

Supplementary Information

Trace metal contamination impacts predicted functions more than structure of marine prokaryotic biofilm communities in an anthropized coastal area

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1. Supplementary materials

1.1. 16S rRNA gene amplicon sequencing of biofilm samples

The PCR reaction (50 μ L) contained 25 μ L of 2X GoTaq® Long PCR Master Mix (Promega), 1 μ M of each primer, and approximately 2 ng of DNA. The following thermal cycling scheme was used: initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 1min, followed by extension at 72°C for 1 min. The final extension was carried out at 72°C for 10 min. PCR products were checked on a 1.5% agarose gel. No amplification was yielded for negative controls consisting in the reaction mixture with MilliQ water as template.

1.2. DNA extraction, 16S rRNA gene amplicon sequencing and quantification of seawater samples

DNA was extracted from the Millipore filters by a combination of enzymatic cell lysis (Ghiglione et al., 2009) and AllPrep DNA/RNA Mini Kit (QIAGEN) according to the manufacturer's instructions. The protocol for the DNA extraction is fully described in Coclet *et al.* (2019).

REFERENCES

- Coclet, C., Garnier, C., Durrieu, G., Omanović, D., D'onofrio, S., Poupon, C. Le, et al. (2019). Changes in bacterioplankton communities resulting from direct and indirect interactions with trace metal gradients in an urbanized marine coastal area. *Front. Microbiol.* 10, 250. doi:10.3389/fmicb.2019.00257.
- Ghiglione JF, Conan P, Pujo-Pay M (2009) Diversity of total and active free-living vs. particle-attached bacteria in the euphotic zone of the NW Mediterranean Sea. *FEMS Microbiol Lett* 299:9–21. <https://doi.org/10.1111/j.1574-6968.2009.01694.x>

2. Supplementary Figures

Fig. S1 Alpha-diversity descriptors including Simpson, Shannon, PD_Whole, Observed OTU, equitability and Chao1 calculated from 16S rRNA OTU table along each sampling sites (i.e. 6ext, MIS, Pt12, Pt15 and 41p) in surface (light) and bottom (dark) samples. Upper and lower lines correspond to the first and third quartile of the distribution of values. The median values are shown with horizontal black wide lines. Outliers are displayed as dots. *P-values* correspond to the results of one-way ANOVA analyses.

Fig. S2 Biofilm densities (cell.cm²) in surface (light) and bottom (dark) polycarbonate plates along the different sampling sites determined by qPCR. *P-values* and “a,” “b,” “c” indexes correspond to the results of one-way ANOVA analyses and post hoc tests (HSD Tukey’s test), respectively.

Fig. S3 Nonmetric dimensional scaling (nMDS) ordination based on Bray-Curtis dissimilarity for normalized 16S rRNA gene libraries by 16S copy number (A), for 16S rRNA gene libraries (B), and for level 3 of KEGG metabolic pathways (C) between the different sampling sites and sampling depths.

Fig. S4 Bar graph representing relative proportions of significant families in bottom (blue) and surface (yellow) biofilm samples. Extended error bar plots showed pairwise comparison of significant family proportions (Welch’s t-test; $p < 0.05$) between bottom (blue) and surface (yellow) biofilm samples. Corrected p-value is determined using Fisher’s exact test.

Fig. S5 Bar graph representing relative proportions of significant families between the sampling sites (41p: green; Pt15: blue; Pt12: yellow; MIS: grey; 6ext: red) in surface (A) and bottom biofilm samples. Extended error bar plots showed pairwise comparison of significant family proportions (Welch’s t-test; $p < 0.01$) between sites in surface (A) and bottom (B) biofilm samples. Corrected p-value is determined using Fisher’s exact test.

Fig S6 Alpha-diversity descriptors including Simpson, Shannon, PD_Whole, Observed OTU, equitability and Chao1 calculated from 16S rRNA OTU table along each sampling sites (i.e. 6ext, MIS, Pt12, Pt15 and 41p) in seawater (blue) and biofilm (green) samples. Upper and lower lines correspond to the first and third quartile of the distribution of values. The median values are shown with horizontal black wide lines. Outliers are displayed as dots.

Fig. S7 Bar graph representing relative proportions of significant families in seawater (blue) and biofilm (green) samples. Extended error bar plots showed pairwise comparison of significant family proportions (Welch's t-test; $p < 0.01$) between seawater (blue) and biofilm (green) samples. Corrected p-value is determined using Fisher's exact test.

Fig. S8 Bar graph representing relative proportions of significant KEGG Pathways at level 2 of SEED metabolic hierarch in biofilms (green) and seawater (blue). Extended error bar plots showed pairwise comparison of significant KEGG Pathway proportions (Welch's t-test; $p < 0.01$) at level 2 of SEED metabolic hierarchy between biofilms (green) and seawater (blue). Corrected p-value is determined using Fisher's exact test.

Fig. S9 Bar graph representing relative proportions of significant KEGG Pathways at level 2 of SEED metabolic hierarchy in biofilms from contaminated (6ext; red) and uncontaminated (41p; green) sites. Extended error bar plots showed pairwise comparison of significant KEGG Pathway proportions (Welch's t-test; $p < 0.01$) at level 2 of SEED metabolic hierarchy between biofilms from contaminated (6ext; red) and uncontaminated (41p; green) sites. Corrected p-value is determined using Fisher's exact test.

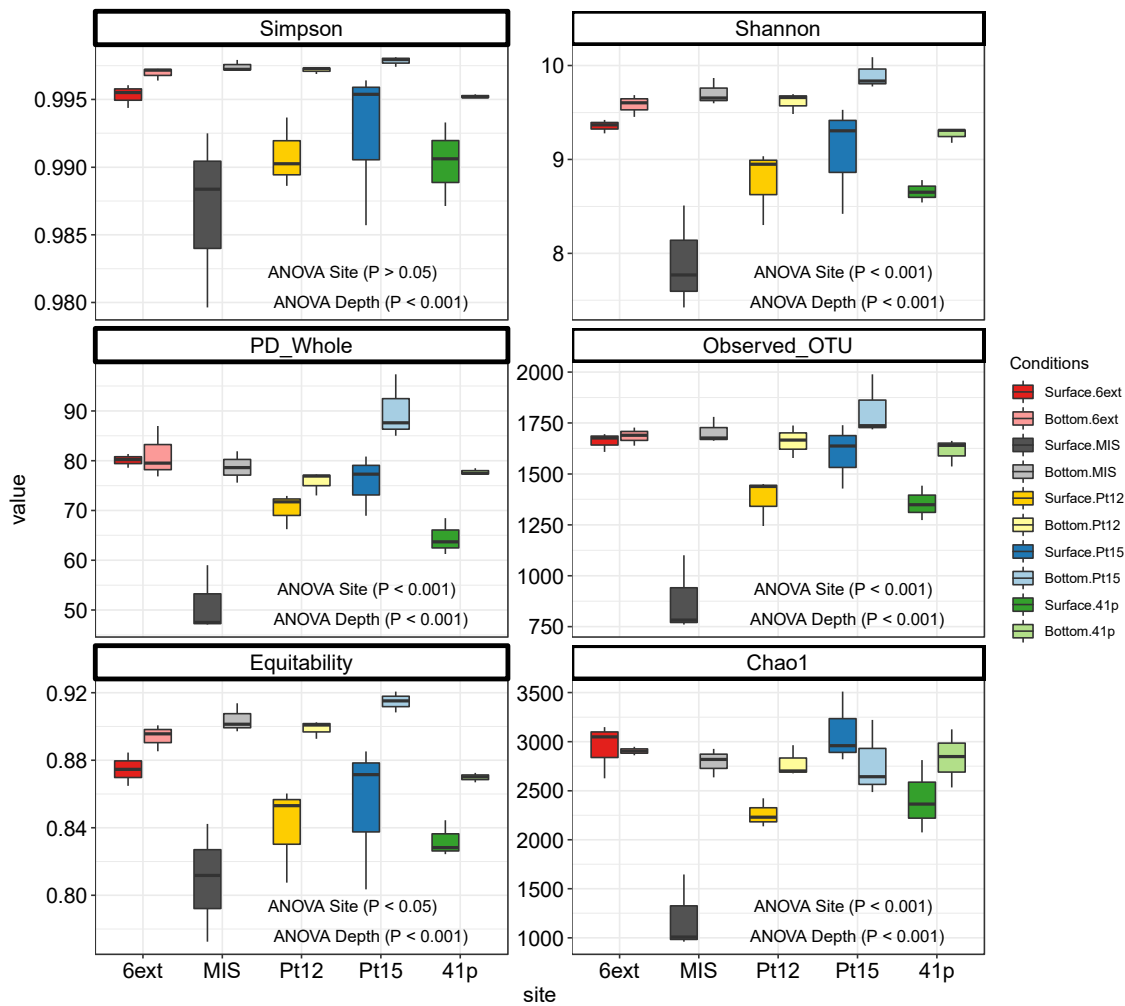


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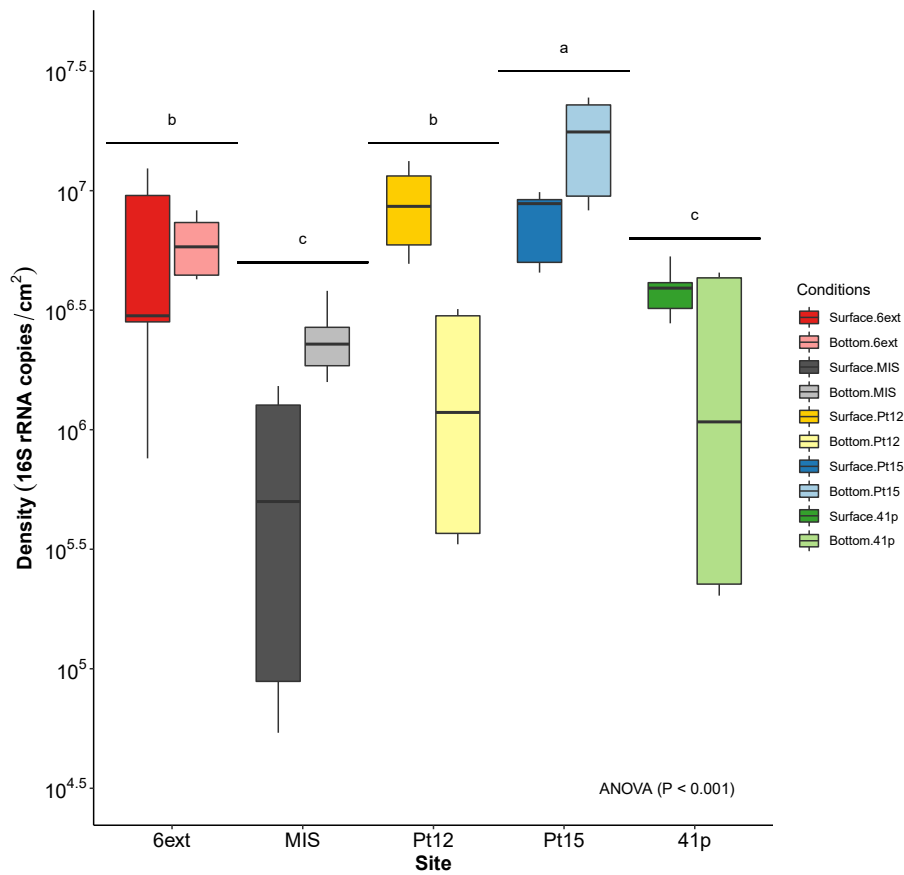
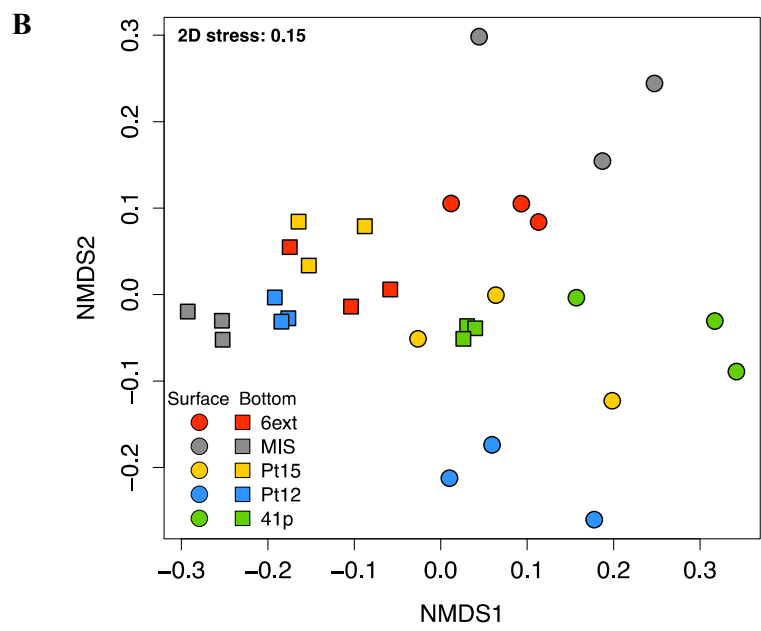
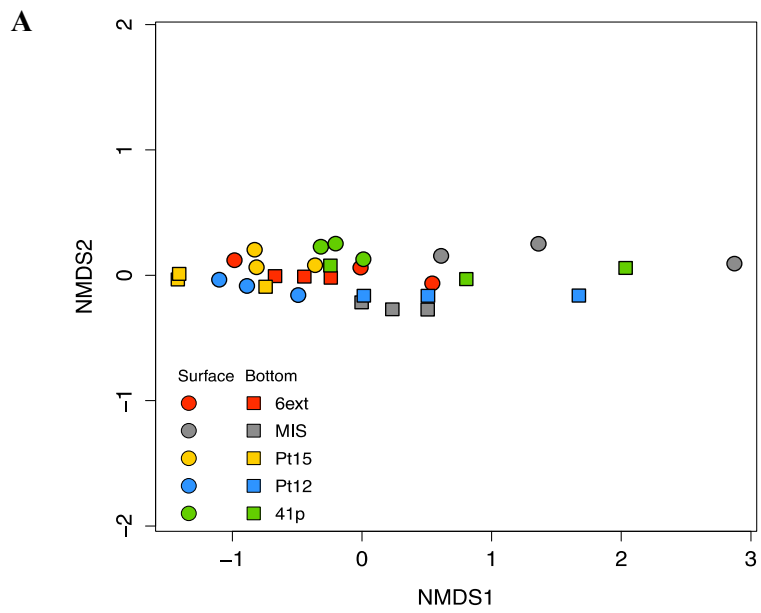


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C

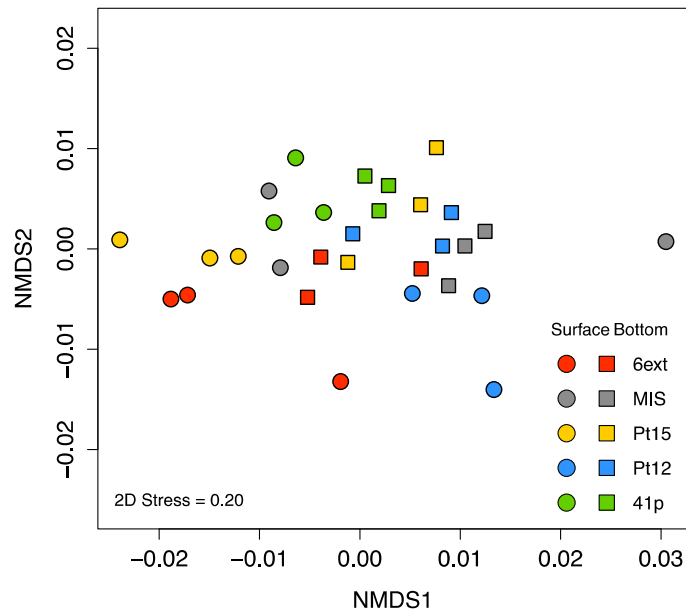


Fig. S3 Nonmetric dimensional scaling (nMDS) ordination based on Bray-Curtis dissimilarity for normalized 16S rRNA gene libraries by 16S copy number (A), for 16S rRNA gene libraries (B), and for level 3 of KEGG metabolic pathways (C) between the different sampling sites and sampling depths.

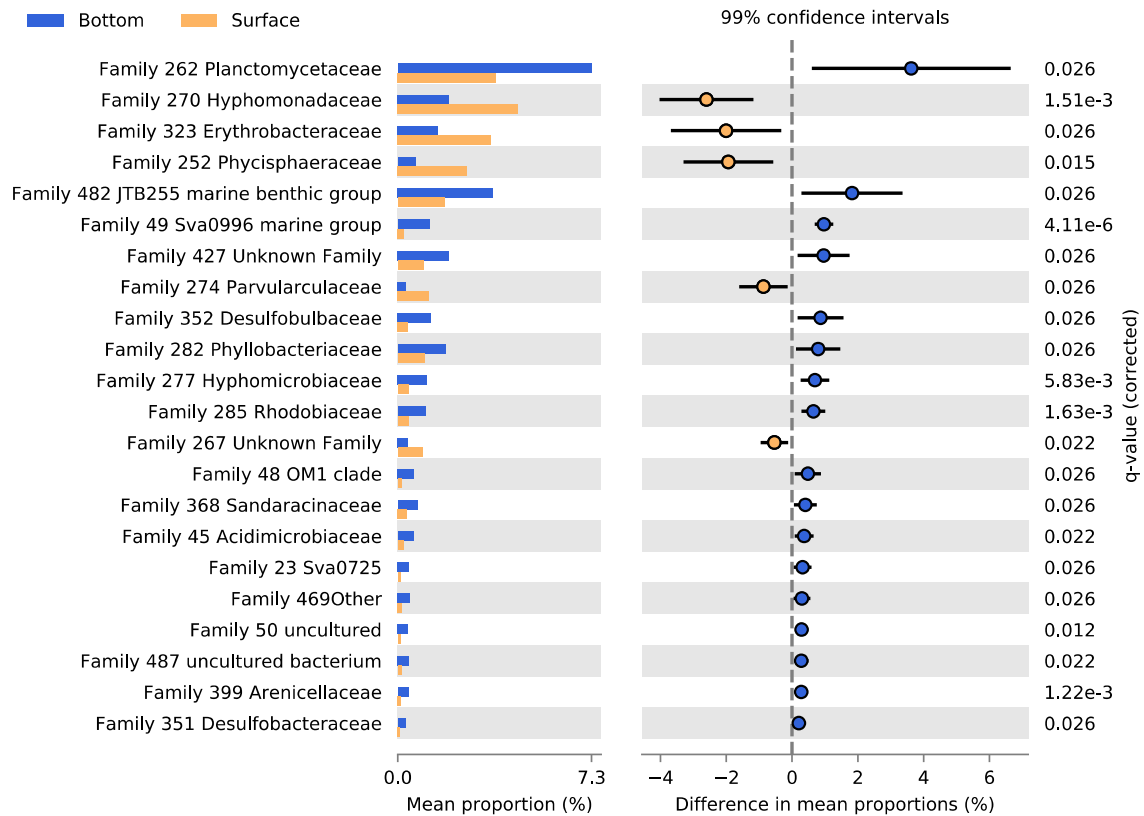
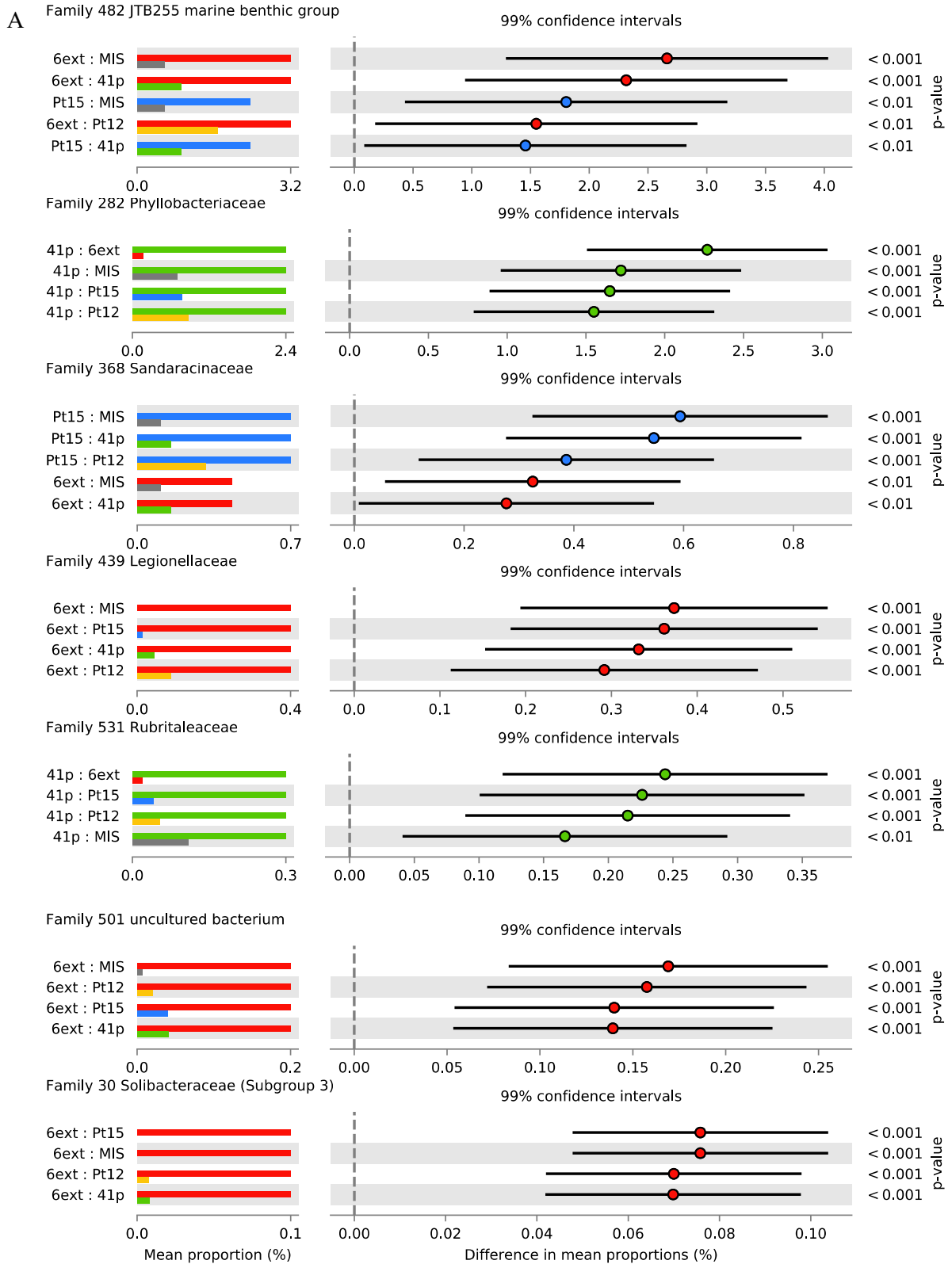
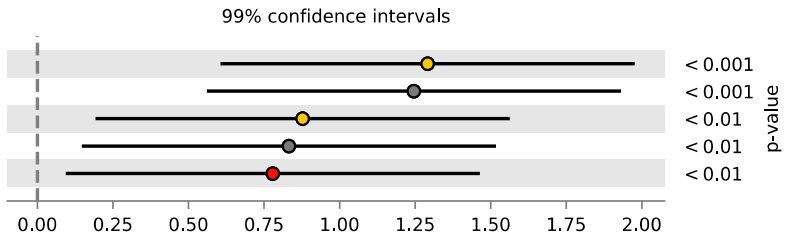
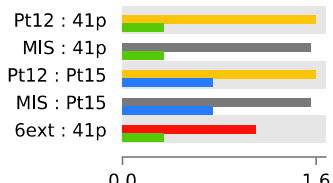


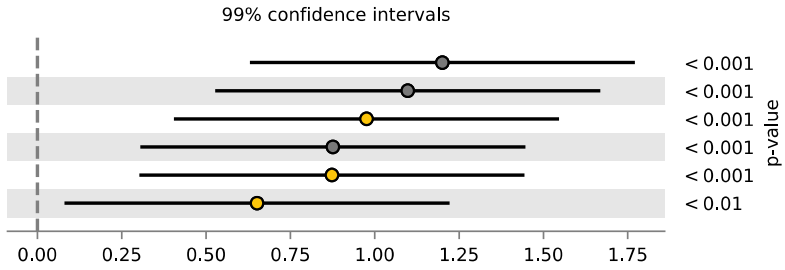
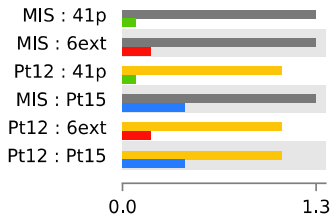
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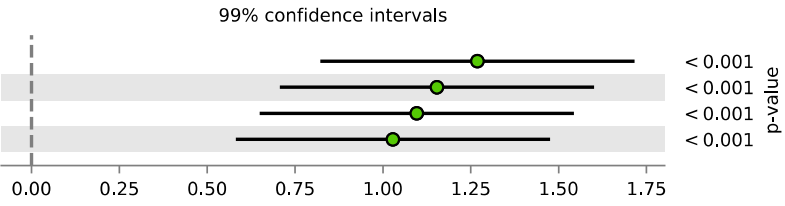
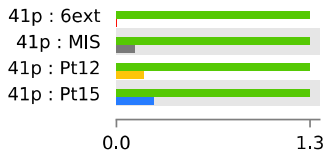
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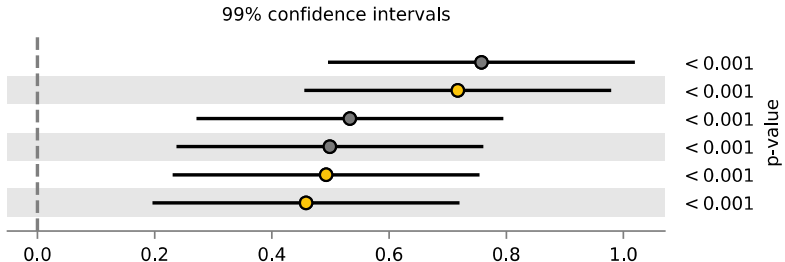
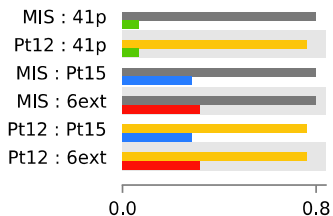
Family 48 OM1 clade



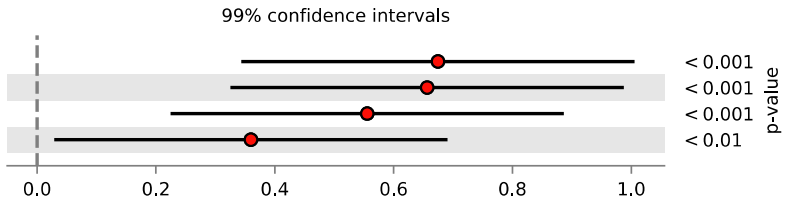
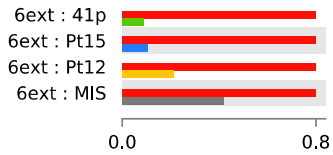
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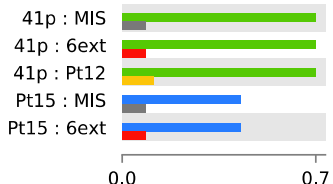
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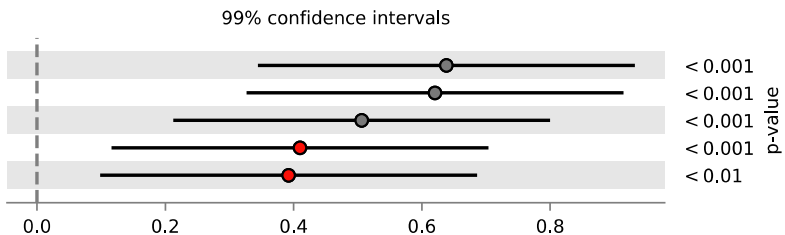
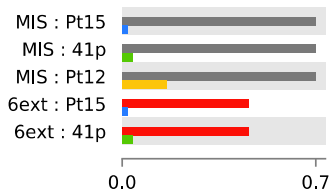
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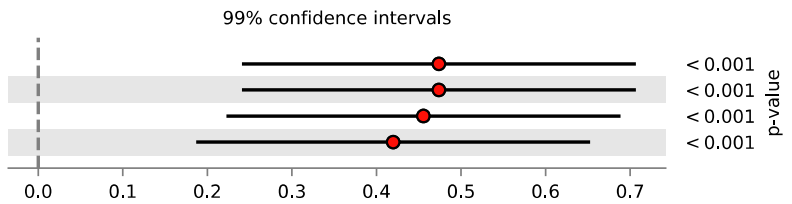
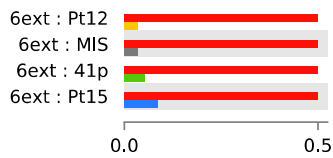
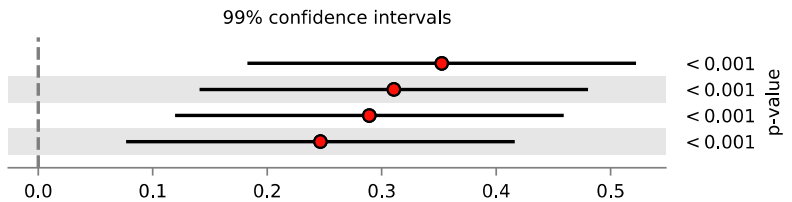
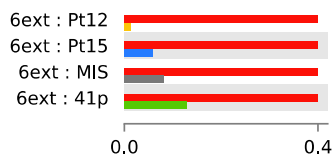
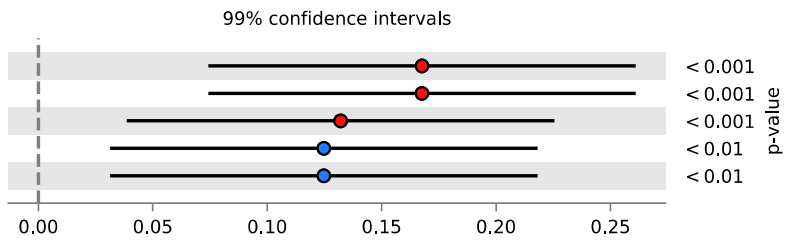
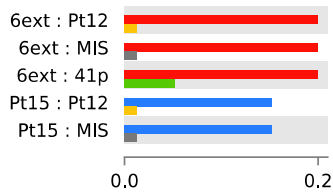
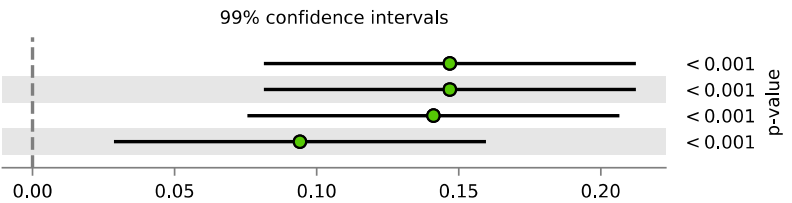
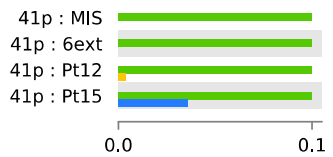
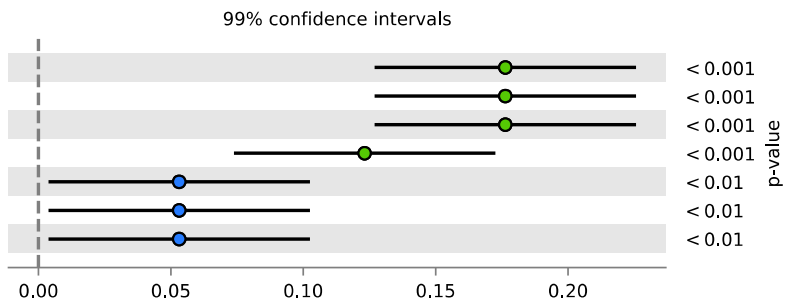
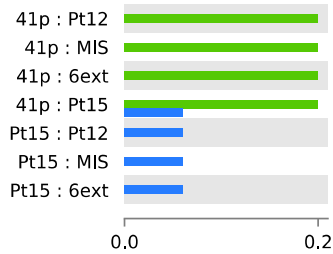
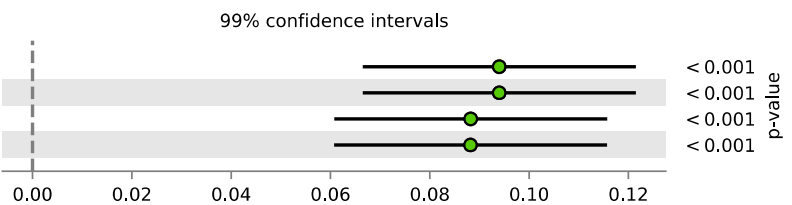
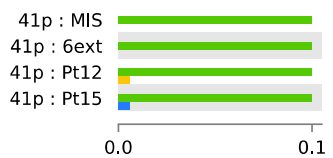
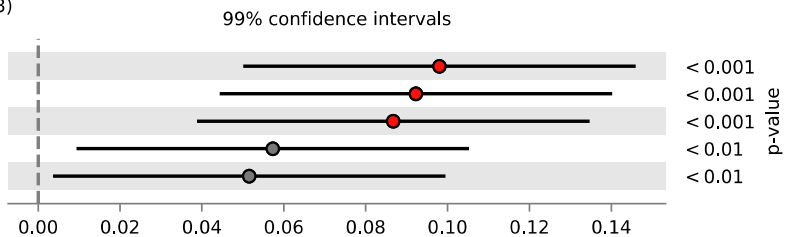
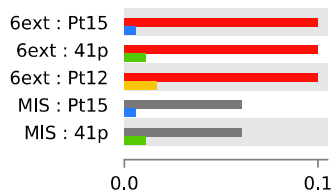


Family 477 Vibrionaceae



Family 501 uncultured bacterium



Family 409 Porticoccaceae

Family 268 uncultured

Family 446 Alcanivoracaceae

Family 448 CrystalBog021C3

Family 398 Shewanellaceae

Family 95 Prolixibacteraceae

Family 30 Solibacteraceae (Subgroup 3)


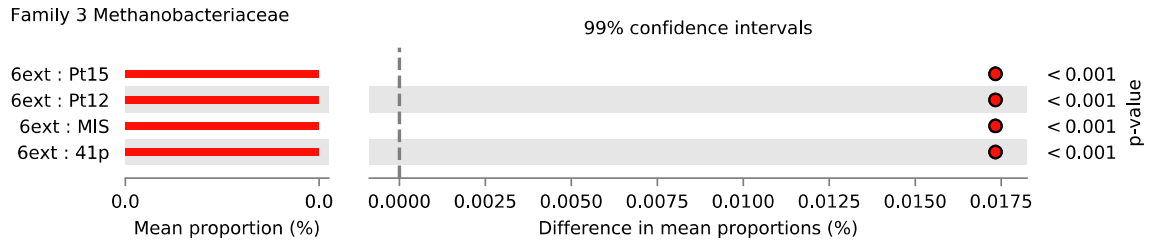
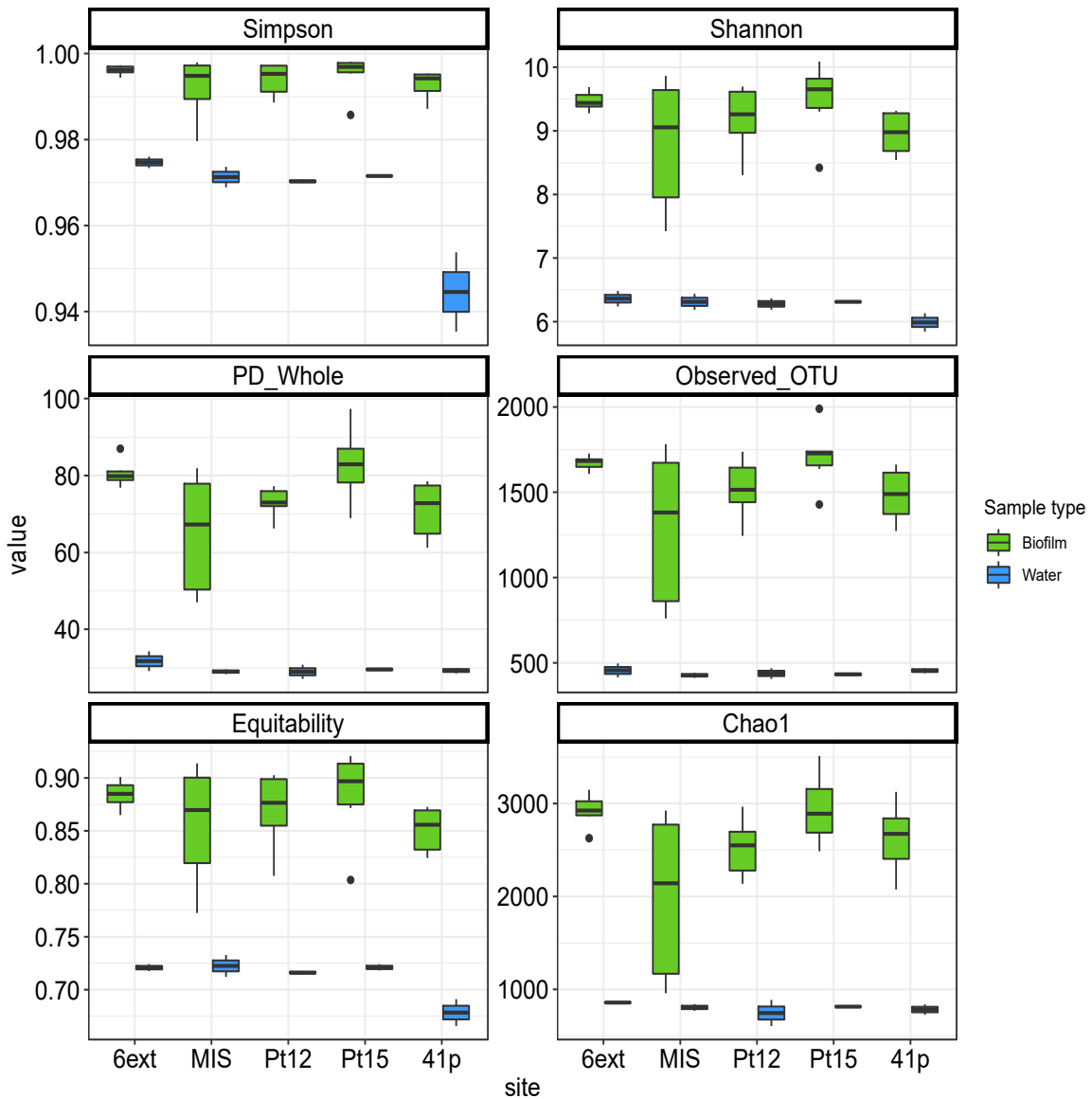


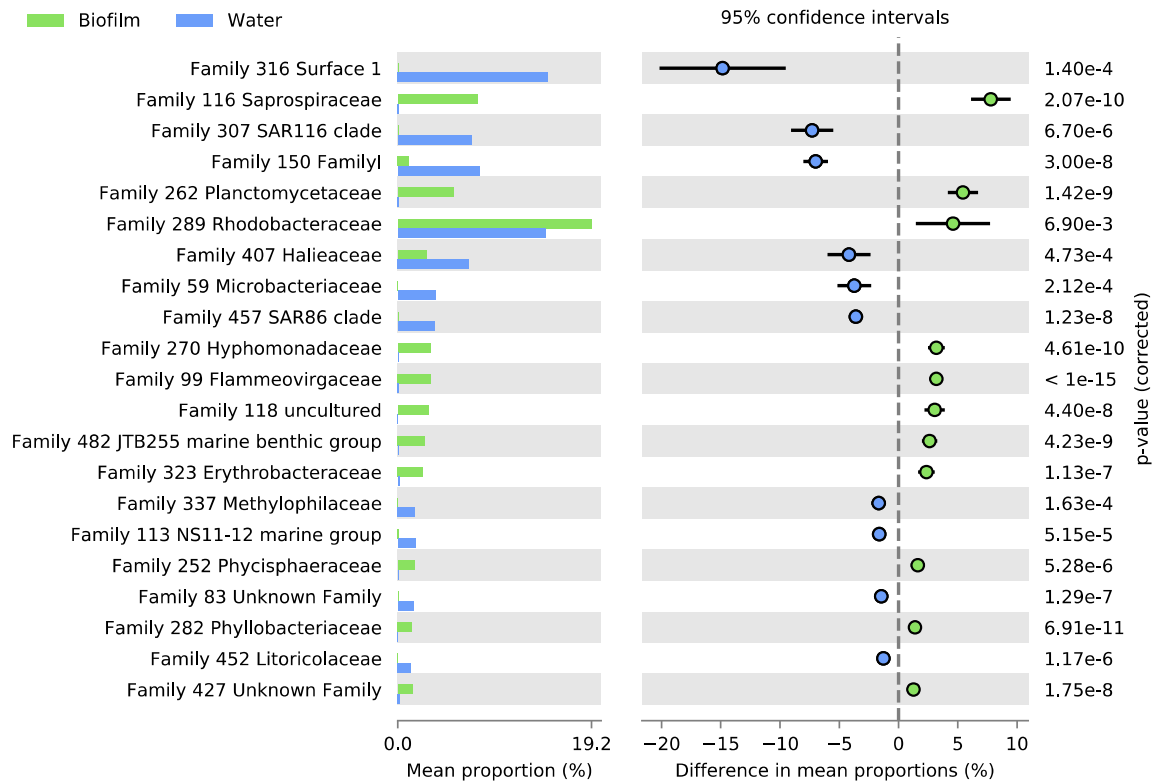
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1

2 **Fig S6** Alpha-diversity descriptors including Simpson, Shannon, PD_Whole, Observed OTU,
 3 equitability and Chao1 calculated from 16S rRNA OTU table along each sampling sites (i.e.
 4 6ext, MIS, Pt12, Pt15 and 41p) in seawater (blue) and biofilm (green) samples. Upper and lower
 5 lines correspond to the first and third quartile of the distribution of values. The median values
 6 are shown with horizontal black wide lines. Outliers are displayed as dots.

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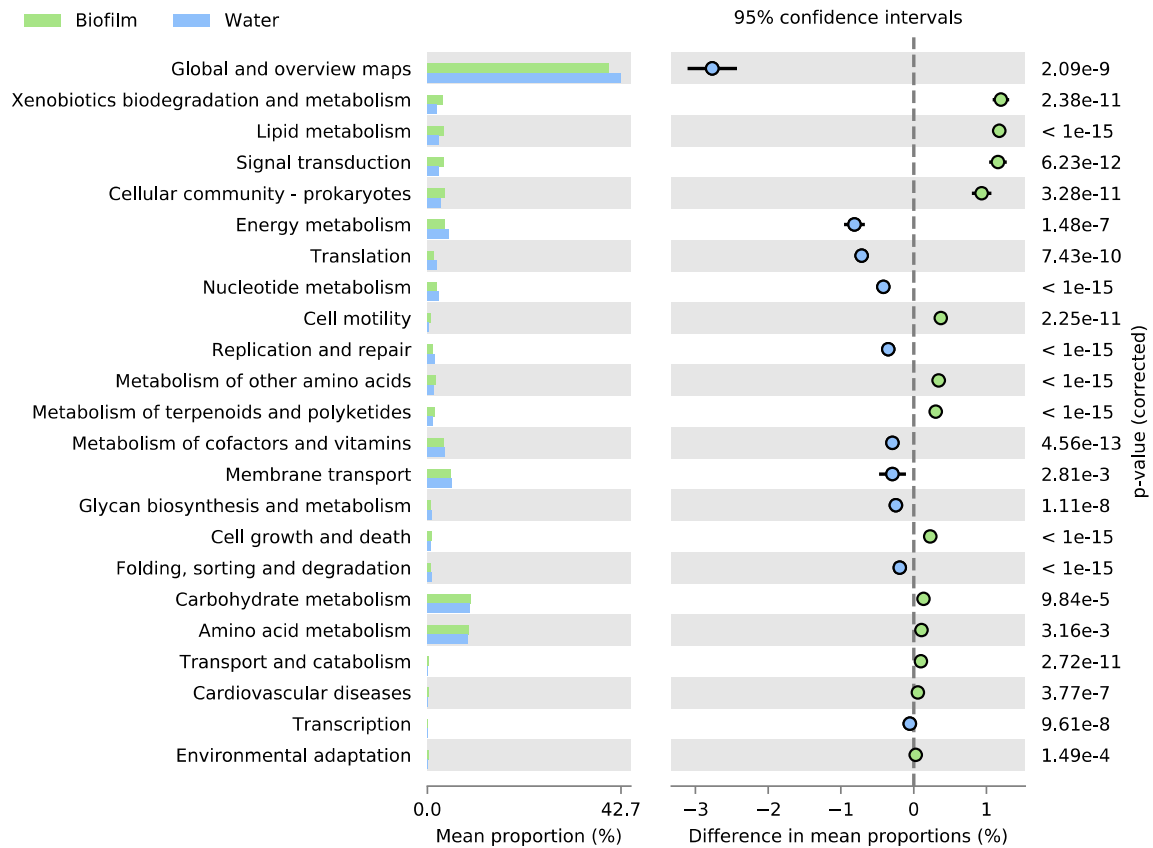
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9 **Fig. S7** Bar graph representing relative proportions of significant families in seawater (blue)

10 and biofilm (green) samples. Extended error bar plots showed pairwise comparison of

11 significant family proportions (Welch's t-test; $p < 0.01$) between seawater (blue) and biofilm

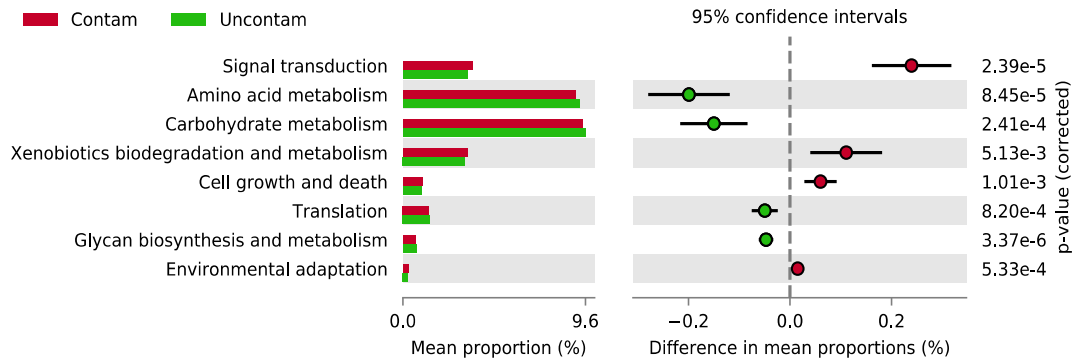
12 (green) samples. Corrected p-value is determined using Fisher's exact test.



13

14 **Fig. S8** Bar graph representing relative proportions of significant KEGG Pathways at level 2 of
 15 SEED metabolic hierarchy in biofilms (green) and seawater (blue). Extended error bar plots
 16 showed pairwise comparison of significant KEGG Pathway proportions (Welch's t-test; $p <$
 17 0.01) at level 2 of SEED metabolic hierarchy between biofilms (green) and seawater (blue).
 18 Corrected p-value is determined using Fisher's exact test.

19



20 **Fig. S9** Bar graph representing relative proportions of significant KEGG Pathways at level 2 of
 21 SEED metabolic hierarchy in biofilms from contaminated (6ext; red) and uncontaminated (41p;
 22 green) sites. Extended error bar plots showed pairwise comparison of significant KEGG
 23 Pathway proportions (Welch's t-test; $p < 0.01$) at level 2 of SEED metabolic hierarchy between
 24 biofilms from contaminated (6ext; red) and uncontaminated (41p; green) sites. Corrected p-
 25 value is determined using Fisher's exact test.

26

27 **3. Supplementary tables**

28 **Table S1.** Samples characteristics in Toulon Bay.

29 **Table S2.** Environmental (biotic and abiotic) characteristics of seawater samples in June 2015.

30 The values represent the mean between sampling dates ($n = 5$) and the standard deviation for
31 each parameter.

32 **Table S3.** Permutational multivariate analysis of variance (PERMANOVA) examining the
33 effects of the factors “Site”, “Lifestyle” and “Depth” on the bacterial communities. Key to
34 abbreviations and column headings: D.f, degrees of freedom; MS, mean square; F, F ratio; R²,
35 coefficient of determination; P, p-value. Probabilities are marked as follows ‘****’ $p < 0.001$;
36 ‘***’ $p < 0.01$; ‘*’ $p < 0.05$.

37 **Table S4.** Permutational multivariate analysis of variance (PERMANOVA) examining the
38 effects of the factors “Site”, and “Depth” on the normalized prokaryotic communities by cell
39 densities. Key to abbreviations and column headings: D.f, degrees of freedom; MS, mean
40 square; F, F ratio; R², coefficient of determination; P, p-value. Probabilities are marked as
41 follows ‘****’ $p < 0.001$; ‘***’ $p < 0.01$; ‘*’ $p < 0.05$.

42 **Table S5.** Permutational multivariate analysis of variance (PERMANOVA) examining the
43 effects of the factors “Site”, and “Depth” on functional profiles of KEGG Pathway at level 3 of
44 SEED metabolic hierarchy. Key to abbreviations and column headings: D.f, degrees of
45 freedom; MS, mean square; F, F ratio; R², coefficient of determination; P, p-value. Probabilities
46 are marked as follows ‘****’ $p < 0.001$; ‘***’ $p < 0.01$; ‘*’ $p < 0.05$.

47

48 **Table S1.** Samples characteristics in Toulon Bay.

Sample name	Site	Depth	Latitude (°N)	Longitude (°E)	No. of raw amplicons	No. of amplicons after filtering	No. of OTUs
41p_S	41p	Surface	43° 4'21.74"	5°57'21.48"	31090	24391	470
41p_S1	41p	Surface	43° 4'21.74"	5°57'21.48"	19130	15186	1273
41p_S2	41p	Surface	43° 4'21.74"	5°57'21.48"	24135	19041	1349
41p_S3	41p	Surface	43° 4'21.74"	5°57'21.48"	38830	28240	1442
41p_B	41p	Bottom	43° 4'21.74"	5°57'21.48"	27021	21754	439
41p_B1	41p	Bottom	43° 4'21.74"	5°57'21.48"	41518	29254	1662
41p_B2	41p	Bottom	43° 4'21.74"	5°57'21.48"	38056	15811	1537
41p_B3	41p	Bottom	43° 4'21.74"	5°57'21.48"	29235	20540	1640
Pt15_S	Pt15	Surface	43° 5'24.82"	5°54'39.05"	48244	36675	441
Pt15_S1	Pt15	Surface	43° 5'24.82"	5°54'39.05"	65410	37044	1739
Pt15_S2	Pt15	Surface	43° 5'24.82"	5°54'39.05"	42561	31438	1637
Pt15_S3	Pt15	Surface	43° 5'24.82"	5°54'39.05"	43641	30362	1428
Pt15_B	Pt15	Bottom	43° 5'24.82"	5°54'39.05"	28125	21500	424
Pt15_B1	Pt15	Bottom	43° 5'24.82"	5°54'39.05"	20433	10336	1719
Pt15_B2	Pt15	Bottom	43° 5'24.82"	5°54'39.05"	25131	8647	1737
Pt15_B3	Pt15	Bottom	43° 5'24.82"	5°54'39.05"	27684	15758	1989
Pt12_S	Pt12	Surface	43° 6'34.96"	5°55'41.09"	23279	17308	406
Pt12_S1	Pt12	Surface	43° 6'34.96"	5°55'41.09"	18631	12258	1450
Pt12_S2	Pt12	Surface	43° 6'34.96"	5°55'41.09"	15387	10164	1438
Pt12_S3	Pt12	Surface	43° 6'34.96"	5°55'41.09"	22721	16207	1244
Pt12_B	Pt12	Bottom	43° 6'34.96"	5°55'41.09"	38367	26656	469

Pt12_B1	Pt12	Bottom	43° 6'34.96"	5°55'41.09"	25947	19365	1666
Pt12_B2	Pt12	Bottom	43° 6'34.96"	5°55'41.09"	25992	18822	1578
Pt12_B3	Pt12	Bottom	43° 6'34.96"	5°55'41.09"	30137	18778	1738
MIS_S	MIS	Surface	43° 7'9.37"	5°54'40.07"	26720	21205	442
MIS_S1	MIS	Surface	43° 7'9.37"	5°54'40.07"	11297	5828	782
MIS_S2	MIS	Surface	43° 7'9.37"	5°54'40.07"	19638	11971	1100
MIS_S3	MIS	Surface	43° 7'9.37"	5°54'40.07"	10571	6279	760
MIS_B	MIS	Bottom	43° 7'9.37"	5°54'40.07"	32551	25934	412
MIS_B1	MIS	Bottom	43° 7'9.37"	5°54'40.07"	34347	25032	1663
MIS_B2	MIS	Bottom	43° 7'9.37"	5°54'40.07"	19239	14098	1676
MIS_B3	MIS	Bottom	43° 7'9.37"	5°54'40.07"	16679	11618	1780
6ext_S	6ext	Surface	43° 7'8.65"	5°54'57.31"	31290	22615	415
6ext_S1	6ext	Surface	43° 7'8.65"	5°54'57.31"	22281	15624	1608
6ext_S2	6ext	Surface	43° 7'8.65"	5°54'57.31"	32959	22598	1677
6ext_S3	6ext	Surface	43° 7'8.65"	5°54'57.31"	43457	24926	1695
6ext_B	6ext	Bottom	43° 7'8.65"	5°54'57.31"	28337	21647	496
6ext_B1	6ext	Bottom	43° 7'8.65"	5°54'57.31"	24539	17898	1639
6ext_B2	6ext	Bottom	43° 7'8.65"	5°54'57.31"	25837	17138	1690
6ext_B3	6ext	Bottom	43° 7'8.65"	5°54'57.31"	33373	17531	1727

50 **Table S2.** Environmental (biotic and abiotic) characteristics of seawater samples in June 2015. The values represent the mean between sampling
 51 dates (n = 5) and the standard deviation for each parameter.

Site	41p		Pt15		Pt12		MIS		6ext	
	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
Temperature	22 ± 1.4	19 ± 1.7	22 ± 1.6	21 ± 1.5	22 ± 1.6	21 ± 1.4	23 ± 1.3	21 ± 1.4	23 ± 1.3	21 ± 1.8
Salinity	39 ± 0.13	39 ± 0.05	39 ± 0.49	39 ± 0.15	39 ± 0.58	39 ± 0.15	38 ± 0.68	39 ± 0.07	38 ± 0.60	39 ± 0.09
Chlorophyll a	0.20 ± 0.06	0.37 ± 0.11	0.89 ± 0.30	1.3 ± 0.20	0.90 ± 0.57	2.0 ± 1.0	0.99 ± 0.31	2.6 ± 1.1	1.2 ± 0.51	3.5 ± 1.8
DOC	1.2 ± 0.14	1.1 ± 0.09	1.2 ± 0.15	1.2 ± 0.10	1.3 ± 0.12	1.2 ± 0.10	1.3 ± 0.24	1.2 ± 0.10	1.4 ± 0.16	1.2 ± 0.08
TN	0.08 ± 0.04	0.06 ± 0.02	0.08 ± 0.01	0.07 ± 0.02	0.09 ± 0.04	0.07 ± 0.02	0.15 ± 0.04	0.08 ± 0.02	0.11 ± 0.04	0.09 ± 0.02
Al	4788 ± 4436	4852 ± 4581	4419 ± 4955	4483 ± 5136	4742 ± 5191	4312 ± 5093	4157 ± 4651	4142 ± 4630	4216 ± 4475	3472 ± 4969
As	25 ± 2.4	27 ± 4.4	28 ± 2.7	27 ± 4.4	25 ± 5.0	24 ± 4.7	25 ± 4.9	26 ± 3.0	30 ± 3.8	27 ± 5.9
Ba	59 ± 2.2	59 ± 2.9	61 ± 2.3	60 ± 2.1	61 ± 2.5	62 ± 7.9	63 ± 3.7	60 ± 2.1	69 ± 4.0	68 ± 8.3
Be	46 ± 25	45 ± 26	48 ± 26	44 ± 5.9	49 ± 19	48 ± 14	52 ± 12	59 ± 28	63 ± 32	63 ± 33
Cd	0.063 ± 0.017	0.060 ± 0.022	0.088 ± 0.017	0.083 ± 0.0081	0.12 ± 0.029	0.13 ± 0.11	0.14 ± 0.056	0.13 ± 0.053	0.15 ± 0.019	0.17 ± 0.043
Cr	7.2 ± 4.7	7.0 ± 2.7	7.1 ± 3.4	7.0 ± 1.9	6.7 ± 2.1	8.0 ± 3.5	7.7 ± 2.0	6.4 ± 2.4	6.1 ± 1.4	5.0 ± 1.1
Cs	2.5 ± 0.10	2.5 ± 0.12	2.5 ± 0.09	2.5 ± 0.09	2.5 ± 0.09	2.5 ± 0.07	2.5 ± 0.13	2.5 ± 0.04	2.6 ± 0.11	2.5 ± 0.05
Cu	5.1 ± 1.2	4.2 ± 0.95	25 ± 6.6	12 ± 1.8	34 ± 7.9	30 ± 23	72 ± 19	28 ± 16	93 ± 22	26 ± 10
Fe	64 ± 41	64 ± 48	68 ± 50	130 ± 92	63 ± 52	66 ± 54	63 ± 42	66 ± 39	70 ± 37	69 ± 46
Li	28806 ± 2049	28921 ± 3253	29658 ± 4865	29403 ± 4373	29393 ± 4157	28757 ± 4732	27747 ± 3817	28355 ± 3057	28235 ± 2619	27813 ± 2526
Mn	14 ± 1.8	14 ± 4.3	24 ± 7.9	24 ± 7.1	25 ± 7.2	28 ± 14	26 ± 7.5	24 ± 6.0	30 ± 5.6	48 ± 6.5
Mo95	164 ± 9.6	162 ± 7.1	162 ± 8.7	161 ± 7.4	160 ± 7.5	160 ± 4.9	159 ± 6.0	162 ± 6.1	164 ± 8.7	161 ± 5.0

52 **Table S2. Continued**

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Site	41p		Pt15		Pt12		MIS		6ext	
Depth	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
Pb	0.26 ± 0.13	0.32 ± 0.086	1.1 ± 0.19	0.65 ± 0.17	1.5 ± 0.35	2.2 ± 2.3	1.4 ± 0.19	1.7 ± 0.18	4.6 ± 0.87	7.2 ± 6.1
Rb	1476 ± 20	1477 ± 46	1479 ± 73	1475 ± 61	1468.60 ± 56.33	1461 ± 63	1437 ± 54	1460 ± 40	1460 ± 49	1455 ± 35
Sb	3.6 ± 0.27	3.5 ± 0.23	4.1 ± 0.79	4.0 ± 0.79	4.1 ± 0.69	4.6 ± 1.3	4.3 ± 0.90	4.2 ± 0.84	5.4 ± 0.82	5.9 ± 1.9
Sn	1.7 ± 0.40	1.8 ± 0.46	1.8 ± 0.49	1.7 ± 0.48	2.2 ± 1.6	1.7 ± 0.56	1.7 ± 0.50	1.6 ± 0.45	1.9 ± 0.48	1.8 ± 0.37
Sr	106041 ± 1881	105845 ± 3392	106071 ± 4756	105787 ± 3868	105608 ± 4101	104734 ± 3787	103183 ± 4011	104936 ± 2494	104297 ± 3280	104835 ± 2195
Ti	16 ± 12	13 ± 10	16 ± 13	13 ± 12	14 ± 14	13 ± 15	15 ± 15	17 ± 15	14 ± 12	13 ± 15
U	16 ± 1.3	15 ± 1.6	15 ± 2.0	15 ± 1.9	15 ± 1.7	15 ± 2.1	15 ± 1.4	16 ± 1.3	16 ± 1.1	16 ± 0.40
V	43 ± 2.2	44 ± 4.5	44 ± 5.2	44 ± 4.1	43 ± 4.6	42 ± 4.4	42 ± 4.3	42 ± 3.4	43 ± 4.1	42 ± 3.4
Zn	18 ± 8.5	15 ± 5.1	49 ± 10	24 ± 4.7	61 ± 13	108 ± 152	130 ± 28	70 ± 38	290 ± 46	166 ± 81

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56 **Table S3.** Permutational multivariate analysis of variance (PERMANOVA) examining the
 57 effects of the factors “Site”, “Lifestyle” and “Depth” on the prokaryotic communities. Key to
 58 abbreviations and column headings: D.f, degrees of freedom; MS, mean square; F, F ratio; R2,
 59 coefficient of determination; P, p-value. Probabilities are marked as follows ‘***’ p<0.001;
 60 ‘**’ p<0.01; ‘*’ p<0.05.

Taxonomic rank	Source of variation	D.f	MS	F	R2	P	Significance
Phylum	Site	4	0.019737	7.490	0.13659	0.001	***
	Lifestyle	1	0.224852	85.326	0.38903	0.001	***
	Depth	1	0.029882	11.339	0.05170	0.002	**
	Site:Lifestyle	4	0.027082	10.277	0.18743	0.001	***
	Site:Depth	4	0.012060	4.577	0.08346	0.001	***
	Lifestyle:Depth	1	0.010746	4.078	0.01859	0.020	*
	Site:Lifestyle:Depth	4	0.006069	2.303	0.04200	0.015	*
	Residuals	20	0.002635	0.09119			
Class	Site	4	0.04588	8.316	0.13310	0.001	***
	Lifestyle	1	0.55500	100.607	0.40255	0.001	***
	Depth	1	0.10884	19.730	0.07894	0.001	***
	Site:Lifestyle	4	0.04704	8.528	0.13648	0.001	***
	Site:Depth	4	0.03424	6.206	0.09933	0.001	***
	Lifestyle:Depth	1	0.05365	9.726	0.03891	0.001	***
	Site:Lifestyle:Depth	4	0.01057	1.916	0.03066	0.045	*
	Residuals	20	0.00552	0.08002			
Order	Site	4	0.05791	9.147	0.09456	0.001	***
	Lifestyle	1	1.41571	223.626	0.57794	0.001	***

	Depth	1	0.11711	18.499	0.04781	0.001	***
	Site:Lifestyle	4	0.07389	11.672	0.12066	0.001	***
	Site:Depth	4	0.03965	6.264	0.06475	0.001	***
	Lifestyle:Depth	1	0.04722	7.460	0.01928	0.002	**
	Site:Lifestyle:Depth	4	0.01428	2.255	0.02332	0.039	*
	Residuals	20	0.00633	0.05169			
Family	Site	4	0.04076	6.820	0.08150	0.001	***
	Lifestyle	1	1.12012	187.399	0.55986	0.001	***
	Depth	1	0.11508	19.253	0.05752	0.001	***
	Site:Lifestyle	4	0.06283	10.512	0.12562	0.001	***
	Site:Depth	4	0.03443	5.760	0.06883	0.001	***
	Lifestyle:Depth	1	0.04781	7.998	0.02390	0.002	**
	Site:Lifestyle:Depth	4	0.01152	1.927	0.02303	0.054	.
	Residuals	20	0.00598	0.05975			

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63 **Table S3.** Continued

Taxonomic rank	Source of variation	D.f	MS	F	R2	P	Significance
Genus	Site	4	0.04057	9.369	0.08104	0.001	***
	Lifestyle	1	1.31058	302.628	0.65437	0.001	***
	Depth	1	0.13668	31.562	0.06825	0.001	***
	Site:Lifestyle	4	0.02516	5.809	0.05024	0.001	***
	Site:Depth	4	0.02733	6.310	0.05458	0.001	***
	Lifestyle:Depth	1	0.06001	13.858	0.02996	0.001	***
	Site:Lifestyle:Depth	4	0.00917	2.117	0.01831	0.037	*
	Residuals	20	0.00433	0.04325			

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65 **Table S4.** Permutational multivariate analysis of variance (PERMANOVA) examining the
 66 effects of the factors “Site”, and “Depth” on the normalized prokaryotic communities by cell
 67 densities. Key to abbreviations and column headings: D.f, degrees of freedom; MS, mean
 68 square; F, F ratio; R2, coefficient of determination; P, p-value. Probabilities are marked as
 69 follows ‘****’ p<0.001; ‘***’ p<0.01; ‘*’ p<0.05.

	Source of variation	D.f	MS	F	R2	P	Significance
Level 3	Site	4	0.392	3.5430	0.293	0.001	***
	Depth	1	0.184	1.6652	0.034	0.188	NS
	Site:Depth	4	0.349	3.1529	0.260	0.001	***
	Residuals	20	0.111				

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71 **Table S5.** Permutational multivariate analysis of variance (PERMANOVA) examining the
 72 effects of the factors “Site”, and “Depth” on functional profiles of KEGG Pathway at level 3 of
 73 SEED metabolic hierarchy. Key to abbreviations and column headings: D.f, degrees of
 74 freedom; MS, mean square; F, F ratio; R2, coefficient of determination; P, p-value. Probabilities
 75 are marked as follows ‘***’ p<0.001; ‘**’ p<0.01; ‘*’ p<0.05.

	Source of variation	D.f	MS	F	R2	P	Significance
Level 3	Site	4	0.0006	5.21	0.34	0.001	***
	Depth	1	0.0009	8.24	0.13	0.002	**
	Site:Depth	4	0.0003	3.07	0.20	0.003	**
	Residuals	20	0.0001				

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