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Microbial Ecology and Geo-electrical Responses

across a Groundwater Plume

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ABSTRACT

We use geophysics, microbiology and geochemistry to link large scale (30+m) geophysical self-potential responses at a groundwater contaminant plume with its chemistry and microbial ecology of groundwater and soil from in and around it. We show that microbially mediated transformation of ammonia to nitrite, nitrate and nitrogen gas is likely to have promoted a well-defined electrochemical gradient at the edge of the plume which dominates the self-potential response. Phylogenetic analysis demonstrates that the plume fringe or anode of the geobattery is dominated by electrogens and biodegradative micro-organisms including *Proteobacteria* alongside *Geobacteraceae, Desulfobulbaceaese* and *Nitrosomonadaceae.* The uncultivated Candidate Phylum *OD1* dominated uncontaminated areas of the site. We define the redox boundary at the plume edge, using calculated and observed electrical self-potential (SP) geophysical measurements. Conductive soils and waste act as an electronic conductor, which is dominated by abiotic iron cycling processes that sequester electrons generated at the plume fringe. We suggest that such geo-electrical phenomena can act as indicators of natural attenuation processes that control groundwater plumes. Further work is required to monitor electron transfer across the geo-electrical dipole to fully define this phenomenon as a geobattery. This approach can be used as a novel way of monitoring microbial activity around the degradation of contaminated groundwater plumes or to monitor *in situ* bioelectrical systems designed to manage groundwater plumes.

INTRODUCTION

Management of complex contaminant plumes in groundwater that undergoes natural or engineered bioremediation can quickly become unsustainable due to the economic and technical constraints associated with long term monitoring. As contaminant groundwater plumes age, microbial communities dominate this polluted environment and degrade the contaminants to the extent that plumes can reach a steady state where contaminant transport is matched by degradation (Prommer, Tuxen, and Bjerg 2006). This can produce a static plume with a well-defined boundary and concentration gradient with the surrounding groundwater. Anaerobic degradation by micro-organisms within contaminant groundwater plumes provides a source of electrons that can be exploited by other micro-organisms. It has been proposed that where there is a redox boundary and a subsurface electronic conductor (e.g., iron precipitates, sulphide or graphite deposits) a naturally occurring, large microbial fuel cell, commonly known as a geobattery may be generated. This phenomenon was originally associated with sulphide and graphite ore deposits (Sato and Mooney 1960), then applied to groundwater plumes (Bigalke 1997; Timm and Möller 2001) where the idea of a biological contribution became of interest (Revil et al. 2010; Doherty et al. 2010).The ability to accurately reproduce these conditions in the laboratory is a source of concern (Hubbard et al. 2011), however recent work with anaerobic marine sediments has revived interest in the biogeobattery concept (Nielsen et al. 2010; Risgaard-Petersen et al. 2014). In these bio-electrochemical systems electrons are produced in an anaerobic anode domain and travel across a redox boundary to a cathode domain where they are consumed. Electrical current flow arises between the positive and the negative pole of the geobattery, generating an electrical field that is readily measured with the electrical self-potential (SP) geophysical method. Previous in-situ field as well as laboratory studies (Naudet et al. 2004)

ascertained that such microbially-mediated SP signals in redox environments are commonly

characterized by a linear relationship between SP (ψ_{redox}) and redox potential (E_H) :

$$\psi_{redox} = C_H\left(E_H - E_H^{ref}\right) \qquad (1)$$

Where E_H^{ref} is E_H measured at a suitable reference electrode located outside of the area affected

by contamination and C_H is the redox coupling coefficient. This relationship is based upon a

model of the source located close to the water table with a C_H value of 0.5 shown to represent a

vertical dipole geometry created by a shallow organic contaminant plume (Linde and Revil

2007; Arora et al. 2007). The geometry of the coupling coefficient is related to the solid angle of

the source in relation to the observation electrodes which varies from C_H 0.5 in field studies as

stated above to 0.18 to 0.2 in 2D laboratory studies (Revil et al. 2010).

This represents one of four common mechanisms capable of generating natural electrical

current flow and associated SP signals. The other three include electrical streaming potentials

due to subsurface water flow, thermal processes (e.g in volcanic areas and coal seam fires) and

electrochemical diffusion. Electrochemical diffusion may arise due to the proximity of waters

with very different ionic strengths that can produce an electric field parallel to the concentration

gradient, such as glacial meltwaters (Kulessa 2003), groundwater tracers (Davis et al. 2010),

engineered bioelectrical systems (Kim and Logan 2011) or groundwater plumes. In an isotropic

heterogeneous porous media the geo-electrical effect of groundwater flow that drags excess

charge in coupled with the electrochemical effect of dissolved contaminants or brines is

described by Revil & Linde (2006) as:

$$\mathbf{j}s = \bar{Q}_V\boldsymbol{u} - k_b T \sum_{i=1}^{N} \frac{T_i \sigma_0}{q_i} \nabla\ln\{i\} \qquad (2)$$

Where $\mathbf{j}_s$ is the source current density in (Am^{-2}), k_b is the Boltzmann constant (1.381×10^{-23}

JK^{-1}), $\{i\}$ is the activity of species i (taken equal to the concentration of species i for an ideal

solution), σ_0 is the DC-electrical conductivity of the porous material (in Sm^{-1}) $\bar{Q}_V$ is the volumetric (moveable) charge per unit pore volume at saturation (expressed in Cm^{-3} and related to the electrical diffuse layer), $\mathbf{u}$ represents the Darcy velocity (in ms^{-1}), q_i is the charge of species i dissolved in water (in C) and T_i (dimensionless) is the macroscopic Hittorf number of the ionic species i in the porous material (i.e. the fraction of electrical current carried by this species). While thermal SP signals are not normally expected to be of concern at contaminated land sites, the presence of streaming and electrochemical potentials must be diagnosed and apportioned where they superimpose on the microbially-mediated redox driven SP signals of interest. It is expected that the electrochemical and redox inputs would have a cumulative effect on the measured self-potential values observed at contaminated sites (i.e. $\psi_{measured} \approx \psi_{EC} + \psi_{redox}$).

The microbial ecology of field scale environments where geobatteries may occur are of considerable interest. Extracellular electron transfer of microbiological origin has been shown to occur at a variety of scales (μm–cm) in the laboratory through different direct and indirect mechanisms. These range from conductive filament electron transfer across redox zones at the centimeter scale by *Desulfobulbaceae* (Pfeffer et al. 2012), to nanowires e.g. *Geobacter sulfurreducens* and *Shewanella oneidensis* (Reguera et al. 2005; Ntarlagiannis et al. 2007), interspecies electron shuttling of conductive materials e.g. *Geobacter sulfurreducens* to *Thiobacillus denitificans* (Kato, Hashimoto, and Watanabe 2012), interaction with iron oxide minerals e.g. *Geobacter* (Kato et al. 2010), and direct electron transfer across cell cytochromes e.g. by *Geobacter sulfurreducens* and *Shewanella oneidensis* (Shi et al. 2007) on the micrometer scale. As yet none of these mechanisms have been observed or inferred to occur over large distances (cm-m) in the field but could potentially contribute (alongside dominant mechanisms such as redox and electrochemistry) to the geobattery phenomenon.

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Our study combines a down borehole SP survey alongside groundwater chemistry and detailed metagenomic microbiological analysis of the 16S rRNA gene to the taxonomic resolution of genus level to characterize the microbial community at a previous Manufactured Gas Plant site in Portadown, Northern Ireland. Comprehensive geological, hydrological and biogeochemical descriptions of the site have been reported (Davis et al. 2010; Ferguson et al. 2003; Doherty et al. 2006; Doherty et al. 2002; Doherty et al. 2010). A risk management strategy previously installed a cement-bentonite wall to contain the contamination within the site, and a sparged permeable reactive barrier (PRB) to treat the contaminated ground waters at the plume fringe. Sparging ceased when levels of contamination dropped significantly, so that the PRB environment is now anaerobic.

METHODS

Characterizing the subsurface environment

Six boreholes across the site and three piezometers in the PRB were monitored over a four month period (Figure 1). Groundwater levels and parameters (redox, conductivity,) were measured with a Multi-parameter Field Logger (TPS 90FL-T) that was calibrated daily. Each borehole was purged until stable parameters were obtained. Groundwater samples were stored immediately at 4°C for microbial (QUB and University of Cambridge, UK) and chemical analysis (QUB and Scientific Analysis Laboratories, Manchester UK). DNA extractions from groundwater samples were carried out within 24 hours of sampling using a standard kit (Powerwater DNA Isolation kit, Mo Bio Lab Inc.). SP monitoring was conducted down boreholes in groundwater. To ensure that the SP electrodes were in groundwater that was representative of the site conditions, all the electrodes were placed midway in the saturated zone of the borehole prior to purging of the wells to obtain groundwater chemistry. A roving electrode

method was used; with the reference borehole located in a pristine area of the site. Standard practice was followed in collecting and identifying drift relative to the reference electrode (Naudet et al. 2004). Data were acquired using miniature non polarizing Ag/AgCl electrodes (Petiau 2000) made in house, modified from a design by (Zhang et al. 2010) with a METRA HIT 22S high impedance voltmeter ($20M\Omega$) with a rugged single core wire. The electrodes were tested for variation in the lab before and after use in the field by measuring the voltage between the electrodes, electrodes indicating a variation of over 2mV were discarded and any results obtained were not used. No appreciable groundwater temperature variation was noted. Scanning Electron Micrographs were also taken from an electrode that was recovered from the cathode groundwater at a depth of 2m.

   Three soil samples at (1 at 6m, 2 at 0.3m) were sampled from within the vicinity of the contaminated plume. DNA was extracted in triplicate and then pooled with Powersoil DNA Isolation kit (Mo Bio Lab Inc.) and stored at -20°C until analysis. A 2-step bcPCR approach was taken to amplify 16S rRNA gene of bacteria. The amplicons were prepared as previously described (Berry et al. 2011) using universal primers for the V6-V9 variable regions, 909F and 1492R including adaptors (LibL/A and LibL/B) and tags(Hamady et al. 2008). PCR was carried out in a final volume of 25μl containing 0.4μm of each 16S primer (HPLC pure from IDT, Inc.) and 1μl of DNA template. PCR conditions were 95°C for 4min and 25 cycles of 95°C for 30s, 52°C for 45s, 72°C for 1min 30s, and a final extension of 72°C for 7min. PCR product was used as the template in the second round amplification (1:25 dilution) where fusion primers were used (adaptors (LibL/A and LibL/B), tags(Hamady et al. 2008) and 16S primers). PCR conditions were 95°C for 4min and 5 cycles of 95°C for 30s, 52°C for 45s, 72°C for 1min 30s and a final extension of 72°C for 7min. PCR reactions were purified using High pure PCR purification kit

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(Roche). DNA was quantified using a spectrophotometer (Nanodrop), with 250ng sent for

sequencing. Amplicons were pooled and sequenced at DNA Sequencing Facility, (Department of

Biochemistry, University of Cambridge, UK) by using a One-way read strategy from the reverse

primer, Titanium chemistry on a 454 GS Junior Devise (Roche) Quantitative sequences analysis

was carried out in QIIME pipeline v 1.7 (Caporaso, Kuczynski, et al. 2010). Sequences were

filtered to exclude reads shorter than 200 bp and with mismatches in the barcode or primer.

Quality filter was made with USEARCH (Edgar 2010) and UCHIME (Edgar et al. 2011) ref base

was used to exclude chimeras, singletons were removed as well. USEARCH was also used to

pick Operational Taxonomic Units (OTUs) based on 97% similarity with a *de novo* method.

Alignment was done using PyNAST (Caporaso, Bittinger, et al. 2010).Taxonomy was assigned

against Greengenes reference database(DeSantis et al. 2006) (released on May 2013) by using

RDP classifier v2.2 (McDonald et al. 2012) (ribosomal database project) (Wang et al. 2007) with

a confidence limit of 0.8. Commands included in QIIME were used to obtain rarefaction curves

and to calculate alpha diversity metrics, whereas Unifrac(Lozupone and Knight 2005) was used

to calculate beta diversity. For visual representation minor groups of less than 1% abundance

were grouped. Raw sequences were archived in the European Nucleotide Archive (ENA) under

the study accession number; PRJEB6020. Direct link to the data can be found at

http://www.ebi.ac.uk/ena/data/view/PRJEB6020.

RESULTS

Groundwater Plume and Apportionment of SP Mechanisms

Chemical groundwater parameters (Supporting Information Table 1 & 2) indicate the

contaminated plume to contain high levels of organic contaminants, ammonium and sulphate,

thus sustaining an anaerobic environment with a high electrical conductivity compared to the rest of the site (Figure 1a). Areas of relatively low redox, high conductivity and high positive SP were characteristic of the groundwater plume, termed the anode of the geobattery (Figure 1a-c). The water and soil samples taken from the contaminated plume were phylogenetically the least diverse (Supporting Information Figure 1) and contained a high proportion of *Proteobacteria* and *Bacteroidetes* (Figure 2a). A high proportion of *Geobacteraceae* and *Desulfobulbaceaese* was present in samples from the anode domain (plume). This reduction in diversity and selection for *Geobacteraceae* and *Proteobacteria* is similar to other examples of soil based MFCs where anthropogenically affected soils have lesser diversity (Dunaj et al. 2012). Members of *Geobacteraceae* have been shown to partake in the degradation of organic contaminants and several types of extracellular electron transfer including Fe(II) (Lovley 2008; Reguera et al. 2005; Kato et al. 2010; Kato, Hashimoto, and Watanabe 2012). Here we see communities such as the soil sample at 6m depth from the center of the plume dominated by 31.0% of the family *Geobacter* and 12.0% of family *Desulfobulbaceae* (Figure 2b). *Desulfobulbaceae* are a strictly anaerobic family that have members who are toluene degraders (Pilloni et al. 2011) as well as members which are capable of long range electron transfer over centimeters (Pfeffer et al. 2012). The large relative abundance of *Geobacteraceae* and *Desulfobulbaceaese* may be due to niche partitioning of iron reducing and sulphate reducing bacteria with *Geobacter* utilizing acetate and some *Desulfobulbaceaese* consuming fermentation end products (Wrighton et al. 2014). *Bacteroidetes* have been associated with later stage biodegradation of Polycyclic Aromatic Hydrocarbons (Viñas et al. 2005) with the order *Bacteroidales* capable of anaerobic fermentation of organic macromolecules (Dawson et al. 2012; Wrighton et al. 2014) and these occur in samples throughout the contaminant plume (5.1 - 29.8%) (Figure 2b). The anode soil and

groundwater samples also contain the phyla *Firmicutes* (2.0 - 12.6%), members of which are also

associated with the anaerobic degradation of organic macromolecules (Dawson et al. 2012), have

been shown to be present in soil remediation bioelectrical systems (Lu et al. 2014) and to have

electrogenic properties (Wrighton et al. 2008). *Syntrophaceae*, is a syntrophic family of bacteria

and these are present in the anode groundwater and in turn may be interacting with the other

degraders to facilitate the full cycling of carbon (Gray et al. 2011). The anaerobic degradation of

typical organic gasworks contaminants such as Polycyclic Aromatic Hydrocarbons and BTEX

compounds within the plume and at the plume fringe would produce electrons which can be

consumed by a variety of electron acceptors. *Pseudomonadaceae* were also abundant in plume

with greatest levels at the plume fringe (2.3%). These are aerobic degraders of organic

contaminants. Some members are iron oxidizers and denitrifiers that would compete with strict

anaerobes at the plume fringe. This selection of electrogens and degraders at a potential anode of

the geobattery was also observed in the anode of a bioelectrical system using soil samples to

demonstrate bioremediation capability (Lu et al. 2014). Conversely, areas of high redox, low

conductivity and low SP were measured in relatively unpolluted 'pristine' areas of the site

(Figure 1a-c) and in an area of dumped ash and iron clinker that was previously identified to

serve as the cathode of a microbial fuel cell (Doherty et al. 2010) (Figure 2a). The reference

borehole in the uncontaminated area has the highest level of microbial diversity with a mixture

of typical environmental soil and groundwater bacteria (Ferguson et al. 2007) (Figure 2a). The

diversity here would reflect the lack of contamination. The reference borehole water also had the

lowest proportions of *Proteobacteria* (1% *Gammaproteobacteria*) with all other samples from

within the plume (up to 59.1%) and geobattery cathode (25.1%) containing a significant

proportion of *Proteobacteria* throughout. Interestingly members of the uncultivated Candidate

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Division OD1 are considered to be strictly anaerobic and likely to be fermentative bacteria, some members of which can reduce sulphur (Wrighton et al. 2012). These made up a high proportion of bacteria in aerobic areas of the site and at the plume fringe but not in highly contaminated areas (Figure 2b) suggesting that the gasworks contamination may have caused its inhibition. The reference borehole contains species which are not seen anywhere else on the site with high levels of OTUs (21.5%) and Minor Genus (19.1%). There are high levels of Minor Genus in all the sample locations (13-32%); these are collated sequences which are individually present at less than 1% within the sample.

The positive and negative field SP values form a dipole (Figure 1c) which represents the poles of a geobattery. To identify and eliminate electrokinetic effects such as ground water flow the hydraulic gradient (-0.009) and SP were calculated over a pristine area of the site (Figure 1c), with a negligible SP effect due to streaming potentials of ~5mV over 20m in the direction of groundwater flow were noted. If extrapolated across the site we may expect a maximum range of ~30mV attributed to streaming potentials. This value is probably an over estimate due to the presence of a cement bentonite slurry wall that controls groundwater flow in the northern half of the site.

Using field redox values it is possible to calculate SP values (ψ_{redox}) using Equation (1) and compare with observed SP signals that were measured at the same time. A comparison of the measured and calculated SP values across the site is shown in Figure 1c-d, Figure 3a and in Figure S3 of the supporting information. Using a reference borehole (E_H^{ref}) located outside the contaminant plume in the pristine aquifer, a linear relationship ($R^2 = 0.9$; $\psi = 0.38[E_H-E_H^{ref}]$) in the SP calculated from E_H data recorded only in boreholes outside the contaminant plume (Figure 3a). Allowing for inherent uncertainties the slope of this relationship is close to the

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value of ~ 0.5 expected from others who developed Equation (1) (Naudet et al. 2004). This inference is consistent with the dominance of redox-driven SP signals (i.e. $\psi \approx \psi_{redox}$) in the cathode region of the pristine aquifer and the absence of strong streaming or electrochemical potentials.

When the reference borehole outside the plume ($E_H{}^{ref}$) was initially used to calculate SP within the plume no immediate correlation was observed. To test if electrochemical factors were also influencing SP within the plume we selected an second reference borehole ($E_H{}^{ref2}$) that was representative of conditions in the plume and a distinctly different linear relationship ($R^2 = 0.8$; $\psi = 0.68[E_H-E_H{}^{ref2}]$) between calculated SP and redox is observed (Figure 3a), characterized by a positive offset of > 200 mV and a steeper slope compared the pristine-aquifer data.

The marked offset in the observed SP-redox relationship across the plume boundary (Figure 1b,c) suggests that electrochemically-driven SP signals may superimpose on redox-driven SPs in the plume region of the site (i.e. $\psi_{measured} \approx \psi_{redox}+ \psi_{EC}$). To further test this, measured SP ($\psi_{measured}$) was plotted against SP calculated from redox (ψ_{redox}) using the two reference electrode approach. The trend wasn't as obvious ($R^2 = 0.66$ $C_H=0.6$), also suggesting another source mechanism (electrochemical) that could be superimposing (Supporting Information Figure S3) To estimate the electrochemical contribution using other field data we considered Equation 2 introduced earlier. It has been demonstrated (Revil 1999) that the gradient of the logarithm of the conductivity of a salt is equivalent to the gradient of the logarithm of the activity of a salt, if we assume that electrochemical self-potential signals in a plume are dominated by a single contaminant cation this allows the total source current to be rewritten as (Revil and Jardani 2013):

$$\boldsymbol{js} = \bar{Q}_V\boldsymbol{u} - \frac{k_bT}{e}\sigma_0(2T_{(+)} - 1)\nabla ln\, \sigma_p \qquad\qquad (3)$$

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Where T is a macroscopic Hittorf number and σ_p is electrical conductivity of the plume pore

water. This assumes that the self-potential field is related to the pathway of plume migration

within an aquifer. However this approach would not be valid within a steady state plume where

plume migration is countered by degradation mechanisms resulting in a plume that does not

advance or may recede with groundwater flow. If the empirical approach that was used to

benchmark the above model by (Woodruff et al. 2010)   is used and the effect of groundwater is

be discounted due to a low hydraulic gradient, this allows a simple method to contrast the

electrical potential between the centre of a simple contaminant plume σ_p and the surrounding

uncontaminated aquifer σ_w by

$$\Delta\psi_{EC} = -\frac{k_b T}{e}(2T_{(+)} - 1)\nabla ln\left(\frac{\sigma_w}{\sigma_p}\right) \qquad (4)$$

If we were to assume that macroscopic Hittorf numbers ($T_{(+)}$) were equal to microscopic Hittorf

numbers ($t_{(+)}$) and =1 when an electronic conductor is present (presence of conductive gasworks

waste). Then the following equation could be used to highlight the self-potential generated

between the conductivity of pore water within the plume to that of the uncontaminated aquifer

$$\Delta\psi_{EC} = -\frac{k_b T}{e} ln\left(\frac{\sigma_w}{\sigma_p}\right) \qquad (5)$$

This approach now discounts material properties and is dependant on the conductivity of pore

water and is equivalent to that of (Kulessa 2003)

$$\Delta\psi_{EC} = C_{chem} ln\left(\frac{\sigma_1}{\sigma_2}\right) \qquad (6)$$

Where C_{chem} is the electrochemical coupling coefficient combining Nernst and diffusion

potentials, and σ_1 and σ_2 are respectively the fluid conductivities within the pristine aquifer and

contaminated plume region. To isolate the two components ($\psi_{redox} + \psi_{EC}$) we first predict the

redox-driven SP signal (ψ_{redox}) in this region using the relationship $\psi_{redox} = 0.38[E_H - E_H^{ref}]$

inferred for the pristine aquifer. We subtract the predicted from the measured SP signals, obtaining residual SP data for the groundwater plume suspected to be of electrochemical origin (ψ_{EC}). Plotting ψ_{EC} against $ln(\sigma_1/\sigma_2)$ according to Equation (6) yields a statistically highly significant relationship ($R^2 \approx 0.99$) that corroborates the presence of strong electrochemical potentials (ψ_{EC}) superposing on redox-driven SP signals (ψ_{redox}) within the plume (Figure 3b). This suggests that the residual electrochemical SP based on differences in fluid conductivity between anode (plume) and cathode (aquifer) of the geobattery also dominates the large SP signals (up to 200mV - Figure 3a,b) at the site with microbially mediated redox reactions contributing around 90mV to the overall SP signal. This is of a similar magnitude to other redox dominated SP signals observed across groundwater plumes (Giampaolo et al. 2014).

Geochemical data also reveals that the electrochemical potentials correlate with the reduction of a single cationic contaminant, ammonium (Figure 3b) providing some confidence that our assumption in Equation 3 is valid. Ammonium is a key biogeochemical contaminant of gasworks plumes. It can limit the aerobic degradation of organic contaminants by preferentially sequestering oxygen and often defines the aerobic / anaerobic boundary of the plume fringe (Torstensson, Thornton, and Broholm 1998). This correlates with the increase in the proportion ammonium degraders from the plume center to plume edge. The aerobic ammonium oxidizer *Nitrosomonadaceae* is dominant in the plume fringe (PRB Inlet) and the cathode monitoring well contains the aerobic ammonium oxidizers *Nitrospira* (8.5%) and *Nitrosomonadaceae* (5.2%) and members of the Candidate Phylum OD1 (3.1%) which have also been postulated to oxidize ammonium to nitrite (Vishnivetskaya et al. 2013). This nitrite can in turn be used as an electron acceptor by anammox organisms such as *Candidatus scalindua* (3.5%) which then produce dinitrogen gas or by other denitrifiers such as members of the *Pseudomonas* (Carlson

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and Ingraham 1983) and *Thiobacillus* genus which occur throughout the plume. The transformation of ammonia to nitrite and ultimately nitrogen gas would remove the suite of nitrogen compounds from the dissolved phase altogether reducing the ionic conductivity of groundwater helping to enhance the electrochemical gradient between plume (anode) and aquifer (cathode) across a conductor generating the Nernst or shale potential as described above.

The SP dipole outside the plume

A steady state plume is expected to have a well-defined plume fringe where degradation of contaminants matches plume production. The two dissimilar though distinct linear relationships of redox and SP inside and outside of the plume mark the position of the plume fringe and redox boundary between -60mV and -30mV (Figure 3a). This correlates well with the position of plume defined by conductivity (Figure 1a and 1c) as well as mid-range of the SP signals (Figure 1b). This suggests that redox driven cycling of iron rather than predominately aerobic processes may be of interest at the cathode (Borch et al. 2010; Schmidt, Behrens, and Kappler 2010; Weber, Achenbach, and Coates 2006). Previous site investigation using induced polarization (chargeability) normalized with resistivity techniques identified the presence of a large zone (30m+) of conductive soil material (up to 29mS/m) in this area (Doherty et al. 2010) that is made of iron rich ash and clinker that acts as, or contributes to, the electronic conductor (Figure 2a). The reduction of iron-rich ash and clinker readily sequestering electrons near the eastern edge of the plume acts as an electron sink or cathode (Equation 7):

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \qquad\qquad (7)$$

The groundwater sample from the cathode monitoring well was predominately aerobic and did not provide much evidence of Fe (III) reduction. Nearby samples from the monitoring wells of the PRB which sits on the plume fringe did have a significant proportion of *Rhodoferax* (6.10%) and members of *Geothrix (7.5%)* which are capable of reducing Fe(III) (Lovley 2008; Finneran

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2003) .Members of the *Geobacter* family also take part in dissimilatory Fe(III) reduction are found in all monitoring wells (1.4-10.6%) and 6m soil sample (30.4%) within the plume. This suggests that the plume fringe is an active area of microbially mediated iron reduction and where this overlaps with conductive areas of dumped iron waste there is an opportunity for electron transfer to occur (Figure 5). The continued presence of ingression of aerobic water from rainfall results in the oxidation of the previously reduced iron compounds in the conductive area of the cathode to iron-oxides (Equation 8) allowing continuous iron cycling to occur.

$$Fe^{2+} + O_2 + 4H^+ \rightarrow Fe^{3+} + 2H_2O \qquad (8)$$
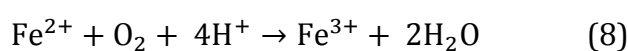
We recognize that such ingression would provide the opportunity for competition to sequester electrons generated in the anode domain by aerobes, suggesting that the point of contact between the anode domain and the electronic conductor must be permanently in an anaerobic condition below the water table. In addition, reddish precipitates observed on sampling tubes combined with SEM images with EDAX analysis show iron precipitation on an electrode at the cathode borehole (Figure 4a-c). These iron precipitates often nucleate around filaments that were observed travelling over 100μm (Supporting Information Figure S2). Bacteria in laboratory experiments can utilize nano-sized iron-oxides as electrical conduits to facilitate electron transfer to a distant electron acceptor (Kato et al. 2010; Nakamura et al. 2013), and models of bacterial-aquifer interaction with the transport of electrons have been proposed (Revil et al. 2010), however more evidence is required to ascertain if these precipitates are actually involved in the transport of electrons over the distances observed.

<div align="center">SUGGESTIONS FOR FURTHER STUDY</div>

To further define the roles of the redox, electrochemical and biological parameters highlighted by this study we would suggest using sediments or water from this or similar gasworks sites in sandbox experiments (Fachin et al. 2012; Revil et al. 2015). This would allow detailed

monitoring of the contribution of individual contaminants such as cations like ammonium which

may influence the electrochemical contribution to SP. Further detailed monitoring and alteration

of redox conditions in such a sandbox with a similar approach to (Risgaard-Petersen et al. 2014)

would offer a simple way to disturb the redox chemistry and to see the effect on SP signals. This

would allow for a more robust modelling approach rather the simple empirical methods that may

not always adhere to the geometry of the electric field that are adopted here. Such experiments

could also allow changes in water table which could mimic the seasonal changes noted by

(Giampaolo et al. 2014) which could also have a significant role in iron cycling (Rittgers et al.

2013) where our long term datasets at this site were lacking. Unclassified OTUs also represent a

significant gap in our full understanding of the microbial ecology of the site these could

represent additional degradative and electrogenic species. Further work is required to investigate

functionality of populations in the consortium of degraders and electrogens which contribute to

the source and transfer of charge. Gene specific metagenomic approaches may (Suenaga 2012)

allow a more targeted approach to identify degradation and electrogenic mechanisms helping to

validate geophysical approaches.

<div align="center">CONCLUSIONS</div>

Conceptual model of geo-electrical mechanisms

   There is a complex system of microbially mediated redox reactions related to the anaerobic

degradation of inorganic and organic compounds within the plume and the subsequent electron

transfer within the plume and at the plume edge. Most bacterial growth proliferates at the redox

boundaries of contaminated plumes (Prommer, Tuxen, and Bjerg 2006). The presence of

potentially electrogenic bacteria and degraders within the plume (Figure 2) suggests the ability of

the indigenous microbial community to produce and transfer electrons at the plume fringe to a

conductive soil body that is rich in iron waste compounds such as ash and clinker (Figure 5). This would allow the anaerobic groundwater plume fringe to produce electrons that are transferred across a redox boundary (Figure 5). The microbially mediated transformation of ammonia to nitrite, nitrate and eventually nitrogen gas is likely to promote a well-defined electrochemical gradient at the edge of the plume which also contributes a dominant electrochemical potential to the geobattery operation. This reduction and removal of contaminant mass from the groundwater plume also helps to maintain its steady state.

Previous models position the electrical field and SP dipoles vertically with a cathode in the aerobic vadose zone directly above the anaerobic anode which usually lies below the water table (Timm and Möller 2001; Revil et al. 2010; Sato and Mooney 1960; Naudet et al. 2004). In this particular case there is lateral dipole across a redox gradient / plume boundary (Figures 1b & 5). Cathodic reactions driving electrical currents have been demonstrated in geobattery models (Castermant et al. 2008) and in MFCs (Rabaey and Keller 2008; Oh, Min, and Logan 2004), where electron consumption at the cathode acts as driving force of the current generated. Potentially in this case, iron cycling within the dumped electrically conductive waste could be driving the degradation of contaminants within the plume enhancing natural attenuation processes. The importance of an established microbial population of degraders to produce electrons and electrogens to transfer electrons within and around contaminant plumes can be highlighted where contaminated plumes have no associated SP signals (Forté, Bentley, and Forte 2013). Plumes that are relatively new would not have developed to a steady state condition, and therefore would not contain established degradative and electrogenic microbial populations capable of supplying a source of electrons and possibly maintaining a distinct electrochemical gradient for compounds like ammonium.

The electric field and resulting dipole is generated around the conductor under potential difference across microbially-mediated redox and electrochemical conditions within the plume and redox boundary at the plume edge. The conductor links the plume with the dumped iron-rich ash and clinker this connection with the plume fringe probably contributes the redox SP signal, this divides the subsurface into domains (Timm and Möller 2001) (Figure 5). The plume domain is probably dominated by an electrochemical SP signal defined by a positive SP polarity relative to a reference electrode in a pristine area of the aquifer (Greenhouse and Harris 1983; Revil et al. 2010) usually observed by down-borehole roving and reference non-polarizing electrodes below the electric field. The cathode domain is at or above the water table and the electric field is in the aerobic vadose zone, this usually exhibits negative SP polarity relative to a reference station in a pristine area of the site (Nyquist and Corry 2002; Naudet et al. 2004). Interestingly at this particular site previous work using surface SP measurements identified the same dipole with a different polarity (Doherty et al. 2010) to the down borehole subsurface SP measurements presented here. This is explained by the relative position of the measuring electrodes to the electric field generated around the electronic conductor (Figure 5). The subsurface SP measurements are all observed below the electronic conductor producing a positive domain in the plume and a negative domain outside the plume. The previous surface SP measurements were observed above the electronic conductor producing a negative domain inside the plume and a positive domain outside the plume. In the phylogenetic analysis the contaminant plume is dominated by *Proteobacteria*, alongside *Geobacteraceae, Desulfobulbaceaese* and *Nitrosomonadaceae* as well as *OD1* dominating the uncontaminated areas of the site. The down-borehole SP method along with microbiological and geochemical analysis shows promise as a low cost, low resource and sustainable method to monitor electron transfer across plume and

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redox boundaries in real time. This approach could be applied to monitor large scale microbially

mediated electron transfer in natural and engineered environments such as enhanced monitored

attenuation and the use of engineered bioelectrical systems to manage groundwater plumes *in*

situ.

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ASSOCIATED CONTENT

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LIST OF FIGURES

Figure 1. Field Chemistry and Self Potential, a) Conductivity b) Redox c) Field self-potential with black box highlighting where electrokinetic contribution was estimated in a pristine area and d) Self potential calculated from field redox using 2 reference boreholes one in a pristine area of the site (Ref) and the other in the center of the groundwater plume (Ref 2), data captured November 2011.

Figure 2. Site outline with sampling locations marked with +. The contaminant plume is indicated by the contour grey scale shading (modelled Solvent Extractable Matter (Doherty et al. 2010)), the area of the conductive soil is outlined in red (previous geophysics investigation (Doherty et al. 2010)). a) Microbial diversity at phylum level for each sample is presented. b) Distribution of potential electrogenic and degradative genus at sample locations across the site. All are water samples with the exception of a soil sample denoted by *.

Figure 3 a) SP calculated from observed redox measurements over a four month period with reference boreholes inside and outside the plume plotted against concurrent field SP measurements. b) Residual SP of measurements taken within the plume taking into account fluid conductivity, plotted against ammonia concentration in groundwater from samples taken at the same time.

Figure 4 a) precipitates on a sampling tube from the cathode borehole, b) Scanning Electron Micrograph image of precipitates from an electrode from the cathode borehole showing nucleation of iron precipitates around filaments c) EDAX analysis of the precipitate highlighted in (b)

Figure 5. Conceptual model of the geo-electrical mechanisms operating in the subsurface. +ve and -ve circles represent the polarity of the SP signals relative to the electric field (dashed lines) generated around the electronic conductor. The plume or anode domain is dominated by degraders of organic contaminants and electrogens at the plume fringe coupled with a microbially mediated electrochemical gradient due to conversion of ammonia to nitrate/nitrite and finally nitrogen gas. This provides a significant electrochemical potential. The cathode

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domain is dominated by Fe (II) / Fe (III) cycling, where Fe (III) is reduced by electrons from the

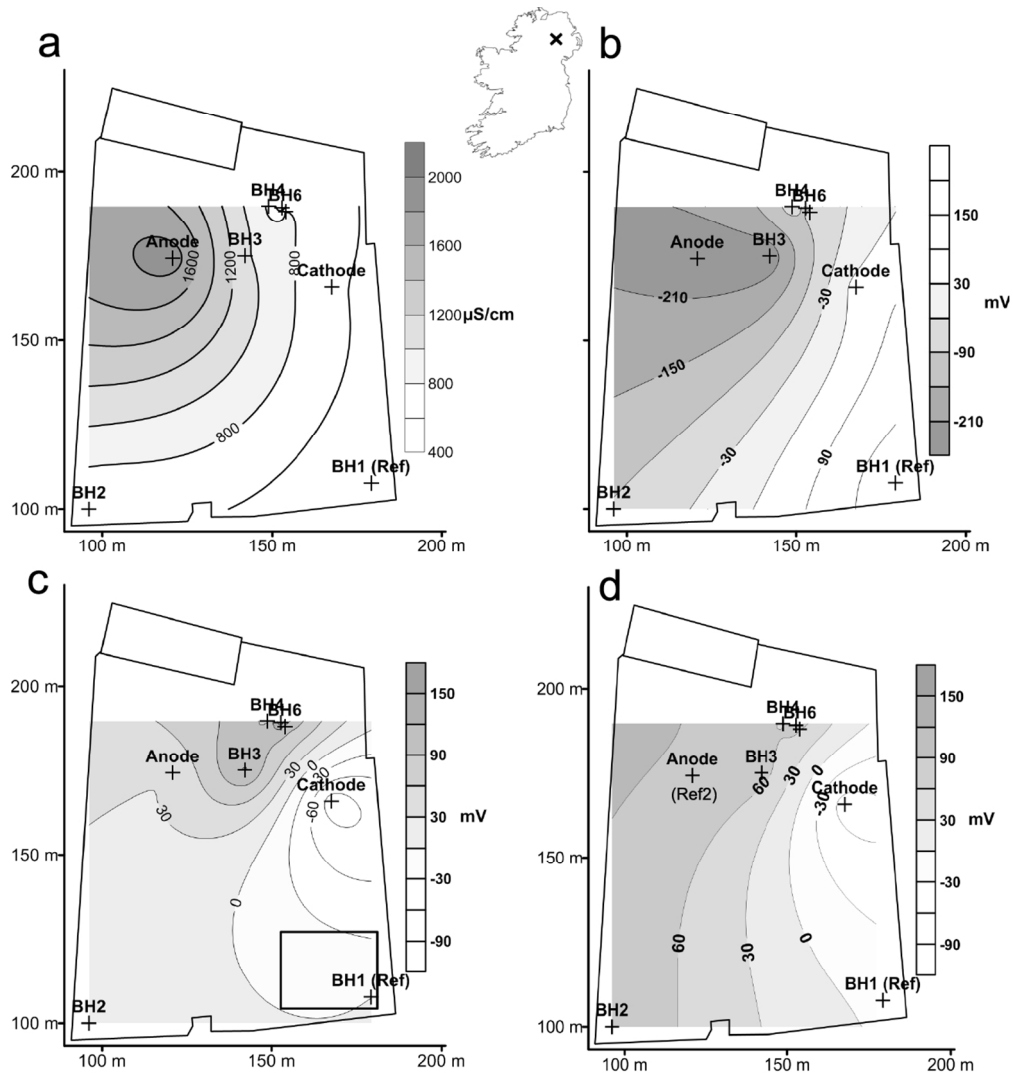anode domain to Fe (II) and oxidised to back to Fe(III) by ingression of aerobic water.

Figure 1. Field Chemistry and Self Potential, a) Conductivity b) Redox c) Field self-potential with black box highlighting where electrokinetic contribution was estimated in a pristine area and d) Self potential calculated from field redox using 2 reference boreholes one in a pristine area of the site (Ref) and the other in the center of the groundwater plume (Ref 2), data captured November 2011.
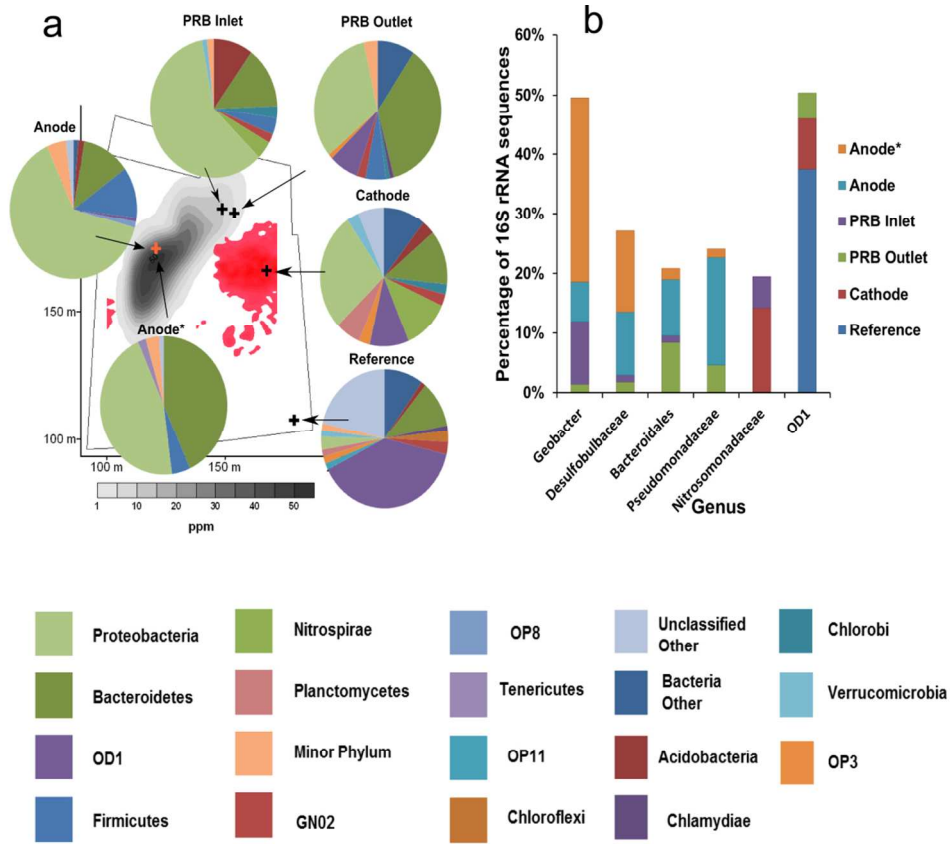110x117mm (300 x 300 DPI)

Figure 2. Site outline with sampling locations marked with +. The contaminant plume is indicated by the contour grey scale shading (modelled Solvent Extractable Matter (Doherty et al. 2010)), the area of the conductive soil is outlined in red (previous geophysics investigation (Doherty et al. 2010)). a) Microbial diversity at phylum level for each sample is presented. b) Distribution of potential electrogenic and degradative genus at sample locations across the site. All are water samples with the exception of a soil sample denoted by *.
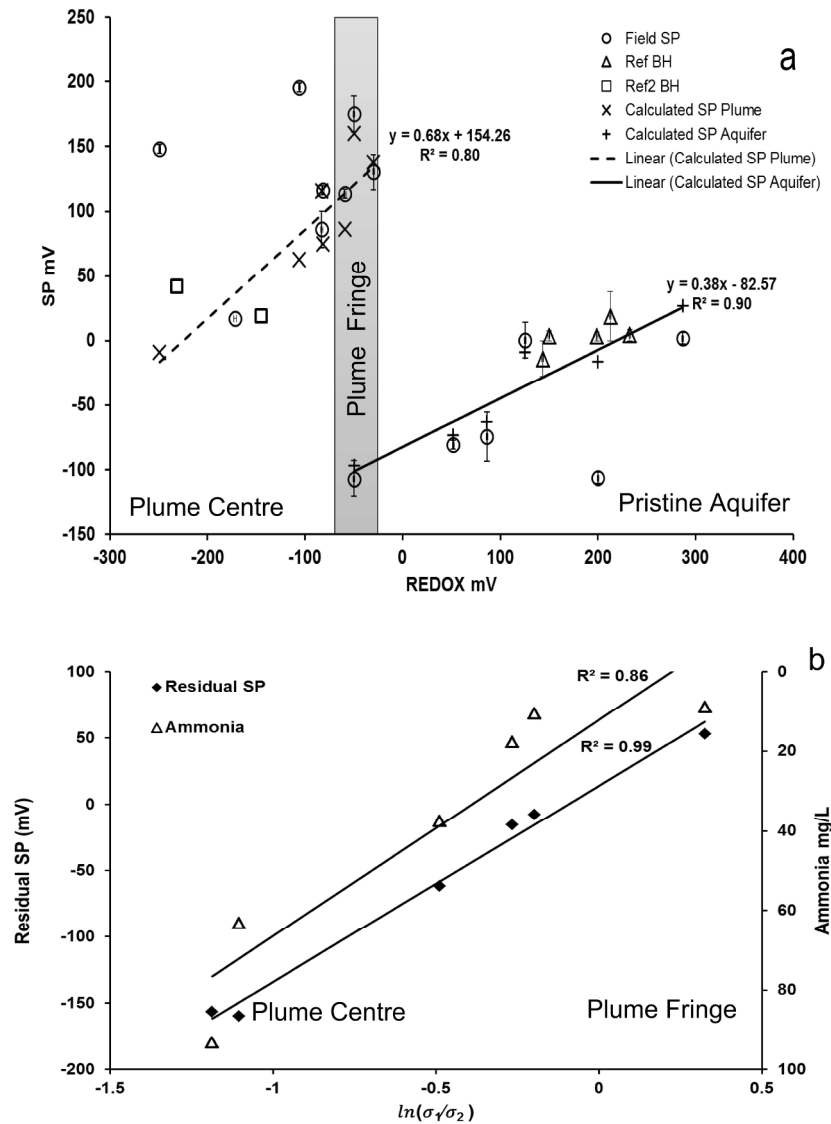103x88mm (300 x 300 DPI)

Figure 3 a) SP calculated from observed redox measurements over a four month period with reference boreholes inside and outside the plume plotted against concurrent field SP measurements. b) Residual SP of measurements taken within the plume taking into account fluid conductivity, plotted against ammonia concentration in groundwater from samples taken at the same time.
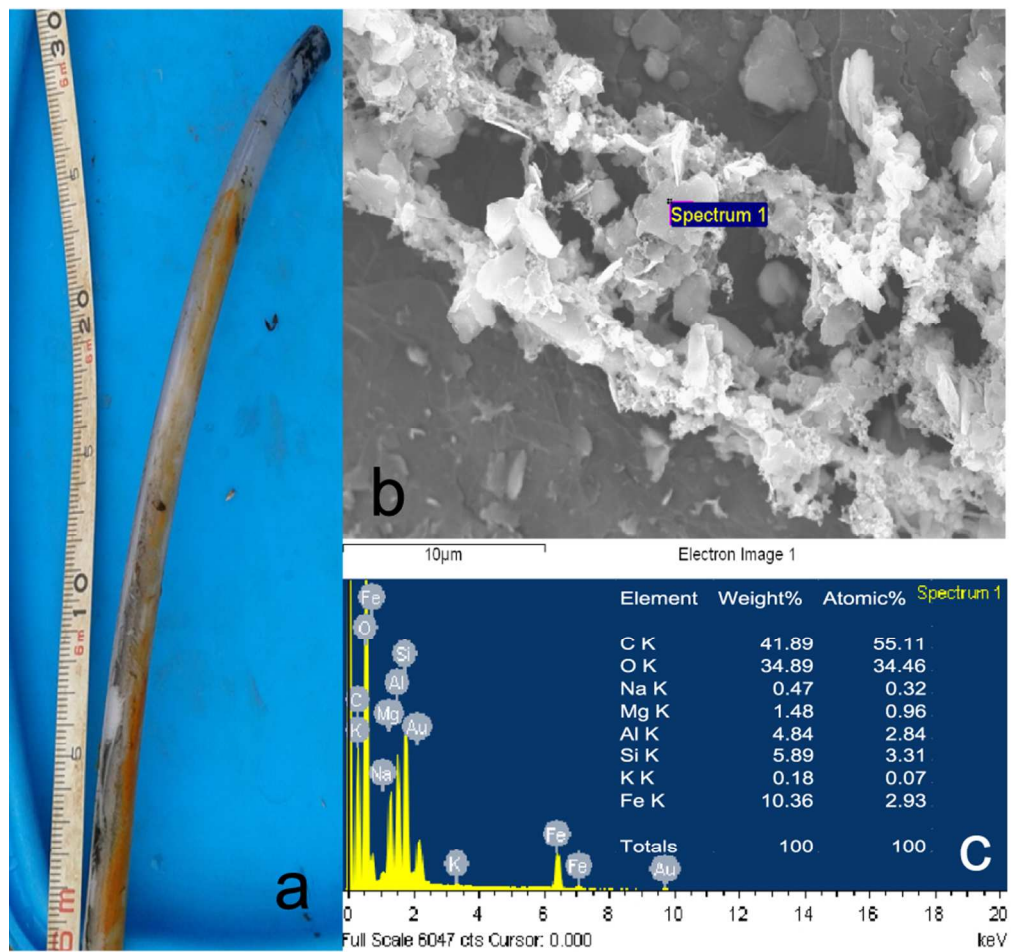110x145mm (600 x 600 DPI)

Figure 4 a) precipitates on a sampling tube from the cathode borehole, b) Scanning Electron Micrograph image of precipitates from an electrode from the cathode borehole showing nucleation of iron precipitates around filaments c) EDAX analysis of the precipitate highlighted in (b)
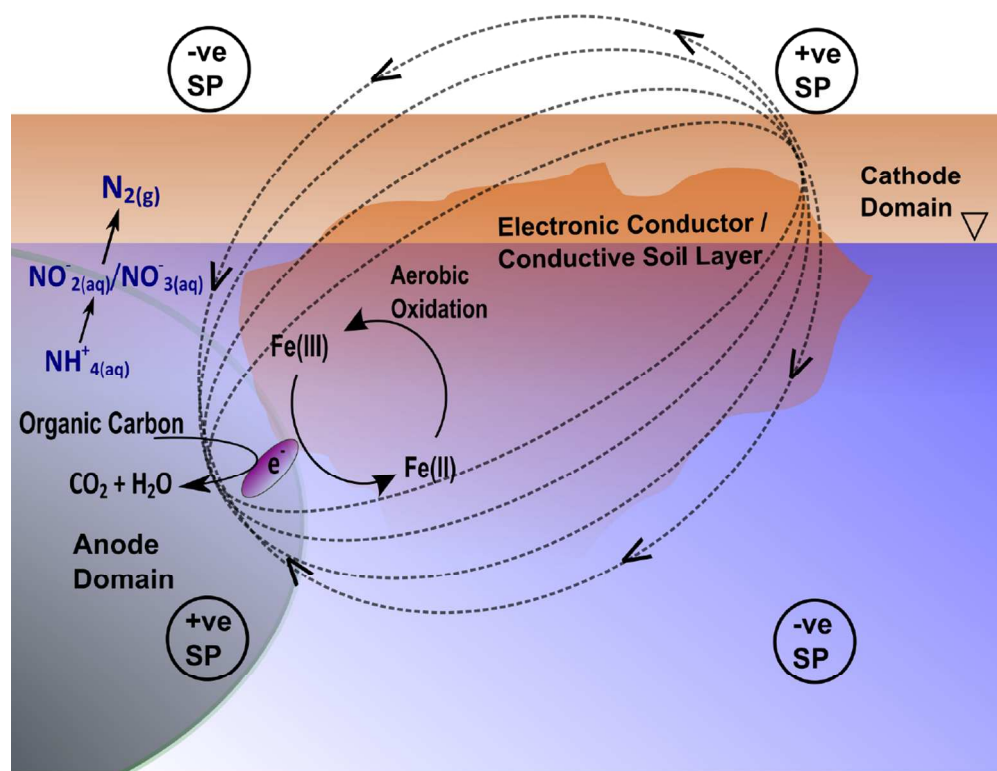84x80mm (300 x 300 DPI)

Figure 5. Conceptual model of the geo-electrical mechanisms operating in the subsurface. +ve and -ve circles represent the polarity of the SP signals relative to the electric field (dashed lines) generated around the electronic conductor. The plume or anode domain is dominated by degraders of organic contaminants and electrogens at the plume fringe coupled with a microbially mediated electrochemical gradient due to conversion of ammonia to nitrate/nitrite and finally nitrogen gas. This provides a significant electrochemical potential. The cathode domain is dominated by Fe (II) / Fe (III) cycling, where Fe (III) is reduced by electrons from the anode domain to Fe (II) and oxidised to back to Fe(III) by ingression of aerobic water. 110x87mm (300 x 300 DPI)