Supplementary Materials

Supplementary Materials and Methods

Animals and diet

The cynomolgus monkeys were housed at the Suzhou Non-human Primate Facility of the Institute of Neuroscience (Jiangsu Province, China). In total, 41 female monkeys were selected and divided into four groups according to their age: group A, eight infant monkeys (1 to 3 months old); group B, 10 young monkeys (2 to 5 years old); group C, 14 adult monkeys (7 to 10 years old); group D, nine middle-aged monkeys (12 to 15 years old). The monkeys were not exposed to antibiotics within 2 months of sample collection. All monkeys were cohoused under a controlled 12-h light/12-h dark schedule, temperature (21–25 °C), and humidity (50%–60%), with continuous access to municipal water. The infant monkeys (1 to 3 months old) were breastfed by their mothers, while the remaining monkeys (young, adult, and middle-age groups) were fed with nutritional granules twice a day (200 g per monkey at 08:00 am and 15:00 pm) and vegetables and fruits (100 g per monkey at 10:00 am) to provide essential nutrients and vitamins. All experimental procedures were performed according to the ethical guidelines of the Institute of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (ION-2019043).

Fecal sample collection

The monkeys were separated in single cages for fecal collection. Fresh fecal samples were collected in sterile tubes, frozen with liquid nitrogen, and stored at −80 °C.

16S rRNA gene sequencing

Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB)/sodium dodecyl sulfate (SDS) method. The 16S rRNA V4 region was amplified with primers 515F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). The sequences were barcoded with the Phusion® High-Fidelity PCR Master Mix (New England Biolabs, USA). Sequencing libraries were constructed using an Ion Plus Fragment Library Kit (Thermo Scientific, USA). The Ion S5TM XL platform was used to sequence the single-end reads.

Data analysis

UPARSE software (Edgar, 2013) was used to analyze the sequencing data. Sequences with more than 97% similarity were assigned to the same operational taxonomic unit (OTU). A representative sequence of each OTU was selected for further annotation according to the SILVA database (Quast et al., 2013). Both α-diversity and β-diversity were determined using QIIME (v1.7.0). Uniform Manifold Approximation and Projection (UMAP) was used to compare differences in gut microbiota among the four monkey groups. Statistically significant biomarkers were identified using linear discriminant analysis (LDA) effect size (LEfSe). The LDA score threshold was set to 3.5 to identify distinguishing taxa. Microbial function was predicted based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database using PICRUSt 2.0 (Phylogenetic Investigation of Communities by Reconstruction of

Unobserved States) (Langille et al., 2013).

Statistical analysis

Statistical significance of α-diversity, β-diversity, and gut microbial changes at the phylum, family, and genus levels was analyzed using one-way analysis of variance (ANOVA). For functional capabilities of microbial communities, statistical significance corresponding to level 1 and level 2 KEGG Orthology between two groups was analyzed using *t*-test.

REFERENCES

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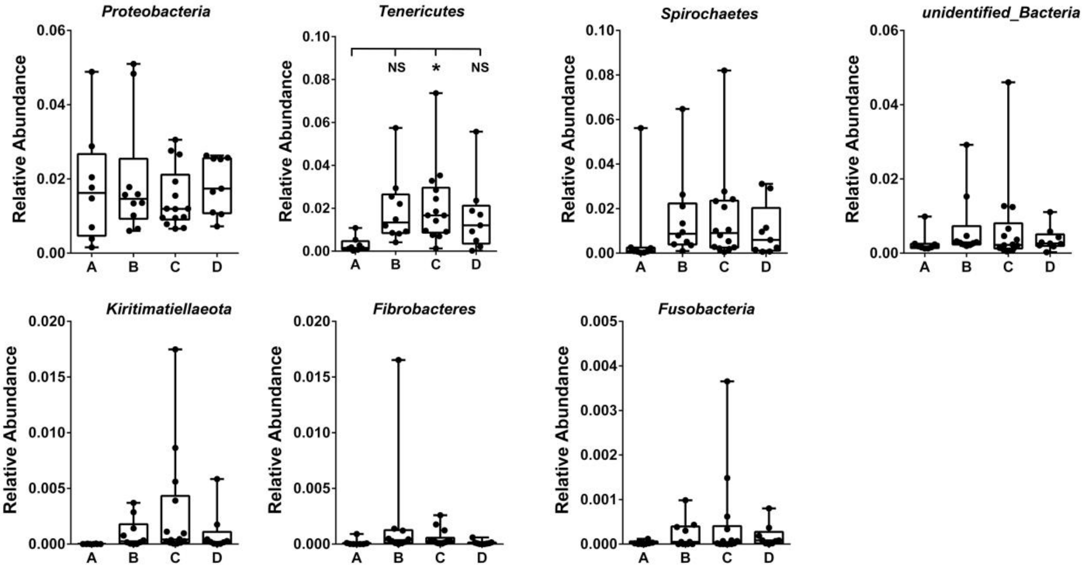
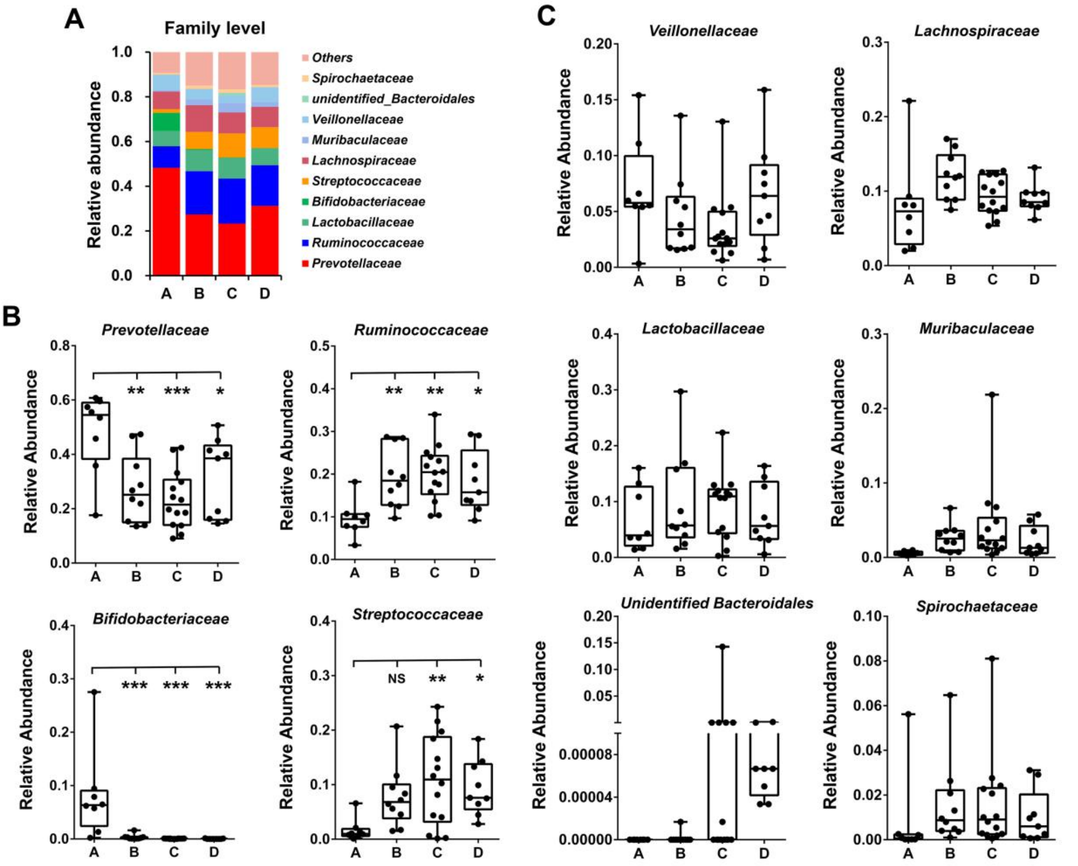
Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Research*, **41**(D1): D590–D596.



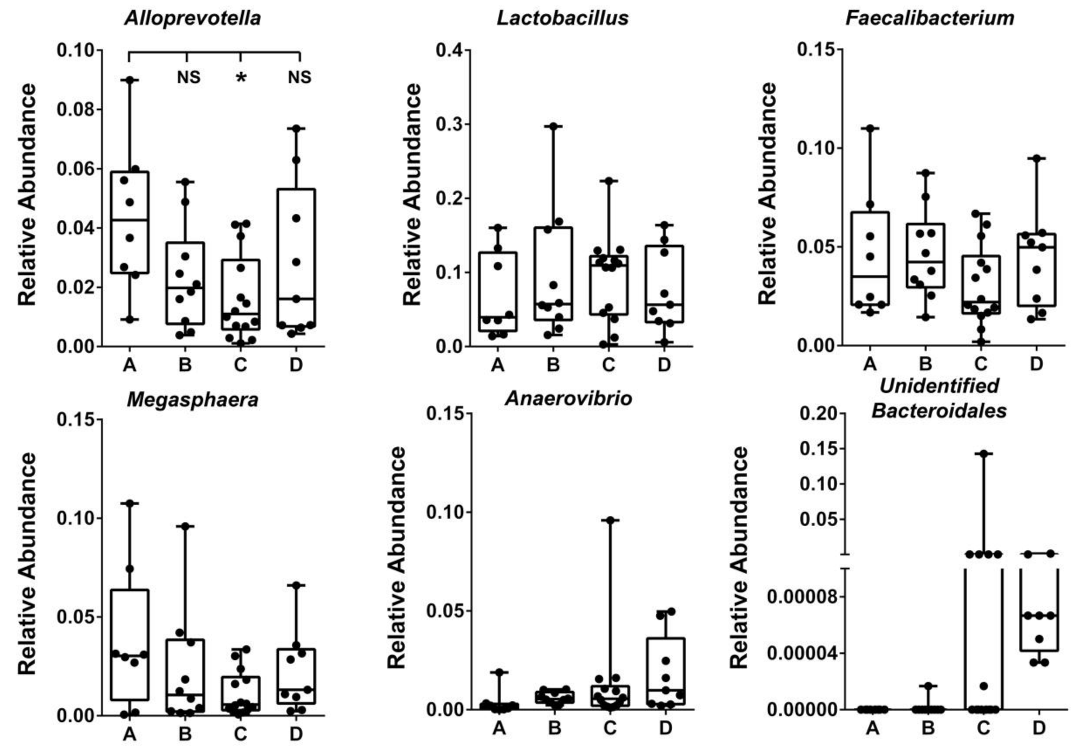
Supplementary Figure S1

Comparison of microbial composition among infant (group A), young (group B), adult (group C), and middle-aged (group D) monkeys at phylum level.



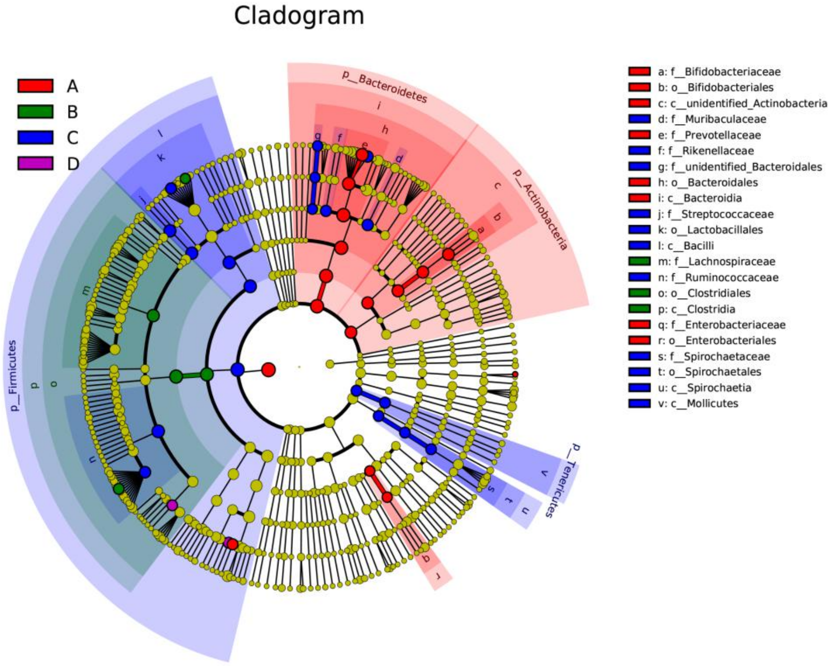
Supplementary Figure S2

Comparison of microbial composition among infant (group A), young (group B), adult (group C), and middle-aged (group D) monkeys at family level. (A) Relative abundance of OTUs assigned at family level. (B) Relative abundances of *Prevotellaceae*, *Ruminococcaceae*, *Bifidobacteriaceae*, and *Streptococcaceae* among four groups. (C) Relative abundances of *Veillonellaceae*, *Lachnospiraceae*, *Lactobacillaceae*, *Muribaculaceae*, unidentified *Bacteroidales*, and *Spirochaetaceae* among four groups.



Supplementary Figure S3

Comparison of microbial composition among infant (group A), young (group B), adult (group C), and middle-aged (group D) monkeys at genus level.



Supplementary Figure S4

Cladogram illustrating distribution of taxa with differential abundances among infant (group A), young (group B), adult (group C), and middle-aged (group D) monkeys.

Supplementary Table S1

Comparison of relative abundances of gut microbiota among infant (group A), young (group B), adult (group C), and middle-aged (group D) monkeys at phylum level.

Group	**Taxonomy**	***Firmicutes***	***Bacteroidetes***	***Actinobacteria***	***Spirochaetes***	***Tenericutes***	***Proteobacteria***	**unidentified_*Bacteria***	***Kiritimatiellaeota***	***Fibrobacteres***	***Fusobacteria***	**Others**
A	A.1	0.321	0.578	0.015	0.056	0.001	0.015	0.010	0.000	0.001	0.000	0.004
	A.2	0.539	0.369	0.062	0.003	0.003	0.020	0.003	0.000	0.000	0.000	0.001
	A.3	0.378	0.478	0.082	0.001	0.011	0.049	0.002	0.000	0.000	0.000	0.000
	A.4	0.365	0.622	0.003	0.001	0.005	0.002	0.002	0.000	0.000	0.000	0.000
	A.5	0.335	0.563	0.068	0.002	0.001	0.029	0.001	0.000	0.000	0.000	0.000
	A.6	0.326	0.585	0.067	0.001	0.001	0.018	0.001	0.000	0.000	0.000	0.001
	A.7	0.510	0.191	0.290	0.000	0.002	0.004	0.002	0.000	0.000	0.000	0.001
	A.8	0.282	0.610	0.099	0.000	0.000	0.007	0.002	0.000	0.000	0.000	0.000
B	B.1	0.600	0.271	0.002	0.065	0.029	0.006	0.005	0.000	0.017	0.000	0.006
	B.2	0.433	0.499	0.002	0.005	0.004	0.051	0.003	0.000	0.000	0.001	0.002
	B.3	0.656	0.313	0.004	0.004	0.012	0.007	0.003	0.000	0.000	0.000	0.001
	B.4	0.764	0.188	0.005	0.008	0.015	0.010	0.002	0.001	0.000	0.000	0.006
	B.5	0.635	0.335	0.003	0.001	0.009	0.014	0.002	0.000	0.000	0.000	0.001
	B.6	0.610	0.316	0.006	0.013	0.025	0.016	0.002	0.001	0.001	0.000	0.008
	B.7	0.573	0.338	0.002	0.009	0.022	0.018	0.029	0.003	0.000	0.000	0.005
	B.8	0.557	0.394	0.018	0.004	0.009	0.013	0.003	0.000	0.000	0.000	0.002
	B.9	0.390	0.504	0.004	0.026	0.008	0.048	0.015	0.000	0.001	0.000	0.002
	B.10	0.661	0.209	0.003	0.021	0.057	0.016	0.003	0.004	0.000	0.000	0.025
C	C.1	0.548	0.382	0.003	0.024	0.028	0.007	0.004	0.000	0.000	0.000	0.003
	C.2	0.582	0.378	0.003	0.002	0.017	0.016	0.001	0.000	0.000	0.001	0.001
	C.3	0.476	0.476	0.003	0.003	0.008	0.031	0.001	0.000	0.000	0.001	0.002

	C.4	0.607	0.334	0.002	0.021	0.018	0.010	0.001	0.000	0.002	0.000	0.005
	C.5	0.632	0.241	0.003	0.028	0.033	0.010	0.002	0.017	0.001	0.000	0.032
	C.6	0.646	0.272	0.003	0.001	0.001	0.028	0.046	0.000	0.000	0.000	0.002
	C.7	0.792	0.165	0.005	0.002	0.024	0.007	0.001	0.000	0.000	0.000	0.003
	C.8	0.542	0.407	0.003	0.010	0.017	0.012	0.007	0.000	0.000	0.000	0.002
	C.9	0.529	0.437	0.002	0.010	0.009	0.008	0.001	0.000	0.000	0.000	0.004
	C.10	0.662	0.299	0.001	0.003	0.007	0.019	0.005	0.001	0.000	0.000	0.003
	C.11	0.703	0.152	0.002	0.023	0.074	0.012	0.013	0.004	0.000	0.000	0.018
	C.12	0.527	0.305	0.001	0.082	0.035	0.009	0.012	0.009	0.003	0.000	0.017
	C.13	0.696	0.260	0.003	0.005	0.014	0.012	0.002	0.001	0.000	0.004	0.004
	C.14	0.443	0.498	0.001	0.008	0.010	0.027	0.002	0.006	0.000	0.000	0.006
D	D.1	0.695	0.244	0.002	0.003	0.024	0.026	0.003	0.000	0.000	0.000	0.003
	D.2	0.644	0.244	0.002	0.011	0.056	0.025	0.004	0.002	0.000	0.000	0.012
	D.3	0.468	0.517	0.002	0.001	0.002	0.007	0.003	0.000	0.000	0.000	0.000
	D.4	0.659	0.289	0.001	0.006	0.012	0.017	0.003	0.006	0.000	0.000	0.007
	D.5	0.596	0.319	0.002	0.031	0.019	0.026	0.002	0.000	0.001	0.000	0.004
	D.6	0.525	0.407	0.000	0.029	0.017	0.017	0.002	0.000	0.000	0.000	0.003
	D.7	0.522	0.432	0.001	0.010	0.009	0.011	0.011	0.000	0.000	0.001	0.002
	D.8	0.488	0.477	0.003	0.001	0.000	0.025	0.006	0.000	0.000	0.000	0.000
	D.9	0.550	0.430	0.002	0.002	0.005	0.011	0.000	0.000	0.000	0.000	0.001

Supplementary Table S2

Comparison of relative abundances of gut microbiota among infant (group A), young (group B), adult (group C), and middle-aged (group D) monkeys at family level.

Group	**Taxonomy**	***Prevotellaceae***	***Ruminococcaceae***	***Lactobacillaceae***	***Bifidobacteriaceae***	***Streptococcaceae***	***Lachnospiraceae***	***Muribaculaceae***	***Veillonellaceae***	***unidentified_Bacteroidales***	***Spirochaetaceae***	**Others**
A	A.1	0.556	0.182	0.014	0.013	0.001	0.020	0.010	0.054	0.000	0.056	0.094
	A.2	0.359	0.079	0.109	0.057	0.005	0.221	0.004	0.056	0.000	0.003	0.109
	A.3	0.458	0.107	0.036	0.079	0.010	0.081	0.006	0.060	0.000	0.000	0.163
	A.4	0.608	0.089	0.133	0.002	0.066	0.045	0.009	0.003	0.000	0.001	0.044
	A.5	0.535	0.100	0.043	0.065	0.021	0.082	0.005	0.055	0.000	0.002	0.093
	A.6	0.574	0.104	0.016	0.062	0.013	0.065	0.004	0.066	0.000	0.000	0.095
	A.7	0.176	0.076	0.160	0.275	0.008	0.093	0.003	0.111	0.000	0.000	0.097
	A.8	0.596	0.033	0.035	0.094	0.009	0.023	0.003	0.154	0.000	0.000	0.051
B	B.1	0.153	0.194	0.169	0.001	0.045	0.124	0.029	0.017	0.000	0.065	0.204
	B.2	0.468	0.125	0.059	0.001	0.056	0.118	0.007	0.054	0.000	0.005	0.108
	B.3	0.219	0.175	0.056	0.002	0.207	0.144	0.037	0.030	0.000	0.004	0.126
	B.4	0.139	0.161	0.297	0.004	0.065	0.170	0.021	0.038	0.000	0.008	0.097
	B.5	0.235	0.287	0.158	0.001	0.017	0.089	0.066	0.017	0.000	0.001	0.129
	B.6	0.267	0.204	0.053	0.004	0.116	0.120	0.020	0.074	0.000	0.013	0.127
	B.7	0.287	0.282	0.024	0.001	0.015	0.161	0.032	0.018	0.000	0.009	0.171
	B.8	0.357	0.129	0.083	0.016	0.084	0.107	0.010	0.136	0.000	0.004	0.075
	B.9	0.474	0.096	0.040	0.003	0.095	0.075	0.007	0.060	0.000	0.026	0.124
	B.10	0.135	0.285	0.016	0.001	0.072	0.088	0.036	0.016	0.000	0.021	0.331
C	C.1	0.275	0.231	0.107	0.001	0.006	0.105	0.049	0.023	0.000	0.024	0.180
	C.2	0.332	0.135	0.223	0.001	0.082	0.085	0.026	0.021	0.000	0.002	0.093
	C.3	0.424	0.102	0.131	0.001	0.044	0.127	0.015	0.052	0.000	0.003	0.101

	C.4	0.233	0.159	0.112	0.001	0.147	0.073	0.027	0.054	0.000	0.021	0.174
	C.5	0.141	0.340	0.113	0.001	0.002	0.080	0.020	0.021	0.000	0.028	0.254
	C.6	0.243	0.104	0.107	0.001	0.198	0.059	0.012	0.130	0.000	0.001	0.147
	C.7	0.138	0.219	0.119	0.001	0.216	0.125	0.008	0.049	0.000	0.002	0.121
	C.8	0.299	0.251	0.116	0.001	0.001	0.100	0.073	0.014	0.000	0.010	0.135
	C.9	0.417	0.176	0.037	0.001	0.116	0.123	0.004	0.028	0.000	0.010	0.088
	C.10	0.184	0.206	0.129	0.000	0.184	0.078	0.068	0.026	0.000	0.003	0.122
	C.11	0.104	0.268	0.012	0.000	0.131	0.122	0.013	0.013	0.000	0.023	0.314
	C.12	0.090	0.204	0.003	0.000	0.103	0.074	0.018	0.026	0.143	0.081	0.259
	C.13	0.186	0.180	0.053	0.000	0.243	0.111	0.038	0.031	0.000	0.005	0.153
	C.14	0.197	0.240	0.045	0.000	0.040	0.053	0.219	0.006	0.000	0.008	0.190
D	D.1	0.145	0.293	0.164	0.000	0.028	0.132	0.058	0.007	0.000	0.003	0.171
	D.2	0.190	0.291	0.006	0.000	0.076	0.098	0.016	0.041	0.000	0.011	0.271
	D.3	0.507	0.139	0.056	0.001	0.044	0.081	0.006	0.075	0.000	0.001	0.089
	D.4	0.163	0.187	0.144	0.000	0.133	0.084	0.050	0.017	0.000	0.006	0.216
	D.5	0.155	0.157	0.127	0.000	0.143	0.085	0.035	0.046	0.000	0.031	0.220
	D.6	0.385	0.221	0.035	0.000	0.065	0.079	0.005	0.085	0.000	0.029	0.097
	D.7	0.401	0.137	0.071	0.000	0.075	0.098	0.013	0.099	0.001	0.010	0.096
	D.8	0.451	0.091	0.031	0.001	0.099	0.062	0.011	0.159	0.000	0.001	0.094
	D.9	0.415	0.118	0.048	0.000	0.184	0.097	0.005	0.064	0.000	0.002	0.068

Supplementary Table S3

Comparison of relative abundances of gut microbiota among infant (group A), young (group B), adult (group C), and middle-aged (group D) monkeys at genus level.

Group	**Taxonomy**	**unidentified_*Prevotellaceae***	***Lactobacillus***	***Bifidobacterium***	***Streptococcus***	**unidentified_*Bacteroidales***	***Faecalibacterium***	***Megasphaera***	***Anaerovibrio***	***Alloprevotella***	**unidentified_*Ruminococcaceae***	**Others**
A	A.1	0.347	0.014	0.013	0.001	0.000	0.110	0.031	0.000	0.009	0.017	0.458
	A.2	0.134	0.109	0.057	0.005	0.000	0.017	0.002	0.019	0.060	0.008	0.591
	A.3	0.247	0.036	0.079	0.010	0.000	0.045	0.030	0.002	0.056	0.007	0.487
	A.4	0.229	0.133	0.002	0.066	0.000	0.055	0.001	0.002	0.049	0.013	0.450
	A.5	0.351	0.043	0.065	0.021	0.000	0.025	0.027	0.001	0.027	0.022	0.420
	A.6	0.251	0.016	0.062	0.013	0.000	0.072	0.031	0.000	0.090	0.003	0.461
	A.7	0.079	0.160	0.275	0.008	0.000	0.021	0.074	0.003	0.037	0.007	0.336
	A.8	0.329	0.035	0.094	0.009	0.000	0.021	0.107	0.000	0.024	0.002	0.378
B	B.1	0.090	0.169	0.001	0.045	0.000	0.025	0.004	0.003	0.005	0.051	0.608
	B.2	0.196	0.059	0.001	0.056	0.000	0.057	0.018	0.010	0.056	0.016	0.531
	B.3	0.112	0.056	0.002	0.207	0.000	0.034	0.009	0.009	0.025	0.037	0.511
	B.4	0.072	0.297	0.004	0.065	0.000	0.031	0.012	0.005	0.009	0.032	0.474
	B.5	0.121	0.158	0.001	0.017	0.000	0.075	0.001	0.004	0.016	0.077	0.529
	B.6	0.101	0.053	0.004	0.116	0.000	0.057	0.037	0.010	0.021	0.024	0.578
	B.7	0.122	0.024	0.001	0.015	0.000	0.087	0.001	0.002	0.019	0.036	0.692
	B.8	0.174	0.083	0.016	0.084	0.000	0.047	0.096	0.007	0.030	0.027	0.437
	B.9	0.238	0.040	0.003	0.095	0.000	0.038	0.042	0.005	0.049	0.012	0.478
	B.10	0.093	0.016	0.001	0.072	0.000	0.014	0.002	0.006	0.004	0.054	0.739
C	C.1	0.136	0.107	0.001	0.006	0.000	0.039	0.003	0.005	0.012	0.060	0.631
	C.2	0.175	0.223	0.001	0.082	0.000	0.055	0.006	0.007	0.027	0.018	0.406
	C.3	0.181	0.131	0.001	0.044	0.000	0.019	0.024	0.016	0.041	0.021	0.522

	C.4	0.118	0.112	0.001	0.147	0.000	0.021	0.034	0.016	0.010	0.028	0.512
	C.5	0.093	0.113	0.001	0.002	0.000	0.015	0.005	0.002	0.002	0.089	0.677
	C.6	0.078	0.107	0.001	0.198	0.000	0.042	0.007	0.096	0.037	0.008	0.427
	C.7	0.074	0.119	0.001	0.216	0.000	0.017	0.030	0.001	0.007	0.042	0.492
	C.8	0.151	0.116	0.001	0.001	0.000	0.061	0.002	0.009	0.015	0.060	0.584
	C.9	0.187	0.037	0.001	0.116	0.000	0.067	0.002	0.011	0.041	0.018	0.521
	C.10	0.084	0.129	0.000	0.184	0.000	0.034	0.016	0.004	0.017	0.043	0.488
	C.11	0.040	0.012	0.000	0.131	0.000	0.024	0.001	0.006	0.007	0.039	0.741
	C.12	0.063	0.003	0.000	0.103	0.143	0.008	0.004	0.002	0.001	0.047	0.626
	C.13	0.098	0.053	0.000	0.243	0.000	0.018	0.018	0.001	0.008	0.040	0.519
	C.14	0.159	0.045	0.000	0.040	0.000	0.002	0.004	0.002	0.003	0.046	0.699
D	D.1	0.075	0.164	0.000	0.028	0.000	0.017	0.002	0.002	0.007	0.062	0.643
	D.2	0.117	0.006	0.000	0.076	0.000	0.038	0.003	0.010	0.004	0.050	0.695
	D.3	0.260	0.056	0.001	0.044	0.000	0.095	0.036	0.007	0.063	0.009	0.428
	D.4	0.097	0.144	0.000	0.133	0.000	0.013	0.009	0.003	0.007	0.038	0.555
	D.5	0.077	0.127	0.000	0.143	0.000	0.024	0.013	0.016	0.006	0.029	0.565
	D.6	0.184	0.035	0.000	0.065	0.000	0.050	0.028	0.025	0.016	0.033	0.564
	D.7	0.197	0.071	0.000	0.075	0.001	0.057	0.011	0.048	0.043	0.016	0.481
	D.8	0.241	0.031	0.001	0.099	0.000	0.056	0.066	0.050	0.029	0.013	0.414
	D.9	0.191	0.048	0.000	0.184	0.000	0.052	0.032	0.003	0.074	0.018	0.400