AEM
Journals.ASM.org

Geogenic Factors as Drivers of Microbial Community Diversity in Soils Overlying Polymetallic Deposits

Frank Reith,[a,b] Carla M. Zammit,[c] Rebecca Pohrib,[a] Adrienne L. Gregg,[b] Steven A. Wakelin[d]

The University of Adelaide, School of Biological Sciences, The Sprigg Geobiology Centre, Adelaide, South Australia, Australia[a]; CSIRO Land and Water, Environmental Contaminant Mitigation and Technologies, PMB2, Glen Osmond, South Australia, Australia[b]; University of Queensland, Earth Sciences, St. Lucia, Brisbane, Queensland, Australia[c]; AgResearch Ltd., Lincoln Science Centre, Christchurch, New Zealand[d]

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 land uses, i.e., agriculture versus natural bushland. One hundred sixty-eight 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 land use ($P < 0.05$). At Hillside, communities in surface soils (0.03- to 0.2-m depth) differed significantly with landform and land use ($P < 0.05$). Communities in deeper soils (>0.2 m) differed significantly with lithology and 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*, *Betaproteobacteria*, and *Epsilonproteobacteria*. In conclusion, long-term geogenic factors can be just as important as land use in determining soil microbial community diversity.

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). Land use, 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 practices 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, and 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

Received 9 June 2015 **Accepted** 29 August 2015

Accepted manuscript posted online 4 September 2015

Citation Reith F, Zammit CM, Pohrib R, Gregg AL, Wakelin SA. 2015. Geogenic factors as drivers of microbial community diversity in soils overlying polymetallic deposits. Appl Environ Microbiol 81:7822–7832. doi:10.1128/AEM.01856-15.

Editor: F. E. Löffler

Address correspondence to Frank Reith, Frank.Reith@csiro.au.

Supplemental material for this article may be found at http://dx.doi.org/10.1128/AEM.01856-15.

anthropogenic land use 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 land use (24, 25). This has been illustrated in a study assessing the impact of short-term changes of land use, e.g., by oversowing 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 land use 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 as important as those of anthropogenic land use. Therefore, the aims of this study are to (i) assess if differences in land use 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 region, hosting a 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 multiplex terminal restriction fragment length polymorphism (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 (see Fig. S1 in the supplemental material). The Fifield Au and Pt field is situated approximately 380 km northwest of Sydney, Australia, at 32°50′33.48″S 147°28′5.38″ E (see Fig. S1). It was the largest Australian producer of Pt in the 1900s (27). The climate at Fifield is semiarid, 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 metasediments and metal-rich intrusions), landforms (erosional, colluvial, alluvial, and depositional), and differing land use (grassland for cattle and natural eucalypt bushland) in May 2011 from the A horizon (at 0.1- to 0.15-m depth). 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 1,000 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 (see Fig. S1 in the supplemental material). 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, and nodular and hardpan carbonates (32). The climate at Hillside is semiarid, with an 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 the landscape. The primary metal deposit occurs in Proterozoic basement rocks, 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). Ninety-three soil samples were collected across four transacts in April 2011. Transacts covered areas of differing land use (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) (by following reference 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 data set of soil geochemical parameters was used (32). Geochemical parameters (data are 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 (total C [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) lithophila (Al, Ca, Cr, Cs, Hf, K, Li, Mg, Na, Nb, Rb, Sc, Sr, Th, U, and V), and (vi) siderophile (Fe, Co, Mn, Ni, and Te) elements.

Assessment of community assemblages and functional potential. (i) Nucleic acid extraction, quantification, and quality control. For multiplex terminal restriction fragment length polymorphism (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 (ND-1000; NanoDrop, 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 double-stranded DNA reagent (Invitrogen, USA) on an MX3000*P* quantitative PCR (qPCR; Stratagene, USA); unknown concentrations were compared against a standard curve derived from known concentrations of λ-phage DNA.

(ii) 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 95°C for 30 s, 55°C for 30 s, 72°C for 60 s, and a final extension step for 10 min at 72°C (35). PCR products were

purified (WizardSV; Promega), and 100 ng was digested with 20 U of MspI, HaeIII, and TaqI for 3 h at 37°C or 65°C. Capillary separation of TRFs was conducted at the Australian Genome Research Facility, and TRFs were scored using GeneMarker software (SoftGenetics, USA) at a detection limit of 200 fluorescent units; TRFs differing by ±0.5 bp were binned together (23). Relative peak heights were used as a measure of abundance. Richness was based on the number of unique TRF lengths obtained.

(iii) 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., land use, soil depth, landform, underlying mineral deposits, and lithology. DNA from three replicates with matching factor combinations was pooled and analyzed on one array. (As soils had been extracted in duplicate and pooled, each array was run with a mixed sample from six individual DNA extractions, i.e., three factor replicates and two extraction repeats.) The amplification of 16S rRNA genes, purification of products, labeling of DNA, and hybridization were conducted by following Brodie et al. using the reaction chemistry described in Wakelin et al. (22, 36). Hybridized arrays were stained and washed on an Affymetrix fluidic station (36). After scanning, data were processed by following the method outlined by Brodie et al. and DeSantis et al. (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 of ≥0.9.

Statistical analyses. Multivariate analyses were conducted in the PRIMER software package with the permutational multivariate analysis of variance (PERMANOVA) add-on using statistical approaches described 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, land use, lithologies, and underlying mineral deposits were calculated. Significance levels were determined using Student's *t* tests ($P < 0.05$). PERMANOVA (40) for TRFs was used to test if between-group variation (i.e., location, soil depth, land use, landform, lithology, and mineral deposits) can explain a significant proportion of the total system variation (i.e., if natural groupings can be detected). Balanced PERMANOVAs were conducted using partial sums of squares on 9,999 permutations of residuals under a reduced model. CAP (canonical analysis of principal coordinates) analysis (40) was used to determine if principal coordinates axe could be found that separate *a priori*-defined treatments (i.e., CAP analysis attempted to seek out predefined 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 9,999 permutations (random allocations) of samples (40). Distance-based linear modeling (DISTLM) was used to assess the geochemical parameters best explaining the variability within the microbial data set (23). SIMPER (similarity percentage) analysis was used to identify taxa/classes/phyla that discriminate between locations, landforms, land use, 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), interpretations of treatment effects on bacterial communities were conducted at the phylum/class level. Unweighted pair group method using average linkages (UPGMA) and maximum-likelihood (with 1,000 bootstrap 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, land use, 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 versus Fifield, $P = 0.0003$), depths (Hillside, $P = 0.01$), across different land uses ($P = 0.003$), and with different underlying mineral deposits ($P = 0.009$) (Fig. 1 and 2a, Table 1); a strong interactive effect of landform and mineral deposits also was observed (Table 1).

At Fifield, soil geochemical properties varied significantly with lithology (square root of the component of variation [$\sqrt{\text{CV}}$] = 4.1; $P < 0.001$), landform ($\sqrt{\text{CV}} = 3.2$; $P < 0.001$), and mineral deposits ($\sqrt{\text{CV}} = 5.3$; $P < 0.001$); no significant differences between the grazing land and bushland were detectable (Table 2). Bacterial community assemblages (based on M-TRLFP) varied significantly with land use, landform, lithology, and mineral deposits; lithology ($\sqrt{\text{CV}} = 14.1$; $P = 0.01$) and land use ($\sqrt{\text{CV}} = 13.9$; $P < 0.02$) were the primary discriminators (Table 2). Fungal communities varied significantly with land use ($\sqrt{\text{CV}} = 14.9$; $P = 0.01$) and underlying lithology ($\sqrt{\text{CV}} = 16.7$; $P < 0.01$) but not landform or mineral deposits (Table 2). Archaeal communities varied significantly with all geogenic factors, but not land use (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 and 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 the most with soil depth; e.g., the bacterial community displayed a $\sqrt{\text{CV}}$ of 16.5 ($P < 0.01$) (Table 3) across topsoils (A-horizon; 0.03 to 0.2 m) and subsurface soils (B-horizon; >0.2 m). No influences of either land use or geogenic factors were detected when surface and deeper soils were analyzed collectively (Table 3). Analyzed independently, bacterial and fungal communities in topsoil varied significantly with land use ($\sqrt{\text{CV}} = 12.5$ and 4.7, respectively; $P < 0.001$) and landform ($\sqrt{\text{CV}} = 9.4$ and 3.4, respectively; $P < 0.05$). Archaeal communities were not linked to any of the factors tested (Table 3). In subsurface soils, bacterial communities varied most significantly with lithology ($\sqrt{\text{CV}} = 9.2$; $P = 0.05$) and mineral deposit ($\sqrt{\text{CV}} = 14.2$; $P < 0.001$) but not land use or landform (Table 3). Fungal communities varied with lithology and mineral deposit as well as land use (Table 3). An interactive

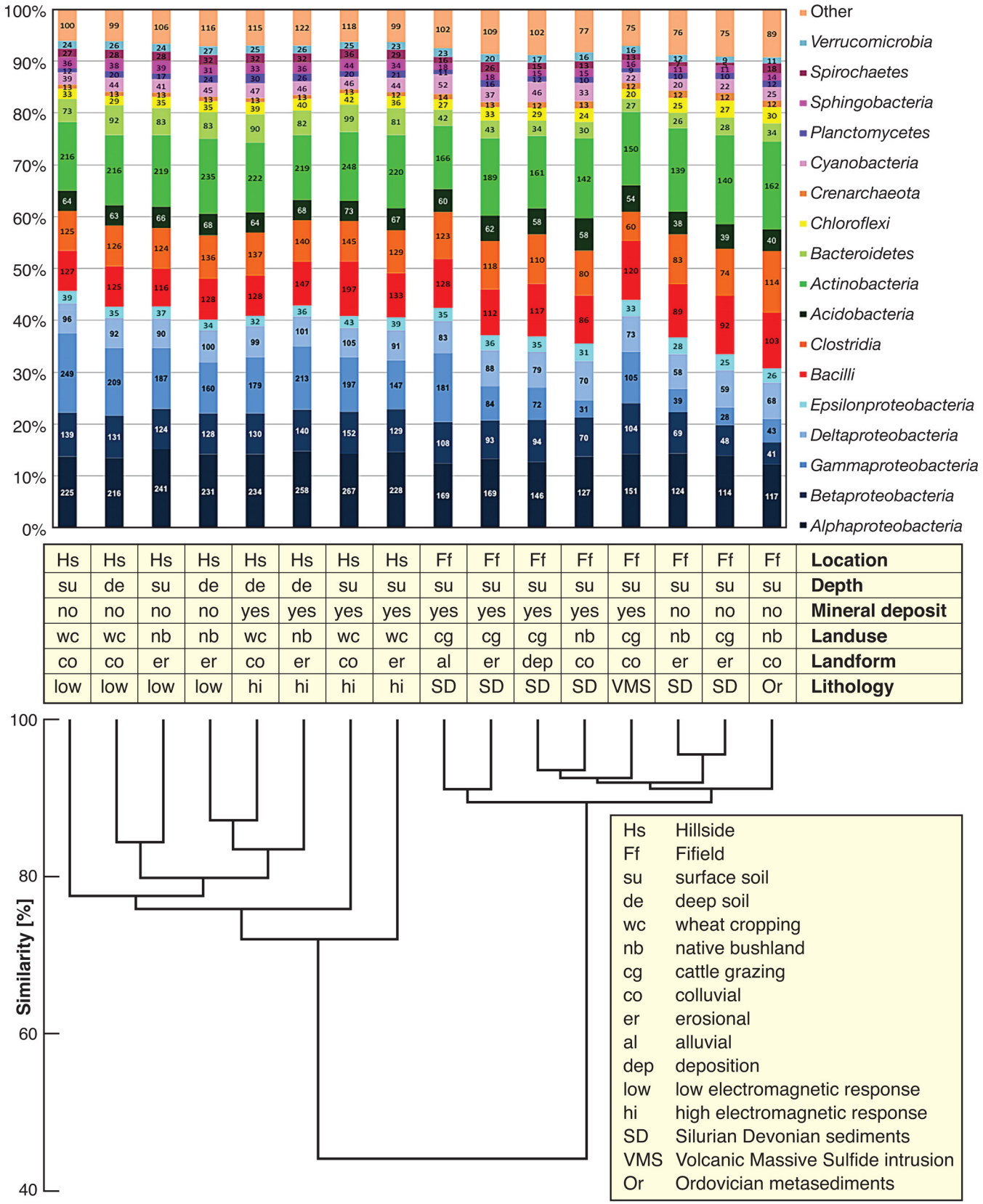

FIG 1 Distribution of dominant prokaryotic phyla/classes (number of OTUs in this group given in bars) and cluster analyses of community data based on individual taxa. Classes are shown for *Proteobacteria* and *Firmicutes*; phyla with <1% coverage are aggregated into "other."

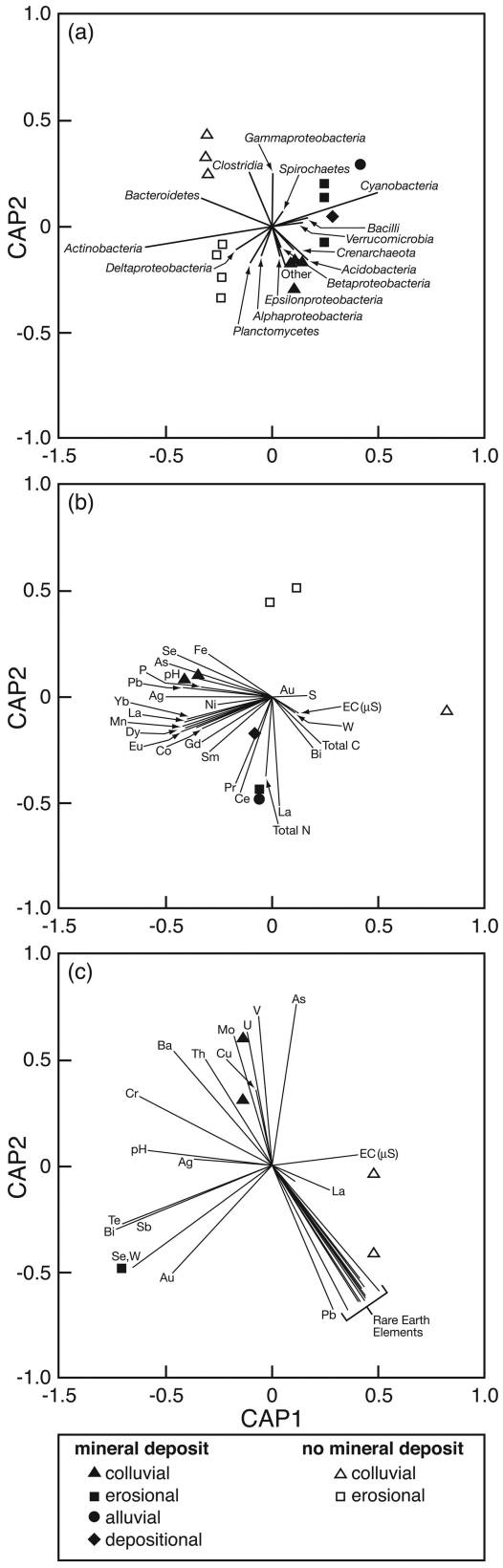

FIG 2 Ordination plots of the first two results produced by CAP of PhyloChip data analyzed for differences in community assemblages in relation to landform and underlying mineral deposits. Vectors of Pearson's correlations of classes/phyla (a) and geochemical parameter (b and c) are overlain.

TABLE 1 Summary of CAP of the examined factors on microbial community assemblages at the Fifield and Hillside sites[a]

Factor	CAP_{trace}[b]	P_{perm}[c]
Location	**0.97**	**0.0003**
Depth	**0.91**	**0.01**
Land use	**0.63**	**0.003**
Mineral deposit	**0.95**	**0.009**
Landform	0.31	0.9
Landform × mineral deposit	**2.82**	**0.05**

[a] Significance is indicated by boldface font ($P < 0.05$).
[b] CAP_{trace} indicates the strength of the association between the data cloud and the hypothesis of interest, shown as squared canonical correlations; values are between 0 (no correlation) and 1 (perfect correlation) (40).
[c] P_{perm} significance level based on a permutation test based on 999 or 9,999 permutations (random allocations) of samples.

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 el-

TABLE 2 Summary of PERMANOVA testing of the influence of geogenic factors on microbial community assemblages (M-TRLFP data) and geochemical parameters at the Fifield site[a]

Community parameter and factor	PERMANOVA value	
	$\sqrt{CV}$[b]	P_{perm}
Bacteria		
Land use	**13.9**	**0.02**
Lithology	**14.1**	**0.01**
Landform	**8.0**	**0.05**
Mineral deposit	**6.1**	**0.01**
Residual	27.3	
Fungi		
Land use	**14.9**	**0.01**
Lithology	**16.4**	**0.004**
Landform	4.1	0.36
Mineral deposit	4.8	0.16
Residual	40.6	
Archaea		
Land use	13.4	0.1
Lithology	16.7	0.08
Landform	**22.6**	**0.004**
Mineral deposit	**12.5**	**0.01**
Residual	50.8	
Geochemistry		
Land use	1.1	0.23
Lithology	**4.1**	**0.0003**
Landform	**3.2**	**0.0004**
Mineral deposit	**5.3**	**0.0001**
Residual	4.4	

[a] Significance is indicated by boldface font ($P < 0.05$).
[b] $\sqrt{CV}$ is the square root of the component of variation, which is a data set-dependent measure of the effect of size in units of the community dissimilarities (i.e., increasing positive values); negative values indicate zero components (40).

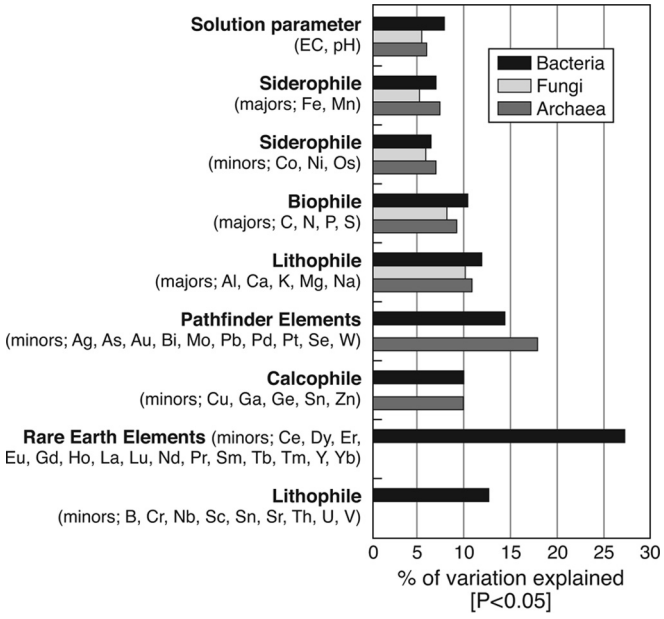

FIG 3 Percentage of bacterial, fungal, and archaeal soil community diversity explained by geochemical parameters; results of distance-based linear regression modeling (DISTLM), based on M-TRLFP data versus geochemical parameters of Fifield samples with significance levels ($P < 0.05$).

ements were capable of explaining 24.2% of variation in fungal communities; 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, land use, lithology, and mineral deposits. Across all samples, 45 phyla, 90 classes, 173 orders, and 306 families were detected (Fig. 1; also see Table S1 in the supplemental material). Between 879 and 1,879 individual taxa were observed (Fig. 1; also see Table S1). Bacterial communities were dominated by *Proteobacteria* (*Alpha*, *Beta*, *Gamma*, *Delta* and *Epsilon* subdivisions; 35.5 to 45.7%), *Firmicutes* (15.1 to 22.6%), *Actinobacteria* (12.2 to 17.1%), *Acidobacteria* (3.8 to 6.3%), *Bacteroides* (2.6 to 5.7%), *Chloroflexi* (1.8 to 3.3%), and *Cyanobacteria* (2.1 to 4.0%) (Fig. 1). *Sphingobacteria*, *Verrucomicrobiae*, *Anaerolineae*, *Planctomycetacia*, *Catabacter*, *Spirochaetes*, and *Archaea* represented between 0.1 and 2.0% of the composition of prokaryotic communities (Fig. 1).

Prokaryotic communities at Hillside were richer in taxa (1,660 ± 101) than those at Fifield (1,007 ± 194) (see Table S1 in the supplemental material). All phyla/classes displayed higher numbers of taxa in Hillside than Fifield soils. Of these, *Alpha*-, *Beta*-, and *Gammaproteobacteria*, *Actinobacteria*, *Bacteroides*, *Sphingobacteria*, and *Spirochaetes* were the most discriminatory taxa ($P < 0.05$) (see Table S1). At Hillside, the total number of taxa in different phyla/classes did not differ between soils depth, land use, and underlying mineral deposits. However, 208 taxa were only detected in soils overlying the mineral deposit (see Table S1).

TABLE 3 Summary of PERMANOVA testing of the influence of the examined factors and the underlying Cu-Au-U deposit on microbial community assemblages and geochemical parameters at the Hillside study site[a]

	PERMANOVA result for:							
	Bacteria		Fungi		Archaea		Geochemistry	
Sample type and factor	$\sqrt{CV}$[b]	P_{perm}	$\sqrt{CV}$	P_{perm}	$\sqrt{CV}$	P_{perm}	$\sqrt{CV}$	P_{perm}
All								
Depth	**16.5**	**0.01**	**20.3**	**0.0006**	**17.4**	**0.0001**		
Land use	9.6	0.3	11.1	0.2	4.8	0.35		
Landform, lithology, mineral deposit	−9.0	0.9	−1.7	0.5	7.6	0.21		
Residual	33.5		28.9		29.2			
Surface								
Lithology	2.9	0.4	5.2	0.27	−5.4	0.54		
Landform	**9.4**	**0.04**	**15.1**	**0.02**	7.8	0.24		
Mineral deposit	−5.8	0.8	−5.4	0.56	−7.5	0.66		
Land use	**12.5**	**0.001**	16.6	0.0001	14.8	0.031		
Residual	15.4		22.0		23.5			
Deep[c]								
Lithology	**9.2**	**0.05**	18.7	0.08	7.0	0.30	**3.12**	**0.001**
Landform	6.9	0.16	10.9	0.18	13.9	0.07	**2.51**	**0.05**
Mineral deposit	**14.2**	**0.006**	**9.6**	**0.04**	6.4	0.3	−4.43	0.4
Land use	6.1	0.17	7.5	0.1	4.2	0.4	NT[d]	NT
Landform × mineral deposit	7.7	0.25	**20.0**	**0.05**	**30.0**	**0.04**	**2.51**	**0.02**
Residual	37.7		28.7		30.9		7.2	

[a] Significance is indicated by boldface font ($P < 0.05$).

[b] $\sqrt{CV}$ is the square root of the component of variation, which is a data set-dependent measure of the effect of size in units of the community dissimilarities (i.e., increasing positive values); negative values indicate zero components (40).

[c] A company data set was used for analyses, which only provided data for deep samples.

[d] NT, not tested, as all geochemical analyses were derived from wheat cropping sites.

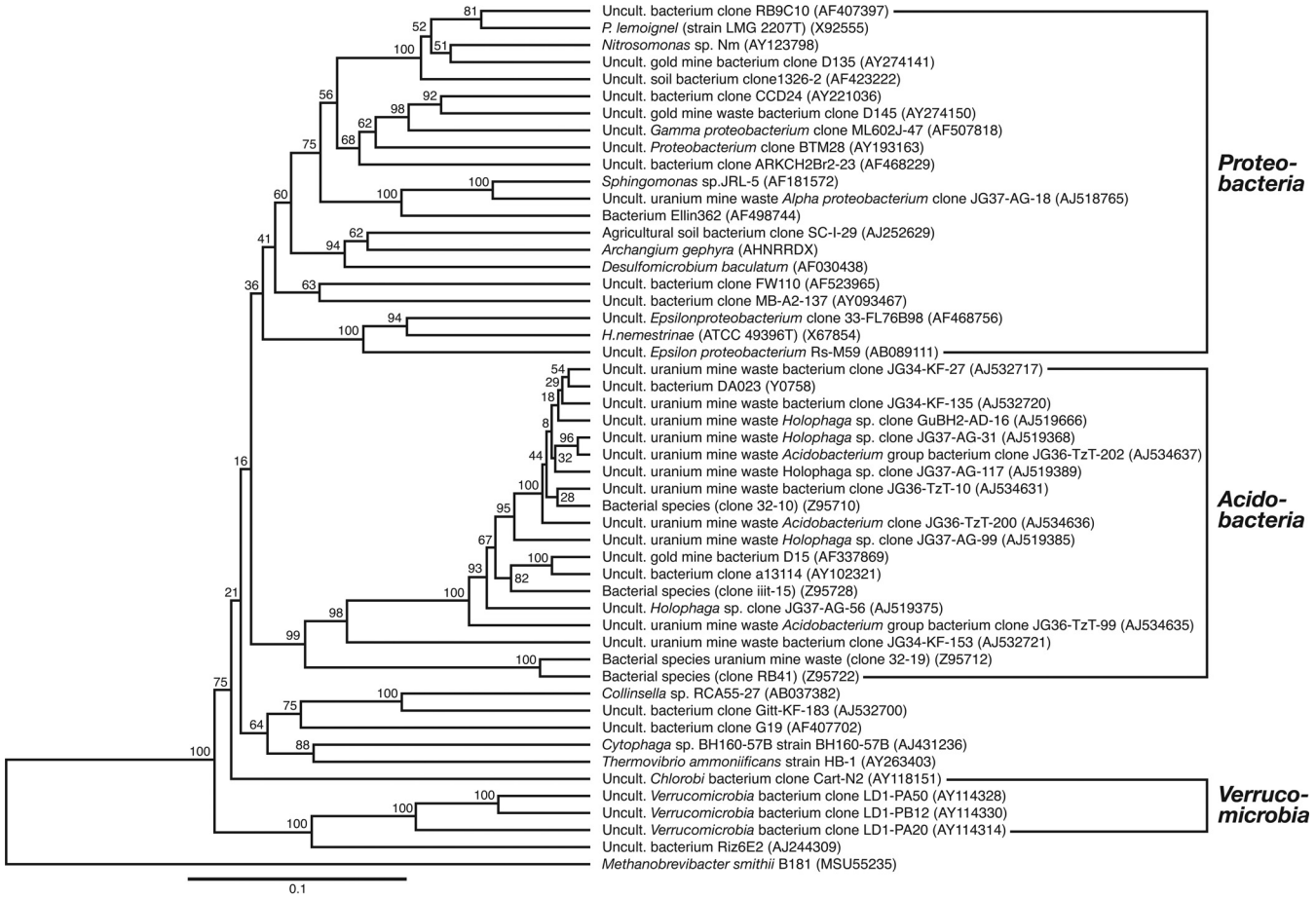

FIG 4 16S rRNA gene maximum-likelihood phylogenetic tree (1,000 bootstrap replicates) of the 50 taxa (PhyloChip) that best discriminate soils overlying mineral deposits from background soils at both sites; taxa were identified by SIMPER analyses. One representative (probe-targeted) sequence per taxum was used. The tree does not represent sequences from the field sites but is a close approximation based on probe matches. Sequences were obtained from GenBank. Uncult., uncultured.

At Fifield, significantly higher numbers of taxa ($P < 0.05$) were observed in samples overlying mineral deposits (1,153 ± 170 taxa) than in background soils (881 ± 72 taxa, with 463 taxa occurring only in soils overlying mineral deposits (see Table S1 in the supplemental material). In particular, *Alpha-*, *Beta-*, *Delta-*, and *Epsilonproteobacteria*, as well as *Acidobacteria*, *Sphingobacteria*, and *Verrucomicrobiae*, 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, 10 belonged to *Bacilli* (see Fig. S2).

At Hillside, higher numbers of *Betaproteobacteria* and *Bacilli* were associated with colluvial than with erosional landforms (see Table S1 in the supplemental material). At Fifield, overall abundances between erosional and colluvial landforms were similar, and alluvial sites contained higher numbers of *Alpha-*, *Beta-*, *Gamma-*, and *Epsilonproteobacteria*, *Firmicutes* (*Bacilli* and *Clostridia*), and *Cyanobacteria* (see Table S1). In soils from cattle grazing sites, significantly more *Bacilli* were detected than in native bushland soils (see Table S1). Whereas *Acidobacteria*, *Beta-* and *Epsilonproteobacteria*, *Cyanobacteria*, *Spirochaetes*, and *Bacilli* correlate well with underlying mineral deposits, *Actinobacteria*, *Deltaproteobacteria*, and *Bacteriodetes* correlated most strongly with background soils (Fig. 2a). SIMPER analyses on individual taxa also showed that 19 and 20 of the 50 most discriminating taxa between soils overlying mineral deposits and background sites were *Acidobacteria* and *Proteobacteria*, respectively (Fig. 4). *Gammaproteobacteria* correlated with colluvial landforms. *Actinobacteria* were closely linked to erosional landforms (Fig. 2a; also see Table S1). At alluvial sites a larger abundance and diversity of *Cyanobacteria* was observed (Fig. 2a; also see 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 land use. This, we hypothesize, is a 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). Reprecipitation 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 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 land use, 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; for examples, see references 26 and 50–52).

The data presented here support our hypothesis that longer *in situ* weathering periods lead to larger differences in community diversity driven by geogenic factors. At Hillside, the highly weathered Paleoproterozoic lithology and associated metal deposit are covered by Aeolian (i.e., <10,000-year-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*, and *Alpha-* and *Gammaproteobacteria*) occurred only in soils overlying the Cu-Au-U-REE deposit. The assessment of overall taxon abundances at the phylum/class levels showed few differences between different factors tested (see Table S1 in the supplemental material). 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 times 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 the class/phylum level were observed with *Alpha-*, *Beta-*, *Delta-*, and *Epsilonproteobacteria* 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 metagenome of a wide range (continental scale) of soils with established histories of land use, 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 land uses can be tested to assess if communities in older soils react differently than 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 (Fig. 2a and 4; also see Table S1 in the supplemental material). 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 show 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* organism first identified by Geissler and Selenska-Pobell from U waste piles near Johanngeorgenstadt (Germany) (57). Other taxa first were identified in samples obtained from Au mines. *Verrucomicrobia* also have been linked to U mining environments (58), with the taxa identified here belonging to the ammonia-oxidizing 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 for 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 previous studies, elevated numbers of *B. cereus* spores also were 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* also was significantly higher in Au-amended than in unamended soils (23). These data suggest that *Bacilli* are particularly well adapted to persist in commonly dry semiarid soils displaying elevated concentrations of heavy metals.

Active *Bacilli* from semiarid Australian environments also may 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 (for example, see reference 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 coprecipitation 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 affects community composition.

Proteobacteria, especially *Alphaproteobacteria*, were the dominant phylum across the study sites and also are often the 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 also have 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 are 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, 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) land use at the study sites was not uniform and/or was minimal, and intensely agriculturally utilized soils were compared to native bushland soils; (ii) soils overlying polymetallic Cu-Au-U-REE and Au-Pt deposits were assessed and 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) versus 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; hence, they may contribute to variation in the soil microbial community diversity at a far wider range of sites than previously suggested.

ACKNOWLEDGMENTS

We thank the Australian Research Council (LP100200212 to F.R.), Rex Minerals, and Rimfire Ltd. Pty.

We thank Colin Plumridge, John Kaminski, Byron J. Dietman, and M. Dhamarajh for their assistance. We also thank the two anonymous reviewers for their thoughtful comments.

REFERENCES

1. **Fierer N, Jackson RB.** 2006. The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A **103:**626–631. http://dx.doi.org/10.1073/pnas.0507535103.
2. **Acosta-Martinez V, Dowd S, Sun Y, Allen V.** 2008. Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. Soil Biol Biochem **40:**2762–2770. http://dx.doi.org/10.1016/j.soilbio.2008.07.022.
3. **Lauber CL, Strickland MS, Bradford MA, Fierer N.** 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol Biochem **40:**2407–2415. http://dx.doi.org/10.1016/j.soilbio.2008.05.021.
4. **Drenovsky RE, Steenwerth KL, Jackson LE, Scow KM.** 2010. Land use and climatic factors structure regional patterns in soil microbial communities. Glob Ecol Biogeogr **19:**27–39. http://dx.doi.org/10.1111/j.1466-8238.2009.00486.x.
5. **Garbeva P, van Veen J, van Elsas JD.** 2004. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. Annu Rev Phytopathol **42:**243–227. http://dx.doi.org/10.1146/annurev.phyto.42.012604.135455.
6. **Lazzaro A, Abegg C, Zeyer J.** 2009. Bacterial community structure of glacier forefields on siliceous and calcareous bedrock. Eur J Soil Sci **60:**860–870. http://dx.doi.org/10.1111/j.1365-2389.2009.01182.x.
7. **Guan X, Wang J, Zhao H, Wang J, Luo X, Liu F, Zhao F.** 2013. Soil bacterial communities shaped by geochemical factors and land use in a less-explored area, Tibetan Plateau. BMC Genomics **14:**820. http://dx.doi.org/10.1186/1471-2164-14-820.
8. **Meola M, Lazzaro A, Zeyer J.** 2014. Diversity, resistance and resilience of the bacterial communities at two alpine glacier forefields after a reciprocal soil transplantation. Environ Microbiol **16:**1918–1934. http://dx.doi.org/10.1111/1462-2920.12435.
9. **Ehrlich HL.** 1998. Geomicrobiology: its significance for geology. Earth Sci Rev **45:**45–60. http://dx.doi.org/10.1016/S0012-8252(98)00034-8.
10. **Ehrlich HL, Newman DK.** 2009. Geomicrobiology, 5th ed. CRC Press, Boca Raton, FL.
11. **Tyson G, Chapman J, Hugenholtz P, Allen EE, Ram RJ, Richardson PM.** 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature **428:**37–43. http://dx.doi.org/10.1038/nature02340.
12. **Baker BJ, Banfield JF.** 2003. Microbial communities in acid mine drainage. FEMS Microbiol Ecol **44:**139–152. http://dx.doi.org/10.1016/S0168-6496(03)00028-X.
13. **Diaby N, Dold B, Pfeifer HR, Holliger C, Johnson DB, Hallberg K.** 2007. Microbial communities on a porphyry copper tailings impoundment and their impact on the geochemical gradients of the mine waste. Environ Microbiol **9:**298–307. http://dx.doi.org/10.1111/j.1462-2920.2006.01138.x.
14. **Kock D, Schippers A.** 2008. Quantitative microbial community analysis of three different sulfidic mine tailing dumps generating acid drainage. Appl Environ Microbiol **74:**5211–5219. http://dx.doi.org/10.1128/AEM.00649-08.
15. **Denef VJ, Mueller RS, Banfield JF.** 2010. AMD biofilms: using model communities to study microbial evolution and ecological complexity in nature. ISME J **4:**599–610. http://dx.doi.org/10.1038/ismej.2009.158.
16. **Aspandiar MF, Anand RR, Gray DJ.** 2008. Geochemical dispersion mechanisms through transported cover: implications for mineral exploration in Australia. Cooperative Research Centre for Landscape Evolution and Mineral Exploration, Open File Rep 246. Wembley West, Australia.
17. **Leslie K, van Geffen PW, MacFarlane B, Oates CJ, Kyser TK, Fowle DA.** 2013. Biogeochemical indicators of buried mineralization under cover, Talbot VMS Cu–Zn prospect, Manitoba. Appl Geochem **37:**190–202. http://dx.doi.org/10.1016/j.apgeochem.2013.07.013.
18. **Neybergh H, Moureau Z, Gerard P, Verraes G, Sulten E.** 1991. Utilisation des concentrations de Bacillus cereus dans les sols comme technique de prospection des gites auriferes. Chron Rech Min **502:**37–46.
19. **Melchior A, Cardenas J, Dejonghe L.** 1994. Geomicrobiology applied to mineral exploration in Mexico. J Geochem Explor **51:**193–212. http://dx.doi.org/10.1016/0375-6742(94)90017-5.
20. **Reith F, McPhail DC, Christy AG.** 2005. Bacillus cereus, gold and associated elements in soil and regolith samples from Tomakin Park Gold Mine in south-eastern New South Wales, Australia. J Geochem Explor **85:**81–89. http://dx.doi.org/10.1016/j.gexplo.2004.11.001.
21. **Wakelin SA, Anand RR, Reith F, Gregg AL, Noble RRP, Goldfarb KC.** 2012. Bacterial communities associated with a mineral weathering profile at a sulphidic mine tailings dump in arid Western Australia. FEMS Microbiol Ecol **79:**298–311. http://dx.doi.org/10.1111/j.1574-6941.2011.01215.x.
22. **Wakelin SA, Anand RR, Reith F, Macfarlane C, Rogers S.** 2012. Biogeochemical surface expression over a deep, overburden-covered VMS mineralization. J Geochem Explor **112:**262–271. http://dx.doi.org/10.1016/j.gexplo.2011.09.005.
23. **Reith F, Brugger J, Zammit CM, Gregg AL, Goldfarb KC, Andersen GL, Desantis TZ, Piceno YM, Brodie EL, Lu Z, He Z, Zhou J, Wakelin SA.** 2012. Influence of geogenic factors on microbial communities in metallogenic Australian soils. ISME J **6:**2107–2118. http://dx.doi.org/10.1038/ismej.2012.48.
24. **Viles HA.** 2012. Microbial geomorphology: a neglected link between life and landscape. Geomorphology **157:**6–16.
25. **Viles HA, Naylor LA, Carter NEA, Chaput D.** 2008. Biogeomorphological disturbance regimes: progress in linking ecological and geomorphological systems. Earth Surf Proc Landforms **33:**1419–1435. http://dx.doi.org/10.1002/esp.1717.
26. **Wakelin SA, Barratt BIP, Gerard E, Gregg AL, Brodie EL, Andersen GL, DeSantis TZ.** 2013. Shifts in the phylogenetic structure and functional capacity of soil microbial communities follow alteration of native tussock

grassland ecosystems. Soil Biol Biochem **57:**675–682. http://dx.doi.org/10.1016/j.soilbio.2012.07.003.
27. **Johan Z, Ohnenstetter M, Slansky E, Barron LM, Suppel D.** 1989. Platinum mineralization in the Alaskan-type intrusive complexes near Fifield, New-South-Wales, Australia 1 Platinum-group minerals in clinopyroxenites of the Kelvin Grove prospect, Owendale intrusion. Mineral Petrol **40:**289–309. http://dx.doi.org/10.1007/BF01164604.
28. **Isbell RF.** 2002. The Australian soil classification (revised ed). CSIRO Publishing, Collingwood, Australia.
29. **Brugger J, Etschmann B, Grosse C, Plumridge C, Kaminski J, Paterson D, Shar SS, Ta C, Howard DL, de Jonge MD, Ball AS, Reith F.** 2013. Can biological toxicity drive the contrasting behavior of platinum and gold in surface environments? Chem Geol **343:**99–110. http://dx.doi.org/10.1016/j.chemgeo.2013.02.010.
30. **Pain CF.** 2008. Regolith description and mapping, p 281–306. *In* Scott KM, Pain CF (ed), Regolith science. CSIRO Publishing, Melbourne, Australia.
31. **Pillans B.** 2008. Regolith through time, p 7–31. *In* Scott KM, Pain CF (ed), Regolith science. CSIRO Publishing, Melbourne, Australia.
32. **Dietman BJ.** 2009. Regolith and associated geochemical and biogeochemical expression of buried copper gold mineralisation at the Hillside Prospect, Yorke Peninsula. B.S. thesis, University of Adelaide, Adelaide, Australia.
33. **Goldschmidt VM.** 1954. Geochemistry. Oxford University Press, Oxford, United Kingdom.
34. **McQueen KG.** 2008. Regolith geochemistry, p 73–104. *In* Scott KM, Pain CF (ed), Regolith science. CSIRO Publishing. Melbourne, Australia.
35. **Singh BK, Nazaries L, Munro S, Anderson IC, Campbell CD.** 2006. Use of multiplex terminal restriction fragment length polymorphism for rapid and simultaneous analysis of different components of the soil microbial community. Appl Environ Microbiol **72:**7278–7285. http://dx.doi.org/10.1128/AEM.00510-06.
36. **Brodie EL, DeSantis TZ, Joyner DC, Baek SM, Larsen JT, Andersen GL.** 2006. Application of a high-density oligonucleotide microarray approach to study bacterial population dynamics during uranium reduction and reoxidation. Appl Environ Microbiol **72:**6288–6298. http://dx.doi.org/10.1128/AEM.00246-06.
37. **DeSantis TZ, Brodie EL, Moberg JP, Zubieta IX, Piceno YM, Andersen GL.** 2007. High density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment. Microb Ecol **53:**371–383. http://dx.doi.org/10.1007/s00248-006-9134-9.
38. **Schatz MC, Phillippy AM, Gajer P, DeSantis TZ, Andersen GL, Ravel J.** 2010. Integrated microbial survey analysis of prokaryotic communities for the PhyloChip microarray. Appl Environ Microbiol **76:**5636–5638. http://dx.doi.org/10.1128/AEM.00303-10.
39. **Clarke KR, Warwick RM.** 2001. Change in marine communities: an approach to statistical analysis and interpretation. Primer-E Ltd., Plymouth, United Kingdom.
40. **Anderson MJ, Gorley RN, Clarke KR.** 2008. PERMANOVA+ for PRIMER–guide to software and statistical methods. Primer-E Ltd., Plymouth, United Kingdom.
41. **Bray JR, Curtis JT.** 1957. An ordination of the upland forest communities of southern Wisconsin. Ecol Monograph **27:**325–349. http://dx.doi.org/10.2307/1942268.
42. **Gower JC.** 1966. Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika **53:**325–338.
43. **Reith F, Dürr M, Welch S, Rogers SL.** 2008. The geomicrobiology of the Regolith, p 127–159. *In* Scott K, Pain CF (ed), Regolith science. CSIRO Press, Melbourne, Australia.
44. **Southam G, Saunders JA.** 2005. The geomicrobiology of ore deposits. Econ Geol **100:**1067–1084. http://dx.doi.org/10.2113/gsecongeo.100.6.1067.
45. **Lejon DPH, Nowak V, Bouko S, Pascault N, Mougel C, Martins JMF.** 2007. Fingerprinting and diversity of bacterial copA genes in response to soil types, soil organic status and copper contamination. FEMS Micro Ecol **61:**424–437. http://dx.doi.org/10.1111/j.1574-6941.2007.00365.x.
46. **Haferburg G, Kothe E.** 2007. Microbes and metals: interactions in the environment. J Basic Microbiol **47:**453–467. http://dx.doi.org/10.1002/jobm.200700275.
47. **Duxbury T, Bicknell B.** 1983. Metal-tolerant bacterial populations from natural and metal-polluted soils. Soil Biol Biochem **15:**243–250. http://dx.doi.org/10.1016/0038-0717(83)90066-4.
48. **Doelman P, Jansen E, Michels M, Vantil M.** 1994. Effects of heavy metals in soil on microbial diversity and activity as shown by the sensitivity-resistance index, an ecologically relevant parameter. Biol Fertil Soils **17:**177–184. http://dx.doi.org/10.1007/BF00336319.
49. **Ranjard L, Echairi A, Nowak V, Lejon DPH, Nouaim R, Chaussod R.** 2006. Field and microcosm experiments to evaluate the effects of agricultural Cu treatment on the density and genetic structure of microbial communities in two different soils. FEMS Microbiol Ecol **58:**303–315. http://dx.doi.org/10.1111/j.1574-6941.2006.00157.x.
50. **Steenwerth KL, Jackson E, Calderon FJ, Stromberg MR, Scowd KM.** 2002. Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biol Biochem **34:**1599–1611. http://dx.doi.org/10.1016/S0038-0717(02)00144-X.
51. **Chim Chan O, Casper P, Sha LQ, Feng ZL, Fu Y, Yang XD, Ulrich A, Zou XM.** 2008. Vegetation cover of forest, shrub and pasture strongly influences soil bacterial community structure as revealed by 16S rRNA gene T-RFLP analysis. FEMS Microbiol Ecol **64:**449–458. http://dx.doi.org/10.1111/j.1574-6941.2008.00488.x.
52. **Shange RS, Ankumah RO, Ibekwe AM, Zabawa R, Dowd SE.** 2012. Distinct soil bacterial communities revealed under a diversely managed agroecosystem. PLoS One **7**(7)**:**e40338. http://dx.doi.org/10.1371/journal.pone.0040338.
53. **Quaiser A, Ochsenreiter T, Lanz C.** 2003. Acidobacteria form a coherent but highly diverse group within the bacterial domain: evidence from environmental genomics. Mol Microbiol **50:**563–575. http://dx.doi.org/10.1046/j.1365-2958.2003.03707.x.
54. **Barns SM, Cain EC, Sommerville L, Kuske CR.** 2007. *Acidobacteria* phylum sequences in uranium-contaminated subsurface sediments greatly expand the known diversity within the phylum. Appl Environ Microbiol **73:**3113–3116. http://dx.doi.org/10.1128/AEM.02012-06.
55. **Bergmann GT, Bates ST, Eilers KG, Lauber CL, Caporaso JG, Walters WA, Knight R, Fierer N.** 2011. The under-recognized dominance of Verrucomicrobia in soil bacterial communities. Soil Biol Biochem **43:**1450–1455. http://dx.doi.org/10.1016/j.soilbio.2011.03.012.
56. **Ward NL, Challacombe JF, Janssen PH, Henrissat B, Coutinho PM, Wu M, Xie G, Haft DH, Sait M, Badger J, Barabote RD, Bradley B, Brettin TS, Brinkac LM, Bruce D, Creasy T, Daugherty SC, Davidsen TM, DeBoy RT, Detter JC, Dodson RJ, Durkin AS, Ganapathy A, Gwinn-Giglio M, Han CS, Khouri H, Kiss H, Kothari SP, Madupu R, Nelson KE, Nelson WC, Paulsen I, Penn K, Ren Q, Rosovitz MJ, Selengut JD, Shrivastava S, Sullivan SA, Tapia R, Thompson LS, Watkins KL, Yang Q, Yu C, Zafar N, Zhou L, Kuske CR.** 2009. Three genomes from the phylum *Acidobacteria* provide insight into the lifestyles of these microorganisms in soils. Appl Environ Microbiol **75:**2046–2056. http://dx.doi.org/10.1128/AEM.02294-08.
57. **Geissler A, Selenska-Pobell S.** 2005. Addition of U (VI) to a uranium mining waste sample and resulting changes in the indigenous bacterial community. Geobiology **3:**275–285. http://dx.doi.org/10.1111/j.1472-4669.2006.00061.x.
58. **Radeva G, Kenarova A, Bachvarova V, Flemming K, Popov I, Vassilev D, Selenska-Pobell S.** 2013. Bacterial diversity at abandoned uranium mining and milling sites in Bulgaria as revealed by 16s RRNA genetic diversity study. Water Air Soil Pollut **224:**1748. http://dx.doi.org/10.1007/s11270-013-1748-1.
59. **Selenska-Pobell S.** 2002. Diversity and activity of bacteria in uranium waste piles, p 225–254. *In* Keith-Roach MJ, Livens FR (ed), Interactions of microorganisms with radionuclides. Elsevier Sciences Ltd., Oxford, United Kingdom.
60. **Fritze D.** 1990. The taxonomy of alkaliphilic Bacillus strains. Int J Syst Bacteriol **40:**92–97. http://dx.doi.org/10.1099/00207713-40-1-92.
61. **Nicholson WL, Munakata N, Horneck G, Melosh H, Setlow P.** 2000. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. Microbiol Mol Biol Rev **64:**548–572. http://dx.doi.org/10.1128/MMBR.64.3.548-572.2000.
62. **Lintern MJ, Sheard MJ, Chivas AR.** 2006. The source of pedogenic carbonate associated with gold-calcrete anomalies in the western Gawler Craton, South Australia. Chem Geol **235:**299–324. http://dx.doi.org/10.1016/j.chemgeo.2006.08.001.
63. **Reith F, Wakelin S, Gregg AL, Schmidt Mumm A.** 2009. A microbial pathway for the formation of gold anomalous calcrete. Chem Geol **258:**315–326. http://dx.doi.org/10.1016/j.chemgeo.2008.10.023.
64. **Reith F, Etschmann B, Dart RC, Brewe DL, Vogt S, Schmidt Mumm A, Brugger J.** 2011. Distribution and speciation of gold in biogenic and

abiogenic calcium carbonates–implications for the formation of gold anomalous calcrete. Geochim Cosmochim A **75:**1942–1956. http://dx.doi.org/10.1016/j.gca.2011.01.014.
65. **Nies DH.** 2000. Heavy metal-resistant bacteria as extremophiles: molecular physiology and biotechnological use of Ralstonia sp. CH34. Extremophiles **4:**77–82. http://dx.doi.org/10.1007/s007920050140.
66. **Mergeay M.** 2006. Metallophiles and acidophiles in metal-rich environments, p 1–19. *In* Extremophiles (life under extreme environmental conditions) in Encyclopedia of life support systems (EOLSS). EOLSS Publishers, Oxford, United Kingdom.
67. **Johnston CW, Wyatt MA, Li X, Ibrahim A, Shuster J, Southam G, Magarvey NA.** 2013. Gold biomineralization by a metallophore from a gold-associated microbe. Nat Chem Biol **9:**241–243. http://dx.doi.org/10.1038/nchembio.1179.
68. **Wiesemann N, Mohr J, Grosse CM, Herzberg M, Hause G, Reith F, Nies DH.** 2013. Influence of copper resistance determinants on gold transformation by *Cupriavidus metallidurans* strain CH34. J Bacteriol **195:**2298–2308. http://dx.doi.org/10.1128/JB.01951-12.
69. **Reith F, Rogers SL.** 2008. Assessment of bacterial communities in auriferous and non-auriferous soils using genetic and functional fingerprinting. Geomicrobiol J **25:**203–215. http://dx.doi.org/10.1080/01490450802081846.