

Supplementary material

1. Supplementary figures

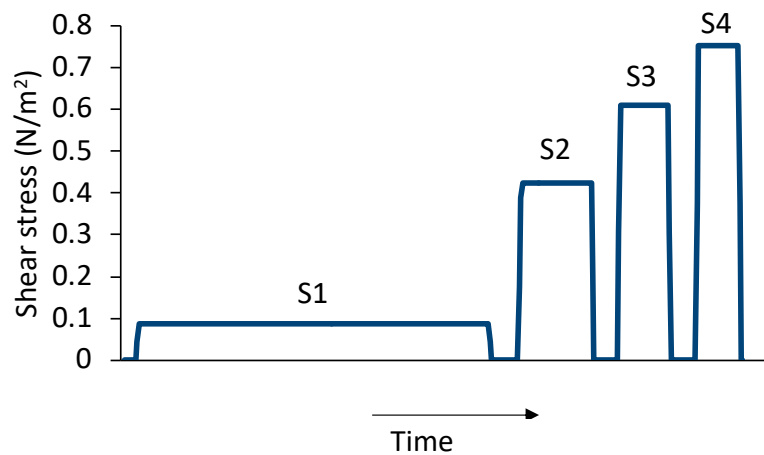


Figure A. Flushing gradual steps (S) that were applied for a duration of 3 turnovers (i.e., the time that the total volume of the water needs for recirculate in the loop 3 times to mix all material mobilised into the water).

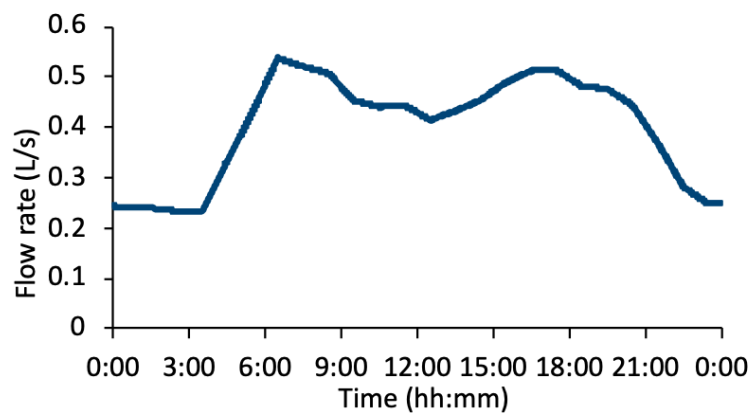


Figure B. Low varied flow profile used during the growth phases (modified from Husband, Boxall and Saul, 2008).

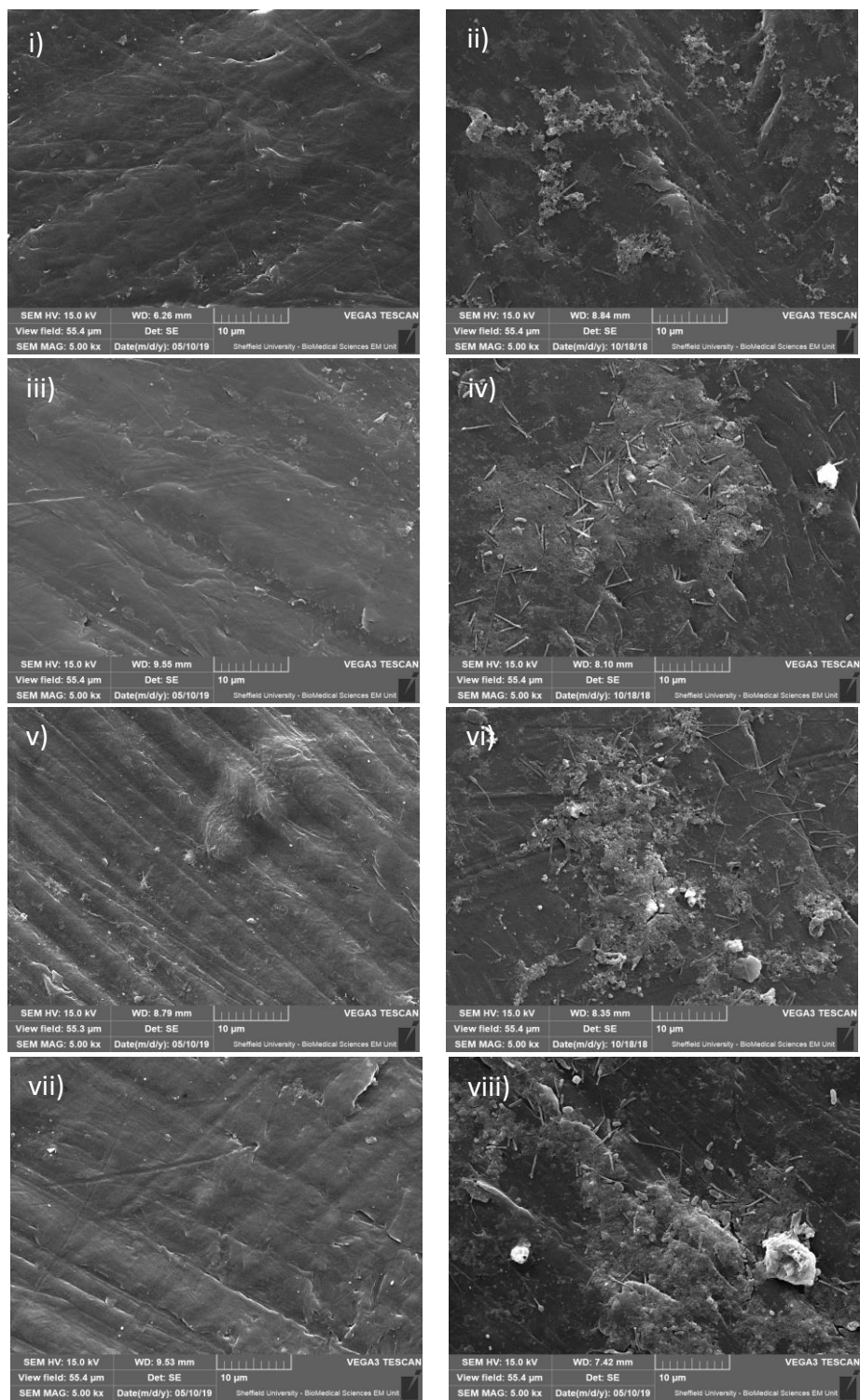


Figure C. SEM micrographs of developed biofilm in modified PWG coupons at 16°C on day 0 (i) and on day 30 (ii); at 24°C after flushing-hyperchlorination on day 0 (iii) and on day 30 (iv); at 24°C after only flushing on day

0 (v) and on day 30 (vi); and at 24°C after transition on day 0 (vii) and on day 30 (viii). MAG=5.00 kx in all micrographs.

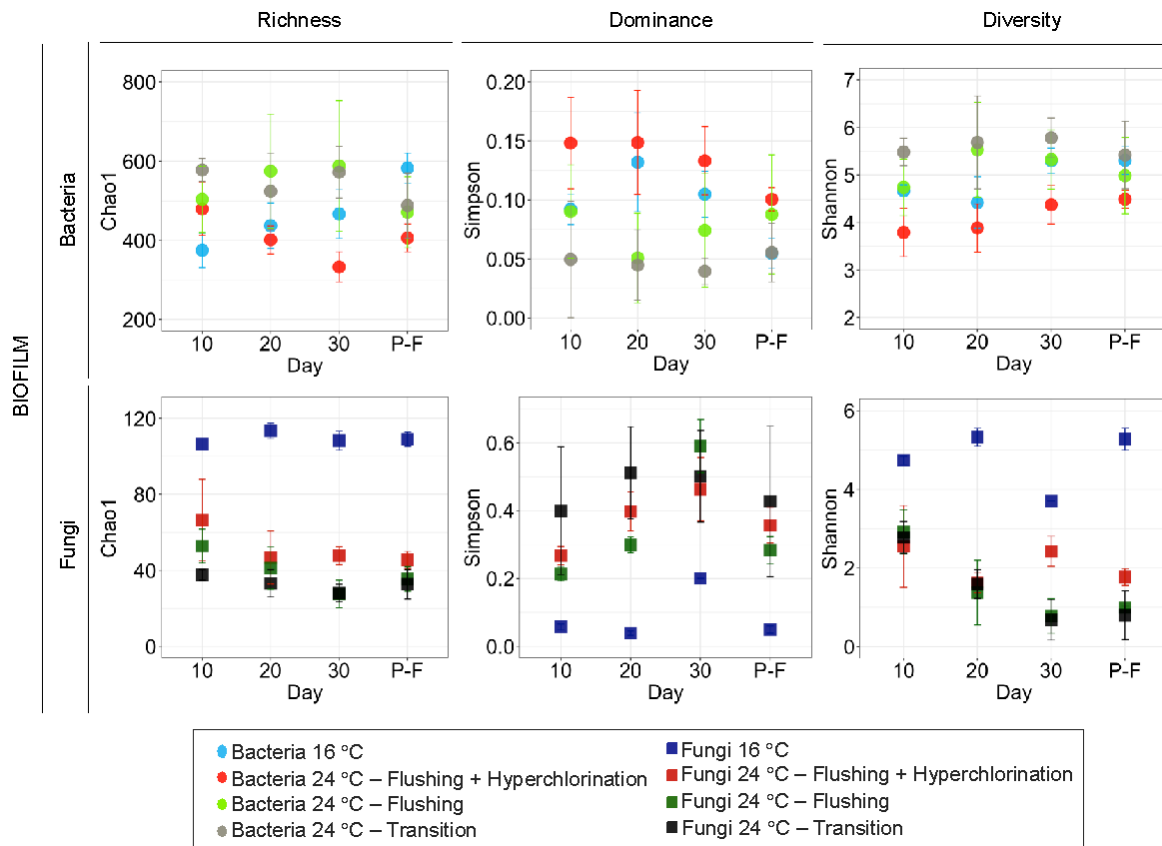


Figure D. Chao 1 (richness), Simpson (dominance) and Shannon (diversity) indices for OTUs at 97 % cut off for bacteria and fungi in biofilm samples calculated for each sampling day during the growth phases and post-flushing events (P-F). All values represent an average (n=3) and errors bars are $\pm$ standard deviation.

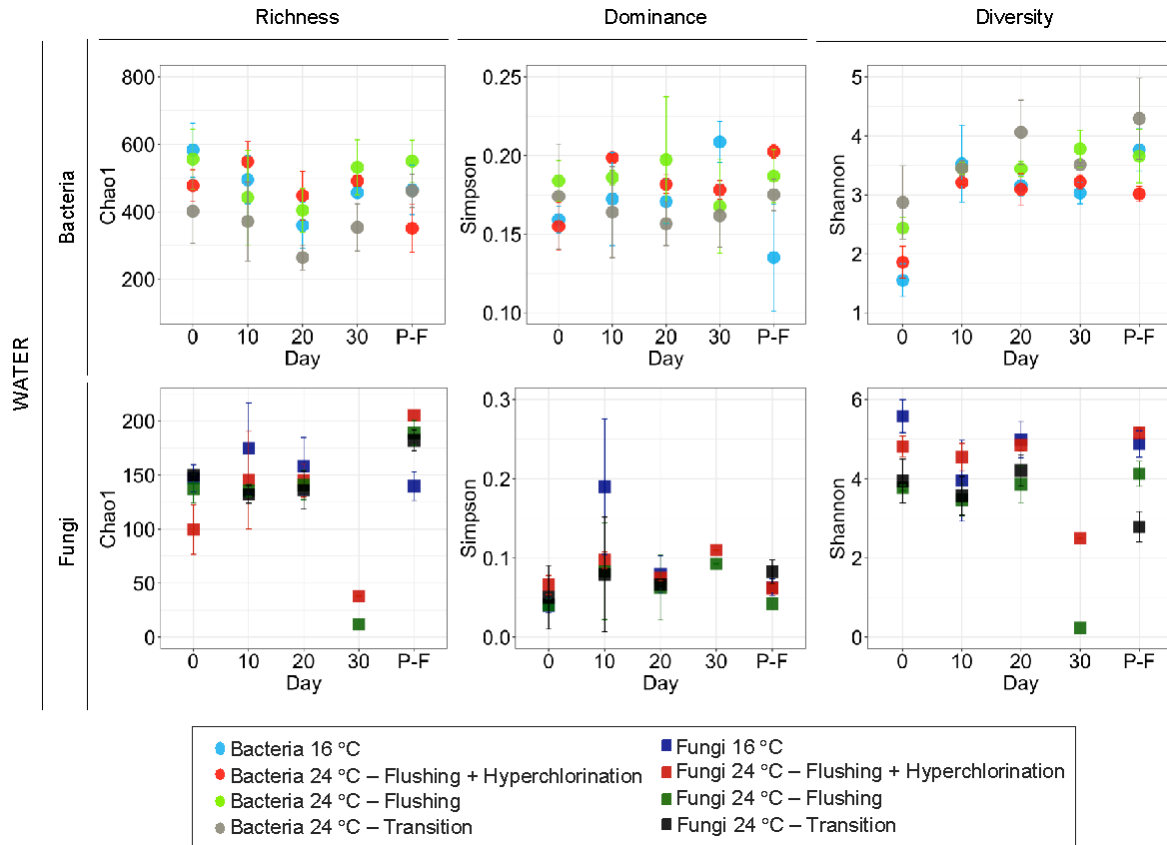


Figure E. Chao 1 (richness), Simpson (dominance) and Shannon (diversity) indices for OTUs at 97 % similarity cut off for bacteria and fungi in water samples calculated for each sampling day during the growth phases and post-flushing events (P-F). All values represent an average ($n=3$) $\pm$ standard deviation, except for fungi on day 30 where just 1 replicate amplified at 24°C after flushing-hyperchlorination and at 24°C after flushing. Neither replicate at 16°C and at 24°C transition amplified; thus, statistics could not be performed.

2. Supplementary Tables

Supplementary Table A. Flow rate conversions applied during the growth phases and in each flushing stage.

	Flow (Q) - (L/s)	Velocity (v) - (m/s)	Shear stress (τ) - (N/m ²)
Growth phase	0.2 - 0.5	0.04 – 0.10	0.1 – 0.3
Step 1 Flushing	0.74	0.15	0.4
Step 2 Flushing	3.58	0.72	2.3
Step 3 Flushing	5.10	1.03	3.4
Step 4 Flushing	6.24	1.27	4.3

Supplementary Table B. Sequence count during each step of bioinformatic analysis of the of the bacterial 16S rRNA gene in all biofilm and water samples at 16 °C and 24°C after different management strategies. (-) Samples that did not amplify or were excluded during 845 the analysis for not having enough readings.

Sample ID	Raw reads	Filtered and imported to QIIME2	After join pair-ends	After dereplication	After clustering (97%)	After chimera filtering	Rarefied	Total OTUs observed
B 16 °C D10 R1	90654	57811	25498	25498	25498	23371	8131	307
B 16 °C D10 R2	86375	53532	26774	26774	26774	24408	8131	191
B 16 °C D10 R3	58467	25624	10383	10383	10383	9802	8131	305
B 24 °C D10 L1 R1	119559	86716	40053	40053	40053	36170	8131	215
B 24 °C D10 L1 R2	100450	67607	32266	32266	32266	28722	8131	248
B 24 °C D10 L1 R3	143669	110826	48742	48742	48742	45693	8131	143
B 24 °C D10 L2 R1	124924	92259	37141	37141	37141	33174	8131	314
B 24 °C D10 L2 R2	100549	67884	27233	27233	27233	24201	8131	286
B 24 °C D10 L2 R3	129204	96539	45850	45850	45850	42798	8131	214
B 24 °C D10 L3 R1	90605	57940	22720	22720	22720	19944	8131	264
B 24 °C D10 L3 R2	85950	53285	22002	22002	22002	18818	8131	307
B 24 °C D10 L3 R3	99470	66805	23378	23378	23378	19785	8131	316
B 16 °C D20 R1	63306	30641	12533	12533	12533	11310	8131	143
B 16 °C D20 R2	100292	67627	25541	25541	25541	23960	8131	317
B 16 °C D20 R3	102204	69539	29148	29148	29148	25206	8131	343
B 24 °C D20 L1 R1	92378	59713	21657	21657	21657	20828	8131	110
B 24 °C D20 L1 R2	148416	115751	47890	47890	47890	44287	8131	187
B 24 °C D20 L1 R3	123752	91087	39391	39391	39391	34614	8131	217
B 24 °C D20 L2 R1	127732	95067	36055	36055	36055	32081	8131	239
B 24 °C D20 L2 R2	95963	63298	26382	26382	26382	23574	8131	419

B 24 °C D20 L2 R3	86587	53922	20812	20812	20812	18541	8131	320
B 24 °C D20 L3 R1	80386	47721	17108	17108	17108	14972	8131	306
B 24 °C D20 L3 R2	101143	68478	24514	24514	24514	21168	8131	342
B 24 °C D20 L3 R3	69182	36517	11923	11923	11923	10523	8131	284
B 16 °C D30 R1	98498	68448	24660	24660	24660	22790	8131	371
B 16 °C D30 R2	103155	73105	21179	21179	21179	19364	8131	265
B 16 °C D30 R3	83209	53159	21081	21081	21081	18530	8131	250
B 24 °C D30 L1 R1	63053	33003	12601	12601	12601	11970	8131	243
B 24 °C D30 L1 R2	106780	76730	26345	26345	26345	23942	8131	194
B 24 °C D30 L1 R3	74908	44858	13987	13987	13987	12423	8131	180
B 24 °C D30 L2 R1	84378	54328	17275	17275	17275	15507	8131	275
B 24 °C D30 L2 R2	60940	30890	9532	9532	9532	8674	8131	298
B 24 °C D30 L2 R3	80726	50676	16150	16150	16150	14344	8131	398
B 24 °C D30 L3 R1	70504	40454	11868	11868	11868	10170	8131	365
B 24 °C D30 L3 R2	69593	39543	11904	11904	11904	9682	8131	307
B 24 °C D30 L3 R3	62018	31968	9149	9149	9149	8131	8131	298
B 16 °C AF R1	101126	68461	33367	33367	33367	31079	8131	297
B 16 °C AF R2	63313	30648	15250	15250	15250	14338	8131	331
B 16 °C AF R3	86112	53447	24243	24243	24243	21691	8131	322
B 24 °C AF L1 R1	102228	69563	28974	28974	28974	25892	8131	188
B 24 °C AF L1 R2	98473	65808	28939	28939	28939	25570	8131	211
B 24 °C AF L1 R3	82766	50101	22055	22055	22055	19342	8131	207
B 24 °C AF L2 R1	96640	63975	26805	26805	26805	24010	8131	327
B 24 °C AF L2 R2	120743	88078	31388	31388	31388	29115	8131	219
B 24 °C AF L2 R3	109367	76702	28270	28270	28270	25590	8131	341

B 24 °C AF L3 R1	95399	62734	23482	23482	23482	20532	8131	250
B 24 °C AF L3 R2	87682	55017	20378	20378	20378	17378	8131	296
B 24 °C AF L3 R3	105268	72603	26946	26946	26946	22934	8131	373
W 16 °C D0 R1	103546	70881	46666	46666	46666	42013	8131	737
W 16 °C D0 R2	108289	75624	40007	40007	40007	36130	8131	708
W 16 °C D0 R3	110607	77942	41583	41583	41583	37358	8131	568
W 24 °C D0 L1 R1	92612	59947	41714	41714	41714	36998	8131	185
W 24 °C D0 L1 R2	97295	64630	44141	44141	44141	39859	8131	203
W 24 °C D0 L1 R3	113712	81047	54482	54482	54482	49800	8131	219
W 24 °C D0 L2 R1	94594	61929	40985	40985	40985	36451	8131	247
W 24 °C D0 L2 R2	94968	62303	41537	41537	41537	36007	8131	229
W 24 °C D0 L2 R3	95757	63092	42372	42372	42372	37420	8131	227
W 24 °C D0 L3 R1	98070	65405	39819	39819	39819	35052	8131	160
W 24 °C D0 L3 R2	106337	73672	44189	44189	44189	39325	8131	175
W 24 °C D0 L3 R3	89698	57033	34437	34437	34437	30619	8131	204
W 16 °C D10 R1	90973	58130	30835	30835	30835	28998	8131	169
W 16 °C D10 R2	87096	54253	26617	26617	26617	24211	8131	244
W 16 °C D10 R3	88686	55843	25465	25465	25465	21380	8131	252
W 24 °C D10 L1 R1	103164	70321	45353	45353	45353	44438	8131	222
W 24 °C D10 L1 R2	111030	78187	49330	49330	49330	48216	8131	243
W 24 °C D10 L1 R3	99111	66268	42349	42349	42349	41438	8131	277
W 24 °C D10 L2 R1	110704	77861	47037	47037	47037	44143	8131	174
W 24 °C D10 L2 R2	88890	56047	34146	34146	34146	32222	8131	202
W 24 °C D10 L2 R3	75001	42158	25778	25778	25778	24445	8131	217
W 24 °C D10 L3 R1	95868	63025	35803	35803	35803	32566	8131	139

W 24 °C D10 L3 R2	106418	73575	41720	41720	41720	37462	8131	175
W 24 °C D10 L3 R3	110580	77737	43984	43984	43984	39505	8131	208
W 16 °C D20 R1	145135	112292	51047	51047	51047	47628	8131	145
W 16 °C D20 R2	116166	83323	39837	39837	39837	36159	8131	145
W 16 °C D20 R3	240753	207910	65752	65752	65752	61400	8131	134
W 24 °C D20 L1 R1	90042	57199	37592	37592	37592	35309	8131	203
W 24 °C D20 L1 R2	103226	70383	46158	46158	46158	43292	8131	238
W 24 °C D20 L1 R3	91276	58433	38999	38999	38999	35709	8131	163
W 24 °C D20 L2 R1	114441	81598	50714	50714	50714	45087	8131	203
W 24 °C D20 L2 R2	131510	98667	59363	59363	59363	52245	8131	194
W 24 °C D20 L2 R3	102214	69371	43629	43629	43629	38293	8131	215
W 24 °C D20 L3 R1	78295	45452	28202	28202	28202	26932	8131	146
W 24 °C D20 L3 R2	141037	108194	67274	67274	67274	62095	8131	144
W 24 °C D20 L3 R3	122985	90142	55596	55596	55596	51139	8131	152
W 16 °C D30 R1	148856	118806	54499	54499	54499	50216	8131	147
W 16 °C D30 R2	123441	93391	42593	42593	42593	39600	8131	155
W 16 °C D30 R3	104777	74727	34978	34978	34978	32019	8131	135
W 24 °C D30 L1 R1	89902	59852	38456	38456	38456	35505	8131	195
W 24 °C D30 L1 R2	99843	69793	48328	48328	48328	43479	8131	176
W 24 °C D30 L1 R3	79748	49698	33604	33604	33604	30700	8131	193
W 24 °C D30 L2 R1	100970	70920	48381	48381	48381	44509	8131	234
W 24 °C D30 L2 R2	62436	32386	21738	21738	21738	20200	8131	240
W 24 °C D30 L2 R3	80646	50596	34072	34072	34072	31400	8131	236
W 24 °C D30 L3 R1	95746	65696	45165	45165	45165	42522	8131	100
W 24 °C D30 L3 R2	103186	73136	50309	50309	50309	46714	8131	109

W 24 °C D30 L3 R3	83032	52982	36539	36539	36539	34426	8131	120
W 16 °C AF R1	121214	91164	46360	46360	46360	43527	8131	140
W 16 °C AF R2	111750	81700	37497	37497	37497	34811	8131	190
W 16 °C AF R3	125104	95054	43827	43827	43827	41546	8131	188
W 24 °C AF L1 R1	94876	64826	37638	37638	37638	35179	8131	147
W 24 °C AF L1 R2	104108	74058	43199	43199	43199	40471	8131	136
W 24 °C AF L1 R3	100457	70407	41122	41122	41122	38459	8131	124
W 24 °C AF L2 R1	92766	62716	37646	37646	37646	35381	8131	280
W 24 °C AF L2 R2	97763	67713	39932	39932	39932	36972	8131	292
W 24 °C AF L2 R3	93850	63800	38314	38314	38314	35504	8131	267
W 24 °C AF L3 R1	90917	60867	39008	39008	39008	38085	8131	198
W 24 °C AF L3 R2	114275	84225	54761	54761	54761	52899	8131	208
W 24 °C AF L3 R3	126626	96576	63074	63074	63074	60683	8131	188

Supplementary Table C. Sequence count during each step of bioinformatic analysis of the fungal ITS1-2 region in all biofilm and water samples at 16°C and 24°C after different management strategies. (-) Samples that did not amplify or were excluded during the analysis for not having enough readings.

Sample ID	Raw reads	Filtered and imported to QIIME2	After join pair-ends	After dereplication	After clustering (97%)	After chimera filtering	Rarefied	Total OTUs observed
B 16 °C D10 R1	33476	20363	10160	10160	10160	10138	1011	64
B 16 °C D10 R2	35243	22130	11015	11015	11015	11006	1011	46
B 16 °C D10 R3	51327	38214	19107	19107	19107	19017	1011	66
B 24 °C D10 L1 R1	36289	18140	9140	9140	9140	9123	1011	47
B 24 °C D10 L1 R2	66985	64876	31472	31472	31472	31096	1011	20
B 24 °C D10 L1 R3	39603	26490	13204	13204	13204	12701	1011	48
B 24 °C D10 L2 R1	99098	79823	38458	38458	38458	38024	1011	40
B 24 °C D10 L2 R2	78109	58834	27968	27968	27968	27818	1011	19
B 24 °C D10 L2 R3	117500	98225	47211	47211	47211	47760	1011	91
B 24 °C D10 L3 R1	72100	52825	26072	26072	26072	26892	1011	20
B 24 °C D10 L3 R2	75271	55996	25148	25148	25148	24157	1011	18
B 24 °C D10 L3 R3	113864	94589	43040	43040	43040	42544	1011	24
B 16 °C D20 R1	119118	99843	49921	49921	49921	49123	1011	92
B 16 °C D20 R2	83599	64324	32110	32110	32110	32030	1011	90
B 16 °C D20 R3	-	-	-	-	-	-	-	-
B 24 °C D20 L1 R1	82637	63362	31446	31446	31446	31276	1011	23
B 24 °C D20 L1 R2	99175	79900	39526	39526	39526	39226	1011	29
B 24 °C D20 L1 R3	74288	55013	27236	27236	27236	27123	1011	13
B 24 °C D20 L2 R1	100536	81261	40024	40024	40024	39754	1011	21
B 24 °C D20 L2 R2	85192	65917	32671	32671	32671	32304	1011	62

B 24 °C D20 L2 R3	111357	92082	45785	45785	45785	45259	1011	82
B 24 °C D20 L3 R1	64244	44969	22248	22248	22248	21622	1011	23
B 24 °C D20 L3 R2	127660	108385	54912	54912	54912	54434	1011	41
B 24 °C D20 L3 R3	65347	46072	22389	22389	22389	22772	1011	28
B 16 °C D30 R1	72130	50525	1195	1195	1195	1011	1011	73
B 16 °C D30 R2	48577	26972	482	482	482	417	-	-
B 16 °C D30 R3	50784	29179	492	492	492	435	-	-
B 24 °C D30 L1 R1	155620	134015	6258	6257	6257	5969	1011	37
B 24 °C D30 L1 R2	224547	202942	4016	4016	4016	3609	1011	29
B 24 °C D30 L1 R3	54948	33343	649	649	649	562	-	-
B 24 °C D30 L2 R1	181244	159639	3248	3248	3248	2995	1011	31
B 24 °C D30 L2 R2	167160	145555	9662	9662	9662	9382	1011	24
B 24 °C D30 L2 R3	130964	109359	2204	2204	2204	1901	1011	47
B 24 °C D30 L3 R1	147988	126383	5695	5695	5695	5411	1011	42
B 24 °C D30 L3 R2	150688	129083	10863	10863	10863	10595	1011	39
B 24 °C D30 L3 R3	149519	127914	2890	2890	2890	2753	1011	40
B 16 °C AF R1	127405	108130	51574	51574	51574	51299	1011	112
B 16 °C AF R2	71460	52185	25049	25049	25049	25183	1011	81
B 16 °C AF R3	107740	88465	43141	43141	43141	43983	1011	114
B 24 °C AF L1 R1	217800	198525	93673	93673	93673	92710	1011	21
B 24 °C AF L1 R2	72890	53615	26802	26802	26802	26673	1011	21
B 24 °C AF L1 R3	184030	164755	80930	80930	80930	80187	1011	23
B 24 °C AF L2 R1	396309	377034	187496	187496	187496	186916	1011	19
B 24 °C AF L2 R2	74979	55704	27818	27818	27818	27300	1011	22
B 24 °C AF L2 R3	89400	70125	33755	33755	33755	33587	1011	43

B 24 °C AF L3 R1	72332	53057	25334	25334	25334	25191	1011	18
B 24 °C AF L3 R2	93903	74628	36357	36357	36357	36110	1011	26
B 24 °C AF L3 R3	86073	66798	32528	32528	32528	32238	1011	54
W 16 °C D0 R1	121078	101803	47311	47311	47311	47170	1011	131
W 16 °C D0 R2	88977	69702	33967	33967	33967	33741	1011	100
W 16 °C D0 R3	51542	32267	15470	15470	15470	15431	1011	96
W 24 °C D0 L1 R1	105273	85998	41474	41474	41474	41310	1011	96
W 24 °C D0 L1 R2	141050	121775	58559	58559	58559	58205	1011	68
W 24 °C D0 L1 R3	119732	100457	49244	49244	49244	49111	1011	78
W 24 °C D0 L2 R1	235055	215780	102713	102713	102713	102052	1011	102
W 24 °C D0 L2 R2	147900	128625	62423	62423	62423	62165	1011	115
W 24 °C D0 L2 R3	148739	129464	73611	73611	73611	72076	1011	121
W 24 °C D0 L3 R1	142316	123041	58323	58323	58323	58018	1011	102
W 24 °C D0 L3 R2	183135	163860	79156	79156	79156	78588	1011	102
W 24 °C D0 L3 R3	196381	177106	85187	85187	85187	84750	1011	87
W 16 °C D10 R1	92193	79080	38360	38360	38360	37941	1011	170
W 16 °C D10 R2	250662	237549	117611	117611	117611	116806	1011	72
W 16 °C D10 R3	157941	144828	70127	70127	70127	69873	1011	57
W 24 °C D10 L1 R1	63939	50826	24615	24615	24615	24562	1011	45
W 24 °C D10 L1 R2	138901	125788	59520	59520	59520	58706	1011	111
W 24 °C D10 L1 R3	116915	103802	49168	49168	49168	48694	1011	137
W 24 °C D10 L2 R1	91892	78779	37555	37555	37555	37279	1011	86
W 24 °C D10 L2 R2	103522	90409	42357	42357	42357	42039	1011	101
W 24 °C D10 L2 R3	87941	74828	35231	35231	35231	34933	1011	86
W 24 °C D10 L3 R1	72392	59279	28626	28626	28626	28313	1011	91

W 24 °C D10 L3 R2	83486	70373	33503	33503	33503	33265	1011	83
W 24 °C D10 L3 R3	94444	81331	38335	38335	38335	38083	1011	101
W 16 °C D20 R1	69449	56336	27550	27550	27550	27054	1011	118
W 16 °C D20 R2	66311	53198	25566	25566	25566	25247	1011	98
W 16 °C D20 R3	52672	39559	17196	17196	17196	16779	1011	124
W 24 °C D20 L1 R1	70515	57402	27734	27734	27734	27409	1011	117
W 24 °C D20 L1 R2	69113	56000	26014	26014	26014	25852	1011	81
W 24 °C D20 L1 R3	90444	77331	36033	36033	36033	35403	1011	112
W 24 °C D20 L2 R1	69267	56154	26033	26033	26033	26638	1011	106
W 24 °C D20 L2 R2	108579	95466	45785	45785	45785	44527	1011	125
W 24 °C D20 L2 R3	89301	76188	36343	36343	36343	35497	1011	111
W 24 °C D20 L3 R1	141292	128179	62335	62335	62335	62091	1011	68
W 24 °C D20 L3 R2	156037	142924	69895	69895	69895	69526	1011	62
W 24 °C D20 L3 R3	181004	167891	82212	82212	82212	81967	1011	48
W 16 °C D30 R1	-	-	-	-	-	-	-	-
W 16 °C D30 R2	-	-	-	-	-	-	-	-
W 16 °C D30 R3	-	-	-	-	-	-	-	-
W 24 °C D30 L1 R1	-	-	-	-	-	-	-	-
W 24 °C D30 L1 R2	-	-	-	-	-	-	-	-
W 24 °C D30 L1 R3	96667	75062	14351	14351	14351	13356	1011	38
W 24 °C D30 L2 R1	-	-	-	-	-	-	-	-
W 24 °C D30 L2 R2	79534	57929	6615	6615	6615	6263	1011	41
W 24 °C D30 L2 R3	-	-	-	-	-	-	-	-
W 24 °C D30 L3 R1	-	-	-	-	-	-	-	-
W 24 °C D30 L3 R2	-	-	-	-	-	-	-	-

W 24 °C D30 L3 R3	-	-	-	-	-	-	-	-
W 16 °C AF R1	81171	68058	32326	32326	32326	32079	1011	116
W 16 °C AF R2	174353	161240	76121	76121	76121	75648	1011	81
W 16 °C AF R3	-	-	-	-	-	-	-	-
W 24 °C AF L1 R1	51868	38755	18248	18248	18248	18080	1011	147
W 24 °C AF L1 R2	55335	42222	19283	19283	19283	19120	1011	116
W 24 °C AF L1 R3	39451	26338	12320	12320	12320	12183	1011	105
W 24 °C AF L2 R1	78879	65766	30726	30726	30726	30551	1011	101
W 24 °C AF L2 R2	83440	70327	33311	33311	33311	33043	1011	115
W 24 °C AF L2 R3	104023	90910	38559	38559	38559	38211	1011	107
W 24 °C AF L3 R1	145434	132321	63883	63883	63883	63637	1011	52
W 24 °C AF L3 R2	165247	152134	73076	73076	73076	72783	1011	77
W 24 °C AF L3 R3	148222	135109	65617	65617	65617	65327	1011	70

Supplementary Table D. Water physico-chemical parameters measured on all sampling points. 16°C values are the average $\pm$ standard deviation of the 3 replicates of the 3 loops during the growth phase (n = 9), and of the loops 1 and 2 post-flushing (n = 6, loop 3 was not flushed). The rest of values represent the average of 3 water replicates analysis $\pm$ standard deviation.

	Sampling day	Temperature (°C)	pH	Total Cl (mg/L)	Free Cl (mg/L)	TOC (mg/L)	Fe (µg/L)	Mn (µg/L)
16 °C	0	16.0 $\pm$ 0.05	7.29 $\pm$ 0.08	0.91 $\pm$ 0.06	0.89 $\pm$ 0.05	1.16 $\pm$ 0.03	44.6 $\pm$ 0.64	0.39 $\pm$ 0.03
	10	15.7 $\pm$ 0.07	7.11 $\pm$ 0.09	0.17 $\pm$ 0.03	0.09 $\pm$ 0.01	1.20 $\pm$ 0.04	41.3 $\pm$ 0.22	0.48 $\pm$ 0.01
	20	16.0 $\pm$ 0.06	6.98 $\pm$ 0.23	0.19 $\pm$ 0.01	0.13 $\pm$ 0.00	1.23 $\pm$ 0.03	46.3 $\pm$ 0.92	0.43 $\pm$ 0.04
	30	15.8 $\pm$ 0.10	6.71 $\pm$ 0.19	0.21 $\pm$ 0.03	0.14 $\pm$ 0.02	1.15 $\pm$ 0.05	44.6 $\pm$ 0.97	0.45 $\pm$ 0.00
	Post-flushing	15.7 $\pm$ 0.05	6.67 $\pm$ 0.08	0.19 $\pm$ 0.02	0.17 $\pm$ 0.01	1.51 $\pm$ 0.06	52.3 $\pm$ 0.41	0.69 $\pm$ 0.06
24°C Flushing + Chlorine	0	23.1 $\pm$ 0.41	6.79 $\pm$ 0.19	0.80 $\pm$ 0.01	0.68 $\pm$ 0.03	1.35 $\pm$ 0.05	49.0 $\pm$ 0.63	0.39 $\pm$ 0.01
	10	24.0 $\pm$ 0.03	7.47 $\pm$ 0.21	0.13 $\pm$ 0.03	0.08 $\pm$ 0.04	1.44 $\pm$ 0.06	49.9 $\pm$ 0.54	0.47 $\pm$ 0.01
	20	23.8 $\pm$ 0.07	6.92 $\pm$ 0.09	0.13 $\pm$ 0.01	0.08 $\pm$ 0.01	1.34 $\pm$ 0.06	59.3 $\pm$ 0.83	0.42 $\pm$ 0.02
	30	23.9 $\pm$ 0.06	6.80 $\pm$ 0.16	0.13 $\pm$ 0.05	0.10 $\pm$ 0.02	1.31 $\pm$ 0.02	45.1 $\pm$ 1.07	0.39 $\pm$ 0.02
	Post-flushing	23.4 $\pm$ 0.03	6.93 $\pm$ 0.13	0.21 $\pm$ 0.01	0.11 $\pm$ 0.03	1.50 $\pm$ 0.02	81.2 $\pm$ 1.34	1.20 $\pm$ 0.04
24°C Flushing	0	23.1 $\pm$ 0.07	6.98 $\pm$ 0.16	0.12 $\pm$ 0.01	0.08 $\pm$ 0.01	1.37 $\pm$ 0.04	48.5 $\pm$ 0.32	0.43 $\pm$ 0.00
	10	24.0 $\pm$ 0.06	7.00 $\pm$ 0.02	0.11 $\pm$ 0.00	0.07 $\pm$ 0.00	1.32 $\pm$ 0.03	51.6 $\pm$ 0.85	0.47 $\pm$ 0.01
	20	23.7 $\pm$ 0.18	6.82 $\pm$ 0.11	0.15 $\pm$ 0.01	0.06 $\pm$ 0.01	1.40 $\pm$ 0.05	52.8 $\pm$ 1.03	0.42 $\pm$ 0.01
	30	24.2 $\pm$ 0.10	6.65 $\pm$ 0.01	0.12 $\pm$ 0.04	0.10 $\pm$ 0.01	1.23 $\pm$ 0.06	48.6 $\pm$ 0.37	0.39 $\pm$ 0.04
	Post-flushing	23.5 $\pm$ 0.03	6.91 $\pm$ 0.11	0.16 $\pm$ 0.00	0.10 $\pm$ 0.02	1.34 $\pm$ 0.06	85.49 $\pm$ 1.69	1.08 $\pm$ 0.07
24°C Transition	0	23.8 $\pm$ 0.18	6.8 $\pm$ 0.04	0.13 $\pm$ 0.01	0.07 $\pm$ 0.03	1.37 $\pm$ 0.06	47.8 $\pm$ 0.37	0.39 $\pm$ 0.01
	10	24.3 $\pm$ 0.07	7.05 $\pm$ 0.02	0.12 $\pm$ 0.02	0.03 $\pm$ 0.01	1.34 $\pm$ 0.03	52.7 $\pm$ 0.24	0.48 $\pm$ 0.03
	20	23.4 $\pm$ 0.07	7.25 $\pm$ 0.03	0.15 $\pm$ 0.00	0.05 $\pm$ 0.02	1.32 $\pm$ 0.01	45.5 $\pm$ 1.38	0.58 $\pm$ 0.12
	30	23.9 $\pm$ 0.28	6.99 $\pm$ 0.12	0.12 $\pm$ 0.01	0.05 $\pm$ 0.00	1.35 $\pm$ 0.03	48.7 $\pm$ 1.61	0.55 $\pm$ 0.02
	Post-flushing	22.9 $\pm$ 0.09	6.96 $\pm$ 0.04	0.15 $\pm$ 0.01	0.08 $\pm$ 0.00	1.34 $\pm$ 0.05	87.8 $\pm$ 1.96	1.24 $\pm$ 0.20

Supplementary Table E. Results from the Kruskal-Wallis test to determine statistical differences in water-physico chemical parameters between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Kruskal-Wallis test	Total chlorine	Free chlorine	Fe	Mn	TOC
Day 0	$X^2 = 9.4$ p-value = 0.22	$X^2 = 9.5$ p-value = 0.23	$X^2 = 6.2$ p-value = 0.09	$X^2 = 10.4$ p-value = 0.15	$X^2 = 8.2$ p-value = 0.06
Day 10	$X^2 = 8.7$ p-value = 0.17	$X^2 = 7.3$ p-value = 0.06	$X^2 = 10.3$ p-value = 0.07	$X^2 = 10.4$ p-value = 0.06	$X^2 = 6.7$ p-value = 0.08
Day 20	$X^2 = 8.8$ p-value = 0.03	$X^2 = 5.9$ p-value = 0.11	$X^2 = 9.9$ p-value = 0.10	$X^2 = 8.1$ p-value = 0.06	$X^2 = 6.00$ p-value = 0.11
Day 30	$X^2 = 5.7$ p-value = 0.12	$X^2 = 9.1$ p-value = 0.27	$X^2 = 8.9$ p-value = 0.30	$X^2 = 9.5$ p-value = 0.06	$X^2 = 7.2$ p-value = 0.06
P-F	$X^2 = 9.2$ p-value = 0.09	$X^2 = 8.1$ p-value = 0.06	$X^2 = 9.4$ p-value = 0.02	$X^2 = 8.81$ p-value = 0.03	$X^2 = 6.7$ p-value = 0.08

Supplementary Table F. Results from the Mann-Whitney U test for pairwise comparisons to determine statistical differences in Fe and Mn concentrations between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Mann-Whitney U test	Fe	Mn
16 °C vs 24 °C Flushing-Hyperchlorination	W = 0 p-value = 0.05	W = 0 p-value = 0.05
16 °C vs 24 °C Flushing	W = 0 p-value = 0.05	W = 0 p-value = 0.05
16 °C - 24 °C Transition	W = 0 p-value = 0.05	W = 0 p-value = 0.032
24 °C Flushing-Hyperchlorination vs 24 °C Flushing	W = 0 p-value = 0.1	W = 4 p-value = 1
24 °C Flushing-Hyperchlorination vs 24 °C Transition	W = 0 p-value = 0.1	W = 0 p-value = 0.06
24 °C Flushing vs 24 °C Transition	W = 5 p-value = 1	W = 0 p-value = 0.06

Supplementary Table G. Results from Kruskal-Wallis test to determine statistical differences in turbidity response between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Kruskal-Wallis test	Turbidity
Last 24 hours	$\chi^2 = 245.8$ p-value = 0.067
Step 1	$\chi^2 = 187.6$ p-value = 0.058
Step 2	$\chi^2 = 117.3$ p-value = $<2e-16$
Step 3	$\chi^2 = 127.9$ p-value = $<2e-16$
Step 4	$\chi^2 = 87.8$ p-value = $<2e-16$

Supplementary Table H. Results from the Mann Whitney U test to determine statistical differences in turbidity response between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Mann-Whitney U test	Step 2	Step 3	Step 4
16 °C vs 24 °C Flushing-Hyperchlorination	W = 1201.1 p-value = 0.12	W = 404.0 p-value = 0.03	W = 277.0 p-value = 0.003
16 °C vs 24 °C Flushing	W = 1705.5 p-value = 0.09	W = 1447.0 p-value = 3.07e-13	W = 179.0 p-value = 1.71e-6
16 °C – 24 °C Transition	W = 0 p-value = < 2.2e-16	W = 0 p-value = 3.07e-14	W = 0 p-value = 2.07e-12
24 °C Flushing-Hyperchlorination vs 24 °C Flushing	W = 1205.6 p-value = 0.09	W = 1479.0 p-value = 0.7	W = 3.71e+02 p-value = 0.06
24 °C Flushing-Hyperchlorination vs 24 °C Transition	W = 0 p-value = < 2.2e-16	W = 0 p-value = 3.07e-14	W = 17 p-value = 1.39e-11
24 °C Flushing vs 24 °C Transition	W = 100.0 p-value = < 2.2e-16	W = 0 p-value = 2.98e-13	W = 0 p-value = 3.01e-12

Supplementary Table I. Results from Kruskal-Wallis test to determine statistical differences in bacterial alpha diversity indices between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Kruskal-Wallis test		Bacteria		
		Chao1	Simpson	Shannon
Biofilm	Day 10	$\chi^2 = 5.6$ p-value = 0.13	$\chi^2 = 7$ p-value = 0.07	$\chi^2 = 8.7$ p-value = 0.06
	Day 20	$\chi^2 = 5.2$ p-value = 0.15	$\chi^2 = 5.9$ p-value = 0.12	$\chi^2 = 6.0$ p-value = 0.11
	Day 30	$\chi^2 = 7.0$ p-value = 0.05	$\chi^2 = 6.4$ p-value = 0.05	$\chi^2 = 5.4$ p-value = 0.05
	Post-flushing events	$\chi^2 = 5.7$ p-value = 0.02	$\chi^2 = 5.2$ p-value = 0.05	$\chi^2 = 4.1$ p-value = 0.02
Water	Day 0	$\chi^2 = 4.8$ p-value = 0.18	$\chi^2 = 9.4$ p-value = 0.2	$\chi^2 = 10.4$ p-value = 0.1
	Day 10	$\chi^2 = 3.2$ p-value = 0.36	$\chi^2 = 5.4$ p-value = 0.14	$\chi^2 = 4.4$ p-value = 0.2
	Day 20	$\chi^2 = 5.5$ p-value = 0.14	$\chi^2 = 7.82$ p-value = 0.06	$\chi^2 = 9.3$ p-value = 0.2
	Day 30	$\chi^2 = 7.8$ p-value = 0.06	$\chi^2 = 4.12$ p-value = 0.08	$\chi^2 = 8.8$ p-value = 0.7
	Post-flushing events	$\chi^2 = 4.9$ p-value = 0.17	$\chi^2 = 7.5$ p-value = 0.06	$\chi^2 = 9.5$ p-value = 0.02

Supplementary Table J. Results from Mann-Whitney U test for pairwise comparisons to determine statistical differences in alpha diversity indices of bacteria biofilm day 30 samples between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Mann-Whitney U test Bacteria – Biofilm Day 30	Chao1	Simpson	Shannon
16 °C vs 24 °C Flushing-Hyperchlorination	W = 2 p-value = 0.4	W = 2 p-value = 0.2	W = 9 p-value = 1
16 °C vs 24 °C Flushing	W = 1 p-value = 0.05	W = 0 p-value = 0.05	W = 4 p-value = 0.05
16 °C – 24 °C Transition	W = 0 p-value = 0.1	W = 9 p-value = 0.1	W = 0 p-value = 0.1
24 °C Flushing-Hyperchlorination vs 24 °C Flushing	W = 0 p-value = 0.2	W = 7 p-value = 0.4	W = 0 p-value = 0.1
24 °C Flushing-Hyperchlorination vs 24 °C Transition	W = 0 p-value = 0.1	W = 9 p-value = 0.1	W = 0 p-value = 0.1
24 °C Flushing vs 24 °C Transition	W = 5 p-value = 1	W = 7 p-value = 0.4	W = 2 p-value = 0.4

Supplementary Table K. Results from Mann-Whitney U test for pairwise comparisons to determine statistical differences in alpha diversity indices of bacteria biofilm post-flushing samples between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Mann-Whitney U test Bacteria – Biofilm Post-flushing	Chao1	Simpson	Shannon
16 °C vs 24 °C Flushing-Hyperchlorination	W = 9 p-value = 0.05	W = 0 p-value = 1	W = 9 p-value = 0.05
16 °C vs 24 °C Flushing	W = 8 p-value = 0.2	W = 2 p-value = 0.4	W = 5 p-value = 1
16 °C – 24 °C Transition	W = 7 p-value = 0.4	W = 4 p-value = 1	W = 4 p-value = 1
24 °C Flushing-Hyperchlorination vs 24 °C Flushing	W = 2 p-value = 0.4	W = 6 p-value = 0.7	W = 3 p-value = 0.7
24 °C Flushing-Hyperchlorination vs 24 °C Transition	W = 1 p-value = 0.2	W = 9 p-value = 0.1	W = 0 p-value = 0.1
24 °C Flushing vs 24 °C Transition	W = 4 p-value = 1	W = 6 p-value = 0.7	W = 4 p-value = 1

Supplementary Table L. Results from Mann-Whitney U test for pairwise comparisons to determine statistical differences in Shannon index of bacteria water post-flushing samples between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Mann-Whitney U test Bacteria- Water port-flushing	Shannon
16 °C vs 24 °C Flushing-Hyperchlorination	W = 9 p-value = 0.05
16 °C vs 24 °C Flushing	W = 6 p-value = 0.7
16 °C – 24 °C Transition	W = 0 p-value = 0.1
24 °C Flushing-Hyperchlorination vs 24 °C Flushing	W = 0 p-value = 0.1
24 °C Flushing-Hyperchlorination vs 24 °C Transition	W = 0 p-value = 0.1
24 °C Flushing vs 24 °C Transition	W = 0 p-value = 0.1

Supplementary Table M. Results from Kruskal-Wallis test to determine statistical differences in fungal alpha diversity indices between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Kruskal-Wallis test		Fungi		
		Chao1	Simpson	Shannon
Biofilm	Day 10	$\chi^2 = 5.8$ p-value = 0.12	$\chi^2 = 7.2$ p-value = 0.05	$\chi^2 = 7.7$ p-value = 0.05
	Day 20	$\chi^2 = 3.4$ p-value = 0.05	$\chi^2 = 5.75$ p-value = 0.02	$\chi^2 = 6.28$ p-value = 0.05
	Day 30	$\chi^2 = 1.19$ p-value = 0.04	$\chi^2 = 2.54$ p-value = 0.05	$\chi^2 = 1.89$ p-value = 0.05
	P-F	$\chi^2 = 6.5$ p-value = 0.05	$\chi^2 = 6.9$ p-value = 0.05	$\chi^2 = 7.77$ p-value = 0.04
Water	Day 0	$\chi^2 = 3.9$ p-value = 0.26	$\chi^2 = 5.15$ p-value = 0.16	$\chi^2 = 4.12$ p-value = 0.24
	Day 10	$\chi^2 = 0.43$ p-value = 0.9	$\chi^2 = 3.8$ p-value = 0.2	$\chi^2 = 3.2$ p-value = 0.36
	Day 20	$\chi^2 = 2.4$ p-value = 0.48	$\chi^2 = 4.6$ p-value = 0.20	$\chi^2 = 5.6$ p-value = 0.13
	Day 30	-	-	-
	P-F	$\chi^2 = 9.8$ p-value = 0.05	$\chi^2 = 1.6$ p-value = 0.4	$\chi^2 = 7.6$ p-value = 0.04

Supplementary Table N. Results from Mann-Whitney U test for pairwise comparisons to determine statistical differences in alpha diversity indices of fungi-biofilm day 10 samples between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Mann-Whitney U test Fungi – Biofilm Day 10	Chao1	Simpson	Shannon
16 °C vs 24 °C Flushing-Hyperchlorination	W = 6 p-value = 0.65	W = 0 p-value = 0.1	W = 9 p-value = 0.1
16 °C vs 24 °C Flushing	W = 1 p-value = 0.05	W = 0 p-value = 0.05	W = 9 p-value = 0.05
16 °C – 24 °C Transition	W = 9 p-value = 0.03	W = 0 p-value = 0.05	W = 9 p-value = 0.05
24 °C Flushing-Hyperchlorination vs 24 °C Flushing	W = 5 p-value = 1	W = 2 p-value = 0.4	W = 7 p-value = 0.4
24 °C Flushing-Hyperchlorination vs 24 °C Transition	W = 8 p-value = 0.18	W = 2 p-value = 0.4	W = 8 p-value = 0.2
24 °C Flushing vs 24 °C Transition	W = 9 p-value = 0.07	W = 4 p-value = 1	W = 5 p-value = 1

Supplementary Table O. Results from Mann-Whitney U test for pairwise comparisons to determine statistical differences in alpha diversity indices of fungi-biofilm day 20 samples between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Mann-Whitney U test Fungi – Biofilm Day 20	Chao1	Simpson	Shannon
16 °C vs 24 °C Flushing-Hyperchlorination	W = 1 p-value = 0.05	W = 5 p-value = 0.05	W = 1 p-value = 0.05
16 °C vs 24 °C Flushing	W = 0 p-value = 0.05	W = 0 p-value = 0.05	W = 0 p-value = 0.05
16 °C – 24 °C Transition	W = 9 p-value = 0.03	W = 4 p-value = 0.05	W = 5 p-value = 0.05
24 °C Flushing-Hyperchlorination vs 24 °C Flushing	W = 2.5 p-value = 0.5	W = 7 p-value = 0.4	W = 1 p-value = 0.2
24 °C Flushing-Hyperchlorination vs 24 °C Transition	W = 5 p-value = 1	W = 8 p-value = 0.2	W = 1 p-value = 0.2
24 °C Flushing vs 24 °C Transition	W = 7 p-value = 0.4	W = 6 p-value = 0.7	W = 4 p-value = 1

Supplementary Table P. Results from Mann-Whitney U test for pairwise comparisons to determine statistical differences in alpha diversity indices of fungi-biofilm day 30 samples between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Mann-Whitney U test Fungi - Biofilm Day 30	Chao1	Simpson	Shannon
16 °C vs 24 °C Flushing-Hyperchlorination	W = 0 p-value = 0.05	W = 5 p-value = 0.05	W = 1 p-value = 0.05
16 °C vs 24 °C Flushing	W = 1 p-value = 0.05	W = 9 p-value = 0.05	W = 1 p-value = 0.05
16 °C - 24 °C Transition	W = 0 p-value = 0.05	W = 1 p-value = 0.05	W = 1 p-value = 0.05
24 °C Flushing-Hyperchlorination vs 24 °C Flushing	W = 7 p-value = 0.4	W = 6 p-value = 0.7	W = 4 p-value = 1
24 °C Flushing-Hyperchlorination vs 24 °C Transition	W = 7 p-value = 0.4	W = 6 p-value = 0.7	W = 4 p-value = 1
24 °C Flushing vs 24 °C Transition	W = 4 p-value = 1	W = 7 p-value = 0.4	W = 2 p-value = 0.4

Supplementary Table Q. Results from Mann-Whitney U test for pairwise comparisons to determine statistical differences in alpha diversity indices of fungi-biofilm post-flushing samples between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Mann-Whitney U test Fungi - Biofilm Post-flushing	Chao1	Simpson	Shannon
16 °C vs 24 °C Flushing-Hyperchlorination	W = 9 p-value = 0.05	W = 0 p-value = 0.05	W = 9 p-value = 0.05
16 °C vs 24 °C Flushing	W = 9 p-value = 0.05	W = 0 p-value = 0.05	W = 9 p-value = 0.05
16 °C - 24 °C Transition	W = 9 p-value = 0.05	W = 0 p-value = 0.05	W = 9 p-value = 0.05
24 °C Flushing-Hyperchlorination vs 24 °C Flushing	W = 4 p-value = 1	W = 3 p-value = 0.7	W = 6 p-value = 0.7
24 °C Flushing-Hyperchlorination vs 24 °C Transition	W = 5 p-value = 1	W = 4 p-value = 1	W = 5 p-value = 1
24 °C Flushing vs 24 °C Transition	W = 6.5 p-value = 0.50	W = 6 p-value = 0.7	W = 3 p-value = 0.7

Supplementary Table R. Results from Mann-Whitney U test for pairwise comparisons to determine statistical differences in alpha diversity indices of fungi-water post-flushing samples between temperatures and management strategies. Differences were considered statistically significant when p-value was ≤ 0.05 .

Mann-Whitney U test Fungi - Water Post-flushing	Chao1	Shannon
16 °C vs 24 °C Flushing-Hyperchlorination	W = 1 p-value = 0.05	W = 2 p-value = 0.2
16 °C vs 24 °C Flushing	W = p-value = 0.05	W = 3 p-value = 0.8
16 °C - 24 °C Transition	W = 1 p-value = 0.05	W = 2 p-value = 0.2
24 °C Flushing-Hyperchlorination vs 24 °C Flushing	W = 6 p-value = 0.7	W = 5 p-value = 0.05
24 °C Flushing-Hyperchlorination vs 24 °C Transition	W = 9 p-value = 0.1	W = 7 p-value = 0.05
24 °C Flushing vs 24 °C Transition	W = 7 p-value = 0.4	W = 7 p-value = 0.05

Supplementary Table S. ANOSIM statistics calculated using the Bray-Curtis distance matrixes to test beta-diversity significant differences. Differences were considered statistically significant when p-value was ≤ 0.05 . Global-R statistic determine the level of differentiation between treatments, indicating 0 no differentiation and 1 a total differentiation of the samples. * p-value ≤ 0.05 .

BIOFILM	Bacteria		Fungi	
	p-value	global-R	p-value	global-R
16°C vs 24°C Flushing-Hyperchlorination	0.001*	0.661	0.001*	0.723
16°C vs 24°C Flushing	0.001*	0.571	0.001*	0.691
16°C - 24°C Transition	0.001*	0.774	0.001*	0.667
24°C Flushing-Hyperchlorination vs 24°C Flushing	0.001*	0.280	0.293	0.024
24°C Flushing-Hyperchlorination vs 24°C Transition	0.001*	0.797	0.429	0.004
24°C Flushing vs 24°C Transition	0.001*	0.194	0.521	0.015
WATER	Bacteria		Fungi	
	p-value	global-R	p-value	global-R
16°C vs 24°C Flushing-Hyperchlorination	0.001*	0.492	0.093	0.062
16°C vs 24°C Flushing	0.001*	0.407	0.427	0.008
16°C - 24°C Transition	0.002*	0.310	0.086	0.136
24°C Flushing-Hyperchlorination vs 24°C Flushing	0.061	0.115	0.450	0.005
24°C Flushing-Hyperchlorination vs 24°C Transition	0.083	0.209	0.187	0.059
24°C Flushing vs 24°C Transition	0.101	0.118	0.790	0.037