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Research Article

Seasons and vertical dynamics influence community composition in a flooded and abandoned mica mine

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Abstract

Artificial lakes formed from past mining activities represent unique but underexplored ecosystems that support diverse microbial communities. This study examined how seasonal variation and depth influence bacterial, archaeal, and microeukaryotic assemblages in the stratified water column of the Blackburn mine (Outaouais, Quebec, Canada). Water and biofilm samples were collected by technical divers from the surface to 52 m during spring, summer, and autumn of 2021–2022, and analysed by 16S/18S rRNA gene sequencing. Seasonal changes had little effect on physicochemical parameters but strongly shaped microbial community composition, together with depth. Archaeal taxa displayed greater stability across depths compared to bacteria and eukaryotes. Oxygen profiles defined three ecological zones: an oxic layer dominated by Actinobacteria and the methanogen Methanosarcina; a transition zone enriched in Chlorobium and methanogens such as Methanospirillum and Methanosaeta; and an anoxic layer containing sulfur-reducing (Desulfomonile and Desulfobacca), sulfur-oxidizing (Sulfuricurvum), and methane-cycling archaea. Eukaryotic communities included algae, particularly Chrysophyceae, and diverse protists. These findings suggest that microbial communities in the mine are integral to sulfur and carbon cycling, emphasizing the ecological significance of such stratified, mining-associated aquatic systems. The Blackburn mine provides valuable insight into how anthropogenic legacies shape microbial diversity and ecosystem functioning in artificial aquatic environments.

Keywords: microbial ecology; mining lake; seasonality; stratification

Introduction

Emerging from human industrial activity, mining lakes or pit mine lakes are deep, artificial aquatic ecosystems. While their presence may be notable, their environmental characteristics remain largely underexplored. Following the cessation of mining activities in times when there was no legal requirement for site restauration, tunnels and open-pit mines were flooded by groundwater and surface water, forming lakes that created aquatic environments overtime, capable of supporting diverse microbial communities (Blanchette and Lund 2016). Each pit mine lake exhibits unique conditions influenced by its geology, hydrology, contamination levels, mining history, and anthropogenic activities (Wolkersdorfer 2008). Characterizing and generalizing the biogeochemical properties of these pit mine lakes remain challenging, but they can share certain characteristics with natural lakes. Indeed, compared to other temperate lakes, the composition of aquatic communities in these artificial environments is shaped by abiotic factors, such as temperature, dissolved oxygen, pH, depth and, metal trace elements (Ostrand and Wilde 2002, Essl et al. 2015). These variables fluctuate with seasonal changes and external inputs, creating vertical gradients within the water column (Tobias-Hünefeldt et al. 2019). In stratified open-pit lakes, these gradients not only affect abiotic factors but also drive significant differentiation in microbial communities between oxic and anoxic zones (Lehours et al. 2005, Diao et al. 2018). The transition zone is often marked by major shifts in microbial community structure and biogeochemical processes (Diao et al. 2018). Aquatic microorganisms play a critical role in biogeochemical cycles, catalysing reactions that drive organic matter decomposition, nutrient cycling, and contaminant biodegradation (Wang et al. 2022). Despite their ecological significance, a comprehensive understanding of the diversity and dynamics of aquatic microbial communities across different depths and seasons in former mine pit lakes remains limited. However, the microbial community characterization in freshwater lakes has been well studied to investigate the factors controlling the microbial community structure like abiotic factors, trophic status, depth, and stratification of the lake. Many studies have observed a correlation between microbial community and environmental factors, responding to seasons variation. Microbial community structure exhibited temporal changes at the phylum and genus levels in oxic and anoxic zones (Shang et al. 2022).

Water from abandoned mines is often characterized by acidic conditions, resulting from high metal content and low organic matter concentrations, a phenomenon commonly referred to as acid mine drainage (AMD) (Chen et al. 2016, Mesa et al. 2017,

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Bonilla et al. 2018). However, some mines exhibit circumneutral pH and are classified as neutral mine drainage (NMD) (Webb and Sasowsky 1994, Jarvis et al. 2023). While NMD has received less attention than AMD due to its comparatively lower environmental impact, its conditions often evolve over time, requiring microbial communities to adapt. Furthermore, NMD environments may serve as valuable reservoirs of microorganisms with potential applications in bioremediation. Although few studies have characterized the microbial communities in pit mine lakes, existing research has primarily focused on bacterial taxa. These studies report a diverse array of bacteria, including Actinobacteria and Betaproteobacteria, commonly found in freshwater systems, as well as sulfur-metabolizing bacteria such as green sulfur bacteria (GSB) of the genus *Chlorobium* and sulfur-reducing bacteria (SRB) (Edberg et al. 2012, Kisková et al. 2019, Prepilková et al. 2023). Despite increasing research on microbial communities in pit mine lakes, the roles of archaeal and eukaryotic microorganisms remain insufficiently characterized. Blanchette and Lund (2021) investigated archaeal assemblages in an Australian pit lake and reported a dominance of the phylum *Thaumarchaeota*, which is typically more abundant in extreme environments compared to neutral lakes. Consideration of the three microbial domains (Bacteria, Archaea, and Eukaryotes) is essential to understand the complexity and interaction of microbial communities in these unique freshwater ecosystems. Over the past decade, significant progress has been made in studying the diversity and dynamics of archaeal communities in freshwater ecosystems (Casamayor et al. 2001, Pouliot et al. 2009, Auguet et al. 2011, Vissers et al. 2013). However, the influence of physicochemical and biological variables on archaeal community composition remains poorly understood, with limited data available to explain their distribution, dynamics, and ecological functions in freshwater ecosystems. Similarly, while microbial eukaryotes in lakes are relatively well-studied, few investigations have focused on their diversity in anoxic or low-oxygen environments. Notably, Lepère et al. (2016) demonstrated a high genetic diversity of microbial eukaryotes in the anoxic waters of a meromictic lake, including groups such as *Chlorophyta*, *Alveolata*, and fungi. These findings highlight the importance of studying microeukaryote in diverse aquatic environments to gain a more comprehensive understanding of ecosystem functionality.

The Blackburn mine is a former phosphate and mica mine, in operation for 60 years and abandoned in the 1940s in the Outaouais region (Québec, Canada). Today the mine is a flooded open pit, stratified, and connected to two major tunnels up to 52 m deep, that are still being mapped. As observed in aquifer ecosystems, we know subsurface microorganisms can adopt one of two lifestyles: either they float in the water column as planktonic cells or attach to substrate surfaces as sessile communities, where they can form biofilms (Marshall 2006). Both communities may constitute different functional groups of microorganisms with specific roles (Marshall 2006, Dos Santos et al. 2018). In the subsurface ecosystem, planktonic and sessile microbial communities have been shown to differ significantly in taxonomic composition and to exhibit temporal variability, although they can interact, and microorganisms can even switch from one lifestyle to another (Flynn et al. 2013, Rajala and Bomberg 2023). Studies have reported that planktonic microbial communities were more diverse than sessile communities, and the dynamics structure of biofilms could be affected by season and depth (Patel et al. 2024, Robinson et al. 2025). But only a few studies can be found on microbial diversity of planktonic and sessile communities, and even lesser have focused on pit lakes. Nevertheless, understanding these communities is fundamental to assess which microorganisms have adapted to these habitats, and subsequently their potential for cultivation and bioremediation applications.Therefore, the goals of this study were to: (1) determine the diversity and relative abundance of the planktonic and sessile microbial communities (bacteria, archaea, and eukaryote) in the Blackburn open-pit and subsurface tunnels; (2) investigate the microbial stratification through the depth gradient of the mine and through three seasons; and (3) determine the correlation between physicochemical variables and microbial communities. Here, we provide a better understanding of the diversity and dynamics of the mine microbiome. For this purpose, water and rock biofilm samples from different depths were collected over 2 years, and 16S/18S rRNA gene amplicon sequencing was applied to identify the diversity and composition of the bacterial, archaeal and eukaryote communities. These results were correlated to spatio-temporal changes in environmental parameters.

Material and methods

Site description and sample collection

Until the closing of the Blackburn mine (Quebec, Canada), in the 1940s, the water had been continuously pumped by the mining company to prevent the pit from being filled by groundwater. It is likely that several years after the mine closed, the open-pit lake of 2416.5 m^2 was formed, and the associated ecosystem was established. For this study, samples were collected manually in spring, summer, and autumn seasons (years 2021 and 2022) by the PTO Exploration team, technical divers specialized in extreme conditions (Table S1). Unfortunately, no sampling could be carried out during the winter season due to an impossible access to the mine. Eight sampling depths (0, 6, 12, 18, 24, 37, 43, and 52 m) were selected to cover a depth gradient from the surface waters of the open pit to the deeper tunnels in the mine (Fig. 1). The depth sampling strategy was designed to span the entire water column, prior to having any information on the hydrodynamics or mixing regime of the lake. Physicochemical parameters (pH, temperature, and dissolved oxygen) were measured on site using a YSI multiparameter probe (model 10 102 030, Yellow Springs, OH, USA). Although the instantaneous YSI measurements did not allow us to identify a clear oxycline during the field campaign, the absence of seasonal variation in our environmental dataset, combined with later long-term autonomous logger measurements (Pastier et al., in preparation), confirmed that Blackburn mine lake is permanently stratified. Hydrogeochemical analyses by Elisa et al. (in preparation) further delineated three stable layers: an oxic epilimnion, a metalimnion encompassing the thermocline and chemocline, and a permanently anoxic monimolimnion. Our sampling depths align with this structure, supporting the three-layer (oxic, transition, and anoxic) framework used in this study. For each water depth, samples were taken in two 1 l polyethylene bottles, previously sterilized (Nalgene®, Rochester, NY, USA), for microbial identification.

Biofilm samples were taken from rock solid surfaces for all depths, by the divers, using sterile syringes. All samples were transported under cold and dark conditions to the laboratory. Water samples for DNA analysis were filtered on 0.2 µm, 47 mm polyethersulfone filter (Sterlitech, Washington, USA) on the same day as sampling. Biofilm samples were manually filtered on 0.2 µm, 25 mm polyethersulfone filter. All filters were stored at −80°C until further processing.

Physicochemical analysis

Water samples for dissolved organic and inorganic carbon (DOC and DIC) were filtered on 0.45 µm polyethersulfone filters

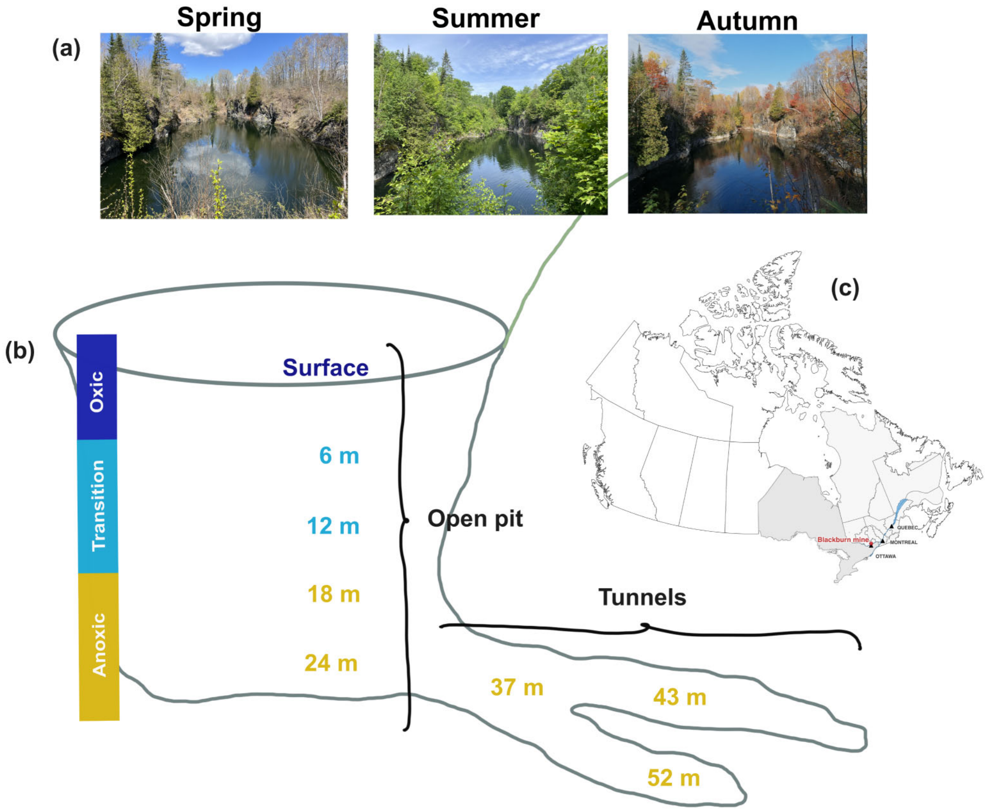

Figure 1. (a) Seasonal changes in the mine landscape. (b) Schema of the Blackburn mine with the depth gradient, from the open pit to the underground mine tunnels. (c) Localization map of the Blackburn mine in Québec. Canada (QGIS).

(Sarstedt®, Germany) in the field and analysed with an OI Analytical Aurora 1030 W TOC Analyzer (College Station, TX, USA) using a persulfate oxidation method at the GRIL-UQAM (Groupe de Recherche Interuniversitaire en Limnologie-Université du Québec à Montréal, Montreal) analytical laboratory. Nitrite (NO_2^-) was analysed with a continuous flow analyzer (OI Analytical Flow Solution 3100 ©, College Station, TX, USA) using an alkaline persulfate digestion method, coupled with a cadmium reactor, following a standard protocol. For ammonium (NHx) analyses, water samples were filtered through 0.20 µm polyethersulfone filters (Sarstedt®, Numbrecht, Germany) and analysed with a Flow Solution 3100 autosampler using a chloramine reaction with salicylate to form indophenol blue dye (EPA Method 350.1). Cations (Ca, Na, Mn, Mg, and S) were analysed into triplicate water samples collected in the Blackburn (Dives: B9, B10, B12, B13, and B14). Water samples were filtered through 0.45 µm polyethersulfone filters (Sarstedt®, Germany) into 15 ml polypropylene trace metal-free tube (VWR®). Each sample was adjusted to a final volume of 10 ml by adding 9.714 ml of mine water and 286 µl pure nitric acid (HNO3; Fisher Scientific) to achieve a final concentration of nitric acid of 2% (v/v), and they were analysed with Inductively Coupled Plasma Triple Quadrupole Mass Spectrometer (Agilent 8900 ICP-QQQ, USA) by the Environmental Analysis Laboratory of the Institute of Environmental Sciences (UQAM).

Isotopic analysis

For all samples, water stable isotopes (δ^2H and δ^{18}O) were analysed at the Research Center on the Dynamics of the Earth System laboratory (GEOTOP-UQAM). Measurements were made using a dual inlet Micromass Isoprime™ isotope ratio mass spectrometer coupled to an Aquaprep™ system for δ^{18}O and using Isotopic Water Analyzer for δ^2H. Isotopic composition are reported in delta notation (δ), calculated as $\delta = (\frac{^{R}sample}{^{R}standard} - 1) \times 1000$, where R represents the heavy to light isotope ratio. For oxygen and hydrogen isotopic analyses, 1 ml of water was pipetted in a 2 ml vial and closed with a septum cap. The samples were analysed with an LGR (Los Gatos Research) model T-LWIA-45-EP Off-Axis Integrated Cavity Output Spectroscopy (OA-ICOS, San Jose, CA, USA). Each sample was injected (1 µl) and measured 10 times. The isotopic compositions of samples were corrected using three internal reference waters (δ^{18}O = 0.2 ± 0.06‰, −13.7 ± 0.07‰ and −20.4 ± 0.10‰; δ^2H = 1.3 ± 0.3‰, −98.9 ± 1.12‰, and −155.7 ± 0.7‰; δ^{17}O = 0.03 ± 0.04‰, −7.3 ± 0.06‰, and −10.8 ± 0.06‰) calibrated on the Vienna Standard Mean Ocean Water–Standard Light Antarctic Precipitation (VSMOW-SLAP) scale (Cozannet et al. 2021). The overall analytical uncertainty (1 s) is better than ±0.1‰ for δ^{18}O, ±1.0‰ for δ^2H, and ± 0.1‰ for δ^{18}O. Values are reported in per mil units (‰) against the Vienna Standard Mean Ocean Water standard (VSMOW).

DNA extraction, 16S and 18S rRNA gene sequencing

DNA was extracted from the filters using the DNeasy power water kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All extracted DNA samples were stored at −80°C until further use. Bacterial, archaeal, and eukaryote 16S/18S rRNA gene sequencing was carried with Illumina Miseq using a MiSeq Reagent Kit v.3 (600 cycles, Illumina) at the CERMO-FC genomic platform (Centre excellence en recherche sur les maladies orphelines fondation Courtois, UQAM). The 16S and 18S rRNA genes were amplified using the Phusion Hot Start II polymerase (Thermo Scientific™), following the manufacturer's instructions. The bacterial V3–V4 region of the 16S rRNA gene was amplified using the primer pair B341F (5′-CCTACGGGAGGCAGCAG-3′) and B785R (5′-GACTACHVGGGTATCTAATCC-3′). The archaeal V3–V5 region of the 16S rRNA gene was amplified using the primer pair A340F (5′-CCCTACGGGGYGCAS-CAG-3′) and A915R (5′-GTGCTCCCCCGCCAATTCCT-3′). The Eukaryotic V5 region of the 18S rRNA was amplified using the primer pair E960F (5′-GGCTTAATTTGACTCAA- CRCG-3′) and NSR1438R (5′-GGGCATCACAGACCTGTTAT-3′) (Table S2). Negative controls ($n =$ 2) for the DNA extraction kit were done from sterilized MiliQ water filtered in same condition than the samples, were sequenced for all three domains. PCR (polymerase chain reaction) amplification negative controls were also sequenced for all three domains. The raw reads have been deposited into the National Center for Biotechnology Information (NCBI) under the SUB15518975 accession number.

Bioinformatic analyses

To identify amplicon sequence variants (ASVs), 16S/18S rRNA gene sequences were filtered, processed, and analysed using a modified DADA2 pipeline (v.1.32.0) (Callahan et al. 2016) in R v4.4.0, to manage the quality of forward and reverse reads. For bacterial sequences, forwards and reverse reads were truncated at positions 250 and 200, respectively. For eukaryote sequences, forwards and reverse reads were truncated at positions 260 and 200, respectively. For archaeal sequences, because of the low quality of the reverse reads, only forward reads were kept and truncated at position 260. Taxonomy was assigned with the DADA2 package using the assign taxonomy function and the SILVA SSU database (v.138.1 for bacterial and archaeal sequences, v.132 for eukaryotes sequences). Nonclassified archaeal and eukaryotic sequences were further classified using a personal database for the Archaea based on Liu et al. (2018) and Zhou et al. (2018) and the PR2 SSU database (v.5.0.0) for the Eukaryotes. ASVs in negative controls (extraction kits and PCR) were removed from all output sequences, with the decontam R package (v.1.24.0, Davis et al. 2018) using the is Contaminant function. Then, bacterial, archaeal, and eukaryotes community tables were rarified to 3457, 6343, and 2012 ASV, respectively. Only samples containing ASVs assigned to the three domains were kept (Table S3).

Statistical analysis

Statistical analyses were performed with Rstudio Server 2022.07.2, and for all tests, differences were considered statistically significant if P-value $<$ .05. To investigate the microbial stratification through the depth gradient of the mine and the seasonality we used a Bray–Curtis matrix related to taxonomy, including the relative abundance taxa for each depth, lifestyle community (sessile or planktonic), and seasonality, using the Phyloseq package (v.1.48.0, McMurdie and Holmes 2013). The relationship between environmental factors and depth or seasonality were determined through Spearman correlation analyses using the cor.test function (v.3.5.1) of the Stats package. The comparison of the environmental variables between the three seasons and open pit zones was performed using the nonparametric Kruskal–Wallis (KW) test, implemented with the kruskal.test function from the Stats package (v 3.6.2). When the KW test yielded a P- value $<$ .05, a *post hoc* pairwise comparisons were conducted using a Dunn test with the dunnTest function from the FSA package (v 0.9.6).

To visualize dissimilarities of bacterial, archaeal, or eukaryotes communities through the depth gradient, the seasonality and between lifestyles (β-diversity), a principal coordinates analysis (PCoA) was performed on the rarefied relative abundance data using a Bray–Curtis dissimilarity matrix with the Vegan package and the vegdist funstion (v.2.6.6.1; Oksanen et al. 2019). Variation partitioning analysis was done based on a PERMANOVA to test whether community composition varied significantly depending on lifestyles, depth, pit zones (oxic, transition, and anoxic), and seasonality, using the adonis2 function of the vegan package. We used distance-based redundancy analysis (db-RDA) to determine which environmental variables had a significant impact on community composition. Spearman correlation analyses were performed using the cor.test function (Stats package, v.3.5.1) to evaluate the relationship between environmental parameters (including depth). When two parameters exhibited a strong positive or negative correlation ($|\rho| > 0.8$), only one of them was retained for further redundancy analysis (Fig. S1). The db-RDA was applied to the distance matrix and the set of explanatory variables using the capscale function of the vegan package, and significance of explanatory variables (DIC, NO_2, NH_X, and seasonality) was assessed with the ANOVA function in R. The unique and shared contributions of each significant variable to community composition was determined using variance partitioning with the varpart function of the vegan package. To determine microbial taxa explaining the differences between sample groups, linear discriminant analysis effect size (LEfSe) was applied at the genus level on rarefied data (LDA threshold > 2, $P < .05$) with the run_lefse function of microbiomMarker package (v 1.10.0). The relative abundance (%) of each taxon identified as discriminant was calculated for its respective zone. Then, to characterize the microbial communities interactions between the surface water and the groundwater, we performed fast expectation-maximization microbial source tracking (FEAST) using the Feast package (v.1.12.0, Su et al. 2021).This method estimated the contribution (proportion) of communities of one depth as a source to another as a sink (Shenhav et al. 2019).

Results

Environmental characteristics of the mine: season and depth effects

The pH in the Blackburn mine water was almost a uniform neutral pH (7.02 ±0.6) over the depth gradient and the three covered seasons. The mine lake was characterized by vertical gradients in temperature, DIC, NH_X, and dissolved O_2 (DO) (Fig. 2, Fig. S2). Surface waters of the open pit were characterized by high DO and low DIC concentrations, whereas the anoxic deep waters were characterized by low (6°C) temperature, and high concentrations of NH_X and DIC.

Based on these results and data generated by Pastier et al. (personal communication; Fig. S2), we determined that the water column of the mine open-pit could be separated in three zones: the oxic zone (0 m), the transition zone (6–12 m), including the thermocline and chemocline, and the anoxic zone (12–52 m) also comprising the underground tunnels within the mine itself.

We observed that DIC and NH_X were significantly and positively correlated with the depth gradient, unlike DOC and temperature which were negatively correlated (Fig. S3). Overall, most environmental parameters in the mine remained stable both seasonally and across depth profiles, unless NH^+ consistently exhibited a strong vertical gradient in all seasons and depths (Figs S4 and S5). Seasonality had no significant effect on these environmental pa-

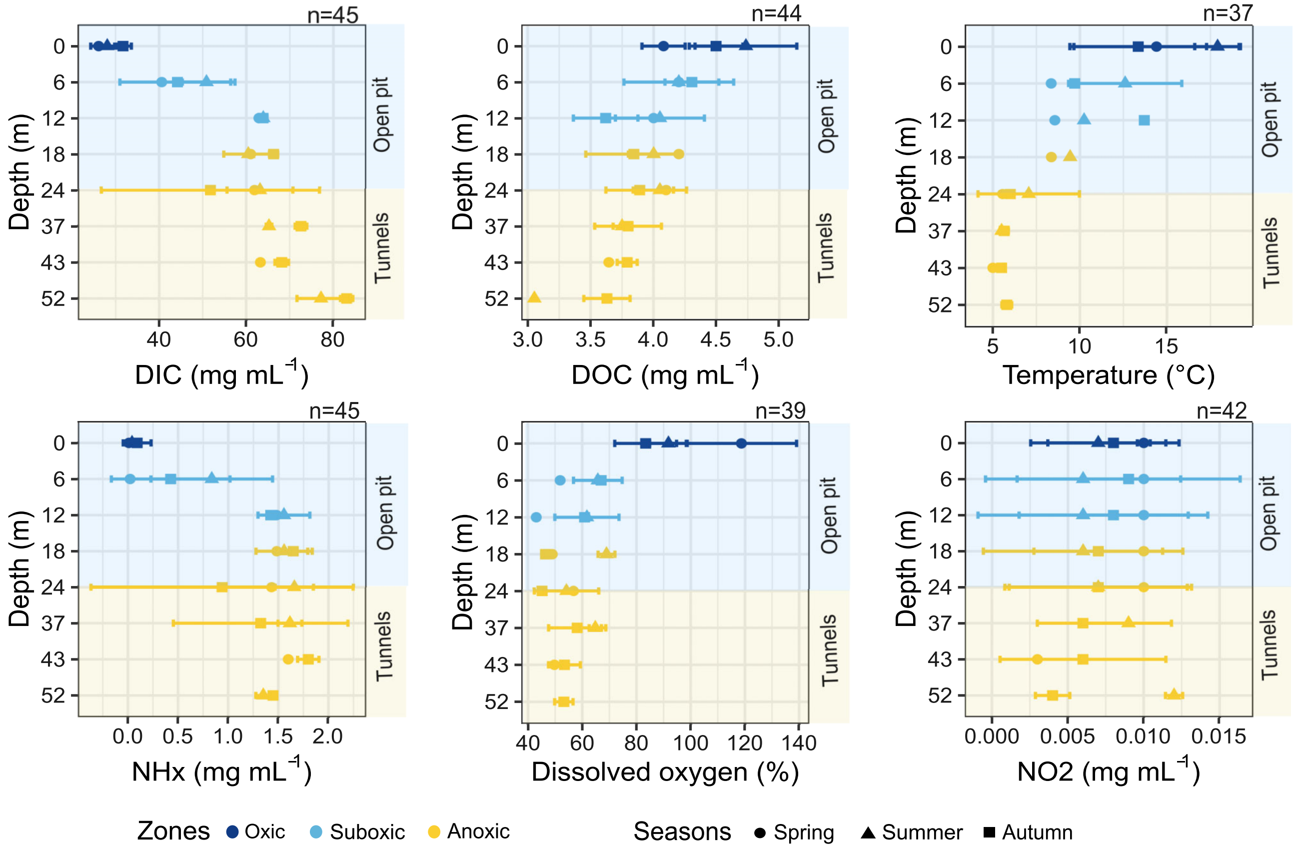

Figure 2. Physicochemical profile of mine water along the depth gradient (0 m $n = 9$; 6 m $n = 6$; 12 m $n = 5$; 18 m $n = 6$; 24 m $n = 5$; 37 m $n = 6$; 43 m $n = 4$; and 52 m $n = 4$) and the season (spring $n = 7$; summer $n = 17$; and autumn $n = 21$). DIC, dissolved inorganic carbon; DOC, dissolved organic carbon; NH_4, ammonium; and NO_2, nitrite.

rameters (Table S4). However, significant differences in environmental variables were observed between the three zones. All environmental variables differed significantly different between the oxic and anoxic zones. Between the anoxic and transition zones, only DIC, temperature, and NH_X showed significant differences (Table S5).

The water sources of the Blackburn mine lake were investigated using the water isotopic composition. The mine water data deviated from the Global Meteoric Water Line (GMWL), and most of it was located above the GMWL and along the line with a slope of 3.56 ($\delta^2H = 3.56 \times \delta^{18}O - 37.057$; $R^2 = 0.94$) (Fig. S6). Water from the underground tunnels displayed $\delta^{18}O$ and δ^2H values of −10.05±0.83‰ and −72.35±2.9‰, respectively, and was not affected by seasons, unlike the surface water. Open pit water sources were mixed between groundwater and surface water. Surface water from the spring season displayed $\delta^{18}O$ and δ^2H values (−10.65±0.9‰ and −76.27±3.2‰, respectively) that were lower than for the two other seasons (−10.04±0.8‰ and −72.42±2.9‰, respectively) indicating a higher contribution from isotopically light water from the snowmelt (Fig. S6).

Microbial source tracking along the depth gradient of the mine

We used FEAST to characterize the potential flows of water from the surface to the deeper tunnels, or *vice versa*, which could transfer microbial cells from one depth to the other (Fig. 3). For each depth, we used the planktonic and sessile communities from the previous depths as potential sources. The FEAST suggested transfers between communities from all three domains, from the surface layers to the deeper layers, especially from surface to 24 m deep, and inversely (in the open pit).

From the surficial to deeper layers, we observed a similar contribution for bacterial and archaeal communities. The bacterial planktonic community in the surface does not seem to contribute to the formation of the deeper layer communities. At 37 m deep, the sources of the bacterial planktonic communities were mostly from the 18 m layer, while for archaeal and eukaryote communities from all superior layers contributed. At 52 m deep, the sources of the planktonic and sessile communities were diversified for the three domains. Bacterial and archaeal communities showed similar patterns, with communities from 52 m depth contributing most from opposite lifestyles. In contrast, eukaryote planktonic communities were primarily influenced by communities from 18 m deep, while sessile communities were influenced by 37 m deep communities. Then, at 24-m deep, the planktonic and sessile eukaryote communities were enriched by planktonic communities from 18 m.

From the deeper to surficial layer, overall, we observed a depletion of the contribution of archaeal and bacterial communities from the inferior layers. At 24 m deep, the sources of planktonic or sessile communities in the rock were mostly from the 37 m layer, except for the eukaryote sessile communities that 52 m sources. The sources of planktonic communities in the surficial layers were more diverse for the eukaryote communities, with 52 m layers as sources, than the bacterial communities with a large contribution from 6 m layers.

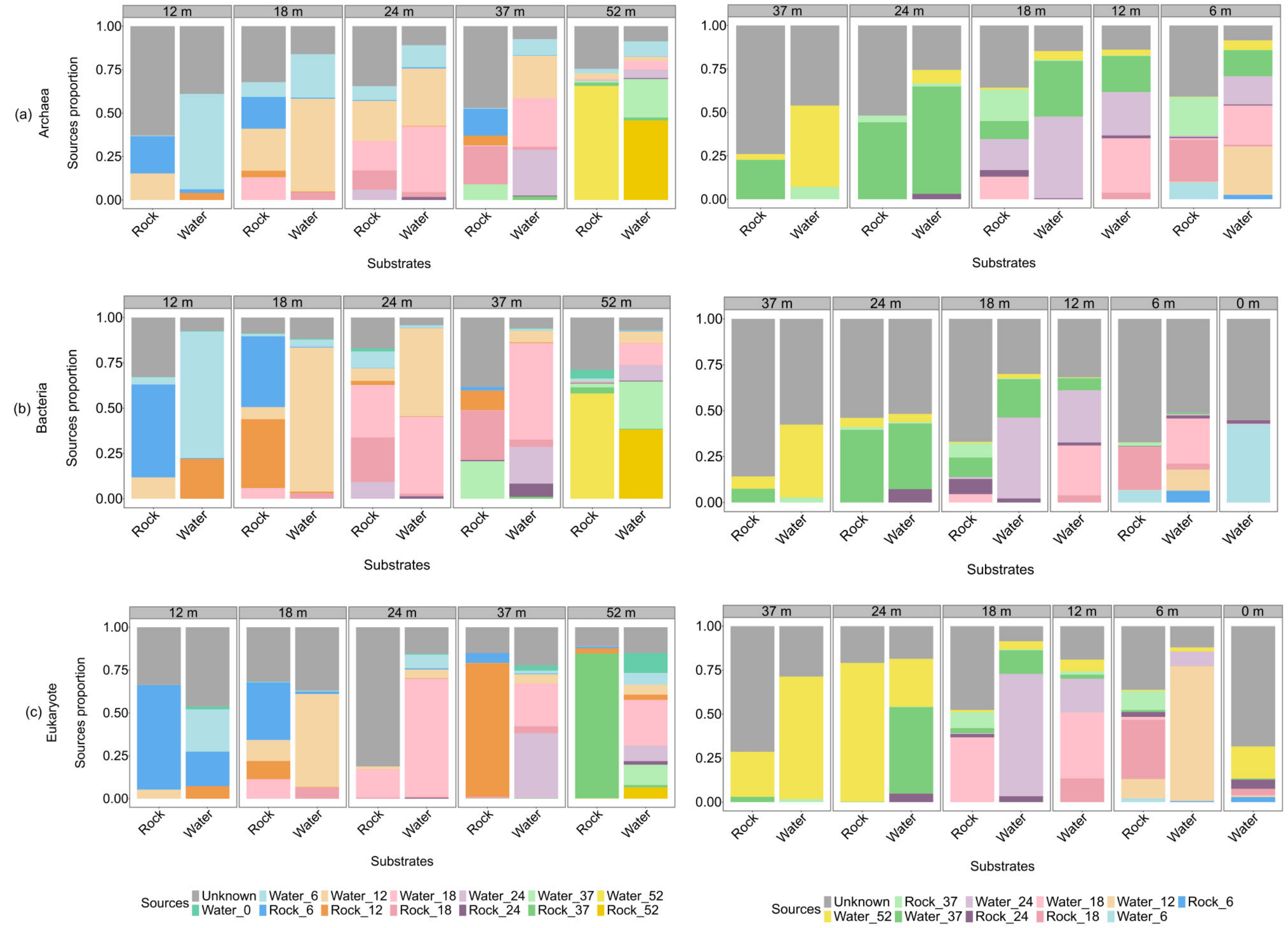

Figure 3. Microbial source tracking estimating the contribution of planktonic and sessile archaeal (a), bacterial (b), and eukaryote (c) communities from surficial into the deeper layers communities, and from the deeper into the surficial layers across the depth gradient and in the different substrates. Each color indicates a source corresponding to a substrate at a specific depth. Unknown sources are indicated in gray.

Influence of lifestyles on microbial community composition

The PCoA graph demonstrated a separation of communities following lifestyle (planktonic/sessile) for the archaeal, bacterial, and eukaryote communities (Fig. 4). PERMANOVA confirmed that the lifestyle adopted by the microbial communities was a significant variable, explaining 11%, 12.9%, and 13.3% of the community variance for each domain (Table S6). The results of the lefse analysis comparing the lifestyles of bacterial communities, all depths combined, showed that the most significant explanatory planktonic genera were *Desulfomonile* (12.9% relative abundance), unclassified (*unc.*) *Arcobacteraceae* (10.3%), *Polynucleobacter* (5.9%), *Desulfobacca* (4.2%), *Anaerovorax* (0.9%), and *WCHB1-32* Prolixibacteraceae *(0.6%)* genera (Fig. S5). For the sessile bacterial communities, the lefse analysis demonstrated that unc. OPB41 Anaerosomatales (<0.1%), *Actinotalea* (1.6%), unc. *Thermodesulfovibrionia* (1.1%), *Syntrophus* (1.3%), unc. Sva0485 Proteobacteria (1.2%), and unc. *Desulfobacterota* (0.3%) were the most significant genera (Fig. S7).

For archaeal communities, the discriminant genera were also the most relatively abundant in the two communities. For the planktonic archaeal communities, discriminant genera belonged to DPANN as unc. AAA0011-D5 Nanoarchaeota (31.4%), and unc. CG1-02-32–21 Micrachaeaota (2.3%) (Fig. S7). Genera belonging to the Woesearchaeales phylum, such as unc. *GW2011-GWC1_47_15* (14.6%), and unc. Woesearchaeales (14.6%) were also discriminant, as well as *Methanosarcina* (2.5%). The unclassified archaea represented 1.7% of the relative abundance of the planktonic community and was a discriminant group. Discriminant sessile archaea were mostly methanogens: *Methanoregula* (20.7%), unc. Methanomassiliicoccales (3.2%), unc. Methanomassiliicoccaceae (1.8%), unc. Methanomicrobiales (1.4%); but also, unc. Bathyarchaeia (14%), and marine Benthic groupe D (5.3%) (Fig. S7).

For the planktonic eukaryote community, the unc. Eukaryota were discriminant and represented 11.4% of the relative abundance of the community (Fig. S7). Genera belonging to the Chrysophyceae class were discriminant too, as well as *Uroglenopsis* (5.8%), unc. Chrysophyceae (3.8%), and *Dinobryon* (2.5%). Half of the eukaryote sessile community belonged to *Apocalathium* (50.1%), a dinoflagellate. The other less abundant discriminant genera were *Chytriodinium* (2.3%), Chrysophyceae clade D (1.7%), Protaspa-lineage (0.9%), *Chrysosphaerella* (2%), and *Aphamonas* (0.6%). Then, we were interested in understanding separately the influence of seasons and depth on the planktonic and sessile microbial communities.

Bacterial community composition and effects of season and depth

In this study, the main bacterial phyla in the open pit and tunnels of the Blackburn mine were Bacteroidota (31.5%), Desul-

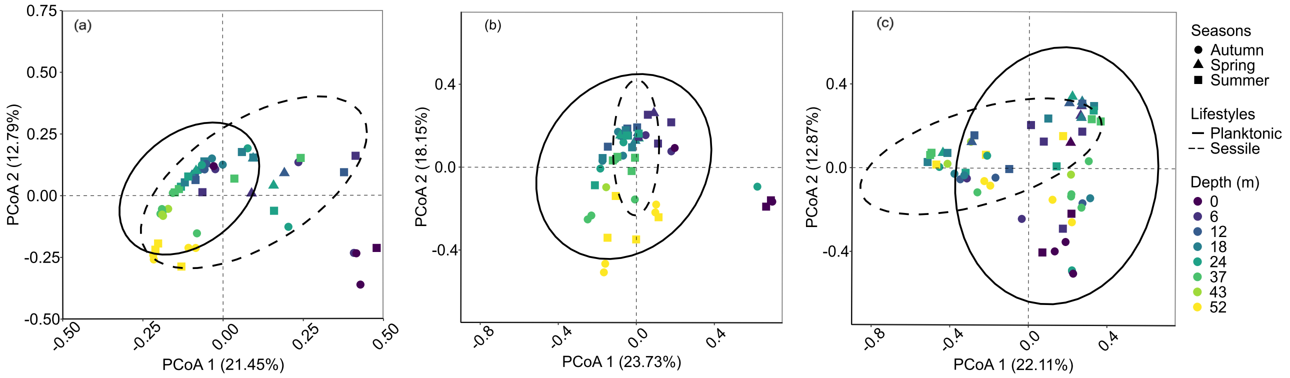

Figure 4. PCoA ordination of archaeal (a), bacterial (b), and eukaryote (c) community variance based on a Bray–Curtis dissimilarity matrix.

fobacterota (20.5%), Proteobacteria (19.3%), and Actinobacteriota (10.6%) (Fig. S8). The planktonic bacterial phyla were globally similar through the depth gradient and seasonality. Some phyla seemed to be characteristic of certain depths, like Actinobacteriota phylum at the surface and 6 m deep (Fig. S9). The SAR324 phyla was exclusively present in autumn at 37 and 43 m deep, and the Dependentiae phylum was only found at 52 m deep. For the sessile bacterial phyla, Acidobacteriota and Mixococcota are relatively more abundant at 6 m in summer and autumn, or only in summer, respectively (Fig. S9).

The PCoA based on Bray–Curtis dissimilarities revealed clusters following the depth gradient along the second axis, which explained 18.15% of the variation in the composition of bacterial communities (Fig. 4). PERMANOVA confirmed that depth was a significant variable on the microbial structure, explaining 23.4% and 28.8% of the variance for the planktonic and sessile communities, respectively (Table S7). However, seasons significantly affected only the variance of the sessile bacterial community structure, accounting for 16.8% of the observed variance, while planktonic communities remained unaffected (Table S7). Furthermore, PERMANOVA confirmed that the structure of the communities was significantly different between the three zones of the mine (oxic, transition, and anoxic), for planktonic and sessile bacteria (Table S8). Autumnal sessile communities were significantly distinct of spring and summer communities (Table S9). The db-RDA revealed that DIC, NH^+, and seasons significantly influenced both planktonic and sessile bacterial communities (Table S10). The ordination plot indicated that bacterial community composition from 0 to 12 m depth, particularly the sessile fraction was primarily shaped by summer conditions, while communities at 52 m depth were more strongly associated with autumn samples (Fig. 5).

The results of the lefse analysis comparing the different mine's zones showed that the most significant explanatory genera in the oxic zone (0–6 m) were *HgCl clade* (21.1%), CL500−29 marine group (12.8%), *Limnohabitants* (6.6%), unc. *Rhodoferax* (2.4%), unc. *Sporichthyaceae* (4.1%), and *Candidatus (cand.) Planktophila* (2.6%) genera (Fig. S11). In the transition zone (6–12 m), *Chlorobium* was discriminant and at a high relative abundance (35.2%). Other genera were discriminating in this zone, such as *Methylobacter* (1.9%) and unc. *Arcobacteraceae* (10.8%). Unc. *Lentimicrobiaceae*, *Desulfatirhabdium*, and *Thiodictyon* were detected as discriminators, but their relative abundance was lower (3.4%, 1.1% and 2.0%, respectively). In the anoxic zone, *Desulfomonile* (13.1%), *Desulfobacca* (4.5%), *Sulfuricurvum* (3.4%), *Syntrophus* (1.4%), *and Actinotalea* (1.1%) were the most discriminant taxa (Fig. S11).

Archaeal community composition and effects of season and depth

The main archaeal phyla in all samples of the mine water column were Nanoarchaeota (53.5%), Halobacterota (25.6%), Thermoplasmatota (5.9%), and Crenarchaeota (7.2%) (Fig. S8). The dominant archaeal phyla and genera were globally similar in terms of the depth gradient, lifestyles, and seasonality (Figs S9 and S10). Indeed, four genera were mostly dominant in all depths and seasons: unc_SCGCAAA011-D5 (23.3%), *Methanoregula* (21.4%), unc. *Woeserachaeales* (12.5%), and unc. *GW2011_GWC1_47_15* (10.9%) (Fig. S10).

The PCoA based on Bray–Curtis dissimilarities revealed a gradient of depth along the first axis, which explained 21.45% of the variation in the composition of archaea (Fig. 4). PERMANOVA confirmed that the depth gradient was a significant variable on the planktonic and sessile archaeal structure, explaining 19% and 28.7% of the variance, respectively (Table S7). As for bacteria, only sessile communities were affected by seasons, explaining 12% of the variation of the structure of sessile archaeal communities. PERMANOVA results also indicated a significant interaction between depth or mine zones and season. As regards to the stratification zones of the mine, PERMANOVA confirmed that the structure of the communities was significantly different between the three zones, for planktonic and sessile archaea (Table S8). No significant values were found for comparison of community composition between seasons (Table S9). The db-RDA analysis showed that the DIC variable was the only one significantly influencing the community structure (Table S10), affecting the structure of deeper archaeal communities (Fig. 5).

In the oxic zone, the lEfSE analysis showed that *Methanosarcina* (13.8%), *unc. CG1−02−57−44* (1.4%), *Methanobacterium* (3.8%), *Methanobrevibacter* (0.78%), *Rice Cluster I* (0.4%), *unc. Methanocellaceae* (0.4%), and were the most significant discriminating taxa (Fig. S11). For the transition zone, *Methanosaeta* (2.3%), *Methanomicrobiales* (1.4%), *unc AR15* (0.3%), *and unc. Thermoplasmata* (0.13%) were significantly discriminating. Then, for the anoxic zone, *Marine Benthic Group D/DHVEG−1* (3%), *unc. Methanomassiliicoccales* (2%), *unc. CG1−02−32−21* (2.1%), *cand. Methanoperedens* (1.8%), and unc. *Methanofastidiosales* (0.8%) were significantly discriminating (Fig. S11).

Eukaryote community composition and effects of season and depth

Globally, the two major eukaryotes phyla were Dinoflagellata (36.6%) and Ochrophyta (28.3%) followed by unc. Eukaryota (9.2%)

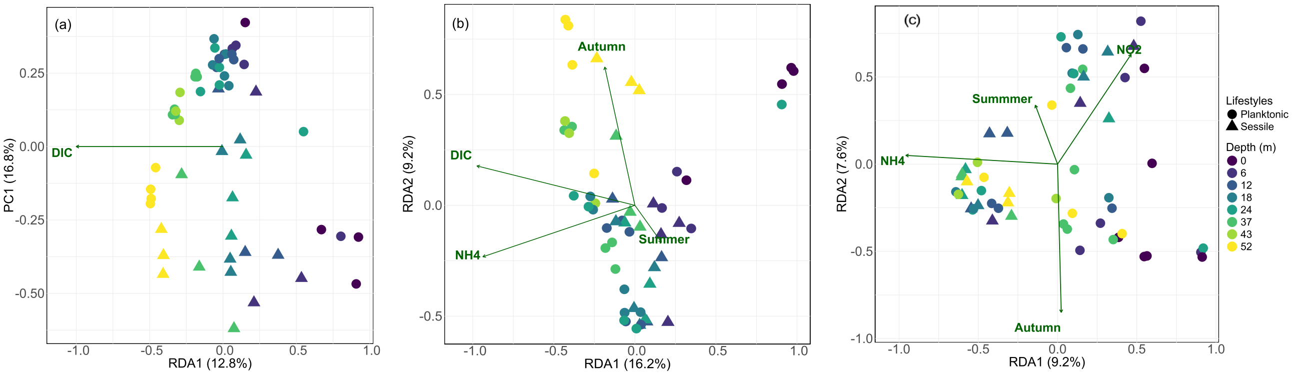

Figure 5. Visualization of db-RDA between archaeal (a), bacterial (b), and eukaryote (c) community and significant environmental factors.

and Cercozoa (6.3%) (Fig. S8). At the genus level, we observed much more variability between seasons and dives than for bacterial and archaeal populations (Fig. S9). Overall, for both lifestyles, *Apocalathium* (27.3%), *Chrysophyceae* (6%), and *Mallomonas* (5.1%) were taxa most abundant in the mine.

The PCoA based on Bray–Curtis dissimilarities revealed clustering of samples based on the depth gradient which could explain 22.11% of the variation in the composition of eukaryote (Fig. 4). PERMANOVA confirmed that the depth was a significant variable on the planktonic and sessile microbial structure, explaining 15.9% and 26.8% of the variance, respectively (Table S7). Seasons significantly explained 19.7% and 16.1% of the variance of planktonic and sessile eukaryote communities. As regards to the stratification zones of the mine, PERMANOVA confirmed that the structure of the communities was significantly different between the three zones, only for planktonic eukaryote communities (Table S8). The three seasons significantly affected the structure of planktonic communities unlike sessile communities, where we observed that the communities found in autumn were significantly different from those in spring and summer (Table S9). The db-RDA analysis showed that NH^+ concentrations and seasonal variation significantly influenced eukaryotic community composition (Table S10). According to the ordination plot, NO_2^- concentration explained the variation observed in some surface communities, while NH^+ was shaping the composition of sessile eukaryotic communities. Summer and autumn conditions explained the variation of distinct communities (Fig. 5).

In the oxic zone, the lefse analysis showed that many unc. Chrysophyceae (11.9%), unc. Ochrophyta (5%), unc. Maxillopoda (4.7%), *Pseudopedinella* (3.9%), and unc. Colpodellida (5.4%) were significantly abundant (Fig. S11). In the transition zone, the most significant explanatory genera were not the most generally abundant: *Mallomonas* (7%), *Cryptomonas* (3%), *Borghiella* (2.3%), and *Bicosoeca* (1.6%). *Haptoglossa* and *Paraphysomonas* were also two significant genera, but they had a relative abundant inferior to 0.1%. In the anoxic zone, lefse analysis showed that discriminating taxa were *Apocalathium* (33.9%), *Chrysoshaerella* (1.6%), *Spumella* (1.7%), *Mastigamoeba* (1%), and unc. *Peronosporales* (2.1%; Fig. S11).

Discussion

The microorganisms found in the Blackburn mine probably came from several sources, such as the groundwater that flooded the underground tunnels and open pit overtime, the surface water (rain or snow melt), or microorganisms living in or on the rocks and soils composing the mine and open pit. The inflow of ground- and surface-water formed an artificial stratified lake in the open pit separating this artificial aquatic ecosystem into three zones which we named: oxic, transition, and anoxic zones. We explored here the observed diversity and structure of microbial communities belonging to all three domains of life found in open pit and underground tunnel water and biofilms. Then we assessed which environmental variable influenced the microbial community structure, be it seasons, the water depth gradient, or the geochemical properties of the water in the tunnels and open pit.

How lifestyles affected the microbial communities in the mine

Distinct differences were observed between planktonic and sessile microbial communities within the mine environment. Similar patterns have been reported in other aquatic ecosystems. For example, in the Forsyth mine, Lhoste et al. (2023) documented comparable contrasts, while in Poyong Lake (China), Ma et al. (2023) reported clear taxonomic divergence between particle attached and free-living bacterial communities. In marine systems, Briand and Coclet (2021) highlighted strong taxonomic differentiation between biofilm-associated and planktonic assemblages, with some taxa exclusively associated with biofilms.

Taxonomic analysis further revealed a clear lifestyle-based partitioning: the planktonic community was dominated by *Desulfomonile* and unc. *Arcobacteraceae*, while sessile communities were enriched in *Actinotalea* and *Syntrophus*. Notably, members of the phylum *Acidobacteriota* were exclusively detected in sessile samples, suggesting niche specialization among attached microbial populations. This observation aligns with Robinson et al. (2025), who found *Acidobacteria* exclusively in biofilms. *Acidobacteria* is known to negatively correlate with pH, with optimal growth around 5.5 (Jones et al. 2009). Despite the neutral pH of the mine pit lake, its presence in biofilms suggests a preference for protective microhabitats, such as biofilms over the planktonic phase. They support the influence of seasonal dynamics on microbial community structure. These findings highlight the importance of examining both planktonic and sessile lifestyles to fully understand microbial community dynamics, few studies have directly compared microbial lifestyles (sessile vs. planktonic) while incorporating a seasonal perspective, highlighting a critical knowledge gap (Berdjeb et al. 2013, Zwirglmaier et al. 2015, Ferrera et al. 2024, Robinson et al. 2025). Research on sessile microbial communities has primarily focused on riverine and marine ecosystems, particularly on algal and bacterial components (Zhang et al. 2021, Gautam et al. 2022) while sessile archaeal communities and those in lentic environments such as mine pit lakes remain comparatively underexplored. Most archaeal biofilm studies have been carried out in laboratory or in AMD environments (van Wolferen et al. 2018). In the Blackburn mine, several discriminant and abundant archaeal genera belong to methanogens, which are well-documented in anaerobic biofilms, such as marine biofilms (Zhang et al. 2003) and human microbiome (Bang et al. 2014). Regarding the eukaryotes, previous studies have highlighted that mine biofilms host diverse taxa. Zirnstein et al. (2012) and Lhoste et al. (2023) documented a broader diversity of eukaryotes including ciliates, flagellates, amoebae, fungi, stramenopiles, rotifers in uranium and iron mine biofilms. In contrast, biofilms from the Blackburn mine were dominated by dinoflagellates along the depth gradient, particularly *Apocalathium*, a genus that remains largely understudied. Dinoflagellates show marked genomic, ecological, and metabolic differences between sessile and planktonic forms (Sitong Lin et al. 2024). Planktonic populations generally maintain higher genetic diversity, whereas biofilm-associated growth can enhance productivity (Yue et al. 2025, Tsirigoti 2020). Although coral symbioses are well documented, much less is known about dinoflagellates living freely in the water column or forming biofilms outside host organisms. The genus *Apocalathium* can form resistant resting cysts that can sediment and contribute to their long-term survival (Hinners 2017). Despite these observations, microeukaryotic biofilm communities remain poorly characterized, and their ecological roles in subsurface aquatic systems are still unclear.

Origin of water and seasonal variation influence on environmental stability and microbial community composition in the open pit and mine tunnels

Isotopic analyses of water (δ^{18}O and δ^{2}H) along the depth gradient revealed that surface waters were seasonally influenced, particularly in spring, when snowmelt contributed to lower isotopic values. In contrast, water from underground tunnels remained isotopically stable, originating from groundwater. The transition zone, enriched in sulfur, likely limits vertical exchange, reinforcing the lake's stratification. Water in the open pit zone (0–24 m) appeared to originate from a mixture of groundwater and surface water. This mixing zone corresponds to the region just above the tunnel entrance, where microbial exchange is most dynamic. However, stable isotope analyses alone are not sufficient to fully characterize water flow within the mine and should be complemented by additional hydrodynamic approaches. However, stable isotope analyses alone are not sufficient to fully characterize water flow within the mine and should be complemented by additional hydrodynamic approaches.

FEAST analysis confirmed both vertical and horizontal microbial fluxes between the water column and biofilms. For the three domains of life, results suggest no detectable microbial exchange between the oxic zone (surface) and the lower transition zone (12 m), indicating limited water circulation across these stratified layers. However, due to the FEAST method limitations, potential microbial exchange between the surface and 6 m depth could not be assessed. In contrast, a substantial overlap in microbial communities between 12 m and 18 m, and between 18 and 24 m (e.g. the anoxic zone of the open pit), implied an active flow or vertical transport in these layers, as seen in meromictic systems like Fayetteville Green Lake (Block et al. 2021). In the tunnels, microbial communities proportion from the open pit, except at the surface, could be attributed to sedimentation processes, as previously described in similar stratified aquatic environments (Phillips et al. 2021). However, a significant proportion of microbial communities remained unclassified, indicating the presence of unknown or poorly characterized sources. Potential contributors may include soil, vegetation, precipitation, or snowmelt.

Although microbial source tracking and stable isotopes provide complementary perspectives on water changes, they represent only part of the hydrodynamic picture. More detailed assessments of flow direction or velocity would require additional hydrological tools. Furthermore, microbial source tracking has important limitations, since planktonic microorganisms are largely transported passively by water flow, and DNA-based detection reflects their presence rather than their activity or absolute abundance. These factors can influence the reliability of source tracking results. Even in the absence of such datasets, the patterns observed are consistent with the isotopic structure of the mining lake.

Our results indicated that seasonal variation had minimal influence on key environmental parameters, such as dissolved oxygen, DIC, DOC, NH^{+}, and pH, as well as on the lake's stratification. Only surface temperature showed seasonal fluctuations. Physical and chemical stability are characteristics of meromictic lakes, where persistent stratification separates oxic and anoxic layers via a transition chemocline. This chemocline acts as a barrier to vertical mixing, creating sharp gradients in nutrients, dissolved gases, and temperature (Zadereev et al. 2017). Such systems offer a unique opportunity to study microbial communities adapted to distinct ecological niches.

The lack of seasonal variation in our study is intriguing given the widespread influence of seasonality on microbial ecosystems. Despite the absence of measurable seasonal effects on environmental parameters, seasonality did impact the microbial communities in the Blackburn mine, as similarly observed by David et al. (2021). Seasonal influences in the Blackburn mine varied by domain and lifestyle: while both planktonic and sessile eukaryotes responded to seasonal changes, only sessile bacterial and archaeal communities did so. This absence of seasonality in planktonic prokaryotes is surprising, as numerous studies have shown temporal variability in these groups (Berdjeb et al. 2013). However, Berdjeb et al. (2013) also noted a lack of seasonal reproducibility among planktonic archaea in Lake Annecy and Lake Bourget, suggesting that the response of planktonic microbial communities to seasonality may vary depending on the ecosystem and environmental context. In our case, the limited seasonal signal could be explained by the presence of a persistent transition layer, which may act as a physical and chemical barrier limiting vertical mixing and, consequently, the exposure of planktonic communities to surface-driven seasonal variations. For the eukaryotes, the seasonal variation corroborates with other findings. In the meromictic lake Alatsee, where the protist community in the oxic mixolimnion mainly exhibited structural changes over the seasons while in the oxic–anoxic interface, the seasonal variation was less pronounced, unlike in the anoxic minomolimnion where the protist community was stable over the season (Oikonomou et al. 2015).

Furthermore, our findings suggest that not only seasonality but also depth significantly influenced both environmental parameters and microbial community structure. Depth-related gradients, such as oxygen, light, and nutrient availability, likely interact with seasonal dynamics to shape the composition and function of microbial communities (Diao et al. 2018, Zhu et al. 2019, Alegría-Gómez et al. 2023). Therefore, the subsequent paragraphs will discuss the effect of depth on microbial communities, following the three zones that were determined in this study (oxic, transition, and anoxic).

Presence of a basal community regardless of depth and seasons

A basal microbial community, largely unaffected by depth or season, was observed in the mine. At the genus level, four archaeal genera demonstrated similar relative abundances within the mine. Among these, *Methanoregula* was particularly present across the depth gradient. This hydrogenotrophic genus, which utilizes H_2/CO_2 for methane production, is a dominant methanogen in freshwater lakes (Borrel et al. 2011). Methanogenesis, a widely occurring and active process in freshwater systems, depends on several factors, including organic carbon availability, primary production, and the methanogen community structure (Thauer et al. 2008). Other nanoarchaeota taxa, such as SCGC AAA011-D5, Woesearchaeales and GW2011 showed consistent presence and abundance in the mine, which was previously observed in low-energy ecosystems such as groundwater and an abandoned mine (Lhoste et al. 2023, Patel et al. 2024) suggesting potential metabolic versatility. These findings highlight a core microbial assemblage in a legacy-impacted environment, allowing a better understanding of the microbial resilience and biogeochemical cycling in artificial lakes.

Among the bacterial taxa, the genus *Polynucleobacter* was consistently detected throughout the mine. As a cosmopolitan and ubiquitous genus in freshwater ecosystems, its resilience to environmental variability explains its ecological adaptability (Jezbera et al. 2006, Newton et al. 2011, Jezbera et al. 2012, Nuy et al. 2020). In contrast, for the eukaryote communities, no permanent taxa are observed along the mine gradient. Eukaryotic communities appeared to be more sensitive to physicochemical variability, suggesting that their composition is more strongly influenced by environmental factors than bacterial or archaeal communities (Oikonomou et al. 2015, Blanchette et al. 2020). Surprisingly, the Ciliophora class did not dominate the planktonic protists in the mine. This is not consistent with other findings in freshwater ecosystems (Šlapeta et al. 2005, Stock et al. 2009, Oikonomou et al. 2015). Furthermore, a top-down effect could eliminate ciliates by predation pressure from zooplankton. Indeed, daphnia, chaoborus larvae are abundant in the surficial layers of the mine. Complementary studies are realizing by Gonnet et al. (in preparation) about how diel, intraseasonal, and depth-related abiotic variations influenced the basal microbial communities acting as dietary resource and the dietary niche of a keystone grazer, *Daphnia pulicaria*. These findings highlight the differential responses of microbial domains to environmental conditions and suggest that eukaryotic taxa are more dynamic and less stable in stratified aquatic systems.

Microbial community structure in the oxic zone

The oxic zone (0–6 m) at the surface of the Blackburn mine open pit was characterized by the presence of dissolved oxygen, light penetration, and temperature fluctuations ranging from 8.5°C to 18.8°C. The microbial taxa identified in this zone are commonly found in freshwater ecosystems, reflecting their ecological versatility and adaptability to these environmental conditions.

Seasonal variation, particularly during summer, appeared to influence microbial community structure. Elevated temperatures and extended photoperiods during this period were associated with shifts in bacterial communities, consistent with previous findings that temperature is a key driver of bacterial community dynamics (Adams et al. 2010). In contrast, nitrite concentrations seemed to play a more prominent role in shaping eukaryotic community composition. The presence of nitrite and oxygen may serve as exogenous electron acceptors, potentially supporting specific metabolic pathways in eukaryotic microorganisms (Barton 2005).

Among the Bacteria, the Actinobacteria phylum was highly dominant, particularly in surface waters and at a depth of 6 m. This phylum is known for its wide distribution across various habitats such as cave soils (Wiseschart et al. 2019) or freshwater lakes (Edberg et al. 2012, Ávila et al. 2017, Morrison et al. 2017). Several bacterial genera characteristic of the oxic zone belong to Actinobacteria, including the CL500-29 marine group, the Hgcl clade, and cand. *Planktophila*. These lineages are widely recognized as core members of freshwater bacterial communities (Jezbera et al. 2009). Additionally, the chemoheterotrophic genus *Limnohabitans* was also prominent in this zone, and is known for its ecological versatility, inhabiting surface freshwater systems, groundwater habitats, and mine pit lakes (Hahn et al. 2011, Blanchette and Lund 2021, Villeneuve et al. 2023). Its rapid growth and high metabolic activity make it a key prey for bacterivorous flagellates, such as members of the *Chrysophyceae* suggesting its crucial role in the microbial food web and energy transfer within the oxic zone (Šimek et al. 2013). *Chrysophyceae* are an important primary producer and a dominant bacterivore. Many lineages are mixotroph, i.e. they have the ability for photosynthetic carbon fixation (photoautotrophy) and to regularly ingest bacteria (chemoheterotrophy) (Olefeld et al. 2018). Several studies have shown that certain protists, such as *Chrysophyceae*, appear to avoid Actinobacteria. Their selective grazing (by size or physiology) can influence bacterial growth conditions and shape bacterial competition by eliminating specific taxa (Jezbera et al. 2005, 2006, Ivanković et al. 2023). That could explain the dominance of Actinobacteria in the oxic zone of the mine.

Among the microeukaryotes of the mine, the presence of *Pedinellales* family (planktonic or sessile) from the *Dictyochophyceae* class was not surprising, as they are widespread in oligotrophic marine and freshwater systems (Unrein et al. 2005, Gerea et al. 2016, Choi et al. 2020), with a large functional diversity, notably a role as bacterivores and herbivores. This is the case for Maxillopoda belongs to copepod, an herbivore widespread in lakes and groundwater (Pop et al. 2023) and colpodellida, a phagotrophic predator in lakes (Mylnikov 2009) both important protists in the mine. The structure of protist community in the oxic zone changed throughout the different seasons, corroborating with the seasonal environmental changes. The ecological versatility of dinoflagellates makes them an abundant component of the oxic zone of freshwater ecosystem.

Among the Archaea, *Methanosarcina*, acetoclastic methanogens, were important taxa in the oxic zone, as seen elsewhere (Ferry 2011). This genus was highly dominant in the surface waters of the mine and has detected in other oxic freshwater environments (Lavergne et al. 2021, Lyautey et al. 2021). Previous studies have suggested that acetoclastic methanogenesis in oxic freshwater systems may be enhanced by nutrient inputs and increased algal production, which provide substrates for methanogens (Grossart et al. 2011, Bogard et al. 2014). In contrast, the methanogenic taxon *Methanobacterium*, also present in the mine, is primarily hydrogenotrophic, utilizing H_2 and CO_2 as substrates for methanogenesis (Lavergne et al. 2021). This suggests that multiple methanogenic pathways may coexist in the oxic zone, influenced by environmental conditions and substrate availability. *Rice Cluster I*, another methanogen group, was also identified in the mine. This ubiquitous and abundant lineage, part of the Euryarchaeota, branches deeply within the methanogenic orders *Methanosarcinales* and *Methanomicrobiales*. Both orders were prominently detected in the transition zone of the mine, highlighting their potential role in the methane cycle within stratified aquatic systems (Conrad et al. 2006). In contrast, *Methanobrevibacter* was a discriminating genus detected in the mine but at a relatively low abundance, suggesting an important ecological role. Genera from the DPANN superphylum, such as *Micrarchaeales* and *CG1-02-57–44* (*Woesearchaeales*), were particularly discriminating in the surface waters of the mine. These taxa are notable for their presence in a wide range of habitats, including groundwater (Patel et al. 2024) and caves (Lauzon et al. 2024). These results highlight the complex and dynamic interplay between bacterial, archaeal, and protistan communities in the oxic zone of the mine (Fig. 6), where niche specialization, trophic interactions, and seasonal environmental shifts shape a unique diverse microbial ecosystem. These findings underscore the ecological importance of oxic surface waters as active biogeochemical interfaces, supporting key microbial processes such as carbon cycling, methanogenesis, and microbial grazing that contribute to the overall functioning and stability of the stratified mine lake.

Microbial community structure in the transition zone

The transition zone (6–12 m) from oxic to anoxic water is accompanied by significant shifts in microbial diversity and biogeochemical parameters, decreasing the temperature, DO, and DOC while DIC and NH_X increased, making it an important zone for characterization (Diao et al. 2018).

In the mine, bacteria involved in sulfur cycling were mainly found in the transition zone. Indeed, *Thiodictyon*, an anoxygenic phototrophic purple sulfur bacterium (PSB), was identified in this zone. Previous studies, such as Diao et al. (2018), reported a negative correlation between *Thiodictyon* abundance and sulfate concentrations in stratified lakes, likely reflecting high SRB activity that reduces sulfate to H_2S, serving as an electron donor for PSB. Its presence in this transition zone suggests an active sulfur cycle linked to the specific geochemical environment. *Chlorobium*, a genus belonging to GSB, was a key taxon in this zone, extending from 6 to 52 m in depth. GSB are typically found in anoxic waters, where they thrive under low light conditions. They play an important role in sulfur cycling by oxidizing sulfide to sulfur species, which can then serve as electron acceptors for SRB such as *Desulfobacter* and *Desulfobulbus*, both abundant in the hypolimnion (anoxic) of the mine (Bryant et al. 2012). In addition to sulfide, *Chlorobium* can utilize Fe(II) and other inorganic sulfur compounds present in mining environments (Weber et al. 2006). Diao et al. (2018) observed a positive correlation between *Chlorobium* abundance and sulfide concentrations, alongside a negative correlation with dissolved oxygen levels. These findings align with the environmental conditions in the Blackburn mine, where a strong sulfide odor indicated high sulfide concentrations, although this was not directly measured. The physical and chemical characteristics of this zone, including low oxygen, elevated sulfide, and stratified light availability, likely support the persistence of *Chlorobium*. Notably, *C. clathratiforme*, a species within this genus, has been documented in similar environments, such as stratified lakes and former uranium open-pit mines (Gregersen et al. 2009, Edberg et al. 2012, Danza et al. 2018).

Among the key archaeal taxa in the Blackburn mine, the methanotrophic genus *Methylobacter* was as a significant taxon. Members of this genus, belonging to the family *Methylococcaceae* (type I methanotrophs), are widely distributed in freshwater systems, with an occurrence rate of 86% in lake environments (Borrel et al. 2011). Their dominance is often associated with their adaptation to low temperatures, giving them a competitive advantage in such ecosystems (Sundh et al. 2005, Kojima et al. 2009). As aerobic methanotrophs, *Methylobacter* are functionally linked to methanogenic archaea, consuming methane produced in anaerobic zones and contributing to the methane cycle in stratified aquatic systems.

In the Blackburn mine, methanogenic archaea were abundant, particularly in the transition zone. This zone hosted hydrogenotrophic and acetoclastic methanogens, with their distribution shaped by key environmental parameters (Lyautey et al. 2021), such as organic matter availability, which controls methanogenesis, while oxygen availability influences the structure and activity of methanotrophic communities in lake sediments. Hydrogenotrophic methanogens, which are cytochrome-deficient and adapted to diverse ecosystems, were notably represented in the transition zone. Taxa such as unclassified genera within the *Methanomicrobiales* order and *Methanospirillum* were characteristic of this zone. *Methanospirillum*, a hydrogenotrophic genus, has been positively correlated with increasing depth in studies of eutrophic lakes, such as the work of Alegría-Gómez et al. (2023). Among acetoclastic methanogens, *Methanosaeta* appeared as a dominant and discriminant taxon, particularly at 6 and 12 m within rock biofilm communities. This genus is the second most frequently encountered methanogen in freshwater lakes, with a prevalence rate of 78.9% (Borrel et al. 2012). Acetoclastic methanogenesis is typically influenced by oxygen depletion, organic matter availability, and temperature (Lyautey et al. 2021), all of which likely contribute to the observed distribution of methanogens in the mine. Two additional archaeal taxa of interest, though less abundant in the transition zone, were unc. Thermoplasmata, which are involved in carbon mineralization and may participate in sulfate reduction (Lhoste et al. 2023), and AR15 genus belonging to the Woesarchaeota phylum potentially involved in anaerobic carbon cycling (Liu et al. 2018). Their presence in former mining environments highlights their roles in nutrient cycling and adaptation to hostile conditions.

For eukaryotes, the transition zone exhibited high relative abundances of algal classes such as Chrysophyceae, Cryptomonadales, all of which are common in freshwater systems, cave waters but also in cold and depth environments (Cairano 2020, Lauzon et al. 2024). Among them, Haptoglossales, belonging to the division Oomycota, were detected. These taxa are known as endobiotic parasites of rotifers and nematodes, or as parasites of phytoplankton and algae (Buaya and Thines 2020). While their ecological roles remain poorly understood, they likely play an important role in maintaining food web stability within these ecosystems. These findings highlight the ecological complexity of the transition zone, where sulfur and methane cycles are tightly coupled by diverse and specialized microorganisms, contributing to the biogeochemical functioning of the mining pit lake ecosystem, illustrating in a schematic overview provided in Fig. 6.

Microbial community structure in the anoxic zone

The anoxic zone of the Blackburn mine (18–52 m) was characterized by high concentrations of NH^+ and DIC, the absence of both light and oxygen, and a stable temperature (~5°C).

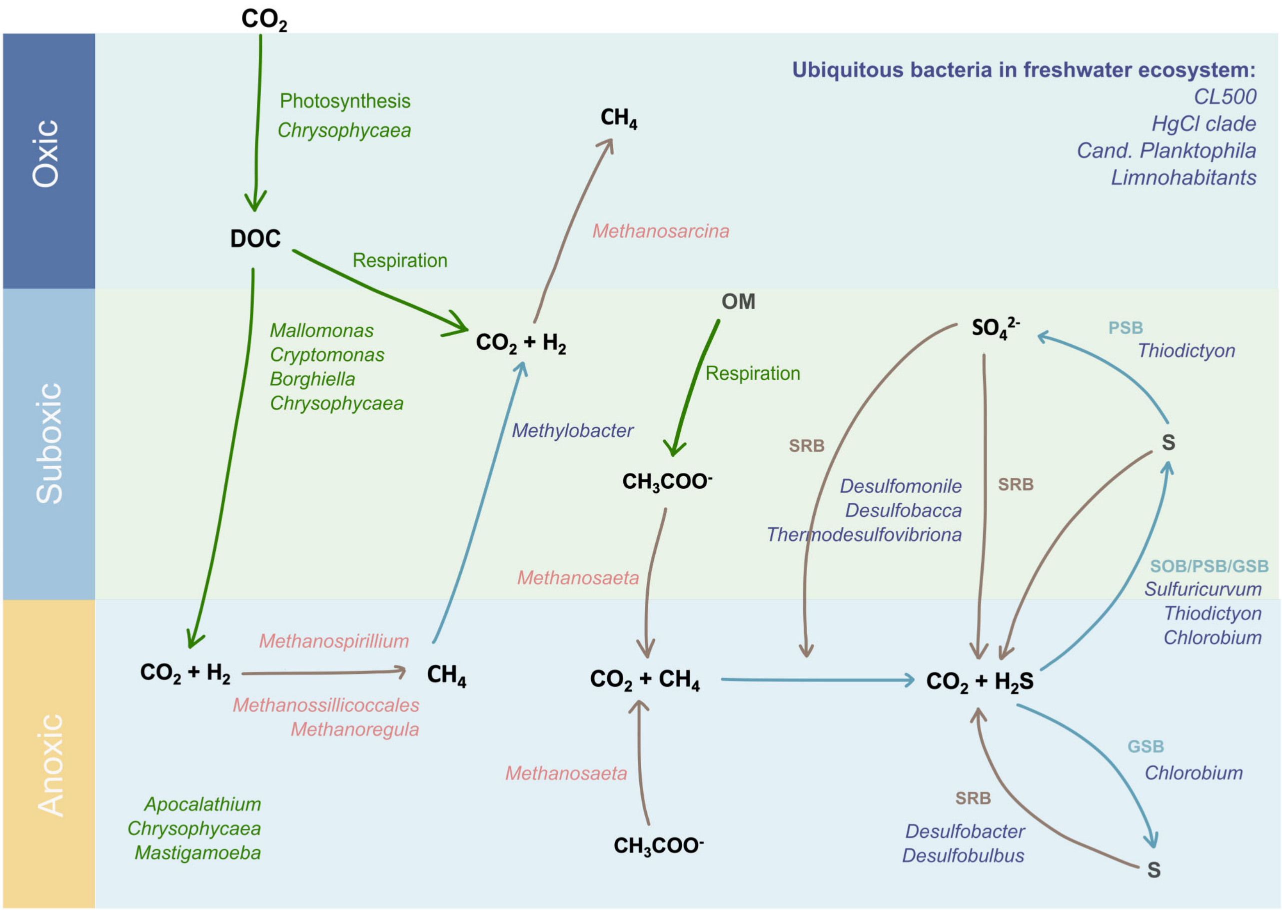

Figure 6. Schema of microbial discriminating taxa in the three zones of the mine, involved in carbon and sulfur cycles. Eukaryote, bacteria, and archaea are represented in green, blue, and pink, respectively. Blue arrows represent oxidative reactions, brown arrows represent reductive reactions, and green arrows represent photosynthesis and organic matter (OM) degradation.

These physicochemical conditions supported the development of specialized microbial communities adapted to anaerobic and chemolithotrophic metabolisms. The db-RDA revealed that the bacterial community composition at 52 m was significantly shaped by autumnal conditions, with both free-living and sessile responding to environmental gradients. NH^+ and DIC emerged as major structuring variables. In anoxic environments, NH^+ and DIC play distinct but complementary roles in microbial metabolism. DIC serves as a major carbon source for chemoautotrophs and methanogens (Wang et al. 2022), whereas NH^+ support microbial growth via assimilatory pathways. In the mining lake, NH^+ strongly influenced both bacterial and eukaryotic communities, whereas DIC influenced archaeal assemblages. Comparable observations have been reported in other stratified freshwater systems, such as Lake Vechten, where NH^+ accumulation drives the succession of ammonia-oxidizing, denitrifying communities (Diao et al. 2018). Similarly, Heiss et al. (2022) documented elevated ammonia and nitrite oxidation rates at depth, correlated with increased NH^+ concentrations. These findings reflect the importance of the limited uptake of NH^+ in deeper, light-limited zones. The detection of an ammonia-oxidizing nanoarchaeon (GW2011) in the mine further supports the role of NH^+ as a key ecological driver. Besides, the anoxic zone is mainly characterized by microbial communities involved in carbon and sulfur cycles, like SRB/SOB and methanogens/methanotrophs. The seasonal variation in the abundance of *Desulfomonile*, particularly its higher abundance in autumn, aligns with studies showing that this genus thrives under conditions of increased depth, lower temperatures, and limited dissolved oxygen. Its activity is positively correlated with sulfide concentrations (Diao et al. 2018, Chen et al. 2020) contributing to the eutrophication of stratified water. The genera *Desulfomonile* and *Desulfobacca* represent sulfate-reducing bacteria (SRB) that reduce sulfate (SO^{2-}) to hydrogen sulfide (H_2S), a process critical to sulfur cycling. While sulfate concentrations were not measured in this study, the characteristic “rotten egg” odor strongly indicates the presence of H_2S, and active sulfate reduction metabolism. Depth-related decrease in sulfur concentrations and sulfate oxygen isotopes was observed in the Blackburn mine (Pastier et al., personal communication), indicating a reduction process. This H_2S can serve as an energy source for sulfur-oxidizing bacteria (SOB) such as *Sulfuricurvum*, which were abundant at 52 m depth. Similar interactions between SRB and SOB have been documented in meromictic lakes, revealing complex ecological networks (İnceoğlu et al. 2015, Inceolu et al. 2015). Also, SOB, including *Sulfuricurvum*, are chemolithoautotrophs that oxidize reduced sulfur compounds using oxygen or nitrate as electron acceptors (Kodama and Watanabe 2004, Kadnikov et al. 2019).These findings highlight a tightly coupled sulfur cycle in the anoxic zone of the Blackburn mine, where sulfate-reducing and SOB form a dynamic microbial network that mediates redox transformations and contributes to the regulation of elemental fluxes.

Hydrogenotrophic methanogenesis, a dominant pathway in the anoxic zone of the mine, was represented by taxa such as the *Methanofastidiosales* order, which performs methyl-dependent hydrogenotrophic methanogenesis (Cozannet et al. 2021). *Methanospirillum*, a hydrogenotrophic methanogen within the *Methanomicrobiales* order, was particularly abundant and discriminant, consistent with its preference for anaerobic environments (Villeneuve et al. 2023) (on 2005). Acetoclastic methanogenesis was dominated by *Methanosaeta*, a genus frequently encountered in freshwater ecosystems and contributing to methane production in sediment biofilms (Borrel et al. 2011). Anaerobic oxidation of methane (AOM) processes was also evident, with cand. *Methanoperedens* exclusively found at a depth of 52 m. This taxon is known to oxidize methane anaerobically using nitrate or iron as electron acceptors (Haroon et al. 2013, Guerrero-cruz et al. 2018). Such AOM-SRB coupling may further contribute to methane cycling, as observed in other freshwater systems (Timmers et al. 2017). The cooccurrence of *Thermoprofundales* (formerly MBG-D) and methanotrophic archaea (e.g. ANME) suggests potential syntrophic relationships or shared selective pressures, as noted in other anoxic ecosystems (Zhou et al. 2019). The genus *Syntrophus*, specific to 52 m depth, represents syntrophic bacteria that form mutualistic relationships with methanogens such as *Methanospirillum*. This syntrophic interaction facilitates secondary fermentation processes and methane production under anoxic conditions (Griebler and Lueders 2009). Additionally, an unclassified genus from the OPB41 lineage, part of the *Actinobacteria* phylum, was identified and discriminated. This lineage is widely distributed in diverse habitats, including volcanic environments, deep aquifers, and subsurface petroleum regions (Khomyakova et al. 2022). As shown in Fig. 6, the coexistence of diverse methanogenic, methanotrophic, and syntrophic taxa reflects a complex and spatially structured methane cycle in the anoxic zone, driven by interdependent microbial processes. This metabolic complexity parallels the sulfur cycle in the same environment, where sulfate-reducing and SOB similarly engage in tightly coupled redox interactions. These microbial networks underscore the complexity of anaerobic energy metabolism and its central role in modulating the biogeochemical dynamics of the Blackburn mine.

Regarding, the eukaryotic communities in the anoxic zone, it contained unique eukaryotic taxa such as *Mastigamoebidae*, anaerobic protists from the Pelobiont group that are typically found in anoxic habitats (Walker et al. 2001, Ptáčková et al. 2013). *Thoracosphaeraceae*, a family within the dinoflagellates, displayed seasonal variation, with higher abundance in autumn. These dinoflagellates are known for their dual life stages—motile planktonic forms and nonmotile calcareous cysts—with the latter being predominant in the mine (Rengefors and Kremp 2018). Among this family, the *Apocalathium* genus was significant in the anoxic zone of the mine, specially the *Apocalathium aciculiferum,* cold-adapted species, capable of tolerating broad environmental gradients which may explain their persistence and dominance in the Blackburn mine (Obertegger et al. 2020, Haraguchi 2023). They can take advantages of cold and dark waters to grow more due to less competition and grazing (Rose and Caron 2007). Interestingly, *Chrysophyceae*, an algal class typically associated with phototrophic or mixotrophic lifestyles was observed in the anoxic zone of the mine. These microorganisms may adopt bacterivorous or mixotrophic strategies under such conditions (absence of light) and highlighting their ecological flexibility (Olefeld et al. 2018, Bock et al. 2022). The diversity and functional specialization of microbial taxa in the anoxic zone highlight its role as a key biogeochemical, where tightly coupled carbon and sulfur processes. These functions complement those observed in the transition zone, suggesting a vertical continuum of microbial interactions that shapes the overall functioning of this artificial aquatic system.

Conclusion

Overall, this study carried out in the Blackburn mining lake revealed a vertical stratification, particularly in oxygen concentration and temperature, delineating three distinct physicochemical zones (oxic, transition, and anoxic). The microbial community was dynamic, with key taxa including methanogenic archaea and sulfur-cycling bacteria. Shifts in microbial composition were closely associated with protist activity, which varied seasonally. Notably, microbial communities differed between planktonic and sessile lifestyles, with sessile communities, from rock biofilms, showing greater seasonal sensitivity, especially among archaeal and bacterial taxa. In contrast, a basal microbial assemblage, largely unaffected by depth or season, was consistently present throughout this ecosystem. Our findings provide further evidence for the central role of carbon and sulfur cycling in structuring microbial assemblages within this artificial aquatic ecosystem. Future research should aim to quantify key chemical drivers such as sulfide, methane, and trace metals to further clarify the ecological functions of the identified taxa. Incorporating multiple hydrodynamic approaches in future work would also help strengthen and refine the interpretations drawn from FEAST. Combining with metagenomic and metatranscriptomic approaches will enhance our understanding of microbial interactions and ecosystem functioning in anthropogenically influenced lake environments. From a microbial perspective, the mine appears as a dynamic ecosystem that has evolved considerably and may still be changing over time. Once shaped by human exploitation as an extractive environment, it has since transitioned back toward natural conditions, giving rise to unique microbial assemblages. Environmental and climatic changes could influence the surface-associated communities and potentially modify the stratification of the system.

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Author contributions

Elise Lhoste (Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft), Jaclin David (Funding acquisition, Project administration), Violaine Ponsin (Funding acquisition, Project administration, Writing – review & editing), Rosabal Maikel (Methodology, Supervision, Writing – review & editing), Cassandre Lazar (Conceptualization, Formal Analysis, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing).

Supplementary data

Supplementary data is available at FEMSEC *Journal* online.

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