

## **Supplemental Data Summary**

**Bacterioplankton communities respond to cadmium (Cd) exposure in coastal water microcosms with high temporal variability**

**Table S1** Two-way analysis of variance (ANOVA) for environmental variables of seawater across different sampling days and Cd levels.

Sampling day	Cd level	pH	DO (mg/L)	DOC (mg/L)	Chl- <i>a</i> (μg/L)	PO <sub>4</sub> <sup>3-</sup> -P (μM)	NO <sub>3</sub> <sup>-</sup> -N (μM)	NH <sub>4</sub> <sup>+</sup> -N (μM)	NO <sub>2</sub> <sup>-</sup> -N (μM)
0d	Origin	7.95 ±0.01	8.52 ±0.47	2.18 ±0.47	14.72 ±0.93	0.60 ±0.06	28.3 ±2.2	6.0 ±1.0	1.36 ±0.10
1d	CK	8.01 ±0.01	8.59 ±0.28	4.43 ±0.26	13.53 ±1.24	0.33 ±0.04	26.8 ±2.5	11.6 ±0.5	1.42 ±0.14
	Cd1	8.01 ±0.01	8.34 ±0.04	6.85 ±2.76	13.02 ±0.26	0.38 ±0.09	24.1 ±5.8	11.5 ±0.3	1.42 ±0.14
	Cd2	7.99 ±0.01	8.22 ±0.04	5.97 ±0.74	8.51 ±1.53	0.47 ±0.02	27.4 ±5.1	11.3 ±0.4	1.42 ±0.17
	Cd3	8.00 ±0.01	8.35 ±0.07	5.23 ±0.46	8.09 ±1.16	0.48 ±0.03	27.3 ±7.0	11.0 ±0.6	1.37 ±0.11
4d	CK	8.05 ±0.02	7.62 ±0.03	6.31 ±0.33	1.30 ±0.08	0.16 ±0.04	32.4 ±6.3	4.1 ±1.1	1.46 ±0.05
	Cd1	8.09 ±0.05	7.96 ±0.33	7.75 ±1.69	2.36 ±1.61	0.14 ±0.05	33.7 ±6.0	4.6 ±0.7	1.53 ±0.26
	Cd2	8.03 ±0.02	8.29 ±0.09	5.02 ±0.87	3.03 ±0.75	0.40 ±0.06	36.6 ±2.1	11.0 ±1.4	1.48 ±0.19
	Cd3	8.03 ±0.02	8.28 ±0.16	4.25 ±0.64	3.21 ±1.40	0.46 ±0.06	34.9 ±2.5	13.9 ±0.4	1.31 ±0.11
7d	CK	8.01 ±0.01	7.22 ±0.26	5.29 ±0.63	1.12 ±0.79	0.31 ±0.02	20.9 ±2.4	12.8 ±0.6	1.45 ±0.22
	Cd1	8.05 ±0.01	7.28 ±0.17	4.35 ±2.02	0.93 ±0.26	0.22 ±0.04	22.0 ±2.5	10.6 ±2.1	1.61 ±0.11
	Cd2	8.03 ±0.01	7.36 ±0.10	4.37 ±1.02	1.40 ±0.32	0.45 ±0.02	20.9 ±5.1	16.3 ±0.7	1.55 ±0.26
	Cd3	8.03 ±0.01	7.28 ±0.08	3.78 ±0.41	1.12 ±0.64	0.41 ±0.06	26.2 ±3.5	17.5 ±0.3	1.33 ±0.12
14d	CK	8.21 ±0.18	8.11 ±0.61	5.67 ±0.64	5.02 ±2.73	0.16 ±0.02	18.1 ±2.2	2.4 ±0.4	1.71 ±0.35
	Cd1	8.09 ±0.04	7.63 ±0.41	4.31 ±0.27	3.49 ±0.84	0.40 ±0.09	14.2 ±2.8	6.3 ±1.2	1.73 ±0.11
	Cd2	8.08 ±0.01	7.40 ±0.06	3.43 ±0.12	1.84 ±1.04	0.80 ±0.07	21.7 ±1.3	10.8 ±0.7	1.57 ±0.21
	Cd3	8.07 ±0.00	7.44 ±0.16	4.41 ±1.02	0.84 ±0.32	0.79 ±0.28	21.0 ±5.0	11.7 ±0.7	1.46 ±0.11
<i>Time</i>	F	14.69	62.83	7.123	234.561	24.49	39.70	5.938	3.999
	<i>P</i>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.016</b>	<b>0.013</b>
<i>Cd</i>	F	2.103	0.353	5.380	10.77	54.24	2.852	5.643	3.567
	<i>P</i>	0.112	0.787	<b>0.003</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>0.047</b>	<b>0.019</b>	<b>0.021</b>
<i>Time</i> × <i>Cd</i>	F	2.030	5.301	3.232	9.114	7.632	0.782	30.78	0.416
	<i>P</i>	0.056	<b>&lt;0.001</b>	<b>0.004</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.634	<b>&lt;0.001</b>	0.920

DO: dissolved oxygen; DOC: dissolved organic carbon; Chl-*a*: chlorophyll *a*.The data represent the mean ± standard deviation (n = 4); Bold values represent significant influence of *Time*, *Cd*, or *Time* × *Cd* on variables (*P* < 0.05).

**Table S2** Two-way analysis of variance (ANOVA) for alpha-diversity metrics (per 3380 sequences) of bacterioplankton communities across different sampling days and Cd levels.

Sampling day	Cd level	Observed richness	Chao1	Phylogenetic diversity	Shannon	Pielou's <i>J</i>
0d	Origin	600 ± 60	1338 ± 191	58.1 ± 5.5	6.73 ± 0.28	0.729 ± 0.019
1d	CK	629 ± 53 abc	1476 ± 276 bcd	59.4 ± 5.1 ab	6.79 ± 0.17 abcd	0.731 ± 0.012 abcd
	Cd1	556 ± 59 cde	1237 ± 134 cde	53.2 ± 5.0 bcde	6.43 ± 0.28 cdef	0.705 ± 0.021 bcdef
	Cd2	581 ± 53 cde	1344 ± 173 cde	55.7 ± 4.5 abc	6.57 ± 0.33 abcde	0.715 ± 0.026 abcde
	Cd3	631 ± 142 abc	1510 ± 445 bc	58.1 ± 11.3 abc	7.09 ± 0.47 ab	0.764 ± 0.025 a
4d	CK	511 ± 70 e	1238 ± 211 cde	44.3 ± 6.5 f	5.61 ± 0.67 h	0.623 ± 0.062 hi
	Cd1	500 ± 78 e	1269 ± 210 cde	44.3 ± 6.5 f	5.46 ± 0.56 h	0.610 ± 0.051 i
	Cd2	689 ± 57 ab	1671 ± 207 ab	59.8 ± 6.0 ab	6.98 ± 0.51 abc	0.740 ± 0.047 abc
	Cd3	722 ± 16 a	1855 ± 40 a	63.3 ± 2.6 a	7.16 ± 0.21 a	0.754 ± 0.021 ab
7d	CK	613 ± 78 bcd	1465 ± 207 bcd	54.3 ± 6.6 bcd	6.31 ± 0.64 defg	0.681 ± 0.056 defg
	Cd1	566 ± 85 cde	1424 ± 394 bcde	49.8 ± 7.6 cdef	6.05 ± 0.63 efgh	0.662 ± 0.055 efghi
	Cd2	558 ± 77 cde	1405 ± 280 bcde	50.3 ± 6.8 cdef	6.26 ± 0.34 defg	0.687 ± 0.023 cdefg
	Cd3	519 ± 21 de	1262 ± 39 cde	45.4 ± 2.0 ef	6.27 ± 0.21 defg	0.695 ± 0.020 cdef
14d	CK	538 ± 110 cde	1134 ± 238 e	50.0 ± 7.4 cdef	5.92 ± 0.53 fgh	0.654 ± 0.041 fghi
	Cd1	516 ± 36 de	1113 ± 130 e	45.3 ± 1.9 ef	5.78 ± 0.33 gh	0.641 ± 0.034 ghi
	Cd2	504 ± 29 e	1156 ± 93 de	46.8 ± 1.8 def	6.02 ± 0.37 efgh	0.670 ± 0.042 efgh
	Cd3	556 ± 34 cde	1342 ± 132 cde	49.8 ± 2.9 cdef	6.49 ± 0.26 bcdef	0.712 ± 0.023 abcde
<i>Time</i>	F	4.111	5.504	6.561	6.683	7.682
	<i>P</i>	<b>0.011</b>	<b>0.003</b>	<b>0.001</b>	<b>0.001</b>	<b>&lt; 0.001</b>
<i>Cd</i>	F	2.955	2.984	3.245	10.67	12.79
	<i>P</i>	<b>0.042</b>	<b>0.040</b>	<b>0.030</b>	<b>&lt; 0.001</b>	<b>&lt; 0.001</b>
<i>Time × Cd</i>	F	3.578	2.257	3.885	3.256	2.720
	<i>P</i>	<b>0.002</b>	<b>0.034</b>	<b>0.001</b>	<b>0.004</b>	<b>0.012</b>

The data represent the mean ± standard deviation (n = 4); Different lowercase letters in the same column indicate significant differences at 0.05 level (based on LSD); Bold values represent significant influence of *Time*, *Cd*, or *Time × Cd* on metrics ( $P < 0.05$ ).

**Table S3** Pearson's correlation coefficients (*r*) between Cd level and relative abundances of top phylotypes primarily driving bacterial community variation caused by Cd addition at each sampling day (*n* = 16). Only the OTUs with significant coefficients are listed. Contribution: percentages of the phylotypes contributing to the overall dissimilarity along the Cd gradient at each time point calculated by similarity percentage (SIMPER) analysis. Taxonomy of the OTUs was identified in Greengenes database.

OTU ID	Taxa	<i>r</i>	<i>P</i>	Contribution %
1d				
OTU3983	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Unclassified	0.971	< 0.001	2.84
OTU10908	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Tenacibaculum	0.837	< 0.001	0.98
OTU6594	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Winogradskyella	0.799	< 0.001	0.72
OTU9866	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Unclassified	0.832	< 0.001	0.71
OTU11304	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Polaribacter	0.883	< 0.001	0.70
OTU12733	c_Alphaproteobacteria; o_Rhodobacterales; f_Rhodobacteraceae; g_Octadecabacter	0.544	0.029	0.85
OTU4372	c_Alphaproteobacteria; o_Rhodobacterales; f_Rhodobacteraceae; g_Pseudoruegeria	0.517	0.040	0.78
OTU11417	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Unclassified	-0.564	0.023	7.08
OTU3859	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Unclassified	-0.666	0.005	4.21
OTU6529	p_Bacteroidetes; c_Unclassified	-0.676	0.004	1.60
OTU5995	p_Bacteroidetes; c_Sphingobacteria; o_Sphingobacteriales; f_Unclassified	-0.927	< 0.001	1.12
OTU551	p_Bacteroidetes; c_Sphingobacteria; o_Sphingobacteriales; f_Unclassified	-0.733	0.001	0.71
4d				
OTU8594	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Sediminicola	0.810	< 0.001	2.56
OTU6594	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Winogradskyella	0.682	0.004	0.91
OTU9866	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Unclassified	0.872	< 0.001	0.73
OTU7500	c_Alphaproteobacteria; o_Rickettsiales; f_Pelagibacteraceae; g_Unclassified	0.766	0.001	1.95
OTU6182	c_Alphaproteobacteria; o_Sphingomonadales; f_Erythrobacteraceae; g_Unclassified	0.524	0.037	0.78
OTU4028	c_Alphaproteobacteria; o_Sphingomonadales; f_Erythrobacteraceae; g_Unclassified	0.808	< 0.001	0.71
OTU11469	c_Gammaproteobacteria; o_Thiotrichales; f_Piscirickettsiaceae; g_Unclassified	0.695	0.003	0.85
OTU10579	c_Alphaproteobacteria; o_Rhodobacterales; f_Rhodobacteraceae; g_Phaeobacter	-0.865	< 0.001	4.19
OTU3392	c_Alphaproteobacteria; o_Rhodobacterales; f_Rhodobacteraceae; g_Anaerospora	-0.672	0.004	2.32
OTU11689	c_Alphaproteobacteria; o_Rhodobacterales; f_Rhodobacteraceae; g_Unclassified	-0.515	0.041	0.84

7d

OTU3983	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Unclassified	0.798	< 0.001	5.67
OTU6594	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Winogradskyella	0.880	< 0.001	1.99
OTU9148	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Algibacter	0.932	< 0.001	0.78
OTU8594	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Sediminicola	0.943	< 0.001	0.76
OTU6182	c_Alphaproteobacteria; o_Sphingomonadales; f_Erythrobacteraceae; g_Unclassified	0.753	0.001	1.79
OTU4028	c_Alphaproteobacteria; o_Sphingomonadales; f_Erythrobacteraceae; g_Unclassified	0.955	< 0.001	1.77
OTU4372	c_Alphaproteobacteria; o_Rhodobacterales; f_Rhodobacteraceae; g_Pseudoruegeria	0.797	< 0.001	0.90
OTU9175	c_Gammaproteobacteria; o_Alteromonadales; f_Alteromonadaceae; g_Glaciecola	0.850	< 0.001	1.36
OTU11469	c_Gammaproteobacteria; o_Thiotrichales; f_Piscirickettsiaceae; g_Unclassified	0.705	0.002	0.93
OTU8528	p_Bacteroidetes; c_Sphingobacteria; o_Sphingobacteriales; f_Saprospiraceae; g_Unclassified	-0.513	0.042	0.80
OTU3392	c_Alphaproteobacteria; o_Rhodobacterales; f_Rhodobacteraceae; g_Anaerospira	-0.645	0.007	4.32
OTU10579	c_Alphaproteobacteria; o_Rhodobacterales; f_Rhodobacteraceae; g_Phaeobacter	-0.796	< 0.001	2.18

14d

OTU8594	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Sediminicola	0.823	< 0.001	5.79
OTU6594	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Winogradskyella	0.910	< 0.001	1.32
OTU4028	c_Alphaproteobacteria; o_Sphingomonadales; f_Erythrobacteraceae; g_Unclassified	0.612	0.012	1.82
OTU2109	p_Bacteroidetes; c_Flavobacteria; o_Flavobacteriales; f_Flavobacteriaceae; g_Unclassified	-0.727	0.001	11.63
OTU8528	p_Bacteroidetes; c_Sphingobacteria; o_Sphingobacteriales; f_Saprospiraceae; g_Unclassified	-0.589	0.016	0.79
OTU4834	c_Alphaproteobacteria; o_Rhodobacterales; f_Rhodobacteraceae; g_Dinoroseobacter	-0.579	0.019	2.08

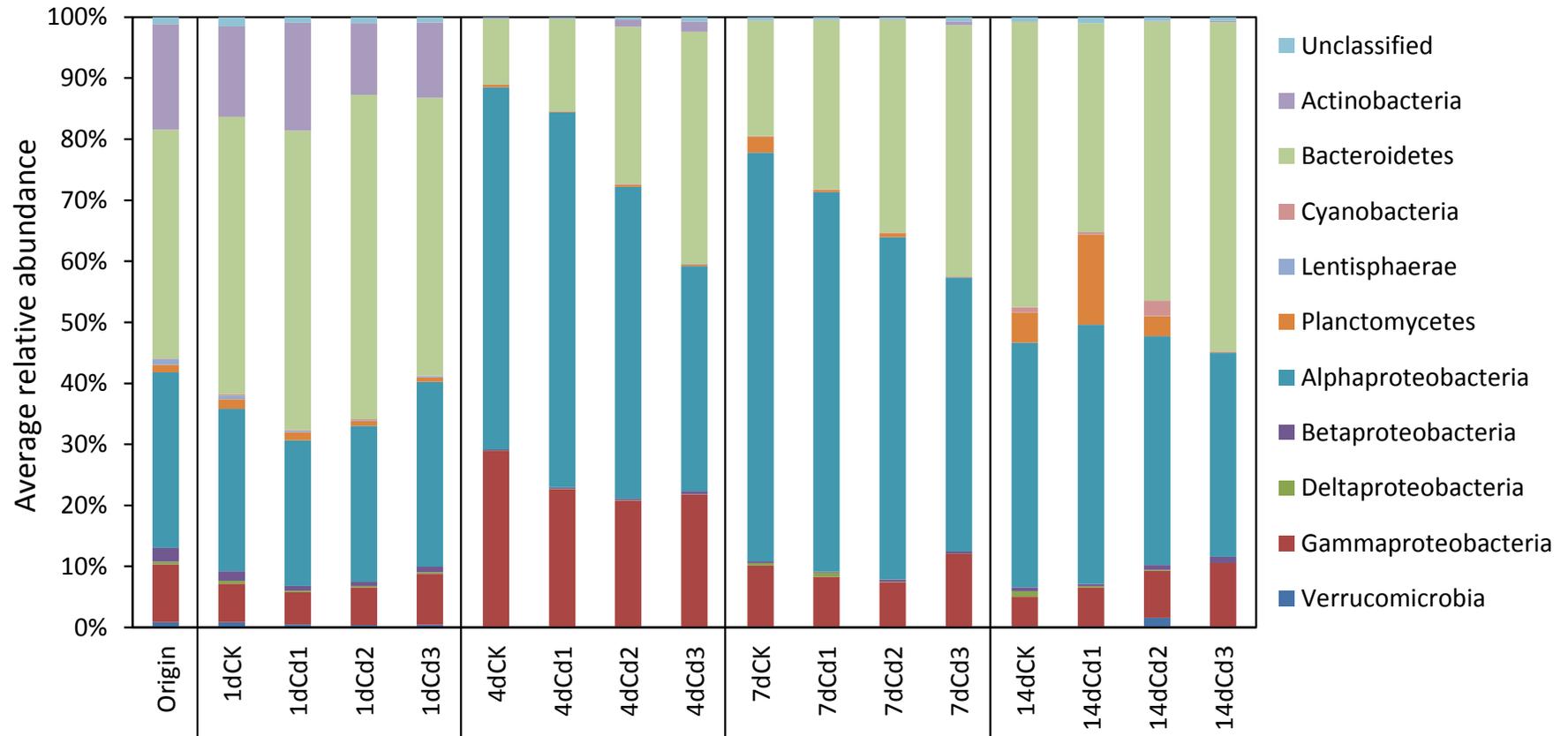
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**Table S4** Closest matched type strains of the representative phylotypes associated with Cd level based on 16S rRNA gene in EzTaxon database.

OTU ID	Length (bp)	Closest match	Similarity (%)	Accession	Taxonomy
Positive group					
OTU3983	388	<i>Polaribacter porphyrae</i> LNM-20 <sup>T</sup>	<b>98.97</b>	AB695286	Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Polaribacter
OTU10908	419	<i>Tenacibaculum adriaticum</i> B390 <sup>T</sup>	95.7	AM412314	Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Tenacibaculum
OTU6594	390	<i>Winogradskyella litorisediminis</i> DPS-8 <sup>T</sup>	<b>100</b>	JQ432561	Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Winogradskyella
OTU9866	421	<i>Dokdonia diaphoros</i> MSKK-32 <sup>T</sup>	94.54	AB198089	Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Dokdonia
OTU11304	388	<i>Polaribacter reichenbachii</i> 6Alg 8 <sup>T</sup>	<b>99.74</b>	HQ891656	Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Polaribacter
OTU8594	393	<i>Maribacter antarcticus</i> CL-AP4 <sup>T</sup>	94.4	EU512921	Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Maribacter
OTU9148	388	<i>Algibacter aestuarii</i> KYW371 <sup>T</sup>	<b>100</b>	HQ405792	Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Algibacter
OTU12733	423	<i>Planktomarina temperata</i> RCA23 <sup>T</sup>	95.74	GQ369962	Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Planktomarina
OTU4372	423	<i>Lentibacter algarum</i> ZX100 <sup>T</sup>	96.22	FJ436732	Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Lentibacter
OTU7500	388	<i>Candidatus Pelagibacter ubique</i> HTCC1062	<b>99.48</b>	CP000084	Alphaproteobacteria; SAR11; SAR11-1; Pelagibacter
OTU6182	393	<i>Altererythrobacter namhicola</i> KYW48 <sup>T</sup>	<b>97.96</b>	FJ935793	Alphaproteobacteria; Sphingomonadales; Erythrobacteraceae; Altererythrobacter
OTU4028	393	<i>Erythrobacter seohaensis</i> SW-135 <sup>T</sup>	<b>97.46</b>	AY562219	Alphaproteobacteria; Sphingomonadales; Erythrobacteraceae; Erythrobacter
OTU11469	393	<i>Methylophaga marina</i> DSM 5689 <sup>T</sup>	96.95	X95459	Gammaproteobacteria; Thiotrichales; Methylophaga; Methylophaga
OTU9175	393	<i>Aliiglaciecola lipolytica</i> E3 <sup>T</sup>	<b>100</b>	EU183316	Gammaproteobacteria; Alteromonadales; Alteromonadaceae; Aliiglaciecola
Negative group					
OTU11417	421	<i>Corallibacter vietnamensis</i> KMM 6217 <sup>T</sup>	94.54	HQ257254	Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Meridianimaribacter
OTU3859	417	<i>Marinitalea sucinacia</i> JC2131 <sup>T</sup>	95.68	FJ387163	Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Marinitalea
OTU6529	421	<i>Owenweeksia hongkongensis</i> DSM 17368 <sup>T</sup>	87.17	CP003156	Bacteroidetes; Flavobacteria; Flavobacteriales; Cryomorphaceae; Owenweeksia
OTU5995	421	<i>Mucilagibacter herbaticus</i> DR-9 <sup>T</sup>	85.51	JN695632	Bacteroidetes; Sphingobacteria; Sphingobacteriales; Sphingobacteriaceae; Mucilagibacter
OTU551	422	<i>Solitalea korensis</i> R2A36-4 <sup>T</sup>	85.04	EU787448	Bacteroidetes; Sphingobacteria; Sphingobacteriales; Sphingobacteriaceae; Solitalea
OTU8528	382	<i>Lewinella nigricans</i> NBRC 102662 <sup>T</sup>	90.31	AB301615	Bacteroidetes; Sphingobacteria; Sphingobacteriales; Saprospiraceae; Lewinella
OTU2109	390	<i>Kordia antarctica</i> IMCC3317 <sup>T</sup>	<b>97.44</b>	JX456458	Bacteroidetes; Flavobacteria; Flavobacteriales; Flavobacteriaceae; Kordia
OTU10579	392	<i>Phaeobacter arcticus</i> DSM 23566 <sup>T</sup>	<b>99.23</b>	AXBF01000003	Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Phaeobacter
OTU3392	392	<i>Roseovarius litoreus</i> GSW-M15 <sup>T</sup>	<b>99.49</b>	JQ390520	Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Roseovarius
OTU11689	392	<i>Tropicibacter phthalicicus</i> KU27E1 <sup>T</sup>	<b>99.23</b>	AB636139	Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Tropicibacter
OTU4834	392	<i>Pseudoruegeria lutimaris</i> HD-43 <sup>T</sup>	<b>98.47</b>	FJ374173	Alphaproteobacteria; Rhodobacterales; Rhodobacteraceae; Pseudoruegeria

Bold values represent similarity > 97%

**Figure S1** Average relative abundances of the dominant bacterial phyla (> 1%) in seawater samples across different sampling days and Cd levels (n = 4). Relative abundances are based on the proportional frequencies of those DNA sequences that could be classified at phylum level except Proteobacteria, which were grouped at class level. Unclassified means that the taxa cannot be assigned to any known phylum in the current Greengenes database.



**Figure S2** Mean ( $\pm$  standard error) unweighted UniFrac distance (**Upper**) and Bray-Curtis dissimilarity (**Bottom**) between communities of parallel samples within each Cd level and samples among the different Cd levels. At each time point,  $P < 0.01$  unless indicated (Student  $t$ -tests, ns: not significant).

