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TECHNICAL ARTICLE

Potential for Passive Treatment of Coal Mine-derived Acid Mine Drainage in Abandoned Stream Channels

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Abstract

Passive treatment of coal mining derived acid mine drainage (AMD) utilizes natural processes to neutralize acidity and remove dissolved metal contaminants. In some cases, microbial communities develop without human intervention that can induce the removal of harmful AMD components. To better understand how these beneficial processes might develop, we studied the Huff Run system in eastern Ohio, where a portion of the stream was artificially diverted to prevent direct entry of AMD into the stream. There are now two abandoned stream channels that receive raw AMD and we hypothesized that the increased residence time of the AMD in these abandoned channels could reduce the adverse effects of AMD on Huff Run. We tracked seasonal changes in the aqueous chemistry and microbiology in the two abandoned channels, referred to as Farr and Lyons. The Fe, Al, and Mn were partially removed from solution as AMD moved through the Farr channel, with net alkaline water, and abundant *Bacillus* and *Paenibacillus* phylotypes. Dissolved Fe was partially removed in the Lyons channel, but neither Al nor Mn were, and the sediments contained abundant phylotypes attributable to *Alicyclobacillus* sp., which are capable of oxidative precipitation of Fe(II) under acidic conditions. Our results indicate that enhanced AMD retention in abandoned channels can induce contaminant removal and that abiotic and biotic reactions in the channels are influenced by the AMD chemistry.

Keywords Water quality · Passive treatment · Microbial community · Stream restoration · Iron oxidation

Introduction

Acid mine drainage (AMD) derived from coal mining is produced when oxygenated water infiltrates abandoned or operating mine voids, whereby pyrite (FeS_2) and other FeS phases react (abiotically or catalyzed by microbiological activity) with dissolved O_2, yielding dilute sulfuric acid with abundant dissolved metals (Stumm and Morgan 1996).

In the Appalachian coal mining regions of eastern Ohio, Kentucky, western Pennsylvania, and West Virginia, the most abundant dissolved metals in the AMD are Fe^{2+}, Mn^{2+}, and Al^{3+}, and these species represent the greatest threat to surface water quality and remediation efforts (Cravotta 2008; Herlihy et al. 1990). A general theme of coal mine derived AMD treatment is to provide some form of aeration and pH neutralization to facilitate the oxidation of Fe^{2+} and Mn^{2+} (Skousen et al. 2017) and subsequent hydrolysis and precipitation of Fe^{3+}, $Mn^{3+/4+}$, and Al^{3+} as a variety of (hydr)oxides (Stumm and Morgan 1996).

This is generally achieved through some combination of diversion of AMD from a surface water body, provision of a neutralizing agent (e.g. addition of limestone), and partial retardation of water flow to facilitate aeration of the water and allow metal (hydr)oxides to settle out of the water (Cravotta and Trahan 1999; Moodley et al. 2018). These treatment strategies can range from systems requiring extensive

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maintenance (referred to as "active treatment") to systems that can operate with minimal human intervention (referred to as "passive treatment) (Coulton et al. 2003; Johnson and Hallberg 2005; Kleinmann et al. 1998). Remediation of AMD in Pennsylvania alone is estimated to cost $5 billion (Sibrell et al. 2005). Given the high costs of active treatment, passive treatment is preferred when it is feasible (Johnson and Hallberg 2005; Naidu et al. 2019).

The Huff Run watershed of southeastern Ohio, USA has been severely impacted by several abandoned surface and underground mines (Kinney 2013). Several passive treatment systems have been installed, along with other remedial efforts, costing ≈ $7 million (Hedin et al. 1994a; Kinney 2013). Two of these treatment systems (referred to as Lyons and Farr treatment systems; Fig. 1) have exceeded their lifetime for effective operation (Fleming 2000). The AMD discharging from Farr into Huff Run contains Fe concentrations and total acidity as high as 35 and 52 mg/L, respectively, while the AMD discharging from Lyons into Huff Run contains Fe concentrations and total acidity as high as 50 mg/L and 95 mg/L, respectively (Kinney 2013). Together, they contribute 81% (38% Farr and 43% Lyons) of Huff Run's iron load (Kinney 2013). In response, rather dramatic action was taken to divert the stream from the AMD discharges with ≈ 2 km of new stream channel (Fig. 1; Kinney 2013). After this intervention, the "old" Huff Run stream channels received AMD from either Lyons or Farr, and eventually discharged back into Huff Run after they traveled an additional 700 m in the old stream channel (Fig. 1). We now refer to the "old" Huff Run channels receiving AMD from the Lyons and Farr treatment systems based on the system from which they receive AMD (i.e. the "Lyons channel" and "Farr channel").

The modification of the Huff Run flow path presented an opportunity to determine if biogeochemical processes taking

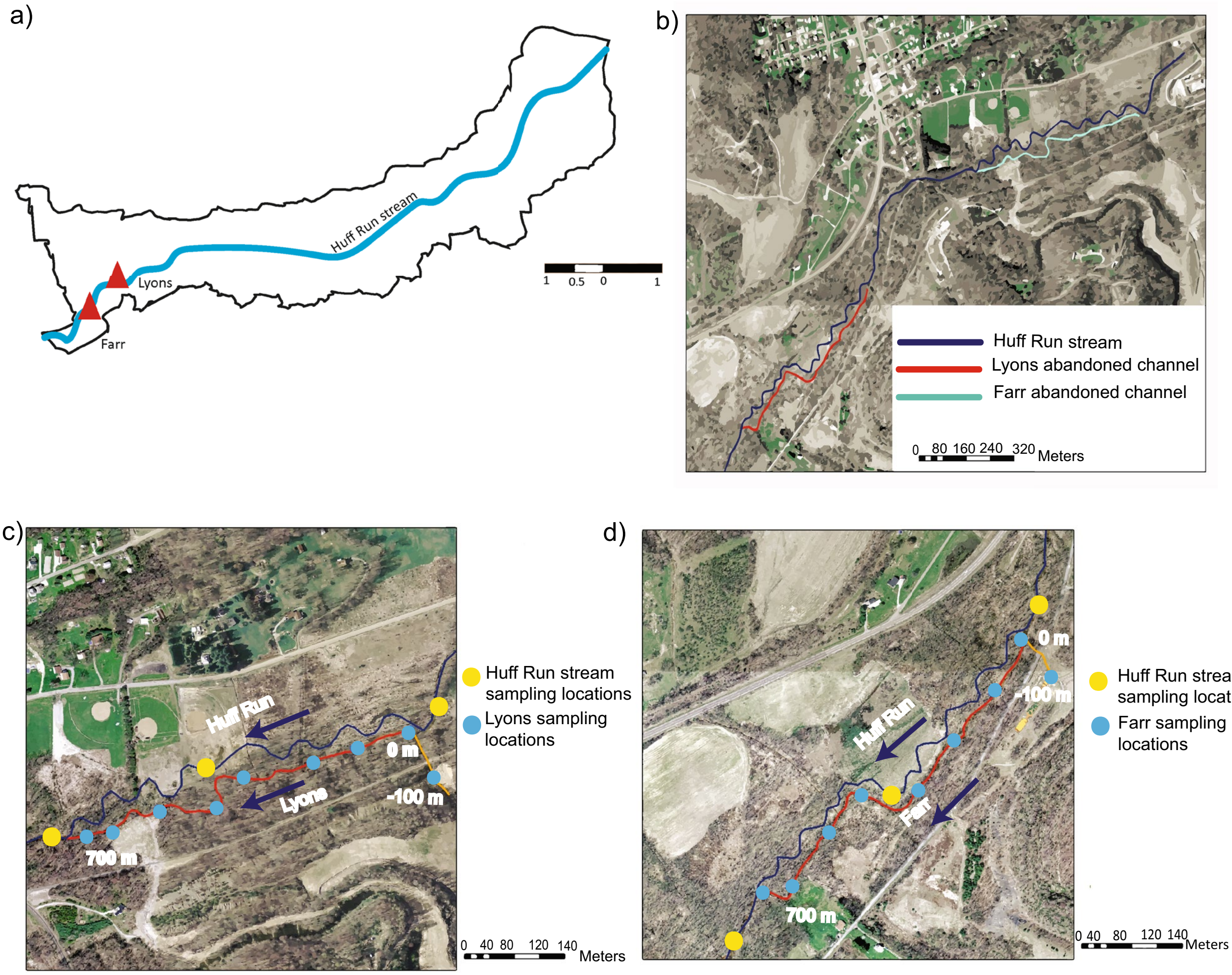

Fig. 1 Maps of **a** Huff Run watershed location with Farr and Lyons AMD treatment discharge points, **b** Huff Run stream with abandoned Lyons and Farr channels, **c** Lyons abandoned channel (red) parallel to new Huff Run stream (blue) with sampling locations and **d** Farr abandoned channel (red) parallel to new Huff Run stream (blue) with sampling locations

place in the old Huff Run channel were enhancing water quality. In many cases, the goals of AMD treatment (e.g. Al, Mn, Fe removal, pH neutralization) can be achieved without the major interventions involved in typical passive treatment systems, thus minimizing cost and enhancing sustainability (Brantner et al. 2014; Senko et al. 2008). While no intervention was performed with the specific goal of using the abandoned channels to enhance water quality, we hypothesized that the increased residence time of the AMD in the abandoned channels could reduce the adverse effects of AMD on Huff Run. This increased residence time could provide the benefit of enhanced aeration, allow precipitation of metal (hydr)oxides, and enhance microbiological contributions to the oxidative precipitation of Fe^{2+} and Mn^{2+} (DeSa et al. 2010; Hallberg and Johnson 2005; Senko et al. 2008). A survey of the chemistry and microbial communities of the Lyons and Farr channels, as well as the “new” Huff Run was conducted to determine the influence of increased AMD retention time on water quality and changes in biogeochemical process. The ultimate goal of this work was to determine if and to what extent abandoned stream channels receiving AMD can help minimize adverse effects on surface waters.

Methods

Sample Collection

Samples for chemical and microbiological analysis were collected throughout two 700 m long now-abandoned channels of Huff Run that receive AMD effluent from the Farr and Lyons treatment systems, and the current Huff Run, part of which is a manmade channel that was constructed to bypass the AMD effluent from the Farr and Lyons treatment systems (Fig. 1). Samples for evaluation of aqueous chemistry were collected in the mornings on March 12, 2017, July 11, 2017, and January 28, 2018, and designated spring, summer, and winter, respectively. Sampling location and distance were consistent among the seasons and in the channels, guided by the global positioning system (GPS). Water samples were collected from: (1) the effluent coming from the treatment systems, (2) within and throughout the abandoned stream channels, and (3) from the Huff Run stream channel. Within each abandoned channel, water samples were collected at the surface level in 50 mL centrifuge tubes with ≈ 100 m between sampling points (Fig. 1c and d). Water samples from the new Huff Run stream parallel to the abandoned channels were also collected in 50 mL centrifuge tubes at three different locations. Sampling locations were upstream from where the old channels were dammed off, the midpoint of the stream, and downstream from the confluence of the abandoned channel and Huff Run stream (Fig. 1b). All samples for aqueous chemical analysis were field-filtered using a 0.2 μM syringe filter. Samples for cation analysis were acidified by the addition of three drops of concentrated (70%) HNO_3 /50 mL samples in the field, while samples for measurement of total dissolved solids, anions, alkalinity, and total acidity were not. Samples for measurement of alkalinity and total acidity were stored in 50 mL centrifuge tubes with no headspace. Samples were transported on ice to the University of Akron before transfer to an Ohio Department of Natural Resources (ODNR) Division of Mineral Resources Management (DMRM) laboratory (a U.S. EPA-certified chemical laboratory in Cambridge, OH) for chemical characterization. Sediment samples were collected at the beginning, middle, and end of each abandoned channel and Huff Run to compare microbial community structure and stream sediment mineralogy (Fig. 1). Grab sediment samples were collected for microbial community analysis and transported on ice to the University of Akron, where they were then stored in a − 80 °C freezer before further processing.

Chemical Analysis

Chemical species to quantify were chosen based on the Ohio Department of Natural Resources acid mine drainage abatement and treatment plan (AMDAT) guidance document (Borch 2010). Field pH, dissolved oxygen (DO), temperature, and electrical conductivity (EC) measurements were taken using a YSI Professional Plus Probe (Yellow Springs Instruments, Yellow Springs, OH). Quantification of total Fe, Mn, Al, sulfate, total alkalinity, and total acidity were carried out at the ODNR laboratory in Cambridge, OH by inductively coupled plasma optical emission spectrometry. Sulfate was quantified using a Dionex (Thermo Fisher Scientific Inc, Sunnyvale, CA) ion chromatography system with an AS22 column and conductivity detector. Alkalinity and total acidity were measured by titration, following ASTM (D1067) protocol (Kirby and Cravotta 2005a, b). The mineralogy of sediments was determined by x-ray diffraction (XRD) using a Rigaku Ultima IV automated diffractometer using CuKα radiation, scanning at 2 of 2–70° and accelerating voltage of 40 kV at 35 mA.

Nucleic Acid Based Microbial Community Analysis

We used a MoBio Powerbiofilm DNA isolation kit (MoBio Laboratories Inc., Carlsbad, CA) to extract DNA from the sediment samples. The DNA was sequenced using 16S rRNA gene amplicon sequencing conducted on MiSeq Illumina using bacterial tag-encoded FLX amplicon sequencing by Molecular Research Laboratories, LP (Shallowater, TX) (Callaway et al. 2010; Dowd et al. 2008). A HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) was used for 28-step single cycle PCR (Forward GTGCCAGCMGCCGCGGTAA and reverse GGACTACHVGGGTWTCTAAT primer sequence) with the following parameters: initial denaturation at 94 °C for 3 min, 28 cycles of 94 °C for 30 s, 53 °C for 40 s, 72 °C for 1 min, and a final elongation step at 72 °C for 5 min. Amplified PCR products were further mixed in equal concentration and purified with calibrated Agencourt AMPure XP beads (Agencourt Bioscience Corporation, MA, USA). Amplicon sequencing was done on a MiSeq Illumina platform at Molecular Research Laboratories, LP (Shallowater, TX). After removal of barcodes and primers from Illumina derived sequences, all sequences that were less than 150 base pairs (bp), including chimeras, ambiguous base calls, and homopolymers > 6 bp were discarded (Gontcharova et al. 2010). These sequence libraries were then analyzed using QIIME scripts in a MacQIIME environment (www.wernerlab.org/software/macqiime; Caporaso et al. 2010a). De novo operational taxonomic units (OTU) were assigned to sequences using a 97% similarity cutoff; taxonomic classifications were assigned using a RDP classifier with a SILVA database, with PyNAST alignment algorithm (Caporaso et al. 2010b; Edgar 2010; Quast et al. 2013). Weighted UniFrac metric and principal coordinate analysis (PCoA) was used to calculate and visualize beta-diversities of aligned OTUs among samples (Kuczynski et al. 2012; Lozupone and Knight 2005; Lozupone et al. 2007). Canonical correspondence analysis (CCA) was conducted with the vegan package in R (Paliy and Shankar 2016; Torondel et al. 2016). PERMANOVA (permutational multivariate analysis of variance using distance matrices) in Adonis vegan package of R v.3.1.3 project was used to find statistically significant environment variables affecting microbial community composition ($p > 0.05$) (Anderson 2001). The visual output of CCA is a tri-plot comparing relationships between sample sites, abundant OTUs, and quantitative environment variables (with arrows), where the direction of arrows point in the direction of maximum change in the value of the associated variable (Paliy and Shankar 2016).

Results and Discussion

Aqueous Chemistry of Lyons, Farr and Huff Run

To determine if passage of AMD through abandoned stream channels enhances treatment, a suite of geochemical water and sediment analyses were conducted. Aqueous chemistry was evaluated in the Lyons and Farr channels and in stretches of Huff Run parallel to the now-abandoned channels to investigate factors (e.g. DO, pH, alkalinity) influencing removal of dissolved metals (Fig. 1). Water quality in the new Huff Run stream was superior to that of the old channels, with higher pH and alkalinity, and less acidity, sulfate, and dissolved Fe, Al, and Mn (Fig. 2). DO in the Lyons and Farr channels were similar to those of Huff Run (Fig. 2a xi, x, and xii), indicating that the AMD was sufficiently aerated as it flowed through the channels. In all cases, the DO was highest in the spring, when the AMD temperature was lowest (2–6 °C), due to the greater solubility of O_2 at relatively low temperatures (Fig. 2a xi, x, and xii). The available O_2 could support oxidation of Fe(II) and precipitation of Fe(III) phases, resulting in partial removal of dissolved Fe in both channels (Fig. 2b vii, viii, ix). Indeed, goethite was detected in both channels (supplemental Figs. S-1 and S-2). Fe(II) removal was most extensive in Lyons during summer (30–10 mg/L) over the course of the channel (Fig. 2b vii). During summer, hydrolysis and precipitation of Fe in the channels led to a decrease in pH (Fig. 2b i, ii), even though total acidity decreased as water moved through the channels (Fig. 2a i, ii) due to removal of dissolved Fe. Fe removal was more efficient in summer than in winter and spring (Fig. 2b vii, viii, ix), likely due to enhanced kinetics of both chemical and microbiological processes at higher temperatures, agreeing with Goyal et al. (2003).

While DO, pH, and acidity followed similar patterns in the Farr and Lyons channels, dissolved Fe, Al, Mn, and sulfate behaved differently (Fig. 2). In the Lyons channel, dissolved Al, Mn, and sulfate concentrations all increased as the AMD traveled through the channel (Fig. 2b). In the Farr channel, dissolved Al increased in the early parts of the channel, while Mn concentrations decreased slightly, if at all, relative to the AMD emerging from the treatment system, as did the sulfate concentration (Fig. 2b v, xi, xiv). The increase in sulfate concentration as AMD moved through the Lyons channel indicated that AMD is infiltrating into the channel from sources other than the old treatment system (Fig. 2b iv). Indeed, Al and Mn concentrations increased concurrently with the increased sulfate concentrations (Fig. 2b iv, x, xiii). Two anomalous Mn concentrations were observed in the spring sampling campaign, ≈ 250 m downstream of AMD emergence (Fig. 2b xiii), and the reason for this is not clear, but may be attributable to sampling error. In the Lyons channel, adequate aeration of the AMD could support oxidative removal of Fe despite intrusion of AMD into the channel from points other than the treatment system effluent (Fig. 2b vii). In contrast, sulfate concentrations decreased as AMD moved through the Farr channel, indicating that the sole source of AMD to the channel was from the defunct Farr treatment system (Fig. 2b v). Here, it appeared that Al was released from sediments as oxidative precipitation of Fe(II) induced a decreased $\mathrm{pH} < 6$ and leached Al from clays (e.g. halloysite, $Al_2Si_2O_5(OH)_4$) in the channel bed (Fig. S-2). Similarly, while a small amount of Mn removal occurred in the Farr channel, Mn concentrations increased in the Lyons channel. Here, the decrease in pH that accompanied oxidative precipitation of Fe likely limited substantial Mn oxidation and precipitation (Fig. 2b vii, viii, xiii, xiv; Johnson and Hallberg 2002). Finally, no Fe oxide/

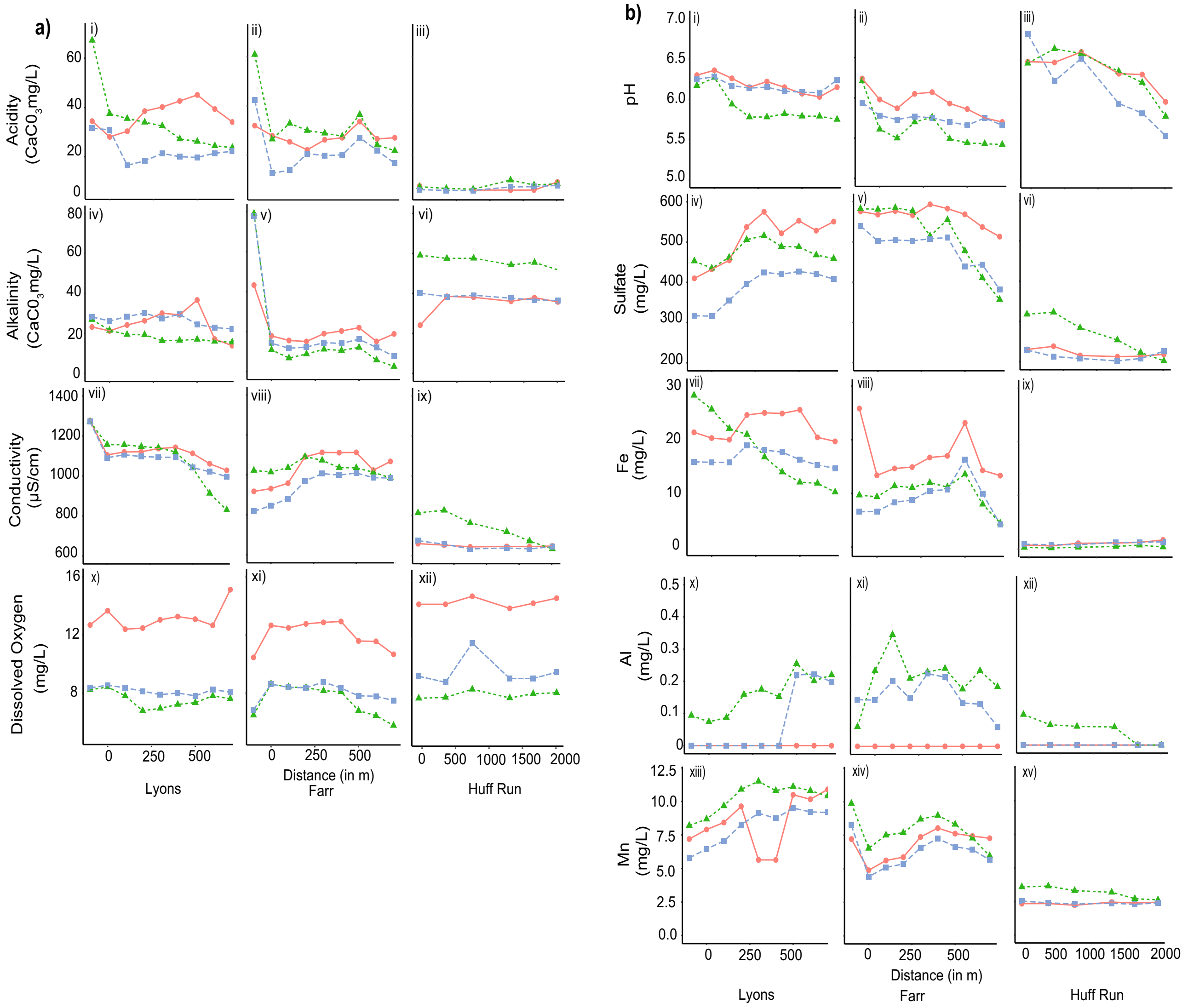

Fig. 2 **a** Water chemistry comparing total acidity (i, ii, iii), total alkalinity (iv, v, vi), conductivity (vii, viii, ix), dissolved oxygen (x, xi, xii), **b** pH (i, ii, iii), sulfate (iv, v, vi), dissolved Fe (vii, viii, ix), dissolved Al (x, xi, xii) and Mn (xiii, xiv, xv) between Lyons, Farr and Huff Run. Blue square, green triangles, and red circle indicate data from sampling events in winter, summer, and spring, respectively

hydroxide minerals associated with AMD were detected in the Huff Run stream samples, suggesting Fe oxidation and precipitation was limited to the Lyons and Farr channels (supplemental Fig. S-3).

Microbial Communities of Lyons, Farr, and Huff Run

To understand how AMD chemistry influenced microbial communities in the Lyons and Farr channels (with Huff Run as an unaffected control), as well as the potential roles of microorganisms in removing Fe and Mn from the AMD, we carried out 16S rRNA gene surveys of the three systems. From the perspective of taxonomic composition, each of the three channels contained distinct microbial communities (Fig. 3a). However, a comparison of the microbial communities using the weighted UniFrac metric indicated that the microbial community of the Lyons channel was distinct from both the Farr channel and Huff Run (Fig. 3b) (Lozupone and Knight 2005; Lozupone et al. 2007).

The microbial communities associated with the Lyons sediments, which received AMD with relatively high total acidity and Fe concentrations throughout the year (Fig. 2a (i) and 2b (vii)), were dominated by Actinobacteria (≈55%) at the 0 and 100 m sampling points (Fig. 3a), of which *Leifsonia*- and *Streptomyces*-attributable phylotypes comprised 95% of the OTU at these sampling points. Both taxa are commonly detected in contaminated soil and can facilitate metal and acidity tolerance by plants (Hottenstein et al. 2019; Rajkumar et al. 2010; Xu et al. 2006). Downstream, at the 700 m sampling point of Lyons, the microbial

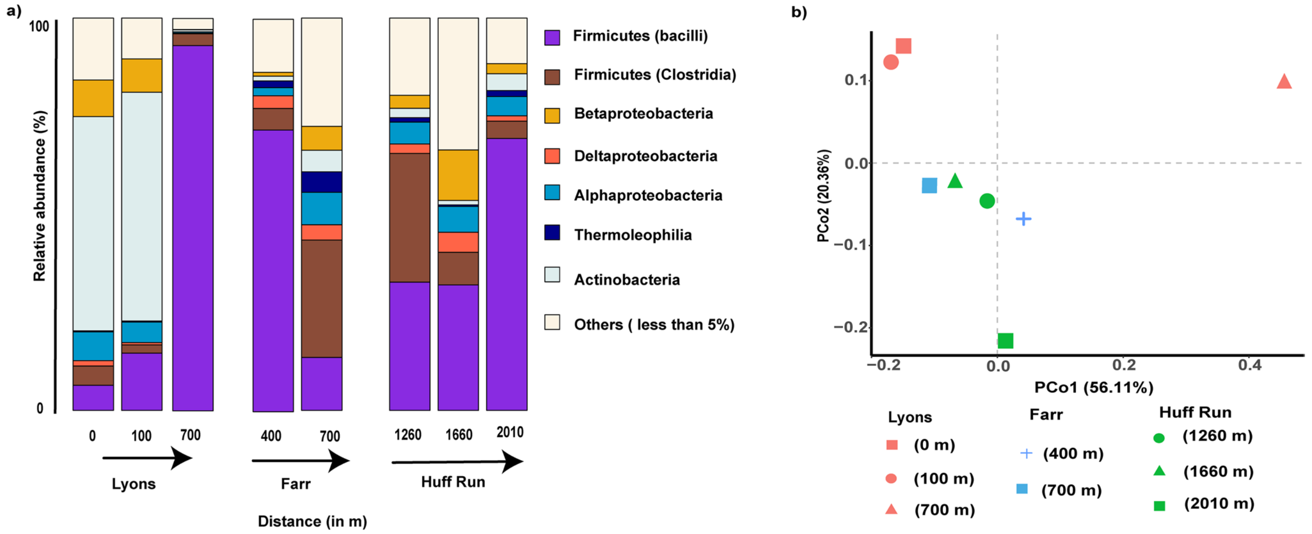

Fig. 3 **a** Relative abundances at phylum and class level (for *Proteobacteria*) of 16S rRNA gene OTU detected in libraries from Lyons at distance 0, 100 and 700 m, Farr at distance 400 and 700 m, Huff Run at distance 1260, 1660, and 2010 m. **b** PCoA of microbial communities associated with Lyons (orange) at distance 0 m (■), 100 m (●), and 700 m (▲), Farr (blue) at distance 400 m (+) and 700 m (), Huff Run (green) at distance 1260 m (●), 1660 m (▲), and 2010 m (■) (Green) using weighted UniFrac (Lozupone and Knight 2005)

community was composed primarily of Firmicutes (96%; Fig. 3a and b), with most of these attributable to *Alicyclobacillus* sp., a genus with several members capable of Fe(II) oxidation and shown to be responsible for oxidative precipitation of Fe from AMD (Burwick et al. 2017; Guo et al. 2009; Joe et al. 2007; Kinnunen et al. 2003; Lu et al. 2010). The development of this *Alicyclobacillus*-dominated community indicates that these organisms may be responsible for the observed removal of Fe (Figs. 2b vii and 4). These results indicate that a microbial community that was able to efficiently oxidize Fe(II) developed in the Lyons sediments due to intrusion of the AMD, as has been observed in laboratory experiments (Brantner and Senko 2014; Sharma et al. 2020).

Most of the OTUs observed in the “new” Huff Run sediments were also attributable to Firmicutes, with an abundance of *Bacillus*-attributable microorganisms (Fig. 3a), which are common neutrophilic organoheterotrophs in freshwater and soil systems (McSpadden Gardener 2004; Nicholson 2002). Similarly, the microbial communities associated with Farr channel contained abundant Firmicutes, with high relative abundance of *Bacilli* and *Clostridia* at 400 and 700 m, respectively (Fig. 3a). Further downstream, the common soil and freshwater phyla (Roesch et al. 2007), Proteobacteria and Actinobacteria were more abundant components of the community (Fig. 3a). Here, sulfate reducing Deltaproteobacteria could be responsible for sulfate removal in the Farr channel (Fig. 2b (v)). Viewing the microbial communities of Huff Run and the Farr channel in total, they were composed of cosmopolitan phylotypes that are frequently detected in aerobic, geochemically non-extreme settings (Grady et al. 2016; McSpadden Gardener 2004; Nicholson 2002). This differs from the Lyons channel, which contained phylotypes attributable to lineages of organisms detected in AMD. Indeed, the PCoA indicated that the microbial communities associated with Farr clustered together with the “new” Huff Run, suggesting a similar community composition (Fig. 3b).

Our results suggest that the water chemistry of Lyons AMD imposed greater control on the sediment microbial community composition than the Farr AMD. In other words, using the UniFrac metric, the Farr AMD did not induce a microbial community dramatically different community than the unimpacted Huff Run (Lozupone and Knight 2005; Lozupone et al. 2007). To further examine the chemical influences of AMD on sediment microbial community composition, we performed CCA to identify environmental variables affecting microbial community compositions (Fig. 4). This analysis indicated a correspondence between *Bacillus* (Bac4, Bac5, Bac6, and Bac7), *Peptostreptococcaceae* (Pep1), *Alicyclobacillus* (Ali2), and the overall microbial communities in Huff Run and the low acidity and sulfate concentrations in that stream (Fig. 4). Conversely, a positive correlation was observed between *Leifsonia* (Lei), *Streptomyces* (Str1, Str2, and Str3), and the overall microbial communities of the Lyons channel and total acidity and sulfate concentrations, indicating their tolerance to high acidity in Lyons channel (Fig. 4). In the case of the Farr channel, *Paenibacillus* (Pae1, Pae2), *Bacillus* (Bac1, Bac2, Bac3, and Bac4) and *Cohnella* (Coh) were positively correlated with high sulfate concentrations in the upstream portions of the Farr channel (Figs. 2b (v) and 4), but further downstream

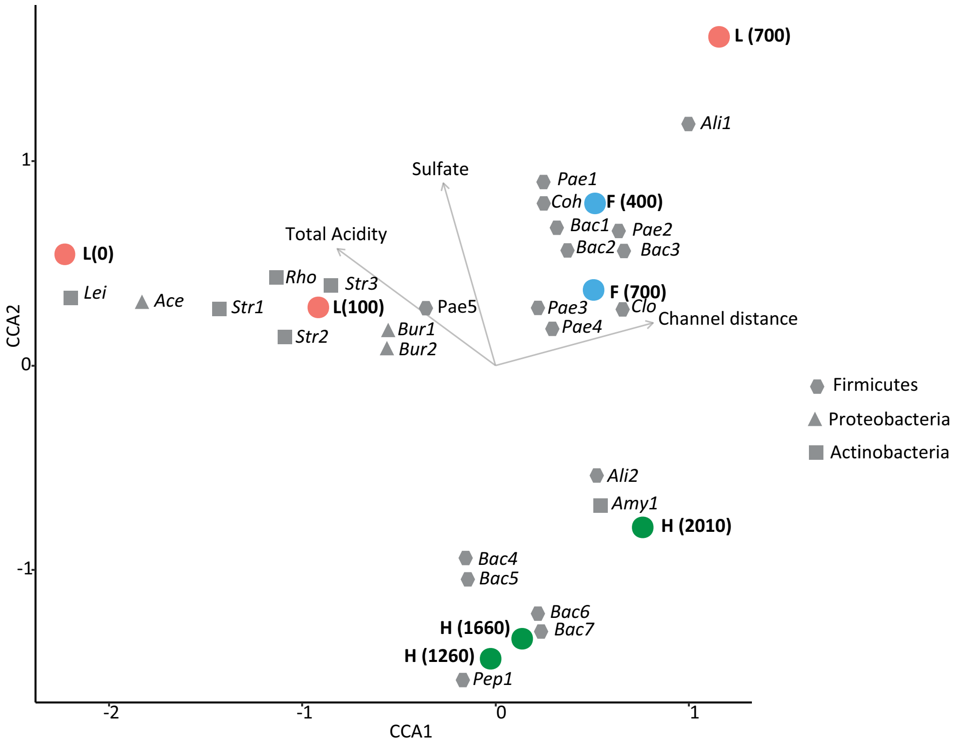

Fig. 4 CCA triplot displaying the relationship among channels (circles), abundant OTUs (triangles, hexagons and squares) and environmental variables (arrows). Sites labels L (0), L (100), and L (700) represents Lyons (orange) at distance 0 m, 100 m and 700 m, respectively; F (400) and F (700) represent Farr (blue) at distance 400 m and 700 m, respectively; H (1260), H (1660), and H (2010) represents Huff Run (green) at 1260 m, 1660 m, and 2010 m, respectively. Grey triangle, hexagons, and squares represents species distribution among sites from *Proteobacteria*, *Actinobacteria* and *Firmicutes* phylotypes, respectively. Select genus displaced on the plot are abbreviated as follows: *Ali* (1,2)- *Alicyclobacillaceae, Pae* (1–5)- *Paenibacillaceae, Coh- Cohnella, Bac* (1–7) -*Bacillus, Clo- Clostridium* sensu stricto 1, *Amy- Amycolatopsis, pep- Peptostreptococcaceae, Bur* (1,2)- *Burkholderia, Lei-Leifsonia, Ace- Acetobacteraceae, Str* (1,2)-*Streptomyces* and *Rho* (1,2)-*Rhodococcus*

(700 m), *Paenibacillus* (Pae3, Pae4) and *Clostridium* (Clo) were positively correlated with comparatively low acidity and sulfate concentrations (Fig. 4). These results suggest that AMD from Farr and Lyons exerted differing controls on the composition of the microbial communities in the AMD streams.

Prospects for the Use of Abandoned Channels for AMD Treatment

The development of successful passive treatment depends on AMD chemistry, flow rate, local topography, and site characteristics (Hedin et al. 1994b; Skousen and Ziemkiewicz 2005; Skousen et al. 1998). Additionally, the physicochemical characteristics of AMD and the passive treatment system may influence microbial community and activities leading to successful treatment (Rakotonimaro et al. 2018; Senko et al. 2008; Sharma et al. 2020). In this field-based study, we found that the Lyons and Farr channels provided an additional 700 m of passage for AMD that had already been passively treated with neutralizing media (i.e. limestone or steel slag), albeit with diminished effectiveness (Kinney 2013). As AMD passed through both channels, dissolved Fe (and Al and Mn in the case of Farr) were partially removed from solution, but the extents of removal fluctuated seasonally and dissolved metal concentrations still exceeded the accepted U.S. EPA National Secondary Drinking Water maximum contaminant levels, which the Ohio EPA uses as their stream remediation standards (Fe < 0.3 mg/L, Al < 0.05–2 mg/L, and Mn < 0.05 mg/L; USEPA 2009).

This field-scale work is consistent with previous laboratory-scale work illustrating that AMD chemistry influences the development of microbial communities in the sediment challenged by AMD (Sharma et al. 2020). Dissolved Fe, Al, and Mn were removed from AMD in the Farr channel (Fig. 2), with net alkaline AMD and a microbial community similar to circumneutral soil and sediments unaffected by AMD (e.g. *Bacillus* and *Paenibacillus*). While the ability to oxidize Fe(II) may be more taxonomically widespread than previously observed (Chan et al. 2018; Sharma et al. 2020) given the AMD chemistry and microbial community composition, it appears that dissolved Fe removal (as well as

Al and perhaps Mn) in the Farr channel is largely an abiotic process. Conversely, in the Lyons channel, the AMD was net acidic, and while dissolved Fe was removed from AMD, neither Al nor Mn were (Fig. 2). Additionally, the features of the Lyons channel microbial community were consistent with a system in which Fe removal was due to biooxidation (e.g. Brantner et al. 2014). These observations indicate that in the case of the Lyons system, Fe removal was largely a microbiologically mediated process.

Our results indicate that AMD chemistry shapes the development of differing sediment microbial communities, depending on AMD chemistry, thus resulting in differing extents of geochemically- or microbiologically-driven AMD attenuation. Therefore, monitoring the changes in the chemistry and microbial communities in an AMD treatment effluent and neighboring waterways can aid in designing sustainable and effective AMD treatment solutions.

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