Supplementary Material

Impact of host DNA and sequencing depth on the taxonomic resolution of whole metagenome sequencing for microbiome analysis

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Supplementary Table S1. Composition of the mock microbial community (B: HM-277D, Staggered, High Concentration, v5.2H), showing the expected and the observed relative abundance of species after WMS. The ratio of observed to expected relative abundance of species is also shown. Grey shading indicates a ≥ 2-fold change**.**

Microbial species	**NCBI assembly accession**	**16S rRNA copies**	**16S rRNA copies per genome [1]**	**No. genome copies**	**GC content [2] (%)**	**Expected relative abundance [3] (%)**	**Observed relative abundance (%)**	**Ratio observed/ expected**
Rhodobacter sphaeroides ATCC 17023	GCF_003324715.1	10,000,000	3	3,333,333	69	34.43	12.79	0.37
Streptococcus mutans ATCC 700610	GCF_000007465.2	10,000,000	5	2,000,000	37	20.66	36.21	1.75
Staphylococcus epidermidis ATCC 12228	GCF_000007645.1	10,000,000	5	2,000,000	32	20.66	27.10	1.31
Escherichia coli ATCC 700926	GCF_002843685.1	10,000,000	7	1,428,571	51	14.75	10.81	0.73
Pseudomonas aeruginosa ATCC 47085	GCF_000006765.1	1,000,000	4	250,000	67	2.58	1.47	0.57
Staphylococcus aureus ATCC BAA-1717	GCF_000017085.1	1,000,000	5	200,000	33	2.07	4.27	2.07
Streptococcus agalactiae ATCC BAA-611	GCF_000007265.1	1,000,000	7	142,857	36	1.48	2.80	1.90
Bacillus cereus ATCC 10987	GCF_000008005.1	1,000,000	12	83,333	36	0.86	0.99	1.14
Clostridium beijerinckii ATCC 51743	GCF_000016965.1	1,000,000	14	71,429	30	0.74	1.14	1.55
Helicobacter pylori ATCC 700392	GCF_000307795.1	100,000	2	50,000	39	0.52	0.81	1.58
Propionibacterium acnes DSM 16379	GCF_000008345.1	100,000	3	33,333	60	0.34	0.19	0.55
Neisseria meningitidis ATCC BAA-335	GCF_000008805.1	100,000	4	25,000	52	0.26	0.20	0.77
Acinetobacter baumannii ATCC 17978	GCF_001593425.2	100,000	6	16,667	39	0.17	0.19	1.13
Listeria monocytogenes ATCC BAA-679	GCF_000196035.1	100,000	6	16,667	38	0.17	0.16	0.93
Lactobacillus gasseri ATCC 33323	GCF_000014425.1	100,000	6	16,667	35	0.17	0.23	1.32
Actinomyces odontolyticus ATCC 17982	GCF_000154225.1	10,000	2	5,000	65	0.05	0.02	0.44
Deinococcus radiodurans ATCC 13939	GCF_001638825.1	10,000	3	3,333	67	0.03	0.02	0.56
Streptococcus pneumoniae ATCC BAA-334	GCF_000006885.1	10,000	4	2,500	40	0.03	0.04	1.55
Enterococcus faecalis ATCC 47077	GCF_000172575.2	10,000	4	2,500	38	0.03	0.04	1.57
Bacteroides vulgatus ATCC 8482	GCF_000012825.1	10,000	7	1,429	42	0.01	0.02	1.65

[1] Ribosomal RNA Database Curated by the Schmidt Laboratory (https://rrndb.umms.med.umich.edu/search/);
[2] NCBI genome database (https://www.ncbi.nlm.nih.gov/genome/genomes/714);
[3] Expected relative abundance = No. genome copies of each species/ sum of genome copies of all species.

Supplementary Table S2. Summary of the sequencing data pre-processing of synthetic samples (SS) metagenomes.

Sample	Total number of raw single-end reads	Total number of quality-filtered reads	Total number of quality-filtered and host decontaminated reads
Microbial sample (MS)	39,682,202	33,309,964	33,309,243
Synthetic sample with 10% host DNA (SS10)	40,087,736	32,489,550	29,899,628
Synthetic sample with 90% host DNA (SS90)	50,846,240	41,297,894	5,535,588
Synthetic sample with 99% host DNA (SS99)	36,098,546	30,214,162	746,018

Supplementary Table S3. Ratio of relative abundances of species from synthetic samples (SS) to MS. Grey shading indicates a ≥ 2-fold change**.**

Microbial species	16S rRNA copies	MS	SS10/MS	SS90/MS	SS99/MS
Rhodobacter sphaeroides ATCC 17023	10,000,000	1	0.805	0.814	1.053
Streptococcus mutans ATCC 700610	10,000,000	1	1.081	1.061	0.877
Staphylococcus epidermidis ATCC 12228	10,000,000	1	1.069	1.053	0.776
Escherichia coli ATCC 700926	10,000,000	1	0.816	0.774	0.886
Pseudomonas aeruginosa ATCC 47085	1,000,000	1	0.742	0.798	0.714
Staphylococcus aureus ATCC BAA-1717	1,000,000	1	1.106	1.085	0.796
Streptococcus agalactiae ATCC BAA-611	1,000,000	1	1.048	1.022	0.711
Bacillus cereus ATCC 10987	1,000,000	1	1.021	1.237	0.574
Clostridium beijerinckii ATCC 51743	1,000,000	1	1.064	1.070	0.589
Helicobacter pylori ATCC 700392	100,000	1	0.911	0.907	0.561
Propionibacterium acnes DSM 16379	100,000	1	0.625	0.875	0
Neisseria meningitidis ATCC BAA-335	100,000	1	0.952	1.011	0.169
Acinetobacter baumannii ATCC 17978	100,000	1	0.967	0.962	0
Listeria monocytogenes ATCC BAA-679	100,000	1	0.943	0.824	0
Lactobacillus gasseri ATCC 33323	100,000	1	1.091	0.910	0
Actinomyces odontolyticus ATCC 17982	10,000	1	0.812	0.435	0
Deinococcus radiodurans ATCC 13939	10,000	1	0.626	0	0
Streptococcus pneumoniae ATCC BAA-334	10,000	1	1.006	0.608	0
Enterococcus faecalis ATCC 47077	10,000	1	1.065	0.310	0
Bacteroides vulgatus ATCC 8482	10,000	1	0.935	0.181	0

Supplementary Table S4. Ratio of relative abundances of species from each SS90 subset (SS90D50, SS90D25, SS90D10, SS90D5) to the SS90 original dataset (SS90D100). Random subsampling to generate each subset was performed in five independent experiments. Grey shading indicates a ≥ 2-fold change. nd: not detected in the SS90 original dataset.

Microbial Species	16S rRNA copies	SS90D100	SS90D50/ SS90D100	SS90D25/ SS90D100	SS90D10/ SS90D100	SS90D5/ SS90D100
Streptococcus mutans ATCC 700610	10,000,000	1	1.004	0.999	1.002	0.625
Staphylococcus epidermidis ATCC 12228	10,000,000	1	1.004	1.023	1.037	0.621
Rhodobacter sphaeroides ATCC 17023	10,000,000	1	0.995	0.995	1.019	0.585
Escherichia coli ATCC 700926	10,000,000	1	1.029	1.009	0.961	0.622
Staphylococcus aureus ATCC BAA-1717	1,000,000	1	1.008	0.997	0.989	0.588
Streptococcus agalactiae ATCC BAA-611	1,000,000	1	0.988	0.994	0.936	0.568
Pseudomonas aeruginosa ATCC 47085	1,000,000	1	0.950	0.922	0.794	0.329
Clostridium beijerinckii ATCC 51743	1,000,000	1	0.990	0.975	0.864	0.382
Bacillus cereus ATCC 10987	1,000,000	1	1.000	0.950	0.971	0.465
Helicobacter pylori ATCC 700392	100,000	1	0.957	0.964	0.776	0.257
Lactobacillus gasseri ATCC 33323	100,000	1	0.949	0.677	0.105	0
Neisseria meningitidis ATCC BAA-335	100,000	1	0.994	0.811	0.328	0
Acinetobacter baumannii ATCC 17978	100,000	1	0.861	0.690	0.156	0
Propionibacterium acnes DSM 16379	100,000	1	0.871	0.772	0.011	0
Listeria monocytogenes ATCC BAA-679	100,000	1	0.850	0.483	0	0
Enterococcus faecalis ATCC 47077	10,000	1	0.090	0	0	0
Streptococcus pneumoniae ATCC BAA-334	10,000	1	0.271	0	0	0
Bacteroides vulgatus ATCC 8482	10,000	1	0	0	0	0
Actinomyces odontolyticus ATCC 17982	10,000	1	0	0	0	0
Deinococcus radiodurans ATCC 13939	10,000	nd	nd	nd	nd	nd

Supplementary Table S5. Statistical analysis (*P*-values) of the results presented in Table S4. The Kruskal-Wallis non-parametric test followed by multiple comparisons (SS90D50, SS90D25, SS90D10, or SS90D5) versus a control group (SS90D100) using Dunn's test was performed for each species. NA: not applicable, as it was not detected in the SS90 original dataset.

Microbial species	16S rRNA copies	SS90D100 vs. SS90D50	SS90D100 vs. SS90D25	SS90D100 vs. SS90D10	SS90D100 vs. SS90D5
Streptococcus mutans ATCC 700610	10,000,000	> 0.9999	> 0.9999	> 0.9999	0.0203
Staphylococcus epidermidis ATCC 12228	10,000,000	> 0.9999	0.2548	0.0341	0.7829
Rhodobacter sphaeroides ATCC 17023	10,000,000	> 0.9999	> 0.9999	> 0.9999	0.0102
Escherichia coli ATCC 700926	10,000,000	0.9766	> 0.9999	> 0.9999	0.1242
Staphylococcus aureus ATCC BAA-1717	1,000,000	> 0.9999	> 0.9999	> 0.9999	0.0203
Streptococcus agalactiae ATCC BAA-611	1,000,000	0.8729	> 0.9999	0.0382	0.0004
Pseudomonas aeruginosa ATCC 47085	1,000,000	> 0.9999	> 0.9999	0.0231	0.0004
Clostridium beijerinckii ATCC 51743	1,000,000	> 0.9999	0.6831	0.223	0.0009
Bacillus cereus ATCC 10987	1,000,000	> 0.9999	> 0.9999	> 0.9999	0.0048
Helicobacter pylori ATCC 700392	100,000	> 0.9999	> 0.9999	0.0292	0.0006
Lactobacillus gasseri ATCC 33323	100,000	> 0.9999	0.1874	0.0025	0.0004
Neisseria meningitidis ATCC BAA-335	100,000	> 0.9999	0.7944	0.0242	0.0009
Acinetobacter baumannii ATCC 17978	100,000	0.9033	0.2575	0.0024	0.0003
Propionibacterium acnes DSM 16379	100,000	> 0.9999	0.6463	0.0015	0.0015
Listeria monocytogenes ATCC BAA-679	100,000	> 0.9999	0.1586	0.0006	0.0006
Enterococcus faecalis ATCC 47077	10,000	0.006	0.0007	0.0007	0.0007
Streptococcus pneumoniae ATCC BAA-334	10,000	0.4097	0.0011	0.0011	0.0011
Bacteroides vulgatus ATCC 8482	10,000	0.0005	0.0005	0.0005	0.0005
Actinomyces odontolyticus ATCC 17982	10,000	0.0005	0.0005	0.0005	0.0005
Deinococcus radiodurans ATCC 13939	10,000	NA	NA	NA	NA

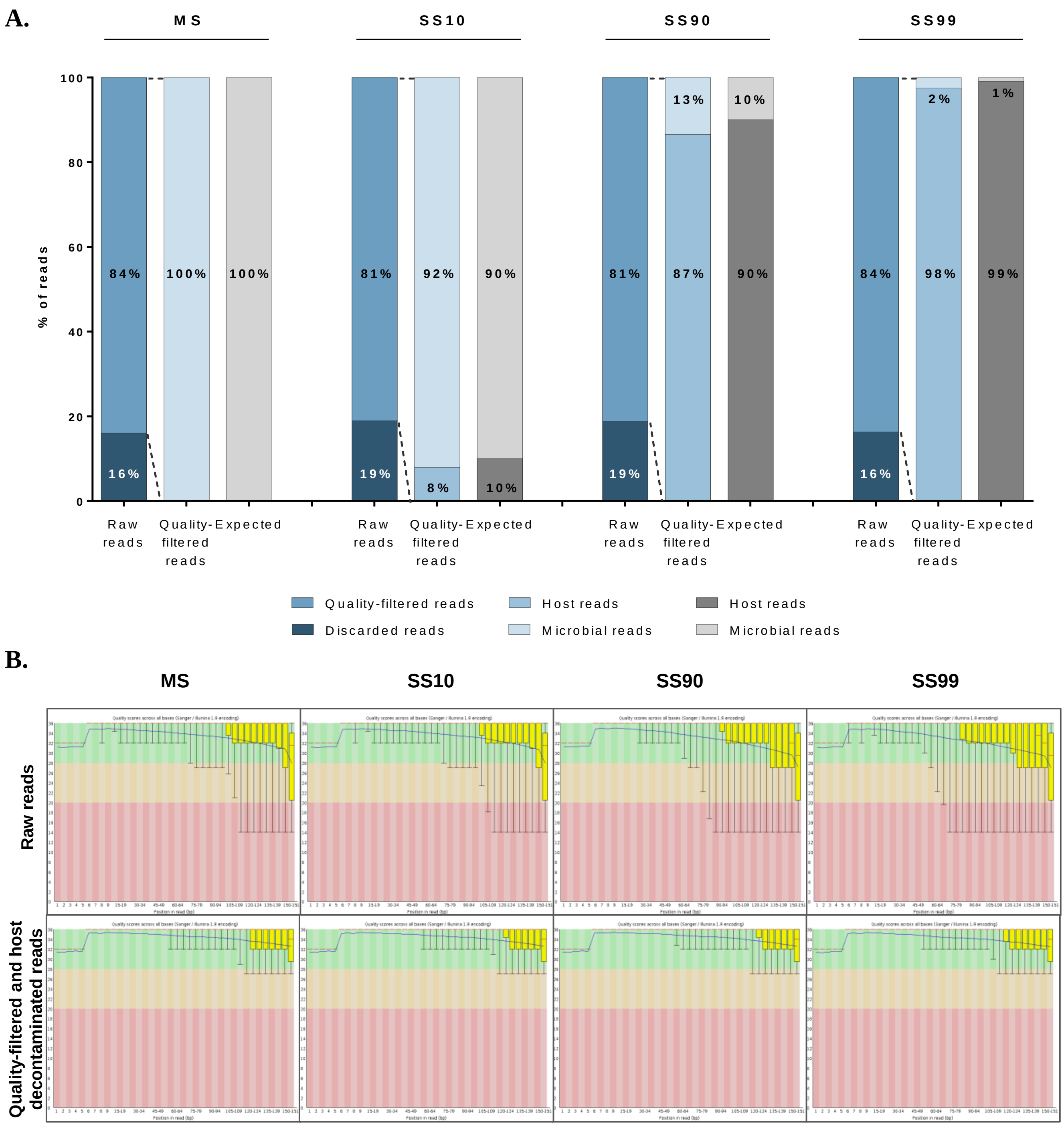
Supplementary Table S6. Ratio of relative abundances of species from each simulated dataset (SD) to MS dataset. Grey shading indicates a ≥ 2-fold change. Random subsampling to generate each simulated dataset was performed in five independent experiments.

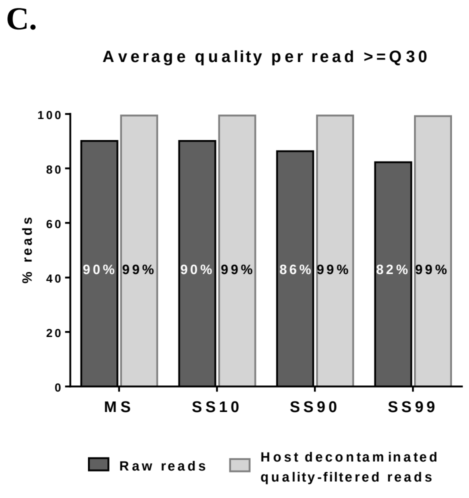
Microbial Species	**16S rRNA copies**	**MS**	**SD10 /MS**	**SD20 /MS**	**SD30 /MS**	**SD40 /MS**	**SD50 /MS**	**SD60 /MS**	**SD70 /MS**	**SD80 /MS**	**SD90 /MS**	**SD91 /MS**	**SD92 /MS**	**SD93 /MS**	**SD94 /MS**	**SD95 /MS**	**SD96 /MS**	**SD97 /MS**	**SD98 /MS**	**SD99 /MS**
Streptococcus mutans	10,000,000	1	1.001	1.003	0.999	1.001	0.999	0.994	0.998	1.002	0.995	1.006	0.997	1.011	1.008	1.011	1.015	0.639	0.590	0.584
Staphylococcus epidermidis	10,000,000	1	1.001	1.005	1.003	1.005	1.005	1.006	1.002	1.005	1.015	1.002	1.003	1.027	1.013	1.005	1.038	0.659	0.572	0.603
Rhodobacter sphaeroides	10,000,000	1	1.002	0.993	0.996	1.000	1.001	1.001	0.998	0.994	0.988	1.000	1.005	0.985	1.001	0.983	1.005	0.638	0.570	0.572
Escherichia coli	10,000,000	1	1.003	1.014	0.997	0.987	0.974	0.987	1.008	0.960	0.913	0.946	0.965	0.911	0.982	0.959	0.940	0.597	0.520	0.499
Staphylococcus aureus	1,000,000	1	1.001	1.008	1.007	1.010	1.015	1.001	1.003	0.996	1.009	1.017	0.986	0.999	0.983	0.981	0.989	0.620	0.530	0.525
Streptococcus agalactiae	1,000,000	1	0.998	1.004	1.002	0.998	0.994	1.000	0.996	1.007	1.014	0.966	0.960	0.977	0.941	0.971	0.959	0.601	0.532	0.395
Pseudomonas aeruginosa	1,000,000	1	0.977	0.994	0.980	0.997	0.990	1.009	0.985	0.982	0.920	0.880	0.918	0.879	0.902	0.809	0.919	0.478	0.411	0.139
Clostridium beijerinckii	1,000,000	1	0.989	0.999	1.007	1.003	0.979	0.979	0.996	0.944	0.946	0.943	1.000	0.920	0.901	0.911	0.911	0.538	0.484	0.304
Bacillus cereus	1,000,000	1	1.039	0.977	1.037	0.978	1.036	1.023	0.991	1.003	0.903	0.979	1.065	0.997	1.011	1.015	0.851	0.698	0.568	0.435
Helicobacter pylori	100,000	1	0.992	0.982	0.990	0.990	0.955	0.963	0.939	0.923	0.909	0.953	0.967	0.851	0.806	0.827	0.798	0.435	0.301	0.072
Lactobacillus gasseri	100,000	1	0.941	0.939	0.919	0.924	0.910	0.903	0.906	0.890	0.698	0.588	0.509	0.413	0.424	0.305	0.295	0.033	0	0
Neisseria meningitidis	100,000	1	1.026	1.097	1.034	1.051	1.043	1.006	1.067	0.988	0.950	0.929	0.892	0.875	0.884	0.670	0.710	0.113	0.045	0
Acinetobacter baumannii	100,000	1	0.978	0.957	0.971	0.951	0.963	0.874	0.888	0.744	0.533	0.675	0.601	0.485	0.397	0.241	0.123	0	0	0
Propionibacterium acnes	100,000	1	0.780	0.830	0.749	0.674	0.843	0.760	0.712	0.858	0.676	0.736	0.659	0.460	0.320	0.381	0.078	0.034	0	0
Listeria monocytogenes	100,000	1	0.895	0.932	0.931	0.975	0.979	0.883	0.948	0.875	0.515	0.573	0.479	0.576	0.292	0.216	0.106	0	0	0
Enterococcus faecalis	10,000	1	0.858	0.820	0.837	0.705	0.820	0.588	0.325	0.210	0	0	0	0	0	0	0	0	0	0
Streptococcus pneumoniae	10,000	1	0.885	0.757	0.848	0.852	0.641	0.711	0.532	0.177	0	0	0	0	0	0	0	0	0	0
Bacteroides vulgatus	10,000	1	0.819	0.782	0.627	0.699	0.581	0.505	0.483	0.030	0	0	0	0	0	0	0	0	0	0
Actinomyces odontolyticus	10,000	1	0.778	0.655	0.685	0.538	0.319	0.157	0.015	0	0	0	0	0	0	0	0	0	0	0
Deinococcus radiodurans	10,000	1	0.704	0.831	0.662	0.523	0.428	0.212	0	0	0	0	0	0	0	0	0	0	0	0

Supplementary Table S7. Statistical analysis (*P*-values) of the results presented in Table S6. The Kruskal-Wallis non-parametric test followed by multiple comparisons (SD10, SD20, SD30, SD40, SD50, SD60, SD70, SD80, SD90, SD91, SD92, SD93, SD94, SD95, SD96, SD97, SD98 or SD99) versus a control group (MS) using Dunn's test was performed for each species.

Microbial species	**16S rRNA copies**	**MS vs. SD10**	**MS vs. SD20**	**MS vs. SD30**	**MS vs. SD40**	**MS vs. SD50**	**MS vs. SD60**	**MS vs. SD70**	**MS vs. SD80**	**MS vs. SD90**	**MS vs. SD91**	**MS vs. SD92**	**MS vs. SD93**	**MS vs. SD94**	**MS vs. SD95**	**MS vs. SD96**	**MS vs. SD97**	**MS vs. SD98**	**MS vs. SD99**
Streptococcus mutans	10,000,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.172	0.2236	0.1963
Staphylococcus epidermidis	10,000,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.1454	>0.999	>0.999	0.1608	>0.999	>0.999	>0.999
Rhodobacter sphaeroides	10,000,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.8504	0.1066	0.0994
Escherichia coli	10,000,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.0832	0.0195	0.0166
Staphylococcus aureus	1,000,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.6271	0.3169	0.3169
Streptococcus agalactiae	1,000,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.2796	>0.999	0.6096	0.0412	0.018	0.0053
Pseudomonas aeruginosa	1,000,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.7215	>0.999	>0.999	0.3926	>0.999	0.0747	>0.999	0.0085	0.0038	0.0007
Clostridium beijerinckii	1,000,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.8975	>0.999	0.5757	>0.999	0.0153	0.0105	0.0028
Bacillus cereus	1,000,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.3371	0.4981	0.1312
Helicobacter pylori	100,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.8736	>0.999	0.7417	>0.999	>0.999	0.9721	0.0462	0.2796	0.1899	0.0015	0.0005	0.0002
Lactobacillus gasseri	100,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.4485	0.0702	0.0371	0.012	0.0154	0.0044	0.0039	0.0001	<0.0001	<0.0001
Neisseria meningitidis	100,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.1467	0.1057	0.081
Acinetobacter baumannii	100,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.9099	0.1078	0.4353	0.1922	0.0841	0.0305	0.0078	0.0019	0.0002	0.0002	0.0002
Propionibacterium acnes	100,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.7519	0.0449	0.0154	0.0181	0.0004	0.0002	0.0001	0.0001
Listeria monocytogenes	100,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.1784	0.3442	0.1697	0.7687	0.0247	0.0123	0.0035	0.0005	0.0005	0.0005
Enterococcus faecalis	10,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.5228	0.1474	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007
Streptococcus pneumoniae	10,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.7679	0.1013	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005
Bacteroides vulgatus	10,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.9671	0.0035	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008	0.0008
Actinomyces odontolyticus	10,000	>0.999	>0.999	>0.999	>0.999	>0.999	>0.999	0.0036	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006	0.0006
Deinococcus radiodurans	10,000	>0.999	>0.999	>0.999	>0.999	>0.999	0.6434	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014

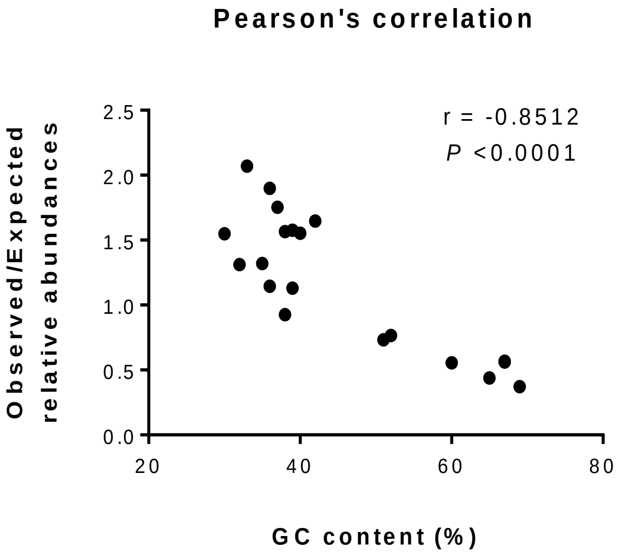
Supplementary Figure S1.





Supplementary Figure S1. Overview of the sequencing data pre-processing from synthetic samples metagenomes. A. Summary view of the fraction of quality-filtered, discarded, microbial and host reads per sample. The *raw reads* bar represents the percentage of quality-filtered and discarded reads upon Trimmomatic quality filtering. The *quality-filtered reads* bar constitutes the fraction of host and microbial quality-filtered reads obtained after performing both quality-filtering and host sequences decontamination steps. The *expected* bar consists in the theoretical percentages of host and microbial reads expected in each synthetic sample. **B.** FastQC graphs showing per base sequence quality in the raw sequence data and in the host decontaminated quality-filtered data from the MS, SS10, SS90 and SS99. Quality scores values across all bases at each position are shown. **C.** Percentage of reads with an average quality of ≥Q30 in the MS, SS10, SS90 and SS99, in the raw and host decontaminated quality-filtered data.

Supplementary Figure S2.



Supplementary Figure S2. Correlation between genomic GC content and ratio of observed to expected relative abundances for the MS reference sample. Pearson's correlation coefficient (r) and *P*-value for the association are shown in the graph. Since it has been shown that the GC content introduces a bias during Illumina Nextera XT library preparation and sequencing (Jones *et al.* 2015), this potential bias was evaluated in the MS dataset. Pearson's correlation analysis showed that the GC content was negatively correlated with the ratio of observed to expected relative abundances (r = -0.8512, *P* <0.0001). This suggests that under and overestimated relative abundances were due to this bias.