

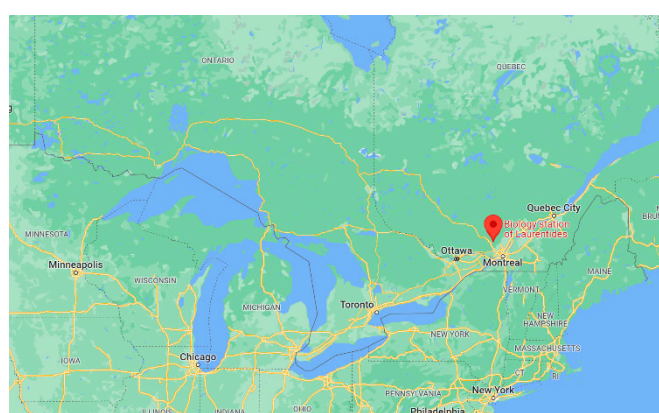


## Article

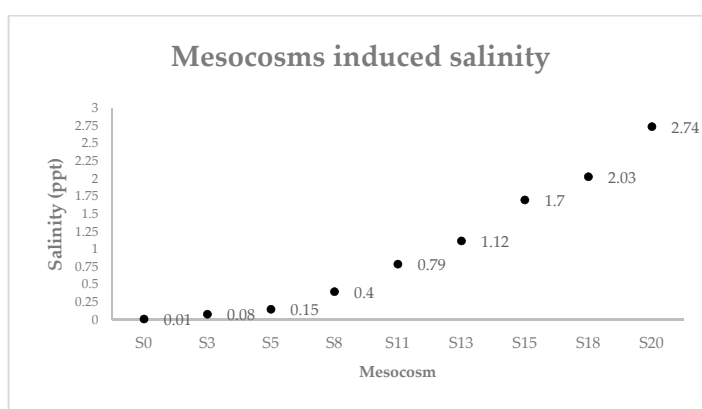
# Response of Prokaryotic Communities to Freshwater Salinization

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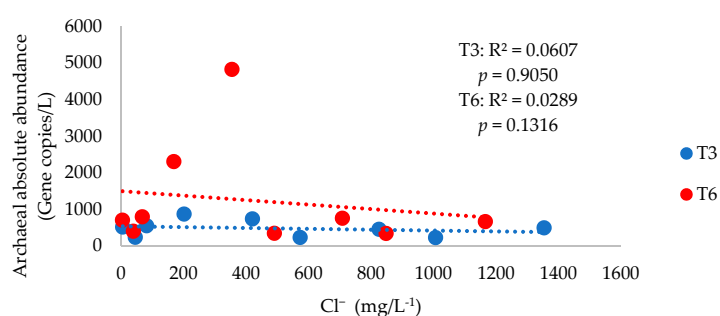


(a)

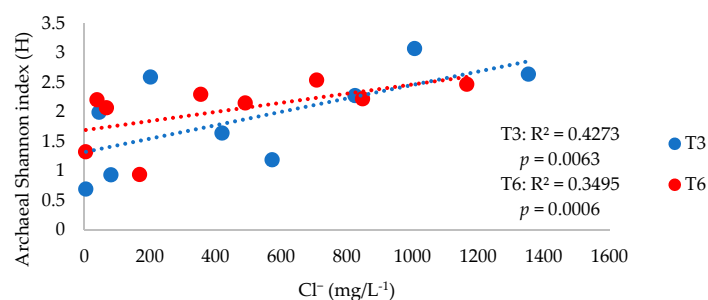


(b)

**Figure S1.** (a) Location of the Laurentians Biological Station, Quebec, Canada, site of the mesocosm installations. (b) Salinity levels in the mesocosms.

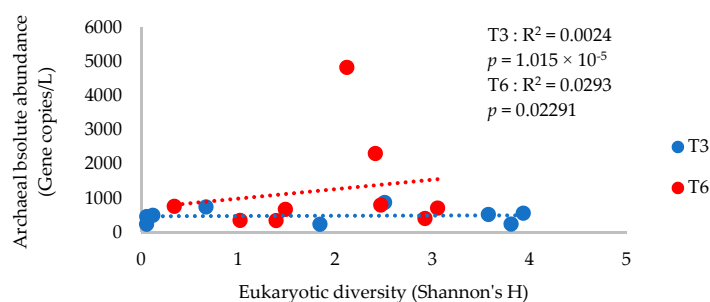


(a)

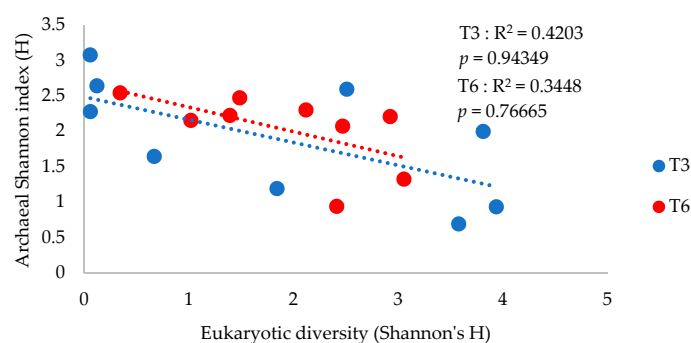


(b)

**Figure S2. (a)** Regression of archaeal absolute abundance and chloride ( $\text{Cl}^-$ ) in mesocosms at T3 and T6 **(b)** Regression of archaeal diversity indices (Shannon's H) and  $\text{Cl}^-$  in mesocosms at T3 and T6.

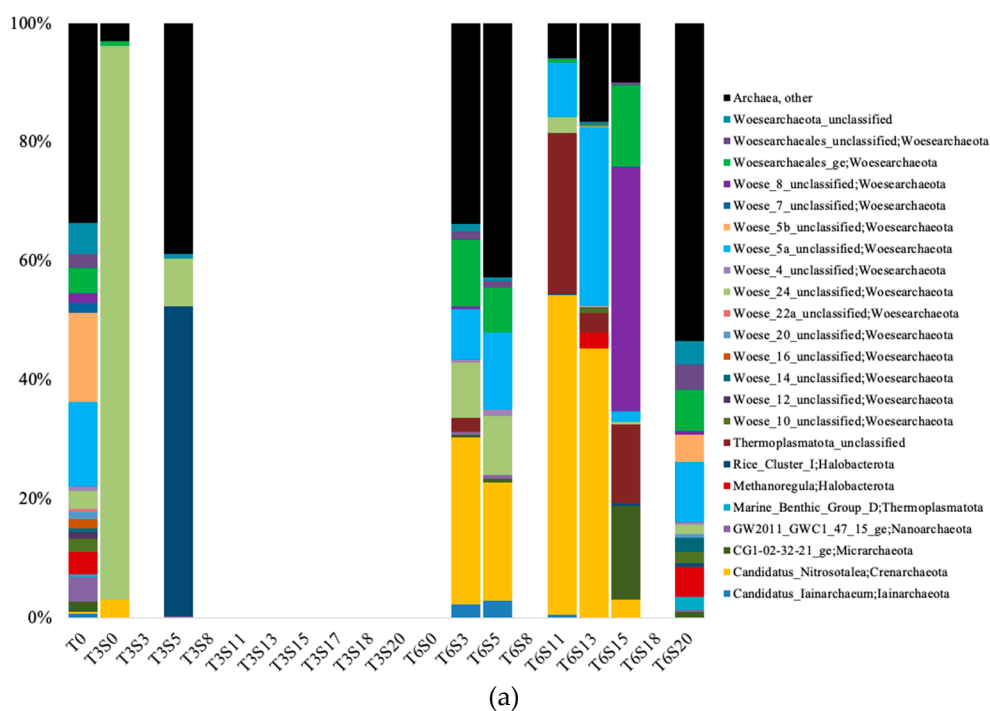


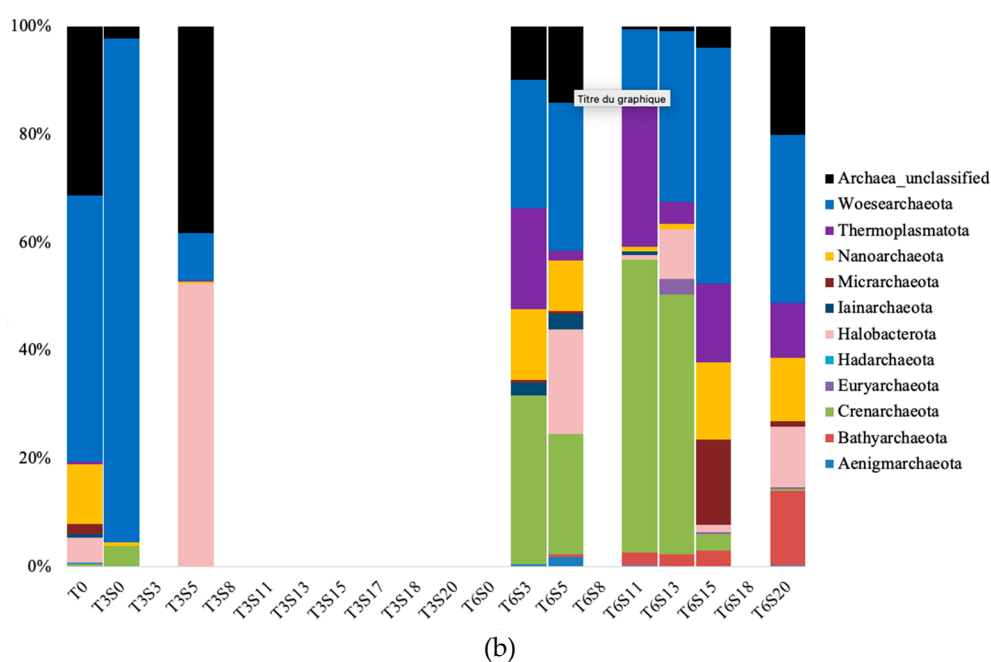
(a)



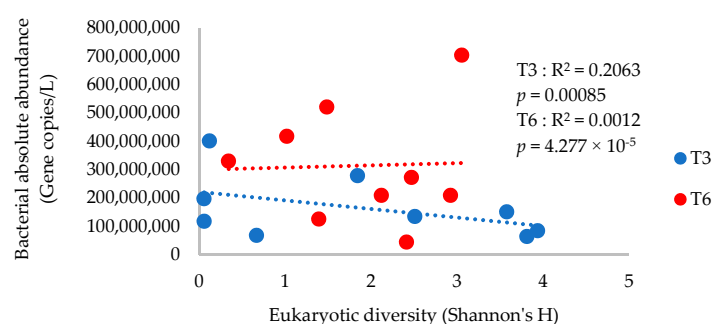
(b)

**Figure S3. (a)** Regression of archaeal absolute abundance and eukaryotic diversity (Shannon's H) in mesocosms at T3 and T6. **(b)** Regression of archaeal diversity (Shannon's H) and eukaryotic diversity (Shannon's H) in mesocosms at T3 and T6

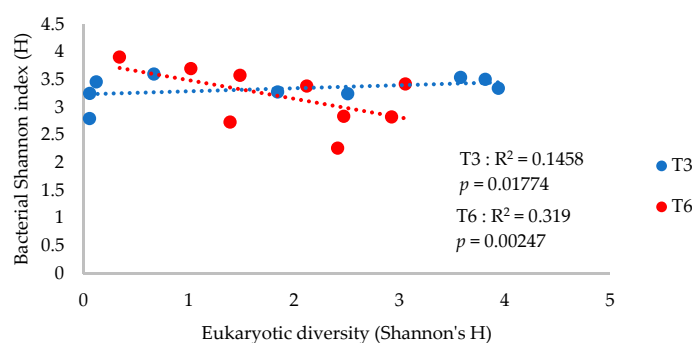




**Figure S4.** Taxonomical classification of the archaeal 16S rRNA genes based on the SILVA database v.138 (Glöckner, Bremen, Germany). (a) Genus-level identification and ; (b) Phylum-level identification.



(a)

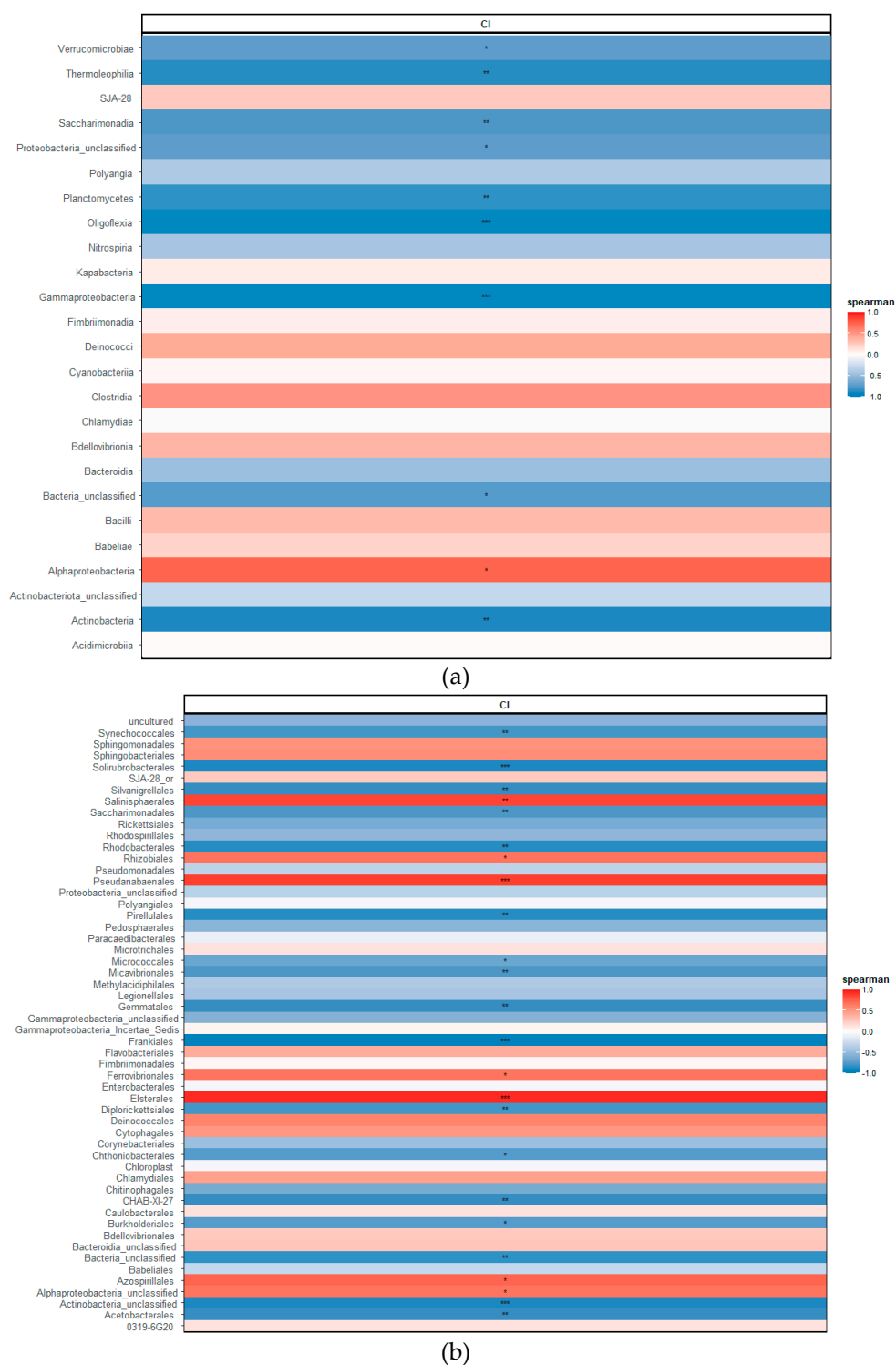


(b)

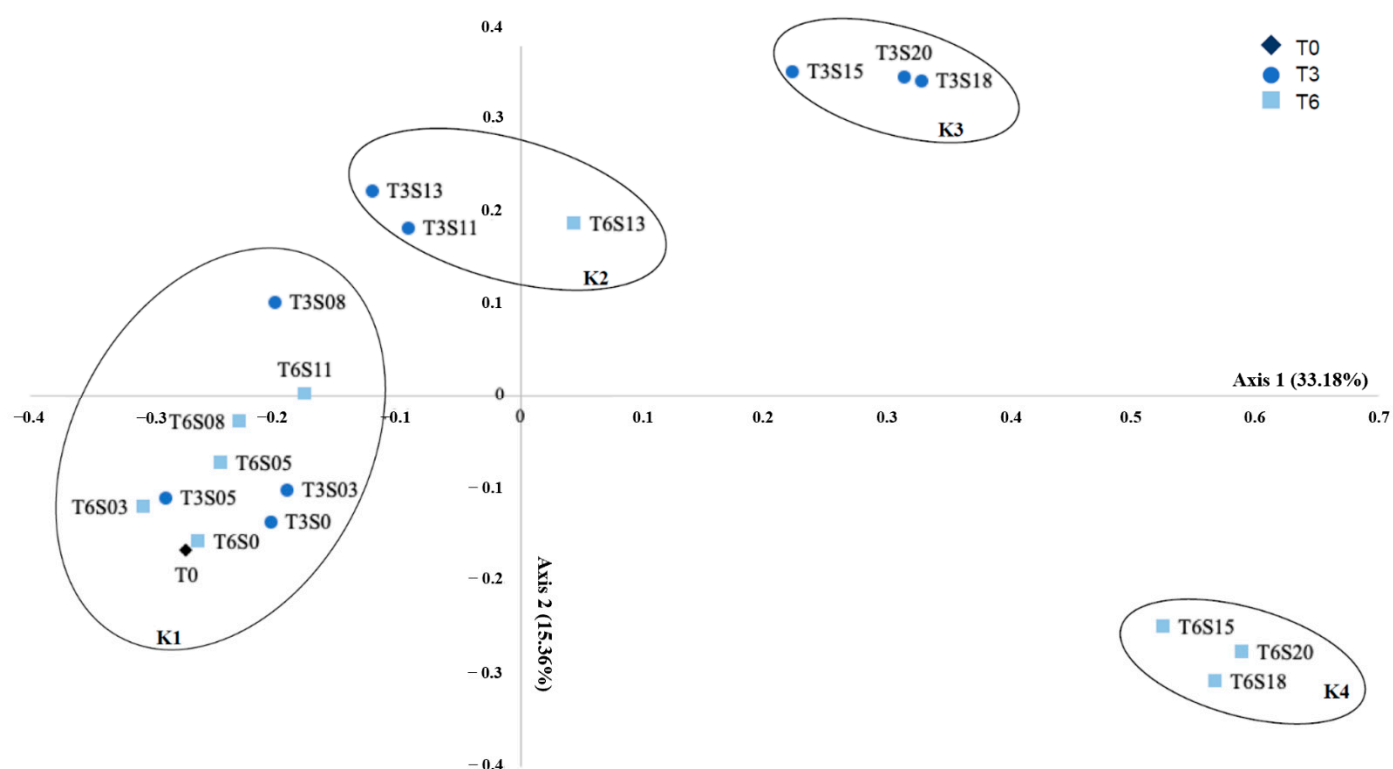
**Figure S5.** (a) Regression of bacterial absolute abundance and eukaryotic diversity (Shannon's H) in mesocosms after three (T3) and six (T6) week incubation. (b) Regression of bacterial diversity (Shannon's H) and eukaryotic diversity (Shannon's H) in mesocosms after three (T3) and six weeks (T6) exposition.



**Figure S6.** Composition of bacterial communities representing a total of more than 2% at the level of **(a)** Classes and ; **(b)** Orders.



**Figure S7.** Heatmap of correlations (Spearman) between CI- values and the most abundant **(a)** classes and **(b)** order, representing the 500 most abundant taxa, in the mesocosms, after three (T3) weeks incubation. None of the classes present showed significance after six (T6) weeks incubation, and were therefore not shown in the figure.



**Figure S8.** Principal coordinate analysis based on a distance matrix between bacterial mesocosm communities. Variance explained by each axis is shown with the axis label. T3, 3 weeks exposure to the salinity gradient; T6, 6 weeks exposure.

**Table S1.** Table of the reaction mixture used for each chip in the context of PCRd.

Material	Volume per chip (μL)	Final concentration
QuantStudio™ 3D Digital PCR Master Mix	7.25	1X
20X SYBR Green 1 dye in TE buffer, pH 8	1.45	2X
Forward and reverse primer	0.3	200nM
cDNA sample	1	50ng total
Water	Bring to 14.5	-

**Table S2.** Table of steps carried out during digital polymerase chain reaction (dPCR), depending on the primers used. The phases, temperatures, times and number of cycles are presented.

Eukaryotic primers E960F - NSR1438R				
	Phase	Temperature	Number of cycles	Number of cycles
Step 1	Initial denaturation	96°C	00:10:00	1X
Step 2	Denaturation	58°C	00:02:00	39X
	Hybridization	98°C	00:00:30	
	Elongation	60°C	00:02:00	
Step 3	Conservation	10°C	∞	1X

Bacterial primers B341F - B785R				
	Phase	Temperature	Number of cycles	Number of cycles
Step 1	Initial denaturation	96°C	00:10:00	1X
Step 2	Denaturation	56°C	00:02:00	39X
	Hybridization	98°C	00:00:30	
	Elongation	60°C	00:02:00	
Step 3	Conservation	10°C	∞	1X
Archaeal primers A340F - A915R				
	Phase	Temperature	Number of cycles	Number of cycles
Step 1	Initial denaturation	96°C	00:10:00	1X
Step 2	Denaturation	58°C	00:02:00	39X
	Hybridization	64°C	00:00:30	
	Elongation	60°C	00:02:00	
Step 3	Conservation	10°C	∞	1X

**Table S3.** Water physico-chemical values measured using a YSI multi-parameter probe in the different mesocosms, at the beginning of the incubation, with measured Cl<sup>-</sup> values, at T0.

Sample	Temp (°C)	Cond (SPC)	Sal (ppt)	pH	DO (%)	Measured Cl <sup>-</sup> (mg/L <sup>-1</sup> )
S0	21.3	17.4	0.01	6.26	99	0.265
S3	21.3	168.9	0.08	6.45	98.8	39.905
S5	21.3	305.5	0.15	6.68	99.3	106.675
S8	21.3	806	0.4	6.76	99.3	202.715
S11	21.4	1564	0.79	6.45	100.8	351.365
S13	21.3	2191	1.12	6.45	100.5	455.585
S15	21.4	2283	1.7	6.42	100.7	511.225
S18	21.4	3845	2.03	6.38	102.1	875.93
S20	21.4	5096	2.74	6.31	101.9	1110.86

**Table S4.** Biotic and abiotic factor values used to carry out multivariate analyses, shown for every sample, composing the different mesocosms at the different incubation times. For T3 and T6 samples, Cl<sup>-</sup> values were calculated following a linear regression using Cl<sup>-</sup> and conductivity ( $y = 1 \times 10^{-20} * x^3 + 0.0000000000000001 * x^2 + 0.2279 * x$ ).

Sample	Sampling (week)	time Temp. (°C)	pH	Cl <sup>-</sup> (mg/L <sup>-1</sup> )	Total phosphorus (µg.L <sup>-1</sup> )	Dissolved oxygen (%)	Bacterial abundance (Gene copies/L)	Eucaryotic abundance (Gene copies/L)	Archaeal abundance (Gene copies/L)	Eukaryotic diversity (H)	Archaeal diversity (H)	Bacterial diversity (H)
T0	0	21.3	6.26	0.27	3.574	99	111549000	12319200	1578	3.793332	4.883306	3.650698

T3S0	3	24.2	7.28	4.71753	2.945	96.3	150300000	17022000	514.8	3.579116	0.685688	3.537383
T3S03	3	24.2	7.26	45.3749	5.31	96.6	63334500	1539750	234	3.812996	1.99047	3.504182
T3S05	3	24.2	7.17	81.4743	7.23	96.2	83961000	2110800	552.6	3.938634	0.926154	3.341875
T3S08	3	24.3	6.98	201.692	4.033	97.3	133900500	2299875	870	2.508806	2.588274	3.242708
T3S11	3	24.5	6.7	420.02	7.36	96.8	67179000	29994900	738	0.668207	1.637801	3.598676
T3S13	3	24.3	6.77	572.713	8.38	94.4	277905000	19275000	229.8	1.842517	1.18385	3.274357
T3S15	3	24.3	7.64	825.454	12.99	97.7	116964000	30273000	456.6	0.058999	2.272586	3.249111
T3S18	3	24.2	7.32	1006.63	5.95	99.5	196560000	117834000	229.5	0.057467	3.07065	2.796754
T3S20	3	24.2	7.07	1353.73	7.93	94.2	400350000	95694000	494.4	0.120876	2.636184	3.457347
T6S0	6	24.6	6.87	4.07941	4.34	116.9	702945000	55291500	702.9	3.056106	1.31744	3.419336
T6S03	6	24.6	7.1	39.0393	4.085	115.3	208181250	18837000	401.85	2.924541	2.201943	2.825572
T6S05	6	24.6	7.06	67.5268	4.851	118.7	271800000	63058500	795.6	2.470229	2.066033	2.837106
T6S08	6	24.5	6.64	168.646	4.532	114.8	43832250	9610425	2304	2.413287	0.933618	2.260369
T6S11	6	24.6	7.75	354.84	4.723	119.8	208692000	33484950	4824	2.119586	2.293485	3.38135
T6S13	6	24.5	6.98	490.441	3.766	115.3	416790000	31560750	343.35	1.019819	2.148207	3.697428
T6S15	6	24.6	6.82	708.085	5.68	116.9	329305500	28736100	756	0.342372	2.536009	3.906411
T6S18	6	24.6	7.13	848.016	3.319	119	125055000	13992075	339.3	1.391766	2.218366	2.726809
T6S20	6	24.5	6.95	1165.94	4.085	117.6	519975000	9024750	665.1	1.486213	2.467024	3.577321

**Table S5.** First 2 axes' values of a principal Coordinates Analysis (PCoA) computed using the eukaryotic operational taxonomic unit (OTU) table (the sequence data is available in Astorg *et al.* [6]).

group	axis1	axis2
T0	-0.198837	0.257246
T3S0	-0.207731	0.261959
T3S03	-0.222076	0.238455
T3S05	-0.280860	0.278656
T3S08	-0.297501	-0.419953
T3S11	-0.291871	-0.518987
T3S13	-0.034741	0.044833
T3S15	0.543059	-0.033957
T3S18	0.545052	-0.033708
T3S20	0.542808	-0.032694
T6S0	-0.208055	0.279988
T6S03	-0.255759	0.325092
T6S05	-0.209272	0.210855
T6S08	-0.225563	0.184650
T6S11	-0.297997	-0.445981
T6S13	-0.296064	-0.510137
T6S15	0.538738	-0.033616
T6S18	0.354396	-0.029152
T6S20	0.502275	-0.023549



**Table S6.** Significant explanatory variables explaining bacterial mesocosm community composition variance, tested using distance-based redundancy analysis (db-RDA). Eukaryotic community composition was represented based on the scores of axes 1 and 2 (A1, A2) of a PCoA (see Table S5).

	Df	SumOfSqs	F	Pr(>F)
Total Phosphorus	1	0.40077	2.1830	0.012
Temperature	1	0.41658	2.2691	0.017
Salinity	1	0.97199	5.2945	0.001
pH	1	0.20366	1.1094	0.317
Dissolved oxygen	1	0.26580	1.4478	0.126
Eukaryote community A1	1	0.55439	3.0198	0.001
Eukaryote community A2	1	0.21777	1.1862	0.244
Absolute abundance Eukaryote	1	0.32528	1.7718	0.044
Absolute abundance Bacteria	1	0.14999	0.8170	0.650
Absolute abundance Archaea	1	0.22345	1.2171	0.244
Chloride	1	0.20694	1.1272	0.299
Residual	6	1.10151		

**Table S7.** Variation in bacterial mesocosm community composition explained by exposure time (3 and 6 weeks) and salinity groups (K1, K2, K3, and K4) defined in the linkage tree (LINKTREE) analysis, tested using permutational multivariate analysis of variance (PERMANOVA).

	Df	SumOfSqs	R2	F	Pr(>F)
Exposure time	1	0.5952	0.11381	4.2359	0.002
Salinity group	3	2.5743	0.49223	6.1067	0.001
Exposure × Salinity	1	0.3742	0.07154	2.6627	0.003
Residual	12	1.6862	0.32242		
Total	17	5.2299	1.00000		

**Table S8.** Similarity percentages (SIMPER) and Kruskal-Wallis analysis table showing the OTUs that are significantly different according to the clustering established by the PCoA. The table shows the compared Clusters, the result of the SIMPER analysis, the OTU group, the initial Kruskal-Wallis p-value (Krusk.p.val) and corrected for false discovery rate (Fdr.krusk. p.val), the taxonomy associated with OTU, the mean relative abundances and standard deviations associated with the two clusters compared.

Clusters	SIMPER	OTU	Krusk p.val	fdr_krusk p.val	Taxonomy	Left mean abund	Left stdev	Right mean abund	Right stdev
K1 / K2	0.014965	Otu05	0.011230	0.028075	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Frankiales(100);Sporichthyaceae(100);Sporichthyaceae_unclassified(74);	0.025580	0.013154	0.002187	0.002308
K1 / K2	0.011071	Otu08	0.017960	0.038487	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Micrococcales(100);Microbacteriaceae(100);Microbacteriaceae_unclassified(84);	0.006505	0.002749	0.022223	0.012884
K1 / K2	0.010568	Otu04	0.027992	0.054367	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Corynebacteriales(100);Mycobacteriaceae(98);Mycobacterium(98);	0.027225	0.007603	0.010748	0.006358

K1 / K2	0.010234	Otu03	0.090969	0.097467	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Frankiales(100);Sporichthyaceae(100);hgcI_clade(88);	0.030314	0.023934	0.013330	0.005392
K1 / K3	0.017633	Otu12	0.005766	0.028075	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Azospirillales(98);Azospirillaceae(98);Azospirillum(96);	0.000386	0.000803	0.029241	0.026043
K1 / K3	0.016101	Otu02	0.011230	0.028075	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Frankiales(100);Sporichthyaceae(100);Sporichthyaceae_ge(75);	0.031136	0.016975	0.003542	0.001080
K1 / K3	0.015186	Otu03	0.011230	0.028075	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Frankiales(100);Sporichthyaceae(100);hgcI_clade(88);	0.030314	0.023934	0.003578	0.000382
K1 / K3	0.014947	Otu08	0.011230	0.028075	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Micrococcales(100);Microbacteriaceae(100);Microbacteriaceae_unclassified(84);	0.006505	0.002749	0.030731	0.014375
K1 / K3	0.014221	Otu10	0.026922	0.054367	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100);Pseudomonadales(100);Moraxellaceae(100);Acinetobacter(100);	0.005450	0.009761	0.027423	0.007042
K1 / K3	0.013751	Otu05	0.011230	0.028075	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Frankiales(100);Sporichthyaceae(100);Sporichthyaceae_unclassified(74);	0.025580	0.013154	0.002695	0.001554
K1 / K3	0.012012	Otu16	0.010897	0.028075	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100);Burkholderiales(100);Comamonadaceae(100);Ideonella(62);	0.001776	0.001284	0.021082	0.019667
K1 / K3	0.011699	Otu01	0.017960	0.038487	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100);Burkholderiales(100);Burkholderiaceae(100);Polynucleobacter(100)	0.037327	0.012281	0.017277	0.007419
K1 / K3	0.011290	Otu11	0.005766	0.028075	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Ferrovibrionales(85);Ferrovibrionales_unclassified(51);Ferrovibrionales_unclassified(51);	0.000357	0.000627	0.018477	0.014816
K1 / K3	0.011152	Otu17	0.017179	0.038487	Bacteria(100);Bacteroidota(100);Bacteroidia(100);Flavobacteriales(100);Flavobacteriaceae(100);Flavobacterium(100);	0.003852	0.008619	0.020506	0.013357
K1 / K3	0.011143	Otu07	0.011230	0.028075	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Micrococcales(100);Microbacteriaceae(100);Candidatus_Planktoluna(82);	0.019798	0.006877	0.001693	0.001004
K1 / K4	0.021400	Otu06	0.008903	0.028075	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100);Beijerinckiaceae(100);Bosea(97);	0.000485	0.000542	0.039979	0.023661
K1 / K4	0.017610	Otu01	0.011230	0.028075	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100);Burkholderiales(100);Burkholderiaceae(100);Polynucleobacter(100);	0.037327	0.012281	0.003421	0.003080
K1 / K4	0.017223	Otu09	0.007290	0.028075	Bacteria(100);Bacteroidota(100);Bacteroidia(100);Cytophagales(100);Spirosomaceae(100);Emticia(100);	0.000415	0.000592	0.031707	0.004035
K1 / K4	0.014792	Otu03	0.011118	0.028075	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Frankiales(100);Sporichthyaceae(100);hgcI_clade(88);	0.030314	0.023934	0.000662	0.001146
K1 / K4	0.014346	Otu02	0.011230	0.028075	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Frankiales(100);Sporichthyaceae(100);Sporichthyaceae_ge(75);	0.031136	0.016975	0.002880	0.001848
K1 / K4	0.012804	Otu04	0.011230	0.028075	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Corynebacteriales(100);Mycobacteriaceae(98);Mycobacterium(98);	0.027225	0.007603	0.003363	0.000607
K1 / K4	0.012714	Otu05	0.011230	0.028075	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Frankiales(100);Sporichthyaceae(100);Sporichthyaceae_unclassified(74);	0.025580	0.013154	0.001286	0.001670
K1 / K4	0.012432	Otu11	0.005766	0.028075	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Ferrovibrionales(85);Ferrovibrionales_unclassified(51);Ferrovibrionales_unclassified(51);	0.000357	0.000627	0.023082	0.005709
K1 / K4	0.010501	Otu07	0.011118	0.028075	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Micrococcales(100);Microbacteriaceae(100);Candidatus_Planktoluna(82);	0.019798	0.006877	0.000255	0.000442
K2 / K3	0.017999	Otu02	0.049535	0.054367	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Frankiales(100);Sporichthyaceae(100);Sporichthyaceae_ge(75);	0.029020	0.015371	0.003542	0.001080
K2 / K3	0.017215	Otu12	0.049535	0.054367	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Azospirillales(98);Azospirillaceae(98);Azospirillum(96);	0.003000	0.002090	0.029241	0.026043
K2 / K3	0.013107	Otu01	0.126630	0.129508	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100);Burkholderiales(100);Burkholderiaceae(100);Polynucleobacter(100);	0.034943	0.013243	0.017277	0.007419
K2 / K3	0.012202	Otu10	0.126630	0.129508	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100);Pseudomonadales(100);Moraxellaceae(100);Acinetobacter(100);	0.009966	0.011963	0.027423	0.007042
K2 / K3	0.012201	Otu11	0.046302	0.054367	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Ferrovibrionales(85);Ferrovibrionales_unclassified(51);Ferrovibrionales_unclassified(51);	0.000318	0.000550	0.018477	0.014816
K2 / K3	0.011948	Otu17	0.049535	0.054367	Bacteria(100);Bacteroidota(100);Bacteroidia(100);Flavobacteriales(100);Flavobacteriaceae(100);Flavobacterium(100);	0.002355	0.001029	0.020506	0.013357
K2 / K3	0.010332	Otu16	0.275234	0.275234	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100);Burkholderiales(100);Comamonadaceae(100);Ideonella(62);	0.006549	0.004671	0.021082	0.019667
K2 / K4	0.020949	Otu06	0.049535	0.054367	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100);Beijerinckiaceae(100);Bosea(97);	0.001684	0.001351	0.039979	0.023661
K2 / K4	0.017946	Otu01	0.049535	0.054367	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100);Burkholderiales(100);Burkholderiaceae(100);Polynucleobacter(100)	0.034943	0.013243	0.003421	0.003080
K2 / K4	0.016387	Otu09	0.046302	0.054367	Bacteria(100);Bacteroidota(100);Bacteroidia(100);Cytophagales(100);Spirosomaceae(100);Emticia(100);	0.002213	0.003833	0.031707	0.004035
K2 / K4	0.015028	Otu02	0.049535	0.054367	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Frankiales(100);Sporichthyaceae(100);Sporichthyaceae_ge(75);	0.029020	0.015371	0.002880	0.001848
K2 / K4	0.012593	Otu11	0.046302	0.054367	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Ferrovibrionales(85);Ferrovibrionales_unclassified(51);Ferrovibrionales_unclassified(51);	0.000318	0.000550	0.023082	0.005709

<b>K2 / K4</b>	0.010729	Otu08	0.049535	0.054367	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Micrococcales(100);Microbacteriaceae(100);Microbacteriaceae_unclassified(84);	0.022223	0.012884	0.003508	0.001603
<b>K3 / K4</b>	0.018689	Otu09	0.049535	0.054367	Bacteria(100);Bacteroidota(100);Bacteroidia(100);Cytophagales(100);Spirosomaceae(100);Emticicia(100);	0.001797	0.001961	0.031707	0.004035
<b>K3 / K4</b>	0.017284	Otu12	0.049535	0.054367	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Azospirillales(98);Azospirillaceae(98);Azospirillum(96);	0.029241	0.026043	0.000637	0.000584
<b>K3 / K4</b>	0.016970	Otu06	0.049535	0.054367	Bacteria(100);Proteobacteria(100);Alphaproteobacteria(100);Rhizobiales(100);Beijerinckiaceae(100);Bosea(97);	0.012505	0.006186	0.039979	0.023661
<b>K3 / K4</b>	0.016864	Otu10	0.036904	0.054367	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100);Pseudomonadales(100);Moraxellaceae(100);Acinetobacter(100);	0.027423	0.007042	0.000000	0.000000
<b>K3 / K4</b>	0.016599	Otu08	0.049535	0.054367	Bacteria(100);Actinobacteriota(100);Actinobacteria(100);Micrococcales(100);Microbacteriaceae(100);Microbacteriaceae_unclassified(84);	0.030731	0.014375	0.003508	0.001603
<b>K3 / K4</b>	0.012075	Otu16	0.046302	0.054367	Bacteria(100);Proteobacteria(100);Gammaproteobacteria(100);Burkholderiales(100);Comamonadaceae(100);Ideonella(62);	0.021082	0.019667	0.001393	0.001157
<b>K3 / K4</b>	0.011726	Otu17	0.049535	0.054367	Bacteria(100);Bacteroidota(100);Bacteroidia(100);Flavobacteriales(100);Flavobacteriaceae(100);Flavobacterium(100);	0.020506	0.013357	0.001156	0.001194
<b>K3 / K4</b>	0.010544	Otu27	0.036904	0.054367	Bacteria(100);Bacteroidota(100);Bacteroidia(100);Chitinophagales(100);Chitinophagaceae(100);Edaphobaculum(100);	0.000000	0.000000	0.017107	0.012023