

Supplementary Materials

Metabarcoding under Brine: Microbial Ecology of Five Hypersaline Lakes at Rottnest Island (WA, Australia)

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Table S1. Labels, ID, GPS coordinates (latitude and longitude) per each sampling point considered in the study.

Lake	Lake ID	GPS coordinates	Sample ID		
			Probe data	Water (nutrients)	Water (microbes)
Vincent	VIN	−31.99972, 115.51444	VIN_1-PD	VIN_1-WN	VIN_1-WM
Garden	GAR	−31.99555, 115.53666	GAR_1-PD	GAR_1-WN	GAR_1-WM
Herschel	HER	−31.99845, 115.52617	HER_1-PD		HER_1-WM
	HER	−31.99638, 115.53138	HER_2-PD	HER_2-WN	HER_2-WM
Baghdad	BAG	−31.99472, 115.52361	BAG1-PD	BAG1-WN	BAG1-WM
	BAG	−31.99714, 115.5187	BAG2-PD		BAG2-WM
	BAG	−31.99372, 115.51351	BAG3-PD		BAG3-WM
Serpentine	SER	−32.00694, 115.52027	SER1-PD	SER1-WN	SER1-WM
	SER	−32.00277, 115.52583	SER2-PD		SER2-WM
	SER	−32.0018, 115.53046	SER3-PD		SER3-WM

Table S2. Results of the Tukey's HSD tests (pairwise comparisons) on the hydrochemical data across the lakes. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$. DO, Dissolved Oxygen; NH₃, ammonia; TDS, Total Dis-solved solids; Cl[−], chloride; Br[−], bromide; SO₄^{2−}, sulphates; DOC, Dissolved Organic Carbon; TN, Total Nitrogen.

	pH	DO	Temperature	Alkalinity	NH ₃	TDS	Cl [−]	Br [−]	SO ₄ ^{2−}	DOC	TN
Garden-Baghdad	***	ns	ns	ns	*	***	***	**	**	***	***
Herschel-Baghdad	***	*	ns	*	ns	***	***	***	***	***	ns
Serpentine-Baghdad	***	ns	***	**	ns	***	***	**	***	*	**
Vincent-Baghdad	***	ns	***	ns	ns	ns	ns	ns	**	**	***
Herschel-Garden	ns	ns	ns	**	**	***	***	***	***	***	***
Serpentine-Garden	ns	ns	***	***	ns	***	***	***	***	***	***
Vincent-Garden	*	ns	***	ns	*	***	***	**	***	***	***
Serpentine-Herschel	ns	ns	***	ns	ns	ns	**	ns	***	***	***
Vincent-Herschel	ns	ns	*	*	ns	***	***	***	***	***	ns
Vincent-Serpentine	ns	ns	ns	**	ns	***	***	***	***	***	***

Table S3. Results of the Tukey's HSD tests (pairwise comparisons) on the values of the diversity indices across the lakes. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$. H, Shannon diversity index; J, Pielou's evenness index.

	α -diversity	H	J
Garden-Baghdad	***	***	***
Herschel-Baghdad	***	***	***
Serpentine-Baghdad	***	***	***
Vincent-Baghdad	ns	ns	ns
Herschel-Garden	***	***	***
Serpentine-Garden	***	***	***
Vincent-Garden	***	***	***
Serpentine-Herschel	**	ns	ns
Vincent-Herschel	***	***	***
Vincent-Serpentine	***	***	***

Table S4. Lower, medium and upper quantiles (2.5%, 50% and 97.5%) and median of the r^2 values obtained from 1000 random replicates.

Variable	Quantiles		
	2.50%	50.00%	97.50%
pH	0.50	0.52	0.53
DO	0.16	0.36	0.55
Temp	0.28	0.33	0.37
Alk	0.78	0.85	0.90
NH ₃	0.27	0.36	0.48
TDS	0.93	0.94	0.95
Cl ⁻	0.94	0.95	0.95
Br ⁻	0.90	0.92	0.94
SO ₄ ²⁻	0.03	0.03	0.03
DOC	0.62	0.63	0.64
TN	0.70	0.73	0.76

Table S5. Results of the Tukey's HSD tests (pairwise comparisons) on the phylogenetic indices across the lakes. *, $p < 0.05$; **, $p < 0.005$; ***, $p < 0.0005$. PD, phylogenetic diversity; MTD, mean pairwise distance; MNTD, mean nearest taxon distance.

	PD	MTD	MNTD
Garden-Baghdad	***	*	*
Herschel-Baghdad	***	***	ns
Serpentine-Baghdad	***	***	ns
Vincent-Baghdad	ns	ns	ns
Herschel-Garden	*	***	***
Serpentine-Garden	***	***	***
Vincent-Garden	***	ns	***
Serpentine-Herschel	ns	ns	ns
Vincent-Herschel	***	***	ns
Vincent-Serpentine	***	***	ns

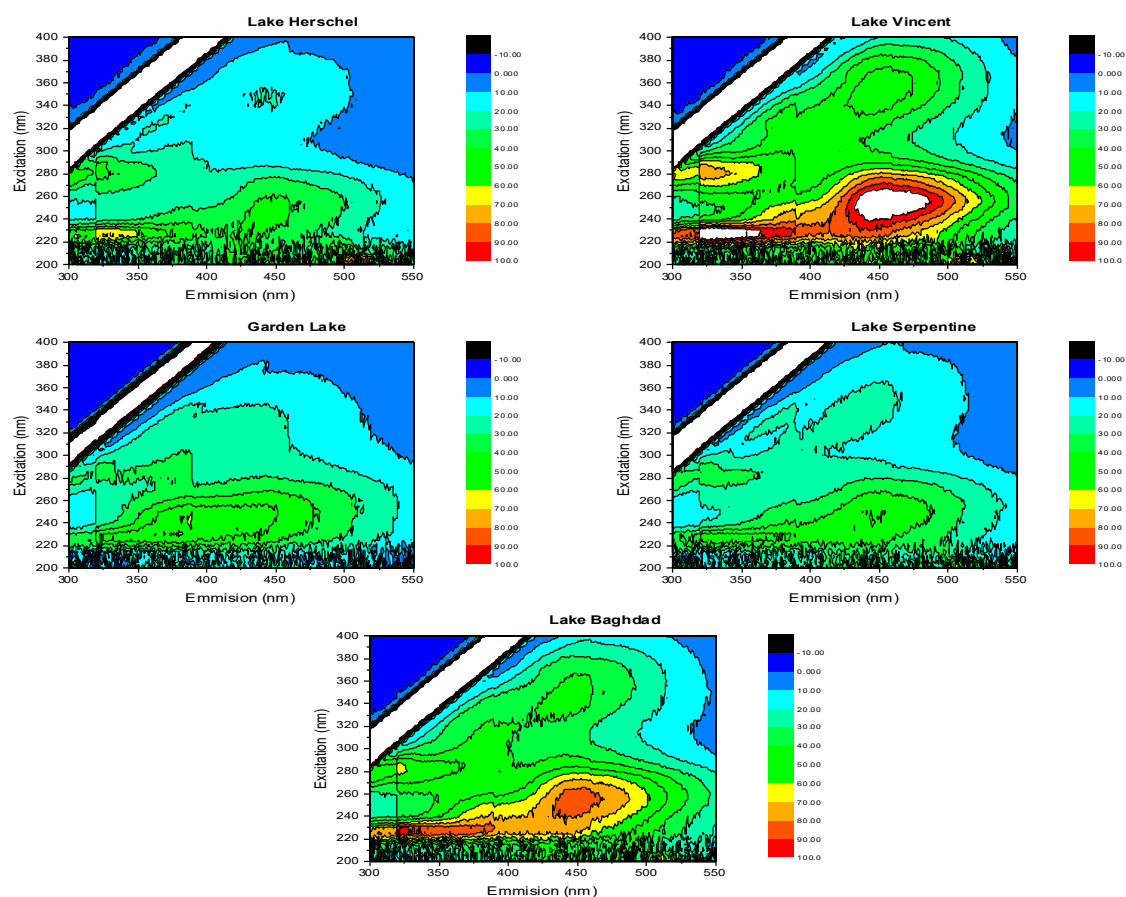


Figure S1. Comparison of the EEM spectra of the five lakes. Plots were performed with Origin 9.1 64 bit software.

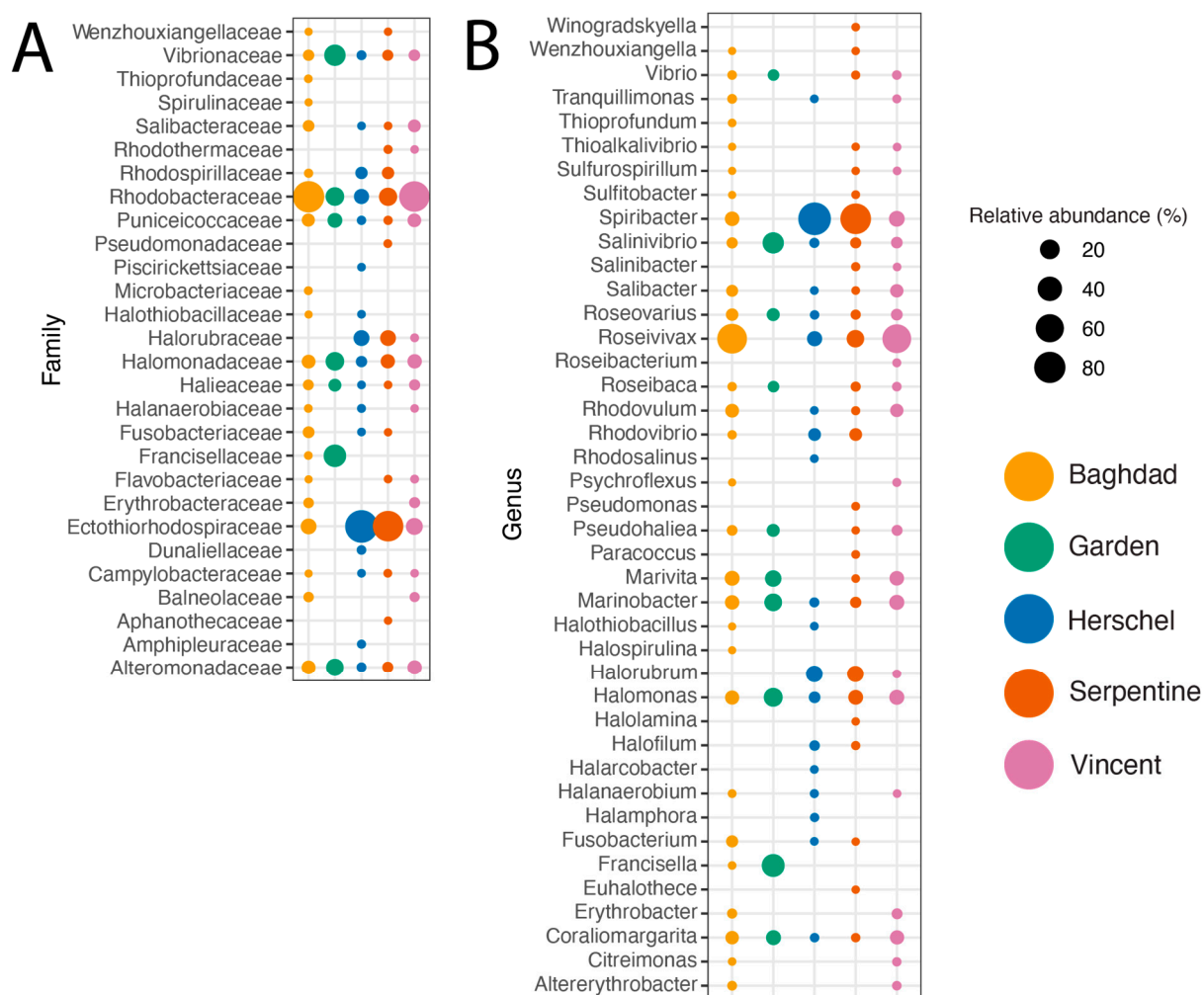
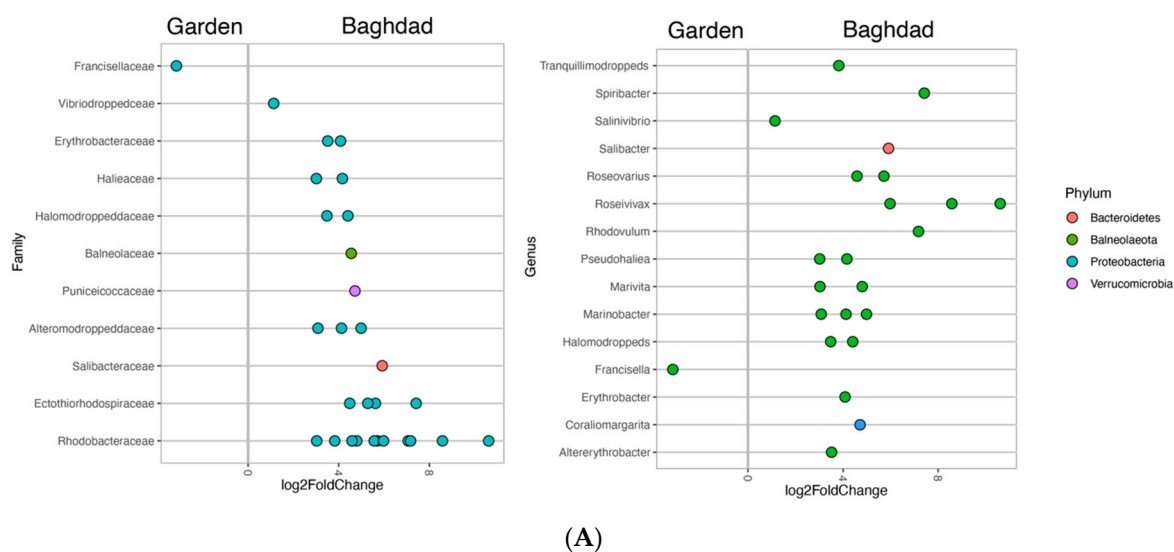
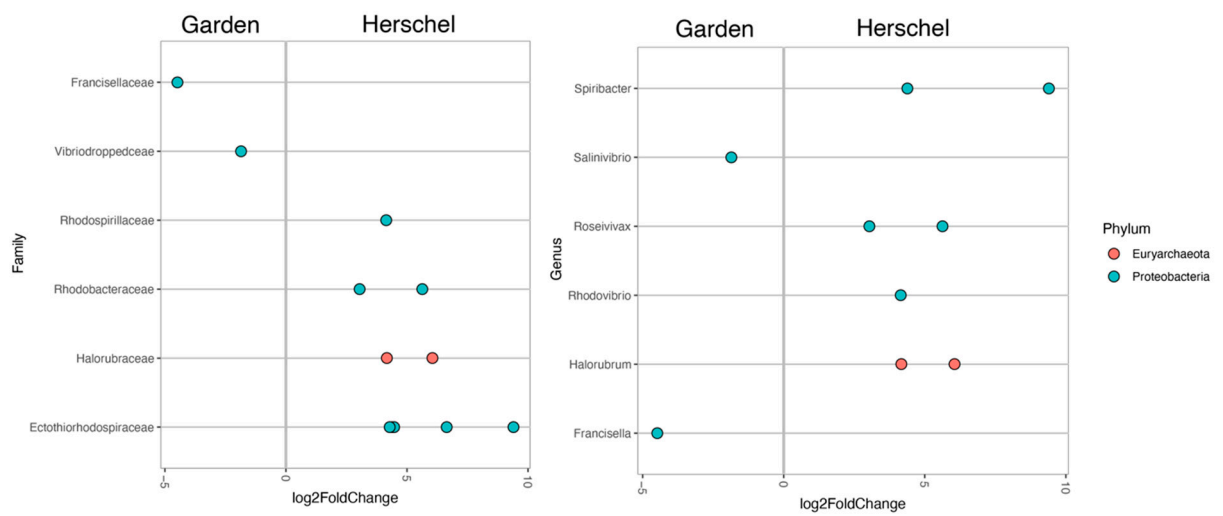


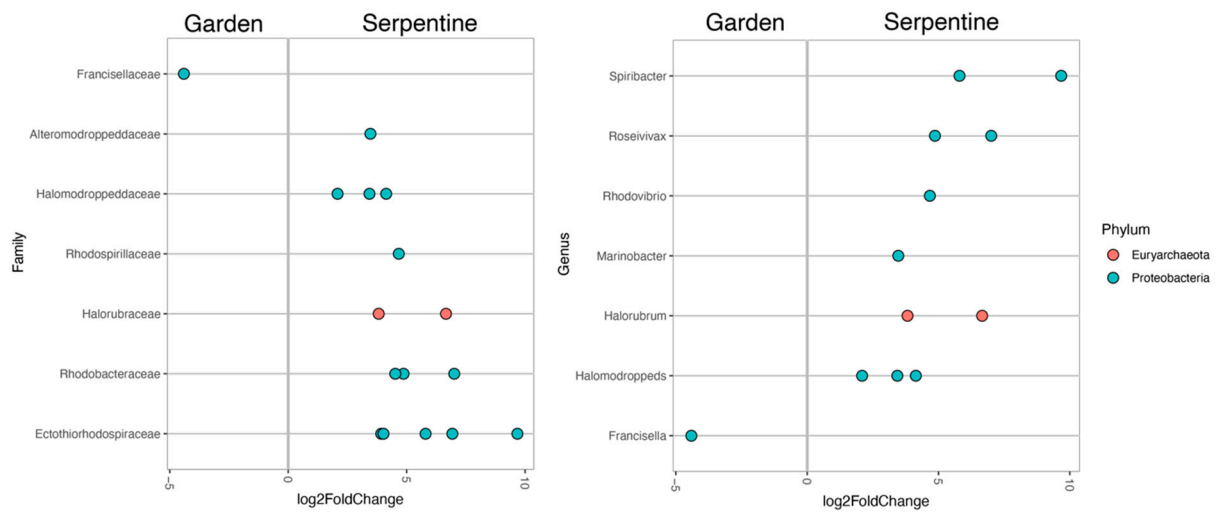
Figure S2. Distribution of the relative abundances at (A) family and (B) genus level across the five lake.



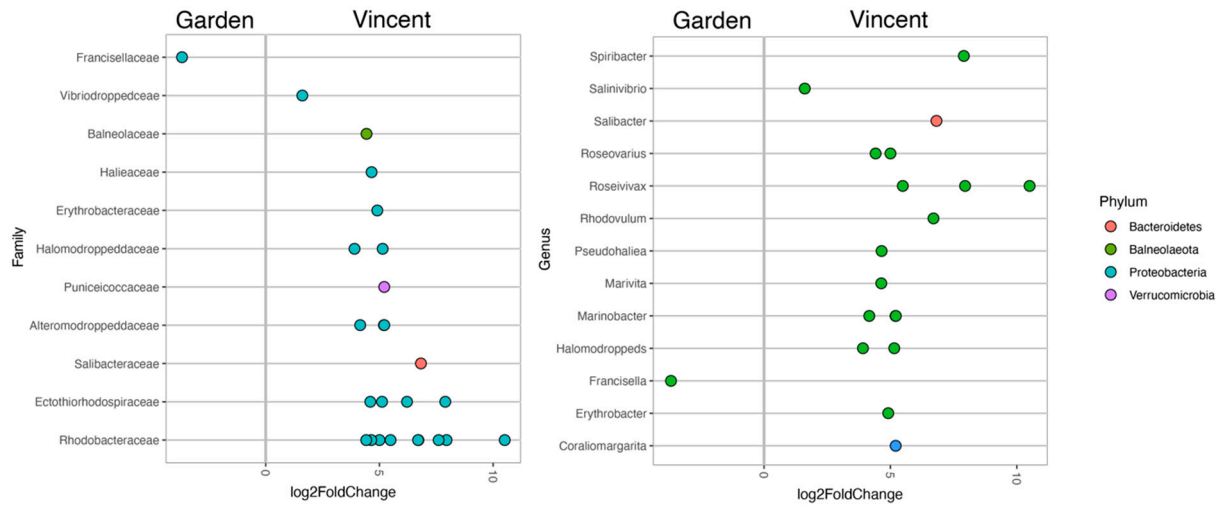
(A)



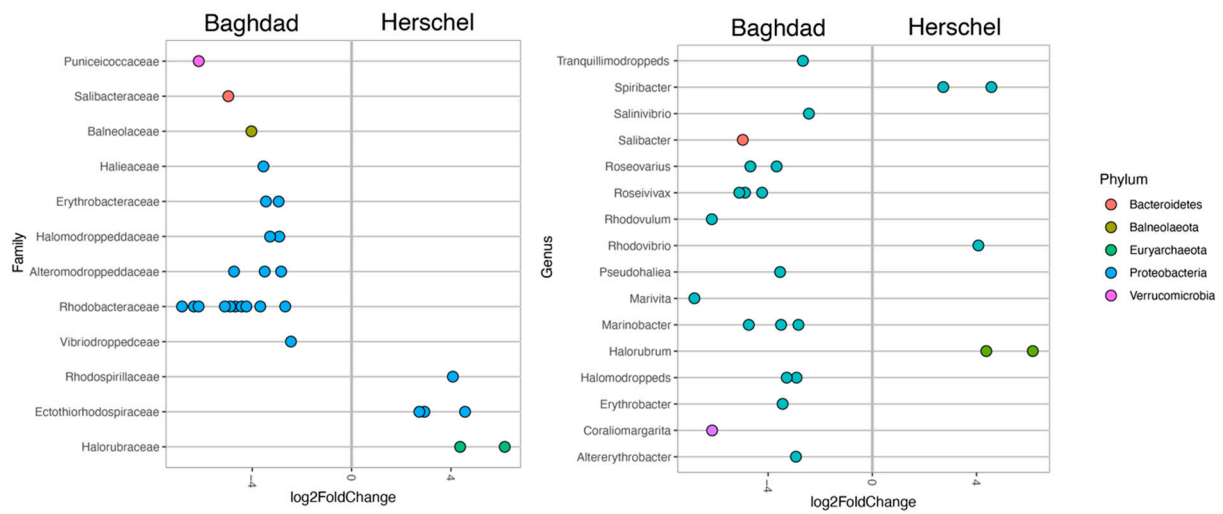
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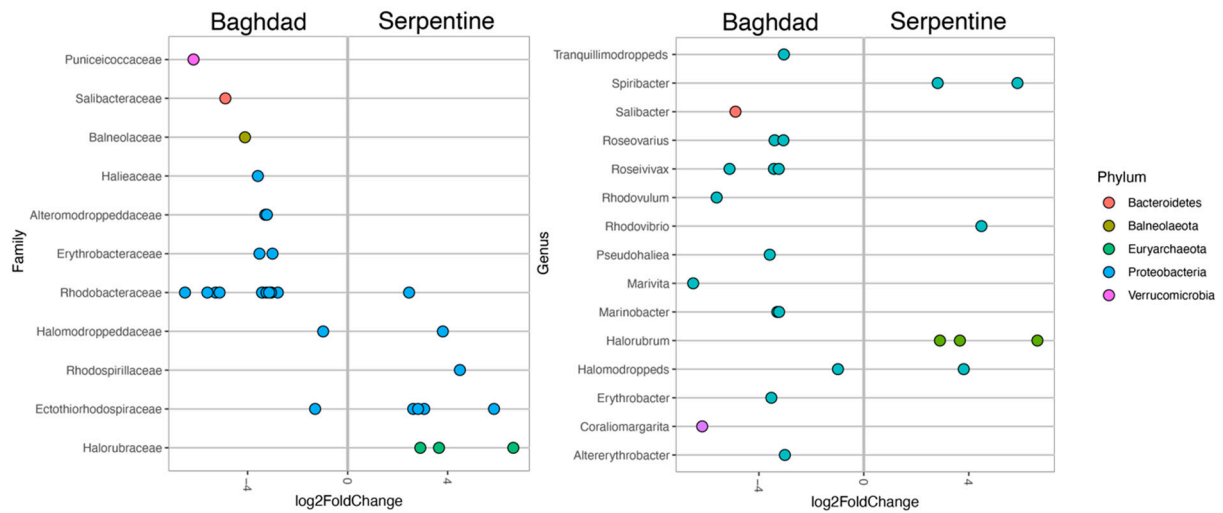
(C)



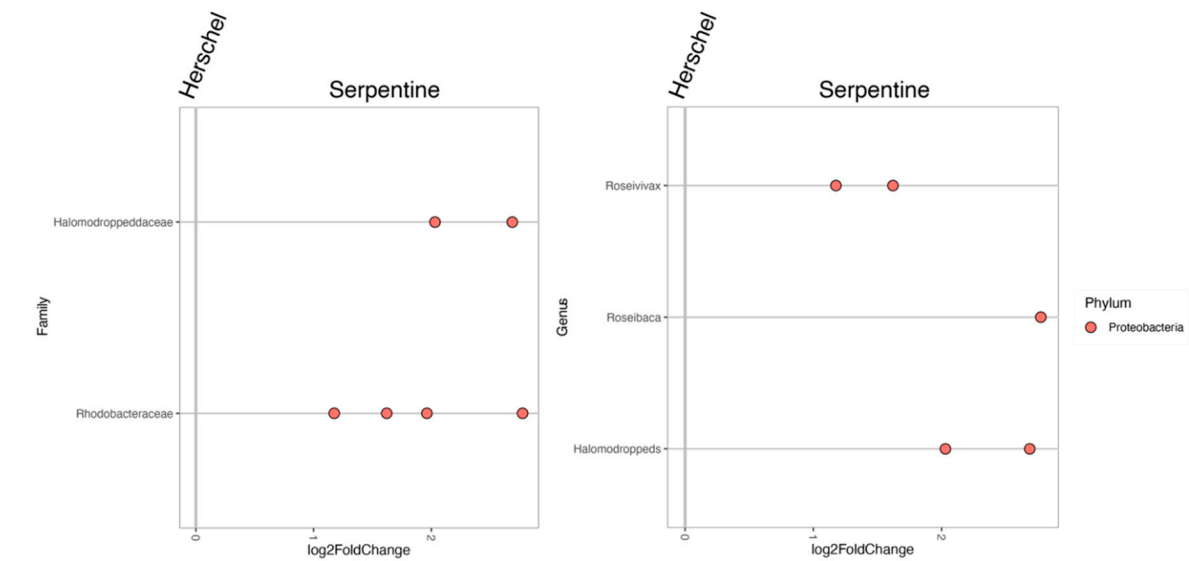
(D)



(E)



(F)



(G)

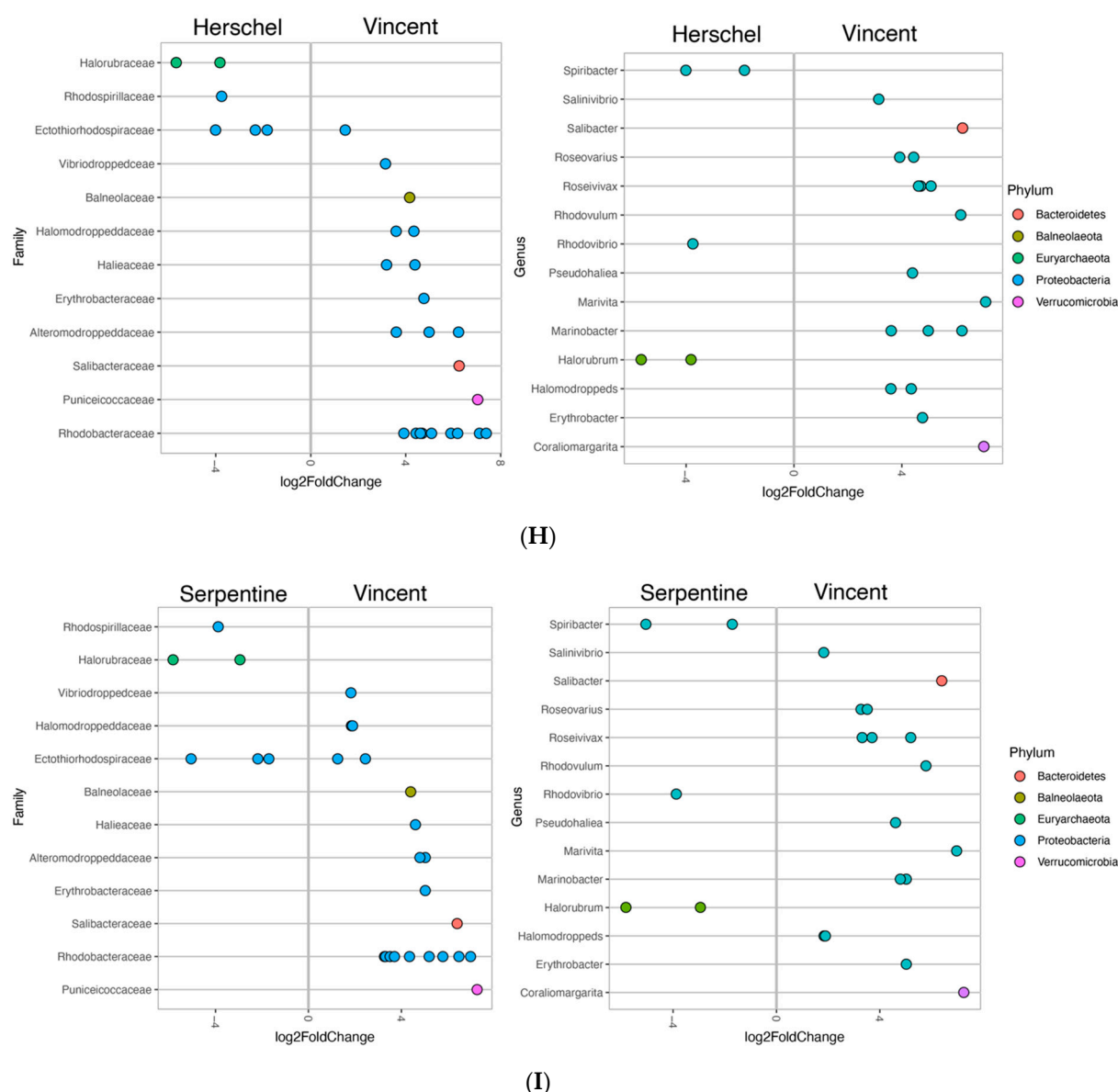


Figure S3. (A) Differentially abundant OTUs (agglomerated by species level) ($p < 0.05$) between Garden Lake and Lake Baghdad at family and genus level. Negative log₂-fold-change indicates taxa that were more abundant at Garden Lake, whereas a positive log₂-fold-change indicates taxa that were more abundant at Lake Baghdad. Each circle represents one taxon that was enriched in one of the two lakes; different colours represent different phyla. (B) Differentially abundant OTUs (agglomerated by species level) ($p < 0.05$) between Garden Lake and Herschel Lake at family and genus level. Negative log₂-fold-change indicates taxa that were more abundant at Garden Lake, whereas a positive log₂-fold-change indicates taxa that were more abundant at Herschel Lake. Each circle represents one taxon that was enriched in one of the two lakes; different colours represent different phyla. (C) Differentially abundant OTUs (agglomerated by species level) ($p < 0.05$) between Garden Lake and Serpentine Lake at family and genus level. Negative log₂-fold-change indicates taxa that were more abundant at Garden Lake, whereas a positive log₂-fold-change indicates taxa that were more abundant at Serpentine Lake. Each circle represents one taxon that was enriched in one of the two lakes; different colours represent different phyla. (D) Differentially abundant OTUs (agglomerated by species level) ($p < 0.05$) between Garden Lake and Lake Vincent at family and genus level. Negative log₂-fold-change indicates taxa that were more abundant at Garden Lake, whereas a positive log₂-fold-change indicates taxa that were more abundant at Lake Vincent. Each circle represents one taxon that was enriched in one of the two lakes; different colours represent different phyla. (E) Differentially abundant OTUs (agglomerated by species level) ($p < 0.05$) between Lake Baghdad and Herschel Lake at family and genus level. Negative log₂-fold-change indicates taxa that were more abundant at Lake Baghdad, whereas a positive log₂-fold-change indicates taxa that were more abundant at Herschel Lake. Each circle represents one taxon that was enriched in one of the two lakes; different colours represent different phyla. (F) Differentially abundant OTUs (agglomerated by species level) ($p < 0.05$) between Lake Baghdad and Serpentine Lake at family and genus level. Negative log₂-fold-change indicates taxa

that were more abundant at Lake Baghdad, whereas a positive log₂-fold-change indicates taxa that were more abundant at Serpentine Lake. Each circle represents one taxon that was enriched in one of the two lakes; different colours represent different phyla. **(G)** Differentially abundant OTUs (agglomerated by species level) ($p < 0.05$) between Herschel Lake and Serpentine Lake at family and genus level. Negative log₂-fold-change indicates taxa that were more abundant at Herschel Lake, whereas a positive log₂-fold-change indicates phyla that were more abundant at Serpentine Lake. Each circle represents one taxon that was enriched in one of the two lakes; different colours represent different phyla. **(H)** Differentially abundant OTUs (agglomerated by species level) ($p < 0.05$) between Herschel Lake and Lake Vincent at family and genus level. Negative log₂-fold-change indicates taxa that were more abundant at Herschel Lake, whereas a positive log₂-fold-change indicates taxa that were more abundant at Lake Vincent. Each circle represents one taxon that was enriched in one of the two lakes; different colours represent different phyla. **(I)** Differentially abundant OTUs (agglomerated by species level) ($p < 0.05$) between Serpentine Lake and Lake Vincent at family and genus level. Negative log₂-fold-change indicates taxa that were more abundant at Serpentine Lake, whereas a positive log₂-fold-change indicates phyla that were more abundant at Lake Vincent. Each circle represents one taxon that was enriched in one of the two lakes; different colours represent different phyla.

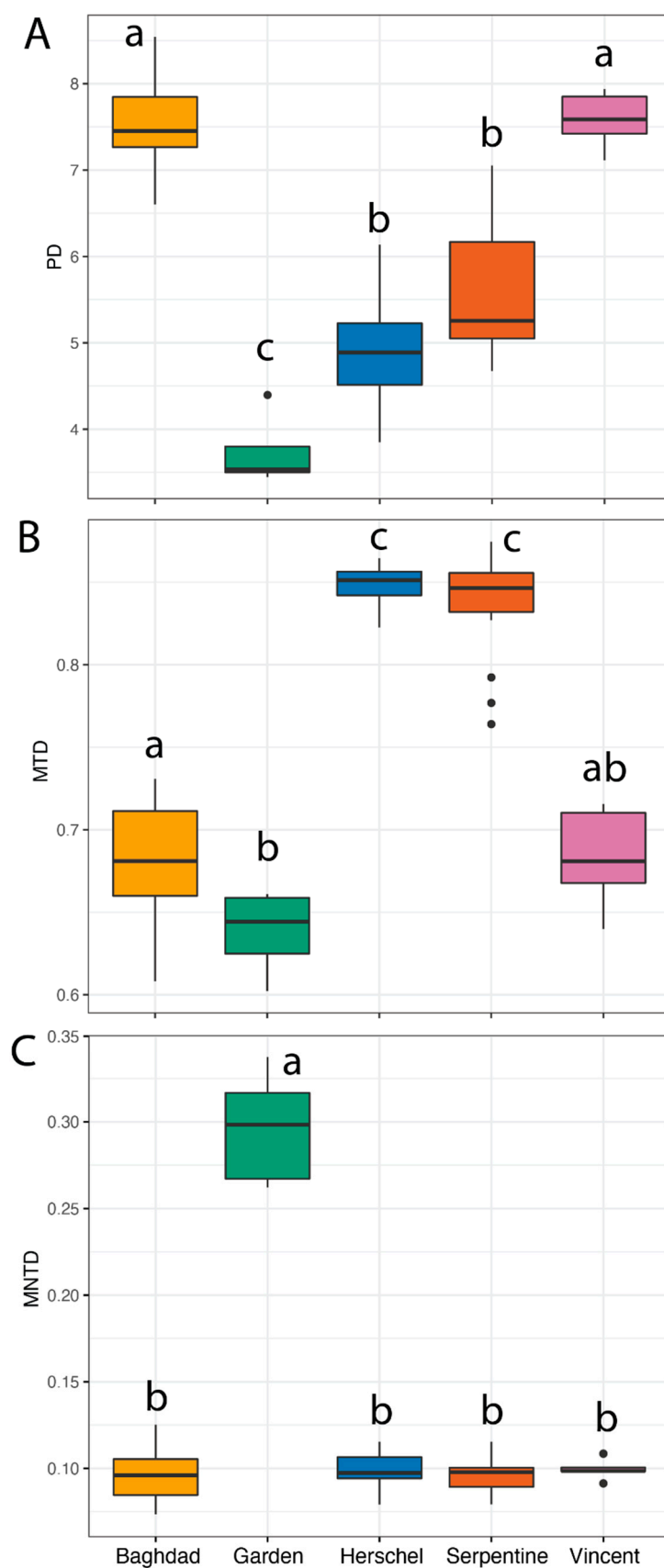


Figure S4. Phylogenetic analyses (A) Phylogenetic diversity (PD) between the microbial communities of the five lakes; (B) Mean pairwise distance distance (MTD) and (C) mean nearest taxon distance (MNTD). different letters (a, b, c) indicate statistically significant results (see Table S5 for the significances of the pairwise comparisons).