

High-throughput amplicon sequencing and stream benthic bacteria: identifying the best taxonomic level for multiple-stressor research

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Supplementary Information

This document contains information on DNA extraction and amplification procedures, descriptions of all observed significant interactions for bacterial response variables with corresponding figures, and full taxonomic information on the common phyla, orders, genera and species (OTU with clustering at 97% similarity).

1. DNA extraction and amplification

DNA was extracted from the 64 samples using a bead beater extraction protocol (Miller et al., 1999). Amplification of the V3 and V4 hypervariable regions of the 16S rRNA gene was performed using oligonucleotide primers (Klindworth et al., 2013) modified by the addition of Illumina Tags (underlined): S-D-Bact-0341-b-S-17 (5'-ACGACGCTCTTCCGATCTCCTAC GGGNGGCWGCAG-3') and S-D-Bact-0785-a-A-21 (5'-CGTGTGCTCTTCCGATCTGACT ACHVGGGTATCTA-3'), resulting in amplicons that contained a 16S rRNA gene region of ~440 bp. The HiFi HotStart PCR kit (KAPA Biosystems, Boston, MA, USA) was used with the following protocol: 0.2 mM dNTPs, 40 ng of DNA template, 0.02 U/ μ l KAPA HiFi Taq, 0.2 μ M of each primer in a total volume of 50 μ l. PCR conditions were: initial denaturation at 95°C for 5 min; followed by 27 cycles of 95°C for 1 min, 56°C (1 min) and 72°C (1 min); final extension at 72°C (5 min). Amplified products were purified using Agencourt AMPure XP purification kit (protocol 000387v001; Beckman Coulter, Brea, CA, USA) and quantified using Qubit dsDNA High Sensitivity Assay (Agilent Technologies, Palo Alto, CA, USA). Then a second round of PCR was performed using TruSeq® RNA PCR Primers (RPI1-33; Illumina, San Diego, CA, USA) to attach 6-bp multiplexing indices and Illumina sequencing adapters using the following protocol: 0.2 mM dNTPs, 2 ng of the purified PCR product, 0.02 U/ μ l KAPA HiFi Taq, 0.2 μ M of each primer in a total volume of 50 μ l. PCR conditions were: initial denaturation at 95°C for 20 sec; 10 cycles of 95°C (20 sec), 55°C (20 sec) and 68°C (20 sec); final extension at 68°C (40 sec). Amplicons were

purified and quantified as above and run on the MiSeq Illumina sequencing platform, producing 2 × 250 bp reads (Illumina, San Diego, CA, USA).

References

Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M et al. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Research* 41: e1 doi:10.1093/nar/gks1808.

Miller D, Bryant J, Madsen E & Ghiorse W (1999). Evaluation and optimization of DNA extraction and purification procedures for soil and sediment samples. *Applied and Environmental Microbiology* 65: 4715-4724.

2. Taxonomic classifications of the abundant and common phyla, orders, genera and species

Abundant Phyla (8)

Phylum	% of all sequences
Proteobacteria	57.5
Bacteroidetes	16.0
Verrucomicrobia	6.3
Planctomycetes	5.2
Actinobacteria	3.4
Cyanobacteria	3.1
Acidobacteria	2.4
Firmicutes	2.2
Total	96.2

Abundant Orders (21)

Phylum	Class	Order	% of all sequences
Proteobacteria	Alphaproteobacteria	Sphingomonadales	8.0
Proteobacteria	Alphaproteobacteria	Rhodobacterales	4.7
Proteobacteria	Alphaproteobacteria	Rhizobiales	3.9
Proteobacteria	Alphaproteobacteria	Caulobacterales	1.8
Proteobacteria	Betaproteobacteria	Burkholderiales	12.6
Proteobacteria	Betaproteobacteria	SC-I-84	1.8
Proteobacteria	Gammaproteobacteria	Pseudomonadales	12.4
Proteobacteria	Gammaproteobacteria	Enterobacteriales	3.6
Proteobacteria	Gammaproteobacteria	Xanthomonadales	2.8
Proteobacteria	Gammaproteobacteria	Legionellales	1.4
Bacteroidetes	[Saprospirae]	[Saprospirales]	4.8
Bacteroidetes	Cytophagia	Cytophagales	2.0
Bacteroidetes	Flavobacteriia	Flavobacteriales	6.7
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	2.3
Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	1.4
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	4.6
Planctomycetes	Phycisphaerae	WD2101	1.0
Planctomycetes	Planctomycetia	Pirellulales	2.4
Actinobacteria	Actinobacteria	Actinomycetales	2.2
Cyanobacteria	Oscillatoriophyceae	Oscillatoriales	1.8
Firmicutes	Bacilli	Bacillales	2.1
		Total	84.4

Abundant Genera (25)

Phylum	Class	Order	Family	Genus	% of all sequences
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	UA	1.3
Proteobacteria	Alphaproteobacteria	Rhizobiales	UA	UA	1.4
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	<i>Rhodobacter</i>	4.2
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	<i>Novosphingobium</i>	1.7
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	<i>Sphingomonas</i>	1.2
Proteobacteria	Alphaproteobacteria	Sphingomonadales	UA	UA	1.5
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	UA	2.8
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	<i>Massilia</i>	4.7
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	<i>Janthinobacterium</i>	3.5
Proteobacteria	Betaproteobacteria	SC-I-84	UA	UA	1.8
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	UA	1.5
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	<i>Erwinia</i>	1.5
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i>	3.7
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	6.9
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	UA	1.7
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	UA	1.7
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	UA	3.2
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	<i>Flavobacterium</i>	2.8
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Chitinophagaceae	UA	4.1
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	<i>Pedobacter</i>	1.8
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	<i>Luteolibacter</i>	3.3
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	UA	1.3
Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	UA	2.2
Cyanobacteria	Oscillatoriothycideae	Oscillatoriales	Phormidiaceae	<i>Phormidium</i>	1.8
Firmicutes	Bacilli	Bacillales	[Exiguobacteraceae]	<i>Exiguobacterium</i>	1.9
				Total	63.5

Abundant Species (16)

Phylum	Class	Order	Family	Genus	Species	% of all sequences
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	<i>Rhodobacter</i>	UA	2
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	<i>Novosphingobium</i>	UA	1.3
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	<i>Sphingopyxis</i>	UA	1
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	UA	UA	1.1
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	<i>Janthinobacterium</i>	<i>lividum</i>	1.3
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	<i>Massilia</i>	<i>varians</i>	2.2
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	<i>Erwinia</i>	<i>billingiae</i>	1.3
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i>	UA	1.4
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	UA 1	3
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	UA 2	1.8
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	UA 3	1.5
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	<i>Flavobacterium</i>	UA	3.4
Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	<i>Pedobacter</i>	UA	1.3
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	<i>Luteolibacter</i>	UA	1.8
Cyanobacteria	Oscillatoriothycideae	Oscillatoriales	Phormidiaceae	<i>Phormidium</i>	UA	1.7
Firmicutes	Bacilli	Bacillales	Exiguobacteraceae	<i>Exiguobacterium</i>	<i>sibiricum</i>	1.8
Total						28.0

Common Species (27)

Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	UA	UA	0.9
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	<i>Janthinobacterium</i>	UA	0.9
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	UA	UA	0.8
Proteobacteria	Alphaproteobacteria	Sphingobacteriales	Sphingomonadaceae		UA	0.6
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	UA	UA	0.6
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	UA	UA	0.6
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i>	UA	0.5
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	<i>Rhodobacter</i>	UA	0.5
Proteobacteria	Alphaproteobacteria	Sphingobacteriales	Sphingomonadaceae	<i>Kaistobacter</i>	UA	0.5
Proteobacteria	Alphaproteobacteria	Sphingobacteriales	Sphingomonadaceae	<i>Sphingomonas</i>	<i>echinoides</i>	0.5
Proteobacteria	Betaproteobacteria	Betaproteobacteria	SC-I-84	UA	UA	0.5
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	UA	UA	0.5
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	<i>Pseudomonas</i>	UA	0.5
Bacteroidetes	Saprospirae	Saprospirales	Chitinophagaceae	UA	UA	0.4
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	<i>Flavobacterium</i>	UA	0.4
Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	UA	UA	0.4
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	UA	UA	0.4
Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	<i>Flavobacterium</i>	UA	0.4
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	<i>Rhodobacter</i>	UA	0.4
Cyanobacteria	Synechococcophycideae	Synechococcales	Synechococcaceae	<i>Synechococcus</i>	UA	0.4
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	UA	UA	0.4
Proteobacteria	Alphaproteobacteria	Sphingobacteriales	Sphingomonadaceae	<i>Sphingomonas</i>	UA	0.3
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	<i>Acinetobacter</i>	UA	0.3
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	UA	UA	0.3
Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	UA	UA	0.3
Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	<i>Luteolibacter</i>	UA	0.3
Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	<i>Janthinobacterium</i>	UA	0.3
Total (abundant plus common)						40.8

GENUS LEVEL	DCD			Sediment			Flow			Nutrients			DCD×Sed		DCD×Flow		DCD×Nut		Sed×Flow		Sed×Nut		Nut×Flow	
	P	ES	dir.	P	ES	dir.	P	ES	dir.	P	ES	dir.	P	ES	P	ES	P	ES	P	ES	P	ES	P	ES
Taxon richness	0.591			0.156			0.412			0.413			0.292	0.384	0.375		0.662		0.358		0.382			
Simpsons evenness	<0.001	0.53	+	0.516			0.249			0.965			0.780	0.371	0.547		0.363		0.223		0.763			
<i>MANOVA</i> (genera > 1%, n=25)	<0.001	0.89		<0.001	0.94		<0.001	0.89		0.198			0.070	0.67	0.332		0.242		<0.001	0.84	0.486		0.122	
Abundant Genera	%																							
<i>Pseudomonas</i>	6.9	<0.001	0.33	-	0.810		0.540			0.215			0.407	0.331	0.215		0.786		0.304		0.489			
<i>Massilia</i>	4.7	<0.001	0.31	-	0.012	0.13	-	0.027	0.10	+	0.215		0.511	0.309	0.179		0.989		0.612		0.158			
<i>Rhodobacter</i>	4.2	0.001	0.20	+	0.176		0.047	0.08	-	0.850			0.392	0.384	0.818		0.433		0.483		0.586			
Chitinophagaceae UAG	4.1	<0.001	0.25	+	0.005	0.16	+	0.622		0.370			0.779	0.209	0.539		0.582		0.241		0.181			
<i>Acinetobacter</i>	3.7	<0.001	0.29	-	0.006	0.15	-	0.733		0.755			0.057	0.07	0.227		0.530		0.516		0.690		0.231	
<i>Janthinobacterium</i>	3.5	<0.001	0.40	-	0.351		0.102			0.961			0.524	0.903	0.984		0.572		0.611		0.544			
<i>Luteolibacter</i>	3.3	<0.001	0.31	+	0.568		0.162			0.136			0.487	0.016	0.12	0.156		0.337		0.653		0.073	0.07	
Flavobacteriaceae UAG	3.2	0.057	0.08	+	0.630		0.334			0.682			0.824	0.507	0.547		0.975		0.413		0.686			
<i>Flavobacterium</i>	2.8	0.001	0.20	-	0.004	0.16	+	<0.001	0.27	+	0.393		0.327	0.292	0.627		0.299		0.773		0.376			
Comamonadaceae UAG	2.8	0.177			0.016	0.12	+	<0.001	0.30	-	0.223		0.841	0.109	0.449		0.551		0.593		0.226			
Pirellulaceae UAG	2.2	<0.001	0.42	+	<0.001	0.42	-	0.706		0.073	0.07	-	0.028	0.10	0.341		0.528		0.624		0.487		0.313	
<i>Exiguobacterium</i>	1.9	<0.001	0.23	-	0.011	0.13	+	0.128		0.068	0.07	+	0.064	0.07	0.109		0.140		0.139		0.291		0.827	
<i>Phormidium</i>	1.8	0.431			0.005	0.16	+	0.903		0.365			0.756	0.297	0.927		0.278		0.335		0.151			
<i>Pedobacter</i>	1.8	0.303			0.275		0.093	0.06	+	0.662			0.493	0.958	0.829		0.959		0.439		0.568			
SC-I-84 UAG	1.8	<0.001	0.40	+	0.048	0.08	+	0.022	0.11	-	0.470		0.343	0.307	0.467		0.301		0.550		0.525			
Xanthomonadaceae UAG	1.7	<0.001	0.38	+	0.060	0.07	+	0.158		0.716			0.923	0.091	0.06	0.468		0.132		0.560		0.870		
<i>Novosphingobium</i>	1.7	0.064	0.07	+	<0.001	0.31	+	0.736		0.065	0.07	-	0.092	0.06	0.828		0.457		0.666	0.014	0.12	0.980		
Pseudomonadaceae UAG	1.7	<0.001	0.44	-	0.544		0.784			0.309			0.391	0.416	0.329		0.762		0.671		0.496			
Sphingomonadales UAG	1.5	<0.001	0.36	+	0.835		0.800			0.389			0.689	0.373	0.982		0.636		0.118		0.946			
Enterobacteriaceae UAG	1.5	<0.001	0.34	-	0.024	0.10	-	0.058	0.07	-	0.083	0.06	+	0.175	0.052	0.08	0.502		0.969		0.539			
<i>Erwinia</i>	1.5	<0.001	0.28	-	0.014	0.12	-	0.002	0.18	-	0.391		0.117	0.011	0.13	0.939		0.319		0.985		0.831		
Rhizobiales UAG	1.4	<0.001	0.34	+	0.239		0.039	0.09	-	0.809			0.292	0.275	0.400		0.355		0.651		0.912			
Caulobacteraceae UAG	1.3	0.713			0.134		0.005	0.16	+	0.899			0.246	0.390	0.757		0.996		0.836		0.447			
Verrucomicrobiaceae UAG	1.3	<0.001	0.40	+	0.065	0.07	-	0.002	0.19	-	0.874		0.290	0.050	0.08	0.184		0.818		0.984		0.815		
<i>Sphingomonas</i>	1.2	0.003	0.17	+	0.218		0.693			0.089	0.06	-	0.097	0.06	0.465		0.267		0.119		0.332		0.137	

63.5%

3. Descriptions of significant interactions

ABUNDANT PHYLA (>1.0% of all sequences)

DCD×sediment: Proteobacteria showed a negative, antagonistic DCD by sediment interaction (i.e. the combined effect of both stressors was less negative than predicted additively) (Fig. S1).

DCD×flow velocity: DCD had a stronger positive effect on the relative abundance of Verrucomicrobia at fast flow velocity than at reduced velocity (Fig. S1).

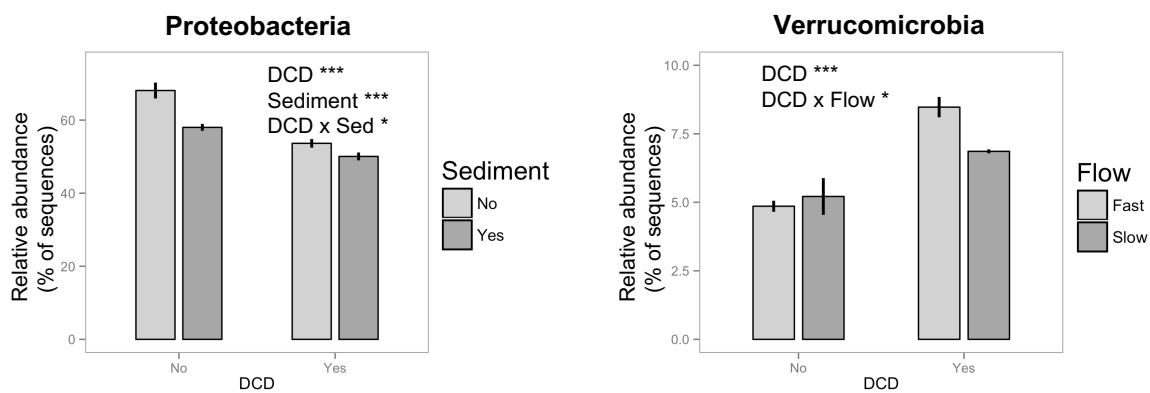


Figure S1. Mean relative abundances (\pm SE) of the abundant phyla that showed significant DCD by sediment (Proteobacteria) or DCD by flow velocity interactions (Verrucomicrobia). $P < 0.001 = ***$, $P < 0.01 = **$, $P < 0.05 = *$.

ABUNDANT ORDERS (>1.0% of all sequences)

DCD×flow velocity: DCD had a stronger positive effect on Verrucomicrobiales and Legionellales at fast flow velocity than at reduced velocity (Fig. S2). Enterobacteriales showed a double-negative, antagonistic response to DCD and reduced flow velocity, with the combined effect of both stressors being less negative than predicted additively (Fig. S2).

DCD×sediment: Sediment addition weakened the positive effect of DCD on the relative abundances of Pirellulales and Legionellales (Fig. S2).

Nutrients×flow velocity: Reduced flow velocity increased the relative abundance of Cytophagales mainly in mesocosms where nutrients were not enriched (Fig. S2).

Sediment×flow velocity: Sediment addition increased the relative abundance of WD2101 (Phycisphaerae) in mesocosms with faster flow velocity, but this pattern was reversed at reduced velocity (Fig. S2).

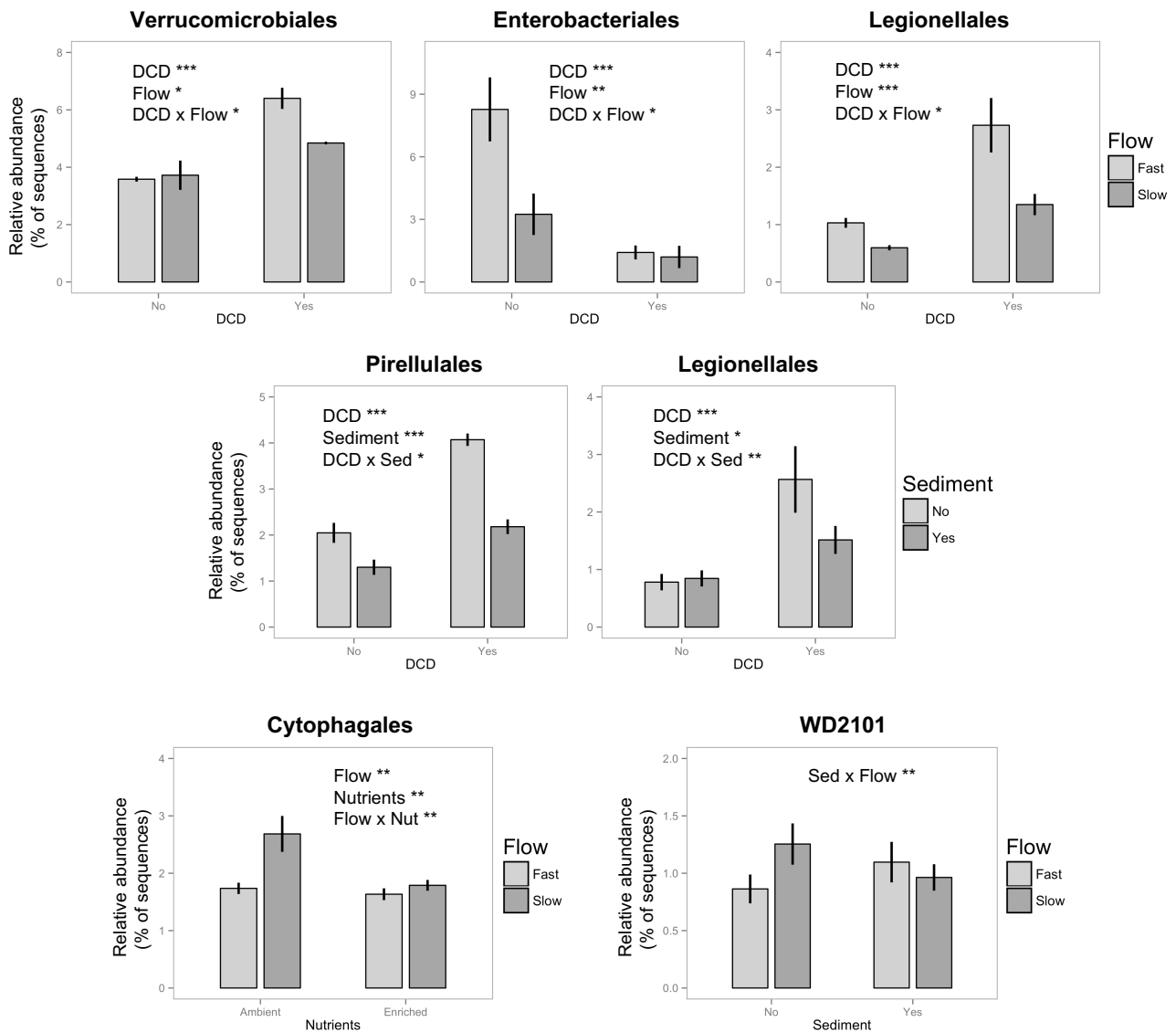


Figure S2. Mean relative abundances (± SE) of the abundant orders that showed significant DCD by flow velocity (Verrucomicrobiales, Enterobacteriales, Legionellales), DCD by sediment (Pirellulales, Legionellales), flow velocity by nutrient (Cytophagales), or sediment by flow velocity interactions (WD2101 Phycisphaerae). $P < 0.001 = ***$, $P < 0.01 = **$, $P < 0.05 = *$.

ABUNDANT GENERA (>1.0% of all sequences)

DCD×flow velocity: Reduced flow velocity weakened the positive effect of DCD on *Luteolibacter* and another unassigned genus of Verrucomicrobiaceae. *Erwinia* showed a double-negative, antagonistic response to DCD and reduced flow velocity, with the combined effect being less negative than predicted additively (Fig. S3).

DCD×sediment: Sediment addition decreased the positive effect of DCD on an unassigned genus of Pirellulaceae (Fig. S3).

Sediment×nutrients: Sediment addition had a stronger positive effect on *Novosphingobium* in mesocosms where nutrients were not enriched (Fig. S3).

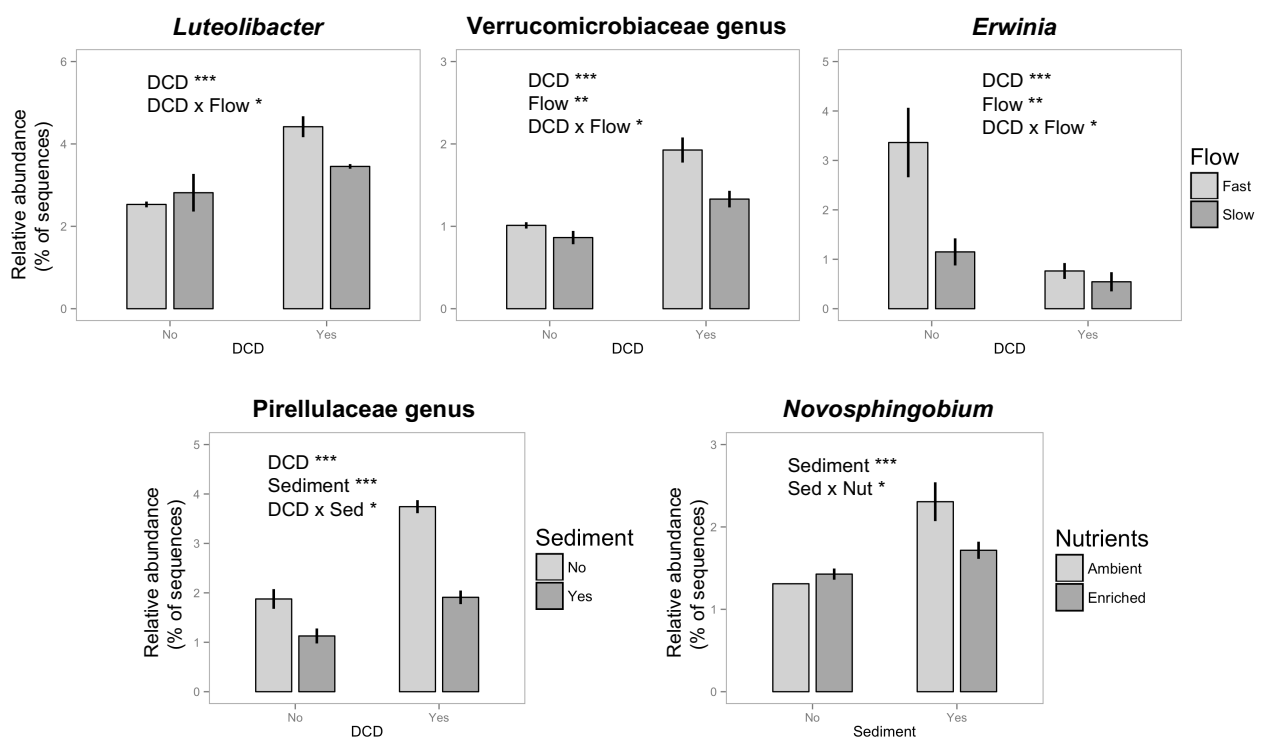


Figure S3. Mean relative abundances (\pm SE) of the abundant genera that showed significant DCD by flow velocity (*Luteolibacter*, an unassigned genus of the family Verrucomicrobiaceae, and *Erwinia*), DCD by sediment (a genus in the family Pirellulaceae), or sediment by nutrient interactions (*Novosphingobium*). $P < 0.001 = ***$, $P < 0.01 = **$, $P < 0.05 = *$.

ABUNDANT SPECIES (>1.0% of all sequences)

DCD×flow velocity: *Luteolibacter* sp. 1 responded more positively to added DCD at fast than at reduced flow velocity. *Erwinia billingiae* decreased with both DCD and reduced flow velocity in a negative antagonistic interaction (combined stressor effects less negative than predicted additively) (Fig. S4).

DCD×sediment: *Acinetobacter* sp. 1 also showed a double-negative, antagonistic response, but to DCD combined with sediment (Fig. S4).

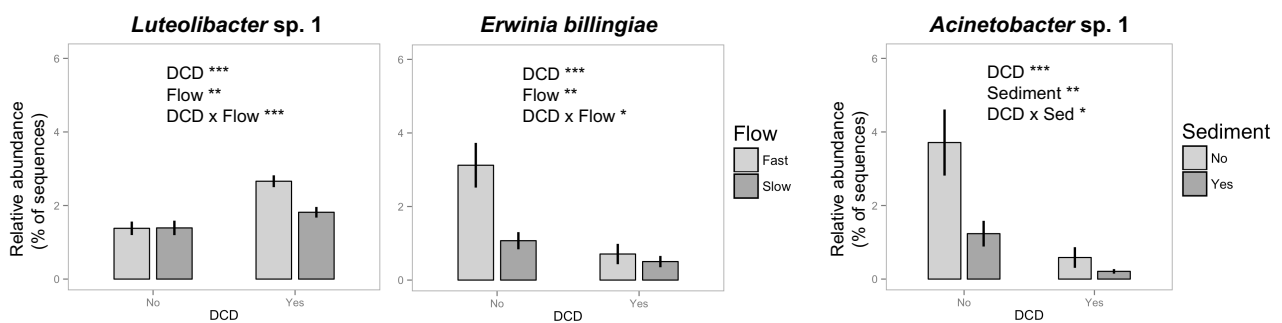


Figure S4. Mean relative abundances (± SE) of the abundant species that showed significant DCD by flow velocity (*Luteolibacter* species and *Erwinia billingiae*) or DCD by sediment interactions (*Acinetobacter* species). $P < 0.001 = ***$, $P < 0.01 = **$, $P < 0.05 = *$.

COMMON SPECIES (>0.3% of all sequences)

DCD×flow velocity: Two species of Enterobacteriaceae (sp. 1 and 2) showed double-negative, antagonistic responses to DCD and reduced flow velocity, with the combined effect of the two stressors being less negative than predicted additively (Fig. S5).

DCD×sediment: Sediment addition weakened the positive effect of DCD on a species of *Zymomonas*, a species of Pirellulaceae (sp. 1) and a species of *Synechococcus*. By contrast, sediment addition increased the relative abundance of *Flavobacterium* sp. 2 in mesocosms without DCD but not in those with DCD addition (Fig. S5).

Sediment×flow velocity: *Flavobacterium* sp. 3 showed a double-positive synergistic response to sediment addition and reduced flow velocity, with the combined effect being more positive than predicted additively (Fig. S5).

Sediment×nutrients: Enterobacteriaceae sp. 3 showed a greater increase with nutrient enrichment in mesocosms without sediment addition compared to those where sediment was added (Fig. S5).

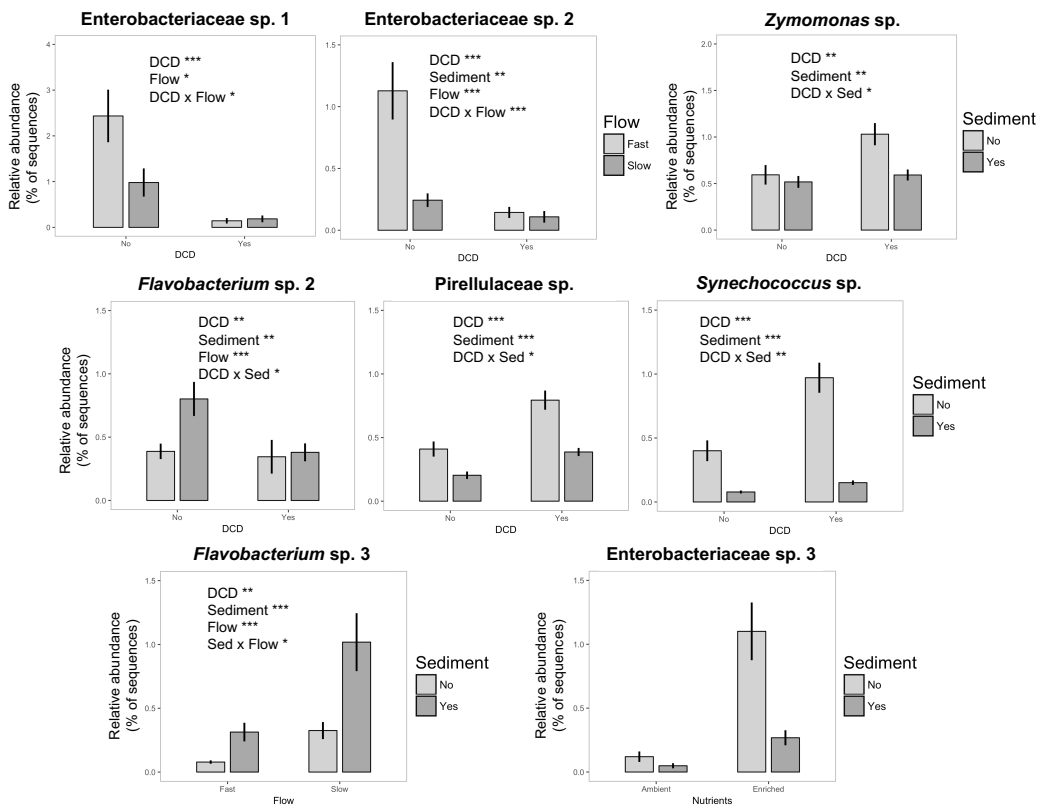


Figure S5. Mean relative abundances (\pm SE) of the abundant species that showed significant DCD by flow velocity (*Enterobacteriaceae* sp. 1 and 2), DCD by sediment (*Zymomonas* sp., *Flavobacterium* sp. 2, *Pirellulaceae* sp., *Synechococcus* sp.), flow velocity by sediment (*Flavobacterium* sp. 3) or sediment by nutrients interactions (*Enterobacteriaceae* sp. 3). $P < 0.001 = ***$, $P < 0.01 = **$, $P < 0.05 = *$.

DCD×nutrients and DCD×flow velocity: *Verrucomicrobiaceae* sp. and *Luteolibacter* sp. 2 showed greater increases with DCD addition in mesocosms with fast flow velocity compared to those where velocity was reduced. *Verrucomicrobiaceae* sp. also responded more positively to DCD when

nutrients were enriched as well, whereas *Luteolibacter* sp. 2 showed a negative response to nutrient enrichment in mesocosms without DCD but not when DCD was added (Fig. S6).

Kaistobacter sp. showed a positive synergistic response to DCD, sediment and nutrients, with abundances being highest when all three stressors were added (Fig. S6). This was the only significant 3-way interaction in our data.

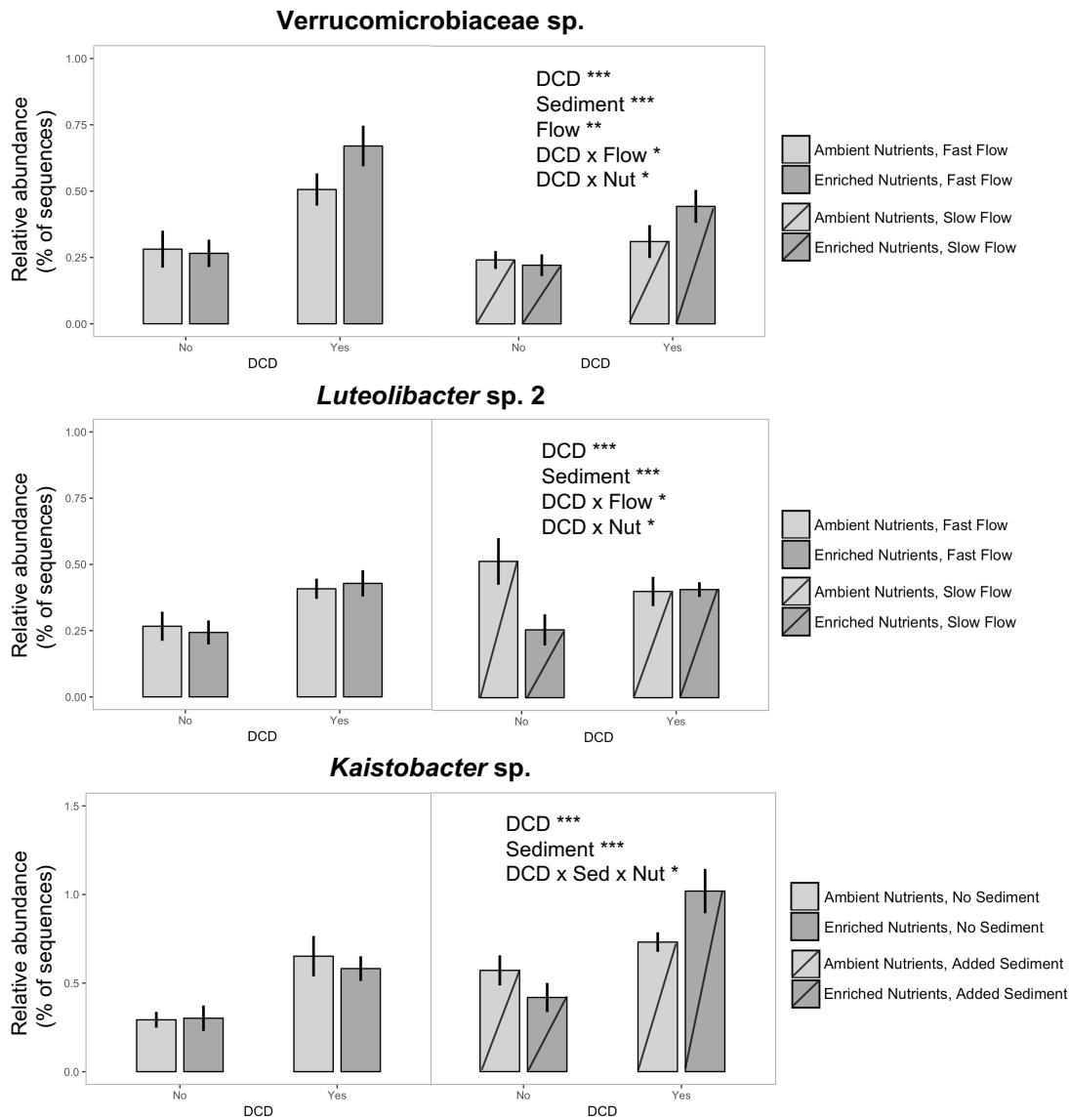


Figure S6. Mean relative abundances (\pm SE) of the abundant species that showed significant DCD by nutrients and DCD by flow velocity interactions (*Verrucomicrobiaceae* sp. and *Luteolibacter* sp. 2), and the only significant three-way interaction (DCD \times sediment \times nutrients; *Kaistobacter* sp.). $P < 0.001 = ***$, $P < 0.01 = **$, $P < 0.05 = *$.