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Soil uranium concentration at Ranger Uranium Mine Land Application Areas drives changes in the bacterial community

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ABSTRACT

Soil microorganisms may respond to metal stress by a shift in the microbial community from metal sensitive to metal resistant microorganisms. We assessed the bacterial community from low (2–20 mg kg^{-1}), medium (200–400 mg kg^{-1}), high (500–900 mg kg^{-1}) and very high (> 900 mg kg^{-1}) uranium soils at Ranger Uranium Mine in northern Australia through pyrosequencing. *Proteobacteria* (28.85%) was the most abundant phylum at these sites, followed by *Actinobacteria* (9.31%), *Acidobacteria* (7.33%), *Verrucomicrobia* (2.11%), *Firmicutes* (2.02%), *Chloroflexi* (1.11%), *Cyanobacteria* (0.93%), *Planctomycetes* (0.82%), *Bacteroidetes* (0.46%) and *Candidate_division_WS3* (*Latescibacteria*) (0.21%). However, 46.79% of bacteria were unclassified. Bacteria at low U soils differed from soils with elevated uranium. Bacterial OTUs closely related to *Kitasatospora* spp., *Sphingobacteria* spp. and *Rhodobium* spp. were only present at higher uranium concentrations and the bacterial community also changed with seasonal and temporal changes in soil uranium and physicochemical variables. This study using next generation sequencing in association with environmental variables at a uranium mine has laid a foundation for further studies of soil-microbe-metal interactions which may be useful for developing sustainable management and rehabilitation strategies. Furthermore, bacterial species associated with higher uranium may serve as useful indicators of uranium contamination in the wet-dry tropics.

1. Introduction

Microorganisms respond quickly to environmental stress compared to higher organisms which makes them ideal for studying the effects of elevated metals on living organisms (Nielsen and Winding, 2002). Changes to biomass, community structure and specific functions may serve as a useful indicator of the changes in soil physical and chemical properties, thereby providing an early sign of soil improvement or an early warning of soil deterioration (Cardoso et al., 2013; Pankhurst et al., 1995). Changes in microbial community composition and its functions reflect the environmental effects resulting from metals contamination (Hattori, 1992; Smejkalova et al., 2003).

Speciation of metals has an effect on microorganisms (Hughes and Poole, 1991). Soluble metal species tend to be mobile and bioavailable (Olaniran et al., 2013) and they are therefore likely to affect the microbial community. However, microorganisms may be able to survive under elevated metal levels because they possess a variety of mechanisms to resist high concentrations of metals (Bruins et al., 2000). These mechanisms may involve reduction (Lovley et al., 1991), bioaccumulation (Merroun et al., 2006), biosorption (Ohnuki et al., 2005) and/or biomineralization (Martinez et al., 2007) of metals into forms that are less soluble and hence less bioavailable or less toxic. In this way, microorganisms also play an important role in metal speciation, which influences migration and toxicity of metals (Merroun et al., 2006).

Microorganisms present in metals contaminated environments are often associated with particular metals, which make them useful bioindicators for that metal and for environmental forensics (Haq and Shakoori, 2000; Smith et al., 2015). For instance, an increase in the population of U associated bacteria may represent high U concentration in soils.

Detailed studies of microbial communities in metal contaminated environments can provide insights into the role of microorganisms in

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metal speciation and mobility. It also allows us to assess their potential as bioindicators. A number of such studies have investigated microbial communities in uranium contaminated environments (Chen et al., 2012; Yan and Luo, 2015; Yan et al., 2016). These studies show that extremely diverse groups of bacteria can exist in uranium contaminated environments.

There is some information about bacterial communities in U-spiked sediments in the wet-dry tropics of northern Australia (Sutcliffe et al., 2017). However, there have been no detailed investigations of bacterial communities in uranium contaminated soils in this region. This also includes seasonal and temporal changes in bacterial community composition in uranium contaminated environments (Mondani et al., 2011). To our knowledge, studies of bacterial communities across a range of soil uranium concentrations under field conditions are also not available. Ranger Uranium Mine Land Application Areas (RUM LAAs) in the wet-dry tropics provide an ideal study site to fill these knowledge gaps because this site is subjected to extreme seasonal changes and has a range of U and other metal concentrations in soils as a result of continuous application of waste water over decades. From 1985 to 2008–09, runoff water from low-grade ore, waste stockpiles and other areas on the mine-site, stored in a retention pond 2 (RP2) during the wet season was irrigated to RUM LAAs during subsequent dry seasons. In addition to radionuclides (U, Ra and Th), the water also contained S as SO_4^{2-}, Ca, Fe and Mn as major solutes, and low levels Cu and Pb. The pH of the water ranges from 7 to 9. Some of the RP2 water was passed through a treatment wetland. This 'polished' RP2 water had less U compared to unpolished water (Overall and Parry, 2004; Zimmermann and Lu, 2011). The RUM LAAs soils have different concentrations of metals and radionuclides, not only because they received water of different quality (unpolished vs polished), but also because the irrigation does not apply water evenly, and some areas have been irrigated longer than others (Table 1). The soils are oxisols having low cation exchange capacities (< 10 cmol [+]/kg), undetectable anion exchange capacity and are expected to have aerobic conditions (Willett et al., 1993; Hollingsworth et al., 2005). *Eh* values of soils were however not measured in the present study. Moreover, soils are permeable with high gravel content (20–50% of soil mass), low clay (< 20%) and soluble salt contents and acidic pH (with irrigation of basic RP2 water neutralizing the soil pH) (Chartres et al., 1991). However, Mn^{+2}, UO^{+2} and Ra^{+2} ions in RP2 water are retained in the soil due to high affinity and adsorption by the ferruginous gravels (Chartres et al., 1991; Willett and Bond, 1995).

Based on historic U levels, sites were categorized as low U (2–20 mg kg^{-1} equivalent to background levels), medium (200–400 mg kg^{-1}), high (500–900 mg kg^{-1}) and very high (> 900 mg kg^{-1}) (Mumtaz et al 2013, 2015). The low U sites were significantly different from the other site categories based on their levels of U, S, Fe, Ca, Al, Cu, Zn, Th, Ni, Pb, Mn, U, TOC, TN, moisture and bulk density (Mumtaz et al., 2015). These sites also showed a significant seasonal and temporal change in soil U and physicochemistry mainly due to changes in the concentrations of soil U, Ca, Fe and S (Mumtaz et al., 2015). Since community level changes in bacteria have been reported in response to changes in physicochemical parameters (Akob et al., 2007; Islam et al., 2011), we predicted that the bacterial community at low U sites would be significantly different from medium, high and very high U sites and there would also be a seasonal and temporal change in the composition of the bacterial community. We also predicted that the bacterial community at RUM LAAs would be different from U contaminated sites in other parts of the world (Selenska-Pobell et al., 2002) due to the unique climatic conditions.

To test these predictions, we studied the microbial community composition at low, medium, high and very high U sites at the RUM LAAs. Culture-dependent techniques capture less than 1% of the cultivable microorganisms (Merroun, 2007), so we used high throughput DNA pyrosequencing (Sogin et al., 2006) to study the bacterial community present in RUM LAAs. Greater sampling depth (number of sequences per sample) obtained by pyrosequencing, allows a better coverage of individual samples, increasing the chances of detecting rare species which provides data that are more reliable for comparisons (Siqueira et al., 2012). The data provided a comprehensive assessment of the microbial community diversity and community change across a range of U and other metals concentrations at the RUM LAAs study sites.

2. Materials and methods

2.1. Sampling sites and collection of soil samples

Soil samples were collected over two years, 2008–09 (year 1) and 2009–10 (year 2). During each of those years, samples were collected in the dry season (September/October) and in the wet season (February/March). Five sub-samples were collected from each of the 18 selected sites at RUM LAAs (Fig. 1, Table 2) using a core sampler with diameter 5 cm and depth 10 cm (Chan et al., 2006). Ninety samples were collected each season and transported to the laboratory on ice. Samples were stored at 4 °C before further analysis.

2.2. Soil bacterial community processing and analysis

Total DNA was extracted from approximately 10 g of soil per sample using the MoBio PowerMax Soil DNA Extraction kit (Geneworks, SA, Australia) according to the manufacturer's instructions. The V6 hypervariable region of the bacterial 16S ribosomal RNA gene was amplified using A-967F and B-1046R primers (Sogin et al., 2006) according to the Roche FastStart High Fidelity PCR System, dNTPack protocol (Roche Diagnostics, NSW, Australia). Amplifications using a Labnet Multigene Thermal Cycler (Labnet International, Inc. Edison, NJ, USA) included an initial denaturation at 92 °C for 2 min, followed by 30 cycles of 94 °C/1 min, 57 °C/45 s, 72 °C/30 s and a final extension at 72 °C for 2 min. Each sample was subjected to a minimum of four amplification reactions. PCR products from these four reactions were pooled and purified using QIAquick PCR Purification kit (Qiagen, Hilden, Germany), and eluted with sterilized distilled water (SDW). Polymerase chain reaction products were quantified by separation through 2% agarose gel electrophoresis and were compared with low DNA mass ladder (Invitrogen CA, USA). Purified samples were

Table 1
Ranger Uranium Mine Land Application Areas (LAAs) (adapted from Akber et al., 2011a).

Land Application Area and abbreviation	Water quality	Irrigation method	Total area (ha)	Year commissioned
Magela (MLAA)	Unpolished RP2 water	Spray	33	1985
Magela Extension (MLAA-EXT)	Unpolished RP2 water	Spray	20	1994
RP1	Polished RP2 water	Flood	46	1995
Djalkmara (DLAA)	Polished RP2 water	Flood	18	1997
Djalkmara Extension (DLAA EXT)	Polished RP2 water	Flood	20	1999
Jabiru East (JELAA)	Unpolished RP2 water	Spray	52	2006
RP1 Extension (RP1 EXT)	Unpolished RP2 water	Spray	8	2006
Corridor Creek (CCLAA)	Unpolished RP2 water	Spray	141	2007