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ORIGINAL PAPER

Microbial adaptations to acidic, nutrient- and metal-rich lakes in Aotearoa New Zealand

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Abstract

Four lakes in the same region of Aotearoa New Zealand were investigated to characterize sediment microbial communities and functions under contrasting environmental conditions. Two lakes, an acidic lake (Rototai) and a lake with elevated metals and nutrients (Killarney) were impacted by extreme stressors, while the lowland mesotrophic lake (Kaihoka East) and an alpine lake (Peel) were used as reference lakes. Using metabarcoding and metagenomics analysis, we profiled community composition, functional pathways, and resistance mechanisms in the lake sediments. Rototai contained high abundances of genes involved in sulfur cycling (assimilatory and dissimilatory sulfate reduction, sulfur oxidation) and acid tolerance (kdp potassium-transport system, ClcA antiporters). In contrast, Killarney had elevated abundances of genes involved in methanogenesis, however despite high metal concentrations, no enrichment of metal-resistance genes was detected. Kaihoka East contained the highest prokaryotic diversity and an elevated abundance of genes involved in nitrification. Although community taxonomic differences were modest across lakes, functional analyses revealed distinct metabolic adaptations. These findings highlight the utility of using metagenomic approaches to identify biogeochemical processes and stress-response strategies in lakes. Improved understanding of microbial functional diversity in surface sediments has implications for lake management, particularly in systems impacted by acidification, high nutrient loading, and metal contamination.

Keywords Extreme lakes · Metagenomics · Metabarcoding · Surface sediments · Microbial community

Introduction

Freshwater lakes vary widely in their physical and chemical characteristics. They range from high-altitude, oligotrophic lakes, which host unique biodiversity, to lowland, coastal, hypertrophic lakes that are nutrient-rich but often support only specific, highly tolerant species. Additionally, some lakes have distinct features, such as low pH levels, which further shape their ecological communities (Scheffers and Kelletat 2016). Biological communities within lakes are affected by a variety of natural (e.g. temperature, light) and anthropogenic pressures (e.g., agricultural runoffs, sedimentation), especially shifts in nutrient concentrations commonly associated with diffuse pollution related to land use change (Pearman et al. 2023). Surface sediments in lakes act as a sink for nutrients and environmental contaminants (Li et al. 2024), that often reflect the conditions of the lake and surrounding catchment. These sediments also contain a high abundance and diversity of microbes which are vital for freshwater biogeochemical cycling, with microbial communities responding to variations in lake conditions (Rohwer et al., 2025). Knowledge on how these microbial communities shift, both taxonomically and functionally, with variations in environmental conditions is still relatively unexplored but is vital to understanding freshwater microbial ecology, evolution, and biogeochemical processes.

Evaluating the functions of bacterial communities has been challenging in the past, and most molecular studies have focused on characterising their composition using techniques, such as metabarcoding (Caporaso et al. 2010). Metabarcoding has limitations (e.g., taxonomic biases)

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but more importantly, does not provide information on the metabolic functions of the organisms studied (Semenov 2021; Wilson et al. 2019). Metagenomics is defined as the direct genetic analysis of genomes, and the characterisation of thousands of genes, contained within an environmental sample (Prosser 2015; Semenov 2021). Metagenomic studies allow for the comprehensive study of microbial diversity, metabolic pathways, and ecological interactions without the need for cultivation, overcoming the limitations of traditional microbiological methods (Abia et al. 2018; Taş et al. 2021). The growing number of metagenomic studies is providing important insights into the ecology and evolution of microbes and can be used as an invaluable tool for understanding microbial communities (Grossart et al. 2020). Metagenomics has been used to investigate freshwater environments and understand how the composition and functional potential of microbes is affected by changing environmental conditions (Biessy et al. 2022; Fang et al. 2022; Linz et al. 2018; Liu et al. 2022). As well as gaining an understanding of metabolic potential of microbes in lake ecosystems, metagenomics has the ability to investigate the functional responses of microbes to extreme conditions (Cowan et al. 2015).

Extreme lakes, which are characterized by harsh conditions such as high salinity, extreme pH or temperatures, or high heavy metals and nutrient concentrations, represent some of the most challenging habitats for microbial life (Peña-Ocaña et al. 2022; Vincent 2018). Extreme environments have been the focus of various studies using molecular techniques to investigate the novel genes, functional pathways, and microbial interactions that are essential for survival in extreme environments (Aerts et al. 2019; Albarracín et al. 2016; Aszalós et al. 2016). For example, the effect of acidification on microbial communities has been investigated in an acidic salt lake in Australia generating a microbial profile of the depositional environment associated with the sulfur-rich sediments (Johnson et al. 2015), and to examine the microbial composition and putative function in extreme acid brine environment (Zaikova et al. 2018). Metagenomic analysis has also shown different microbial metal tolerance mechanisms (e.g. transports, redox reactions and DNA repair) exists in samples from rivers (Yan et al. 2020) and mangroves (Puthusseri et al. 2021).

In this study, metagenomics was used to investigate the microbial communities and their functional potential in surface sediments collected from four lakes in the same region (Tasman, top of the South Island) in Aotearoa New Zealand. Two of these lakes have extreme conditions: an acid lake (pH = 4; Rototai) and a nutrient and metal-rich lake (Lake Killarney). The aim of this research was to describe the microbial diversity and their functional profiles in these two extremes lakes and compare them to two lakes from the same region without extreme environmental selective pressures. We hypothesised that: (1) Microbial community composition and functional potential would differ across lakes due to unique environmental conditions, and (2) Pathways for acid resistance, nutrient cycling and metal tolerance will be elevated in the acid lake and the metal- and nutrient-rich lake.

Materials and methods

Sampling sites

Four lakes in the Tasman Region in Aotearoa New Zealand (Fig. 1), spanning a range of characteristics (Table 1) and environments (extreme, normal and alpine) were selected for this study.

Lake Killarney (Fig. 2) is a small lake (0.8 ha, 12 m max. depth) with no permanent in- or outflows and is surrounded by parkland and residential houses. At the time of sampling stormwater from the nearby town had been directed into the lake for a number of years, and it experiences severe algal blooms in summer (James and McCallum 2015). Lake Killarney is supertrophic, with an anoxic bottom and is in a highly degraded condition and its surface sediment contain very high concentrations of heavy metals (cadmium, chromium, copper, lead, nickel and zinc) and phosphorus (Wood et al. 2023). In 1942, the land around the lake was almost completely devegetated and is now surrounded by grasses and a number of large non-native deciduous trees, that are likely contributing to the organic load (Wood et al. 2023).

Lake Rototai (Fig. 2), also called Blue Lake because of distinctive turquoise colour, is a flooded sink hole with a high surface area (0.57 ha) to depth ratio (14 m max. depth), filled with groundwater (James and McCallum 2015). The pH of this lake is extremely low (pH 4), and the reasons for this remain unknown. The most likely reason is that the lake is situated in a coal seam (see Blodau (2006)). Historical pollen data indicated some removal of native trees during the post-European settlement era and the lake is now partly surrounded by grass and non-native vegetation (Wood et al. 2023).

Lake Peel (Fig. 2) is a small shallow alpine cirque lake (4.7 ha, 9 m max. depth, 1,350 m altitude), mostly surrounded by native alpine vegetation. The remote location of this lake has meant that evidence of human presence or impact on this lake is minimal. This lake was used as a control and reference lake for this study.

Lake Kaihoka East (Fig. 2) is a relatively unmodified coastal lake. It has no outlets or permanent inflows, with the hydrology mainly controlled by rainfall and seepage (Schallenberg 2011). Lake Kaihoka East is mesotrophic, has

Fig. 1 Locations (yellow points) of lakes studied in the Tasman Region of Aotearoa New Zealand. Lakes Killarney (high metals and nutrients) and Rototai (low pH) are characterised as extreme lakes in this study

a maximum depth of 14.8 m and its catchment is dominated by low-producing grassland and native vegetation. It is used in this study to represent a typical lowland lake in Aotearoa New Zealand that has experienced some modification and degradation (Thomson-Laing et al. 2025).

Surface sediment analysis

Surface sediment collection for nutrient and metal characterisation

Samples for surface sediment geochemistry samples were collected by Uwitech gravity corer (90 mm diameter core) at the deepest point in the lake. Five cores were taken, and the top 2 cm of sediment removed using a core cutter and combined in a 500 mL container. These were stored chilled

Table 1 Physical characteristics, catchment land use and sediment bacterial trophic index (SBTI; Pearman et al., 2022) for the four-study lake. ND = not determined

Lakes	Max. depth (m)[1]	Secchi Disc (m)[1]	Altitude (m)[2]	Area (ha)[2]	Catchment (%)[3]						SBTI[1]
					High productivity exotic grass	Low productivity exotic grass	Native	Native Forest	Urban	Other (gravel)	
Kaihoka East	13.7	4	52	5.3	0.0	51.2	0.0	48.8	0.0	0.0	Mesotrophic
Rototai	14	7.8	15	0.57	80.5	0.0	0.0	19.0	0.5	0.0	ND
Killarney	11.8	1.8	11	0.87	54.2	0.0	8.5	0.8	36.5	0.0	Supertrophic
Peel	8.9	8.4	1,353	4.7	0.0	0.0	86.7	0.0	0.0	13.3	Mesotrophic

[1] Data from Our lakes' health: past, present, future (Lakes380) field sampling (Lakes - Our Lakes Our Future/)

[2] Data from Freshwater Ecosystems of New Zealand (FENZ) Geodatabase (Leathwick et al. 2010)

[3] Data from Land Cover Database v5 (https://lris.scinfo.org.nz/layer/104400-lcdb-v50-land-cover-database-version-50-mainland-new-zealand/)

Fig. 2 Photographs of the four study lakes and their surrounding environments. Killarney = supertrophic, nutrient-rich lake; Rototai = acid lake (pH 4); Kaihoka East = unmodified coastal lake, used as a typical lowland lake for this study; Peel = alpine lake, used as control for this study

(4 °C) and shipped to the laboratory within 48 h for nutrient and elemental characterisation.

Surface sediment samples for nutrient and elemental analyses were homogenised, centrifuged (3,000 × g, 40 min, 4 °C) and the pore water decanted. The sediments were analysed for metals using the methods described in Pearman et al. (2020). Briefly, the sediment was dried, passed through a 2 mm sieve and analysed for the following metals using acid digestion followed by inductively coupled plasma mass spectrometry, based on the US EPA method 200.8 for iron (Fe), manganese (Mn), aluminium (Al), calcium (Ca), lead (Pb), copper (Cu), zinc (Zn), cadmium (Cd), phosphorus (P) and sulphur (S). Total nitrogen and total organic carbon (TOC) were analysed using catalytic combustion (900 °C, O_2) and separated using a thermal conductivity detector. Oven drying of a known sediment volume was used to calculate bulk density, while oven drying followed by ashing

(550 °C and 950 °C) and gravimetric determination were used to measure organic matter and carbonate content.

The surface sediment geochemistry of the study lakes was compared to national statistics from 214 lakes analysed during a previous research programme (Lakes - Our Lakes Our Future/).

Surface sediment collection for DNA and metabarcoding processing and analysis

Surface sediment samples for environmental DNA (eDNA) analysis were taken in triplicate at the deepest point of each lake using a ponar grab. Using sterile spatulas, approximately 2 g of the undisturbed surface sediment layer (top 1–2 mm) was placed in sterile tubes and stored in lifeguard (Qiagen, Hilden, Germany) before being transferred to a -80 °C freezer in the laboratory for later DNA extraction.

DNA for metabarcoding was extracted from 0.25 g of sediment in triplicate using the DNeasy PowerSoil Kit (Qiagen). The V3-V4 region of the bacterial 16 S rRNA gene was amplified by polymerase chain reaction (PCR) using the primers: 341 F: 5-CCT ACG GGN GGC WGC AG-3 and 805R: 5-GAC TAC HVG GGT ATC TAA TCC-3 (Herlemann et al. 2007; Klindworth et al. 2013). Conditions for the PCR reactions and subsequent library construction for sequencing on an Illumina Miseq are as described in Pearman et al. (2020). Raw reads from the sequencing machine were demultiplexed and processed using cutadapt (Martin 2011) and Amplicon Sequence Variants (ASVs) were inferred using the DADA2 (Callahan et al. 2016) using the pipeline detailed in Pearman et al. (2020). Reads were taxonomically classified using the RDP classifier (Wang et al. 2007) against the CyanoSeq version of the SILVA 138 database (Lefler et al. 2023; Pruesse et al. 2007) with a minBoot threshold of 70. The raw sequences are available under accession PRJNA750120 of the NCBI SRA database.

Metagenomics analysis

DNA from the surface sediment was extracted using an alkaline buffer extraction protocol as described in Thomson-Laing et al. (2022). Briefly, 3–5 g of sediment was transferred to 6 mL (0.33 M) sodium hydroxide and 3 mL (pH 6.7) Tris-EDTA in a 50 mL Falcon tube and vortexed. The sediment was incubated for 50 min at 65 °C before cooling for 5 min and centrifuging for one hour (15,000 × g) at 15 °C. The supernatant was transferred to a new tube and the DNA was precipitated overnight at -20 °C with 7.5 mL of Tris HCl (1 M, pH 6.7), 1.5 mL sodium acetate (3 M, pH 5.2) and 100% molecular grade ethanol. DNA was collected by centrifugation (10,000 x g, 1 h, 4 °C). Further purification was undertaken on the resulting DNA using DNeasy PowerSoil Kits (Qiagen) following the manufacturer's instructions using an automated homogenizer (1600 MiniG Automated Tissue Homogenizer and Cell Lyser, SPEX SamplePrep, NJ, USA) and a robotic workstation for DNA extraction (QIAcube, Qiagen). A VAHTS Universal Plus DNA Library Prep Kit was used for library preparation and samples were sequenced on an Illumina (San Diego, CA, USA) NovaSeq 6000 machine by Azenta Life Science (Suzhou, China). The raw sequences are available under accession of the Genomics Aotearoa Database (https://doi.org/10.57748/3jdy-rc84).

Sequenced reads were trimmed using Trimmomatic (LEADING:5; TRAILING:5; SLIDING WINDOW:10:15; MINLEN:30 (Bolger et al. 2014). Samples were individually assembled using Megahit (Li et al. 2015) using the meta-large preset. The individual assemblies were filtered using seqmagick (Yu 2024) to contain contigs greater than 500 base pairs (bp). Coding sequences were predicted using prodigal (Hyatt et al. 2010) and a non-redundant gene catalogue of the four lakes was constructed and clustering was undertaken using CD-HIT-EST (c = 0.95 -aS 0.9) (Li and Godzik 2006) and further filtered to coding sequences greater than 100 bp. Quality trimmed reads were mapped against the non-redundant gene catalogue using sqm_mapper.pl (Tamames and Puente-Sánchez 2019) with bowtie2. Annotation of the non-redundant gene catalogue of the four lakes was undertaken using EggNOG-mapper (Cantalapiedra et al. 2021; Huerta-Cepas et al. 2019) using DIAMOND (Buchfink et al. 2015). Classification of the coding sequences was undertaken against NCBI's non redundant (nr) database using blastp within DIAMOND (max target seqs = 10). The least common ancestor for each coding sequence was determined by finding the common ancestor of classifications that had bitscores within 10% of the highest bitscore for that sequence.

Results

Lake surface sediment chemistry

The surface sediment of Killarney was enriched in nitrogen, organic carbon and many metals compared with the national median (Fig. 3). Sulfur was extremely high in Rototai (21,000 mg kg^{-1} dry weight (dw); national median = 2,875 mg kg^{-1} dw) and aluminium in Kaihoka East (28,400 mg kg^{-1} dw; national median = 20,500 mg kg^{-1} dw). In Rototai, total nitrogen (7,500 mg kg^{-1} dw) and phosphorus (1,150 mg kg^{-1} dw) were lower than the national median (9,100 mg kg^{-1} dw and 1,310 mg kg^{-1} dw, respectively).

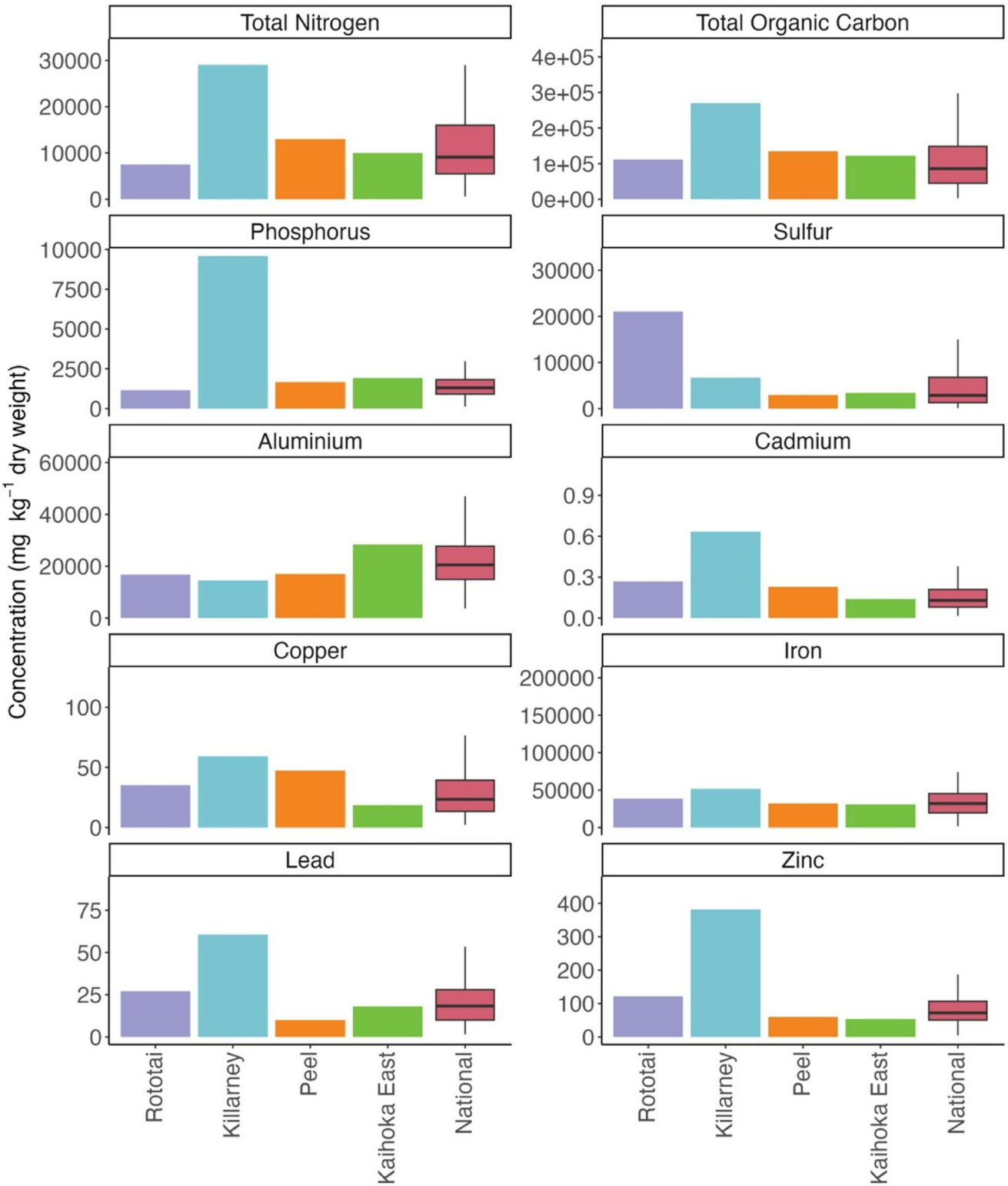

Fig. 3 Nutrient and elemental concentrations in sediment chemistry for samples from the top 0–2 cm of the lakebed sediments from four selected lakes in the Tasman region of Aotearoa New Zealand compared to a national median ($n = 214$ lakes). Elemental results are total recoverable contents. Note y-axis scale varies

Microbial composition and diversity

The 16 S rRNA metabarcoding results contained a total of 3,360 ASVs for the four lakes. The highest diversity was observed in Kaihoka East (1,513 ASVs) and the lowest was in Killarney (741 ASVs). Peel and Rototai had 846 and 783 ASVs respectively. In total, there were only 13 ASVs shared between all the lakes belonging to five phyla (Verrucomicrobiota (6 ASVs), Bacteroidota (3 ASVs), Chloroflexota (2 ASVs) and a single ASV for Desulfobacterota and Spirochaetota).

The metabarcoding analysis (Fig. 1A) revealed that Pseudomonadota was the most prevalent phyla averaging 17.6% of the total community followed closely by Bacteroidota (ave. 17.4%). Pseudomonadota had the highest proportion in Lake Peel (31.5%) while Lake Killarney had the highest proportion of Bacteroidota (ave. 25.4%). Acidobacteriota, Desulfobacterota and Chloroflexota were present in all the lakes and cyanobacteria were only absent in Rototai. Investigation of the metabarcoding data at the genus level showed differences in the predominant classified genera for each lake (Supplementary Table 1). In Rototai, the genus with the highest relative abundance was *Geothrix* (phylum Acidobacteriota) with 12.2% of the reads with this genera accounting for <0.5% of reads in the other lakes. Similar patterns were observed for the next two most abundant genera in Rototai with *Sulfuricurvum* (phylum Campylobacterota) accounting for 11.2% and *Paludibacter* (phylum Bacteroidota) contributing 7.1% with negligible relative abundances (<0.02%) in the other lakes. In Killarney, the classified genera with the highest relative abundance was *Chlorobium* (6.6%) followed by *Smithella* (5.6%) and *Cyanobium* (4.9%). For *Chlorobium* and *Smithella*, the relative abundances in Killarney were higher than the other lakes and especially Kaihoka East and Peel. In Kaihoka East, *Cyanobium* had the highest contribution to the microbial community (11%) while in Peel, *Crenothrix* accounted for the highest relative proportion (11.7%).

In the metagenomics analysis, a higher proportion of taxa could not be classified at the phylum level (Fig. 4B). Similar to the metabarcoding data, Pseudomonadota was the most prevalent phylum (ave. 20.4%), having the highest relative abundance in Lake Peel (29.6%). Cyanobacteriota relative abundances were negligible in Rototai (0.12%) but were most prominent in Lake Killarney (14.2%). Bacteroidota had lower relative abundances, with the highest relative abundance found in Rototai with 5.0%. In Killarney, a higher proportion of Archaea (Euryarcaeota) was observed (3.1%) compared to the other lakes. While the metabarcoding only efficiently targeted the bacterial component of the community due to the use of the 16 S rRNA primers, the metagenomics analysis also showed the presence of the eukaryotic phyla Chlorophyta and the archaea Euryarchaeota, especially in Lake Killarney (9.5% and 3.1% respectively).

Multivariate analysis showed that the four lakes were distinct from each other in both the metabarcoding ($F = 15.23$; $p < 0.001$; Fig. 4C) and the functional profile using metagenomic analysis ($F = 30.16$; $p < 0.001$; Fig. 4D) datasets. Visualisation showed especially distinct taxonomic and functional communities for Rototai and Lake Killarney, although there was insufficient statistical power for significant differences to be noted in pairwise comparisons.

Functional analysis

Rototai had the highest relative abundance of genes associated with sulfur metabolism (assimilatory sulfate reduction, ASR; Sulfur oxidation, SOX; and dissimilatory sulfate reduction, DSR), nitrogen metabolism (denitrification, nitrogen fixation and apparent recovery of nitrogen, ANR) and the lowest relative abundance of genes associated with nitrification (Fig. 5; Supplementary Table 2). Lake Killarney had the highest relative abundance of genes associated with methanogenesis and reverse Krebs cycle (rTCA), and Lake Peel had the lowest relative abundance of these genes (Fig. 5). Lake Killarney also had the lowest relative abundance of genes associated with respiration, SOX and denitrification. Finally, Lake Kaihoka East had the highest relative abundance of the genes associated with dissimilatory nitrate reduction to ammonium (DNRA), nitrification and the Wood-Ljungdahl (WL) carbon fixation pathway (Fig. 5).

The analysis of the microbial communities associated with genes related to sulfur metabolism showed that for all pathways there was a positive relationship to sulfur concentrations in the sediment with highest values in Rototai (Figs. 5 and 6). For ASR and DSR, Pseudomonadota was the most prevalent taxa, although a large proportion of the coding sequences could not be assigned to specific taxa (Suppl Fig. 1A). Acidobacteriota made a small contribution to DSR in Rototai and Kaihoka East. Sulfur oxidation was dominated by Pseudomonadota, predominantly Alphaproteobacteria or Betaproteobacteria, in all lakes with levels highest in Rototai (Suppl Fig. 1A).

Indicative genes for nitrogen fixation *nifD* (K02586), *nifH* (K02588) and *nifK* (K02591) were higher in Rototai compared with the other lakes and lowest in Killarney (Fig. 5). Taxonomic classifications showed that Pseudomonadota was the predominant phyla across all lakes, for the sequences that could be classified to the phylum level (Suppl Fig. 1B). In Rototai, Pseudomonadota was predominantly comprised of Alphaproteobacteria (26 counts per million (cpm)) and Betaproteobacteria (8 cpm) while Gammaproteobacteria contributed mainly to the Pseudomonadota in

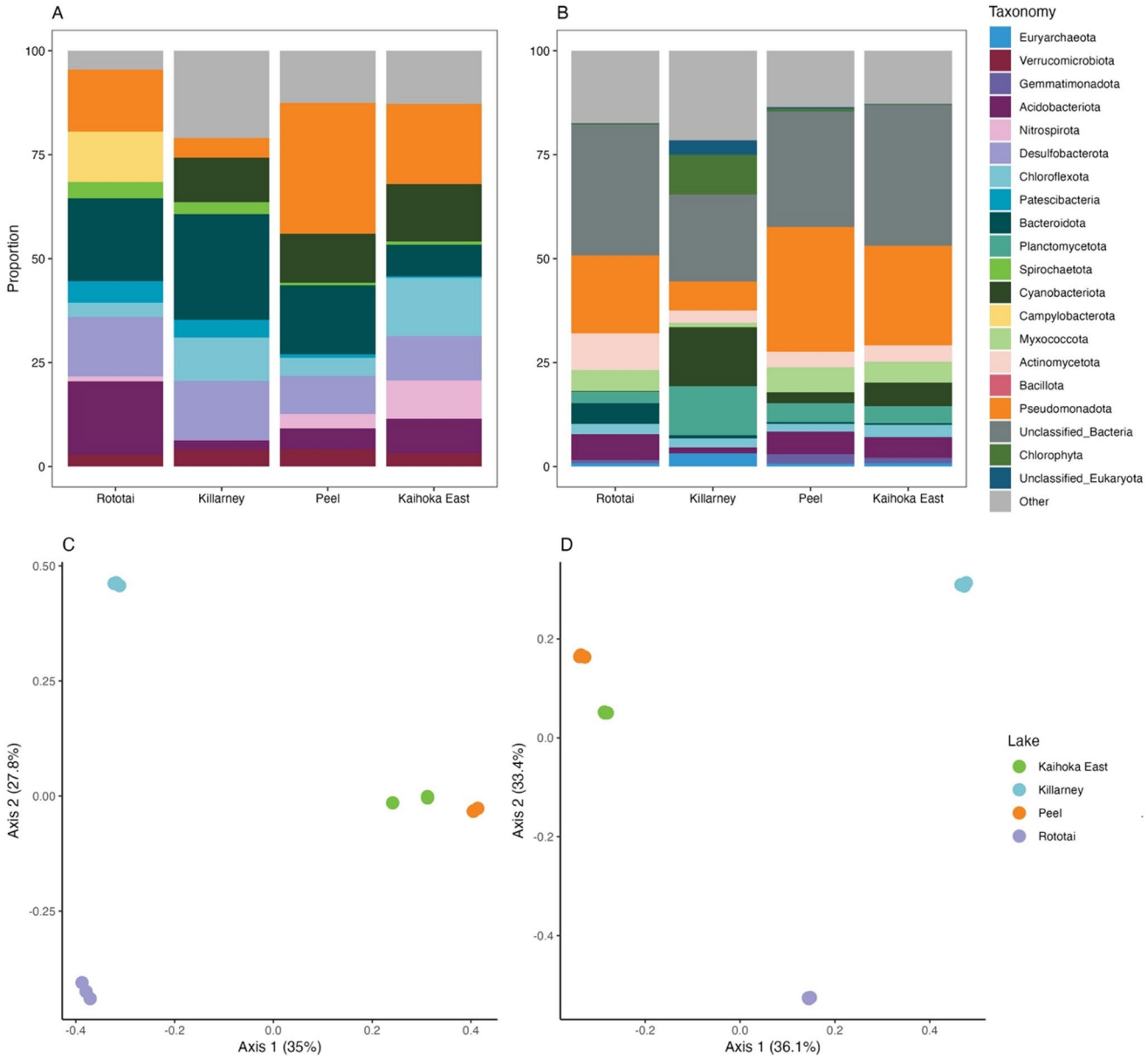

Fig. 4 Taxonomic composition of the four study lakes at the phyla level for; (A) 16 S rRNA gene metabarcoding, and (B) metagenomics analysis. Only the phyla which had an abundance of greater than 2% in at least 1 lake for either the metabarcoding or metagenomics are plotted. Principal Coordinate plot of community composition of, (C) 16 S rRNA metabarcoding analysis, and (D) functional analysis using metagenomics, based on Bray-Curtis dissimilarity matrix

Peel (47 cpm), Kaihoka (16 cpm) and Killarney (3 cpm). Cyanobacteriota made up minor portions of the nitrogen fixation especially in Killarney (3 cpm), Peel (0.4 cpm) and Kaihoka East (1 cpm) (Suppl Fig. 1B).

Resistance and tolerance related genes

The relative abundances of coding sequences for phosphorus-related gene categories (*ppk*, *ppX*, *phoR*, *phoB*, *Ugp*, *Phn*, *AlkP*, *Pst*) showed similar patterns across the lakes with negative relationships with both TN and TP (Fig. 6). However, these negative relationships were not significant for *Ugp*, *Phn* and *AlkP*. With the exception of *AlkP*, the lowest relative abundance of phosphorus-related genes was in Killarney which has the highest levels of phosphate (9,590 mg kg^{-1} dry weight) and lowest TN: TP ratio (2.62). Phosphorus-related gene categories were all positively correlated to the TN: TP ratio but these relationships were not significant.

Copper resistance genes (*copA*, *cusA*, *cusR* and *scsB*; Fig. 6; Supplementary Table 2) were negatively correlated to the concentration of copper in the sediment with *copA* and

cusA being significant correlated. The zinc/cadmium transporters (*czcA* and *czcB*) were not significantly negatively correlated to the concentrations of either zinc or cadmium but strongly positively correlated to sulfur. *ZntA* a gene indicative of zinc resistance was positively correlated to the zinc concentration in the surface sediment although not significantly. In contrast, *znuC*, *zitB* and *zur* were negatively correlated although only *zur* had a significant correlation.

Gene abundance analysis showed that acid resistance genes (Boase et al. 2022) cpm for the high affinity potassium transporter (*Kdp*) were elevated in Rototai compared to the other lakes (Fig. 7; Supplementary Table 2). Similar patterns were observed for chloride-hydrogen (Cl^-/H^+) antiporters (*ClcA*) with highest values in Rototai and lowest levels in Lake Killarney. The average cpm for the glutamate decarboxylase system (*GadA*, *GadB* and *GadC*) were slightly higher in Rototai although not substantially over Lake Killarney.

Discussion

This study investigated the diversity and functional profiles of microbial surface sediment communities in four lakes located in the same region in Aotearoa New Zealand. Two of these lakes experience extreme conditions: an acid lake (pH=4; Rototai) which has very high levels of cadmium, and a nutrient/metal/sulphur-rich lake (Lake Killarney). These two lakes were compared to two lakes from the same region not experiencing extreme environmental selective pressures (Lake Kaihoka East and the alpine Lake Peel).

We first hypothesised that microbial community composition and functional potential would differ across lakes due to unique environmental conditions. We observed that the non-extreme Lake Kaihoka East had almost twice as many ASVs in the metabarcoding dataset as the other lakes with the two extreme lakes having the lowest diversity. A lower diversity in extreme environments is not unexpected as selective pressures may favour specialist taxa (Baker and Banfield 2003). Lake Peel had also low microbial diversity but this was also expected for an alpine lake (Füreder et al. 2006) where dispersal limitations may play a part in reducing diversity (Heino et al. 2021). The taxonomic analysis at the phylum level did not identify marked differences between the lakes, apart from cyanobacteria being absent from Rototai, however some differences were noted in the genera present in the lakes. *Paludibacter* (phylum Bacteroidota) was present in Rototai with a relative abundance of 7%, while it was absent from the dataset in Killarney and Kaihoka East (with negligible 0.2% in Peel). This anaerobic genus includes sulfate reducing species which have been speculated to play key roles in sulfate removal from wastewater sludge (Liang et al. 2013) at low pH. The presence of this genus in the sediment of Rototai, which has both high sulfate and low pH, would indicate that this taxon is playing a key role in the biogeochemical cycling in lake sediments as well as wastewaters. The genus *Sulfuricurvum* is a sulfur oxidizer, using nitrate as an electron acceptor in anaerobic environments, and was present in the sediments of Rototai, while absent from the data in the other three lakes. Previous work has shown this genus to contribute substantially to the microbial community in bioreactors especially in those with varying pH (pH 2–6) (Salo and Bomberg 2022). It has also been shown to be negatively correlated with total nitrogen concentrations, which would agree with the current results as Rototai has the lowest levels of total nitrogen in the current study (Wang et al. 2019). Heavy metals have been shown to impact the microbial community structure of lakes (Zhang et al. 2018) and work in a shallow lake system in China have shown that the genus *Smithella* was positively correlated to several heavy metals (zinc, cobalt and lead; (Wang et al. 2019). This is in agreement with our results where the relative abundance of *Smithella* was highest in Killarney which is characterised by elevated levels of heavy metals in the sediments and indicates that this genus is heavy metal tolerant. In recent times, blooms of cyanobacteria in lakes are becoming an increasing problem (Picard et al. 2022). In three of the study lakes, with the exception of Rototai, the picocyanobacterial genus *Cyanobium* was present with highest relative abundances in Kaihoka East. The absence of cyanobacteria in Rototai agrees with previous work showing that planktonic cyanobacteria are absent from lakes with low pH (Brock 1973; Steinberg et al. 1998). As well as these genera level differences, both the taxonomic and functional multivariate analysis showed that there were significant differences in the communities (at the ASV/gene level) amongst lakes. While the pairwise comparisons did not have the statistical power to evaluate which lakes were different from each other, the multivariate plots indicated that Rototai (low pH) and Killarney (high metal concentrations) were distinct from each other, and the other two lakes. This confirms our hypothesis that these two lakes, which are experiencing extreme environmental pressures, showed distinct taxonomic and functional profiles.

Secondly, we hypothesised that pathways for acid resistance, nutrient cycling and metal tolerance would be elevated in the acid lake and the metal- and nutrient-rich lake. We observed that the number of genes indicative of sulfur cycling were highest in Rototai which also had the highest concentration of sulfur in the sediment. Considerably higher abundances of sulfur cycling genes for both dissimilatory sulfur reduction and sulfur oxidation were observed in Rototai, while gene abundance for assimilatory sulfur reduction was also highest in Rototai. Sulfate

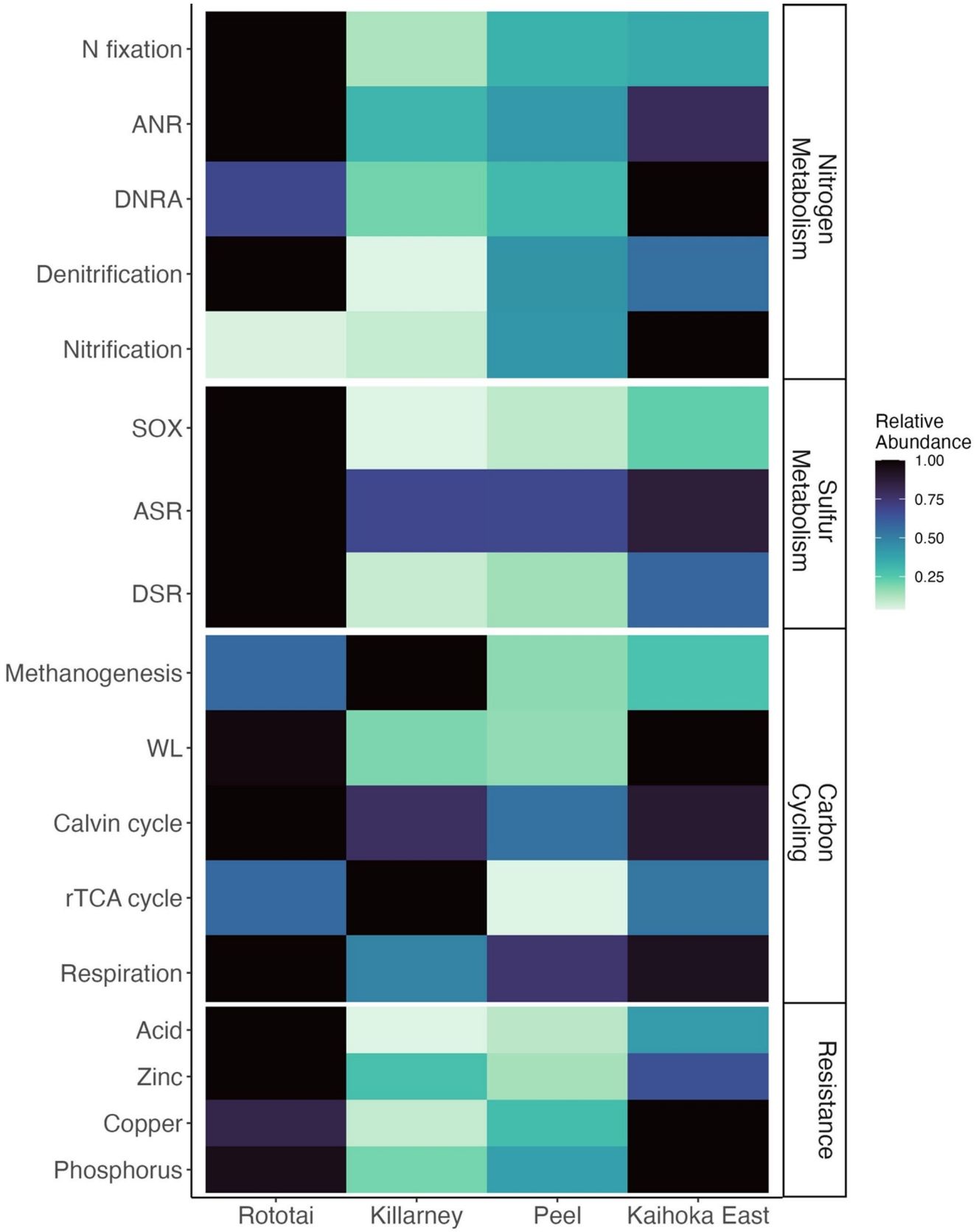
N fixation
ANR
DNRA
Denitrification
Nitrification
SOX
ASR
DSR
Methanogenesis
WL
Calvin cycle
rTCA cycle
Respiration
Acid
Zinc
Copper
Phosphorus
Rototai
Killarney
Peel
Kaihoka East
Nitrogen Metabolism
Sulfur Metabolism
Carbon Cycling
Resistance
Relative Abundance
1.00
0.75
0.50
0.25

Fig. 5 Heatmap of the differentially expressed genes relating to selected metabolic processes in the four study lakes (Tasman region, Aotearoa New Zealand). See Supplementary Table 2 for values. N = nitrogen; ANR = Assimilatory Nitrate Reduction; DNRA = Dissimilatory Nitrate Reduction; SOX = sulfur oxidation; ASR = assimilatory sulfur reduction; DSR = dissimilatory sulfur reduction; rTCA = reverse tricarboxylic acid; WL = Wood–Ljungdahl pathway

reducing bacteria are predominantly anaerobes, and while genus level identification of the sulfate reducing genes could not be obtained in the current study the high relative abundance of *Paludibacter* in the metabarcoding data suggests that this genus could play a vital role in sulfur cycling in anoxic regions of the sediment. Sulfur oxidation was also observed to be higher in Rototai, with the metabarcoding results suggesting that *Sulfuricurvum* was the predominant sulfur oxidizer in the lake. This genus can utilise nitrate as an electron acceptor in anaerobic environments for the oxidation of sulfur although cannot be ruled out that other taxa are responsible for sulfur oxidation in oxic/anoxic transition zones, as reduced sulfur produced in the anoxic zones diffuses into the oxic regions where it can be oxidised (Holmer and Storkholm 2001). Indeed, studies have shown that often high proportions of reduced sulfur are re-oxidised in lakes (reviewed in Holmer and Storkholm (2001). Sulfur reduction can play an important role in the decomposition of organic matter in lakes if the amounts of sulfate, or other suitable electron acceptors (e.g., thiosulfate, nitrate, iron) and organic matter are sufficient, however, methanogenesis often plays a greater role when suitable electron acceptor levels are insufficient (Purdy et al. 2003; Sinke et al. 1992). In Lake Killarney, the methanogenesis genes were related to archaeal methanogens in general and indicate in this lake environmental conditions are increasing the contribution of methanogenesis to the decomposition of organic matter compared to the other lakes.

Phosphorus is a key element in cell membranes and genetic material, and can be a limiting factor to growth in freshwater systems (Reynolds and Davies 2001). Lake sediments often comprise a large reservoir of legacy nutrients, which can play critical roles in lake water quality and health (Waters et al. 2023). The ratio of TN: TP can act as an indicator of which nutrient is deficient, with higher ratios indicating phosphorus as a limiting factor (Guildford and Hecky 2000). This aligns with our current results where a positive relationship between the abundance of phosphate related genes and the TN: TP ratio was observed, despite these trends being mainly not significant. Further work across a broader selection of lakes would be required to ascertain if this observation can be generalised beyond the lakes studied and if the trend becomes significant when more lakes are included. It should also be noted that the lack of statistical significance in the current study could also be due to differences in the amount of bioavailable phosphorus, as a fraction of the total phosphorus. Further work is also required to understand the role of phosphorus bioavailability on the abundance of phosphate transporters.

Metals are non-degradable pollutants that can exist in the sediment as free cations, soluble complexes with inorganic and organic ligands or associated with colloidal material (Muñoz-García et al. 2022). While metals are important for biochemical functions, they can also have toxic effects on organisms and stimulate the production of damaging reactive oxygen species (Muñoz-García et al. 2022). In the current study, negative correlations were observed between copper resistance genes and the concentration of copper in the sediment. This is a result that has previously been observed in mangroves (Muñoz-García et al. 2022) however no trend was observed in marine and river sediments (Roosa et al. 2014). Firstly, the concentrations of the metal ions in the sediment may not reflect the bioavailability of that metal (Roosa et al. 2014). As the resistance genes would only be affected by concentrations of bioavailable metals, this could alter the correlations observed. Secondly, the resistance genes are not totally specific to a single metal but are also responding to other metals (e.g., nickel, cobalt or cadmium; (Muñoz-García et al. 2022). While nickel and cobalt were not measured in the current study, cadmium concentrations followed a similar trend to copper so is unlikely to explain the lack of a positive correlation between the resistance genes and metal concentrations in the sediments. Lastly, other environmental factors may be playing a role in the prevalence of resistance genes in the metagenome. For instance, *copA* requires oxygen to function (Roosa et al. 2014) which is likely to be less prevalent in anoxic sediments compared to oxic environments. Lake Killarney had very high levels of zinc compared with the other lakes. Because of this, we investigated the metagenomic responses to this metal, as well as any resistance/tolerance response. The genes *zntA* and *czcB* showed very low positive/no correlation with zinc concentrations. These genes have been shown to be involved in the efflux of zinc from the cytoplasm when zinc levels are high in the environment (Hantke 2001). The lack of correlation could be due to other metals stimulating the activity of the transporter (for instance lead in the case of *zntA* (Blencowe and Morby 2003) and cadmium for *czcB*), thus affecting specific correlations. As metagenomics only looks at the functional potential, future work using metatranscriptomics could investigate if there is a correlation between the transcription of the resistance genes and zinc concentrations. The other zinc efflux mechanism, *zitB*, had a non-significant negative correlation with zinc but also transports cobalt and cadmium so is likely to be affected by concentrations of these metals as well as zinc. The negative correlation of *znuC* with zinc is expected as this gene is involved in the uptake of zinc into the cell and

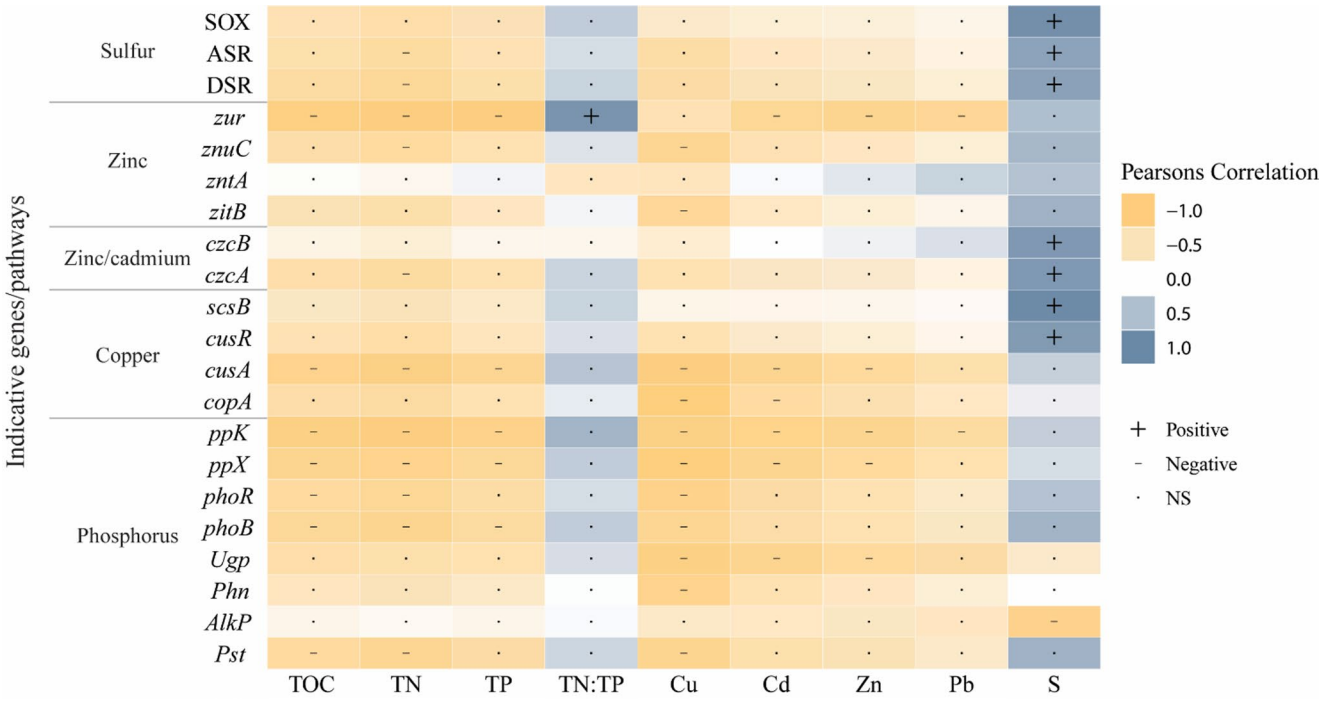

Fig. 6 Pearsons' correlations analysis between various indicative genes/pathways and lake surface sediment geochemistry for the four lakes combined. DSR– dissimilatory sulfate reduction; ASR– Assimilatory sulfate reduction; SOX– sulfur oxidation; TOC– total organic carbon; TN– total nitrogen; TP– total phosphate; Cu– copper; Cd– Cadmium; Zinc– Zinc; Pb– Lead; S– sulfur. **NS–** non-significant

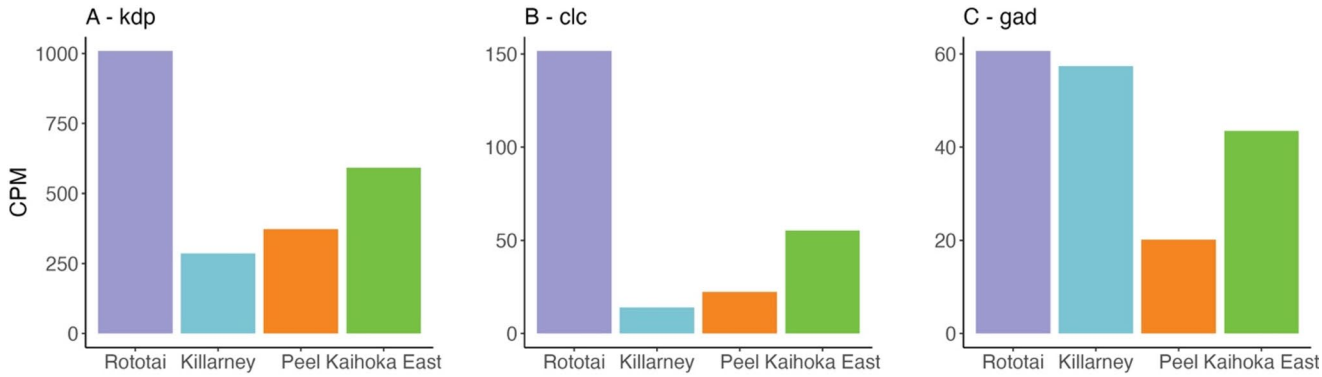

Fig. 7 Number (total count per million - CPM) of genes related to acid resistance in the four study lakes. (A) potassium-transporting ATP-ase (*kdp*), (B) Chloride-hydrogen (Cl^-/H^+) antiporters (*clcA*), and (C) glutamate decarboxylase system (*gad*)

has been shown to be regulated by zinc concentrations (Pederick et al. 2015).

Rototai is a flooded sinkhole with a pH of 4 and organisms inhabiting this lake are expected to show responses to acid stress. Acid tolerance is often created by maintaining a circumneutral cytoplasmic pH despite the proton gradient between the cytoplasm and environment (Boase et al. 2022). One of the first line of defence to acid stress is the development of a positive membrane potential that inhibits positively charged protons from crossing the cell membrane (Vergara et al. 2020). This is often generated by the accumulation of potassium ions (K^+) through transporters such as kdp channel proteins (Vergara et al. 2020). The current study, showed that *kdp* related genes were more abundant in Rototai compared to the other lakes. This aligns with other studies showing that many acidophiles actively transcribe the *kdp* system in acidic environments (Rivera-Araya et al. 2020; Vergara et al. 2020). Another potential mechanism that has been observed in acidophiles to maintain a circumneutral cytoplasm is the removal of H^+ ions from the cytoplasm using proton pumps such as voltage gated ClC-type Cl^-/H^+ transporters (e.g., *clcA*) (Boase et al. 2022). Similarly to the kdp system, the highest counts per million were observed in Rototai with considerably lower abundances in the other lakes and indicating this is another potential mechanism for acid tolerance in the lake. A further method for reducing

protons in the cytoplasm is through the use of the glutamate decarboxylase system (gad) (Foster 2004). This system uses H^+ to replace the *a*-carboxyl group of glutamate, thus reducing the level of H^+ in the cytoplasm (Boase et al. 2022). In this study, while Rototai had the highest abundances for the *gad* genes, it was not substantially different from the other lakes. While the role of glutamate decarboxylase in acid stress is well documented and is likely to play a role in acid tolerance in Rototai, it may be responding to other stresses (e.g., oxidative stress; (Boura et al. 2020) in the other lakes thus accounting for its elevated abundances in these lakes.

In conclusion, this study demonstrates how environmental stressors such as low pH and elevated metal concentrations can shape microbial communities and their metabolic potential in lake sediments. In relation to hypothesis one, we showed that there was a distinct community structure and function in Rototai (pH 4) and Killarney (heavy metal- and nutrient-rich) compared to Kaihoka East and Peel. This reflects the distinct ecological pressures that are being exerted on the communities in those lakes which is driving the presence of specialized taxa and functional adaptations. For the second hypothesis, we showed that there was a higher abundance of acid resistance genes in Rototai, as we expected. However, the relationship between metal resistance genes and metal concentrations in the sediment was less clear with various factors potentially affecting the correlations. These findings highlight the complex interplay between environmental drivers and microbial communities, demonstrating the utility of metagenomic approaches in examining microbial responses to varied freshwater habitats.

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Declarations

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