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

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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

(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 wat

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

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/

in the Mt Todd region was 30–39 °C during 2008–

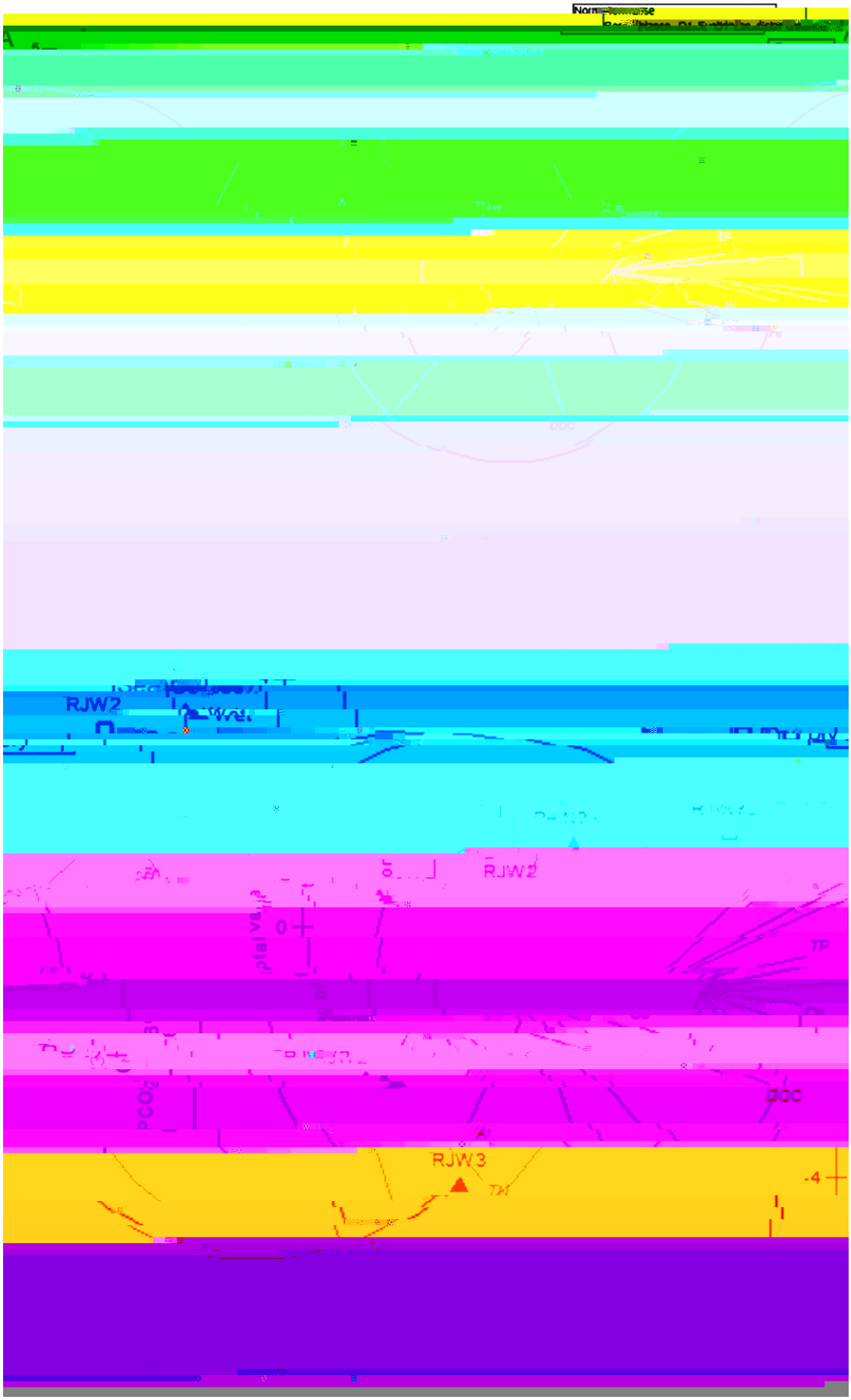

Fig. 6. Principal component analysis (PCO) based on dissolved elemental concentrations and pH in (A) AMD and (B) water samples collected from Rum Jungle in wet and dry seasons.

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 signifi

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

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

(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

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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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