Bacterially-mediated iron cycling and associated biogeochemical processes in a subtropical shallow coastal aquifer: implications for groundwater quality

Chaofeng Lin[1,2,*], Eloise I. Larsen[1,2], Genevieve R. Larsen[1,3], Malcolm E. Cox[1,2,3] and James J. Smith[1,2]

[1] School of Earth, Environmental and Biological Sciences, Science and Engineering Faculty, Queensland University of Technology, Brisbane 4001 Australia; [2] Institute for Sustainable Resources, Queensland University of Technology, Brisbane 4001 Australia; [3] National Centre for Groundwater Research and Training, Australia

*Correspondence address: Department of Geological Sciences, College of Earth, Ocean and Environment, University of Delaware, Newark, DE 19716 US; E-mail: chflin@gmail.com; Tel: 1-302-831-3420

Keywords Alluvial aquifer; iron transport; organic matter oxidation; biofouling; subtropical Australia

Abstract Bacterially-mediated iron redox cycling exerts a strong influence on groundwater geochemistry, but few studies have investigated Fe biogeochemical processes in coastal alluvial aquifers from a microbiological viewpoint. The shallow alluvial aquifer located adjacent to Poona estuary on the subtropical Southeastern Queensland coast represents a redox-stratified system where iron biogeochemical cycling potentially affects water quality. Using a 300 m transect of monitoring wells perpendicular to the estuary, we examined groundwater physico-chemical conditions and the occurrence of cultivable bacterial populations involved in iron (and manganese, sulfur) redox reactions in this aquifer. Results showed slightly-acidic and near-neutral pH, suboxic conditions and an abundance of dissolved iron consisting primarily of iron(II) in the majority of wells. The highest level of dissolved iron(III) was found in a well proximal to the estuary most likely a result of iron curtain effects due to tidal intrusion. A number of cultivable, (an)aerobic bacterial populations capable of diverse carbon, iron or sulfur metabolism coexisted in groundwater redox transition zones. Our findings indicated aerobic, heterotrophic respiration and bacterially-mediated iron/sulfur redox reactions were integral to carbon cycling in the aquifer. High abundances of dissolved iron and cultivable iron and sulfur bacterial populations in estuary-adjacent aquifers have implications for iron transport to marine waters. This work demonstrated bacterially-mediated iron redox cycling and associated biogeochemical processes in subtropical coastal groundwaters using culture-based methods.

Introduction

Iron (Fe) is a transition-metal element and naturally undergoes active reactions between ferrous and ferric states in circumneutral-pH, redox-stratified aquifers. Due to the instability of dissolved Fe(II) and the adsorptive capability of insoluble Fe(III) compounds, active Fe cycling exerts a strong influence on groundwater geochemistry. Associated redox reactions can be substantially driven by bacterial activities, which promote Fe accumulation to high levels (>1.0 mg L^{-1}) in coastal groundwater redox transition zones (Chapelle & Lovley, 1992). In the presence of trace amounts of O_2 (<0.1–1.0 mg L^{-1}), dissolved Fe can support substantial bacterial growth, particularly Fe(II)-oxidizing/Fe(III)-depositing bacteria such as stalked *Gallionella* and sheathed *Leptothrix* (Tyrrel & Howsam, 1997; Stuetz & McLaughlan, 2004). Conversely, in anaerobic microsites of coastal aquifers, the presence of Fe(III)-reducing bacteria such as *Geobacter* spp. can reduce Fe oxyhydroxides, mobilizing large quantities of Fe and increasing dissolved Fe loads that can be transported to marine waters (Lovley, 1997).

In addition to diverse Fe(II)-oxidizing and Fe(III)-reducing bacteria capable of Fe metabolism under (sub)oxic and anoxic conditions (Kappler & Straub, 2005; Weber et al., 2006), manganese (Mn) and sulfur (S) bacteria also influence Fe cycling via biological (direct) and abiotic (indirect) mechanisms. For example, most Mn(IV)-reducing bacteria and some sulfate-reducing bacteria can utilize Fe(III) as an alternative electron acceptor (Lovley, 2006), whereas microbially-produced Mn(IV) and sulfide abiotically react with aqueous Fe(II)/(III), affecting Fe re-distribution and transport in groundwater-dependent ecosystems (Chapelle & Lovley, 1992; Brown et al., 1999). However, research regarding bacterially-mediated Fe (and Mn, S) cycling in coastal systems has focused largely on sequential reduction processes linked to organic carbon (C) mineralization and mineral mobilization, and overlooked oxidation processes which have the potential to promote elemental redox cycling (Canfield et al., 1993; Lovley, 1997). In addition, previous work regarding coastal groundwater Fe (and Mn, S) cycling has primarily studied the geophysical and chemical mechanisms of seawater intrusion, mineral precipitation and/or ion exchange (Charette and and Sholkovitz~~et al.~~, 2002; Testa et al., 2002; Spiteri et al., 2006; Weng et al., 2007; Johnston et al., 2010). There have been few bacterial culture-based studies relating to Fe biogeochemical processes in coastal alluvial aquifers (Chapelle & Lovley, 1992).

The focus of this study is a shallow alluvial aquifer on the subtropical southeast Queensland (SEQ, Australia) coastal lowland, where large-scale clearing of native vegetation preceded establishment of exotic pine plantation in the 1950s. Recent plantation harvesting and replanting practices, combined with natural seasonal flooding, may contribute to mineral soil disturbance and mobilization of limiting nutrients causing off-site

pollution (Costantini & Loch, 2002; Lin et al., 2011). Of concern in this coastal setting is that the growth of toxin-producing marine cyanobacterium, *Lyngbya majuscula*, can be promoted by high levels of nutrients and dissolved Fe, and the presence of humic substances from land runoff that make the Fe bioavailable (Pointon et al., 2003). *L. majuscula* intermittently blooms on the coast of Queensland and worldwide (Ahern et al., 2006; Al-Shehri & Mohamed, 2007; Bell & Elmetri, 2007) adversely affecting marine ecosystem health through smothering benthic habitats. It is hypothesized that abundant dissolved Fe and other substrates support dynamic bacterial populations and elemental cycling in redox-stratified coastal aquifers which affect the quality of these groundwaters.

Using a 300 m transect of monitoring wells from the plantation-forested lowland to an estuary, we examined groundwater physico-chemical conditions and the abundances of cultivable Fe- (and Mn-, S-) oxidizing and reducing bacteria in the shallow aquifer. We specifically focused on a group of neutrophilic Fe(II)-oxidizing bacteria which have recently been found ubiquitous in surface and subsurface waters encompassing the catchment study area (Lin et al., 2012). The objectives were to identify environmentally significant functional bacterial groups contributing to biogeochemical processes in this estuary-adjacent ecosystem, and to evaluate implications for groundwater Fe (and Mn, S) cycling and potential water quality impacts.

Materials and methods

Site description

The shallow, alluvial aquifer under study is adjacent to the mouth of Poona estuary located 300 km north of Brisbane, on the Fraser Coast of SEQ (Fig. 1). Local climate is subtropical maritime. Annual average rainfall is 1 148 mm, mostly during warmer months (Nov–Mar). Mean monthly maximum temperatures range from 22.0°C in Jul to 30.7°C in Jan; with monthly minima 8.6°C in Jul to 20.6°C in Jan. The small catchment of Poona Creek (ca. 100 km^2) consists of a flat to gently undulating coastal plain with elevations mostly <50 m above the Australian Height Datum (AHD). Catchment gradients gently decrease towards the eastern coastline, with tidal creeks discharging to the Ramsar-, and UNESCO-listed Great Sandy Strait. Surface drainages are ephemeral in upper, but perennial in lower, reaches. The presence of discontinuous clay layers results in water-logging of overlying topsoils in much of the region. Exotic *Pinus* plantation forestry was first established in the 1950s and presently covers over half the catchment area. Native vegetation consisting of *Melaleuca* and *Eucalyptus* spp., tufted native grasses and sedges remains in buffer zones adjacent to waterways.

Groundwater monitoring wells were drilled in June 2007 along a 300 m transect perpendicular to the Poona estuary, near its mouth (Fig. 2). The original aim of the drilling for this study was to locate continuous shallow aquifers within the study area for general characterization of ground and surface waters in the Poona catchment. As there were no previous studies focusing on aquifer location and mapping in the Poona catchment, drilling was chiefly exploratory. Much of the geology in the area was found to consist of shallow topsoil overlying weathered bedrock. However, borelogs from exploratory drilling in the study area between the Poona Creek estuary and pine plantation compartments contained alluvial sands and gravels of high hydraulic conductivity. This shallow aquifer is contained within a meandering bank of Poona creek, the result of gradual infilling from south to north during channel migration.

Wells were constructed using a hydraulic rotary drilling rig with bentonite drilling mud. Well depths were 6–12 m, with a three-meter slotted PVC screen at the bottom. Wells were capped, and housed with galvanized steel casing set in a concrete surface seal. During the drilling process, sediment samples were collected from wells at 0.5 m intervals. A typical sediment profile on the transect (except for W8) consisted of (a) topsoil consisting of sands and organics (some aeolian sand layers), (ca. $\leq$ 1 m thickness) (b) discontinuous gleyed semi-confining to confining clay layer (ca. 1-3 m thickness), (c) alluvial sands and gravels of ca. 5 to 8 m thickness (aquifer materials), (d) thin peat layer (ca. $\leq$ 1 m thickness), and (e) weathered bedrock. Based on the cross-section (Fig. 2), aquifer thickness can be up to ca. 8 m. However, the full extent of this aquifer has not been mapped; only that associated with the transect from the south towards the estuary.

The aquifer was estimated from the geomorphology of the area at ca. 0.26 km^2. Supratidal flats consisting of silty sands and a high proportion of organics occur between the estuary and the transect monitoring wells. W8 is located where the clay layer dips towards the estuary and limits tidal intrusion further inland (marking the northern boundary of the alluvial aquifer), and is at the transition between these two lithologies. A topsoil layer of ca. 1 m and ca. 3.5 m of organic silty sands (similar to those in the supratidal flats) overly the clay layer at this point (Fig. 2).

The aquifer itself can be considered semi-confined. Although the shallow clay layer is expected to have low hydraulic conductivities and reduce the rate of vertical percolation, it is discontinuous and rainfall recharge is relatively fast. On one data collection field trip during a high rainfall event, purging time limits had to be imposed as recharge was continuous and the monitoring wells could not be completely purged. Flow direction in the aquifer is southwards or downhill (Fig. 2). However, at W8, flow direction is controlled by tidal flux during

steady state conditions, i.e. times of low rainfall (Larsen & Cox, 2011). Water levels in the transect vary between 1.0 and 2.5 metres below ground surface (bgs) or -0.5 to 1.5 m AHD.

Sampling

Groundwater samples were collected from seven wells (W1–W8) in December 2008. W3 was excluded as it was screened in a confining clay layer and considered to not be representative of groundwater flows in the area. To ensure representative sampling of aquifer water, wells were purged using a submersible pump prior to *in situ* measurements and sample collection, with continuously recharged wells purged for at least 10 min. For laboratory chemical analysis, samples were collected using a deionized water-rinsed reusable bailer or submersible pump and filtered in the field using 0.45 μm pore size polycarbonate filter paper. For cation tests, samples were acidified to pH <2 using 50% nitric acid and stored in high-density polyethylene bottles (Eaton et al., 2005). For anion tests, sample bottles were not acidified but filled to eliminate air space. Cation and anion samples were stored at 4ºC prior to analysis. Biological samples were aseptically collected using an 80% (v/v) ethanol-rinsed reusable bailer and sealed in autoclaved glass bottles leaving no headspace. Biological samples were transported to the laboratory within 2 d of collection and processed for bacterial cultivation within 24 h upon arrival, or stored at 4°C prior to other analyses.

To yield sufficient microbial biomass for 16S rRNA-based molecular biological analysis, a second round of groundwater sampling was conducted in Feb 2009 specifically for laboratory cultivation and enrichment of neutrophilic Fe(II)-oxidizing bacteria using semi-solid FeS gradient medium. In addition, a sample of rust-colored flocculent microbial mat material from estuarine sediment surface water was aseptically collected. The mat sample was collected in a foil-wrapped sterile 70 mL Sarstedt plastic specimen container and transported to the laboratory within 2 d of collection for microscopic examination.

Groundwater physico-chemical analysis

Groundwater temperature (°C), pH, redox potential (Eh), dissolved oxygen (DO) and electrical conductivity (EC) were measured *in situ* using a calibrated TPS 90FL field multimeter. Water levels were measured with a Solinst dip-tape water level meter in meters below ground surface and later converted to m AHD by determination of topographic heights from LiDAR data in ESRI Arcmap. Water samples were analyzed for dissolved organic carbon (DOC) using the combustion method with a Shimadzu TOC-5000A analyzer; cations including Mn, magnesium (Mg), calcium (Ca), sodium (Na) and potassium (K) using Inductively Coupled

Plasma–Optical Emission Spectrometry (Varian Vista-MPX); anions including, chloride (Cl), sulfate (SO_4^{2-}), phosphate (PO_4^{3-}) and nitrate (NO_3^-) using a Dionex DX300 ion chromatograph; dissolved inorganic C (DIC, bicarbonate as alkalinity) colorimetrically using an AQ2+ Seal discrete analyser; sulfide (S^{2-}) colorimetrically using a manual spectrophotometric methylene blue method (Lovley & Phillips, 1987; Eaton et al., 2005). Total Fe and Fe(II) were determined colorimetrically on the AQ2+ Seal discrete analyser using the phenanthroline method (Eaton et al., 2005).

Bacterial cultivation and enumeration

A variety of laboratory media were employed for cultivation and enumeration of Fe, Mn and S bacteria (Table 1). One-to-ten dilution-to-extinction (10^{-1}–10^{-10}) was performed using (an)oxic broth media for (an)aerobic bacteria, i.e., one mL groundwater was directly inoculated into 9.0 mL of each broth medium, and all dilutions were made directly in respective media. For enumeration of neutrophilic, microaerophilic Fe(II)-oxidizing bacteria that have been found abundant in slightly-acidic, iron-rich environments (Emerson & Moyer, 1997; Emerson & Weiss, 2004; Weiss et al., 2007), Modified Wolfe's Mineral Medium was used to prepare the one-to-ten dilution series (10^{-1}–10^{-6}), with 0.1 mL diluted groundwater inoculated into CO_2-buffered, liquid FeS gradient medium and incubated under suboxic conditions. To increase surface area for bacterial growth and facilitate microscopy, a sterile glass rod was inserted into each tube of the liquid FeS gradient medium before inoculation. For molecular biological analysis, laboratory enrichment cultures were obtained using semi-solid FeS gradient medium by directly inoculating 0.5, 0.1 or 0.01 mL groundwater. For enumeration of aerobic, heterotrophic bacteria (HPC) and Mn(II)-oxidizing bacteria, groundwater dilution series (10^{-1}–10^{-6}) were prepared in sterile phosphate buffered saline (pH 6.8), with 0.1 mL spread on R2A and PC media for plate counts (colony-forming units, CFU). An uninoculated control was included for each medium during laboratory cultivation. Presumptive positive growth was recorded based on observation of specific growth reactions (Table 1) after two (FeS gradient medium) or four weeks incubation (all other media) in the dark at 25°C (FeS gradient media) or 28°C (all other media). The presence of bacterial cells and filaments was confirmed using light microscopy (1000× total magnification). Single colonies were subcultured from laboratory gradient enrichment cultures after four weeks incubation, with cells harvested from the fourth successive subculture prepared for electron microscopy or stored at −80°C prior to molecular biological analysis (Emerson & Moyer, 1997).

Light microscopy was performed directly on a subsample of suspended microbial mat material to identify bacterial morphology *in situ*. Electron microscopy was performed on the fourth transfer of laboratory gradient

enrichment cultures to examine the association of putative Fe(II)-oxidizing bacterial cells with iron oxides. For scanning electron microscopy (SEM), a glass rod with presumptive colonies was selected from the liquid FeS gradient medium showing positive bacterial growth. The glass rod was aseptically cut into 1 cm lengths, fixed using 1–3% glutaraldehyde for a minimum of 2 h at 4°C, washed with 0.1 M cacodylate buffer (pH 7), and dehydrated with an ethanol series (70%, 90% and 100%) and amyl acetate. SEM samples were dried in a Denton Vacuum critical point dryer, mounted on aluminum stubs and coated with gold. Imaging and energy-dispersive X-ray spectroscopy (EDS) were performed using an FEI Quanta 200 Environmental SEM under high vacuum at 5–15 kV. For transmission electron microscopy (TEM), a sterile syringe or Pasteur pipette was used to extract 0.5–1 mL cell material from the Fe(II) oxidation band in the semi-solid FeS gradient medium. A droplet of suspended cell material was mounted on a copper grid without staining. Direct imaging was performed using a JEOL 1200EX microscope operating at 80 kV.

16S rDNA-based molecular biological analysis

Total genomic DNA (gDNA) was extracted from semi-solid gradient enrichment cultures using a MoBio PowerSoil™ DNA isolation kit following manufacturers instructions. Extracted gDNA was checked for quality and quantity using 1% (w/v) agarose gel electrophoresis before storage at –20°C prior to further molecular biological analysis. For Temperature Gradient Gel Electrophoresis (TGGE) analysis, a 16S rDNA fragment of approximately 550 bp was amplified from bacterial gDNA extracts using primers 341F-GC/907R (Muyzer et al., 1995). The PCR reaction contained 0.5 U Roche Taq DNA polymerase, 2.5 µL 10× PCR buffer, 3.0 mM $MgCl_2$, 0.4 mM each dNTP, 0.25 µM each primer, and 1–2 µL template DNA; the final volume was adjusted to 25 µL with sterile Gibco UltraPure™ distilled water. *Acidithiobacillus ferroxydans* was used as a positive control and PCR amplifications were performed in an Eppendorf Mastercycler S following a touchdown protocol (Muyzer et al., 1995). Final primer extension was carried out at 72°C for 3 minutes, with 30 total cycles.

PCR products of low yield were concentrated with cold ethanol before TGGE analysis. The Diagen TGGE system contained horizontal polyacrylamide gels made of 5% (w/v) acrylamide/bis (37.5:1), 8 M urea and 2% (v/v) glycerol in 1× ME electrophoresis buffer (20 mM 4-morpholinepropanesulfonic acid, 1 mM EDTA, pH 8.0). An aliquot of 5 µL PCR product was applied to the gel for electrophoresis. After running at 300 V for 3.5 h (48–62°C), gels were silver stained with DNA bands excised using a sterile scalpel blade. Excised bands were transferred into microcentrifuge tubes containing 30–50 µL elution buffer (0.5 M ammonium acetate, 1 mM EDTA, pH 8.0), and incubated at 4°C overnight. Eluted DNA was concentrated to 10 µL using ethanol and re-

amplified using bacterial primers 341F/907R and the same PCR reaction and touchdown protocol described above. Re-amplified DNA was cleaned using a MoBio UltraClean™ PCR clean-up kit. Automated DNA sequencing was performed using an ABI 3500 Genetic analyzer.

Sequence data was manually checked and modified with 4Peaks (A. Griekspoor and Tom Groothuis, http://mekentosj.com), then compared with existing sequences in the GenBank database using BLAST (http://blast.ncbi.nlm.nih.gov/). Sequences were automatically aligned with closest relatives in the Greengene database (Nov, 2008) using ARB 5.1 (Ludwig et al., 2004). Alignment was manually checked and corrected if necessary. A phylogenetic tree was constructed using the Neighbor-joining algorithm and ARB 5.1. Sequences were deposited in GenBank under accession No. JQ712491–JQ712497 and HQ117915.

Data analysis

Data were examined for normality using the K-S test and variables log-transformed when necessary. Spearman-rank order correlation was performed with *p*-value 0.05 as the significance threshold. Data analysis was performed with SPSS 16.

Results

Groundwater physico-chemistry

During the sampling period in December 2008, there was an overall downward slope towards the estuary going from 1.1 m AHD at W1 to –0.6 m AHD at W8 except at W4 (1.9 m AHD) where the water table was elevated relative to the adjacent wells W2 and W5 which were 0.6 and 0.2 m respectively (Table 2). Associated temperatures ranged from 18.5 to 24.6°C, mostly >21°C and varied diurnally. The pH was slightly acidic in landward wells and gradually increased towards the estuary to a near neutral level (5.2–6.6). DO and Eh were within the microaerobic range, 0.1 to 1.0 mg L^{-1} and –50 to +150 mV, respectively, except for W1 (DO 1.8 mg L^{-1}). There was an increase in EC from W1 towards the estuary, with a substantial EC variation between W7 and W8 (Table 2).

DOC levels were between 3.5 and 7.6 mg L^{-1}, except for in W6 where it was approximately one order-of-magnitude higher (47 mg L^{-1}). Similarly, the highest DIC level was in central wells W4–W6 (31–55 mg L^{-1}, Table 2). Total dissolved Fe was the lowest in W1 (1.6 mg L^{-1}), between 4.2–9.0 mg L^{-1} in central wells W2–W6, and >10 mg L^{-1} in W7 and W8 (Table 3). A similar trend was observed in dissolved Fe(II) (1.2–28.0 mg L^{-1}), which comprised the majority of total dissolved Fe in all wells. Total dissolved Mn was below minimum

detection limit (MDL, 0.02 mg L^{-1}) in most wells, except W2 and W8 (Table 2). Sulfate was below 8.3 mg L^{-1} in W1–W6, but reached substantially higher levels in W7 and W8 (42.1 and 35.5 mg L^{-1}, respectively). Sulfide levels ranged from 0.2 to 0.6 mg L^{-1} in well waters, showing no clear trend (Table 3). Nitrate and phosphate were <MDL (0.05 mg L^{-1}) for all samples analyzed. Sodium and chloride were the dominant cation and anion, respectively, for all samples (Table 3). Regarding total dissolved ions (TDI), there was a large increase between W7 and W8 from 403 to 1564 mg L^{-1} over a distance of 20 m (gradient = 58 mg L^{-1} m^{-1}) with a more gradual increase between W1 and W7 of 97 to 403 mg L^{-1} over 270 m (gradient = 1.13 mg L^{-1} m^{-1}) (Tables 2 and 3).

Bacterial cultivation and enumeration

Laboratory cultivation demonstrated the presence of (micro)aerobic and anaerobic bacteria capable of putatively chemoheterotrophic or chemolithotrophic C, Fe or S metabolism in well waters (Table 4). HPC was consistently between 7.5×10^3 and 3.4×10^4 CFU mL^{-1}. A strong correlation was observed between HPC and DOC levels ($r^2 = 0.79$, $p < 0.05$). Neutrophilic, microaerophilic Fe(II)-oxidizing and aerobic Fe(III)-depositing bacterial levels ranged from 10 to 10^4 cells mL^{-1}, except for substantially higher level of the latter in W8 (10^9 cells mL^{-1}). Neutrophilic, anaerobic Fe(III)-reducing bacteria were also present in all wells. Highest Fe(III)-reducing bacterial levels were in central wells W4–W6 (10^6–10^8 cells mL^{-1}), which contained the highest Fe(II)-oxidizing bacterial levels (10^4 cells mL^{-1}. Levels of neutrophilic Fe(II)-oxidizing and Fe(III)-reducing bacteria (log_{10}-transformed) were both correlated with DIC ($r^2 = 0.79$, $p < 0.05$ and $r^2 = 0.81$, $p < 0.05$, respectively). Acidophilic Fe(II)-oxidizing and neutrophilic Mn(II)-oxidizing bacteria were <MDL (<10 cells mL^{-1}).

In addition, several cultivable bacteria capable of sulfide, elemental S or thiosulfate oxidation were detected in well waters (Table 4). Of these, sulfide-oxidizing bacterial levels were <MDL in landward W1 and W2 (<10 cells mL^{-1}), increasing from W5 (10 cells mL^{-1}) towards the estuary, reaching four orders-of-magnitude higher in W8 (10^5 cells mL^{-1}). The levels of elemental S- and thiosulfate-oxidizing bacteria both fluctuated, with the former up to 5–6 orders-of-magnitude higher than the latter in landward W2 and W8 proximal to the estuary. Sulfate-reducing bacteria occurred at 10 to 10^3 cells mL^{-1}, with the lowest level in central well W5, increasing towards W8 near the estuary.

SEM-EDS analysis indicated Fe and O as the primary elements in both biogenic and abiotic Fe precipitates in FeS gradient media (data not shown). SEM of laboratory enrichment cultures from the liquid FeS gradient medium showed abundant unicellular bacterial cells proximal to Fe oxide precipitates (Fig. 3). TEM of laboratory enrichment cultures from the semi-solid FeS gradient medium showed unicellular bacterial cells of

similar morphology in close association with Fe oxide deposition (Supplemental material). Together our experience observing the proximity and the close association of bacterial cells (curved rods) with Fe oxides (Lin et al., 2012) lead us to conclude that these cells are associated with Fe oxidation. *Gallionella*-like stalks and *Sphaerotilus* or *Leptothrix*-like sheaths were not observed in the microbial mat sample using light microscopy. Neither was this filamentous bacterial morphology observed in Fe and S bacterial enrichment cultures.

16S rDNA-based PCR-TGGE analysis

PCR-TGGE of laboratory enrichment cultures from the semi-solid FeS gradient medium demonstrated the presence of *Sideroxydans lithotrophicus*-related bacteria (PN001 and PN015, 96% sequence identity, SI) in four of seven wells (W1, W2, W4, W7), and a *Gallionella capsiferriformans*-related bacterium in W6 (PN0013, 96% SI) (Fig. 4, ~~and~~5). Other bacteria detected included *Ralstonia pickettii* (PN030, 98% SI), *Burkholderia kururiensis* (PN0029, 100% SI), *Massilia timonae* (PN003, 99% SI), *Burkholderia tropica* (PN008, 100% SI) and *Dyella koreensis* (PN002, 98% SI) (Figs. 4, ~~and~~ 5).

Discussion

Groundwater biogeochemistry

The Poona estuary-adjacent aquifer features slightly-acidic and near-neutral pH (Table 2). Groundwater temperature was higher when compared with other research (e.g. Taylor et al., 1997), largely due to the subtropical climate and shallow water depths. Relatively higher DO and positive Eh values in landward W1 (Table 2) indicated advection of oxic freshwater from the landward end of the transect. Although seawater is also advecting landward from the estuary, the semi-confining clay layer between W7 and W8 appears to limit intrusion inland, resulting in a large difference in total dissolved ions (TDI) between these two wells (Tables 2 and 3). The large differences in Eh and pH between W7 and W8 (Tables 2 and 3) indicate the presence of a groundwater redox transition near the fresh–seawater interface.

DOC content (Table 2) was generally higher than the average range for natural or pristine aquifers (0.5–2.0 mg L^{-1}), but comparable to organic-rich coastal and estuarine groundwaters (2–10 mg L^{-1}) (Brown et al., 1999; Goldscheider et al., 2006; Pavelic et al., 2007; Perera & Jinno, 2010). Similarly, laboratory cultivation demonstrated HPC comparable to cultivable counts of aerobic, heterotrophic bacteria in contaminated or fouled organic-rich groundwaters (ca. 10^3–10^5 cells mL^{-1}), and 2–4 orders-of-magnitude higher than those in pristine shallow unconsolidated aquifers (ca. <10–10^3 cells mL^{-1}) (Marxsen, 1988; Taylor et al., 1997; Stuetz &

McLaughlan, 2004; Ultee et al., 2004). Together these findings suggested aerobic, heterotrophic respiration was of significance to organic matter mineralization, accounting for the correlation between HPC and DOC (p <0.05) in the aquifer. However, a corresponding increase in HPC was not seen in W6 (Table 4) despite substantial DOC input possibly related to the buried peat layer (Table 2). We postulate the majority of organic matter in W6 was oxidized via anaerobic respiration, leading to highest DIC levels in adjacent wells (W4–W6, Table 2).

Anaerobic reduction of metals, particularly Fe(III) has been reported as the primary mechanism for organic matter mineralization in coastal groundwater redox transition zones (Chapelle & Lovley, 1992; Snyder et al., 2004). This work also showed high abundances of dissolved Fe(II) and Fe(III)-reducing bacteria in well waters, whereas a lack of dissolved Mn was associated with an absence of cultivable Mn(II)-oxidizing bacteria (Table 4). Due to the abundance of dissolved Fe(II) and the tendency of abiotic Mn(IV) reduction by dissolved Fe(II), Mn(IV) oxides were unlikely to occur in aquifer sediments. Consequently, microbial Mn metabolism was limited via a lack of bioavailable substrates, and bacterial Fe(III) reduction dominated organic matter mineralization, particularly in central wells W4–W6 which had lowest Eh and highest DIC and Fe(III)-reducing bacterial levels.

In addition, unicellular, neutrophilic, microaerophilic Fe(II)-oxidizing and aerobic Fe(III)-depositing bacteria were found ubiquitous in the aquifer (Table 4). The culturable Fe(II)-oxidizing bacteria were related to *Sideroxydans lithotrophicus* and *Gallionella capsiferriformans* (96% SI), which thrive in Fe-rich subsurface environments and compete with abiotic Fe(II) oxidation by O_2 at the oxic–anoxic interface (Emerson & Moyer, 1997; Emerson & Weiss, 2004; Weiss et al., 2007). Despite the proximity and the close association of putative Fe(II)-oxidizing bacterial cells with crystalline Fe oxides (Fig 3), we observed few Fe precipitates on the cell surface, suggesting the production of soluble/colloidal Fe(III) compounds in slightly-acidic micro-environments. Associated cell surface encrustation with Fe(III) precipitates could be retarded due to the excretion of metal-binding ligands by these bacteria, as reported by Roden et al. (2004).

Despite being several orders-of-magnitude less than Fe(II)-reducing bacteria, neutrophilic Fe(II)-oxidizing bacteria could be highly active and compete successfully with abiotic Fe(II) oxidation, particularly in the central wells W4–W6 with abundant dissolved Fe(II) and DIC (Table 2). Such bacterially-mediated Fe(II) oxidation has the potential to promote bacterial Fe(III) reduction at the redox interface via supplying limiting Fe(III) substrates such as amorphous or poorly-crystalline ferrihydrite (Sobolev & Roden, 2002), accelerating groundwater Fe cycling. On the other hand, the correlation between DIC and Fe(II)-oxidizing/Fe(III)-reducing bacterial levels (p <0.05) suggested that putatively chemolithotrophic Fe(II)-oxidizing bacteria that utilize CO_2 were supported by

bacterial Fe(III) reduction linked to organic C oxidation. Such bacterial involvement could be of increasing significance to Fe redox cycling at lower pH (e.g. < 5) due to the increasing stability of Fe substrates. However, the rate of Fe redox cycling was likely controlled by neutrophilic Fe bacteria in Poona catchment aquifer due to the pH range of 5.2–6.6 and a lack of culturable, acidophilic Fe bacteria.

Aerobic S-oxidizing and anaerobic sulfate-reducing bacteria co-occurred in all wells (Table 4). Increasing S bacterial numbers towards the estuary indicated bacterial S cycling was promoted by seawater intrusion, which supplied a limiting sulfate substrate. The associated dissolved Fe(III) is most likely in an organically-complexed form, either transported in this form or complexed *in situ*. Organic complexation will stabilize Fe(III) in the presence of abundant Fe(III)-depositing bacteria at near neutral pH (Langmuir 1997; Krachler 2005).

Seawater is generally characterized by low Fe (10–100 μg L^{-1}) (Armstrong 1957) and Fe is generally terrestrially sourced. Löhr et al. (2010) found extensive areas of high Fe soils within Poona catchment containing a large proportion of ferricrete and Fe-concretions, most of which were in the form of sparely soluble Fe-oxides. Iron is widespread throughout this study area, although readily extractable Fe concentrations are low overall. However, localized anoxic conditions and input of organic matter in waterlogged soils and stream sediments enable transformation of Fe-oxides to more readily available forms (Löhr et al. 2010). Dissolved Fe(II) is transported into aquifer sediments from water logged areas, along with stable organically-complexed Fe(III). Elevated Fe levels at W7 and W8 were most likely a result of 'iron curtain' effects at this saline/fresh interface. With increasing pH there is a precipitation of groundwater-borne dissolved Fe(II) and subsequent accumulation of Fe oxides onto subsurface sands at the groundwater-seawater interface (Charette and Sholkovitz 2002; Spiteri et al. 2006). However, a parallel Poona catchment study by Larsen (2012) indicated redox conditions vary with depth where fresh lower pH/Eh waters overly more saline higher pH/Eh waters. Consequently, both oxidation and reduction processes occur, resulting in high levels of both Fe(II), organically-complexed Fe(III) and particulate ferrihydrite for subsequent reduction.

Implications for groundwater quality

The abundances of Fe(II)-oxidizing and Fe(III)-reducing bacteria in the shallow aquifer demonstrate the potential for active groundwater Fe cycling, which can promote the mobility of Fe in groundwaters by maintaining Fe species in soluble forms or suspended, poorly-crystalline oxyhydroxides within the sediment profile. Together the results of high dissolved Fe levels in the majority of these monitoring wells has

implications for Fe transport to marine waters, a potential contributor to cyanobacterial blooms. Larsen (2012) found groundwaters within the supratidal flats between the transect and the estuary also contained abundant Fe (1.4 to 12.2 mg L^{-1} total Fe) although substantially reduced from W8 (32.8 mg L^{-1}) due to increased pH and DO associated with saline intrusion. These results indicate groundwater Fe loads were being transported to within 85 m of the estuary. The high organic content of sediments in the supratidal flats, humic and fulvic acids maintain the pH (6.3 to 7.2, variable with depth) at a level where dissolved Fe(II) and/or organically-complexed Fe remain stable. However, quantities of Fe reaching the estuary and the fate of this Fe once within the drainage system remain to be determined.

In addition, biofilm formation by neutrophilic, microaerobic Fe(II)-oxidizing/Fe(III)-depositing bacteria and/or sulfide production by anaerobic sulfate-reducers are considered to be important mechanisms causing well clogging and fouling (Taylor et al., 1997). The abundance of Fe and SO_4^{2-}/S bacterial populations, as well as dissolved Fe (1.0–26 mg L^{-1}) in the Poona estuary-adjacent aquifer demonstrates potential for the development of turbidity and color (Fe >0.05–0.1 mg L^{-1}), and adverse taste and household staining (Fe >0.3 mg L^{-1}), as well as microbially-mediated clogging and biocorrosion of metallic infrastructure including well casings, screens, and pumps (Taylor et al., 1997, WHO, 1996; NHMRC, 2004). Together the detection of several heterotrophic bacteria generally known as plant pathogens or soil bacteria in well waters (Fig 4 and 5) (~~1995;~~ Scola et al., 1998; Sintchenko et al., 2000; Reis et al., 2004; Deris et al., 2010) indicate the Poona estuary-adjacent aquifer or a comparable aquifer should not be used for potable groundwater abstraction.

Conclusions

The subtropical Poona estuary-adjacent alluvial aquifer provided a profile of slightly-acidic and redox-stratified groundwater. Abundant DOC/DIC and Fe/S substrates supported a large number of neutrophilic, (micro)aerobic and anaerobic bacterial populations capable of diverse C, Fe and S metabolism in this aquifer. Aerobic, heterotrophic respiration and bacterial Fe/S oxidation-reduction reactions most likely functioned as the primary driver of groundwater electron flow. High abundances of dissolved Fe and associated Fe (and S) bacterial populations have implications for groundwater quality and abstraction, as well as mineral dissolution and Fe mobilization in coastal alluvial aquifers. Dissolved Fe can pass through the estuary-adjacent saline gradient and discharge into marine habitats, where it may trigger potentially toxic blooms of cyanobacteria.

Acknowledgements

This project was funded by the Australian Research Council (Linkage Project LP0669786), Forestry Plantations Queensland (FPQ), and Institute for Sustainable Resources at the Queensland University of Technology (ISR, QUT). We thank Sue Gill for assistance with microbiological media; Luke Nothdurft and Deb Stenzel for SEM/TEM assistance. C. Lin was supported by a QUT–China Scholarship Council (CSC) Joint Postgraduate Scholarship.

References

Ahern, K. S., J. W. Udy & S. M. Pointon, 2006. Investigating the potential for groundwater from different vegetation, soil and landuses to stimulate blooms of the cyanobacterium, *Lyngbya majuscula*, in coastal waters. Marine and Freshwater Research 57: 177–186.

Al-Shehri, A. M. & Z. A. Mohamed, 2007. Mass occurrence and toxicity of the cyanobacterium *Lyngbya majuscula* under phosphorus-limited conditions in the Red Sea. Ecohydrology & Hydrobiology 1: 51–57.

Armstrong, F. A. J., 1957. The iron content of sea water. Journal of the Marine Biological Association of the United Kingdom 36: 509–517.

Bell, P. R. F. & I. Elmetri, 2007. Some chemical factors regulating the growth of *Lyngbya majuscula* in Moreton Bay, Australia: importance of sewage discharges. Hydrobiologia 592: 359–371.

Brown, C. J., J. D. Coates & M. A. A. Schoonen, 1999. Localized sulfate-reducing zones in a coastal plain aquifer. Ground Water 37: 505–516.

Canfield, D. E., B. Thamdrup & J. W. Hansen, 1993. The anaerobic degradation of organic matter in Danish coastal sediments: iron reduction, manganese reduction, and sulfate reduction. Geochim Cosmochim Acta 57: 3867–3883.

Chapelle, F. H. & D. R. Lovley, 1992. Competitive exclusion of sulfate reduction by Fe(lll)-reducing bacteria: a mechanism for producing discrete zones of high-iron ground water. Ground Water 30: 29–36.

Charette, M. A. & E. R. Sholkovitz, 2002. Oxidative precipitation of groundwater-derived ferrous iron in the subterranean estuary of a coastal bay. Geophysical Research Letters 10: 1–4.

Costantini, A. & R. Loch, 2002. Effects of site preparation on runoff, erosion, and nutrient losses from *Pinus* plantations established on the coastal lowlands of south-east Queensland, Australia. Australian Journal of Soil Research 40: 1287–1302.

Deris, Z. Z., H. V. Rostenberghe, H. Habsah, R. Noraida, G. C. Tan, Y. Y. Chan, A. R. Rosliza & M. Ravichandran, 2010. First isolation of *Burkholderia tropica* from a neonatal patient successfully treated with imipenem. International Journal of Infectious Diseases 14: e73–74.

Eaton, A. D., L. S. Clesceri, E. W. Rice, A. E. Greenberg & M. A. H. Franson, 2005. Standard methods for the examination of water and wastewater, APHA-AWWA-WEF, Washington, DC: 1368.

Emerson, D. & C. L. Moyer, 1997. Isolation and characterization of novel iron-oxidizing bacteria that grow at circumneutral pH. Applied Environmental Microbiology 63: 4784–4792.

Emerson, D. & J. V. Weiss, 2004. Bacterial iron oxidation in circumneutral freshwater habitats: findings from the field and the laboratory. Geomicrobiol Journal 21: 405–414.

Goldscheider, N., D. Hunkeler & P. Rossi, 2006. Review: microbial biocenoses in pristine aquifers and an assessment of investigative methods. Hydrogeology Journal 14: 926–941.

Johnston, S. G., A. F. Keene, R. T. Bush, E. D. Burton, L. A. Sullivan, L. Isaacson, A. E. McElnea, C. R. Ahern, C. D. Smith & B. Powell, 2010. Iron geochemical zonation in a tidally inundated acid sulfate soil wetland. Chemical Geology 280(3-4): 257-270.

Kappler, A. & K. L. Straub, 2005. Geomicrobiological cycling of iron, in Banfield, J. F., J. Cervini-Silva & K. M. Nealson (eds), Reviews in Minerology and Geochemistry. The Mineralogical Society of America, Chantilly: 85–108.

Krachler, R., F. Jirsa & S. Ayromlou, 2005. Factors influencing the dissolved iron input by river water to the open ocean. Biogeosciences 4: 311–315.

Langmuir, D., 1997. Aqueous Environmental Geochemistry. New Jersey, Prentice-Hall, Inc.

Larsen, G. & M. Cox, 2011. Hydrochemical and isotopic characterisation of groundwaters to define aquifer type and connectivity in a sub-tropical coastal setting, Fraser Coast, Queensland. Environmental Earth Sciences 7: 1885-1909.

Lin, C., Larsen, E. I., Grace, P. R. & J. J. Smith, 2011. Occurrence and distribution of iron and associated bacterial populations in soils of a forested subtropical coastal catchment. European Journal of Soil Biology 47, 322–332.

Lin, C., Larsen, E. I., Nothdurft, L. D. J. J. Smith, 2012. Neutrophilic, microaerophilic Fe(II)-oxidizing bacteria are ubiquitous in aquatic habitats of a subtropical Australian coastal catchment. Geomicrobiology Journal 29, 1–12.

Löhr, S. C., M. Grigorescu, J. H. Hodgkinson, M. E. Cox & S. J. Fraser, 2010. Iron occurrence in soils and sediments of a coastal catchment: A multivariate approach using self organising maps. Geoderma 156: 253-266.

Lovley, D. R., 1997. Microbial Fe(III) reduction in subsurface environments. FEMS Microbiology Reviews 20: 305–313.

Lovley, D. R., 2006. Dissimilatory Fe(III)- and Mn(IV)-reducing prokaryotes, in Dworkin, M., S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt (eds), The Prokaryotes. Springer, New York: 635–658.

Lovley, D. R. & E. J. P. Phillips, 1987. Rapid assay for microbially reducible ferric iron in aquatic sediments. Applied Environmental Microbiology 53: 1536–1540.

Marxsen, J., 1988. Investigations into the number of respiring bacteria in groundwater from sandy and gravelly deposits. Microbial Ecology 16: 65–72.

Muyzer, G., A. Teske, C. O. Wirsen & H. W. Jannasch, 1995. Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. Archives of Microbiology 164: 165–172.

NHMRC (Australian National Health and Medical Research Council), 2004. Australian drinking water guidelines, http://www.nhmrc.gov.au/publications/synopses/eh19syn.htm.

Pavelic, P., P. J. Dillon, K. E. Barry, J. L. Vanderzalm, R. L. Correll & S. M. Rinck-Pfeiffer, 2007. Water quality effects on clogging rates during reclaimed water ASR in a carbonate aquifer. Journal of Hydrology 334: 1–16.

Perera, E. D. P. & K. Jinno, 2010. A numerical approach to simulate the biogeochemical involvement in a coastal reduced groundwater environment. Water, Air, and Soil Pollution 207: 369–389.

Pointon, S. M., P. W. Moody, M. Preda, M. Cox, K. M. Hey, C. R. Ahern & B. Powell, 2003. Investigation of terrestrial sources of nutrients supporting *Lyngbya majuscula* growth in Moreton Bay - Land Sources. Indooroopilly, Queensland, Department of Natural Resources and Mines.

Reis, V. M., P. E. Santos, S. Tenorio-Salgado, J. Vogel, M. Stoffels & S. Guyon et al, 2004. *Burkholderia tropica* sp. nov., a novel nitrogen-fixing, plant-associated bacterium. International Journal of Systematic and Evolutionary Microbiology 54: 2155–2162.

Ryan, M., J. Pembrok & C. Adley, 2006. *Ralstonia pickettii*: a persistent Gram-negative nosocomial infectious organism. Journal of Hospital Infection 62: 278–284.

Scola, B. L., R. J. Birtles, M.-N. Mallet & D. Raoult, 1998. *Massilia timonae* gen. nov., sp. nov., isolated from blood of an immunocompromised patient with cerebellar lesions. Journal of Clinical Microbiology 36: 2847–2852.

Sintchenko, V., P. Jelfs, A. Sharma, L. Hicks, G. L. Gilbert & C. Waller, 2000. *Massilia timonae*: an unusual bacterium causing wound infection following surgery. Clinical Microbiology Newsletter 22: 149–151.

Snyder, M., M. Taillefert & C. Ruppel, 2004. Redox zonation at the saline-influenced boundaries of a permeable surficial aquifer: effects of physical forcing on the biogeochemical cycling of iron and manganese. Journal of Hydrology 296: 164–178.

Sobolev, D. & E. E. Roden, 2002. Evidence for rapid microscale bacterial redox cycling of iron in circumneutral environments. Antonie Van Leeuwenhoek 81: 587–597.

Spiteri, C., P. Regnier, C. P. Slomp & M. A. Charette, 2006. pH-dependent iron oxide precipitation in a subterranean estuary. Journal of Geochemical Exploration 88: 399–403.

Stuetz, R. M. & R. G. McLaughlan, 2004. Impact of localised dissolved iron concentrations on the biofouling of environmental wells. Water Science and Technology 49: 107–113.

Taylor, S. W., C. R. Lange & E. A. Lesold, 1997. Biofouling of contaminated ground-water recovery wells: characterization of microorganisms. Ground Water 35: 973–980.

Testa, J. M., M. A. Charette, E. R. Sholkovitz, M. C. Allen, A. Rago, C. W. Herbold, 2002. Dissolved iron cycling in the subterranean estuary of a coastal bay: Waquoit Bay, Massachusetts. Biological Bulletin 203: 255–256.

Tyrrel, S. F. &P. Howsam, 1997. Aspects of the occurrence and behaviour of iron bacteria in boreholes and aquifers. Quarterly Journal of Engineering Geology and Hydrogeology 30: 161–169.

Ultee, A., N. Souvatzi, K. Maniadi & H. Konig, 2004. Identification of the culturable and nonculturable bacterial population in ground water of a municipal water supply in Germany. Journal of Applied Microbiology 96: 560-568.

Weber, K. A., L. A. Achenbach & J. D. Coates, 2006. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction. Nature Reviews Microbiology 4: 752–764.

Weiss, J. V., J. A. Rentz, T. Plaia, S. C. Neubauer, M. Merrill-Floyd, T. Lilburn, C. Bradburne, J. P. Megonigal & D. Emerson, 2007. Characterization of neutrophilic Fe(II)-oxidizing bacteria isolated from the rhizosphere of wetland plants and description of *Ferritrophicum radicicola* gen. nov. sp. nov., and *Sideroxydans paludicola* sp. nov. Geomicrobiol Journal 24: 559–570.

Weng, H., Y. Qin & X. Chen, 2007. Elevated iron and manganese concentrations in groundwater derived from the Holocene transgression in the Hang-Jia-Hu Plain, China. Hydrogeology Journal 15: 715–726.

WHO, 1996. Iron in drinking water – Guidelines for drinking-water quality, second ed. Vol. 2. Health criteria and other supporting information. World Health Organization, Geneva.

Table 1. Laboratory enrichment media used in bacterial cultivation and enumeration

Medium	**pH**	**Target bacteria**	**Growth reaction**	**Reference(s)**
9K	2.5	Aerobic, acidophilic Fe(II)-oxidizing, e.g. *Acidithiobacillus ferrooxydans*	Rust-colored Fe(III) oxide precipitate	Lazaroff (1963)
Liquid FeS gradient medium	4.8	Neutrophilic, microaerophilic Fe(II)-oxidizing, e.g. stalk-forming *Gallionella ferruginea*	Rust-colored colonies attached to tube wall	Hanert (2006)
Semi-solid FeS gradient medium	6.3	Neutrophilic, microaerophilic Fe(II)-oxidizing, e.g. unicellular *Sideroxydans*	Dense rust-colored Fe(II) oxidation band at the oxic–anoxic interface	Emerson and Floyd (2005)
Fe(III) medium	7.0	Aerobic, neutrophilic Fe(III)-depositing, e.g. *Sphaerotilus* and *Leptothrix*	Turbidity and filamentous growth	Atlas (2004)
Fe(III)-EDTA medium	7.0	Anaerobic, neutrophilic Fe(III)-reducing	Ferrozine indicator turns purple[a]	Gould et al. (2003)
S medium	4.8	Aerobic, acidophilic elemental S-oxidizing, e.g. *Thiobacillus thiooxidans*	pH indicator bromophenol blue turns yellow[b]	Unz (2005)
Thiosulfate medium	7.8	Aerobic, neutrophilic thiosulfate-oxidizing, e.g. *Thiobacillus thioparus*	pH indicator bromothymol blue turns yellow[b]	Unz (2005)
MP broth	7.0	Neutrophilic, microaerophilic sulfide-oxidizing, e.g. filamentous *Beggiatoa* and *Thiothrix*	pH indicator bromothymol blue turns yellow[b]	Unz (2005)
API	7.5	Anaerobic, neutrophilic sulfate-reducing, e.g. *Desulfovibrio*	Black FeS precipitate	Tanner (1989)
PC	7.0	Aerobic, neutrophilic Mn(II)-oxidizing, e.g. hyphal, budding *Pedomicrobium*	Dark brown or black Mn(IV) precipitate on colonies	Tyler and Marshall (1967)
R2A	7.2	Aerobic, heterotrophic	Bacterial colonies	Reasoner and Geldreich (1985)

a. Ferrozine reacts with Fe(II) produced via bacterial Fe(III) reduction; and b. pH decreases due to S oxidation and H_2SO_4 production

Table 2. Physico-chemistry, DOC and DIC of monitoring wells in Poona estuary-adjacent shallow groundwaters (December 2008)

Well	Distance from estuary (m)	Tm[a] (ºC)	pH	Eh[b] (mV)	DO[c] (mg L^{-1})	EC[d] (mS cm^{-1})	DOC[e] (mg L^{-1})	DIC[f] (mg L^{-1})
W1	600	22.7	5.4	90	1.85	0.17	6.7	3.7
W2	520	21.3	5.2	118	0.40	0.28	4.0	2.1
W4	420	18.5	5.4	-21	0.69	0.28	7.6	30.6
W5	390	21.8	5.6	-3	0.59	0.43	7.3	39.7
W6	360	24.6	5.9	-32	0.56	0.39	47.3	54.6
W7	320	21.2	5.7	-16	0.12	0.72	3.7	3.7
W8	300	22.2	6.6	30	0.56	2.34	3.5	6.7

a. Temperature; b. redox potential; c. dissolved oxygen; d. electron conductivity; e. dissolved organic carbon; and f. dissolved inorganic carbon

Table 3. Ionic concentrations and TDI for monitoring wells in Poona estuary-adjacent shallow groundwaters (mg L^{-1})

Well	Fe_{total}	Fe(II)	Mn_{total}	SO_4^{2-}	S^{2-}	NO^{3-}	PO_4^{3-}	Ca	Mg	Na	K	Cl	TDI
W1	1.6	1.2	0.01	8.3	0.5	<0.05	<0.05	0.5	5.9	38	0.6	8	96
W2	9.0	7.4	0.1	7.8	ND[a]	<0.05	<0.05	3.0	7.9	43	0.5	75	147
W4	5.4	4.6	0.01	8.1	0.6	<0.05	<0.05	1.0	5.0	55	1.5	70	177
W5	3.0	2.6	0.02	6.8	0.2	<0.05	<0.05	1.5	6.3	75	2.0	107	242
W6	4.2	4.2	0.02	4.2	0.5	<0.05	<0.05	1.5	7.0	68	2.7	90	227
W7	15.0	11.6	0.02	42.1	0.4	<0.05	<0.05	2.2	14	120	3.2	206	406
W8	32.8	28.0	0.1	35.5	0.2	<0.05	<0.05	16	120	310	14	932	1467

a. not determined

Table 4. Cultivable bacterial numbers of Poona estuary-adjacent shallow groundwater (CFU or cells mL^{-1})

Well	HPC	Neutrophilic Fe(II)-oxidizing bacteria	Fe(III)-depositing bacteria	Fe(III)-reducing bacteria	Sulfide-oxidizing bacteria	Elemental sulfur-oxidizing bacteria	Thiosulfate-oxidizing bacteria	Sulfate-reducing bacteria
W1	1.4×10^4	10^1	10^3	10^4	<10	10^3	10	10^2
W2	9.8×10^3	10^2	10^2	10^2	<10	10^7	10^2	10^2
W4	1.2×10^4	10^4	10^3	10^9	10	10^3	10^3	10^3
W5	3.4×10^5	10^4	10^4	10^8	10	10^2	10	10
W6	3.0×10^4	10^4	10^3	10^7	10^2	10^4	10^2	10^2
W7	7.5×10^3	10^3	10^2	10^2	10^3	10^3	10	10^2
W8	9.6×10^3	10^2	10^9	10^4	10^5	10^7	10	10^3

Acidophilic Fe(II)-oxidizing and neutrophilic Mn(II)-oxidizing bacteria were below 10 cells or CFU mL^{-1} (MDL)

Figure captions:

Figure 1. Location of Poona estuary on the Fraser Coast of Southeast Queensland.

Figure 2. Schematic diagram of the transect of sampling wells (W1–W8, except W3) near Poona estuary mouth (1:60 vertical exaggeration); W1 and W8 are approximately 950 and 650 m from the estuary northern bank, respectively; topographic heights extracted from Light Detection and Ranging (LIDAR) data supplied by Forestry Plantations Queensland.

Figure 3. Neutrophilic Fe(II)-oxidizing bacterial enrichment culture recovered from Poona estuary-adjacent shallow groundwater (W5) using liquid FeS gradient medium (A–B) Photographs of rust-colored colonies attached to tube wall and glass rod; (C) TEM image of unicellular bacterial cells in the vicinity of Fe oxide precipitates.

Figure 4. TGGE of neutrophilic Fe(II)-oxidizing bacterial enrichment cultures recovered from Poona estuary-adjacent shallow groundwater using semi-solid FeS medium.

Figure 5. Neighbor-joining tree of bacterial enrichment cultures (PN) recovered from Poona estuary-adjacent shallow groundwater using semi-solid FeS gradient medium. Bootstrap values shown at nodes for frequencies at or above a 40% threshold (1000 bootstrap resampling). *Acidithiobacillus ferrooxydans* used as outgroup. Bar indicates 10% sequence variance.

Figure 6. Profile of the sampling site illustrating potential for bacterially-mediated groundwater Fe cycling in the Poona estuary-adjacent shallow aquifer (not to scale; well designations as per Fig. 2).

Supplemental material

Figure 1. Neutrophilic Fe(II)-oxidizing bacterial enrichment culture recovered from Poona estuary-adjacent shallow groundwater (W7) using semi-solid FeS gradient medium (A) Photograph of rust-colored Fe(II) oxidation band due to bacterial growth (arrows); (B) TEM image of unicellular bacterial cells (arrows) associated with Fe oxide precipitates; and (C) TEM image of unicellular cell showing polar, electron dense areas (arrows) due to Fe oxide deposition.