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Bacterial and eukaryal diversity in soils forming from acid mine drainage precipitates under reclaimed vegetation and biological crusts

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ABSTRACT

Low-pH and low-nutrient conditions make the establishment of vegetation in mining-impacted environments particularly challenging. This study was conducted on soils forming from secondary Fe mineral precipitates in experimental plots at a 50-year-old acid mine drainage (AMD) barrens in Central Pennsylvania, U.S.A. Our objective was a qualitative assessment of bacterial and eukaryal diversity in reclaimed precipitates supporting dense successional vegetation six years after a single addition of lime (to raise pH from 2.5 to 4.5) and a single incorporation of compost. At the time of sampling, the pH of reclaimed precipitates had declined to initial values (2.5–2.7) and was similar to that of control precipitates supporting indigenous biological crusts. Microbial diversity was compared using 454 pyrosequencing of 16S rRNA genes (V1-V5) and 18S rRNA (V4-V5) in upper layers (adhering to roots and crusts) and lower layers (below roots and crusts). A total of 1721 bacterial OTUs at 97% similarity and 307 eukaryal OTUs at 97% similarity were recognized in the entire dataset. Despite similar pH, reclaimed precipitates had more diversity than control precipitates. *Proteobacteria* and *Actinobacteria* were the most abundant bacterial phyla in reclaimed and control precipitates, respectively. *Acidobacteria* were more abundant in root-and crust-enriched layers than in the underlying precipitates containing less carbon. *Basidiomycota* fungi were the most abundant classified eukaryotes in reclaimed precipitates, while *Bryophyta* dominated control precipitates. *Glomeromycota* were observed in reclaimed but not in control precipitates, where *Ascomycota* were the most abundant fungi. After the one–time reclamation approach, bacterial richness in AMD precipitates was three-fold lower than that determined for acid sulfate soils using closed reference OTU picking. These results suggested the need for further lime and compost incorporation to increase soil functionality.

1. Introduction

Mining-impacted soils and tailings provide poor substrates for plant growth due to extremes in pH, lack of organic matter, and high levels of acidity-generating metals (Iverson and Maier, 2009; Moynahan et al., 2002). Such soil materials typically exhibit low microbial diversity and activity (Schippers et al., 2000; Southam and Beveridge, 1992), which is consistent with observations that acidic soils (pH < 4) support lower bacterial richness than neutral soils (Lauber et al., 2009; Nacke et al., 2011; Rousk et al., 2010). Because pH exerts such a strong effect on soil bacterial richness, increasing the pH of mining-impacted substrates is an important prerequisite for enhancing microbial diversity and activity during vegetative reclamation (Moynahan et al., 2002).

In a previous report, we described how acidic iron oxy(hydr) oxides (pH 2.5–2.7) could be vegetated in place at an acid mine drainage (AMD) barrens in Central Pennsylvania, U.S.A. (Lupton et al., 2013). These AMD precipitates, which were composed mainly of ferrihydrite and goethite, averaged about 70% Fe by weight and thus represented an atypical parent material for soil formation. The barrens had supported no vegetation for the previous 50 years except for bryophyte-dominated biological crusts in wetter areas. In 2006 experimental plots at the barrens were reclaimed with addition of lime to pH 4.5, incorporation of compost into the upper 15 cm, and a first-year oats nurse crop. By 2011, this experiment had demonstrated that the one-time reclamation treatment could rehabilitate AMD precipitates into

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an incipient soil that supported >90% vegetative cover for the next five years, thus meeting the U.S. Surface Mining Control and Reclamation Act requirements for mine reclamation (Lupton et al., 2013; Rojas et al., 2014).

The objective of the present study was a qualitative survey of bacterial and eukaryal diversity in the vegetated soil six years after reclamation compared to a control soil covered by a biological crust. At the time of sampling in 2011, the pH values of reclaimed precipitates had declined to those of control precipitates (2.5–2.7). We expected reclaimed precipitates to exhibit greater bacterial and eukaryal diversity than control precipitates and aimed to see how the single intervention had affected microbial composition. We also expected that both types of precipitates would host relatively few taxa associated with other studied AMD systems, such as subterranean biofilms at Richmond Mine in California (Bond et al., 2000) and flowing surface waters of the Tinto River in Spain (Amaral-Zettler et al., 2011). These taxa include lithoautotrophs such as *Acidithiobacillus* and *Leptospirillum* spp. and acid-tolerant microscopic eukaryotes (Amaral-Zettler et al., 2011; Baker and Banfield, 2003; Johnson, 2012; Palacios et al., 2008; Tyson et al., 2005). The presence of such taxa have not been studied in a reclaimed AMD system such as this one, which has been exposed to sunlight, drying, and carbon enrichment by incipient vegetation (Lupton et al., 2013).

We used 454 barcoded FLX pyrosequencing of 16S and 18S rRNA genes to compare bacterial and eukaryal taxa in precipitates at two different depths differing in organic carbon enrichment from plant roots and moss rhizoids. This study thus enabled assessment of microbial diversity in soils having similar low pH but different carbon inputs and management histories.

2. Materials and methods

2.1. Background and site description

The research site lies within a 50-year-old AMD barrens (0.5 ha) approximately 5 km north of Kylertown, PA, 41°01′22.00′N; 78° 09′ 08.064′ W (Lupton et al., 2013). Native soils at this site are classified as Brinkerton (Typic Fragiaqualfs) and Ernest (Aquic Fragiudults) soil series developed from mixed colluvium. Fragipan layers at depths of 55–70 cm impede permeability of both soils, resulting in fluctuating water tables and shallow subsurface flows (Lupton et al., 2013). In this area vegetation had been killed by massive overland flow of AMD from a natural spring emerging from an abandoned deep mine complex (Lower Kittanning coal seam). As overland flow eventually became channelized, areas were left with thick (up to 35 cm) layers of ferric iron (oxyhydr)oxide precipitates that had accumulated on surfaces of native soils. These precipitates consisted mainly of ferrihydrite and goethite and had extremely high Fe contents ranging from 641 to 751 g kg^{-1} (Rojas et al., 2014). The only observable vegetative cover in the barrens were mossy biological crusts in wetter areas with shallow subsurface flow (Prasanna et al., 2011). Concentrations of As, Cd, and Co in these precipitates were similar to those reported for other AMD systems, while other metal concentrations were within ranges reported for unpolluted soils (Rojas et al., 2014).

In 2006, experimental plots (3 m × 3 m) were established in crust-covered areas by rototilling lime and compost into the upper 15 cm of the plots. A mixture of $MgCO_3$ and $Ca(OH)_2$ (11 t ha^{-1}) was added to achieve pH of 4.5 (similar to adjacent forest soils). Compost (2.8% N) was added at 27 t ha^{-1} to provide a total N addition of 756 kg N ha^{-1}, most of which was organic N. After rototilling, plots were mulched with oat straw (10 t ha^{-1}), containing viable seed that served as a nurse crop during establishment of sown legumes and grasses (Lupton et al., 2013). For details on soil properties and successional vegetative cover see supplementary material (Table S1). Five years following reclamation, indigenous woody species consisting of *Betula* and *Populus,* as well as *Acer, Pinus, Spiraea* and *Crataegus* spp., had replaced the sown plant species in reclaimed plots (Rojas et al., 2014). After the 2006 reclamation treatment, successional plant growth remained at >90% of areal cover, even though soil pH had declined to 2.8, similar to the pH of control precipitates (2.5).

2.2. Sample collection and processing

One plot each of reclaimed and control precipitates (5 m apart) were sampled in July 2011 by excising one random square section (0.25 m^2, 8 cm thickness) from the central portion of the plot. Plant roots in reclaimed precipitates were concentrated in the upper 5-cm layer, while moss-rhizoid layers in control precipitates were 2–3 mm thick. In the field, upper and lower layers were separated for separate processing. For the reclaimed precipitates, the topmost 5-cm layer adhering to plant roots was labeled RR for "reclaimed root-adherent." The underlying 3-cm layer was labeled RB for "reclaimed below-root-adherent" precipitates. For the control precipitates, the topmost 2-cm layer of precipitates adhering to the biological crust was labeled CC for "control crust-adherent", after being scraped aseptically from the crust. The underlying 6-cm layer was labeled CB for "control below-crust-adherent". Samples were placed on ice for transport to the lab, where plants, roots, and large organic debris were removed. Bulked precipitates were mixed and split to obtain three subsamples from each of four layers (RR, RB, CC, CB). Subsamples were analyzed separately for gravimetric moisture content, total organic carbon (TOC) and nitrogen (N), pH, EC as described previously (Rojas et al., 2014). Microbial community DNA was extracted from three 0.35-g subsamples (moist weight) per layer and pooled for pyrosequencing analysis. DNAs were extracted with the MoBio PowerLyzer™ Power Soil® DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA), following manufacturer's instructions except that use of Solution 3 (inhibitor removal) was omitted to improve yield. The DNA concentrations were determined using a NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific Inc.).

2.3. Pyrosequencing of soil DNA extracts

Community DNAs were subjected to bar-coded amplicon library preparation (i.e. targeted sequences of DNA) and pyrosequencing using the 454 Genome Sequencer FLX Titanium System and molecular biology reagents according to manufacturer's protocols (Roche Diagnostics, Indianapolis, IN, USA) at The Pennsylvania State University Genomics Core Facility. PCR amplicon libraries were prepared for each sample using universal bacterial primers (27F-907R), complementary to regions V1–V5 of the 16S rRNA gene (Lane, 1991), and eukaryotic primers (518F-1193F), complementary to V4 and V5 regions of the 18S rRNA (Baker et al., 2004). Bacterial forward and reverse primers, respectively, were (5′-AGAGTTTGATCMTGGCTCAG-3′) and (5′-CCCCGTCAATTCMTTTGAGTTT-3′). Eukaryal forward and reverse primers were (5′-GAGGRCMAGTCTGGTGC-3′) and (5′-GGGCATMACDGACCTGTT-3′), respectively. Each primer oligonucleotide contained an upstream 10-bp barcode, a 454 Roche-adaptor and a 4-bp key sequence. The PCR reactions contained 1 μL (5 μM) of each forward and reverse primer, 0.5 μL of dNTP mix (10 mM each), 0.25 μL of 5 U FastStart HiFi polymerase, 2.5 μL FastStart buffer and 1 μL of DNA extract per 25 μL reaction volume. Reaction conditions consisted of an initial denaturation step at 94 °C for 3 min followed by 35 cycles, each consisting of denaturation at 94 °C for 15 s, primer annealing at 55 °C for 45 s, and extension at 72 °C for 1 min with a final extension at 72 °C for 8 min. After PCR amplification, products were treated with 70% ethanol and AMPure magnetic beads as directed by the manufacturer and selected for sizes of at least 500 bp