Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Supplementary information

Fecal pollution source characterization in the surface waters of recharge and contributing zones of a karst aquifer using general and host-associated fecal genetic markers

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Table S1. Description and land-use information of sampling sites selected for the current study.

Sampling Site	Latitude	Longitude	Zone	Site description
Leon Creek-1 (L-1)	29.5422	-98.6320	Recharge	Located in a residential area and the furthest downstream side of the Edward's Aquifer recharge zone. The waste discharge point is located at around 100m from this site.
Leon Creek-2 (L-2)	29.6489	-98.6216	Contributing	Located near Dominion neighborhood and the Dominion Country Club and golf course; A waste discharge point is located about 10m to the North.
Leon Creek-3 (L-3)	29.6494	-98.6219	Contributing	Located about 100m upstream of the L-2 and near Dominion neighborhood and the Dominion Country Club and golf course; A waste discharge point is located about 10m to the South.
Leon Creek-4 (L-4)	29.5793	-98.6074	Contributing Zone within Transition Zone	Located near the University of Texas at San Antonio (UTSA) and next to a student housing apartment complex; An outfall is located about 100m NE of the sampling site.
Balcones Creek-1 (B-1)	29.7331	-98.7035	Contributing	Located in a rural area in the northern boundary of Bexar County.
Balcones Creek-2 (B-2)	29.7385	-98.6880	Contributing	Located in a rural area in the northern boundary of Bexar County; about 1.5km from the B-1 site.
Balcones Creek-3 (B-3)	29.7411	-98.6446	Recharge	Located near the City of Fair Oaks Ranch in the northern boundary of Bexar County.
Balcones Creek- 4 (B-4)	29.7420	-98.6456	Recharge	Located near the City of Fair Oaks Ranch in the northern boundary of Bexar County; Around 1km from site B-3. Cibolo creek converges with Balcones Creek about 1.5km upstream of this site.
San Geronimo Creek- 1 (S-1)	29.6207	-98.7946	Contributing	Located on the farthest west side to Bexar County and samples were collected from the east side stream of the creek (to the east of Bandera Road).
San Geronimo Creek- 2 (S-2)	29.6208	-98.7956	Contributing	Located on the farthest west side to Bexar County and samples were collected from the west side upstream of the creek that convergence with the S-1 site.
Helotes Creek-1 (H-1)	29.6061	-98.6859	Contributing	Located in a rural village. This site is closer (<2mi.) to Edwards aquifer recharge zone.

Table S2. List of primers and probes used in this study.

Assay	Primer/probe sequences (5'-3') [a]	Reference
E. coli (EC23S857)	F: GGTAGAGCACTGTTTTGGCA R: TGTCTCCCGTGATAACTTTCTC P: FAM-TCATCCCGACTTACCAACCCG-TAMRA	Chern et al., 2011[1]
General *Enterococcus* (Entero1)	F: AGAAATTCCAAACGAACTTG R: CAGTGCTCTACCTCCATCATT P: FAM-TGGTTCTCTCCGAAATAGCTTTAGGGCTA-TAMRA	Ludwig & Schleifer, 2000[2]
Universal *Bacteroidales* (BacUni)	F: CGTTATCCGGATTTATTGGGTTTA R: CAATCGGAGTTCTTCGTGATATCTA P: FAM-TGGTGTAGCGGTGAAA-TAMRA-MGB	Kildare et al., 2007[3]
Human-associated *Bacteroidales* (BacHum)	F: TGAGTTCACATGTCCGCATGA R: CGTTACCCCGCCTACTATCTAATG P: FAM-TCCGGTAGACGATGGGGATGCGTT-TAMRA	Kildare et al., 2007[3]
Human-associated *Bacteroidales* (HF183)	F: ATCATGAGTTCACATGTCCG R: CGTAGGAGTTTGGACCGTGT P: FAM-CTGAGAGGAAGGTCCCCCACATTGGA-TAMRA	Haugland et al., 2010[4]
Cow-associated *Bacteroidales* (BacCow)	F: CCAACCTTCCCGATACTC R: GGACCGTGTCTCAGTTCCAGTG P: FAM-TAGGGGTTCTGAGAGGAAGGTCCCCC-TAMRA	Kildare et al., 2007[3]
Ruminant-associated *Bacteroidales* (Rum2Bac)	F: ACAGCCCGCGATTGATACTGGTAA R: CAATCGGAGTTCTTCGTGAT P: FAM-ATGAGGTGGATGGAATTCGTGGTGT-TAMRA	Mieszkin et al., 2010[5]
Dog-associated *Bacteroidales* (BacCan)	F: GGAGCGCAGACGGGTTTT R: CAATCGGAGTTCTTCGTGATATCTA P: FAM-TGGTGTAGCGGTGAAA-MGB	Kildare et al., 2007[3]
Chicken-Duck associated *Bacteroidales* (Bird-specific)	F: AATATTGGTCAATGGGCGAGAG R: CACGTAGTGTCCGTTATTCCCTTA P: FAM-TCCTTCACGCTACTTGG-MGB	Kobayashi et al., 2013[6]
Avian associated marker (GFD)	F: TCGGCTGAGCACTCTAGGG R: GCGTCTCTTTGTACATCCCA	Green et al., 2012[7]

[a] F, forward primer; R, reverse primer; P, probe.

Table S3. Precipitation (inches) recorded at different sites during each sampling event (rainfall received within 7 days before sampling event).

Sampling date	L-1	L-2	L-3	L-4	B-1	B-2	B-3	B-4	S-1	S-2	H-1
3/1/2019	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.1
3/18/2019	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.1
3/29/2019	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4/12/2019	0.2	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.0	1.0	0.7
4/26/2019	0.1	0.8	0.8	0.8	0.8	0.8	0.8	0.8	1.7	1.7	0.1
5/10/2019	0.6	2.2	2.2	2.2	2.2	2.2	2.2	2.2	3.1	3.1	0.0
5/24/2019	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.5	0.5	0.0
6/7/2019	0.7	2.5	2.5	2.5	2.5	2.5	2.5	2.5	0.6	0.6	0.0
6/21/2019	0.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.8	1.8	0.0
7/8/2019	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0
7/19/2019	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8/2/2019	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8/16/2019	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0
8/30/2019	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.3	0.3	0.0
9/13/2019	0.3	1.6	1.6	1.6	1.6	1.6	1.6	1.6	0.1	0.1	0.0
9/27/2019	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10/11/2019	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0
10/25/2019	1.0	6.9	6.9	6.9	6.9	6.9	6.9	6.9	1.8	1.8	0.2
11/8/2019	0.3	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.1	0.1	0.1
11/22/2019	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
12/6/2019	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
12/20/2019	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
1/10/2020	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
1/24/2020	0.2	0.7	0.7	0.7	0.7	0.7	0.7	0.7	1.1	1.1	0.3
2/7/2020	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

2/21/2020	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0
3/6/2020	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.6	0.6	0.1

Table S4. Land-use variables within 1 km radius of study sites.

Site	Septic tank density (tanks/km2)	Human population	Impervious surface (%)
L-1	15	16776	32
L-2	30	1488	15
L-3	30	1488	15
L-4	1	4401	31
B-1	10	850	6
B-2	9	722	6
B-3	39	452	7
B-4	36	801	7
S-1	1	2238	11
S-2	1	2200	11
H-1	51	8670	15

Table S5.1 Nitrate (NO_3-N) concentrations (mg/L) in the water samples collected from different sites of the study area.

Sampling date	L-1	L-2	L-3	L-4	B-1	B-2	B-3	B-4	S-1	S-2	H-1
03/01/2019	BDL	1.12	1.26	0.60	BDL	0.43	BDL	BDL	BDL	0.27	0.53
03/18/2019	BDL	BDL	BDL	0.77	BDL	BDL	0.30	BDL	BDL	BDL	BDL
03/29/2019	BDL	0.44	0.32	0.45	BDL	0.46	BDL	BDL	BDL	0.24	0.27
04/12/2019	0.25	BDL	BDL	0.55	BDL	BDL	BDL	BDL	BDL	BDL	0.34
04/26/2019	0.27	0.36	0.48	BDL	BDL	BDL	0.88	0.56	BDL	0.42	0.22
05/10/2019	0.52	0.69	0.66	0.58	0.29	0.46	0.57	0.45	BDL	BDL	0.73
05/24/2019	BDL	1.30	1.51	0.23	0.23	0.31	BDL	BDL	BDL	BDL	0.71
06/07/2019	BDL	BDL	0.41	0.57	BDL	0.25	BDL	BDL	BDL	BDL	0.29
06/21/2019	0.27	BDL	0.28	BDL	BDL	BDL	BDL	BDL	BDL	0.29	0.26
07/08/2019	0.72	BDL	0.53	0.24	0.23	BDL	BDL	BDL	BDL	BDL	BDL
07/19/2019	BDL	0.20	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.22	0.52
08/02/2019	0.23	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.26
08/16/2019	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.23	BDL	BDL	BDL
08/30/2019	BDL	BDL	BDL	0.13	BDL	BDL	BDL	BDL	BDL	BDL	BDL
09/13/2019	0.29	0.38	0.56	0.64	BDL	0.48	BDL	0.24	BDL	BDL	0.63
09/27/2019	0.32	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
10/11/2019	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
10/25/2019	1.48	0.73	0.67	1.18	BDL	1.09	BDL	1.42	BDL	BDL	BDL
11/08/2019	0.47	0.30	0.60	0.42	0.07	0.10	0.07	0.08	0.08	0.09	0.14
11/22/2019	0.07	0.19	0.29	0.28	0.07	0.09	0.07	BDL	0.07	0.07	0.14
12/06/2019	BDL	BDL	0.11	0.48	BDL	BDL	BDL	BDL	BDL	BDL	BDL
12/20/2019	BDL	BDL	0.07	0.41	0.13	0.07	BDL	BDL	BDL	BDL	BDL
01/10/2020	BDL	BDL	BDL	0.45	BDL		BDL	BDL	0.07	BDL	0.16
01/24/2020	0.06	0.07	0.17	0.15	0.06	0.14	0.07	0.07	0.07	0.09	0.13
02/07/2020	0.07	BDL	0.17	0.11	0.06	0.07	0.16	BDL	0.07	0.12	0.08
02/21/2020	BDL	0.05	0.09	0.12	BDL	BDL	0.07	BDL	BDL	BDL	BDL
03/06/2020	BDL	BDL	0.29	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

BDL, Below detection limits.

Table S5.2 Nitrite (NO_2-N) concentrations (mg/L) in the water samples collected from different sites of the study area.

Sampling date	L-1	L-2	L-3	L-4	B-1	B-2	B-3	B-4	S-1	S-2	H-1
03/01/2019	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
03/18/2019	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
03/29/2019	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
04/12/2019	BDL	BDL	BDL	0.02	BDL	BDL	BDL	BDL	BDL	BDL	BDL
04/26/2019	BDL	0.02	0.03	0.03	BDL	BDL	0.02	0.02	BDL	0.02	0.01
05/10/2019	BDL	BDL	0.02	0.02	BDL	BDL	0.02	BDL	BDL	BDL	BDL
05/24/2019	BDL	0.03	0.04	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
06/07/2019	BDL	0.03	0.02	0.03	BDL	BDL	BDL	BDL	BDL	BDL	BDL
06/21/2019	0.03	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
07/08/2019	BDL	BDL	0.02	BDL	0.02	BDL	BDL	BDL	BDL	BDL	BDL
07/19/2019	BDL	0.02	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
08/02/2019	BDL	BDL	BDL	BDL	BDL	BDL	0.03	BDL	BDL	BDL	BDL
08/16/2019	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
08/30/2019	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
09/13/2019	BDL	0.04	0.04	0.04	BDL	0.02	BDL	BDL	BDL	BDL	BDL
09/27/2019	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
10/11/2019	0.42	BDL	BDL	0.58	BDL	BDL	BDL	BDL	BDL	BDL	BDL
10/25/2019	BDL	BDL	BDL	BDL	BDL	0.05	BDL	BDL	BDL	BDL	BDL
11/08/2019	0.12	0.12	0.12	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
11/22/2019	BDL	0.12	0.12	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
12/06/2019	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
12/20/2019	BDL	BDL	BDL	BDL	BDL	0.04	BDL	0.09	BDL	BDL	BDL
01/10/2020	BDL	BDL	BDL	0.08	BDL		BDL	BDL	BDL	BDL	BDL
01/24/2020	0.12	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
02/07/2020	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
02/21/2020	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
03/06/2020	BDL	BDL	0.02	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

BDL, Below detection limits.

Table S5.3 Ammonia nitrogen (NH_4-N) concentrations (mg/L) in the water samples collected from different sites of the study area.

Sampling date	L-1	L-2	L-3	L-4	B-1	B-2	B-3	B-4	S-1	S-2	H-1
03/01/2019	BDL	BDL	BDL	0.02	BDL	BDL	0.02	BDL	BDL	BDL	BDL
03/18/2019	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.02
03/29/2019	0.02	BDL	BDL	BDL	BDL	BDL	BDL	BDL	0.05	0.03	BDL
04/12/2019	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
04/26/2019	BDL	0.03	0.03	BDL	BDL	BDL	0.03	0.02	0.02	0.11	0.16
05/10/2019	BDL	0.02	BDL	0.02	BDL	0.02	0.04	0.02	BDL	BDL	0.02
05/24/2019	BDL	0.03	0.02	0.03	0.02	BDL	BDL	BDL	BDL	BDL	0.03
06/07/2019	0.04	BDL	0.05	0.02	0.02	0.02	BDL	BDL	BDL	0.02	0.02
06/21/2019	0.03	0.02	BDL	0.02	0.02	BDL	BDL	0.02	0.02	0.03	0.03
07/08/2019	BDL	BDL	BDL	0.02	0.02	BDL	BDL	BDL	BDL	0.20	BDL
07/19/2019	0.05	BDL	0.02	0.02	BDL	BDL	BDL	BDL	BDL	0.02	0.02
08/02/2019	0.04	0.02	BDL	0.03	BDL	0.02	0.03	0.02	BDL	0.02	BDL
08/16/2019	0.02	0.16	BDL	0.03	BDL	BDL	BDL	BDL	BDL	0.02	0.02
08/30/2019	0.03	0.03	0.02	0.10	BDL	BDL	BDL	BDL	BDL	0.02	0.03
09/13/2019	0.04	0.02	0.03	0.15	0.04	0.06	0.03	0.09	BDL	BDL	0.03
09/27/2019	0.02	BDL	BDL	0.07	0.02	0.15	BDL	0.03	BDL	0.02	0.04
10/11/2019	0.04	0.22	BDL	0.09	BDL	0.09	0.02	0.79	BDL	BDL	0.04
10/25/2019	0.03	BDL	BDL	BDL	BDL	0.18	BDL	BDL	BDL	BDL	BDL
11/08/2019	BDL	BDL	BDL	BDL	BDL	0.16	BDL	BDL	BDL	BDL	BDL
11/22/2019	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
12/06/2019	BDL	0.03	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
12/20/2019	BDL	0.02	BDL	0.09	BDL	0.05	0.03	BDL	BDL	BDL	0.04
01/10/2020	BDL	0.04	0.04	0.02	0.02		0.02	0.03	0.03	0.03	0.03
01/24/2020	BDL	BDL	BDL	0.03	BDL	BDL	BDL	BDL	BDL	BDL	BDL
02/07/2020	BDL	BDL	BDL	BDL	BDL	BDL	0.07	0.06	BDL	BDL	BDL
02/21/2020	BDL	BDL	BDL	BDL	BDL	BDL	0.11	BDL	BDL	BDL	BDL
03/06/2020	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

BDL, Below detection limits.

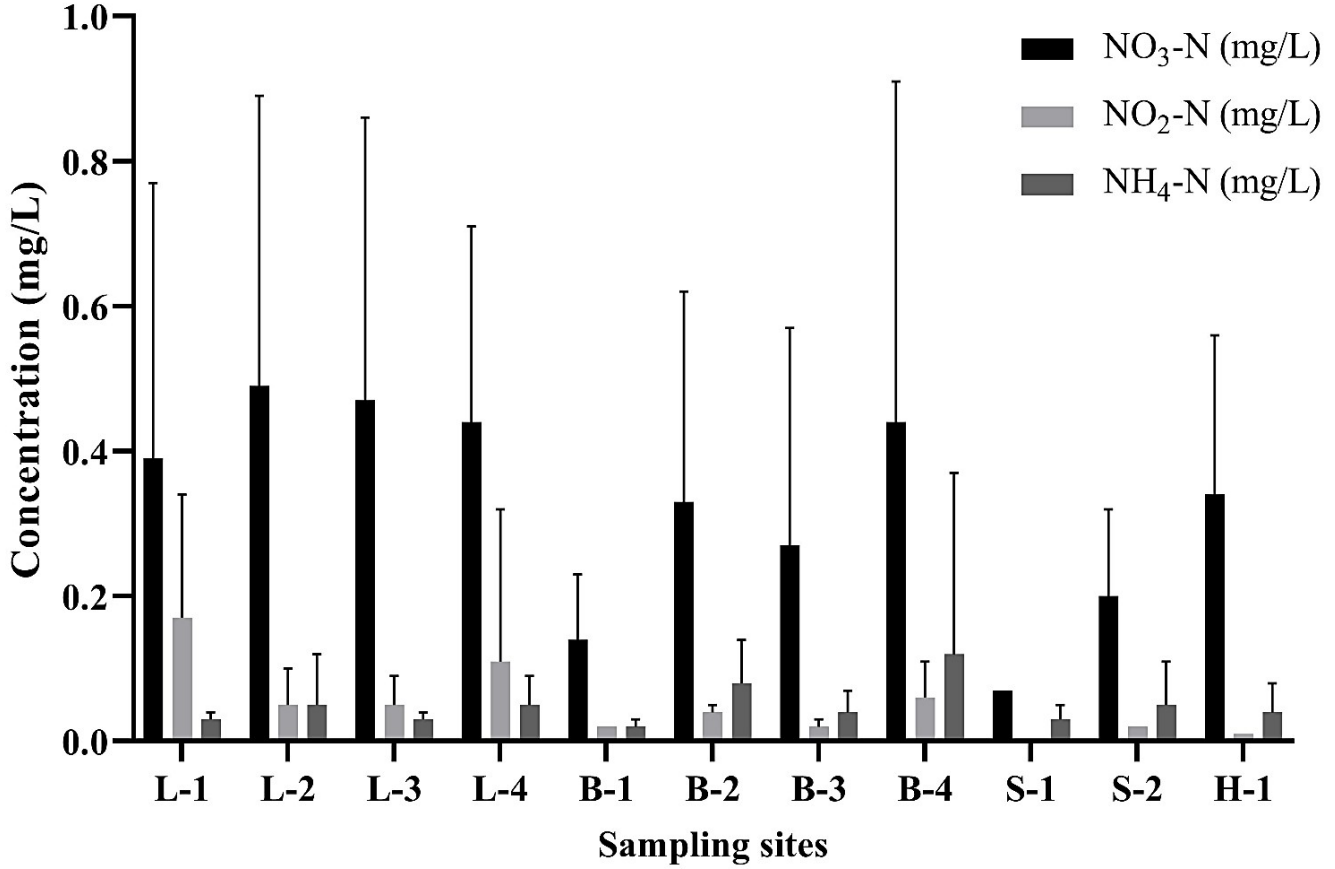

Figure S1. Median concentration (mg/L) of nitrate (NO3-N), nitrite (NO2-N), and ammonia (NH4-N) detected in the water samples collected from different sites of Leon (L), Balcones (B), San Geronimo (S), and Helotes (H) creeks.

Table S6. Performance characteristics (range) of qPCR assays were carried out in this study.

Assay	Efficiency (%)	R^2	Slope	LOQ (GC/reaction)	LOD[a] (GC/reaction)
E. coli	98.4 to 107	0.985 to 0.998	-3.16 to -3.36	10	3
Entero1	93.2 to 99.7	0.996 to 0.999	-3.33 to -3.49	20	3
BacUni	95.8 to 109	0.980 to 0.990	-3.10 to -3.31	10	3
BacHum	97.0 to 102	0.996 to 0.998	-3.25 to -3.39	10	3
HF183	92.3 to 99.8	0.984 to 0.999	-3.35 to -3.52	10	3
Rum2Bac	97.1 to 105	0.980 to 0.989	-3.19 to -3.39	10	3
BacCow	91.6 to 104	0.976 to 0.998	-3.22 to -3.54	10	3
BacCan	89.4 to 101	0.993 to 0.996	-3.30 to -3.60	10	3
Chicken/Duck-Bac	97.0 to 99.5	0.997 to 0.999	-3.35 to -3.39	10	3
GFD	91.2 to 102	0.997 to 0.998	-3.28 to -3.55	10	3

[a] based on according to [8] and [9].

Table S7. Correlation (Pearson's) between nutrients, FIB and MST markers detected in all sampling sites of Edwards aquifer.

	NO_2-N	**NO_3-N**	**NH_4-N**	***E.coli***	***Entero1***	**BacUni**	**BacHum**	**HF183**	**Rum2Bac**	**BacCow**	**BacCan**	**CDBac**	**GFD**
NO_2-N	1												
NO_3-N	0.554	1											
NH_4-N	0.042	0.377	1										
E.coli	0.362	0.354	0.012	1									
Entero1	0.462	0.199	-0.245	**.678***	1								
BacUni	0.307	0.464	0.204	**.884****	0.542	1							
BacHum	0.544	0.352	0.602	0.205	0.177	0.308	1						
HF183	0.397	0.561	0.270	0.345	0.277	0.348	0.583	1					
Rum2Bac	0.399	-0.043	0.356	-0.158	-0.005	-0.168	**.802****	0.169	1				
BacCow	0.353	0.533	**.614***	0.079	0.296	0.259	**.645***	0.333	0.394	1			
BacCan	0.295	**.672***	0.296	0.393	0.371	0.481	0.150	0.452	-0.351	**.626***	1		
CDBac	0.113	**.804****	0.419	0.286	0.214	0.336	0.136	0.509	-0.234	0.578	**.755***	1	
GFD	-0.374	0.118	0.000	0.336	0.205	**.614***	-0.118	0.120	-0.452	0.036	0.216	0.229	1

* Correlation is significant at $p < 0.05$; ** Correlation is significant at $p < 0.01$ level; CD-Bac, Chicken/Duck-Bac.

Table S8.1 Correlation [a] (Pearson's) between nutrients, FIB and MST markers in sampling sites clustered based on precipitation data.

Cluster-1			**Cluster-2**			**Cluster-3**		
Variables	***r* value**	***p* value**	**variables**	***r* value**	***p* value**	**variables**	***r* value**	***p* value**
NO_2-N - *Entero1*	-0.929	0.022	NO_3-N - *Entero1*	0.606	0.022	NO_3-N - *E. coli*	0.635	0.026
NO_3-N – BacHum	0.376	0.045	NO_3-N - BacCan	0.585	0.028	NO_3-N - BacUni	0.565	0.006
NO_3-N - Rum2Bac	0.444	0.016	NO_3-N - GFD	-0.743	0.002	NO_3-N - HF183	0.737	0.05
E. coli – BacUni	0.557	0.000	*E. coli* - BacUni	0.392	0.003	NO_3-N - CDBac	0.748	0.003
E. coli - HF183	0.506	0.000	*E. coli* - BacCow	0.344	0.011	NH_4-N - BacHum	0.948	0.001
E. coli – BacCow	0.417	0.002	*E. coli* - GFD	0.564	0.000	NH_4-N - Rum2Bac	0.830	0.021
E. coli – BacCan	0.451	0.001	*Entero1* - BacUni	0.482	0.000	NH_4-N - BacCow	0.752	0.041
E. coli – CDBac	0.293	0.032	*Entero1* - Rum2Bac	0.473	0.000	*E. coli* - *Entero1*	0.775	0.047
E. coli – GFD	0.436	0.001	*Entero1* - BacCow	0.308	0.023	*E. coli* - BacUni	0.927	0.003
Entero1 - Rum2Bac	0.421	0.002						

[a] only significantly positive or negative results were reported here.

Table S8.2 Correlation (Pearson's) between nutrients, FIB and MST markers in sampling sites clustered based on septic tank data.

Cluster-1			Cluster-2			Cluster-3		
variables	***r* value**	***p* value**	**variables**	***r* value**	***p* value**	**variables**	***r* value**	***p* value**
NO_3-N - Rum2Bac	0.322	0.019	NO_2-N - *Entero1*	-0.759	0.000	NO_3-N - *Entero1*	0.455	0.010
NO_3-N - BacCan	0.304	0.027	NO_3-N - *Entero1*	0.279	0.040	NO_3-N - Rum2Bac	0.366	0.043
NO_3-N - GFD	-0.283	0.040	NO_3-N - BacHum	0.424	0.005	NO_3-N - CDBac	-0.435	0.014
NH_4-N - *E. coli*	0.288	0.050	NO_3-N - Rum2Bac	0.321	0.036	*E. coli* - *Entero1*	0.251	0.024
E. coli - BacUni	0.503	0.000	NH_4-N - BacUni	0.360	0.045	*E. coli* - BacUni	0.548	0.000
E. coli - HF183	0.175	0.043	NH_4-N - GFD	0.597	0.000	*E. coli* - HF183	0.288	0.010
E. coli - BacCow	0.552	0.000	*E. coli* - BacUni	0.567	0.000	*E. coli* - Rum2Bac	0.347	0.002
E. coli - BacCan	0.298	0.000	*E. coli* - BacHum	0.251	0.024	*E. coli* - BacCow	0.491	0.000
E. coli – CDBac	0.196	0.023	*E. coli* - HF183	0.405	0.000	*E. coli* - BacCan	0.266	0.017
E. coli – GFD	0.545	0.000	*E. coli* - BacCow	0.555	0.000	*E. coli* - GFD	0.554	0.000
Entero1 - BacUni	0.489	0.000	*E. coli* - BacCan	0.609	0.000	*Entero1* - BacUni	0.647	0.000
Entero1 - BacHum	0.221	0.010	*E. coli* – CDBac	0.487	0.000	*Entero1* - BacHum	0.292	0.009
Entero1 - Rum2Bac	0.240	0.005	*E. coli* – GFD	0.423	0.000	*Entero1* - Rum2Bac	0.236	0.035
Entero1 - BacCow	0.178	0.041	*Entero1* - BacUni	0.278	0.012	*Entero1* - GFD	0.236	0.035
			Entero1 - BacHum	0.314	0.004			
			Entero1 - HF183	0.255	0.022			
			Entero1 - Rum2Bac	0.377	0.001			
			Entero1 - BacCan	0.267	0.016			

[a] only significantly positive or negative results were reported here.

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