally, the abundance of *Subdoligranulum variabile* only accounts for less than 1% of *Subdoligranulum* species. So, maybe other species of this genus were responsible for reported metabolic benefits (Van Hul and Le Roy, 2020).

Moreover, 2 feature ASVs in cecum, ASV552 (*Christensenellaceae R-7 group* spp.) and ASV656 (*Erysipelatoclostridium* spp.), are obviously positively and negatively related with the absolute and relative length of cecum respectively. According to a recent research, there was a strong positive correlation between proportion of *Erysipelatoclostridium* spp. and severity of Parkinson's disease (Rosario et al., 2021). *Erysipelatoclostridium* spp. contributed to the secretion of tryptophan, whose microbial metabolite, indolepropionic acid as an anti-inflammatory factor, was significantly increased in Parkinson's disease (Rosario et al., 2021). Christensenellaceae spp. were reported negatively related with body mass index, inflammatory bowel disease, and some metabolic diseases, like obesity and metabolic syndrome (Waters and Ley, 2019). To sum up, ASV552 and ASV656 might be the candidate bacteria to mirror the development and health status of cecum.

In crop, *Avibacterium endocarditidis* is the Top 1 feature ASV based on random forestry analysis.

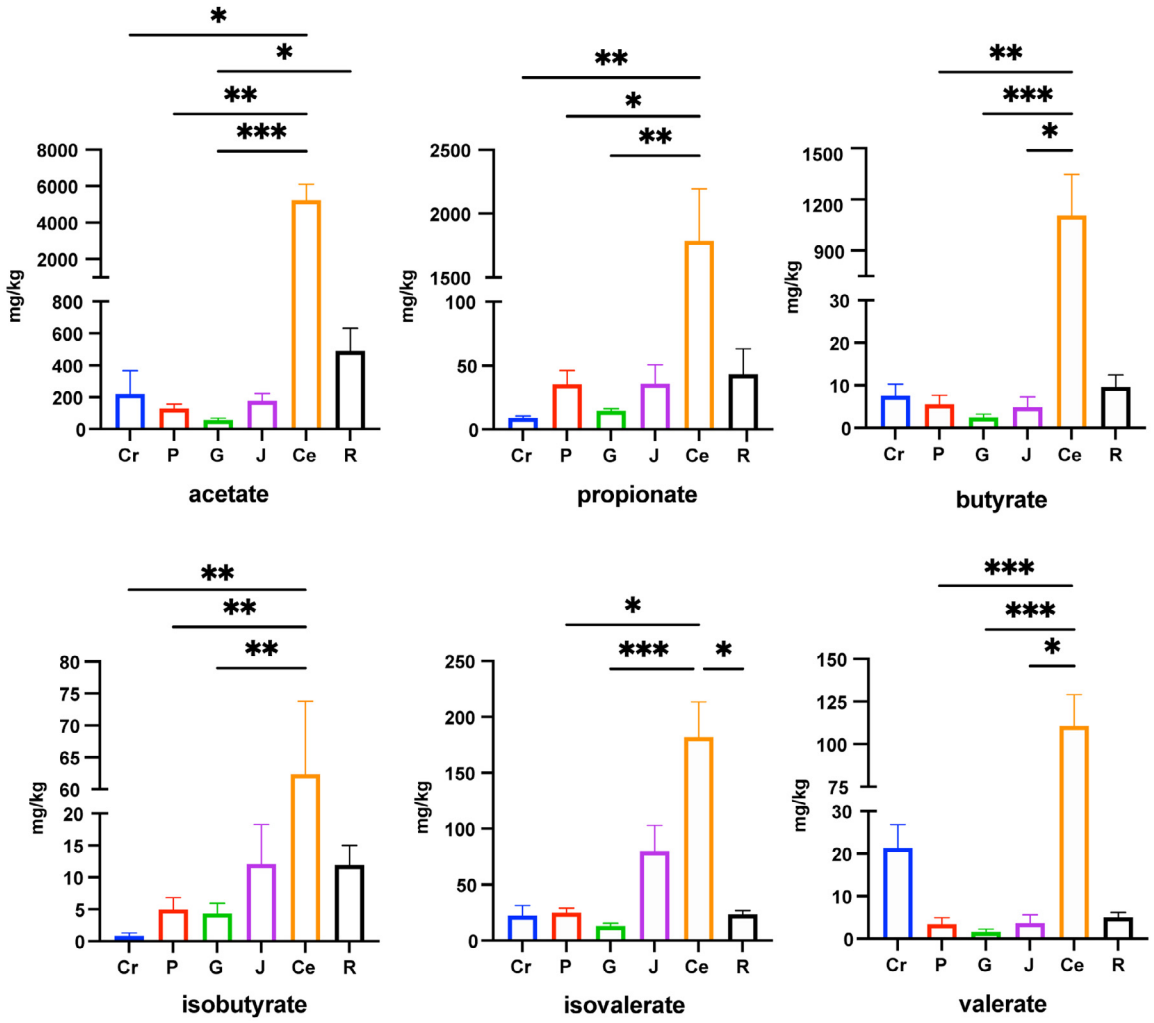

Figure 8. The gastrointestinal SCFA pattern of goose.

Avibacterium is a genus within the family Pasteurellaceae. *Avibacterium* spp. are routinely found in respiratory tract or digestive tract of birds and *Avibacterium paragallinarum* is the only one member reported as the pathogen causing infectious coryza in birds (Xu et al., 2019). Except *Avibacterium endocarditidis,* another 2 feature ASVs in crop, ASV1037 (*Pasteurellaceae bacterium 199871*) and ASV5 (*Rothia* spp.), whose proportions are positive correlation with the body weight of goose, are also expected as the representative bacteria to distinguish high-growth-performance goose.

More interestingly, the number of feature ASVs in crop is only less than cecum, indicating the microbiota structure in crop is very characteristic along the GI tract

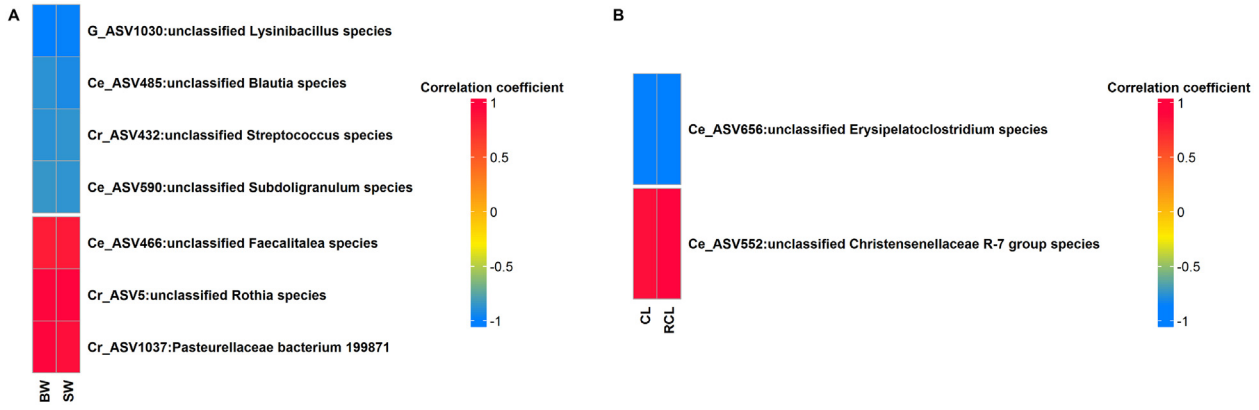

Figure 9. The association analysis between physiological characters and specific ASVs. (A) Correlation between weight and specific ASVs in each section; (B) correlation between cecal development parameters and cecal specific ASVs. BW, body weight; CL, length of cecum; CRL, relative length of cecum; SW, slaughter weight.

of goose. The structure of goose crop is not typical comparing with chickens and the study on it is rare. In chickens, food may remain and be fermented primarily by *Lactobacillus* spp. (10^8–10^9 cfu/g) in crop for 6 h after esophageal transport (Chen, 2005; Stanley et al., 2012). But different from chickens, the predominant bacteria in crop of Changle geese are *Streptococcus, Gallibacterium*, and *Enterococcus* spp. The genus *Gallibacterium* consists of 7 species and one of them is *Gallibacterium anatis*, which was isolated from the upper respiratory, lower genital, and digestive tracts of healthy chickens and could adhere to epithelial cells and cause infection as an opportunistic pathogen (Bojesen et al., 2003; Narasinakuppe Krishnegowda and Dhama, 2020). Based on the adaptive evolution theory, the enrichment of genus *Gallibacterium* instead of *Lactobacillus* in crop of geese may attribute to its atypical structure and shorter food transit time comparing with chickens.

The contents of SCFAs in each section were consistent with the microbial fermentation pattern along GI tract. Cecum is the biggest contributor. Abundant cellulose degraders in cecum, like *Subdoligranulum, Faecalibacterium*, and *Bacteroides*, produce the highest yield of SCFAs. The result is in agreement with several studies conducted in Taihu geese and muscovy ducks (Yang et al., 2018,2020). An integrated analysis of the host genome and the gut metagenome of Sichuan White geese found many expanded and rapidly evolving metabolism-related gene families enriched in the goose genome and there were strong symbiotic interactions between goose and its gut microbiota (Gao et al., 2016). Therefore, the above results clearly illustrate the co-evolution of the microbial genomes and host genome, from the perspective of regionally diverse populations and fermentation pattern.

In conclusion, different locations along GI tract possess distinct physiological characters and microbiota structure and may play distinguishing roles in the growth and intestinal health of Changle goose.

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DISCLOSURES

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

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