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Variation in microbial community structure correlates with heavy-metal contamination in soils decades after mining ceased

Rachelle E. Beattie[a,1], Wyatt Henke[a,2], Maria F. Campa[b], Terry C. Hazen[b,c], L. Rex McAliley[a,3], James H. Campbell[a,*]

[a] Department of Natural Sciences, Northwest Missouri State University, Maryville, MO, USA
[b] Bredesen Center, University of Tennessee, Knoxville, TN, USA
[c] Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA

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ABSTRACT

Microorganisms play vital roles in Earth's biogeochemical cycles. Identifying disturbances in microbial communities due to anthropogenic contamination can provide insights into the health of ecosystems. Picher, Oklahoma, was the site of large-scale mining operations for Pb, Zn, and other heavy metals until the mid-1950s, operating within the Tri-State Mining District (TSMD) of Missouri, Kansas and Oklahoma. Although mining ceased decades ago, high concentrations of heavy metals (> 1000 ppm) remain in area soil and water systems. Previously, we mapped metal concentrations on samples collected from mine tailings in Picher and along cardinal-direction transects within an 8.05-km radius of the town. To elucidate changes in microbial community structure due to regional metal contamination, 16S rRNA gene sequences and qPCR calculations of total *Bacteria* and *Archaea* were analyzed against these metal concentrations. *Bacteria* were negatively and significantly correlated with Pb, Cd, Zn, and Mg; however, *Archaea* was only significantly and positively correlated with pH. Illumina sequencing of 16S rRNA genes showed significant differences in microbial communities of chat and west transect samples. Comparison of soil chemistry with community structure indicated that Al, Pb, Cd, and Zn significantly impacted community composition and distribution of individual OTUs. Mapping the distribution of heavy-metal contamination and microbial communities in these soils represents the first step in understanding effects of long-term, heavy-metal contamination at a basic trophic level.

1. Introduction

Microorganisms are vital to biogeochemical cycles necessary for healthy ecosystems (Madsen, 2011; Smith et al., 2015). As the most abundant and diverse form of life on Earth, changes in microbial communities provide insight into the health and functioning of complex environments (Whitman et al., 1998; Horner-Devine et al., 2004). Heavy-metal contamination has been implicated frequently in altering microbial community structure by reducing biomass and diversity; these effects have been well documented for aerobic soil microbial communities in cultivation-based studies, and, more recently, with 16S rRNA gene sequencing (Bardgett et al., 1994; Leita et al., 1995; Kandeler et al., 1996; Konopka et al., 1999; Sobolev and Begonia, 2008).

Anthropogenic activities such as mining and smelting are primary sources of heavy-metal contamination in soils. Factors including soil pH and moisture can affect mobility and distribution of metal contamination (John and Leventhal, 1995; Violante et al., 2010). Aided by natural processes such as wind, rain, and erosion, soil particles containing heavy-metal contaminants can travel more than 20 km from their origins, affecting soils and water systems indirectly linked to the original site (Arditsoglou and Samara, 2005; Zota et al., 2009).

Soil microbial communities play a critical role in the development of stable soil systems (Torsvik and Øvreås, 2002; Schulz et al., 2013). Soil vitality, in turn, influences terrestrial and aquatic ecosystems. Therefore, heavy-metal contamination in soils may potentially have system-wide implications. Microbial populations in soils drive production of carbon and nitrogen sources, in addition to other nutrients, aid

* Corresponding author.
E-mail address: jhcamp@nwmissouri.edu (J.H. Campbell).
[1] Current address: Department of Biological Sciences, Marquette University, Milwaukee, Wisconsin, USA.
[2] Current address: Department of Microbiology, Molecular Genetics & Immunology, University of Kansas Medical Center, Kansas City, Kansas, USA.
[3] Current address: DESCO Environmental Consulting, Magnolia, Texas, USA.

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in establishing plant communities, and help develop food web structures (Aneja et al., 2006; Schulz et al., 2013). Disturbances in microbial communities caused by heavy-metal contamination can disrupt these essential biogeochemical processes. However, long-term exposure of heavy metals in soils may produce communities with higher levels of tolerance and resistance (Feris et al., 2004; Joynt et al., 2006). Therefore, mapping community shifts in contaminated zones is vital to understanding the diversity present and potentially recovering microbes beneficial for remediation efforts.

Picher, Oklahoma, is an abandoned mining town that was once part of the larger Tri-State Mining District (TSMD) of Kansas, Missouri, and Oklahoma (McKnight and Fischer, 1970). The town is located within the larger Picher mine field (PMF) which occupies approximately 40 square miles (Andrews et al., 2000-2006; United States Geological Survey, 2015). Mining began in 1904 after the discovery of abundant Pb and Zn in deposits of galena and sphalerite, respectively (McKnight and Fischer, 1970, Beyer et al., 2005). Mining and smelting activities in the PMF created extensive contamination. On the ground surface, contamination is dominated by large piles of milled mine waste, termed chat, that is mostly composed of chert and traces of mined metals (McKnight and Fischer, 1970, Dames and Moore, 1993, Beyer et al., 2005). Chat piles within the PMF can reach 60 m in height (Wasiuddin et al., 2010; Wasiuddin et al., 2011; Johnson et al., 2016) and cover over 35 million cubic meters of surface area, amounting to nearly 68 million metric tons of waste. Pb, Zn, and Cd are heavy metals found in the highest concentration in chat, but traces of other hazardous metals (e.g. Al, Fe, Ni) have been discovered, as well (Zota et al., 2009; Johnson et al., 2016). All mining activity in the area ended by 1970 after the discovery of elevated blood lead levels (BLLs) in local children (United States Environmental Protection Agency, 2016). In 1983, the United States Environmental Protection Agency declared the PMF a “Superfund” site (Johnson et al., 2016; United States Environmental Protection Agency, 2016).

Our earlier work characterized local soil chemistry (Beattie et al., 2017) and documented unequal distributions of heavy-metal contamination in soils at distances of up to 8.05-km from chat. This study focuses on the use of molecular techniques to examine impacts of heavy-metal contamination on soil microbial community structure. Mapping the distribution of heavy-metal contamination and its effects on microbial communities in these soils represents the first step in understanding the effects of long-term heavy-metal contamination at a basic trophic level.

2. Materials and methods

2.1. Description of sampling sites and sample collection

Topsoil samples (n = 111; 0–10 cm depth) were collected within an 8.05-km radius of Picher in Ottawa County, Oklahoma, in August 2015, as part of a comprehensive study on chemical characterization and microbial community structure within the PMF (Beattie et al., 2017). Details of all measured environmental variables can be found in Beattie et al. (2017). Samples were collected using an ethanol-cleaned metal hand spade at intervals of 0.32 km in each of the cardinal directions (n = 100) extending from and directly from mine tailings found within chat piles of Picher (n = 11). Samples were stored on ice at the time of collection (< 24 h) and subsequently stored at −80 °C until further analysis.

2.2. Soil chemistry

Soil pH, moisture, and heavy-metal concentrations of samples (n = 111) were collected in an associated study (Beattie et al., 2017). Concentrations of 20 metals (Al, Ar, B, Cd, Cr, Co, Cu, Fe, Mg, Mn, Mo, Ni, Pb, K, Na, Te, Ti, W, V, and Zn) were analyzed following a modified EPA method (3050B) using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; Varian 710-ES) (United States Environmental Protection Agency, 1996).

2.3. DNA extraction and qPCR

Genomic DNA was extracted from 0.25 g of soil (dry-weight equivalent) using the MoBio Power Soil DNA Isolation Kit. Domain-wide, SYBR green assays for *Bacteria* and *Archaea* were used to determine abundances of *Bacteria* and *Archaea* using quantitative-PCR (qPCR). Standards were generated by amplifying full-length 16S rRNA genes cloned into pGEM T-Easy vectors (Promega). *Escherichia coli* B (ATCC 8739) served as the bacterial standard with primers 338F and 518R (Fierer et al., 2005). *Pyrococcus furiosus* (DSM 3638) was used for the archaeal standard using primers 915F and 1059R (Reysenbach et al., 2006). Clones were confirmed by Sanger sequencing (University of Tennessee, Knoxville). Plasmids were purified by alkaline lysis (Sambrook and Russel, 2001) and quantified using a Qubit fluorometer (Invitrogen). qPCR reaction mixtures contained 18 µL of Master Mix and 2 µL of gDNA, as described previously (Gihring et al., 2011; Shakya et al., 2013). Resulting quantifications were converted into gene copies g^{-1} of soil. MATLAB statistical software was used to calculate Pearson correlations between *Bacteria* and *Archaea* quantifications with previously measured environmental variables (Beattie et al., 2017).

2.4. Amplicon library preparation and sequencing

Amplicon libraries of the V4 region of the 16S rRNA gene were obtained using 515F and 806R barcoded primers. Sixty samples were randomly selected for sequencing using MATLAB statistical software (available in Table S1). PCR was conducted using Platinum Taq High Fidelity components from Invitrogen and dNTPs from Roche. Cycling conditions consisted of an initial denaturation at 94 °C for 2 min, followed by 25 amplification cycles of 94 °C for 45 s, 55 °C for 30 s, 68 °C for 1 min 30 s, then a final extension cycle of 72 °C for 5 min. Following PCR, samples were visualized on agarose gels, combined into 11 pools based upon similarity of concentration, and quantified using a fluorometer. Illumina sequencing was performed according to manufacturer suggestions. Sequences were subjected quality control and operational taxonomic unit (OTU) clustering using mothur bioinformatics software (version 1.37.4; Schloss et al., 2009) and Schloss's MiSeq SOP (Kozich et al., 2013) using the Comet Supercomputer (San Diego Supercomputing Center; SDSC) accessed through the XSEDE initiative (www.xsede.org).

2.5. Statistical analyses

Singleton, doubleton, and non-microbial OTUs were discarded (Shakya et al., 2013). Raw counts of remaining OTUs within each sample were converted into percentages, square-root transformed, and a Bray-Curtis resemblance matrix was calculated (Campbell et al., 2012; Clarke and Gorley, 2006). This matrix was used for nonmetric multidimensional scaling (NMDS), distance-based linear modeling (DistLM), and distance-based redundancy analysis (dbRDA) when coupled with soil-chemistry measurements (Anderson et al., 2008). All samples and variables were selected for DistLM analysis and dbRDA plot. Individual OTUs were analyzed for correlations to soil chemistry using canonical correspondence analysis (CCA; ter Braak, 1986) and Spearman's correlation coefficients. These analyses used PRIMER (version 6.1.15) with the PERMANOVA + (version 1.0.5) add-on package (Clarke and Gorley, 2006), Matlab running an open-source library (Strauss, 2010), and R packages vegan (Oksanen et al., 2017) and psych (Revelle, 2017). R was accessed on the Stampede Supercomputer (Texas Advanced Computing Center; TACC) and the Bartik computational cluster (NWMSU).

2.6. Sequence deposition

Nucleotide sequences generated in this study have been deposited in the NCBI Sequence Read Archive (Accession no. SRP153081).

3. Results

3.1. Occurrence of bacteria and archaea within the PMF

Samples were randomly selected ($n = 60$) from 111 sampling sites (Beattie et al., 2017) for qPCR and Illumina analysis (Table S1). Multiple attempts were made to amplify all 11 samples from chat; however, low DNA yields left us with amplicons from only two chat samples. This was likely due to low biomass in pure chat. Total bacteria and archaeal counts ranged from 2.30×10^8–1.36×10^{10} copies g^{-1} and 1.98×10^6–7.73×10^7 copies g^{-1}, respectively (Table S2). Mean values were 4.74×10^9 copies g^{-1} for *Bacteria* and 3.51×10^7 copies g^{-1} for *Archaea*.

3.2. Correlations of bacterial and archaeal 16S rRNA gene copies and environmental factors

Correlations of bacterial and archaeal 16S rRNA gene copies to each other and environmental factors are shown in Table 1. Gene copies of bacteria and archaea were significantly and positively correlated to one another ($p = 6.01 \times 10^{-4}$; Fig. 1), an interesting trend as neither group subsequently correlated with the same environmental parameters (Table 1). Bacterial numbers were significantly and negatively correlated with Pb, Cd, Zn, and Mg (Figure S1). Archaeal copy numbers showed a significant positive correlation with soil pH; however, archaeal numbers were not significantly correlated with any other measured environmental factor, including heavy metals.

3.3. Diversity indices of PMF microbial communities

Analysis of 16S rRNA gene sequences in 60 samples provided a total of 5,636,726 sequences clustered in 53,273 OTUs at a 0.03 genetic distance. Removal of OTUs that were singletons, doubletons (Shakya et al., 2013) and non-microbial left 5,582,368 sequences in 27,082 OTUs that were included in subsequent analyses. Shannon-Wiener and Simpson diversity indices were calculated to determine if selected metals (Al, Pb, Zn, Cd) significantly impacted whole community richness and evenness (Table S3). Only Pb significantly correlated with diversity measurements ($p < 0.05$, Figure S2). However, Shannon-Wiener diversity indices indicated a reduction in diversity while Simpson diversity indices indicated an increase in diversity with increasing Pb concentration indicating conflicting results ($r^2 = -0.377$, $r^2 = 0.267$, respectively; Figure S2).

Table 1
Pearsons's correlations of qPCR assays of total *Bacteria,* total *Archaea*, and bacteria:archaea ratio.

Variable	Bacteria		Archaea		Bacteria:Archaea	
	r	*p*	r	*p*	r	*p*
H3O+	0.06	0.64	**0.54**	**< 0.01**	−0.06	0.67
% Moisture	0.18	0.16	0.15	0.27	0.10	0.45
Te	−0.12	0.37	−0.08	0.53	−0.05	0.68
W	−0.06	0.63	0.04	0.76	−0.13	0.34
K	0.17	0.19	0.10	0.46	0.02	0.86
Mn	−0.03	0.82	−0.16	0.23	0.09	0.49
Ni	−0.03	0.84	−0.04	0.78	0.06	0.68
Al	0.22	0.09	0.13	0.32	0.07	0.59
Cd	**−0.33**	**0.01**	−0.15	0.26	−0.07	0.61
Zn	**−0.35**	**0.01**	−0.17	0.19	−0.07	0.59
Fe	−0.01	0.96	−0.07	0.62	0.08	0.54
Pb	**−0.32**	**0.01**	−0.20	0.12	0.03	0.81
V	0.20	0.12	0.07	0.60	0.12	0.35
B	−0.11	0.39	−0.05	0.68	−0.06	0.67
As	−0.04	0.76	−0.11	0.42	0.12	0.35
Cu	−0.23	0.08	−0.14	0.28	0.06	0.64
Co	0.02	0.90	−0.04	0.79	−0.06	0.63
Cr	−0.01	0.95	−0.09	0.50	0.14	0.28
Ti	0.16	0.23	0.17	0.21	−0.04	0.76
Na	0.00	0.99	0.01	0.92	−0.12	0.35
Mg	**−0.42**	**< 0.01**	−0.12	0.34	−0.25	0.05

Bold text indicates statistical significance as controlled by Benjamini-Hochberg adjustment.

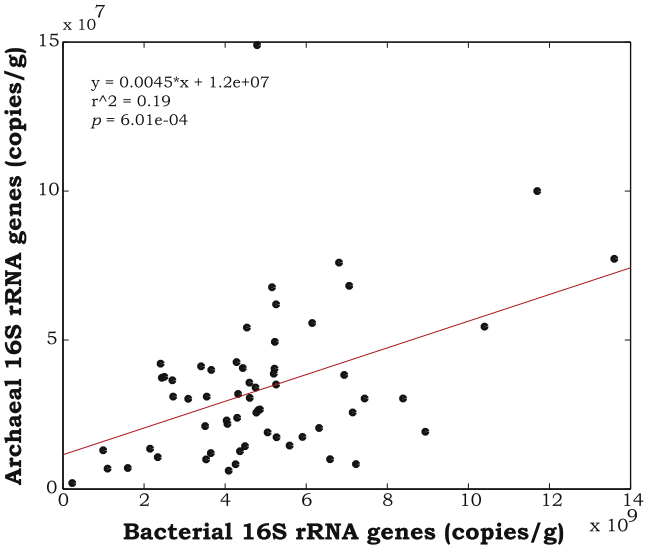

Fig. 1. Correlation of bacterial to archaeal 16S rRNA gene counts.

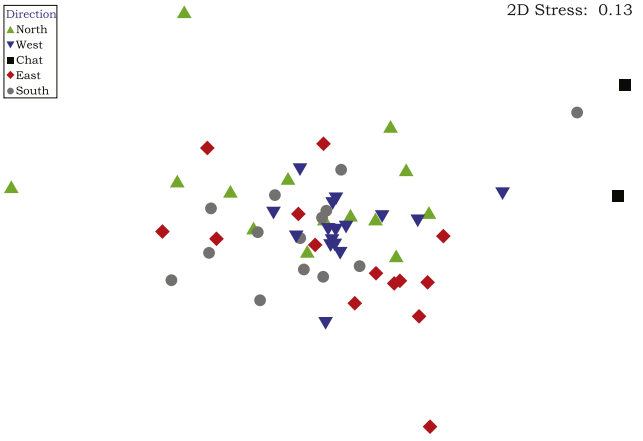

Fig. 2. Nonmetric multidimensional scaling (NMDS) representation of OTU-based clustering (0.03 genetic distance). Counts for each OTU within each sample were standardized to percentage, square-root transformed and a Bray-Curtis similarity matrix was calculated.

3.4. Nonmetric multidimensional scaling and PERMANOVA analysis of PMF microbial community structure

Microbial community composition was compared among samples using a Bray-Curtis similarity matrix (Clarke and Gorley, 2006). To elucidate relationships of microbial communities within the PMF, a nonmetric multidimensional scaling (NMDS) plot (Fig. 2) indicated that east, south, and west samples showed low levels of dissimilarity both within and among sampling direction, as evidenced by their overlap. Samples from the north transect were more dissimilar from both one another and from all other directions as indicated by the horizontal

spread (Fig. 2). Additionally, the microbial communities of the chat were most dissimilar from all directional transects; however, it can be noted that the microbial community composition of S02 and W01 (the two sampling locations closest to the chat locations on the plot) were more similar to chat communities than other sampling sites. The sampling location of these two samples, taken at distances of 0.64 km and 0.32 km from the center of Picher, respectively, indicates that proximity to surface chat piles in Picher likely plays a role in the composition of microbial communities within the PMF. Close inspection of the individual samples plotted on the NMDS also revealed samples more similar to chat (located on the right side of the plot) are all located within 4.025-km of the town center of Picher which is the maximum distance of surface chat piles surrounding Picher. Together, these results indicate that metal contamination influences microbial community composition in soils surrounding the PMF.

As the NMDS plot revealed directional patterns of microbial communities, we conducted a PERMANOVA to determine if the microbial community structure of sampling directions was significantly different. PERMANOVA and subsequent *post-hoc* test determined that the west transect and chat were significantly different from all other sampling transects (Tables S4 and S5, $p < 0.05$).

3.5. Distance-based redundancy analysis of measured environmental variables and PMF microbial communities

Although NMDS and PERMANOVA determined that significant differences in microbial communities within the PMF exist, this analysis does not specify which environmental variables explain these differences. To elucidate the environmental factors that best explain the microbial community variation, distance-based linear models (distLM) and distance-based redundancy analysis (dbRDA) were used. The proportion of variation in microbial community profile caused by individual environmental factors are provided in Table 2. Soil moisture percentage, pH, and nine heavy metals including Pb, Zn, and Cd, explained a significant proportion of variation. This result supports qPCR data which demonstrated correlations between bacterial gene number and many of the above environmental factors (e.g. Pb, Zn, Cd, etc.).

Ordination of microbial community profiles in response to measured environmental factors using dbRDA (Fig. 3) explained significant amounts of variation in community structure. Mg, Zn, Al, and percent moisture are the primary environmental factors accounting for dbRDA1 and horizontal spread of sampling sites across the plot while Pb, Ti, Cu, Cd, and K are the primary environmental factors accounting for dbRDA2 and vertical spread of sampling sites across the plot. Although most samples from each sampling direction are observably heterogeneous, chat samples and those samples found within 4.025 km of the town center are again closely ordinated on the upper right-hand corner of the plot, and Zn and Mg are the primary environmental factors accounting for their variation.

Table 2
DistLM marginal tests of soil chemistry on microbial community structure.

Variable	*Pseudo-F*[a]	Proportion of variation explained	*p*[b]
Al	5.037	7.99E-02	**0.000**
Zn	4.795	7.64E-02	**0.000**
Pb	4.336	6.96E-02	**0.000**
Mg	4.121	6.63E-02	**0.000**
H_3O^+	4.100	6.60E-02	**0.000**
V	3.888	6.28E-02	**0.000**
Cd	3.505	5.70E-02	**0.000**
Cu	3.449	5.61E-02	0.000
K	3.366	5.49E-02	**0.000**
Ti	2.462	4.07E-02	**0.001**
Moisture	2.107	3.51E-02	**0.007**
Mn	1.649	2.77E-02	0.037
Co	1.554	2.61E-02	0.060
Cr	1.366	2.30E-02	0.110
Fe	1.286	2.17E-02	0.132
Te	1.283	2.16E-02	0.140
Ni	1.110	1.88E-02	0.276
W	1.087	1.84E-02	0.299
Na	0.926	1.57E-02	0.529
B	0.925	1.57E-02	0.521
As	0.887	1.51E-02	0.589

[a] Pseudo-*F* value from permuted tests ($n = 9999$).
[b] Bold text indicates statistical significance as controlled by Benjamini-Hochberg adjustment.

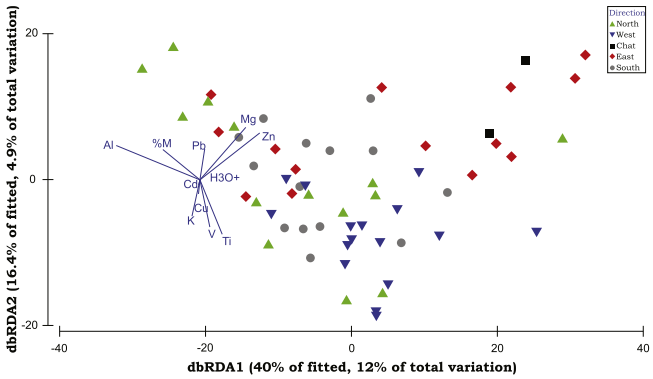

Fig. 3. Distance-based redundancy analysis (dbRDA) of soil microbial communities in response to measured soil chemistry. Variables found to explain significant amounts of variation in communities by DistLM (Table 2) were included in the model.

3.6. Correlations of individual OTUs with metals of historical importance in the PMF

To determine the impact of all measured environmental variables on individual OTUs, a Canonical Correspondence Analysis (CCA; Fig. 4) was used. Individual OTUs appear to be most strongly influenced by pH, Mg, Co, and Al. Metals associated with mine tailings in Picher (Pb, Cd, Zn) influence OTUs similarly as shown by the close proximity of their vectors on the CCA and distribution of OTUs around them. Additionally, Spearman's rho correlations of OTUs with the metals of economic and mining importance (Al, Cd, Pb and Zn) were analyzed. Seven phyla containing 100 or more individual OTUs were found to significantly correlate with at least one of these metals including *Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Planctomycetes,*

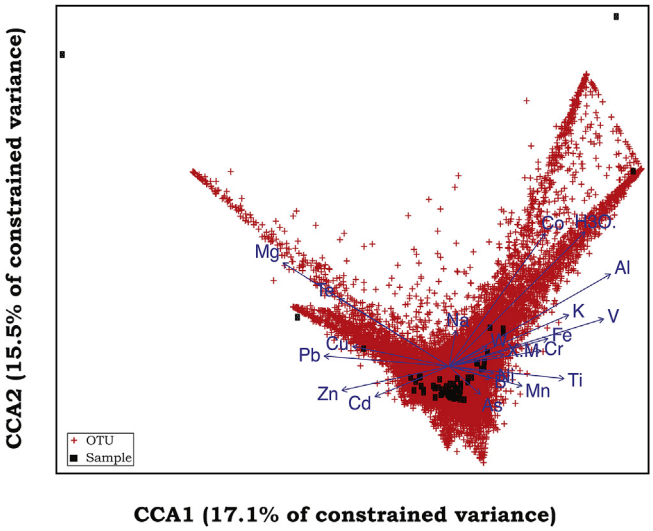

Fig. 4. Canonical correspondence analysis (CCA) of OTUs in relation to measured soil chemistry.

Proteobacteria, and *Verrucomicrobia*. Within these phyla, multiple genera were identified to significantly and positively correlate with increased metal concentration including *Streptomyces* and *Amycolatopsis* within *Actinobacteria*, *Flavisolibacter* within *Bacteroidetes*, *Sphingomonas* and *Geobacter* within *Proteobacteria*, and *Gemmata* and *Planctomyces* within *Planctomyetes*. Additionally, of the 2591 OTUs that correlated with at least one element of interest (Al, Cd, Pb, and Zn), 60% (1569 OTUs) correlated with two or more of the listed metals and 28% (715 OTUs) correlated with all four listed metals (Table S6).

4. Discussion

4.1. Bacteria and archaea 16S rRNA gene copies correlate differentially with measured environmental factors

Results from qPCR data indicated that *Bacteria* and *Archaea* were significantly and positively correlated in the PMF soil communities. Interestingly, *Archaea* was only significantly correlated with soil pH, a phenomenon that is well documented for a variety of soil types (Fierer and Jackson, 2006; Wardle, 1992). This was surprising as we anticipated some significant impact of measured heavy metals on archaeal gene copy numbers, especially as *Archaea* significantly correlated with *Bacteria*. Studies have shown that genes for metabolism, resistance and detoxification are widespread throughout the archaeal domain, particularly in the phylum *Crenarchaeota* (Bini, 2010; Yin et al., 2015). However, OTU analysis of 16S rRNA genes revealed low numbers of the *Crenarchaeota* phylum. It is possible that *Bacteria* and *Archaea* are simply affected differently by heavy-metal contamination, as this result has been identified between *Bacteria* and *Fungi* (Rajapaksha et al., 2004).

Significant, negative correlations of *Bacteria* gene copy numbers and four metals (Pb, Cd, Zn, and Mg) were also found within the PMF samples. These negative correlations were anticipated as heavy metals exert a toxic effect on soil microorganisms, particularly at high concentrations (Hiroki, 1992; Wang et al., 2007). Pb, Cd, and Zn are known anthropogenic contaminants from mining activity in the area. Each of these three metals has been shown to reduce microbial biomass and productivity at concentrations as low as 1 ppm for Pb, 2 ppm for Cd, and 5 ppm for Zn. (Bollag and Stotzky, 1990; Choudhury and Srivastava, 2001; Sobolev and Begonia, 2008; Trevors et al., 1985). PMF samples contained Pb, Cd, and Zn in concentrations ranges from 3 to 1115 ppm, 0.08–43 ppm, and 8–4486 ppm, with mean values of 76 ppm, 4 ppm, and 711 ppm, respectively (Beattie et al., 2017). Excessive quantities of these heavy-metals are likely causing the reduction in observed bacterial 16S rRNA gene copies.

Although Mg was also significantly and negatively correlated with bacterial numbers, mean values of Mg in the PMF soils (~303 ppm) were lower than area means (1500 ppm), according to the United States Geological Survey for Ottawa County, OK (McKnight and Fischer, 1970). Mg is a cofactor of many enzymes (Gottschalk, 1986) and it is difficult to reach toxic levels within the cell (Chamnongpol and Groisman, 2002); therefore, the relationship between Mg and bacterial concentrations in PMF soils was unexpected. One possible explanation for this negative correlation is that Mg is an elemental component of dolomite rock. Dolomite rock is found within Mississippi-Valley type deposits of the PMF, which also contain the mineral precursors of the primary contaminating metals in Picher (Johnson et al., 2016; van der Perk, 2013). Therefore, the observed bacteria-Mg correlation is more likely due to an underlying correlation of Mg to Pb, Cd, and Zn. However, the assumption that Pb, Cd, and Zn are bioactive and therefore influencing microbial community structure while Mg is not needs to be tested further.

4.2. Microbial community structure is impacted by patterns of heavy-metal contamination in the PMF

Studies conducted at the PMF have focused on correlations between heavy-metal concentrations and changes within organisms of higher tropic levels including, fish, waterfowl, and blood lead levels in children (Beyer et al., 2005; Brumbaugh et al., 2005; United States Environmental Protection Agency, 2016). No soil microbial community research has been conducted in the PMF; therefore, connections between this study and previous work is difficult. However, research on other heavy-metal contaminated soils has shown that microbial community structure varies greatly in conjunction with changes in contaminant concentration (Azarbad et al., 2015; Gough and Stahl, 2011; Hemme et al., 2010). Variation within samples is evident from NMDS analysis (Fig. 2). In particular, samples from chat are dissimilar from other sampling directions; however, samples in close proximity to surface chat (within 4.025-km of the town center) are more similar to chat than samples collected at further distances. This result is congruent with soil chemistry data collected in our associated study, as chat soil chemistry was found to be significantly different from all other sampling directions.

One difference noted between soil chemistry data and the microbial community structures is that north soil chemistry was significantly different while west microbial communities were significantly different from all other sampling directions. Visually, samples from the north transect appear more dissimilar than other samples, so initially this result was surprising (Fig. 2). However, upon further investigation, the plot clearly displays that samples from the west transect are more *similar* to each other than any other group. This result is likely due two possible factors: relatively low soil moisture content of west transect samples and proximity to surface chat piles directly adjacent to sampling locations. dbRDA (Fig. 3) indicated that soil moisture percentage (along with pH and nine heavy metals including Pb, Zn, and Cd) explained a significant proportion of microbial community variation (Table 2). These data are supported by both our qPCR and environmental factor correlations in addition to previous field research. Soil moisture content is an important factor for regulating diffusion of oxygen and other soluble substrates, and low soil moisture content (< 60%) has been shown to decrease microbial activity in soils (Csonka, 1989; Linn and Doran, 1984; Stark and Firestone, 1995). Thus, low soil moisture content may be a driving factor of significant differences between west transect samples and all other directions. Additionally, west transect samples were closest to roadside chat piles at distances further from the center of Picher than all other transects (see Beattie et al., 2017).

The importance of pH in soil microbial community structure was denoted above. Although pH did not significantly correlate with bacterial gene number in our study, it was correlated with archaeal gene number, and it has been well documented that pH explains variation in microbial communities for a variety of soil types (Fierer and Jackson, 2006; Wardle, 1992). Metals that explained significant variation in microbial communities included the three primary contaminating metals in the PMF (Pb, Zn, Cd), as well as other metals of importance (Al and Mg). Mg negatively correlated with bacterial gene numbers in this study and was highly correlated with Zn in our previous soil chemistry study, and Al is a common metal found in northeastern Oklahoma soils (McKnight and Fischer, 1970). These trends were supported by correlation data (Table 1) and the dbRDA plot (Fig. 3).

4.3. Diversity indices of whole communities reveal conflicting correlations with metals of importance in the PMF

Whole community OTU diversity indices revealed either no correlations or conflicting correlations with four metals of importance in Picher (Al, Pb, Zn, and Cd). Conflicting results for Pb correlations may be due to a difference in the calculation of each index. Although

statistically significant, correlations were weak and were likely impacted by an outlying sample (E06) that can be seen in Figure S2. In general, these metals appear to have little effect on microbial community richness and evenness. One likely explanation for this phenomenon is that the long-term presence of heavy metal contamination in these soils has altered the microbial communities to the point at which they are now stable; similar trends have been found in other heavy metal contaminated regions (Joynt et al., 2006; Markowicz et al., 2016). If this is the case, a lack of previous studies of PMF microbes precludes us from determining the length of time required to reach a climax community.

4.4. Individual OTUs correlate with metals of historical importance in the PMF

In contrast to whole community correlations, individual OTU correlations between heavy-metal concentration and OTU abundance identified significant relationships within seven phyla (*Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Proteobacteria,* and *Verrucomicrobia*) with at least one of four metals selected for analysis (Al, Cd, Pb and Zn). Species within these phyla have previously been identified as dominating in heavy-metal contaminated soils due to either inherent or acquired tolerances and resistances to toxic metals (El Baz et al., 2015; Gu et al., 2017; Kielak et al., 2016; Shen et al., 2016). At the genus level, *Gemmata* and *Planctomyces* were recently identified as dominant genera in agricultural soils with high heavy-metal stress (Shen et al., 2016), similar to our study, and genera *Streptomyces* and *Amycolaptopsis* were dominant in isolates found within abandoned Zn and Pb mining areas (El Baz et al., 2015). Interestingly, 60% of OTU abundances identified to correlate with at least one of the four selected metals correlated with two or more metals, supporting the interplay of these heavy metals on the microbial community. Collectively, these data indicate that soil microbial community structure has been strongly influenced by mining activity within the PMF, particularly by economically relevant heavy metals (Pb, Cd, and Zn). Also, remediation of these soils might be possible using a diverse collection of bacteria.

5. Conclusions

The aim of this study was to determine microbial community structure within heavy-metal contaminated soils at the PMF. Results indicated that Al, Cd, Pb, and Zn correlated with a decrease in total numbers of 16S rRNA genes of bacteria, but no such correlations were found with archaeal gene numbers. This result was unprecedented and warrants additional study. In addition, overall community composition analyses indicated that variation in community structure is present, with nine metals, pH, and soil moisture explaining a significant proportion of community variation. These results indicate that historical Pb and Zn mining activities have affected microbial community structure within PMF soils. This result is supported by 16S rRNA gene sequencing, indicating that these metals also correlate to significant increases in OTUs within the phyla *Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Proteobacteria,* and *Verrucomicrobia*.

Results from this study provide a better understanding of how distribution of heavy-metal contamination affects the composition of soil microbial communities. Although microorganisms are considered a basic trophic level, changes in microbial community structure has the potential to alter biogeochemical cycling, affecting ecosystems on a much larger scale. Future research in the PMF should focus on identifying microbial species capable of proliferation in high heavy-metal concentrations and elucidating microbial functional genes present to aid in remediation efforts. These data provide a basis for evaluating changes in ecological system structure as a result of heavy-metal contamination and should be utilized to elucidate trends at higher trophic levels.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.soilbio.2018.08.011.

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