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ORIGINAL ARTICLE

Bacterial populations within copper mine tailings: long-term effects of amendment with Class A biosolids

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Abstract

Aim: This study evaluates the effect of surface application of dried Class A biosolids on microbial populations within copper mine tailings.
Methods and Results: Mine tailing sites were established at ASARCO Mission Mine close to Sahuarita Arizona. Site 1 (December 1998) was amended with 248 tons ha^{-1} of Class A biosolids. Sites 2 (December 2000) and 3 (April 2006) were amended with 371 and 270 tons ha^{-1}, respectively. Site D, a neighbouring native desert soil, acted as a control for the evaluation of soil microbial characteristics. Surface amendment of Class A biosolids showed a 4 log_{10} increase in heterotrophic plate counts (HPCs) compared to unamended tailings, with the increase being maintained for 10-year period. Microbial activities such as nitrification, sulphur oxidation and dehydrogenase activity were also sustained throughout the study period. 16S rRNA clone libraries obtained from community DNA suggest that mine tailings amended with biosolids achieve diversity and bacterial populations similar to native soil bacterial phyla, 10 years postapplication.
Conclusion: Addition of Class A biosolids to copper mine tailings in the desert south-west increased soil microbial numbers, activity and diversity relative to unamended mine tailings.
Significance and Impact of the Study: The amended tailings resulted in a functional soil with respect to microbial characteristics, which were sustainable over a 10-year period enabling the development of appropriate vegetation.

Introduction

Mining of ores for copper is a significant industry throughout many areas of Arizona and many other regions around the world. During this process, large amounts of material are initially removed to provide access to the ore itself. These deposits are termed 'overburden' and result in human-made mountains of material often resembling natural mesas. In addition to these disturbances, there is also a need to deposit the processed ore following extraction of the copper-containing minerals. These so-called mine tailings are often placed in desert areas to a depth of 35 m. Such degradation of land is a major concern in many countries (Harris 2003).

Mine tailings are essentially crushed rock, and as such are a perfect inorganic matrix resembling soil; however, unweathered mine tailings are unique, unlike any natural soil. Tailings have very low cation exchange capacities, minimal microbial populations and almost zero organic matter content. Left alone, mine tailings are unsightly and only allow for scant vegetative growth. This in turn leads to erosion via wind, which can create dust storms. Tailings have very low concentrations of macronutrients for plant growth, especially nitrogen, and conversely can contain high concentrations of heavy metals, which can be particularly problematic in tailings where the pH is low. In such instances, soil fertility and microbial communities are drastically reduced (Seaker and Sopper 1988).

Therefore, there is a need for the reclamation of tailings to allow for subsequent revegetation and ecosystem stability. One potential solution allowing for the reclamation of mine tailings is the use of biosolids that are produced at every municipality throughout the state and nation (over 8 million tons per year) (Marx *et al.* 1995). Biosolids interestingly represent another human-made waste that needs to be disposed of or utilized. The term 'biosolids' implies treatment to produce Class A or Class B biosolids that meet the land application standards in the Part 503 Environmental Protection Agency regulations (US EPA 1994). Class A biosolids are treated so that they contain nondetectable levels of pathogens (NRC 2002).

In recent years, there has been a growing recognition and interest in the use of biosolids to restore metal-affected ecosystems (Brown *et al.* 2003). In 1994, the Arizona Mined Land Reclamation Act was passed that required reclamation of all mining disturbances on private land to a predetermined postmining use. In 1996, the Arizona Department of Environmental Quality (ADEQ) adopted new rules allowing for the use of biosolids during reclamation.

The use of biosolids for land reclamation has been researched extensively for at least two decades. As of 1993, approximately 72 sites in the United States had been reclaimed using biosolids (Sopper 1993). However, most of this research has been conducted at sites in the coal mining region of Pennsylvania, and only limited research has been conducted in the Western United States (Thompson and Rogers 1999). More research is needed on reclamation of copper mine tailings in the arid south-west. In particular, microbial community populations, structure and activity are important indicators of ecosystem recovery that need to be evaluated during the reclamation process (Seaker and Sopper 1988; Kelly and Tate 1998; Yin *et al.* 2000).

The goal of mine tailing reclamation is to ensure self-sustainable microbial and plant communities (Pond *et al.* 2005; Rosario *et al.* 2007). As such, activities of soil micro-organisms are essential to maintain a healthy ecosystem. Microbial communities play a key role in restoring mine sites by promoting plant growth through the mineralization of inorganic nutrients (Xu and Johnson 1995), development of soil structure (Maier *et al.* 2008), nutrient cycling (Burd *et al.* 2000) and reduction in metal toxicity (Salt *et al.* 1999; Sandrin *et al.* 2000). Micro-organisms are also known to be resistant, resilient and functionally redundant in response to disturbances or changes in the environment (Allison and Martiny 2008).

The purpose of this study was to monitor the microbial ecological structure and diversity as a function of time following biosolids land application onto mine tailings located in southern Arizona. The hypothesis of this study was that a sustainable soil bacterial population could be established following amendment of mine tailings with biosolids following which significant revegetation of the tailings would occur. Biosolids containing approximately 90% solids was added to different mine tailing sites in 1998, 2000 and 2006. The objectives of this work were to evaluate long-term effects of land application of biosolids on (i) bacterial numbers, (ii) common microbial transformations or activity (nitrification, sulphur oxidation and dehydrogenase activity), and (iii) microbial diversity post-biosolid amendment. Note that the assays for bacterial numbers and transformation were all carried out on samples taken from two sites (Sites 1 and 2). The diversity analyses were conducted on multiple sites that included sites 1, 2 and 3 (See Table 1).

Materials and methods

Site description and soil sampling

The present study was conducted near a copper mine (Mission Mine) site located at Sahuarita, Arizona, (24 km) south of Tucson (31°58′45·46″N, 111°02′58·69″

Table 1 List of study sites, amount of biosolids added to the tailings, year of biosolid application and when sites were sampled

			Date of samples when collected for analysis	
Sampling site	BS applied (tons ha^{-1})	Date of BS application	Microbial numbers (HPCs) and activity	Microbial diversity
Mine tailings	–	–	December 1998*	December 2009†
Site 1	248	December 1998	December 2008	December 2009
Site 2	371	December 2000	December 2008	December 2009
Site 3	270	April 2006	–	April 2006 & December 2009
Desert soil	–	–	December 2008	December 2009

BS, Biosolids; HPCs, heterotrophic plate counts.

*Soil samples were analysed only for microbial numbers (HPCs).

†PCR on extracted community DNA was not successful on unamended tailing samples that were collected from mine tailings site.

W), within the arid Sonoran Desert environment where average summer temperatures are 35°C and rainfall is minimal (<300 mm annually) (Fig. 1). Sites varied in terms of when the biosolids were added and the amount of biosolids added. The specific sites are shown in Table 1. An additional difference between the sites was that at Site 1, the biosolids were tilled into the soil, whereas for sites 2 and 3, the biosolids were not tilled into the soil and remained on the surface of the tailings.

Two other control areas encompassing approximately 10 ha each were also part of the studies: unamended tailings between the two biosolids plots and undisturbed desert immediately east of the tailings (Fig. 1). The applied biosolids were a cake with moisture content of approximately 10% and contained approximately 10^8 CFU g^{-1} dry weight of biosolids of culturable heterotrophic bacteria (HPC).

The aim of the study was to determine the influence of land application of biosolids on culturable (HPC) and soil moisture content in mine tailings amended with biosolids at Sites 1 and 2. In December 2008, soil samples were collected from Sites 1 and 2; a pristine desert soil located adjacent to the mine tailings and unamended mine tailings. The soil samples consisted of soil cores taken with a 7·5-cm-diameter soil auger. At each location, cores were taken to a depth of 150 cm in 30 cm increments, that is, five separate samples per core. Soil cores were composited in a sterile ziploc freezer bag and transported back to the laboratory. Samples were sieved through a 2-mm mesh screen, and gravimetric water content was determined before further analysis. All soil core samples were subsequently analysed for microbial characteristics. Additional surface samples were collected from Site 3 in April 2006, 3 weeks after biosolid amendment for microbial community analysis via construction of clone libraries. All sites were sampled in December 2009 for bacterial diversity analysis. Therefore, diversity estimates within biosolid-amended tailings were made ≃1 month, 3·5 years, 9 years and 11 years after biosolid addition. PCR amplification was not successful on the unamended tailings. This is most likely due to PCR inhibition by heavy metals because the total Cu concentrations of the mine tailings ranged from 570 to 2500 ppm and total Mo concentrations ranged from 45 to 195 ppm. Therefore, sequence data were determined for Site 3 (3 weeks after application), Site 3 (3·5 years after application), Site 2 (9 years after application), Site 1 (11 years after application) and natural desert soil.

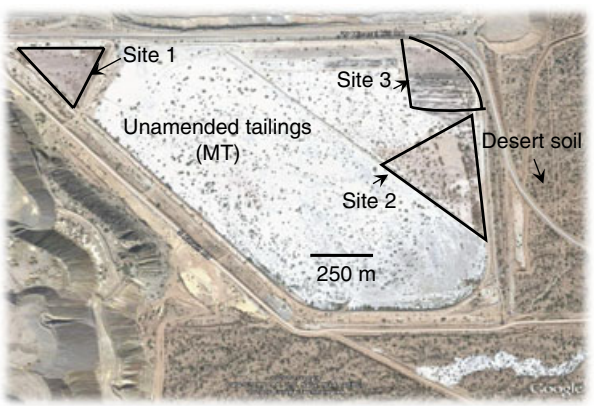

Figure 1 Twenty-one November 2009 Quickbird image of the study site showing the three biosolids plots, unamended tailings and undisturbed desert soil east of the tailings. Site 1, sampled 10 and 11 years after application; Site 2, sampled 8 and 9 years after application; Site 3, sampled 3 weeks and 3·5 years after application. Also shown are natural desert soil areas and white coloured unamended mine tailings = MT.

Heterotrophic plate counts microbial activity and soil moisture content

Heterotrophic plate counts (HPCs) were determined within samples at all sites. From all samples, 10 g of soil from each site was placed in a 250-ml jar containing 95 ml 0·1% peptone solution (EMD Chemicals Inc. Gibbstown, NJ, USA) and was shaken vigorously for 10 min. Culturable cells were enumerated by 10-fold serial dilutions with physiological saline (0·85% NaCl) (EMD Chemicals Inc.) followed by plating on R_2A agar (Difco Co., Sparks, MD, USA) and incubation at 27°C for 7 days. Counts were reported as colony forming units (CFU) per gram dry weight of each sample.

Soil samples were analysed to evaluate microbial activity using analyses for nitrification, sulphur oxidation and dehydrogenase activity. The nitrification potential was determined using a method that assessed the maximum rate of nitrification within soil samples (Hart *et al.* 1994). The method ('shaken soil-slurry method for assessing the nitrification potential in soil') involves shaking fifteen grams of a sieved field moist soil sample in a dilute ammonium phosphate solution (approximately 1 : 7 soil/solution ratio) and the oxidation of ammonium to nitrate in a two-step transformation by nitrifying organisms.

The rate of sulphur oxidation in soil samples was determined by measuring the amount of sulphate (SO_4^{2-}) produced during a specific period after application of S° to soil samples. Sieved field moist soils (240 g) were amended with 0·5 g S°. Sulphate was extracted using a NaCl extraction solution, which aids in dispersing soil particles. The rate of sulphur oxidation was determined by measuring changes in the soil sulphate concentration over a period of three weeks by measuring absorbance via a spectrophotometer at 470 nm (Hart *et al.* 1994; Pepper and Gerba 2004).

Living organisms found in soils have dehydrogenase enzymes (enzymes catalysing dehydrogenation) involved in oxidation of organic compounds. Therefore, the result of the dehydrogenase assay could be regarded as average activity of microbial population in soil (Maier *et al.* 2008). Dehydrogenase activity was determined in duplicate using a procedure modified from Pepper and Gerba (2004). The method involves colorimetric determination of 2, 3, 5-triphenyl formazan (TPF) produced by the reduction in 2, 3, 5-triphenyltetrazolium chloride (TTC) by soil micro-organisms. Six grams of the sieved field moist soil was mixed with 3% TTC (v/v) in sealed test tubes. The tubes were incubated at 30°C for 4 days followed by methanol extraction and quantification of water-insoluble red dye (TPF) using a spectrophotometer at 485 nm.

Soils were also monitored for moisture content on a dry weight basis from 2000 to 2008 with multiple (3–5) analyses made each year.

Analysis of variance (one-way ANOVA) was performed on data from all sites using the statistical software package (SYSTAT Software, Inc, San Jose, CA, USA). Results of all tests were considered significant at $P < 0{\cdot}05$.

Bacterial community DNA extraction, PCR amplification, cloning and sequencing of 16S rRNA

Soil community DNA was extracted using a MO BIO PowerMax® Soil DNA Isolation kit (MoBio Laboratories, Carlsbad, CA, USA). A total of 10 g soil (soil cores were taken from a depth of 0–30 cm) was used for community DNA extraction. Extracted DNA was repurified and concentrated to a 30 μl DNA solution using a Qiagen PCR purification kit (Qiagen Inc., Valencia, CA, USA). Presequencing PCR amplification of the 16S rRNA with the community DNA was performed in an Applied Biosystems GeneAmp PCR system 2700 (Applied Biosystems, Foster City, CA, USA) under the following conditions: 1× PCR buffer, 2·5 mmol l^{-1} $MgCl_2$, 0·2 mmol l^{-1} dNTP (equimolar each of dNTPs), 1·2 U of *Taq* Gold DNA Polymerase (Applied Biosystems), primers 341F (5′-CTCCTACGGAGGCAGCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (0·4 μmol l^{-1} each) (Integrated DNA Technologies, Coralville, IA, USA), dH_2O and 0·25 ng template DNA concentration per reaction tube. PCR cycling conditions: 10 min at 95°C (initial denaturation), then cycled 35 times at 95°C (denaturation) for 40 s, 51°C (annealing) for 45 s and 72°C (extension) for 1 min 10 s, followed by a final extension at 72°C for 5 min and hold at 4°C. PCR products (approximately 1151 bp) were purified with a Qiagen PCR purification kit (Qiagen Inc., Valencia, CA, USA) and eluted with 50 μl elution buffer.

The entire volume was then electrophoresed through a 1·5% (w/v) agarose and 1× Tris Borate gel at 120 V for 60 min. The gels were stained with ethidium bromide and visualized under an ultra violet (UV) light box operated at approximately 312 nm. The PCR products were excised from the gel then purified using a Qiagen Gel Purification kit (Qiagen).

PCR product (≃1151 bp) was ligated into a transport plasmid TOPO TA Cloning® kit, containing pCR®2.1-TOPO vector. Direct cloning of PCR product using these commercially available kits is often difficult because proofreading polymerase removes the 3′ adenine (A)-overhang necessary for TA cloning. Therefore, the purified PCR product was incubated with additional dATP for 60 min at 72°C. After the addition of 3′-overhangs, 4 μl of the postamplification product was used for ligation to the TOPO TA Cloning vector (TOPO TA Cloning® vectors, Carlsbad, CA, USA) for 30 min at room temperature (22–23°C) and then tubes were placed on ice. Once ligated, the vectors were then transformed into the TOPO10 One Shot™ (Invitrogen Corp., Carlsbad, CA, USA) *Escherichia coli*, chemically competent cells (each 50 μl) following the manufacturer's procedure. Individual colonies were screened for cloning efficiency and confirmation of the insertion of the 16S rRNA PCR product into the vector. More than 270 clones per sample were screened for sequence analysis using M13F (5′-CTGGCCGTTTTAC-3′) and M13R (5′- GTCATAGCTGTTTCCTG-3′) primers. The 16S rRNA products were purified and quantified prior to sequencing using primer 341F primer and a 3730XL DNA analyzer (Applied Biosystems) at the University of Arizona Genetics Core sequencing laboratory.

16S rRNA sequence analysis

Sequences were trimmed at fixed intervals (<30 and >650 bp) using FAKTORY (Biotechnology Computing Facility, University of Arizona) (Miller and Myers 1999), and only sequences with at least 600 bp of readable sequence were used for further analysis. The clones were characterized by 16S rRNA sequence analysis and compared to known sequences in GenBank using BLAST (Basic Local Alignment Search Tool, National Center for Biotechnology Information) (Altschul *et al.* 1990). BLASTN results were then viewed and organized using Sequence Comparison Output Organizing Tool (SCOOTaR) (Arizona Research Biotechnology Computing Facility, University of Arizona). Sequences that showed at least 96% homology were used for identification at the genus level. All sequences were deposited in a public database (GenBank, Bethesda, MD, USA) with the following accession numbers: JN693517–JN694583.

The 16S rRNA gene sequences were classified using the Ribosomal Database Project II (RDP), naïve Bayesian (Wang *et al.* 2007). MOTHUR (Schloss *et al.* 2009) was

also used to assign sequences to operational taxonomic units (OTU) defined at the 0·05 level. Comparisons were made between biosolids-amended mine tailings and desert soil (control) using library pairwise analyses. Analyses comprised rarefaction, chao-richness, Shannon-diversity, libshuff and parsimony tests. Statistical significance was assessed using a Tukey-corrected $\alpha = 0{\cdot}025$ (Schloss *et al.* 2009).

Results

Heterotrophic plate counts microbial activity

The bacterial HPC count in the biosolids-amended tailings increased from $<10^3$ CFU g^{-1} dry soil (unamended mine tailings) to $\simeq 10^7$ CFU g^{-1} dry soil following Class A biosolid addition (Fig. 2). This indicates that the influence of biosolids ($1{\cdot}8 \times 10^7$ CFU g^{-1} dry biosolid) was effective in initially providing a large bacterial population within the tailings. In addition, the data also show that quantitatively, the enhanced bacterial populations have been effectively maintained for more than 10 years (Site 1) following biosolid amendments (Fig. 2). Soil moisture content in the high-rate biosolids-amended tailings plots (Site 2) was frequently higher than in the low-rate biosolid-amended mine tailings (Site 1) and desert soil (Table 2).

At both sites, amendment of tailings with Class A biosolids was shown to enhance microbial activity relative to values measured in natural desert soils (Table 3). Both biosolid-amended Sites 1 and 2 had higher nitrification activity and sulphur oxidation than adjacent desert soils, indicated by the higher nitrate and sulphate concentrations, respectively. Interestingly, Site 1 had consistently higher values than Site 2 for both assays. In contrast, the dehydrogenase assay showed that the formation of TPF was higher in the surface (0–30 cm) of the desert soil than in the biosolid-amended tailings (Table 3). In general, microbial activity diminished at the lower (90–150 cm) depths of the soil profile except Site 1, which showed elevated sulphate concentrations at lower depths.

Soil moisture content differed significantly by soil depth ($F = 58{\cdot}81$ $P = 0{\cdot}001$), year of sampling ($F = 2{\cdot}68$ $P = 0{\cdot}015$) and site ($F = 8{\cdot}53$ $P = 0{\cdot}004$). Overall mean soil moisture content (averaged over all depths) at Site 1 was 6·55% (SE = 0·22) compared to 8·11% (SE = 0·54) at Site 2.

16S rRNA sequence analysis

The results from the Ribosomal Database Project II (RDP II) 16S rRNA gene sequence analysis are shown in Table 4, with most (approximately 90%) sequences identified to known bacterial taxon levels of bacterial origin. The RDP II 16S rRNA gene sequence analyses have indicated that members of the major phyla including *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Acidobacteria* and *Bacteroidetes* were widespread in all sites (copper mine tailings and desert soil samples). Sequences belonging to the bacterial phyla, *Proteobacteria* (23·9–31·4%)

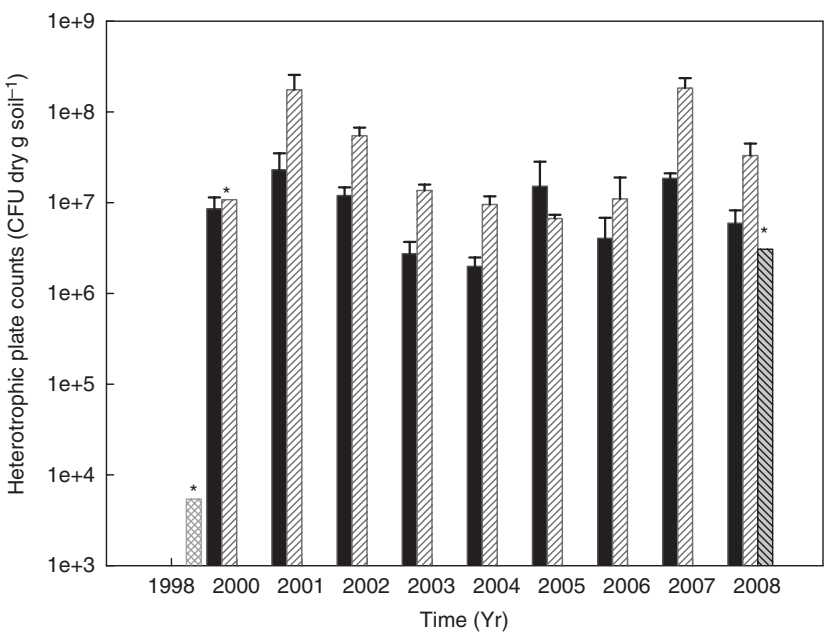

Figure 2 Heterotrophic Plate Count (HPC) Populations in Surface (0–30 cm) Mine Tailings and natural desert soil (December 2008). Data represent mean of HPCs on soil samples collected from December 1998 to December 2008. (means values ± SE). *Sites were sampled once per year. Site 1 and Site 2, biosolid amended tailings; D, natural Desert soil; and MT, unamended mine tailings. (■) Site 1; (▨) Site 2; (▧) D; (▩) MT.

Table 2 Soil moisture (%) in mine tailings amended with biosolids at Sites 1, 2 and desert soil

		Depth (cm)				
Site	Year	0–30	30–60	60–90	90–120	120–150
Site 1	2000	7·4	6·3	8·2	11·2	na
	2001	5·6	5·1	6·6	8·4	10·2
	2002	5·2	4·0	5·4	6·7	8·8
	2003	6·6	4·8	6·1	7·1	8·7
	2004	6·0	4·5	6·3	7·1	8·6
	2005	7·6	4·1	5·1	6·2	7·1
	2006	2·7	5·5	3·4	2·6	3·4
	2007	2·9	3·2	4·7	5·1	8·7
	2008	4·5	3·5	4·0	4·7	8·0
	Mean	5·4	4·6	5·5	6·6	7·9
Site 2	2000	na	na	na	na	na
	2001	13·5	10·6	9·1	8·6	10·6
	2002	11·1	7·5	8·0	7·3	9·0
	2003	13·2	10·7	7·6	7·9	8·9
	2004	8·8	8·6	6·0	6·0	8·4
	2005	9·0	8·4	5·9	6·3	7·4
	2006	3·7	6·9	3·9	6·7	6·7
	2007	7·2	7·1	7·2	8·0	8·9
	2008	6·8	5·8	4·3	4·9	6·3
	Mean	9·2	8·2	6·5	7·0	8·3
Desert soil	2008	4·1	4·5	3·9	3·2	2·5

na, data not available.

and *Actinobacteria* (9·3–41·6%), were found to be the most dominant groups in all sites with the exception of sequences affiliated with the phylum *Firmicutes* which were significantly higher (52·6%) at Site 3 where samples were taken 3·5 years after biosolids application (Table 4). The class Alphaproteobacteria comprised the largest

Table 3 Microbial activities of amended tailings and desert soil. (means values ± Standard deviation, Site 1 = 10 years after application, Site 2 = 8 years after application and natural desert soil)

Site	Depth (cm)	Nitrification (NO_3, mg kg^{-1})	Sulphur oxidation (μgSO_4-S per g dry soil)	Dehydrogenase (μgTPF per g dry soil)
Site 1	0–30	71·2 ± 1·6	2253·9	11·0 ± 3·0
	30–60	42·3 ± 0·6	2351·4	11·9 ± 2·6
	60–90	13·5 ± 1·1	2377·8	11·4 ± 1·7
	90–120	3·3 ± 0·1	3037·5	6·8 ± 1·3
	120–150	2·0 ± 0·0	3170·5	3·4 ± 0·9
Site 2	0–30	4·6 ± 0·00	1603·1	10·1 ± 0·9
	30–60	0·4 ± 0·05	1682·8	1·6 ± 1·7
	60–90	0·2 ± 0·00	1586·9	9·8 ± 3·8
	90–120	<0·1 ± 0·00	1824·3	8·9 ± 1·7
	120–150	<0·1 ± 0·00	1422·3	8·6 ± 2·1
Desert soil	0–30	1·9 ± 0·01	113·1	60·4 ± 12·4
	30–60	0·3 ± 0·02	36·2	8·6 ± 2·1
	60–90	0·9 ± 0·01	25·4	7·1 ± 0·9
	90–120	0·7 ± 0·00	34·5	3·7 ± 1·3
	120–150	0·7 ± 0·03	100·2	2·8 ± 3·4

Table 4 Ribosomal Database Project II (RDP) Classification of 16S rRNA Bacterial Clone Libraries (sequence clone isolates% distribution per sample) for the copper mine tailings amended with Class A biosolids and natural desert soil

	Treatment (Site)				
Phylum	Site 3*	Site 3†	Site 2	Site 1	Desert soil
Proteobacteria	23·9	25·2	30·7	31·4	29·0
Proteobacteria (alpha)	21·1	49·3	63·9	57·6	48·1
Proteobacteria (beta)	59·2	13·4	10·8	12·9	26·6
Proteobacteria (delta)	1·4	1·5	7·2	1·2	8·9
Proteobacteria (gamma)	16·9	31·3	12·0	28·2	12·7
Unclassified *Proteobacteria*	1·4	4·5	6·0	0·0	3·8
Actinobacteria	23·9	9·3	31·4	41·6	25·7
Firmicutes	16·2	52·6	5·8	2·2	1·1
Acidobacteria	0·0	1·1	14·4	3·6	17·3
Bacteroidetes	26·6	2·6	3·6	9·9	12·1
Chloroflexi	3·0	3·0	3·6	1·5	0·7
TM7	0·3	0·7	1·1	3·6	0·7
Gemmatimonadetes	0·0	0·4	0·4	2·6	0·4
Deinococcus-Thermus	0·0	1·5	0·0	0·0	0·0
Verrucomicrobia	0·0	0·0	0·0	0·4	0·7
OD1	0·0	0·7	0·0	0·0	0·0
Nitrospira	0·0	0·0	0·0	0·4	0·4
Ktedonobacteria (class)	0·0	0·0	0·0	0·0	0·4
Planctomycetes	0·3	0·0	0·0	0·4	0·0
Cyanobacteria	0·0	0·0	0·0	0·0	0·4
Unclassified bacteria	5·7	3·0	9·0	2·6	11·0
Total number of clones	297	270	277	274	272

*Site 3 sampled 3 weeks after biosolids application.
†Site 3 sampled 3·5 years after biosolids application.

percentage of the Proteobacteria (21·1–63·9%) of the clones but soil sampled 3 weeks after biosolid application was dominated by Betaproteobacteria (59·2%). This high percentage found soon after land application could have been due to the presence of nitrifying bacteria known to oxidize ammonia (Wells *et al.* 2009).

Species richness and diversity measurements, conducted using MOTHUR, are shown in Table 5. The results show that among the five populations, Site 3† and Site 2 had the greatest estimated richness according to the chao-estimator at a 0·05 OTU definition level, with the desert soil closely following. Site 1 and Site 3‡ both demonstrated lower estimated richness. The diversity index (calculated by Shannon-estimation) demonstrated that Site 2 and the desert site were more diverse than other sites. Parsimony and libshuff pairwise comparisons demonstrated that sequence libraries were all significantly different from one another.

Vegetative cover

The extent of vegetation within the sites was estimated using both traditional vegetation transects and via

Table 5 Summary of 16S rRNA bacterial clone libraries and diversity estimates for the copper mine tailings amended with Class A biosolids and natural desert soil

Site	Number of clones	Unique OTUs*	Richness-estimation Chao	Diversity index Shannon
Site 3†	297	143	428·4	4·60
Site 3‡	270	139	258·3	4·22
Site 2	277	174	549·3	4·92
Site 1	274	129	210·4	4·63
Desert Soil	272	170	369·6	4·91

*Operational taxonomical units (OTUs) defined at the 0·05 level.
†Site 3 sampled 3 weeks after biosolids application.
‡Site 3 sampled 3·5 years after biosolids application.

panchromatic Quickbird satellite imagery. At the time of soil sampling for this study, the percentage vegetative cover on biosolid-amended plot was 56–59% compared to 25–27% for indigenous Sonoran Desert vegetative cover.

Discussion

Soil amendment with biosolids has been shown to increase both the numbers and the activity of micro-organisms (Seaker and Sopper 1988). Biosolid amendment has also been shown to enhance soil fertility, plant growth and other physical properties of amended tailings (Brown *et al.* 2003; Pond *et al.* 2005). The present study showed a significant increase in HPC counts in the biosolids-amended tailings. HPC counts increased from $<10^3$ CFU g^{-1} dry tailings to 10^7 CFU g^{-1} dry soil following Class A biosolid addition. This indicates that biosolids were effective in promoting large bacterial populations, which have since lasted over a decade.

Soil moisture contents in the high-rate biosolids-amended tailings, Site 2, were frequently higher than in the low-rate biosolid-amended mine tailings (Site 1) and desert soil (Table 2). In addition, data from the present study showed a trend of increased microbial activity in tailings following biosolid amendment. However, it is of interest that microbial activities were higher at Site 1 than at Site 2 despite the fact that Site 2 received a higher loading rate of biosolids. These differences may be due to the fact that biosolids were incorporated into the soil by tilling at Site 1, whereas at Site 2, the biosolids remained on the soil surface. The fact that microbial activities were higher in the biosolid-amended tailings than in soils may be due to the fact that the vegetative cover on the biosolid-amended tailings was greater than that within desert soil. Vegetation is known to promote bacterial populations and activity through root oxidation which results in the formation of rhizosphere soil. Microbial activity assays were not conducted on the unamended tailings because of a lack of carbon substrate (0·03% total organic carbon) and a lack of microbes ($5·4 \times 10^3$ heterotrophic plate count bacteria/g). The observed increase in microbial activity in these biosolid-amended mine tailings has been reported in other study (Brown *et al.* 2003). Similarly, other studies have reported that amendment of Class B biosolids into agricultural lands (Sullivan *et al.* 2006; Zerzghi *et al.* 2009) and mine tailings (Chander *et al.* 2001; Pond *et al.* 2005) resulted in enhanced microbial activity.

A successful reclamation plan for copper mine tailings using biosolids needs to address the specific characteristics of each site and continued monitoring over time is necessary to ensure that no adverse environmental impacts occur. Cultural assays on soil microbial numbers and activity are useful to determine the effects of specific soil amendments on soil bacteria, but they fail to give a complete picture of the status of the soil system. Combined conventional and molecular analyses of bacterial communities are needed to gain useful information on the effectiveness of restoration process, because of the presence of large unculturable soil bacterial populations (Harris 2003).

The RDP II 16S rRNA gene sequence analysis was carried out to identify dominant bacterial population, and data showed that the most persistent bacterial populations were members of dominant soil bacterial phyla including *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes* and *Acidobacteria* (Tables 4 and 5). This is consistent with most studies of soil bacterial communities (Kuske *et al.* 1997; Sun *et al.* 2004; Janssen 2006). Studies have shown that by analysing the diversity of these bacterial communities, it is possible to determine whether the microbial communities that develop in amended mine tailings are similar to those found within indigenous desert soils (Chen *et al.* 1988; Mendez *et al.* 2008). In this study, both the reclaimed mine tailings and the desert soil comprised soil bacterial organisms similar to members found in desert soils from the south-western United States reported by Kuske *et al.* (1997) and in most typical soils (Maier *et al.* 2008). It is also of interest that the percentage of unclassified bacteria from the desert soil was in fact higher than the percentage from the other sites. This illustrates the fact that our knowledge of desert soil bacteria is limited and warrants further research.

Initially, sequences affiliated with the phylum *Firmicutes* seemed to dominate at the early stage of the study (Site 3) but eventually their prevalence diminished with time. This might be due to the fact that *Firmicutes*, *Bacteroidetes* and *Actinobacteria* are known to dominate 80% of identified bacteria in the human gut (Mariat

et al. 2009). Thus it could be expected that after the initial application of biosolids, biosolid-associated bacteria (not soil) would dominate microbial populations. This confirms the hypothesis of this study in which amendment of Class A biosolid into nutrient-poor mine tailings would ultimately lead to the establishment of a functionally redundant soil bacterial population followed by subsequent revegetation.

Overall, analyses based on OTUs using MOTHUR indicated that biosolids treated mine tailings have eventually acquired diversity levels approaching that of the desert soil (Table 5). Shannon calculations showed that Sites 3† (3 weeks after application), 2 (9 years after application) and the desert soil samples had a slightly higher level of diversity, while the others were all similar to one another, possibly due to each site eventually reaching a level of diversity similar to the desert soil. Diversity and chao-estimated richness levels at Site 3‡ (3 years after application) were among the lowest of the libraries, despite site 3† (3 weeks after application) demonstrating high chao-richness and second lowest diversity. Site 2 demonstrated the largest chao-estimated richness and likewise yielded the highest diversity. This site was collected 9 years following land application and may indicate a peak richness and diversity before final establishment of a stable desert-like soil microbial community. Community comparisons demonstrated that all populations were comprised of variable constituents and structure and yielded significantly different populations.

These analyses demonstrated that even 3 weeks after amendment with biosolids, mine tailings have diverse bacterial populations of both biosolid and soil origin. Finally, such bacterial populations might produce process rates similar to a typical desert soil original community. Soil microbial measurements have demonstrated that there are changes in bacterial numbers, activity and diversity. These are positive signs for a successful reclamation process and illustrate a useful means to the dispose biosolids. This is important because many states produce copper in the United States as well as large amounts of mine tailings. The study has shown that amendment of Class A biosolids has the potential to improve the quality of these 'useless' copper mining wastes by providing plant growth medium that allows for revegetation and reduces air pollution.

Summary

The current study showed that the use of high-quality biosolids (Class A biosolids) has been effective in restoring disturbed soils. All sites have been subjected to long-term monitoring continually for a decade at one site, and measurements of soil microbial characteristics have been used to determine the success of the reclamation process. Data suggest that increased heterotrophic bacterial numbers, enhanced microbial activity and shifts in dominant bacterial populations all occurred postbiosolid application. All measured changes were sustained over time, indicating that amendment with Class A biosolids is an effective way of restoring mine tailings. In conclusion, the current study shows that surface amendment of Class A biosolids into copper mine tailings was able to establish a typical soil bacterial population capable of functioning as a normal soil, which is a key factor in providing long-term ecosystem stability.

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