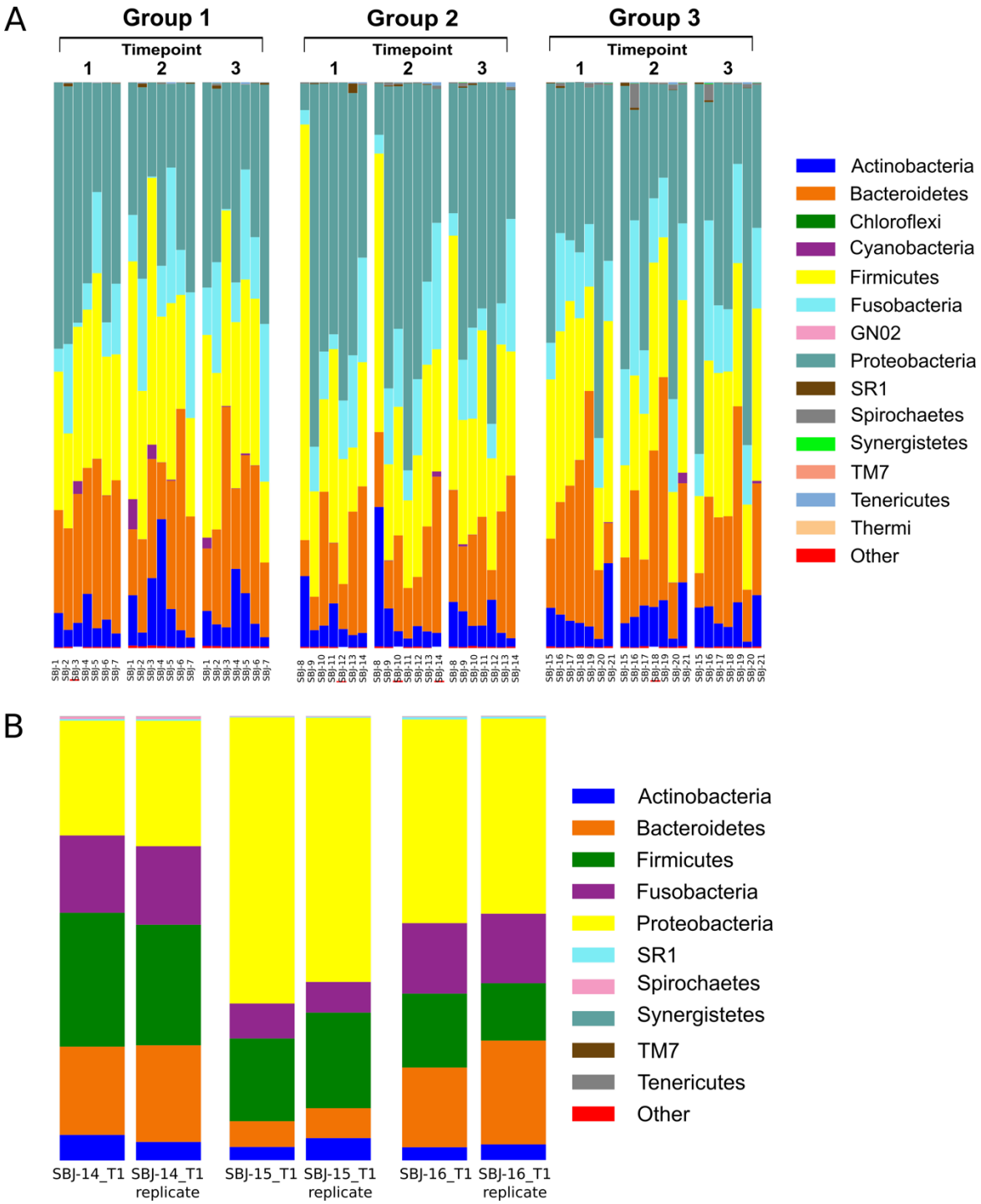
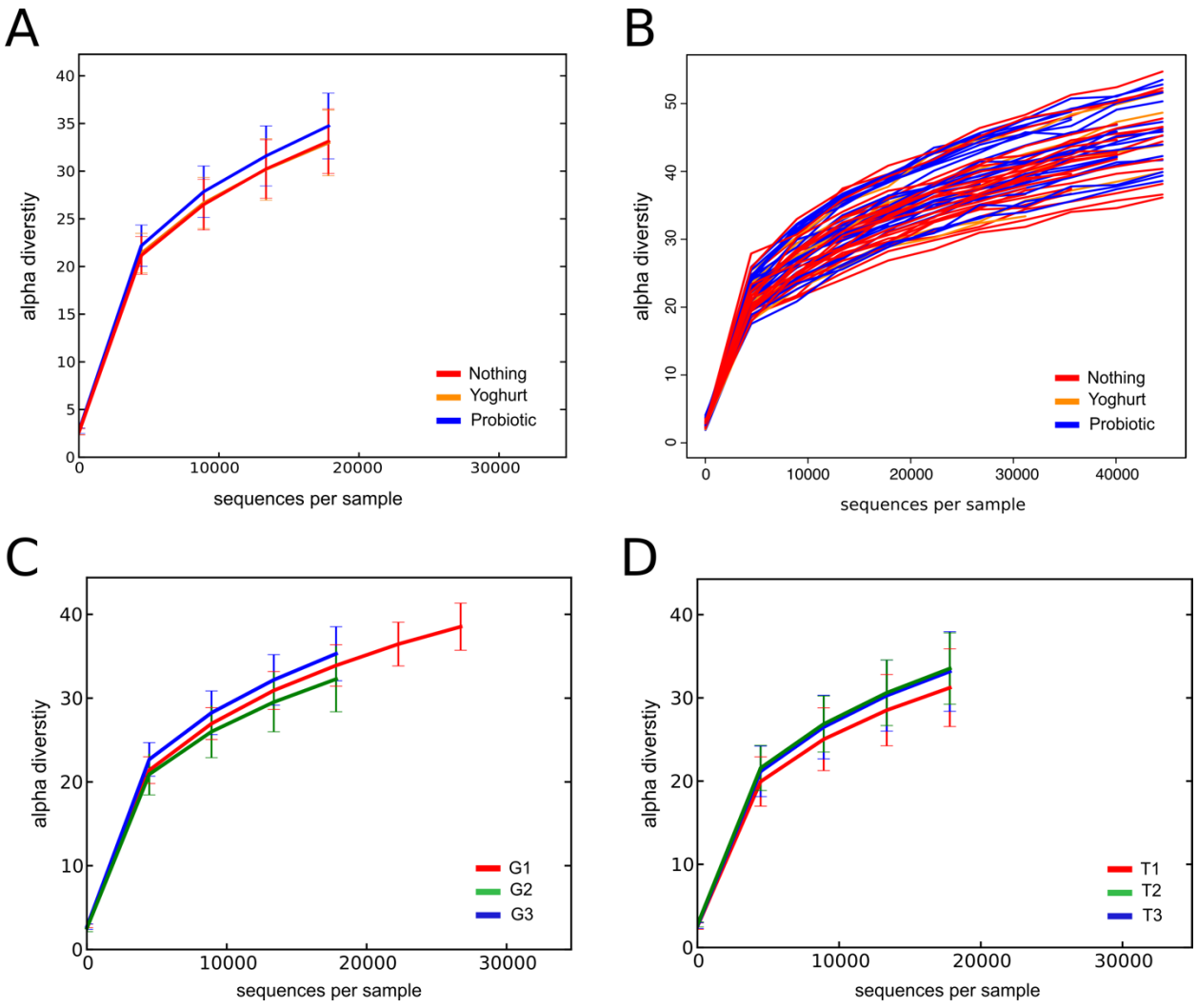
The short-term impact of probiotic consumption on the oral cavity microbiome

Erik Dassi[^1], Pamela Ferretti[^1], Giuseppina Covello[1], HTM-CMB-2015, Roberto Bertorelli[2], Michela A. Denti[1], Veronica De Sanctis[2], Adrian Tett[1], Nicola Segata*[,1]

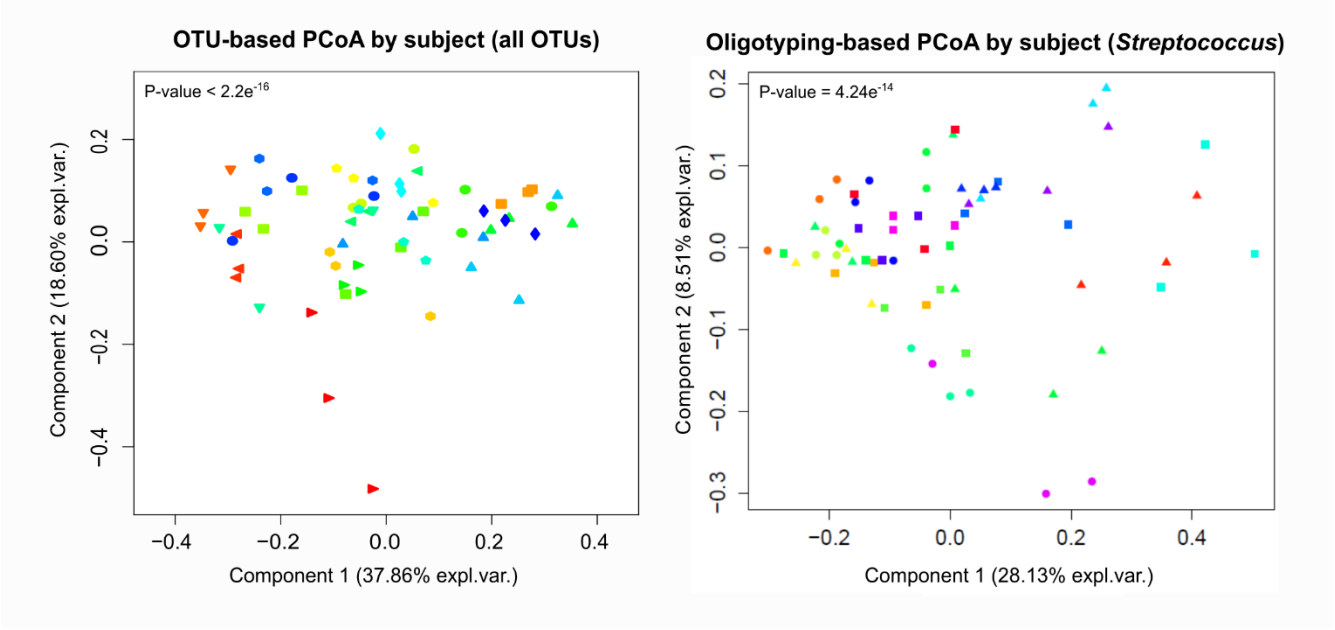
Supplementary Figures



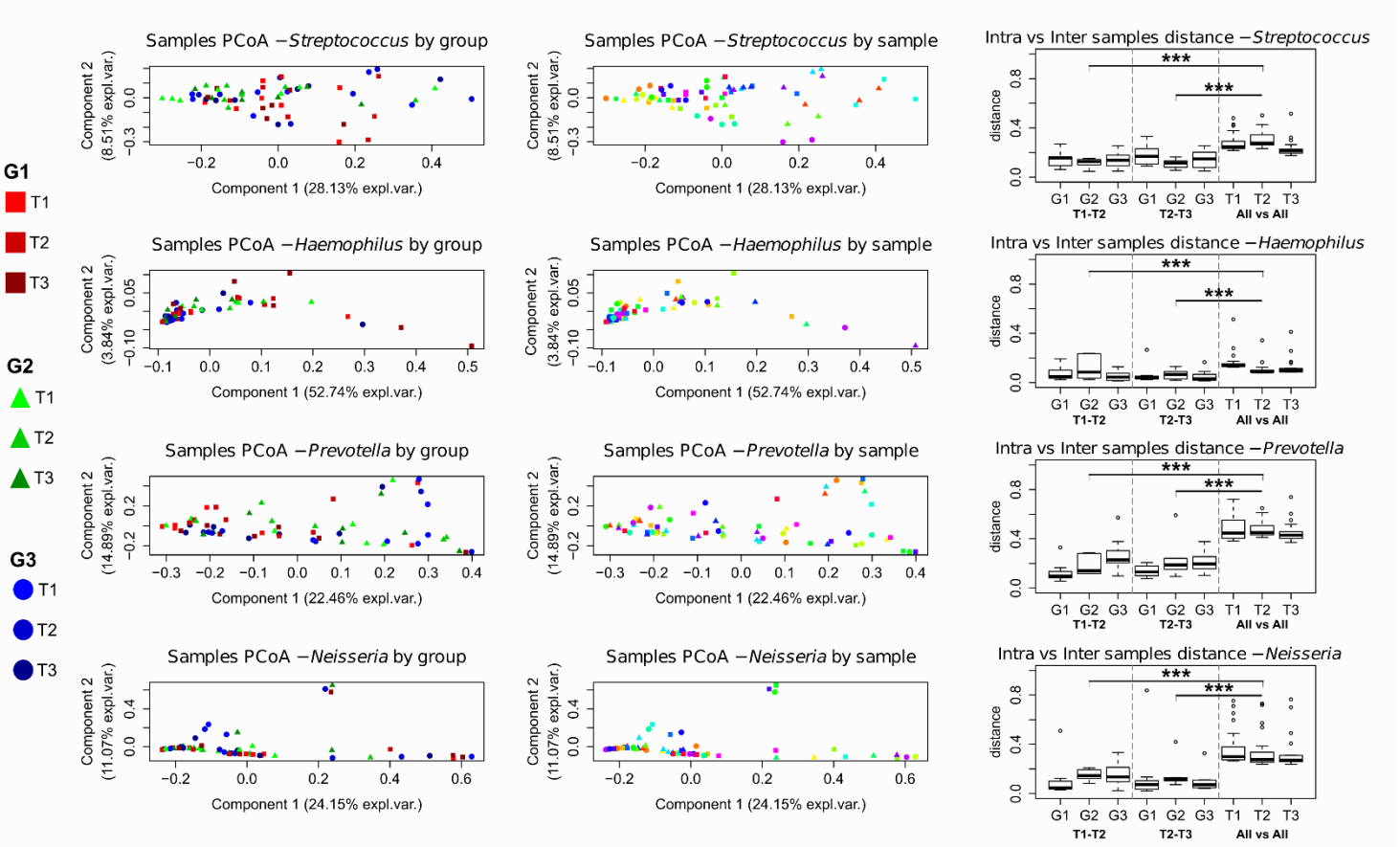
Supplementary Figure 1. (**A**) Taxonomic profiling of all samples at the phylum level. (**B**) Comparison of replicates taxonomic composition at the phyum level.



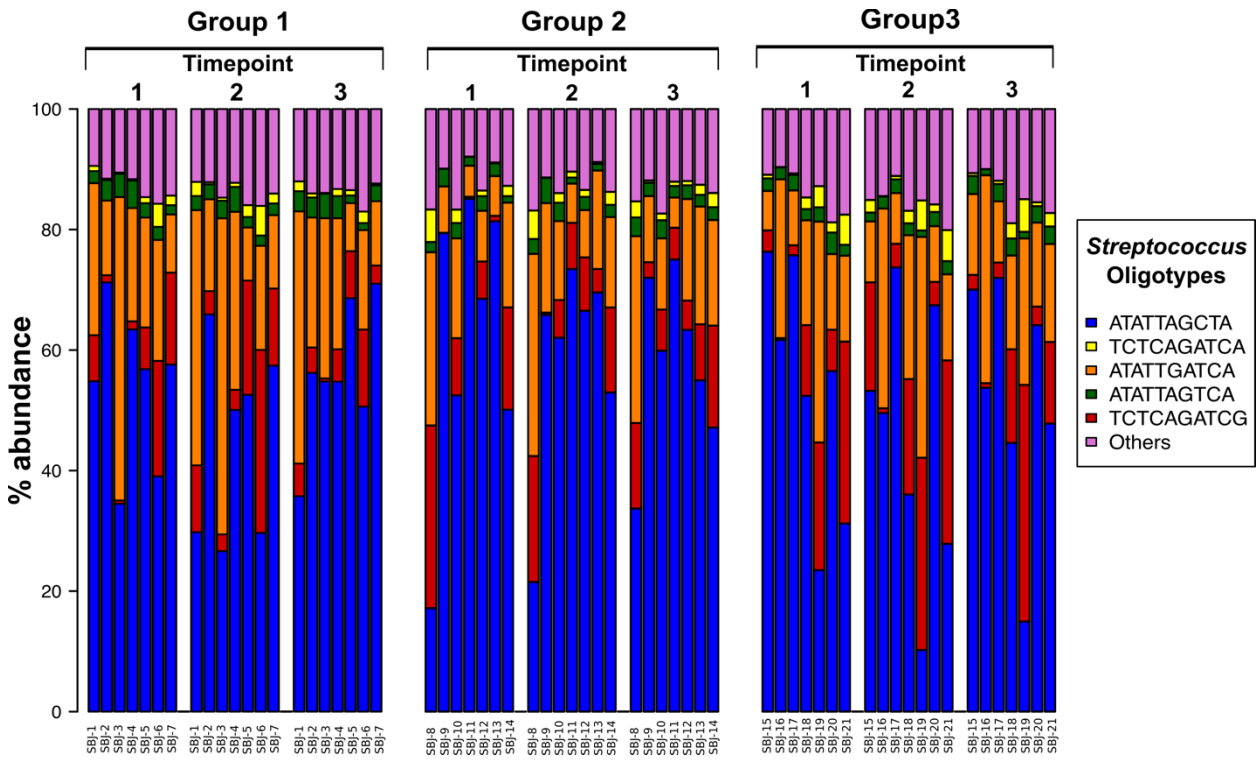
Supplementary Figure 2. Alpha rarefaction curves for the PD-Whole Tree metric, with samples colored by intake (**A** and **B**), by group (**C**), and timepoint (**D**).



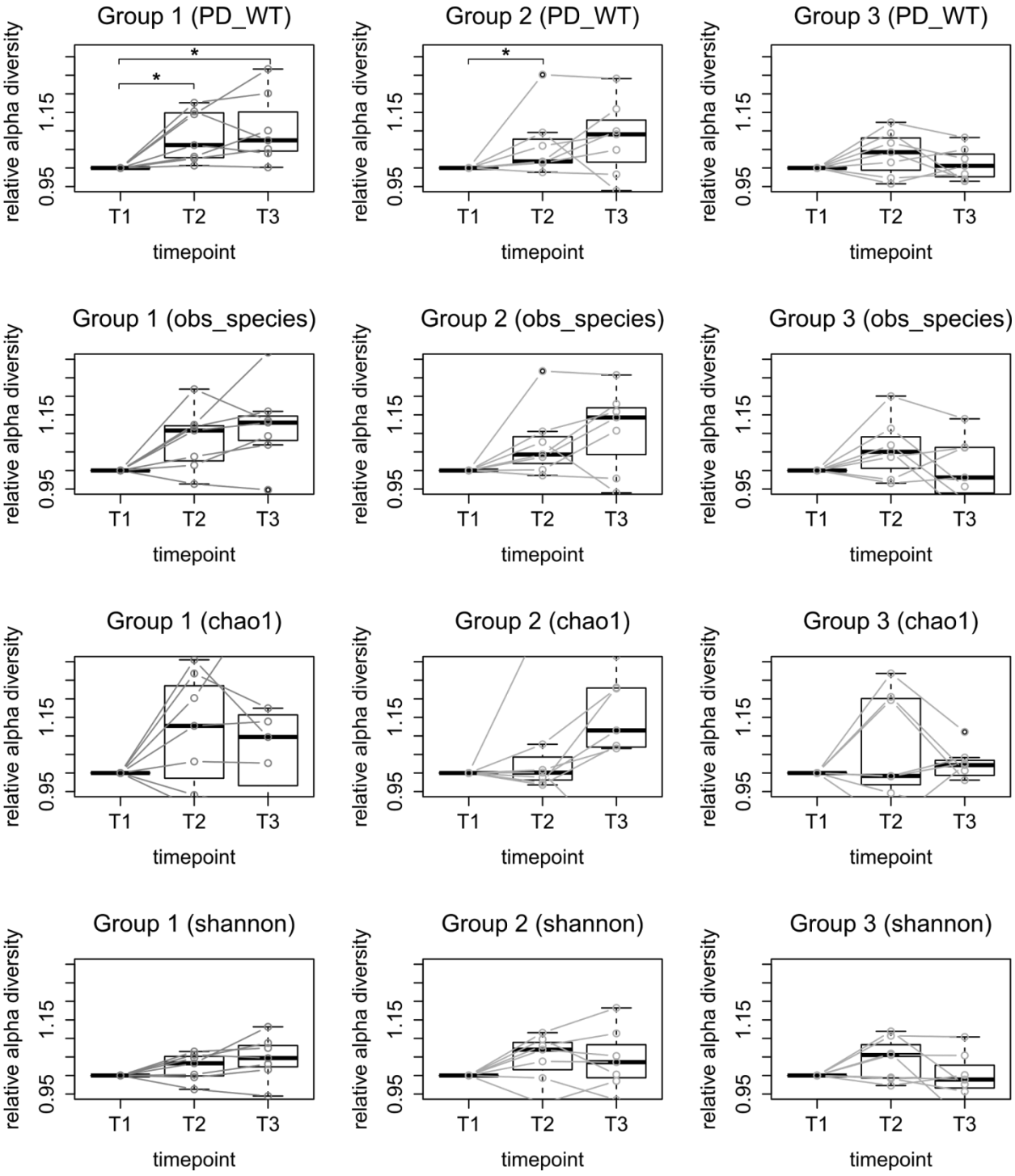
Supplementary Figure 3. OTU-based (including all OTUs, left) and oligotype-based PCoA by subject (including only *Streptococcus* oligotypes, right). OTU-based PCoA employs the weighted Unifrac metric while the oligotyping PCoA uses the Bray-Curtis metric.The proportion of explained variance for both principal components is indicated next to the component name. The indicated Wilcoxon test P-value represents the significance of the lower beta diversity observed within samples of the same subject with respect to samples of other subjects (intra subject vs inter subject beta diversity).



Supplementary Figure 4. Oligotype-based PCoA (Bray-Curtis metric) divided by group and sample (left and center columns), intra- and inter-samples distance comparison (right column) for the four main relevant genera (Streptococcus, Haemophilus, Prevotella and Neisseria). Intra-samples distance is defined as the distance between two time points of the same group, while the inter-samples distance is based on single timepoints across all groups. The proportion of explained variance for each principal component is indicated next to the component name. Distances diversity (Bray-Curtis metric) P-values are computed between intra-subject beta diversity and all diversity values (i.e. T1-T2 vs All, T2-T3 vs All) by using the Wilcoxon test (*** indicates P-value <.001).



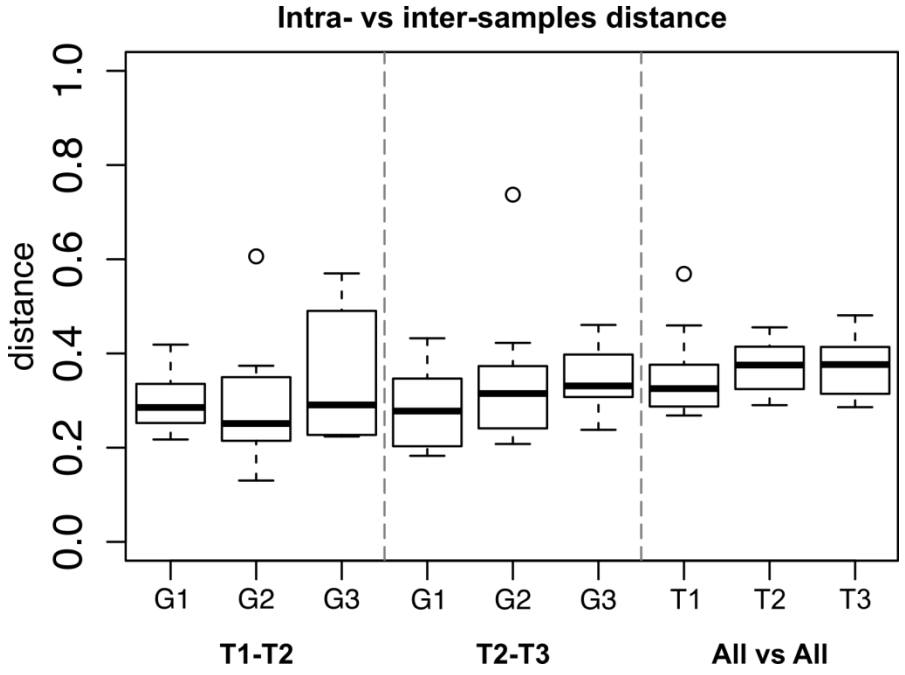
Supplementary Figure 5. Probiotic *Streptococcus* oligotypes across timepoints and study groups.


Group 1 (PD_WT)
Group 2 (PD_WT)
Group 3 (PD_WT)
Group 1 (obs_species)
Group 2 (obs_species)
Group 3 (obs_species)
Group 1 (chao1)
Group 2 (chao1)
Group 3 (chao1)
Group 1 (shannon)
Group 2 (shannon)
Group 3 (shannon)
relative alpha diversity
0.95
1.15
T1
T2
T3
timepoint
*

69

70

Supplementary Figure 6. Alpha diversity computed with four different metrics (PD_Whole Tree, Observed species, Chao1 and Shannon) on the three study groups (* for Wilcoxon test P-values <.05).



Supplementary Figure 7. OTU-based (beta diversity computed with the weighted Unifrac metric) inter-sample (difference between samples in each timepoint across groups) and intra-sample distances (variation of each group between timepoints). No statistically significant values were found.

Supplementary Tables

#SampleID	BarcodeSequence	Treatment	Timepoint	CombinedTreatmentTimepoint	Subject
S1	ACTTCCAACTTC	2	3	2_3	1
S2	TATACCGCTGCG	2	2	2_2	1
S3	GGCCAGTTCCTA	2	1	2_1	1
S4	GAGCCATCTGTA	1	3	1_3	2
S5	CAACTCCCGTGA	1	2	1_2	2
S6	CGAGCAATCCTA	1	1	1_1	2
S7	TAACGTGTGTGC	3	3	3_3	3
S8	TACAGCGCATAC	3	2	3_2	3
S9	CTGCTATTCCTC	3	1	3_1	4
S10	TGCGCTGAATGT	NA	1	NA_1	5
S11	ATGGGTTCCGTC	H2O	-	H2O_-	-
S12	ACGAGACTGATT	3	1	3_1	3
S13	CGTAATTGCCGC	3	1	3_1	6
S14	AGGCTTACGTGT	1	2	1_2	7
S15	TGTCGCAAATAG	1	1	1_1	7
S16	CATTCGTGGCGT	3	2	3_2	6
S17	TCGACATCTCTT	2	3	2_3	8
S18	AGTTACGAGCTA	H2O	-	H2O_-	-
S19	GCAACACCATCC	2	3	2_3	9
S20	GAATACCAAGTC	2	2	2_2	9
S21	GATCCCACGTAC	YOGURT	-	YOGURT_-	-
S22	GGACTTCCAGCT	1	3	1_3	7
S23	GTGGTGGTTTCC	3	3	3_3	6
S24	AGCGGAGGTTAG	2	1	2_1	9
S25	GATTCCGGCTCA	1	3	1_3	10
S26	GCTCGAAGATTC	1	2	1_2	10
S27	GTCGACAGAGGA	1	1	1_1	10
S28	TGTGAATTCGGA	NA	3	NA_3	11
S29	ACCATAGCTCCG	NA	2	NA_2	11
S30	AGATTGACCAAC	NA	1	NA_1	11
S31	CAAACAACAGCT	H2O	-	H2O_-	-
S32	ACCAGTGACTCA	NA	3	NA_3	12
S33	TTGGCTCTATTC	3	3	3_3	13
S34	GTTGTTCTGGGA	3	2	3_2	13

S35	GTACGATATGAC	3	1	3_1	13
S36	GTCGTGTAGCCT	NA	2	NA_2	12
S37	GTGTTGTCGTGC	3	1R	3_1R	14
S38	ACAATAGACACC	3	2	3_2	14
S39	CTATCTCCTGTC	3	3	3_3	14
S40	AAGGCGCTCCTT	-	-	-_-	-
S41	TACGAGCCCTAA	-	-	-_-	-
S42	GTATCTGCGCGT	-	-	-_-	-
S43	CCAATACGCCTG	-	-	-_-	-
S44	AATTGTGTCGGA	-	-	-_-	-
S45	TGTAACGCCGAT	H2O	-	H2O_-	-
S46	GTTCTCTTCTCG	H2O	-	H2O_-	-
S47	AACTAGTTCAGG	3	1	3_1	14
S48	ATCACCAGGTGT	-	-	-_-	-
S49	TCTCTACCACTC	2	1R	2_1R	15
S50	CATCCCTCTACT	2	1	2_1	15
S51	TACTACGTGGCC	H2O	-	H2O_-	-
S52	GAACACTTTGGA	1	3	1_3	16
S53	GCATATGCACTG	1	2	1_2	16
S54	GCGATATATCGC	1	1R	1_1R	16
S55	GTAGATCGTGTA	1	1	1_1	16
S56	ATCCTTTGGTTC	2	2	2_2	8
S57	CTCACAACCGTG	PROBIO	-	PROBIO_-	-
S58	TAGTATGCGCAA	2	3	2_3	15
S59	GGTGACTAGTTC	2	2	2_2	15
S60	TCCCTTGTCTCC	2	1	2_1	8
S61	CCACAGATCGAT	1	3	1_3	17
S62	CGGTCAATTGAC	1	2	1_2	17
S63	ACTCACAGGAAT	1	1	1_1	17
S64	TAATACGGATCG	1	3	1_3	18
S65	CACTACGCTAGA	1	2	1_2	18
S66	CGAGGGAAAGTC	1	1	1_1	18
S67	GATCTGCGATCC	2	3	2_3	19
S68	TGCATACACTGG	2	2	2_2	19
S69	AGCAGAACATCT	2	2	2_2	20
S70	CGTAAGATGCCT	2	1	2_1	20
S71	ATTCTGCCGAAG	H2O	-	H2O_-	-
S72	TGGTCAACGATA	2	1	2_1	19
S73	TATCGACACAAG	3	1	3_1	21

S74	GTGGAGTCTCAT	1	2	1_2	22
S75	ATGATGAGCCTC	1	1	1_1	22
S76	TCGGAATTAGAC	3	1	3_1	23
S77	TGCAGTCCTCGA	1	3	1_3	22
S78	CAAATTCGGGAT	H2O	-	H2O_-	-
S79	CAGCTCATCAGC	2	3	2_3	20
S80	AGTCGAACGAGG	3	3	3_3	23
S81	TGGAGTAGGTGG	NA	1	NA_1	12
S82	GCGTTCTAGCTG	3	3	3_3	21
S83	AGCATGTCCCGT	3	2	3_2	21
S84	ATCGCACAGTAA	3	2	3_2	23
S85	CTCACCTAGGAA	-	-	-_-	-
S86	AGTTGAGGCATT	NA	3	NA_3	24
S87	GATGTTCGCTAG	2	3	2_3	25
S88	TTGGGTACACGT	3	3	3_3	4
S89	TTGCGTTAGCAG	NA	2	NA_2	24
S90	AGTCGTGCACAT	2	2	2_2	25
S91	CATTATGGCGTG	3	2	3_2	4
S92	ACCGGTATGTAC	NA	1	NA_1	24
S93	ATGTCACCGCTG	-	-	-_-	-
S94	ATGGCTGTCAGT	-	-	-_-	-
S95	TAGGCATGCTTG	-	-	-_-	-
S96	GCTGTACGGATT	2	1	2_1	25

Supplementary Table 1. Complete list of sample-specific barcodes

Sample	Reads number
S1	50526
S10	46977
S11	6665
S12	51079
S13	20495
S14	27616
S15	54144
S16	52966
S17	48616
S18	5689

S19	27388
S2	35639
S20	45784
S21	11971
S22	57370
S23	38848
S24	48008
S25	44994
S26	45549
S27	37001
S28	49413
S29	42653
S3	46768
S30	53523
S31	8907
S32	43243
S33	50998
S34	45130
S35	44395
S36	36871
S37	25443
S38	45526
S39	18892
S4	43149
S45	5094
S46	9432
S47	38238
S49	28819
S5	44005
S50	53621
S51	3454
S52	49281
S53	53320
S54	39694
S55	54313
S56	46405
S57	56272
S58	46044
S59	54384

S6	42087
S60	54205
S61	45554
S62	51480
S63	36904
S64	33864
S65	44308
S66	32996
S67	54979
S68	19663
S69	54256
S7	40795
S70	45037
S71	1319
S72	55541
S73	36903
S74	50243
S75	49103
S76	46690
S77	43081
S78	5738
S79	49973
S8	39161
S80	48575
S81	24173
S82	38928
S83	42462
S84	40829
S86	35517
S87	45406
S88	50658
S89	44453
S9	38436
S90	45953
S91	27628
S92	38070
S96	32260

106

Supplementary Table 2. Total million reads per sample