

SUPPLEMENTAL FIGURES AND TABLES FOR:

Temporal Variability is an Inherent Feature of the Healthy Canine Microbiome Assessed by Full-Length 16S rRNA Gene Sequencing

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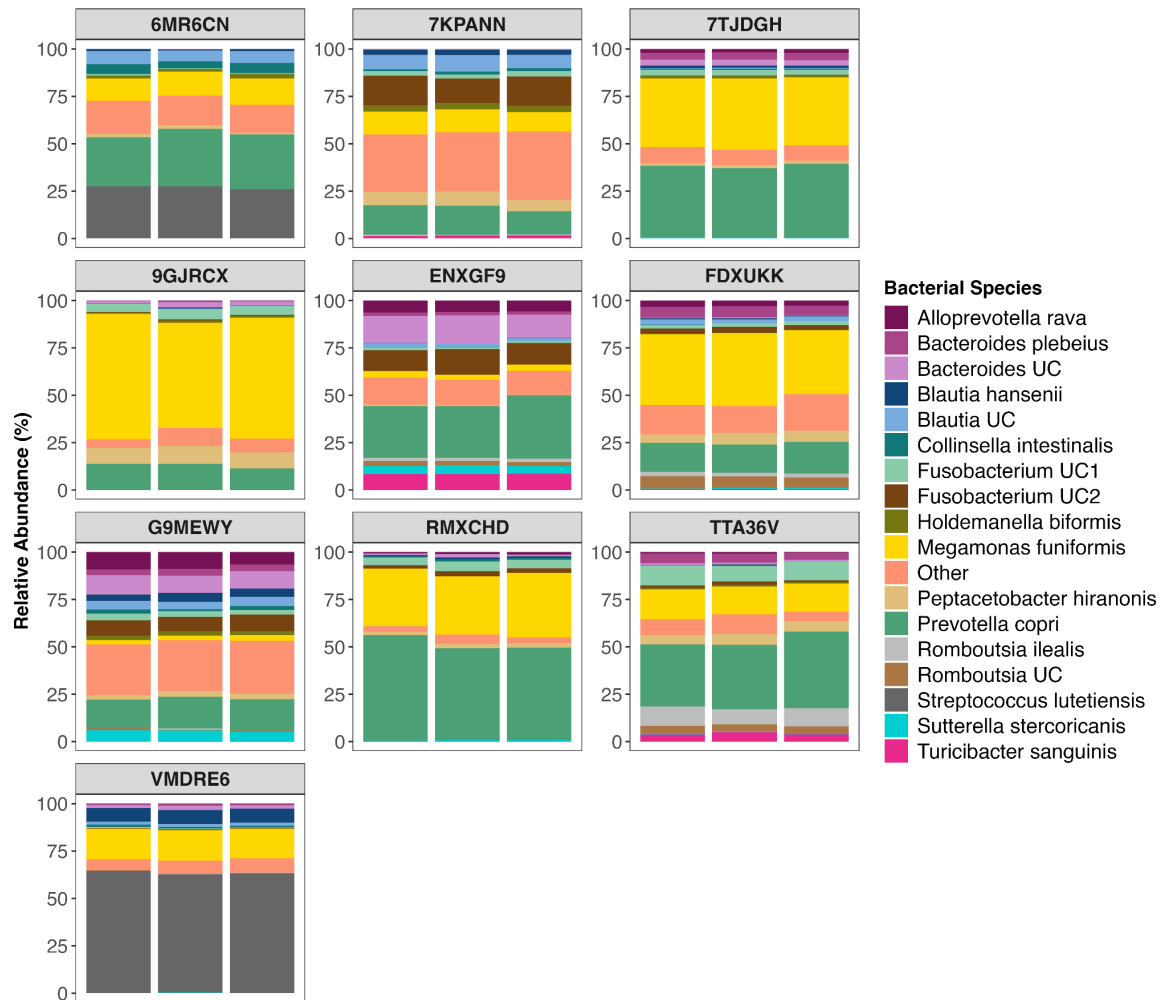
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Figure S1. DNA Extractions automated with the QIAGEN QIAcube HT instrument show high reproducibility. Ten fecal samples, each from a different donor, were randomly selected and extracted three separate times, followed by PCR amplification and sequencing on the PacBio Sequel IIe. **A)** Microbiome composition for the 10 samples across the three extraction replicates. **B)** PCoA ordination based on Aitchison distances, with replicates color-coded by Sample ID.

A)



B)

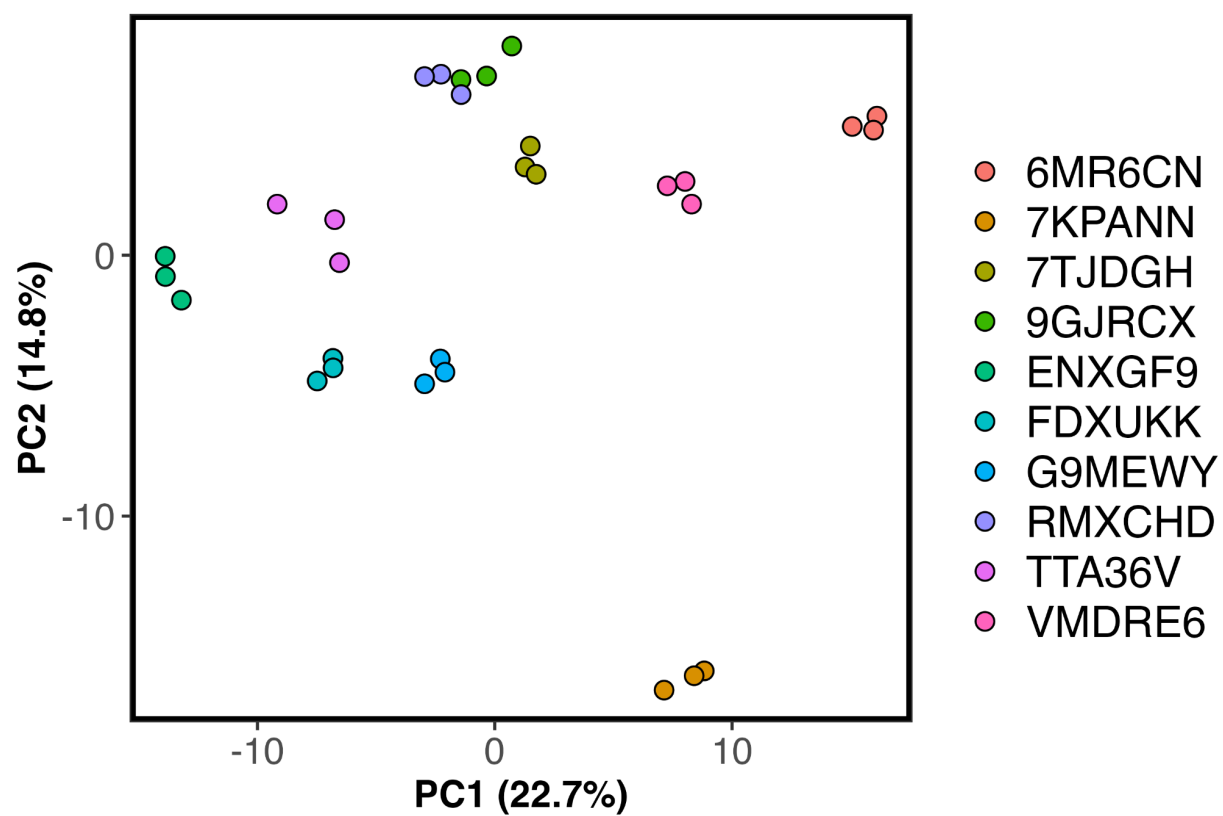


Figure S2. PacBio Sequel IIe sequencing shows low technical variability across sequencing runs. A) Composition of the Zymo Microbial Community DNA Standard across sequencing runs. These runs were run consecutively. **B)** Boxplot of microbiome dissimilarity (Aitchison distance) comparing samples sequenced in different runs versus within the same run (no difference, $p>0.05$).

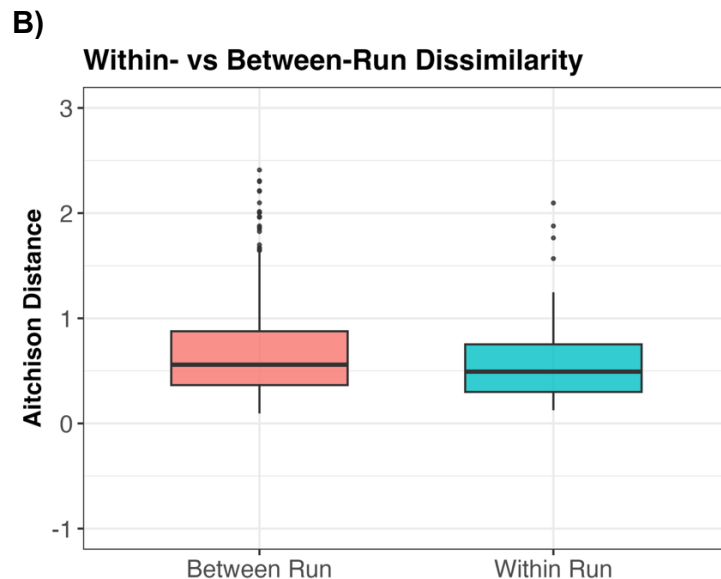
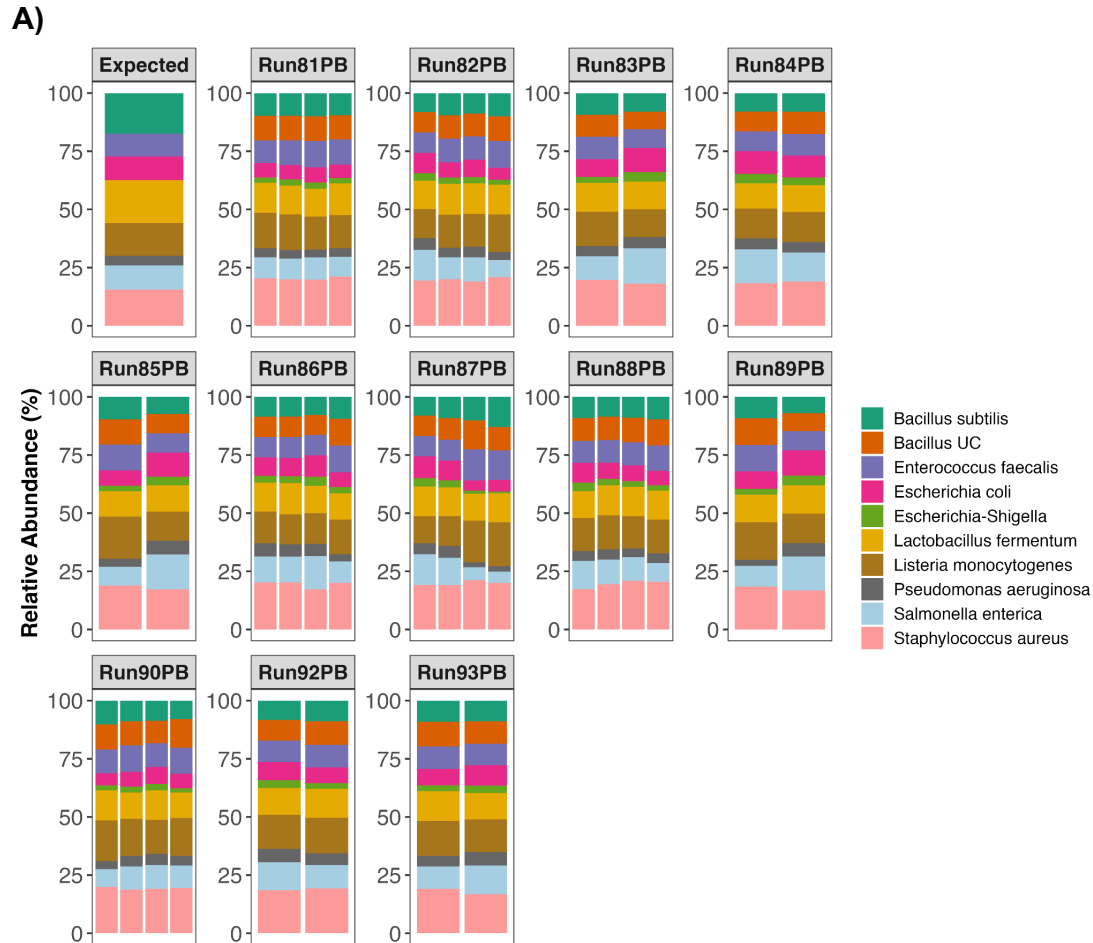
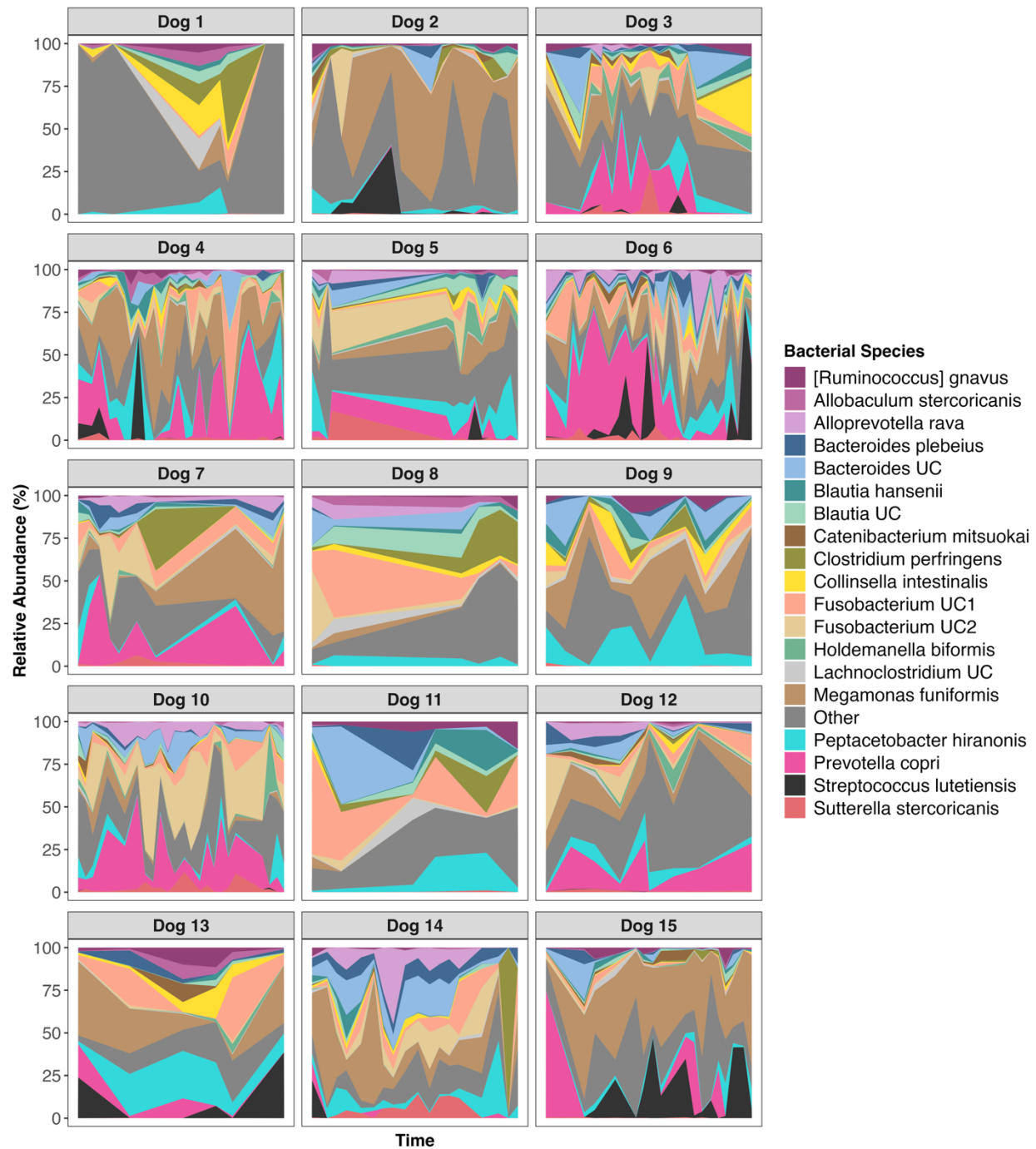
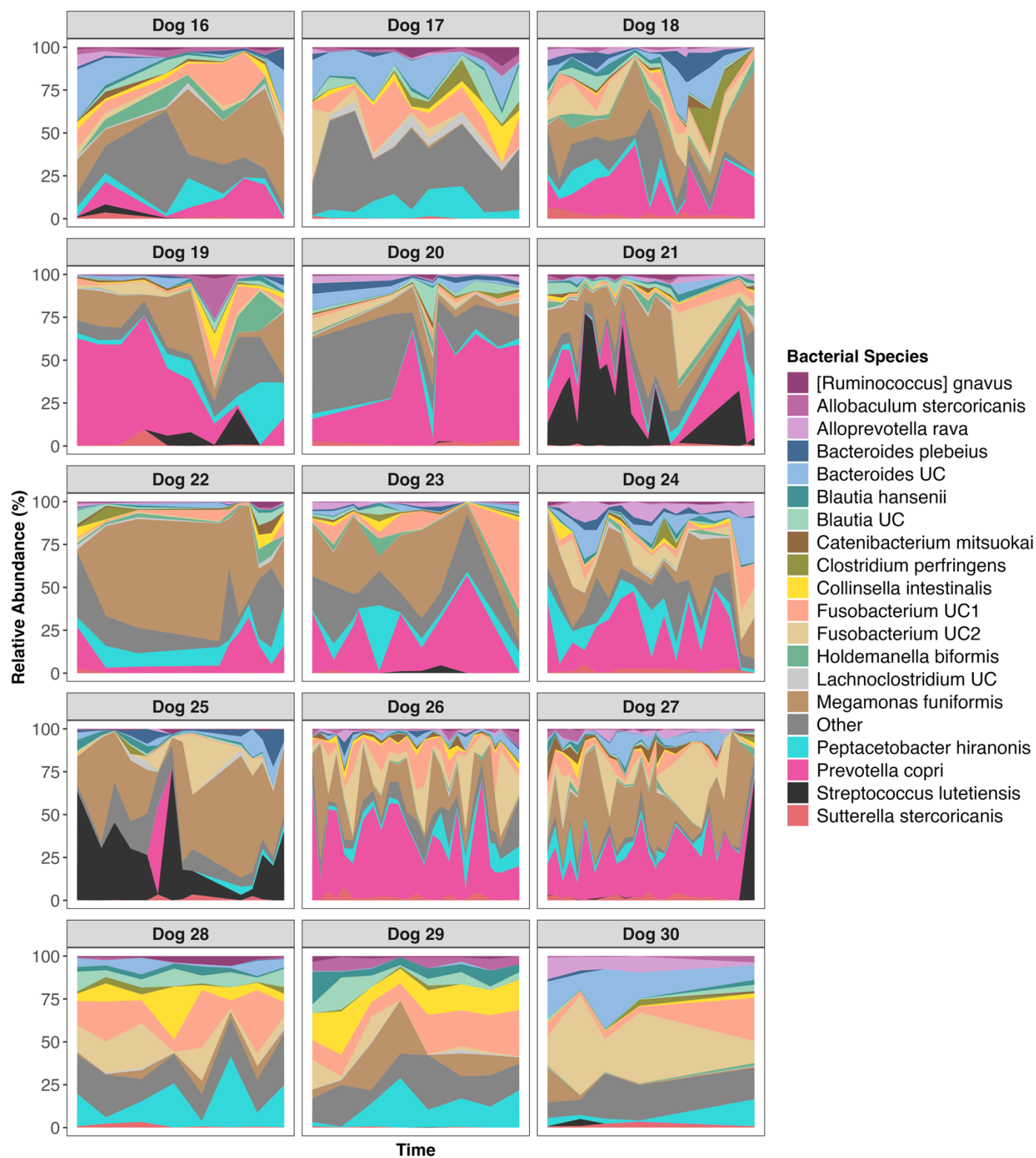
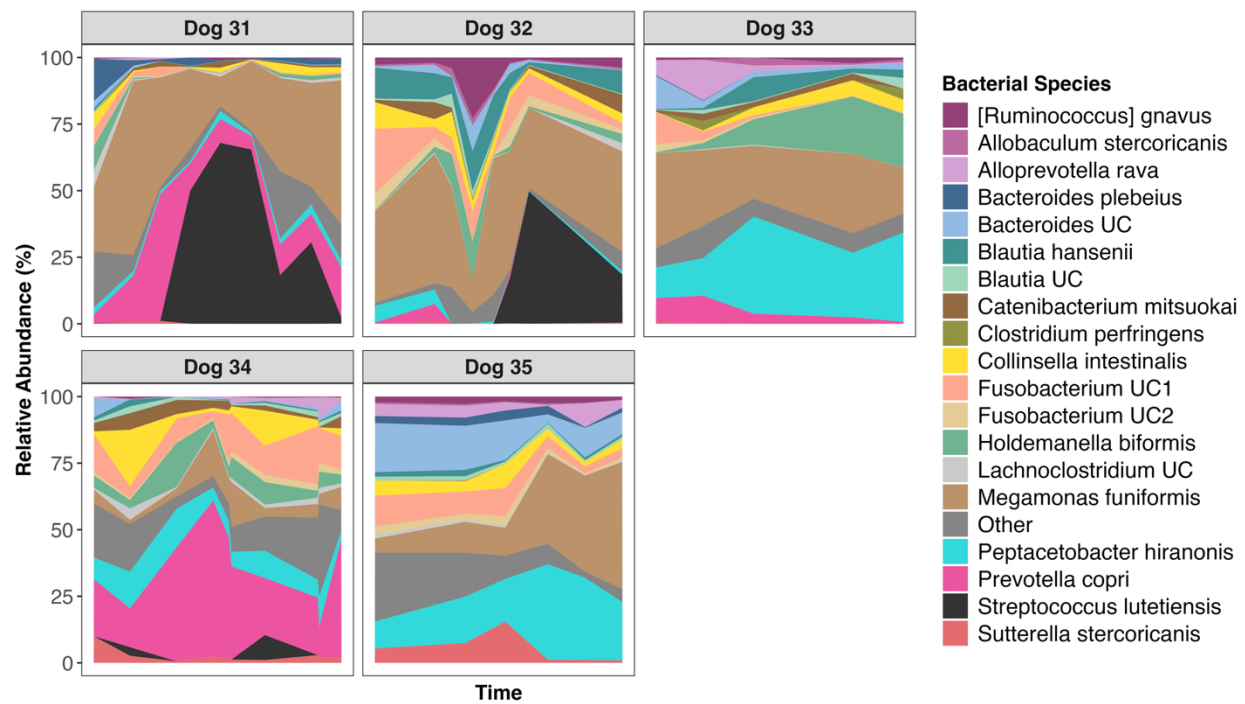


Figure S3. Fecal microbiome composition (species-level) for 35 healthy dogs sampled longitudinally over 1-2 years. Samples are grouped by Dog ID and ordered by date of collection. Dates span 1–2 years for each dog. Bacterial taxa with relative abundances >1% are displayed, while all others are collapsed into 'Other'. UC – unclassified. **(B)** Mean relative abundances of the 42 most abundant bacterial taxa across all fecal samples in the dataset.

A)



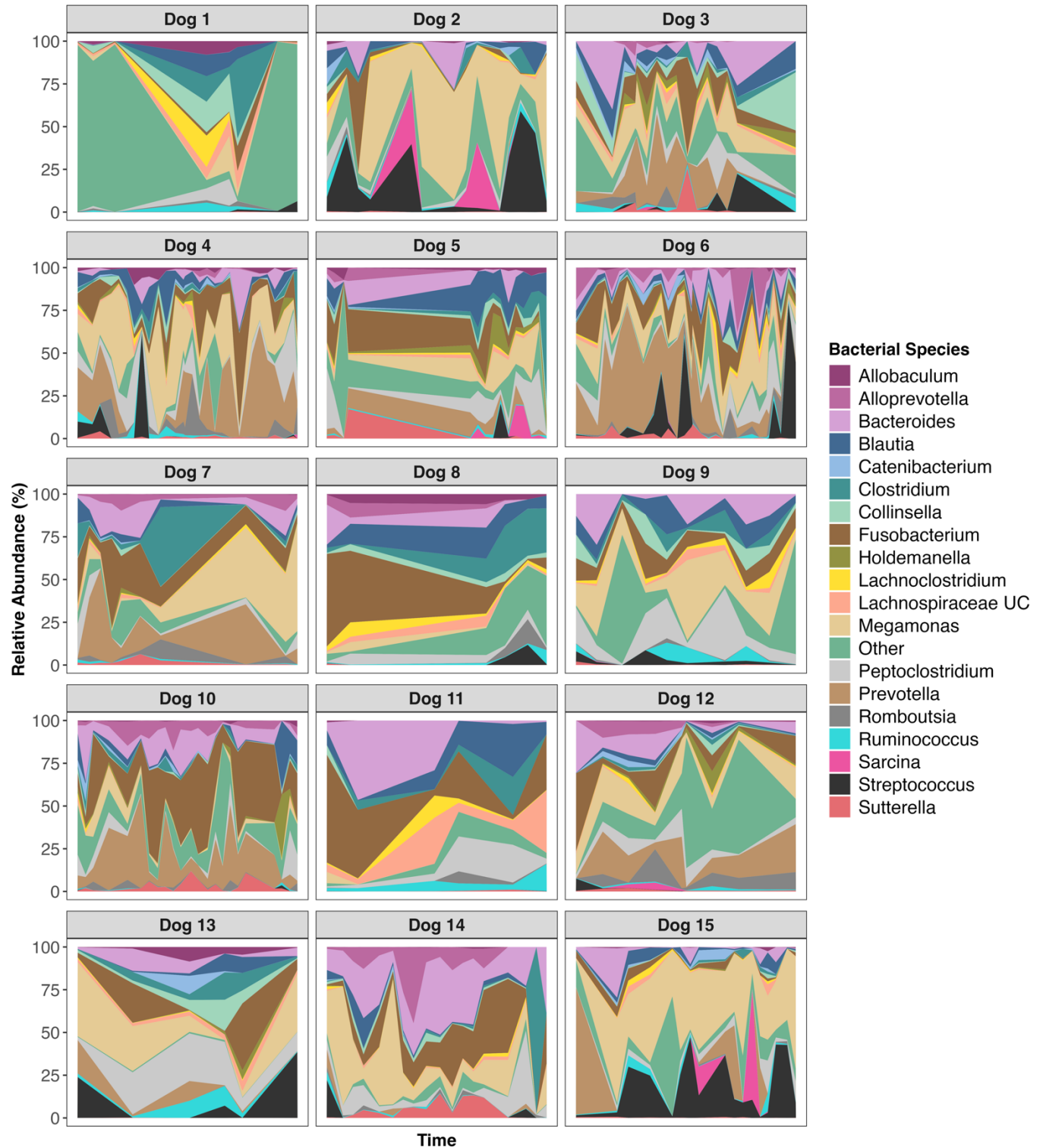


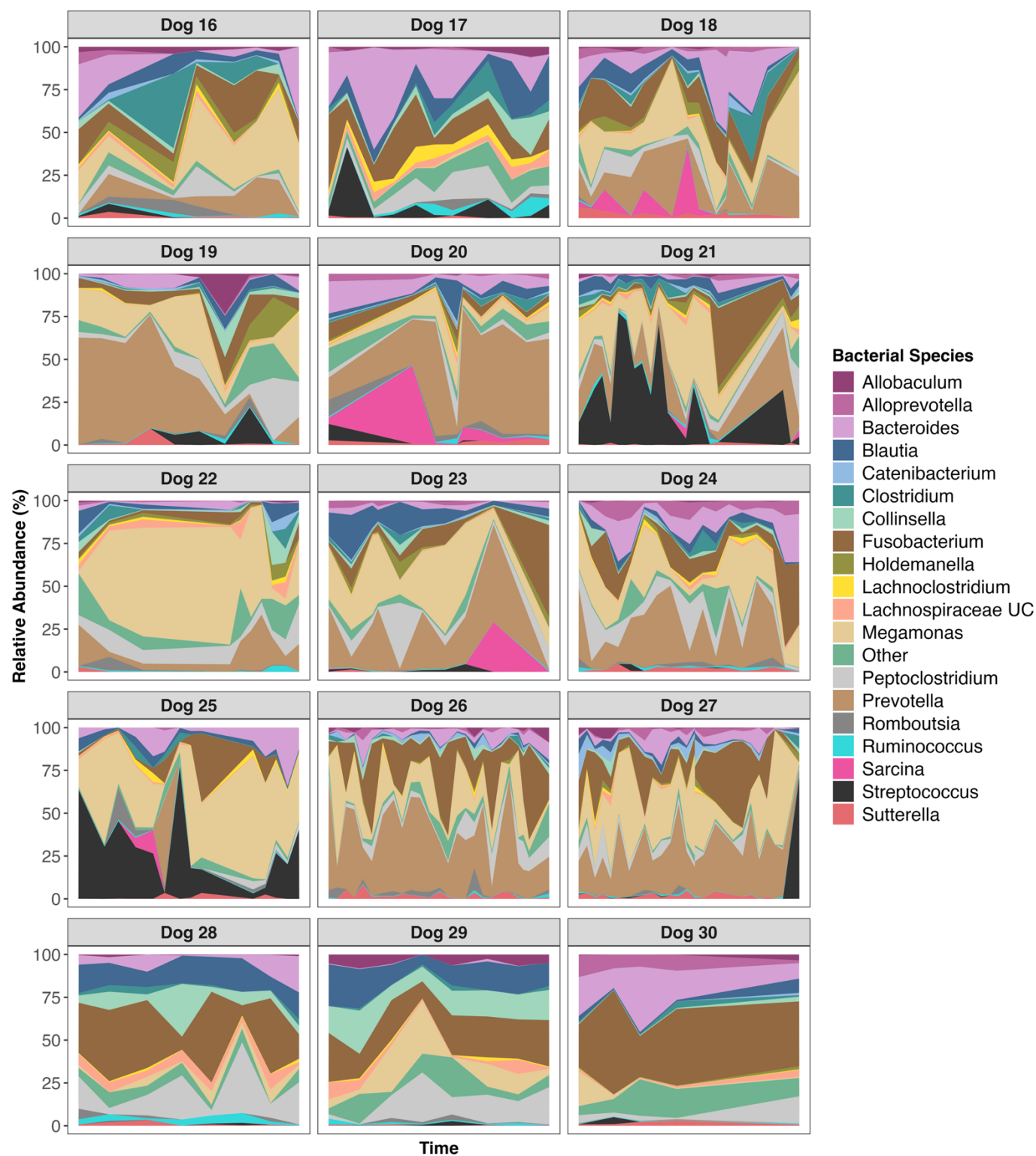


B)

Species	Avg.Abun	Species	Avg.Abun	Species	Avg.Abun
Megamonas funiformis	18.829	Clostridium perfringens	1.348	Clostridium sporogenes	0.491
Prevotella copri	14.849	[Ruminococcus] gnavus	1.181	Blautia glucerasea	0.420
Peptacetobacter hiranonis	7.190	Lachnoclostridium	1.019	Turicibacter sanguinis	0.393
Fusobacterium UC2	6.417	Catenibacterium mitsuokai	0.979	Enterococcus faecium	0.390
Fusobacterium UC1	6.231	Allobaculum stercoricanis	0.964	Parasutterella secunda	0.349
Streptococcus lutetiensis	4.918	Faecalimonas umbilicata	0.929	Blautia wexlerae	0.337
Bacteroides UC	4.340	Bacteroides coprocola	0.884	Faecalibacterium prausnitzii	0.335
Collinsella intestinalis	2.332	Sarcina ventriculi	0.746	Fusobacterium mortiferum	0.330
Alloprevotella rava	2.138	Lachnospiraceae	0.725	Succinivibrio dextrinosolvens	0.328
Blautia UC	2.126	Escherichia coli	0.552	Clostridium UC	0.326
Holdemanella bififormis	1.719	Blautia marasmi	0.535	[Clostridium] dakarensis	0.324
Bacteroides plebeius	1.700	Romboutsia UC	0.523	Lactobacillus reuteri	0.323
Blautia hansenii	1.592	Streptococcus gallolyticus	0.517	Acidaminococcaceae	0.322
Sutterella stercoricanis	1.414	Romboutsia ilealis	0.495	[Eubacterium] tenue	0.313

Figure S4. Fecal microbiome composition (genus-level) for 35 healthy dogs sampled longitudinally over 1-2 years. Samples are grouped by Dog ID and ordered by date of collection. Dates span 1–2 years for each dog. Bacterial taxa with relative abundances >1% are shown and all others are collapsed into 'Other'.





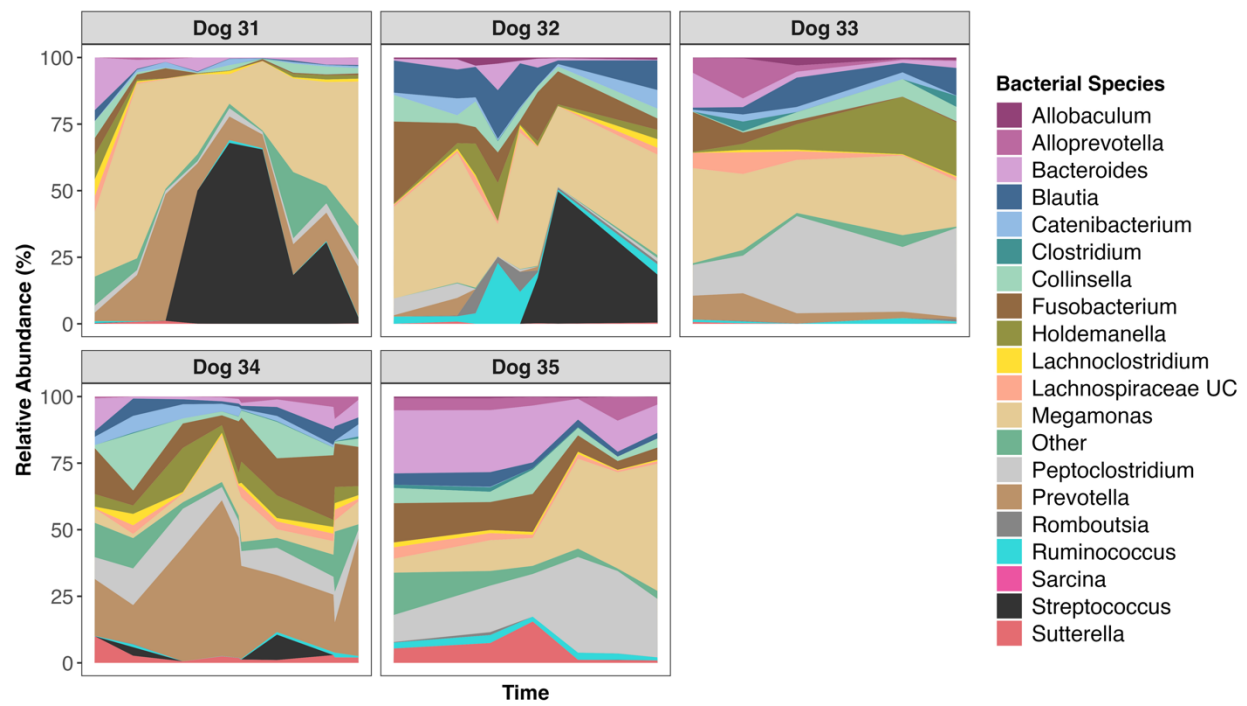
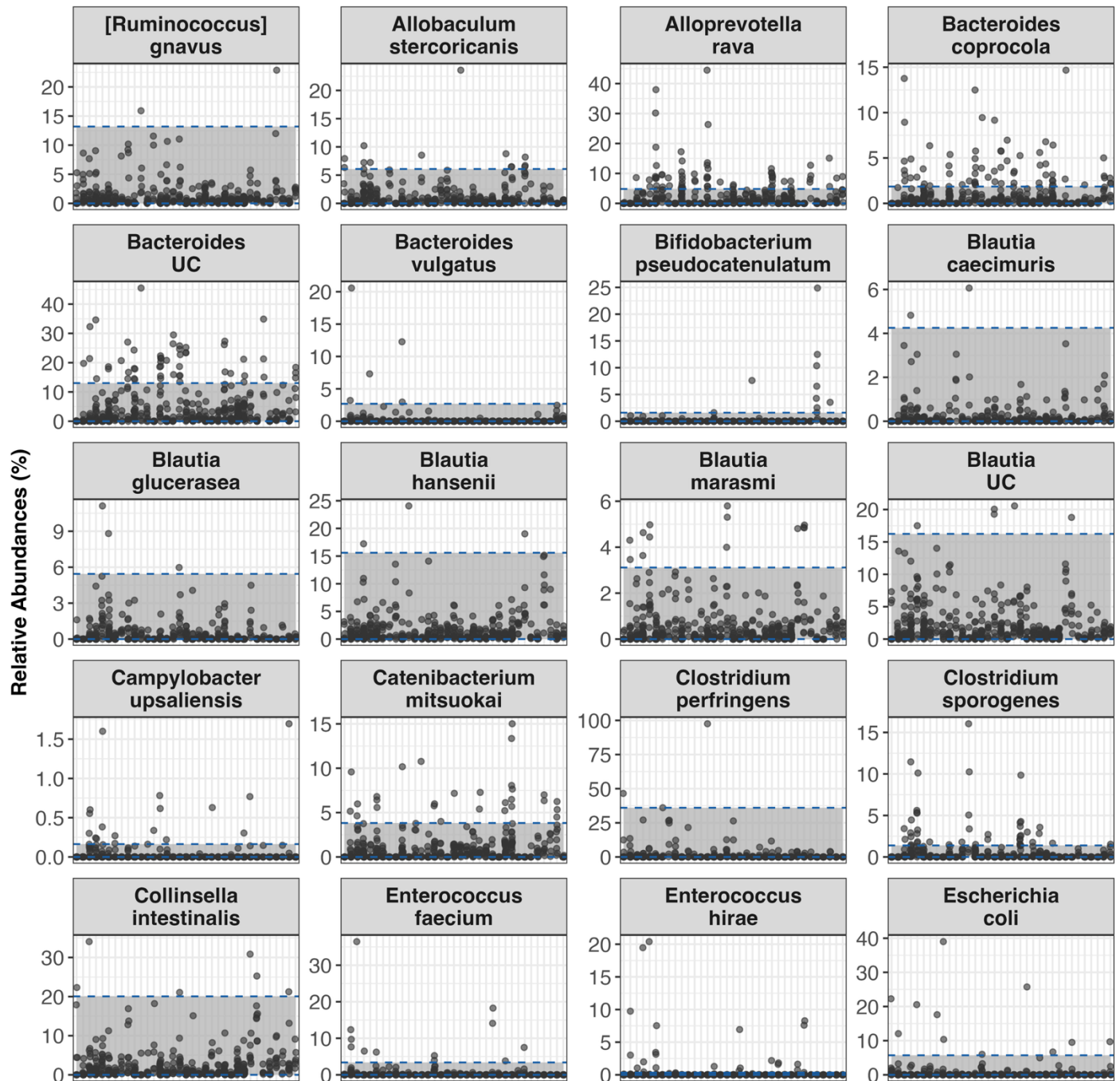


Figure S5. Bacterial relative abundances compared with population reference ranges.

Points represent the relative abundance of each taxon in study samples, ordered by dog. The shaded band shows the 95% reference interval derived from 286 healthy dogs (Rojas et al. [1]). Samples falling within the shaded region lie within the expected range for a healthy population.



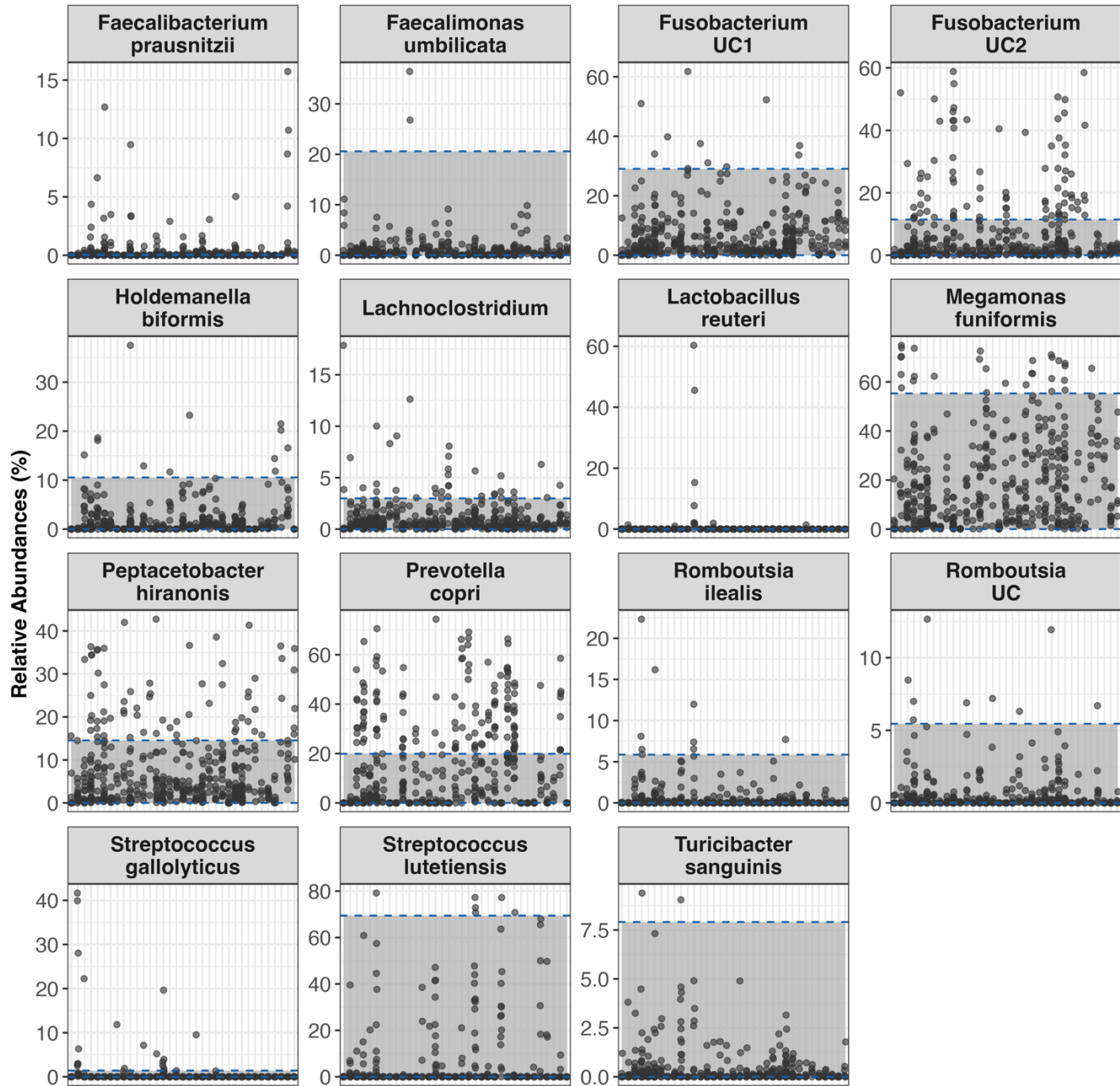


Figure S6. Month-to-month variation in the relative abundance of prominent bacterial species in the canine microbiome. LOESS smoothing curves were used to visualize temporal fluctuations in bacterial taxon abundances by fitting a locally weighted regression directly to the raw abundance values. This summarizes the overall temporal trend while smoothing short-term fluctuations. Shaded areas indicate the 95% confidence interval. Year 2022 was selected as it had the most samples, which was sufficient to illustrate month-to-month variation. Only taxa with a mean relative abundance greater than 1% were included in the analysis.

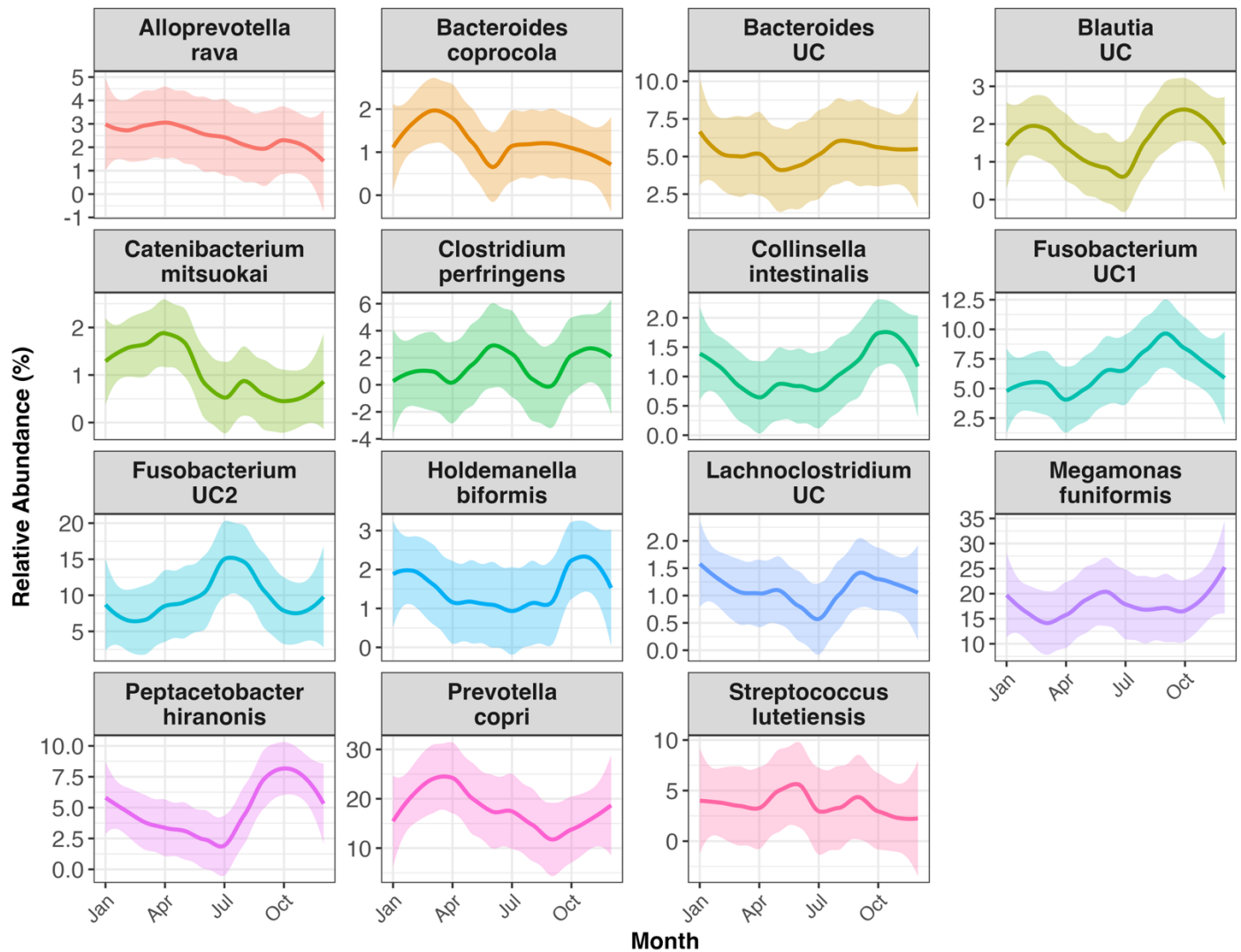


Table S1. Do microbiome temporal changes follow a stochastic or time-structured pattern? For each dog, microbiome similarity (Bray-Curtis and Aitchison distances) was correlated with the temporal proximity of samples (pairwise time differences) using Spearman-based Mantel tests with 999 permutations. P-values were adjusted using FDR. R-values indicate the strength of the correlation. P-values < 0.05 suggest a time-structured (distance decay) pattern, while p-values > 0.05 indicate more stochastic fluctuations.

	A	B	C	D	E	F	G
1	Dog ID	Mantel r (Bray-Curtis)	p.value (Bray-Curtis)	adj.p.value (Bray-Curtis)	Mantel r (Aitchison)	p.value (Aitchison)	adj.p.value (Aitchison)
2	Dog 21	0.48298567	0.001	0.007	0.70331374	0.001	0.0042
3	Dog 14	0.414828927	0.001	0.007	0.53256498	0.001	0.0042
4	Dog 6	0.323236179	0.001	0.007	0.25144237	0.003	0.007875
5	Dog 27	0.237443529	0.004	0.012	0.40177151	0.001	0.0042
6	Dog 5	0.372456823	0.003	0.012	0.66766392	0.001	0.0042
7	Dog 17	0.400332003	0.003	0.012	0.27912097	0.038	0.049875
8	Dog 19	0.482954407	0.004	0.012	0.25881978	0.053	0.0654706
9	Dog 10	0.188342613	0.022	0.056	0.25288702	0.009	0.0171818
10	Dog 3	0.273297796	0.038	0.0798	0.39023738	0.007	0.0147
11	Dog 26	0.137863614	0.042	0.080181818	0.24089942	0.002	0.006
12	Dog 34	0.159364912	0.162	0.2268	0.47572304	0.002	0.006
13	Dog 15	0.122407696	0.162	0.2268	0.32695329	0.005	0.0116667
14	Dog 2	0.108994191	0.152	0.2268	0.27010046	0.018	0.027
15	Dog 22	0.094516227	0.245	0.3215625	0.50474565	0.013	0.021
16	Dog 18	0.051913819	0.309	0.381705882	0.2918052	0.01	0.0175
17	Dog 24	0.01386548	0.363	0.4235	0.19526899	0.033	0.0462
18	Dog 4	0.015869844	0.387	0.427736842	0.29938405	0.001	0.0042
19	Dog 7	0.326261697	0.024	0.056	-0.25635789	0.943	0.943
20	Dog 23	0.25676346	0.117	0.20475	0.35403832	0.064	0.0746667
21	Dog 9	-0.034989458	0.559	0.58695	0.18977333	0.125	0.1381579
22	Dog 25	-0.034936271	0.593	0.593	0.00812286	0.421	0.44205

Table S3. Spearman correlations between taxon relative abundances and month for 14 abundant taxa. Spearman rank correlations were used to assess the relationship between bacterial relative abundance and month. Year 2022 was selected for analysis as it had the most samples, and taxon abundances were averaged by month to best capture overall seasonal trends. Only taxa with mean relative abundances >1% in 2022 were included. P-values were adjusted using the false discovery rate (FDR).

taxa	spearman_rho	p_value	p_adj
Catenibacterium mitsuokai	-0.64335664	0.02795455	0.4193183
Alloprevotella rava	-0.51048951	0.09360538	0.6054241
Bacteroides coprocola	-0.47552448	0.12131936	0.6054241
Prevotella copri	-0.43356643	0.16144643	0.6054241
Streptococcus lutetiensis	-0.35664336	0.25601254	0.6451729
Peptacetobacter hiranonis	0.32867133	0.29738508	0.6451729
Fusobacterium UC1	0.29370629	0.35433253	0.6451729
Holdemanella bifformis	-0.27972028	0.37870923	0.6451729
Fusobacterium UC2	0.25874126	0.41693843	0.6451729
Megamonas funiformis	0.25174825	0.43011529	0.6451729
Lachnoclostridium UC	-0.16083916	0.61935617	0.7939249
Collinsella intestinalis	0.15384615	0.63513996	0.7939249
Blautia UC	0.10489510	0.74947904	0.8647835
Bacteroides UC	0.04195804	0.90373884	0.9682916
Clostridium perfringens	0.00000000	1.00000000	1.0000000