

Supplement 1

Table S1. Table of primers.

Primer pair	Sequences	Amplicon size	Reference
16S 27F + 338R	F: 5'-AGA GTT TGA TCM TGG CTC AG-3'	311 bp	Ludwig et al. 1993, Suzuki and Giovannoni 1996
V1-V2	R: 5'-ATT ACC GCG GCT GCT GG-3'		
18S UNonMetF+ UNonMetR	F: 5'-GTG CCA GCA GCC GCG-3'	600 bp	
V4	R: 5'-TTT AAG TTT CAG CCT TGC G-3'		Bower et al. 2004
E572 + E1009R	F: 5'-CYG CGG TAA TTC CAG CTC-3'	400 bp	Comeau et al. 2011
	R: 5'-CRA AGA YGA TYA GAT ACC RT-3'		

Table S2. Thermocycling protocols for each target.

	UNonMetF/ UNonMetR	E572/E1009R
<i>PCR conditions</i>	1. 94°C, 2 min	1. 94°C, 2 min
	2. 94°C, 10 s	2. 94°C, 30 s
	3. 51,1 °C, 30 s	3. 55 °C, 30 s
	4. 72°C, 1 min	4. 72°C, 1 min
	5. 72°C, 5 min	5. 72°C, 5 min

} 35x
} 30x

Table S3. Target specific M13-linker primer and fusion primer sequences. Each sample was barcoded with 10-12 bp long unique barcode (marked with N) attached to IonA-forward fusion primer.

M13_27F	TGTAAAACGACGGCCAGTAGAGTTTGATCMTGGCTCAG
M13_E572F	TGTAAAACGACGGCCAGTCYGCGGTAATTCCAGCTC
IonP1_338R	CCTCTCTATGGGCAGTCGGTGATTGCTGCCTCCCGTAGGAGT
IonP1_E1009R	CCTCTCTATGGGCAGTCGGTGATCRAAGAYGATYAGATACCRT
IonA_key_bc_M13	CCATCTCATCCCTGCGTGTCTCCGACTCAGNNNNNNNNNTGTAAAACGACGGCCAGT

Table S4. Environmental variables measured at all samplings.

Site	Sample time	Salinity	Temp.	C/N ratio	Organic matter	Water-content	Porosity	M. grain size	Sorting
List	August 2018	33	22	9.48	0.940	17.59	0.34	1.41	1.27
Saltö	August 2018	26	18	10.46	1.16	24.03	0.43	2.24	1.76
	November 2018	25	3	8.86	1.24	27.22	0.50	2.82	0.82
	April 2019	23	11	8.57	1.07	21.20	0.40	2.35	1.53
	August 2019	22	18	7.77	1.34	25.17	0.47	2.16	1.90
Herslev	August 2018	15	23	6.31	1.06	21.56	0.40	1.37	0.70
	November 2018	16	5	4.40	1.16	23.35	0.48	2.15	0.85
	April 2019	15	15	1.94	1.02	22.46	0.40	2.35	0.85
	August 2019	15	17	8.30	1.23	20.37	0.37	2.06	1.05
Gollwitz	August 2018	13	23	9.83	1.42	27.63	0.49	2.78	0.89
Öland	August 2018	7	21	9.89	0.89	22.69	0.43	2.40	0.63

November 2018	8	1	3.83	0.61	24.15	0.44	2.70	0.41	
April 2019	9	14	7.06	0.55	23.42	0.44	2.50	0.46	
August 2019	8	18	6.90	0.70	20.75	0.37	2.48	0.49	
Tvärminne 2018	August	7	13	6.82	1.40	32.41	0.56	2.60	0.67
Pori 2018	August	6	18	8.64	0.24	22.42	0.40	2.49	0.63

Table S5a. Differences in Shannon index between sites in spatial study by TukeyHSD test. Different letters indicate significantly different sites. No significant differences were found by Tukey HSD test for OTU richness of bacteria and eukaryotes.

Spatial Dataset	Shannon_Bacteria
Herslev	ab
List	ab
Pori	ab
Saltö	a
Tvärminne	ab
Öland	b
Gollwitz	ab

Table S5b. Differences in Shannon index and OTU richness between sites in temporal study tested by TukeyHSD test. Different letters for each site indicate significantly different sites.

Temporal Dataset	Bacteria		Eukaryote	
	Shannon	OTU richness	Shannon	OTU richness
Saltö	a	a	a	ab
Öland	b	a	ab	a
Herslev	a	a	b	b

Table S6. ANOVA test of alpha diversity measured by OTU richness and Shannon against salinity. Note salinity is grouped to three categories as low (<10), medium (>10-20) and high (>20). Values indicate p value, asterisk indicate significance level, *=p<0.01, **p=0.001, ns=not significant.

Dataset	OTU richness	Shannon
Spatial Bacteria	ns	0.06
Temporal Bacteria	ns	0.00 *
Spatial Eukaryotes	ns	ns
Temporal Eukaryotes	0.04 **	0.03 **

Table S7a. Envfit of dbRDA to environmental factors, for bacteria, spatial dataset.

Factor	dbRDA1	dbRDA2	r2	Pr(>r)
Salinity	-0.99855	-0.05375	0.9366	0.001
Temperature	-0.49502	0.86888	0.3725	0.019
C/N ratio	-0.94493	-0.32726	0.1949	0.133
Organic content	-0.41533	0.90967	0.4321	0.009
Water content	0.97625	0.21663	0.1885	0.151
Porosity	0.94187	0.33598	0.2223	0.102
Mean grain size	0.95360	-0.30109	0.2676	0.064
Sorting	-0.98570	-0.16854	0.7862	0.001

Table S7b. Envfit of dbRDA to environmental factors, for bacteria, temporal dataset.

Factor	dbRDA1	dbRDA2	r2	Pr(>r)
Salinity	-0.99980	0.02015	0.9167	0.001
Temperature	0.67618	0.73674	0.0545	0.385
C/N ratio	-0.54336	-0.83950	0.3427	0.001
Organic content	-0.88020	0.47461	0.8040	0.001
Water content	-0.82895	-0.55933	0.2229	0.015

Porosity	-0.93201	-0.36243	0.1390	0.087
Mean grain size	0.10028	-0.99496	0.4259	0.001
Sorting	-0.99893	-0.04624	0.6669	0.001

Table S7c. Envfit of dbRDA to environmental factors, for eukaryotes, spatial dataset.

Factor	dbRDA1	dbRDA2	r2	Pr(>r)
Salinity	0.58199	-0.81320	0.2273	0.086
Temperature	0.81118	-0.58480	0.3911	0.014
C/N ratio	-0.39504	-0.91866	0.2331	0.099
Organic content	-0.05752	-0.99834	0.2801	0.046
Water content	-0.99289	-0.11907	0.3091	0.026
Porosity	-0.99450	-0.10471	0.2568	0.069
Mean grain size	-0.99365	-0.11251	0.5344	0.002
Sorting	0.15724	-0.98756	0.3445	0.021

Table S7d. Envfit of dbRDA to environmental factors, for eukaryotes, temporal dataset.

Factor	dbRDA1	dbRDA2	r2	Pr(>r)
Salinity	-0.12322	-0.99238	0.3962	0.001
Temperature	0.93328	-0.35916	0.3138	0.005
C/N ratio	0.10791	-0.99416	0.5917	0.001
Organic content	0.22063	-0.97536	0.2798	0.010
Water content	-0.46710	-0.88421	0.0422	0.480
Porosity	-0.93906	-0.34376	0.0415	0.515
Mean grain size	-0.99793	-0.06436	0.1948	0.023

Sorting	0.32393	-0.94608	0.5221	0.001
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Table S8. See Supplement 2 (xlsx). Correlation between OTUs and salinity.

Table S9a. Keystone OTUs of high salinity site Saltö, temporal samples. OTU identity listed as botu = bacterial OTU, eotu = eukaryote OTU.

OTU	Connections	Phylum	Order	Genus
botu515	60	Gammaproteobacteria	Haliaceae	Proteobacteria
botu607	55	Alphaproteobacteria	Rhodobacteraceae	Proteobacteria
botu331	53	Gammaproteobacteria	BD2-7	Proteobacteria
botu613	52	Gammaproteobacteria	BD7-8_fa	Proteobacteria
botu700	50	Gammaproteobacteria	Saccharospirillaceae	Proteobacteria
botu239	47	Alphaproteobacteria	uncultured	Proteobacteria
eotu228	45	Ochrophyta	Bacillariophyta	Stramenopiles
botu1484	44	Bacteroidia	Crocinitomicaceae	Bacteroidota
botu3889	43	Bacteroidia	Flavobacteriaceae	Bacteroidota
botu183	42	Gammaproteobacteria	Alteromonadaceae	Proteobacteria
eotu520	41	Unclassified	Unclassified Eukaryota	Unclassified Eukaryota
botu2817	41	Bacteroidia	Flavobacteriaceae	Bacteroidota
botu929	41	Alphaproteobacteria	Rhodobacteraceae	Proteobacteria
eotu1227	40	Unclassified	Unclassified Eukaryota	Unclassified Eukaryota
eotu356	40	Ochrophyta	Phaeophyceae	Stramenopiles

Table S9b. Keystone OTUs of medium salinity site Herslev, temporal samples. OTU identity listed as botu = bacterial OTU, eotu = eukaryote OTU.

OTU	Connections	Phylum	Order	Genus
botu1673	51	Bacteroidota	Flavobacteriales	Unclassified Flavobacteriaceae
eotu933	45	Alveolata	Dinophyceae	Dinophyceae
eotu3734	40	Alveolata	Dinophyceae	Unclassified Dinophyceae
eotu34	37	Stramenopiles	Bacillariophyta	Unclassified Raphid pennate
eotu1174	36	Opisthokonta	Ascomycota	Unclassified Pezizomycotina
botu607	35	Proteobacteria	Rhodobacterales	Unclassified Rhodobacteraceae
eotu113	30	Unclassified Eukaryota	Unclassified Eukaryota	Unclassified Eukaryota

eotu3124	28	Alveolata	Dinophyceae	Unclassified Dinophyceae
eotu4284	28	Alveolata	Dinophyceae	Unclassified Dinophyceae
botu370	28	Patescibacteria	Candidatus Campbellbacteria	Candidatus Campbellbacteria_ge
eotu363	27	Unclassified Eukaryota	Unclassified Eukaryota	Unclassified Eukaryota
eotu681	27	Stramenopiles	Unclassified Stramenopiles	Unclassified Stramenopiles
botu1222	27	Bacteroidota	Chitinophagales	uncultured
botu290	27	Bacteroidota	Flavobacteriales	Lutibacter
botu4620	27	Bacteroidota	Flavobacteriales	Aquibacter
eotu852	26	Alveolata	Unclassified Alveolata	Unclassified Alveolata

Table S9c. Keystone OTUs of low salinity site Öland, temporal samples. OTU identity listed as botu = bacterial OTU, eotu = eukaryote OTU.

OTU	Connections	Phylum	Order	Genus
eotu368	34	Alveolata	Dinophyceae	Unclassified Gymnodiniaceae
eotu338	32	Unclassified Eukaryota	Unclassified Eukaryota	Unclassified Eukaryota
eotu67	30	Archaeplastida	Chlorophyceae	Unclassified Chlamydomonadales
eotu3341	29	Stramenopiles	Phaeophyceae	Unclassified Phaeophyceae
eotu231	26	Stramenopiles	Bacillariophyta	Unclassified Raphid-pennate
eotu135	22	Alveolata	Unclassified Alveolata	Unclassified Alveolata
eotu176	22	Stramenopiles	Bacillariophyta	Unclassified Raphid-pennate
eotu497	21	Stramenopiles	Bacillariophyta	Unclassified Raphid-pennate
eotu393	20	Stramenopiles	Unclassified Ochrophyta	Unclassified Ochrophyta
eotu863	20	Stramenopiles	Bacillariophyta	Unclassified Raphid-pennate
eotu730	17	Stramenopiles	Unclassified Sagenista	Unclassified Sagenista

Fig. S1. Pearson correlation of alpha diversity and salinity for spatial samples.

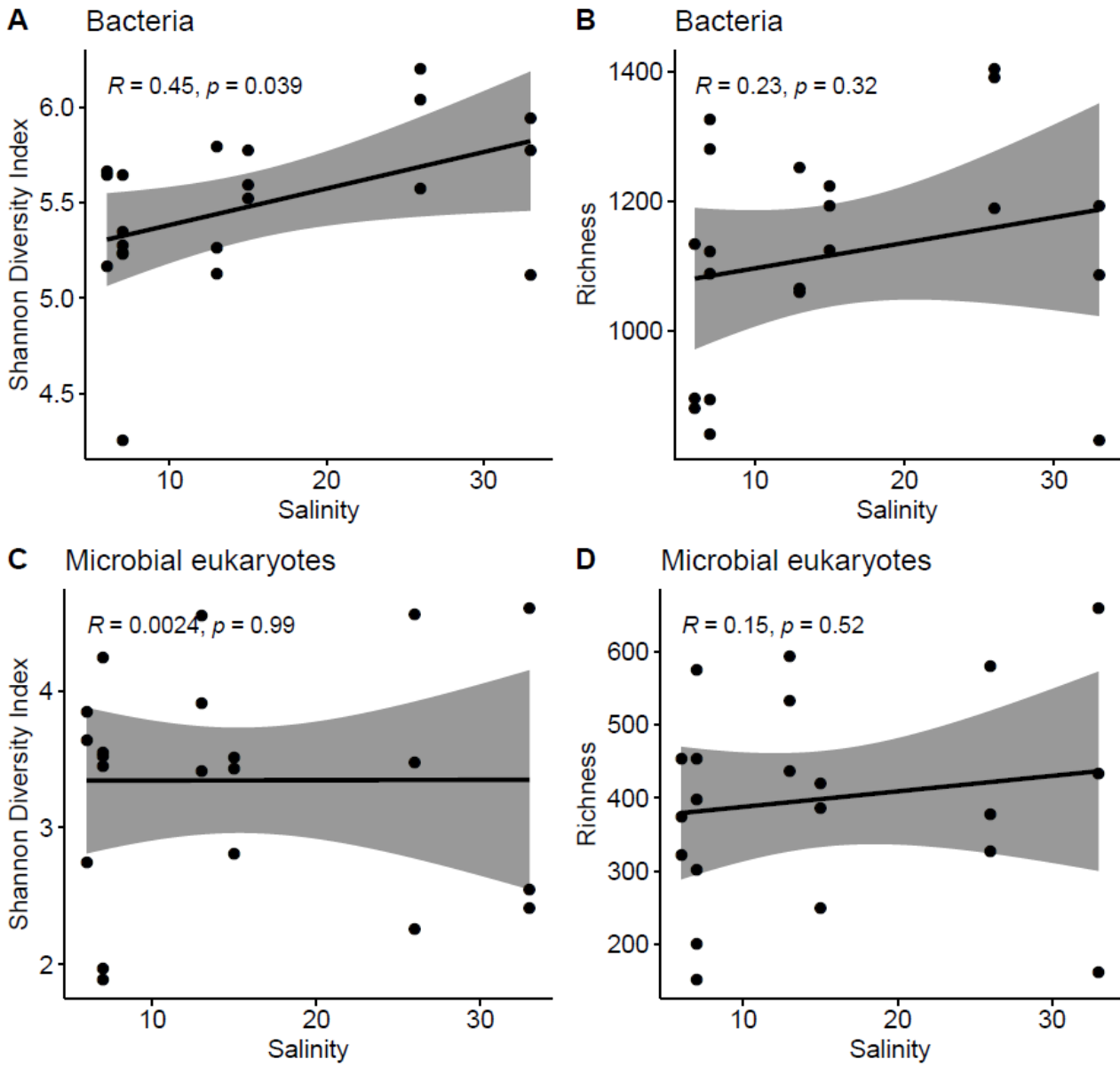


Fig. S2a. Analysis of distance-decay for spatial bacterial dataset.

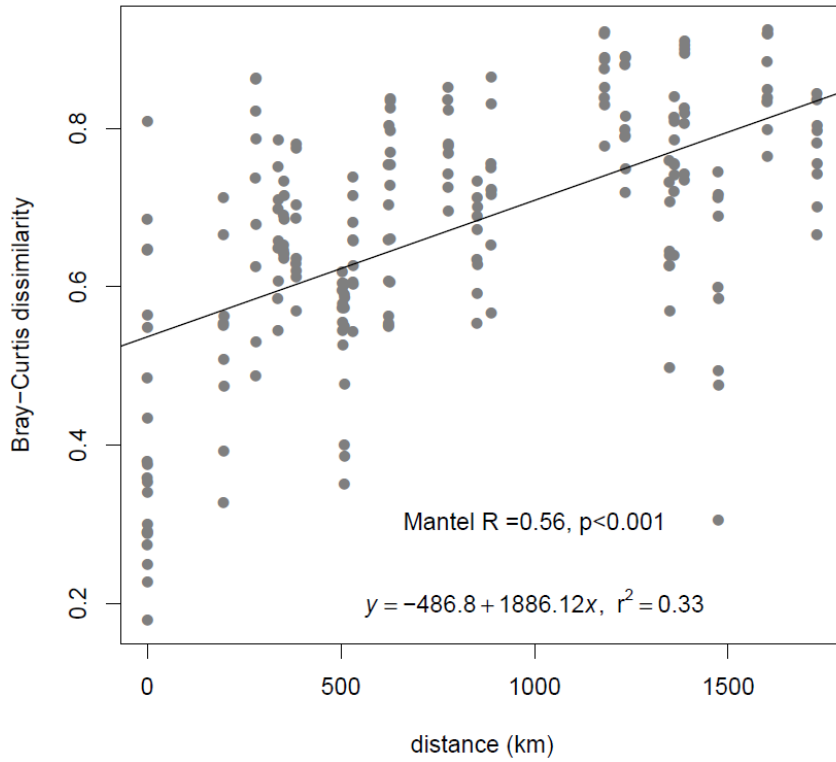


Fig. S2b. Analysis of distance-decay for temporal bacterial dataset.

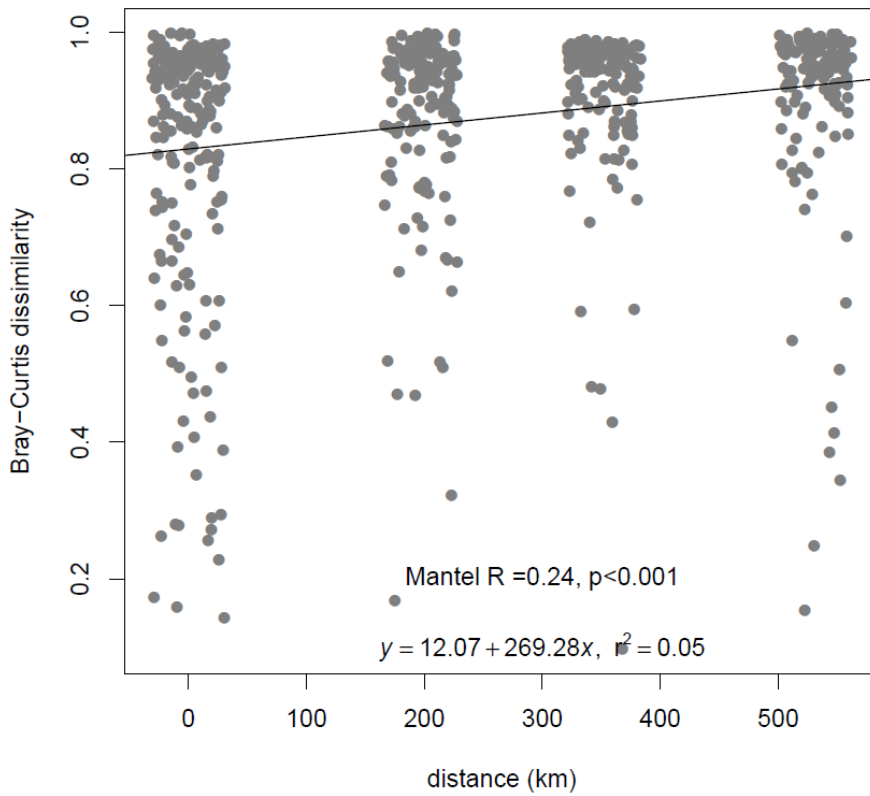


Fig. S2c. Analysis of distance-decay for temporal microbial eukaryote dataset.

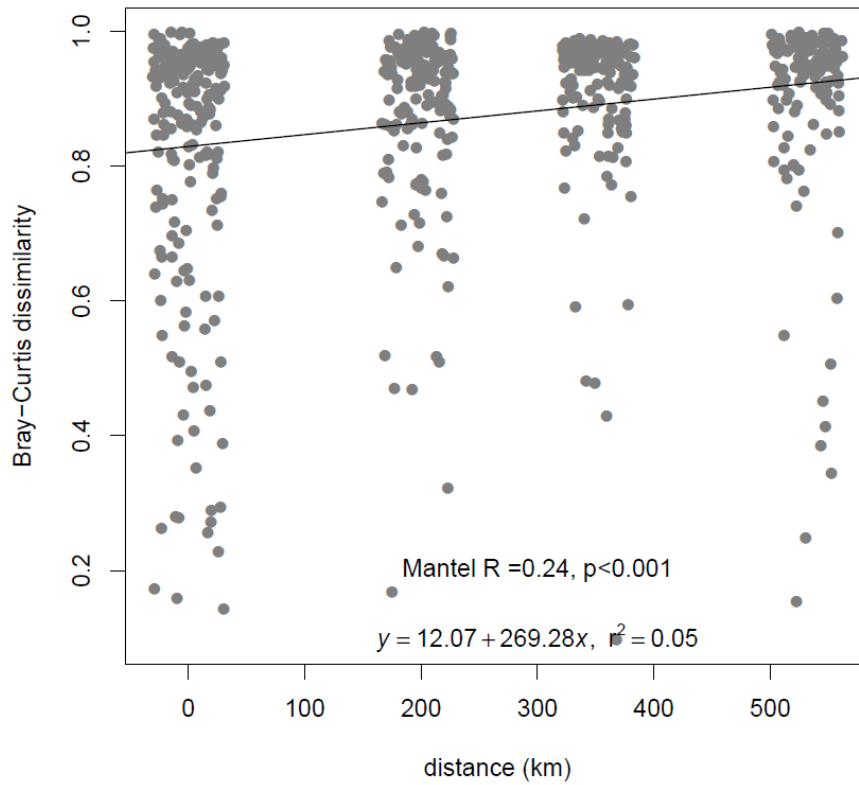


Fig. S3. Pearson correlation of alpha diversity and salinity for temporal samples.

