The following supplement accompanies the article

Experimentally induced habitat filtering in marine bacterial communities

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SUPPLEMENT.

Additional results on community differences (Morisita's index analyses), community structure (diversity, evenness, richness and MPD) and community phylogenetic structure (phylocom) done on different RAMI threshold values. Also, additional information about our methodological approach is provided

Table S1. Morisita's index values for all pair-wise library comparisons for RAMI threshold values 0.06, 0.04 and 0.02. For abbreviations see 'Materials and methods: Sampling and experimental setup' in the main article

	SKA-T exp1	SKA-T exp2	SKA-C exp2	Ska exp1	SKA exp2	BAL-T exp1	BAL-T exp2	BAL-C exp2	BAL exp1
RAMI (0.06)									
BALexp2	0.00	0.00	0.00	0.21	0.10	0.01	0.01	0.00	0.25
BALexp1	0.07	0.00	0.00	0.67	0.28	0.05	0.00	0.00	
BAL-C exp2	0.00	0.00	0.00	0.00	0.00	0.21	0.46		
BAL-T exp2	0.27	0.09	0.16	0.00	0.00	0.30			
BAL-T exp1	0.32	0.01	0.00	0.00	0.00				
SKAexp2	0.00	0.00	0.00	0.39					
Skaexp1	0.01	0.00	0.00						
SKA-C exp2	0.05	0.52							
SKA-T exp2	0.06								

	SKA-T exp1	SKA-T exp2	SKA-C exp2	Ska exp1	SKA exp2	BAL-T exp1	BAL-T exp2	BAL-C exp2	BAL exp1
RAMI (0.04)									
BALexp2	0.00	0.00	0.00	0.20	0.14	0.01	0.00	0.00	0.16
BALexp1	0.06	0.00	0.00	0.11	0.05	0.03	0.00	0.00	
BAL-C exp2	0.00	0.00	0.00	0.00	0.00	0.01	0.43		
BAL-T exp2	0.28	0.12	0.13	0.00	0.00	0.17			
BAL-T exp1	0.27	0.01	0.00	0.00	0.00				
SKAexp2	0.00	0.00	0.00	0.33					
Skaexp1	0.01	0.00	0.00						
SKA-C exp2	0.02	0.55							
SKA-T exp2	0.08								

	SKA-T exp1	SKA-T exp2	SKA-C exp2	Ska exp1	SKA exp2	BAL-T exp1	BAL-T exp2	BAL-C exp2	BAL exp1
RAMI (0.02)									
BALexp2	0.00	0.00	0.00	0.12	0.20	0.01	0.00	0.00	0.03
BALexp1	0.04	0.00	0.00	0.11	0.07	0.02	0.00	0.00	
BAL-C exp2	0.00	0.00	0.00	0.00	0.00	0.00	0.39		
BAL-T exp2	0.19	0.09	0.09	0.00	0.00	0.10			
BAL-T exp1	0.14	0.00	0.00	0.00	0.00				
SKAexp2	0.00	0.00	0.00	0.22					
Skaexp1	0.01	0.00	0.00						
SKA-C exp2	0.01	0.45							
SKA-T exp2	0.01								

Table S2. Number of operational taxonomic units (OTUs) (species), Simpson's evenness values and Margalef's index of species richness for the Sanger and 454 (non-singletons) libraries, respectively. Mean pair-wise phylogenetic distance (MPD) for Sanger libraries. Analysis made on OTUs constructed with RAMI threshold values of 0.02 and 0.06. Note that MPD values are unavailable for 454-data; the data size exceeded the limit for the methodological approach. For abbreviations see 'Materials and methods: Sampling and experimental setup' in the main article

		Sanger (0.02)				Sanger (0.06)			
		No. of OTU	**Evenness**	**Richness**	**MPD**	**No. of OTU**	**Evenness**	**Richness**	**MPD**
BAL→SKA 1	**Start**	46	1.03	11.08	1.08	33	0.60	7.88	1.42
	Transferred	40	0.31	9.18	0.71	30	0.31	6.83	0.84
BAL→SKA 2	**Start**	44	0.48	9.53	0.96	41	0.39	8.87	1.21
	Transferred	38	0.75	8.65	0.68	18	0.67	3.98	0.70
SKA→BAL 1	**Start**	31	0.17	6.65	0.90	26	0.20	5.54	1.13
	Transferred	39	0.40	8.40	1.01	32	0.42	6.86	1.29
SKA→BAL 2	**Start**	31	4.52	8.51	1.04	20	0.65	5.39	1.39
	Transferred	38	0.78	9.00	0.73	17	0.39	3.89	0.94
Controls	**BAL-C 2**	30	0.21	6.51	0.96	19	0.23	4.04	1.18
	SKA -C 2	35	0.17	7.54	0.74	17	0.28	3.55	0.99
		454 data (0.02)				454 data (0.06)			
BAL→SKA 2	**BAL 454**	341	0.10	37.77	–	228	0.03	25.11	–
	BAL-T 454	122	0.10	13.51	–	51	0.12	5.57	–

Table S3. The degree of clustering (net related index, NRI, and nearest taxa index, NTI) for the non-treated bacterial communities sampled in the Baltic Sea and Skagerrak. Positive and negative values indicate clustered and ovedispersed communities respectively. *p < 0.1, **p < 0.05; n: number of OTUs in the sample; and NRI_gt and NTI_gt denotes the number of random permutations that resulted in higher values than the analyzed sample. RAMI value for clustering of 0.02 (left) and 0.06 (right). Alpha: *Alphaproteobacteria*; Bacter: *Bacteroidetes*; Beta: *Betaproteobacteria*; Gamma: *Gammaproteobacteria*; Delta: *Deltaproteobacteria*. For other abbreviations see 'Materials and methods: Sampling and experimental setup' in the main article

	Sanger data (0.02)					Sanger data (0.06)				
Sample	**n**	**NRI**	**NRI_gt**	**NTI**	**NTI_gt**	**n**	**NRI**	**NRI_gt**	**NTI**	**NTI_gt**
All bacteria										
BAL1	49	–2.15	987	2.40**	10	34	–2.35**	993	1.71	43
BAL2	43	0.21	405	–0.99	835	42	0.73	234	0.01	521
SKA1	34	1.21	114	2.34**	10	27	1.43	82	1.69*	37
SKA2	31	–0.98	834	4.50**	0	22	–1.38	912	3.82**	0
Alpha										
BAL1	9	1.06	142	1.76*	29	7	0.14	506	1.84**	17
BAL2	12	–0.21	581	1.18	118	10	–0.86	802	1.32	78
SKA1	14	–1.17	889	2.15**	14	11	–0.64	755	1.46*	46
SKA2	11	1.02	152	2.8**	0	5	–0.79	855	1.26	91
Bacter										
BAL1	14	0.75	255	1.79*	27	9	0.32	392	0.60	291
BAL2	12	–0.45	683	–0.70	757	12	–0.28	621	–0.19	576
SKA1	4	1.26	77	1.61*	46	3	0.76	261	0.96	165
SKA2	7	2.17**	5	2.28**	6	6	1.87**	11	2.22**	6
Beta										
BAL1	4	0.14	625	0.39	403	3	0.14	713	–0.43	796
BAL2	2	0.14	558	0.14	558	2	0.29	507	0.29	507
SKA2	3	0.89	63	1.19*	43	2	0.48	329	0.48	329
Gamma										
BAL1	4	–1.22	881	–0.74	773	4	–0.73	802	–0.30	693
BAL2	6	–1.54	925	–1.69	937	6	–1.15	847	–1.29	889
SKA1	5	1.28*	46	0.82	203	5	1.35*	40	1.29	79
SKA2	5	1.43*	34	1.08	125	5	1.51**	13	1.46*	41

	454 data (0.02)					454 data (0.06)				
Sample	**N**	**NRI**	**NRI_gt**	**NTI**	**NTI_gt**	**N**	**NRI**	**NRI_gt**	**NTI**	**NTI_gt**
BAL2										
Actinobacteria	67	6.87**	15	7.27**	15	29	5.31**	25	5.14**	0
Alpha	51	–0.985	830	0.3454	350	33	1.37	158	1.29	156
Bacteroidetes	61	1.1012	188	2.29*	28	46	1.76	68	2.17*	34
Beta	35	–0.628	739	0.8289	197	16	–0.08	479	0.63	265
Cyanobacteria	23	4.03*	45	3.66*	45	14	3.55	63	2.99	63
Delta	18	1.93	111	2.09**	0	17	1.88	118	1.97	67
Firmicutes	20	0.6618	106	0.6313	106	20	0.71	99	0.68	99
Gamma	50	–2.773	997	0.1915	424	41	–0.91	812	–0.41	633
Verrucomicrobia	16	3.49	65	3.07	65	12	3.21	78	2.81	78

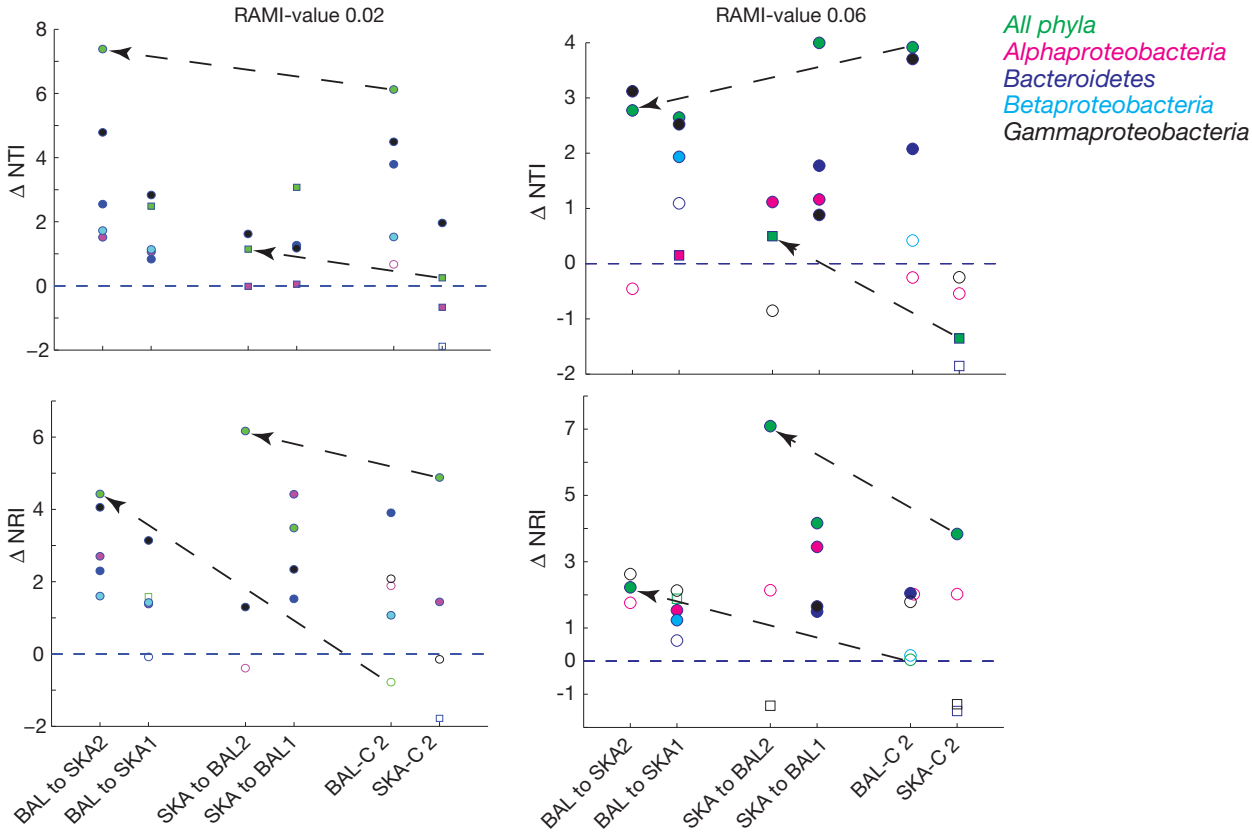

Fig. S1. Change in NTI and NRI for the different Sanger-data treatments, measured as the difference between NTI and NRI values (*y*-axis) between a natural and its corresponding transplanted community (denoted on the *x*-axis). BAL to SKA1: Baltic community (sampled in June) transplanted into Skagerrak water; BAL to SKA2: Baltic community (sampled in August) transplanted into Skagerrak water; SKA to BAL1: Skagerrak community (sampled in June) transplanted into Baltic water; SKA to BAL2: Skagerrak community (sampled in August) transplanted into Baltic water; BAL-C 2: Baltic community (sampled in August), no water transplant; SKA-C 2: Skagerrak community (sampled in August), no water transplant. Color

coding denotes analyses done on data subsets defined by OTUs (species) that were assigned to the same phylum (except green, which denotes all phyla). Filled squares denote significant values for both the naturally sampled (non-treated) and treated (transplant and control) communities. Filled circles denote significant values for the treated community only. Open squares denote significant values for the natural community only. Open circles denote no significant structuring. Dashed arrows indicate NRI and NTI differences between transplanted (bottle effect + change in salinity- and DOC- concentrations) and control treatments (bottle effects) for data including all phyla. RAMI values 0.02 (left column) and 0.06 (right column)

DNA extraction, PCR, clone libraries and 454-pyrosequencing

DNA was extracted (from Sterivex filters (5 l Baltic and Skagerrak inoculum, respectively) and Supor filters (community DNA from the continuous cultures)) using an enzyme/phenol-chloroform protocol (Riemann et al. 2000) but with a 30 min lysozyme digestion (5 mg ml^{-1} final conc.) at 37°C and an overnight proteinase K digestion (100 µg ml^{-1} final conc.) at 55°C (Boström et al. 2004).

Clone libraries and 454-sequencing of 16S rRNA genes were used to compare community composition and structure before and after chemostat treatment. For clone libraries, bacterial (constructed from all 10 communities) 16S rRNA genes were amplified using primers 27F and 1492R. After purification (Gel Extraction Kit [E.Z.N.A] followed by the Cycle-Pure Kit [E.Z.N.A]), the products were cloned (TOPO TA Cloning® Kit, Invitrogen), the plasmid DNA was extracted (R.E.A.L Prep 96 Plasmid Kit, Qiagen), and the inserts were sequenced with primer 27F (commercially by Macrogen). 454-pyrosequencing was performed from the Baltic inoculums sampled in August and its transplanted counterpart (equivalent to BAL→SKA2). Partial bacterial 16S rRNA genes (including the variable V4 to V6 regions) were amplified using a primer cocktail containing the degenerate primers 530F (‘5-GTGCCAGCMGCNGCGGTA-3’) (Dowd et al. 2008), but with TA added at the 3-prime end to increase specificity, and 1061R (‘5-CRRCACGAGCTGACGAC-3’) (Andersson et al. 2008). Pyrosequencing was performed at the University of Copenhagen on a 454 GS FLX system (Roche Applied Science) according to the manufacturer’s instructions.

LITERATURE CITED

Andersson AF, Lindberg M, Jakobsson H, Bäckhed F, Nyren P, Engstrand L (2008) Comparative analysis of human gut microbiota by barcoded pyrosequencing. PLoS ONE 3:e2836

Boström KH, Simu K, Hagström A, Riemann L (2004) Optimization of DNA extraction for quantitative marine bacterioplankton community analysis. Limnol Oceanogr Methods 2:365–373

Dowd SE, Sun Y, Wolcott RD, Domingo A, Carroll JA (2008) Bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP) for microbiome studies: bacterial diversity in the ileum of newly weaned *Salmonella*-infected pigs. Foodborne Pathog Dis 5:459–472

Riemann L, Steward GF, Azam F (2000) Dynamics of bacterial community composition and activity during a mesocosm diatom bloom. Appl Environ Microbiol 66:578–587