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Applied and Environmental Microbiology, 2015; 81(22):7822-7832

AEM Accepted Manuscript Posted Online 4 September 2015
Appl. Environ. Microbiol. doi:10.1128/AEM.01856-15

Confidential Information:	**Revised manuscript re-submitted to Applied and Environmental Microbiology**

GEOGENIC FACTORS AS DRIVERS OF MICROBIAL COMMUNITY DIVERSITY IN SOILS OVERLYING POLYMETALLIC DEPOSITS

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Running Title: Microbial communities in metal-rich Australian soils

Keywords: Microbial communities, landform, landuse, lithology, mineral deposits, metals

Number of Pages: 41

Number of Figures: 4

Number of Tables: 3

Number of Supplementary materials: 3

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Abstract

This study shows that the geogenic factors landform, lithology and underlying mineral deposits (expressed by elevated metal concentrations in overlying soils) are key-drivers of microbial community diversity in naturally metal-rich Australian soils with different landuse, *i.e.*, agriculture *vs*. natural bushland. 168 soil samples were obtained from two metal-rich provinces in Australia, *i.e.*, the Fifield Au-Pt-field (New South Wales) and the Hillside Cu-Au-U-rare-earth-element (REE) deposit (South Australia). Soils were analyzed using three-domain multiplex terminal-restriction-fragment-length-polymorphism (M-TRFLP) and PhyloChip microarrays. Geogenic factors were determined using field-mapping techniques and analyses of >50 geochemical parameters. At Fifield, microbial communities differed significantly with geogenic factors and equally with landuse ($P<0.05$). At Hillside, communities in surface soil (0.03-0.2 m depth) differed significantly with landform and landuse ($P<0.05$). Communities in deeper soils (>0.2 m) differed significantly with lithology and the mineral deposit ($P<0.05$). Across both sites, elevated metal contents in soils overlying mineral deposits were selective for a range of bacterial taxa, most importantly *Acidobacteria, Bacilli,* and *Beta-* and *Epsilon-Proteobacteria*. In conclusion, long-term geogenic factors can be equally important in determining soil microbial community diversity than landuse.

INTRODUCTION

Determining the drivers of microbial community composition in soils is challenging, because soils are complex ecosystems containing numerous ecological niches. This results in an immense diversity with up to thousands of taxa per gram of soil (1). Landuse, climate, vegetation, soil-type and anthropogenic pollution are strongly linked to soil microbial community structures, functions and activities at many sites (2-4). In particular, agricultural practises are known to drive differences in soil microbial communities (5). Influences of geogenic factors, *e.g.*, landform, underlying lithologies and mineral deposits on soil microbial communities have received little attention. However, three studies of soils from Switzerland and Nepal have shown that geological/mineralogical factors can significantly affect species assemblages and functions (6-8).

A primary goal of geomicrobiological research is to link microbial communities and metal cycling in metallogenic environments, and to determine how communities are structured due to metal-associated drivers (9). Microorganisms play a pivotal role in the biogeochemical cycling of many metals, particularly those essential for cell function, *e.g.*, Co, Cu, Fe, Mg, Mn, Mo, Na, Ni, V, W and Zn, and those oxidized or reduced in catabolic reactions to gain metabolic energy, *e.g.*, As, Fe, Mn, Mo, Sb, Se, Sn, Te, U and V (10). Therefore, metal-rich environments are useful model systems allowing links between microbial taxa and geochemical parameters to be identified (11).

To date, mine tailing- and acid mine drainage sites have been primary *foci* for assessing community compositions and functions at metal-rich sites (12-15). In contrast, few studies have assessed microbial communities in naturally metal-rich soils with low to moderate metal contents. These sites are highly abundant and typical examples include soils overlying/surrounding buried mineral deposits, where physical and (bio)geochemical cycling has led to the formation of metal enrichment zones (16). A recent Canadian study has shown that distinct microbial community assemblages were present in the glacial cover overlying a buried volcanogenic massive sulfide (VMS) deposit (17). In this study, a strong correlation between Zn and Cu concentrations, total biomass and abundances of methanotrophic bacteria was observed (17). Using culture-based approaches, correlations between abundances of *Bacillus cereus* spores and the presence of Au and its pathfinder elements (*i.e.*, As, Ag, Bi, Cu, Mo, Se, Te) in soils overlying Au deposits in Belgium, the United States and Australia have been observed (18-20). In Western Australian soils overlying Cu-Pb-Zn-deposits the solubilization, transport and deposition of metals is mediated by resident plant- and microbial communities (21, 22). Subsequently, elevated concentrations of mobile metals in the soils are related to changes in the microbial community composition. In particular, highly mobile elements, *e.g.*, S, Zn, Cl and Al, were implicated as drivers of bacterial community structures across these sites (21, 22). A study of 187 soils collected at four naturally auriferous (*i.e*., Au-containing) areas in remote Australia has shown that microbial communities and functional potentials differ significantly with landform, soil depth, lithology and Au-deposits (23). This demonstrated that geogenic factors are important drivers of microbial community diversity at sites where anthropogenic landuse is minimal and uniform across sampling localities. However, as geogenic influences are likely to develop over extended 'geological' time periods, their effects may be masked by short-term changes in landuse (24, 25). This has been illustrated in a study assessing the impact of short-term changes of landuse, *e.g.*, by over-sowing with exotic grasses and legumes, on soil microbial community structures and functional potentials at four tussock-based grassland sites in

New Zealand (26). Results have shown that soil bacterial and fungal communities and functional capabilities were strongly influenced by landuse, but unaffected by sampling locality, *i.e.*, geographic distance and environmental setting.

We hypothesize that influences of geogenic factors (*i.e.*, landform, underlying lithology and mineral deposits) on soil microbial community assemblages are equally important than those of anthropogenic landuse. Therefore, the aims of this study are to i) assess if differences in landuse mask influences of geogenic factors, and ii) determine the dominant bacterial taxa differentiating soils overlying metal deposits from adjacent background soils. To achieve this, samples were collected from two metallogenic sites in Australia. The Fifield Pt-Au-field site is Australia's largest platinoferrous regions hosting range of Alaskan-type primary Pt-, VMS- and hydrothermal Au deposits. The Hillside deposit is located in the Gawler Craton, which also hosts one of the world's largest Cu-Au-U deposits, Olympic Dam. Soil microbial communities were characterized using M-TRFLP and high-density phylogenetic microarrays (PhyloChip G2), field-mapping/geochemical analyses were used to determine geogenic factors and multivariate statistical approaches were used to test the hypotheses.

MATERIALS AND METHODS

Field sites and sampling

Soils with elevated natural metal contents and adjacent background soils were collected from two Australian sampling areas, *i.e.*, Hillside and Fifield (Fig. S1). The Fifield Au- and Pt-field is situated approximately 380 km north-west of Sydney, Australia at 32°50'33.48" S 147°28'5.38" E (Fig. S1). It was the largest Australian producer of Pt in the 1900s (27). The climate at Fifield is semi-arid with most plant growth occurring in summer and temperature limiting growth in winter. Soils are largely residual, with little material being transported into the area (28). They are classified as Red Sodosols following the Australian soil classification scheme (28). Landscape evolution in the area commenced in the Early to Middle Devonian (29). Subsequent periods of weathering, laterization and fluvial erosion have occurred since (29). This has resulted in an undulating landscape with distinct erosional, colluvial, alluvial and depositional landforms (Australian landscape classification system; 30). At Fifield, 75 soil samples were collected across a 20 km transect covering Au, Pt and base metal deposits with differing underlying lithologies (Ordovician and Silurian-Devonian (meta)-sediments and metal-rich intrusions), landforms (erosional, colluvial, alluvial and depositional), and differing landuse (grassland for cattle and natural *Eucalypt* bushland) in May 2011 from the A-horizon (at 0.1-0.15 m depth). Note: Due to the extended weathering history and long periods of tectonic stability of the Australian continent, landscape evolution and weathering have occurred for millions to hundreds of millions of years leaving behind deeply weathered (down to 1000 m) *in situ* or transported weathered materials, with landscapes looking to the “untrained” eye flat with little or no relief (31). Therefore a specific classification system was developed to classify these landscapes in a geomorphological context; this system was used for this study (30).

The Hillside site is located close to the township of Ardrossan in South Australia, at 34°32’04.32” S 137°52’41.81” E (Fig. S1). The site is situated in the Gawler Craton, which also hosts the Olympic Dam Fe-oxide Cu-Au-U-REE deposit. The highly weathered *in situ* lithology is covered by well-sorted, rounded, Aeolian sediments consisting of spherical quartzose, calcareous sands, nodular- and hardpan carbonates (32). The climate at Hillside is semi-arid, with average yearly minimum of 10.6°C and maximum of

22.6°C. Much of the Hillside area has been cleared for agriculture, especially wheat production. Prior to clearing, the vegetation consisted mostly of mallee woodland dominated by red mallee (*Eucalyptus socialis*); remnants of this vegetation still occur throughout landscape. The primary metal deposit occurs in Proterozoic basement rocks and ore metals are hosted by the sulfides bornite and chalcopyrite; in the oxidized zone overlying the sulfidic zone secondary Cu carbonates and U-minerals have formed (32). The contemporary landscape consists of subdued relief and is lower at the coast and higher inland (32). 93 soil samples were collected across four transacts in April 2011. Transacts covered areas of differing landuse (wheat cropping and native Mallee woodland), landforms (erosional, colluvial and depositional), geophysical responses (airborne electromagnetic indicative of different lithologies) and depths. Surface soils were collected from 0.03 to 0.2 m depths. Deeper soils were collected directly above the carbonate hardpan, below depths of 0.2 to 0.5 m.

At each sampling site, six 50 mL centrifuge tubes of soil were collected under field-sterile conditions. Samples for DNA-extraction were frozen on-site. Tubes stored at ambient temperature were used for geochemical analyses.

Geochemical characterization

After homogenization, Fifield soil samples were microwave digested in concentrated *aqua regia*. Concentrations of major metals were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; Spectro ARCOS SOP, Germany); minor- and trace metals were determined by inductively coupled plasma-mass spectrometry (ICP-MS; Agilent 7500ce, USA; following 23). Total C and N were determined by high-temperature combustion (Formacs analyser; Skalar Inc., USA);

electrical conductivity (E.C.) and pH were measured in 1:5 soil to water extracts. For Hillside samples, an existing dataset of soil geochemical parameters was used (32). Geochemical parameters (data available on request) were categorized into seven groups, including solution parameters (E.C. and pH), and six elemental groups based on a modified Goldschmidt element classification system (33, 34): (i) Pathfinder- (Fifield: Ag, As, Au, Bi, Mo, Pb, Pd, Pt, Se and W; Hillside: As, Ag, Au, Ba, Bi, Cu, Mo, Pb, Sb, Se, Te, Th, U, V and W), (ii) biophile- (C_{tot}, N_{tot}, P and S), (iii) rare earth (Be, Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nb, Nd, Pr, Sm, Tb, Tm, Y and Yb), (iv) chalcophile- (Cd, Cu, Ga, Ge, Sn, Ti, Tl and Zn), (v) lithophile- (Al, Ca, Cr, Cs, Hf, K, Li, Mg, Na, Nb, Rb, Sc, Sr, Th, U, and V), and (vi) siderophile- (Fe, Co, Mn, Ni, Te) elements.

Assessment of community assemblages and functional potential

Nucleic acid extraction, quantification and quality control

For M-TRFLP, DNA was extracted in duplicate from 0.25 g of homogenized field-fresh soils using the PowerSoil DNA Isolation kit (MoBio, USA) with a mechanical disruption step of 5 m s^{-1} for 20 s on a Bio101 FastPrep bead-beater. Duplicate extracts were pooled and used for further analyses. For microarray analyses, DNA was extracted in duplicate from 10 g of soil using the PowerMAX Soil Mega Prep DNA Isolation kit (MoBio, USA). DNA quality was assessed spectrophotometrically (NanoDrop ND-1000, USA): Only DNA with 260 : 280 and 260 : 230 ratios of 1.8 and 1.5, respectively, was pooled prior to further analyses. The total amount of DNA extracted was quantified using Quant-it Picogreen dsDNA reagent (Invitrogen, USA) on a MX3000*P* qPCR (Stratagene, USA); unknown concentrations were compared against a standard curve derived from known concentrations of λ-phage DNA.

Multiplex terminal restriction fragment length polymorphism (M-TRFLP)

Bacterial, archaeal and fungal communities were characterized using M-TRFLP of the small subunit rRNA gene (35). Multiplex-PCR (25 µL volume) used Qiagen HotStar *Taq* chemistry and thermocycling consisted of 30 cycles of 95ºC for 30 s, 55ºC for 30 s, 72ºC for 60 s and a final extension step for 10 min at 72ºC (35). PCR products were purified (Wizard®SV; Promega), and 100 ng digested with 20 U of *MspI*, *HaeIII* and *TaqI* for 3 h at 37ºC or 65ºC. Capillary separation of TRF's was conducted at the Australian Genome Research Facility and TRF's scored using GeneMarker software (SoftGenetics, USA) at a detection limit of 200 fluorescent units; TRF's differing ± 0.5 base-pairs (bp) were binned together (23). Relative peak heights were used as measure of abundance. Richness was based on the number of unique TRF lengths obtained.

PhyloChip analysis

The PhyloChip G2 microarray was used to further characterize the bacterial and archaeal community compositions (36). Key-samples were chosen based on environmental factors, *i.e.*, landuse, soil depth, landform, underlying mineral deposits and lithology. DNA from three replicates with matching factor combinations was pooled and analyzed on one array. Note: as soils had been extracted in duplicate and pooled, each array was run with a mixed sample from six individual DNA extraction (*i.e*., three factor replicates and two extraction repeats). Amplification of 16S rRNA genes, purification of products, labelling of DNA and hybridisation were conducted following Brodie *et al.* (2006) using the reaction chemistry described in Wakelin *et al.* (2012b; 22, 36). Hybridized arrays were stained and washed on an Affymetrix fluidic station (36). After scanning, data were processed following the method outlined by Brodie *et al.* (2006) and DeSantis *et al.* (2007; 36, 37). Data were imported into PhyloTrac for scoring of taxa (38). Operational taxonomic units (OTUs) were deemed detected by a positive fraction (PF) of probe-pair matches ≥0.9.

Statistical analyses

Multivariate analyses were conducted in the PRIMER software package with the PERMANOVA add-on using statistical approaches described in previously (23, 39, 40). Soil geochemical data (except pH) were log-transformed to remove skew. Data were normalized and similarity matrices based on Euclidean distances were calculated. For bacterial, fungal and archaeal community analyses, each TRF was treated as an OTU and the peak height was inferred as representing the relative abundance of that OTU. Similarity matrices were generated on square-root transformed abundance data using the Bray-Curtis method (41). For PhyloChip data (log values), a similarity matrix was created using the Gower method (42). The taxonomical hierarchy for each taxon was determined and the distribution of phyla/classes plotted; taxa representing <1% of the total abundance were combined as 'other'. Differences in overall abundances of phyla/classes between soils from different landforms, landuse, lithologies and underlying mineral deposits, were calculated. Significance levels were determined using student's t-tests ($P<0.05$). PERMANOVA (permutational multivariate ANOVA; 40) for TRFs was used to test if between group variation (*i.e.*, location, soil depth, landuse, landform, lithology and mineral deposits) can explain a significant proportion of the total system variation (*i.e.,* if natural groupings can be detected). Balanced PERMANOVA analyses were conducted using partial sums of squares on 9999 permutations of residuals under a reduced model.

CAP analysis (canonical analysis of principal coordinates; 40), was used to determine if principal coordinates axes could be found that separate *a priori* defined treatments (*i.e.*, CAP analysis attempted to 'seek-out' pre-defined groups within the data cloud). Vector overlays, based on Pearson correlations, were used to explore relationships between significant individual variables and the ordination axes. CAP analyses were conducted based on the respective resemblance matrices; the significance of test effects was determined against null distributions based on 999 or 9999 permutations (random allocations) of samples (40). Distance-based linear modelling (DISTLM) was used to assess the geochemical parameters best explaining the variability within the microbial dataset (23). SIMPER (similarity percentage) analysis was used to identify taxa/classes/phyla that discriminate between locations, landforms, landuse and underlying lithology and mineral deposits. For phylum-/class-level SIMPER analysis, data were normalized to take into account differences in probe numbers between the different phyla represented on the array. Given the high number of PhyloChip variables (OTUs), interpretation of treatment effects on bacterial communities were conducted at the phylum/class level. UPGMA and maximum-likelihood (with 1000 boot strap replicates) phylogenetic trees based on PhyloChip probes for 50 OTUs that best discriminate soils overlying mineral deposits from background soils as well as taxa detected only in soils overlying the mineral deposits were constructed using GENEIOUS v. 7.0.

RESULTS

Linking community profiles, geochemistry and environmental factors

Significant links between bacterial, fungal and archaeal community assemblages, landuse, landform, underlying lithology and mineral deposits, expressed through the geochemical properties of soils, were present at both locations. CAP and cluster analyses of microarray data showed that bacterial and archaeal community assemblages varied significantly between sites (Hillside *v.* Fifield; P=0.0003), depths (Hillside; P=0.01), across different landuses (P=0.003) and with underlying mineral deposits (P=0.009; Figs. 1 and 2A; Table 1); a strong interactive effect of landform and mineral deposits was observed was also observed (Table 1).

At Fifield, soil geochemical properties varied significantly with lithology ($\sqrt{CV}=4.1$; $P<0.001$), landform ($\sqrt{CV}=3.2$; $P<0.001$) and mineral deposits ($\sqrt{CV}=5.3$; $P<0.001$); no significant differences between the grazing- and bushland, were detectable (Table 2). Bacterial community assemblages (based on M-TRLFP) varied significantly with landuse, landform, lithology and mineral deposits; lithology ($\sqrt{CV}=14.1$; $P=0.01$) and landuse ($\sqrt{CV}=13.9$; $P<0.02$) were the primary discriminators (Table 2). Fungal communities varied significantly with landuse ($\sqrt{CV}=14.9$; $P=0.01$) and underlying lithology ($\sqrt{CV}=16.7$; $P<0.01$), but not landform or mineral deposits (Table 2). Archaeal communities varied significantly with all geogenic factors, but not landuse (Table 2). No significant interactive effects were observed. All groups of geochemical parameters showed significant relationships with the microbial data, the strongest being the solution parameters as well as biophile- and siderophile elements (Fig. 3). Levels of Au/Pt pathfinder and REE in samples explained 14.4% and 27.3% of variation in the bacterial community composition, respectively (Fig. 3). Gold/Pt pathfinder elements explained 12.8% and 18.0% of variation in fungal and archaeal communities, respectively (Fig. 3). Solution parameters, biophile, siderophile and pathfinder elements also explained most of the variation in community assemblages detected with high density microarrays ($P<0.05$; Fig. 2B).

At Hillside, microbial communities varied with most with soil depth; *e.g.*, the bacterial community displayed a √CV=16.5 (P<0.01; Table 3) across top-soils (A-horizon; 0.03-0.2 m) and sub-surface soils (B-horizon; >0.2 m). No influences of either landuse or geogenic factors were detected when surface- and deeper soils were analyzed collectively (Table 3). Analyzed independently, bacterial and fungal communities in top-soil varied significantly with landuse (√CV=12.5; √CV=4.7; P<0.001, respectively) and landform (√CV=9.4; √CV=3.4; P<0.05, respectively). Archaeal communities were not linked to any of the factors tested (Table 3). In sub-surface soils, bacterial communities varied most significantly with lithology (√CV=9.2; P=0.05) and the mineral deposit (√CV=14.2; P<0.001), but not landuse or landform (Table 3). Fungal communities varied with lithology and the mineral deposit as well as landuse (Table 3). An interactive effect between landform and mineral deposit was observed for bacterial and fungal communities. Across all Hillside soils, geochemical properties varied with soil depth. The landform was a significant influence in deep soils. Pathfinder elements and solution parameters were capable of explaining 70.4% and 20.6% (P<0.05) of variation in the bacterial community, respectively; this was confirmed by analyses linking geochemical properties and microarray data (Fig. 2C). Lithophile major elements were capable of explaining 24.2% of in fungal community; other associations were not significant.

Linking taxa, geochemistry and environmental factors

PhyloChip G2 microarrays were used to compare bacterial and archaeal communities representative of location, landform, landuse, lithology and mineral deposits. Across all samples, 45 phyla, 90 classes, 173 orders and 306 families were detected (Fig. 1; Table S1). Between 879 and 1879 individual taxa were observed (Fig. 1; Table

S1). Bacterial communities were dominated by *Proteobacteria* (*Alpha*-, *Beta*-, *Gamma*, *Delta* and *Epsilon*- subdivisions; 35.5-45.7%), *Firmicutes* (15.1-22.6%), *Actinobacteria* (12.2–17.1%), *Acidobacteria* (3.8-6.3%), *Bacteriodes* (2.6-5.7%), *Chlorofexi* (1.8-3.3%), and *Cyanobacteria* (2.1–4.0%) (Fig. 1). *Sphingobacteria*, *Verrucomicrobiae*, *Anaerolineae*, *Planctomycetacia*, *Catabacter*, *Spirochaetes*, and *Archaea* represented between 0.1 and 2.0% to the composition of prokaryotic communities (Fig. 1).

Prokaryotic communities at Hillside were richer in taxa (1660±101) compared to Fifield (1007±194; Table S1). All phyla/classes displayed higher numbers of taxa in Hillside compared to Fifield soils. Of these, *Alpha*-, *Beta*- and *Gamma-Proteobacteria, Actinobacteria, Bacteroides*, *Sphingobacteria* and *Spirochaetes* were the most discriminatory taxa ($P<0.05$; Table S1). At Hillside the total number of taxa in different phyla/classes did not differ between soils depth, landuse and underlying mineral deposit. However, 208 taxa were only detected in soils overlying the mineral deposit (Table S1).

At Fifield, significantly higher numbers of taxa ($P<0.05$) were observed in samples overlying mineral deposits (1153±170 taxa) compared to background soils (881±72 taxa; with 463 taxa occurring only in soils overlying mineral deposits (Table S1). In particular, *Alpha-, Beta-, Delta-* and *Epsilon-Proteobacteria* as well as *Acidobacteria*, *Sphingobacteria* and *Verrucobacteria* were significantly more abundant in soils overlying mineral deposits. Twenty-eight taxa occurred in soils overlying the mineral deposits at Fifield as well as Hillside; of these ten belonged to *Bacilli* (Fig. S2).

At Hillside higher numbers of *Beta-Proteobacteria* and *Bacilli* were associated with colluvial compared to erosional landforms (Table S1). At Fifield overall abundances between erosional and colluvial landforms were similar, alluvial sites contained higher number of *Alpha-, Beta-, Gamma-* and *Epsilon-Proteobacteria*, *Firmicutes* (*Bacilli* and

Clostridia) and *Cyanobacteria* (Table S1). In soils from cattle grassing sites significantly more *Bacilli* were detected compared to native bushland soils (Table S1). Whereas *Acidobacteria*, *Beta*- and *Epsilon-Proteobacteria, Cyanobacteria*, *Spirochaetes* and *Bacilli* correlate well with underlying mineral deposits, *Actinobacteria*, *Delta-Proteobacteria,* and *Bacteriodetes* correlated most strongly with background soils (Fig. 2A). SIMPER analyses on individual taxa also showed that of the 19 and 20 of the 50 most discriminating taxa between soils overlying mineral deposits and background sites were *Acidobacteria* and *Proteobacteria*, respectively (Fig. 4). *Gamma-Proteobacteria* correlated with colluvial landforms. *Actinobacteria* were closely linked to erosional landforms (Fig. 2A; Table S1). At alluvial sites a larger abundance and diversity of *Cyanobacteria* was observed (Fig. 2A; Table S1).

DISCUSSION

We show that landform, underlying lithology and mineral deposits are closely related to microbial community assemblages in geologically 'older', naturally metal-rich soils. At our study sites geogenic factors are as important in explaining the variation in community assemblages as anthropogenic landuse. This, we hypothesize, is a likely result of the extended history of weathering and metal cycling at these sites. Over extended periods of weathering, metal-bearing minerals are decomposed through the interaction of biogenic and abiogenic factors (43). As a result heavy metals (*e.g.*, Au, Hg, Pb, Ag, Cd, Cu, Zn, Ni and U) are mobilized and become bioavailable in soils (43). For example, Fe- and S-oxidizing microorganisms alter metal sulfides leading to the production of acid and the mobilization of heavy metals (44). Re-precipitation and biomineralization of metals leads to the formation of metal enrichment zones, which in-turn affect microbial communities (16, 21-23). Elevated concentrations of mobile metals can select for community assemblages that are better able to deal with metal toxicity and are therefore better suited to survive in these environments (45, 46). This is often expressed as an increase in the diversity and/or abundance of metal-resistant populations (47-49) and may not be easily masked by differences/changes in landuse, as observed in soils from New Zealand, Europe and North America evolving from unweathered rock since the extensive Upper Pleistocene glaciations (*i.e.*, geologically "young" soils; *e.g.*, 26, 50-52).

The data presented here supports our hypothesis that longer *in-situ* weathering periods lead to larger differences in community diversity driven by geogenic factors. At Hillside, the highly-weathered Palaeoproterozoic lithology and associated metal deposit are covered by Aeolian (*i.e.*, <10,000 years old) sediments, consisting of quartzose, and pedogenic carbonates. During this time, decomposition of Cu-, Au- and U-bearing bornite, pyrite and chalcopyrite, combined with the biogeogenic cycling of these metals and their pathfinders, has led to the formation of secondary metal enrichment zones, which strongly influenced community assemblages. In total, 208 bacterial taxa (mostly *Bacilli*, *Acidobacteria*, *Alpha-* and *Gamma-Proteobacteria*) occurred only in soils overlying the Cu-Au-U-REE deposit. Assessment of overall taxa abundances at the phylum/class levels showed little differences between different factors tested (Table S1). This suggests that individual taxa were replaced at sites overlying the deposit, but that the concentration of toxic, mobile heavy metals in combination with "geologically shorter" exposure time was not sufficient to alter community composition at this level. At Fifield soils have evolved continuously from *in situ* materials for millions of years (29), leading to soils highly enriched in mobile metals. Microbial communities at this site have been subjected to elevated metal concentrations for very long periods of time (potentially millions of years). Here all of the measured elemental groups were significantly linked to microbial community assemblages, with 462 taxa detected only in soils overlying the mineral deposits. Significant differences on class/phylum level were observed with *Alpha*-, *Beta*-, *Delta*- and *Epsilon-Proteobacteria* as well as *Acidobacterial* and *Cyanobacterial* taxa, which were more numerous and abundant in the metal-rich soils.

To further test this hypothesis and identify key-drivers affecting community assemblages, a range of studies and experiments can be conducted, including: i) determining the soil meta-genome of wide range (continental-scale) of soils with established histories of landuse, age, lithology, landform and mineral deposits; this will be important to assess if correlations observed in our study occur in general in Australia and internationally; ii) establishment of soil micro- and mesocosm experiments with well-defined model communities and *in-situ* communities from geologically "young" and "old" soils; these can be incubated with a range/combination of mobile metal ions and effects on community composition and function can be assessed; iii) in field trials diversity-disturbance responses of soil microbial communities can be measured after amendment of soils with increasing metal doses; here geologically "young" and "old" soils with different landuse can be tested to be assess, if communities in older soils react differently to those from younger environments.

While communities at both sites were strongly affected by location, particular groups of bacteria contributed strongly to the differences between metal-rich and background soils across both sites (Figs. 2A and 4; Table S1). The abundance/occurrence of *Acidobacteria*, *Verrucomicrobia*, *Bacilli and Proteobacteria* contributed most strongly to the differences between metal-rich and background soils across both sites These taxa are known for their ability to withstand elevated concentrations of mobile heavy metals and/or affect the speciation and mineralogical association of these metals as discussed below. Few *Acidobacteria* and *Verrucomicrobia* have been cultured to date, yet sequence data shows that both phyla are ubiquitous and highly abundant in many soils (53-55). A recent study completed the genomes of three *Acidobacterial* strains (56). Based on the combination of physiological and genomic evidence, the authors suggested that *Acidobacteria* are long-lived, divide slowly, exhibit slow metabolic rates under low-nutrient conditions, and are well equipped to tolerate fluctuations in hydration and high contents of mobile metals observed at both study sites (56). Hence, they are well adapted to survive at both study sites. In particular, *Acidobacteria* were commonly identified in soils and sediments overlying or containing U deposits, respectively, as well as in waste rock and mill tailings from U mines (36, 54, 56). In our study, many of the key-organisms differentiating metal-rich from background sites matched probes of an *Acidobacteria* first identified by Geissler and Selenska-Pobell (2005) from a U waste piles near Johanngeorgenstadt (Germany; 57). Other taxa were first identified in samples obtained from Au mines. *Verrucomicrobia* have also been linked to U mining environments (58), with the taxa identified here belonging to the ammonia-oxidising group. The ubiquity and abundance of *Acidobacteria* and *Verrucomicrobia* in metal-rich soils at the study sites, combined with their ability to survive in meal-polluted extreme environments, suggest that they serve functions important to biogeochemical metal cycling at the study sites, similar to those observed other U-rich sites (59).

Bacilli are halotolerant, alkaliphile/alkalitolerant and capable of forming endospores; these can persist in a dormant state and tolerate extreme conditions (60,61). In a previous studies, elevated number of *B. cereus* spores were also detected in auriferous soils from Belgium, China, Argentina and Mexico, and the use of *B. cereus* as a bioindicator for Au-exploration has been proposed (18, 19). A significant increase in the number of *B. cereus* spores in auriferous soils from Tomakin was observed (20). In microcosm experiments, the abundance of *Bacilli* were also significantly higher in Au-amended compared to unamended soils (23). These data suggest that *Bacilli* are particularly well adapted to persist in commonly dry semi-arid soils displaying elevated concentrations of heavy metals.

Active *Bacilli* from semi-arid Australian environments may also have another capability affecting metal cycling. Research has shown that the formation of metal-anomalous pedogenic carbonates is biomediated through the activity of resident *Bacilli*, and is not simply the result of passive nucleation on inactive cells or evapotransporative processes as previously thought (*e.g.*, 62). Enrichment cultures from South Australian pedogenic carbonates from an area adjacent to Hillside with similar environmental conditions, consisted of *Bacillus* and related *Paenibacillus* and *Lysinibacillus* taxa (63). These cultures were shown to induce Ca-carbonatogenesis as well as the co-precipitation and subsequent enrichment of Au, U and Cu in pedogenic carbonates (63, 64). This suggested that *Bacilli* contribute strongly to the formation of metal anomalous zones in carbonate rich soils, which in turn effects community composition.

Proteobacteria, especially *Alpha-Proteobacteria*, were the dominant phylum across the study sites and are also often dominant class of bacteria in metal-contaminated Australian soils (21-23). The *Proteobacteria* contain well-characterized metallophillic bacterial genera, *e.g.*, *Cupriavidus* and *Pseudomonas*, which have also been detected in the metal-rich soils at the Fifield and Hillside sites (65-66). In addition to surviving under high metal conditions, some of these microorganisms have been shown to play a functional role in the bioprecipitation and biomineralization of metals, *e.g.*, Cu and Au (67, 68). The presence of these organisms at the study sites suggests that microbial communities may be well adapted to high contents of mobile metals at the sites.

In conclusion, the results of this study show that geogenic factors, *i.e.*, landform, lithology and mineral deposits and associated geochemical parameters are can strongly affect the composition of microbial community assemblages in naturally metal-rich soils. The study expands on the results of earlier works (21-23, 69) in a number of important ways: (i) landuse at the study sites was not uniform and/or minimal, instead intensely agriculturally utilized soils were compared to native bushland soils; (ii) soils overlying polymetallic Cu-Au-U-REE and Au-Pt deposits were assessed, compared to earlier studies that featured soils overlying economic Au deposits; and (iii) soils from sites with strongly differing histories of landscape evolution were assessed, *i.e*., soils resulting from *in situ* weathering (Fifield) *vs*. soils formed from Aeolian materials overlying heavily weathered terrain (Hillside). This indicates that geogenic factors are important for the selection of microbial community assemblages in ‘geologically old’ soils and landscapes, and hence may contribute to variation in the soil microbial community diversity at a far wider range of sites than previously suggested.

Acknowledgements

The authors acknowledge: the Australian Research Council (LP100200212 to F.R.), Rex Minerals, Rimfire Ltd. Pty. We thank Colin Plumridge, John Kaminski, Byron J. Dietman and M. Dhamarajh for their assistance; we thank the editor Prof. Dr. F. Loeffler for the handling of the manuscript and the two anonymous reviewers for their thoughtful comments.

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Figure legends

Figure 1 Distribution of dominant prokaryotic phyla/classes (number of OTUs in this group given in bars) and cluster analyses of community data based on individual taxa; note: classes are shown for *Proteobacteria* and *Firmicutes*; phyla with <1 % coverage are aggregate into "other".

Figure 2 Ordination plots of the first two Cap produced by CAP of Phylochip data analysed for differences in community assemblages in relation to landform and underlying mineral deposits. Vectors of Pearson's correlations of classes/phyla (A) and geochemical parameter (B,C) overlain.

Figure 3 Percentage of bacterial, fungal and archaeal soil community diversity explained by geochemical parameters; results of distance based linear regression modelling (DISTLM), based on M-TRLFP data *v.* geochemical parameters of Fifield samples with significance level ($P<0.05$)

Figure 4 16s rRNA gene maximum-likelihood phylogenetic tree (1000 boot strap replicates) of the 50 taxa (PhyloChip) that best discriminate soils overlying mineral deposits from background soils at both sites; taxa were identified by SIMPER analyses. Note: One representative (probe-targeted) sequence per taxum was used; the tree does not represent sequences the from field sites, but is a close approximation based on probe matches; sequences were obtained from GenBank.

TABLES

Table 1

Summary of CAP of the factors location, depth, landuse, lithology and mineral deposits on microbial community assemblages at the Fifield and Hillside sites; significant P<0.05 (bold font).

Factor	**CAP**	
	Trace^{c}	$\mathbf{P_{perm}}$
Location	**0.97**	**0.0003**
Depth	**0.91**	**0.01**
Landuse	**0.63**	**0.003**
Mineral deposit	**0.95**	**0.009**
Landform (Lf)	0.31	0.9
M x Lf	**2.82**	**0.05**

Table 2 Summary of PERMANOVA testing of the influence of the factors landuse, lithology, landform and minerals deposits on microbial community assemblages (M-TRLFP data) and geochemical parameters at the Fifield site; significant P<0.05 (bold font).

	PERMANOVA (√) CV[a]	**P_{perm}**
Bacteria		
Landuse	**13.9**	**0.02**
Lithology	**14.1**	**0.01**
Landform	**8.0**	**0.05**
Mineral	**6.1**	**0.01**
Residual	27.3	-
Fungi		
Landuse	**14.9**	**0.01**
Lithology	**16.4**	**0.004**
Landform	4.1	0.36
Mineral	4.8	0.16
Residual	40.6	-
Archaea		
Landuse	13.4	0.1
Lithology	16.7	0.08
Landform	**22.6**	**0.004**
Mineral	**12.5**	**0.01**
Residual	50.8	-
Geochemistr		
Landuse	1.1	0.23
Lithology	**4.1**	**0.0003**
Landform	**3.2**	**0.0004**
Mineral	**5.3**	**0.0001**
Residual	4.4	-

[a] (√)CV is the square root of the component of variation, which is a dataset dependent measure of the effect of size in units of the community dissimilarities (*i.e.*, increasing positive values); negative values indicate zero components (40).

Table 3

Summary of PERMANOVA testing of the influence of the factors depth, landuse, lithology, landform and the underlying Cu-Au-U deposit on microbial community assemblages and geochemical parameters at the Hillside study site; significant P<0.05 (bold font).

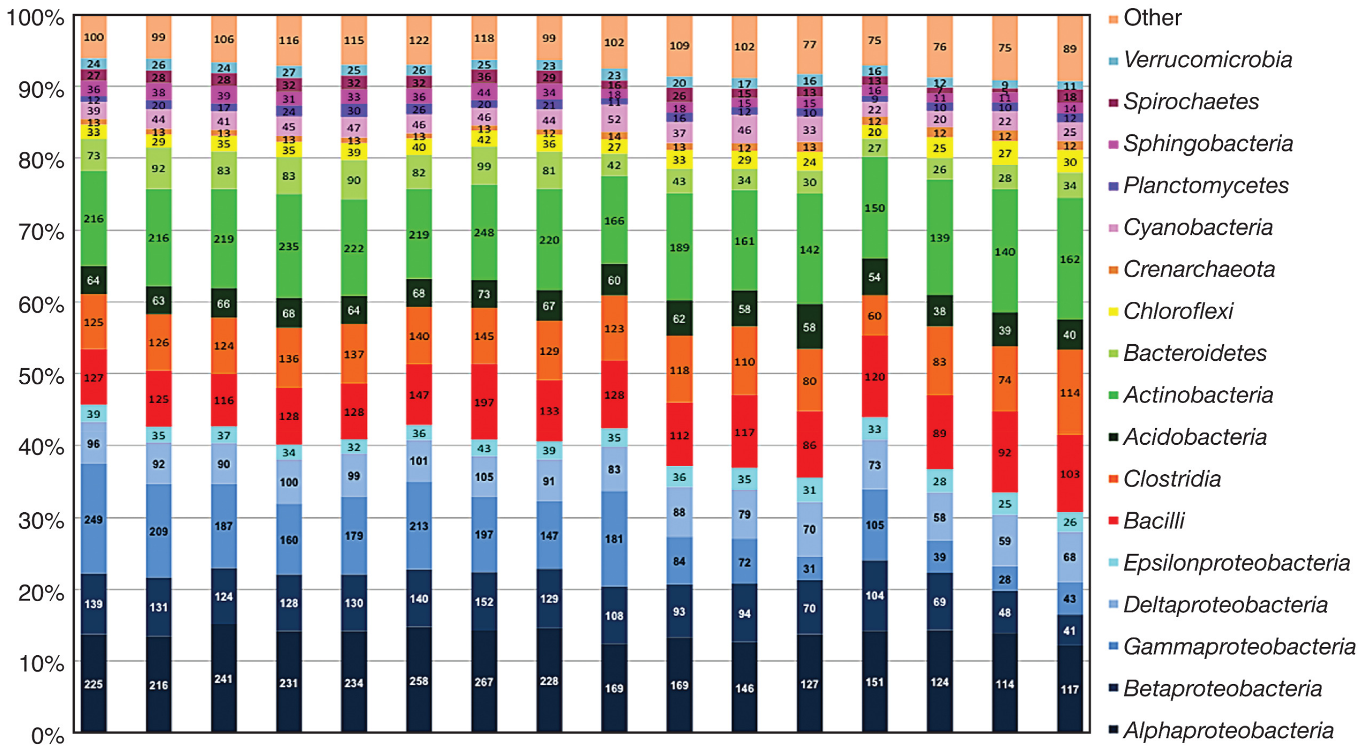
	Bacteria		***Fungi***	
	PERMANOVA		**PERMANOVA**	
	(√) CV[a]	P_{perm}	**(√) CV**	P_{perm}
All samples				
Depth	**16.5**	**0.01**	**20.3**	**0.0006**
Landuse	9.6	0.3	11.1	0.2
Landform, lithology, mineral deposit	-9.0	0.9	-1.7	0.5
Residual	33.5	-	28.9	-
Surface samples				
Lithology	2.9	0.4	5.2	0.27
Landform	**9.4**	**0.04**	**15.1**	**0.02**
Mineral deposit	-5.8	0.8	-5.4	0.56
Landuse	**12.5**	**0.001**	16.6	0.0001
Residual	15.4	-	22.0-	-
Deep samples				
Lithology	**9.2**	**0.05**	18.7	0.08
Landform (Lf)	6.9	0.16	10.9	0.18
Mineral deposit (M)	**14.2**	**0.006**	**9.6**	**0.04**
Landuse	6.1	0.17	7.5	0.1
Lf x M	7.7	0.25	**20.0**	**0.05**
Residual	37.7	-	28.7-	-

	Archaea		***Geochemistry***	
	PERMANOVA		**PERMANOVA**	
	(√) CV	P_{perm}	**(√) CV**	P_{perm}
All samples				
Depth	**17.4**	**0.0001**	-	-
Landuse	4.8	0.35	-	-
Landform, lithology, mineral deposit	7.6	0.21	-	-
Residual	29.2	-		-
Surface samples				
Lithology	-5.4	0.54	-	-
Landform	7.8	0.24	-	-
Mineral deposit	-7.5	0.66	-	-
Landuse	14.8	0.031	-	-
Residual	23.5	-	-	-
Deep samples[b]				
Lithology	7.0	0.30	**3.12**	**0.001**
Landform (Lf)	13.9	0.07	**2.51**	**0.05**
Mineral deposit (M)	6.4	0.3	-4.43	0.4
Landuse	4.2	0.4	n.t.[c]	n.t.
Lf x M	**30.0**	**0.04**	**2.51**	**0.02**
Residual	30.9	-	7.2	-

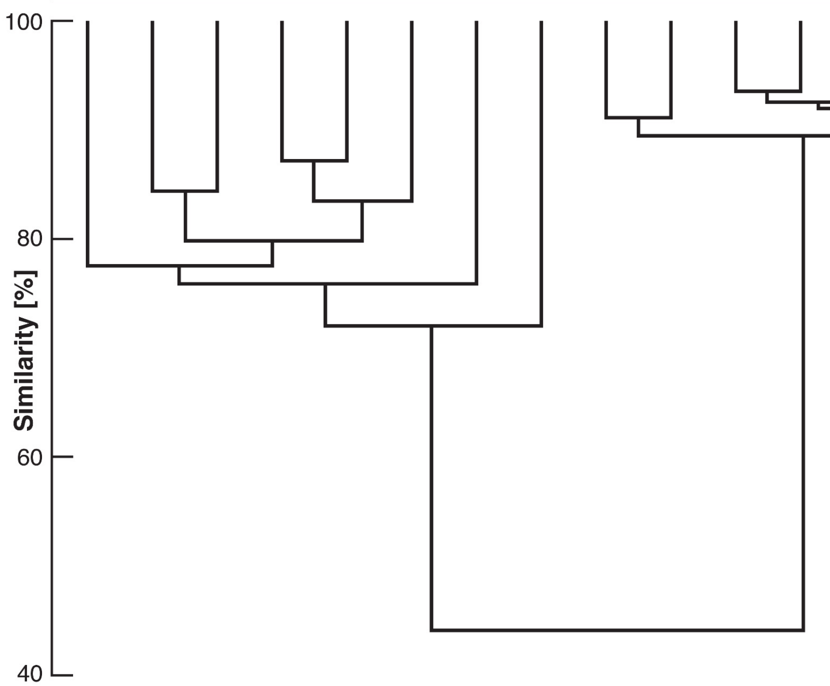
[a] **(√)**CV is the square root of the component of variation, which is a dataset dependent measure of the effect of size in units of the community dissimilarities (*i.e.*, increasing positive values); negative values indicate zero components (40).

[b] a company dataset was used for analyses, which only provided data for deep samples.

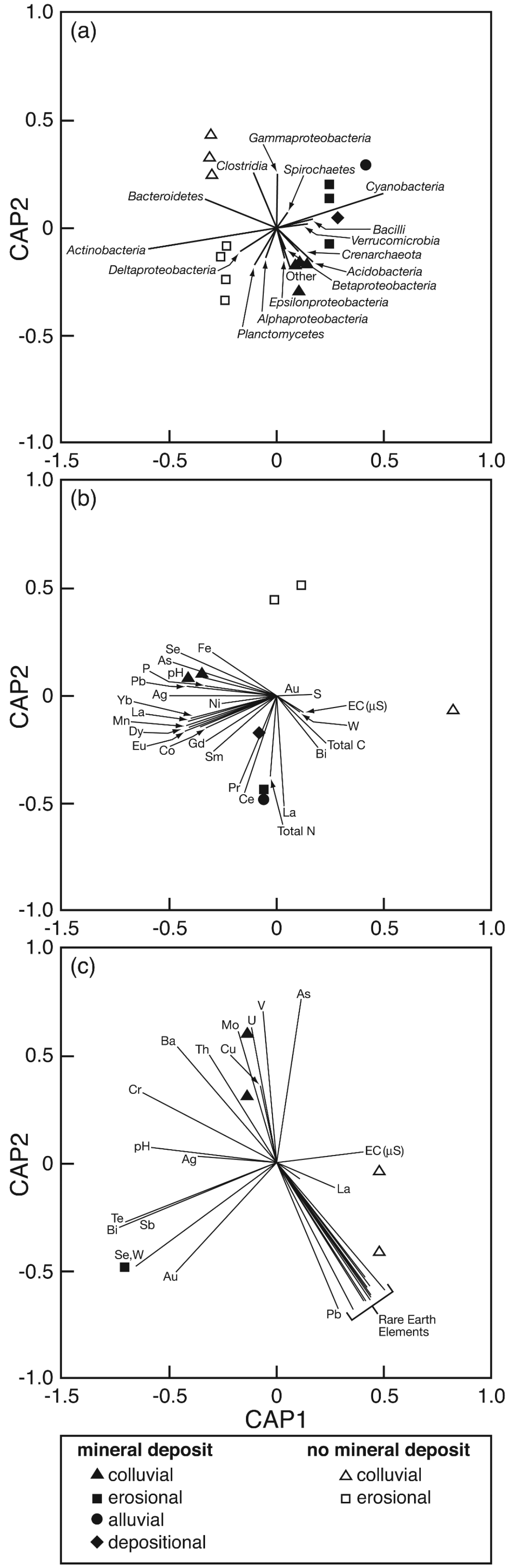
[c] not tested, as all geochemical analyses were derived from wheat cropping sites

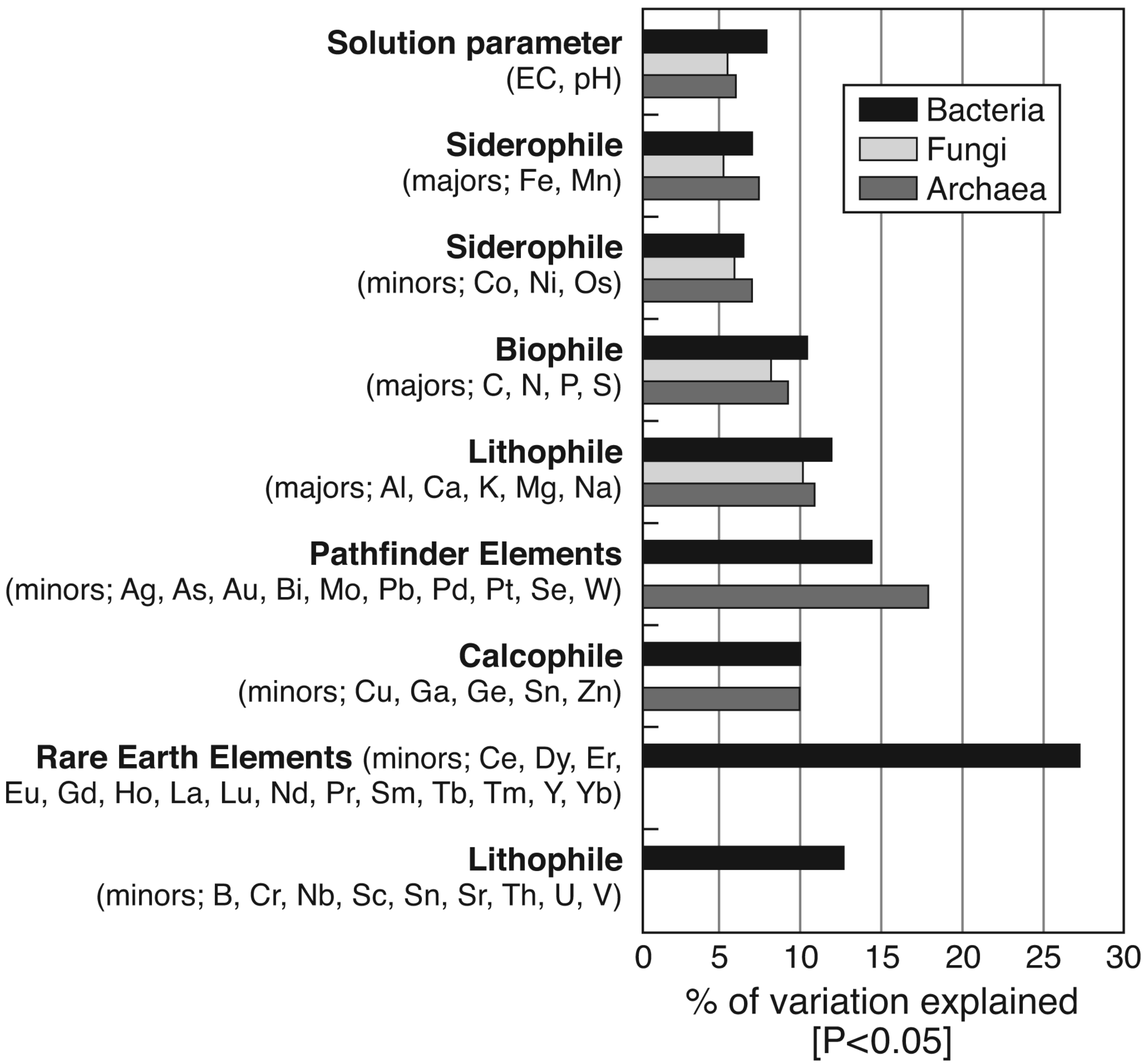


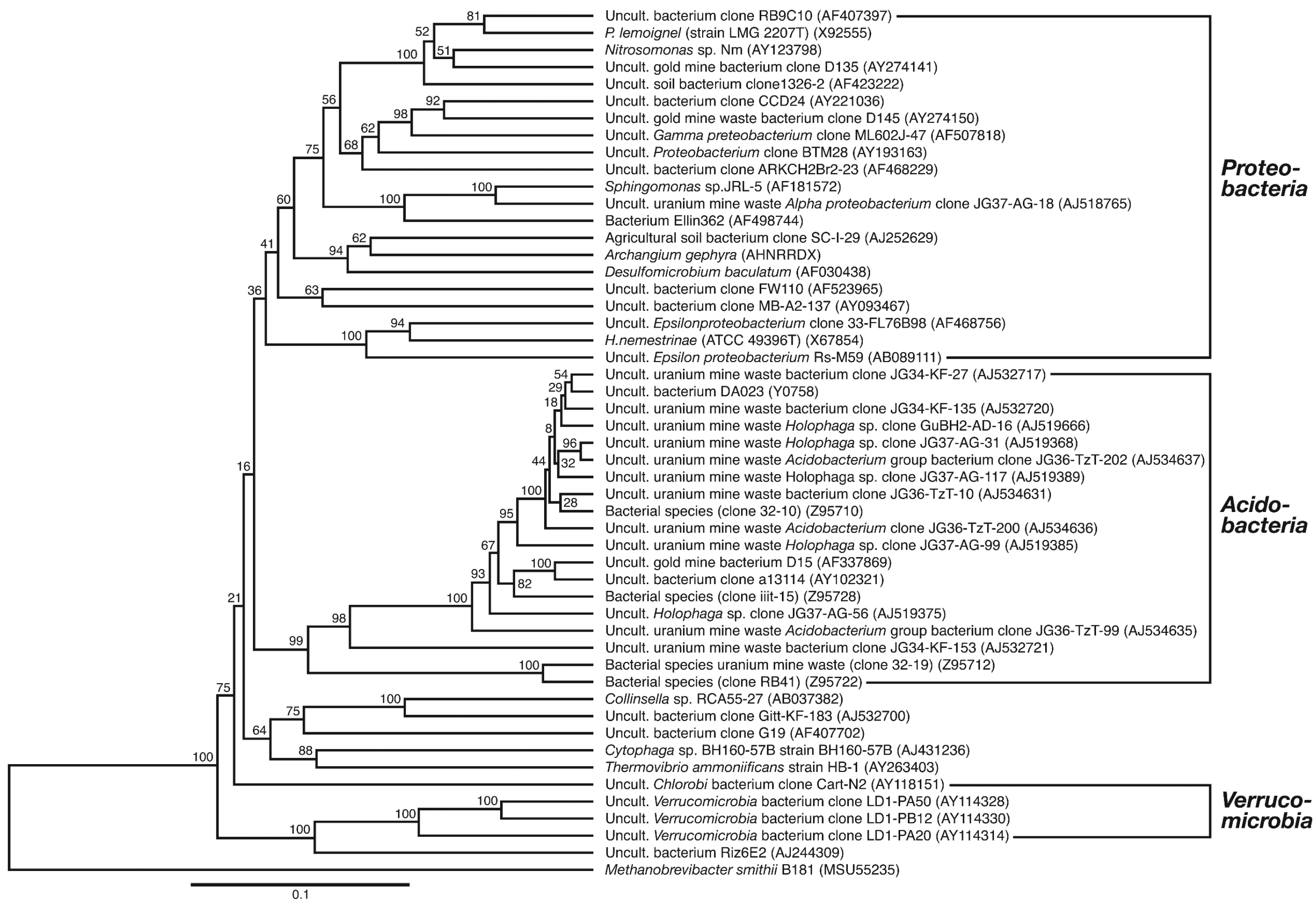
Hs	Hs	Hs	Hs	Hs	Hs	Hs	Hs	Ff	Ff	Ff	Ff	Ff	Ff	Ff	Ff	**Location**
su	de	su	de	de	de	su	su	su	su	su	su	su	su	su	su	**Depth**
no	no	no	no	yes	yes	yes	yes	yes	yes	yes	yes	yes	no	no	no	**Mineral deposit**
wc	wc	nb	nb	wc	nb	wc	wc	cg	cg	cg	nb	cg	nb	cg	nb	**Landuse**
co	co	er	er	co	er	co	er	al	er	dep	co	co	er	er	co	**Landform**
low	low	low	low	hi	hi	hi	hi	SD	SD	SD	SD	VMS	SD	SD	Or	**Lithology**



Hs	Hillside
Ff	Fifield
su	surface soil
de	deep soil
wc	wheat cropping
nb	native bushland
cg	cattle grazing
co	colluvial
er	erosional
al	alluvial
dep	deposition
low	low electromagnetic response
hi	high electromagnetic response
SD	Silurian Devonian sediments
VMS	Volcanic Massive Sulfide intrusion
Or	Ordovician metasediments


(a)
Gammaproteobacteria
Clostridia
Spirochaetes
Cyanobacteria
Bacteroidetes
Bacilli
Verrucomicrobia
Crenarchaeota
Actinobacteria
Acidobacteria
Betaproteobacteria
Deltaproteobacteria
Other
Epsilonproteobacteria
Alphaproteobacteria
Planctomycetes
(b)
Se
Fe
As
P
pH
Pb
Ag
Au
S
EC (µS)
Ni
Yb
La
W
Mn
Dy
Eu
Co
Gd
Sm
Total C
Bi
Pr
Ce
La
Total N
(c)
As
V
Mo
U
Ba
Th
Cu
Cr
pH
Ag
EC (µS)
La
Te
Bi
Sb
Se,W
Au
Pb
Rare Earth Elements
CAP2
CAP1
1.0
0.5
0
-0.5
-1.0
-1.5
mineral deposit
colluvial
erosional
alluvial
depositional
no mineral deposit
colluvial
erosional


Solution parameter
(EC, pH)
Siderophile
(majors; Fe, Mn)
Siderophile
(minors; Co, Ni, Os)
Biophile
(majors; C, N, P, S)
Lithophile
(majors; Al, Ca, K, Mg, Na)
Pathfinder Elements
(minors; Ag, As, Au, Bi, Mo, Pb, Pd, Pt, Se, W)
Calcophile
(minors; Cu, Ga, Ge, Sn, Zn)
Rare Earth Elements (minors; Ce, Dy, Er,
Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Tb, Tm, Y, Yb)
Lithophile
(minors; B, Cr, Nb, Sc, Sn, Sr, Th, U, V)
Bacteria
Fungi
Archaea
0
5
10
15
20
25
30
% of variation explained
[P<0.05]


Uncult. bacterium clone RB9C10 (AF407397)
P. lemoignel (strain LMG 2207T) (X92555)
Nitrosomonas sp. Nm (AY123798)
Uncult. gold mine bacterium clone D135 (AY274141)
Uncult. soil bacterium clone1326-2 (AF423222)
Uncult. bacterium clone CCD24 (AY221036)
Uncult. gold mine waste bacterium clone D145 (AY274150)
Uncult. Gamma preteobacterium clone ML602J-47 (AF507818)
Uncult. Proteobacterium clone BTM28 (AY193163)
Uncult. bacterium clone ARKCH2Br2-23 (AF468229)
Sphingomonas sp.JRL-5 (AF181572)
Uncult. uranium mine waste Alpha proteobacterium clone JG37-AG-18 (AJ518765)
Bacterium Ellin362 (AF498744)
Agricultural soil bacterium clone SC-I-29 (AJ252629)
Archangium gephyra (AHNRRDX)
Desulfomicrobium baculatum (AF030438)
Uncult. bacterium clone FW110 (AF523965)
Uncult. bacterium clone MB-A2-137 (AY093467)
Uncult. Epsilonproteobacterium clone 33-FL76B98 (AF468756)
H.nemestrinae (ATCC 49396T) (X67854)
Uncult. Epsilon proteobacterium Rs-M59 (AB089111)
Uncult. uranium mine waste bacterium clone JG34-KF-27 (AJ532717)
Uncult. bacterium DA023 (Y0758)
Uncult. uranium mine waste bacterium clone JG34-KF-135 (AJ532720)
Uncult. uranium mine waste Holophaga sp. clone GuBH2-AD-16 (AJ519666)
Uncult. uranium mine waste Holophaga sp. clone JG37-AG-31 (AJ519368)
Uncult. uranium mine waste Acidobacterium group bacterium clone JG36-TzT-202 (AJ534637)
Uncult. uranium mine waste Holophaga sp. clone JG37-AG-117 (AJ519389)
Uncult. uranium mine waste bacterium clone JG36-TzT-10 (AJ534631)
Bacterial species (clone 32-10) (Z95710)
Uncult. uranium mine waste Acidobacterium clone JG36-TzT-200 (AJ534636)
Uncult. uranium mine waste Holophaga sp. clone JG37-AG-99 (AJ519385)
Uncult. gold mine bacterium D15 (AF337869)
Uncult. bacterium clone a13114 (AY102321)
Bacterial species (clone iiit-15) (Z95728)
Uncult. Holophaga sp. clone JG37-AG-56 (AJ519375)
Uncult. uranium mine waste Acidobacterium group bacterium clone JG36-TzT-99 (AJ534635)
Uncult. uranium mine waste bacterium clone JG34-KF-153 (AJ532721)
Bacterial species uranium mine waste (clone 32-19) (Z95712)
Bacterial species (clone RB41) (Z95722)
Collinsella sp. RCA55-27 (AB037382)
Uncult. bacterium clone Gitt-KF-183 (AJ532700)
Uncult. bacterium clone G19 (AF407702)
Cytophaga sp. BH160-57B strain BH160-57B (AJ431236)
Thermovibrio ammoniificans strain HB-1 (AY263403)
Uncult. Chlorobi bacterium clone Cart-N2 (AY118151)
Uncult. Verrucomicrobia bacterium clone LD1-PA50 (AY114328)
Uncult. Verrucomicrobia bacterium clone LD1-PB12 (AY114330)
Uncult. Verrucomicrobia bacterium clone LD1-PA20 (AY114314)
Uncult. bacterium Riz6E2 (AJ244309)
Methanobrevibacter smithii B181 (MSU55235)
Proteo-bacteria
Acido-bacteria
Verruco-microbia
0.1