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Microbial community signature in Lake Coeur d'Alene: Association of environmental variables and toxic heavy metal phases

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ABSTRACT

The water and sediments of Lake Coeur d'Alene in northern Idaho (USA) have been impacted by decades of mining operations within the Coeur d'Alene mining district. Using a multivariate statistical approach, correlations were explored between the microbial community (via 16S rDNA microarray) in sediment cores and operationally defined heavy metal phases (via continuous sequential extractions). Candidate phyla *NC10*, *OP8* and *LD1PA* were only detected in metal contaminated cores and diversity doubled among *Natronoanaerobium* in metal contaminated cores compared to the uncontaminated control site. This may suggest some increased fitness of these phyla in contaminated sediments. In contrast, diversity within the phyla *Aquificae*, *Coprothermobacteria*, and *Synergistes* was at least double in the uncontaminated control site. In linear models composed of two geochemical variables from the presumed sulfate reducing lineages detected in this study, orders *Desulfobacterales*, *Desulfuromonadales*, *Desulfotomaculum,* and *Syntrophobacterales* were highly correlated with Pb (positive influence) and Zn (negative influence) in the operationally defined residual fraction, and most taxa within orders from *Desulfovibrionales. Bdellovibrionales* highly correlated with Pb in the exchangeable/carbonate (negative influence) and oxyhydroxide (positive influence) phases. Diversity within families from metal reducing bacterial lineages *Shewanellaceae*, *Geobacteraceae*, and *Rhodocyclaceae* showed high correlation with Pb in the exchangeable/carbonate (negative influence) and oxyhydroxide (positive influence) phases. To our knowledge, this is the first time these techniques have been used in combination to describe a contaminated system. Resulting correlations suggest the diversity of the microbial community was influenced primarily by partitioning of heavy metals into exchangeable Pb over other Pb phases and, to a lesser extent, residual Pb to residual Zn phase partitioning.

1. Introduction

The Coeur d'Alene (CDA) Mining district has had 90 mines in operation producing Pb, Zn, Ag, and Sb (Balistrieri et al., 2003; Horowitz et al., 1992, 1995; Tonkin et al., 2002). Over seven million tons of Pb, three million tons of Zn, and 34 thousand tons of Ag have been mined from the CDA Mining district, which stretches from Coeur d'Alene, Idaho to Superior, Montana (Leach et al., 1985).

The history of this district as well as the type of ore deposits and mineralogy has been summarized by Leach and others (Fleck et al., 2002; Leach et al., 1985; Mauk and White, 2004; Panneerselvam et al., 2006; Rosenberg and Larson, 2000). Briefly, the mineralogy of the CDA Mining District consists primarily of quartz [SiO_2] and siderite [$FeCO_3$] veins containing deposits of galena [PbS], sphalerite [ZnS], and tetrahedrite [$Cu_{12}Sb_4S_{13}$] (Leach et al., 1985). Pyrite [FeS_2], chalcopyrite [$CuFeS_2$], and pyrrhotite [Fe_xS x = 0.8,1] are also locally abundant (Leach et al., 1985). Fe minerals including siderite, magnetite, pyrite, pyrrhotite, goethite, hematite, and ferrihydrite have been reported in the sediments of Lake Coeur d'Alene (LCDA) and the mining district (Cummings et al., 2000; Farrand and Harsanyi, 1997; Toevs et al., 2006).

Horowitz et al. (1995) reported the Coeur d'Alene River (CDAR) and adjacent lake sediments showed the greatest level of contamination with heavy metals. Batch sequential extractions indicate that heavy metals in the delta region of LCDA appear to be associated with an operationally defined sulfidic phase (Harrington et al., 1998b), while those elsewhere in LCDA appear to be

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predominantly associated with the more mobile hydroxides (Horowitz et al., 1995; Woods and Beckwith, 1997), though there is some controversy on this point (Horowitz et al., 1999). Haus et al. (2008) employed sequential extractions in combination with high resolution mass spectrometry and electron microscopy to study seasonal influences on arsenic speciation in the lateral lakes surrounding the CDAR. They found that stable water column height was a better predictor for partitioning of arsenic to less mobile phases when compared to a seasonally fluctuating water column. Additionally, studies of mixing and neutralization of acidic, metal contaminated water with uncontaminated surface waters (Balistrieri et al., 1999; Paulson and Balistrieri, 1999) and surface complexation of metals onto oxyhydroxides (Balistrieri et al., 2003; Tonkin et al., 2002) have been performed to predict metal fate and transport in this contaminated system. Sengör et al. (2007) developed models that incorporated microbial influences with surface complexation and chemical species changes during neutralization.

Concerns over repartitioning of extracted elements during selective chemical extractions have given rise to continuous sequential extraction (CSE) techniques. CSE are thought to limit transient metals redistribution during chemical extractions due to continuous removal of extracted elements when compared to equilibrium batch processes (Miro et al., 2005; Shiowatana et al., 2001; Wisotzky and Cremer, 2003). While a variety different analytical methods have been employed to study metal contamination and heavy metal phase association in and around LCDA, CSE techniques have not been employed at this site. This study utilizes CSE to measure heavy metal phase association while limiting repartitioning of metals during extraction processes.

The microbial ecology of LCDA has been studied through culture dependent (Cummings et al., 1999, 2000; Harrington et al., 1998a; Niggemyer et al., 2001; Sass et al., 2009) and independent techniques (Cummings et al., 2003; Ramamoorthy et al., 2009; Rastogi et al., 2009). Novel organisms have been cultivated from LCDA, including *Ferribacterium limneticum* (Cummings et al., 1999), *Desulfovibrio idahonensis* (Sass et al., 2009), *Arthrobacter* sp. (Moberly et al., 2010), and *Geobacter* sp. (Cummings et al., 2000). Using taxa specific primers for real-time polymerase chain reaction (rt-PCR) and denaturing gradient gel electrophoresis (DGGE), Cummings et al. (2003) reported that *Geobacteraceae* were abundant and diverse in LCDA across metal contaminated and uncontaminated sites sampled. Similarly, Ramamoorthy et al. (2009) used most probable numbers and quantitative PCR of the a-adenosine 5′-phosphosulfate reductase to estimate sulfate reducing bacteria (SRB) populations in LCDA, and reported non-culture based estimates of SRB populations were higher in contaminated sites than pristine sites. Utilizing clone libraries of *rpoB* and 16S rRNA genes, Rastogi (Rastogi et al., 2011, 2009) found β-*Proteobacteria* and *Crenarchaeota* to be the dominant bacterial and archaeal communities in the CDAR, respectively. However with advancements in microbial community analysis tools, larger scale, community level analyses have yet to be reported in the LCDA system.

In the past, molecular approaches to characterize diversity and abundance of microbial community have relied on amplification of a portion of the 16S rRNA gene, followed by cloning and sequencing of the amplified product. The number of clones needed to describe a majority of the community taxa can be large (Brodie et al., 2006) and this method is relatively expensive. Gene-based microarrays allow for rapid characterization of community assemblages by hybridizing amplified sample DNA onto short sequences of known probes bonded to the microarray surface; producing a florescent signal when bound. Microarray based techniques can capture diversity not generally observed by cloning methods (Bohorquez et al., 2012; Brodie et al., 2006) but only detect DNA with similar nucleotide composition to the designed probes. Advanced sequencing techniques, also known as next generation sequencing, have become the "gold standard" for microbial community analysis and through rapid advancements in recent years continue to decrease cost while providing increased information. Microarray based community analysis was employed in this study to characterize microbial community composition and was compared to clone libraries for limited samples.

Microbes are known to catalyze reactions that alter their environment resulting in detoxification (e.g. precipitation, reduction, (de)methylation, production of metal-binding proteins, cell surface complexation) and adaptation (e.g. horizontal gene transfer of metal resistant genes, mutation) (Lloyd and Lovley, 2001). Additionally, metal toxicity has been shown to depend on chemical speciation and geochemical factors, such as surface area for adsorption and redox active phases (e.g. hematite, goethite, and ferrihydrite) (Balistrieri et al., 2015; Gadd, 1992; Hoang and Tong, 2015; Meyer et al., 2015, 2006; Moberly et al., 2010; Morton et al., 2000; Sani et al., 2003; Tipping and Lofts, 2015; Van Genderen et al., 2015). To better understand the influence of toxic metals on microbial communities, simultaneous characterization of both heavy metal speciation and microbial community is important. Effects of anthropogenic heavy metal contamination on microbial community structure have been studied in other systems (Ancion et al., 2013; Feris et al., 2003, 2004a, 2004b; Gillan et al., 2005; Gough and Stahl, 2011), but are lacking in the LCDA system. In this study, we examine correlations between operationally defined heavy metal phases and microbial community diversity at the phylum and family levels using linear models and GGE biplots (Yan et al., 2001, 2007), with inferences to metabolic function.

2. Methods

2.1. Sediment sampling

Core samples were collected in July 2008 from the LCDA delta region reported to be most contaminated with heavy metals (Horowitz et al., 1992). As a comparison site with similar geochemistry, samples were also collected from the relatively uncontaminated St. Joe River delta, also in LCDA. Core sleeves were disinfected with ethanol prior to sampling. Sealed sediment cores were flash frozen immediately after removal from the lake bed by submersion in a mixture of dry ice and ethanol. Cores were transported on dry ice to Montana State University and frozen (−25 °C) for approximately two days until sectioning. Samples were sectioned into sub-cores while frozen according to visible stratification and divided for geochemical and molecular analyses. Samples for molecular analyses were taken with sterile spatula from the inner diameter of the core to avoid contamination from both sample handling and spatial contamination from organisms transported between different levels of the core while sampling. Samples destined for geochemical analysis were sealed in serum bottles under nitrogen and frozen (−25 °C) until analysis. See Tables 1 and 2 for further information on core and subcore samples.

2.2. Microbial community characterization

Genomic DNA was extracted from 1.0 g of sediment from each stratified sub-core using a PowerSoil DNA extraction kit (Molecular Biosciences, Carlsbad, CA). Extracted DNA was divided between a 16S rDNA microarray (PhyloChip) analysis developed by Lawrence Berkley National Lab (Brodie et al., 2006) and, as a comparative tool, clone libraries to provide another means of microbial community comparison that includes relative abundance. DNA for PhyloChip analysis was prepared as previously described by Brodie et al. (2006). An equal mass of DNA amplicons were added to each