Developmental differences in the intestinal microbiota of Chinese 1-year-old infants and 4-year-old children

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The microbiota profile of children changes with age. To investigate the differences in the gut microbiota profile of 1- and 4-year-old children, we collected fecal samples and sequenced the V3–V4 hypervariable region of the 16S rRNA gene via high-throughput DNA sequencing. From phylum to species level, the microbiota underwent significant changes with age. The abundance of phyla Proteobacteria and Actinobacteria declined with age, whereas phyla Firmicutes and Bacteroidetes increased with age and dominated the gut microbiota of 4-year-olds. The intestinal environment of children at age four is closer to maturity. Hence, the abundance of Bifidobacterium significantly decreased in the gut of 4-year-olds, whereas Akkermansia muciniphila increased from 0.14% in 1-year-olds to 4.25% in 4-year-olds. The functional change in gut microbiota is consistent with changes in infant food, as microbiota participating in amino acid and vitamin metabolism were enriched in 1-year-olds, whereas microbiota involved in lipid metabolism increased with age.

Abbreviations
GI Gastrointestinal
S-MBCS Shanghai-Minhang Birth Cohort Study
RDP Ribosomal Database Project
OTU Operational Taxonomic Units
ANOSIM Analysis of similarities
PICRUSt Phylogenetic Investigation of Communities by Reconstruction of Unobserved States
KEGG Kyoto Encyclopedia of Genes and Genomes
PCoA Principal coordinate analyses
HMO Human milk oligosaccharide

Intestinal microbiota play an important role in human health, and dysbiosis (an imbalance in microbiota) could cause immunologic dysregulation1 and various chronic diseases, including diabetes2, obesity3, inflammatory bowel disease4, rheumatoid arthritis5, autism spectrum disorders6, and cancers7. The gut–brain axis8, gut–liver axis9 and gut–lung axis10 imply a bidirectional interaction between microbiota and tissues of human body, emphasizing the role of microbiota in both health and disease.

Strikingly, intestinal microbiota imbalance early in life may lead to disease conditions at a later age11,12. The gut microbiota taxa of 2-year-old infants showed an increasingly strong association with the BMI of 12-year-old children13, and reduced bacterial diversity in both 1- and 12-month-old infants' intestinal microbiota was associated with an increased risk of allergic sensitization in the first 6 years of life14. These findings drew more attention on the development of pediatric intestinal microbiota15.

The formation of infant gastrointestinal (GI) microbiota is affected by many factors, including mode of delivery16,17, type of feeding18–20, race and cord blood vitamin D levels21. Most studies support the opinion that vaginal delivery and breast-feeding contribute to healthy gut microbiota22,23, but growing infants displayed increased alpha-diversity and reduced beta-diversity in the gut microbiota24. Analyses have shown that infants

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SIM analysis suggested that the microbial composition was significantly different (p < 0.001, R = 0.731) between coordinate analyses (PCoA) showed two significant parts divided by two different point of age (Fig. 1). ANO-
taken antibiotics in one month, and had not developed eczema. All samples were stored at − 80°C before DNA
Fecal samples were collected from 1-year-old infants and 4-year-olds who were healthy, not overweight, had not
participants involved in this study. All methods were performed in accordance with the Declaration of Helsinki.
Planned Parenthood Research (IRB00008297). Written informed consents were obtained from the parents of all
(S-MBCS), which was reviewed and approved by the ethics committee board of the Shanghai Institute of
extraction.

The online software, Ribosomal Database Project (RDP) classifier30 was used for taxonomy assignment for each
ness, and diversity (Shannon, Shannoneven, ACE, Chao, and Good’s coverage) were also assessed using Mothur.
clustered into OTUs at 97% similarity with reads number normalizing to 17,436. Community richness, even-
99.8% for both groups (1- and 4-year-olds, Table 1), indicating that the sequencing depth was sufficient for gut
microbiota investigation in children of two different ages.

DNA extraction, PCR amplification and 16S rRNA gene sequencing. DNA extraction and PCR
amplification were performed as described previously27, with some modifications. In brief, the genomic DNA
was extracted from 300 mg of feces using a QIAamp DNA stool mini kit (Qiagen, Hilden, Germany) according
the manufacturer's instructions. The integrity of extracted genomic DNA was checked by 1.5% agarose gel
electrophoresis. To generate 16S rRNA gene amplicons, the V3-4 hypervariable region of the 16S rRNA genes
was amplified using the primers 338F (5'-CCT ACG GGNGGC WGC AG-3') and 806R (5'-GACTACHVGGG
TATCTAATCC-3') with a TransStart Fastpfu DNA Polymerase (TransGen, Beijing, China) in 20 cycles. All
amplicons were purified using the QIAquick PCR Purification Kit (Qiagen), quantified on Qubit (Life Technolo-
gies), then pooled into equal concentrations. The pooled amplicons were ligated with adaptors using TruePrep
DNA Library Prep Kit V2 for Illumina (Vazyme,China), then 2 × 300 bp paired-end sequencing was performed
on an Illumina MiSeq instrument with MiSeq Reagent Kit v3.

Bioinformatics and statistical analysis. Paired-end 16S rRNA sequences were assembled using Mothur
(version 1.41.1)28. DNA sequences were discarded using the following criteria: containing ambiguous bases, or
containing chimeric or contaminant sequences, or homopolymers of > 8 nucleotides, or with lengths shorter than
350 bp. The chimeric sequences were identified by VSEARCH algorithm, and non-16S contaminants sequences
were filtered based on the RDP database. Using SILVA reference databases (V132)29, the DNA sequences were
clustered into OTUs at 97% similarity with reads number normalizing to 17,436. Community richness, even-
ness, and diversity (Shannon, Shannoneven, ACE, Chao, and Good’s coverage) were also assessed using Mothur.
The online software, Ribosomal Database Project (RDP) classifier30 was used for taxonomy assignment for each
Operational Taxonomic Units (OTU)31 using default parameters. Differences in bacterial diversity were assessed
using analysis of similarities (ANOSIM), based on the unweighted UniFrac distance metrics using Mothur. The
prediction of microbiome functions were analyzed using Phylogenetic Investigation of Community
metabolic pathways (KEGG)34.

Representative OTUs were identified as species against the SILVA SSU database (132) and the NCBI online
database with > 99% identity and highest total score29,35.

Results

Gut bacterial populations in 1- and 4-year-old children. A total of 10,210,871 (17,436–395,495)
high-quality reads were obtained by high-throughput sequencing of 16S rRNA genes from 153 fecal samples (40
and 113 from 1- and 4-year-olds, respectively). To normalize data and avoid statistical bias, 17,436 16S rRNA
genes of each sample were selected to calculate bacterial species richness, evenness, and diversity at 97% similarity.
A total of 7195 OTUs (371 OTUs per sample on average) were obtained, and the Good’s coverage was over
99.8% for both groups (1- and 4-year-olds, Table 1), indicating that the sequencing depth was sufficient for gut
microbiota investigation in children of two different ages.

Bacterial composition changes with age. Based on the unweighted UniFrac distance metrics, principal
coordinate analyses (PCoA) showed two significant parts divided by two different point of age (Fig. 1). ANO-
SIM analysis suggested that the microbial composition was significantly different (p < 0.001, R = 0.731) between

| Group | Sample | OTUs | Coverage | Richness | Evenness | Diversity |
|-------|--------|------|----------|----------|----------|-----------|
| 1-year | 40     | 1159 | 0.998975 | 1580.63  | 1584.31  | 0.566114  | 3.99411   |
| 4-years | 113   | 7089 | 0.999931 | 7103.50  | 7106.58  | 0.56353   | 4.99643   |

Table 1. The gut microbiota diversity evaluation of 1-year and 4-years old children.
From phylum to species level, the microbiota differed significantly between 1- and 4-year-olds. At the phylum level, a total of 12 phyla were confirmed, with five major phyla (Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, and Verrucomicrobia) composing > 99% of gut microbiota (Fig. 3), and three phyla unique to 4-year-olds. Phyla Actinobacteria and Proteobacteria showed significant enrichment in 1-year-olds (q < 0.05), while phyla Firmicutes, Synergistetes, and Verrucomicrobia were significantly enriched in 4-year-olds (Fig. 4A).

At the class level, a total of 20 classes were revealed; 16 classes were identified in both 1- and 4-year-olds and nine classes were significantly different between both groups (Figure S1), with Actinobacteria, Bacilli, Erysipelotrichia and Gammaproteobacteria significantly enriched in 1-year-olds, and Betaproteobacteria, Clostridia, Deltaproteobacteria, Synergistia and Verrucomicrobia significantly increased in 4-year-olds.

At the order level, 38 orders were identified in total; 28 orders were identified in both groups, and nine orders were significantly different between both groups (Figure S2), including Bifidobacteriales, Enterobacterales, Erysipelotrichales and Lactobacillales enriched in 1-year-olds and Burkholderiales, Clostridiales, Desulfovibrionales, Synergistales, and Verrucomicrobiales enriched in 4-year-olds.

At the family level, 81 families were identified in total; 58 families were identified in both groups, 19 families were significantly different between the groups (Fig. 4B). Eight families, Actinomyceaeae, Bifidobacteriaceae, Clostridiaceae 1, Enterobacteraeae, Enterococcaceae, Erysipelotrichaceae, Peptostreptococcaceae and Streptococaceae were significantly enriched in 1-year-olds, while the other 11 families, like Lachnospiraceae. Ruminococaceae and Verrucomicrobiaceae were significantly enriched in 4-year-olds.

At the genus level, 203 genera were identified in total; 133 genera were identified in both groups, 18 of which were major genera in both groups (> 1% per group Table 2). The fecal microbiome of 1-year-olds was generally dominated by Bifidobacterium, Escherichia/Shigella, and Bacteroides, each genus composing > 12% (total 47.4%) of the microbiome. While in 4-year-olds, the dominant genera were Bacteroides (19.3%) and Faecalibacterium (10.2%). A total of 46 genera (including 11 major genera) were significantly different between both groups (Fig. 4C). Noteworthy, 12 genera including Actinomyces, Blautia, Clostridium sensu stricto, Clostridium XVIII, Eggerthella, Intestinibacter, Klebsiella, Streptococcus, Bifidobacterium, Escherichia/Shigella and Veillonella) were significantly enriched in 1-year-olds. The other 34 genera, including Akkermasia, Dialister, Faecalibacterium, Gemmiger, Roseburia, etc., were significantly enriched in 4-year-olds. The dominant genus, Bacteroides, maintained a stable population with age (12.9% and 19.3% in 1- and 4-year-olds, respectively).

At the species (OTU) level, 23 of the top 30 OTUs were significantly different between 1- and 4-year-olds (Table S1), with 19 of them confirmed as known species via NCBI BLAST (Fig. 4D). Ten species were significantly enriched in 1-year-olds, including Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium pseudocatenulatum, Blautia wexlerae, Fusicatenibacter saccharivorans, Romboutsia timonensis, Ruminococcus gnavus, Streptococcus salivarius, Escherichia coli, and Veillonella dispar, which occupied 39.9% of gut microbiota in 1-year-olds. The abundance of the other nine species, including Akkermansia muciniphila, Bacteroides uniformis, Bacteroides xylanisolvens, Gemmiger formicilis, Roseburia faecis, Roseburia inulinivorans, Ruminococcus bromii, Faecalibacterium prausnitzii, and Dialister in avidus, increased significantly in 4-year-olds compared to that in 1-year-olds.
Predicted functional change of gut microbiota between 1-year-old and 4-year-old children. We used PICRUSt software to predict the potential functional changes with age. Sixty-nine predicted Metabolism Pathways, 17 pathways related to Genetic Information Processing, two pathways belonging to Cellular Processes, and three pathways participating Environmental Information Processing, were identified as having significant differences (q-value < 0.05) between 1- and 4-year-olds (Figure S3). Analysis revealed the relative abundance of genes involved in O-glycan biosynthesis, steroid biosynthesis, secondary bile acid biosynthesis, carotenoid biosynthesis, etc., were significantly increased in 4-year-olds. Genes participating in cell motility, membrane transport, galactose metabolism, folate biosynthesis, glutathione metabolism, etc., were significantly decreased in 4-year-olds.

Discussion
In this study, we compared the gut microbiota of 1- and 4-year-old Chinese children by investigating the V3–V4 hypervariable region of the 16S rRNA gene via high-throughput DNA sequencing. Our results revealed that the gut microbiota in children increased significantly from age 1 to 4. In terms of population, species richness, species evenness, and diversity were mainly dominated by five phyla (Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes and Verrucomicrobia). These findings are consistent with previous reports that the gut microbiome of children gradually mature as that of adults during the first three years of life.

A lot of research in the past decades have focused on the development of the infant gut microbiota during the first 3 years of life; only, few reports have investigated the variation in gut microbiota in children above 3 years of age, and further studies are needed. For example, Fiona et al. indicated that the gut microbiota of children was dynamic before age four due to the effects of perinatal factors, and Ringel-Kulka et al. revealed that by age four the microbiota of children were still not as mature as those of adults, suggesting that the microbiota of children continue to progress after age 4.

Hence, our research paid attention to the development of children's gut microbiota at age four for a deeper understanding of the intestinal microbiota of children. Through the unweighted UniFrac distance metrics, we demonstrated that there was a significant difference in gut microbiota compositions between 1- and 4-year-old children, suggesting that the gut microbiota of infants matures with age.

We analyzed specific differences from the phylum to the species level. At the phylum level, Actinobacteria and Proteobacteria were significantly reduced in the intestines of 4-year-olds. This result is consistent with previous reports that Actinobacteria, represented by Bifidobacterium, declined after weaning due to decreased protein requirements. However, Firmicutes, Synergistetes, and Verrucomicrobia increased significantly in
the intestines of 4-year-olds. It was recently noted that the abundance of *Firmicutes* is suppressed while children receive breast milk\(^2\). Once weaning begins, *Firmicutes* increase in abundance and dominate gut microbiota. It is supposed that the introduction of solid foods can increase bacterial load and short-chain fatty acid levels, which may be due to the ability of *Firmicutes*, such as *Roseburia* spp., to metabolize carbohydrates in the diet\(^4\). *Bacteroides*, which can breakdown complex plant polysaccharides\(^4\), maintain dominance of the gut microbiota with age, indicating that *Bacteroides* already attained stability at infancy. These results are consistent with previous reports that *Firmicutes* and *Bacteroidetes* are the most dominant phyla in healthy adult subjects\(^4\), suggesting a maturation of gut microbiota at age 4.

At the genus level, *Bifidobacterium*, *Escherichia/Shigella*, and *Veillonella* were enriched in the intestines of 1-year-olds. *Bifidobacterium* levels declined with age, in agreement with reports that *Bifidobacterium* is more abundant in children than in adults\(^4\). It is generally known that *Bifidobacterium* has several subspecies relating to infants. *Bifidobacterium longum* subsp. is a kind of archetypical bacteria capable of using human milk oligosaccharide (HMO) as substrates. *B. longum* subsp. *infantis* is an infant commensal that thrives in the presence of milk\(^4\). Our results showed that the abundance of *Bifidobacterium* decreased significantly in the gut microbiota of 4-year-olds, coinciding with the beginning of weaning and the introduction of table foods. In the gut

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**Figure 3.** The gut microbiota composition of 1-year-old and 4-years-old children on different taxa level. The main bacteria phylum, class, order, family and genus in children gut were illustrated in different colors.
microbiota of 4-year-olds, the abundance of *Faecalibacterium*, *Dialister*, *Gemmiger*, and *Akkermansia* increased significantly. Notably, *Akkermansia* is a probiotic, and may be more beneficial for growing children as they face a more complex diet and other environmental factors. *Akkermansia* helps regulate the thickness of intestinal mucus and maintain intestinal barrier integrity to reduce sugar absorption. It is beneficial for weight loss, blood sugar regulation and diabetes mellitus management.

At the species level, *F. prausnitzii*, *D. invisus*, *A. muciniphila*, *R. bromii*, *B. xylanisolvens*, *G. formicilis*, *R. faecis* and *R. inulinivorans* increased significantly with age, while *E. coli*, *B. pseudocatenulatum*, *B. longum*, *B. wexlerae*, *F. saccharivorans*, *R. timonensis*, *R. gnavus*, *S. salivarius*, *V. dispar* and *B. breve* were more abundant in the intestines of 1-year-olds than in those of 4-year-olds. *F. prausnitzii* is one of the most abundant microorganisms in the intestinal tract of healthy people; it can generate butyrate as an anti-inflammatory to help slow down inflammatory bowel disease.

*A. muciniphila*, a mucin-degrading probiotic, increased from 0.14% in 1-year-olds to 4.25% in 4-year-olds. *A. muciniphila* can regulate immune responses by promoting relevant gene expression, and reverse high-fat diet-induced metabolic disorders by improving host metabolism. *R. inulinivorans* belong to the genus Roseburia, which was reported to produce short-chain fatty acids and play a major role in maintaining gut health and immune defense. The increased levels of these profitable species suggest that the intestinal environment of 4-year-olds is attaining adult-like maturity.

*Bifidobacterium* is able to thrive on HMOs and is dominant in infant gut microbiota before weaning. The abundance of these milk-related *Bifidobacterium*, including *B. pseudocatenulatum*, *B. longum*, and *B. breve* significantly decreased in 4-year-olds due to the change in their diet (from milk to solid food). The onset of weaning is usually associated with an increase in a diversity of intestinal microbiota, with Bifidobacteria-dominated...
intestinal microbiota gradually being replaced by more complex microbial communities capable of degrading carbohydrates from plant and animal sources.

In this study, PICRUSt software was used to predict the potential function of the gut microbiota of 1- and 4-year-olds. Bacteria involved in galactose metabolism, amino acid metabolism, cofactor and vitamin metabolism like folate biosynthesis, nicotinate and nicotinamide metabolism, vitamin B6 metabolism, etc., were significantly enriched in 1-year-olds. While the abundance of microbiota participating in lipid metabolism, metabolism of

| Genus Feature | 1-year: mean rel. freq. (%) | 1-year: std. dev. (%) | 4-years: mean rel. freq. (%) | 4-years: std. dev. (%) | q-values Enriched in |
|---------------|--------------------------|----------------------|----------------------------|-----------------------|-------------------|
| Actinomyces Difference | 0.1017 | 0.1187 | 0.0239 | 0.0296 | 0.0021 | 1-year |
| Akkermansia Major and difference | 0.1368 | 0.7098 | 4.6110 | 13.4165 | 0.0052 | 4-years |
| Alistipes Difference | 0.0612 | 0.2742 | 0.8799 | 1.8020 | 0.0002 | 4-years |
| Anaerofilis Difference | 0.0006 | 0.0035 | 0.0034 | 0.0091 | 0.0268 | 4-years |
| Anaerotruncus Difference | 0.0194 | 0.0529 | 0.0750 | 0.1206 | 0.0015 | 4-years |
| Barnesella Difference | 0.0000 | 0.0000 | 0.0307 | 0.1228 | 0.0576 | 4-years |
| Bifidobacterium Major and difference | 22.4657 | 21.6951 | 2.4992 | 4.4353 | 0.0001 | 1-year |
| Bilophila Difference | 0.0016 | 0.0661 | 0.0827 | 0.1719 | 0.0001 | 4-years |
| Blautia Major and difference | 4.8976 | 5.9816 | 2.2828 | 2.9867 | 0.0458 | 1-year |
| Butyricicoccus Difference | 0.0978 | 0.2197 | 0.3386 | 0.0657 | 0.2022 | 4-years |
| Butyricimonas Difference | 0.0003 | 0.0167 | 0.0185 | 0.0763 | 0.0099 | 4-years |
| Christensenella Difference | 0.0000 | 0.0000 | 0.0070 | 0.0177 | 0.0009 | 4-years |
| Cloacibacillus Difference | 0.0000 | 0.0000 | 0.0117 | 0.0063 | 0.0293 | 4-years |
| Clostridium IV Major and difference | 0.0136 | 0.0587 | 1.8773 | 3.7870 | 0.0001 | 4-years |
| Clostridium sensu stricto Major and difference | 1.9968 | 3.8008 | 2.728 | 6.705 | 0.0324 | 4-years |
| Clostridium XIVa Major and difference | 0.5308 | 0.7931 | 1.1052 | 1.8900 | 0.0381 | 4-years |
| Clostridium XIVb Difference | 0.0067 | 0.0195 | 0.4134 | 0.9732 | 0.0004 | 4-years |
| Clostridium XVIII Major and difference | 1.9694 | 3.7634 | 0.9881 | 1.562 | 0.0186 | 1-year |
| Coprococcus Difference | 0.0324 | 0.1297 | 0.2113 | 0.5193 | 0.0073 | 4-years |
| Dialister Major and difference | 0.5084 | 2.8026 | 4.8636 | 10.4218 | 0.0011 | 4-years |
| Eggerthella Difference | 0.1535 | 0.2235 | 0.0381 | 0.0516 | 0.0151 | 4-years |
| Eisenbergiella Difference | 0.0000 | 0.0000 | 0.0294 | 0.0620 | 0.0001 | 4-years |
| Enterococcus Difference | 0.2863 | 0.4723 | 0.0060 | 0.0254 | 0.0051 | 1-year |
| Escherichia/Shigella Major and difference | 11.9353 | 14.2319 | 3.9337 | 10.4966 | 0.0126 | 1-year |
| Esakella Difference | 0.0008 | 0.0038 | 0.0105 | 0.0362 | 0.0284 | 4-years |
| Faecalibacterium Major and difference | 2.4513 | 4.7935 | 10.2261 | 13.3559 | 0.0000 | 4-years |
| Gemmiger Major and difference | 0.7854 | 2.5049 | 4.2201 | 8.4445 | 0.0018 | 4-years |
| Holdemania Difference | 0.0177 | 0.0735 | 0.0756 | 0.1338 | 0.0074 | 4-years |
| Hungatella Difference | 0.0011 | 0.0040 | 0.0010 | 0.0356 | 0.0214 | 4-years |
| Intestinibacter Major and difference | 1.4863 | 1.6740 | 0.1925 | 0.3523 | 0.0009 | 4-years |
| Intestimonas Difference | 0.0000 | 0.0000 | 0.0595 | 0.1416 | 0.0008 | 4-years |
| Klebsiella Major and difference | 1.3742 | 2.7721 | 0.1372 | 0.4604 | 0.0355 | 1-year |
| Lactomurphy Difference | 0.0008 | 0.0037 | 0.0051 | 0.0116 | 0.0050 | 4-years |
| Odoribacter Difference | 0.0000 | 0.0000 | 0.0048 | 0.1476 | 0.0182 | 4-years |
| Oscillobacter Difference | 0.0125 | 0.0326 | 0.8457 | 1.0766 | 0.0000 | 4-years |
| Parabacteroides Difference | 0.2177 | 0.5702 | 0.7776 | 1.0870 | 0.0011 | 4-years |
| Parasutterella Difference | 0.0118 | 0.0562 | 0.2985 | 0.9139 | 0.0085 | 4-years |
| Parvimonas Difference | 0.0011 | 0.0032 | 0.0058 | 0.0148 | 0.0112 | 4-years |
| Phascolarctobacterium Difference | 0.0089 | 0.0474 | 0.7095 | 1.9686 | 0.0023 | 4-years |
| Pseudoflavonifractor Difference | 0.0000 | 0.0000 | 0.0011 | 0.0038 | 0.0111 | 4-years |
| Pyramidobacter Difference | 0.0003 | 0.0017 | 0.0062 | 0.0245 | 0.0454 | 4-years |
| Roseburia Major and difference | 0.5940 | 1.6508 | 4.4477 | 8.4708 | 0.0002 | 4-years |
| Ruminococcus2 Major and difference | 0.3787 | 0.9060 | 1.0312 | 1.6200 | 0.0144 | 4-years |
| Scardovia Difference | 0.0000 | 0.0000 | 0.0040 | 0.0040 | 0.0379 | 4-years |
| Streptococcus Major and difference | 4.8186 | 6.7029 | 0.8335 | 1.3996 | 0.0182 | 1-year |
| Bacteroides Major | 12.9731 | 19.2120 | 19.29113 | 19.1440 | 0.2184 | 4-years |
| Veillonella Major and difference | 5.5698 | 6.5315 | 1.2074 | 3.7846 | 0.0023 | 1-year |

Table 2. Dominant genera and significant difference between 1-year and 4-years old children.
terpenoids and polyketides pathways increased with age. This functional change in microbiota is consistent with children’s diet changes.

Nevertheless, the current study has limitations as this study used fecal samples. As is known, the fecal microbiota does not fully represent the luminal or mucosal communities of the GI tract. Although previous studies have already revealed that fecal microbial community has a good potential to identify most taxa in the chicken gut, non-invasively sampling at different gut locations would be preferred, and recently developed smart sampling capsule would achieve this goal. Another limitation of our study is lacking of paying attention to the factors influencing the development of intestinal microbiota in children, such as breast milk feeding, dietary habits and antibiotic use.

In conclusion, the first 4 years of life is a crucial period for the formation of intestinal microbiota in young children, and has a profound impact on subsequent physical development and health. Our study demonstrates that the intestinal microbiota composition of infants changed from Bifidobacteria-dominated to a more complex microbiota, and attained adult-like intestinal microbiota maturation by age four. It is worthy of note that owing to various influencing factors, there are great differences in the composition and development of intestinal microbiota among different populations; our study is scientifically relevant among existing studies involving other ethnic groups.

Data availability
The microbiota sequence data for the 1- and 4-year-old children have been deposited in the National Omics Data Encyclopedia (NODE, https://www.bioinxio.org/node/index) under the accession numbers OEX010570 and OEX010571, respectively.

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Author contributions

W.Y. and H.Z. designed the project. Material preparation was performed by M.M., F.Y. and Y.C. DNA extraction and sequencing was performed by M.G. and M.D. Bioinformatics analysis was performed by M.G., M.D. and W.Y. The first draft of the manuscript was written by M.G., W.Y. and H.Z., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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