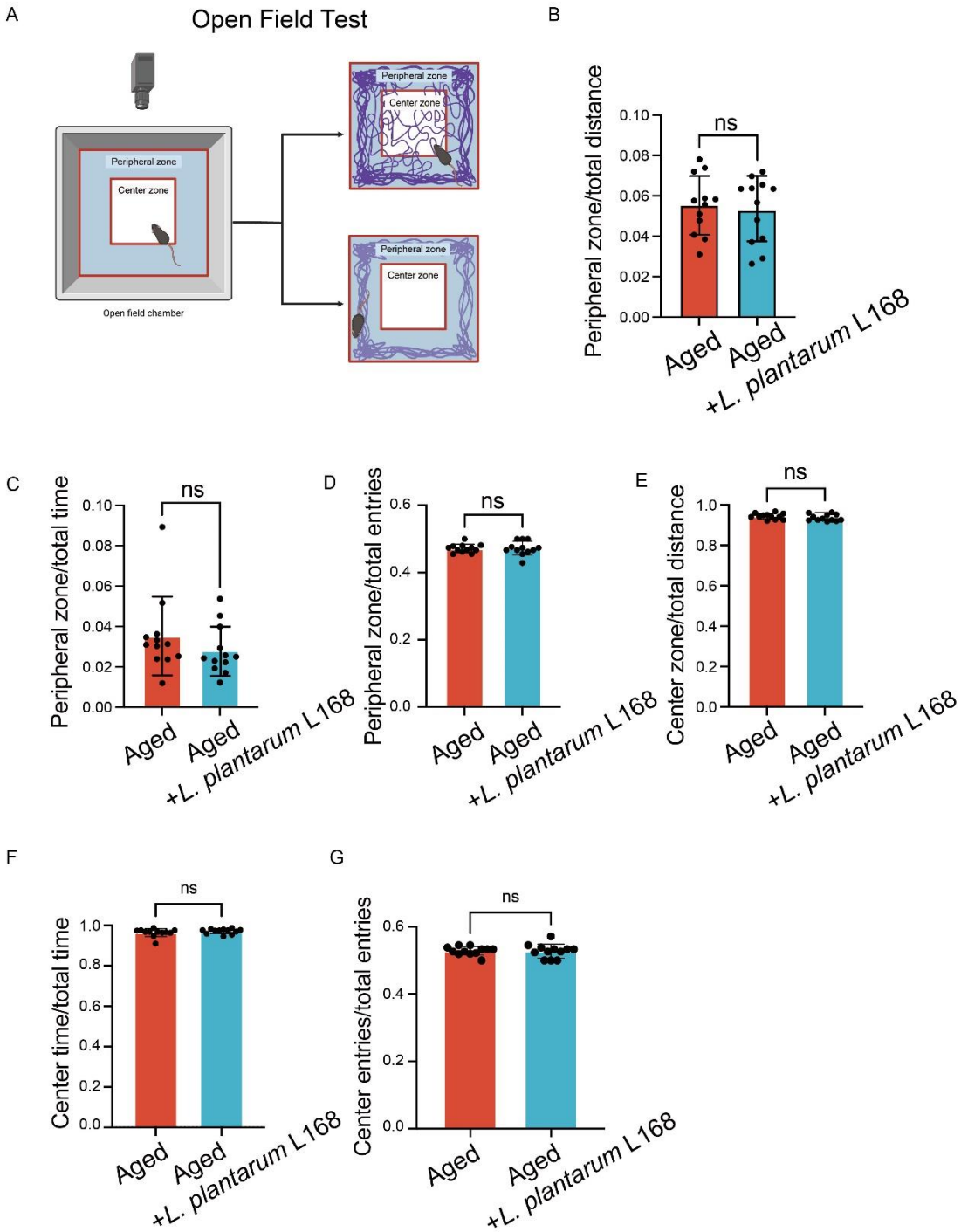
Supplementary Materials

Prolonged supplementation with *Lactiplantibacillus plantarum* alleviates age-associated phenotypes in mice

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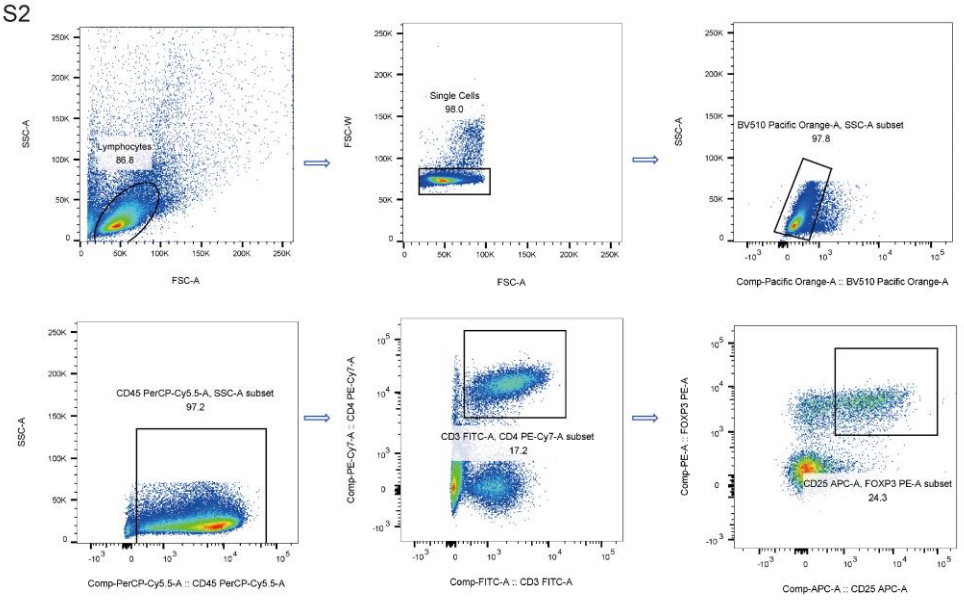
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Supplementary Figure 1. Open-field performance in aged mice with or without long-term *L. plantarum* L168 supplementation. (A) Schematic of the open-field test and representative movement tracks. Created with BioRender. (B-D) Proportions of total distance traveled (B), total time spent (C), and total entries (D) in the peripheral zone. (E-G) Corresponding proportions of distance traveled (E), time spent (F), and entries (G) in the center zone. Each dot represents one mouse. Data are presented as mean ± SEM (n = 12 mice per group). Groups were compared using two-sided

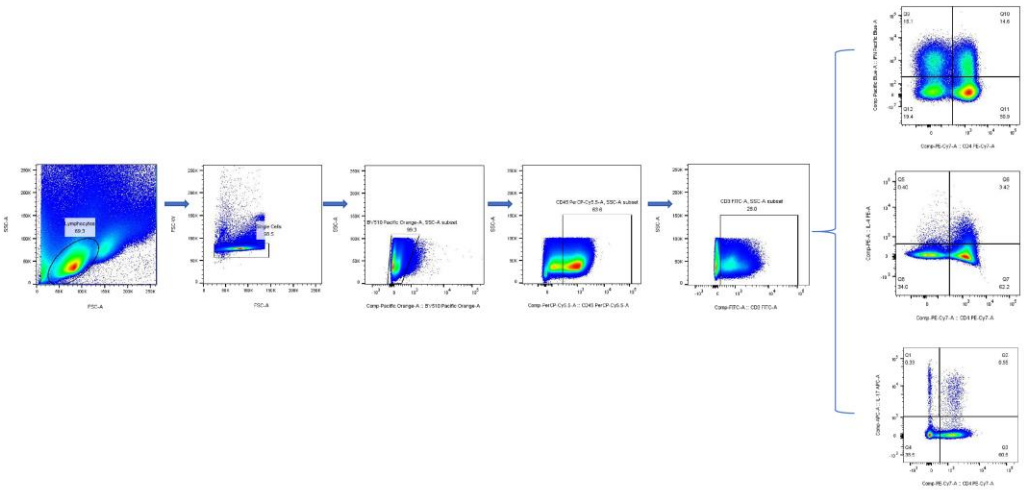
unpaired Student's t-tests. Unadjusted P values were 0.6978 (B), 0.2927 (C), 0.9025 (D), 0.6909 (E), 0.2927 (F), and 0.9025 (G). ns, not significant.

Figure S2



Supplementary Figure 2. Representative flow-cytometric gating strategy for the identification of regulatory T cells. Splenic lymphocytes were identified by forward- and side-scatter characteristics (FSC-A versus SSC-A), followed by singlet selection using FSC-A versus FSC-W. Viable cells with low fluorescence in the viability-dye channel were selected, followed by $CD45^+$ leukocytes. $CD3^+CD4^+$ T cells were identified within the viable $CD45^+$ population, and regulatory T cells were defined as $CD25^+Foxp3^+$ cells within the $CD3^+CD4^+$ compartment. Numbers indicate the percentage of cells relative to the corresponding parent population.

Figure S3



Supplementary Figure 3. Representative flow-cytometric gating strategy for the identification of Th1, Th2 and Th17 cells. Splenic lymphocytes were identified by forward- and side-scatter characteristics (FSC-A versus SSC-A), followed by singlet selection using FSC-A versus FSC-W. Viable cells with low fluorescence in the viability-dye channel were selected, followed sequentially by $CD45^+$ leukocytes and $CD3^+$ T cells. Within viable $CD45^+CD3^+$ T cells, Th1, Th2 and Th17 cells were identified as $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}4^+$ and $CD4^+IL\text{-}17A^+$ cells, respectively. Gate and quadrant percentages are relative to the corresponding parent population; the three cytokine-positive $CD4^+$ frequencies use viable $CD45^+CD3^+$ cells as the denominator.

Supplementary Table 1. Sequencing yield and quality control of the 15 negative controls used for five-region 16S rRNA gene sequencing

Sample	Raw reads	Raw bases (Mb)	Valid reads	Valid bases (Mb)	Valid (%)	Q20 (%)	Q30 (%)	GC (%)
DNA_Control1	15,806	4.74	11,785	3.08	74.56	97.53	93.62	59.20
DNA_Control2	21,781	6.53	17,367	4.65	79.73	97.42	93.22	58.44
DNA_Control3	23,736	7.12	19,964	5.48	84.11	97.93	94.43	58.06
DNA_Control4	11,012	3.30	8,255	1.94	74.96	95.95	90.31	64.06
DNA_Control5	15,710	4.71	13,012	3.53	82.83	97.86	94.37	58.20
PCR_Control1	10,952	3.29	7,143	1.75	65.22	96.62	91.53	61.76
PCR_Control2	12,807	3.84	8,990	2.22	70.20	95.86	89.80	59.84

PCR_Control3	11,522	3.46	7,690	1.78	66.74	95.03	87.92	64.18
PCR_Control4	6,057	1.82	3,181	0.85	52.52	97.77	94.17	57.41
PCR_Control5	8,997	2.70	4,350	1.07	48.35	96.28	90.72	58.31
NC1	58,599	17.58	53,764	15.43	91.75	98.62	96.17	55.71
NC2	59,263	17.78	54,662	16.13	92.24	99.28	97.60	55.62
NC3	56,358	16.91	53,302	13.29	94.58	95.98	90.69	63.03
NC4	56,024	16.81	52,329	12.74	93.40	95.62	89.62	64.44
NC5	55,912	16.77	51,352	12.66	91.84	96.56	91.78	62.96

DNA_Control1-5, DNA-extraction controls; PCR_Control1-5, no-template PCR controls; NC1-5, sampling blanks. Mb, million bases. Valid reads and bases refer to the sequencing provider's quality-filtered output. Valid (%) is the percentage of raw reads retained. Q20 and Q30 denote the percentages of bases with Phred scores of at least 20 and 30, respectively; GC denotes GC content. Percentages and base yields retain the precision of the sequencing report. These metrics describe sequencing quality control rather than taxon-level contaminant removal.

Supplementary Table 2. Taxonomic records identified as experiment-associated or sampling-associated contaminants by the TMB-based workflow

Category	Feature ID	Domain	Phylum	Class	Order	Family	Genus	Species
Experiment-associated contaminants	149	Bacteria	Actinomycetota	Actinomycetes	Propionibacteriales	Propionibacteriaceae	Cutibacterium	Cutibacterium_acnes
Experiment-associated contaminants	164	Bacteria	Actinomycetota	Actinomycetes	Streptomycetales	Streptomycetaceae	Streptomyces	Streptomyces_unclassified
Experiment-associated	193	Bacteria	Bacillota	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus_unclassified

contaminants								
Experiment-associated contaminants	204	Bacteria	Bacillota	Bacilli	Bacillales	Planococcaceae	Lysinibacillus	Lysinibacillus_unclassified
Experiment-associated contaminants	230	Bacteria	Bacillota	Bacilli	Lactobacillales	Aerococcaceae	Aerococcus	Aerococcus_unclassified
Experiment-associated contaminants	241	Bacteria	Bacillota	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	Enterococcus_faecalis
Experiment-associated contaminants	290	Bacteria	Bacillota	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	Streptococcus_unclassified
Experiment-associated contaminants	307	Bacteria	Bacillota	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus	Staphylococcus_epidermidis
Experiment-associated contaminants	311	Bacteria	Bacillota	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus	Staphylococcus_hominis

Experiment-associated contaminants	314	Bacteria	Bacillota	Bacilli	Staphylococcales	Staphylococcaceae	Staphylococcus	Staphylococcus_unclassified
Experiment-associated contaminants	374	Bacteria	Bacillota	Clostridia	Lachnospirales	Lachnospiraceae	Lachnospiraceae_unclassified	Lachnospiraceae_unclassified
Experiment-associated contaminants	415	Bacteria	Bacillota	Clostridia	Oscillospirales	Oscillospiraceae	Oscillospiraceae_unclassified	Oscillospiraceae_unclassified
Experiment-associated contaminants	464	Bacteria	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified	Bacteria_unclassified
Experiment-associated contaminants	489	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Phocaeicola	Phocaeicola_vulgatus
Experiment-associated contaminants	514	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Marinilabiliaceae	Marinilabiliaceae_unclassified	Marinilabiliaceae_unclassified
Experiment-associated	531	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Porphyromonadaceae	Porphyromonas	Porphyromonas_pogonae

contaminants								
Experiment-associated contaminants	578	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Weeksellaceae	Cloacibacterium	Cloacibacterium_normanense
Experiment-associated contaminants	610	Bacteria	Cyanobacteriota	Chloroplast	Chloroplast_unclassified	Chloroplast_unclassified	Chloroplast_unclassified	Chloroplast_unclassified
Experiment-associated contaminants	696	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Methylobacterium_unclassified
Experiment-associated contaminants	721	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Phyllobacterium	Phyllobacterium_myrsinacearum
Experiment-associated contaminants	762	Bacteria	Pseudomonadota	Alphaproteobacteria	Rickettsiales	Mitochondria	Mitochondria_unclassified	Mitochondria_unclassified
Experiment-associated contaminants	775	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	Novosphingobium_unclassified

Experiment-associated contaminants	814	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	Sphingomonas_unclassified
Experiment-associated contaminants	833	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderia	Burkholderia_unclassified
Experiment-associated contaminants	834	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderiaceae_unclassified	Burkholderiaceae_unclassified
Experiment-associated contaminants	848	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Herbaspirillum	Herbaspirillum_huttiense
Experiment-associated contaminants	874	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Pelomonas	Pelomonas_unclassified
Experiment-associated contaminants	876	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Ralstonia	Ralstonia_mannitolilytica
Experiment-associated	910	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Neisseriaceae	Neisseria	Neisseria_unclassified

contaminants								
Experiment-associated contaminants	912	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Neisseriaceae	Uruburuella	Uruburuella_suis
Experiment-associated contaminants	916	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Rhodocyclaceae	Azospira	Azospira_oryzae
Experiment-associated contaminants	919	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Rhodocyclaceae	Hydrogenophilus	Hydrogenophilus_thermoluteolus
Experiment-associated contaminants	921	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Rhodocyclaceae	Oryzomicrobium	Oryzomicrobium_terrae
Experiment-associated contaminants	923	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Rhodocyclaceae	Tepidiphilus	Tepidiphilus_succinatimandens
Experiment-associated contaminants	931	Bacteria	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Alteromonadaceae	Pseudoalteromonas	Pseudoalteromonas_unclassified

Experiment-associated contaminants	980	Bacteria	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Vibrionaceae	Vibrio	Vibrio_rotiferianus
Experiment-associated contaminants	982	Bacteria	Pseudomonadota	Gammaproteobacteria	Enterobacterales	Vibrionaceae	Vibrio	Vibrio_unclassified
Experiment-associated contaminants	995	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	Acinetobacter_baumannii
Experiment-associated contaminants	1015	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	Acinetobacter_unclassified
Experiment-associated contaminants	1023	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	Moraxella_unclassified
Experiment-associated contaminants	1034	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas_carnis
Experiment-associated	1050	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas_protegens

contaminants								
Experiment-associated contaminants	1056	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas_unclassified
Experiment-associated contaminants	1062	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Stutzerimonas	Stutzerimonas_unclassified
Experiment-associated contaminants	1079	Bacteria	Pseudomonadota	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas_unclassified
Sampling-associated contaminants	77	Bacteria	Actinomycetota	Actinomycetes	Mycobacteriales	Mycobacteriaceae	Corynebacterium	Corynebacterium_accolens
Sampling-associated contaminants	105	Bacteria	Actinomycetota	Actinomycetes	Mycobacteriales	Mycobacteriaceae	Corynebacterium	Corynebacterium_tuberculostearicum
Sampling-associated contaminants	149	Bacteria	Actinomycetota	Actinomycetes	Propionibacteriales	Propionibacteriaceae	Cutibacterium	Cutibacterium_acnes
Sampling-associated	180	Bacteria	Bacillota	Bacilli	Alicyclobacillales	Alicyclobacillaceae	Alicyclobacillus	Alicyclobacillus_

contaminants								acidocaldarius
Sampling-associated contaminants	193	Bacteria	Bacillota	Bacilli	Bacillales	Bacillaceae	Bacillus	Bacillus_unclassified
Sampling-associated contaminants	465	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Bacteroidaceae	Alloprevotella	Alloprevotella_unclassified
Sampling-associated contaminants	514	Bacteria	Bacteroidota	Bacteroidia	Bacteroidales	Marinilabiliaceae	Marinilabiliaceae_unclassified	Marinilabiliaceae_unclassified
Sampling-associated contaminants	578	Bacteria	Bacteroidota	Bacteroidia	Flavobacteriales	Weeksellaceae	Cloacibacterium	Cloacibacterium_normanense
Sampling-associated contaminants	593	Bacteria	Bacteroidota	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	Pedobacter	Pedobacter_quisquiliarum
Sampling-associated contaminants	610	Bacteria	Cyanobacteriota	Chloroplast	Chloroplast_unclassified	Chloroplast_unclassified	Chloroplast_unclassified	Chloroplast_unclassified
Sampling-associated contaminants	651	Bacteria	Pseudomonadota	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	Acetobacter	Acetobacter_unclassified
Sampling-associated	668	Bacteria	Pseudomonadota	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	Brevundimonas_

contaminants								mongoliensis
Sampling-associated contaminants	669	Bacteria	Pseudomonadota	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	Brevundimonas_unclassified
Sampling-associated contaminants	696	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Methylobacterium	Methylobacterium_unclassified
Sampling-associated contaminants	708	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Agrobacterium	Agrobacterium_unclassified
Sampling-associated contaminants	714	Bacteria	Pseudomonadota	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	Brucella	Brucella_unclassified
Sampling-associated contaminants	762	Bacteria	Pseudomonadota	Alphaproteobacteria	Rickettsiales	Mitochondria	Mitochondria_unclassified	Mitochondria_unclassified
Sampling-associated contaminants	775	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	Novosphingobium_unclassified
Sampling-associated contaminants	784	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	Sphingomonas_aquatilis
Sampling-associated	811	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	Sphingomonas_sanguinis

contaminants								
Sampling-associated contaminants	814	Bacteria	Pseudomonadota	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	Sphingomonas_unclassified
Sampling-associated contaminants	834	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Burkholderiaceae_unclassified	Burkholderiaceae_unclassified
Sampling-associated contaminants	848	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Herbaspirillum	Herbaspirillum_huttiense
Sampling-associated contaminants	853	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Hydrogenophaga	Hydrogenophaga_unclassified
Sampling-associated contaminants	876	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	Ralstonia	Ralstonia_mannitolilytica
Sampling-associated contaminants	916	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Rhodocyclaceae	Azospira	Azospira_oryzae
Sampling-associated contaminants	921	Bacteria	Pseudomonadota	Gammaproteobacteria	Burkholderiales	Rhodocyclaceae	Oryzomicrobium	Oryzomicrobium_terrae
Sampling-associated	1002	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	Acinetobacter_johnsonii

contaminants								
Sampling-associated contaminants	1015	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	Acinetobacter_unclassified
Sampling-associated contaminants	1021	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	Moraxella_cinereus
Sampling-associated contaminants	1023	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Moraxella	Moraxella_unclassified
Sampling-associated contaminants	1034	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas_carnis
Sampling-associated contaminants	1050	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas_protegens
Sampling-associated contaminants	1056	Bacteria	Pseudomonadota	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	Pseudomonas_unclassified
Sampling-associated contaminants	1079	Bacteria	Pseudomonadota	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Xanthomonas	Xanthomonas_unclassified

Feature ID is the identifier in the supplied contaminant tables. The experiment-associated list contains 45 records representing 39 genera, and the sampling-associated list contains 35 records representing 27 genera. The table reports taxonomy

records identified for removal and does not contain per-taxon read counts or prevalence values