A.M. Cornall et al.

Microbial indicators of metal-impacted marine sediment

MOLECULAR SCREENING OF MICROBIAL COMMUNITIES FOR CANDIDATE
INDICATORS OF MULTIPLE METAL IMPACTS IN MARINE SEDIMENTS FROM
NORTHERN AUSTRALIA

ALYSSA M. CORNALL,[†] ALEA ROSE,[†] CLAIRE STRETEN,^{†‡} KEITH MCGUINNESS,[†] DAVID
PARRY,^{†‡} and KAREN GIBB*[†]

[†]Charles Darwin University, Darwin, Northern Territory, Australia

[‡]Australian Institute of Marine Science, Darwin, Northern Territory, Australia

*(Submitted 15 December 2014; Returned for Revision 29 January 2015; Accepted 11 August
2015)*

Abstract

Coastal sediments accumulate metals from anthropogenic sources and as a consequence industry is required to monitor sediment health. The total concentration of a metal does not necessarily reflect its potential toxicity or biological impact, so biological assessment tools are useful for monitoring. Rapid biological assessment tools sensitive enough to detect relatively small increases in metal concentrations would provide early warning of future ecosystem impact. The author's investigated in situ populations of Archaea and Bacteria as potential tools for rapid biological assessment in sediment at 4 northern Australian coastal locations over 2 yr, in both wet and dry seasons. The 1 M HCl-extractable concentrations of metals in sediment were measured, and Archaeal and Bacterial community profiles were obtained by next-generation

sequencing of sediment deoxyribonucleic acid (DNA). Species response curves were used to identify several taxonomic groups with potential as biological indicators of metal impact. Spatial variation, sediment grain size, water depth, and dissolved oxygen also correlated with microbial population shifts. Seasonal variation was less important than geographic location. Metal-challenge culture trials supported the identification of metal-resistant and -sensitive taxa. In situ Archaea and Bacteria are potentially sensitive indicators for changes in bioavailable concentrations of metals; however, the complexity of the system suggests it is important to identify metal-specific functional genes that may be informed by these sequencing surveys, and thus provide a useful addition to identity-based assays.

Keywords: Sediment quality, Biomonitoring, Metals, Bioavailability, Benthic ecology

This article includes online-only Supplemental Data.

* Address correspondence to Karen.Gibb@cdu.edu.au

Published online 14 August 2015 in Wiley Online Library (www.wileyonlinelibrary.com).

DOI: 10.1002/etc.3205

INTRODUCTION

It has been clearly demonstrated that physical and chemical characteristics alone are insufficient to measure or estimate the ecological impact of changes to marine sediments, including increased metal loading [1,2]. Consequently, the Australian and New Zealand Environment and Conservation Council (ANZECC) guidelines for sediment and water quality recommend the use of in situ organisms endemic to the region to measure ecological impacts [1,3].

Communities of sediment Archaea and Bacteria are known to respond to changes in metal concentrations in a number of measurable ways. Responses can include a decrease in

microbial biomass [4], changes in diversity (long-term or short-term) [5], changes in community structure including changes in the dominant species and/or emergence of new or underrepresented species [6,7], and increased abundance of genetic elements conferring metal resistance [8]. Because of their sensitivity to change, their critical roles in nutrient cycling, and their ubiquitous presence in all environmental niches, Archaea and Bacteria should be considered as potential indicators of the ecological impact of changes in sediment metal concentrations.

There are significant impediments to assessing organism responses to environmental metals, particularly for Archaea and Bacteria. As yet, only a small number of Bacterial species have been considered as bioindicators, and for the most part these have been laboratory-modified strains [9–11] that may not accurately represent the reactions of naturally occurring organisms across a wide range of environments, including marine sediments. Microcosm experiments are often used to test community responses to stressors in higher organisms; however, in sediments it is difficult to replicate appropriate bioavailable metal concentrations in the laboratory [12]. In addition, Archaea are very difficult to culture, which further confounds attempts to conduct microcosm experiments. In situ screening is advantageous as it allows location-specific monitoring of community changes [13]. This approach also circumvents the difficulty of trying to duplicate the complex multiple site- and season-specific physical and chemical characteristics of marine sediments, all of which can influence metal bioavailability and toxicity.

Mass sequencing technology provides a more comprehensive snapshot of microbial communities than culture-based or cloning-based methods [14–16]. Similarly, next-generation sequencing is a useful tool for measuring changes in the abundance and expression of functional genes [14,17]. There are, however, limitations to both approaches. Community structure is sensitive to spatial and temporal drift, seasonal fluctuation, and the effects of other

environmental factors such as nutrient availability [18,19]. The detection of functional genes is problematic because many resistance genes are not specific to metals, and may also have homologues in nonmicrobial organisms. Therefore an approach that combines both methods may prove more effective than either alone.

It is also critical that appropriate environmental data be considered when one is selecting in situ indicators. It is known that concentrations of total metals are not equivalent to bioavailable metal concentrations in marine sediments [20–23]. Increasingly, evidence indicates that marine Bacteria are predominantly interacting with metals in solid substrates such as sediment particles, and have limited interaction with dissolved metals in porewater [4,6,24]. Analytical and mechanistic based approaches to routine monitoring of bioavailability are scarce because assessment methods are operationally rather than functionally defined [25]. For example, metal levels are most correctly referred to in terms of the extractant used (e.g., 1 M HCl-extractable element [fraction]) and not as the bioavailable fraction. Previously, we showed that changes in bacterial populations in coastal sediment were best correlated with the 1 M HCl-extractable fraction of metals [6], and thus 1 M HCl-extractable concentrations of metals appear to be a suitable surrogate for microbial-bioavailable concentrations of metals in sediments and will be referred to as the bioavailable form in the present study. It will, however, be necessary to make the connection between operational and functional metal levels if mechanistic based approaches to routine monitoring of bioavailability are to be achieved.

In the present study, we hypothesize that community structure and/or membership of benthic microbes change in response to stress-inducing concentrations of bioavailable metals in coastal marine sediments, and that changes in microbial communities identified by sequencing surveys can be used to identify potential candidate indicator organisms for further study. We

have generated species response curves across a range of bioavailable concentrations of target metals, and have identified groups of microbial organisms that have the potential to inform the selection of molecular indicators of metal impact in coastal sediments. The present study describes a developmental approach for a potential molecular screening method [26] that may be useful as the initial steps in the formation of a comprehensive algorithm for sediment monitoring that may ultimately include identity- and function-based tests of in situ communities.

MATERIALS AND METHODS

Study sites

Four coastal study sites were selected in northern tropical and subtropical Australia (Figure 1). Two transects, with 6 sampling stations along each, were located at Gove Harbour and Drimmie Arm (latitudes 12°S). All stations were nearby or adjacent to mangrove sediments, and water depth increased with distance from shore. Stations closest to shore were located adjacent to a wastewater outlet (IH8, IH6) or a site of potential run-off from waste storage facilities (F7.0) at an alumina refinery. Nine sampling stations were located at Bing Bong, in the southwest Gulf of Carpentaria (latitude 15°S), and included 2 types of sediment: anoxic sediment at stations 5, 6, and 7 inside a loading swing basin and shipping channel adjacent to a storage and loading facility for Pb and Zn concentrate; and very shallow sandy sediment associated with sparse mangroves at sampling stations 1 to 4, 8, and 9. Six stations were located at Western Basin, Gladstone Harbour (latitude 23°S). Two stations (RT40 and RT41) were located near an industrial wharf that was colocated with a diffuser pipe for wastewater from an alumina refinery, and the other sampling stations were located to the north (NT21 and NT22) and south (ST10 and ST28) of the wharf and contained sparse seagrass beds. Sediment was sampled twice in each of the wet (October–April) and dry (May–September) seasons between 2008 and 2010. Because of

serious adverse weather, and changes in operating conditions at the adjacent industrial facility in 2009, only 1 wet season sample was collected from Bing Bong (2010).

Sampling stations at each study site were selected to provide a gradient of increasing sediment metal concentrations for the metals of interest (Al, V, Zn, Cu, Ga, Pb, Cd, and As). Relatively pristine reference stations were selected based on the following factors: relative location to the source of impact; reduced likelihood of metal contamination based on prevailing wind direction and available hydrodynamic modeling data for current direction [27]; sediment metal concentration data collected from the same stations in routine monitoring studies (D. Parry and K. Gibb, Charles Darwin University and Australian Institute of Marine Science, Darwin, Northern Territory, Australia, unpublished data); and sediment data from other northern Australian coastal sediments [28]. Reference stations were also selected based on location and physicochemical factors, which were matched as closely as feasible to the test stations.

Physical and chemical analysis of sediments

Marine sediment samples were obtained using a Van Veen stainless steel sediment grab deployed from a boat, or using an acid-washed Teflon-coated spoon from the shore. Duplicate sediment samples were collected at each station. Samples up to 10 cm deep were collected, and the temperature, pH, and redox were measured in situ using a Hydrolab model MS5, which was calibrated immediately before use. The range of error for redox readings in the field is ± 50 mV. Samples were stored in zip-lock plastic bags and acid-washed polypropylene centrifuge tubes inside double, sealed bags containing anaerobic Anaerogen sachets (Oxoid) to minimize oxidation. Samples were placed on ice for transport and subsamples for deoxyribonucleic acid (DNA) extraction or chemical analysis were stored at -20°C prior to processing and analysis. Particle size, total organic carbon (TOC) content, and 1 M HCl-extractable concentrations of

metals were determined using standard methodologies as previously described [6]. Acid volatile sulfide (AVS) concentrations were determined as previously described [29]. Data from duplicate samples were averaged for analysis.

Pyrosequencing of 16S rRNA gene V6 amplicon libraries

Total DNA was extracted from approximately 10 g of sediment per sample using the Powermax Soil DNA Extraction kit (MoBio). The DNA was further purified by sodium chloride–ethanol precipitation and resuspended in sterile distilled water. The V6 hypervariable region of the bacterial *16S rRNA* gene was amplified by polymerase chain reaction (PCR) using a forward primer containing a 454 adaptor sequence (Roche), a unique barcode for each sample [30], and the A967F sequence [31], and a single reverse primer for all samples containing the 454 adaptor sequence and the 1046R sequence [31]. The V6 hypervariable region of the archaeal *16S rRNA* gene was amplified by PCR using a forward primer containing a 454 adaptor sequence (Roche), a unique barcode for each sample and the archaeal forward primer sequence (958arcF), and an equal quantity of the major (1048arcRmajor) and minor (1048arcRminor) reverse primers containing a 454 adaptor and the archaea reverse primer sequences [32]. Template DNA was added to primers (final concentration 0.4 μ M each) and reagents from the FastStart High Fidelity PCR system (Roche). Dimethyl sulfoxide (DMSO) was added to optimize product amplification. Reactions were carried out in a thermocycler under the following conditions: 94 °C for 3 min; followed by 32 cycles of 94 °C for 30 s, annealing as described below for 45 s and 72 °C for 1 min; followed by 72 °C for 2 min. Annealing temperatures for bacterial sequences were optimized for each sample and ranged from 66.3 °C to 69.6 °C to allow amplification of difficult samples. The annealing temperature for archaeal sequences was maintained at 57 °C for all samples. A minimum of 4 amplification reactions was conducted for each sample; these were

combined and purified using a QIAquick PCR Purification kit (QIAGEN), and eluted with sterile distilled water. The PCR products were quantified by 2% agarose gel electrophoresis and where necessary concentrated to >100 ng/ μ L by sodium acetate–ethanol precipitation. Barcoded PCR products were sequenced at the Australian Genome Research Facility, Brisbane, Australia, using the 454 GS-FLX (dry season 2008 samples) and 454 GS-FLX Titanium (wet and dry seasons 2009, wet season 2010 samples) Sequencing System (Roche). Sequence data were sorted by barcode and barcode sequences removed at the Australian Genome Research Facility. Sequences were pretreated to remove poor-quality sequence with the RDP pyrosequencing pipeline [33,34]. Sequences containing more than 1 ambiguous base and/or comprising less than 70 nucleotides were removed at this stage. Sequences were further processed using Mothur Ver 1.13.0 [35] to remove poor-quality, poorly aligned, and suspected chimeric sequences, to assign operational taxonomic units (OTUs) to sequences within 97% similarity, and to classify OTUs using the SILVA database [36]. The alpha diversity in each combined sample was calculated using the Shannon Index. The richness of species representation was calculated by dividing the observed number of OTUs by the expected number of OTUs (Chao value). The Shannon Index and the Chao value were calculated in Mothur. The 10 most abundant Archaeal and Bacterial OTUs were also subjected to a BLASTN search of the nucleotide collection (nr/nt) database and matched to the closest listed isolate. Sequences with matches to the 16S ribosomal RNA sequences (Bacteria and Archaea) database of over 90% identity and 100% query coverage (Bacteria), or over 87% identity and 97% query cover (Archaea), and E values below $1e-20$ were classified to genus level where possible.

Statistical analysis of community structure

For all of the following statistical analyses, data generated from all samples were combined into a single dataset each for Archaea and Bacteria. Rare OTUs (those that occurred less than 10 times) were omitted. Counts for the remaining OTUs in each sample were standardized by total and square root transformed in Primer 6 Ver 6.1.12 software [37]. Resemblance matrices were generated using Bray–Curtis similarity. Two-factor permutational analyses of variance/multivariate analyses of variance (PERMANOVA) were performed comparing study site and season (sums of squares type III [partial], fixed effects sum to zero for mixed terms, unrestricted permutation of raw data; number of permutations: 999).

For all the subsequent analyses, the remaining OTUs were grouped into larger taxonomic groups. Archaea were split into 2 phyla, Crenarchaeota and Euryarchaeota. To improve coverage and generate more reliable species response curves (described below in *Generation of species response curves*), OTU counts were clustered into larger taxonomic groups. The OTUs in phyla Crenarchaeota were clustered into Class (class 3), and the OTUs in phyla Euryarchaeota were clustered by Genus (class 6), as these OTUs were assigned to taxonomic groupings by Mothur with more depth than Crenarchaeota. Bacteria were clustered by Family (class 5). Samples were standardized by total in Primer 6 and analyzed in Canoco Ver 4.56 [38]. Using species, environmental (1 M HCl-extractable metal concentrations) and covariable (physicochemical) data, direct gradient analysis was performed with the following options selected: unimodel (canonical correspondence analysis) with interspecies scaling (Hill's scaling), square-root transformation, and no forward selection. Unrestricted Monte Carlo permutation of the first ordinate axis and canonical axes together with a reduced model of 499 permutations was also performed. The canonical correspondence analysis plots were prepared using CanoDraw, to

visualize the relationships between community structure and bioavailable metal concentration, sediment physicochemical characteristics, season, and study site location.

Generation of species response curves

Species response curves to each metal, in the form of generalized additive models with Poisson distribution, were generated in CanoDraw using normalized but untransformed abundance data for each taxonomic group (described above in *Statistical analysis of community structure*). To avoid overfitting the data, 2 degrees of freedom were selected. The shapes of the curves and the predicted optimal concentration of each metal were recorded for each taxonomic group. Because a total of 1880 curves were generated, the number of significant ($p < 0.05$) results that might be generated by random chance was 94. The p values from each curve were therefore adjusted using an estimated false discovery rate to generate q values using the R software package Q-VALUE. A lambda of 0.2 and pi_0 of 0.45 were selected. Results where the relationship between metal concentration and species abundance was not significant, as evidenced by q values greater than 0.05, were excluded from further analysis.

Cluster analysis of environmental variables

A hierarchical cluster dendrogram of the 1 M HCl-extractable concentrations of target metals, and the physicochemical characteristics of present study sediments, was prepared using Primer 6. A resemblance matrix (Pearson correlation) of variables was subjected to hierarchical cluster analysis (group average).

Media preparation for metal challenge experiments

Peptone and yeast extract for bacterial culture media were sourced from Oxoid. Metal compounds for culture media were sourced from Sigma Aldrich, Amresco, and Ajax Chemical. Chemicals were of the highest purity available and were all >99% pure. Anderson's Marine Agar

(2.5 g/L peptone; 2.5 g/L yeast extract; 0.1 g/L FePO_4 ; 15 g/L agar; pH 7.5; 750 mL/L aged seawater) was prepared as previously described [39]. Stocks of metal salts were dissolved in double-distilled water, filter-sterilized to avoid precipitation, and stored in sterile, opaque polypropylene bottles. Metal concentrations of stock solutions were confirmed by inductively coupled plasma–mass spectrometry (ICP-MS), and stock solutions were added to molten media prior to pouring into sterile Petri dishes. The following combinations were mixed to generate solid culture media containing high concentrations of metals: 4 metals (478.7 mg/L NaVO_3 ; 252.5 mg/L GaCl_3 ; 208.2 mg/L $\text{NaH}_3\text{AsO}_4 \cdot 7\text{H}_2\text{O}$ or 86.7 mg/L AsNaO_2 ; 12.6 mg/L $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$); 6 metals (478.7 mg/L NaVO_3 ; 252.5 mg/L GaCl_3 ; 208.2 mg/L $\text{NaH}_3\text{AsO}_4 \cdot 7\text{H}_2\text{O}$ or 87.6 mg/L AsNaO_2 ; 12.6 mg/L $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$; 833.9 mg/L ZnCl_2 ; 2.03 mg/L $\text{CdCl}_2 \cdot 2\frac{1}{2}\text{H}_2\text{O}$). To obtain medium concentrations of metals, these quantities were halved. The pH of the molten agar was adjusted to 7.5 with HCl or NaOH prior to pouring.

Bacterial culture

Whole sediment samples were diluted 1/10 000 in sterile 0.9% NaCl solution. A 100- μL aliquot of each sample was inoculated onto Anderson's Marine Agar, with and without metals as described above in *Media preparation for metal challenge experiments*, and incubated at 28 °C for up to 7 wk to allow recovery of slow-growing and small colonies. The morphology and pigmentation of visible colonies on each plate were recorded as previously described [40,41], and representatives of each morphotype were amplified in broth culture.

Sanger sequence identification of cultured bacteria

The DNA was extracted from pelleted pure cultures using the MoBio Microbial DNA extraction kit. The *16S rRNA* gene was amplified from whole sediment isolates by MacroGen using 27F [5'-AGAGTTTGTATCCTGGCTCAG-3'] and 1492R [5' TACCTTGTTACGACTT 3']

primers and the following thermocycler program: 95 °C for 5 min; followed by 32 cycles of 92 °C for 1 min, 57 °C for 1 min, and 72 °C for 1 min; 72 °C for 2 min. The resulting gene products were sequenced using the 518F primer [5'-CCAGCAGCCGCGGTAATACG-3'] in the forward direction and the 800R primer 5'-TACCAGGGTATCTAATCC-3'] in the reverse direction. Sequence reads were checked for quality, trimmed, and assembled into contigs using Macvector with Assembler 12.0.3 (MacVector). Isolates with matches to the 16S ribosomal RNA sequences (Bacteria and Archaea) database of over 97% identity and 100% query cover, and E values below 1e-20, were assigned to the closest genus.

Deposition of DNA sequence data

Raw pyrosequence data was deposited in the US National Center for Biotechnology Information (NCBI) Sequence Read Archive under BioProject accession number SRP010979. All 16S rRNA gene Sanger sequences were deposited in the Genetic sequence database at the NCBI (GenBank IDs: JQ032266–JQ032305 and JQ032342–JQ032618).

RESULTS

Physical and chemical analysis of study sites

Physicochemical data and 1 M HCl-extractable concentrations of target metals and phosphorous at each sampling station are listed in Table 1 and Table 2, respectively. At all stations, metals were below ANZECC and Agriculture and Resource Management Council of Australia and New Zealand sediment quality guideline-high values (formerly interim sediment quality guideline-high values), where guidelines exist (for Cd, Cu, Pb, Zn, and As; 10 mg/kg, 270 mg/kg, 220 mg/kg, 410 mg/kg, and 70 mg/kg dry wt, respectively) [1,3]. The sediment quality guideline-high value represents a 50% probability of effect on test organisms [42]. At most stations metal concentrations were also below sediment quality guideline values (formerly

interim sediment quality guideline-low values, representing a 10% probability of effect on test organisms; for Cd, Cu, Pb, Zn, and As; 1.5 mg/kg, 65 mg/kg, 50 mg/kg, 200 mg/kg, and 20 mg/kg dry wt, respectively), with the following exceptions: Zn, As, and/or Pb were elevated above sediment quality guideline values at 1 or 2 stations inside the swing basin (barge loading wharf) at Bing Bong on all 3 sampling visits; and As was elevated at 1 station during 1 sampling visit each in Drimmie Arm and Gladstone Harbour.

In general, the stations closest to shore contained finer particles and lower redox than those further out, and ranged from -365 meV to 222 meV, with no apparent changes between wet and dry seasons. Sediment temperatures ranged from 20.9 °C to 28.8 °C during the dry season and from 23.3 °C and 32.7 °C during the wet season. The pH of the sediments ranged from 6.3 to 8.9 and did not appear to vary between wet and dry seasons. The TOC content ranged between 0.054% and 2.68% .

Community structure influenced by location and season

Across all samples, the combined total number of Archaeal and Bacterial sequence reads of sufficient quality for analysis were $625\,857$ and $614\,435$, and the total numbers of OTUs were $18\,545$ OTUs and $53\,547$ OTUs, respectively. The number of OTUs per sample ranged from 247 archaeal OTUs to 1849 archaeal OTUs, and from 316 bacterial OTUs to 4978 bacterial OTUs (Supplemental Data, Table S1). The expected richness of each sample was between 2-fold and 5-fold higher than the actual observed number of OTUs. As expected, coverage (an estimate of what proportion of the total number of species present was actually detected) and number of OTUs detected were lower for samples sequenced using the GS-FLX platform than for those sequenced using GS-FLX Titanium. Even so, alpha-diversity, as measured by Shannon indices, indicated that all samples contained highly diverse communities of Archaea and Bacteria, with

no apparent differences observed between sequencing platforms (Supplemental Data, Table S2). Evenness values for Archaeal and Bacterial communities prior to removal of rare OTUs averaged between 0.80 and 0.84, respectively, indicating that the contribution of rare species was small. Beta-diversity, as indicated by multidimensional scaling, showed well-defined clustering of reference and impacted stations (Supplemental Data, Figures S1A and S1B). After all rare OTUs were removed, the total number of OTUs across all samples was reduced to 2179 (Archaea) and 3514 (Bacteria). Two-factor PERMANOVA analysis of OTU data indicated that all study sites were significantly different from each other, both within and between seasons ($p < 0.05$). Both Archaeal and Bacterial communities clustered by study site (Figure 2A and B). Samples from Gove Harbour and Drimmie Arm, being located very close together, were intermingled in the canonical correspondence analysis, and contained Bacterial communities similar to those at Bing Bong. Sampling stations at Gladstone Harbour formed a very distinct cluster. Gladstone Harbour Archaeal communities were most closely related to those at Bing Bong. Microbial community shifts at each station sampled during the wet season (open circles) and dry season (filled black circles) are shown in Figures 2C and D (top panels). The canonical correspondence analysis plots suggested some subtle shifts between wet and dry seasons, but there was substantial overlap between wet and dry season community structure. No individual taxonomic groups appeared to be strongly associated with either wet season or dry season. By grouped taxa, stations that were near each other were also most closely related in community structure on canonical correspondence analysis plots, with 55% (Archaeal) and 43% (Bacterial) of variation explained by axes 1 and 2.

Metal concentrations affected community structure

Figure 2C and D (bottom panels) contains vectors for 1 M HCl-extractable metal concentrations and physicochemical characteristics of the sediment in relation to Archaeal and Bacterial community structure. Both Archaeal and Bacterial community structures at Gladstone Harbour were less strongly correlated with environmental conditions (1 M HCl-extractable metal concentrations and physicochemical variables) than at other study sites. Gove Harbour and Drimmie Arm had a greater abundance of (Archaea) Thermoplasmata and Methanobacteria than other sites, and these were associated with increased concentrations of 1 M HCl-extractable Cd, Ga, and Al, whereas Bing Bong and Gladstone Harbour had a greater abundance of Methanomicrobia and Halobacteria, and these were associated with increased concentrations of Cu and Pb. Bacteria that were moderately associated with increased concentrations of Cu included Gemmatimonadetes and Gammaproteobacteria, which were more abundant at Gove Harbour. Bacteria that were strongly associated with increased concentrations of the other 7 target metals included Lentisphaerae, Verrucomicrobia, Chlamydiae, and Firmicutes (most strongly associated with Bing Bong) and Deferribacteres and Deinococcus-Thermus. Bacteria that were strongly associated with decreasing concentrations of metals included Alphaproteobacteria, Betaproteobacteria, and Deltaproteobacteria, Spirochaetes, Planctomycetes, Acidobacteria, Nitrospirae, Bacteroidetes, Chloroflexi, Cyanobacteria, and Actinobacteria.

Responses of individual taxa to multiple metal impact

Species response curves to 1 M HCl-extractable concentrations of metals were generated for taxonomic groups as described above in *Generation of species response curves*. Response curves were generated by combining data from all sampling stations. Five different types of unimodal response to 1 M HCl-extractable metal concentrations were consistently observed, as shown in Figure 3, similar to those reported previously [43]. In addition to the previously

described response curves, 3 types of bimodal response were observed. Models A and B (decreasing monotonic and skewed unimodal with low optima) indicate a predominantly sensitive population; model C (symmetrical unimodal) indicates a more moderate response; models D, E, and F (bimodal and skewed bimodal) indicate distinct sensitive and resistant subpopulations within a population; models G and H (skewed unimodal with high optima, and increasing monotonic) indicate a predominantly resistant population. The optima of each curve are defined as the metal concentration at which the curve peaks, and indicate the metal concentration at which the species (taxonomic group) are most abundant. The predicted optimal metal concentrations, shapes of the response curves, and resistance profiles for each taxonomic group of Crenarchaeota, Eukaryarchaea, and Bacteria are shown in the Supplemental Data, Tables S3 to S11. Only statistically significant models ($q < 0.05$) are listed.

Taxa with response curves predicting resistance or tolerance to high concentrations of multiple metals are shown in Table 3. Of the Archaea, 20 of 57 genera of Euryarchaeota were predicted to be resistant to multiple metals. As a group, Thermoplasmata contained the highest proportion of predicted multiple-resistant genera, including genera resistant to as many as 6 of the 8 target metals. The genus *Methanothermus* (Methanobacteria) was modeled as resistant to all target metals except Pb; and of the Halobacteria, *Haloquadratum* and *Halogeometricum* were modeled as resistant to 4 metals (Cu, Pb, V, Zn). Table 3 also shows Bacterial classes that contained multiple-metal-resistant families including Chlamydiae, Deferribacteres, Lentisphaerae, Planctomycetes, and Verrucomicrobia (except Verrucomicrobiales DEV007). Deferribacteres in particular were predicted to be resistant to all 8 target metals. Of the Proteobacteria, Deltaproteobacteria, Candidatus Thiobis, and Gammaproteobacteria (Oceanospirillales) were predicted to be resistant to multiple metals (Table 3). Several Classes of

Crenarchaeota were predicted to be resistant to single metals, including Group C3 (Ga), Soil Crenarchaeotic Group, and Miscellaneous Crenarchaeotic Group (Cu; Supplemental Data, Tables S3–S5). Seven of 15 genera in Methanomicrobia were resistant to Cu (Supplemental Data, Tables S6–S8).

Taxa with response curves indicating a preference for low concentrations of multiple metals are shown in Table 4. Most Crenarchaeota were sensitive to multiple metals, with Cd being the least well tolerated. Of the Eukaryarchaeota, members of Methanomicrobia were sensitive to multiple metals. Of the Bacteria, Chloroflexi and Cyanobacteria were mostly sensitive to high concentrations of Al, Cu, Pb, V, and Zn. Almost all genera in Halobacteria were modeled as being sensitive to both Al and Ga. Pseudoalteromonadaceae were sensitive to Al, Pb, V, and Zn.

Several taxa had bimodal responses to 1 or more metals, suggesting 2 distinct populations with metal sensitivity (Table 5). Of the Crenarchaeota, the response curves of marine benthic group B were bimodal to multiple metals (Pb, V, and Zn), with strong responses to both high and low concentrations of these metals. Two genera from the Eukaryarchaeota gave bimodal responses: marine benthic group D (Thermoplasmata) to As and Zn; and *Methanolobus* (Methanomicrobia) to Zn. Of the bacteria, Actinobacteria were modeled with bimodal responses to up to 4 (V, Cu, Pb, Ga, and/or Zn) of the target metals; and Bacteroidetes Sphingobacteriales to Cd, Cu, and Pb.

Responses of abundant OTUs to multiple metal impact

The 10 most abundant Archaeal OTUs were *Methanothermobacter* sp., *Methanopyrus* sp. (3 OTUs), uncultured Thermoplasmatales, *Ignicoccus* sp. (3 OTUs), *Thermofilum* sp., and miscellaneous uncultured Crenarchaeota. The 10 most abundant Bacterial OTUs were

Pseudoalteromonas sp., *Marichromatium* sp., *Thioalkalivibrio* sp. (2 OTUs), *Thiohalospira* sp., *Microbulbifer* sp. (2 OTU), Enterobacteriaceae, *Cobetia* sp., and Syntrophaceae. Species response curves to 1 M HCl-extractable metal concentrations were generated for the 10 most abundant Archaeal and Bacterial OTUs; only models where $p < 0.05$ are shown. Of the Archaeal OTUs (Figure 4A), *Methanothermobacter* sp. (OTU1) was resistant to As, Cu, and V. One *Methanopyrus* sp. (OTU3) was sensitive to Al, Pb, and Zn, whereas another *Methanopyrus* sp. (OTU4) was sensitive to Cd but resistant to As and Zn. The 3 *Ignicoccus* sp. (OTU 2, 6, and 8) had varying resistance profiles, with OTU2 demonstrating resistance to Al, As, Cu, and V; OTU6 was sensitive to Al, As, and V, and also resistant to Cu; whereas OTU8 was sensitive to both Cu and V. The uncultured Thermoplasmatales sp. (OTU5) was resistant to Al, As, Cd, and V; showed a bimodal resistant response to Zn; and was sensitive to Pb. *Thermofilum* sp. (OTU9) was sensitive to Al, As, V, and Zn, and demonstrated a bimodal response to Cd. The miscellaneous uncultured Crenarchaeota (OTU10) was sensitive to Cd and Pb but moderately resistant to Al and As.

Of the Bacterial OTUs (Figure 4B), *Pseudoalteromonas* sp. (OTU1) was very sensitive to Al, Cu, Pb, V, and Zn. *Thiohalospira* sp. (OTU4) and 1 *Microbulbifer* sp. (OTU5) were resistant to 5 metals each, 1 *Thioalkalivibrio* sp. (OTU9) displayed weak bimodal responses to Al and Cd, and another *Thioalkalivibrio* sp. (OTU3) was moderately resistant to Cu. Enterobacteriaceae (OTU10) and *Cobetia* sp. (OTU11) were both sensitive to multiple metals.

Correlations between environmental variables

Sediment chemistry variables were subjected to cluster analysis to identify closely related metals and physicochemical characteristics (Supplemental Data, Figure 2). Metals clustered into 2 main groups, comprising 1 M HCl-extractable Al and Ga; and Cu, Zn, V, and As. Lead and Cd

remained separate from the other metals. Concentrations of 1 M HCl-extractable Ga and Al were closely related to concentrations of phosphorous. Concentrations of V and As were also closely related to each other. Because of the close associations of the concentrations of these metals across sampling stations, 1 of each pair of metals Al and Ga, and V and As, may be considered redundant variables. Aluminum and Ga are also chemically similar, and both V and As are commonly found in marine sediments as oxides (vanadate and arsenate). Of the nonmetal physicochemical variables, AVS, particle size, and TOC were most strongly linked to metal concentrations; however, none of these variables were closely linked to particular metals. Dissolved oxygen was the most distantly linked variable from all of the others.

Decreased diversity of culturable bacteria with increased metals

Culture media containing high concentrations of metals were generated by adding approximately the same concentrations of each target metal (to the nearest 50 mg/kg) as the highest concentrations observed in the test sediments. Culture media containing medium concentrations of metals were generated by adding half of these concentrations. Sediment bacteria from impacted and reference sediments were cultured by inoculating spiked and control media with diluted sediment samples. From 392 colonies isolated on Anderson's Marine Media, good-quality sequence data were obtained for 324 isolates. Losses were observed because of inability to grow pure cultures in liquid culture, inability to amplify good-quality PCR products of the *16S rRNA* gene, and poor or short sequence for some isolates. From the sequences that were obtained, 19 different genera were identified by *16S rRNA* gene sequence comparison (Table 6). The 3 most frequently observed genera were *Halomonas*, *Idiomarina*, and *Marinobacter*. *Halomonas*, *Kordiimonas*, and *Marinobacter* grew on media spiked with up to 6 metals; *Idiomarina*, *Pseudidiomarina*, *Pseudomonas*, and *Sulfitobacter* grew on 4 metals; and

Bacillus, *Hyphomonas*, *Limnobacter*, *Planococcus*, *Pseudoalteromonas*, *Rheinheimera*, *Rhodobacter*, *Thalassospira*, and *Vibrio* only grew on media containing no additional metals. Three genera *Maricaulis*, *Muricauda*, and *Roseivirga* only grew on media containing 6 metals. *Roseivirga* sp., *Muricauda* sp., *Maricaulis* sp., and *Limnobacter* sp. were isolated predominantly or exclusively from impacted sediments; *Sulfitobacter* sp., *Pseudoalteromonas* sp., and *Planococcus* sp. were isolated predominantly or exclusively from reference sediments.

DISCUSSION

Monitoring ecotoxicological impacts of sediment metals

Currently available ANZECC guidelines for metals in sediments are based largely on sensitivity testing for a limited number of species [1,3,42]. The ANZECC guidelines provide national recommendations for sediment metal concentrations but also call for location-specific values to be determined where possible. Most of the sediments in the present study were below national sediment guideline values for most metals—with a few exceptions for Zn, As, and/or Pb—and are considered to be moderately impacted by elevated metal concentrations. The ANZECC guidelines have recently been reviewed and they now include discussion of new methods for analyzing ecosystems, including the use of ecogenomics [3,44]. The present study presents an ecogenomic approach incorporating metagenetics to select potential location-specific indicator species for north Australian coastal sediments impacted by metals.

Increased metal concentrations affect community structure

The community structures of both Bacterial and Archaeal communities changed in association with increases in bioavailable (1 M HCl-extractable) metal concentrations. Community structures at Bing Bong, Gove Harbour, and Drimmie Arm were strongly correlated with increasing 1 M HCl-extractable metal concentrations of multiple metals. Location-specific

relationships were also observed, which appeared to be influenced in part by physicochemical factors as well as by several specific metals. Archaea and Bacteria at Gladstone Harbour were distinct from those at Gove and Drimmie Arm, being more strongly correlated with lower pH and not correlated with increasing metal concentrations, except for Cu. Archaea at Bing Bong responded differently from Bacteria, with Archaea clustering with those at Gladstone Harbour and Bacteria clustering with those at Gove Harbour and Drimmie Arm. Changes in Archaeal and Bacterial community structure may be indicative of metal impact and suggest the presence of individual taxa that may act as indicators.

Microbial responses to sediment metals

Based on their correlation with changes in sediment bioavailable metal concentrations, there were 2 types of Archaeal and Bacterial candidate indicator species. Candidate indicators included those that demonstrated sensitivity, or resistance, to multiple metals. These 2 types of indicators have been identified by the Marine Strategy Framework Directive (European Commission 2010) as important indicators of seafloor integrity and benthic community condition [16]. A reduction in the prevalence of sensitive organisms, or an increase in the prevalence of resistant organisms, may indicate increased metal stress in the environment. Taxa models that indicate a higher tolerance to multiple metals included the Archaea marine benthic group B, Thermoplasmata, Methanobacteria, and Methanomicrobia (gen. *Haloquadratum* and *Halogeometricum*); and Bacteria: Chlamydiae, Deferribacteres, Deinococcus-Thermus (Truepera), Lentisphaerae, Planctomycetes, Verrucomicrobia, Proteobacteria (Candidatus-Thiobis), and some Deltaproteobacteria and Gammaproteobacteria (Oceanospirillales). A number of these candidate metal-resistant indicators have previously been described as such; these include Methanobacteria, Deferribacteres, *Haloquadratum*, and *Halogeometricum* [45–48].

Chlamydiae, being obligate intracellular organisms, are most likely related to human or bird waste at sampling stations that are also cocontaminated with metals, and may not be consistent indicators of sediment metals [49]. Thermoplasmata were very abundant across all stations and would therefore not make good indicators of moderate metal impact. They may be more useful as indicators of more extreme impact because their absence as a result of more extreme ecological stress would be easily detected.

Taxa that were identified as sensitive to multiple metals included most Crenarchaeota and Methanomicrobia (Archaea); and Cyanobacteria, Chloroflexi, Pseudoalteromonadaceae, and Verrucomicrobiales DEV007 (Bacteria). However, some of these sensitive taxa (particularly Cyanobacteria) have been previously well described as metal tolerant [50–54], and the metal susceptibility profiles described in the present study may have been influenced by confounding factors, as discussed below in the section *Confounding factors*. Because of the complexity of these sediments, and the potential for confounding factors that may bias analysis, culture and resistance testing of organisms identified in this screening process are required to confirm metal resistance profiles. In addition, linear models were not tested in the present study, as responses to metals tend to follow bimodal patterns; however, given the relatively low concentrations of metals in the present study sediments, there may be some organisms that would be better modeled with a linear response.

Despite the differences in taxonomic depth, and biases such as the absence of anaerobic organisms isolated by culture, there was some overlap between the cultured organisms and those identified by pyrosequencing. In metal challenge trials in culture, *Halomonas* sp. were confirmed as metal resistant; being of the order Oceanospirillales, this matches the sequencing results.

Pseudoalteromonas isolates were confirmed as sensitive to metals in the culture media. These

were cultured from the same stations from which *Pseudoalteromonas* sequences were detected by pyrosequencing, and were also modeled as metal sensitive in the resistance models. This result is interesting as *Pseudoalteromonas* sp. are frequently associated with metal resistance [55], and it suggests that the local isolates are sensitive to the target metals at these stations. Other metal-sensitive aerobic heterotrophs isolated by culture included *Limnobacter*, *Planococcus*, *Rheinheimera*, and *Rhodobacter* species. By contrast, *Maricaulis*, *Muricauda*, and *Roseivirga* species were only isolated from metal-spiked media and demonstrate resistance to multiple metals; however, they may be outcompeted at low metal levels as a consequence of physical or metabolic adaptations that confer resistance to metals [56]. They may become more abundant in the sediment as the growth of metal-sensitive, competing organisms is inhibited, and are therefore candidate indicator organisms for metal impact. Other tolerant genera included *Idiomarina*, *Pseudidiomarina*, *Pseudomonas*, *Sulfitobacter*, *Halomonas*, *Kordiimonas*, and *Marinobacter*, which may also be useful as indicators of metal impact. These metal challenge trials complemented the in situ approach by providing further clarity about the metal-response behavior of particular taxa. Metal interactions in marine sediment are highly complex, so it is unlikely that culture studies will ever be able to replicate this complexity. We isolated bacteria directly from marine sediment straight into the challenge plates without multiple passages to obtain pure cultures in an attempt to accommodate this complexity. We did not calculate growth curves from pure isolates in microcosm studies because this approach is even further removed from the reality of complex metal–marine sediment interactions present in situ.

Acquisition of resistance in previously sensitive populations

Although some taxa appeared to be inherently metal resistant, other microbial taxa demonstrated bimodal responses, suggesting that these populations contain 2 divergent

subpopulations, 1 of which has acquired resistance because of exposure. It may also indicate different responses from different genera within the larger phylogenetic groups; however, examination of response curves of individual genera within a subset of these phyla suggests that this is not the case. These organisms are likely to have acquired mobile genetic elements that confer resistance to multiple metals [8,57,58]. Taxa with bimodal responses to multiple metals included Archaea (marine benthic group B and Thermoplasmatales) and Bacteria (Acidimicrobiales, unclassified Actinobacteria, and Sphingobacteriales; Table 5). These taxa appear to contain subpopulations resistant to bioavailable Cd, Pb, Cu, Zn, and/or V. Mobile genetic resistance elements for multiple metals (particularly Pb, Cd, and Zn) are common, and it is likely that members of these subpopulations have acquired resistance to all 3 of these metals [59]. Further investigation into taxa such as those identified may reveal functional genetic indicators of metal stress in marine sediments that are transferred between taxa, and that may form the basis of genetic testing for marine sediment health.

Functional resistance genes as candidate indicators

Resistance to metals such as Zn(II), Cd(II), Pb(II), Cu(I), and As(III) rely predominantly on controlling influx or enhancing efflux [60]. There are 4 families of constitutive metal efflux systems found in bacteria, and 3 of these are also found in Archaea [60]. The most common of these are the P-type adenosine triphosphatase (ATP)ases [59], and P-type ATPases specific for 1 or more cations have been characterized and published for at least Thermoplasmata and Methanobacteria (Archaea), and Chlamydiae (Bacteria), Planctomycetes, and Lentisphaerae phyla [61,62] (example accession numbers [NCBI] ADY60643.1 and [Uniprot] A6DTT3). Genes for the P-type ATPase proteins are can be found on transposable elements such as plasmids and transposons, and horizontal transfer of these genes between bacterial species has

been observed in heavy-metal-impacted soils [58]; they therefore have potential as functional indicators of metal impact. Taxa with resistance profiles for Al and Ga and/or V have also been characterized, and these included *Thermoplasmata* and *Methanohalophilus* (Archaea); and *Candidatus-Thiobios*, *Oceanospirillales*, and *Verrucomicrobia* (Bacteria). The bioavailability of Ga and Al is very low in sediments at near-neutral pH [63], and there are no known resistance genes to Ga. Bacterial genes for Al resistance have been described, including members of the aminotransferase superfamily [64], and some species may also demonstrate noninducible resistance or tolerance to Al [65].

Based on the candidate indicator species identified in the present study, known functional genes from these taxa could be targets for metagenomic or transcriptomic surveys of sediments to identify functional indicators of metal impact. Because many resistance genes are not specific to metals, and may also have homologues in nonmicrobial organisms, an informed and more targeted approach to characterizing the profiles of taxa-specific genes or transcripts in sediments is likely to be more successful than a nontargeted approach.

Confounding factors

Changes in sediment metals chemistry are often associated with other alterations to the sediment such as redox and organic carbon content, sulfide production, pH, and grain size [66]. All these factors can also impact on microbial community structure, but their effects can be difficult to differentiate from the toxic effects of metals. Detailed resistance profiling of candidate indicator species in the laboratory, or characterization of specific metal-resistance genes or transcripts, would be required to determine whether the effects measured in situ were reproduced by physicochemical (i.e., nontarget) stressors. Across the study sites, the most closely related sediment characteristics were phosphorous concentrations with Al and Ga; and

particle size with organic carbon content, which was linked to Cu, Zn, V, and As concentrations. Sulfide concentrations (AVS) were not strongly correlated to any metals and are not strongly correlated with microbial populations [6].

In addition, naturally occurring, metal-independent changes such as water depth also impacted on particular benthic microbes. The most obvious example of this was the response of Cyanobacteria. Cyanobacteria, in particular *Synechococcus* sp., which were the most prevalent group of Cyanobacteria identified by culture in the present study sediments (data not shown), are recognized as highly metal-resistant [54]. In the present study, however, Cyanobacteria were predicted to be sensitive to multiple metals. In fact, Cyanobacterial prevalence was strongly inversely correlated with water depth, suggesting that the limiting factor in this case is most likely to have been reduced exposure to sunlight at deeper [67], more heavily impacted sampling stations, particularly at the Bing Bong study site. The impact of reduced sunlight may have had a stronger influence on Cyanobacterial prevalence than the moderate impact of sediment-associated metals, thus confounding the analysis. Because normal environmental variation appears to have a stronger impact on the prevalence of Cyanobacteria than the target source of impact (metals), these organisms would likely make poor indicator species for low to moderate metal impact. Overall, the relatively small number of taxa that demonstrated responses to multiple metals, and the strong influence of normal physicochemical changes on community structure and individual taxa, would suggest that microbes at these study sites have been subject to only a moderate level of metal impact. These sediments are complex and will require multiple lines of evidence to support selection of indicator species. Complex interspecies interactions within sediment communities also affect the responses of individual taxa. For these reasons,

ultimately a gene-specific indicator will likely be most appropriate for assessing metal impact across multiple locations.

Next-generation sequence technology is advancing rapidly, but, despite these advances, the technology is still confounded by the potential bias of rare species or taxa that are spatially constrained to specific locations or specific types of sediments. Rare species will, in general, not make good indicators, unless they experience a significant selective advantage in the presence of a particular pollutant(s) [68], and location-specific indicators may only be useful within limited geographical ranges. Diversity assessment based on DNA should not stand alone as a test of ecological impact and should be used in conjunction with culture-dependent resistance testing and/or further development of functional genetic testing for specific resistance markers.

CONCLUSIONS

An analysis of community changes derived using molecular tools is a useful screening tool that, in combination with careful analysis of the physicochemical characteristics of the sediment, can be used to identify bioindicators from Archaeal and Bacterial communities. These data have provided candidate Bacteria and Archaea for the next phase, which would include high-throughput quantitative culture-independent tests such as quantitative PCR. These candidates could lead to the development of in situ bioindicators for metal and general pollution in marine sediments. This ecosystem-scale assessment provides a solid framework for selecting indicators and is an excellent evidence-based approach for selecting indicators of metal impact.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3205.

Acknowledgment—The present study was funded by the Australian Research Council Project LP0776402, Rio Tinto Alcan Yarwun, Rio Tinto Alcan Gove, Xstrata Zinc McArthur River

Mine (now Glencore MRM), Northern Land Council, Northern Territory Research and Innovation Board, and the Northern Territory Government. The authors thank S. Beyer, K. Strobel, M. Neave, M.J. Buscot, and K. Chambers for helping with field sampling and sample preparation, and we thank J. Manning, D. Campbell, and F. Foti for metal analysis. We also acknowledge with thanks the field support provided by staff from the Dhimurru Aboriginal Corporation.

Disclaimer—The authors declare that they have no conflicts of interest.

Data availability—Raw pyrosequence data were deposited in the US National Center for Biotechnology Information (NCBI) Sequence Read Archive under BioProject accession number SRP010979. All 16S rRNA gene Sanger sequences were deposited in the Genetic sequence database at the NCBI (GenBank IDs: JQ032266–JQ032305 and JQ032342–JQ032618).

References

1. Australian and New Zealand Environment and Conservation Council, Agriculture and Resource Management Council of Australia and New Zealand. 2000. An introduction to the Australian and New Zealand guidelines for fresh and marine water quality. In *National Water Quality Management Strategy*, 4th ed, Canberra, ACT, Australia, pp 3.5–4.
2. van Dam RA, Harford AJ, Houston MA, Hogan AC, Negri AP. 2008. Tropical marine toxicity testing in Australia: A review and recommendations. *Australas J Ecotoxicol* 14:55–88.
3. Simpson SL, Batley GB, Chariton AA. 2013. Revision of the ANZECC/ARMCANZ sediment quality guidelines. CSIRO Land and Water Science Report 08/07. CSIRO Land and Water, Canberra, ACT, Australia.
4. Gillan DC, Pernet P. 2007. Adherent bacteria in heavy metal contaminated marine sediments. *Biofouling* 23:1–13.

5. Sun MY, Dafforn KA, Brown MV, Johnston EL. 2012. Bacterial communities are sensitive indicators of contaminant stress. *Mar Pollut Bull* 64:1029–1038.
6. Cornall A, Beyer S, Rose A, Streten-Joyce C, McGuinness K, Parry D, Gibb K. 2013. HCl-extractable metal profiles correlate with bacterial population shifts in metal-impacted anoxic coastal sediment from the wet/dry tropics. *Geomicrobiol J* 30:48–60.
7. Gough HL, Stahl DA. 2011. Microbial community structures in anoxic freshwater lake sediment along a metal contamination gradient. *ISME J* 5:543–558.
8. Ryan RP, Ryan DJ, Dowling DN. 2005. Multiple metal resistant transferable phenotypes in bacteria as indicators of soil contamination with heavy metals. *J Soil Sediments* 5:95–100.
9. Bulich A, Isenberg D. 1981. Use of the luminescent bacterial system for the rapid assessment of aquatic toxicity. *ISA Trans* 20:29–33.
10. Claxton LD, Umbuzeiro GdA, DeMarini DM. 2010. The *Salmonella* mutagenicity assay: The stethoscope of genetic toxicology for the 21st century. *Environ Health Perspect* 118:1515–1522.
11. Kurvet I, Ivask A, Bondarenko O, Sihtmäe M, Kahru A. 2011. LuxCDABE—Transformed constitutively bioluminescent *Escherichia coli* for toxicity screening: Comparison with naturally luminous *Vibrio fischeri*. *Sensors* 11:7865–7878.
12. Hughes MN, Poole RK. 1991. Metal speciation and microbial growth—The hard (and soft) facts. *J Gen Microbiol* 137:725–734.
13. Kuperman RG, Siciliano SD, Römbke J, Oortsk K. 2014. Deriving site-specific soil clean-up values for metals and metalloids: Rationale for including protection of soil microbial processes. *Integr Environ Assess Manag* 10:388–400.

14. Edwards R, Rodriguez-Brito B, Wegley L, Haynes M, Breitbart M, Peterson D, Saar M, Alexander S, Alexander EC, Rohwer F. 2006. Using pyrosequencing to shed light on deep mine microbial ecology. *BMC Genomics* 7:57.
15. Liu Z, Lozupone C, Hamady M, Bushman FD, Knight R. 2007. Short pyrosequencing reads suffice for accurate microbial community analysis. *Nucleic Acids Res* 35:e120.
16. Bourlat SJ, Borja A, Gilbert J, Taylor MI, Davies N, Weisberg SB, Griffith JF, Lettieri T, Field D, Benzie J, Glöckner FO, Rodríguez-Ezpeleta N, Faith DP, Bean TP, Obst M. 2013. Genomics in marine monitoring: New opportunities for assessing marine health status. *Mar Pollut Bull* 74:19–31.
17. Gilbert JA, Field D, Huang Y, Edwards R, Li W, Gilna P, Joint I. 2008. Detection of large numbers of novel sequences in the metatranscriptomes of complex marine microbial communities. *PLoS One* 3:e3042.
18. Pringault O, Viret H, Duran R. 2012. Interactions between Zn and bacteria in marine tropical coastal sediments. *Environ Sci Pollut Res Int* 19:879–892.
19. Volant A, Desoeuvre A, Casiot C, Lauga B, Delpoux S, Morin G, Personne JC, Hery M, Elbaz-Poulichet F, Bertin PN, Bruneel O. 2012. Archaeal diversity: Temporal variation in the arsenic-rich creek sediments of Carnoules Mine, France. *Extremophiles* 16:645–657.
20. Jong T, Parry DL. 2004. Heavy metal speciation in solid-phase materials from a bacterial sulfate reducing bioreactor using sequential extraction procedure combined with acid volatile sulfide analysis. *J Environ Monit* 6:278–285.
21. Ogendi GM, Brumbaugh WG, Hannigan RE, Farris JL. 2007. Effects of acid-volatile sulfide on metal bioavailability and toxicity to midge (*Chironomus tentans*) larvae in black shale sediments. *Environ Toxicol Chem* 26:325–334.

22. Tessier A, Campbell PGC, Bisson M. 1979. Sequential extraction procedure for the speciation of particulate trace metals. *Anal Chem* 51:844–851.
23. Turner A, Singh N, Millard L. 2008. Bioaccessibility and bioavailability of Cu and Zn in sediment contaminated by antifouling paint residues. *Environ Sci Technol* 42:8740–8746.
24. Albrechtsen H Jr. 1994. Distribution of bacteria, estimated by a viable count method, and heterotrophic activity in different size fractions of aquifer sediment. *Geomicrobiol J* 12:253–264.
25. Peijnenburga WJGM, Zablotskajaa M, Vijver MG. 2007. Monitoring metals in terrestrial environments within a bioavailability framework and a focus on soil extraction. *Ecotoxicol Environ Saf* 67:163–179.
26. Chariton AA, Ho KT, Proestou D, Bik H, Simpson SL, Portis LM, Cantwell MG, Baguley JG, Burgess RM, Pelletier MM, Perron M, Gunsch C, Matthews RA. 2014. A molecular-based approach for examining responses of eukaryotes in microcosms to contaminant-spiked estuarine sediments. *Environ Toxicol Chem* 33:359–369.
27. Herzfeld M, Parslow J, Andrewartha J, Sakov P, Webster IT. 2004. Hydrodynamic modelling of the Port Curtis region. CRC for Coastal Zone, Estuary and Waterway Management, Brisbane, Australia.
28. Munksgaard NC, Parry DL. 2002. Metals, arsenic and lead isotopes in near-pristine estuarine and marine coastal sediments from Northern Australia. *Mar Freshw Res* 53:719–729.
29. Greenberg A, Clesceri L, Eaton A. 1992. *Standard Methods for the Examination of Water and Wastewater*, 18th ed. APHA, Washington, DC.
30. Parameswaran P, Jalili R, Tao L, Shokralla S, Gharizadeh B, Ronaghi M, Fire AZ. 2007. A pyrosequencing-tailored nucleotide barcode design unveils opportunities for large-scale sample multiplexing. *Nucleic Acids Res* 35.

31. Sogin ML, Morrison HG, Huber JA, Welch DM, Huse SM, Neal PR, Arrieta JM, Herndl GJ. 2006. Microbial diversity in the deep sea and the underexplored “rare biosphere”. *Proc Natl Acad Sci U S A* 103:12115–12120.
32. Huber JA, Welch DBM, Morrison HG, Huse SM, Neal PR, Butterfield DA, Sogin ML. 2007. Microbial population structures in the deep marine biosphere. *Science* 318:97–100.
33. Cole JR, Chai B, Farris RJ, Wang Q, Kulam-Syed-Mohideen AS, McGarrell DM, Bandela AM, Cardenas E, Garrity GM, Tiedje JM. 2007. The ribosomal database project (RDP-II): Introducing myRDP space and quality controlled public data. *Nucleic Acids Res* 35:D169–D172.
34. Cole JR, Wang Q, Cardenas E, Fish J, Chai B, Farris RJ, Kulam-Syed-Mohideen AS, McGarrell DM, Marsh T, Garrity GM, Tiedje JM. 2009. The Ribosomal Database Project: Improved alignments and new tools for rRNA analysis. *Nucleic Acids Res* 37:D141–D145.
35. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Horn DJV, Weber CF. 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol* 75:7537–7541.
36. Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glockner FO. 2007. SILVA: A comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* 35:7188–7196.
37. Clarke K, Warwick R. 2001. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*, 2nd ed. PRIMER-E, Plymouth, UK.

38. ter Braak CJF, Smilauer P. 2002. *CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (Ver 4.5)*, 4.5 ed. Microcomputer Power, Ithaca, NY, USA.
39. Atlas RM. 2005. *Handbook of Media for Environmental Microbiology*, 2nd ed. CRC, Boca Raton, FL, USA.
40. Pelczar MJJ. 1957. *Manual of Microbiological Methods*. McGraw-Hill, New York, NY, USA.
41. Smibert RM, Krieg NR. 1994. Phenotypic characterization. In Gerhardt P, Murray R, Wood W, Krieg N, eds, *Methods for General and Molecular Bacteriology*. ASM, Washington, DC, p 615.
42. Long ER, Macdonald DD, Smith SL, Calder FD. 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environ Manage* 19:81–97.
43. Horsak M. 2006. Mollusc community patterns and species response curves along a mineral richness gradient: A case study in fens. *J Biogeogr* 33:98–107.
44. Commonwealth of Australia. 2013. *National Water Quality Management Strategy*, vol 2014. Canberra, ACT, Australia.
45. Abelson PH, Aldous E. 1950. Ion antagonisms in microorganisms: Interference of normal magnesium metabolism by nickel, cobalt, cadmium, zinc, and manganese. *J Bacteriol* 60:401–413.
46. Greene AC, Patel BKC, Sheehy AJ. 1997. *Deferribacter thermophilus* gen. nov., sp. nov., a novel thermophilic manganese- and iron-reducing bacterium isolated from a petroleum reservoir. *Int J System Bacteriol* 47:505–509.

47. Kim BK, Macario ECd, Nölling J, Daniels L. 1996. Isolation and characterization of a copper-resistant methanogen from a copper-mining soil sample. *Appl Environ Microbiol* 62:2629–2635.
48. Vidyasagar M, Prakash SB, Sreeramulu K. 2006. Optimization of culture conditions for the production of haloalkaliphilic thermostable protease from an extremely halophilic archaeon *Halogeometricum* sp. TSS101. *Lett Appl Microbiol* 43:385–391.
49. Wyrick PB. 2000. Intracellular survival by *Chlamydia*. *Cell Microbiol* 2:275–282.
50. Andrade L, Keim CN, Farina M, Pfeiffer WC. 2004. Zinc detoxification by a cyanobacterium from a metal contaminated bay in Brazil. *Braz Arch Biol Technol* 47:147–152.
51. Baptista MS, Vasconcelos MT. 2006. Cyanobacteria metal interactions: Requirements, toxicity, and ecological implications. *Crit Rev Microbiol* 32:127–137.
52. Lavorante AF, Gine MF, Gervasio APG, Miranda CES, Fiore MF, Bellato CM, Carrilho E. 2003. Identification of a metallothionein in *Synechococcus* by capillary electrophoresis hyphenated with inductively coupled plasma mass spectrometry. *Anal Sci* 19:1611–1616.
53. Lefebvre DD, Kelly D, Budd K. 2007. Biotransformation of Hg(II) by cyanobacteria. *Appl Environ Microbiol* 73:243–249.
54. Ybarra GR, Webb R. 1999. Effects of divalent metal cations and resistance mechanisms of the cyanobacterium *Synechococcus* sp. strain PCC 7942. *J Hazard Substance Res* 2.
55. Ivanova E, Gorshkova N, Kurilenko V. 2001. Tolerance of marine proteobacteria of the genera *Pseudoalteromonas* and *Alteromonas* to heavy metals. *Microbiology* 70:283–285.
56. Renella G, Mench M, Landi L, Nannipieri P. 2005. Microbial activity and hydrolase synthesis in long-term Cd-contaminated soils. *Soil Biol Biochem* 37:133–139.

57. Diels L, Mergeay M. 1990. DNA probe-mediated detection of resistant bacteria from soils highly polluted by heavy metals. *Appl Environ Microbiol* 56:1485–1491.
58. Martinez RJ, Wang Y, Raimondo MA, Coombs JM, Barkay T, Sobecky PA. 2006. Horizontal gene transfer of PIB-type ATPases among bacteria isolated from radionuclide- and metal-contaminated subsurface soils. *Appl Environ Microbiol* 72:3111–3118.
59. Rensing C, Ghosh M, Rosen BP. 1999. Families of soft-metal-ion-transporting ATPases. *J Bacteriol* 181:5891–5897.
60. Nies DH. 2003. Efflux-mediated heavy metal resistance in prokaryotes. *FEMS Microbiol Rev* 27:313–339.
61. De Hertogh B, Lantin A-C, Baret PV, Goffeau A. 2004. The archaeal P-type ATPases. *J Bioenerget Biomembr* 36:135–142.
62. Nies DH. 1999. Microbial heavy-metal resistance. *Appl Microbiol Biotechnol* 51:730–750.
63. Pina RG, Cervantes C. 1996. Microbial interactions with aluminium. *BioMetals* 9:311–316.
64. Jo J, Jang Y-S, Kim K-Y, Kim M-H, Kim I-J, Chung W-I. 1997. Isolation of ALU1-P gene encoding a protein with aluminum tolerance activity from *Arthrobacter viscosus*. *Biochem Biophys Res Commun* 239:835–839.
65. Fischer J, Quentmeier A, Gansel S, Sabados V, Friedrich CG. 2002. Inducible aluminum resistance of *Acidiphilium cryptum* and aluminum tolerance of other acidophilic bacteria. *Arch Microbiol* 178:554–558.

66. Chariton AA, Roach AC, Simpson SL, Batley GE. 2010. Influence of the choice of physical and chemistry variables on interpreting patterns of sediment contaminants and their relationships with estuarine macrobenthic communities. *Mar Freshw Res* 61:1109–1122.
67. Olson RJ, Chisholm SW, Zettler ER, Armbrust EV. 1990. Pigments, size, and distribution of *Synechococcus* in the North Atlantic and Pacific Oceans. *Limnol Oceanogr* 35:45–58.
68. Gray JS, Clarke KR, Warwick RM, Hobbs G. 1990. Detection of initial effects of pollution on marine benthos: An example from the Ekofisk and Eldfisk oilfields, North Sea. *Mar Ecol Prog Ser* 66:285–299.

Figure 1. Locations of the present study sites in northern Australia. Insets show the sample transects at each study site.

Figure 2. Canonical correspondence analysis of the community structure of archaea (A, C) and bacteria (B, D) based on relative abundance of operational taxonomic units (OTUs). Figures A and B show the present study site-specific relationships (Bing Bong, circles; Gladstone, crosses; Drimmie Arm and Gove Harbour - combined for clarity, diamonds). Figures C and D, wet season samples are indicated by open circles, dry season samples are indicated by filled circles. In the top panels, arrows indicate increasing abundance of selected taxonomic groupings; in the bottom panels, red arrows indicate increasing concentrations of 1 M HCl-extractable metals, and grey arrows indicate increases in the measures of physicochemical parameters. Percentages indicate axis contributions to species-environment relationships. Individual OTUs are not displayed.

Figure 3. Eight distinct shapes were repeatedly observed in species response curves generated for each target metal. The 1 M HCl-extractable metal concentration which corresponds to the highest species abundance represents the optimal concentration of that metal for the species under investigation. A, Decreasing monotonic; B, skewed unimodal with low optima; C, symmetrical unimodal; D, bimodal; E and F, skewed bimodal; G, skewed unimodal with high optima; H, increasing monotonic. The Y-axes represent the amplitude of species responses and the X-axes represent metal concentrations. Abundance peaks at predicted optimal metal concentrations are indicated with arrows. S = sensitive; R = resistant; R* = bimodal/resistant.

Figure 4. (A) Indicator species response curves of the 10 most abundant Archaeal operational taxonomic units (OTUs) to 1 M HCl-extractable concentrations of Al, As, Cd, Cu, Pb, V, and Zn. Only statistically-significant ($p < 0.05$) models are shown. No models were significant for Ga responses. Operational taxonomic unit1 *Methanothermobacter* sp., OTU2, OTU6, and OTU8 *Ignicoccus* sp., OTU3, OTU4, and OTU7 *Methanopyrus* sp., OTU5 *Thermoplasma* sp., OTU9 *Thermophilum* sp., OTU10 Miscellaneous uncultured Crenarchaeota.

(B) Indicator species response curves of the 10 most abundant Bacterial OTUs to 1 M HCl-extractable concentrations of Al, As, Cd, Cu, Pb, V, and Zn. Only statistically-significant ($p < 0.05$) models are shown. No models were significant for Ga responses. Operational taxonomic unit1 *Pseudoalteromonas* sp., OTU2 *Marichromatium* sp., OTU3 *Thioalkalivibrio* sp., OTU4 *Thiohalospira* sp., OTU5, and OTU7 *Microbulbifer* sp., OTU9 *Thioalkalivibrio* sp., OTU10 *Enterobacteriaceae*, OTU11 *Cobetia* sp., OTU13 *Syntrophaceae*.

Mac OS X 2 F ATTR x x
-W8BNMSWD

Sample	Sediment temp (°C)	Overlying water depth (m)	pH	Redox (meV)	% Particles <63 µm	% TOC
Dry season 2008						
Bing Bong						
1	25.2	0.0	8.1	-126	15.8	0.84
2	25.8	0.0	7.9	143	21.1	0.33
3	24.2	0.0	8.2	33	9.4	0.49
4	23.6	0.0	6.8	104	1.6	0.09
5	30.5	4.0	7.4	-221	40.8	1.85
6	30.1	3.5	7.6	-271	30.6	1.46
7	31.4	2.0	7.3	-375	21.5	1.23
8	32.2	0.1	8.5	-125	0.6	0.05
9	31.5	0.1	8.0	52	1.1	0.06
Gove Harbour						
D5.0	27.1	9.5	7.5	55	27.1	0.61
D2.0	26.5	10.2	7.6	-110	100.0	1.46
C0.25	27.2	10.5	7.6	-340	100.0	1.68
IH4	27.2	9.5	7.7	-368	100.0	1.66
IH6	27.2	7.3	7.6	-309	100.0	1.77
IH8	27.3	4.4	7.4	-351	71.3	1.52
Drimmie Arm						
F1.0	26.5	7.2	7.4	-53	72.6	1.25
F3.0	26.8	6.5	7.2	-118	100.0	1.59
F5.5	26.5	3.0	7.6	-36	8.1	0.34
F6.0	26.4	2.8	7.4	-53	4.3	0.17
F6.5	26.4	2.5	7.2	-110	100.0	2.12
F7.0	25.8	1.0	7.1	-66	33.4	1.32
Yarwun						

ST10	20.0	4.5	7.6	-156	100.0	1.55
NT21	18.9	1.5	7.4	-156	100.0	1.65
NT22	19.4	1.8	7.6	-123	100.0	1.46
ST28	19.3	4.5	7.9	5	16.1	0.67
RT40	20.2	15.0	6.5	-137	66.8	1.02
RT41	20.2	8.0	7.4	-175	96.0	1.90
Wet season 2009						
Gove Harbour						
D5.0	30.2	9.0	7.6	-118	31.0	0.38
D2.0	30.4	10.0	7.4	-180	100.0	1.15
C0.25	30.0	11.0	7.5	-203	100.0	1.33
IH4	30.0	10.5	7.7	-314	100.0	1.53
IH6	30.5	7.5	7.7	-216	100.0	1.78
IH8	31.0	4.4	7.4	-250	59.5	0.59
Drimmie Arm						
F1.0	30.1	7.5	7.6	-165	90.5	1.04
F3.0	30.6	6.0	7.6	-107	100.0	1.13
F5.5	31.2	3.0	7.7	-160	10.0	0.24
F6.0	30.4	2.5	7.6	-170	5.5	0.15
F6.5	30.3	2.7	7.5	-165	100.0	1.79
F7.0	30.4	1.5	7.5	-150	44.0	1.90
Yarwun						
ST10	25.3	3.5	7.5	-135	100.0	1.45
NT21	26.4	8.0	7.4	-146	59.5	1.36
NT22	25.0	2.3	7.1	-61	100.0	1.41
ST28	25.8	2.7	7.1	-99	6.0	0.26
RT40	26.1	18.5	7.5	-145	64.5	1.07
RT41	26.5	10.0	7.6	-171	100.0	1.77
Dry season 2009						
Bing Bong						
1	20.9	0.5	8.0	NM	6.5	0.16
2	22.5	0.5	7.9	NM	2.0	0.12
3	22.8	0.5	7.6	NM	51.0	0.55
4	22.9	0.5	8.0	NM	3.0	0.07
5	24.1	3.5	6.3	NM	100.0	1.95
6	23.7	1.0	7.7	NM	20.0	0.38
7	23.9	3.5	7.9	NM	100.0	1.83
8	23.6	0.0	7.8	NM	3.0	0.11
9	22.4	0.0	7.8	NM	2.5	0.09
Gove Harbour						
D5.0	25.5	7.6	7.5	-130	34.0	0.38

D2.0	25.3	10.4	7.7	−151	100.0	1.12
C0.25	25.7	10.1	7.6	−260	100.0	1.37
IH4	25.4	9.2	7.7	−308	100.0	1.44
IH6	25.3	5.4	7.5	−330	100.0	1.93
IH8	25.6	4.3	7.7	−320	100.0	2.46
Drimmie Arm						
F1.0	25.1	5.5	7.9	−98	100.0	1.08
F3.0	25.5	4.5	7.8	50	100.0	1.12
F5.5	25.7	4.0	7.3	−175	11.0	0.29
F6.0	25.5	0.9	7.8	−150	5.0	0.13
F6.5	25.6	0.8	7.6	−210	33.0	0.83
F7.0	24.0	0.5	7.3	−150	100.0	2.46
Yarwun						
ST10	19.1	3.0	6.5	−158	100.0	1.44
NT21	20.2	2.5	6.5	−15	28.5	0.62
NT22	19.6	3.0	6.5	−172	100.0	1.22
ST28	19.9	3.0	6.8	222	28.0	2.28
RT40	19.7	17.5	6.8	−172	100.0	1.14
RT41	19.3	8.8	6.7	−185	100.0	1.73
Wet season 2010						
Bing Bong						
1	27.5	0.0	7.5	7	20.1	0.90
2	28.8	0.0	7.8	55	23.3	0.55
3	24.0	0.3	7.9	−167	2.1	0.17
4	24.5	0.3	7.4	175	2.9	0.07
5	23.0	5.0	7.6	−184	100.0	2.63
6	24.0	2.5	7.5	−176	100.0	2.40
7	23.9	5.0	7.8	−271	100.0	3.23
8	27.1	0.0	7.9	−139	1.3	0.03
9	25.8	0.0	8.0	−138	1.5	0.00
Gove Harbour						
D5.0	29.1	9.0	7.7	−102	26.6	0.29
D2.0	28.9	10.2	7.6	−111	56.0	1.02
C0.25	28.7	9.7	7.5	−286	75.3	1.34
IH4	28.7	9.0	7.5	−338	82.3	1.48
IH6	28.9	6.0	7.6	−348	70.5	1.81
IH8	29.0	4.0	7.4	−375	55.2	1.70
Drimmie Arm						
F1.0	29.1	6.8	7.5	−128	65.8	1.09
F3.0	28.8	6.0	7.5	−103	74.1	1.06
F5.5	29.1	3.2	7.6	−138	7.7	0.22

F6.0	29.0	2.5	7.8	-140	7.2	0.23
F6.5	29.1	4.0	7.5	-188	39.2	1.00
F7.0	28.5	2.0	7.2	-101	31.6	1.11
Yarwun						
ST10	25.4	3.5	7.4	-197	58.1	1.59
NT21	25.5	9.0	7.3	-157	3.6	1.13
NT22	25.1	4.5	7.5	-192	43.4	1.24
ST28	25.7	5.5	7.7	84	66.6	0.36
RT40	25.2	20.0	7.0	-180	35.8	1.92
RT41	25.4	8.0	7.1	-191	52.4	1.94

TOC = total organic carbon; AVS = acid volatile sulfide; NM = not measured. Table 2. 1M HCl-extractable (mg/kg dry wt) element concentrations in sediment samples

		Distance from closest source (m)	1M HCl-extractable metals, arsenic, and phosphorus					
Status			Al	As	Cd	Cu	Ga	Mo
Dry season 2008								
Bing Bong								
1	Impacted	1800	852	2.1	0.03	1.4	1.8	— ^a
2	Impacted	1000	783	2.5	0.02	1.9	2.0	— ^a
3	Impacted	600	502	1.9	0.01	0.4	1.5	— ^a
4	Impacted	610	467	1.7	0.01	0.4	1.2	— ^a
5	Impacted	1400	2744	5.0	0.03	6.5	2.4	— ^a
6	Impacted	120	2346	4.6	0.09	9.6	2.4	— ^a
7	Impacted	120	2361	4.7	0.16	10.6	2.7	— ^a
8	Reference	650	392	1.7	0.01	0.3	1.3	— ^a
9	Reference	1300	430	1.6	0.01	0.3	1.5	— ^a
Gove Harbour								
D5.0	Reference	4000	1729	1.0	0.02	0.8	1.3	— ^a
D2.0	Reference	2000	3458	1.1	0.05	1.9	2.5	— ^a
C0.25	Impacted	500	6619	2.1	0.17	4.6	8.7	— ^a
IH4	Impacted	300	11 234	3.7	0.26	6.3	17.2	— ^a
IH6	Impacted	180	27 001	7.8	0.39	10.2	47.6	— ^a
IH8	Impacted	20	13 209	5.3	0.18	5.6	23.0	— ^a
Drimmie Arm								
F1.0	Reference	5500	3365	1.7	0.04	1.7	2.8	— ^a
F3.0	Reference	3700	3210	2.4	0.03	1.4	2.8	— ^a
F5.5	Impacted	1300	625	1.2	0.02	0.5	1.3	— ^a
F6.0	Impacted	1000	424	0.9	0.01	0.3	0.8	— ^a
F6.5	Impacted	900	4434	3.0	0.05	2.2	9.7	0.53

F7.0	Impacted	350	2048	1.6	0.03	1.1	4.0	— ^a
Yarwun								
ST10	Reference	3000	1565	2.3	0.01	7.8	2.1	— ^a
NT21	Reference	4050	1573	3.1	0.00	7.1	2.1	— ^a
NT22	Reference	3100	1621	2.4	0.00	6.9	2.0	— ^a
ST28	Reference	3600	842	2.0	0.00	2.4	1.2	— ^a
RT40	Impacted	30	1306	2.1	0.01	6.4	1.9	— ^a
RT41	Impacted	60	2908	2.9	0.01	9.8	5.9	— ^a
Wet season 2009								
Gove Harbour								
D5.0	Reference	4000	1672	1.0	0.02	0.7	1.4	— ^a
D2.0	Reference	2000	3716	1.3	0.04	1.8	2.7	— ^a
C0.25	Impacted	500	8024	3.4	0.20	4.7	11.6	— ^a
IH4	Impacted	300	8914	3.9	0.25	5.6	12.6	— ^a
IH6	Impacted	180	19 896	7.9	0.35	9.1	33.0	1.86
IH8	Impacted	20	7103	3.7	0.10	3.2	13.8	— ^a
Drimmie Arm								
F1.0	Reference	5500	3023	2.1	0.03	1.2	2.5	— ^a
F3.0	Reference	3700	3673	2.8	0.03	1.4	3.1	— ^a
F5.5	Impacted	1300	724	1.4	0.02	0.4	1.4	— ^a
F6.0	Impacted	1000	481	1.1	0.01	0.3	0.9	— ^a
F6.5	Impacted	900	4774	4.0	0.05	2.0	10.2	— ^a
F7.0	Impacted	350	3223	3.8	0.05	1.3	7.8	— ^a
Yarwun								
ST10	Reference	3000	1859	2.1	0.01	9.8	2.3	— ^a
NT21	Reference	4050	2361	3.3	0.01	7.5	2.7	— ^a
NT22	Reference	3100	1539	4.5	0.00	8.2	2.1	— ^a
ST28	Reference	3600	847	2.4	0.00	2.4	1.2	— ^a
RT40	Impacted	30	1574	2.7	0.01	8.1	2.1	— ^a
RT41	Impacted	60	3377	4.2	0.01	10.1	7.1	— ^a
Dry season 2009								
Bing Bong								
1	Impacted	1800	813	2.6	0.01	0.5	1.8	— ^a
2	Impacted	1000	655	2.4	0.01	0.3	1.8	— ^a
3	Impacted	600	1327	3.2	0.02	2.2	1.9	— ^a
4	Impacted	610	677	2.1	0.01	0.2	1.3	— ^a
5	Impacted	1400	2970	4.9	*	6.8	2.7	— ^a
6	Impacted	120	847	2.1	*	2.9	1.6	— ^a
7	Impacted	120	2670	6.3	*	10.5	2.9	— ^a
8	Reference	650	716	2.1	0.01	0.3	1.5	— ^a
9	Reference	1300	672	1.9	0.01	0.3	1.5	— ^a

Gove Harbour									
D5.0	Reference	4000	2480	1.2	0.02	0.8	1.7	— ^a	
D2.0	Reference	2000	4780	1.4	0.04	2.2	3.1	— ^a	
C0.25	Impacted	500	8105	2.7	0.19	4.5	9.1	0.49	
IH4	Impacted	300	13 150	4.2	0.27	6.8	18.6	0.46	
IH6	Impacted	180	18 100	5.8	0.38	10.3	25.6	0.88	
IH8	Impacted	20	20 250	7.0	0.25	10.0	31.4	0.56	
Drimmie Arm									
F1.0	Reference	5500	4540	2.2	0.04	1.5	3.2	— ^a	
F3.0	Reference	3700	5290	3.0	0.03	1.6	3.7	— ^a	
F5.5	Impacted	1300	1125	1.3	0.02	0.4	1.7	— ^a	
F6.0	Impacted	1000	533	1.0	0.01	0.2	0.9	— ^a	
F6.5	Impacted	900	3100	2.4	0.03	1.1	5.6	— ^a	
F7.0	Impacted	350	6560	4.1	0.08	2.2	12.4	— ^a	
Yarwun									
ST10	Reference	3000	2205	2.2	0.00	7.4	2.6	— ^a	
NT21	Reference	4050	1955	2.9	0.00	8.2	2.4	— ^a	
NT22	Reference	3100	1455	2.8	0.01	4.3	1.8	— ^a	
ST28	Reference	3600	2210	2.8	0.00	3.5	2.3	— ^a	
RT40	Impacted	30	1865	2.5	0.01	8.2	2.5	— ^a	
RT41	Impacted	60	3895	3.9	0.01	10.2	9.7	— ^a	
Wet season 2010									
Bing Bong									
1	Impacted	1800	876	3.0	0.06	1.6	2.1	— ^a	
2	Impacted	1000	1022	3.4	0.02	1.9	2.0	— ^a	
3	Impacted	600	1170	2.1	0.02	2.2	1.9	— ^a	
4	Impacted	610	568	1.8	0.01	0.2	1.1	— ^a	
5	Impacted	1400	2020	4.9	0.04	6.2	2.4	— ^a	
6	Impacted	120	1785	5.7	0.08	9.5	2.4	— ^a	
7	Impacted	120	1120	3.3	0.20	8.7	2.5	— ^a	
8	Reference	650	428	1.8	*	0.2	1.2	— ^a	
9	Reference	1300	475	1.9	0.01	0.2	1.4	— ^a	
Gove Harbour									
D5.0	Reference	4000	1580	1.4	0.02	0.6	1.3	— ^a	
D2.0	Reference	2000	3185	1.4	0.04	1.6	2.5	— ^a	
C0.25	Impacted	500	5890	2.6	0.17	4.1	7.4	— ^a	
IH4	Impacted	300	9490	3.9	0.24	5.9	13.8	— ^a	
IH6	Impacted	180	14 800	6.2	0.31	8.5	24.2	1.21	
IH8	Impacted	20	16 600	7.4	0.27	7.7	30.4	— ^a	
Drimmie Arm									

F1.0	Reference	5500	3275	3.9	0.03	1.4	2.9	— ^a
F3.0	Reference	3700	3395	3.7	0.03	1.4	3.1	— ^a
F5.5	Impacted	1300	658	1.3	0.01	0.3	1.3	— ^a
F6.0	Impacted	1000	645	1.1	0.01	0.2	1.1	— ^a
F6.5	Impacted	900	2520	2.8	0.03	1.2	5.6	— ^a
F7.0	Impacted	350	1995	2.8	0.05	1.0	4.9	— ^a
Yarwun								
ST10	Reference	3000	1680	2.6	0.01	9.3	2.3	— ^a
NT21	Reference	4050	2225	3.1	0.01	7.7	2.6	— ^a
NT22	Reference	3100	2150	2.1	0.01	8.3	2.7	— ^a
ST28	Reference	3600	797	2.6	— ^a	2.5	1.2	— ^a
RT40	Impacted	30	2230	2.4	0.01	14.8	2.5	— ^a
RT41	Impacted	60	2380	2.8	0.01	13.9	3.3	— ^a

^a

Table 3. Multiple metal-resistant taxa^a

	Al	As	Cd	Cu
Eukaryarchaeota → Thermoplasmata				
Thermoplasmatales → marine group III → unclassified	R	R	R	
Thermoplasmatales → CCA47 → unclassified	R	R	R	S
Thermoplasmatales → ASC21 → unclassified			S	R
Thermoplasmatales → AMOS1A-4113-D04 → unclassified	R	R	R	
Thermoplasmatales → 20c-4 → unclassified	R	R	R	S
Thermoplasmatales → → unclassified	R	R	R	S
Kazan-3A-21 → → unclassified	R	R	S	R
Eukaryarchaeota → Methanobacteria				
Methanobacteriales → Methanothermaceae → <i>Methanothermus</i>	R	R	R	R
Eukaryarchaeota → Halobacteria				
Halobacteriales → Halobacteriaceae → <i>Haloquadratum</i>	S	S	S	R
Halobacteriales → Halobacteriaceae → <i>Halogeometricum</i>	S	S	S	R
Deferribacteres				
Deferribacterales → <i>Caldithrix</i> → unclassified	R	R	R*	R*
Deferribacterales → LCP-89 → unclassified	R	R	R	R
Deinococcus-Thermus				
Deinococcales → Trueperaceae		R*		

Lentisphaerae					
	Lentisphaeria → ML1228J-2 → unclassified		R*		R*
	Lentisphaeria → WCHB1-41 → unclassified		R	S	R*
Planctomycetes					
	MBMPE71 → → unclassified		R	S	R
Proteobacteria					
	Candidatus Thiobios → → unclassified	R	R	S	S
Proteobacteria → Deltaproteobacteria					
	Desulfarculales → Desulfarculaceae				R
	Desulfobacterales → Desulfobacteraceae	R*	R	S	R*
	Desulfovibrionales → Desulfomicrobiaceae			S	R
	Desulfuromonadales → Desulfuromonadaceae	S	S		R
	Desulfuromonadales → Geobacteraceae	R		R*	
Proteobacteria → Gammaproteobacteria					
	Oceanospirillales → Hahellaceae		S		R
	Oceanospirillales → Oleiphilaceae	R	R	R	
	Oceanospirillales → ZA2525c	R	R	R	S
Verrucomicrobia					
	OPB35 → → unclassified				R
	Opitutae → Puniceicoccales → Puniceicoccaceae	R	R	R*	R
	Unclassified	R		R	R

^a Sensitivity and resistance profiles of multiple-metal-resistant Archaea and Bacteria, based on response curves.

S = sensitive; R = resistant; R* = bimodal population including resistant.

<

Table 4. Multiple-metal-sensitive taxa^a

	Al	As	Cd	Cu	C
Crenarchaeota					
	AK31	S	S	S	
	Marine benthic group A	S	S	S	
	Marine group I		S	S	S
	pMC2A209	S	S	S	
	Unclassified Crenarchaeota	S	S	S	S
Eukaryarchaeota → Halobacteria					

Halobacteriales → Halobacteriaceae → unclassified	S			
Halobacteriales → Halobacteriaceae → <i>Natronomonas</i>	S			
Halobacteriales → Halobacteriaceae → <i>Natronolimnobius</i>	S		S	
Halobacteriales → Halobacteriaceae → <i>Natrinema</i>	S			
Halobacteriales → Halobacteriaceae → <i>Natrialba</i>	S			
Halobacteriales → Halobacteriaceae → <i>Halovivax</i>	S			
Halobacteriales → Halobacteriaceae → <i>Halorhabdus</i>	S			
Halobacteriales → Halobacteriaceae → <i>Halococcus</i>	S	S	S	R
Halobacteriales → Halobacteriaceae → <i>Haloarcula</i>	S			
Halobacteriales → Halobacteriaceae → <i>Halalkalicoccus</i>	S			
Halobacteriales → Halobacteriaceae → <i>Haladaptatus</i>	S		S	
Halobacteriales → deep sea Euryarcheotic group → unclassified	S			S
Eukaryarchaeota → Methanomicrobia				
Methanosarcinales → Methanosarcinaceae → <i>Methanosalsum</i>	S		S	
Methanosarcinales → Methanosarcinaceae → ANME-3	S	S	S	S
Unclassified Methanomicrobia	S	S		S
Chloroflexi				
KD4-96 → → unclassified	S			S
S085 → → unclassified	S	S		S
Unclassified		S	S	
Cyanobacteria				
Chloroplast → → unclassified	R*			S
Subsection I → → unclassified	S			S
Unclassified	S			S
Proteobacteria → Gammaproteobacteria				
Alteromonadales → Pseudoalteromonadaceae	S			R*
Verrucomicrobia				
Verrucomicrobiae → Verrucomicrobiales → DEV007	S		S	S

Sensitivity and resistance profiles of multiple-metal-sensitive Archaea and Bacteria, based on response curves.

OS = sensitive; R = resistant; R* = bimodal population including resistant.

.

Table 5. Bimodal responsive taxa^a

	Al	As	Cd
Crenarchaeota			

Marine benthic group B				
Eukaryarchaeota → Thermoplasmata				
Thermoplasmatales → marine benthic group D and DHVEG-1 → unclassified	R	R*	R	
Eukaryarchaeota → Methanomicrobia				
Methanosarcinales → Methanosarcinaceae → <i>Methanolobus</i>				
Actinobacteria				
Acidimicrobidae → Acidimicrobiales				
Unclassified	S			
Bacteroidetes				
Sphingobacteria → Sphingobacteriales → unclassified	S		R*	

^a Sensitivity and resistance profiles of Archaea and Bacteria with bimodal responses to target metals, based on response curves.

2S = sensitive; R = resistant; R* = bimodal population including resistant.

Table 6. Metal sensitivity and resistance of Bacteria isolated from sediment in culture media spiked with metals

Bacteria	Grew in culture with:			Response characterized as:
	0 metals	4 metals	6 metals	
<i>Maricaulis</i> ^a	☐	☐	☐	Require metals for growth
<i>Muricauda</i> ^a	☐	☐	☐	
<i>Roseivirga</i> ^a	☐	☐	☐	
<i>Halomonas</i>	☐	☐	☐	Highly resistant to metals but do not require them for growth
<i>Kordiimonas</i>	☐	☐	☐	
<i>Marinobacter</i>	☐	☐	☐	
<i>Idiomarina</i>	☐	☐	☐	Resistant to metals but do not require them for growth
<i>Pseudidiomarina</i>	☐	☐	☐	
<i>Pseudomonas</i>	☐	☐	☐	
<i>Sulfitobacter</i> ^b	☐	☐	☐	
<i>Bacillus</i>	☐	☐	☐	
<i>Hyphomonas</i>	☐	☐	☐	
<i>Limnobacter</i> ^a	☐	☐	☐	
<i>Planococcus</i> ^b	☐	☐	☐	
<i>Pseudoalteromonas</i> ^b	☐	☐	☐	
<i>Rheinheimera</i>	☐	☐	☐	Sensitive to metals
<i>Rhodobacter</i>	☐	☐	☐	
<i>Thalassospira</i>	☐	☐	☐	
<i>Vibrio</i>	☐	☐	☐	

Isolated predominantly or exclusively from impacted sediments.

^b Isolated predominantly or exclusively from reference sediments.
g/kg.

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Cornall, A;Rose, A;Streten, C;McGuinness, K;Parry, D;Gibb, K

Title:

Molecular screening of microbial communities for candidate indicators of multiple metal impacts in marine sediments from northern Australia

Date:

2016-02-01

Citation:

Cornall, A., Rose, A., Streten, C., McGuinness, K., Parry, D. & Gibb, K. (2016). Molecular screening of microbial communities for candidate indicators of multiple metal impacts in marine sediments from northern Australia. ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY, 35 (2), pp.468-484. <https://doi.org/10.1002/etc.3205>.

Persistent Link:

<http://hdl.handle.net/11343/290807>