

Supplementary Information

Homogeneous selection promotes microdiversity in the glacier-fed stream microbiome

6 Stilianos Fodelianakis¹, Alex D. Washburne^{2,3}, Massimo Bourquin¹, Paraskevi Pramateftaki¹,
7 Tyler J. Kohler¹, Michail Styllas¹, Matteo Tolosano¹, Vincent De Staercke¹, Martina Schön¹,
8 Susheel Bhanu Busi⁴, Jade Brandani¹, Paul Wilmes⁴, Hannes Peter¹ & Tom J. Battin¹

10 ¹Stream Biofilm & Ecosystem Research Lab, ENAC Division, Ecole Polytechnique Federale de
11 Lausanne

12 ²Department of Microbiology and Immunology, Montana State University, USA

13 ³Selva Analytics, LLC, Bozeman, Montana, USA

14 ⁴Systems Ecology Research Group, Luxembourg Centre for Systems Biomedicine, University of
15 Luxembourg, Esch-sur-Alzette, Luxembourg

17 **Supplementary Results**

18

19 **Detailed taxonomic diversity**

20 The communities in the different glacier-fed streams were both rich in terms of the
21 number of detected SVs and in terms of the number of taxonomic groups. We detected 8186 SVs
22 in total and 640 ± 60 SVs per glacier-fed stream on average. In terms of taxonomy, there were 36
23 assigned bacterial Phyla, 112 Classes, 162 Orders, 156 Families and 159 Genera present in the
24 sampled communities. The most diverse and abundant Phyla were Proteobacteria (3267 SVs,
25 34.4-90.2% of total cells per sample), Bacteroidetes (1324 SVs, 1.8-31.9% of total cells per
26 sample) and Planctomycetes (728 SVs, 0.5-9.9% of total cells per sample) and the most diverse
27 and abundant Classes within these three phyla were Betaproteobacteria (1429 SVs, 15.2-78.8%
28 of total cells per sample), Saprospirae (481 SVs, 0.09-13.4% of total cells per sample) and
29 Planctomycetia (539 SVs, 0.4-8.5% of total cells per sample), respectively (Fig. S6).

30

31 **Core microbiome**

32 The core microbiome, which we defined as the taxonomic units present in at least one
33 sample at every reach, included 11 Phyla, 20 Classes, 29 Orders, 18 Families and 11 Genera
34 (Fig. 1, Table S2). The 11 Genera within the core microbiome included a total of 1133 SVs
35 (13.9% of the total SVs) that comprised on average 34.8% (13.6 – 62%) of the total cells per
36 gram of dry sediment per community. Six core Genera were taxonomically affiliated to
37 Betaproteobacteria; *Methylotenera*, *Polaromonas*, *Rhodoferax*, *Leptothrix*, *Methylibium* and
38 *Rubrivivax*, containing 541 SVs and comprising on average 25.1% of the total cells per gram of
39 dry sediment per community. Two core Genera were affiliated to Planctomycetes; *Gemmata* and

40 *Planctomyces*, containing 258 SVs and comprising on average 1.3% of the total cells per gram of
41 dry sediment per community. Two core Genera were affiliated to Alphaproteobacteria;
42 *Hyphomicrobium* and *Novosphingobium*, containing 113 SVs and comprising on average 3.5%
43 of the total cells per gram of dry sediment per community. One core Genus was affiliated to
44 Bacteroidetes; *Flavobacterium*, containing 205 SVs and comprising on average 3% of the total
45 cells per gram of dry sediment per community, and one Genus was affiliated to Nitrospirae;
46 *Nitrospira*, containing 20 SVs and comprising on average 1.9% of the total cells per gram of dry
47 sediment per community.

48

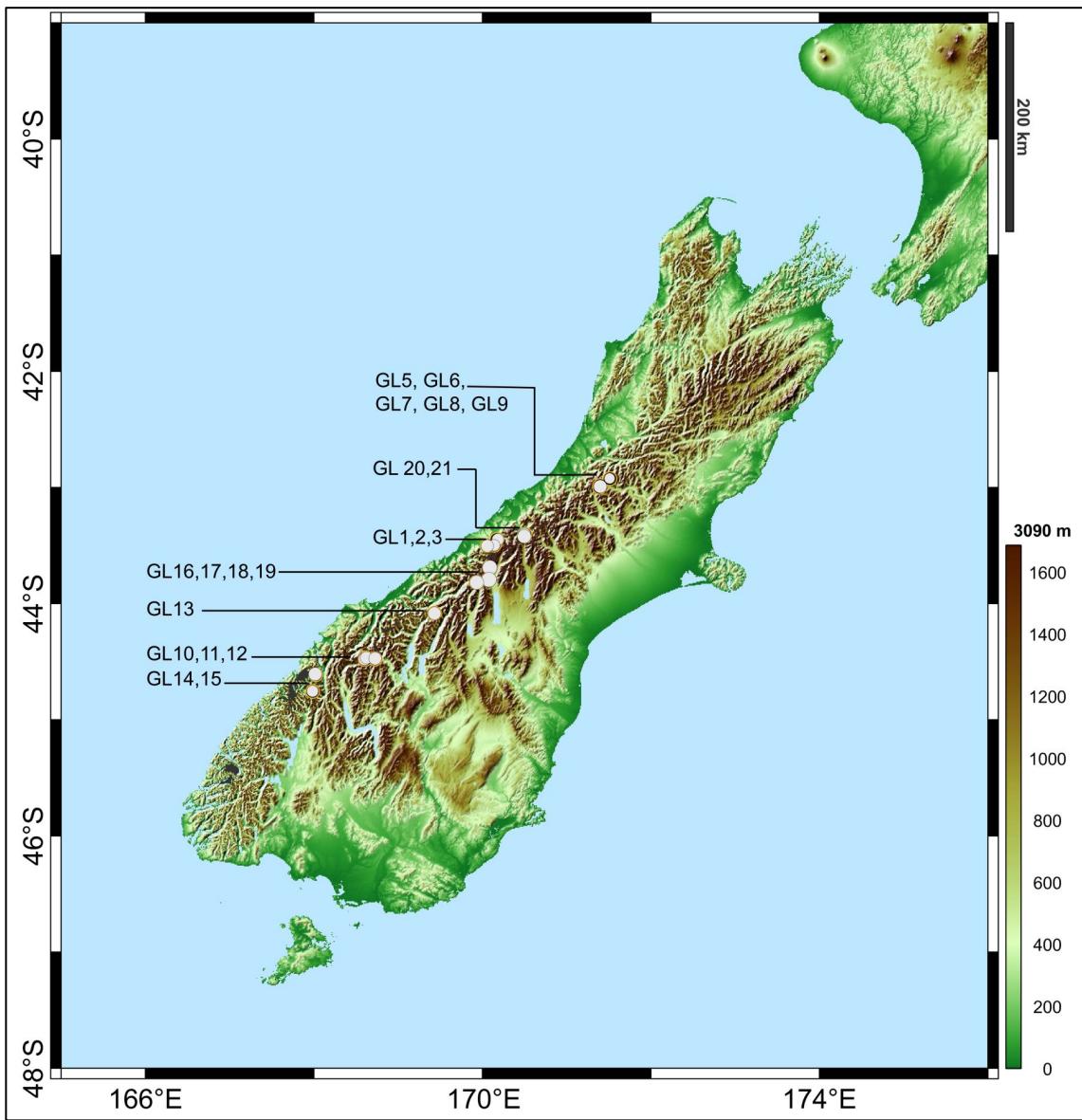
49 **Environmental drivers of bacterial β -diversity**

50 Using distance-based redundancy analysis and a forward stepwise model building, we
51 found that seven of the recorded environmental parameters, namely conductivity, dissolved
52 oxygen, latitude, chl- α content, pH, water temperature and turbidity, could collectively explain
53 43.5% of the variance in the Bray-Curtis (BC) dissimilarity among the samples (Fig. S4, Table
54 S6). Conductivity had the highest proportion of variance explained (14.1%), followed by
55 dissolved oxygen (8.1%), latitude (5.7%), chl- α content (5.6%) and pH (4.3%) while the rest of
56 the variables explained low proportions of variance (1.4-2.3%).

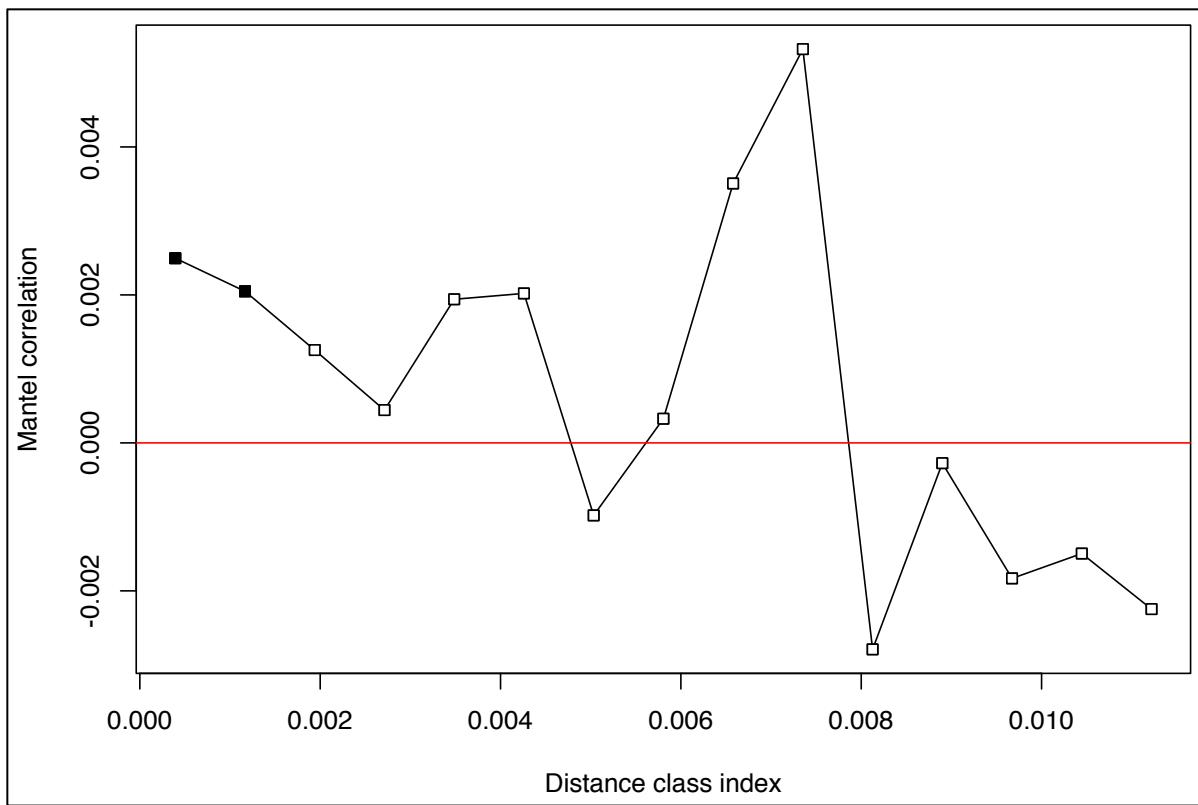
57 The samples had a distinct clustering pattern along the first constrained axis that
58 explained 18.8% of the total BC variance (Fig. S4). The left axis side was characterized by a
59 low-dispersion cluster of samples from turbulent streams with high conductivity, pH and
60 dissolved oxygen, and the right axis side was characterized by a high-dispersion cluster of
61 samples from warmer streams with higher chl- α content. The second constrained axis explained
62 8.7% of total BC variance and was mostly associated with latitude, suggesting that a part of

63 community variability can be explained by geographic legacies. Samples from the same stream
64 but at different reaches (UP and DN) clustered mostly together and the variance explained by the
65 factor “reach” was non-significant.

66 **Supplementary Figures**

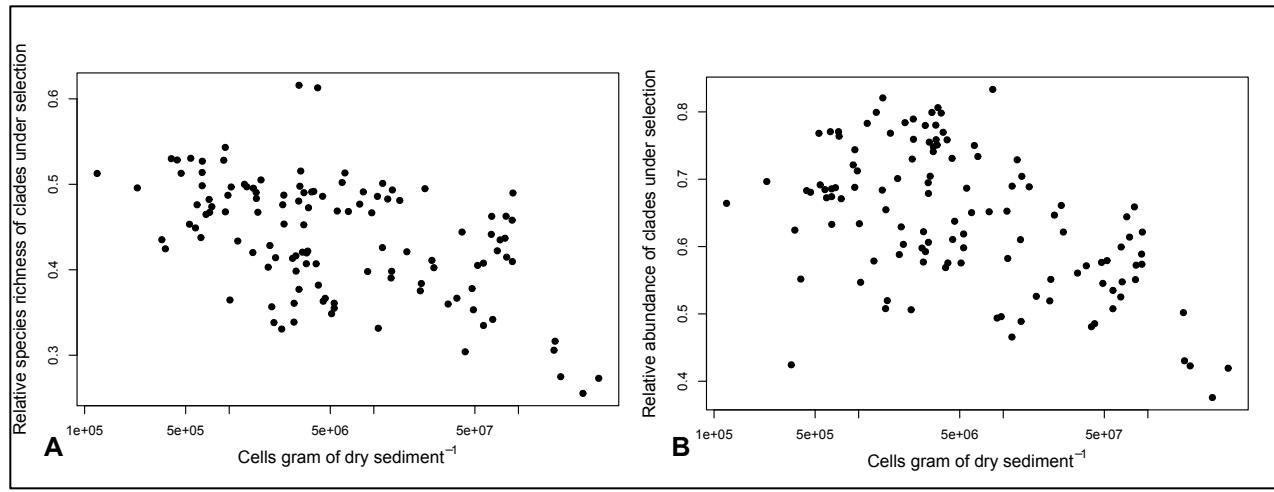


67
68 **Figure S1. The location of the sampled glacier-fed streams (GFS) at the Southern Alps of**
69 **New Zealand.** GFS are assigned numbers from 1 to 21 (excluding 4) for operational purposes.
70 Map colors correspond to the altitude as per the legend on the right.

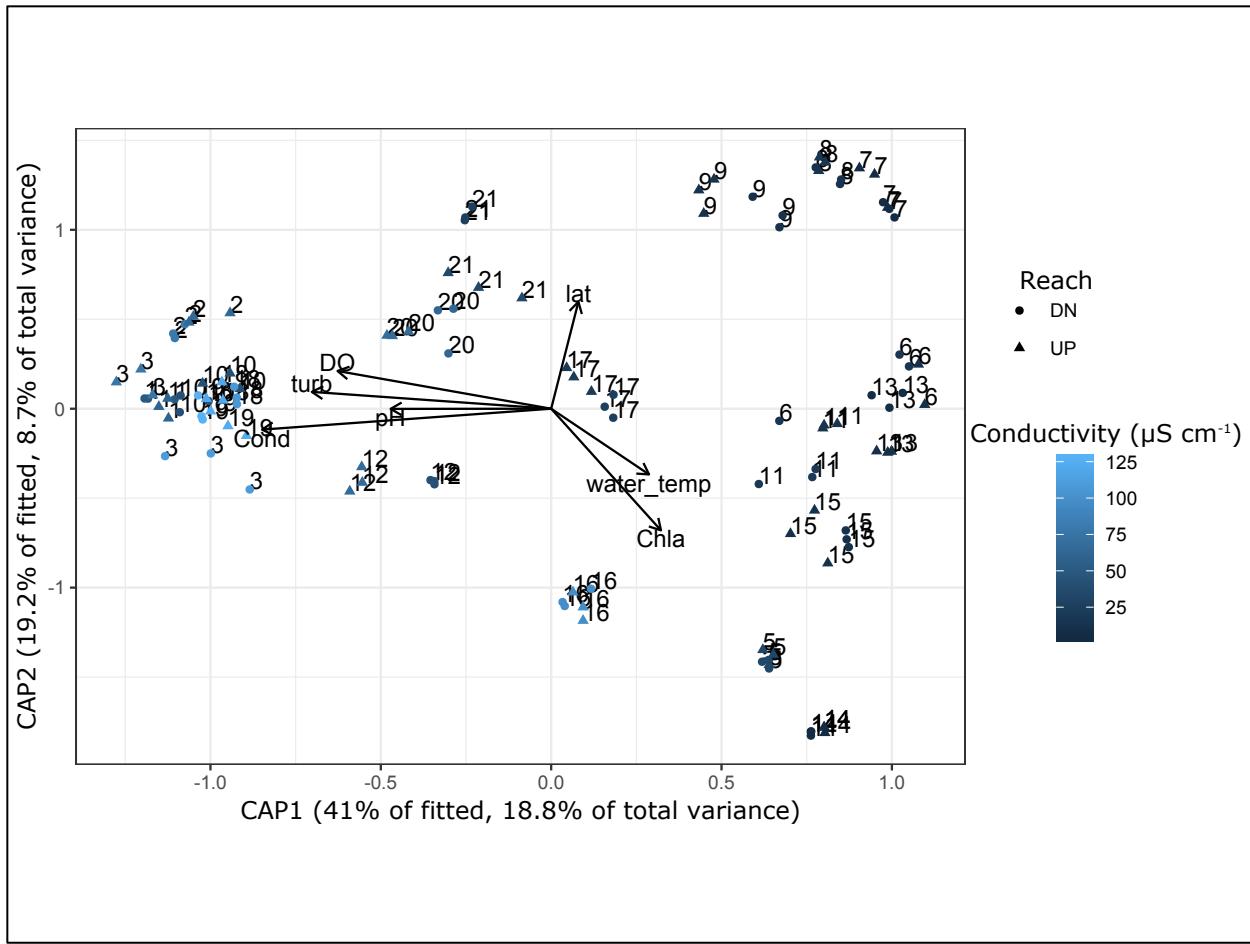


71
72 **Figure S2. Mantel correlogram between phylogenetic distances and niche distances among**
73 **all the Sequence Variants.** Filled squares correspond to significant correlations and empty
74 squares correspond to non-significant correlations. The phylogenetic distances were Hellinger-
75 transformed before the correlations. The niche distances were calculated as the Euclidean
76 distances of standardized niche optima regarding the environmental parameters that explained a
77 significant proportion of β -diversity as per the db-RDA analysis, namely conductivity, dissolved
78 oxygen, latitude, chl- α , pH, water temperature and turbidity.

79

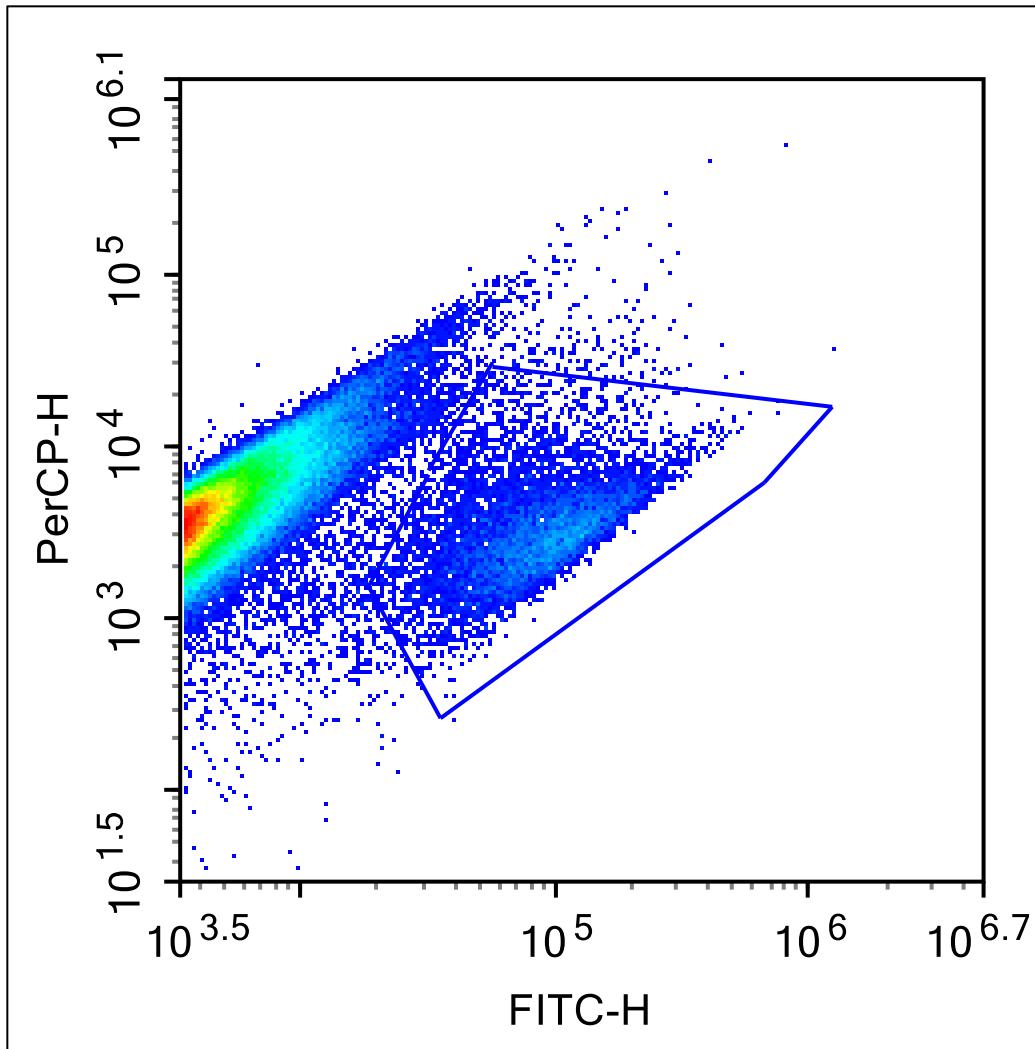


80 **Figure S3. The inverse relationship between the relative species richness (A) and the**
81 **relative abundance (B) of phylogenetic clades under environmental selection and total**
82 **bacterial cell density A. The relative species richness of the clades under environmental**
83 **selection (y-axis) as a function of total bacterial cell density (x-axis, in log-scale). B. The**
84 **relative abundance of the clades under environmental selection (y-axis) as a function of**
85 **total bacterial cell density (x-axis, in log-scale). Adjusted R²= 0.252, and 0.282, for panels A**
86 **and B, respectively. For both panels p<<0.001 and n=119.**



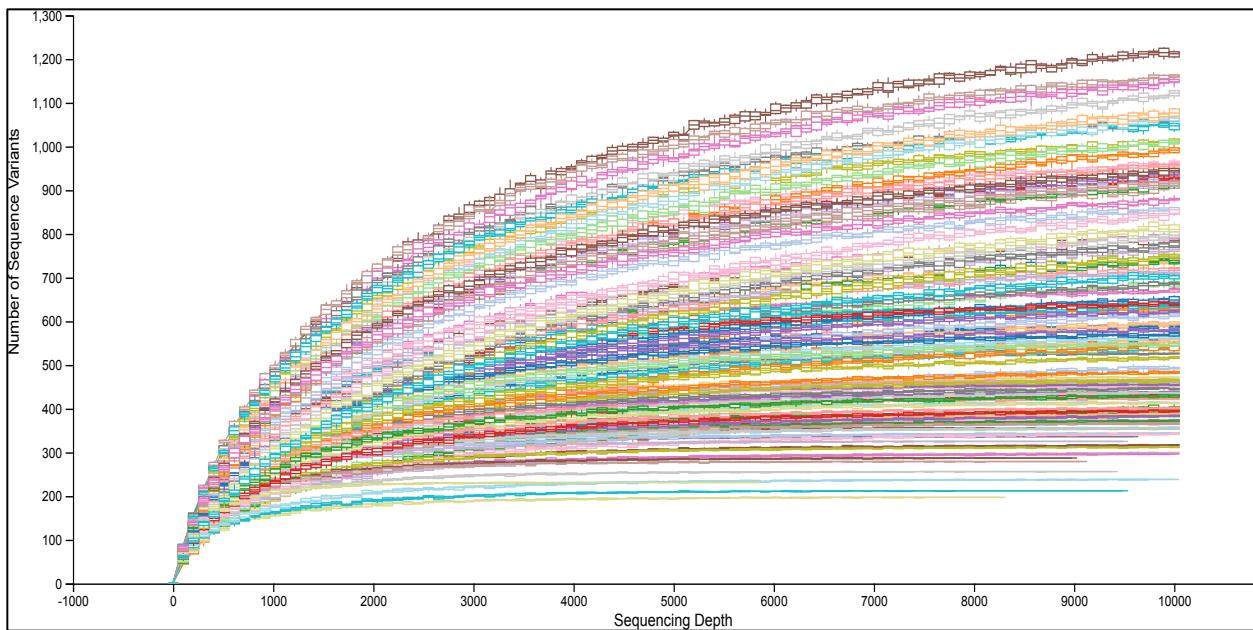
87

88 **Figure S4. The constrained ordination plot of the distance-based redundancy analysis on**
 89 **the BC dissimilarity of bacterial communities from all the sampled streams.** Factors
 90 included in the model are plotted as vectors. Samples are colored based on conductivity that was
 91 the factor explaining most of the BC variance, as per the legend on the middle right. Sample
 92 symbols are given according to the reach as per the legend on the upper right. Numbers above
 93 samples indicate the location of the stream as per Figure S1. Axes are scaled based on the
 94 variance they explain. Cond: conductivity, DO: dissolved oxygen, lat: latitude, Chla: chl- α
 95 content, water_temp: water temperature, turb: turbidity.

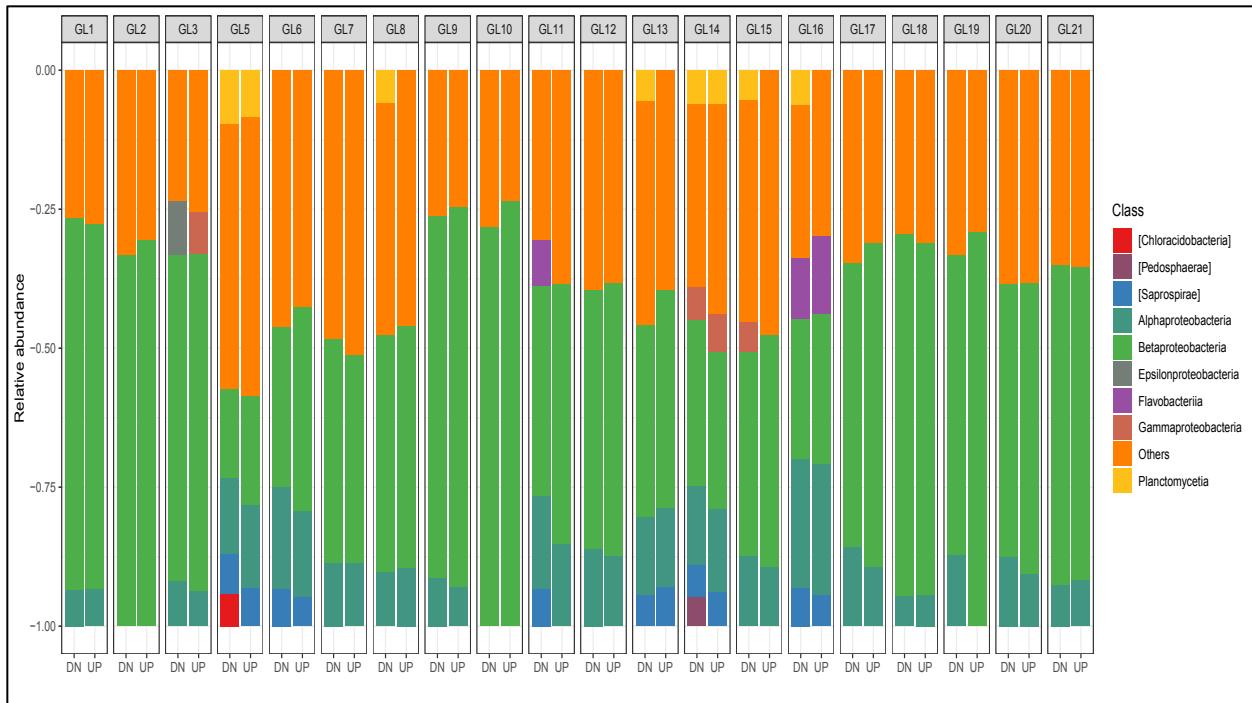


96

97 **Figure S5. Flow cytometry scatterplot of green (FITC-H – x-axis) versus red (PerCP-H – y-**
98 **axis) fluorescence of a typical sediment fixed extract.** The polygon delineates the gate used for
99 counting cells, which is based on events with disproportionately more green fluorescence
100 compared to red fluorescence because of SybrGreen staining. On the contrary, debris is shown
101 on the middle left as a dense cluster of events with higher red:green fluorescence ratio.



103 **Figure S6. The rarefaction curves of the generated 16S rRNA gene amplicons from all the**
104 **samples.** The number of sequences is shown on the x-axis and the number of observed sequence
105 variants (SVs) are shown on the y-axis. Rarefaction was performed at intervals of 100 sequences
106 with 10 permutations per sequence interval at a sequencing depth of up to 10,000 reads. The
107 range of the obtained values is represented with boxplots. Different colors represent different
108 samples.



109

110 **Figure S7. Taxonomical composition of the sampled communities at the Class level.** The
 111 sampled streams are ordered by ascending operational number. Each barplot corresponds to a
 112 different reach (UP or DN) within a given stream and represents the mean of three (or two in the
 113 case of GL6 – UP) within-reach samples. Classes with low mean relative abundances are
 114 grouped together in the “Others” category. Candidate classes are named in brackets.

115 **Supplementary Tables**

116

117 **Table S1.** The identified phylogenetic groups under homogeneous selection, i.e., with
 118 significantly lower total z-scores compared to outgroups. Group 1 corresponds to the
 119 phylogenetic group having different scores and Group 2 to the phylogenetic outgroup that the
 120 comparison is made against.

Factor	Group 1 Consensus Taxonomy	Number of SVs in Group 1	Group 2 Consensus Taxonomy	Number of SVs in Group 2	Contrast test p-value
1	Betaproteobacteria (Class)	1418	All the rest	6758	6.8E-255
2	Novosphingobium (Genus)	5	All present Bacteria except Betaproteobacteria	6753	8.5E-148
3	Nitrospira (Genus)	18	All present Bacteria except Betaproteobacteria	6731	3.4E-67
4	Alphaproteobacteria (Class)	602	All present Bacteria except Betaproteobacteria	6129	6.4E-70
5	[Saprospirae] (Candidate Class)	338	All present Bacteria except Alpha- and Betaproteobacteria	5791	3.3E-59
6	Methylotenera (Genus)	48	Betaproteobacteria	1359	2.3E-17
7	Comamonadaceae (Family)	575	Betaproteobacteria except Methylotenera	784	3.3E-16
8	Ellin606 (Uncultured Order)	54	Betaproteobacteria except Methylotenera and Comamonadaceae	730	2.5E-18

121

122

123 **Table S2. The core microbiome, i.e., the taxonomic units present in at least one sample at**
 124 **every reach, at different taxonomic levels.**

Core Level	Number of Taxa	Taxa names	Number of SVs within the taxa	Mean relative abundance (min-max)
Phylum	11	Proteobacteria, Nitrospirae, Bacteroidetes, Planctomycetes, Acidobacteria, Actinobacteria, OD1, Verrucomicrobia, Chloroflexi, Gemmatimonadetes, Cyanobacteria	7554	94.3% (78-97.1%)
Class	20	Chloracidobacteria, Acidobacteria-6, Solibacteres, Acidimicrobia, Actinobacteria, Thermophilia, Saprospirae, Cytophagia, Flavobacteriia, Sphingobacteriia, Chloroplast, Gemmatimonadetes, Nitrospira, ZB2, Planctomycetia, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Spartobacteria	6103	91% (77.1-96.3%)
Order	29	Rhizobiales, Sphingomonadales, Rhodobacterales, Caulobacterales, Pseudomonadales, MND1, Ellin6067, IS-44, KD8-87, Acidimicrobiales, Solibacterales, iii1-15, Gemmatales, Sphingobacteriales, RB41, Gaiellales, Chthoniobacterales, Burkholderiales, Methylophilales, Actinomycetales, Flavobacteriales, Cytophagales, Saprospirales, Planctomycetales, Pirellulales, Nitrospirales, Myxococcales, Xanthomonadales	4514	72.5% (30.4-91%)
Family	18	Hyphomicrobiaceae, Sphingomonadaceae, Caulobacteraceae, Chthoniobacteraceae, Saprospiraceae, Ellin6075, Gemmataceae, Sinobacteraceae, Oxalobacteraceae, Comamonadaceae, Methylophilaceae, Flavobacteriaceae, Cytophagaceae, Chitinophagaceae, Planctomycetaceae, Pirellulaceae, Nitrospiraceae, Xanthomonadaceae	2724	49.2% (19.8-72.2%)
Genus	12	Methylotenera, Polaromonas, Rhodoferax, Leptothrix, Methylibium, Rubrivivax, Novosphingobium, Hyphomicrobium, Nitrospira, Flavobacterium, Planctomyces, Gemmata	1133	34.8% (13.6-62%)

126 **Table S3. The properties of the detected genera across the whole dataset that had at least 2**
 127 **Sequence Variants (SVs) and that are formally assigned in the scientific nomenclature.**

	Number of SVs	Mean Nucleotidic Similarity (%) [#]	Mean Niche Breadth (B)	Mean relative abundance
Flavobacterium	201	93,02	3,91	2,95E-02
Planctomyces	153	86,46	4,18	1,00E-02
* Methylibium	130	96,59	4,62	1,58E-02
* Rhodoferax	112	96,66	4,93	2,87E-02
Gemmata	105	86,13	3,56	3,27E-03
* Leptothrix	98	96,45	4,98	2,46E-02
* Methylotenera	84	95,42	6,99	1,24E-01
Bdellovibrio	84	85,46	2,73	1,91E-03
* Hypomicrobium	82	95,16	5,24	1,27E-02
* Polaromonas	73	96,55	6,93	4,94E-02
* Rhodobacter	69	95,93	5,50	1,30E-02
* Thiobacillus	48	95,09	7,37	4,67E-02
* Rubrivivax	44	95,81	4,78	8,82E-03
DA101	44	93,90	3,33	2,14E-03
Hymenobacter	36	94,58	2,35	8,57E-04
* Novosphingobium	31	96,58	6,07	2,20E-02
* Janthinobacterium	26	97,37	2,15	7,56E-04
* Herminiimonas	24	96,12	3,11	1,85E-03
* Rhodoplanes	22	95,64	3,38	1,09E-03
* Kaistobacter	22	97,18	6,02	6,91E-03
* Sediminibacterium	20	94,51	5,79	2,86E-03
* Nitrospira	20	96,53	8,12	1,90E-02
Luteolibacter	20	97,16	2,81	1,09E-03
Opitutus	19	95,94	2,71	3,67E-04
Candidatus Solibacter	17	94,26	3,68	8,83E-04
Gemmatimonas	15	96,70	3,51	4,27E-04
CM44	15	94,13	3,71	6,40E-04
Deinococcus	15	91,93	2,55	2,87E-04
* Segetibacter	12	93,87	5,92	1,29E-03
A17	12	93,74	3,51	5,13E-04
Candidatus Koribacter	11	92,67	3,22	2,66E-04
Fimbrimonas	11	90,08	4,07	5,37E-04
* Phenylobacterium	10	95,78	4,50	9,00E-04
* Paucibacter	10	98,26	4,09	5,69E-04
Chthoniobacter	10	96,86	4,22	8,80E-04
* Devosia	9	96,47	3,18	3,35E-04
Geobacter	9	95,96	2,67	2,94E-04

Phormidium	8	96,55	2,91	2,52E-04
Pirellula	8	90,23	3,46	1,39E-04
*Gallionella	8	95,25	7,60	4,79E-03
Pseudomonas	8	97,96	3,03	4,78E-04
Lysobacter	8	96,40	3,90	5,53E-04
Rudanella	7	92,11	2,00	5,34E-05
*Flavisolibacter	7	94,70	2,95	1,35E-04
*Sphingomonas	7	96,06	3,68	3,95E-04
*Ramlibacter	7	95,45	3,11	1,97E-04
*Variovorax	7	96,45	4,30	9,50E-04
Leptospira	7	92,54	3,86	1,69E-04
Cytophaga	6	92,75	3,00	1,28E-04
Spirosoma	6	89,42	2,03	2,94E-05
Pseudanabaena	6	91,41	3,14	1,84E-04
*Hydrogenophaga	6	96,94	1,42	8,03E-04
*Dechloromonas	6	97,49	1,76	1,63E-04
Cellvibrio	6	95,96	3,31	1,28E-04
Aquicella	6	90,38	2,38	4,06E-05
Salinibacterium	5	98,16	3,78	3,15E-04
Fluviicola	5	92,65	2,53	3,60E-05
JG37-AG-70	5	98,23	3,27	1,62E-04
*Pedomicrobium	5	97,96	3,18	8,82E-05
*Zymomonas	5	98,33	2,53	6,96E-05
Sulfuricurvum	5	95,28	4,68	1,45E-02
Leadbetterella	4	97,68	4,71	3,51E-04
Sporocytophaga	4	91,96	1,92	2,14E-05
Leptolyngbya	4	88,99	2,09	6,27E-05
*Pelomonas	4	97,19	7,72	1,47E-03
*Sulfuritalea	4	96,45	3,71	7,73E-05
Crenothrix	4	94,48	4,33	7,69E-05
Bryobacter	3	97,51	8,08	3,67E-04
Armatimonas	3	99,34	5,38	7,13E-04
Paludibacter	3	96,68	2,63	5,25E-05
Emticicia	3	95,90	3,56	1,02E-04
Candidatus Protochlamydia	3	93,99	2,58	1,94E-05
Candidatus Rhabdochlamydia	3	88,29	1,93	5,18E-06
Gloeobacter	3	85,83	1,84	2,59E-05
Calothrix	3	96,86	2,11	6,80E-05
*Bosea	3	97,60	6,13	4,07E-04
*Labrys	3	97,18	2,25	1,22E-05
*Lutibacterium	3	96,93	2,36	4,99E-05
Smithella	3	96,50	3,12	9,75E-06
Arcobacter	3	98,84	1,67	7,38E-04

Gluconacetobacter	3	98,75	5,77	1,84E-04
Acinetobacter	3	96,34	4,30	9,39E-05
Steroidobacter	3	94,54	1,87	1,07E-05
Iamia	2	98,76	1,36	8,01E-06
Demequina	2	97,54	1,60	1,07E-05
Crocinitomix	2	97,87	5,78	5,96E-05
*Niabella	2	99,05	2,59	1,60E-05
Haliscomenobacter	2	99,29	1,46	1,33E-05
Dolichospermum	2	99,01	1,81	2,31E-05
Staphylococcus	2	98,83	1,83	3,85E-06
Clostridium	2	94,53	1,57	8,06E-06
BD2-6	2	90,65	2,35	8,13E-06
*Bradyrhizobium	2	99,75	4,24	1,86E-04
*Methylobacterium	2	96,77	3,70	4,83E-05
*Rubellimicrobium	2	92,79	2,32	9,49E-06
*Phaeospirillum	2	97,51	6,08	2,92E-05
*Sphingobium	2	96,02	1,57	4,32E-05
*Delftia	2	98,59	1,84	9,04E-06
Plesiocystis	2	99,76	2,87	1,25E-05
HTCC	2	99,53	1,51	2,57E-06
Thiofaba	2	98,13	2,37	6,81E-06
Legionella	2	96,49	2,18	1,00E-05
Alkanindiges	2	99,53	2,09	5,59E-05
Perlucidibaca	2	92,04	3,20	7,09E-06
Rhodanobacter	2	98,36	1,64	1,98E-05
Thermomonas	2	98,59	3,45	4,70E-05
Prosthecobacter	2	96,01	3,77	3,15E-05
Pedosphaera	2	93,90	2,03	1,48E-05
Candidatus Xiphinematobacter	2	90,62	2,34	4,17E-06
OR-59	2	81,67	3,41	8,81E-06

128
129
130

*Genus within phylogenetic clades under homogeneous selection [#]Based on the sequenced part of the 16S rRNA gene

131 **Table S4. The properties of the Sequence Variants with constantly low phylogenetic**
 132 **turnover (LPT-SVs).** LPT-SVs are grouped based on their consensus taxonomy and groups are
 133 sorted based on the percent of total score (column 3).

Consensus Taxonomy (Level)	Number of LPT-SVs	Percent of Total Score among LPT-SVs*	Mean Total Score per LPT-SV	Mean contributing community pairs per LPT-SV
*Nitrospira (genus)	16	13.1	-6481	1866
*Methylotenera (genus)	29	12	-3262	1352
*Polaromonas (genus)	20	11.3	-4455	1515
*Ellin6067 (order)	10	10.9	-8615	2147
*Leptothrix (genus)	19	8.8	-3647	1241
*Burkholderiales (order)	13	8.8	-5301	1412
*Novosphingobium (genus)	8	6.8	-6751	1310
Pirellulaceae (family)	3	4.6	-11992	2560
mb2424 (family)	6	2.7	-3562	1119
Sphingobacteriales (order)	3	2.7	-7071	1915
*Betaproteobacteria (class)	9	2.7	-2441	913
*Rhodoferax (genus)	5	2.3	-3601	994
*Thiobacillus (genus)	3	2	-5135	1231
Rhizobiales (order)	2	1.9	-7423	2319
Ellin6075 (family)	2	1.8	-7013	3482
Gaiellaceae (family)	1	1.4	-10893	2051
Xanthomonadaceae (family)	4	1.2	-2385	573
Chitinophagaceae (family)	2	0.8	-3619	1100
Chromatiales (order)	1	0.7	-5457	3074
*Rhodobacter (genus)	1	0.6	-4272	880
*Saprospiraceae (family)	1	0.5	-3849	2346
*MND1 (order)	2	0.4	-1573	971
*Methylibium (genus)	3	0.4	-983	492
*IS-44 (order)	3	0.3	-896	419
Flectobacillus (genus)	1	0.3	-2444	456
Planctomyces (genus)	1	0.2	-1621	348
Sulfuricurvum (genus)	1	0.2	-1592	342
Bacteroidetes (phylum)	1	0.2	-1261	566
*Methylophilaceae (family)	1	0.2	-1229	674
Gammaproteobacteria (class)	1	0.1	-1060	230
Total for *	143	80.5		

134 * Groups residing within phylogenetic clades under homogeneous selection

135

136

137 **Table S5. The measured geographical and physicochemical properties at each sampled
138 glacier-fed stream.**

Glacier	Reach	Altitude (m.a.s.l.)	Water T (°C)	DO (mg l ⁻¹)	pH	Potential (mV)	Conductivity (μS cm ⁻¹)	Turbidity (NTU)	Chl-α (μg g ⁻¹)
GL1	UP	354	2.4	13.3	7.81	-47.7	60.1	363	1.02E-04
GL1	DN	210	3.1	13.4	7.71	-42.9	49.4	347	9.93E-05
GL2	UP	1186	0	12.8	9.11	119.4	64	227	2.3E-04
GL2	DN	1100	1.23	12.75	8.32	n/d	78.8	164.33	2.42E-04
GL3	UP	266	0.33	15.38	8.83	-104	77.2	265.33	3.63E-04
GL3	DN	219	4.3	12.72	8.31	-77.3	109.2	352.67	6.84E-04
GL5	UP	1326	7.7	10.35	7.54	-34.1	32.4	0.05	1.58E-01
GL5	DN	1264	9.35	9.89	7.55	-34.9	32.5	0	2.58E-01
GL6	UP	1362	8.03	10.31	7.6	-39.3	9.2	7.82	1.04E-02
GL6	DN	1117	7.95	10.49	8.15	-72.8	15	0.92	2.95E-03
GL7	UP	1779	3	11.04	6.72	11.5	5.9	37.7	7.48E-04
GL7	DN	1707	3.58	10.98	6.84	5.5	6.3	32.33	7.7E-04
GL8	UP	1670	0.68	11.86	7.34	-22.4	14.9	12.57	6.18E-04
GL8	DN	1604	1.25	11.74	7.17	-12.7	14.2	11.1	7.37E-04
GL9	UP	1391	3.58	11.51	7.19	-12.8	12.5	23.07	5.14E-04
GL9	DN	1246	6.03	10.99	6.78	8.7	12.2	27.23	5.5E-04
GL10	UP	1111	0.65	13.16	8.1	-64	40.7	48.97	7.51E-04
GL10	DN	1077	0.7	12.92	7.43	-27.2	40.8	98.57	8.14E-04
GL11	UP	1581	2.93	11.14	10.18	-180	14.5	4.12	1.1E-02
GL11	DN	1474	5.38	10.65	10.18	-181.6	19	1.92	2.67E-02
GL12	UP	756	6.6	11.35	9.83	-163.1	66.7	9.88	3.33E-03
GL12	DN	714	7	11.39	9.83	-163.1	41.7	8.22	3.4E-03
GL13	UP	1720	0.73	11.58	6.76	15.2	9.25	4.95	1.78E-02
GL13	DN	1662	0.85	11.93	6.7	19	6.9	4.53	8.84E-03
GL14	UP	1079	3.38	11.81	6.41	34.3	4.5	0	1.151E-01
GL14	DN	1056	4.85	11.37	6.23	44.5	4.5	0	7.17E-02
GL15	UP	1281	1.4	11.94	6.47	30.9	10.4	0.81	3.85E-03
GL15	DN	1245	1.6	12.04	6.6	25.8	10.5	1.24	3.63E-02

GL16	UP	1342	5.65	10.63	7.82	-43	95.8	0.66	5.83E-02
GL16	DN	1209	4.93	11.03	7.8	-41.2	97.5	0.43	4.72E-02
GL17	UP	1229	5.9	10.71	7.47	-23.9	21.2	22.97	3.27E-03
GL17	DN	1007	6.9	10.43	7.6	-31.4	26	16.73	2.83E-03
GL18	UP	1204	4.15	11.35	7.98	-51.2	119	2.15	6.63E-04
GL18	DN	1117	5.53	11.04	8	-52.7	106.3	11.17	5.35E-04
GL19	UP	1177	0.13	11.23	8.69	-88.5	122.4	232.67	3.2E-04
GL19	DN	1094	1.8	11.25	8.29	-68.3	126.5	65.53	4.2E-04
GL20	UP	1475	2.63	11.8	7.83	-42.9	68.4	1.46	5.76E-04
GL20	DN	990	5.28	11.43	7.81	-41.2	62.6	2.85	9.84E-04
GL21	UP	1084	3.4	11.99	7.59	-29.9	33.7	2.3	2.44E-03
GL21	DN	1014	2.68	12.25	7.54	-26.9	25.4	4.7	6.8E-04

139

m.a.s.l.: meters above sea level, DO: Dissolved Oxygen, NTU: Nephelometric Turbidity Units, n/d: non-determined

140 **Table S6. The summary of the step-wise model building for the distance-based redundancy**
141 **analysis.** The “+” sign before each variable indicates its step-wise addition to the model formula.

	Cumulative Adjusted R ² (%)	Df	AIC	F	p
Conductivity	14.14	1	414.19	20.45	0.002
+ Dissolved Oxygen	22.25	1	403.37	13.2	0.002
+ Latitude	27.99	1	395.21	10.24	0.002
+ Chl-α	33.61	1	386.5	10.74	0.002
+ pH	37.88	1	380.5	7.86	0.002
+ Water T	40.21	1	375.93	6.35	0.002
+ Turbidity	42.15	1	372.94	4.76	0.002
All variables	43.54				

142 Df: Degrees of freedom, AIC: Akaike Information Criterion