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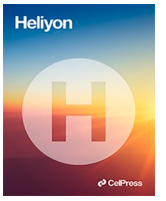

Research article

Unlocking water potential in drylands: Quicklime and fly ash enhance soil microbiome structure, ecological networks and function in acid mine drainage water-irrigated agriculture

Rabelani Munyai [a,b], Henry Joseph Oduor Ogola [b,c,*], Virginia Wambui Kimani [d], David Mxolisi Modise [e]

[a] Department of Agriculture and Animal Health, University of South Africa, Florida Science Campus, Roodepoort, 1709, South Africa
[b] School of Food and Agricultural Sciences, Jaramogi Oginga Odinga University of Science and Technology, P.O Box 210-40601 Bondo, Kenya
[c] Department of Environmental Sciences, University of South Africa, Florida Science Campus, Roodepoort, 1709, South Africa
[d] Industrial Microbiology and Biotechnology Research Centre (IMB-RC), Kenya Industrial Research and Development Institute (KIRDI), Popo Road off Mombasa Road, South C, Nairobi, Kenya
[e] Faculty of Natural and Agricultural Sciences, North West University, Private Bag X6001, Potchefstroom Campus, Potchefstroom, 2520, South Africa

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ABSTRACT

In water-stressed regions, treated acid mine drainage (AMD) water for irrigated agriculture is a potential solution to address freshwater scarcity. However, a significant knowledge gap exists on the short and long-term effects of treated AMD water on soil health. This study used high-throughput Illumina sequencing and predictive metagenomic profiling to investigate the impact of untreated AMD (AMD), quicklime- (A1Q and A2Q) and quicklime and fly ash-treated AMD water (AFQ) irrigation on soil bacterial diversity, co-occurrence networks and function. Results showed that untreated AMD water significantly increased soil acidity, electrical conductivity (EC), sulfate (SO_4^{2-}), and heavy metals (HM), including reduced microbial diversity, disrupted interaction networks, and functional capacity. pH, EC, Cu, and Pb were identified as key environmental factors shaping soil microbial diversity and structure. Predominantly, *Pseudomonas*, *Ralstonia picketti*, *Methylotenera* KB913035, *Brevundimonas vesicularis*, and *Methylobacterium oryzae*, known for their adaptability to acidic conditions and metal resistance, were abundant in AMD soils. However, soils irrigated with treated AMD water exhibited significantly reduced acidity (pH > 6.5), HM and SO_4^{2-} levels, with an enrichment of a balanced bacterial taxa associated with diverse functions related to soil health and agricultural productivity. These taxa included *Sphingomonas*, *Pseudoxanthomonas*, *Achromobacter*, *Microbacterium*, *Rhodobacter*, *Clostridium*, *Massillia*, *Rhizobium*, *Paenibacillus*, and *Hyphomicrobium.* Moreover, treated AMD water contributed to higher connectivity and balance within soil bacterial co-occurrence networks compared to untreated AMD water. These results show that quicklime/fly ash treatments can help lessen impacts of AMD water on soil microbiome and health, suggesting its potential for irrigated agriculture in water-scarce regions.

* Corresponding author. School of Food and Agricultural Sciences, Jaramogi Oginga Odinga University of Science and Technology, P.O Box 210-40601 Bondo, Kenya.
E-mail address: henryogola@gmail.com (H.J.O. Ogola).

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1. Introduction

Water scarcity has emerged as a significant global challenge, with demand for water surpassing the available supply and exerting adverse effects on community development and livelihood. Particularly vulnerable are regions characterized by low rainfall, high evaporation rates, and limited surface and groundwater resources, a situation further exacerbated by climate change [1]. Given water's pivotal role in achieving all 17 United Nations Sustainable Development Goals (UN SDGs) [2], exploring alternative water sources enhanced by existing technologies is crucial to mitigating the impact of water scarcity, regardless of initial quality.

South Africa exemplifies a country grappling with a severe water shortage crisis, attributed to rapid population, urbanization, and climate change [1]. This crisis impedes irrigated agriculture and rural development, jeopardizing food security and economic growth, especially in arid and semi-arid regions. Acid mine drainage (AMD) is another problem that affects the availability of water used for domestic and agricultural purposes in South Africa and around the world [3]. The Witwatersrand Basin in South Africa, with a long history of gold mining, is one of the regions affected by AMD, discharging about 202 million L/day AMD wastewater [4], This water resource has been suggested as an important potential solution to the region's freshwater shortage [4,5], but poses a threat to ecological systems, agricultural land, and human health due to its high acidity and nonbiodegradable heavy metals (HM) contamination. Therefore, it must be treated to a quality suitable for use in irrigated agriculture and non-potable applications [6].

Various AMD treatment technologies have been tested, including active and passive treatment methods, with different degrees of success. For example, active technologies involving the use of different chemicals such as lime and its by-products, zeolites, nanomaterials etc. [7–9], have been widely used for treating AMD due to their effectiveness in neutralizing acidity, removing HMs and SO_4^{2-} from wastewater. According to Skousen [10] and Tolonen et al. [11], quicklime can remove up to 99% of various HMs and 60% of SO_4^{2-} from AMD. Various alkaline materials, including NaOH, $Ca(OH)_2$, $MgCO_3$ (cryptocrystalline magnesite), MgO, Na_2CO_3, $Mg(OH)_2$, $CaCO_3$ and CaO, have also been found to be effective in neutralizing and removing contaminants from AMD [12]. In addition to lime, coal fly ash zeolites potential in the remediation of AMD treatment [13,14], and in reducing acid generation and controlling hydraulic conductivity of mine piles [15] have been demonstrated. These findings suggest that lime and fly ash zeolites are effective and promising methods for treating AMD and reducing its negative impact on the environment. Due to increasing water scarcity, the use of untreated and treated AMD water for irrigation is becoming more common in agriculture, but sustained use requires consideration of the impacts of these waters on both crop production and maintenance of good soil physical properties and health. Particularly, phytotoxicity-related AMD acidity and HM contamination of agricultural soil and crops, and HM entering the food chain through food crop uptake, remain key concerns related to reuse of AMD water for irrigated agriculture [6].

Optimal soil microbiome diversity is vital for sustainable agriculture, as it is crucial for plant growth, soil health, and fertility. Studies have shown that the soil microbiome plays a significant role in AMD-contaminated environments, involving essential processes such as pH neutralization, metal bioremediation, plant growth promotion, sulfates/iron reduction, and biogeochemical N and C recycling [6]. However, there is limited information is available on how the structure and functions of soil microbiome are affected in AMD water-irrigated agriculture. Nevertheless, few available studies have shown that soil acidification, HM, Fe and SO_4^{2-} pollution under such conditions, may significantly reduce microbial biomass, richness, and diversity, leading to impaired soil health, plant growth and productivity impairment [16,17]. Field application of SO_4^{2-} in upland soils has also reported mixed effects on the composition and functions of soil microbiomes [18,19]. While some studies indicate that SO_4^{2-} addition can lead to increased soil acidity and salinity, potentially harming soil microbiota [18,19], others suggest that SO_4^{2-} enrichment can promote the growth of acidophilic, HM, and sulfate-adapted microbial taxa, contributing to optimal plant growth and bioremediation of AMD-polluted agricultural soils [20]. For instance, Wang et al. [21] reported the enrichment of *Acidobacteria* (genera *Candidatus Solibacter and Candidatus Koribacter*) and *Crenarchaeota* phyla, contributing to energy metabolic processes related to C/N pathways in paddy rice soil irrigated with AMD water. An increase of metabolic activities that generate alkalinity and the abundance and the diversity of sulfate reducing bacteria (SRB; genus *Desulfobacca, Desulfovibrio, Syntrophobacter, Desulforhopalus, Desulfarculus,* and *Desulfobulbus*) and iron-reducing bacteria (FeOB) in the AMD-irrigated paddy soil has also been reported [20]. In addition, treatment technologies used to neutralize acidity and remove HM and SO_4^{2-} from AMD water have been found to alter the autochthonous AMD water microbiome, resulting in a shift towards a more diverse bacterial taxa, including sulfate reducing bacteria (SRB) and iron-reducing bacteria (FeOB) [22]. While numerous studies have investigated the impact of AMD contamination on soil microbiomes, a significant gap remains in understanding the specific effects of residual HMs and in treated AMD water on soil microbial diversity and functioning in AMD water-irrigated agriculture. Given the residual levels of HMs and SO_4^{2-} in treated AMD water [11,12], understanding the short- and long-term effects of these waters on soil health (including microbial, physical and hydraulic properties), plant productivity, and potential public health implications is also crucial.

This study explored the intricate interplay between soil microbiome and agricultural practices, specifically focusing on the effects of quicklime- and fly ash-treated AMD water irrigation. The primary objective was to uncover the mechanisms optimizing soil microbiome structure and co-occurrence networks, while also understanding the interplay between bacterial function and the soil environment under treated AMD water-irrigated agriculture. Through high-throughput Illumina sequencing of 16S rDNA gene amplicons, the study analyzed soil bacteria to discern trends in diversity, richness, ecological networks and functional capacity as affected by treated (A1Q, A2Q and AFQ) and untreated AMD water (AMD) in irrigated agriculture. It also evaluated the interrelationship of microbiome structure and function with prevailing soil physicochemical factors properties. With the anticipated rise of AMD water use in irrigated agriculture, this study contributes to establishing a robust theoretical framework that enhances understanding of the broader implications of quicklime and fly ash-treated AMD water irrigation on soil health and agricultural productivity. By exploring the interactions between treated AMD water, soil microbiomes, and agricultural practices, novel insights into the

ecological dynamics of AMD-affected agricultural ecosystems have been uncovered. Understanding these dynamics is crucial not only for optimizing agricultural productivity but also for mitigating potential public health implications associated with the use of treated AMD water in irrigation practices. Ultimately, the practical implications of this study may extend to improve soil management strategies and ensuring food security in water-stressed regions such as South Africa, grappling with AMD wastewater management from the mining industry.

2. Material and methods

2.1. Treated AMD water preparation

AMD water were collected from gold mine tailing dam in Randfontein, Gauteng Province, South Africa. Coal fly ash and quicklime were obtained from Eskom Matla Power Station and All Lime Services Pty located in Mpumalanga and Elandsfontein, South Africa, respectively. The typical physicochemical properties of the AMD water and coal fly ash used has been described previously by Kalu et al. [23] and Alegbe et al. [24]. Certified seeds of Marykies and Royal (determinate) cultivar of potato were obtained from McCain, Delmas, Mpumalanga, South Africa.

The jar test, as described by Othman et al. [25], was used to treat AMD water with quicklime and fly ash before use as irrigation water. The treatments included: 1:99 (10 g quicklime + 990 mL AMD water), (A1Q); 2:98 (20 g quicklime + 980 mL AMD water), (A2Q); and 2:25:73 (20 g quicklime + 250 g fly ash 100 + 730 mL AMD water) (AFQ). In addition, normal tap water was included as the Control and 100% AMD water was used as untreated AMD treatment (AMD) in the experiments. The typical physicochemical and HM composition of the water used for irrigation experiments is summarised in Supplementary Material Table S1.

2.2. Experimental design and sampling

The greenhouse experiments were caried out at Ceres Greenhouse Facility, University of South Africa (UNISA), Florida Science Campus, Roodepoort, Gauteng Province (S 26° 10′ 30″ S, 27° 55′ 22.8″ E), spanning from September to December 2018 (Season 1; conditioning phase) and from March to June 2019 (Season 2; test phase). During the experiment, potato plants were subjected to five irrigation treatments (Control, AMD, A1Q, A2Q and AFQ), arranged in a completely randomized block (CRB) design, with each treatment consisting of 10 plants replicated at least three times. Potatoes were cultivated in 5 L pots with a growing substrate (3:1:1 Culterra topsoil + vermiculite + river sand) and received irrigated 500 mL water every 2 days until harvesting. Throughout the study period, the mean temperatures, relative humidity, and light intensity were maintained at 25 °C, 50%, and 1100 μmol s^{-1} m^{-2}.

Bulk soil samples were collected during the second harvesting in June 2019 for physicochemical and metagenomic analysis. For each treatment plot, three soil cores (5 cm diameter × 20 cm length) were randomly collected from the 0–20 cm layer as replicates. Each sample was divided into two portions: one portion stored at 4 °C for physicochemical analysis and the other at −80 °C for DNA extraction and metagenomic sequencing.

2.3. Soil geochemical properties analyses

To analyze the soil physiochemical properties and HM content, protocols described by Odhiambo et al. [26] were employed. In situ measurements of pH and EC, were conducted using a multi-probe field meter (YSI TM 6 series, Sonde Marion, Germany). Soil SO_4^{2-} and NO_3^- levels were analyzed after ball milling of the dry soil samples and quantified by MQuant® sulfate (Merck KGaA, Darmstadt, Germany) and Nitrite/Nitrate Colorimetric® Assay Kit (Merck KGaA, Darmstadt, Germany), respectively.

The concentration of HMs were determined using an Inductively Coupled Optical Emission Spectrometer (Agilent Technologies 700 series ICP-OES) after microwave digestion of the 0.5 g soil samples.

2.4. DNA extraction, high throughput sequencing and bioinformatic analyses

The genomic DNA extraction, PCR amplification of bacterial 16S rRNA V1–V3 hypervariable region and library preparation for targeted amplicon sequencing were conducted following established protocols [27]. Subsequently, the generated libraries were sequenced on Illumina 2 x 300-bp paired-end run Miseq 250®platform at the Eureka Labs, University of South Africa - Florida Science Campus.

The protocol and pipeline used to process the raw Miseq sequences into operational taxonomic units (OTUs) at 97% sequence similarity across various taxonomic levels has been detailed previously [28]. Diversity indices and richness estimates were calculated using MOTHUR, and the variations in the community structure and diversity between irrigation treatments tested statistically analyzed by ANOVA, Wilcoxon rank sum test, or Kruskal-Wallis test, with Benjamini-Hochberg FDR (false discovery rate) correction applied at $p < 0.05$, depending on the data type. For beta-diversity based on Bray-Curtis dissimilarity distances, vegan package [29] in R was utilized to perform hierarchical clustering, and principal coordinates analysis (PCoA). Redundancy analysis (RDA) was employed to assess the effects of environmental variables on bacterial community structures and identify significant differences in community composition. This analysis was complimented with ANOSIM and adonis PERMANOVA tests in the vegan package to examine the relationship between soil physicochemical characteristics and bacterial community.

To identify significant bacterial taxa (indicator species) associated with different AMD water irrigated agriculture, the indicspecies package [30] in R was used. Venn diagrams, for graphical descriptions of unique and shared bacterial genera between different AMD

water irrigated soils, were generated using the VennDiagram package in R [31]. Co-occurrence network analysis was performed using the R package NetCoMi [32] on the top 0.01% of OTUs. Spearman correlations were calculated between taxa based on CLR-transformed OTUs, where strong positive ($\rho > 0.7$) and negative ($\rho < -0.7$) interactions were visually represented. For predictive functional profiling of bacterial taxa, PICRUSt2 (Phylogenetic study of Communities by Reconstruction of Unobserved States) was used following the developer's instructions [33].

3. Results and discussion

3.1. Quicklime and fly-ash treatment improves soil physicochemical properties

Table 1 presents a summary of the bulk soil physicochemical properties under untreated and treated AMD water, and normal tap water (Control). Untreated AMD water irrigated soils exhibited significantly higher acidity (pH = 3.85; one-way ANOVA, $p < 0.05$) compared to the other treatments. Furthermore, untreated AMD irrigation water resulted in significantly elevated levels of soil EC, SO_4^{2-}, iron, and HM (Al, As, Co, Cu, Ni, and Pb) (one-way ANOVA, $p < 0.05$), consistent with findings of previous studies [21,34]. The acidic pH, coupled with Fe and S recycling and the mineral phase transformation, may influence metalloid mobility via leaching and precipitation in acid sulfate soils, thereby affecting soil properties and crop health [35]. Elevated EC can also stress plant, causing productivity losses [36]. These findings are further supported by Raletsana et al. [37], who noted 46% reduced potato yield with AMD water irrigation, while Choudhry et al. [38] reported a 62% decrease rice yield in rice due to elevated acidity, EC, Fe, Mn, SO_4^{2-}, and diminished availability of P, K, and Zn in AMD-contaminated paddy field. Hence, the phytotoxic effects of AMD acidity and the contamination of agricultural soil and crops, and potential transfer of toxic HM into the food chain, underscore concerns over untreated AMD water agriculture [6].

In remediating AMD, quicklime (CaO) and fly ash has been proposed in addressing AMD water's acidity and elevated HM/SO_4^{2-} during agricultural irrigation [14]. This study assessed 1% (A1Q) and 2% quicklime (A2Q) and quicklime + fly ash (AFQ) treatments effectiveness in mitigating acidity and elevated levels of HMs and SO_4^{2-} in AMD water for agricultural use. Both A1Q, A2Q, and AFQ treatments resulted in soils with circumneutral pH (pH 5–8), significantly distinct from untreated AMD water soils, but akin to Control (tap water irrigation) (Table 1). Notably, A2Q and AFQ achieved higher neutralization, yielding pH > 7.7, complying with South Africa Water Quality (SAWQ) guidelines within the pH 6.5 and 8.4 for agricultural use [39]. These findings are consistent with lime/-limestone [9,11] and fly ash neutralization studies [13,15]. Further, quicklime and fly ash treatment also resulted to substantial reduction in EC reduction, SO_4^{2-}, NO_3^- and HM content (one way ANOVA, $p < 0.05$) (Table 1) in bulk soils compared to untreated AMD water (Table 1). This is consistent with findings of prior research that has established that reduction in SO_4^{2-} contents, including Mg, Cl, K, Ca, Na and NO_3^{2-} can significantly contribute to lower soil EC [34]. Therefore, these results highlight the potential of quicklime and fly ash as neutralizing agents in remediating AMD water during agricultural irrigation. The treatments not only improved soil physicochemical characteristics, but also addresses concerns related to acidity, salinity, and HM contamination, having implications for sustainable agricultural practices in AMD-affected regions.

3.2. Impact of irrigation treatments on bacterial community diversity indices

The description and quality metrics of the metagenomic datasets generated from this study has been previously described by Ogola et al. [28]. Table 2 illustrates the impact of irrigation treatments on alpha diversity indices of soil bacterial communities. In summary, a total of 946,212 high-quality sequences were obtained from 33 samples with an average read and sequence length of 28,670 and 501 bp per sample, respectively (Table 2). Both Good's coverage (Table 2) and rarefaction curves and rank abundance plots

Table 1
Effect of different treatment on soil physiochemical properties.

Parameters	Mean (±SE) concentration				
	Control	AMD	A1Q	A2Q	AFQ
pH	7.13 ± 0.12*a*	3.85 ± 0.14*d*	5.67 ± 0.11*c*	7.70 ± 0.05*a*	7.87 ± 0.06*a*
EC (μS cm^{-1})	5.2 ± 0.09*c*	263 ± 7.7*a*	50 ± 1.49*bc*	72 ± 1.47*b*	85 ± 0.95*b*
NO_3^-(mg kg^{-1})	0.74 ± 0.08*e*	8.78 ± 0.31*a*	4.21 ± 0.08*b*	2.80 ± 0.02*c*	2.17 ± 0.06*c*
SO_4^{2-} (mg kg^{-1})	16.3 ± 0.43*e*	12706 ± 20*a*	1148 ± 1.86*d*	1209 ± 17*c*	1265 ± 9.1*b*
Al (mg kg^{-1})	0.29 ± 0.08*c*	1055 ± 98*a*	6.84 ± 1.09*b*	0.84 ± 0.02*c*	0.47 ± 0.08*c*
As (mg kg^{-1})	0.02 ± 0.01*b*	2.06 ± 0.63*a*	1.78 ± 0.96*a*	0.01 ± 0.00*b*	0.01 ± 0.00*b*
Cd (mg kg^{-1})	<0.01*c*	0.16 ± 0.11*a*	0.06 ± 0.03*b*	0.02 ± 0.00*bc*	0.02 ± 0.00*c*
Co (mg kg^{-1})	0.03 ± 0.01*c*	29.5 ± 4.56*a*	2.4 ± 0.34*b*	0.03 ± 0.00*c*	0.01 ± 0.00*c*
Cu (mg kg^{-1})	0.04 ± 0.00*c*	3.78 ± 0.98*a*	0.66 ± 0.08*b*	0.04 ± 0.01c	0.11 ± 0.04*bc*
Fe (mg kg^{-1})	0.06 ± 0.01*b*	0.57 ± 0.18*a*	0.11 ± 0.03*a*	0.01 ± 0.00*b*	0.09 ± 0.00*a*
Ni (mg kg^{-1})	4.59 ± 0.77*c*	1029 ± 108*a*	23.0 ± 6.11*b*	2.54 ± 0.83*cd*	1.94 ± 0.67*d*
Pb (mg kg^{-1})	<0.01*c*	6.5 ± 1.07*a*	2.33 ± 1.21*b*	2.15 ± 0.98*b*	3.08 ± 0.63*b*
Zn (mg kg^{-1})	0.02 ± 0.00*b*	2.33 ± 0.03*a*	3.68 ± 0.78*a*	1.94 ± 0.19*a*	2.16 ± 0.99*a*

Control, tap water; A1Q, AMD water treated with 1 g quicklime; A2Q, AMD water treated with 2 g quicklime; and AFQ, AMD water treated with fly ash and 2 g quicklime DO: dissolved oxygen, EC: electrical conductivity, NO: nitrate, TDS: total dissolved solids, and SO_4^{2-}: sulfate. Mean ± SE in same row with dissimilar letter are significantly different at $p < 0.05$.

Table 2
Effect of different AMD water irrigation treatments on the richness and diversity of soil bacterial community.

Indices[a]	AMD	A1Q	A2Q	AFQ	Control	One-way ANOVA
Observed OTUs	93 ± 43*c*	561 ± 35*a*	465 ± 10*a*	394 ± 52*b*	689 ± 47*a*	$F_{4,23} = 193.5$, $P < 0.0001$***
Quality reads	48403 ± 40800*a*	16686 ± 3210 *ab*	16068 ± 3840 *ab*	10648 ± 4117*b*	53275 ± 33301*a*	$F_{4,23} = 5.302$, $P = 0.0036$**
Simpson 1_D	0.771 ± 0.070*b*	0.984 ± 0.003*a*	0.967 ± 0.011*a*	0.971 ± 0.011*a*	0.977 ± 0.014*a*	$F_{4,23} = 44.2$, $P < 0.0001$***
Shannon_H	2.20 ± 0.21*b*	4.99 ± 0.09*a*	4.49 ± 0.18*a*	4.47 ± 0.31*a*	4.86 ± 0.41*a*	$F_{4,23} = 209.8$, $P < 0.0001$***
Chao-1	94 ± 49*c*	576 ± 33*a*	485±8 *ab*	419 ± 48*b*	696 ± 45*a*	$F_{4,23} = 221.3$, $P < 0.0001$***
Good's coverage	0.982	0.975	0.991	0.982	0.985	

[a] Diversity indices (observed OTUs, *Chao1*, Shannon and Simpson) were based on rarefied datasets of 8495 sequences representing the lowest number of reads in a sample. AMD, 100% AMD water; Control, tap water; A1Q, AMD water treated with 1 g quicklime; A2Q, AMD water treated with 2 g quicklime; and AFQ, AMD water treated with fly ash and 2 g quicklime.

(Supplementary Material Fig. S1) indicated sufficient sequencing depth, effectively capturing the complete soil bacterial community diversity.

There was significant negative impact of untreated AMD water (AMD) on the richness (observed OTUs and Chao1; $p < 0.0001$) and diversity indices (Shannon and Simpson; $p < 0.0001$) of the bacterial community compared to other treatments. The intricate diversity of soil microbial community, coupled with their dynamic response to ecology shifts, positions them as sensitive indicator of human-induced impacts on soil ecology. Consistent with findings of this study, there are reports suggesting that untreated AMD water irrigation may induce soil alterations related to acidification, HM, and SO_4^{2-} pollution [6,21,40], resulting in adverse negative effects on microbial biomass and richness. Interestingly, the quicklime and fly ash-treated AMD water samples (A1Q, A2Q, and AFQ) showed

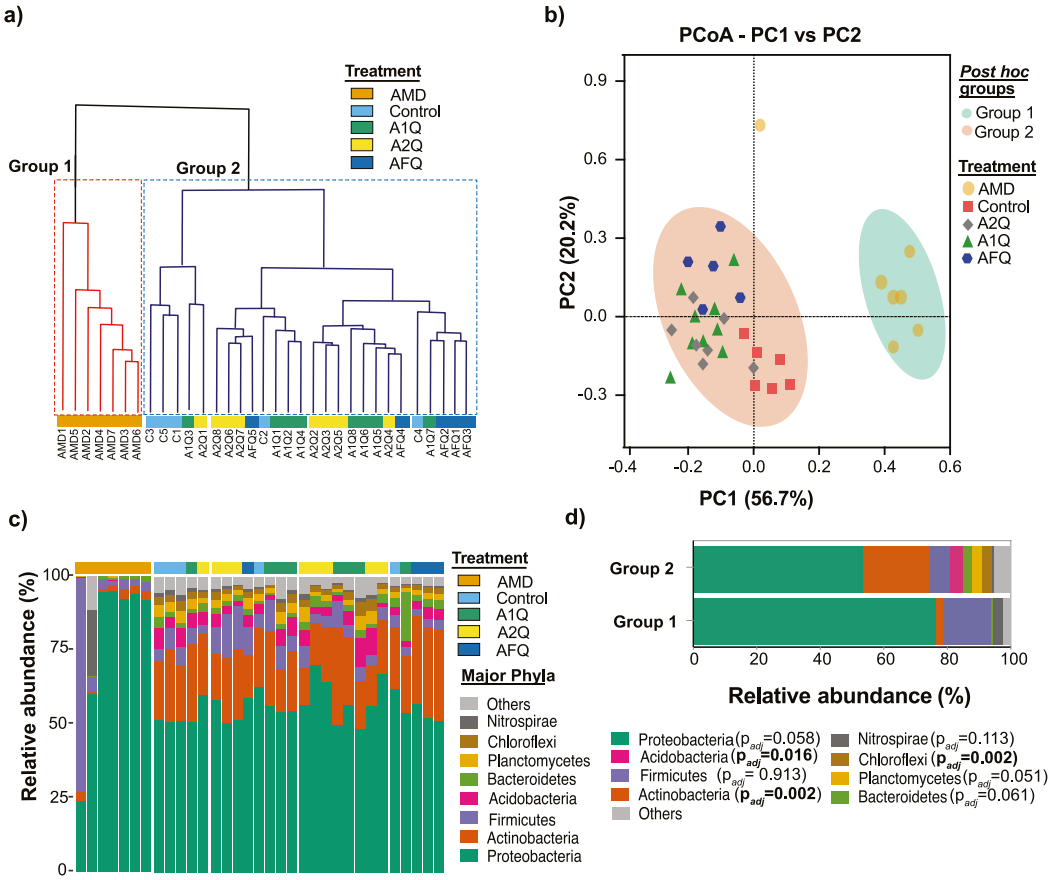

Fig. 1. Phylogenetic diversity of the soil bacteria community composition. a) Dendrogram showing the complete-linkage agglomerative hierarchical clustering (Ward's method) based on a Euclidean distance of the different treatments. b) Principal coordinate analysis (PCoA) of soil bacteria community composition in different treatments of AMD water irrigation. PCoA was calculated using the Bray-Curtis distance matrix. c) The relative abundance of major taxonomic groups at phylum level for the bulk soil bacteria treated with different AMD irrigation water. d) Relative abundance of major taxonomic groups at phylum level for the *post-hoc* groupings of the samples Group 1 (AMD) and Group 2 (A1Q, A2Q, AFQ, and Control). Phyla with significant relative abundance (Wilcoxon rank, with BH FDR correction at $p < 0.05$) between pot hoc Group 1 and 2 are bolded.

improvements in soil bacterial diversity and richness, approaching levels closer to the Control (normal tap water) (Table 2).

Results of beta diversity analysis based on hierarchical clustering (Ward's method) and PCoA (Bray-Curtis dissimilarity distance) are provided in Fig. 1a and b, respectively. Both analyses classified the bulk soil samples into two distinct post hoc groupings: Group 1 (AMD) and Group 2 (Control, A1Q, A2Q, and AFQ) (Fig. 1a and b), forming the basis for subsequent downstream analysis and discussion. PCA analysis revealed that the two axes captured 76.9% composition variation, suggesting that the combination of the PC1 and PC2 effectively captured a substantial amount of the underlying patterns in the data. This divergence was further supported by ANOSIM ($R = 0.5112$, $p = 0.0097$) and adonis PERMANOVA ($F = 3.234$, $p = 0.0211$) tests, that showed significant differences between the irrigation treatments (Table 3). These results indicate that the application of quicklime and fly ash effectively mitigated the adverse effects of excessive acidity and HM content associated with untreated AMD water irrigation. The observed enhancement in microbial diversity, abundance, and composition in treated samples underscores the potential of these treatments in restoring soil health and fostering a more resilient and productive ecosystem [41]. These findings highlight the potential of quicklime and fly ash treatments in promoting a more balanced and diverse bacterial community in AMD water-irrigated agriculture.

3.3. Perturbations of soil bacterial community structure

The study identified a diverse soil microbial community across AMD water irrigation treatments, spanning 46 phyla, 152 classes, 276 orders, 539 families, and 1189 genera. Fig. 1c presents the overall relative abundance of major phyla under different irrigation treatments. Dominant bacterial phyla, including *Proteobacteria* (37.08%–96.30%), *Actinobacteria* (0.64%–20.21%), *Firmicutes* (0.90%–58.80%), *Bacteroidetes* (0.27%–13.94%), *Acidobacteria* (0.00%–6.02%), and *Chloroflexi* (0.00%–4.41%) (Fig. 1c and d), were consistent with their ubiquity in soil habitats in AMD-contaminated agricultural soils [6,21,41]. However, treatment-related variations were observed, with A1Q, A2Q, and AFQ treatments closely resembling the Control group (Fig. 1c and d).

Post hoc Group 1 (AMD) soils exhibited enrichment in *Proteobacteria* (>85% relative abundance), particularly *Gammaproteobacteria* (~36%), *Alphaproteobacteria* (~25.1%), and *Betaproteobacteria* (~21.9%) (Supplementary Information Fig. S2). *Proteobacteria* dominance, mainly *Gammaproteobacteria* and *Betaproteobacteria,* aligns with their extensive degradation capability and adaptability to nutrient-unstable and HM-polluted environments like mining areas [20,42], and AMD-contaminated agricultural soils [19,21,43]. Heatmaps representing the relative distribution of the top 40 OTUs at genus and species level are provided as Supplementary Information Fig S3 and Fig. 2a. Genus-level analysis revealed increased abundance HM-tolerant bacteria like *Pseudomonas* (*Gammaproteobacteria*), *Ralstonia* and *Methylotenera* (*Betaproteobacteria*) and *Brevundimonas* and *Methylobacterium* (*Alphaproteobacteria*) in untreated AMD water-irrigated soils (Fig. 2). This indicates the significant impact of untreated AMD water acidity and HM pollution on shaping microbial community structure, favoring tolerant bacterial taxa [44,45].

In contrast, Group 2 samples (Control, A1Q, A2Q and AFQ), displayed a balanced and diverse microbial structure, characterized by lower abundance of *Gammaproteobacteria* (<11.9%; Kruskal-Wallis test, $p = 0.0096$) and *Betaproteobacteria* (<4.3%; Kruskal-Wallis test, $p < 0.0001$) (Supplementary Information Fig. S2), but increased enrichment of *Alphaproteobacteria* (53.1–61.9%; Kruskal-Wallis test, $p < 0.0001$), *Actinobacteria* (Kruskal-Wallis test, $p = 0.002$), *Acidobacteria* (Kruskal-Wallis test, $p = 0.0016$), *Chloroflexi* (Kruskal-Wallis test, $p = 0.0002$), *Nitrospirae*, and *Plantomycetes* (Fig. 1d). Overall, quicklime and fly ash-treated AMD water (A1Q, A2Q, and AFQ) positively impacted soil microbiome structure at both phylum (Fig. 1c and d), and genus level (Fig. 2), approaching that observed in the Control soil irrigated with tap water. This positive influence may partly be attributed to the effective acidity neutralization and reduction of HM and SO_4^{2-} pollution linked to quicklime and fly-ash treatments reported (Table 1). These results strongly suggest that quicklime and fly-ash treatments positively influenced the soil microbial community diversity (Table 2) and structure (Figs. 1 and 2) in AMD water-irrigated agriculture.

Table 3
Adonis PERMANOVA and envfit results showing the contribution of different AMD irrigation water and physicochemical factors on bulk soil bacterial community variation. Effects were considered to significantly contribute to community variation at $p < 0.05$.

Parameter	adonis PERMANOVA				envfit	
	df	*F*	R^2	*P*	r^2	*P*
Treatment	4	3.234	0.244	**0.02****		
Al	1	2.884	0.085	0.12	0.1700	0.053
As	1	1.819	0.055	0.13	0.1057	0.156
Cd	1	1.168	0.036	0.29	0.2059	0.127
Co	1	1.148	0.036	0.37	0.1874	0.238
Cr	1	1.215	0.038	0.30	0.1297	0.101
Cu	1	2.273	0.168	**0.04***	0.1960	**0.035***
Fe	1	1.520	0.047	0.30	0.0222	0.530
Ni	1	1.615	0.050	0.18	0.1994	0.310
Pb	1	3.787	0.109	**0.01****	0.3215	**0.004****
Zn	1	1.130	0.035	0.26	0.1207	0.125
SO_4	1	1.141	0.036	0.32	0.1357	0.090
pH	1	2.711	0.142	**0.02***	0.2068	**0.025***
EC	1	2.614	0.250	**0.02***	0.2369	**0.013***
NO_3	1	1.051	0.033	0.37	0.2330	0.111

*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.0001$.

(caption on next page)

Fig. 2. Differential abundance of bacterial taxa in soils irrigated by different AMD water. a) Heatmap of the top 40 OTUs at species level. Taxa identified as indicator species (OTUs with an association coefficient *IndVal* > 0.7, at Benjamini-Hochberg (BH) false detection rate correction *q* value < 0.5, are highlighted red and blue for *post-hoc* Group 1 and 2, respectively. b) LEfSe plot of taxa exhibiting differential abundance (LDA >3.0 at BH FDR corrected *q* value < 0.5) between the two post hoc Group 1 (AMD) and Group 2 (A1Q, A2Q, AFQ and Control). c) Relative abundance of the differentially abundant taxa in Group 1 and 2 samples identified by Wilcoxon rank sum test at $p < 0.05$, with BH FDR corrected *q* value < 0.5. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.4. Differential abundance of bacterial taxa in the post hoc groups

To assess the compositions of the soil microbial communities under various irrigation conditions, the study employed a comprehensive approach involving Venn diagram, indicator species analysis (ISA), and LEfSe differential analyses. Results of the Venn Diagram, ISA and LEfSe analyses of the top 50 OTUs are provided in Supplementary Information Fig. S4, Fig. 2b and c, respectively. The Venn diagrams highlighted the limited overlap between AMD and treated samples (A1Q, A2Q, and AFQ), sharing only 5% of the total sequences, whereas the Control and treated samples collectively shared 52% of the total sequences, indicating significant differences in bacterial community composition between post hoc Group 1 and Group 2.

Consistent with the Venn diagram analysis, ISA detected 6 and 35 taxa strongly associated (*IndVal* > 0.70, $p < 0.05$) with post-hoc Group 1 and 2, respectively (Fig. 2a and Supplementary Material Table S2). Group 1 (AMD) exhibited indicator taxa such *Pseudomonas* (*P. migulae* and *P. marginalis*), *Ralstonia picketti*, *Methylotenera* KB913035, *Brevundimonas vesicularis* and *Methylobacterium oryzae*. Stringent LEfSe analysis (LDA effect size >4.0, BH FDR corrected $p < 0.05$) (Fig. 2b) and Wilcoxon rank sum test (Fig. 2c) also identified phyla (*Actinobacteria* and *Acidobacteria*), and 3 classes (*Betaproteobacteria*, *Alphaproteobacteria* and unclassified *Actinobacteria*) as differentially enriched taxa in the post hoc Group 1. In contrast, Group 2 had diverse taxa as the key indicator species, including families *Rhizobiaceae*, *Micrococcaceae*, and *Bradyrhizobiaceae* and genera *Sphingomonas*, *Devosia* and *Pseudolabrys* among others enriched in A1Q, A2Q, AFQ and Control (Fig. 2b). These results provide valuable insights into the distinct microbial compositions influenced by irrigation treatments, laying the foundation for further understanding the ecological implications of these differences in soil health and productivity.

3.5. Relationships between environmental variables and abundant bacterial taxa

In this study, redundancy analysis (RDA), which accounts for both the explanatory power of environmental variables and the inherent variation in microbial community composition [46,47], was employed to identify environmental factors that significantly influenced microbial community structure and quantify the strength and direction of these relationships. The RDA analysis results, depicted in Fig. 3, indicated that all of the environmental variables explained 31.3% of the variation in bacterial community composition. Specifically, RDA1 and RDA 2 accounted for 25.7% (Pseudo-$F = 11.568$, $p = 0.008$) and 6.2% (Pseudo-$F = 5.354$, $p = 0.028$) of the variation, respectively. Using a stepwise model building approach on the constrained ordination (Supplementary Material Table S3), adonis PERMANOVA and envfit test (Table 3), pH, EC, Cu, and Pb were identified as the key environmental factors explaining variation in soil microbial community structure between different treatments.

Soil pH, a well-recognized determinant of bacterial community structure [27,48], has a profound impact on the balance between stochastic and deterministic processes in successional soils [49]. In this study, the observed significant clustering of microbial diversity

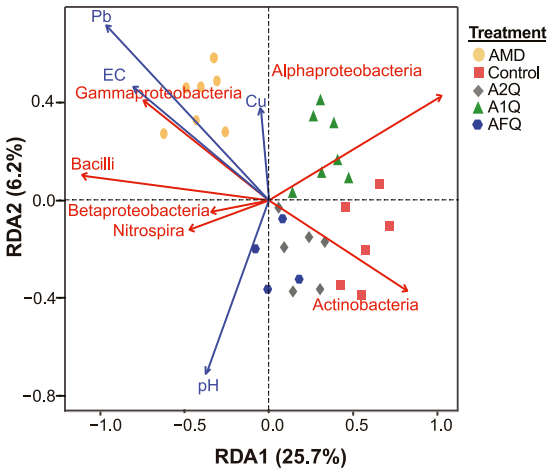

Fig. 3. Redundancy analysis (RDA) triplot of the bacterial community composition at the class level (relative abundance >1 %) and environmental variables in the bulk soil samples. Red arrows indicate the members of the bacterial community. The blue arrows represent environmental variables (Cu, Pb, EC and pH) with significant correlation at, based on ordistep forward selection after BH correction (Supplementary Material Table S2). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

in irrigated soils (Fig. 1b), correlated negatively with acidic pH (Fig. 3). This aligns with the findings of Tripathi et al. [49], who reported that extreme acidic pH conditions drive the assembly of more phylogenetically clustered bacterial communities through deterministic processes influenced by abiotic and biotic factors that affect organismal fitness. The hypothesis posits that the extreme acidic pH conditions observed in Group 1 samples likely impose stringent limits on survival and fitness, exerting strong selective pressures through ecological and evolutionary time. This may explain the low bacterial diversity and structure observed in Group 1 (Table 1, Figs. 1 and 2), with only acid- and HM-tolerant taxa such as *Pseudomonas migulae*, *P. marginalis*, *Ralsonia picketti*, *Methylotenera* KB913035, *Brevundomonas vesicularis* and genus *Methylobacterium* detected as indicator species (Fig. 2b and c). Contrastingly, Group 2 were characterized by pH conditions close to neutral, and exhibited less phylogenetically clustered bacterial communities (Figs. 1 and 3). At species level, *Sphingomonas* (*S. agri*, *S. sedimenicola*, and *Sphingomonas* JN579998), *Pseudolabrys* (unclassified *Pseudolabrys*, PAC001870), *Rhizobium radiobacter*, *Bradyrhizobium japonicum*, *Mesorhizobium loti*, *Sphingobium vermicomposti* and *Arthrobacter globiformis*, were the major indicator taxa identified, many that are adapted to near-neutral pH conditions [49]. Under these circumneutral pH conditions, the phylogenetic assembly of bacterial community is likely shaped by stochastic processes involving random birth, death, and dispersal events, rather than deterministic processes [49,50].

In addition to pH, envfit test indicated that EC ($r^2 = 0.2369$), Cu ($r^2 = 0.1960$), and Pb ($r^2 = 0.3215$) were significantly correlated ($p < 0.05$) with bacterial community composition (Table 3). The untreated AMD water-irrigated soils exhibited a potential link between dissolved HM and SO_4^{2-} concentrations, and EC, Cu, and Pb as key predictors of reduced microbial diversity and composition. Previous studies have emphasized the strong correlation between EC and bacterial community structure, particularly in the contexts with elevated metal concentration [17,51]. Although the reported Pb and Cu concentrations were relatively low (<10 mg/kg), below levels known to cause toxic effects on bacterial diversity and function (>40 mg/kg) [52], it is plausible that the combined effects of high acidity and interactions with other HMs may contribute to the prominence of Cu and Pb in influencing species assemblage, even at these lower concentrations. This perspective aligns with findings of Wang et al. [21], who noted the strong negative influence of low pH and HM (Cu, Pb and Zn) bacterial communities in AMD water-irrigated in rice paddy soils, even under conditions of low HM pollution.

Overall, these findings suggest that pH, EC, Cu, and Pb are important environmental variables that influence the structure and composition of the bacterial community in the soil. These findings collectively suggest that pH, EC, Cu, and Pb are crucial environmental variables influencing the structure and composition of the bacterial community in the soil. This nuanced understanding of the relationship may contribute to a more comprehensive interpretation of the ecological dynamics in response to different AMD water irrigation conditions.

3.6. Structure and composition of bacterial co-occurrence networks

Network plots presented in Fig. 4 delineate the distinction between the ecological networks in the two post hoc groupings. Group 1

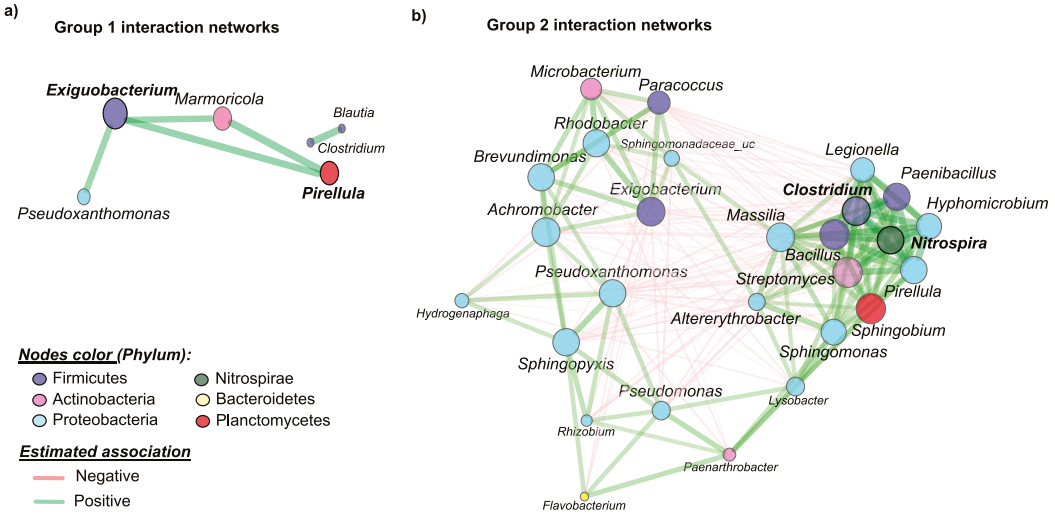

Fig. 4. Comparison of bacterial interaction networks within bulk soils irrigated with acid mine drainage (AMD) water. The SPRING method was utilized to assess associations, and only clusters containing a minimum of two taxa sharing the same color in both bacterial networks of the post hoc groupings, Group 1 (untreated AMD water) and Group 2 (A1Q, A2Q, AFQ, and Control treatments), are depicted in panels (a) and (b), respectively. Node color indicates the phylum, while node sizes represent the cumulative relative frequencies of genera with high Eigenvector centrality (>0.7). Key hubs, identified by bold font and borders, denote nodes with significant Eigenvector centrality (>1, P_{adj} <0.05) within the post-hoc groupings, determined through Netcomi R package co-occurrence network analysis [32]. Green and red edges represent estimated positive and negative associations between nodes and hubs within the two post-hoc groupings. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

exhibited a network with lower complexity and edge density (0.014 vs 0.630) than Group 2, despite having similar clustering coefficients (0.780 vs 0.729). The modularity, indicating network's divisibility into distinct modules or subgroups, was higher for Group 1 (0.320) than Group 2 (0.025). These findings align with Group 1 having a higher degree of bacterial associations, all forming positive edges (100%) and grouped into 23 clusters or modules. On the other hand, Group 2 displayed a single large cluster/module encompassing 27 bacterial taxa, with a notably higher number of nodes but a nearly equal distribution of positive/negative edges (48.9% vs. 51.1%) (Fig. 4). Moreover, the microbial network in Group 2 showed higher vertex and edge connectivity (6 vs. 1), indicating a greater number of connections/edges between nodes compared to Group 1. These results suggest that microbial interactions in Group 2 samples were more diversified and balanced, in contrast to the sparse and random networks observed in Group 1

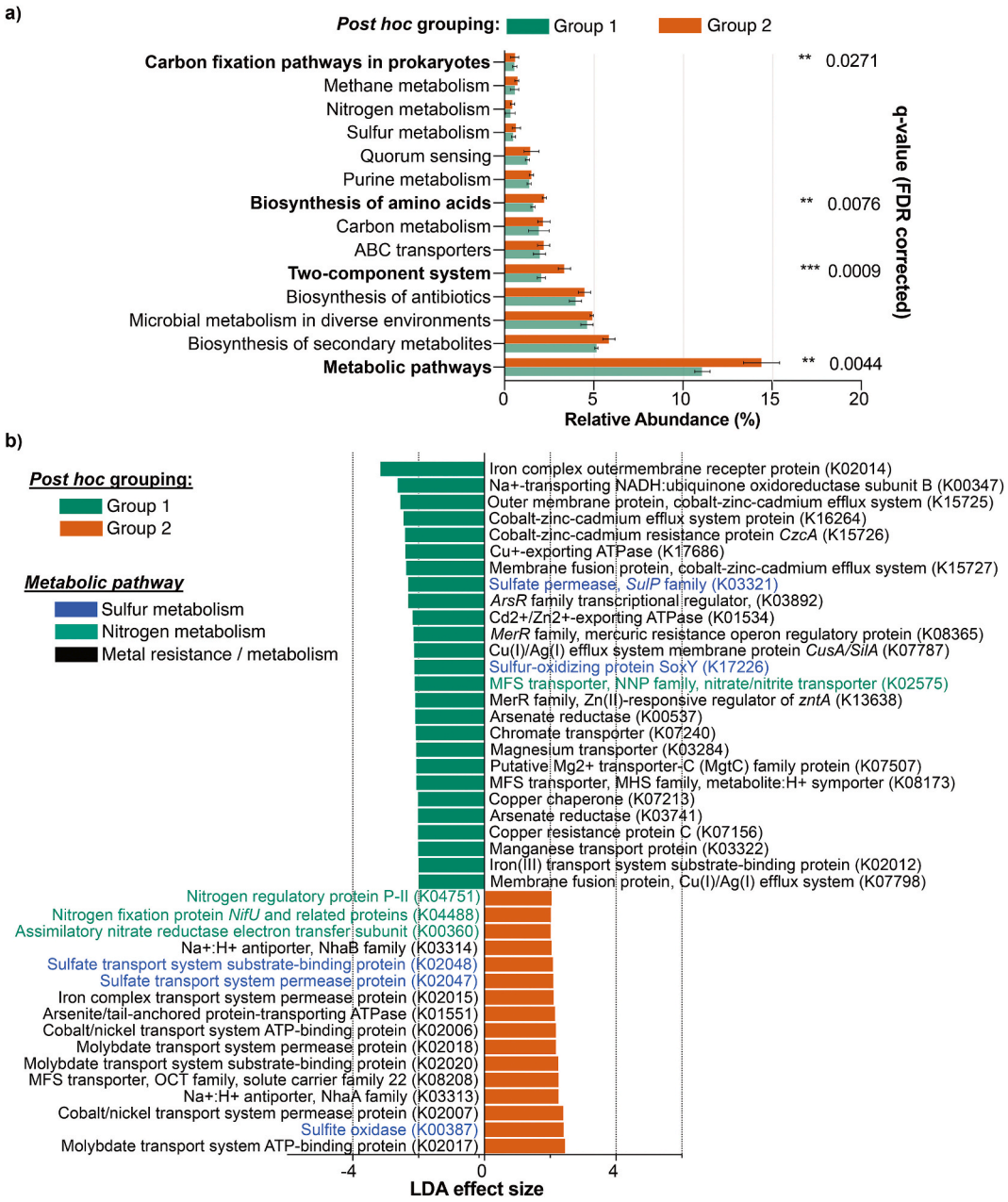

Fig. 5. Microbial functional analysis in soil irrigated with different AMD water. a) Relative abundance of top microbial PICRUSt-predicted KEGG pathways detected in the post-hoc Group 1 (AMD) and Group 2 (A1Q, A2Q, AFQ and Control) samples. The Kruskal-Wallis H test was used to compare the significant difference Benjamini Hochberg (BH) FDR-adjusted P values are reported at the right of the image, and stars indicate Bonferroni-significant differences. b) 42 KO genes that are differentially abundant (LDA effect size >2.0, p(FDR) < 0.05) between posthoc Group 1 and 2 samples based on LEfSe analysis. The metabolic groups the KOs belong to is highlighted.

samples.

Microbial networks in Group 1 and Group 2 also exhibited significant differences in betweenness centrality (Jaccard index, Jacc = 0.000, $p(\leq \text{Jacc}) = 0.0390$) and hub taxa (Jacc = 0.000, $p(\leq \text{Jacc}) = 0.0431$), indicating keystone/hub bacterial taxa divergence with potential functional significance. In Group 1, only *Exiguobacterium*, *Pirellula*, *Marmoricola*, *Nitrospira*, *Hyphomicrobium*, and *Pseudoxanthomonas* were the positive nodes, with *Exiguobacterium* and *Pirellula* as key hubs (Fig. 4a). These taxa thrives extreme environments, including low pH and HM contamination [53,54], suggesting their connection with AMD contaminated soil and ecosystem adaptability. This aligns with Yang et al. [55] findings that pH is a pivotal environmental factor that shapes network topology and the abundance of keystone taxa in agricultural soils. However, Yang et al. [55] noted more complex microbial networks associated with low pH than reported in the current study. It is plausible that the combined effect of high acidity and HM and SO_4^{2-} pollution could account for the observed reduction in microbial networks observed in Group 1 samples.

Group 2 exhibited a diverse interaction involving 118 positive nodes and 133 negative nodes, consisting of 27 taxa. Key hubs in Group 2 comprised *Sphingobium*, *Pseudoxanthomonas*, *Achromobacter*, *Microbacterium*, *Rhodobacter*, *Pirellula*, *Sphingomonas*, *Sphingopyxis*, *Nitrospira*, *Streptomyces*, *Bacillus*, *Clostridium*, *Massillia*, *Legionella*, and *Paenibacillus* (Fig. 4b). Notably, *Nitrospira*, *Streptomyces*, *Pirellula*, *Bacillus*, and *Clostridium* exhibited significantly higher Eigenvector centrality values (>0.7, $P_{adj} < 0.05$), indicating their importance in the bacterial associations of bulk soils irrigated with treated AMD water or tap water (Control). Other taxa in Group 2 samples such as *Masillia*, *Sphingobium*, *Sphingomonas*, *Paenibacillus*, and *Hyphomicrobium* also exhibited higher Eigenvector centrality values (>0.7), albeit without statistical significance ($P_{adj} > 0.05$). In contrast, only *Exiguobacterium* was identified as a key taxon within the associations of AMD-contaminated soil microbial networks (Eigenvector centrality >1, $P_{\text{adj}} < 0.05$). It can be postulated that higher keystone taxa diversity and abundance in Group 2 samples positively relates to soil ecosystem function and stability, rather than being solely tied to microbial taxa adaptability in Group 1 treatment. Thus, these keystone taxa likely play a crucial role in improving and maintaining soil health under treated AMD water-irrigated agriculture. Hence, the more diversified and balanced microbial interactions observed in Group 2, coupled with the higher abundance of keystone taxa, may suggest a positive correlation with soil ecosystem function and stability in treated AMD water-irrigated agriculture.

3.7. PICRUSt2 metagenomic functional profiling in the two post hoc groups

Predictive metabolic and functional profiling of different AMD water irrigation treatments was conducted using PICRUSt2 based on the taxonomic abundance of 16S rDNA gene of soil bacterial community. The weighted nearest sequenced taxon index (NSTI) score was between 0.069 and 0.186, confirming PICRUSt2 accurate prediction capability [33].

Overall, the abundance of level 1 KEGG pathways related to metabolism, nutrient cycling, biosynthetic processes, quorum sensing and regulatory mechanisms were comparable for the two post hoc groups (Fig. 5a). Notably, Group 2 (A1Q, A2Q, AFQ, and Control) displayed higher relative pathway abundance (Fig. 5a), with metabolic pathways, biosynthesis of amino acids, two-component systems, and carbon fixation pathways being significantly enriched (Kruskal-Wallis H test, $p(\text{FDR}) < 0.05$) than in Group 1. These findings suggest that the Control and treated AMD water irrigated soils had higher global metabolic activity than untreated AMD water irrigated soils, indicating metabolic stress due to AMD pollution. Moreover, it could be speculated that the extreme metal-rich and acidic condition might inhibit some enzymatic activities within the microbial communities accounting for the subdued metabolic pathway observed in AMD polluted soils. Similar to these results, Aguinaga et al. [51] observed a shift in the abundance of microbial metabolic pathways in AMD contaminated wetland sediments, linked to the severity of the AMD pollution.

This study predicted 618 KEGG orthologs (level 3), with 42 KEGG orthologs (KO) showing differential abundance between two post-hoc groups (Fig. 5b). Interestingly, Group 1 (AMD) exhibited significant enrichment (LDA effect size >2.0) in 26 KOs, primarily associated with HM resistance/metabolism and cation/proton metabolism. Enriched KOs also included genes related to sulfur metabolism genes, such as sulfate permease (*SulP* family - K03321) and sulfur-oxidizing protein *SoxY* (K17226), along with MFS transporter/NNP family and nitrate/nitrite transporter (K02575). These results suggest that AMD pollution selects for specific tolerant bacterial communities that mediates and sustains processes of acidity and HM adaptation and SO_4^{2-} metabolism in these soils [6,51]. Consistent with the predicted KOs, LEfSe and ISA analysis identified acidophilic, HM, and sulfate-adapted microbial taxa belonging to genus *Pseudomonas, Brevundimonas* and *Ralstonia* as the indicator taxa for Group 1 samples (Fig. 2). Similarly, *Methylotenera* and *Methylobacterium*, known methylotrophic genera that utilizes methanol and other one-carbon compounds as a carbon and energy source, were also the other indicator taxa identified. These taxa encompasses several species known to play important roles in environmental processes such as acidity and metal resistance, sulfur metabolism, organic matter degradation and community interactions, key to their survival in AMD-contaminated soils [56,57]. They are recognized for their contributions to the remediation of contaminated sites such as acid mine drainage (AMD) soils. Therefore, it can be inferred that these indicator bacteria play an essential role in biogeochemical processes and the remediation of AMD-contaminated soils.

In contrast, Group 2 samples exhibited a diverse array of KOs linked to nutrient metabolism and cycling, biosynthetic processes, quorum sensing and regulatory mechanisms (Fig. 5b). Specifically, significant enrichment was observed for N (nitrogen fixation protein *NifU* and related proteins - K04488, assimilatory nitrate reductase electron transfer subunit - K00360, and nitrogen regulatory protein P–II – K04751) and sulfate metabolism (sulfate transport system permease protein - K02047, sulfate transport system substrate-binding protein - K02048, and sulfite oxidase - K00387) (Fig. 5b). Moreover, eight genes associated with HM tolerance/metabolism and cation/proton efflux systems (Na+:H+ antiporter, NhaA family - K03313, and Na+:H+ antiporter, *NhaB* family - K03314) were highly enriched in Group 2. This indicates that the divergent metabolic profile observed between Group 1 and 2, might be attributed to differential exposure to acid and metal stress. In Group 2 samples, where quicklime and fly ash treatment mitigated metal toxicity, the microbial communities require fewer mechanisms for metal and acidic tolerance and detoxification mechanisms, thereby participate

in repertoire of metabolic and biochemical processes linked to maintaining and enhancing soil health.

At taxonomic level, various taxa, including genera *Sphingomonas, Pseudolabrys*, *Arthrobacter, Rhizobium*, *Bradyrhizobium* and *Mesorhizobium*, were identified as indicator taxa (Fig. 2a). Notably, *Sphingomonas* was identified as keystone species/hub for ecological networks in Group 2 samples (Fig. 4). *Sphingomonas*, *Pseudolabrys*, and *Arthrobacter* constitute a diverse group of bacteria found in various environments, renowned for their abilities in degrading xenobiotics, nutrient recycling, plant growth promotion, bioremediation, and microbial interactions, as well as pathogen suppression [58]. In contrast, *Rhizobium*, *Bradyrhizobium* and *Mesorhizobium* are symbiotic N_2-fixing soil bacteria capable of producing various phytohormones and stimulate plant growth [6,59]. Similar to our findings, Narendrula and Nkongolo [59] observed increased abundance of *Bradyrhizobiaceae* family including the nitrogen-fixing *Bradyrhizobium* genus was more abundant in limed AMD-contaminated mining soils compared to unlimed areas. However, it should be noted that *Rhizobium radiobacter*, currently reclassified as *Agrobacterium tumefaciens*, is a known gram-negative pathogen in soils causing crown gall disease in plants. Under AMD-contaminated soils, the abundance of this bacterium is affected by the low pH and HM toxicity [60], that explains its significant enrichment in Group 2 than Group 1 samples.

In this context, it can be hypothesized that the higher microbial diversity and activity in Group 2 treated soil samples provide a greater competition against *A. tumefaciens,* subsequently reducing its abundance and infectivity to the plants. Intriguingly, despite its pathogenic nature, Jian et al. [60] reported that inoculation with *A. tumafaciens* CNWGS0286 and *Sinorhizobium melloti* promotes growth and metal accumulation in alfalfa under Cu and Zn stress. This illustrates positive microbial interactions these strains of the bacterium can have in improving soil health and crop productivity. In this study, the abundance of indicator species *Sphingobium vermicomposti* could be linked to vermiculite, vermicompost additive added to all soil samples. Nevertheless, the significant enrichment of this species in the Control and treated AMD water-irrigated soils (Group 2) than Group 1 indicate its low tolerance to acidic pH and HM toxicity. Anecdotal evidence suggests that the genus *Sphingobium* contains a small number of plant-growth promoting rhizobacteria (PGPR), with most taxa in this genus playing an important role in biodegradation and bioremediation in sediments and sandy soils [61]. This provides a clue of their potential involvement in maintaining and/or improving soil health in Group 2 samples.

The complex interactions between microbial taxa identified in this study suggest that Group 2, treated with quicklime and fly ash, harbors a more balanced and diversified microbial network. These results highlight the essential roles of these communities in biogeochemical processes, soil health, and potentially, AMD-contaminated soil remediation. Unlike previous studies focusing on individual treatments, this study uniquely investigated the combined effects of quicklime and fly ash on AMD-irrigated agriculture. Moreover, this approach provides a more holistic understanding of potential benefits or risks of the treatments for soil health and agricultural productivity by exploring the functional dynamics and specific contributions of different bacterial taxa.

4. Conclusion

In summary, this study shed light on the detrimental impact of untreated AMD water on soil bacterial richness and diversity, while highlighting the efficacy of quicklime and fly ash in mitigating acidity, reducing HM and SO_4^{2-} content, while improving soil microbiome structure, ecological networks and functional capacity. Acidophilic, HM, and sulfate-adapted microbial taxa belonging to genus *Pseudomonas, Brevundimonas* and *Ralstonia* were the key indicator taxa identified in AMD water-irrigated soils. These findings underscore the importance of pH, EC, and HMs as pivotal environmental factors shaping soil microbial communities, particularly in AMD-affected environments. In contrast, genera *Sphingomonas, Pseudolabrys*, *Arthrobacter, Rhizobium*, *Bradyrhizobium* and *Mesorhizobium*, consisting of various bacterial species renowned for their abilities in degrading xenobiotics, nutrient recycling, plant growth promotion, microbial interactions, and pathogen suppression, were identified as indicator taxa quicklime and fly ash-treated AMD water soils. Notably, the observation that quicklime and fly ash-treated soils exhibited a more diverse and balanced microbial community structure with potential benefits for soil health and productivity is a promising avenue for further exploration.

However, certain limitations in this study warrants future research: i) investigate long-term impacts of moderate residual SO_4^{2-} and HM levels in the treated AMD water on microbial succession and soil quality; ii) explore direct interactions between the identified bacterial taxa and plants in terms soil health, nutrient cycling, and plant growth; iii) acknowledge the limitations of inferring soil microbial function from in silico predictions such as PICRUSt algorithm; and iv) validation findings in real-world agricultural scenarios. Despite limitations, this study significantly advances our understanding of untreated AMD water's effects and the benefits of quicklime and fly ash treatments on soil microbial communities. Considering environmental impacts and costs of AMD pollution, this study provides valuable information for shaping policies related to mining wastewater treatment and usage, soil health, and agricultural sustainability in AMD-polluted ecosystems. Overall, the findings holds promise for unlocking water potential in drylands while promoting sustainable agriculture practices, contributing to several UN SDGs in the process.

5. Data availability statement

The raw fastq files generated from Illumina sequencing during this study has been deposited in NCBI SRA database as BioProject ID PRJNA974836 under SRA accession numbers SRX20460999 to SRX20461031. All the data analysis results obtained during this study are included in the manuscript and its Supplementary Information.

CRediT authorship contribution statement

Rabelani Munyai: Methodology, Investigation, Conceptualization. **Henry Joseph Ogola Oduor:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Formal analysis, Data curation. **Virginia Wambui Kimani:** Writing –

review & editing, Writing – original draft. **David Mxolisi Modise:** Supervision, Conceptualization.

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.heliyon.2024.e27985.

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