Supplemental Methods File

16S rRNA gene amplification, amplicon sequencing and sequence data quality assessment and processing, and taxonomic assignments

Sequencing library construction: Sequencing libraries were constructed by amplifying the 16S rRNA gene V3-V4 regions using a protocol previously described[1,2] that comprises a 2-step PCR. In the first step the 16S rRNA gene is amplify, while the second step PCR add a unique dual index (UDI, Illumina) to each amplicon/sample. The V3-V4 regions of the 16S rRNA genes were targeted using bacterial primers 338F and 806R, combined with a heterogeneity spacer of 0 to 7 bp and the Illumina sequencing primers[2]. Forward primer was: ACACTGACGACATGGTTCTACA + heterogeneity spacer (0–7 bp) + ACTCCTRCGGGAGGCAGCAG, with the underline sequence matching to the 16S rRNA gene. Reserve primer was: TACGGTAGCAGAGACTTGGTCT + heterogeneity spacer (0–7 bp) + GGACTACHVGGGTWTCTAAT. Initial 16S rRNA gene amplification PCR contained 1X Phusion Taq master mix (ThermoFisher), forward and reverse primers (0.4µM each), 3% DMSO, and 5µl of genomic DNA. PCR cycling conditions were: an initial denaturation at 94°C for 3 min, 20 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, elongation at 72°C for 1 min, and a final elongation step at 72°C for 7 min. These amplicons were diluted 1:20, and 1µl was used in the barcoding PCR which introduces the 8-bp dual unique indices and the flow-cell linker adaptors to the amplicons by priming to the Illumina sequencing primer sequence introduced in step 1[2]. Forward primer was: AATGATACGGCGACCACCGAGATCTACAC + UDI (8 bp) + ACACTGACGACATGGTTCTACA. Reverse primer was: CAAGCAGAAGACGGCATACGAGAT + UDI (8 bp) + TACGGTAGCAGAGACTTGGTCT. Primers final concentration was 0.4µM in each reaction, with Phusion Taq master mix (1X) and 3% DMSO. Cycling conditions were: an initial denaturation at 94°C for30 sec, 10 cycles consisting of denaturation at 94°C for 30 sec, annealing at 58°C for 30 sec, elongation at 72°Cfor 60 sec, and a final elongation step at 72°C for 5 min.

Amplicon library pooling, sequencing and data pre-processing: Amplicons were pooled according to the procedure described in Holm et al.[2]. The pooled samples were cleaned up with AMPureXP (Agencourt/Beckman Coulter, Brea, CA) beads by following the manufacturer's instructions, and size was selected around 600 bp and eluted in water. Amplicon size was confirmed using an Agilent TapeStation 2200 with a DNA1000 tape for quality assurance. Libraries were sequenced on an Illumina MiSeq instrument using 600 cycles, producing 2X 300-bp paired-end reads. Demultiplication was performed using the UDI and a mapping file linking indices pairs to samples and split_li-braries.py, a QIIME-dependent script[3]. The resulting forward and reverse fastq files were split by sample using the QIIME-dependent script split_sequence_file_on_sample_ids.py, and primer sequences were removed using TagCleaner (version 0.16)[4]. Further processing followed the DADA2 workflow for Big Data and DADA2 (v. 1.5.2) (https://benjjneb.github.io/dada2/bigdata.html)[5].
Sequence read lengths of 255 bp and 225 bp were chosen for hard trimming of forward and reverse reads, respectively. Individual reads were further truncated at the base, where a quality score of 2 was observed, and filtered to contain no ambiguous bases. Additionally, the maximum number of expected errors in a read was set to 2. Reads were assembled only if the overlap between forward and reverse reads, which occurs in the conserved region between V3 and V4, was 100% identical. Chimeras for combined runs were removed per the DADA2 protocol.
Amplicon sequence variants (ASVs) generated by DADA2 were individually taxonomically classified using the RDP naïve Bayesian classifier[6] trained with the SILVA v138.1 16S rRNA gene sequence database[7] (-p-confidence 0.7). Read counts for ASVs assigned to the same taxonomy were summed for each sample.

References

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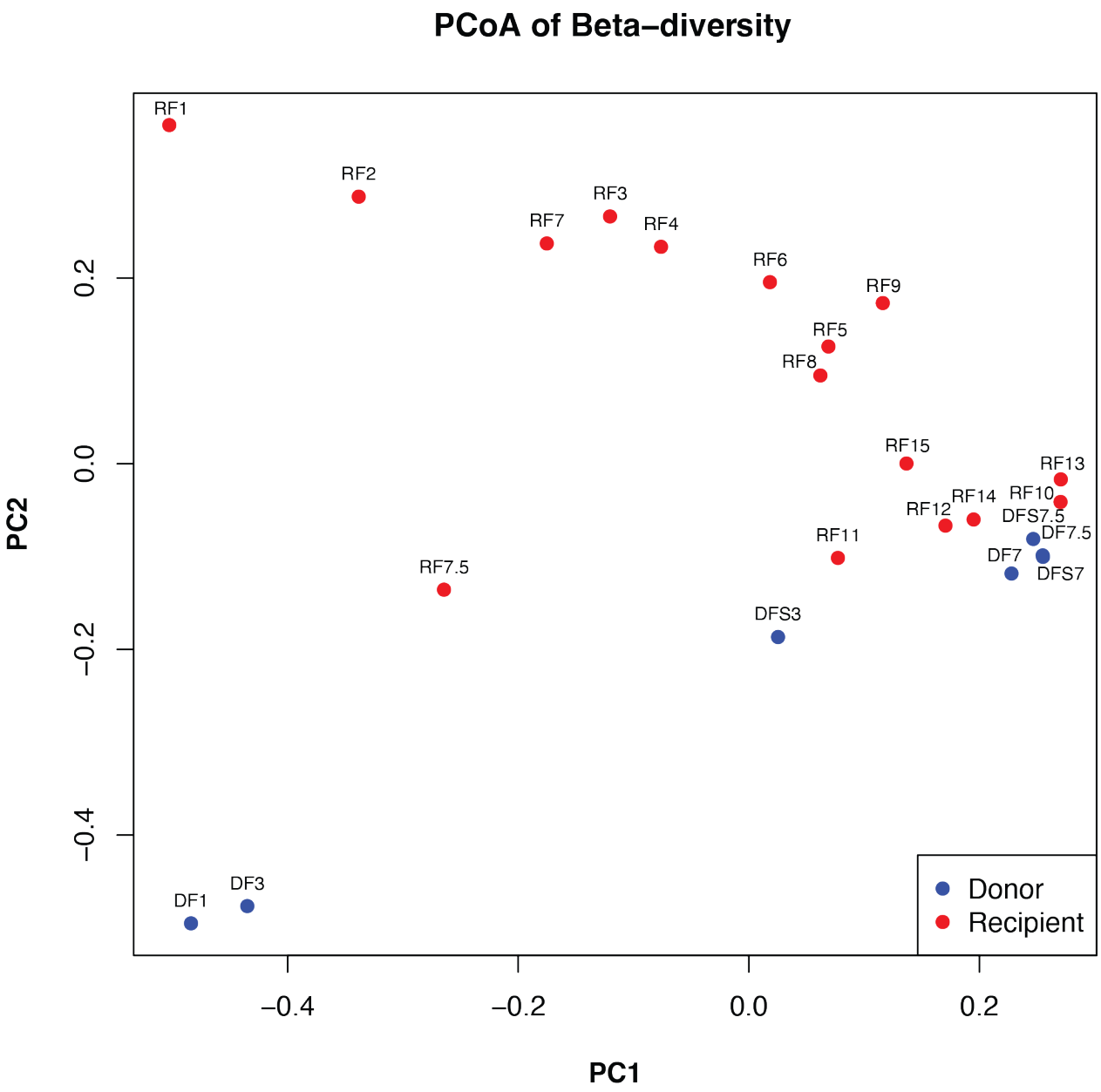
Supplementary Figure 1



Supplementary Figure 1. Principal Coordinate Analysis based on a Bray-Curtis dissimilarity matrix calculated on the 31 taxa identified in the gut microbiota of the donor and recipient.

Supplementary Table 1. Bacterial taxa relative abundance in the donor and recipient stool samples. DF: donor fecal material, DFS: Donor fecal slurry, RF: Recipient fecal material.

sampleID	FF06519474	FF06519480	FF06519487	FF06519491	FF06519482	FF06519488	FF06519490	FF06519476	FF06519478	FF06519481	FF06519483	FF06519484	FF06519485	FF06519486	FF06519489	FF06519492	FF06519493	FF06519494	FF06519495	FF06519497	FF06519498	FF06519499	FF06519500
days	1	3	7	7.5	3	7	7.5	1	2	3	4	5	6	7	7.5	8	9	10	11	12	13	14	15
group	DF	DF	DF	DF	DFS	DFS	DFS	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF	RF
sample_name	DF1	DF3	DF7	DF7.5	DFS3	DFS7	DFS7.5	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF7.5	RF8	RF9	RF10	RF11	RF12	RF13	RF14	RF15
group2	Reference	Reference	Reference	Reference	Reference	Reference	Reference	Before	FMT enema	FMT enema	FMT enema	FMT enema	FMT enema	FMT enema	FMT Oral	FMT Oral	FMT Oral	FMT Oral	FMT Oral	FMT Oral	FMT Oral	Loperamide	Loperamide
sequence_counts	2184	6162	160878	153595	58604	159490	127917	148640	163706	204414	210859	168524	154366	182235	149564	147758	200390	197227	118111	114897	169102	191946	211482
g_Clostridium_sensu_stricto_1	19.4	70.2	41.6	44.6	55.6	45.3	41.8	6.6	15.1	13.8	26.1	37.0	45.7	26.4	9.6	39.3	44.7	53.7	26.1	53.0	44.8	19.5	36.3
g_Paeniclostridium	3.0	1.5	12.9	15.2	5.2	20.3	34.3	0.0	2.6	37.6	7.0	15.1	2.1	0.5	0.1	4.4	26.7	22.3	14.8	6.1	31.9	28.5	3.9
f_Clostridiaceae_1	0.0	18.9	10.0	3.7	12.8	8.9	3.3	0.0	0.0	0.0	4.2	17.2	0.0	20.7	83.1	0.2	0.2	0.4	30.9	17.1	0.2	5.3	0.2
f_Enterobacteriaceae	0.0	0.1	0.0	0.0	0.0	0.0	0.0	24.9	18.2	21.3	37.2	10.7	14.6	20.0	2.1	6.0	10.2	0.1	0.1	0.0	0.1	0.1	0.0
f_Pasteurellaceae	4.1	0.0	0.1	0.2	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	2.1	20.0	7.9	1.5	18.7	32.9	33.4
g_Enterobacter	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.3	10.7	12.6	21.4	6.0	8.1	12.1	1.1	3.4	6.0	0.1	0.1	0.0	0.0	0.1	0.0
g_Paraclostridium	0.0	0.0	0.0	0.0	0.0	0.0	0.1	6.3	0.0	0.0	1.6	4.4	10.8	17.9	0.0	37.5	0.2	1.4	2.4	0.1	2.0	1.9	0.0
g_Terrisporobacter	2.1	1.1	0.9	8.7	2.5	1.4	5.4	0.4	0.3	1.6	0.0	0.2	17.9	0.0	0.0	3.1	1.3	0.7	5.9	10.3	0.7	8.5	0.3
g_Tyzzerella_4	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.1	38.9	4.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	13.4
g_Enterococcus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.9	1.5	6.9	0.6	1.6	0.3	1.4	2.0	0.1	7.0	0.1	0.3	2.3	0.9	1.7	11.7
g_Escherichia/Shigella	0.0	0.0	0.0	0.0	0.0	0.0	0.0	34.6	12.5	0.4	0.3	0.3	0.1	0.1	0.0	0.0	0.1	0.1	0.0	0.1	0.4	0.1	0.0
g_Turicibacter	0.9	0.1	19.0	9.8	0.3	4.1	4.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
f_Peptostreptococcaceae	10.2	6.3	7.3	2.6	21.4	11.7	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
g_Romboutsia	0.4	0.1	3.9	11.9	1.9	3.6	5.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
g_Tyzzerella_3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.3	4.2	0.2	0.2	0.5	1.4	0.8	0.8	4.2	4.6	0.0	0.0	0.0
g_Sarcina	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	2.8	0.0	0.3	0.3	0.0	0.0	0.1	7.1	3.7	0.0	1.1	0.0
g_Epulopiscium	0.0	0.0	2.3	2.3	0.0	0.5	3.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
g_Streptococcus	38.7	0.9	0.7	0.5	0.1	2.7	0.5	0.4	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
g_Oceanobacillus	0.9	0.0	0.1	0.2	0.0	0.2	0.1	0.0	0.0	0.2	0.1	0.2	0.1	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.3	0.1
g_Bifidobacterium	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.5	0.0	0.2	0.0	0.0	0.7	0.0	0.0	0.2
g_Lactobacillus	1.5	0.3	0.0	0.0	0.0	0.0	0.0	0.4	0.1	0.0	0.0	0.1	0.0	0.1	0.6	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.2
g_Clostridium_sensu_stricto_13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
g_Corynebacterium_1	1.8	0.2	0.7	0.1	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
g_Actinomyces	4.9	0.0	0.1	0.1	0.0	0.2	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
g_Gemella	6.0	0.0	0.1	0.0	0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
f_Microbacteriaceae	1.2	0.0	0.1	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
g_Helicobacter	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
g_Bacillus	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
f_Propionibacteriaceae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
g_Cupriavidus	1.1	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
g_Peptostreptococcus	2.93	0.11	0.06	0.01	0.02	0.07	0.01	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Supplementary Table S1A. Bacterial taxa mean relative abundance over different period of the study in the donor and recipient fecal microbiota.

	Donor	Before FMT	FMT Enema	FMT Oral	Loperamide
	Day 1,3,7, 7.5	Day 1	Day 2-7	Day 7.5-13	Day 14-15
g_Clostridium_sensu_stricto_1	45.51	6.63	27.36	38.75	27.89
g_Paeniclostridium	13.19	0.00	10.83	15.19	16.20
f_Clostridiaceae_1	8.24	0.00	7.02	18.88	2.74
f_Enterobacteriaceae	0.02	24.92	20.35	2.64	0.06
f_Pasteurellaceae	0.64	0.00	0.00	7.67	33.15
g_Enterobacter	0.01	14.33	11.80	1.51	0.03
g_Paraclostridium	0.03	6.26	5.79	6.24	0.95
g_Terrisporobacter	3.15	0.36	3.34	3.16	4.39
g_Tyzzerella_4	0.06	0.12	7.16	0.00	6.69
g_Enterococcus	0.00	9.94	2.04	1.80	6.70
g_Escherichia/Shigella	0.00	34.63	2.27	0.11	0.07
g_Turicibacter	5.48	0.00	0.05	0.15	0.01
f_Peptostreptococcaceae	8.76	0.00	0.01	0.00	0.06
g_Romboutsia	3.85	0.00	0.00	0.00	0.01
g_Tyzzerella_3	0.00	0.00	1.01	1.77	0.00
g_Sarcina	0.00	0.00	0.65	1.61	0.56
g_Epulopiscium	1.19	0.00	0.00	0.00	0.00
g_Streptococcus	6.30	0.38	0.02	0.01	0.04
g_Oceanobacillus	0.20	0.01	0.10	0.08	0.18
g_Bifidobacterium	0.00	0.19	0.05	0.20	0.11
g_Lactobacillus	0.25	0.43	0.05	0.10	0.09
g_Clostridium_sensu_stricto_13	0.00	1.78	0.00	0.00	0.00
g_Corynebacterium_1	0.48	0.00	0.00	0.00	0.00
g_Actinomyces	0.78	0.01	0.03	0.02	0.01
g_Gemella	0.92	0.00	0.00	0.00	0.00
f_Microbacteriaceae	0.20	0.00	0.02	0.02	0.02
g_Helicobacter	0.00	0.00	0.02	0.04	0.00
g_Bacillus	0.10	0.00	0.01	0.01	0.03
f_Propionibacteriaceae	0.01	0.01	0.02	0.01	0.01
g_Cupriavidus	0.19	0.00	0.01	0.02	0.00
g_Peptostreptococcus	0.46	0.00	0.00	0.00	0.00

Supplementary Table 1B. Bacterial taxa sequence counts in the donor and recipient fecal samples. DF: donor fecal material, DFS: Donor fecal slurry, RF: Recipient fecal material.

SampleID	DF1	DF3	DF7	DF7.5	DFS3	DFS7	DFS7.5	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF7.5	RF8	RF9	RF10	RF11	RF12	RF13	RF14	RF15
Types	Donor	Donor	Donor	Donor	Donor	Donor	Donor	Recipient	Recipient	Recipient	Recipient	Recipient	Recipient	Recipient	Recipient	Recipient	Recipient	Recipient	Recipient	Recipient	Recipient	Recipient	Recipient
Total Sequence Counts	2184	6162	160878	153595	58604	159490	127917	148640	163706	204414	210859	168524	154366	182235	149564	147758	200390	197227	118111	114897	169102	191946	211482
g_Clostridium_sensu_stricto_1	424	4325	66910	68508	32566	72307	53520	9849	24758	28203	55104	62393	70549	48024	14364	58053	89551	105950	30826	60937	75829	37421	76716
g_Paeniclostridium	65	90	20776	23326	3041	32332	43924	0	4263	76890	14744	25448	3276	960	94	6550	53589	43991	17458	7042	53926	54717	8225
f_Clostridiaceae_1	0	1166	16016	5712	7529	14157	4260	0	52	0	8906	28982	0	37644	124295	222	424	866	36530	19702	255	10080	486
f_Enterobacteriaceae	0	5	6	8	3	22	0	37039	29786	43559	78483	18070	22611	36506	3109	8827	20345	209	137	36	88	202	12
f_Pasteurellaceae	90	0	87	269	2	174	27	0	0	5	4	0	0	0	0	5129	4211	39460	9280	1769	31662	63107	70665
g_Enterobacter	0	3	0	0	0	7	0	21294	17449	25787	45119	10054	12553	21963	1629	4964	11958	120	64	19	48	127	4
g_Paraclostridium	0	0	12	14	0	38	174	9311	13	11	3450	7400	16646	32614	20	55456	472	2827	2860	106	3341	3629	0
g_Terrisporobacter	45	67	1474	13293	1476	2204	6909	535	533	3185	62	381	27595	11	0	4646	2570	1445	7003	11865	1223	16255	654
g_Tyzzerella_4	9	0	0	0	0	0	0	176	63604	8426	8	0	0	0	0	0	0	0	8	0	0	0	28295
g_Enterococcus	0	0	0	0	0	12	0	14771	2381	14074	1164	2748	460	2537	2950	112	14109	180	296	2625	1487	3169	24827
g_Escherichia/Shigella	0	0	0	0	0	8	10	51470	20423	845	710	485	82	117	8	56	214	131	55	98	725	202	92
g_Turicibacter	20	8	30644	15029	188	6546	5172	3	76	427	6	0	10	81	0	1514	0	12	14	0	3	0	60
f_Peptostreptococcaceae	223	390	11763	3952	12553	18609	2327	0	0	42	25	0	16	0	0	0	6	0	0	0	12	166	64
g_Romboutsia	9	8	6353	18352	1114	5676	6476	0	0	0	0	0	0	37	0	0	0	0	0	0	56	0	23
g_Tyzzerella_3	0	0	0	0	0	0	0	0	0	2235	725	7134	300	345	811	2112	1641	1573	4904	5307	0	5	0
g_Sarcina	0	0	0	0	0	0	0	0	0	0	1674	4668	0	569	494	0	0	194	8425	4276	22	2141	0
g_Epulopiscium	0	0	3714	3516	0	877	4051	0	2	10	0	0	0	0	0	0	0	0	0	0	0	0	0
g_Streptococcus	846	53	1051	759	65	4365	681	563	46	9	4	0	8	125	14	5	11	0	11	21	21	6	168
g_Oceanobacillus	19	0	207	273	0	287	87	16	49	354	215	287	113	82	114	50	182	130	84	146	130	556	165
g_Bifidobacterium	0	0	0	0	0	3	0	277	73	25	0	25	0	408	676	0	399	0	26	770	52	6	470
g_Lactobacillus	32	16	2	4	0	29	3	645	135	50	3	204	0	131	875	4	38	0	17	69	14	17	379
g_Clostridium_sensu_stricto_13	0	0	0	0	0	0	0	2652	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0
g_Corynebacterium_1	39	14	1162	227	15	651	59	0	0	10	6	10	0	0	0	0	0	0	25	6	0	0	0
g_Actinomyces	107	3	232	151	16	351	27	12	25	65	166	36	20	29	3	14	62	27	8	6	79	0	28
g_Gemella	130	0	145	74	4	447	114	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
f_Microbacteriaceae	26	0	110	28	0	131	12	6	11	45	73	26	23	23	6	8	60	27	20	30	33	20	73
g_Helicobacter	0	0	0	8	0	0	0	0	2	35	27	75	40	0	0	0	430	6	22	21	14	19	0
g_Bacillus	12	0	59	55	0	41	38	0	0	56	26	42	25	9	49	10	29	35	0	0	32	88	21
f_Propionibacteriaceae	0	0	47	0	0	67	0	17	14	49	120	41	19	14	0	3	62	27	4	4	36	8	30
g_Cupriavidus	24	7	18	20	20	41	28	4	11	17	17	15	8	6	53	9	22	8	34	42	14	0	15
g_Peptostreptococcus	64	7	90	17	12	108	18	0	0	0	18	0	12	0	0	2	5	9	0	0	0	5	10

Fecal Microbiota Transplantation in a Domestic Ferret Suffering from Chronic Diarrhea and Maldigestion–Fecal Microbiota and Clinical Outcome: A Case Report

Code

Initialization Loading packages and libraries

Hide

```
# Install necessary packages if not already installed
if (!requireNamespace("vegan", quietly = TRUE)) install.packages("vegan")
if (!requireNamespace("readxl", quietly = TRUE)) install.packages("readxl")
if (!requireNamespace("ggplot2", quietly = TRUE)) install.packages("ggplot2")
if (!requireNamespace("tidyr", quietly = TRUE)) install.packages("tidyr")
if (!requireNamespace("pheatmap", quietly = TRUE)) install.packages("pheatmap")

library(ggplot2)
library(tidyr)
library(readxl)
library(pheatmap)
library(vegan)
```

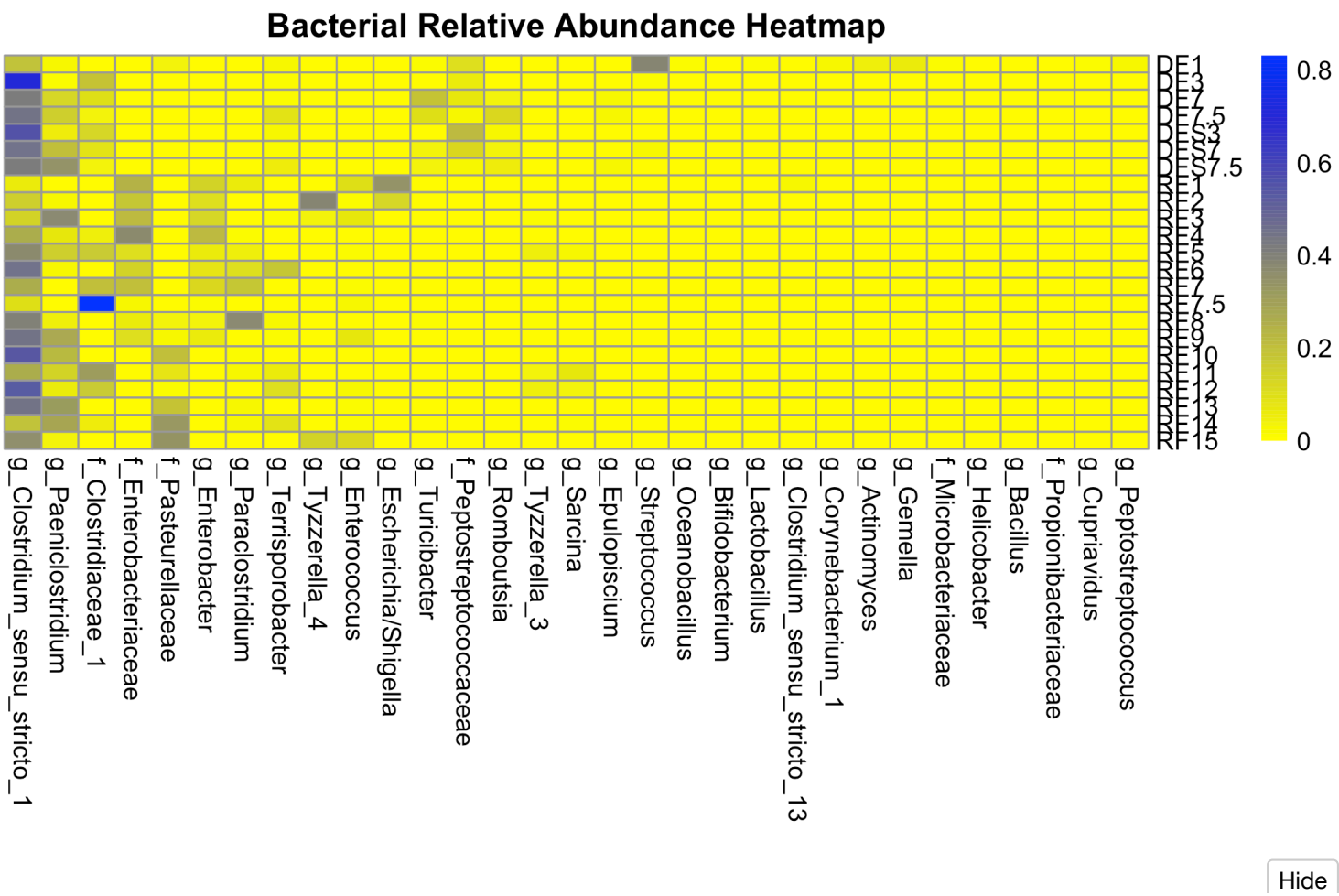
Hide

```
# Read the data from Excel file
data <- read_excel('Ferret_Heatmap.xlsx')
```

Heatmap Relative Abundance Yellow/Blue

Hide

```
# Convert the tibble to a regular data frame
data_relative_abundance <- as.data.frame(data)

# Calculate the relative abundance
data_relative_abundance[, 3:ncol(data_relative_abundance)] <- data_relative_abundanc
e[, 3:ncol(data_relative_abundance)] / data_relative_abundance$Tcount

# Set row names to 'SampleID'
rownames(data_relative_abundance) <- data_relative_abundance$SampleID
data_relative_abundance <- data_relative_abundance[,-1]

# Remove the 'Tcount' column as it's no longer needed for visualization
data_relative_abundance <- data_relative_abundance[,-c(1,2)]

# Create the heatmap
heatmap_obj <- pheatmap(
  data_relative_abundance,
  cluster_rows = FALSE,
  cluster_cols = FALSE,
  scale = "none",  # No scaling of data
  color = colorRampPalette(c("yellow", "blue"))(100),  # Yellow to blue color scheme
  main = "Bacterial Relative Abundance Heatmap",
  show_rownames = TRUE  # Show sample IDs on the y-axis
)
```



Hide

```
# To customize the appearance of the sample IDs on the y-axis:
# heatmap_obj$tree_row$labels_colors <- "black"  # Change the color to black, for exa
mple

# To save the heatmap as an image file (e.g., PNG):
#save_ps("heatmap.ps", heatmap_obj)
```

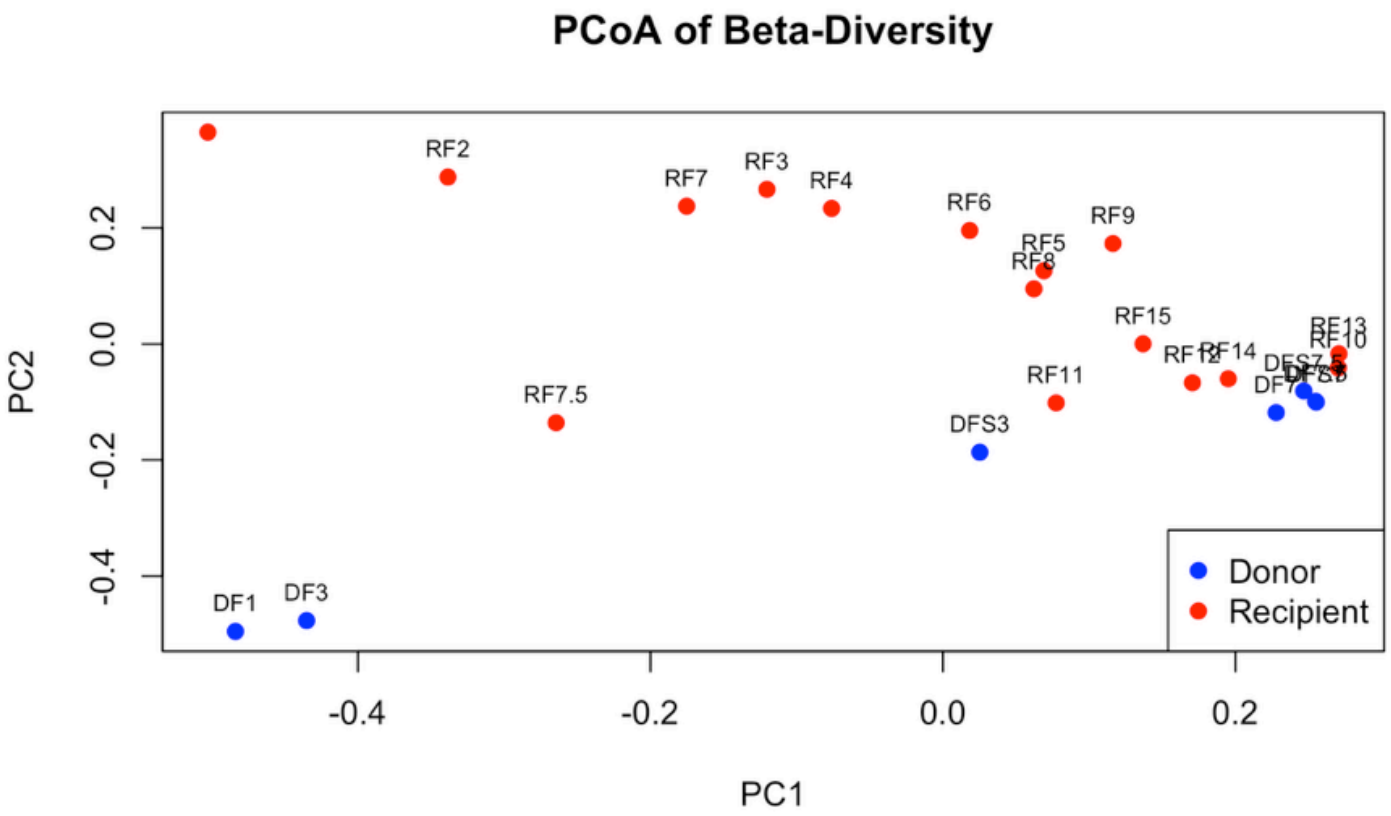
PCoA beta-Diversity

Hide

```
# Prepare the data in new framework call data_beta
# First column is Sample ID, Second column is Type, Third column is Total Sequence Co
unts, we remove them for diversity analysis
data_beta <- data[,-c(1,2,3)]

# Calculate beta-diversity using Bray-Curtis method
beta_div <- vegdist(data_beta, method = "bray")

# Perform PCoA on the distance matrix
pcoa_res <- cmdscale(beta_div, eig = TRUE, k = 2) # k is the number of dimensions

# Create a data frame for plotting
samples <- as.data.frame(pcoa_res$points)
colnames(samples) <- c("PC1", "PC2")

# Add sample ID for labeling
samples$SampleID <- data[,1]
# Extract the Sample Type information for coloring the points
sample_types <- data[,2] # Second column contains the sample type information
samples$Type <- sample_types

# Plot the PCoA with points colored by Sample Type
plot(samples$PC1, samples$PC2, xlab = "PC1", ylab = "PC2", main = "PCoA of Beta-Diver
sity", type = "n")
with(samples, points(PC1, PC2, pch = 19, col = ifelse(Type == "Donor", "blue", "re
d")))
```

Hide

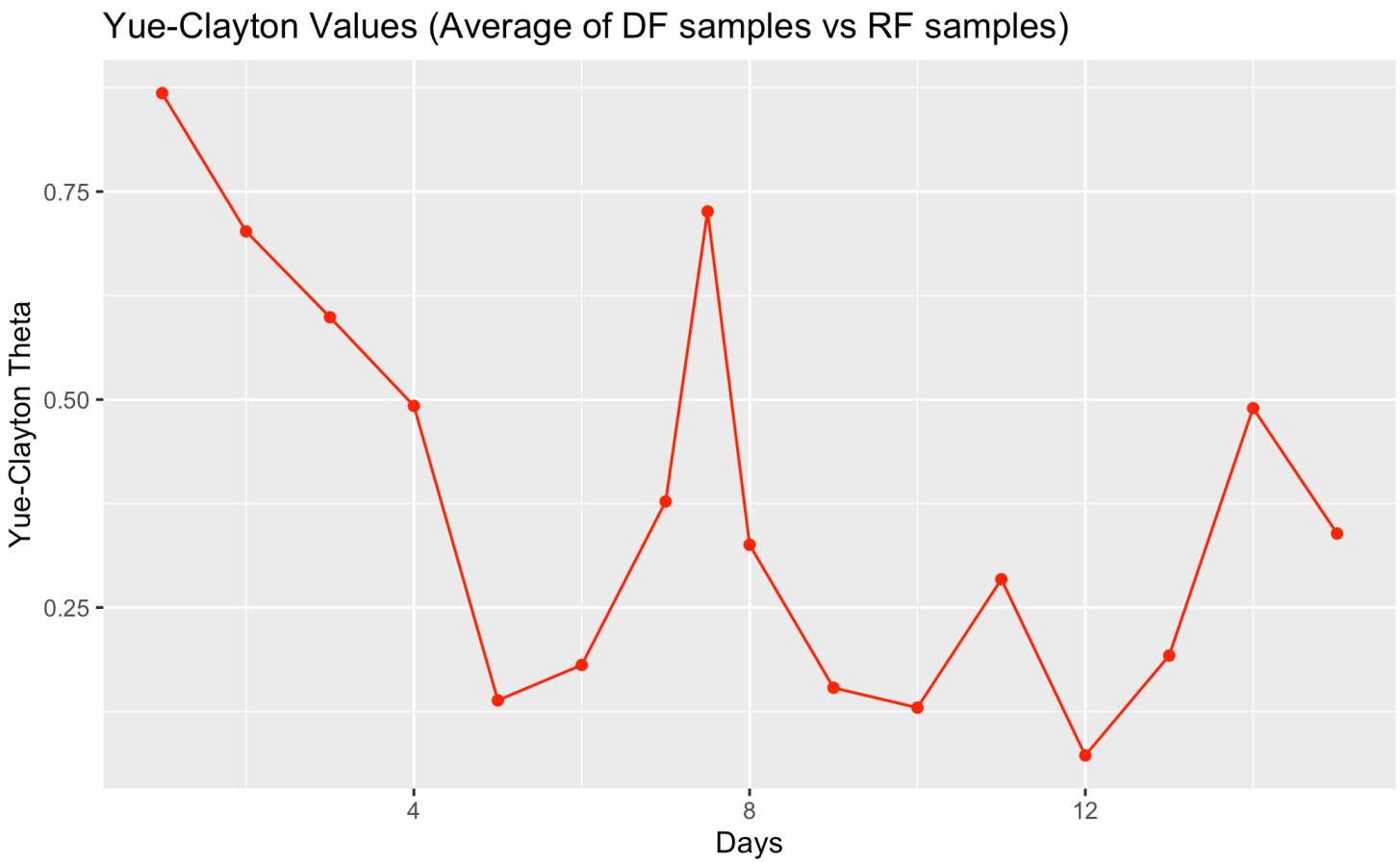
```
with(samples, text(PC1, PC2, labels = samples$SampleID$SampleID, cex = 0.7, pos = 3))

# Add a legend to the plot
legend("bottomright", legend = c("Donor", "Recipient"), col = c("blue", "red"), pch =
19)
```



Yue-Clayton Theta Calcuation (Average DF vs RF samples)

Hide

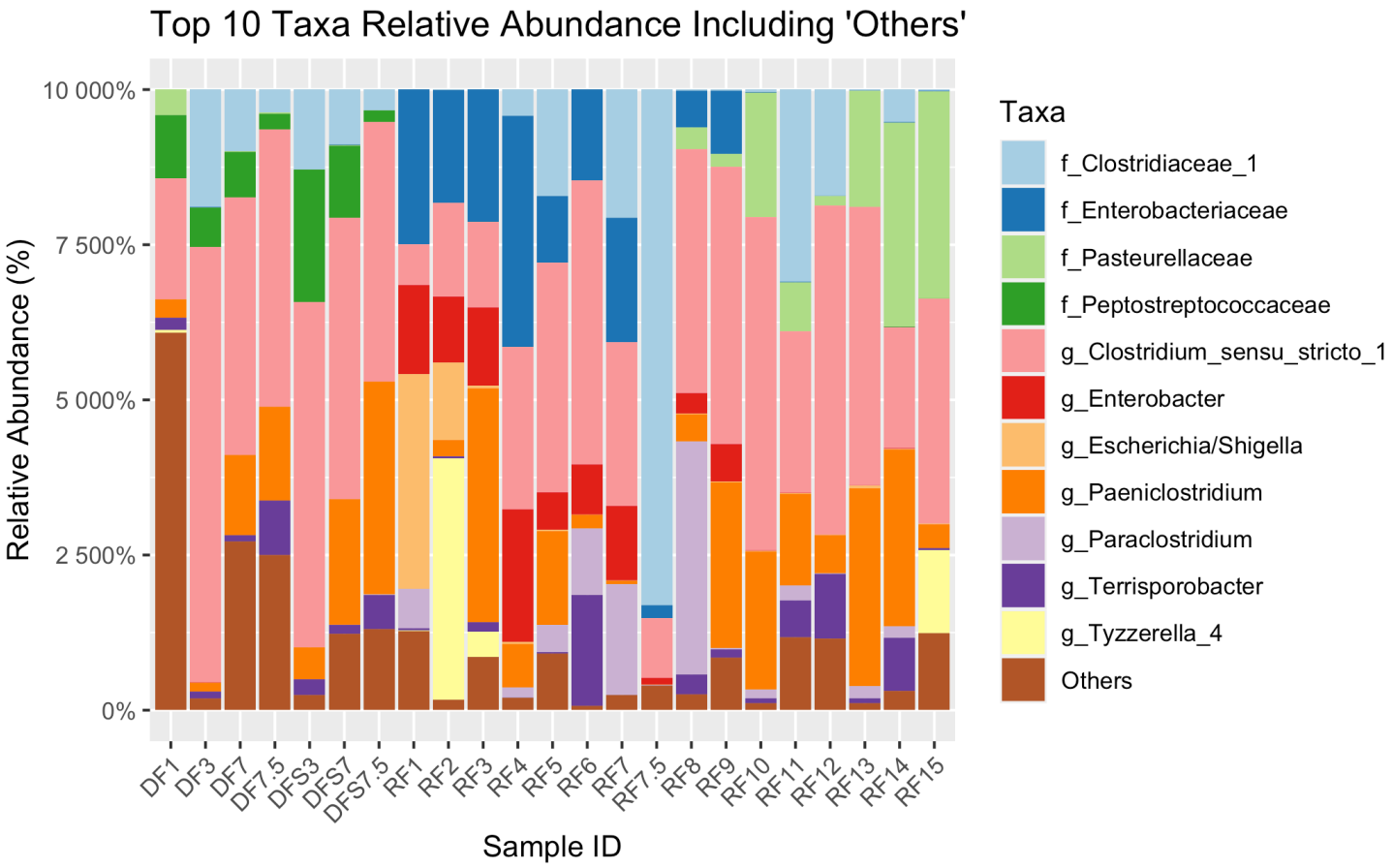
```
# Read the data
dataYC <- read_excel("Ferret_Taxonomic_table_percent_final.xlsx")

# Function to calculate Yue-Clayton theta value
yue_clayton <- function(sample_a, sample_b) {
  numerator <- sum(sample_a * sample_b)
  denominator <- sqrt(sum(sample_a^2) * sum(sample_b^2))
  return(1 - (numerator / denominator))
}

# Calculate the average bacterial proportions for all DF samples
average_df <- colMeans(subset(dataYC, Group == "DF")[, 4:ncol(dataYC)])

# Filter data for RF samples
rf_samples <- subset(dataYC, Group == "RF")

# Calculate Yue-Clayton values for all RF samples using the overall DF average
yue_values <- sapply(1:nrow(rf_samples), function(i) yue_clayton(average_df, rf_sampl
es[i, 4:ncol(rf_samples)]))
plot_data <- data.frame(Day = rf_samples$Day, YueClayton = yue_values)

# Plot the values
ggplot(plot_data, aes(x = Day, y = YueClayton)) +
  geom_point(color = "red") +
  geom_line(color = "red") +
  ggtitle("Yue-Clayton Values (Average of DF samples vs RF samples)") +
  xlab("Days") +
  ylab("Yue-Clayton Theta")
```



Barplot Figure 3B

Hide

```
# Read the data from the Excel file
data2 <- read_excel('Ferret_heatmap.xlsx')

# Calculate relative abundances
data2 <- data2 %>%
  mutate(across(-c(SampleID, type, Tcount), ~ .x / Tcount)) %>%
  select(-Tcount)

# Identify top 10 most abundant taxa
top_10_taxa <- data2 %>%
  select(-SampleID, -type) %>%
  summarise(across(everything(), sum)) %>%
  gather(key = "Taxa", value = "Abundance") %>%
  top_n(10, Abundance) %>%
  arrange(desc(Abundance)) %>%
  pull(Taxa)

# Add g_Escherichia/Shigella to this list
top_taxa <- c(top_10_taxa, "g_Escherichia/Shigella")

# Prepare data, grouping non-top taxa as 'Others'
data_long <- data2 %>%
  pivot_longer(-c(SampleID, type), names_to = "Taxa", values_to = "Abundance") %>%
  mutate(Taxa = ifelse(Taxa %in% top_taxa, Taxa, "Others")) %>%
  group_by(SampleID, type, Taxa) %>%
  summarise(Abundance = sum(Abundance), .groups = 'drop') %>%
  ungroup() %>%
  mutate(RelativeAbundance = Abundance * 100) # Convert to percentage

# Order the samples
data_long$SampleID <- factor(data_long$SampleID, levels = unique(data$SampleID))

# Plot
ggplot(data_long, aes(x = SampleID, y = RelativeAbundance, fill = Taxa)) +
  geom_bar(stat = "identity", position = "stack") +
  scale_y_continuous(labels = scales::percent_format()) +
  theme(axis.text.x = element_text(angle = 45, hjust = 1)) +
  labs(x = "Sample ID", y = "Relative Abundance (%)", fill = "Taxa", title = "Top 10
Taxa Relative Abundance Including 'Others'") +
  #facet_wrap(~type, scales = "free_x") +
  scale_fill_brewer(palette = "Paired") # Use a nice color palette
```


Top 10 Taxa Relative Abundance Including 'Others'
Relative Abundance (%)
10 000%
7 500%
5 000%
2 500%
0%
DF1
DF3
DF7
DF7.5
DFS3
DFS7
DFS7.5
RF1
RF2
RF3
RF4
RF5
RF6
RF7
RF7.5
RF8
RF9
RF10
RF11
RF12
RF13
RF14
RF15
Sample ID
Taxa
f_Clostridiaceae_1
f_Enterobacteriaceae
f_Pasteurellaceae
f_Peptostreptococcaceae
g_Clostridium_sensu_stricto_1
g_Enterobacter
g_Escherichia/Shigella
g_Paeniclostridium
g_Paraclostridium
g_Terrisporobacter
g_Tyzzerella_4
Others