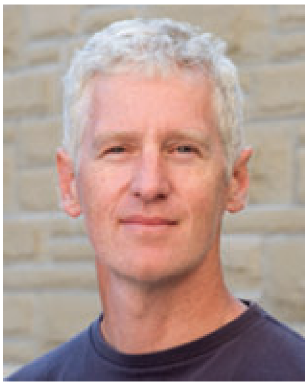
Small but mighty: microorganisms offer inspiration for mine remediation and waste stabilisation





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Understanding the natural microbiological mechanisms that promote iron cycling in iron ore mine environments may provide novel tools for the remediation of the fragile, iron-rich duricrust ecosystems associated with these environments as well as provide a solution for the stabilisation of hillslopes and tailings (waste) dams. A diverse array of microfossils is frequently identified throughout metre-scale duricrusts (canga; >50 wt.% Fe) that cap iron ore deposits in Brazil, shedding light on the intimate role of microorganisms in the evolution of these crusts. Nanoscale secondary ion mass spectrometry revealed that carbon and nitrogen biosignatures are occasionally preserved, and typically associated with the cell envelope structures of microfossils. The microfossils are 1–5 µm in length, with filamentous and rod-shaped morphologies commonly preserved[1,2]. When examined using backscatter electron scanning electron microscopy, canga shows a complex microstructure from repeated dissolution and re-precipitation of iron oxide minerals. Geochronology[3], geochemistry[4] and microbiology[5] provide insights into the past and present-day role of microorganisms in the evolution of canga. These dynamic biogeochemical processes in canga contribute to the continuous formation of new iron cements, preserving some of world's longest-lived continuously exposed surfaces. Harnessing and accelerating the biogeochemical cycling of iron may contribute to the development of novel technologies for mine remediation and waste stabilisation.

To supply the world with building resources, mountains are literally moved. The iron ore mining provinces in Australia and Brazil are vast, with iron ore reserves in each country totalling 10s of billions of tonnes[6]. China's financial development in the last decade has required an exponential production of iron ore: more than 880 and 440 million tonnes of iron ore are now extracted from Australian and Brazilian mines each year respectively[6]. The increased extraction of the iron ore creates serious environmental risks and mine remediation challenges.

The massive iron ore provinces in Australia and Brazil are primarily produced by extensive weathering of 2 billion-year-old banded iron formations[7], leaching away the silica and enriching the relatively immobile iron. During weathering in these systems, a hard ferruginous duricrust, referred to as canga, forms at the surface. Canga caps the iron ore deposits[3] and is extremely resistant to erosion, thereby protecting the iron ore below it from erosion during the relentless weathering over billions of years[8]. Cosmogenic isotope studies have revealed canga to be one of the longest-lived continuously exposed surfaces in the world[9,10]. The key to the longevity of canga is the redox cycling of iron. In canga, Fe(III) oxide minerals are continuously dissolved by the flora and microbiota associated with canga, driving iron into solution (Reaction 1). However, $Fe^{2+}_{(aq)}$ is unstable; it oxidises and re-precipitates to form new iron-rich cements in the presence of oxygen (Reactions 2 and 3). Therefore, chemical weathering of canga is partly responsible for the generation of new iron oxide cements that contribute to the long-term stability of canga and its extreme resistance to erosion[11]. Canga continues to evolve today via the biogeochemical cycling of iron[3].

10.1071/MA19055

$$4FeOOH_{(goethite)} + CH_2O + 8H^{+}_{(aq)} 4Fe^{2+}_{(aq)} + CO_2 + 7H_2O \quad \text{Reaction 1}$$

$$4Fe^{2+}_{(aq)} + O_2 + 4H^{+}_{(aq)} \leftrightharpoons Fe^{3+}_{(aq)} + 2H_2O \quad \text{Reaction 2}$$

$$Fe^{3+}_{(aq)} + 3H_2O \leftrightharpoons Fe(OH)_{3(s)} + 3H^{+}_{(aq)} \quad \text{Reaction 3}$$

Our research aims to understand the role microorganisms play in the redox-based cycling of iron oxide minerals (Reactions 1–3) within iron-rich duricrusts. Understanding the biological processes that contribute to redox-based iron cycling within these duricrusts may provide new technologies to enhance the reformation of iron-rich duricrusts after the completion of iron ore mining. The accelerated re-cementation of iron-rich duricrusts will aim to restore functional hydrogeology[12], provide a substrate for revegetation using native plants[13,14] and assist in the development of new biotechnologies that aim to stabilise degraded slopes. Biological hotspots, including well developed biofilms in streams (Figure 1*a*), ephemeral pools (Figure 1*b*) and rhizospheric biomes, provide ideal sites to understand the modern-day biogeochemical cycling of iron. In these regions, microbially driven iron cycling is accelerated and new cements in the duricrust are formed at a relatively rapid rate. For example, organic acids exuded by plants and microbes may chelate iron, making it more readily available for iron-reduction. These environments also contain higher concentrations of carbon sources for direct iron reduction (Reaction 1). Microorganisms also play an integral role in cement formation. Their cell envelope structures act as active sites for mineral

Figure 1. (*a*) Photograph of the canga plateaus and associated rupestrian vegetation in the Carajás, Pará, Brazil. (*b*) An ephemeral pool, common in depressions in the canga plateaus, with a surface sheen biofilm. (*c*) Iron-enriched fragments that are cemented on a slope, highlighting the relatively fast formation of some iron-rich cements. (*d*) Unstained transmission electron micrograph, revealing the heterogeneous iron oxide minerals that nucleate on the cells' surfaces and extracellular polymeric substances. White arrows highlight mineralised cell envelopes.

nucleation (Reaction 3)[15]. The question then becomes how can microbes growing at the micrometre scale be used to help to recreate these kilometre scale horizons?

Surface sheens that occur on many of the pools and seeps in canga environments provide an insight into the contemporary biogeochemical cycling of iron (Figure 1*b*). Within these surface sheens, microorganisms and extracellular polymeric substances induce the precipitation of poorly crystalline iron oxide minerals at the nanoscale (Figure 1*d*). With high surface to volume ratios, the cell envelopes of microorganisms provide a surfeit of reactive sites for mineral nucleation (Figure 1*d*). Scaling-up nanoscale chemical processes for mine remediation remains a global challenge. However, the size and abundance of microorganisms as well as their ability to form biofilms[16] represent an important aspect of bioremediation efforts. Critically for canga, as these biofilms are continuously exposed to iron solutions they become fossilised (Figure 2). The poorly crystalline iron oxides that nucleated on the cells' surfaces transform to more stable phases (Reaction 4), creating biocements (Figure 2). In addition, microbial biofilms act as an organic scaffold that gives the iron-rich cements a 'direction' for growth, rather than simply coating and enlarging existing grains (Figure 2). Carbon and nitrogen biosignatures, revealed by nanoscale secondary ion mass spectrometry (nanoSIMS), may be preserved with mineral encrusted cell envelopes (Figure 3), likely as a result of the effectively irreversible complexation of aluminium with the cell envelope[2]. Combining these concepts, microbial growth on crushed rock surfaces could be enhanced to form natural biofilm-rock aggregates. Simultaneously, microbially-promoted iron reduction would be optimised, forming iron-rich solutions within anaerobic bioreactors that would be distributed throughout the mine remediation site. Finally, the biofilm-rock aggregates would be exposed to the iron-rich solutions, where the soluble iron would become immobilised as iron oxide precipitates on the microbial cells' surfaces, mineralising the biofilms and forming new biocements. These processes could be repeated until the surface substrates were sufficiently stable to begin revegetation programmes.

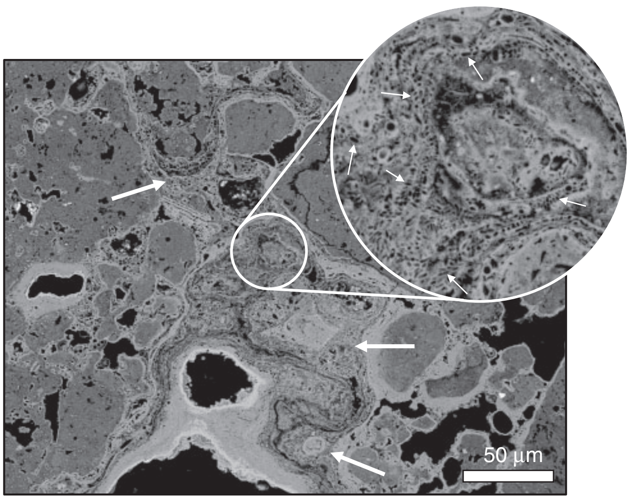

Figure 2. Representative, field-emission backscattered electron micrographs highlighting the preservation of microbial cements throughout a canga drill core subsample, collected from the S11D mine site in the Serra dos Carajás, Pará, Brazil. The circled and enlarged micrograph highlights the preservation of encrusted cell envelopes (thin arrows). Fossilised microorganisms contribute to the formation of biocements within canga (thick arrows).

$$Fe(OH)_{3(s)} \leftrightharpoons FeO(OH)_{(s)} + H_2O \qquad \text{Reaction 4}$$

In tropical regions that experience monsoonal rainfall, timing of cement formation is also a logistical constraint. In the field, cements can form on relatively steep slopes (Figure 1*c*), indicating they can consolidate loose material relatively quickly. These cements may initially form on the scale of decades and continue to be reinforced over the ensuing centuries by the continued precipitation iron oxides. Therefore, understanding and accelerating the natural processes that have previously formed these concretions will provide tools for relatively rapid, long-term landform stabilisation.

One of the driving forces to develop novel strategies for iron ore mine remediation are the unique florae associated with ferruginous duricrusts in Australia and Brazil. More than 900 plant species have been identified on ironstone outcrops in south-western Australia, with 44 species considered ironstone specialists[14]. Similarly, the florae associated with iron ore provinces in Brazil are diverse and naturally rare, with several endemic plant species growing on canga substrates. Canga capping iron ore deposits in the Carajás Mineral Province, Pará, in northern Brazil, supports 856 plant species, while 946 plant species have been collectively identified on canga in the Quadrilátero Ferrífero (QF), Minas Gerais, in south-eastern Brazil[13]. Extraordinarily, only 96 species are common to both the Carajás and the QF[13], highlighting the species richness of canga-associated florae. Accelerating the regeneration of canga-like substrates is essential for the restoration of these naturally rare ecosystems.

Brazil has suffered from two large-scale iron ore tailings dam collapses in recent years[17,18], with disastrous environmental impacts and deaths occurring at each site. Promoting the natural biogeochemical cycling of iron oxide minerals within canga that has preserved iron ore mines in Australia and Brazil may serve as an effective strategy to remediate mine sites in the future. Increasingly, we are looking to our smallest companions, the mighty 'microorganism', to help solve our greatest environmental challenges.

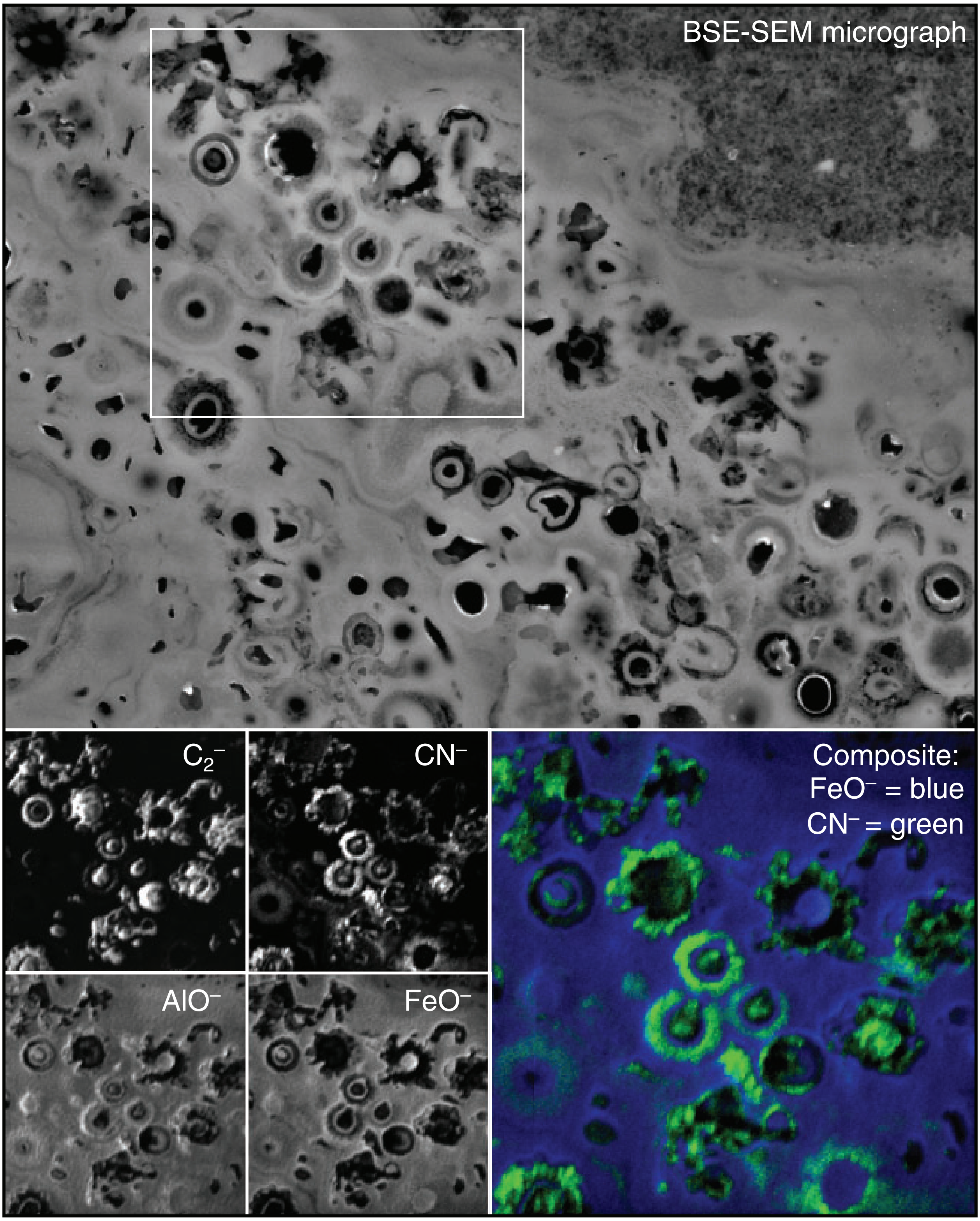

Figure 3. High resolution backscattered electron micrograph of microfossils preserved throughout iron-rich cements. The white square highlights the region analysed using nanoscale secondary ion mass spectrometry, the results of which are displayed below as relative intensity micrographs. Carbon and nitrogen elemental micrographs highlight the preservation of organic carbon and nitrogen associated with the cell envelopes of microfossils. The composite micrograph to the right [FeO^- (blue) and CN^- (green)] shows the distribution of nitrogen and its association with the microfossil structures.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We acknowledge support from the Vale S.A.-UQ Geomicrobiology initiative and the Australian Research Council Linkage Program (LP140100805) to G. Southam. The authors acknowledge the facilities and the scientific and technical assistance of the Australian Microscopy and Microanalysis Research Facility at the Centre of Microscopy and Microanalysis, at the University of Queensland. Alan Levett acknowledges the support from the Australian

Government Research Training Program. We thank Linda Blackall and an anonymous reviewer for valuable suggestions that improved the manuscript.

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Biographies

Alan Levett is a final year PhD candidate in the field of geomicrobiology at the University of Queensland. His research focuses on the biogeochemical cycling of iron within tropical iron ore systems. Alan's research combines high-resolution microscopy, mass spectrometry (NanoSIMS) and synchrotron-based microspectroscopy techniques with ecogenomics to analyse past and present-day interactions of microorganisms with metals and minerals.

Dr Emma Gagen is a Research Fellow at the University of Queensland. Her research focuses on harnessing microbial processes for accelerated mine site rehabilitation (iron ore mines in Brazil, coal mines in central Queensland). Other projects she contributes to relate to microbial colonisation of meteorites, bacterial degradation of anhydrite, microbiology of sulphur rich environments and formation of seafloor iron-manganese crusts. Emma's research interests extend to all areas of environmental microbiology and she is fascinated by the role microorganisms play in geochemical processes.

Professor Gordon Southam is an interdisciplinary researcher who has crossed traditional boundaries between biological and geological sciences. His research, using state-of-the-art microanalyses techniques (e.g. HR-TEM, FIB-SEM and synchrotron methods), focuses on the vital roles that bacteria play in mineral weathering, e.g. acid mine drainage systems, in the formation of mineral signatures in soils and sediments, and the genesis of authigenic secondary minerals, e.g. carbonates, iron oxides, metal sulphides and placer gold.