Supplementary Materials:

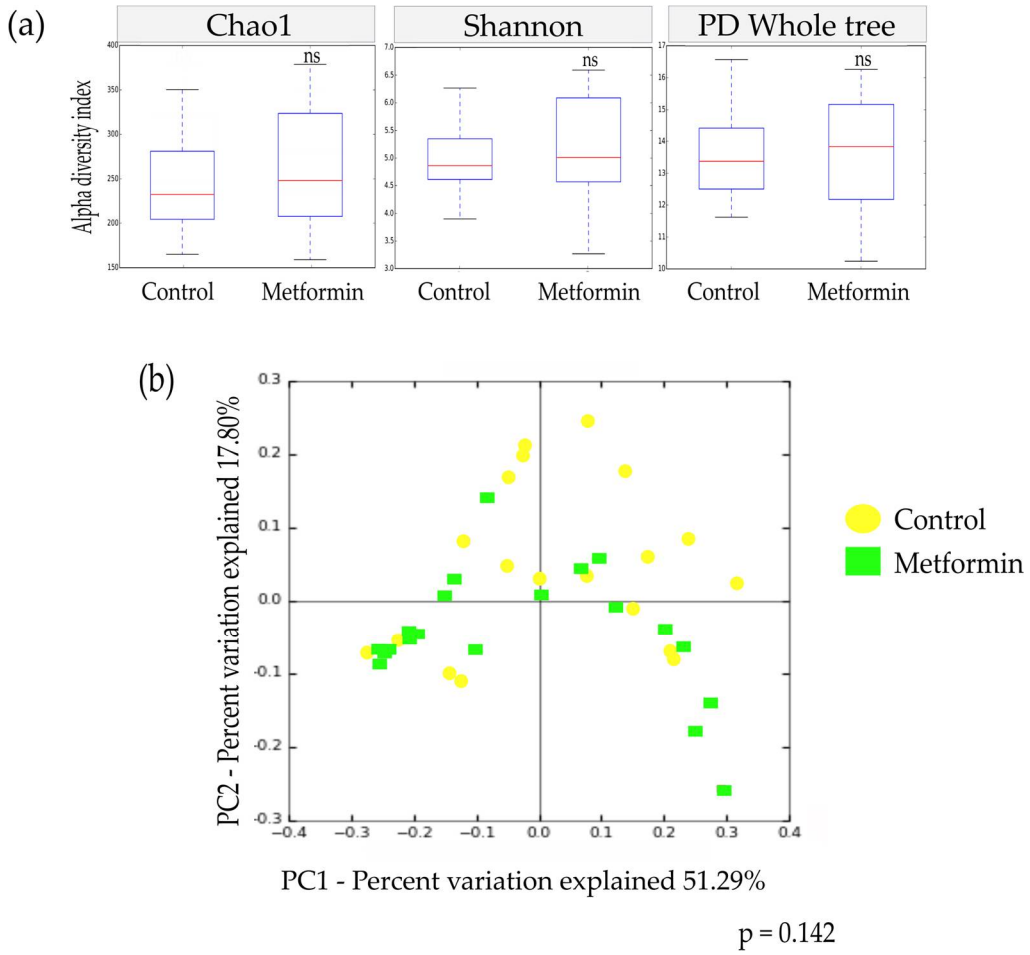


Figure S1. Alpha and beta diversity comparison of fecal microbiota between the metformin and control groups before the beginning of treatment. (**a**) Alpha diversity analyses. Ns, non-significant. Student's *t*-test. (**b**) Principal coordinate analysis (PCoA) plots created using weighted UniFrac distances. Green and yellow dots indicate metformin and control samples, respectively.

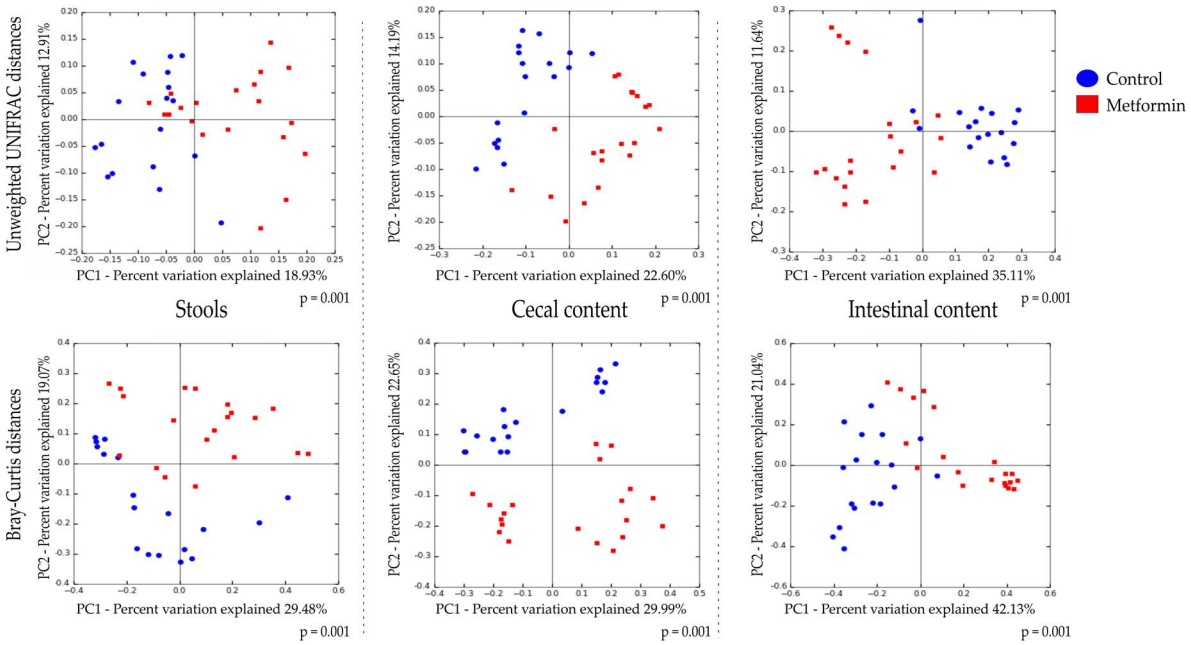


Figure S2. 2D PCoA plots created using unweighted UniFrac and Bray–Curtis distances. Red and blue dots indicate metformin and control samples, respectively. Adonis statistical tests showed significant differences between the two groups (999 permutations; for unweighted Unifrac distances R2 = 0.138, 0.174 and 0.260 for stool, cecal and intestinal content respectively, for Bray-Curtis distances R2 = 0.162, 0.200 and 0.297 for stool, cecal and intestinal content respectively, $p = 0.001$).

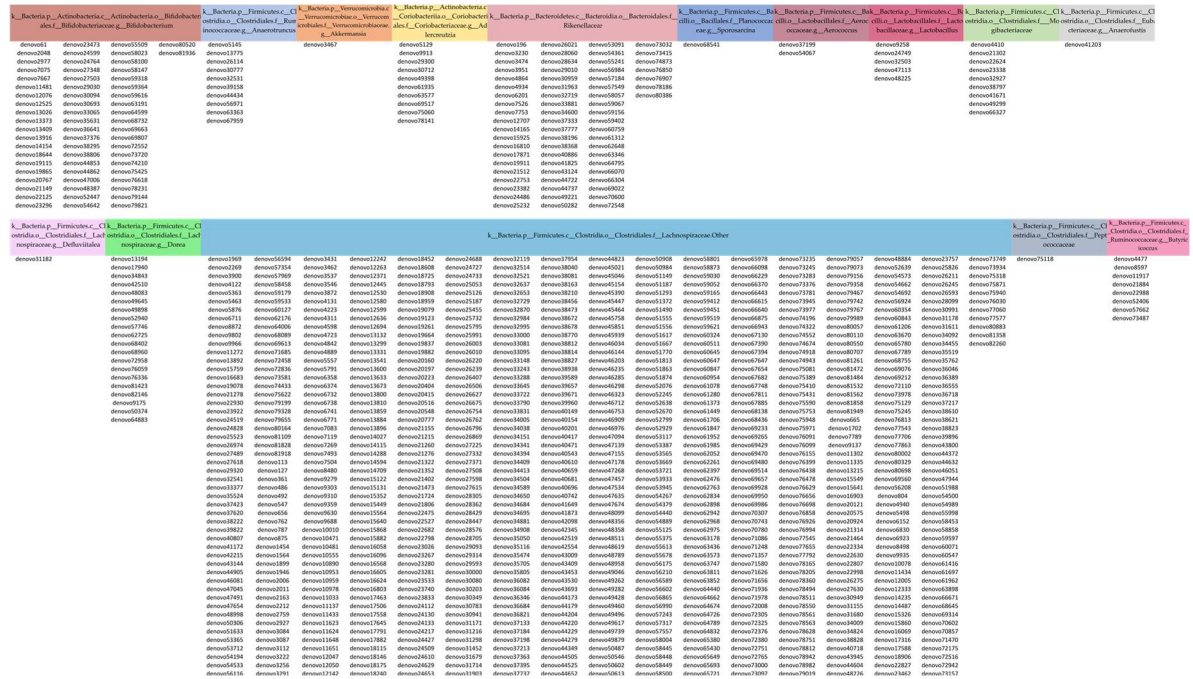
Table S1. Comparison of the relative abundance of bacteria between the metformin and control treatment groups at species level for different digestive sites. The comparison was performed with Multiple t test, NS: non-significant ($p > 0.05$).

	Stools			Caecal content				Intestinal content			
	Control (%)	Metformin (%)	p		Control (%)	Metformin (%)	p		Control (%)	Metformin (%)	p
Species	*Bifidobacterium pseudolongum*	7.28	28.14	2.83E-04	*Corynebacterium stationis*	4.4E-04	0	NS	*Corynebacterium stationis*	2.38E-03	1.64E-03	NS
	Alistipes indistinctus	0	2.77E-03	NS	*Bifidobacterium pseudolongum*	1.92	18.31	9.00E-06	*Bifidobacterium pseudolongum*	20.79	67.44	<1.00E-06
	Jeotgalicoccus psychrophilus	0.148	0.020	6.12E-03	*Jeotgalicoccus psychrophilus*	0.025	9.13E-03	NS	*Bacillus cereus*	0	1.69E-03	NS
	Staphylococcus aureus	0.026	4.28E603	1.31E-02	*Staphylococcus aureus*	3.09E-03	2.20E-03	NS	*Jeotgalicoccus psychrophilus*	0.207	0.042	NS
	Staphylococcus sciuri	4.16E-03	2.19E-03	NS	*Staphylococcus sciuri*	0	6.21E-04	NS	*Staphylococcus aureus*	0.041	8.55E-03	1.75E-02
	Lactobacillus reuteri	1.89E-03	0	NS	*Defluvitalea saccharophila*	0.058	0.023	8.62E-03	*Staphylococcus sciuri*	5.98E-03	3.73E-03	NS
	Defluvitalea saccharophila	0.165	0.030	8.45E-04	*Ruminococcus gnavus*	2.54	2.55	NS	*Lactobacillus reuteri*	0.020	3.38E-03	1.24E-02
	Ruminococcus gnavus	2.42	2.15	NS	*Butyricicoccus pullicaecorum*	1.61	0.83	4.65E-04	*Streptococcus infantis*	2.04E-03	0	NS
	Butyricicoccus pullicaecorum	0.919	0.745	NS	*Clostridium methylpentosum*	0.013	4.23E-03	3.27E-02	*Defluvitalea saccharophila*	0.11	3.85E-03	1.80E-03
	Clostridium methylpentosum	5.40E-03	0.012	NS	*Enterobacter hormaechei*	0	7.56E-03	NS	*Ruminococcus gnavus*	4.9	0.627	8.58E-04
	Oxalobacter formigenes	4.43E-03	0	2.83E-02	*Escherichia coli*	8.80E-04	8.9E-03	NS	*Butyricicoccus pullicaecorum*	0.22	0.031	5.30E-05
	Enterobacter hormaechei	0	8.95E-03	NS	*Akkermansia muciniphila*	0.048	0.21	2.00E-06	*Helicobacter pylori*	0	3.78E-03	NS
	Escherichia coli	9.11E-03	0.018	NS					*Enterobacter hormaechei*	0	9.61E-03	NS
	Akkermansia muciniphila	0.146	0.248	NS					*Escherichia coli*	0.085	0.084	NS
									Acinetobacter johnsonii	0	9.34E-03	NS
									Pseudomonas veronii	1.40E-03	0	NS
									Akkermansia muciniphila	0.26	0.18	NS

Table S2. Bacterial taxa with LDA scores > 2 in at least two of the three digestives sites in the metformin and control groups. S, stool; CC, cecum; IC, intestine.

Bacterial taxa	Digestive site	LDA score	Group	p
k__Bacteria.p__Actinobacteria.c__Actinobacteria.o__Bifidobacteriales.f__Bifidobacteriaceae.g__Bifidobacterium	IC	4.66	Metformin	7.71E-06
k__Bacteria.p__Actinobacteria.c__Actinobacteria.o__Bifidobacteriales.f__Bifidobacteriaceae.g__Bifidobacterium	S	4.32	Metformin	1.62E-04
k__Bacteria.p__Actinobacteria.c__Actinobacteria.o__Bifidobacteriales.f__Bifidobacteriaceae.g__Bifidobacterium	CC	4.22	Metformin	5.76E-07
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Anaerotruncus	S	3.01	Metformin	5.01E-03
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Anaerotruncus	CC	3.07	Metformin	8.60E-04
k__Bacteria.p__Verrucomicrobia.c__Verrucomicrobiae.o__Verrucomicrobiales.f__Verrucomicrobiaceae.g__Akkermansia	S	2.09	Metformin	1.09E-02
k__Bacteria.p__Verrucomicrobia.c__Verrucomicrobiae.o__Verrucomicrobiales.f__Verrucomicrobiaceae.g__Akkermansia	CC	2.32	Metformin	2.47E-05
k__Bacteria.p__Actinobacteria.c__Coriobacteriia.o__Coriobacteriales.f__Coriobacteriaceae.g__Adlercreutzia	CC	2.47	Control	5.03E-04
k__Bacteria.p__Actinobacteria.c__Coriobacteriia.o__Coriobacteriales.f__Coriobacteriaceae.g__Adlercreutzia	IC	3.83	Control	2.67E-07
k__Bacteria.p__Actinobacteria.c__Coriobacteriia.o__Coriobacteriales.f__Coriobacteriaceae.g__Adlercreutzia	S	5.6	Control	5.10E-06
k__Bacteria.p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Rikenellaceae	IC	2.74	Control	2.53E-04
k__Bacteria.p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Rikenellaceae	S	4.05	Control	4.83E-05
k__Bacteria.p__Firmicutes.c__Bacilli.o__Bacillales.f__Planococcaceae.g__Sporosarcina	CC	2.42	Control	1.61E-04
k__Bacteria.p__Firmicutes.c__Bacilli.o__Bacillales.f__Planococcaceae.g__Sporosarcina	IC	3.37	Control	2.22E-08
k__Bacteria.p__Firmicutes.c__Bacilli.o__Bacillales.f__Planococcaceae.g__Sporosarcina	S	2.49	Control	5.94E-05
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Aerococcaceae.g__Aerococcus	IC	3.37	Control	7.09E-03
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Aerococcaceae.g__Aerococcus	S	3.2	Control	2.83E-03
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Lactobacillaceae.g__Lactobacillus	IC	2.81	Control	4.18E-05
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Lactobacillaceae.g__Lactobacillus	S	2.32	Control	2.80E-03
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Mogibacteriaceae	IC	2.84	Control	5.76E-06
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Mogibacteriaceae	S	2.34	Control	3.80E-03
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Eubacteriaceae.g__Anaerofustis	CC	2.87	Control	4.09E-05
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Eubacteriaceae.g__Anaerofustis	IC	3.18	Control	4.82E-06
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Eubacteriaceae.g__Anaerofustis	S	2.64	Control	1.09E-07
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Defluviitalea	CC	2.76	Control	6.04E-03
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Defluviitalea	IC	3,00	Control	5.15E-05
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Defluviitalea	S	2.38	Control	1.30E-03
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Dorea	CC	2.47	Control	5.48E-03
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Dorea	IC	3.23	Control	1.39E-06
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Dorea	S	2.62	Control	5.00E-03
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.Other	CC	2.81	Control	6.25E-04
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.Other	IC	2.92	Control	5.63E-04
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Peptococcaceae	CC	2.6	Control	4.30E-05
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Peptococcaceae	S	2.65	Control	1.14E-03
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Butyricicoccus	CC	2.92	Control	2.86E-03
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Butyricicoccus	IC	3.24	Control	4.30E-05

Table S3. OTUs number of bacterial taxa with LDA scores > 2 in at least two of the three digestives sites in the metformin and control groups.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k__Bacteria.p__Verrucomicrobia.c__Verrucomicrobiae.c__Verrucomicrobiales.f__Verrucomicrobiaceae.g__Akkermansia
k__Bacteria.p__Actinobacteria.c__Coriobacteriia.o__Coriobacteriales.f__Coriobacteriaceae.g__Adlercreutzia
k__Bacteria.p__Bacteroidetes.c__Bacteroidia.o__Bacteroidales.f__Rikenellaceae
k__Bacteria.p__Firmicutes.c__Bacilli.o__Bacillales.f__Planococcaceae.g__Sporosarcina
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Aerococcaceae.g__Aerococcus
k__Bacteria.p__Firmicutes.c__Bacilli.o__Lactobacillales.f__Lactobacillaceae.g__Lactobacillus
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Mogibacteriaceae
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Eubacteriaceae.g__Anaerofustis
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__DefluviitaleaceaeUCG-011
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.g__Dorea
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Lachnospiraceae.Other
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Peptococcaceae
k__Bacteria.p__Firmicutes.c__Clostridia.o__Clostridiales.f__Ruminococcaceae.g__Butyricicoccus

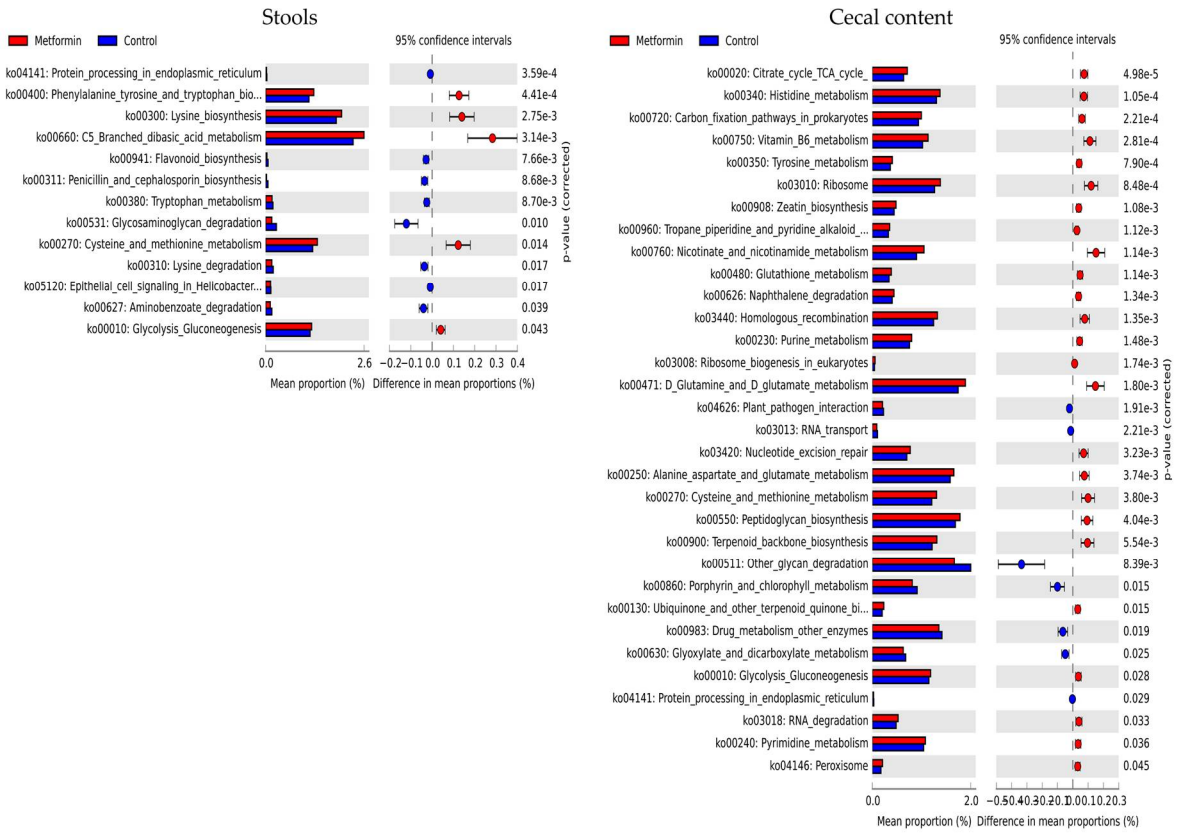

Figure S3. Differentially enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (i.e., relative abundance > 0.001%) in cecal and fecal microbiota ($p < 0.05$).

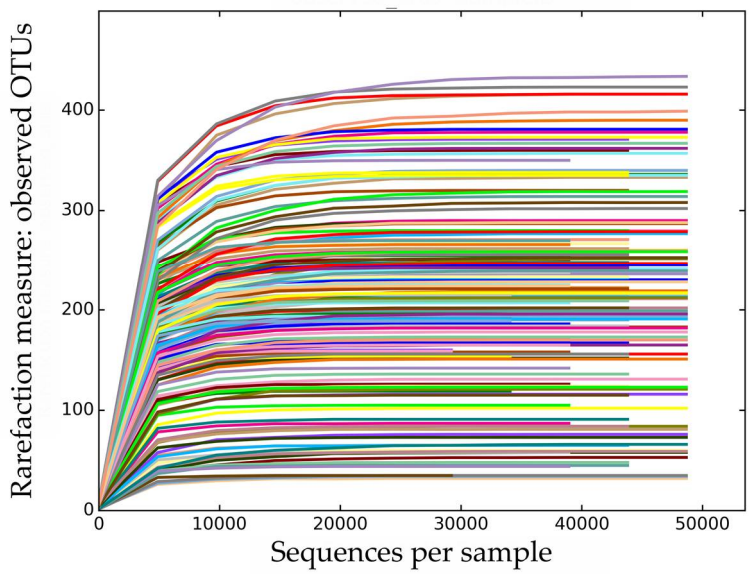

Figure S4. Rarefaction curves showing the number of observed OTU as a function of the number of sequences per samples. All samples (153) analyzed in this study were presented.

Table S4. Number of reads obtained in each sample after preprocessing.

Samples	Number of reads	Samples	Number of reads	Samples	Number of reads	Samples	Number of reads
1	79645	40	64949	79	63410	118	78084
2	55989	41	64579	80	52032	119	102842
3	92015	42	64265	81	57533	120	86872
4	58957	43	57574	82	59439	121	95966
5	77663	44	66610	83	54356	122	78709
6	70640	45	57122	84	50410	123	88562
7	87986	46	83783	85	45497	124	89355
8	66420	47	64824	86	62147	125	86426
9	84913	48	60005	87	64362	126	81709
10	95964	49	63235	88	51869	127	105105
11	91683	50	62651	89	52023	128	95330
12	72678	51	64494	90	59943	129	123933
13	87168	52	69597	91	41804	130	84681
14	66789	53	59757	92	62311	131	89451
15	79866	54	70762	93	54698	132	82373
16	65482	55	68541	94	42045	133	126809
17	66102	56	61862	95	61350	134	110943
18	62044	57	67608	96	44858	135	85467
19	57239	58	55190	97	32606	136	82502
20	87881	59	62301	98	53454	137	85507
21	66821	60	71317	99	45075	138	109549
22	86593	61	53220	100	44837	139	95059
23	104498	62	59081	101	59142	140	45628
24	78768	63	51903	102	51582	141	55684
25	74352	64	69273	103	50626	142	63501
26	53154	65	57854	104	41510	143	60142
27	70604	66	67666	105	52298	144	59139
28	71264	67	60616	106	45921	145	62810
29	71625	68	52588	107	48307	146	63360
30	80648	69	56615	108	51070	147	60401
31	65641	70	60102	109	61448	148	70067
32	65373	71	65017	110	53567	149	63861
33	55860	72	51024	111	58184	150	54678
34	64601	73	58001	112	61833	151	50451
35	74087	74	61585	113	81673	152	56174
36	82083	75	49701	114	77242	153	55721
37	41921	76	54286	115	74677		
38	71215	77	62250	116	66769		
39	63023	78	59153	117	88884		

Supplementary material S1

16SrRNA gene sequencing

DNA quantified using a SYBR Green assay (SYBR Green I, Sigma-Aldrich, Missouri, USA). For all samples, DNAg concentrations were > 2ng/μL and sufficient for analysis. Primers (343F et 803R) targeting the V3–V4 region of the 16S rRNA gene and a Metabiote

kit were used to prepare the amplicon library. Illumina MiSeq paired-end 2 × 250 bp (Illumina, San Diego, CA, USA) sequencing of the corresponding products was performed. On average, 66,333 full-length V3-V4 region of 16S rDNA sequences assembled at 97% nucleic identity were obtained. These full-length V3-V4 region of 16S rDNA sequences were qualitatively and quantitatively sufficient to allow affiliation, to obtain the taxonomic profiles of bacterial populations identified within the samples. The data were processed according to the QIIME pipeline. The pre-processing parameters included the removal of PCR primers and poor quality readings (score below Q30). Then, the minimum overlap area of 30 bases to perform reassociation was search, and finally 97% nucleic identity over the entire overlap area were assembled. Chimeric sequences were detected and eliminated among the full-length 16S rDNA sequences using an internal method based on application of the Usearch 6.1 software. Next, a clustering step was performed on group similar sequences using a defined nucleic identity threshold (97% identity for affiliation at the genus level on the targeted region of the 16S rRNA gene) using the Uclust v1.2.22q program. The number of sequences after preprocessing were available in Table S4. An open reference operational taxonomical unit (OTU) creation process and full-length binding method were used to create groups of sequences. The most abundant sequence of each OTU was then considered, this reference sequence was taxonomically compared to the Greengenes ver. 13_8 reference database (www.greengenes.gov) using the RDP v2.2 classifier. Rarefaction curves of observed OTUs indicating enough sequencing depth (Figure S4). Closed-reference OTUs were also created to allow the use of the PICRUSt software.