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Geochemical and microbial factors driving crustacean assemblages in adjacent aquifer units within the same aquifer

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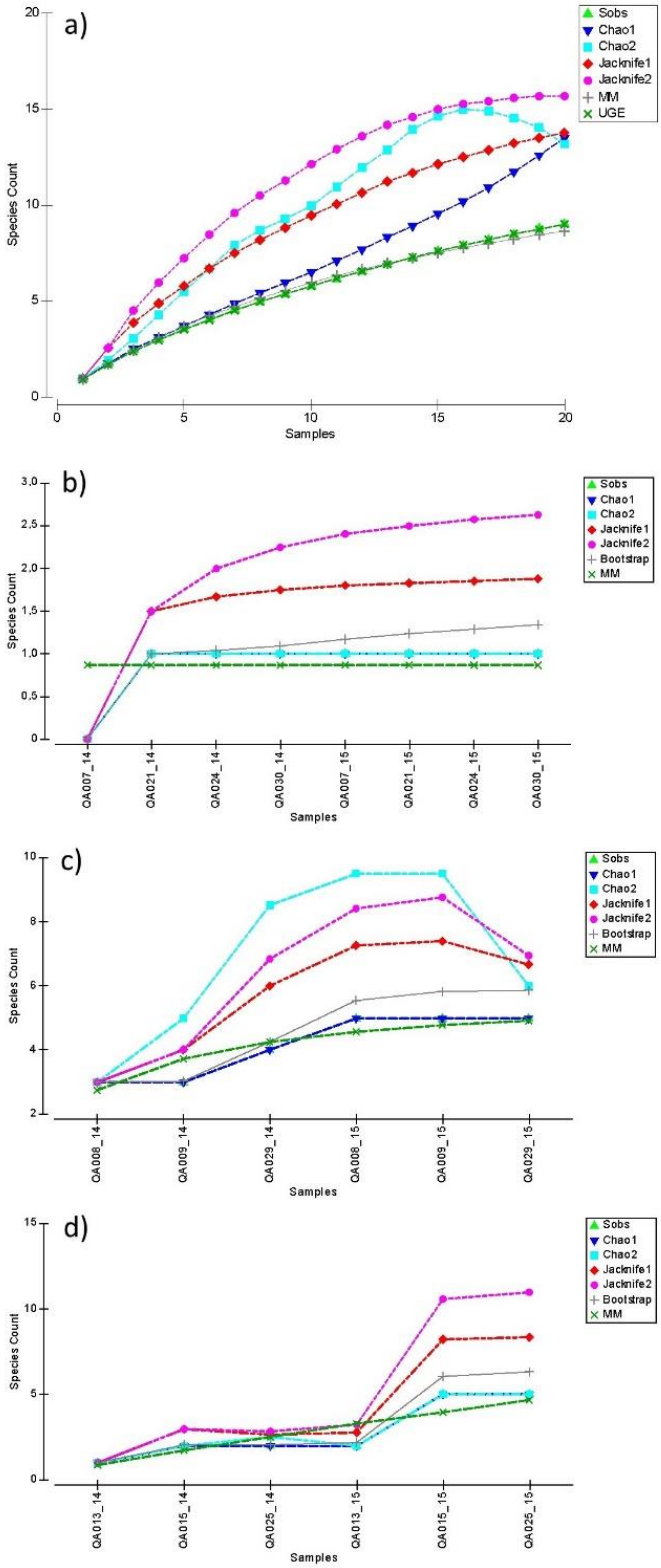

Fig. S1. Species rarefaction and estimators' curves of the crustacean taxa at increasing sample size. S(obs): species rarefaction curve of observed species richness. Other lines represent the estimated species richness using mean values obtained by the non-parametric and parametric estimators. a) Sabatini Mounts aquifer: Sobs: 9; Chao1: 10; Chao2: 9.9; Jacknife1: 11.8; Jacknife2: 10.3; Bootstrap: 10.6; MM: 9.1; b) sulphate-depleted aquifer unit: Sobs: 1; Chao1: 1; Chao2: 1; Jacknife1: 1.8; Jacknife2: 2.6; Bootstrap: 1.3; MM: 0.87; c) earth-alkaline aquifer unit: Sobs: 1; 5; Chao1: 5; Chao2: 6; Jacknife1: 6.6; Jacknife2: 6.9; Bootstrap: 5.6; MM: 5.8; UGE: 4.9; d) K-rich aquifer unit: Sobs: 5; Chao1: 5; Chao2: 5; Jacknife1: 8.3; Jacknife2: 6.1; Bootstrap: 6.3; MM: 4.6.

Table S1. Results of the two-way PERMANOVAs for the environmental and microbial variables, and crustacean assemblage in the Sabatini Mounts aquifer; YEAR (2014 and 2015) and groundwater body (GWB): K-rich (K), earth-alkaline (E-A) and sulfate-depleted (S-D). Permutational post-hoc *t*-tests were performed for significant PERMANOVA outcomes not biased by heterogeneity of variances. * indicates significant PERMDISP. Degree of freedom are indicated in brackets. SB indicates groundwater-obligate species. Significant p-values < 0.05 are reported in bold.

Variable	GWB (2,14)	YEAR (1,14)	GWBxYEAR (2,14)	K/E-A(10)	K/S-D(10)	S-D/E-A(8)
Overall field data	**0.002**	0.435	0.984	**0.043**	**0.001**	**0.004**
WT	0.474	0.972	1.000			
ORP	0.179	0.263	0.814			
T	**0.023**	0.076	0.921	0.348	0.096	**0.006**
pH	**0.002**	0.502	0.553	**0.005**	0.104	**0.011**
DO	0.436	0.798	0.806			
EC	**0.001**	0.902	0.994	**0.001**	**0.001**	**0.038**
Major	**0.001**	**0.019**	0.095	**0.001**	**0.001**	**0.001**
F	**0.011***	0.964	0.992			
Cl	0.524	0.803	0.992			
NO3	**0.012***	0.702	0.947			
SO4	**0.025**	0.984	0.997	0.281	**0.003**	**0.019**
HCO3	**0.001**	0.931	0.997	**0.006**	**0.002**	**0.001**
Na	**0.002**	0.296	0.817	0.071	**0.004**	0.066
Mg	0.113	0.759	0.892			
K	**0.001**	0.075	0.807	**0.007**	**0.002**	0.178
Ca	**0.005***	0.835	0.926			
Si	**0.021**	**0.001**	0.638	0.092	**0.036**	0.954
DOC	0.813	**0.019**	0.401			
Trace	**0.003**	0.535	0.986	0.101	**0.013**	**0.021**
Li	**0.008**	0.921	0.989	**0.019**	**0.016**	0.1
B	**0.001**	0.117	0.956	**0.035**	**0.001**	**0.01**
Al	0.751	0.134	0.785			
V	0.53	0.085	0.62			
Cr	0.423	0.603	0.97			
Mn	0.64	0.505	0.265			
Fe	0.739	0.392	0.439			
Ni	0.309	0.245	0.355			
Cu	0.317*	0.203	0.867			
Zn	0.156	0.473	0.845			
As	0.591	0.552	0.929			
Se	**0.036***	0.466	0.778			
Rb	**0.001**	0.476	0.957	**0.048**	**0.01**	**0.02**
Sr	0.192*	0.948	0.943			
Ba	0.339	0.489	0.459			
Pb	0.866	0.635	0.358			
U	**0.003**	0.965	0.959	0.089	**0.002**	**0.004**
Microbial						
TCC	**0.038**	0.345	0.72	0.781	**0.046**	**0.005**
LNA	**0.034**	0.695	0.836	0.38	**0.038**	**0.006**
HNA	**0.032**	0.825	0.905	0.365	**0.037**	**0.014**
HNA/LNA	**0.036**	0.772	0.905	0.386	**0.048**	**0.016**
Overall substrates	**0.001***	**0.003***	**0.011**			
Carbohydrates	**0.028**	**0.003**	**0.001**			
Polymers	**0.001***	**0.004**	**0.032**			
Carboxylic acids	0.052	**0.006***	**0.024**			
Aminoacids	**0.021***	**0.002**	**0.06**			
Amines	0.127	0.134	0.338			
Crustaceans						
All species	**0.015***	0.754	0.869			
SB	**0.002**	0.615	0.382	0.83	**0.002**	**0.003**
Overall locomotion	**0.001**	0.169	0.646	0.857	**0.001**	**0.001**
burrower	**0.001**	0.434	0.575	0.667	**0.001**	**0.001**
interstitial	0.737	0.45	0.724			
swimmer	0.422	0.216	0.472			
Overall diet	**0.006***	0.457	0.919			
fine sediment + microorg.	**0.007***	0.288	0.728			
living microphytes	0.152	0.999	0.999			
Overall feeding habits	0.111	0.55	0.841			
deposit-feeder	0.078	0.423	0.575			
collector	0.217	0.407	0.513			
grazer	0.153	0.998	0.997			
Overall thermal tolerance	**0.006**	0.161	0.915	0.844	**0.004**	**0.014**
eurythermal	**0.003**	0.217	0.822	0.952	**0.007**	**0.013**
moderately stenothermal	0.443	0.204	0.451			
stenothermal	0.331	0.259	0.597			

Table S2. Results of DistLM (Distance-based Linear Model) tests examining linear relationships between environmental and microbial factors, and crustacean assemblage characteristics. DistLM tests were conducted to assess the associations between a range of environmental and microbial predictor variables and three response variables: overall crustacean assemblage composition, abundances of groundwater-obligate species, and the composition of the assemblage in terms of functional traits.

Conditional DistLM tests report AICc (Akaike Information Criterion corrected for small sample sizes) and R^2 (explained variance) values. Abbreviations as in Table S2.

Var 1	**Var 2**	**AICc**	**R2**	**N. var**	**Var**
Taxonomic composition	Water Table	145.31	0.15	1	WT
Taxonomic composition	Field parameters	141.08	0.32	1	EC
Taxonomic composition	Major ions	136.66	0.52	2	SO4; Ca
Taxonomic composition	Trace elements	137.43	0.71	5	V, Cr, tot, As, Se, U
Taxonomic composition	Mirobial factors	139.07	0.36	1	HNA
Groundwater-obligate abundances	Water Table	132.51	0.25	1	WT
Groundwater-obligate abundances	Field parameters	127.4	0.49	2	EC, pH
Groundwater-obligate abundances	Major ions	99.38	0.91	4	NO3, SO4, K, Ca
Groundwater-obligate abundances	Trace elements	11.54	0.89	5	B, Al, V, Se, Ba
Groundwater-obligate abundances	Mirobial factors	130.42	0.71	3	CARB, LNA
Locomotion	Water Table	102.31	0.03	1	WT
Locomotion	Field parameters	100.54	0.11	1	ORP
Locomotion	Major ions	101.4	0.07	1	DOC
Locomotion	Trace elements	100.77	0.1	1	Rb
Locomotion	Mirobial factors	100.01	0.25	2	CARB_A, AMIN
Diet	Water Table	122.39	0.02	1	WT
Diet	Field parameters	117.84	0.36	2	pH, EC
Diet	Major ions	110.67	0.62	3	SO4, K, Ca
Diet	Trace elements	109.42	0.81	6	Li, B, Cr tot, Ni, As tot, U
Diet	Mirobial factors	120.34	0.28	2	CARB, AMMI
Feeding habits	Water Table	123.18	0.02	1	WT
Feeding habits	Field parameters	119.58	0.33	2	pH, EC
Feeding habits	Major ions	113.66	0.58	3	SO4, K, Ca
Feeding habits	Trace elements	113.6	0.77	6	Li, B, Cr tot, Ni, As tot, U
Feeding habits	Mirobial factors	115.52	0.19	1	CARB
Thermal tolerance	Water Table	119.39	0.02	1	WT
Thermal tolerance	Field parameters	111.46	0.43	2	pH, EC
Thermal tolerance	Major ions	113.28	0.38	2	HCO3, Na
Thermal tolerance	Trace elements	110.07	0.62	2	Li, B, As tot, Rb
Thermal tolerance	Mirobial factors	115.62	0.19	1	POLY

Marginal DistLM tests reported with R^2 and r (Pearson correlation coefficient) values. Significant relationships ($p < 0.05$) are highlighted in bold. Correlation signs are shown for significant models (P: p-values; significant p-values < 0.05). Abbreviations as in Table S2. TAX: taxonomic composition; LOC: locomotion trait.

	TAX				*GW-OBLIGATE*				*LOC*				*DIET*				*FEEDING HABITS*				*THERMAL TOLERANCE*			
	P	**R^2**	**r**		**P**	**R^2**	**r**		**P**	**R^2**	**r**		**P**	**R^2**	**r**		**P**	**R^2**	**r**		**P**	**R^2**	**r**	
WT	0.027	0.15	0.39		0.131	0.25	0.50		0.668	0.03	0.18		0.187	0.08	0.28		0.209	0.01	0.08		0.582	0.03	0.16	
ORP	0.3	0.06	0.25		0.141	0.11	0.34		0.115	0.11	0.34		0.463	0.04	0.19		0.539	0.03	0.18		0.507	0.03	0.18	
T	0.498	0.05	0.22		0.631	0.01	0.11		0.51	0.03	0.18		0.319	0.06	0.24		0.348	0.06	0.24		0.113	0.12	0.34	
pH	0.054	0.13	0.36		0.251	0.07	0.27		0.489	0.03	0.16		0.871	0.01	0.08		0.832	0.01	0.11		0.681	0.02	0.14	
DO	0.055	0.14	0.37		0.056	0.17	0.41		0.579	0.03	0.16		0.213	0.08	0.27		0.215	0.08	0.28		0.179	0.08	0.29	
EC	**0.002**	**0.32**	**0.56**	+	**0.004**	**0.38**	**0.62**	+	0.562	0.02	0.15		0.062	0.15	0.39		0.067	0.15	0.39		**0.007**	**0.26**	**0.51**	+
F	0.107	0.10	0.32		**0.026**	**0.22**	**0.47**	+	0.628	0.02	0.13		**0.038**	0.19	**0.44**	+	**0.034**	0.17	**0.42**	+	0.706	0.02	0.13	
Cl	0.205	0.08	0.28		0.144	0.10	0.32		0.789	0.02	0.14		0.305	0.06	0.25		0.333	0.06	0.24		0.274	0.07	0.26	
NO3	0.384	0.05	0.23		0.323	0.05	0.23		0.593	0.02	0.15		0.552	0.03	0.17		0.616	0.03	0.17		0.219	0.08	0.28	
SO4	**0.001**	**0.34**	**0.58**	+	**0.001**	**0.41**	**0.64**	+	0.879	0.00	0.07		**0.046**	**0.14**	**0.38**	+	**0.048**	0.14	**0.38**	+	**0.029**	0.16	**0.40**	+
HCO3	**0.008**	**0.23**	**0.48**	+	**0.021**	**0.23**	**0.48**	+	0.731	0.02	0.14		0.171	0.09	0.30		0.185	0.09	0.30		**0.026**	0.21	**0.45**	+
Na	**0.002**	**0.25**	**0.50**	+	**0.002**	**0.48**	**0.69**	+	0.898	0.01	0.09		**0.003**	0.30	**0.55**	+	**0.006**	0.27	**0.52**	+	**0.008**	0.23	**0.48**	+
Mg	0.08	0.11	0.33		0.187	0.09	0.30		0.678	0.02	0.13		0.32	0.06	0.24		0.315	0.05	0.23		0.131	0.11	0.33	
K	0.56	0.04	0.19		0.222	0.09	0.29		0.426	0.04	0.21		**0.004**	0.28	**0.53**	-	**0.011**	0.24	**0.49**	-	0.185	0.08	0.28	
Ca	0.077	0.11	0.34		0.501	0.03	0.17		0.999	0.00	0.00		0.65	0.02	0.14		0.663	0.02	0.15		0.311	0.07	0.26	
Si	0.78	0.02	0.15		0.374	0.05	0.21		0.315	0.07	0.26		0.888	0.01	0.07		0.926	0.01	0.08		0.777	0.01	0.12	
DOC	0.873	0.02	0.13		0.838	0.01	0.07		0.231	0.08	0.28		0.409	0.05	0.21		0.41	0.05	0.21		0.672	0.02	0.14	
Li	0.066	0.12	0.35		**0.009**	0.31	**0.55**	+	0.364	0.03	0.18		**0.035**	0.20	**0.45**	+	**0.037**	**0.18**	**0.42**	+	0.187	0.09	0.30	
B	**0.016**	**0.18**	**0.42**	+	**0.007**	0.34	**0.59**	+	0.531	0.04	0.20		**0.018**	0.24	**0.49**	+	**0.033**	**0.21**	**0.46**	+	**0.003**	**0.37**	**0.61**	+
Al	0.648	0.03	0.18		0.459	0.04	0.19		0.511	0.03	0.16		0.419	0.04	0.21		0.426	0.04	0.20		0.796	0.01	0.11	
V	0.455	0.04	0.21		0.879	0.00	0.06		0.61	0.03	0.19		0.521	0.03	0.18		0.444	0.04	0.20		0.823	0.01	0.11	
Cr tot	**0.003**	**0.25**	**0.50**	-	**0.006**	0.38	**0.62**	-	0.887	0.01	0.09		**0.042**	0.18	**0.42**	-	**0.028**	**0.19**	**0.43**	-	0.661	0.02	0.15	
Mn	0.586	0.03	0.17		0.415	0.05	0.22		0.564	0.01	0.09		0.352	0.05	0.22		0.453	0.04	0.21		0.155	0.09	0.29	
Fe	0.576	0.04	0.20		0.322	0.06	0.25		0.643	0.01	0.10		0.441	0.05	0.22		0.489	0.04	0.21		0.193	0.09	0.30	
Ni	0.185	0.09	0.31		**0.028**	0.14	**0.38**	+	0.677	0.00	0.06		0.487	0.04	0.20		0.38	0.04	0.20		0.528	0.04	0.21	
Cu	**0.005**	**0.21**	**0.45**	+	**0.026**	0.25	**0.50**	+	0.659	0.02	0.13		**0.045**	0.15	**0.39**	+	0.076	0.14	0.38		**0.031**	**0.18**	**0.42**	+
Zn	0.249	0.07	0.26		0.109	0.12	0.34		0.457	0.02	0.14		0.16	0.10	0.32		0.169	0.10	0.31		**0.09**	**0.13**	**0.36**	+
As tot	**0.002**	**0.26**	**0.51**	+	**0.002**	0.49	**0.70**	+	0.64	0.03	0.18		**0.007**	0.31	**0.56**	+	**0.006**	**0.29**	**0.54**	+	0.116	0.10	0.32	
Se	**0.039**	**0.14**	**0.38**	+	0.153	0.11	0.33		0.942	0.00	0.06		0.479	0.04	0.19		0.527	0.03	0.18		0.513	0.04	0.20	
Rb	**0.008**	**0.22**	**0.47**	+	**0.001**	0.56	**0.75**	+	0.155	0.10	0.32		**0.002**	0.32	**0.57**	+	**0.006**	**0.28**	**0.53**	+	**0.038**	**0.19**	**0.44**	+
Sr	0.232	0.07	0.27		0.784	0.01	0.09		0.985	0.00	0.04		0.762	0.01	0.11		0.731	0.02	0.13		0.354	0.05	0.23	
Ba	0.396	0.05	0.23		0.089	0.15	0.39		0.359	0.06	0.24		0.209	0.08	0.29		0.256	0.07	0.27		0.407	0.04	0.21	
Pb	0.819	0.02	0.12		0.692	0.02	0.13		0.29	0.03	0.18		0.905	0.01	0.08		0.927	0.01	0.08		0.894	0.01	0.09	
U	**0.012**	**0.22**	**0.46**	+	0.099	0.14	0.37		0.91	0.01	0.09		0.141	0.11	0.33		0.146	0.10	0.32		**0.01**	**0.26**	**0.51**	+
CARB	0.666	0.03	0.17		0.641	0.01	0.12		0.342	0.06	0.25		0.445	0.04	0.20		0.347	0.05	0.22		0.347	0.05	0.22	
POLY	0.152	0.10	0.31		0.139	0.11	0.33		0.966	0.00	0.05		0.212	0.09	0.29		**0.02**	**0.19**	**0.44**	-	**0.02**	**0.19**	**0.44**	+
CARB_A	0.213	0.07	0.27		0.054	0.17	0.41		0.195	0.10	0.31		0.143	0.11	0.34		0.413	0.04	0.20		0.413	0.04	0.20	
AMMI	0.139	0.09	0.30		0.315	0.06	0.24		0.608	0.04	0.20		0.344	0.05	0.23		0.062	0.15	0.39		0.062	0.15	0.39	
AMIN	0.191	0.08	0.28		0.383	0.05	0.21		0.054	0.11	0.34		0.852	0.01	0.09		0.501	0.03	0.18		0.501	0.03	0.18	
TCC	**0.012**	**0.19**	**0.44**	+	**0.022**	0.25	**0.50**	+	0.638	0.02	0.14		0.379	0.05	0.22		0.321	0.06	0.24		0.057	0.15	0.39	
LNA	**0.001**	**0.37**	**0.60**	+	**0.001**	0.66	**0.81**	+	0.568	0.04	0.20		**0.018**	**0.25**	**0.50**	+	**0.012**	**0.24**	**0.49**	+	0.071	0.16	0.40	
HNA	**0.001**	**0.37**	**0.60**	-	**0.001**	0.60	**0.78**	-	0.579	0.04	0.20		**0.013**	**0.24**	**0.49**	-	**0.024**	**0.23**	**0.48**	-	0.05	0.16	0.40	