Microb Ecol (2012) 64:738–749
DOI 10.1007/s00248-012-0020-3

SOIL MICROBIOLOGY

Microbial and Geochemical Assessment of Bauxitic Un-mined and Post-mined Chronosequence Soils from Mocho Mountains, Jamaica

Dawn E. Lewis · Ashvini Chauhan · John R. White · Will Overholt · Stefan J. Green · Puja Jasrotia · Denis Wafula · Charles Jagoe

Received: 24 January 2012 / Accepted: 31 January 2012 / Published online: 4 March 2012

Abstract Microorganisms are very sensitive to environmental change and can be used to gauge anthropogenic impacts and even predict restoration success of degraded environments. Here, we report assessment of bauxite mining activities on soil biogeochemistry and microbial community structure using un-mined and three post-mined sites in Jamaica. The post-mined soils represent a chronosequence, undergoing restoration since 1987, 1997, and 2007. Soils were collected during dry and wet seasons and analyzed for pH, organic matter (OM), total carbon (TC), nitrogen (TN), and phosphorus. The microbial community structure was assessed through quantitative PCR and massively parallel bacterial ribosomal RNA (rRNA) gene sequencing. Edaphic factors and microbial community composition were analyzed using multivariate statistical approaches and revealed a significant, negative impact of mining on soil that persisted even after greater than 20 years of restoration. Seasonal fluctuations contributed to variation in measured soil properties and community composition, but they were minor in comparison to long-term effects of mining. In both seasons, post-mined soils were higher in pH but OM, TC, and TN decreased. Bacterial rRNA gene analyses demonstrated a general decrease in diversity in post-mined soils and up to a 3-log decrease in rRNA gene abundance. Community composition analyses demonstrated that bacteria from the Proteobacteria (α, β, γ, δ), Acidobacteria, and Firmicutes were abundant in all soils. The abundance of Firmicutes was elevated in newer post-mined soils relative to the un-mined soil, and this contrasted a decrease, relative to un-mined soils, in proteobacterial and acidobacterial rRNA gene abundances. Our study indicates long-lasting impacts of mining activities to soil biogeochemical and microbial properties with impending loss in soil productivity.

Dawn E. Lewis and Ashvini Chauhan contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00248-012-0020-3) contains supplementary material, which is available to authorized users.

D. E. Lewis · A. Chauhan (✉) · D. Wafula · C. Jagoe
School of the Environment, Florida A&M University,
1515, S. MLK Blvd., Suite 305B, FSHSRC Building,
Tallahassee, FL 32307, USA
e-mail: ashvini.chauhan@famu.edu

J. R. White
Wetland and Aquatic Biogeochemistry Laboratory, Department of Oceanography and Coastal Science, Louisiana State University,
Baton Rouge, LA 70803, USA

W. Overholt · P. Jasrotia
Department of Earth, Ocean, and Atmospheric Sciences,
Florida State University,
1060 Atomic Way,
Tallahassee, FL 32306, USA

S. J. Green
DNA Services Facility, University of Illinois at Chicago,
835 S. Wolcott Avenue, A310 MSB,
Chicago, IL 60612, USA

C. Jagoe
NOAA Environmental Cooperative Science Center,
Florida A&M University,
Tallahassee, FL 32307, USA

Present Address:
D. E. Lewis
Center for Plasma Science and Technology,
Florida A&M University,
2077 E. Paul Dirac Drive,
Tallahassee, FL 32310, USA

Introduction

Mining of bauxite-rich soils in Jamaica involves stripping the vegetation and stockpiling of the 0–3-cm top soils adjacent to the mined area [37]. Once the bauxite reserves are depleted, the stockpiled soil is spread back onto the mined area and revegetated with plants and shrubs to rehabilitate the ecosystem. There is a significant interest in preparing the post-mined areas for agricultural purposes because Jamaica has limited viable agricultural land [37]. Consequently, regulations in Jamaica require bauxite industries to restore mined areas such that their productivity mirrors those found in un-mined soils. Reclamation of mined areas in Jamaica began in the 1980s, but there is a renewed interest by the mining industry to obtain a comprehensive measure of biodiversity and ecology within the post-mined areas [25].

Even though bauxite mining is a temporary change in land use, criteria for successful restoration prior to public use remain obscure [7]. Previous studies have shown that this temporary change in land use causes long-lasting impacts to soil physicochemical properties [13, 21, 28, 38, 48, 51]. Correlated with soil degradation, there is a significant reduction in plant production, specifically of breadfruit, ginger, tomato, banana, pineapple, coconut, coffee, and sugarcane, across the Jamaican island [27]. Large-scale failure of Jamaican crops grown on previously mined soils is largely attributed to acute nutrient stress and increased susceptibility to soilborne pathogens, leading to a rapid decline of exported crops which is considered to be a leading cause of the island's socioeconomic decline [24].

Because the biological structure and associated loss of functions in the mined soils continues to be poorly understood, establishing a restoration trajectory for such soils is fairly complex [19, 25, 48, 51]. Previously, changes in animal or plant communities have been studied as indicators of ecosystem level changes following anthropogenic disturbances [35, 52]. Studies conducted more recently suggest that changes in microbial activities, structure, and functions can precede detectable changes in soil physicochemical status, thereby providing early signs of environmental degradation [1, 6, 9, 10]. Relationships between diversity and recovery of microbial functions induced by different types of stresses have been characterized, including change in land use, soil organic carbon [22, 23, 33], and other anthropogenic impacts [10–13]. These studies indicate that microorganismal diversity shifts, and resilience is relative to the functions being studied, viable species, their interactions in a particular environment being studied, and physicochemical structure which directly shapes the microorganismal composition and associated biogeochemical functions [23]. However, the effects of mining activities on soil bacterial diversity remain unclear, even though such information will greatly add to our knowledge on anthropogenic affects and reestablishment of soil microbiome within degraded ecosystems.

Previously, we performed soil functional analysis on soils collected from Mocho Mountains which showed that the rate and number of substrates utilized by the un-mined and post-mined soil varied, such that the post-mined sites rehabilitated in 1987 and 2007 showed significantly lower functional response than an un-mined site. To some extent, bacterial metabolic responses varied with seasons, but the least physiological activity was shown by the 1987 post-mined site indicating a long-term perturbation at this particular site [33]. In this study, we provide an assessment of rehabilitation through cultivation-independent molecular analyses of bacterial community structure, bacterial abundances, and geochemistry within un-mined and post-mined chronosequence soils at 1, 10, and 20 years apart in rehabilitation ages. This study provides a comprehensive understanding on the long-term impacts of bauxite mining to soil biogeochemical and microbial properties and provides recommendations for future rehabilitation of post-mined soils.

Methods

Study Sites and Sample Collection

Soil samples from three post-mined chronosequence sites and an adjacent un-mined site were collected from the Mocho Mountains of Jamaica (18°03′33″ N, 77°33′33″ W; map shown in Fig. SI-1), as also described previously [33]. A chronosequence represents variable time series of soils undergoing ecological succession and facilitates assessment of restoration trajectory [17]. The chronosequence sites have been undergoing post-mined rehabilitation since 1987 (site CC24), 1997 (site F25), and 2007 (site C9). These sites are typically hilly with elevations ranging from 397 to 590 m above sea level. The soil texture of un-mined and post-mined soils is stony loam. Vegetation in the un-mined site consisted mainly of mango (*Mangifera indica*) and tamarind (*Tamarindus indicus*) interspersed with shrubs and grasses. Plant species that were documented at the time of sample collection included African star grass (*Cynodon nlemfuensis* Vandercyst), sparse populations of ferns, and tropical shrubs such as black sage (*Cordia curassavica)* in the un-mined site; bamboo (*Bambusa vulgaris*) was observed from site CC24 (1987). In site F25 (1997) and C9 (2007), sparse populations of Guinea grass (*Panicum maximum* Jacq.), and *Brachiaria* species were also present. The first set of soil cores were collected in September 2007 2 weeks after the passage of Hurricane Dean. The second samples were collected in January 2008. These 2 months typically represent the wet and dry seasons in the Jamaican island [45]; however, the passage of Hurricane Dean between the two sample collection periods

certainly marks an extreme, though not an altogether unusual event for the island.

Prior to collection of soils, aboveground plant and detritus layer were removed, and randomly, six to ten soil cores from each of the four sites were collected with an auger measuring 15×7.5 cm as previously reported [33]. Sampling locations were approximately 15–30 m apart, and cores were collected at depths of 0–5 cm (top soil). A total of 36 cores were collected for each sampling period, stored in 1.6-L Whirlpak® bags over ice, and transported to the laboratory at Tallahassee, Florida within 72 h of collection. Upon receipt, the samples were passed through a 2-mm sieve to remove coarse roots and debris and were immediately processed for further analyses.

Biogeochemical Analysis

For biogeochemical analyses, each core collected from the un-mined and post-mined sites was treated and analyzed separately. The soil pH was determined on a 2:1 (water:soil) slurry using a digital pH meter. Total carbon (TC) and nitrogen (TN) were determined on dried, ground subsamples using a Carlo-Erba NA-1500 CNS Analyzer (Haak-Buchler Instruments, Saddle Brook, NJ, USA). For total phosphorus (TP), 0.5 g of dried, ground subsample was combusted at 550°C for 4 h in a muffle furnace followed by dissolution of the ash in 6 M HCl on a hot plate [2]. Total P was analyzed in the digested solution using an automated ascorbic acid method on a Seal AQII discrete analyzer, Method 365.4 [53]. Total sodium (Na) and potassium (K) were determined on samples prepared by nitric acid digestion followed by inductively coupled argon plasma spectrometry (model Vista MPX CCD simultaneous ICP-OES manufactured by Varian, Inc., Walnut Creek, CA, USA; Method 200.7) [53].

DNA Isolation and Quantitative PCR Analysis of Bacterial rRNA Genes

DNA was extracted from individual soil cores using the Power Soil DNA Isolation Kit (MO BIO Inc.); DNA concentrations were measured using a ND-1000 Nanodrop spectrophotometer (Starlab, USA). Quantification of bacterial small subunit rRNA genes (SSU rRNA) was performed as described previously [40], using *Taqman* 2× Gene Expression Master Mix (Invitrogen, Foster City, CA). Primers and probe were ordered from Integrated DNA Technologies (Coralville, IA, USA). Absolute quantification was performed using a standard curve derived from PCR products generated by near-full gene amplification of SSU rRNA genes using the general bacterial primer set 27 F and 1492R [32]. The standard curve was linear across a five-order of magnitude scale (from 2.79E+08 to 2.79E+03 copies/reaction), with a 92% efficiency. All reactions were performed in triplicate and analyzed using the ABI 7900HT Fast Real-Time PCR system.

Denaturing Gradient Gel Electrophoresis

Bacterial SSU rRNA genes were amplified using universal eubacterial primers GM5 forward (GC clamp) and 907 reverse, targeting the V3-V5 regions of the 16S rRNA gene [39]. PCR reaction was prepared using ready-to-go PCR beads (GE Healthcare), with approximately 15 ng of template DNA, PCR grade sterile water, and 0.4 pmol/μL of each primer. Subsequently, the PCR amplicons were separated using denaturing gradient gel electrophoresis (DGGE) using the Dcode™ Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA), as described previously [39]. After electrophoresis, the gel was stained with SYBR Green I (Molecular Probes, Eugene, OR, USA) for 30 min, viewed using a UV transilluminator (302 nm), and analyzed with Quantity One analytical software (Bio-Rrad, Hercules, CA, USA). DGGE band analysis was performed by setting a background subtraction at 15 using the rolling disk method; bands that consisted of intensity lower than 10% were excluded from further analysis. Banding patterns were then used to analyze any differences that exist between microbiota from un-mined and post-mined chronosequence soils by calculating the diversity indices, species richness, and evenness. Bands of interest after DGGE analysis were excised and sequenced as described previously [33].

Comparison of the bacterial diversity within each site (beta diversity) by DGGE indicated no significant differences existed. Therefore, for further microbial analyses, equal concentrations of DNA from each core were pooled to represent one un-mined sample and three post-mined chronosequence samples (1987, 1997, and 2007).

SSU rRNA Gene Sequencing and Phlyogenetic Analyses

Clone libraries of bacterial SSU rRNA genes were generated from PCR amplicons produced with 27 F and 1492R primers. After PCR products were checked for purity and size, they were ligated using the TOPO TA cloning kit, as recommended by the manufacturer (Invitrogen, Carlsbad, CA, USA). The ligated products were then sequenced at the Genome Center in Washington University at St. Louis, MI, USA. All sequences were reviewed and processed using the Sequencher software package version 4.5 (Gene Codes Corporation, Ann Arbor, MI, USA). Sequences were screened for chimeric properties using the Ribosomal Database Project (RDP) (http://rdp.cme.msu.edu.lp.hscl.ufl.edu/html/), and suspected chimeras were eliminated from further analysis. Unaligned sequences were submitted to the Sequence Match program of the Ribosomal Database program and to the Advanced BLAST search program of the National

Center for Biotechnology Information (www.ncbi.nlm.nih.gov) to obtain taxonomic affiliations. Multiple alignments with closely related bacterial sequences were performed using the NAST alignment tool in Greengenes [14]. Phylogenetic and molecular evolutionary analyses were conducted using MEGA4 [49].

Composite samples for pyrosequencing were prepared by separately pooling approximately equal amounts of genomic DNA extracted from each of the eight soil cores from un-mined soils and of the six to ten cores collected from the three post-mined chronosequence soils. DNA extracts were sent to the Research and Testing Laboratory (Lubbock, TX, USA) for bacterial ribosomal RNA (rRNA) gene tag-encoded FLX amplicon pyrosequencing. PCR amplification was performed using primers Gray28F 5′ GAGTTTGATCNTGGCTCAG and Gray519r 5′ GTNTTACNGCGGCKGCTG, and sequencing reactions utilized a Roche 454 FLX instrument (Roche, Indianapolis, IN, USA) with titanium reagents, titanium procedures, a one-step PCR, and a mixture of Hot Start and HotStar high fidelity *Taq* polymerases. Following sequencing, all failed sequence reads, low quality sequence ends, and tags were removed; sequences were depleted of any nonbacterial ribosome sequences and chimeras as has been described previously [15]. Subsequently, all sequences were initially trimmed to 400 bases, and sequences shorter than this were removed from further analysis. Each sample library was randomly subsampled and equalized to 4,300 sequences to reduce potential biases introduced by unequal library sizes [20].

Sequence analysis was accomplished through the software packages quantitative insights into microbial ecology (QIIME) [5] and Primer6 (Primer-E, Lutton, Ivybridge, UK). Sequences were clustered into operational taxonomic units (OTUs) at a 97% sequence identity threshold, and representative sequences from each OTU were picked using scripts in QIIME. Shannon–Weaver [46], Chao1 richness estimator [8], and species richness alpha diversity indices were calculated for each sample. Beta diversity was assessed by generating a phylogenetic tree-based distance matrix using the weighted UniFrac metric and was visualized using a two-dimensional principal coordinate analysis plot (PCoA) in QIIME. Beta diversity was further assessed in Primer6. An OTU table with the number of sequences of each OTU present in each sample was exported from QIIME. Initially, OTU was normalized into relative abundance and summed across all samples. A separate dataset was generated containing only OTU that is composed of more than 1% of all sequences. Both datasets were imported into Primer6, Bray–Curtis resemblance matrices were generated [4], and sample similarity was visualized using nonparametric multidimensional scaling plots using 50 runs and a stress value of 0.01. Reference sequences were classified using the RDP classification algorithm implemented in QIIME at 50% confidence [54]. Sequences were clustered based on this classification at the class, family, and genus levels.

Statistical Analyses

Biogeochemical measurements were taken in triplicate, and mean values are reported in this study. Soil chemistry data were tested for normality using Shapiro–Wilk W tests and transformed (arcsine square root or logarithmic) if necessary. We used a two-way analysis of variance (ANOVA) to test for differences and interactions between seasons and among sites (SAS/JMP 8.02). Differences among means were evaluated using Tukey's honestly significant difference (HSD) test. Additionally, DGGE banding patterns from the un-mined and chronosequence sites were analyzed using Bio-Rad Quantity One™ (version 4.4.0; Bio-Rad Laboratories, Hercules, CA, USA). We excluded bands with intensity <0.05, and patterns were analyzed using principal component analysis (PCA) and cluster analysis from the unweighted pair group method with arithmetic averages to form linkage dendrograms, as reported previously [28]. DGGE patterns were also examined using two indices: the Shannon–Weaver index of diversity, H [46], and the equitability index, E [41], calculated according to previously reported formulas:

$$\mathrm{H} = -\Sigma(n_\mathrm{i}/N)\log(n_\mathrm{i}/N) \text{ and } E = H/\log S$$

where n_i is the relative intensity of each DGGE band, S is the number of DGGE bands, and N is the sum of the relative intensity for all bands in a given sample [18]. Statistical significance of variance was evaluated with a one-way ANOVA using the MVSP software package [30].

Additionally, all sequences obtained from un-mined and post-mined soils were statistically assessed by UniFrac, as previously described [34]. Comparative analyses were run using the P test, UniFrac metric test, and PCA using the scatter plot option. Further analyses were performed by a lineage-specific analysis, applying the G test of significance to determine whether sequences had different distributions over the un-mined and post-mined bauxite soils. Normalized ratios of Proteobacteria:Acidobacteria were calculated to estimate soil nutrient status by taking the sum of all proteobacterial numbers identified from each site and dividing the value by the ratio of Proteobacteria:Acidobacteria, as shown before [26].

Nucleotide Sequence Accession Numbers

The partial SSU rRNA gene sequences obtained in this study are available in GenBank under accession numbers of GU219955-GU219977 (DGGE sequences) and GU219880-GU219954 (clone sequences).

Results

Soil Biogeochemical Properties

Soil carbon concentrations were highest at the un-mined site and did not differ between seasons (Fig. 1a). Soil nitrogen concentrations were also highest at the un-mined site and lowest at the most recently restored sites (Fig. 1b). Soil phosphate concentrations were lowest in the un-mined and recently restored sites and highest in the sites that were restored in 1987 (Fig. 1c). Soil carbon:nitrogen ratios were highest in the most recently restored site and did not differ among the sites restored in 1897 and 1997 and in the un-mined site. Seasonal differences in soil nitrogen, phosphate, and carbon:nitrogen ratios were not significant.

Effect of Mining on Bacterial Abundance and Community Structure

Un-mined soil samples had the highest measured bacterial SSU rRNA gene abundance and, by inference, the highest bacterial abundance. All post-mined samples had significantly lower SSU rRNA abundances than the un-mined soil, with values ranging from 1.41E+05 to 9.04E+07 copies per gram soil (Table 1). Initial analyses of these soils by PCR-DGGE revealed that both the numbers and band intensities changed along the site and seasons. Relative phylotype abundances obtained by PCR-DGGE and cloning are shown in Figs. SI-2a–c and SI-3a–c. PCA (Fig. 2a) and Euclidean distance estimations (Fig. 2b) on this data revealed that, regardless of the seasons, un-mined sites were more similar to the 1997 site but distinctly different than the post-mined sites from 1987 and 2007.

To identify shifts in microbial communities across these soils, DGGE bands were excised and sequenced. The SSU rRNA gene sequence analysis on selected bands indicated that Acidobacteria was the most abundant phylum (35%), followed by Bacteroidetes (21%), Firmicutes (11.5%), Proteobacteria (11.5%), and Actinobacteria (19%) (Figs. SI-2a–c and SI-3a–c). PCA was conducted on the gene sequences, and Shannon's diversity (H') and evenness (E) were calculated (Table 1 and Fig. SI-3a–c). These analyses also indicated that bacterial communities in un-mined and 1997 post-mined soils were more similar to each other than either was to those from the sites rehabilitated in 1987 and 2007. To generate deeper sequence coverage than DGGE, further analyses was performed by clone library sequencing and bar-coded pyrosequencing of bacterial SSU rRNA genes from un-mined and post-mined soil genomic DNA. Details on the nature of bacterial phyla/species identified by cloning and pyrosequencing analyses are provided in Table 2, which clearly established that differences exist between the un-mined and post-mined sites such that the un-mined soil consisted predominantly of sequences from the phyla Proteobacteria (44%), Acidobacteria (27%), Actinobacteria (11%), and Firmicutes (12%) in the wet season (Fig. 3). Dry season did not alter this distribution dramatically; sequences from Proteobacteria predominated at 40%, with sequences from Acidobacteria, Actinobacteria, and Firmicutes represented at 20%, 15%, and 11% of the total analysis. Among Proteobacteria, sequences derived from α-Proteobacteria predominated regardless of seasons or sites, but un-mined soil showed larger relative abundances of α-

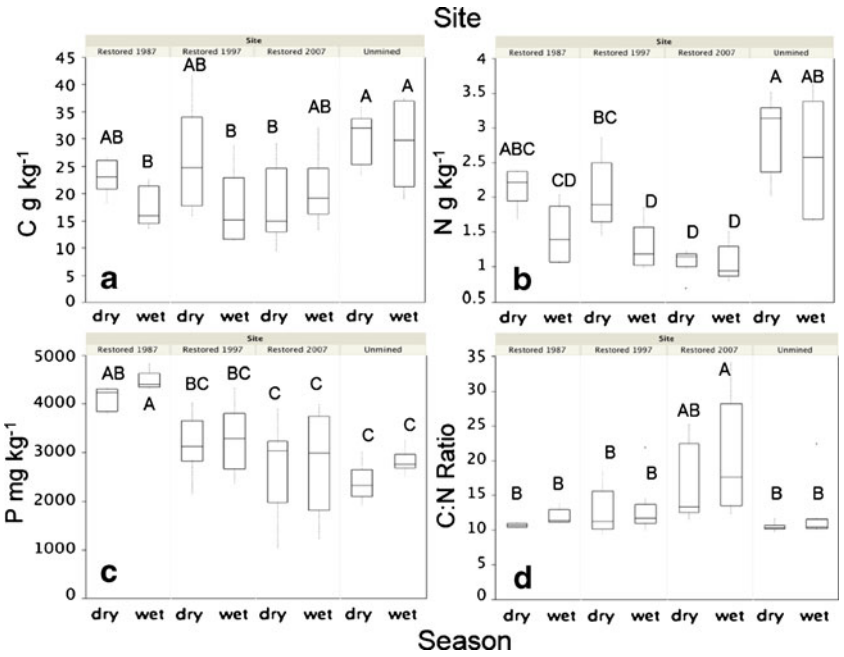

Figure 1 Box and whisker plots of soil biogeochemical properties of un-mined and rehabilitated chronosequence bauxite soils. Shown are **a** soil carbon, **b** soil nitrogen, **c** soil phosphate, and **d** soil carbon: nitrogen ratio. Sites with the same letter are not different by two-way ANOVA ($P>0.05$) and Tukey's HSD test

Table 1 Biogeochemical and microbial analyses from un-mined and rehabilitated bauxite soils in the wet (September 2007) and dry (January 2008) seasons collected from Mocho Mountains, Jamaica

Sample name	J32	BMU23	JC9	C9	JF25	F25	JCC24	CC24
Soil type	Un-mined	Un-mined	2007 rehabilitated	2007 rehabilitated	1997 rehabilitated	1997 rehabilitated	1987 rehabilitated	1987 rehabilitated
Elevation	494	494	590	590	397	397	410	410
Season	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Moisture content	18.79±0.44	20.41±0.82	14.61±0.73	17.14±0.66	18.54±0.45	18.80±0.61	17.89±0.16	18.22±0.26
Biogeochemical analyses								
pH	6.87±0.08	6.66±0.10	7.83±0.04	7.90±0.06	7.42±0.09	7.82±0.11	7.68±0.07	7.48±0.19
Total carbon (g kg^{-1})	30.33±1.64	29.23±2.64	17.74±2.43	20.43±2.13	25.56±2.78	17.40±2.13	23.06±1.26	17.29±1.48
Total nitrogen (g kg^{-1})	2.91±0.19	2.54±0.30	1.08±0.06	1.07±0.09	2.04±0.15	1.30±0.09	2.15±0.11	1.46±0.16
C/N ratio	10.52±0.19	12.14±1.48	16.31±1.94	20.14±2.88	12.54±1.02	13.02±1.19	10.70±0.12	11.98±0.45
Total phosphorous (mg kg^{-1})	2,397±124.6	2,812±82.06	2,720±327.20	2,824±355.40	3,171±172.50	3,271±207.60	4,124±96.19	4,481±82.13
Analysis of rrS genes								
Copies per gram soil (SD%)	1.13E+09 (18.7%)	1.27E+09 (17.2%)	9.34E+06 (8.4%)	1.88E+07 (17.9%)	2.82E+07 (14.6%)	9.04E+07 (12.1%)	1.80E+05 (11.0%)	1.41E+05 (7.0%)
Diversity of rrS gene sequences (4,300 sequences)								
No. of OTU (97%)	1,884	1,782	908	1,354	1,937	1,625	869	655
% Coverage	72	73	93	85	71	78	93	95
Pyrosequence Shanon diversity (*H'*)	6.87±0.05	6.75±0.05	6.17±0.04	6.56±0.04	7.00±0.04	6.7±0.04	6.03±0.04	5.7±0.04
DGGE Shanon diversity (*H'*)	1.510±0.01	1.325±0.02	0.390±0.22	0.855±0.06	1.580±0.02	1.280±0.03	1.495±0.04	0.945±0.04
Pyrosequence evenness (*E*)	0.91	0.9	0.91	0.91	0.91	0.91	0.89	0.88
DGGE evenness (*E*)	0.99	0.93	0.64	0.87	0.99	0.92	0.95	0.83

Values given are±standard error

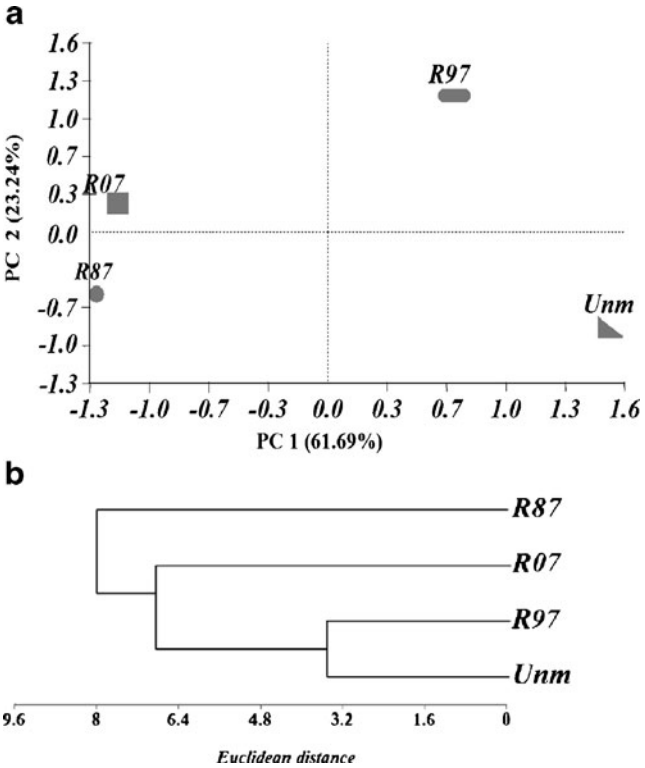

Figure 2 PCA and dendrograms following PCR-DGGE of soil microbiota from un-mined and rehabilitated chronosequence bauxite soils collected from the Mocho Mountains of Jamaica. Shown are **a** PCA with percentages of variation that are explained by the first two principal coordinates and **b** cluster dendrogram representing the degree of association between different sites; the lower the value of the Euclidean distance, the more significant is the association. MUnm refers to Mocho un-mined, R2007 to site rehabilitated in 2007, R1997 to site rehabilitated in 1997, and R1987 to site rehabilitated in 1987

Proteobacteria sequences when compared with post-mined sites. In Acidobacteria, sequences from group 6 were the most abundant, followed by those from groups 4, 5, 7, and 17.

Bacterial sequences obtained from the un-mined and post-mined soils were evaluated by UniFrac analyses. A PCoA was performed using weighted UniFrac distance metric, and both the *P* test and lineage specific *G* test revealed that microbiota from un-mined soil were significantly different than those from post-mined soils (Fig. SI-4). Moreover, bacterial species identified from the 1987 and 1997 post-mined soils clustered together on the same PCA axis, but microbiota from site 2007 clustered separately from other sites. PCA1 explained 66.7% and PCA2, 19.5% of variability, with a cumulative percentage of 86.2%.

Additionally, pyrosequencing data were analyzed using a PCoA plot of a weighted UniFrac resemblance matrix. PCo1 explained 50% of the variance and PCo2 explained 15% of the total variance, placing the microbial communities from the un-mined site in separate axes than the post-mined soils (data not shown). Additional analysis was performed by constructing a nonparametric multidimensional scaling (NMDS) plot using a Bray–Curtis resemblance matrix on all pyrosequence OTUs (Fig. 4a). This plot showed that un-mined sequences from both the seasons clustered together and away from all the post-mined soils. When OTUs >1% of all the pyrosequences used for NMDS analyses, the distinction between the un-mined and post-mined soil bacterial communities became even greater (Fig. 4b).

Alpha diversity measurements on both pyrosequence data and DGGE banding patterns were also calculated (Table 1). The pyrosequence data indicated that the un-mined soil and the 1997 rehabilitated site were the most diverse, and high evenness values were observed for all samples (>0.88) (Table 1). Although the 1987 rehabilitated soils, with the lowest measured bacterial abundance, had the lowest diversity of any of the soils, the diversity was still substantial. A positive but fairly weak correlation between Shannon indices calculated from pyrosequence data and Shannon indices calculated from DGGE patterns was observed (slope=0.45, *r* square value=0.251).

Discussion

Bauxite mining is considered to be a major threat to soil physicochemical and biological quality promoting the destruction of plant cover, deficiency of organic matter, and the degradation of soil structure [13, 21, 28, 38, 48, 51]. With the shortage of arable land in Jamaica, there is a considerable interest in using the reclaimed post-mined areas for sustainable agriculture [37]. Successful post-mining reclamation requires plant species to be reestablished on stable and fertile soil material [13]. Accretion of organic matter is critical to successful restoration because several edaphic properties including soil structure, water retention, nutrient availability, and microbial activity are directly affected by soil organic matter content [55]. Even though mining is a temporary change in land use, our study contributes to a growing body of evidence suggesting long-term ecological impairment of mined soils. Specifically, soil biogeochemical properties across these bauxite-rich soils exhibited three seasonal trends: (a) values increase with age of rehabilitation (pH, C:N ratios), (b) values decrease with age of rehabilitation (TN, TP), and (c) no definitive trend observed (TC, NA, and K) (Table 1). Even after years of rehabilitation, elevated soil pH and decreased C and N were consistently shown in post-mined sites. Likewise, the abundance and diversity of bacteria in the rehabilitated sites remained largely reduced relative to the un-mined site. Consequent to anthropogenic impacts, typically, a reduction of species richness and shifts in bacterial community structure also occur [9–12]. Thus, unperturbed species-rich assemblages are found to be evenly distributed in the environment, which are replaced by species-poor assemblages with high

Table 2 Relative bacterial phylotype abundances identified by clone library and pyrosequencing analyses from un-mined and rehabilitated bauxite soils in the wet (September 2007) and dry (January 2008) seasons collected from the Mocho Mountains, Jamaica

Closest phylogenetic affiliation	Un-mined	1987 rehabilitated	1997 rehabilitated	2007 rehabilitated
α-Proteobacteria				
Bradyrhizobium	+	−	−	−
Hyphomicrobium	+	−	+	+
Pedomicrobium	−	+	+	+
Rhodoplanes	+	−	−	−
Methylobacterium	+	−	−	−
Odyssell	+	+	+	+
Novosphingobium	+	+	+	+
Porphyrobacter	+	−	−	−
Sphingomonas	+	−	−	−
β-Proteobacteria				
Burkholderia	+	−	−	−
Delftia	−	−	−	+
Ramlibacter				
γ-Proteobacteria				
Aquicella	+	+	+	+
Pseudomonas	+	−	−	−
δ-Proteobacteria				
Cystobacteraceae	+	−	+	+
Polyangiaceae	−	+	+	+
Smithella	+	−	−	−
Acidobacteria				
Gp1	+	−	−	+
Gp4	+	+	+	+
Gp5	+	−	+	+
Gp6	+	+	+	+
Gp7	+	+	−	+
Gp11	−	−	−	+
Gp16	+	+	+	−
Gp17	−	+	−	−
Gp25	−	+	−	−
Verrucomicrobia				
Subdivision 3	+	−	−	+
Xiphinematobacteriaceae	+	−	−	−
Actinobacteria				
Corynebacterineae	+	+	+	+
Micrococcineae	+	+	+	+
Micromonosporineae	+	+	+	+
Propionibacterineae	+	−	+	+
Pseudonocardineae	+	−	−	+
Streptomycineae	+	−	+	+
Rubrobacterineae	+	+	+	+
Sphingobacteria				
Terrimonas	+	−	+	+
Niastella	+	−	+	+
Bacilli				
Paenibacillaceae 1	+	−	−	−
Bacillaceae 1	−	+	+	+
Pasteuriaceae incertae sedis	−	+	+	+
Planctomycetacia				
Gemmata	+	−	+	+
Pirellula	+	+	+	−

(+) bacterial species identified from the specific site, (–) not present and/or not identified

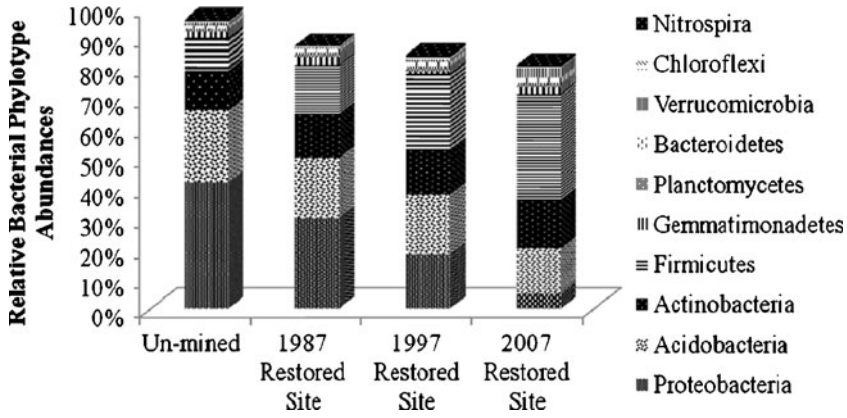

Figure 3 Relative bacterial phylotype abundances from pooled clone library OTUs and pyrosequence OTUs composed of >1% of all sequences from un-mined and post-mined rehabilitated soils of Mocho Mountains, Jamaica

dominance of few species following perturbation [35]. Overall, bacterial community shifts across these bauxite-rich soils followed specific trends, such that (a) an increase in Firmicutes, Actinobacteria, and Verrucomicrobia was shown in newly rehabilitated sites, (b) a decrease of Proteobacteria and Acidobacteria was shown in newly rehabilitated sites, and (c) no consistent trend was shown across sites (Gemmatimonadetes, Planctomycetes, Bacteroidetes, Chloroflexi, *Nitrospira*) (Fig. 3, Table 2, Figs. SI-2 and SI-3). Similar to our study, a significant increase of Firmicutes has been recently shown by Banning et al., in a chronosequence of developing *Eucalyptus* forest in Australia rehabilitated for 18 years after cessation of bauxite mining and, thus, indicates the propensity of this endospore-forming group to outcompete others during secondary succession processes. Surprisingly, the Australian rehabilitated soils also

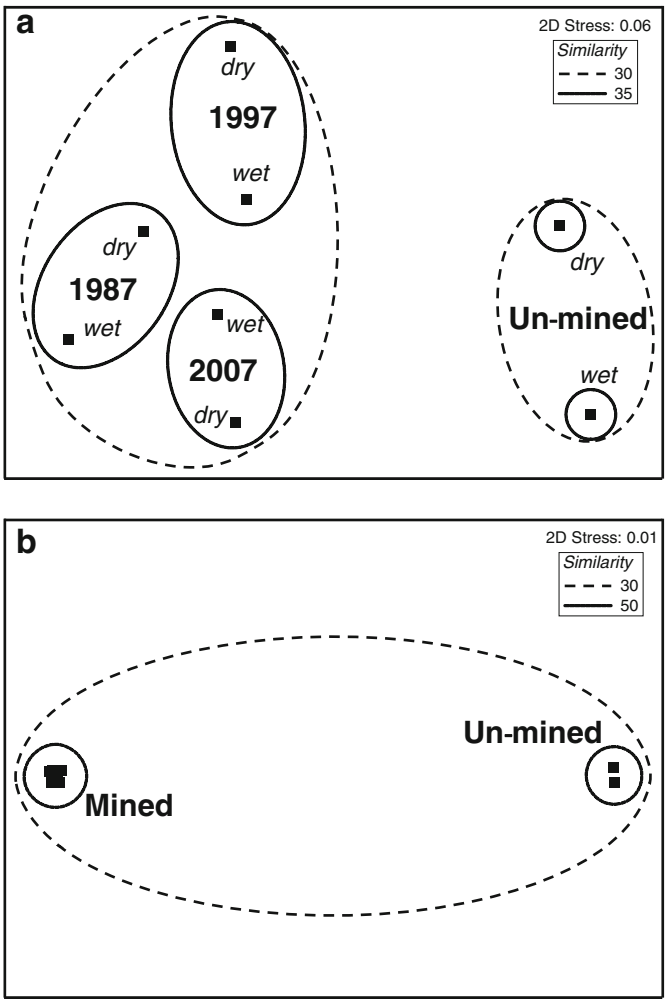

Figure 4 Multidimensional scaling plot of SSU rRNA pyrosequence libraries derived from un-mined and post-mined rehabilitated soils. Sequences were clustered at a 97% similarity threshold, and the clustering output was used to generate a resemblance matrix. The matrix was then used for a nonmetric multidimensional Scaling analysis, visualized in two dimensions within Primer6, using averaged Bray–Curtis distances. Shown are **a** all OTUs included in the analyses and **b** OTUs that constituted >1% of all sequences. Sequence libraries generated from different soils and seasons are indicated

consisted of increasing numbers of Proteobacteria with increasing restoration age. This further suggests a strong likelihood of functional role(s) played by bacteria belonging to Proteobacteria, Firmicutes, and Acidobacteria in soils undergoing post-mining secondary succession [3].

Among Proteobacteria, regardless of soils or seasons, we found α-Proteobacteria to predominate in these bauxitic soils, as shown for other soil environments [47, 50]. Of major interest was the relative dominance of several proteobacterial species including *Bradyrhizobium*, *Rhodoplanes*, *Methylobacterium*, *Porphyrobacter*, *Sphingomonas*, *Burkholderia*, *Pseudomonas*, and *Smithella* in the un-mined soil relative to post-mined soils. These bacteria are known to be metabolically versatile catalyzing several biogeochemical functions in soils such as symbiotic nitrogen fixation, nutrient cycling remineralization, and biodegradation of environmental pollutants [6, 29, 43, 50].

The second predominant group in these soils—Acidobacteria—continues to be poorly characterized despite forming one of the most dominant members in soils [42, 50]. Recently, 26 Acidobacteria subdivisions were proposed, and whole genome sequencing indicates their metabolic versatility, which likely aids in their survival under prolonged dry periods and low pH [6, 44]. Additionally, this group also has a propensity to thrive in oligotrophic conditions, typically coupled with lower plant productivity and reduction in the availability of nutrient-rich plant materials to the soil microbiota, as likely prevalent in the post-mined soils. Specifically, we found Acidobacteria group 6 as the most dominant in these bauxitic soils followed by groups 4, 5, 7, and 17 (Table 3).

Interestingly, Proteobacteria:Acidobacteria ratios have been shown to correlate with soil nutrient status. In oligotrophic soils, this ratio is typically found to be around 0.16 [16, 31] and ranges between 0.34–0.46 in low-productivity soils. Conversely, ratios of ~0.87 have been reported from highly productive soils [36]. More recently, these ratios in the eutrophic Everglades soils were reported to be 0.85 with restored soils having lower ratios of ~0.5 [26]. We found normalized Proteobacteria:Acidobacteria ratios to be lower in the 1987 and 1997 post-mined soils (~0.6 and 0.47, respectively) relative to the un-mined soil (~0.64). These ratios were significantly lower (0.25) in the 2007 post-mined soils, similar to those found in oligotrophic soils. This suggests that despite cessation of mining and decades of rehabilitation, nutrient limitation, among other factors, continues to stress these soils. Given the predominance of Proteobacteria and Acidobacteria in these soils, further studies specific to these groups should be performed to better understand their functional roles in soil restoration processes. It will be of particular interest to investigate whether Proteobacteria, Acidobacteria, and Firmicutes represent early successional stages in post-mined soils and drive the restoration trajectory or that these groups merely colonize and outcompete other microbiota due to their metabolical versatility. Future studies on the biogeochemical role(s) of these soil microorganisms along with soil fungi, especially the arbuscular mycorrhizhae shown to be affected by mining [28], will not only provide early signs of soil perturbation but also indicate restoration success of anthropogenically impacted environments.

The geochemical and microbiological data point to a strong and persistent negative impact of mining on the quality of soil in Jamaica. Rehabilitated sites examined in this study are characterized by lower organic carbon and nitrogen, elevated pH, and reduced bacterial abundance. Bacterial diversity (alpha diversity), although generally lower in rehabilitated soils, is not diagnostic for post-mined sites, due to substantial seasonal variation. DGGE analyses, which favor clustering of the 1997 rehabilitated site with the un-mined soil, most likely have inadequate resolution for discerning shifts in community structure for such highly diverse communities. Comparison of massively parallel amplicon libraries from each sample revealed that all rehabilitated sites were more similar to each other, regardless of season, than they were to the un-mined soils. Coupled with the strong pattern of decrease quality of organic matter in rehabilitated soils, the microbiological data demonstrate that even 20 years of rehabilitation is not sufficient for restoration of soil fertility; paradoxically, the site with the longest period of restoration (1987 rehabilitated site—CC24) was the least improved of all the restored sites examined (Table 1, Fig. 1, Fig. SI-1). We are tempted to speculate that these long-term impacts are likely the direct result of topsoil removal during mining activities, causing displacement of native soil microbiota critical to nutrient cycling. However, other edaphic factors such as drainage, texture, and chemical properties characteristic of the pre-mined soils cannot be ignored. In order to obtain definitive correlations between microorganisms impacted by mining activities along with potential impacts to soil biogeochemical functions, further studies should be performed on sites having similar edaphic factors—before, during, and after mining activities, such that "real-time" effects of mining and rehabilitation during secondary succession can be better understood.

Acknowledgments We thank Mr. John Gardner, Environmental Manager for Mining, ALCOA World Alumina Australia, for facilitating the funding to support this study. Support from the Department of Defense (DoD) grant # W911NF-10-1-0146 is also acknowledged. We also acknowledge the staff at Jamalco for logistics with sample collection, Maverick and Benjamin LeBlanc for their assistance in soil geochemical characterization, and the three anonymous reviewers for their very helpful critique.

References

1. Ager D, Evans S, Li H, Lilley AK, van der Gast CK (2009) Anthropogenic disturbance affects the structure of bacterial communities. Environ Microbiol 12:670–678
2. Andersen JM (1976) An ignition method for determination of total phosphorus in lake sediments. Water Res 10:329–331
3. Banning NC, Gleeson DB, Grigg AH, Grant CD, Andersen GL, Brodie EL, Murphy DV (2011) Soil microbial community successional patterns during forest ecosystem restoration. Appl Environ Microbiol 77(17):6158–6164
4. Bray JR, Curtis JT (1957) An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs 27:325–349
5. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods. doi:10.1038/nmeth.f.303
6. Castro HF, Classen AT, Austin EE, Norby RJ, Schadt CW (2010) Soil microbial community responses to multiple experimental climate change drivers. Appl Environ Microbiol 76:999–1007
7. Chambers JC, Wade GL (1990) Evaluating reclamation success: the ecological consideration. General Technical Report NE-164. U. S. Department of Agriculture, Forest Service, Rodnor, PA
8. Chao A (1984) Nonparametric estimation of the number of classes in a population. Scandinavian J Stat 11:265–270
9. Chauhan A, Cherrier J, Williams HN (2009) Impact of sideways and bottom up control factors on bacterial community succession over a tidal cycle. Proc Natl Acad Sci USA 106:4301–4306
10. Chauhan A, Ogram A, Reddy KR (2006) Syntrophic archaeal associations differ with nutrient impact in a freshwater marsh. J Appl Microbiol 100:73–84
11. Chauhan A, Ogram A, Reddy KR (2004) Syntrophic-methanogenic associations along a nutrient gradient in the Florida Everglades. Appl Environ Microbiol 70:3475–3484
12. Cleveland CC, Nemergut DR, Schmidt SK, Townsend AR (2007) Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. Biogeochemistry 82:229–240
13. Coke LB, Weir CC, Hill VG (1987) Environmental impact of bauxite mining and processing in Jamaica. Soc and Econ Stud 36:289–333
14. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol 72:5069–5072
15. Dowd SE, Sun Y et al (2008) Survey of bacterial diversity in chronic wounds using pyrosequencing, DGGE, and full ribosome shotgun sequencing. BMC Microbiol 8:43
16. Dunbar J, Takala S, Barns SM, Davis JA, Kuske CR (1999) Levels of bacterial community diversity in four arid soils compared by cultivation and 16S rRNA gene cloning. Appl Environ Microbiol 65:1662–1669
17. Dyck WJ, Cole DW (1994) Strategies for determining consequences of harvesting and associated practices on long-term productivity. In: Dyck WJ, Cole DW (eds) Impacts of forest harvesting on long-term site productivity. Chapman & Hall, New York, pp 11–40
18. Fromin N, Hamelin J, Tarnawski S, Roesti D, Jourdain-Miserez K, Forestier N et al (2002) Statistical analysis of denaturing gel electrophoresis (DGE) fingerprinting patterns. Environ Microbiol 4:634–643
19. Gardner JH, Bell DT (2007) Bauxite mining restoration by Alcoa World Alumina Australia in Western Australia: social, political, historical, and environmental contexts. Restoration Ecology 15: S3–S10
20. Gihring TM, Green SJ, Schadt CW (2011) Massively parallel rRNA gene sequencing exacerbates the potential for biased community diversity comparisons due to variable library sizes. doi:10.1111/j.1462-2920.2011.02550.x
21. Greenberg WA, Wilding LP (2007) Pre- and post-mined bauxite soils of Jamaica: physical and chemical properties. Soil Sci Soc Am J 71:181–188
22. Griffiths BS, Bonkowski M, Roy J, Ritz K (2001) Functional stability, substrate utilization and biological indicators of soils following environmental impacts. Appl Soil Ecol 16:49–61
23. Griffiths BS, Hallett PD, Kuan HL, Gregory AS, Watts CW, Whitmore AP (2008) Functional resilience of soil microbial communities depends on both soil structure and microbial community composition. Biol Fertil Soils 44:745–754
24. Hanke S, Schuler K (1995) Alternative monetary regimes for Jamaica. Study for the Private Sector Organization of Jamaica (PSOJ)
25. Harris J (2009) Soil microbial communities and restoration ecology: facilitators or followers? Science 325:573–574
26. Hartman WH, Richardson CJ, Vilgalys R, Bruland GL (2008) Environmental and anthropogenic controls over bacterial communities in wetland soils. PNAS 105:17842–17847
27. Hogg BM (1966) Yield decline on sugar cane on part of Worthy Park Estate. Proceedings of the Jamaica Association of Sugarcane Technologists
28. Jasper DA (2007) Beneficial soil microorganisms of the Jarrah forest and their recovery in bauxite Southwestern Australia. Restoration Ecology 15:S74–S84
29. Kersters K, De Vos P, Gillis M, Swings J, Vandamme P, Stackebrandt E (2006) Introduction to the Proteobacteria. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) The prokaryotes: a handbook on the biology of bacteria, vol 5, 3rd edn. Springer, New York, pp 3–37
30. Kovach WL (1999) MVSP—a multivariate statistical package for windows, version 3.0, Kovach Computing Services, Pentraeth, Wales, UK
31. Kuske CR, Barns SM, Bush JD (1997) Diverse uncultivated bacterial groups from soils of the arid Southwestern United States that are present in many geographic regions. Appl Environ Microbiol 63:3614–3621
32. Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York
33. Lewis D, White JR, Wafula D, Athar R, Williams HN, Chauhan A (2010) Soil functional diversity analysis of a bauxite mined restoration chronosequence. Microb Ecol 59:710–723
34. Lozupone C, Knight R (2005) UniFrac: a new phylogenetic method for comparing microbial communities. Appl Environ Microbiol 71:8228–8235
35. Magurran AE, Phillip DAT (2001) Implications of species loss in freshwater fish assemblages. Ecography 24:645–650
36. McCaig AE, Glover LA, Prosser JI (1999) Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. Appl Environ Microbiol 65:1721–1730
37. Morgan GW (1974) Crop productivity as affected by depth of topsoil spread for reclaiming bauxite mined lands in Jamaica. Tropical Agric 51:332–346
38. Mummey DL, Stahl PD, Buyer JS (2002) Soil microbiological properties 20 years after surface mine reclamation: spatial analysis of rehabilitated and undisturbed sites. Soil Biol Biochem 34:1717–1725

39. Muyzer G, De Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700
40. Nadkarni MA, Martin EF, Jacques NA, Hunter N (2002) Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology 148:257–266
41. Pielou EC (1975) Ecological diversity. Wiley, New York
42. Quaiser A, Ochsenreiter T, Lanz C, Schuster SC, Treusch AH, Eck J, Schleper C (2003) Acidobacteria form a coherent but highly diverse group within the bacterial domain: evidence from environmental genomics. Mol Microbiol 50:563–575
43. Quirino BF, Pappas GJ, Tagliaferro AC, Collevatti RG, Neto EL, da Silva MRSS, Bustamante MMC, Krüger RH (2009) Molecular phylogenetic diversity of bacteria associated with soil of the savanna-like Cerrado vegetation. Microbiol Res 164:59–70
44. Sait M, Davis KER, Janssen PH (2006) Effect of pH on isolation and distribution of members of subdivision 1 of the phylum Acidobacteria occurring in soil. Appl Environ Microbiol 72:1852–1857
45. Scholten JJ, Andriesse W (1986) Morphology, genesis and classification of three soils over limestone. Jamaica Geoderma 39:1–40
46. Shannon CE, Weaver W (1949) In: The mathematical theory of communication. University of Illinois Press, Urbana
47. Spain AM, Krumholz LR, Elshahed MS (2009) Abundance, composition, diversity and novelty of soil Proteobacteria. ISME J 3:992–1000
48. Spain AV, Hinz DA, Ludwig JA, Tibbett M, Tongway DJ (2006) Mine closure and ecosystem development—Alcan Gove bauxite mine. Northern Territory, Australia, ISBN 978-0-9756756-6-3. Proceedings of the first international seminar on mine closure 2006 In: First international seminar on mine closure, Perth, Western Australia, Australia, 13-15 September 2006
49. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
50. Tarlera S, Jangid K, Ivester AH, Whitman WB, Williams MA (2008) Microbial community succession and bacterial diversity in soils during 77 000 years of ecosystem development. FEMS Microbiol Ecol 64:129–140
51. Tibbett M (2009) Large-scale mine site restoration of Australian eucalypt forests after bauxite mining: soil management and ecosystem development. In: Batty L (ed) Ecology of industrial pollution: remediation, restoration and preservation. Cambridge University Press, Cambridge
52. Tokeshi M (1993) Species abundance patterns and community structure. Adv Ecol Res 24:112–186
53. US Environmental Protection Agency (USEPA) (1993) Methods for chemical analysis of water and wastes. Environ Monit Support Lab, Cincinnati
54. Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naïve Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73:5261–5267
55. White JR, Reddy KR (2000) The effects of phosphorus loading on organic nitrogen mineralization of soils and detritus along a nutrient gradient in the northern Everglades, Florida. Soil Sci Soc of Am J 64:1525–1534