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Alkaline mine drainage drives stream sediment microbial community structure and function

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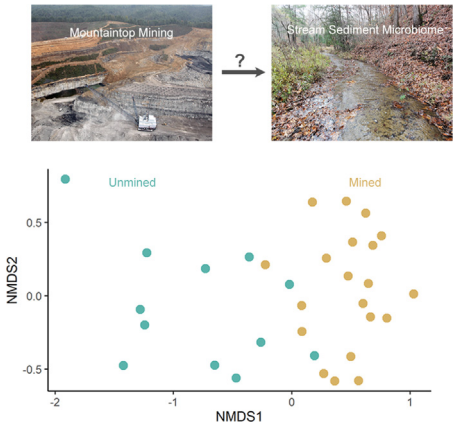
HIGHLIGHTS

- Bacterial richness and diversity highest at alkaline mine drainage impacted sites
- Se, S, %C and %N are main drivers of community structure.
- Indicator taxa are identified along a mining gradient.
- Alkaline mine drainage negatively affects microbial functional pathways.

GRAPHICAL ABSTRACT



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ABSTRACT

With advances in eDNA metabarcoding, environmental microbiomes are increasingly used as cost-effective tools for monitoring ecosystem health. Stream ecosystems in Central Appalachia, heavily impacted by alkaline drainage from mountaintop coal mining, present ideal opportunities for biomonitoring using stream microbiomes, but the structural and functional responses of microbial communities in different environmental compartments are not well understood. We investigated sediment microbiomes in mining impacted streams to determine how community composition and function respond to mining and to look for potential microbial bioindicators. Using 16s rRNA gene amplicon sequencing, we found that mining leads to shifts in microbial community structure, with the phylum Planctomycetes enriched by 1-6% at mined sites. We observed ~51% increase in species richness in bulk sediments. In contrast, of the 31 predicted metabolic pathways that changed significantly with mining, 23 responded negatively. Mining explained 15-18% of the variance in community structure and S, Se, % C and %N were the main drivers of community and functional pathway composition. We identified 12 microbial indicators prevalent in the ecosystem and sensitive to mining. Overall, alkaline mountaintop mining drainage causes a restructuration of the sediment microbiome, and our study identified promising microbial indicators for the long-term monitoring of these impacted streams.

1. Introduction

Microbial diversity is increasingly used as a tool for environmental biomonitoring due to microorganisms' sensitivity to disturbances, ubiquitous presence in the environment, and functional ecological

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importance (Cordier et al., 2019; Fortunato et al., 2013; Lanzén et al., 2020; Simonin et al., 2019). One example is the incorporation of microbial community data into the development of biotic indices for monitoring ecosystem responses to environmental changes (Aylagas et al., 2017; Caruso et al., 2016). Compared to traditional morphology-based approach which targets large organisms such as fish, birds and macroinvertebrates, studying microbiomes is a non-invasive approach which enables a phylogenetically comprehensive characterization of the community residing in a given environment (Cordier et al., 2019). Using high-throughput sequencing techniques, recent advances in eDNA metabarcoding significantly lowers the cost and improves the accuracy of high-throughput species identification from DNA in environmental samples (Taberlet et al., 2012). These molecular technologies make studying microbial communities a cost-effective alternative to the traditional morphology-based methods and enable a more integrative approach to ecosystem monitoring.

Using eDNA methodology, we set out to examine the impact of mountain top mining (MTM) on stream sediment bacterial community structure, and subsequently test if we could identify putative bioindicators for this set of anthropogenically-imposed stressors. MTM is the dominant form of land cover change in Central Appalachia and involves removing mountain ridges with explosives to expose coal seams (Bernhardt et al., 2012; Bernhardt and Palmer, 2011). MTM has profound impacts on regional river and stream ecosystems since the surface mines dispose waste rock into valleys, where they bury headwater streams and cause significant changes in stream hydrology and water quality (Ross et al., 2016). High carbonate content in bedrock leads to alkaline mine drainage (AlkMD) through neutralization of sulfuric acid, leading to an increase in calcium, magnesium, bicarbonate, and sulfate in the receiving waters (Bernhardt et al., 2012; Bernhardt and Palmer, 2011; Lindberg et al., 2011). AlkMD also leads to a significant increase in trace elements such as selenium (Se) in the water column, aquatic organisms, and in riparian spiders (Lindberg et al., 2011; Naslund et al., 2020). AlkMD causes a consistent decline in aquatic diversity across a broad range of organisms (Giam et al., 2018). We speculated that AlkMD would also cause significant changes in microbial assemblages in stream sediment, as microbial diversity and composition are known to be sensitive to pH, nutrients, and metal concentrations (Huang et al., 2016).

Relatively few studies have focused on the impact of AlkMD on microbial communities, in contrast of the large body of literature on acid mine drainage (AMD) (Baker and Banfield, 2003; Denef et al., 2010; Huang et al., 2016; Kuang et al., 2013; Méndez-García et al., 2014). As microbial community diversity and composition vary along environmental gradients, microbial communities in streams receiving AlkMD could be fundamentally different from AMD. Of the few studies that have examined AlkMD-impacted streams, there are inconsistent trends in microbial response, with some studies reporting a negative impact on microbial diversity in biofilms and the water column (Bier et al., 2015; Giam et al., 2018), while others found no impact on riparian soil microbial diversity (Fan et al., 2016). Research on the functional response of microbial communities to AlkMD has also yielded inconsistent results. A few studies found no overall impact of AlkMD on sulfur (S) metabolism despite elevated S concentration in AlkMD-impacted streams (Bier et al., 2015, 2020), whereas other studies found evidence of increased sulfate reduction in riparian soils and sediments (Fan et al., 2016; Kang et al., 2013). Discrepancies among the studies could result from differences in field conditions as well as the environmental compartment studied.

In this study, we examined sediment microbial communities along an environmental gradient spanning a total of 20 mining impacted and reference watersheds in Central Appalachia (0-97% of watershed mined). We asked the following questions: 1) How does AlkMD affect bacterial diversity and composition in the sediments, and what environmental factors are the main drivers of the changes? 2) Which bacterial taxa can be used as bioindicators for monitoring AlkMD pollution in sediments? 3) How does AlkMD affect the predicted functional composition and diversity of the microbial communities in sediments? To address these questions, we analyzed the sediment microbial community profiles obtained through 16S rRNA gene amplicon sequencing. We separated the samples into bulk and fine sediment fractions to examine whether response patterns of the two microbial communities differ since the fine sediment fraction contains more labile organic matter (OM) associated with higher microbial activity. We selected several environmental factors to characterize the physical and chemical gradients caused by AlkMD in the stream sediments, including: % watershed mined, Se, S, manganese (Mn), methyl mercury (MeHg), total mercury (THg), percent carbon (%C) and percent nitrogen (%N). Hg is of interest due to the high amount of Hg in coal and the extremely high weathering rates occurring near MTM (Gerson et al., 2020b; Ross et al., 2016). Se is of interest since Se concentrations in stream macroinvertebrates and emergent insects from these sites are among the highest reported in the literature (Naslund et al., 2020). We compared the putative microbial indicators found in our study with microbial indicators identified in an earlier study conducted in very similar field conditions in the same region by Bier et al. (2015) to see if there is any overlap in the taxa driving the community response patterns.

2. Methods

2.1. Study sites and sample collection

We collected samples in May 2018 from the Mud River watershed and neighboring mined watersheds located in southwestern West Virginia, USA (Boone, Lincoln, Logan, Raleigh, and Wyoming Counties). The Mud River watershed drains the Hobet 21 mine complex, which operated for ~50 years as the largest surface mine in Central Appalachia (Lindberg et al., 2011; Pericak et al., 2018). Many of the site's samples are part of a nearly decade-long monitoring program. Sites include both those that once had MTM within the watershed (hereafter, "Mined"; $n = 13$) and those without MTM (hereafter, "Unmined"; $n = 7$). The percent of the watershed mined was calculated per Naslund et al. (2020), and sampling methodology is described further in Naslund et al. (2020) and Gerson et al. (2020b). Initial site characterization data of the water column is also recorded in Gerson et al. (2020a).

We collected all sediment samples using clean hands-dirty hands protocol (USEPA Method 1669) (USEPA, 1996a) as bulk and fine sediment, with the fine sediment representing the more labile and microbially active portion. We collected one bulk and one fine sediment samples from each sampling location ($n = 20$). Due to sequencing constraints, we analyzed 20 bulk sediment and 12 fine sediment samples for microbial composition. All collected sediments were collected as surficial sediments. We collected fine sediment by vigorously mixing bulk sediment with stream water in a bucket for 30 s, then allowing settling for 30 s. We filled a sterile gallon-sized plastic bag with the water suspension and stored it for 24 h at 4 °C to allow the fine sediment to settle out. We then poured off the water to obtain the fine sediment. Bulk and fine sediment were stored frozen until lyophilization.

2.2. Soil physicochemical characterization

We analyzed homogenized sediments for total Hg via thermal decomposition, catalytic reduction, amalgamation, desorption, and atomic absorption spectroscopy on a Milestone Direct Mercury Analyzer (DMA-80; USEPA Method 7473) (USEPA, 1998). All samples were run in duplicate, and values were accepted if sample values had <10% relative percent difference. Average values between the duplicates are used for all analyses. All standards had average recoveries within 10% of accepted values, and all blanks were below detection limit. Prior to MeHg analysis, we digested sediment samples by microwave digestion with trace metal grade nitric acid (Rahman and Kingston, 2005; Tseng

et al., 1997). We ran digested sediment samples for MeHg by aqueous ethylation with sodium tetraethylborate, purge and trap, and cold vapor atomic fluorescence spectrometry on a Tekran 2500 spectrometer (CVAFS; USEPA Method 1630) (USEPA, 2001). All standards had average recoveries within 15% of accepted values, and all blanks were below detection limit. Prior to Se, S, and Mn analysis, we digested sediment samples using USEPA Method 3050B (USEPA, 1996b). We ran digested samples for Se and Mn using a Perkin Elmer Elan DRCII inductively coupled plasma mass spectrometry (ICP-MS), S using a Perkin Elmer ICP-Optical Emission Spectrometer Model 8000. All standards for Se, Mn, and S had recoveries within 10% of accepted values, and all blanks were below detection limit. Finally, sediment samples were analyzed for CHN using a Perkin Elmer 2400 CHNS Analyzer. More details on quality control for all elements can be found in Gerson et al. (2020b).

2.3. DNA extraction and 16S rRNA gene sequencing

We extracted DNA from fine and bulk sediment samples using the DNeasy PowerSoil Kit (Qiagen) following manufacturer's instructions. We measured total DNA concentration using the Qubit dsDNA BR Assay Kit (Invitrogen). For the sediment microbiome analysis, we targeted the V4 hypervariable region of the bacterial 16S rRNA gene (515F/806R) (Caporaso et al., 2011) and sequenced with Illumina MiSeq (150 bp paired end, V2 chemistry) at the MSU-RTSF. Raw sequences are available at ENA Sequence Read Archive: PRJEB45281.

2.4. Bioinformatic analyses of 16S rRNA gene sequences

The raw sequences were processed through the Quantitative Insights Into Microbial Ecology 2 (Qiime2) pipeline (Bolyen et al., 2019). We used the denoising software DADA2 to remove low-quality reads, putative chimera, and resolve the denoised sequences into amplicon sequence variants (ASVs) with default quality settings (Callahan et al., 2017). We assigned taxonomy to the ASVs using the Silva V132 (99%) reference database (Quast et al., 2013). For the ASVs of interest to downstream indicator analysis, the taxonomic affiliation was refined using Blastn on the National Center for Biotechnology Information (NCBI) website. Only ASVs affiliated with Bacteria and Archaea were kept in the dataset after filtering chloroplast and mitochondrial ASVs. ASVs present only in one sample were removed from the final dataset since they could be remaining sequencing errors not detected by DADA2, resulting in 4951 ASVs and 781,802 reads in final dataset. The samples were rarefied to 10,991 reads per sample.

2.5. Statistical analyses of bacterial community structure

All statistical and diversity analyses were performed in R (version 3.6.2) (R Core Team, 2019) using the rarefied ASV table. The diversity metrics (species' richness, Shannon diversity and Pielou's evenness) were calculated using the vegan package (version 2.5-6) (Oksanen et al., 2019). The differences in physicochemical properties between the mined and the unmined sites were examined using a nonparametric test (Mann-Whitney *U* test) because the sample distributions did not satisfy normality assumptions. Relationships between the diversity indices and measured sediment parameters were investigated using Spearmann linear regressions. Significant differences in community composition among the samples were tested by permutational multivariate ANOVA (perMANOVA) using the adonis() function in the vegan package.

To visualize the impact of mining on sediment bacterial community composition, we conducted nonmetric multidimensional scaling (NMDS) using the metaMDS() function in the vegan package. Distance matrices of the community data were based on Bray-Curtis distances. We examined the correlations between community composition and environmental variables using the envfit() function in the vegan package. Finally, we performed Threshold Indicator Taxa ANalysis (TITAN) using the TITAN2 (version 2.4) package to identify reliable indicator taxa and their environmental thresholds (change points) along the environmental gradients at both individual and community levels (M. E. Baker and King, 2010). We compared the indicator taxa identified in our study versus the indicator taxa identified in Bier et al. (2015) to see whether there was overlap at the family level and if so, whether the same family identified had similar response to mining. We also identified the core taxa defined as ASVs present at >90% of the samples at either the mining impacted or non-mining impacted sites using the microbiome package (version 1.8.0). We compared the overlap between the list of indicator taxa and the list of core taxa to find potential monitoring candidates for AlkMD pollution in sediments.

2.6. Predicted functional profiles

We used the software PICRUSt2 (Langille et al., 2013) that provides a computational approach, which uses a database of reference genomes to predict function profile for each 16S rRNA ASV with the closest relatives, and in turn aggregates this into the functional composition of a metagenome. The predicted composition of KEGG orthogroup (KOs) and MetaCyc metabolic pathway abundance among the samples was visualized using NMDS of Bray-Curtis distance matrices. Sample 181 had an extreme NMDS1 value that was three standard deviations above the average and was later discovered to have unusually large relative abundance of Firmicutes in the phylum composition compared to other samples. We therefore removed this sample from the PICRUSt2 downstream analyses. To examine the difference in relative abundance of the metabolic pathways between mining impacted and non-mining impacted sites, we calculated $\log_2$ fold changes of the predicted pathway abundance using the DESeq2 package (version 1.26.0) (Love et al., 2014).

3. Results

3.1. Chemical characterization of stream sediments

Concentrations of Se and S were significantly higher at the mined sites in both bulk ($p = 0.0049$, $p = 0.0055$, Mann-Whitney *U* test) and fine ($p = 0.00018$, $p = 0.00018$, Mann-Whitney U test) sediment fractions (Table S1). At both mined and unmined sites, fine sediment samples had higher concentrations of trace elements (Se, Mn, THg), S, %C and %N (p-value for Se = 0.00014, all else <0.0001, Mann-Whitney U Test) than bulk sediment samples.

3.2. Sequencing and taxa identification

We obtained 781,802 quality reads after denoising and filtering, from 20 bulk sediment and 12 fine sediment samples in total. The average number of reads per sample was 24,431 (range 10,199–39,253). A total of 4951 unique ASVs were obtained from these reads. Classification of the ASVs yielded 48 phyla, 89 classes, 205 orders, 367 families and 686 genera. Across all the samples, Proteobacteria represented the most dominant phylum (40.6%), followed by Bacteroidetes (11.5%), Acidobacteria (9.5%) and Actinobacteria (8.5%). Other less abundant phyla identified included: Verrucomicrobia (8.0%), Chloroflexi (7.8%), Planctomycetes (5.7%), Firmicutes (2.4%), Gemmatimonadetes (1.6%) and Nitrospirae (1.0%). All the other phyla had relative abundance < 1%. 0.2% of the reads were unclassified at the phylum level. The most abundant classes were Gammaproteobacteria (17.0%), Alphaproteobacteria (16.9%) and Bacteroidia (10.7%). 11.6% of the reads were unclassified at the class level. Of the ASVs, 3.4% were assigned to known species.

3.3. Impact of mining on bacterial diversity and community structure

We found that observed ASV richness was higher at the mined sites, with an average increase of 51% in the bulk sediment (Table S2). Shannon diversity was significantly higher at the mined sites in both

bulk ($p = 0.037$, Mann-Whitney *U* test) and fine sediments ($p = 0.018$, Student's *t*-test) (Table S2). In contrast, mining did not affect community evenness. We noticed that observed ASV richness and Shannon diversity were highly variable across the samples from the unmined sites but consistently higher at the mined sites (Fig. 1a).

At the phylum level, Planctomycetes was enriched (from 1 to 6% in the bulk and from 2 to 5% in the fine sediment) at the mined sites (Fig. 1b). Based on the non-phylogenetic Bray-Curtis distances, the bacterial community composition differed significantly between mined and unmined sites and between bulk and fine sediments (Fig. 2b). Mining ($R^2 = 0.13$, $p = 0.001$) had a larger impact on community composition compared to sediment type ($R^2 = 0.056$, $p = 0.009$), likely due to the overlap between the two communities as fine sediment samples were extracted from the bulk sediment (Fig. S3). Mining (unmined vs mined) explained 15-18% of the variance, and the percent of the watershed mined explained 16-17% of the variance in the community composition for both sediment fractions (Table S3). The NMDS analyses using Bray-Curtis matrix showed that the bacterial communities were structured by the extent of mining in the watershed, with percent watershed mined significantly correlated with NMDS1 (Fig. 2; Table S5). Se and S, which characterized the physicochemical differences between the mined and unmined sites, were also found to be significant drivers of bacterial community composition. Other significant environmental variables include %C, %N and THg (Fig. 2).

3.4. Indicator and core taxa along the mining gradient

On the bulk sediment samples, TITAN analysis identified 83 positive responders to mining that presented individual change points at 26.3 to 51.4% of watershed mined. Among all positive responders, the community-level change point was detected at 46% (Table S4; Fig. 3). In contrast, only 5 indicator taxa were found to respond negatively to mining. These few negative responders significantly decreased in abundance when 6.7-49.0% of the watershed was mined, with a community-

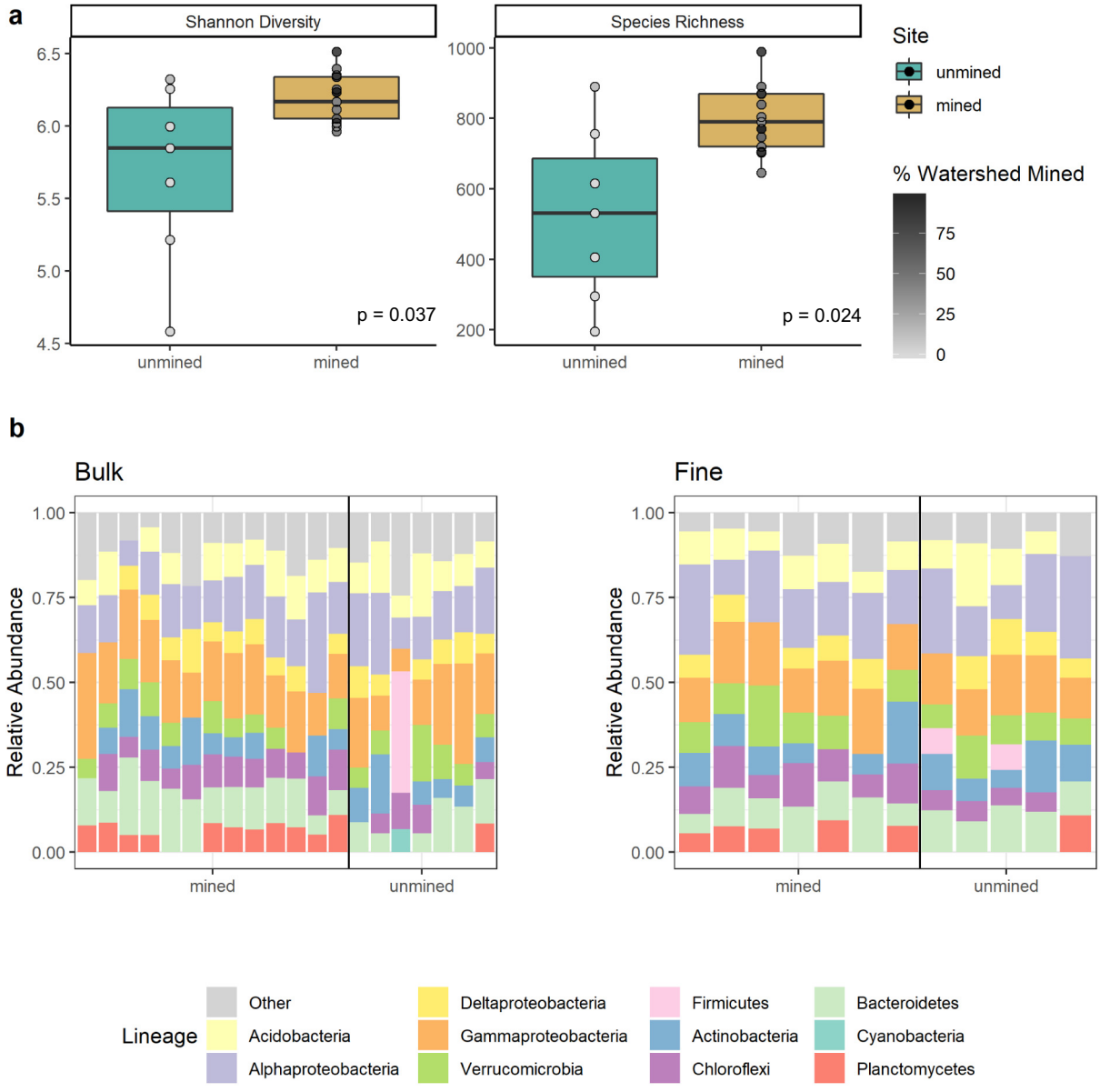

Fig. 1. a) Shannon diversity and observed species richness were significantly higher at the mined sites for bulk sediments. b) Relative abundance of dominant lineages (phylum/class level) for bulk and fine sediment samples; Planctomycetes was enriched at the mined sites in both sediment fractions.

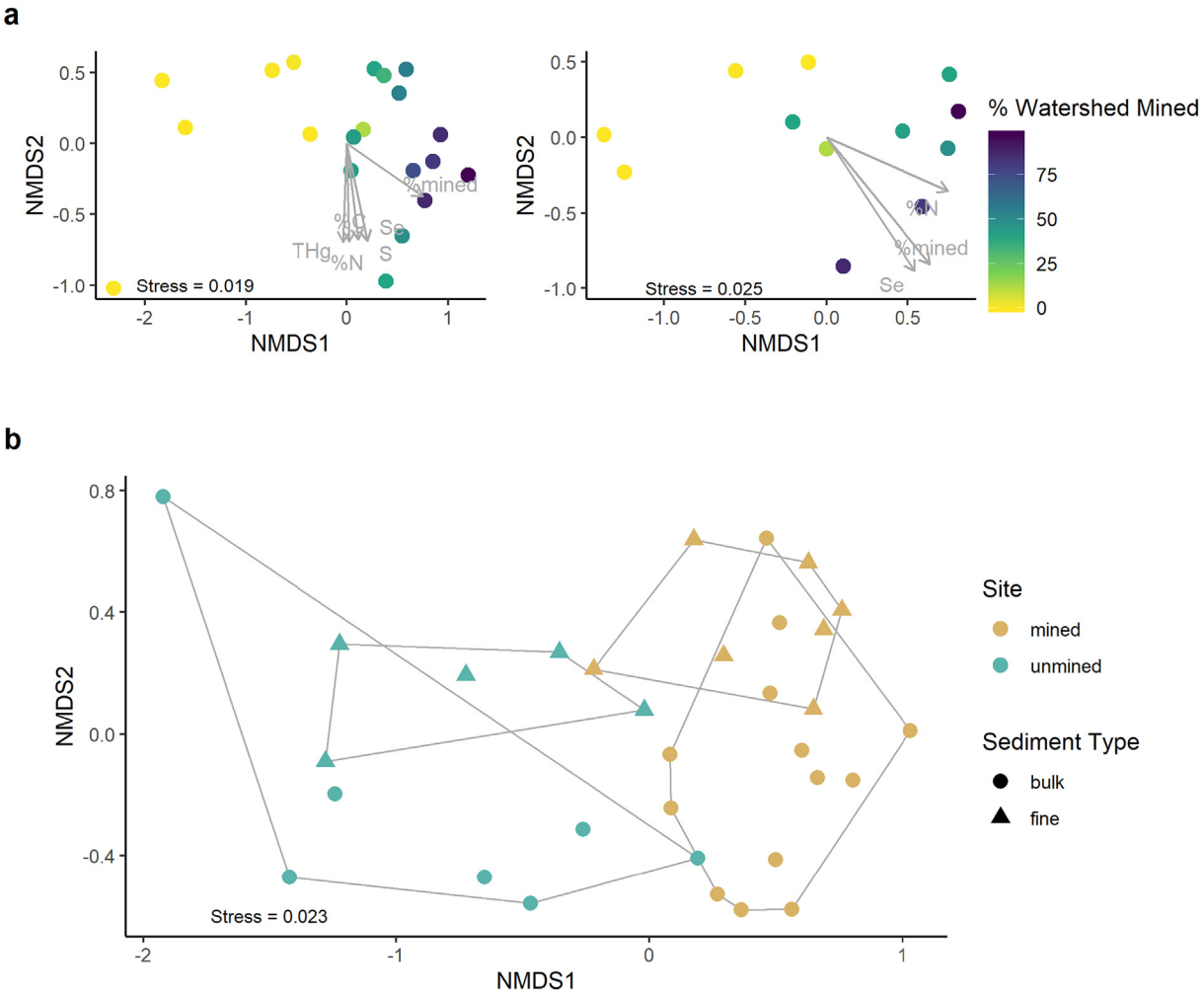

Fig. 2. NMDS plots depicting the impact of percent watershed mined and other environmental variables on microbial community structure for a) bulk and fine sediment samples and b) showing the impact of mining and sediment type on community composition. Only significant vectors are shown.

level change point at 23.4% (Table S4; Fig. 3). The indicator taxa covered a wide range of bacterial lineages, with Alphaproteobacteria (20.5%) as the dominant lineage, followed by Gammaproteobacteria (14.8%), Chloroflexi (13.6%) and Acidobacteria (10.2%) (Fig. 3). Using the same set of samples, TITAN identified 52 indicator taxa with a positive response to Se, a key indicator of MTM and a significant driver of community composition. The positive indicators responded to Se concentrations between 0.36 and 0.50 μg/g, with a community-level change point at 0.42 μg/g (Table S4). The six negative responders had similar thresholds to Se as the positive responders (Table S4). Twenty-five out of 58 indicator taxa to Se were also identified as indicator taxa when using percent of the watershed mined as the gradient variable in the TITAN analysis. TITAN analysis was also performed on the fine sediment samples; the result was not significant due to the lower sample size.

Of the 65 unique families found in the indicator species analysis in Bier et al. (2015) and 31 unique families found in our TITAN analysis, we found 12 indicator families in common. In particular, we found that 6 of the 12 families shared between the two studies had similar responses to mining and all of them were positively associated with mining (Table 2). In contrast, we found 5 indicator taxa responding positively to mining but belonged to families found to be associated with unmined sites in Bier et al. (2015).

We identified 35 core taxa (taxa present at >90% of samples) at the mined sites and 6 at the unmined sites, with 3 core taxa present in both mined and unmined sites for the bulk sediment (Fig. 4a). For the fine sediment, we found 30 core taxa at the mined sites and 19 at the unmined sites with 3 core taxa in common (Fig. 4b). 19 taxa were found to be core taxa at either mined or unmined sites for both sediment fractions (Fig. 4c). To identify ASVs that could be good targets for biomonitoring of the mining impacts, we identified the ASVs that were both highly prevalent at mined or unmined sites (core taxa) and responsive to the mining gradient (indicator taxa). We found that 12 of the bulk sediment's core taxa were also identified as indicator taxa (purity = 0.1, reliability > 0.95) using TITAN (Fig. 3; Table 1). Among the positive indicators of mining, one taxon in the phylum of Gammaproteobacteria and one taxon in the order of Rhizobiales had the highest relative abundance (0.51-0.64%) at the mined sites and high sensitivity to mining (low environmental change point, 6.7%). More details associated with these ASVs, including core type (whether the core taxa are specific to a condition), response type (positive or negative), prevalence and relative abundance of the taxa depending on the core type, and TITAN change point values are summarized in Table 1.

3.5. Impact of mining on predicted metagenome and metabolic features

To investigate the impact of mining on the functional profile of the bacterial communities, we used PICRUSt2 to calculate the relative abundance of the predicted gene orthologs and metabolic pathways in each sample. Mining did not impact the predicted gene and metabolic pathway richness (Fig. S1) or the composition of the predicted metagenome

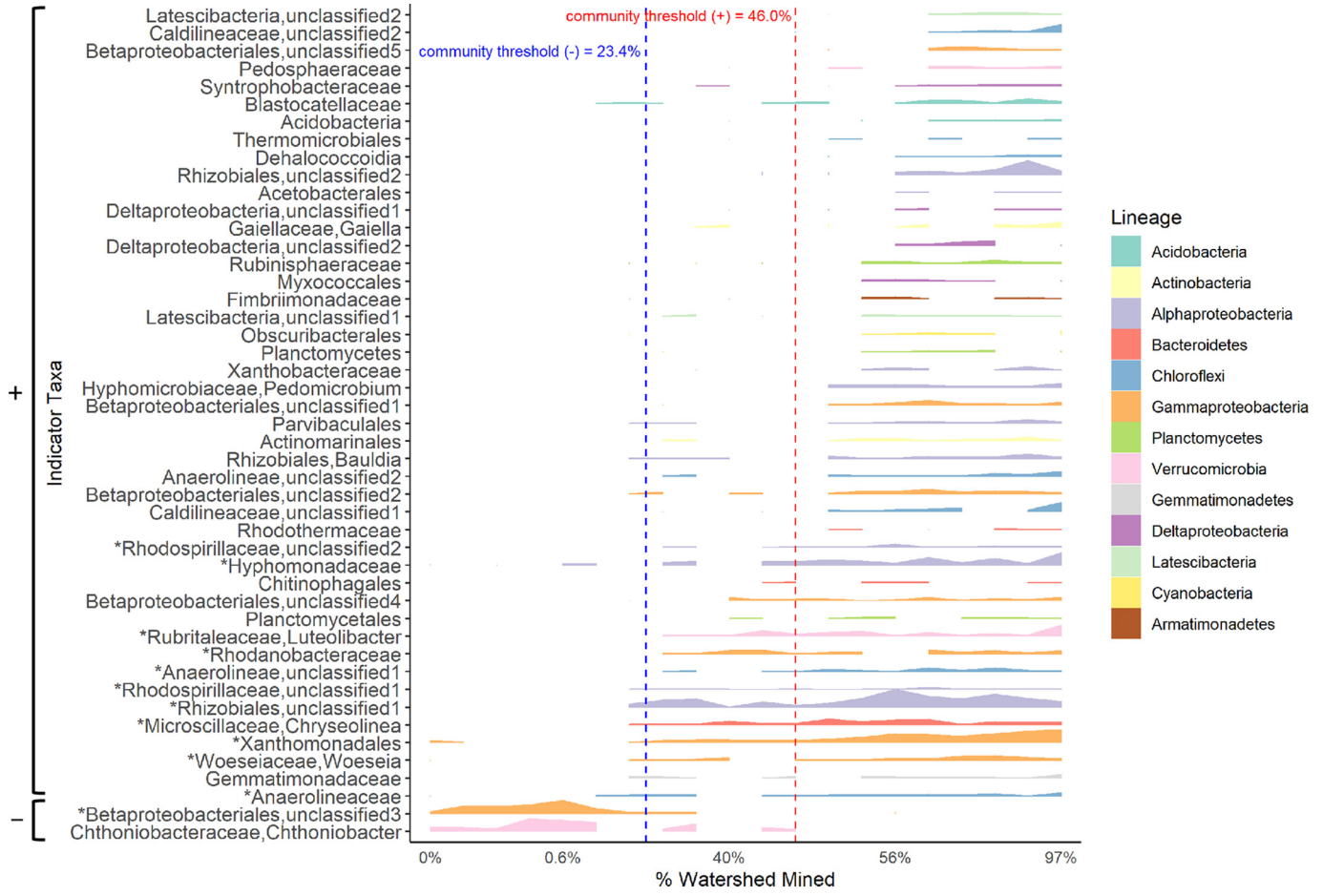

Fig. 3. Indicator taxa identified by TITAN in the bulk sediment along the mining. The height of each curve shows the relative abundance change of each indicator taxon over the mining gradient. Only indicator taxa with purity score = 1 and reliability score > 0.95 are shown here for clarity. Taxa marked with * are also core taxa (taxa present >90% samples). +: positive responders; −: negative responders.

($p > 0.05$, Fig. S2). Mining impacted the composition of the predicted metabolic pathways ($p = 0.002$, $R^2 = 0.15$), with percent watershed mined and other environmental variables including Se, S, %C, %N and THg as the significant factors in the bulk and %C and %N in the fine sediment (Fig. 5a). All of these environmental variables were correlated with NMDS2 (Table S6).

We identified 31 metabolic pathways for the bulk sediment that changed significantly with mining using DeSeq2, with 23 that declined and 8 enriched (Fig. 5b). Five of the 8 enriched pathways are involved with biosynthesis, and 3 are involved with degradation/assimilation/utilization. In contrast, 14 of 23 (60%) pathways involved with degradation/assimilation/utilization and 7 with the generation of precursor metabolites and energy (30%) responded negatively (Fig. 5b). Only 2 pathways involved with biosynthesis (10%) responded negatively (Fig. 5b). The top 4 pathway classes responding to mining are: aromatic compound degradation (16%), carbohydrate degradation (13%),

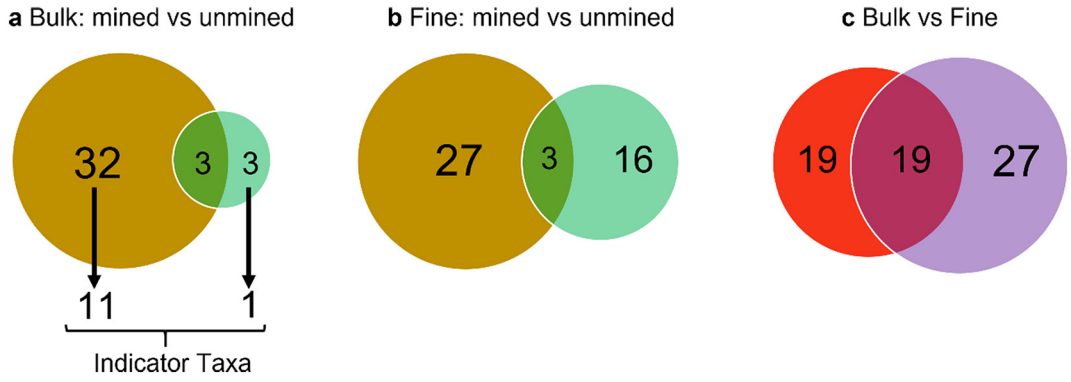

Fig. 4. Number of core taxa at mining impacted (brown) vs non-mining impacted (green) sites in the a) bulk and b) fine sediment; c) overlap of core taxa in the bulk (red) and fine (purple) sediment. Core taxa are defined as taxa present at >90% of the samples at either mined or unmined sites.

Table 1
Identification of ASV candidates for mining biomonitoring: bulk sediment taxa identified in both core microbiome analysis and indicator taxa analysis.

Taxa (at the best available classification)	Core type	Response type	Prevalence	Relative abundance %	Change point
Chloroflexi, Anaerolineae, Anaerolineales, Anaerolineaceae	Mined	+	0.92	0.10 ± 0.07	0.8
Proteobacteria, Alphaproteobacteria, unclassified1	Mined	+	1.00	0.06 ± 0.04	6.7
Bacteroidetes, Bacteroidia, Cytophagales, Microscillaceae, Chryseolinea	Mined	+	1.00	0.24 ± 0.14	6.7
Proteobacteria, Alphaproteobacteria, Rhizobiales, unclassified1	Mined	+	1.00	0.64 ± 0.37	6.7
Gammaproteobacteria, unclassified	Mined	+	1.00	0.51 ± 0.32	6.7
Proteobacteria, Gammaproteobacteria, Sterodobacterales, Woeseiaceae, Woeseia	Mined	+	0.92	0.17 ± 0.12	6.7
Chloroflexi, Anaerolineae, unclassified	Mined	+	0.92	0.12 ± 0.10	23
Proteobacteria, Gammaproteobacteria, Xanthomonadales, Rhodanobacteraceae	Mined	+	0.92	0.17 ± 0.12	23
Verrucomicrobia, Verrucomicrobiales, Verrucomicrobiales, Rubritaleaceae, Luteolibacter	Mined	+	0.92	0.26 ± 0.24	23
Proteobacteria, Alphaproteobacteria, Caulobacterales, Hyphomonadaceae	Mined	+	0.92	0.37 ± 0.26	41
Proteobacteria, Alphaproteobacteria, unclassified2	Mined	+	0.92	0.08 ± 0.08	43
Proteobacteria, Gammaproteobacteria, Betaproteobacteriales, unclassified	Unmined	–	1.00	0.54 ± 0.33	40

Note: Change point is measured in % watershed mined. Relative abundance% is reported mean ± one standard deviation.

nucleotide biosynthesis (13%) and TCA cycle pathway (13%). We also explored denitrification, sulfate reduction and selenate reduction pathways; the results were either not significant or the pathways were not present in our study, likely because the annotations of the pathways are still far from complete and are not yet representative of the metabolic diversity of environmental samples. We did not find any significant pathways in the fine sediment, likely due to insufficient sample size.

4. Discussion

4.1. Increased bacterial richness and shift in community structure at AlkMD-impacted sites

Contrary to previous studies that examined microbial communities in the water column and riparian soil (Bier et al., 2015; Fan et al., 2016), we found that AlkMD had a positive impact on alpha diversity of the sediment bacterial communities. The effects of AlkMD on community diversity and composition patterns were consistent between the bulk and fine sediments. The increase in sediment bacterial diversity may be a result of soil erosion downstream of surface coal mining sites as weathering rates from MTM watersheds in West Virginia are among the highest ever-recorded mineral weathering rates globally, with up to 7600 kg/ha/yr of dissolved sediments delivered to streams (Pericak et al., 2018). This soil erosion can bring a massive influx of microorganisms into the streams and sediments, which explains the increase in alpha diversity as also suggested by other studies (Li et al., 2015; Ruiz-González et al., 2015; Wisnoski et al., 2020).

Community composition differed significantly between mined and unmined sites, with mining explaining 15-18% of the variance in community composition. Only 31% of the ASVs were in common between mined and unmined sites, which suggests an important restructuration and that rare taxa may play an important role in the compositional shift in response to AlkMD (Rocca et al., 2019). Among all the ASVs, 1.8% were identified as statistically reliable indicators to mining using TITAN. We did not observe an increase in the relative abundance of Alpha- and Gammaproteobacteria, consistent with the finding in Bier et al. (2015) but contrary to Feris et al. (2009). One interesting trend we found which has not been discussed in previous studies is the enrichment of the phylum Planctomycetes at AlkMD-impacted sites. Studies have shown that members of Planctomycetes have strong hydrolytic capabilities, enabling them to degrade various biopolymers, and that Planctomycetes diversity is driven by variations in soil organic matter, pH and nitrate concentration (Buckley et al., 2006; Dedysh and Ivanova, 2019; Delmont et al., 2018; Wagner and Horn, 2006). We speculate that the enrichment of Plantomycetes at mined sites may also be related to changes in OM due to mining-induced soil erosion. Though we do not currently have good classification for these Planctomycetes indicator taxa (only 3.4% of the ASVs were assigned to species), further investigation on these microbes through cultivation or shotgun sequencing will likely yield new insights on their roles in mining impacted sediments.

The compositional differences between mined and unmined sites were best explained by Se, S, %C and %N. While the first two factors represent the major differences between mined and unmined sites among all the environmental factors we considered, our results suggest that changes in OM and nutrient availability associated with soil erosion may be important drivers of sediment bacterial community structure in addition to AlkMD-related factors (Beattie et al., 2020).

4.2. Identification of indicator taxa for sediment biomonitoring

Consistent with the increase in alpha diversity in the mined sites, the majority of indicator taxa were positive responders (96%), with the response threshold occurring when 26.3-51.4% of the watershed was mined. This threshold is higher than the 5.4% threshold identified for macroinvertebrates, indicating that these microorganisms are impacted when mining impacts are elevated in the watershed (Bernhardt et al.,

Table 2
Comparison of putative microbial indicators identified in this study versus in Bier et al. (2015) at the family level.

Indicator family with similar response to mining	
Family	Response
Chloroflexi, Anaerolineae, Caldilineales, Caldilineaceae	+
Proteobacteria, Alphaproteobacteria, Caulobacterales, Hyphomonadaceae	+
Bacteroidetes, Ignavibacteria, Ignavibacteriales, Ignavibacteriaceae	+
Nitrospirae, Nitrospira, Nitrospirales, Nitrospiraceae	+
Proteobacteria, Alphaproteobacteria, Rhodobacterales, Rhodobacteraceae	+
Bacteroidetes, Bacteroidia, Chitinophagales, Saprospiraceae	+
Indicator family with differential response to mining	
Family	Response
Proteobacteria, Alphaproteobacteria, Caulobacterales, Caulobacteraceae	+ −[a]
Actinobacteria, Thermoleophilia, Gaiellales, Gaiellaceae	+/− +[a]
Gemmatimonadetes, Gemmatimonadetes, Gemmatimonadales, Gemmatimonadaceae	+ −[a]
Proteobacteria, Alphaproteobacteria, Rhizobiales, Hyphomicrobiaceae	+ −[a]
Proteobacteria, Alphaproteobacteria, Sphingomonadales, Sphingomonadaceae	+ −[a]
Proteobacteria, Gammaproteobacteria, Xanthomonadales, Xanthomonadaceae	+ −[a]

+: associated with mined sites; −: associated with unmined sites.
[a] Result from Bier et al. (2015).

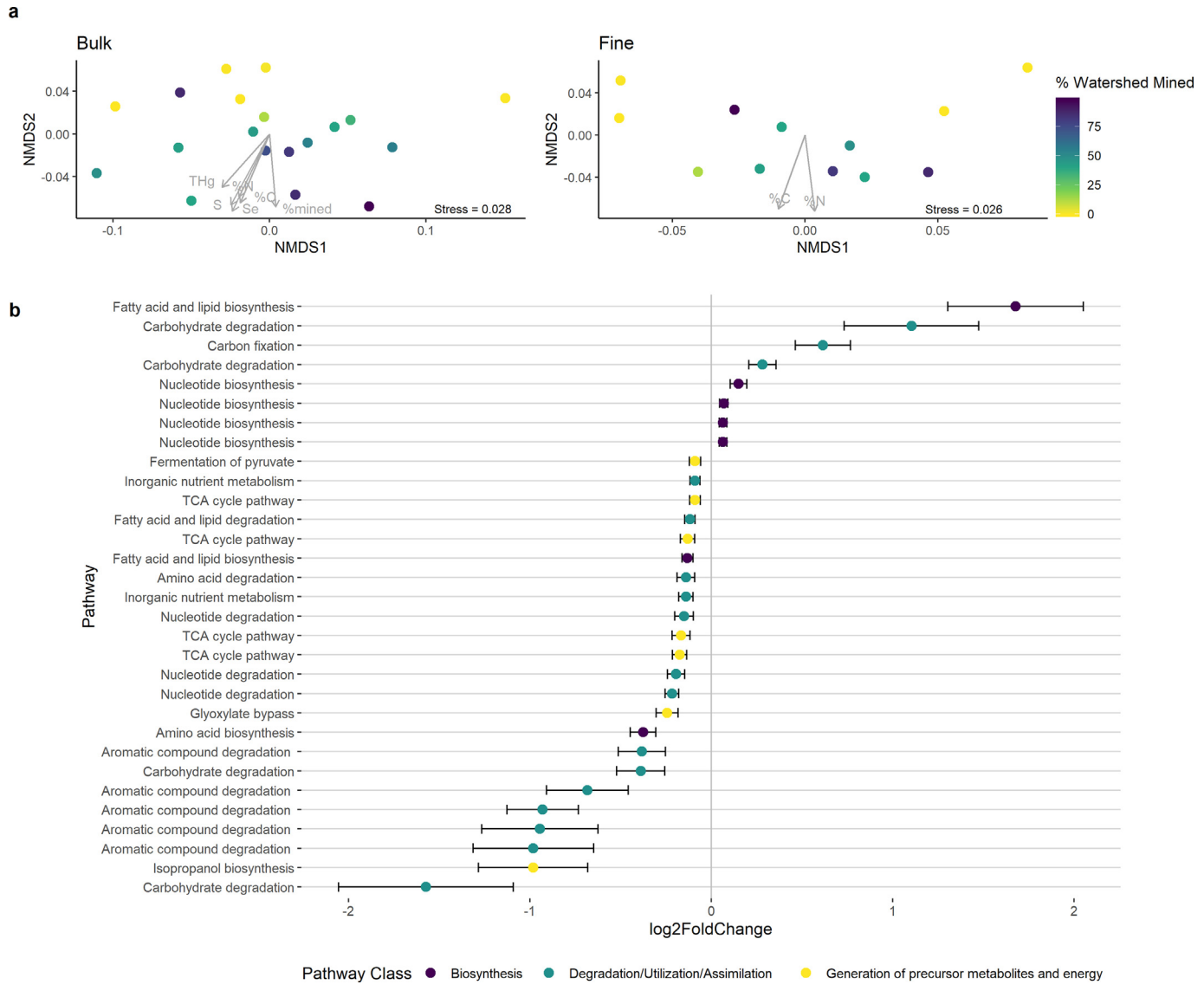

Fig. 5. a) NMDS plots depicting the impact of percent watershed mined and other environmental variables on MetaCyc pathway composition for bulk and fine sediment samples. b) MetaCyc pathways with significant changes in abundance at the mining impacted sites compared with non-mining impacted sites; negative $\log_2$ fold changes indicate lower pathway abundance at the mining impacted sites (Independent Hypothesis Weighted adjusted p values, all $p < 0.05$); error bars represent one standard error.

2012). The community response threshold to Se is 0.36-0.50 μg/g, which is higher than the Se background level (0.29 μg/g) specified in the U.S. National Oceanic and Atmospheric Administration's (NOAA) sediment quick screening reference. While the top two classes of the indicator taxa identified (Alphaproteobacteria and Gammaproteobacteria) were also the most common bacterial classes found in our samples, the third most abundant indicator class, Anaerolineae, had a disproportionately high presence (~10%) in our list of indicator taxa. The high proportion of positively responding Anaerolineae may be attributed to variations in sediment OM, as members of the class encode abundant carbohydrate transport and metabolism genes (Campbell et al., 2014; Zhu et al., 2018). Additionally, we found 6 bacterial families which have been consistently found to be positively associated with mining by comparing our indicator taxa analysis with that in Bier et al. (2015). Among them, the family Hyphomonadaceae and Nitrospiraceae are known to be involved in the nitrogen cycle and the family Rhodobacteraceae and Saprospiraceae are known to be involved in sulfur and carbon biogeochemical cycling (Abraham and Rohde, 2014; Daims, 2014; McIlroy and Nielsen, 2014; Pujalte et al., 2014). While there were multiple differences between the two studies such as the difference in sequencing technology and taxa classification methodology (ASV vs OTU), the fact that similar responding patterns to mining of some bacterial families were found in both studies strengthens our conclusion and highlights the potential ecological importance of these bacterial families in the impacted stream ecosystems.

To further identify potential candidates for biomonitoring, we compared the list of core taxa present at >90% of the samples at either the mined or unmined sites with the indicator taxa identified using TITAN. Taxa that are both core and indicator taxa are promising candidates for biomonitoring because they are both prevalent in the given environment and sensitive to the environmental stressor. We identified 11 positively responding and 1 negatively responding indicator taxa satisfying the two criteria. Identification of indicator taxa that are both strongly associated with mining impacted or reference sites and sensitive to environmental disturbance offers great promise for the incorporation of bacterial assemblage information into ecosystem monitoring analysis such as biotic indices and ecological indicators. The 12 biomonitoring candidates need to be validated on multiple AlkMD sites to assess

their sensitivity in contrasted regions. To facilitate their detection, a multiplex qPCR assay could be designed to offer a cheap and rapid monitoring of the multiple indicator taxa in a single test.

4.3. Microbial functional pathways were negatively affected by AlkMD

We did not find a significant impact of mining on the diversity (observed richness, Pielou's evenness, and Shannon index) of functional genes and MetaCyc functional pathways, similar to Bier et al. (2020). Bier et al. (2020) found that the composition of functional genes and pathways differed significantly between mined and unmined sites, but we observed such difference only in the composition of functional pathways, not functional genes. The significant shift in community composition but not the composition of functional genes is likely a result of functional redundancy in microbial communities (Louca et al., 2018).

Functional pathway composition differed significantly between mined and unmined sites, and the variance in functional pathway composition was best explained by the same environmental factors (Se, S, % C, and %N) as in community composition. Our result supports the findings in experimental studies that C and nutrient supply affect functional attributes of microbial communities (Chodak et al., 2013; Findlay et al., 2003). Of the pathways that changed significantly, the majority (74%) responded negatively to mining, consistent with the finding in Bier et al. (2020). Elevated conductivity and metal concentrations in AlkMD-impacted streams can be potential causes of negative response to mining from many of the functional pathways, as osmotic stress and metal toxicity are well-documented stressors to microbial communities (Chodak et al., 2013; Feris et al., 2009). This decrease in pathway diversity was surprising because we observed an overall increase in bacterial alpha diversity at mined sites, and we expected that the new taxa could have added new functions to the sediment microbiome. These results suggest that the mining-tolerant taxa are highly functionally redundant and that their higher diversity do not compensate the loss of some microbial functional pathways present at unmined sites (Frossard et al., 2012). Together, this trend and our finding that the majority (54.8%) of the responding pathways are involved in C metabolism such as aromatic compound and carbohydrate degradation suggest that the impact of AlkMD on stream sediment bacterial communities can have important implications for ecosystem functioning.

5. Conclusion

Our study demonstrated that environmental microbiomes can be useful in monitoring ecosystem impacts of environmental degradation caused by human activities such as MTM. AlkMD produced from MTM significantly alters the structure of sediment bacterial communities, with the signature chemical pollutants (Se and S) and OM as the main drivers of community structure. The differences in bacterial diversity patterns found in our study versus previous studies emphasize the importance of considering the compartment studied (water column vs sediment) and possible interactive impacts of different environmental factors (AlkMD vs soil erosion). Our results also suggest that AlkMD may impact the functional potential of sediment microbiomes and that the mining-tolerant taxa appear to be highly functionally redundant which leads to the loss of some microbial metabolic pathways. We identified 12 indicator taxa both prevalent at the site and sensitive to the disturbance, some of which belong to the same families as the indicator taxa found in a previous study. These taxa are promising candidates for biomonitoring AlkMD in stream ecosystems.

CRediT authorship contribution statement

Lingrong Jin: Formal analysis, Writing – original draft. **Jacqueline R. Gerson:** Investigation, Writing – review & editing, Supervision, Funding acquisition. **Jennifer D. Rocca:** Investigation, Writing – review & editing. **Emily S. Bernhardt:** Writing – review & editing, Funding acquisition. **Marie Simonin:** Investigation, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.150189.

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