

Supplementary Material

MANUSCRIPT TITLE: Plastisphere microbial succession and predicted functional responses: A 60-day temporal study in wastewater effluent

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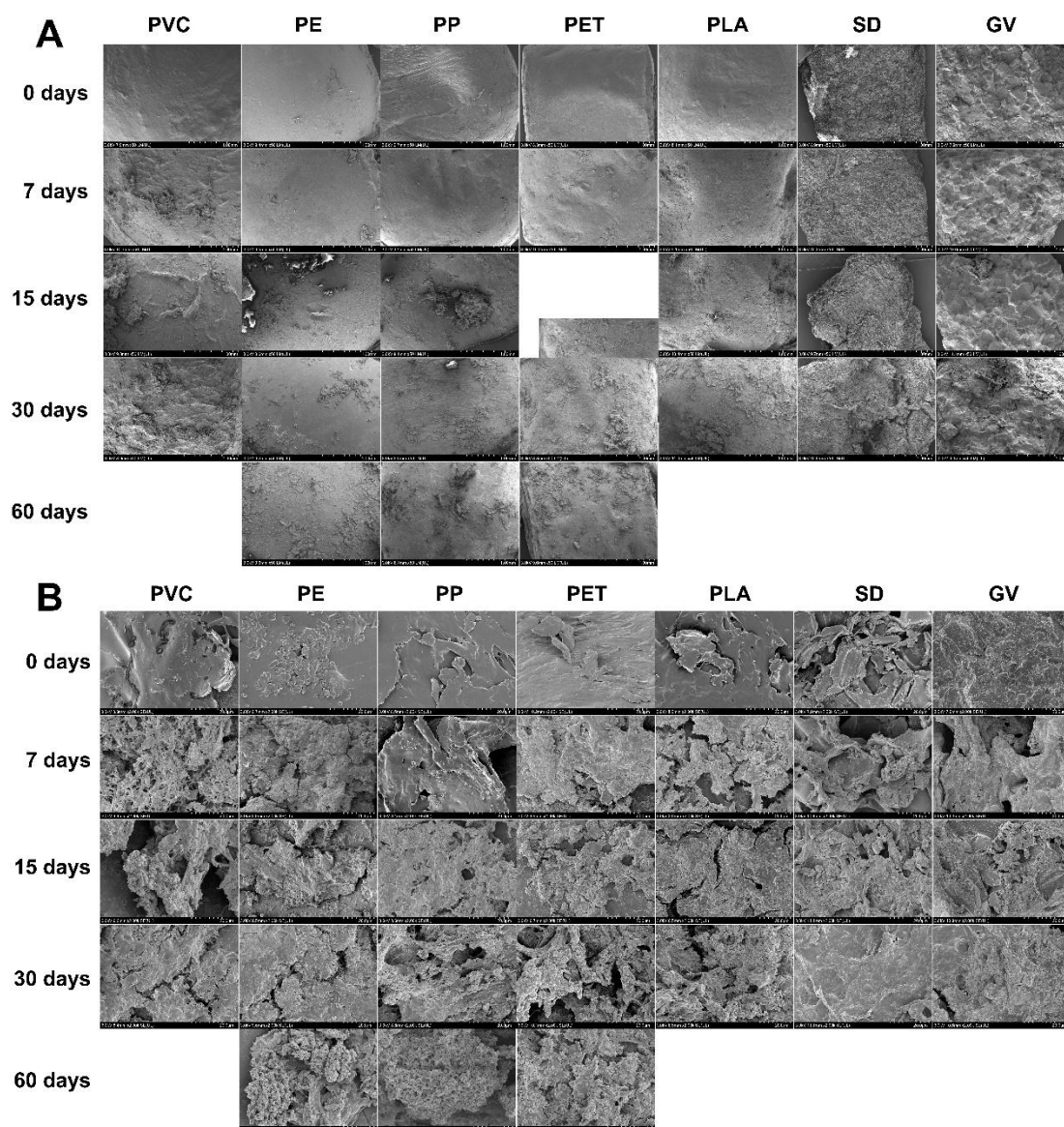


Fig. S1 Biofilm formation on samples during in-situ cultivation at different observational scales. A: 1mm; B: 20µm.

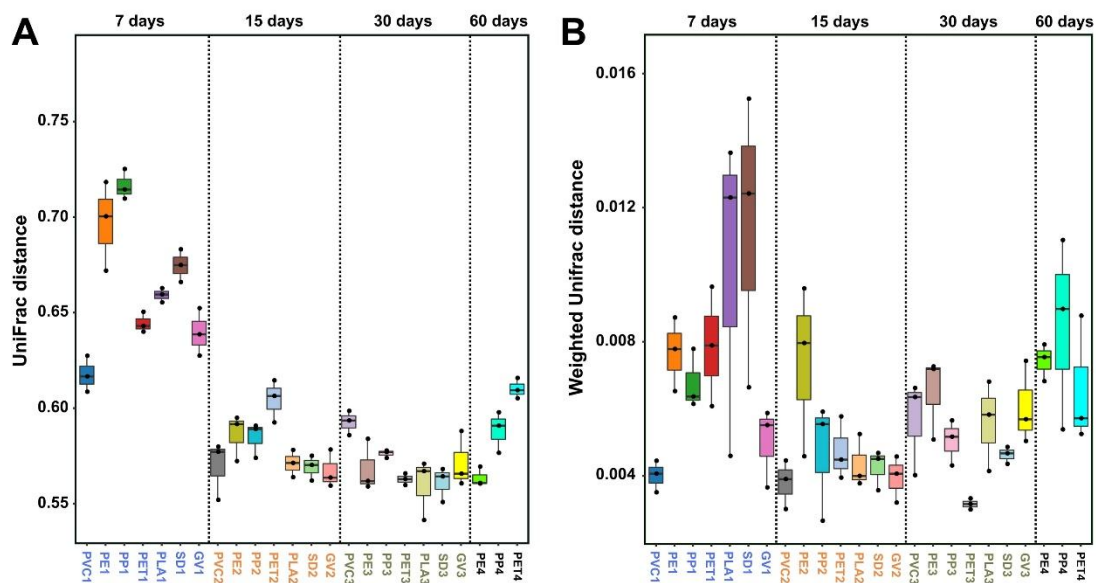


Fig. S2 Differences in UniFrac distances between groups of samples. A: Unweighted; B: Weighted.

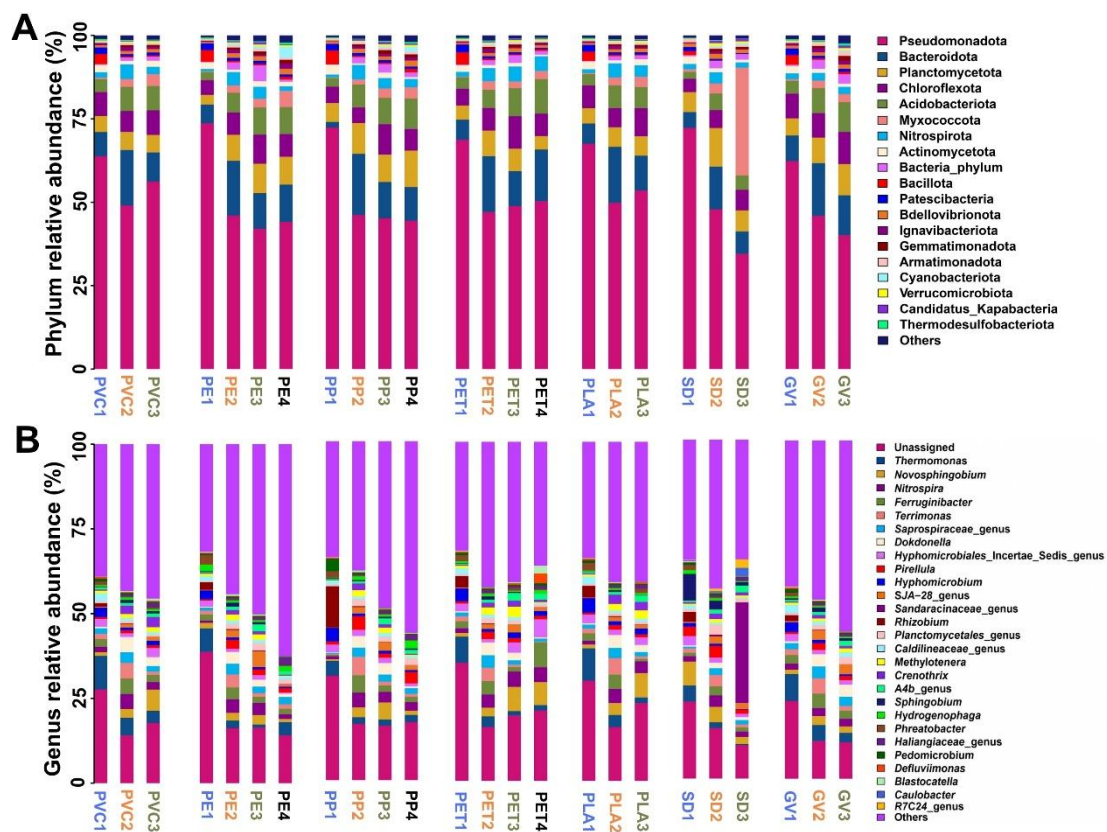


Fig. S3 Bar chart of bacterial community composition at different time points grouped by sample type.

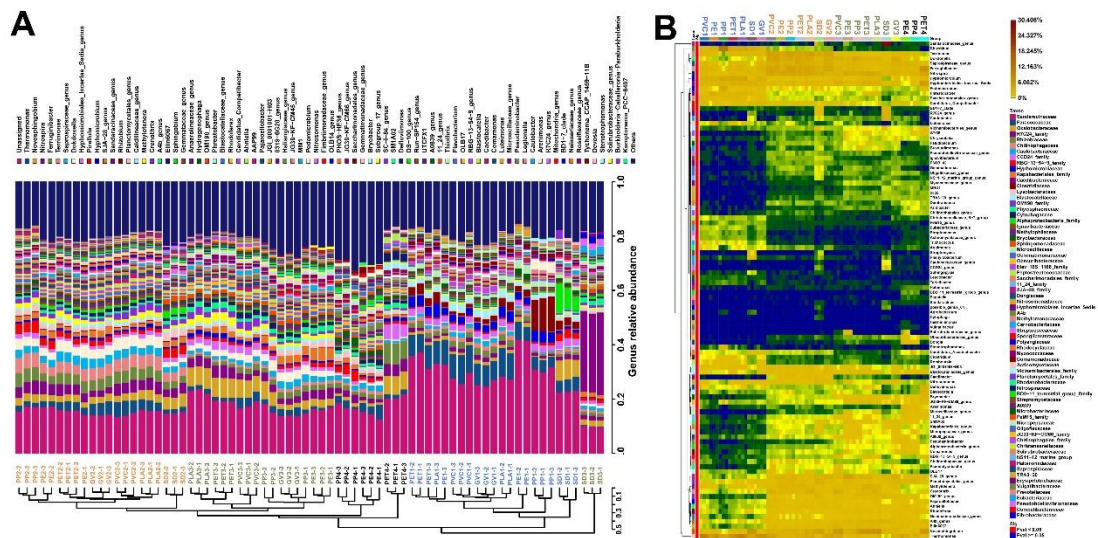


Fig. S4 Overall species composition of the samples. A: cluster diagram of species composition; B: heatmap of relative abundance.

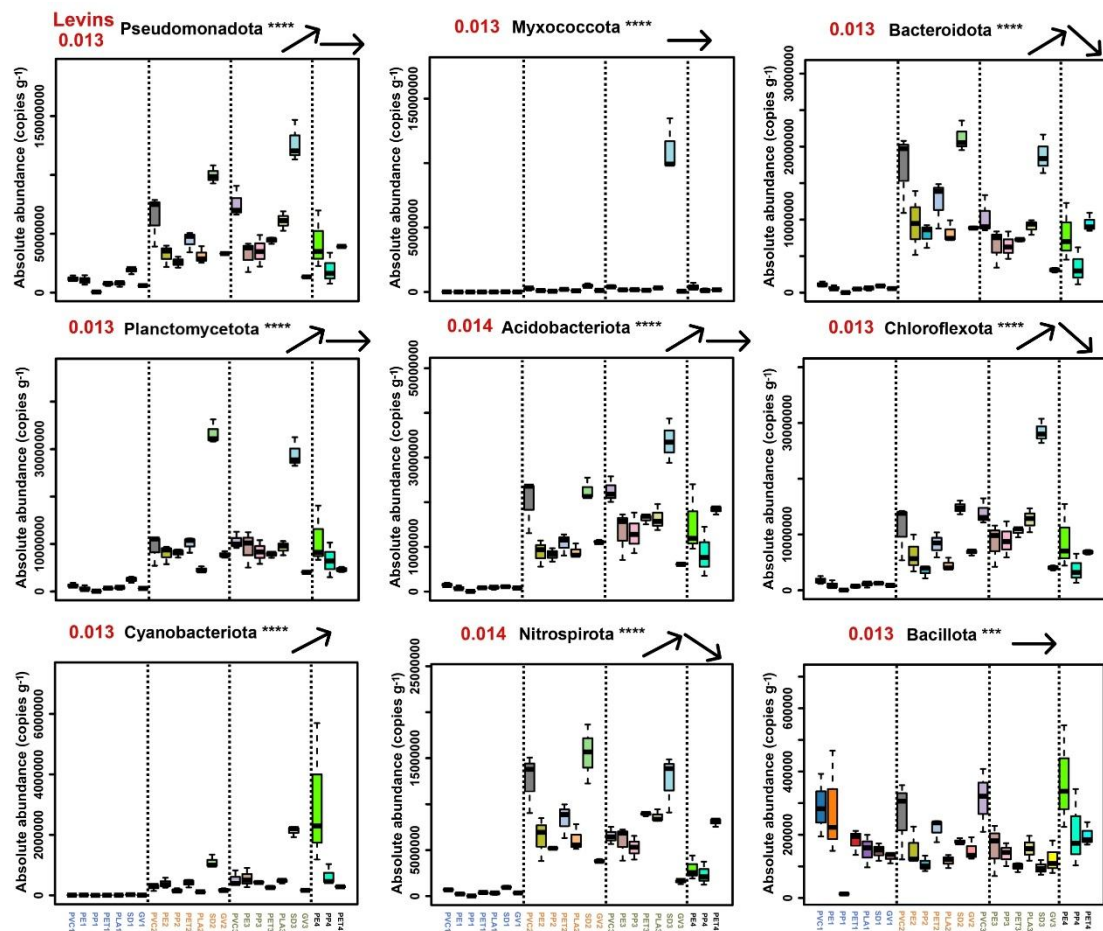


Fig. S5 Absolute abundances of the nine representative phyla shown in **Fig. 3C**. Values are expressed as 16S rRNA gene copies g⁻¹.

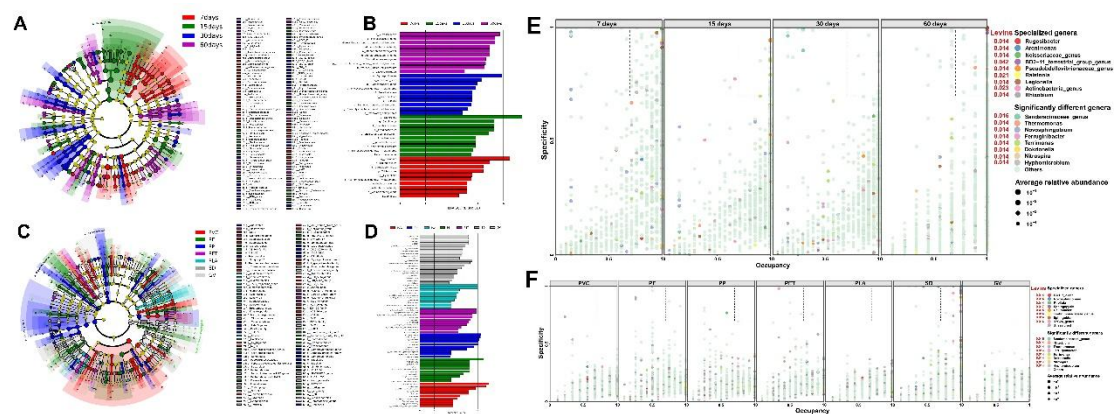


Fig. S7 Differential taxa analysis. A, B: LEfSe and LDA analyses grouped by time; C, D: LEfSe and LDA analyses grouped by sample type; E: Specificity-occupancy analysis grouped by time; F: Specificity-occupancy analysis grouped by sample type.

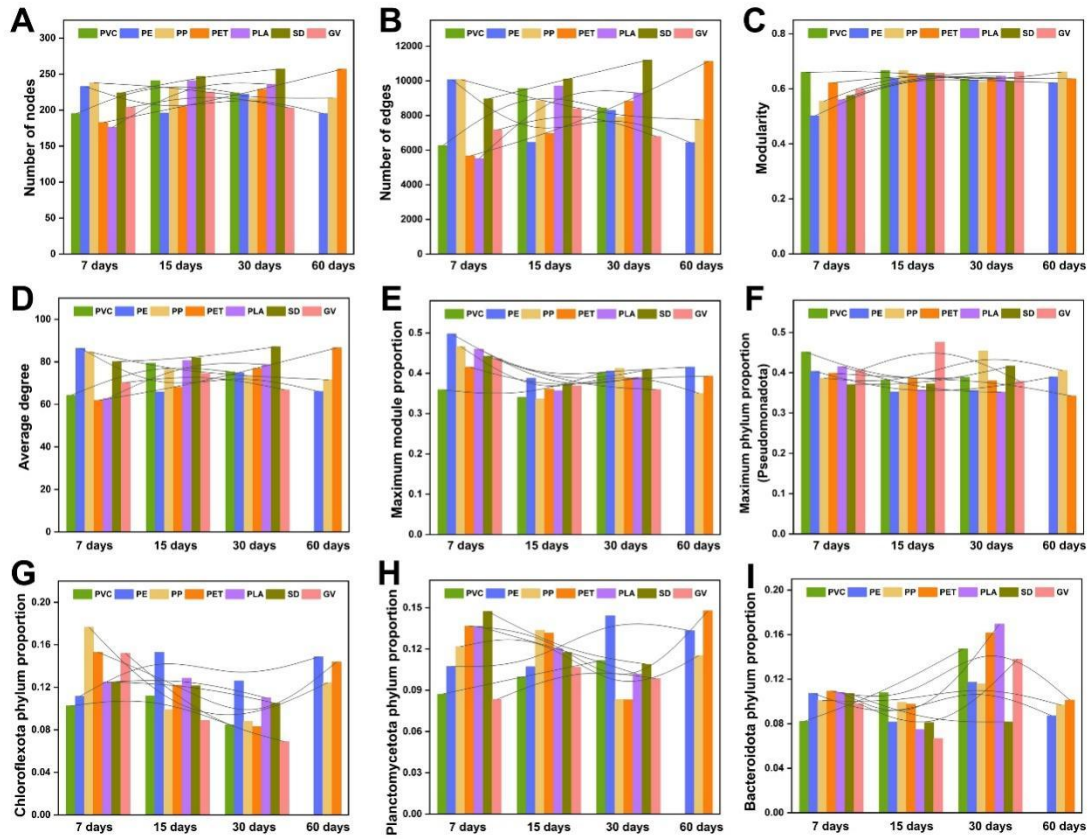


Fig. S9 Changes in basic network metrics across samples. A: Number of nodes; B: Number of edges; C: Modularity; D: Average degree; E: Proportion of the largest module; F: Proportion of the largest phylum (Pseudomonadota); G: Proportion of the phylum Chloroflexota; H: Proportion of the phylum Planctomycetota; I: Proportion of the phylum Bacteroidota.

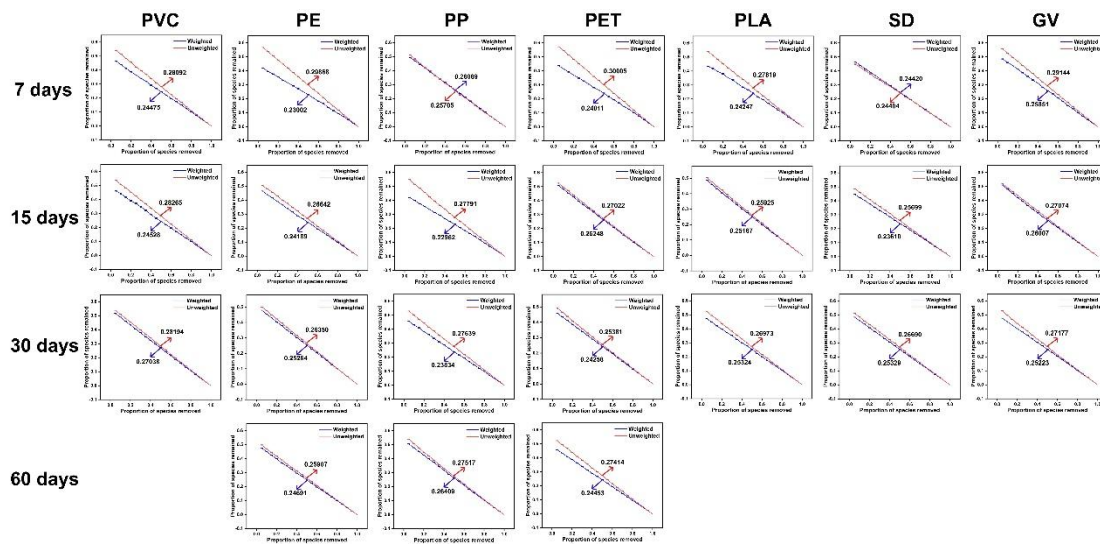


Fig. S10 Changes in robustness of microbial co-occurrence networks.

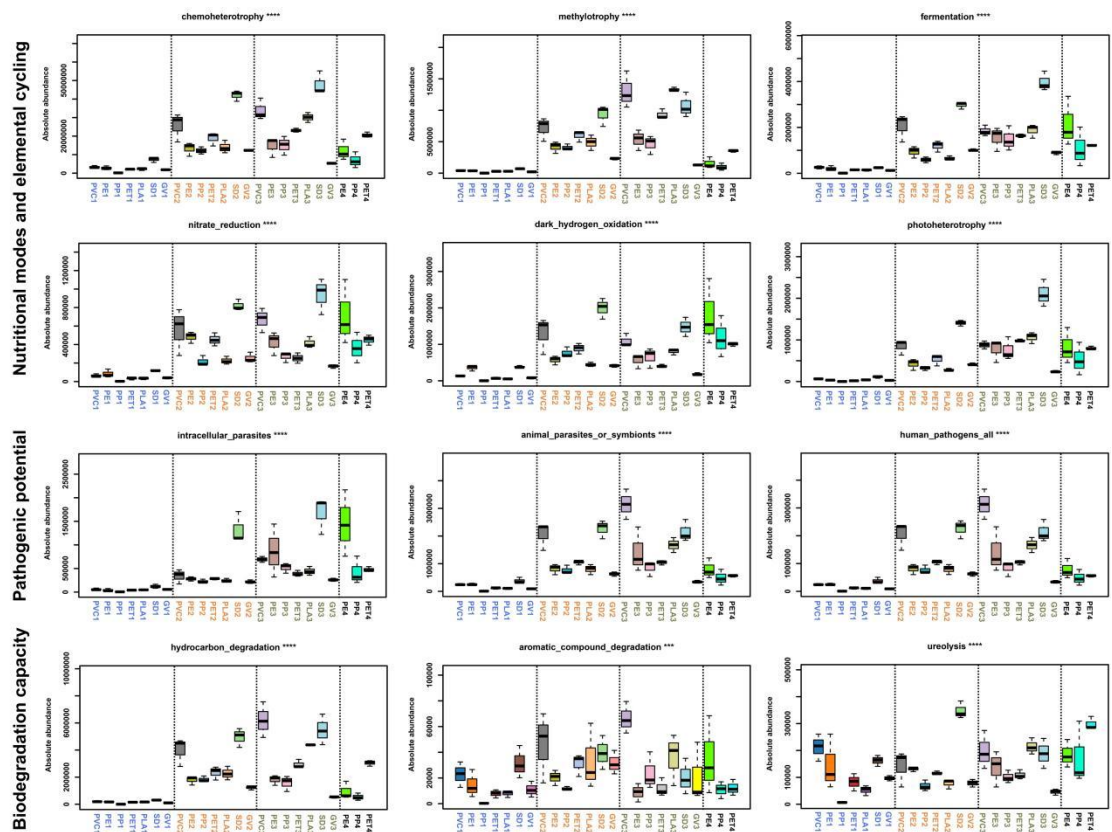


Fig. S12 Absolute abundances of taxa assigned to the 12 selected FAPROTAX-predicted functional categories. Values are expressed as 16S rRNA gene copies g^{-1} .

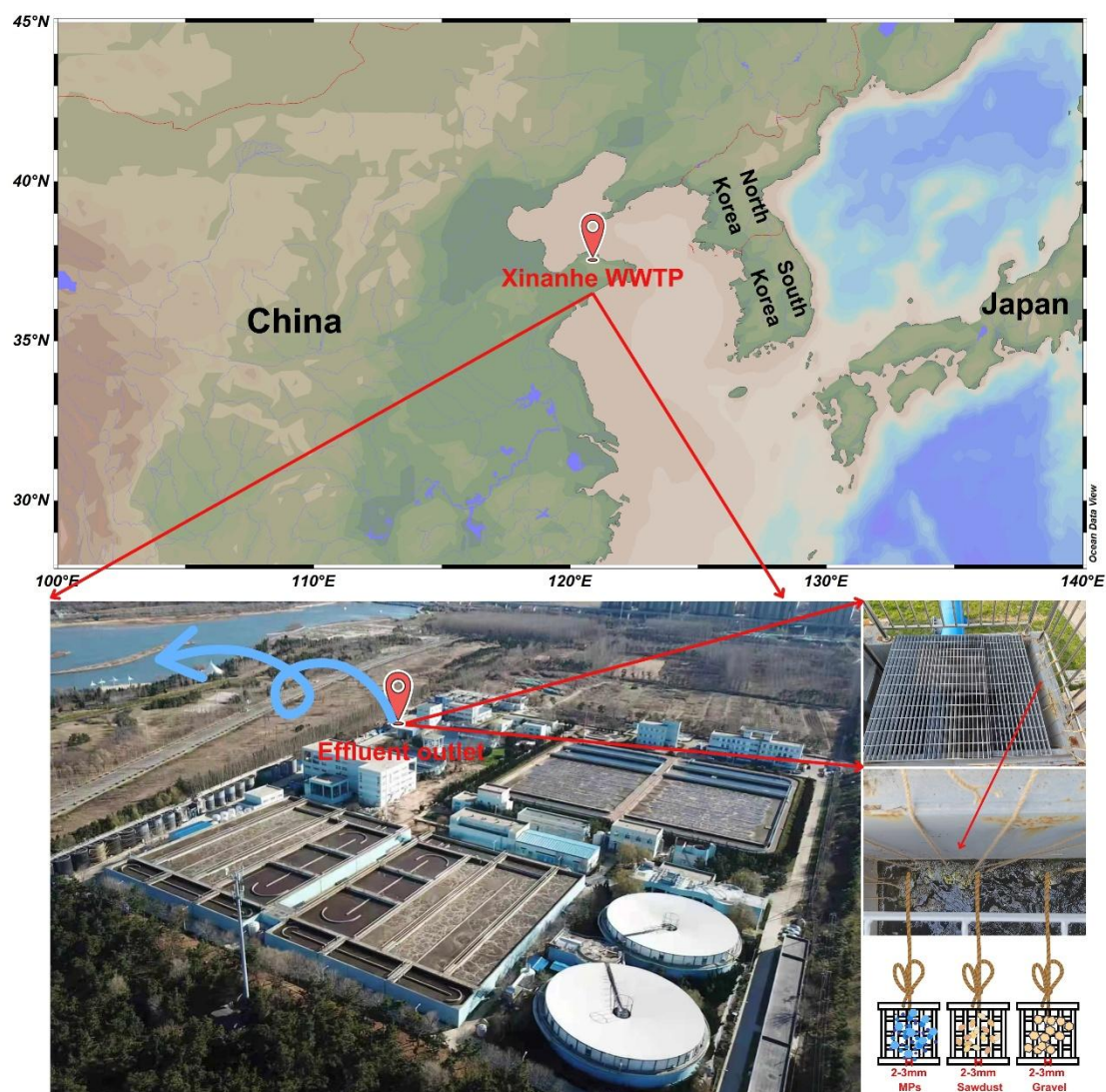


Fig. S13 Experimental site and setup for in-situ incubation.

Note: Xinanhe WWTP is located in the Department of Yantai City, Shandong Province, China, is a government-organized public welfare wastewater treatment institution. The plant primarily treats domestic sewage and industrial wastewater, serving an area of approximately 74.1 square kilometers. Covering a land area of about 10 hectares, it has a designed daily treatment capacity of 130,000 tons and mainly employs the A²O WWTP process.

Table. S1 Water quality during the experiment.

Time	Indicator	Temperature (°C)	pH	Suspended	Chemical	5-day	NH ₄ +N (mg L ⁻¹)	Total	Total	Effluent (m ³)
				solids	Oxygen	Biochemical		Nitrogen	Phosphorus	
				(SS) (mg L ⁻¹)	Demand (COD) (mg L ⁻¹)	Oxygen Demand (BOD ₅) (mg L ⁻¹)		(TN) (mg L ⁻¹)	(TP) (mg L ⁻¹)	
	Standard	GB 13195-91	HJ 1147-2020	GB 11901-1989	HJ 828-2017	HJ 505-2009	HJ 535-2009	HJ 636-2012	GB 1893-1989	
June	Average	24.267	7.17	8.7	29.57	2.8	0.31	12.4	0.24	137583.5
		±1.48	±0.18	±1.18	±7.19	±0.61	±0.32	±1.62	±0.06	±7006.84
	Maximum	29	7.5	10	45	4.0	1.59	14.4	0.37	145200
	Minimum	22	6.9	7	18	1.6	0.06	8.3	0.11	116800
	Cumulative	728.00	215.00	261	887	83.9	9.43	360.07	7.18	4127505
July	Average	26.9	7.28	9.1	24.84	2.15	0.53	12.5	0.32	137882.13
		±0.91	±0.2	±0.91	±7.42	±0.78	±0.51	±1.76	±0.1	±5787.85
	Maximum	28	7.6	10	48	4.1	1.92	14.9	0.49	146872
	Minimum	24	6.9	7	17	1.2	0.09	8.61	0.12	126192
	Cumulative	834	225.7	282	770	66.5	16.52	387.39	9.82	4274346
7 days	Average	22.71	7.23	9.14	24.14	2.73	0.57	13.06	0.24	128057.14
		±0.95	±0.1	±1.07	±6.52	±0.77	±0.55	±1.26	±0.1	±7372
	Maximum	24	7.3	10	37	4	1.59	14.5	0.37	135664

	Minimum	22	7.1	7	18	1.6	0.09	11.3	0.11	116800
	Cumulative	159	50.6	64	169	19.1	4	91.4	1.68	896400
15 days	Average	23.27	7.11	8.6	25.93	2.63	0.43	12.31	0.25	134600.2
		±0.96	±0.16	±1.24	±5.9	±0.64	±0.42	±1.61	±0.07	±8589.97
	Maximum	25	7.3	10	37	4	1.59	14.5	0.37	145768
	Minimum	22	6.9	6	18	1.6	0.10	8.30	0.11	116800
	Cumulative	349	106.6	129	389	39.4	6.45	184.7	3.72	2019003
30 days (June)	Average	24.27	7.17	8.7	29.57	2.8	0.31	12.4	0.24	137583.5
		±1.48	±0.18	±1.18	±7.19	±0.61	±0.32	±1.62	±0.06	±7006.84
	Maximum	29	7.5	10	45	4.0	1.59	14.4	0.37	145200
	Minimum	22	6.9	7	18	1.6	0.06	8.3	0.11	116800
	Cumulative	728.00	215.00	261	887	83.9	9.43	360.07	7.18	4127505
60 days	Average	25.57	7.22	8.88	27.23	2.45	0.43	12.44	0.28	137870.05
		±1.79	±0.2	±1.06	±7.67	±0.75	±0.44	±1.69	±0.09	±6329.4
	Maximum	29	7.6	10	48	4.1	1.92	14.9	0.49	146872
	Minimum	22	6.9	6	17	1.2	0.06	8.3	0.11	116800
	Cumulative	1534	433.3	533	1634	146.7	25.73	746.46	16.55	8272203

Note: All the aforementioned water quality parameters fall within the routine monitoring requirements stipulated by the Chinese National Standard “Discharge Standard of Pollutants for Municipal Wastewater Treatment Plants” (GB18918-2002).

Table. S2 The relationship between latent variables and observed variables in the PLS-PM analysis of the carbon and nitrogen cycle genes

Latent variable	Observed variable	Weight	Loading	Communality	Redundancy
Differential taxa	Pseudomonadota	-0.21261	-0.9479	0.89860	0.00000
	Myxococcota	0.09377	0.2664	0.07095	0.00000
	Bacteroidota	0.16212	0.7751	0.60080	0.00000
	Planctomycetota	0.13658	0.6588	0.43402	0.00000
	Acidobacteriota	0.17379	0.8446	0.71329	0.00000
	Chloroflexota	0.10753	0.5149	0.26511	0.00000
	Cyanobacteriota	0.07614	0.3929	0.15437	0.00000
	Nitrospirota	0.16188	0.7414	0.54969	0.00000
	Bacillota	-0.22466	-0.9159	0.83885	0.00000
Diversity	Observed	0.28608	0.9616	0.92474	0.75726
	Shannon	0.25628	0.9335	0.87147	0.71363
	Pielou_e	0.21905	0.8665	0.75085	0.61486
	NMDS1	0.37749	0.8047	0.64759	0.5303
	NMDS2	0.05753	-0.138	0.01904	0.01559
Biofilm status	Copy number	0.48618	0.9638	0.92899	0.32566
	Biofilm content	0.4828	0.9662	0.93355	0.32726
	Hydrophobicity	-0.22965	-0.2827	0.0799	0.02801
	Nodes	-0.07148	0.4191	0.17562	0.15099
	Edges	-0.04393	0.2765	0.07643	0.06571
	Modularity	-0.15658	0.8038	0.64602	0.55542
	Average degree	-0.021	0.1687	0.02847	0.02448
	Largest module	0.14118	-0.7781	0.60551	0.52059
	Average variation degree	-0.02084	0.0781	0.00611	0.00525
Stability	Unrobustness	0.07193	-0.4701	0.221	0.19001
	Robustness	-0.0375	0.2418	0.05849	0.05029
	Natural connectivity	-0.17455	0.827	0.68397	0.58805
	Positive cohesion	0.17286	-0.8516	0.7252	0.6235
	Negative cohesion	-0.15866	0.7979	0.63661	0.54733
	Network stability	-0.08625	0.3826	0.14635	0.12583
	Maximum vulnerability	0.10575	-0.6699	0.44872	0.38579
	Average vulnerability	0.17498	-0.8695	0.75604	0.65002
	β NIT	0.03943	0.2351	0.05527	0.04453
Community assembly	R ²	0.44874	0.9493	0.90113	0.72605
	m	0.58461	0.966	0.93324	0.75193
	Chemoheterotrophy	0.07418	-0.4988	0.24878	0.2081
Function	Methylotrophy	0.15635	-0.7314	0.53501	0.44752
	Fermentation	-0.15214	0.5727	0.32797	0.27434
	Nitrate_reduction	0.11719	-0.3159	0.09979	0.08347

Dark_hydrogen_oxidation	-0.00581	0.0835	0.00697	0.00583
Photoheterotrophy	0.23848	-0.7983	0.6373	0.53308
Intracellular_parasites	0.00776	0.038	0.00145	0.00121
Animal_parasites_or_symbionts	-0.06272	0.0611	0.00374	0.00312
Human_pathogens_all	-0.06095	0.0542	0.00294	0.00246
Hydrocarbon_degradation	0.1694	-0.765	0.58529	0.48957
Aromatic_compound_degradation	-0.20639	0.8081	0.653	0.54621
Ureolysis	-0.24942	0.9237	0.85328	0.71373

Note: Differential taxa were selected as the top nine phyla with significant differences in relative abundance. Due to the strong similarity among some predicted functions (e.g., chemoheterotrophy and aerobic chemoheterotrophy, as well as phototrophy and photoheterotrophy; see **Fig. 5D**), 12 functions were selected after screening for evaluation, considering both abundance and representativeness.

Table. S3 Information on sample weight and DNA concentration.

Sample ID	Template DNA concentration (ng/uL)	Sample quantity (g)
PVC1-1	1.48	0.2993
PVC1-2	1.86	0.3031
PVC1-3	1.87	0.2959
PE1-1	0.50	0.1525
PE1-2	0.78	0.1596
PE1-3	1.17	0.1572
PP1-1	0.14	0.3255
PP1-2	0.10	0.3140
PP1-3	0.12	0.3045
PET1-1	0.60	0.2047
PET1-2	0.71	0.2098
PET1-3	0.79	0.2041
PLA1-1	1.24	0.3092
PLA1-2	1.05	0.3153
PLA1-3	0.66	0.2911
SD1-1	2.04	0.1483
SD1-2	2.02	0.1516
SD1-3	2.18	0.1527
GV1-1	0.96	0.4016
GV1-2	1.21	0.4024
GV1-3	1.32	0.4109
PVC2-1	19.26	0.3347
PVC2-2	12.20	0.3612
PVC2-3	25.60	0.3771
PE2-1	13.80	0.3283
PE2-2	5.96	0.3282
PE2-3	9.64	0.3270
PP2-1	7.60	0.3044
PP2-2	9.54	0.3683
PP2-3	4.78	0.3303
PET2-1	15.90	0.3513
PET2-2	13.40	0.3437
PET2-3	11.30	0.3636
PLA2-1	14.10	0.4184
PLA2-2	7.58	0.3733
PLA2-3	10.60	0.4137
SD2-1	23.60	0.2240
SD2-2	17.12	0.2006
SD2-3	22.20	0.2093
GV2-1	20.33	0.6049
GV2-2	13.68	0.5751

GV2-3	20.40	0.5902
PVC3-1	27.53	0.2941
PVC3-2	38.00	0.2979
PVC3-3	25.33	0.2937
PE3-1	3.72	0.1592
PE3-2	8.14	0.1674
PE3-3	7.42	0.1478
PP3-1	8.96	0.3038
PP3-2	17.36	0.2942
PP3-3	12.90	0.3059
PET3-1	9.68	0.2009
PET3-2	8.24	0.2061
PET3-3	8.86	0.1986
PLA3-1	18.08	0.3015
PLA3-2	18.56	0.3016
PLA3-3	20.80	0.2992
SD3-1	23.10	0.1504
SD3-2	24.67	0.1527
SD3-3	31.20	0.1534
GV3-1	5.78	0.4092
GV3-2	6.70	0.4105
GV3-3	5.22	0.4102
PE4-1	31.37	0.4622
PE4-2	13.10	0.4577
PE4-3	20.40	0.5256
PP4-1	3.16	0.4518
PP4-2	7.92	0.4363
PP4-3	16.16	0.4310
PET4-1	16.88	0.4630
PET4-2	20.83	0.5376
PET4-3	46.80	0.5066

Note: Taking PVC1-1 as an example, “PVC” denotes the sample type. The number “1” before the hyphen indicates the first sampling timepoint (i.e., 7 days), and the number “1” after the hyphen represents replicate sample 1.

Table. S4 Sample-specific standard curves used for synthetic spike-in-based absolute quantification. In the equation $y = ax + b$, x represents the $\log_{10}$ -transformed recovered read count of a synthetic standard, and y represents its $\log_{10}$ -transformed known input copy number

Sample ID	Linear equation	R ²
PVC1-1	$y=1.0045x-0.1588$	0.992100743
PVC1-2	$y=1.0435x-0.4453$	0.986921121
PVC1-3	$y=1.0008x-0.1877$	0.99122663
PE1-1	$y=1.0426x+0.0113$	0.993544308
PE1-2	$y=0.996x-0.0143$	0.987569621
PE1-3	$y=1.0447x-0.2029$	0.98416482
PP1-1	$y=0.9856x+0.7162$	0.967476318
PP1-2	$y=1.0173x+0.7025$	0.990457524
PP1-3	$y=0.9373x+0.9496$	0.955942974
PET1-1	$y=1.0166x+0.0644$	0.994386087
PET1-2	$y=1.0368x-0.2236$	0.9967262
PET1-3	$y=1.0012x+0.0236$	0.993502821
PLA1-1	$y=1.039x-0.2633$	0.988982933
PLA1-2	$y=1.0713x-0.444$	0.994703164
PLA1-3	$y=1.0296x-0.1057$	0.991359873
SD1-1	$y=1.0332x-0.2114$	0.994128751
SD1-2	$y=1.0037x-0.2246$	0.987747213
SD1-3	$y=0.9627x+0.035$	0.990611707
GV1-1	$y=1.0634x-0.4004$	0.991833966
GV1-2	$y=0.9938x-0.1565$	0.99129498
GV1-3	$y=1.0256x-0.1801$	0.993442957
PVC2-1	$y=1.0391x-0.9378$	0.981937409
PVC2-2	$y=0.9548x-0.6663$	0.981196525
PVC2-3	$y=1.0412x-1.037$	0.990654257
PE2-1	$y=1.0137x-0.91$	0.969464724
PE2-2	$y=1.0406x-0.6696$	0.986726491
PE2-3	$y=0.9405x-0.6561$	0.948660361
PP2-1	$y=1.0701x-0.8986$	0.987648884
PP2-2	$y=0.9735x-0.5989$	0.986598834
PP2-3	$y=1.0023x-0.5823$	0.994804429
PET2-1	$y=1.0115x-1.1107$	0.988280942
PET2-2	$y=0.9992x-1.0009$	0.987403324
PET2-3	$y=0.9778x-0.6926$	0.987062651
PLA2-1	$y=0.9948x-0.87$	0.990645915
PLA2-2	$y=0.9827x-0.5873$	0.991153266
PLA2-3	$y=0.9621x-0.6644$	0.981531497
SD2-1	$y=1.0809x-1.0453$	0.992440245
SD2-2	$y=1.0197x-0.7383$	0.988746658

SD2-3	$y=0.9845x-0.6936$	0.988085451
GV2-1	$y=0.9929x-0.6687$	0.996290944
GV2-2	$y=1.0708x-0.9363$	0.993785956
GV2-3	$y=0.9708x-0.7395$	0.979739972
PVC3-1	$y=1.0438x-0.5932$	0.99089626
PVC3-2	$y=1.0646x-0.7763$	0.993748993
PVC3-3	$y=1.1175x-0.8027$	0.980016637
PE3-1	$y=0.9683x-0.1503$	0.991973679
PE3-2	$y=1.0636x-0.8$	0.996305671
PE3-3	$y=0.9795x-0.4145$	0.994170531
PP3-1	$y=0.978x-0.5274$	0.981246893
PP3-2	$y=0.9939x-0.5342$	0.989009226
PP3-3	$y=1.0819x-1.0102$	0.991175597
PET3-1	$y=1.0129x-0.7024$	0.989984353
PET3-2	$y=0.9817x-0.5388$	0.98728486
PET3-3	$y=1.0342x-0.7364$	0.98423231
PLA3-1	$y=1.0328x-0.6725$	0.990852645
PLA3-2	$y=1.0491x-0.7826$	0.994533611
PLA3-3	$y=1.0677x-0.9196$	0.995937167
SD3-1	$y=0.9976x-0.58$	0.995908905
SD3-2	$y=1.001x-0.5705$	0.989186415
SD3-3	$y=0.983x-0.5926$	0.993676845
GV3-1	$y=1.0742x-0.7475$	0.994467467
GV3-2	$y=0.989x-0.4652$	0.994161662
GV3-3	$y=1.0522x-0.6345$	0.989351419
PE4-1	$y=1.0937x-0.847$	0.987369794
PE4-2	$y=1.0184x-0.8568$	0.988852083
PE4-3	$y=0.9772x-0.6688$	0.967902561
PP4-1	$y=0.9937x-0.2964$	0.9887554
PP4-2	$y=0.9957x-0.5657$	0.971161052
PP4-3	$y=0.9839x-0.5375$	0.983000609
PET4-1	$y=1.0725x-0.8968$	0.992155499
PET4-2	$y=0.9508x-0.5076$	0.984876782
PET4-3	$y=1.003x-0.6824$	0.99304117

Note: Taking PVC1-1 as an example, “PVC” denotes the sample type. The number “1” before the hyphen indicates the first sampling timepoint (i.e., 7 days), and the number “1” after the hyphen represents replicate sample 1.

Table. S5 Statistical validation of differential microbial taxa and predicted functions

Category	Taxon/function	Shapiro-Wilk p	Brown-Forsythe p	One-way ANOVA p	Kruskal-Wallis p
Phylum	Pseudomonadota	0.2070	0.7513	1.351686e-34	2.034718e-06
Phylum	Myxococcota	6.60×10^{-5}	0.3547	1.194158e-57	2.142892e-06
Phylum	Bacteroidota	0.1447	0.8719	1.518371e-31	2.052265e-06
Phylum	Planctomycetota	0.9145	0.9591	2.794509e-26	2.410339e-06
Phylum	Acidobacteriota	2.41×10^{-4}	0.3055	1.262709e-39	1.325327e-06
Phylum	Chloroflexota	0.2325	0.9135	5.332187e-15	1.568204e-05
Phylum	Cyanobacteriota	1.74×10^{-15}	0.1316	6.942872e-06	3.668600e-06
Phylum	Nitrospirota	0.8572	0.9523	4.070876e-25	2.844583e-06
Phylum	Bacillota	5.70×10^{-5}	0.1183	3.572699e-36	3.408010e-06
Genus	<i>Sandaracinaceae_genus</i>	5.35×10^{-16}	0.0026	3.498162e-68	7.241640e-05
Genus	<i>Rhizobium</i>	4.58×10^{-10}	0.0111	8.716394e-43	2.106663e-06
Genus	<i>Thermomonas</i>	0.0132	0.5312	5.636967e-25	3.119507e-06
Genus	<i>Novosphingobium</i>	7.28×10^{-8}	0.0783	2.946473e-30	1.521738e-06
Genus	<i>Ferruginibacter</i>	0.0046	0.8487	3.687513e-30	2.757170e-06
Genus	<i>Terrimonas</i>	0.1374	0.7003	1.062025e-39	2.938471e-06
Genus	<i>Dokdonella</i>	2.37×10^{-7}	0.3641	1.463877e-24	2.467681e-06
Genus	<i>Nitrospira</i>	0.8723	0.9521	4.065501e-25	2.844583e-06
Genus	<i>Hyphomicrobium</i>	6.40×10^{-8}	0.1313	3.967130e-37	1.247983e-06
Function	Chemoheterotrophy	0.9585	0.9466	2.308883e-27	2.480875e-06
Function	Methyлотrophy	0.0056	0.3654	2.260496e-27	1.827452e-06
Function	Fermentation	0.8313	0.8727	4.098555e-19	6.409547e-06
Function	Nitrate_reduction	6.72×10^{-5}	0.2641	9.609241e-16	4.142427e-06
Function	Dark_hydrogen_oxidation	8.65×10^{-10}	0.0235	1.768716e-19	4.381794e-06
Function	Photoheterotrophy	0.2499	0.9874	1.246216e-30	2.044930e-06
Function	Intracellular_parasites	0.0064	0.6976	5.628677e-18	1.014952e-05
Function	Animal_parasites_or_symbionts	1.24×10^{-5}	0.3355	3.535956e-09	6.013101e-05
Function	Human_pathogens_all	1.34×10^{-5}	0.3372	2.634856e-09	4.740921e-05
Function	Hydrocarbon_degradation	0.0417	0.7677	2.723936e-24	2.631161e-06
Function	Aromatic_compound_degradation	3.29×10^{-9}	0.3526	5.957588e-06	1.472690e-05
Function	Ureolysis	2.83×10^{-7}	0.0429	2.916081e-21	1.069931e-05

Text. S1 Synthetic spike-in standard design, sample-specific calibration, and calculation of absolute abundance in 16S rRNA amplicon sequencing^{2,3}

Absolute quantitative 16S rRNA amplicon sequencing was performed by Shanghai G&C Biotechnology Co., Ltd. using a patented synthetic spike-in standard system (Patent No. ZL201910295389.X). Because the original MPs, sawdust, and gravel samples differed substantially in density and other physicochemical characteristics, total genomic DNA was first extracted from each sample. After DNA extraction and quality assessment, synthetic spike-in reference DNA equivalent to approximately 0.1% of the total DNA mass was added to each DNA extract before PCR amplification.

The spike-in standard system consisted of nine synthetic DNA sequences whose nucleotide arrangements differed from naturally occurring microbial gene sequences. These standards were designed to be co-amplified with bacterial 16S rRNA gene templates using the same primer pair. Based on their known input copy numbers, the nine sequences were distributed across four concentration levels separated by 10-fold intervals.

The synthetic spike-in standards and native bacterial 16S rRNA gene templates were amplified in the same PCR reaction using primers 515F and 907R and subsequently underwent the same library preparation, sequencing, and bioinformatic processing procedures. After sequence quality control, reads assigned to the synthetic spike-in standards were identified and removed from the microbial ASV dataset.

For each sample, a sample-specific standard curve was constructed from the relationship between the recovered reads of the synthetic standards and their known input copy numbers. The standard curve was expressed as:

$$\text{Log}_{10}(\text{Cs}) = a \times \text{log}_{10}(\text{Rs}) + b$$

where Rs represents the recovered read count of a synthetic spike-in standard, Cs represents its known input copy number, and a and b denote the slope and intercept, respectively. The read count of each microbial ASV was subsequently substituted into the corresponding sample-specific equation to estimate its 16S rRNA gene copy abundance in the amplification template.

The resulting abundance estimates were normalized according to the amount of DNA used as the PCR template, the total amount of DNA extracted from the sample, and the original sample mass. The absolute abundance per gram of sample was calculated as follows:

Absolute abundance (copies g⁻¹) = estimated copies in the PCR template × total extracted DNA amount / DNA amount used for PCR / original sample mass

The equations and coefficients of determination of all sample-specific standard curves are provided in Table S4.

Compared with conventional target-specific qPCR, which typically requires a separate primer set, amplification assay, and standard curve for each selected gene or taxon, the synthetic spike-in-based amplicon approach enables the simultaneous quantitative profiling of numerous ASVs within a single sequencing workflow. It therefore avoids the need to establish a separate calibration system for every microbial target and may reduce additional between-assay and between-batch variation associated with multiple target-specific qPCR measurements. Because all samples were processed using the same spike-in system, primer pair, sequencing procedure, and sample-specific calibration strategy, this approach provides an internally consistent framework for comparing community-wide temporal and inter-sample trends. However, this methodological advantage does not imply that the approach is universally more accurate than well-validated target-specific qPCR, which remains valuable for the independent validation of selected genes or taxa.

Despite these advantages, synthetic spike-in-based absolute quantification does not eliminate all potential sources of bias. Because the synthetic standards were introduced after DNA extraction, differences in DNA extraction efficiency among MPs, sawdust, and gravel were not corrected. Moreover, taxon-specific primer mismatches, differences in template sequence composition and secondary structure, preferential PCR amplification, and variation in 16S rRNA gene copy number among bacterial taxa may influence the resulting abundance estimates. Therefore, the reported absolute abundance values represent spike-in-calibrated estimates of 16S rRNA gene copy abundance rather than exact microbial cell numbers or completely bias-free true abundances.

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