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Microbial community changes induced by Managed Aquifer Recharge activities: linking hydrogeological and biological processes

Carme Barba et al.

Correspondence to: Carme Barba (carme.barba@upc.edu)

Supplementary material

Hydrochemical analyses of water samples

Samples for Cl^-, NO_3^-, SO_4^{-2} and HCO_3^- analysis were filtered through 0.2-µm nylon filters, stored at 4^oC and analyzed using high performance liquid chromatography (HPLC) with a WATERS 515 HPLC pump, IC-PAC anion columns, and a WATERS 432 detector. Samples for the determination of cations were filtered through a 0.2-µm filter, acidified in the field with 1% $HNO3^-$ and stored at 4^oC. Cations were analyzed using inductively coupled plasma-optical emission spectrometry (ICP-OES, Perkin-Elmer Optima 3200 RL). Samples for DOC analysis were filtered through a 0.45-µm nylon filter and collected in muffled (450^oC, 4.30 h) glass bottles, acidified and stored at 4^oC. In addition, water for TOC determination was sampled and stored at 4^oC. TOC and DOC were analyzed with an infrared detector using the NPOC method (Shimadzu TOC-Vcsh).

Molecular analyses for liquid and soil samples

Liquid samples were filtered through 0.22-µm GV Durapore® membrane filters (Merck Millipore, USA) and stored at -80ºC. Soil samples were centrifuged at 14.000 rpm and the liquid fraction was discarded before pellets were cold-stored at -80ºC. Total DNA extraction was conducted using PowerWater® and PowerSoil® DNA Isolation Kits (MoBio Laboratories, USA) for water (100 mL) and soil (200 mg) samples, respectively. For bacterial analyses, a 550-bp DNA fragment in the 16S region of the small-subunit ribosomal RNA gene was amplified using the primer set 341f/907r (Muyzer et al., 1993) a with a GC clamp added at the 5' end of the forward primer. Final concentrations of the PCR reactions consisted of 1x PCR buffer, 2 mM of $MgCl_2$, 200 µM of each deoxynucleoside triphosphate, 500 nM of each primer, 2.5 U of Taq DNA polymerase (Invitrogen, ThermoFisher Scientific, USA) and 10 ng of template DNA. The amplification protocol consisted of: 94ºC for 5 min; 20 cycles of 94ºC for 1 min, 65ºC for 1 min (-0.5ºC/cycle), 72ºC for 3 min; 15 cycles of 94ºC for 1 min, 55ºC for 1 min, 72ºC for 3 min; and a single final extension of 72ºC for 7 min.

Denaturing gradient gel electrophoresis (DGGE) was performed using the Dcode Universal Mutation Detection System (Bio-Rad, Spain). First, 900 ng of DNA from PCR products were loaded onto 6% (w/v) polyacrylamide gels (acrylamide/bis solution 37.5:1) containing linear chemical gradients of 30-70% denaturant. The 100% denaturing solution contained 7 M urea and 40% (v/v) deionized formamide. Gels were run in 1X Tris acetate-EDTA (TAE) for 16 h at 75 V and 60ºC, stained with 1 µg/mL ethidium bromide solution for 25 min, washed with deionized water for 25 min and photographed with Universal Hood II (Bio-Rad, Spain). DGGE images were analyzed using InfoQuest™ FP software. Dice’s coefficient and the unweighted pair group method with arithmetic averages (UPGMA) were employed for the clustering of DGGE gel profiles. Non-metric Multidimensional Scaling (NMD) was performed using the Vegan package (Oksanen et al., 2017). Prominent bands from the DGGE were excised, re-amplified and sequenced by Macrogen (South Korea). The obtained sequences were trimmed with FinchTV software and checked for chimeras using the UCHIME algorithm (Edgar et al., 2011) integrated in Mothur version 1.38 (Schloss et al., 2009). Each 16S rRNA sequence was assigned to its closest neighbor according to the Basic Local Alignment Search Tool (BLAST) results (Altschul et al., 1997). Curated sequences were deposited in the National Center for Biotechnology Information (NCBI) GenBank database under accession numbers MF471641-MF471667.

Table S1 – Summary of hydrochemical parameters at the Llobregat MAR site in both scenarios Dry and Wet.

Sampling location (depth-*masl*)	P1-*BG* (-0.6)		P8 (1)		P8 (-3)		P2 (3)		P2 (-2)		P2(-5)		P5 (2.3)		P5 (-2.2)		P3 (0.8)		P3 (-4.2)		P10 (-1)		Infiltration basin	
Scenario	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D
pH	7.05	7.36	7.45	7.13	7.39	7.27	7.30			7.34	7.28		7.39	6.98	7.42		7.15	7.15	7.15		7.28	7.35	8.47	
DOC (mg/l)	1.63	1.93	13.65	3.96	8.02	1.652	3.17			1.80	4.70		3.61	1.53	3.76		3.22	2.33	3.15		2.57	1.61	6.6	
TOC (mg/L)	2.48	1.97	7.95	4.05	13.05	1.80	1.99			2.00	3.64		2.96	1.50	2.70		2.13	1.88	2.94		3.58	1.70		
HCO_3^- (mg/L)	354.3	382.6	241.3	379.3	253.8	408.1	261.2			314.2	272.6		256.1	408.1	255.2		399.0	294.3	385.8		327.1	396.4	144.5	
NO_3^- (mg/L)	5.02	17.79	2.75	2.82	2.88	15.49	3.13			12.9	3.64		3.41	17.7	4.08		6.06	18.2	8.73		5.88	16.28	6.14	
$SO4^-_2$ (mg/L)	173.0	181.5	120.0	141.23	152.1	199.54	138.4			140.0	138.4		133.8	200.3	147.8		168.6	177.0	168.7		180.0	174.8	123.4	
Cl^- (mg/L)	224.8	212.4	267.5	186.1	262.8	193.5	267.0			211.8	212.0		274.6	191.7	269.7		241.0	193.2	241.7		265.5	189.1	245.9	
Ca (mg/L)	135.2	142.6	92.5	123.0	99.9	72.3	94.2			121.4	103.5		97.4	141.9	96.7		144.2	133.3	141.3		123.3	132.7	67.6	
Na (mg/L)	138.9	116.3	160.0	110.1	161.2	116.7	153.9			113.5	158.2		163.4	118.0	165.0		142.5	108.8	143.9		162.8	117.5	147.9	
K (mg/L)	19.6	23.7	30.0	23.0	29.4	20.7	30.2			22.9	31.0		38.6	24.4	31.9		24.2	23.9	24.4		26.3	27.5	28.0	
Mg (mg/L)	33.73	36.3	26.7	30.8	28.5	38.3	27.4			31.9	30.0		29.1	38.2	28.8		24.2	33.0	35.2		31.4	37.2	23.0	
S (mg/L)	52.2	63.5	40.5	51.2	47.2	64.3	41.7			58.1	42.6		42.9	63.4	42.7		47.7	58.7	47.9		51.4	59.9	42.2	
Fe (mg/L)	<0.2	<0.2.	<0.2	<0.2	<0.2	<0.2	<0.2			<0.2	<0.2		<0.2	<0.2	<0.2		<0.2	<0.2	<0.2		<0.2	<0.2	<0.2	
Mn (ppb)	28.9	0.87	2.46	145.30	2.77	<0.8	10.79			5.507	8.94		2.17	<0.8	1.46		3.67	0.81	1.74		1.58	<0.8	4.76	

Table S2. Sequence information corresponding to the DGGE bands obtained from bacterial populations' fingerprints in the Llobregat MAR system studied.

Phylotype		Phylogenetic affiliation		
Band code[a]	Accesion number	Taxonomic linage (Phylum, Class, Order, Family, Genus)[b]	Closest match (accesion no.)[c]	Similarity (%)[d]
B1	MF471641	Firmicutes(100); Bacilli(100); Bacillales(100); Planococcaceae(100)	Chryseomicrobium sp. (KX889925)	98
B2	MF471642	Proteobacteria(100); Betaproteobacteria(100); Neisseriales(94); Neisseriaceae(94)	*Vogesella indigofer*a (KF951043)	100
B3	MF471643	Firmicutes(100); Bacilli(100); Bacillales(100); Planococcaceae(100)	*Chryseomicrobium* sp. (KX889925)	99
B4	MF471644	Proteobacteria(94); Betaproteobacteria(87); TRA3-20(54); TRA3-20_fa(54)	Uncultured beta proteobacterium (KF182906)	94
B5	MF471645	Chloroflexi(100); Dehalococcoidia(100); vadinBA26(61); vadinBA26_fa(61)	*Dehalogenimonas alkenigignens* (JQ994267)	100
B6	MF471646	Proteobacteria(100); Gammaproteobacteria(100); Xanthomonadales(100); Xanthomonadaceae(100); *Stenotrophomonas*(98)	*Stenotrophomonas* sp. (LC136883)	97
B7	MF471647	Nitrospirae(100); Nitrospira(100); Nitrospirales(100); Nitrospiraceae(100); *Nitrospira*(100)	Uncultured bacterium (HM445209)	97
B8	MF471648	Chloroflexi(100); Dehalococcoidia(100); vadinBA26(52); vadinBA26_fa(52)	*Dehalogenimonas alkenigignens* (JQ994267)	100
B9	MF471649	Firmicutes(100); Bacilli(100); Bacillales(100); Planococcaceae(100)	*Chryseomicrobium* sp. (KX889925)	99
B10	MF471650	Cyanobacteria(100); Cyanobacteria(100); SubsectionI(100);	Uncultured	99

		FamilyI(100)	cyanobacterium (FJ916292)	
B11	MF471651	Cyanobacteria(100); Cyanobacteria(100); SubsectionI(100); FamilyI(100)	Uncultured cyanobacterium (FJ916292)	97
B12	MF471652	Proteobacteria(97); Betaproteobacteria(86); Nitrosomonadales(38); Nitrosomonadaceae(34)	Uncultured Burkholderiaceae (AM935619)	94
B13	MF471653	Proteobacteria(100); Betaproteobacteria(100); Nitrosomonadales(89); Nitrosomonadaceae(89); uncultured(88)	Uncultured Burkholderiaceae (AM935619)	97
B14	MF471654	Proteobacteria(100); Betaproteobacteria(100); TRA3-20(100); TRA3-20_fa(100); TRA3-20_ge(100)	Uncultured beta proteobacterium (EU979071)	99
B15	MF471655	Proteobacteria(99); Betaproteobacteria(94); Hydrogenophilales(47); Hydrogenophilaceae(47)	Uncultured beta proteobacterium (JN868168)	92
B16	MF471656	Proteobacteria(82); Betaproteobacteria(56); Nitrosomonadales(20); Nitrosomonadaceae(20)	Uncultured beta proteobacterium (AM935274)	88
B17	MF471657	Nitrospirae(100); Nitrospira(100); Nitrospirales(100); Nitrospiraceae(100); *Nitrospira*(100)	Uncultured Nitrospiraceae (EU298577)	97
B18	MF471658	Bacteroidetes(100); Cytophagia(100); Cytophagales(100); Cytophagaceae(85); *Pontibacter*(84)	Uncultured Bacteroidetes (HF564274)	99
B19	MF471659	Chlorobi(80); Chlorobia(80); Chlorobiales(80); OPB56(80); OPB56_ge(80)	Uncultured bacterium (KC666711)	99
B20	MF471660	Actinobacteria(99); Actinobacteria(99); Frankiales(99); Sporichthyaceae(99); Sporichthyaceae_ge(99)	Uncultured actinobacterium (LC018957)	99
B21	MF471661	Actinobacteria(93); Actinobacteria(93); Micrococcales(86); Microbacteriaceae(77)	Uncultured actinobacterium (LC018957)	96

B22	MF471662	Acidobacteria(91); Solibacteres(91); Solibacterales(91); Solibacteraceae_(Subgroup_3)(91)	Uncultured Acidobacteria (KM016273)	97
B23	MF471663	Proteobacteria(100); Betaproteobacteria(100); Burkholderiales(99); Comamonadaceae(99)	Uncultured bacterium (EU465081)	95
B24	MF471664	Proteobacteria(100); Betaproteobacteria(100); Burkholderiales(94); Comamonadaceae(79)	Uncultured Comamonadaceae (LT679549)	94
B25	MF471665	Proteobacteria(96); Alphaproteobacteria(90); Rhizobiales(75); Rhizobiales_Incertae_Sedis(25)	Uncultured alpha proteobacterium (HF584680)	98
B26	MF471666	Proteobacteria(100); Betaproteobacteria(100); Burkholderiales(100); Comamonadaceae(100); *Aquabacterium*(100)	Uncultured bacterium (KF065163)	99
B27	MF471667	Bacteria(100); Proteobacteria(100); Betaproteobacteria(100); Methylophilales(100); Methylophilaceae(100); *Methylophilus*(100)	*Methylotenera mobilis* (AB698738)	99

[a] band numbers correspond to those presented in figure 5

[b] taxonomic string with bootstrap values (in parentheses), generated in mothur using SILVA database reference file release 119

[c] closest relative according to INSA (International Nucleotide Sequence Database)

[d] percentage sequence similarity with closest INSA using BLAST tool

Table S3 – Granulometric composition of sediments in Llobregat MAR site

Sampling location		*>12.7 mm*	*2-12.7 mm*	*0.3-2 mm*	*0.056-0.3 mm*	*<0.056 mm*
Pre-sedimentation basin	Decantation	*39.48*	*6.68*	*20.66*	*28.10*	*5.07*
Infiltration basin	Entrance	54.05	25.43	12.93	6.91	0.68
	Midfield	40.35	30.36	20.90	7.62	0.76
	End	15.36	40.26	31.54	11.47	1.37

References

Altschul, S., Madden, T., Schaffer; AA, Zhang, J., Zhang, Z., Miller, W. and Lipman, D.: Gapped BLAST and PSI-BLAST: a new generation of protein database search programs, Nucleic Acids Res, 25(17), 3389–3402, doi:10.1093/nar/25.17.3389, 1997.

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C. and Knight, R.: UCHIME improves sensitivity and speed of chimera detection, Bioinformatics, 27(16), 2194–2200 [online] Available from: http://dx.doi.org/10.1093/bioinformatics/btr381, 2011.

Muyzer, G., De Waal, E. C. and Uitterlinden, A. G.: Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, Appl Environ Microbiol, 59(3), 695–700, doi:0099-2240/93/030695-06$02.00/0, 1993.

Oksanen, J., Blanchet, F., Kindt, R., Legendre, P., Minchin, P., O'Hara, R., Simpson, G., Solymos, P., Stevens, M. and Wagner, H.: Vegan: Community Ecology Package. R package version 2.4–3. The R Project for Statistical Computing, Vienna, Austria., [online] Available from: https://cran.r-project.org/web/packages/vegan/index.html (Accessed 21 July 2017), 2017.

Schloss, P. D., Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B., Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W., Stres, B., Thallinger, G. G., Van Horn, D. J. and Weber, C. F.: Introducing mothur: open-source, platformindependent, community-supported software for describing and comparing microbial communities., Appl Environ Microbiol, 75(23), 7537–41, doi:10.1128/AEM.01541-09, 2009.