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The chemical composition and bacteria communities in acid and metalliferous drainage from the wet–dry tropics are dependent on season

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HIGHLIGHTS

- The metal concentrations in Rum Jungle AMD changed from the wet to dry season.
- Metals in Mt Todd AMD changed with year rather than with season.
- The bacteria communities in AMD were influenced by elemental composition.
- *Leptospirillum* and *Acidithiobacillus* were not prevalent at Mt Todd and Rum Jungle.

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ABSTRACT

Acid and metalliferous drainage (AMD) occurs when sulphidic minerals, such as arsenopyrite, chalcopyrite and pyrite, are exposed to oxygen and water. Climate, geology and mine site practices can have a significant impact on AMD composition. The elemental composition of the AMD can also affect the bacterial community. Our hypothesis was that in the dry season the AMD at two mine sites, Rum Jungle and Mt Todd, in the Northern Territory, Australia, has a higher concentration of dissolved metals because standing water evaporates during the extended dry period. Our second hypothesis was that the wet and dry season bacteria community in AMD at Rum Jungle and Mt Todd are different, and this difference is correlated to seasonally specific changes in physicochemistry. The first hypothesis was tested by measuring elemental concentrations in AMD during the wet and dry seasons at Mt Todd and Rum Jungle mine sites. The physicochemical properties such as temperature, pH and dissolved oxygen were also measured. To test the second hypothesis, we extracted DNA from AMD samples collected at Rum Jungle and Mt Todd during the wet and dry seasons. The hypervariable V6 region of the bacterial 16S rRNA gene was sequenced by 454 pyrosequencing. The bacterial community composition was examined and related to physiochemical variables. The elemental concentrations in Rum Jungle AMD were higher in the dry season compared to the wet season, but at Mt Todd the elemental composition of AMD changed with year, rather than season. The bacteria community in AMD at Rum Jungle changed between the wet and dry season while in Mt Todd AMD the bacteria community from year 1 was significantly different from year 2. The data showed that the elemental composition and bacteria communities of AMD at Rum Jungle and Mt Todd are influenced by season, mine site practices and geological characteristics of the ore body. In addition, the iron oxidising bacteria *Leptospirillum* and *Acidithiobacillus* typically associated with AMD in temperate regions were not prevalent at out tropical study sites.

1. Introduction

Acid and metalliferous drainage (AMD) (also referred to as acid mine drainage or acid rock drainage) occurs when sulphidic minerals, such as arsenopyrite, chalcopyrite and pyrite, are exposed to oxygen and water. Fig. 1 shows the oxidation of pyrite (Eq. (1)) (GARD, 2011). While AMD occurs naturally, the majority of AMD is anthropogenic and originates from reactive sulphide minerals stored in waste rock dumps (WRDs), ore stockpiles, tailings dams, mine pits and leach pads (GARD, 2011). In the initial stages of sulphidic mineral dissolution, ferric ion (Fe^{3+}) oxidises the sulphidic mineral, which results in the production of sulphate, acidity and ferrous ion (Fe^{2+}) (Fig. 1, Eq. (2)) (Evangelou and Zhang, 1995). At pH values greater than 6, Fe^{2+} is rapidly oxidised to Fe^{3+} due to chemical or prokaryotic processes (Fig. 1, Eq. (3)) (Plumlee et al., 1999; Johnson and Hallberg, 2005). The regenerated Fe^{3+} will oxidise more of the sulphidic mineral and thereby

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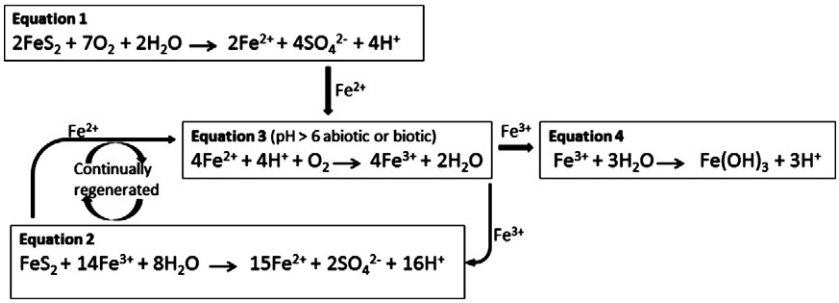

Fig. 1. Schematic representation of pyrite oxidation processes.

increase AMD formation due to more iron being available after each dissolution cycle (Fig. 1, Eq. (2)) (Plumlee et al., 1999), unless the Fe^{3+} reacts with water to form iron oxides (Fig. 1., Eq. (4)). The abiotic oxidation of Fe^{2+} slows at pH below 4 (Plumlee et al., 1999).

At pH<4, iron-oxidising bacteria and archaea drive Fe^{2+} oxidation (Plumlee et al., 1999; Baker and Banfield, 2003) and therefore play a key role in the formation of AMD through the regeneration of Fe^{3+} (GARD, 2011). Iron-oxidising bacteria, such as *Acidithiobacillus ferrooxidans* and *Leptospirillum ferriphilum*, are associated with AMD (Baker and Banfield, 2003; Johnson and Hallberg, 2003). In addition, the iron-oxidising archaea *Ferroplasma acidarmanus* and *Acidianus brierleyi* are also found in AMD (Baker and Banfield, 2003; Johnson and Hallberg, 2003). Which iron-oxidising genera are dominant in AMD depends on climate, and physicochemical variables such as pH, metal concentrations and temperature (Edwards et al., 1999; Bond et al., 2000). Other bacterial genera reported in AMD include *Acidocella*, *Acidobacterium*, *Acidomonas*, *Acidisphaera*, *Pseudomonas* and *Sphingomonas* (Johnson et al., 2001; Yin et al., 2007; Yang et al., 2008). Different bacterial and archaeal community structures in AMD have been reported worldwide, and this is largely attributed to the different chemistries of the AMD at these sites (Walton and Johnson, 1992; Bond et al., 2000; Bruneel et al., 2006; He et al., 2007; Yin et al., 2007; Palacios et al., 2008; Yang et al., 2008). The implication of this close link between bacterial community composition and site chemistry suggests that changes in AMD composition will also affect the bacterial community present (Edwards et al., 1999).

The composition of AMD is influenced by geological characteristics such as: the neutralising capacity in the host ore (carbonate content); rock type hosting the deposit; nature of the ore; trace element concentration in the deposit and host rock; and pyrite and iron sulphide content (Plumlee et al., 1999). The higher the pyrite and sulphidic mineral content (relative to available neutralising capacity) the lower the AMD pH and the higher the metal concentrations (Plumlee et al., 1999). The composition of AMD is also influenced by the climatic regime (Nordstrom and Alpers, 1999; Plumlee et al., 1999). Extended dry periods typical of the wet–dry tropics in this region (http://www.bom.gov.au) produces AMD with a lower pH and higher metal concentrations compared to the wet season AMD (Nordstrom and Alpers, 1999). In the dry period, sulphate salts form, and when the first rains come the salts are hydrated and there is an associated increase in metal concentrations in the AMD (Plumlee et al., 1999). This first rain flush of AMD that accumulates in the WRD during the dry period can also cause a drop in pH and increase in metal concentrations in AMD (Nordstrom, 2009). After this first flush, metal concentrations return to the same concentration as before the rain event (Nordstrom, 2009), but if there is extended rainfall then the metal levels decrease and the pH increases (Plumlee et al., 1999; Nordstrom, 2009).

Mine sites located in the wet–dry tropics of Northern Australia experience an extended dry period between May–October (dry season) (http://www.bom.gov.au). The dry season is followed by a period of heavy rainfall (1000–1700 mm) (wet season) (http://www.bom.gov.au).

Our hypothesis was that in the dry season the AMD at Rum Jungle and Mt Todd, in the Northern Territory has a higher concentration of dissolved metals because of there is no rain diluting AMD and standing water evaporates during the extended dry period. Our second hypothesis was that the wet and dry season bacteria community in AMD at Rum Jungle and Mt Todd are different, and this difference is correlated to seasonally specific changes in physicochemistry variables.

2. Sampling and analytical methods

2.1. Study area

The Mt Todd site is an inactive mine located approximately 230 km south-east of Darwin (van Dam et al., 2008). Pyrite, arsenopyrite, chalcopyrite and pyrrhotite, which comprise less than 5% of Mt Todd's Batman deposit, are located in quartz–sulphide veins hosted in greywacke and siltstone (Hungerford, 1995). The low grade ore and waste rock from Batman pit are in uncapped WRDs. AMD from the WRDs is directed through drainage channels into retention ponds. The AMD at Mt Todd is held in Batman pit, a retention pond (RP1) and tailings storage facility (Fig. 2).

Rum Jungle mine is located approximately 105 km by road south of Darwin, Northern Territory, Australia (Goodman et al., 1981). Uranium was mined from White's and Dyson's open pits from 1954 to 1958, while copper was extracted from the Intermediate pit (Fig. 3) in 1964 (Taylor et al., 2003; http://www.nt.gov.au/d/rumjungle/index.cfm?header=Rum%20Jungle%20Home). Quartz is the dominant constituent (34.3%) in the intermediate WRD and pyrite is a minor component (5.8%) (Taylor et al., 2003). Twenty percent of Dyson's WRD is black pyritic shale and the average pyrite content is 2–3% (Taylor et al., 2003). White's WRD is a mixture of carbonaceous slates, graphitic schists, phosphate, sulphate and sulphide minerals (Goodman et al., 1981). The sulphide minerals, pyrite, chalcopyrite, chalcocite, covellite and galena, constitute 3% of White's WRD (Goodman et al., 1981). All three WRDs at Rum Jungle were covered with clay and gravel during 1983–1986 (Taylor et al., 2003). The clay caps have cracked and water can now enter during the wet season which has led to the generation of AMD (Mudd and Patterson, 2010).

2.2. Sampling

AMD stored at Mt Todd in Batman pit (MTD1) and retention pond 1 (MTD2 and MTD3) (Table 1; Fig. 2) was sampled in 2008 and 2009. Water (MTW1, MTW2 and MTW3) samples were collected from reference sites near Mt Todd (Table 1; Fig. 2). Wet season samples were collected from Mt Todd in April 2008 and January 2009, and dry season samples in July 2008 and September 2009. Additional samples

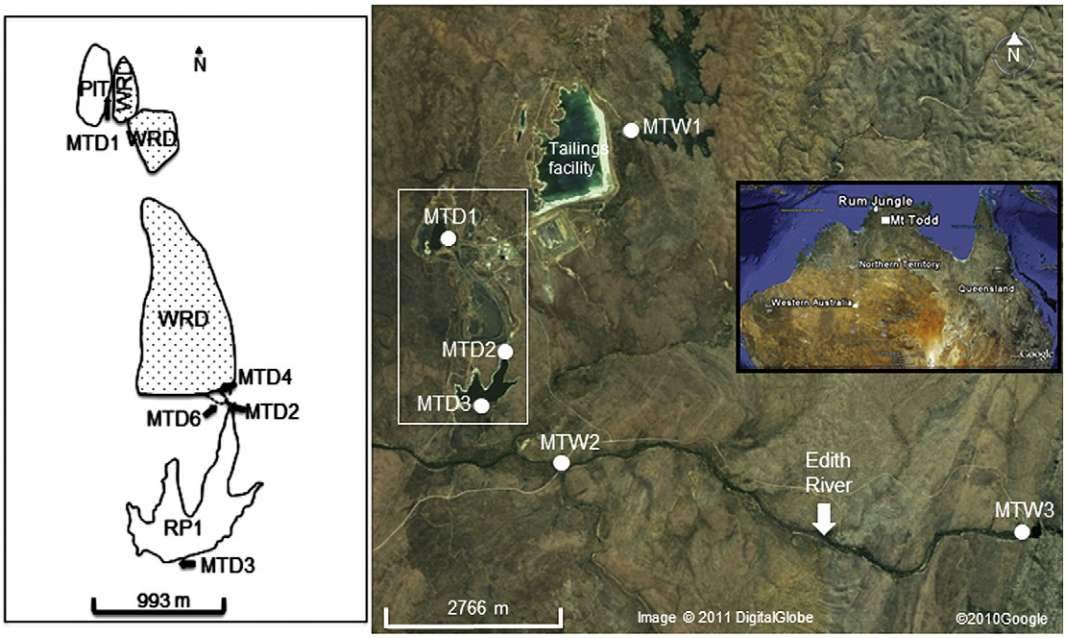

Fig. 2. Site layout at Mt Todd and the sampling sites for current study (RP1 — retention pond 1, pit — Batman pit). White box indicates region that is shown in sketch map.

(D4, D5 and D6) were collected from AMD seeps feeding into RP1 at Mt Todd during the wet season of 2010 (Table 1; Fig. 2).

Rum Jungle wet season 1 AMD samples RJD1, RJD2, and RJD3 and reference water samples RJW1, RJW2 and RJW3 were collected in April 2008 (Table 2, Fig. 3). Rum Jungle AMD RJD3 originates from White's WRD. The RJD2 sample was collected from the East Branch Finniss River diversion and the AMD at this site possibly originates from the Intermediate and White's WRD. RJD1 was collected from White's open cut pit (Fig. 3). Rum Jungle samples were collected from the same sites during July 2008 (dry season 1) except for RJW1, which was replaced with RJW4 water sample (Table 2, Fig. 3). The Rum Jungle sites RJD2, RJD3, RJW4, RJW2 and RJW3 were sampled in January 2009 (wet season), D1 was not sampled at this time and it was replaced by site RJD4 which is the Intermediate open cut pit (Table 2, Fig. 3). A late dry season sampling was performed at Rum Jungle in September 2009. There was no water at reference sites RJW1 and RJW3, so these were replaced by RJW5 and RJW6 sampling sites (Table 2, Fig. 3). The AMD seep RJD3 had stopped flowing in September 2009 so site RJD5 and an additional AMD site RJD6 were sampled (Table 2, Fig. 3).

Temperature, pH, dissolved oxygen (DO) and conductivity were measured in situ using Hydrolab model MS5, which was calibrated immediately before use. Unfiltered and filtered (<0.45 μm filtered in situ), water and AMD were collected in acid washed 250 ml bottles (Nalgene) at all sites. Additional 1 l unfiltered water samples were collected for total suspended solids (TSS) analysis and bacteria analysis. To determine if storage and shipment affected the elemental concentrations in water and AMD, the samples were spiked with reference metal and nutrient solutions immediately after collection (Table 1). Duplicate water and AMD samples were collected at Mt Todd and Rum Jungle.

The Australian Bureau of Meteorology climate data for Mt Todd and Rum Jungle was retrieved from the website http://www.bom.gov.au.

2.3. Analytical methods

AMD and water samples were prepared for elemental analysis (USEPA method 1638 (1995) and USEPA method 6020 CLP-M version 7.0). Briefly, the samples were acidified to pH<2 with ultrapure analytical grade concentrated nitric acid. The unfiltered acidified water and AMD samples were digested overnight at 60 °C to release metals from particulate matter. The following elements were measured in the acidified filtered and unfiltered samples by inductively coupled plasma mass spectrometry (ICPMS, Agilent 7500ce): S, Mg, K, Ca, Al, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb and U. Reference samples were run as quality controls in all ICPMS runs (see Supplementary Table 1).

For total suspended solids analysis, 0.5–1 l of water or AMD was filtered through 0.45 μm filters (Pall) in the laboratory. The filters were weighed pre and post filtering on a UMX2 Ultramicrobalance (Mettler Toledo). The difference in weight pre and post filtering was used to calculate the TSS in samples. The TSS method was developed in house at Charles Darwin University based on the Greenberg et al. (1992) protocol. Total phosphorous (TP) and total nitrogen (TN) were measured in both filtered and unfiltered water and AMD samples according to the Queensland Health Scientific methods. The Queensland Health method is based on the Lachat methods 31-107-04-1-B total nitrogen and 31-115-01-3-B total phosphorous. In brief, TP and TN in the water and AMD samples were measured by digestion with potassium persulphate/sulphate in an autoclave for 1 h. The TP and TN levels were measured using flow injection analysis (Lachat Quickchem 8000 with automated ion analyser). The quality controls used for TP and TN analysis are listed in Table 1. Total organic carbon (TOC) and dissolved organic carbon (DOC) were measured using high temperature combustion method (TOC-VCSH, Shimadzu Scientific Instruments, Oceania Pty Ltd) based on APHA method 5310 (Greenberg et al., 1992). TOC and DOC were measured by the Environmental Research Institute of the Supervising Scientist (ERISS) (Darwin, Australia).

The phenanthroline method (Greenberg et al., 1992) was used to determine iron oxidation states in year 2 dry season water and AMD samples. Stock phenanthroline (0.001 g 1, 10-phenanthroline monohydrate/0.001 l high pure water) was added to filtered or unfiltered samples in the field. The Fe^{2+} was calculated photometrically using a standard curve prepared according to Greenberg et al. (1992). All samples were read on a Helios gamma UV–visible spectrophotometer (Thermo Scientific). The Fe^{3+} concentration was calculated by

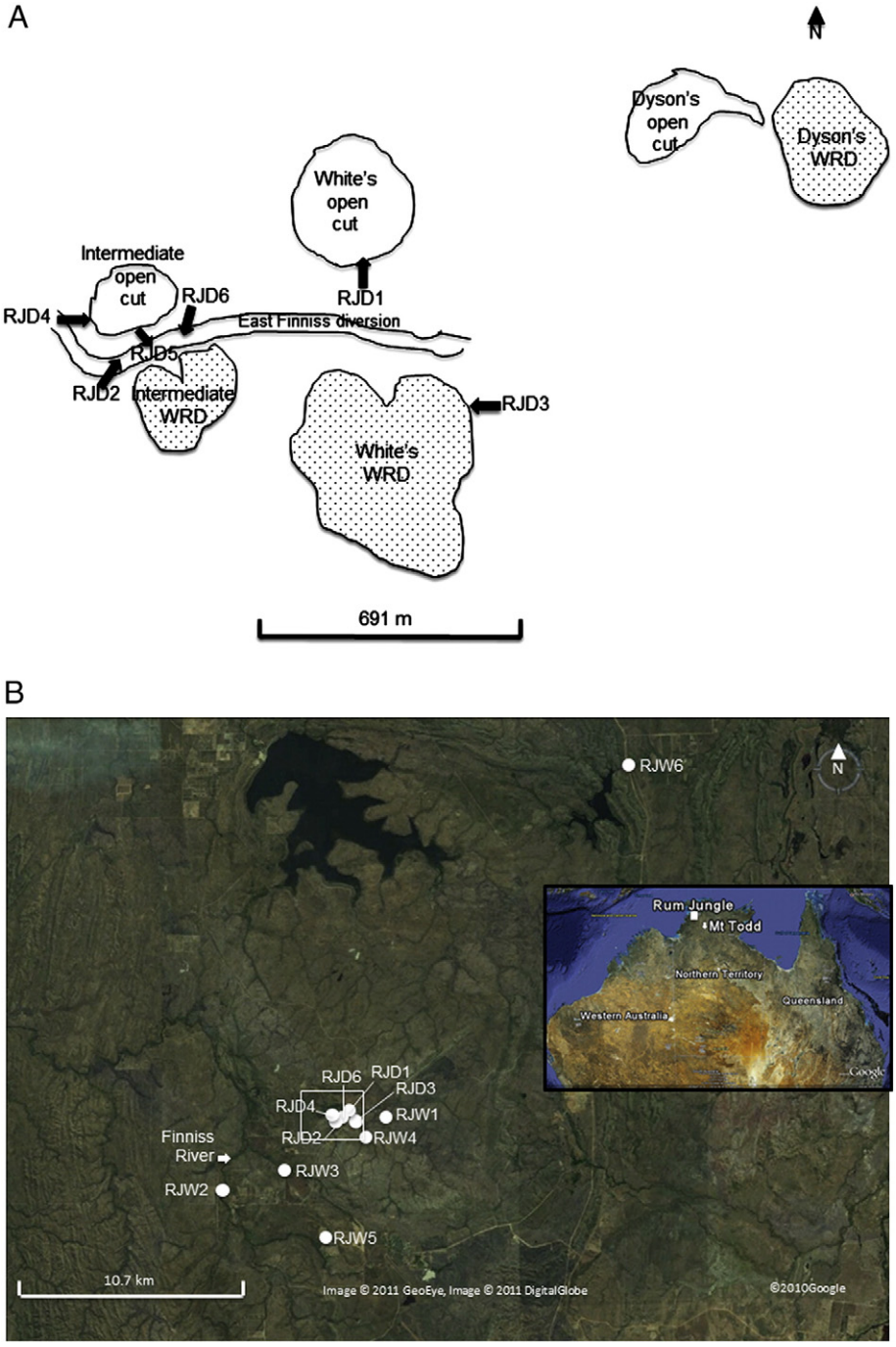

Fig. 3. Rum Jungle site layout (A) and the sampling sites for current study (A and B). A) Site layout and AMD sampling locations. B) All sampling points, white box indicates region that is shown in sketch map.

subtracting the Fe^{2+} concentration from total iron concentration that had been determined by ICPMS analysis.

2.4. Prokaryotic community analysis

For DNA extraction, 200–800 ml water or AMD was filtered to 0.22 μM (Geneworks). Bacteria DNA was extracted from the filters using the MoBio Power water (Geneworks). All steps were performed according to the manufacturer's protocol. DNA quantity was determined by separating DNA on a 1% agarose gel by electrophoresis and comparing to 1 kb plus molecular weight standard (Invitrogen). The gel separated DNA was visualised by staining with SBYR red stain (Invitrogen) and UV transillumination.

The V6 region of bacterial 16S rRNA genes was amplified from samples using the PCR primers A-967F and B-1046R primers (Sogin et al., 2006). Primer sequences contained barcode sequences (Parameswaran et al., 2007) and Roche 454 adaptor sequence (Roche Diagnostics). The target genes were amplified using the FASTSTART high fidelity PCR system all steps were performed according to the manufacturer's protocol (Roche). Negative DNA controls were included in each PCR batch. Quadruplicate PCR reactions were performed for each sample. The PCR products were pooled and purified using Qiaquick purification kit (Qiagen). The concentration of purified PCR products was determined by separating products and comparing to low molecular weight mass ladder (Invitrogen) on 2% agarose gel. The purified products were sent to the Australian Genomic Research Facility (AGRF, Brisbane) for standard or

Table 1
AMD and water sampling details for Mt Todd.

Sample type	Location	Coordinates	Origin of contaminant	Sampling time	Season	Sample code
AMD	Batman pit	14° 08′ 21.4″ 132° 06′ 09.9″	WRD	April 2008 July 2008 January 2009 September 2009	Wet 1 Dry 1 Wet 2 Dry 2	MTD1
	Retention pond 1 northern end	14° 09′ 18.1″ 132° 06′ 41.1″	WRD	April 2008 July 2008 January 2009 September 2009	Wet 1 Dry 1 Wet 2 Dry 2	MTD2
	Retention pond 1 southern end	14° 09′ 47.2″ 132° 06′ 27.3″	WRD	April 2008 July 2008 January 2009 September 2009	Wet 1 Dry 1 Wet 2 Dry 2	MTD3
	Seepage channel 1	14° 09′ 15.6″ 132° 06′ 39.0″	WRD	February 2010	Wet 3	MTD4
	Seepage channel 2	14° 09′ 17.8″ 132° 06′ 38.2″	WRD	February 2010	Wet 3	MTD6
Water	Horseshoe Creek	14° 07′ 25.3″ 132° 07′ 47.0″	None	April 2008 July 2008 January 2009 September 2009	Wet 1 Dry 1 Wet 2 Dry 2	MTC1
	Edith River	14° 10′ 18.5″ 132° 07′ 11.6″	None	April 2008 July 2008 January 2009 September 2009	Wet 1 Dry 1 Wet 2 Dry 2	MTC2
	Edith Falls	14° 10′ 52.9″ 132° 11′ 16.4″	None	April 2008 July 2008 January 2009 September 2009	Wet 1 Dry 1 Wet 2 Dry 2	MTC3

titanium 454 FLX sequencing. The 454 sequence data set was filtered using the Ribosomal database project pyro pipeline (Maidak et al., 1997). Sequences which failed to meet the following criteria were removed from the 454 sequence data set during filtering: sequence less than 70 base pairs after the forward primer, those that contained greater than one ambiguous nucleotide; and had greater than two differences to the forward primer and quality score was less than 30. The raw sequence data is available from the National Centre for Biotechnology Information short read archive.

OTU and phylotype sequence analysis was performed using MOTHUR software (Schloss et al., 2009), unless specified otherwise. For OTU analysis, sequences in the data set were aligned to the reference SILVA bacteria 16S rRNA gene alignments (Pruesse et al., 2007) using the Needleman pairwise algorithm. The bacteria 16S rRNA

Table 2
AMD and water sampling details for Rum Jungle.

Sample type	Location	Coordinates	Origin of contaminant	Sampling time	Season	Sample code
AMD	White's open cut pit	12° 59′ 20.7″ 131° 00′ 32.4″	White's WRD	April 2008 July 2002	Wet 1 Dry 1	RJD1
	Finniss River diversion southern end	12° 59′ 29.1″ 131° 00′ 11.4″	Intermediate and White's WRD	April 2008 July 2008 January 2009 September 2009	Wet 1 Dry 1 Wet 2 Dry 2	RJD2
	Drainage channel Whites WRD	12° 59′ 34.1″ 131° 00′ 42.6″	White's WRD	April 2008 July 2008 January 2009	Wet 1 Dry 1 Wet 2	RJD3
	Intermediate open cut pit	12° 59′ 22.3″ 131° 00′14.2″	Intermediate	January 2009	Wet 2	RJD4
	Finniss River diversion northern end	12° 59′ 28.9″ 131° 00′ 12.7″	Intermediate and White's WRD	September 2009	Dry 2	RJD5
	Finniss River diversion	12° 59′ 28.6″ 131° 00′ 13.2″	Intermediate and White's WRD	September 2009	Dry 2	RJD6
Water	River east of Rum Jungle	12° 59′ 24.7″ 131° 01′ 29.8″	None	April 2008	Wet 1	RJW1
	Finniss River	13° 01′ 19.3″ 130° 57′ 06.3″	None	April 2008 July 2008 January 2009 September 2009	Wet 1 Dry 1 Wet 2 Dry 2	RJW2
	Spring south west of Rum Jungle	13° 00′ 49.3″ 130° 58′ 44.2″	None	April 2008 July 2008 January 2009	Wet 1 Dry 1 Wet 2	RJW3
	River south east of Rum Jungle	12° 59′ 24.7″ 131° 01′ 29.8″	None	July 2008 January 2009	Dry 1 Wet 2	RJW4
	Rum Jungle lake	13° 2′ 36.0″ 130° 59′ 52.1″	None	September 2009	Dry 2	RJW5
	Manton River	12° 50′17.10″ 131° 8′0.20″	None	September 2009	Dry 2	RJW6

gene alignment was filtered to remove sequences that did not align in the V6 region of the bacteria 16S rRNA gene. Chimera sequences were identified using Chimeraslayer; window size was set at 10% of the alignment. A one nucleotide preclustering step (Huse et al., 2010) was performed on the aligned 16S rRNA genes to remove false OTUs. After the removal of chimaeras and false OTUs, the 16S rRNA gene sequences were assigned to OTUs using the average clustering method. To calculate the Chao and Simpson index, the 16S rRNA sequences were subsampled so the total number of sequences in each sample were 447. The rarefaction, coverage, Chao, and Simpson index were calculated using MOTHUR. Representative sequences from each OTU group were assigned to taxonomic groups by comparing sequences to the quality controlled SILVA bacteria 16S rRNA database (http://www.arb-silva.de/, Sponsor: Silva, accessed February 2011) using MOTHUR software.

A conservative approach was taken for community analysis so the OTU matrix for 97% similarity between OTUs (Stackebrandt and Goeble, 1994) was used for multivariate analysis. The total number of reads obtained for each sample was variable because the 454 sequencing chemistry changed from standard to titanium during the course of this study. For this reason sequence data were standardised in PRIMER software (Primer-E Ltd) before undertaking multivariate statistical analysis. The OTU matrix was square root transformed and a resemblance matrix generated using Bray Curtis. The Spearman Rank Correlation Method was used to calculate the relationship between physicochemical parameters and bacteria community structure. PERMANOVA analysis was used to assess the statistical significance of differences in the bacteria or physicochemical parameters of wet and dry season samples from Mt Todd.

3. Results

3.1. Seasonal conditions

At Mt Todd and Rum Jungle, the majority of rain fell between the months of November to April (Fig. 4) which is typical for the wet–dry tropics. During 2008, the total rainfall at Rum Jungle was 1900 mm, while 1400 mm of rain was recorded in 2009 (Fig. 4). The average annual rainfall for Rum Jungle is 1590 mm (http://www.bom.gov.au) therefore the conditions at Rum Jungle were drier than the annual average in 2009. At Mt Todd, 1400 mm of rain was recorded in 2008 and 970 mm in 2009 (Fig. 4). The average annual rainfall for this region is 1200 mm (http://www.bom.gov.au), therefore like Rum Jungle the conditions in 2009 were drier than average. The maximum temperature

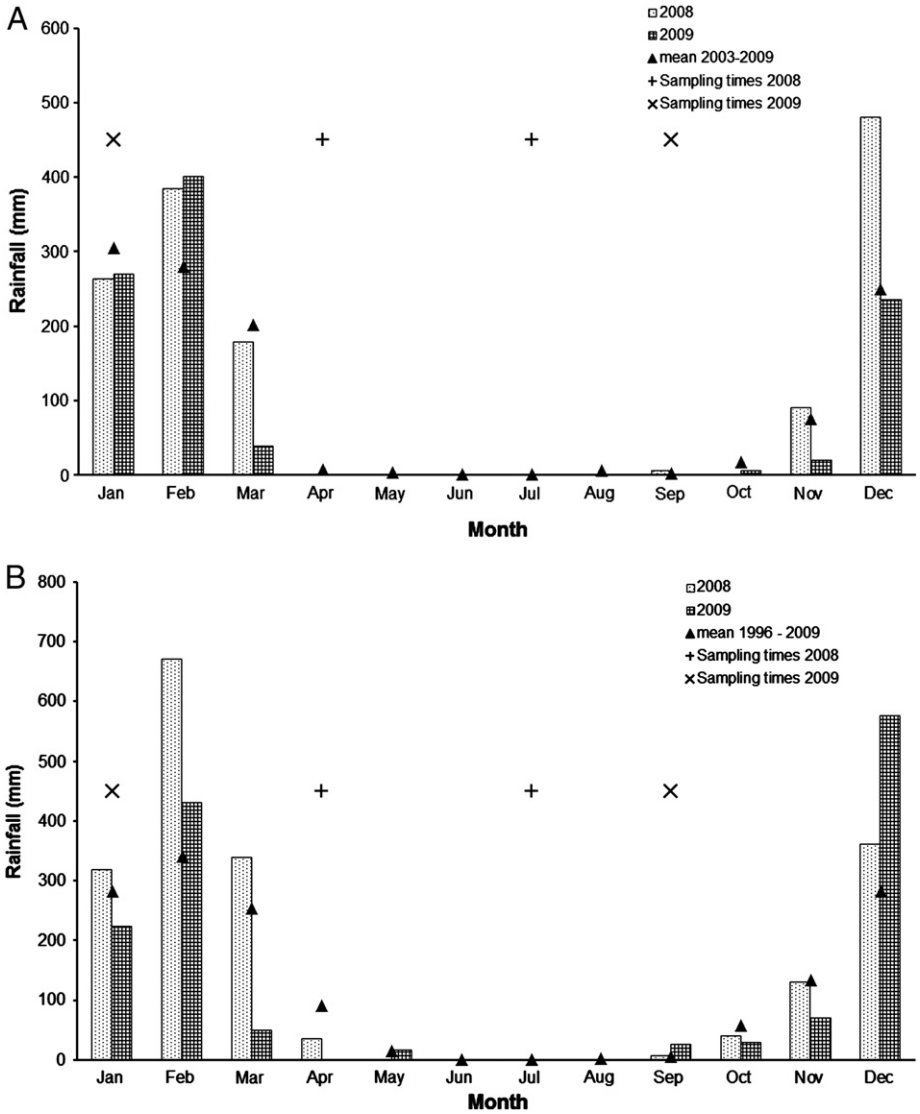

Fig. 4. Rainfall at Mt Todd (A) and Rum Jungle (B) during 2008–2009 compared to mean monthly rainfall (Source: http://www.bom.gov.au; viewed: March 2011). + indicates sampling times in 2008 and X indicates samples in 2009.

Table 3
Dissolved oxygen and pH of water and AMD sites at Mt Todd and Rum Jungle.

Site	Wet 2008			Dry 2008			Wet 2009			Dry 2009		
	Temp (°C)	pH	DO (%)	Temp (°C)	pH	DO (%)	Temp (°C)	pH	DO (%)	Temp (°C)	pH	DO (%)
MTW1	29.4	6.37	81.2	21.5	6.55	80.1	33.1	7.19	89.3	30.2	8.49	115
MTW2	26.5	6.55	90.8	22.2	5.35	85.7	29.9	5.97	76.9	29.4	7.28	73.7
MTW3	27.5	6.78	105	21.5	6.23	97.0	30.1	6.47	98.4	30.0	7.81	98.7
MTD1	33.6	3.05	107	25.1	3.36	99.4	31.2	3.19	87.2	31.0	3.74	97.9
MTD2	34.5	3.27	114	28.1	3.57	107	29.8	3.72	90.9	30.0	3.83	95.6
MTD3	32.5	3.35	109	26.2	3.88	106	33.5	3.89	94.9	30.9	4.01	101
MTD4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
MTD6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
RJW1	24.3	6.75	92.8	nd	nd	nd	nd	nd	nd	nd	nd	nd
RJW2	27.1	7.05	93.1	21.9	7.77	73.1	28.9	7.22	78.1	27.15	7.76	65.2
RJW3	27.5	7.04	91.2	21.4	7.54	36.1	26.9	7.29	69.2	nd	nd	nd
RJW4	nd	nd	nd	25.7	7.35	84.0	28.3	7.51	66.7	nd	nd	nd
RJW5	nd	nd	nd	nd	nd	nd	nd	nd	nd	30.38	6.64	65.9
RJW6	nd	nd	nd	nd	nd	nd	nd	nd	nd	31.83	9.14	85.8
RJD1	27.3	6.6	113	27.3	5.76	98.9	29.4	nd	nd	31.47	6.24	91.2
RJD2	29.6	6.35	91	29.5	3.16	97.3	28.9	6.94	84.1	34.42	3.21	93.5
RJD3	33.6	3.51	112	29.5	3.18	69.2	30.7	3.42	29.1	nd	nd	nd
RJD4	nd	nd	nd	nd	nd	nd	nd	5.41	85.6	nd	nd	nd
RJD5	nd	nd	nd	nd	nd	nd	nd	nd	nd	34.57	2.92	92.7
RJD6	nd	nd	nd	nd	nd	nd	nd	nd	nd	31.34	3.16	87.8

in the Mt Todd region was 30–39 °C during 2008–2009. The mean maximum temperature for this area is 30–37.5 °C. Therefore the maximum temperature at Mt Todd during our study was typical for this region in the wet–dry tropics. While at Rum Jungle, the maximum temperatures were between 31 and 38 °C during and the mean maximum temperature is 28–37 °C therefore during the study the maximum temperature at Rum Jungle was slightly higher than average conditions.

3.2. Characteristics of AMD and water from Mt Todd and Rum Jungle

The temperature of AMD at Mt Todd ranged from 25 to 35 °C while at Rum Jungle the temperature range was 27–35 °C (Table 3). The dissolved oxygen levels in water and AMD samples ranged between 87 and 113% (Table 3). The dissolved oxygen level was generally higher in the wet season due to rainwater. The pH of Mt Todd dry season AMD fell in the range of 3.3–4.0 while wet season AMD was between 3.05 and 3.89 (Table 3). At Rum Jungle, the pH range for AMD2 (RJD2) site in the wet season was 6.3 and 6.9 while dry season samples at this site were 3.16 and 3.20 (Table 3). For the other Rum Jungle AMD sites the pH range was 5.7–2.9 (Table 3). The pH of water from Mt Todd and Rum Jungle was 5.3 to 9.1 (Table 3).

There was 85–100% recovery of metals and nutrients from spiked samples and reference material (data not shown). Based on field duplicates, the percentage relative standard deviations were generally <5% except in cases where elemental concentrations were extremely low and at sites with high particulate levels. The water samples collected at Mt Todd and Rum Jungle represent base line elemental concentrations. Four water samples collected at Mt Todd fit the Plumlee et al. (1999) and GARD guide (2011) the neutral/saline mine drainage classification due to the concentration of dissolved Zn, Cu, Pb, Co, Ni and pH (see Supplementary Fig. S1). Dissolved metal levels in Mt Todd water samples classified as neutral drainage, were 1000 fold lower than the combined dissolved Zn, Cu, Pb, Co, Ni and Cd concentration in Mt Todd AMD (see Supplementary Fig. S1). Based on combined dissolved Zn, Cu, Pb, Co, Ni and Cd concentrations, and pH, all the Mt Todd AMD samples were classified as AMD with high metals (see Supplementary Fig. S1). For Rum Jungle, RJD3 AMD was always classified as acidic with high dissolved metals and so was the RJD2

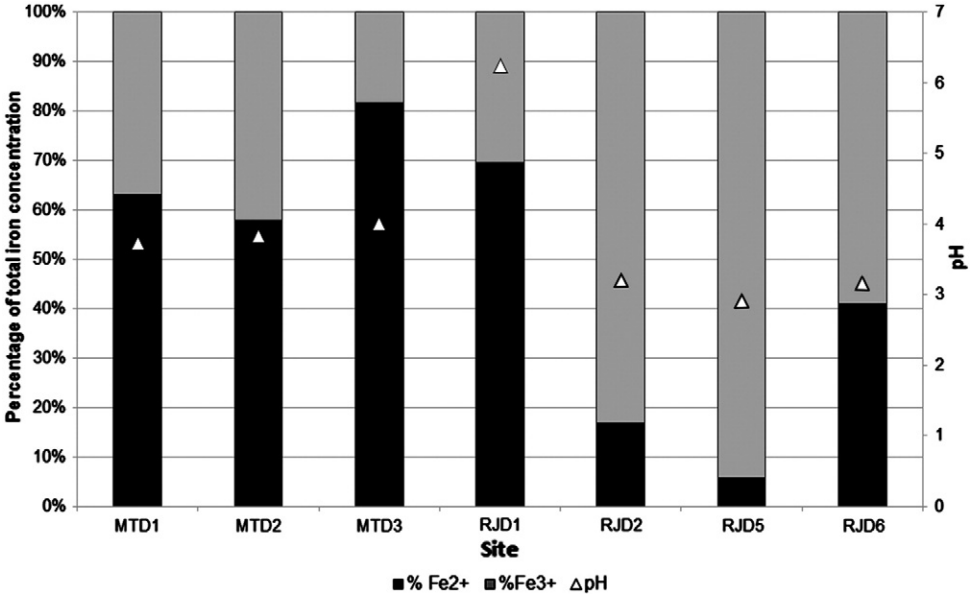

Fig. 5. Percentage Fe^{2+} and Fe^{3+} and pH of Mt Todd (MT) unfiltered and Rum Jungle (RJ) filtered samples collected in dry season of 2009.

AMD sample collected in dry season 1 (see Supplementary Fig. S1). The AMD samples RJD2, RJD5 and RJD6 which were collected in dry season 2 had extreme dissolved metal levels (see Supplementary Fig. S1). The Rum Jungle AMD samples, RJD2 wet seasons 1 and 2, RJD1 and RJD4 were classified as neutral drainage (see Supplementary Fig. S1). Only one water sample collected at Rum Jungle fit the neutral/saline drainage category (see Supplementary Fig. S1).

Fe^{2+} and Fe^{3+}concentrations were measured in unfiltered AMD at Mt Todd and in filtered samples at Rum Jungle. Iron oxidation states were similar in both Mt Todd unfiltered and filtered AMD samples. The unfiltered samples had extremely low TSS levels (data not shown). The concentration of Fe^{2+} and Fe^{3+} varied with site (Fig. 5). Ferrous ion was more prevalent in MTD1, MTD3, MTW1, RJD1 and RJW6 samples (Fig. 5). At RJD2 and RJD5, the dissolved ferric ion was the dominant oxidation state (Fig. 5). For the remaining samples the Fe^{2+} and Fe^{3+} concentrations were almost the same (Fig. 5).

The dissolved iron concentrations in Mt Todd AMD collected from Batman pit (MTD1) were similar to concentrations reported by Plumlee et al. (1999) for AMD samples (see Supplementary Fig. S2). In contrast, dissolved iron concentrations in the other Mt Todd AMD samples MTD2 and MTD3 were similar to levels in reference water samples from Mt Todd and Rum Jungle water samples (see Supplementary Fig. S2). Iron in Rum Jungle AMD fell in the range typical for AMD and was 10–300 times higher than levels measured in Mt Todd AMD samples (see Supplementary Fig. S2).

Mt Todd seep samples (MTD4 and MTD6) had similar dissolved iron concentrations (0.1 mg/l) to AMD collected RP1 (MTD2 and MTD3). These data suggest that iron is being removed from AMD before it exits the waste rock dump. Iron was the only element that was at similar levels in all seep samples (see Supplementary Fig. S3). Dissolved Pb in seep sample MTD4 was double the concentration measured in seep sample MTD6, but dissolved As, U, and total nitrogen in MTD4 seep were 6, 8 and 13 times lower, respectively, than in MTD6 (see Supplementary Fig. S3).

3.3. Differences in elemental concentrations of wet and dry season AMD and water samples

Samples from Rum Jungle sites RJD4, RJD5, RJD6, RJW1, RJW4, RJW5 and RJW6 were not analysed as part of the seasonal comparison because samples were only collected from these sites once or twice during the project. Principal component analysis (PCO) of Rum Jungle AMD and water samples showed that dry season samples had different levels of dissolved elements than the wet season samples (Fig. 6). The dry and wet season dissolved elemental composition of Rum Jungle AMD samples, RJD2 and RJD3, were significantly different (PERMANOVA, $p = 0.04$), but the Rum Jungle reference water samples, RJW2 and RJW3, were not (PERMANOVA, $p = 0.268$). The dry season AMD season samples had higher dissolved Al, Zn, As, Fe, U, TP, S, TN and Cu (Fig. 6a). At the Rum Jungle AMD site RJD2, the metals showed the following increases from wet to dry season, As 40× (0.141–5.5 μg/l), Zn 220× (144–3200 μg/l), Cu 370× (51–18,900 μg/l), Fe 8000× (3.4–27,700 μg/l), S 30× (34–1120 μg/l) and Al 1400× (38–53,800 μg/l) (see Supplementary Fig. S4). At the Rum Jungle AMD site RJD3, metal concentrations were generally 1.5–2 times higher in the dry season except for Al, Fe and Pb, which were higher in the wet season of year 2 (see Supplementary Fig. S4). At Mt Todd, the dissolved Al, DOC, As, U, Cu and Zn concentrations were higher at the AMD site MTD3 during the dry season compared to the wet season sample collected at this site (Fig. 7A). At MTD2 the metal concentrations were higher in year 2 compared to year 1 (Fig. 7A). Dry and wet season dissolved elemental concentrations of Mt Todd AMD were not significantly different (PERMANOVA, $p = 0.30$). This was also true for the reference water samples (PERMANOVA, $p = 0.14$) with the exception of MTW3 (Fig 7B).

3.4. Bacteria community composition in AMD and water samples

Seasonal changes in bacterial community diversity were compared using an OTU dataset that had been edited to remove poor quality reads at 97% similarity. There was generally a decrease in species richness and community diversity (Simpson index) in Mt Todd and Rum Jungle AMD during the dry, particularly for samples collected late in the dry season year 2 (Tables 4 and 5). This decrease in diversity was particularly noticeable at the Rum Jungle AMD (D2) site where the number of predicted OTUs (Chao) decreased from 456 in the wet season to 189 OTUs in the dry season (Table 5). Bacterial diversity also generally decreased in the Mt Todd water from the control sites during the dry season (Tables 4 and 5). The changes in species richness and community diversity from dry and wet season and year 1 to year 2 were significantly different (PERMANOVA = 0.028).

The bacteria communities in water and AMD were significantly different (PERMANOVA, $p = 0.001$) (Fig. 8). Based on SIMPER analysis the OTUs 1, 2, 3, 7 and 44 contributed the most to the differences in the water and AMD bacteria communities (Fig. 8). Based on comparisons to bacteria 16S rRNA gene sequences in the SILVA reference database these OTUs represent the following genera; OTU1 — *Acidiphilium*, OTU3 — *Burkholderia*, OTU2 — *Polynucleobacter*, OTU7 — *Malikia* and OTU44 — *Escherichia*. OTU1 was generally higher in Mt Todd AMD samples in both wet and dry seasons, while at Rum Jungle OTU3 was higher in AMD in the dry season.

The Rum Jungle AMD (RJD1, RJD2, RJD3, RJD4, RJD5 and RJD6) wet and dry season bacteria communities were significantly different (PERMANOVA, $p = 0.037$) (Fig. 9). The bacteria communities in Rum Jungle AMD dry season 2 were different from the wet and dry 1 due to the increased frequency of OTUs 1, 3, 5 and 6 (Fig. 9). Based on comparisons with the SILVA database OTUs 5 is a cyanobacteria but OTU 6 could not be classified. OTU7 was more prevalent in Rum Jungle Wet 2 AMD while OTU 11 increased in dry season 1 AMD (Fig. 9).

PCO analysis showed that the bacteria communities in Mt Todd AMD (MTD1, MTD2 and MTD3) wet and dry season samples were different because they grouped away from each other (Fig. 10), however, PERMANOVA analysis showed the difference was not statistically significant ($p = 0.276$). There was, however, a significant difference in the bacteria communities from year 1 sampling to year 2 sampling (PERMANOVA, $p = 0.002$). This difference was due to a higher level of *Acidiphilium* (OTU 1) in year 2. During the wet season of year 1, *Acidiphilium* (OTU 1) comprised 0.5–5% of the total AMD bacterial community, however, by the year 2 dry season it had increased to 22–43%. Conversely, the frequency of OTU11 (*Opitutae*) and OTU44 (*Sporichthya*) decreased in the Mt Todd AMD during these two years.

There was a significant correlation between the relative abundance of bacterial OTUs and physicochemical variables at MT Todd (Rho 0.476, significance 0.1%) and Rum Jungle (Rho 0.611, significance 0.1%) based on RELATE analysis.

3.5. Iron cycling bacteria

The Gamma-proteobacteria which contains *Acidithiobacillus*, was dominant at Mt Todd AMD sites MTD1 and MTD2 in the year 1 wet season (Fig. 11). The Gamma-proteobacteria was dominant at MTD1 and increased at MTD3 in the year 1 dry season (Fig. 11). In year 2 at Mt Todd AMD sites, the Alpha-proteobacteria were generally dominant (Fig. 11). In year 1 at Rum Jungle, the Gamma-proteobacteria were generally dominant in the AMD, while in year 2, the Beta-proteobacteria were more prevalent (Fig. 11). Further analysis of the V6 region of the bacteria 16S rRNA gene revealed that the Gamma-proteobacteria bacteria sequences isolated from Rum Jungle and Mt Todd were not closely related to *Acidithiobacillus* while the Alpha-proteobacteria were closely related to *Acidiphilium*.

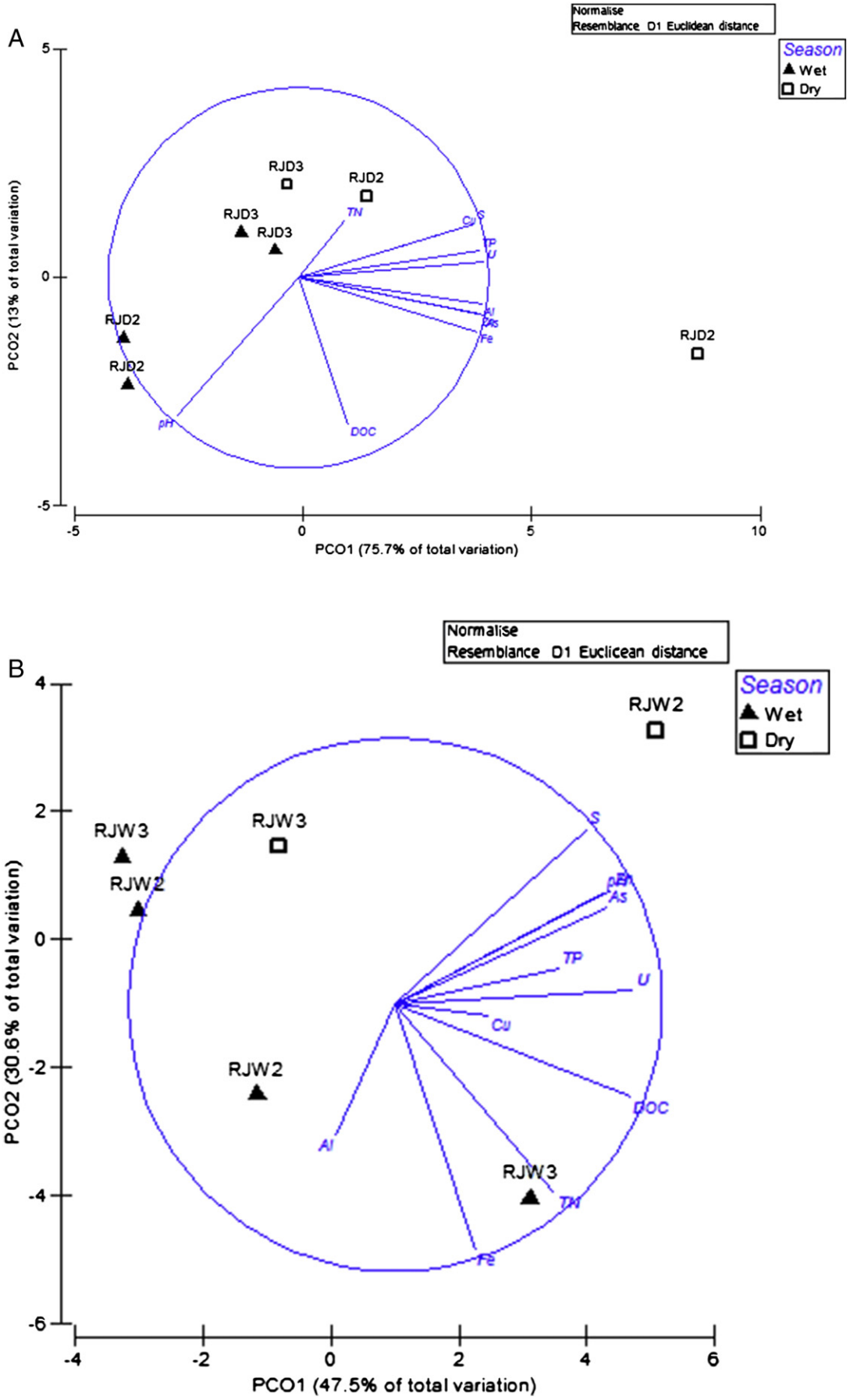

Fig. 6. Principal component analysis (PCO) based on dissolved elemental concentrations and pH in (A) AMD and (B) water samples collected from Rum Jungle in wet and dry seasons.

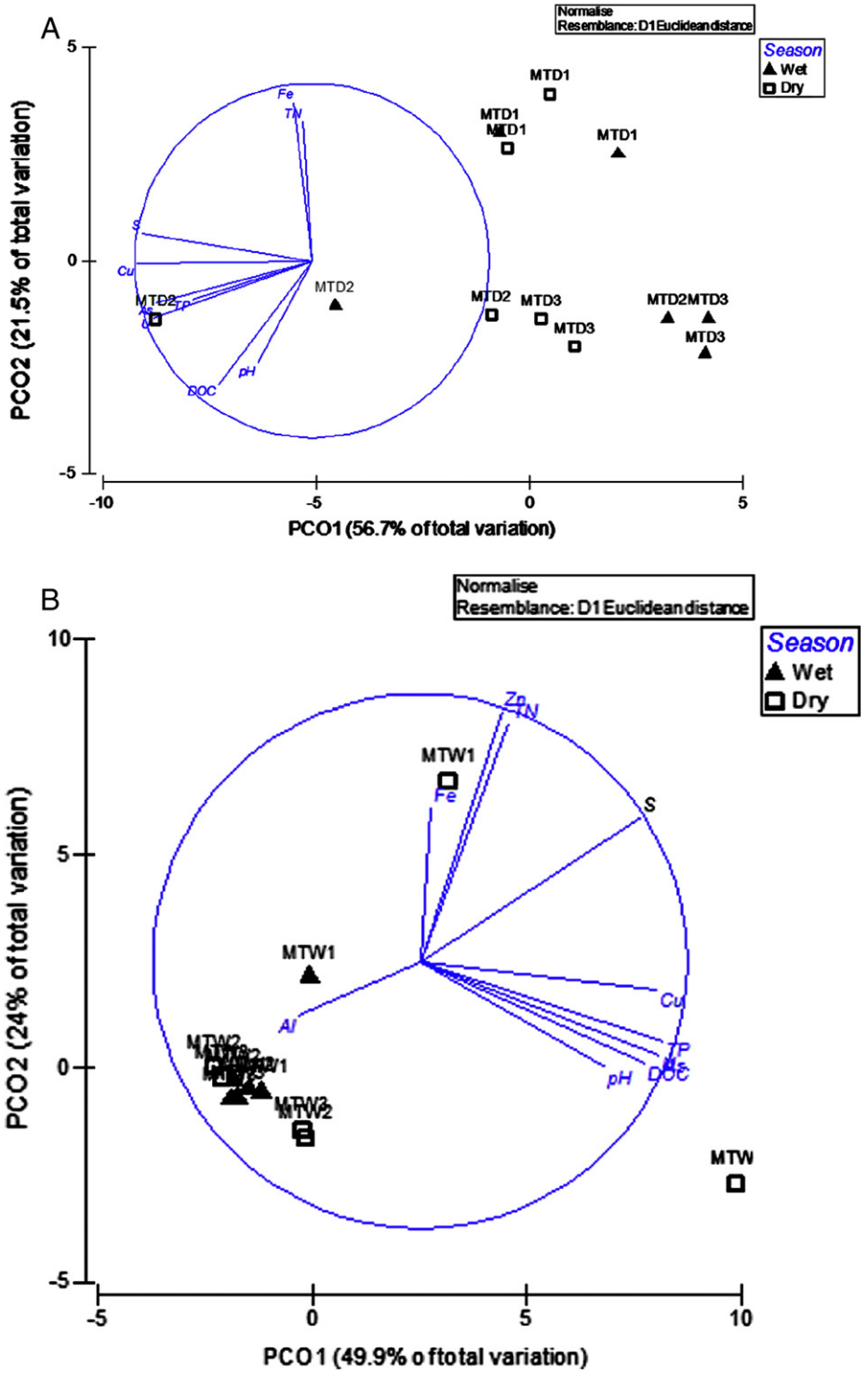

Fig. 7. Principal component analysis (PCO) based on dissolved elemental concentrations and pH in (A) AMD and (B) water samples collected from Mt Todd in wet and dry seasons. Symbol labelled MTW in bottom right side is MTW1.

4. Discussion

Our hypothesis was that the dry season AMD at Rum Jungle and Mt Todd would have higher concentrations of dissolved metals due to the extended dry period in the wet–dry tropics. Our data for Rum Jungle supported this hypothesis because the elemental concentrations were significantly higher in the dry season. One plausible explanation for these changes is that the elements concentrate in the dry season due to evaporation, and then the heavy wet season rainfall dilutes elements in the AMD (Nordstrom, 2009; Plumlee et al., 1999). Our data clearly show that the elemental concentrations in Rum Jungle AMD are influenced by the wet–dry cycle in northern Australia. At Mt Todd there was no significant difference in elemental concentrations of dry and wet season AMD but there was a significant difference between years. The AMD in the two seepage channels, MTD4 and MTD6, had different elemental compositions, which suggests AMD at Mt Todd originates from ore bodies with different geological characteristics. The AMD from these seeps mix at MTD2

Table 4
Summary of coverage, species richness and diversity based on V6 region of bacterial 16S rRNA genes from Mt Todd AMD and water. * number of reads after low quality reads removed in MOTHUR. ns = no sample.

Site	Year	Number of reads*		Good's coverage		Number of observed OTUs		Chao (number of OTUs/sample size 447 sequences)		Simpson index (sample size 447)	
		Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
MTW1	1	696	734	0.59	0.67	351	304	334	277	0.021	0.029
	2	4410	4595	0.94	0.97	436	227	233	123	0.067	0.311
MTW2	1	610	3845	0.66	0.87	266	702	245	761	0.023	0.033
	2	5332	3588	0.89	0.93	761	381	907	207	0.233	0.046
MTW3	1	797	3654	0.75	0.78	257	1145	244	700	0.035	0.008
	2	4720	4050	0.84	0.93	993	466	449	260	0.108	0.032
MTD1	1	714	668	0.71	0.60	251	332	276	347	0.073	0.034
	2	4657	3711	0.95	0.91	342	508	155	284	0.114	0.198
MTD2	1	633	520	0.55	0.78	362	158	442	131	0.027	0.071
	2	3973	3847	0.84	0.96	950	264	623	101	0.034	0.183
MTD3	1	828	648	0.72	0.66	305	293	269	297	0.034	0.019
	2	3473	3768	0.94	0.97	305	173	268	82	0.063	0.102
MTD4	3	2340	ns	0.88	ns	518	ns	413	ns	0.013	ns

therefore AMD composition at this sampling point would depend on rates of leaching and the volume of AMD contributed by each channel. The AMD composition at MTD3 would also be influenced by these factors because AMD from MTD2 contributes to MTD3 AMD. This mixing may mask seasonal changes. Another plausible explanation for the lack of difference between seasons is the mine site water management plan. At this site AMD is pumped between storage areas (pers. comm. Mine Site Manager, Mt Todd Mine, July 2008) so the AMD mixes with water from other sources and this mixing would change the composition of the AMD (Herbert, 1994; Plumlee et al., 1999). Thus, the nature of AMD at Mt Todd is influenced by mixing of AMD from different ore bodies, site location and mine site practices.

Our second hypothesis was that the wet and dry bacteria community in AMD at Rum Jungle and Mt Todd would be different due to changes in the physicochemical parameters. Our data for Rum Jungle support this hypothesis because the bacteria community in Rum Jungle AMD changed from wet to dry season, this change was mainly due to an increase occurrence of bacteria closely related to *Burkholderia* during the dry season. These changes in Rum Jungle AMD bacteria community were correlated with changes in the AMD physicochemical parameters. Our data from Mt Todd only partially supported this hypothesis, because the bacteria communities in wet and dry seasons were not significantly different, but the communities in year 1 were significantly different from those recorded in year 2. The nature of the AMD at Mt Todd changed i.e. the elemental composition, also changed with year and not season. Thus the changes in bacteria communities and physicochemical variables showed the same patterns and are related to each other. The changes in Mt Todd AMD elemental composition most likely showed yearly changes instead of seasonal changes due to mine site management practices and the geological composition of ore body contributing to the AMD. Thus, the bacteria community composition in AMD is most likely also influenced by these factors. This hypothesis is further supported by the finding that Rum Jungle and Mt Todd AMD, which originate from ore bodies with different geological characteristics, had different bacteria communities.

At Rum Jungle site D2 that showed the largest seasonal changes, dissolved zinc concentration increased 220 fold while the dissolved sulphur concentration increased 30 fold from wet to dry season. These increases are in contrast to those reported for AMD in California, which also has a wet and dry season, where sulphate increased fourfold during the dry season (Nordstrom, 2009). This suggests the extreme seasonal changes in the wet–dry tropics contribute to larger variation in the AMD composition compared to those reported in some temperate climates. However, due to the structure of the water body affecting the impact of climate on AMD composition, comparison of these wet–

Table 5
Summary of coverage, species richness and diversity based on V6 region of bacteria 16S rRNA genes from Rum Jungle AMD and water samples. * number of reads after low quality reads removed in MOTHUR. ns = no sample.

Site	Year	Number of reads*		Good's coverage		Number of observed OTUs		Chao		Simpson index	
		Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
RJW1	1	734	Ns	086	ns	143	ns	145	ns	0.089	ns
RJW2	1	670	930	0.86	0.83	141	221	130	191	0.086	0.052
	2	4573	3792	0.94	0.92	428	478	162	342	0.047	0.049
RJW3	1	790	995	0.64	0.87	339	198	387	188	0.056	0.056
	2	4642	ns	0.93	ns	533	ns	187	ns	0.097	ns
RJW4	1	ns	828	ns	0.85	ns	164	ns	212	ns	0.139
	2	2992	ns	0.93	ns	299	ns	168	ns	0.088	ns
RJW5	2	ns	4080	ns	0.95	ns	350	ns	212	ns	0.086
RJW6	2	ns	3615	ns	0.86	ns	726	ns	560	ns	0.034
RJD1	1	579	552	0.63	0.56	276	296	263	285	0.022	0.029
	2	ns	3515	ns	0.93	ns	344	ns	194	ns	0.140
RJD2	1	572	515	0.69	0.78	229	151	213	180	0.030	0.064
	2	4986	3105	0.88	0.97	835	142	456	189	0.080	0.187
RJD3	1	944	447	0.57	0.69	517	174	409	591	0.022	0.071
	2	3579	ns	0.88	ns	745	ns	319	ns	0.111	ns
RJD4	2	4467		0.94		414		159	ns	0.064	ns
RJD5	2		2874		0.97		127	ns	61	ns	0.423
RJD6	2		3727		0.97		148	ns	63	ns	0.383

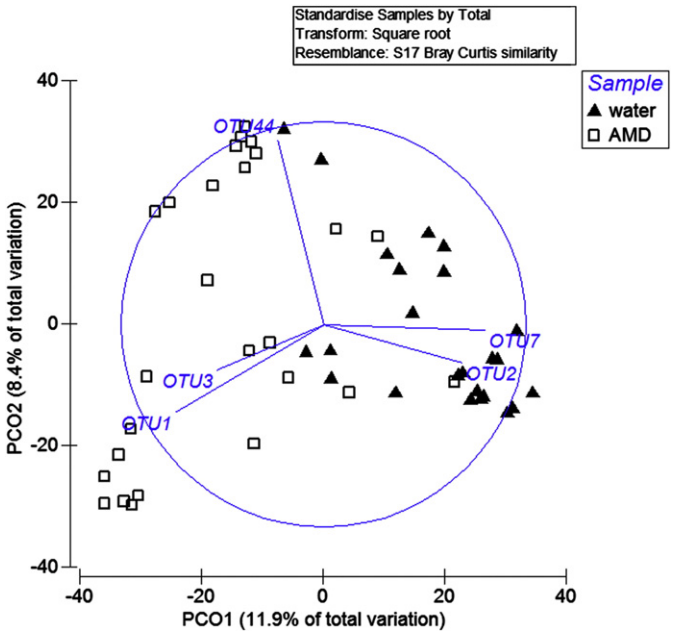

Fig. 8. Principal component analysis (PCO) based on relative abundance of bacteria OTUs in water and AMD samples from both Rum Jungle and Mt Todd during wet and dry seasons. OTU1 = *Acidiphilium*; OTU2 = *Polynucleobacter*; OTU3 = *Burkholderia*; OTU7 = *Malikia*; and OTU44 = *Escherichia*.

dry tropics sites to temperate climates is only valid if the water bodies have similar physical structure.

Element concentrations in Mt Todd AMD were typical of AMD except for iron at MTD2 and MTD3. Iron at these sites ranged from 0.1 to 1 mg/l, which is 10–100 times lower than typical AMD (Plumlee et al., 1999). The iron concentrations may be low because it is adsorbed to the surface of calcite (Mettler et al., 2009) which cross cuts through the quartz–sulphide veins in the Batman deposit (Hein et al., 2006). Alternatively, the low iron may be due to mixing of AMD with groundwater or rainwater (Olyphon et al., 1999) that can result in the formation of insoluble oxides such as goethite and jarosite (Herbert, 1994; Plumlee et al., 1999). Iron oxide formation only occurs if the oxidation reaction is coupled to a reduction reaction such as dissolved oxygen to water or denitrification (Herbert, 1994). The total nitrogen concentration at Mt Todd was 0.5–1.5 mg/l therefore nitrate may be available to couple denitrification with iron oxide formation. The sites at Mt Todd were highly oxygenated (97–101% dissolved oxygen) so oxygen is available for formation of iron oxides. Jarosite may also form due to the activity of the iron oxidising bacteria *A. ferrooxidans* (Daoud and Karamanev, 2006) but since the bacteria closely related to *Acidithiobacillus* were not identified in AMD from Mt Todd we have no evidence for a bacterial driven iron removal process at this site. This suggests that a more likely explanation for the removal of iron from Mt Todd AMD is some abiotic process.

Iron oxidising bacteria play a key role in AMD formation through the regeneration of Fe^{3+}. Bacteria closely related to iron oxidising bacteria were not dominant in Mt Todd AMD. Instead bacteria closely related to the iron reducing bacteria genera, *Acidiphilium* were prevalent. These findings suggest that iron reduction and not iron oxidation is the dominant side of the iron cycle in Mt Todd AMD. This is supported by the iron oxidation ratio in Mt Todd AMD, because Fe^{2+} was dominant. Mt Todd AMD pH was 3.0–4.0 and the sites were oxygenated (87–116% dissolved oxygen). Under these conditions Fe^{2+} should be the dominant oxidation state due abiotic processes (Stumm and Morgan, 1996). Therefore it may be a combination of abiotic and biotic factors driving the Fe^{2+}:Fe^{3+} ratio at Mt Todd. The pH and dissolved oxygen of Rum Jungle AMD were similar to Mt Todd thus Fe^{2+} should be dominant due to abiotic processes (Stumm and Morgan, 1996) but instead Fe^{3+} was dominant which indicates a biological process is influencing iron redox state. However, bacteria closely related to the typical iron oxidising bacteria were not identified in the AMD at these Rum Jungle sites. Acidic sediment bacteria also play a role in iron cycling (Lu et al., 2010), thus the sediment associated bacteria may be driving the iron cycling at Rum Jungle.

One of the reasons that bacteria closely related to iron-oxidising bacteria, *Acidithiobacillus* sp. and *Leptospirillum* sp., were not prevalent in Mt Todd and Rum Jungle AMD may have been due to the high pH. *Leptospirillum* sp. usually grows at extremely low pH (0–3)

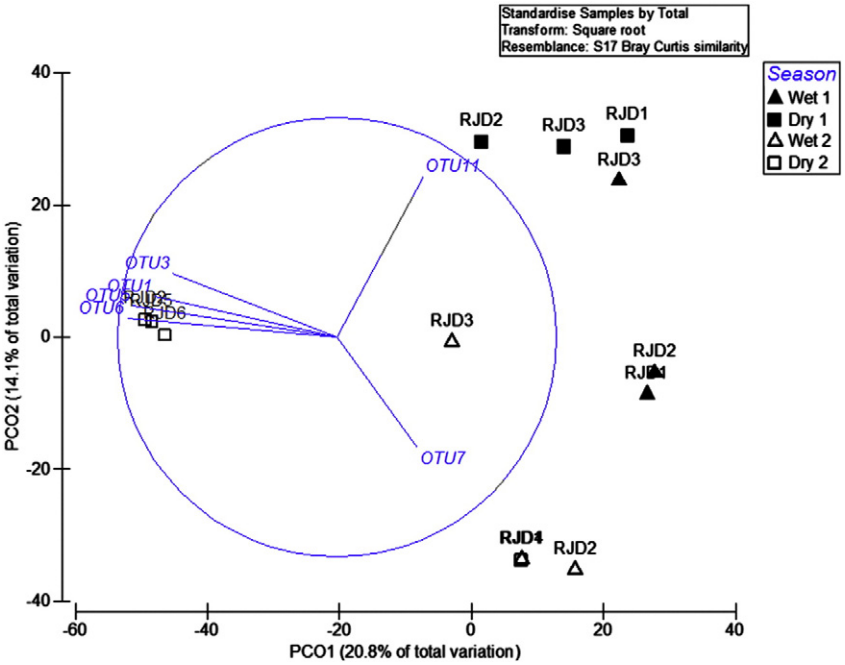

Fig. 9. Principal component analysis (PCO) based on relative abundance of bacteria OTUs in AMD samples from Rum Jungle in wet and dry seasons. OTU1 = *Acidiphilium*; OTU2 = *Polynucleobacter*; OTU3 = *Burkholderia*; OTU7 = *Malikia*; and OTU44 = *Escherichia*.

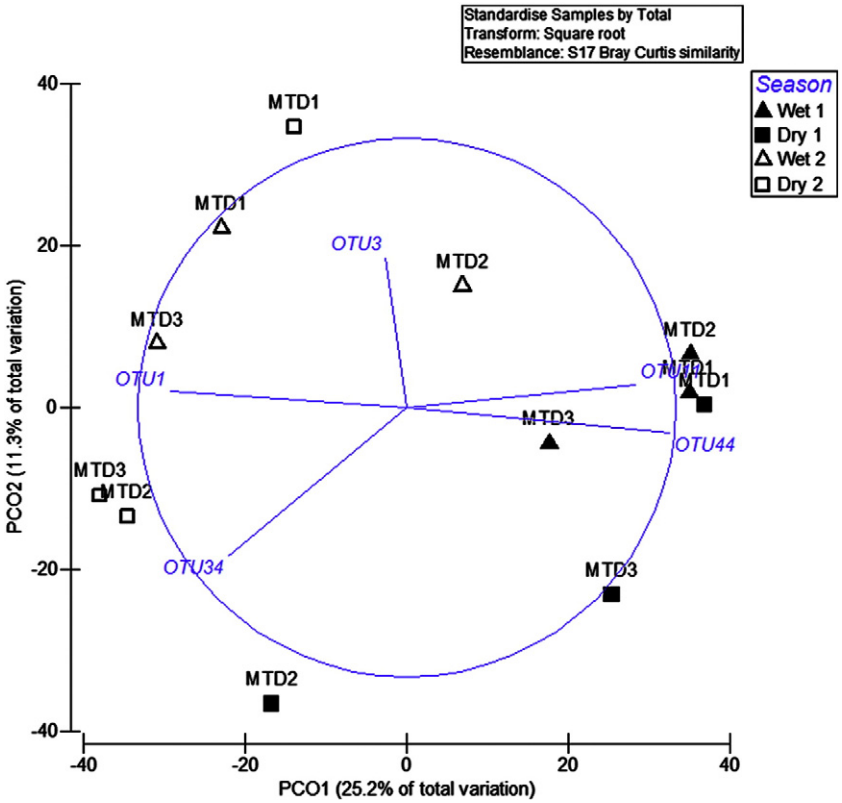

Fig. 10. Principal component analysis (PCO) based on relative abundance of bacteria OTUs in AMD samples from Rum Jungle in wet and dry seasons. OTU1 = *Acidiphilium*; OTU2 = *Polynucleobacter*; OTU3 = *Burkholderia*; OTU7 = *Malikia*; and OTU44 = *Escherichia*.

(Edwards et al., 1999) and the pH at the northern Australia sites ranged between 2.9 and 4. The pH range of the AMD at Mt Todd and Rum Jungle was more suitable for *Acidithiobacillus* sp. but it was only identified in one sample from Mt Todd and this may have been a result of sub-optimal temperature conditions. Growth of *Acidithiobacillus* sp. is optimal at 26 °C (Baker and Banfield, 2003) and the temperature of AMD at Mt Todd and Rum Jungle ranged from 25 to 35 °C. The lower temperatures were recorded in the dry season which may therefore be the only time of the year in which conditions support the growth of *Acidithiobacillus*. In addition, *Acidithiobacillus* sp. may not have been identified at Rum Jungle and Mt Todd because they are prevalent when $Fe^{2+} > 500$ mg/l (Johnson and Hallberg, 2003). Thus, the conditions in Mt Todd and Rum Jungle AMD are not optimal for iron oxidising bacteria, *Leptospirillum* and *Acidithiobacillus*, which are typically associated with AMD formation.

Bacteria closely related to *Acidiphilium* sp. dominated Mt Todd AMD and Rum Jungle AMD at RJD6 in the dry season of year 2. *Acidiphilium* are acidophilic heterotrophs and the availability of organic carbon affects their growth (Harrison, 1983). Organic carbon did not seem to be limiting *Acidiphilium* sp. growth in the Mt Todd and Rum Jungle AMD year one because samples with high levels of this *Acidiphilium* sp. had similar organic carbon levels as AMD samples where this genus was not dominant. This suggested that environmental factors other than organic carbon influence the occurrence of *Acidiphilium* at these sites. The samples that were dominated by *Acidiphilium* sp. at Mt Todd also had the highest elemental (Mg, Al, S, Ca, Mn, Cr, Co, Ni, Cu, Zn, As, Cd, Pb, and U) concentrations. It is possible that the prevalence of these bacteria increase in the dry season because they are more tolerant of metals and out-compete metal-sensitive heterotrophs, such *Escherichia* and *Sporichthya*.

The AMD and reference water bacteria communities were significantly different at Mt Todd and Rum Jungle. At Mt Todd the AMD communities differed from water due to the higher prevalence of putative *Acidiphilium* species. At Rum Jungle, putative *Burkholderia* and *Escherichia* were always more abundant in AMD compared to water samples. Given that *Acidiphilium* only occurred in one water sample (MTW1) which was possibly impacted by AMD, this bacteria genus may be a potential indicator of AMD in natural waterways in northern Australia. Although *Escherichia* and *Burkholderia* were more prevalent in AMD they are not potential indicator species because they were also present in the reference water samples. However, the elevated occurrence of these potentially pathogenic bacteria in AMD suggests that these genera should be monitored in northern Australia waterways impacted by discharge or overflow.

In conclusion, the concentrations of elements in AMD from some Rum Jungle samples were higher in the dry season when water bodies were evaporating, and lower in the wet season when rainfall diluted the discharge. These seasonal changes in Rum Jungle AMD were not as large as those reported in temperate climates. For the AMD samples that did not exhibit seasonal changes the elemental concentrations increased over the course of the two year study. The bacteria communities at Rum Jungle changed between wet and dry season while at Mt Todd the AMD bacteria communities in year 1 were different from year 2. The changes in bacteria communities at Rum Jungle and Mt Todd were correlated with physicochemical variables. Therefore the bacteria are responding to changes in the environmental variables which arise from evaporation, dilution, water management or mixing of AMD from different ore bodies. Bacteria closely related to iron-oxidising bacteria, *Acidithiobacillus* and *Leptospirillum*, which are typically dominant in AMD from temperate climates, were only present at low levels and sometimes not detectable in AMD from Rum Jungle and Mt Todd. Thus the bacterial communities in northern Australia were generally different from those in temperate climates due to the absence of *Acidithiobacillus* sp. and *Leptospirillum* sp.

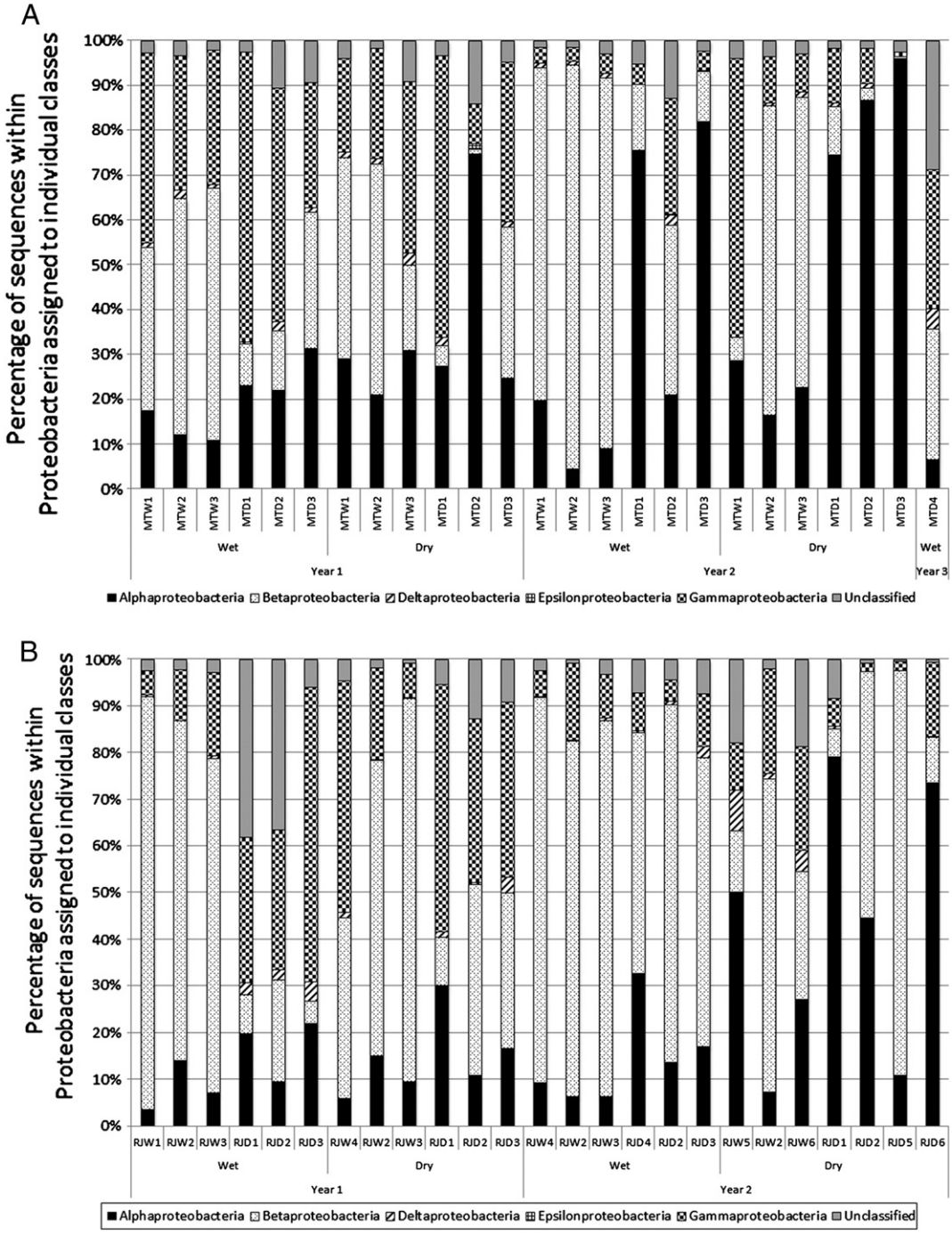

Fig. 11. Relative abundance of Proteobacteria classes in AMD and water from (A) Mt Todd and (B) Rum Jungle in wet and dry seasons.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2012.10.024.

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