Spatial and temporal changes in sulphate-reducing groundwater bacterial community structure in response to Managed Aquifer Recharge

D. A. Reed, S. Toze and B. Chang

ABSTRACT

The population dynamics of bacterial able to be cultured under sulphate reducing condition was studied in conjunction with changes in aquifer geochemistry using multivariate statistics for two contrasting Managed Aquifer Recharge (MAR) techniques at two different geographical locations (Perth, Western Australia and Adelaide, South Australia). Principal component analysis (PCA) was used to investigate spatial and temporal changes in the overall chemical signature of the aquifers using an array of chemical analytes which demonstrated a migrating geochemical plume. Denaturing Gradient Gel Electrophoresis (DGGE) using DNA from sulphate-reducing bacteria cultures was used to detect spatial and temporal changes in population dynamics. Bacterial and geochemical evidence suggested that groundwater at greatest distance from the nutrient source was least affected by treated effluent recharge. The results suggested that bacterial populations that were able to be cultured in sulphate reducing media responded to the migrating chemical gradient and to the changes in aquifer geochemistry. Most noticeably, sulphate-reducing bacterial populations associated with the infiltration galleries were stable in community structure over time. Additionally, the biodiversity of these culturable bacteria was restored when aquifer geochemistry returned to ambient conditions during the recovery phase at the Adelaide Aquifer Storage and Recovery site.

Key words | biogeochemistry, managed aquifer recharge, temporal and spatial variation, water reuse, wastewater

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INTRODUCTION

Managed Aquifer Recharge (MAR) is a technique that can be used to capture and store water in aquifers for later reuse (Dillon *et al.* 2005). MAR can be used to aid the recycling of water that would normally be lost or discarded to the environment. With an increasing demand for freshwater supplies, MAR is a technique that can be used to supplement dwindling resources.

Groundwater studies have historically focused on contaminated aquifers which are segregated into discrete redox zones dominated by different physiologic microbial processes (Stumm & Morgan 1981; Lovely 1991; Lovely & Goodwin 1988; Chapelle 2001). Numerous important geochemical processes in subsurface environments are carried out exclusively by enzyme catalysed microbial processes (Lovely 1991). Groundwater microbial populations are therefore clearly capable of changing the chemical nature of groundwater. Studies that have combined molecular techniques to describe microbial community structure with multivariate statistics to investigate groundwater microbial and geochemical characteristics (Fahy *et al.* 2005; Haack *et al.* 2004) have demonstrated an interdisciplinary approach which comprehensively explores these biogeochemical interactions.

An Aquifer Storage and Recovery (ASR) water reuse scheme in South Australia (Dillon *et al.* 2005) has demonstrated significant geochemical changes and improvements

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in the quality of recovered water (Vanderzalm *et al.* 2006). A MAR water reuse scheme using infiltration galleries in Western Australia (Toze 2006) is also currently examining water quality improvements (Bekele *et al.* 2006; Toze 2006).

Enhancement in the quality of recovered water *via* MAR has been hypothesised to occur through a combination of physical, chemical and biological processes. However no comprehensive investigations studying the microbial population dynamics during MAR have been previously undertaken. Insight into these biogeochemical processes has the potential to assist in the improved removal of contaminants such as nutrients, pathogens and trace organics from the recharged water. In addition, biological clogging processes which can serious impact MAR schemes may be better controlled due to a better knowledge of microbial responses to geochemical changes.

The aim of this study was to investigate changes in groundwater bacterial community structures during MAR and the major influences that drive these population changes. The project used two different MAR techniques utilising wastewater of dissimilar quality in aquifers with contrasting redox and chemical conditions from two different geographical areas. One of several groups within the bacterial populations studied were the bacteria that could be cultured in sulphate reducing media. Sulphate reducing bacteria were a target group due to their known presence at both sites and the different geochemical changes at the two different sites.

METHODS

Field sampling and laboratory analysis

Detailed and comprehensive information on site descriptions and groundwater collection at the two MAR sites are given in Dillon *et al.* (2005) and Bekele *et al.* (2006).

The Adelaide ASR microbial study was undertaken using modified McFeters chambers which were assembled as described by Pavelic *et al.* (1998). The chambers contained background groundwater and were suspended into four observation bores: 0 m, 4 m, 75 m and 300 m distance from the injection well. Representative samples were collected from these chambers at set intervals between 3rd April 2002 and 25th July 2002. At the Perth infiltration gallery site a number of different observation bores were monitored between November 21st 2005 and 3rd April 2006 (Bekele *et al.* 2006). At the Perth site, groundwater was collected directly from the monitoring bores using methods described in ASTM International (2001).

Groundwater collected for microbial analyses from chambers (Adelaide) and non-chambers (Perth) were transferred to sterile anaerobic 100 mL serum bottles and stored on ice prior to inoculating into sulphate reducing bacterial medium. Sulphate-reducing media was prepared according to Atlas (1996) containing Sodium Lactate as an energy and carbon source. A volume 0.2 mL of collected groundwater was used to inoculate the SRB medium which was then incubated at 25°C for 14 days or until a black precipitate (indicating the production of hydrogen sulphide due to sulphate reduction) was observed. Bacterial cells were then harvested by centrifugation at 4,500 rpm for 20 min and stored at −80°C until processed. Groundwater samples were also collected at the same time for a range of geochemical parameters as detailed in Vanderzalm *et al.* (2006) and Toze (2006).

DNA extraction, PCR and DGGE

DNA was extracted from the harvested bacterial cultures using a QIamp Stool Mini Kit (Qiagen) which was modified from the manufacturer's instructions by adding 0.5 μl of sterile zirconia/silica beads (0.1 mm diameter; Biospec) to 1.4 mL ASL buffer (QIAamp Stool Mini Kit) and incubated at 95°C for 5 min. The samples then underwent beadbeating at 3000 rpm in a MINI-BEADBEATER™ (Biospec) for 3 min then cooled on ice for 1 min. The total volume of supernatant was recovered at each step and the volume of DNA extraction kit reagents was proportionally increased to match the increase volume of sample. The final DNA extracts were stored at −80 °C until used in PCR amplifications.

PCR was used to amplify the bacterial DNA in all of the DNA extracts. PCR reaction mixtures contained approximately 50–100 ng of DNA template, 0.4 μl of bacterial forward primer V3-F-GC (20 μm) and 0.4 μl of 907-R (20 μm) reverse bacterial primer (Muyzer *et al.* 2004), 25 μl of BioRad iQ™ supermix (BioRad) and autoclaved ddH_20 to a total volume of 50 μl. Cycling was provided by a

PTC-200 (MJ Research) thermal cycler. A 'touchdown' PCR cycle was modified from Muyzer *et al.* (2004) using 63°C as the initial annealing temperature.

DGGE was performed using the Bio-Rad D-Code™ System as described previously (Muyzer *et al.* 2004). A 30–70% linear denaturing gradient formed in 8% polyacrylamide gels was used for the Perth infiltration gallery samples and a 40–60% linear DNA-denaturing gradient formed in 6% polyacrylamide gels used for the Adelaide ASR samples. Wells of the DGGE gels were loaded with 50 μl of PCR product combined with 8 μl of 6 × gel loading buffer. Each DGGE gel contained two bacterial reference ladders from 'in-house' groundwater sample strains and a further non-PCR standard included a 100 bp ladder (K180-Amresco) diluted 1:2 with 6 × gel loading buffer (Reed 2007). DGGE was performed at 200 V for 4 hr at 60°C in 1 × TAE buffer. Gels were stained for at least 1 hr in 250 mL of 1 × TAE buffer with ethidium bromide (0.5 mg/L) and visualized by UV illumination (254 nm). Gel images were acquired by using the transilluminator MultiImage™ Light cabinet (Alpha Innotech Corporation).

DGGE Gel and Statistical analyses

DGGE tiff files were processed by GelCompar II, version 3.5 (Applied Maths). All gels were normalised using the DGGE reference ladders used and automatic and manual band matching undertaken as described in Reed (2007). A binary matrix table was generated and imported into PRIMER6/PERMANOVA + statistical package and a Bray Curtis similarity matrix undertaken for multidimensional scaling (MDS) and permanova analyses. Geochemical data obtained form the groundwater samples was transferred into PRIMER6/PERMANOVA + and principal component analyses (PCA) undertaken on normalised data (Reed 2007).

RESULTS AND DISCUSSION

Temporal and spatial variation of aquifer solution geochemistry

The average aquifer solution geochemistry for both of the MAR sites is given in Table 1. Changes in aquifer solution geochemistry at the Adelaide ASR site as determined by the overall temporal variation in chemical signature indicated the formation of distinct distance-based PCA clusters (i, ii, iii, iv) during the injection phase (Figure 1). PCA cluster (i) comprised of geochemical samples from the injection well; PCA cluster (ii) included 4 m well samples; PCA cluster (iii) was composed of 50 m well samples and PCA cluster (iv) incorporated native non-impacted background groundwater (300 m). In contrast during the recovery phase (from sampling event 218) groundwater geochemical data from 0 m, 4 m and 50 m did not form distinct distance-based PCA clusters. During the recovery phase, samples from all distances migrated towards the chemical signature of the background native groundwater as previously described by Vanderzalm *et al.* (2006).

The overall chemical signature at the Perth infiltration gallery site predominantly formed three groups indicated as PCA clusters i, ii and iii (Figure 2). PCA cluster (i) included geochemical samples from the infiltration galleries (0 m); PCA cluster (ii) included observation bores −10 m to 8 m from

Table 1 | Average geochemical parameters of wastewater, recovered water and background groundwater for the two MAR sites

MAR site	Water type	Eh mV	EC mS/m	DO mg/L	Cl mg/L	DOC mg/L	Fe mg/L	N_NO_3 mg/L	SO_4 mg/L	TDS mg/L
Floreat	Wastewater*	437	144	3	279	11	0	2.5	64	757
	Rec water†	170	116	4	188	5	3	0.7	60	681
	Groundwater‡	348	102	2	161	3	0	0.05	73	650
Bolivar	Wastewater*	853	3592	6.0	415	20	0.1	2.5	181	2006
	Rec water†	73	2470	0.02	594	11	0.7	0.01	216	1267
	Groundwater‡	86	2265	0.3	1024	1	1	0.05	301	1470

*Waster water used for recharge: tertiary treated for ASR and secondary treated for infiltration galleries.
†Recovered Water.
‡ambient groundwater collected from background bores outside the influence of the MAR schemes.

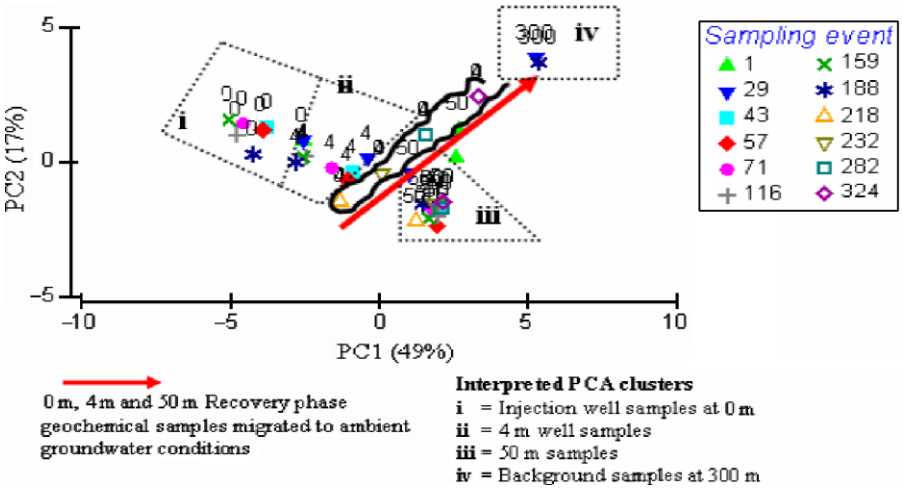

Figure 1 | CA plot of all geochemical data (22nd January to 8th October 2002) from Injection, 4 m, 50 m and 300 m groundwater samples at the Adelaide ASR site over time.

sampling event 68 days onwards; PCA cluster (iii) included samples from −10 m to 8 m for sampling events 2 to 33 days in addition to all observation bores at greater distance including the background (−177 m) and extraction bores (50 m). The chemical signature of the infiltration galleries.

(PCA cluster i) was unique compared with groundwater geochemistry of all observation bores thus indicating the quality differences of the secondary treated effluent prior to infiltration to the aquifer. These results therefore indicate water quality improvements of wastewater *via* infiltration through unsaturated soil into and through an aquifer system.

The PCA clustering (Figure 2) also indicated that samples at a distance greater than 8 m from the infiltration gallery were most similar to the chemical signature of groundwater from the background and extraction wells which did not vary with time (PCA cluster iii). These results thus indicate a distant-dependent lag phase for geochemical plume migration to these observation bores. In contrast, groundwater from the observation bores located between −10 m to 8 m from the infiltration gallery formed a distinct group (PCA cluster ii) after sampling event 33 days which was significantly different to the background and extraction well groundwater (PCA cluster iii). These results suggest the recharged water formed a plume which migrated from the infiltration galleries to these groundwater observation bores between sampling events 33 to 68.

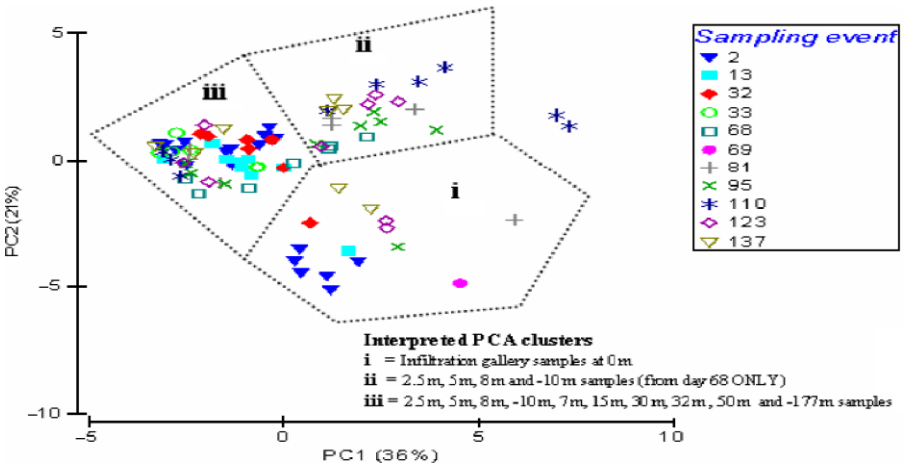

Figure 2 | PCA plot of all geochemical data (2nd November 2005 to 3rd April 2006) from Perth MAR groundwater samples over time.

Spatial and temporal variation in microbial community structure

DNA extracted from the sulphate-reducing cultures isolated from the various sampling locations demonstrated differential DGGE band intensity for both MAR sites. Sulphate-reducing cultures from the Adelaide ASR background groundwater (300 m) displayed more dominant bands compared with those isolated nearer to the nutrient source. In contrast, it was the recharge water prior to infiltration (infiltration gallery at 0 m) which exhibited the most intensive DGGE band class types at the Perth infiltration site. Differences between sulphate-reducing bacterial dominance between nutrient poor (Adelaide 300 m) and nutrient rich (Perth infiltration gallery) environments could possibly be attributed to dissimilarities between the two aquifers used for MAR at Adelaide and Perth. The Adelaide aquifer is deep, confined and anaerobic (Dillon *et al.* 2005) and therefore ambient groundwater may provide advantageous conditions for sulphate-reducing bacterial proliferation due to a preferential redox zone. In contrast, the ambient groundwater from the Perth shallow aerobic aquifer (Bekele *et al.* 2006) may have represented less favourable conditions due to the higher redox concentrations compared to redox conditions at the Adelaide ASR site.

Sulphate-reducing bacteria can be easily detected in Perth groundwater partially due to the available sulphate in the groundwater (Robertson *et al.* 2002). The secondary treated effluent within the infiltration galleries also represented an elevated nutrient source compared to the ambient groundwater samples thus providing more appropriate conditions for the growth of sulphate reducing bacteria. It is also proposed that the dominance of sulphate-reducing bacteria cultured from the infiltration gallery may have been due to the colonisation of these bacterial types in the wastewater treatment process prior to MAR. In contrast it was not always possible to culture sulphate-reducing bacteria from the injection well (nutrient source) at the Adelaide ASR site during the injection phase as very aerobic water was being directly injected into the aquifer, thus raising the redox well above the level where growth and survival of sulphate reducing bacteria was possible. The two MAR methods differ in that the injection of tertiary treated effluent at the Adelaide ASR site introduces oxygen into the injection well, where redox levels were highest compared with all other groundwater samples.

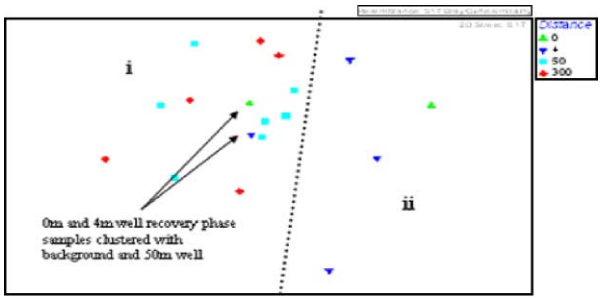

Figure 3 | 2D MDS plot for amplified microbial rDNA/DGGE banding patterns for all Injection well, 4 m, 50 m and 300 m sulphate-reducing cultures from Adelaide ASR.

Multidimensional scaling (MDS) analysis of the bacterial populations cultured in the sulphate-reducing medium between different observation wells over time indicated potential MDS clusters, some of which were distance-based for both MAR sites (Figures 3 and 4). The community structures cultured from the injection well (0 m) and 4 m well formed MDS cluster (ii) in Figure 3 during the injection phase at the Adelaide ASR site. In contrast, during the storage and recovery phases the bacterial populations cultured in the SRB medium at the 0 m and 4 m wells were observed to migrate to MDS cluster (i) which contained all 50 m and background groundwater bacterial community structures. The change in bacterial population dynamics thus supports geochemical data where the chemical signature of the injection and 4 m well returned to ambient conditions. These results therefore suggest that the change in bacterial diversity to the extent that bacteria that could be cultured in the SRB medium returned during the storage and recovery phases of the ASR cycle.

The MDS clusters formed from the cultured bacterial populations from the Perth infiltration site (Figure 4) showed greater variation between distances over time (high MDS stress of 0.27) than described for the Adelaide ASR site. The Perth MAR site differs from the Adelaide ASR site in that there is continual recharge of treated wastewater with continual recovery at distance from the infiltration galleries. Despite the uncertainty of the potential MDS clustering, PERMANOVA analysis determined significant spatial differences between these groundwater sulphate reducing microbial communities (Table 2). PERMANOVA pair-wise comparisons between sulphate-reducing community structures between distances demonstrated smaller

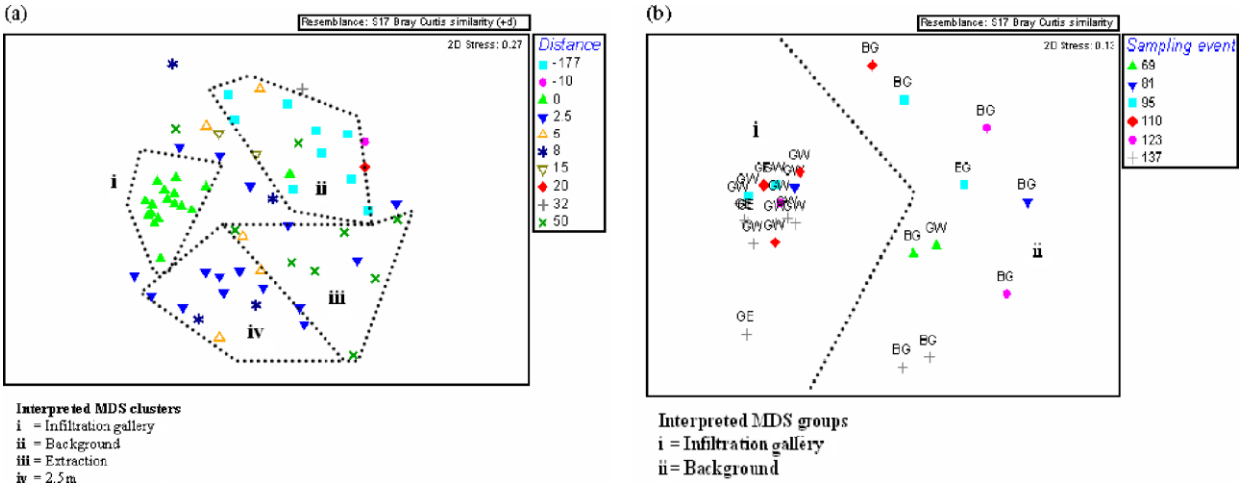

Figure 4 | 2D MDS plot for amplified microbial rDNA/DGGE banding patterns for (a) all Perth MAR sulphate-reducing cultures over time; and (b) Perth MAR background (BG) and infiltration gallery (GW/GE) sulphate-reducing cultures over time.

differences between community structure (> P value) for observation bores in closest proximity to the infiltration gallery (data not shown – Reed 2007). Thus microbial analyses again supports the observed geochemical outcomes in that both the geochemistry and culturable bacterial populations in closest proximity to the infiltration galleries were more greatly impacted from MAR than at distances further from the infiltration galleries.

The sulphate-reducing cultures from the infiltration gallery demonstrated distinct temporal stability in community structure (MDS cluster i, Figure 4(a)) compared with all other distance-based samples. A MDS comparing the infiltration gallery (0 m) and background sulphate reducing cultures only (Figure 4(b)) revealed substantial differences between community dynamics over time. The oligotrophic ambient groundwater demonstrated greater variation in the bacterial community structure in the cultures from the SRB medium over time. It is proposed that the elevated nutrients within the infiltration gallery created an environment that allowed for the establishment of a stable sulphate-reducing bacterial community as a consequence of the constant supply of secondary treated effluent.

The development of a stable sulphate-reducing bacterial community suggests they were able to competitively exclude other bacterial types. In contrast the low nutrient conditions of the Perth ambient groundwater (Table 1) perhaps created an environment for enhanced competition for the limited resources. In addition, nutrient poor groundwater can consist predominantly of refractory carbon compared with the more bioavailable nutrient source from the wastewater (Skjemstad *et al.* 2002). Thus, the differences in organic carbon between the infiltration gallery and background groundwater may also have created an environment for elevated competitiveness. These results suggest that 'energy-poor resources' prevents the establishment of a stable sulphate-reducing bacterial community over time.

Table 2 | PERMANOVA – Perth MAR sulphate-reducing cultures*

Source	df	SS	MS	F	P (permutation)
Distance	5	37181	7436	6.55	0.001
Sampling event	2	3946	1973	1.73	0.07
Distance × Sampling event	10	21554	2155	1.90	0.001
Res	44	49963	1135		
Total	61	1.1538E5			

*Experimental design included time and distance as fixed factors and an interaction of these as a variable factor.

CONCLUSIONS

1) Bacterial populations culturable under sulphate reducing conditions responded to MAR where the variation in microbial community structure at the recharge site was different to the ambient groundwater.
2) Nutrient poor environments produced greater temporal diversity when analysing sulphate-reducing population structures from ambient groundwater while higher nutrient loads caused temporal stability in sulphate reducing bacterial population structure.
3) At return to ambient groundwater conditions as observed during the recovery phase at the ASR site resulted in a concomitant return in sulphate-reducing bacterial population dynamics to the indigenous community structure.
4) The recharge of recycled water to aquifers can cause a change in microbial population structure which has direct links to changes in geochemistry. More understanding of these concomitant processes will aid better prediction of water quality changes during MAR.

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