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## Abstract

Trace metals are required as co-factors in metalloproteins that are essential in microbial metabolism and growth. The microbial requirements of diverse metals and the capabilities of prokaryotic taxa to acquire these metals remain poorly understood. We present here results from metagenomic observations over an entire productive season in the region off Kerguelen Island (Indian Sector of the Southern Ocean). We observed seasonal patterns in the abundance of prokaryotic transporters of 7 trace elements (Zn, Mn, Ni, Mo, W, Cu, Co) and the consecutive spring and summer phytoplankton blooms were strong drivers of these temporal trends. Taxonomic affiliation of the functional genes revealed that the transporters of these trace metals were associated to different microbial assemblages. Our observations suggest that *Rhodobacteraceae* had a broad repertoire of trace metal transporters (Mn, Zn, Ni, W, Mo) and a more restricted set was observed for other prokaryotic groups, such as *Flavobacteriaceae* (Zn), *Nitrincolaceae* (Ni, W) and *Thioglobaceae* (Mo). The prevalence of one or more trace metal transporters within a prokaryotic group, as determined on the family level, was overall confirmed in representative metagenome assembled genomes. We discuss the potential involvement of prokaryotic groups in processes related to organic matter utilization that require these metals and the consequences on carbon and trace metal cycling in surface waters of the Southern Ocean.

## Introduction

The metals zinc (Zn), manganese (Mn), nickel (Ni), cobalt (Co), copper (Cu), molybdenum (Mo) and tungsten (W) are co-factors in metalloenzymes that drive the biogeochemical cycling of major elements. Diverse metal-containing enzymes are involved in the transformation and processing of dissolved organic matter (DOM), the major pool of organic carbon in the ocean. The utilization of different types of organic substrates within the DOM pool, covering a range of size fractions and varying degrees of bioavailability, is mediated by prokaryotes through pathways with distinct metal requirements. Zn is involved in carbohydrate-active enzymes (CAZymes) that play an important role in the degradation of polysaccharides (Hobbs et al. 2023), while Mn is required in the central carbon metabolism (Hansel 2017). Ni and Mo are both essential for nitrogen fixing microbes (Kuypers et al. 2018) and these elements are further involved in the utilization of nitrogen-containing substrates (Ni) and in carbon monoxide oxidation (Mo) (King and Weber, 2007). Methylotrophs require W for the utilization of substrates with no carbon-carbon bonds, including C1 compounds (Chistoserdova 2011). Cu, a catalyst in numerous cuproenzymes, has a major involvement in the respiratory enzyme cytochrome c oxidase in aerobic microbes (Ridge et al. 2008), and Co is mainly used as a key component of cobalamin (vitamin B12) synthesis (Bertrand et al. 2015). Despite the essential role of these elements in microbial metabolism and the cycling of DOM, little is known about the capacities of diverse prokaryotes to acquire these metals.

The key role of the trace element iron (Fe) for microbial communities in large parts of the ocean has motivated research on trace metal uptake mechanisms. The search for genes involved in the acquisition of different forms of Fe in individual genomes and metagenomes revealed that $Fe^{3+}$ transporters were common in marine prokaryotic genomes, while transporters for siderophore-bound Fe and heme were more abundant in *Gammaproteobacteria* and *Bacteroidetes* as compared to *Alphaproteobacteria* (Hopkinson and Barbeau, 2012). Exploring metagenome assembled genomes (MAGs)

from the Southern Ocean extended this view to taxonomy-specific differences in Fe-acquisition strategies (Sun et al. 2021). The use of distinct Fe-uptake mechanisms was further supported by metatranscriptomics approaches (Debeljak et al. 2019; Sun et al. 2021). By contrast, the ecological strategies of prokaryotic taxa for the uptake of other essential trace metals has been less investigated. A genomic study revealed pronounced differences in the capacity of metal transport between *Roseobacter* and SAR11 (Hogle et al. 2016). While *Roseobacter* genomes contained transporters for several metals (Fe, Co, Ni, Cu, Zn), a reduced repertoire was present in SAR11 genomes (Hogle et al. 2016). The potential role of *Rhodobacteraceae* in trace metal cycling was confirmed by gene expression patterns in experimental incubations (Debeljak et al. 2021). *Rhodobacteraceae* were major contributors to the transcripts of genes associated to the transport, sensing and regulation of nine metals (Zn, Mn, Cu, Co, Ni, Cd, Mo, W, and Fe) in Southern Ocean microbial communities (Debeljak et al. 2021). The diverse metabolisms present in marine microbial communities are likely strong drivers of the acquisition strategies of prokaryotic taxa, which in turn has implications on their role in the cycling of these elements in the ocean.

The main objectives of the present study were to identify genes encoding for the transport of seven metals (Zn, Mn, Ni, Mo, W, Cu, Co) in Southern Ocean prokaryotic communities, to investigate their temporal patterns over an entire productive season and to identify the key microbial players for each metal considered. We therefore searched for the respective genes in metagenomes obtained from microbial communities collected with an autonomous sampler in surface waters of the Central Kerguelen Plateau. Our observations are discussed with respect to a previous study in which we investigated the seasonal dynamics of Fe and organic substrate acquisition gene repertoires (Zhang et al. 2023).

## Experimental procedures

### Sample collection, DNA extraction and metagenome sequencing

We used 12 metagenomes obtained from seawater samples collected by the Remote Access Sampler (RAS) in surface waters (40 m) above the Kerguelen plateau (Station A3) in the Indian sector of the Southern Ocean (Blain et al. 2021). The details of DNA extraction and metagenome sequencing are given in the study by Liu et al. (2020) and Zhang et al. (2023). The data sets are available in the European Nucleotide Archive (ENA) repository at https://www.ebi.ac.uk/ena under the project ID PRJEB56376.

### Metagenome assembly

Quality control (QC) passed reads from each sample were individually assembled by MEGAHIT v1.2.9 with default settings and metaSPAdes v3.15.2 (Nurk et al. 2017, parameters used -k 21, 33, 55, 77, 99, 127). After comparing the length of N50 produced by two tools (N50-MEGAHIT=690 bp, N50-metaSPAdes=935 bp), we finally used the metaSPAdes assembly results for analysis.

### Metagenomic functional analysis

Open reading frames (ORFs) were predicted using Prodigal v2.6.3 (Hyatt et al. 2010, parameters used -p meta). CD-HIT v4.8.1 (Li & Godzik, 2006, parameters used -c 1 -aS 1 -g 1) was used to construct a non-redundant protein database. A total of 8.9 Mio non-redundant protein-coding genes were identified for all samples. Genes per kilobase million (GPM) were used as a proxy for gene abundance as described in Zhang et al. (2023). In order to identify the transporter sequences resulting from the individual metagenomic assemblies, predicted protein sequences were aligned using eggNOG 5.0 (Huerta-Cepas et al. 2019) and GhostKOALA (Kanehisa et al. 2016) with default parameters. All KEGG orthologous (KO) numbers defined as “Transporter” in KEGG were retrieved, and metal transport-related genes were verified by reviewing previous literature and manually checking the metal transport information in the KEGG database

(Table S1). We then focused on genes related to the transport of trace metals into the cell, except for Co and Cu (see below). We used the *sitABCD* complex (Mn/Fe; Table S1) to refer to Mn transporters (Papp-Wallace & Maguire, 2006), as other Mn transporter proteins (e.g. *mnt*, *tro*) had low abundances or were undetectable in our sequences. Zn-transporters were identified based on ZIP2 (Grass et al. 2005) and *znuABC* (Hantke et al. 2001). While the ZIP family is known to contain several general trace metal transporters, the ZIP2 we used has been characterised as being specific for Zn (Grass et al. 2005). *Cbik* was used to refer to Ni transporters (Rodionov et al. 2006). Mo-transporters were identified based on *modABC* (Grunden & Shanmugam, 1997) and W-transporters were identified based on *tupAB* (Hagen, 2023). Genes for Co uptake were low and no Cu uptake genes were detected in our metagenomes, we therefore only considered the efflux systems of both metals and present the seasonal patterns for *cusABC* (for Cu) and *cnrABC*, *czcD* (for Co) (Franke et al. 2003; Okamoto & Eltis, 2011).

Metagenomic taxonomic analysis and MAGs analysis

Taxonomic classification of metal-related genes was performed according to the Genome Taxonomy Database (Parks et al. 2022) using Centrifuge v1.0.4 (Kim et al. 2016) and the alignments with highest score were kept. We mainly considered the family level and used metagenome-assembled genomes (MAGs) for a more fully resolved description. One MAG assigned to *Flavobacterium*, *Nitrincolaceae*, *Porticoccaceae*, SAR11, *Sphingomonadaceae* and *Thioglobaceae*, and two MAGs assigned to *Polaribacter* as well as four MAGs assigned to *Rhodobacteraceae* were downloaded from data previously published by Sun et al. (2021). Following the method described in the analysis of MAGs by Zhang et al. (2023), functional annotations of MAGs were performed and the abundances of MAGs and their metal transport-related genes across the 12 metagenomes were calculated. The coverage of the MAGs in each metagenome sample was determined by aligning reads from each sample back to the MAGs using Bowtie2 (Langmead & Salzberg, 2012). The mean coverage for each

MAG was then calculated from these alignments using anvi'o v7.1. This mean represents the average depth of coverage across contig, calculated as the coverage of each nucleotide in a contig divided by the length of the contig. Visualization of data was performed using the packages ggplot2 in the R v4.2.1 and SigmaPlot v14.5 software.

# Results

## Environmental context

The remote autonomous sampler (RAS) was deployed at station A3, located above the Kerguelen plateau southeast of Kerguelen Island (50.63°S, 72.06°E; overall depth 527 m) in the Indian Sector of the Southern Ocean (Fig. 1). Samples were collected at 40 m depth from 25 October 2016 to 24 February 2017 covering the entire productive season. During this time period temperature increased steadily from 4°C in early spring to 6° late summer (Fig. S1). The high concentrations of silicic acid at the beginning of the season (20 µM) rapidly decreased during spring (to 5 µM), while concentrations of nitrate remained elevated over the course of the season (20 to 30 µM) (Blain et al. 2021) (Fig. S1). Two consecutive phytoplankton blooms occurred during the sampling period, a spring bloom with a peak in mid-November and a summer bloom in early January (Fig. 1). Diatoms were the main contributors to phytoplankton biomass and revealed pronounced successional changes in community composition (Blain et al. 2021). Seasonal dynamics in prokaryotic community composition were strongly related to phytoplankton biomass and composition (Liu et al. 2020).

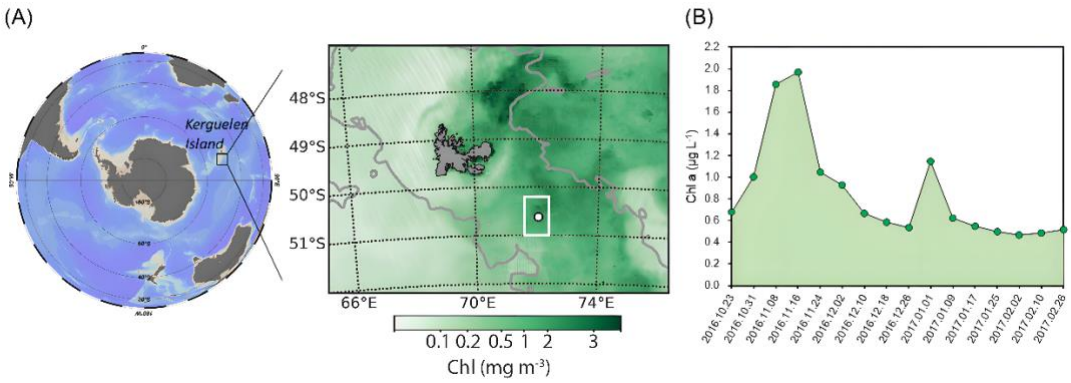


Figure 1. (A) Regional view of the mooring site on the Central Kerguelen plateau. The white dot denotes the position of the RAS mooring overlaying monthly chlorophyll for November 2016. Grey lines are the 1000 m isobaths. (B) Temporal changes of Chlorophyll *a* (green shaded area) during the period of the RAS deployment. Modified from Blain et al. (2021) and Liu et al. (2020).

## Seasonal dynamics in trace element transporters

In a previous study we determined the temporal patterns of genes related to the uptake of different forms of iron (Fe) and of genes involved in the cleavage and uptake of organic substrates (Zhang et al. 2023). We focus here on genes related to the transport of the metals Mn, Zn, Ni, Mo, W, Co and Cu. A total of 89 metal transport related KEGG orthologous (KO) genes were retrieved from the KEGG database. Among these, 67 KO genes were detected in our dataset and their normalized abundances varied between 0.2 to 760 genes per kilobase million (GPM) (Table S1, Fig. S2). We set a threshold for the further analyses with the aim to consider only the most abundant genes. We therefore considered only those KO genes for which the sum of the respective GPM in the 12 metagenomes was > 45 GPM; this resulted in 26 KO genes (Table S1). The majority (84 %) of these transporters are metal uptake genes, and we also detected several efflux genes. In the case of Co, uptake genes had low abundance (< 40 GPM) in our metagenomes, and no Cu uptake genes were detected.

Seasonal patterns of *sitABCD* (Mn/Fe transporters) revealed a sharp increase in GPM during the spring bloom and remained high until the summer bloom, followed by a rapid decline and a second smaller peak after the summer bloom (Fig. 2A). Transporters of Zn (*znuABC* and ZIP2*)*, Ni (*cbiK)* and W (*tupAB*) increased after the spring bloom and their temporal patterns matched those of Mn over the remaining season (Fig. 2B, C, D). By contrast, transporters of Mo (*modABC*) had low GPM during the spring period, and revealed a small increase prior to and a marked peak after the summer bloom (Fig. 2E). As for Cu and Co, their efflux genes (*cusABC* for Cu and *cnrABC*, *czcD* for Co) had seasonally high GPM prior to and after the spring and summer blooms (Fig. 2F).

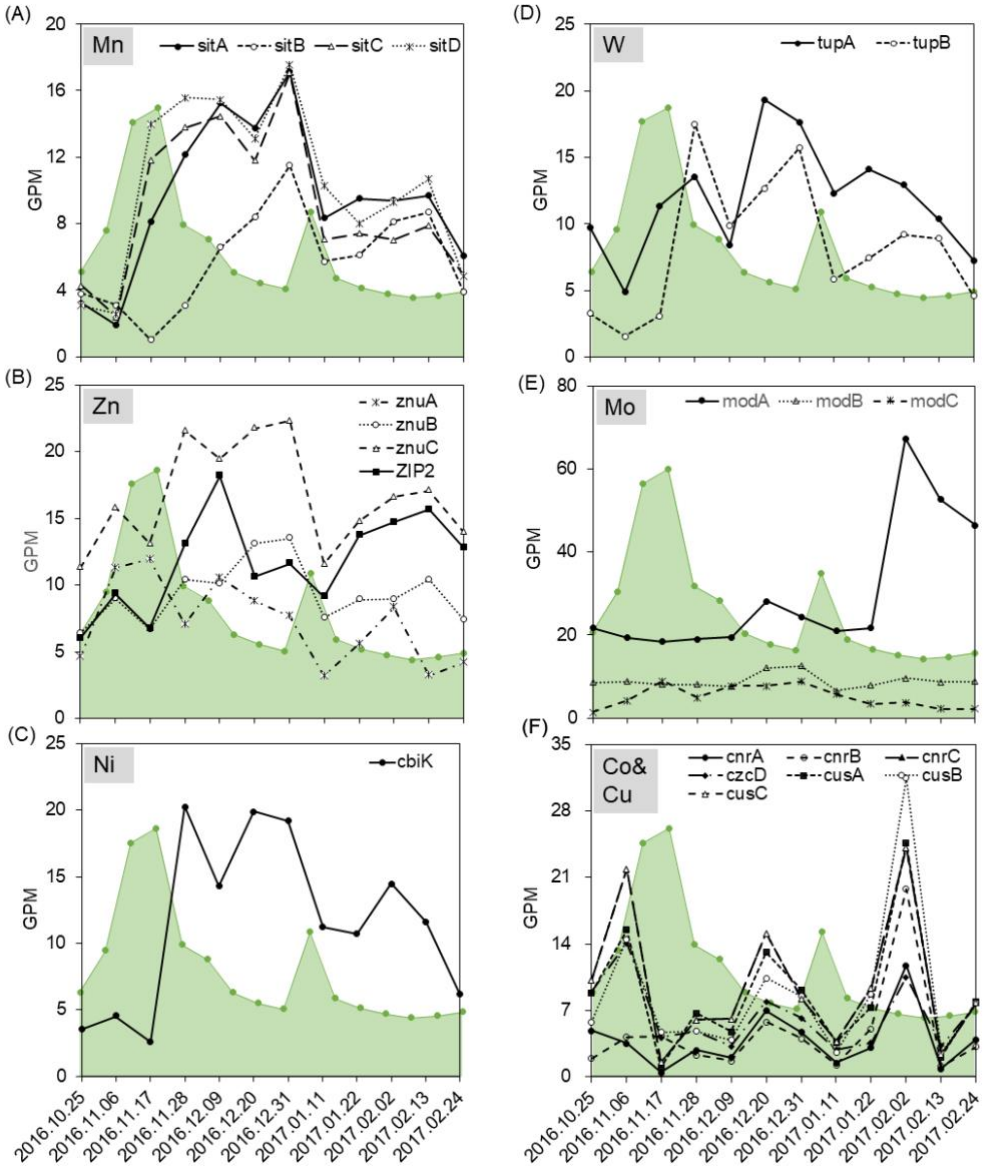


Figure 2. Temporal changes of Chlorophyll a (green shaded area) and abundance of genes for Mn transporters (A), Zn transporters (B), Ni transporters (C), W transporters (D), Mo transporters (E), and Co and Cu efflux transporters (F). Normalized gene abundances are given in genes per kilobase million (GPM).

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200 Taxonomic affiliation of transporter genes

201 The taxonomic affiliations revealed pronounced differences in the relative contribution
202 of prokaryotic groups, at the family level, to the transporters of different trace metals
203 (Fig. 3). *Porticoccaceae* were the dominant contributors to *sitABCD* (Mn/Fe) during

the peak of the spring bloom and its early declining phase and *Rhodobacteraceae* had an equal share during the remaining season. In the case of Zn, a contrasting picture was obtained for the two types of transporters identified in our metagenomes. ZIP2 were predominantly attributed to *Flavobacteriaceae*, with the exception of the start of the spring bloom when *Pseudomonadaceae* had high contributions to ZIP2 transporters. By contrast, taxonomic affiliations were more diverse for *znuABC* with seasonally changing contributions of *Pseudomonadaceae*, *Porticoccaceae*, *Rhodobacteraceae* and *Pelagibacteraceae.* The taxonomic assignments of *cbiK* (Ni) were dominated by *Nitrincolaceae* after the spring bloom and *Rhodobacteraceae* and *Pelagibacteraceae* were dominant contributors during the remaining season. Taxonomic assignments of *tupAB* (W) were similar to those of *cbiK*. Transporters of Mo (*modABC*) were affiliated to *Thioglobaceae*, *Pseudomonadaceae*, *Porticoccaceae*, and *Rhodobacteraceae* throughout the season. *Pseudomonadaceae* and *Sphingomonadaceae* had similar contributions to Co and Cu efflux transporters (Fig. S3).

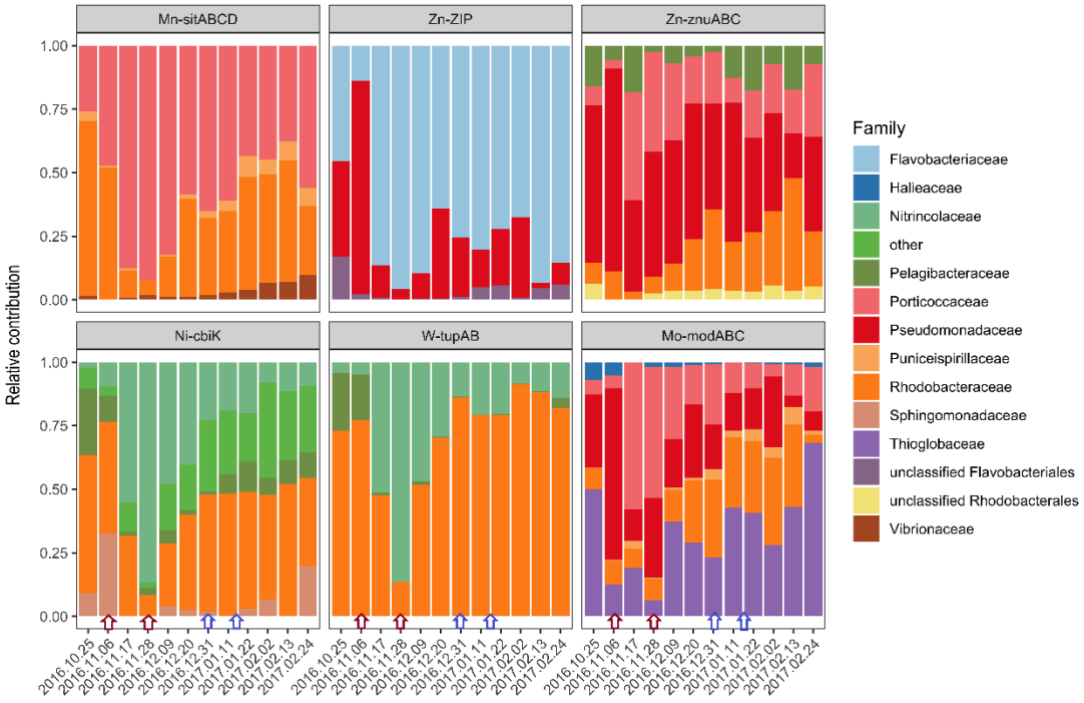


Figure 3. Relative contribution of prokaryotic groups to specific genes associated with Mn (*sitABCD*), Zn (ZIP2 and *znuABC*), Ni (*cbiK*), W (*tupAB*) and Mo (*modABC*) transporters. Prokaryotic groups are based on the family level. Red arrows

indicate the spring phytoplankton bloom, and blue arrows indicate the summer phytoplankton bloom.

Metal acquisition genes in metagenome assembled genomes (MAG)

To identify the inventories of metal acquisition genes in individual prokaryotic taxa, we used previously published MAGs obtained from samples collected in the Kerguelen region, including our study site at the time point of the RAS deployment (Sun et al. 2021). We chose 12 MAGs belonging to *Flavobacteriaceae*, *Nitrincolaceae*, *Porticoccaceae*, *Rhodobacteraceae*, SAR11, *Sphingomonadaceae* and *Thioglobaceae* (Fig. 4). These MAGs were chosen because they belong to prokaryotic groups with a substantial contribution to the different gene categories (Fig. 3). Moreover, ASVs belonging to these prokaryotic groups were shown to be abundant and have distinct seasonal relative abundance patterns (Liu et al. 2020). For some abundant prokaryotic groups with contributions to the genes of interest, as for example *Pseudomonadaceae*, no MAGs were previously annotated (Sun et al. 2023). Our results indicate that the *Rhodobacteraceae* MAGs (MAG 20, 23, 52, 106) had the most diverse and abundant metal transport gene inventories, including transporters of Mn, Zn, Ni, W, Mo, and Co, while MAGs belonging to other families had a reduced set of transporter genes. Two of the *Rhodobacteraceae* MAGs (20 and 52) had relatively high read-based coverage during the late declining stage of the spring bloom, while MAG106 had a less pronounced seasonal pattern. We detected Zn transporters (*znuABC* and ZIP2) in two *Flavobacteriaceae* MAGs (*Flavobacterium* MAG16 and *Polaribacter* MAG88) that had both highest coverage after the spring bloom. In addition, *Flavobacteriaceae* MAG16 harbored genes for Cu and Co efflux transporters. *Nitrincolaceae* MAG115 contained transporters for Zn (*znu*), Ni (*cbik*) and W (*tup*) and had the highest coverage of all MAGs considered here, peaking during the early spring bloom decline. A contrasting seasonal coverage was observed for SAR11 MAG133 for which the W transporter *tup* was detected. *Sphingomonadaceae* MAG116 and *Thioglobaceae* MAG136 harbored both the Ni transporters (*cbik*), and MAG116 also contained *mod*.

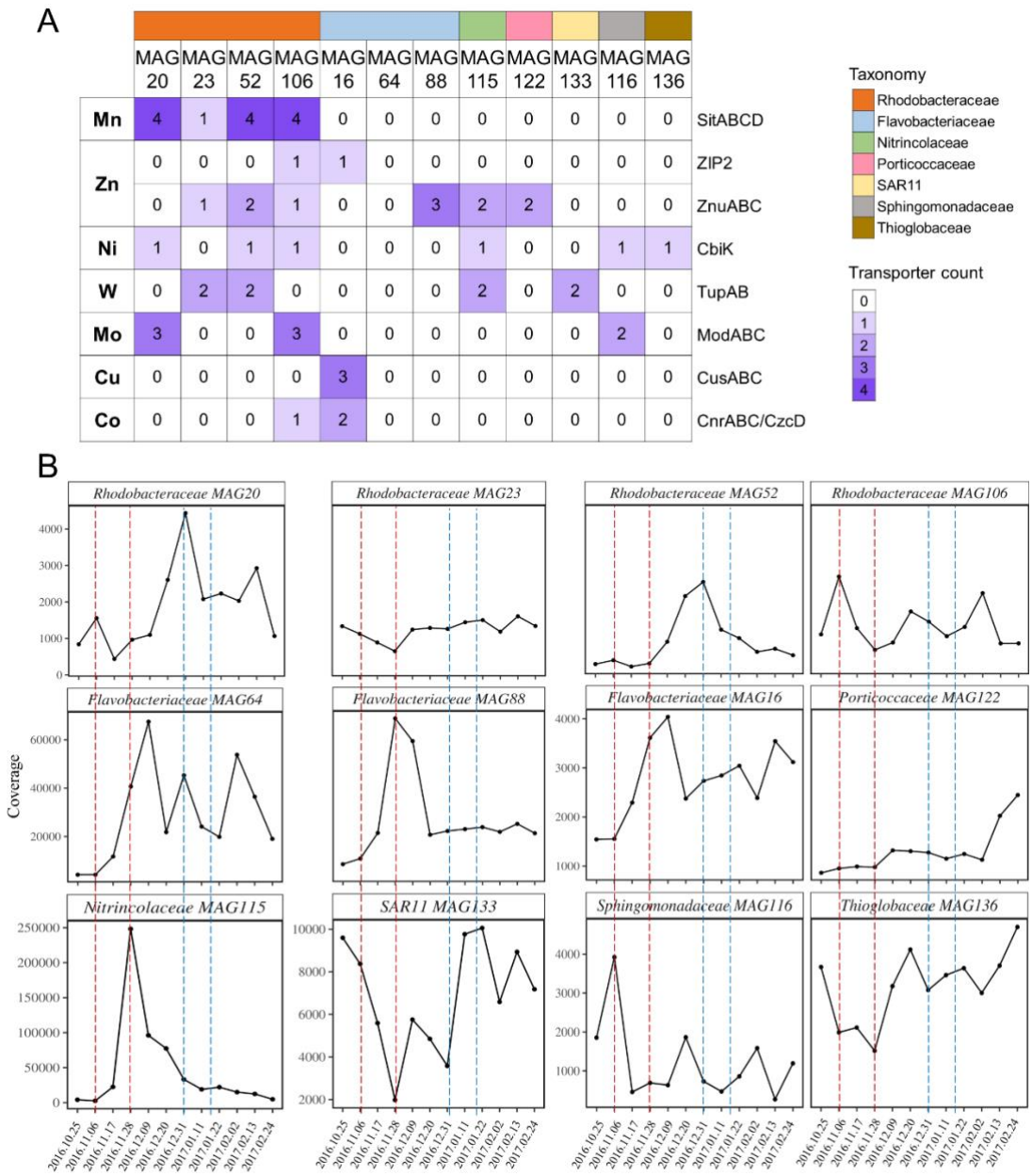


Figure 4. The distribution of specific genes related to Mn, Zn, Ni, W, Mo, Cu and Co transporters in the twelve MAGs. The square blocks are colored according to the gene counts (A). The coverage of MAGs in the twelve samples. The red lines represent the first spring phytoplankton bloom, and blue lines represent the second summer phytoplankton bloom. (B).

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# Discussion

We observed marked seasonal patterns of prokaryotic transporter genes for the metals Mn, Zn, Ni, W and Mo in the Southern Ocean. These metals are key for metalloenzymes involved in diverse metabolic pathways. Among these are pathways linked to the degradation, uptake and processing of the diverse pool of organic substrates that constitute the main source of carbon and energy for heterotrophic microbes. Concentrations of dissolved Fe (~ 0.1-0.3 nM) (Blain et al. 2007, Quéroué et al 2015), Mn (~ 0.3 nM; Zhang et al., under revision) are in the picomolar range in surface waters at our study site, and those of Zn (~ 2 nM) and Ni (~ 6 nM) in the lower nanomolar range (Wang et al. 2019). Mo is present at higher concentrations in marine surface water (~ 100 nM) (Matsuoka et al. 2023) than W (~ 50 pM; Matsuoka et al. 2023). Iron is essential for microbial respiration, and it has been shown that this element can be limiting for microbial growth in Southern Ocean surface waters (Obernosterer et al. 2015, Baltar et al. 2018). However, whether this is the case for the metals considered here is not known. Due to their overall higher concentrations and probably lower cellular requirements due their utilization in specific pathways, as opposed to the wider use of Fe as a redox catalyst, it is unlikely that they are direct limiting factors of microbial growth (Baltar et al. 2018). The seasonal patterns observed in our study therefore rather reflect the requirements of a given trace element for the processing of the seasonally changing amount of DOM and the diversity of organic substrates present within this pool. Our results further allow us to identify abundant prokaryotic groups with the capacity to transport a given trace element. A high contribution of a given prokaryotic group to a trace metal transporter could illustrate its competitiveness driven by trace-metal acquisition under a specific bloom stage. Given the key role of DOM in driving prokaryotic growth and community composition in this ocean region (Landa et al. 2016), we will focus our discussion on a few prominent biogeochemical processes within the marine carbon cycle involving trace metals and the potential microbial key players.

Zn and Mn are contained in enzymes with distinct roles in the cleavage and processing of organic carbon. Zn plays an important role in catalytic processes to access organic matter. About half of the Zn-bound proteins are enzymes, among which hydrolases make up more than 50% (Andreini et al. 2012). The Zn enzymes alcohol dehydrogenase and carboxypeptidase belong to glycoside hydrolase (GH) families of CAZymes, and include GH18 and GH19 responsible for the decomposition of chitin (Nguyen et al. 2019). We identified both the high-affinity Zn-uptake system, encoded by the *znuABC* genes (Hantke 2001) and the low-affinity ZIP2 transporter (Grass et al. 2005). ZIP2 transporters had a seasonal trend that closely matched that of CAZymes (Fig. S4) (Zhang et al. 2023) pointing to increased Zn-requirements for the degradation of complex substrates. *Flavobacteriaceae*, including *Flavobacterium* and *Polaribacter*, were the main contributors to ZIP2 (Fig. 3 and Fig. S5) and this family accounted for up to 30% of the CAZymes during the bloom decline (Zhang et al. 2023). The 3 *Flavobacteriaceae* MAGs all peaked after the spring bloom (Fig. 4B) and 2 MAGs (MAG16 and MAG88) harbored genes for Zn transporters (Fig. 4A). These results highlight that *Flavobacteriaceae*, through their well-documented capacities to degrade various polymeric substrates (Xing et al. 2015; Krüger et al. 2019) could play an important role in the cycling of Zn.

Mn is involved in the central carbon metabolism, phosphorylation and peptide cleavage (Hansel 2017). Phosphoenolpyruvate synthase uses Mn as a cofactor and pyruvate carboxylase is a Mn-containing enzyme, and both are critical in gluconeogenesis (Hansel 2017). We detected the gene *sitABCD* in our metagenomes, a protein transport system that is not specific to Mn, but can potentially transport also Fe (Sabri & Dozois, 2006). Using a set of transporters of $Fe^{3+}$ (*fbp*, *futA1/A2*, *fbpB-futB*) and $Fe^{2+}$ (*feo*, *efeUOB*) (Fig. S8, Zhang et al. 2023) revealed a seasonal pattern that differed from that observed for *sit* in the present study (Fig. 2A), suggesting that the temporal dynamics of *sit* likely reflect Mn transporters. The rapid increase in Mn transporters during the spring bloom and their continuously high levels throughout the declining phase is likely

related to the processing of readily accessible phytoplankton-derived organic substrates. *Porticoccaceae* and *Rhodobacteraceae* were identified as main contributors to *sit* transporters, but at distinct time periods (Fig. 3). The temporal pattern of the contribution of *Porticoccaceae* to *sit* transporters closely matched the dynamics of an ASV belonging to SAR92 (*Porticoccaceae*) that revealed a rapid increase in relative abundances (to up to 10%) during the spring bloom (Liu et al. 2020). These observations suggest a fast response of SAR92 to phytoplankton-derived substrates, an idea that is further supported by previous observations pointing to SAR92 as an abundant and active member of the microbial community during the peak and spring bloom decline (West et al. 2008; Obernosterer et al. 2011). Using the same metagenomic dataset, we observed that *Porticoccaceae* had a low contribution to CAZymes (2-15%) (Zhang et al. 2023), suggesting that this group had a minor implication in the degradation of complex compounds (Kim et al. 2019; He et al. 2023). *Rhodobacteraceae* accounted for a large share of *sit* transporters during the transition phase to the summer bloom consistent with the seasonal patterns of *Rhodobacteraceae* MAGs and abundant ASVs (Liu et al. 2020), suggesting their increased contribution to the processing of DOM at the final stage of the spring bloom decline (Buchan et al. 2019).

Nickel is an essential cofactor of enzymes in nitrogen fixation, nitrogen uptake and carbon fixation. Among nickel-dependent enzymes are urease, carbon monoxide dehydrogenase (CODH**)** and superoxide dismutase (NiSOD**)**. *Nitrincolaceae* had a dominant contribution to the Ni transporters *cbik* during the early spring bloom decline when the respective GPMs were high (Fig. 2). This pattern could be due to the presence of two *Nitrincolaceae* ASVs that together reached 40% relative abundance (Liu et al. 2020), coherent with the remarkably high coverage of *Nitrincolaceae* MAG115 that harbored *cbik* (Fig. 4). *Nitrincolaceae* are specialized in the utilization of amino acids and peptides (Francis et al. 2021; Debeljak et al. 2023) which could explain the high contribution of this family to the peptide/nickel transport system in early spring (*ddp*, PE.P, PE.P1, PE.S) (Fig. S6A). Urea is a nitrogen containing compound and its

utilization requires the Ni-containing enzyme urease. Urea can account for a substantial fraction of the prokaryotic nitrogen demand in marine systems (Jørgensen 2006), including the Southern Ocean (Hollibaugh et al. 2023) and diverse prokaryotic taxa are implicated in this process (Connelly et al. 2014). The increase in the concentrations of ammonium during the bloom decline (Fig. S1) could indicate that reduced forms of nitrogen, including urea, become important sources of this nutrient for certain prokaryotes, potentially including *Nitrincolaceae*. Further in-depth studies are required to confirm this idea.

Tungstoenzymes are required by methylotrophic prokaryotes to metabolize organic substrates containing no carbon-carbon bonds, such as single-carbon (C1) compounds (e.g. methane, methanol), methylated amines and methylated sulfur species (DMS, DMSO) by specialized pathways (Chistoserdova 2011; Maia et al. 2017). W-containing formate dehydrogenase (FDH) catalyzes the oxidation of formate, an intermediate product in methylotrophy, to carbon dioxide and therefore plays an important role in the processing of these compounds (Chistoserdova 2011). Methylotrophy appears to be widespread in marine microbial communities (Sun et al. 2011; Lidbury et al. 2014). In the present study, *Rhodobacteraceae* were the main contributors to W-transporters throughout the season, except for the peak of the spring bloom when W-transporters were mainly affiliated with *Nitrincolaceae* (Fig.3). *Rhodobacteraceae* are known utilizers of methylated sulfur compounds (Buchan et al. 2005) and amines (Chen 2012; Lidbury et al. 2014, Taubert et al. 2017), which could explain their W requirements. Recent observations point to a potential role of *Nitrincolaceae* in C1 metabolism (Francis et al. 2021). The full set of methanesulfonate monooxygenase genes, required for the utilization of methanesulfonate as carbon source and for the oxidation of DMS were present and expressed in *Nitrincolaceae* MAGs from the Southern North Sea (Francis et al. 2021). These observations could point to a possible role of *Nitrincolaceae* members in methylotrophy, but further genome-based investigations would be required to identify the pathways involving W-containing enzymes in members belonging to this family.

Mo is widely known for its role in nitrogenase, the enzyme responsible for $N_2$ fixation (Tuit, 2003). Another metabolism in which Mo is involved is carbon monoxide (CO) oxidation. Mo-containing CO dehydrogenase (CODH, *coxSML*) is an enzyme in aerobic CO oxidizers that can use CO as their carbon source (King and Weber, 2007). The seasonal trend in Mo transporters, peaking in late summer, matched those of *nifH* and CODH (Fig. S4) and suggests that both pathways could potentially be taking place. The main contributors to the *nifH* genes were *Xanthobacteraceae* (Fig. S6C), a family that is widely known to have $N_2$ fixing capacity (Brenner et al. 2005; González-Cortés et al. 2022). *Thioglobaceae*, important contributors to Mo transporters throughout the season, have the potential for carbon fixation (Morris et al. 2022), however, in the present study *cox* was not affiliated with this group. By contrast, *Rhodobacteraceae* contributed to both *mod* and *cox*, suggesting that members of this group could make use of this pathway. Genomic insights (Moran et al. 2004, Simon et al. 2017) and uptake experiments (Tolli et al. 2006) have shown that *Roseobacter* oxidize CO. These Mo-requiring processes are likely to occur concurrently, but carried out by different microbial groups.

Several trace metals could further play a role in superoxide dismutases (SODs). SODs are metalloenzymes that catalyze the conversion of superoxide molecules to hydrogen peroxide and molecular oxygen. SODs are one of the main defense mechanisms of cells against oxidative stress (McCord and Fridovich, 1969). Three types of SODs were described in prokaryotes, that are Ni-containing SODs (NiSOD), Fe- or Mn-containing SODs (FeSOD and MnSOD), and Cu- and Zn-containing SODs (Cu,ZnSODs) (Wang et al. 2018). In our metagenomes, all types of SODs were identified and they peaked during the early spring bloom decline (Fig. S7). Microbial respiration is linked to the formation of reactive oxygen species (ROS) that can be neutralized by SODs (Tiwari et al. 2002). The sharp increase in SODs could be a result of the enhanced microbial metabolism in response to the phytoplankton bloom, including respiration of large

amounts of DOM (Lesser 2006). Our results indicate that different metals could be used for SODs concurrently. The involvement of diverse taxa in this key process in combination with the different capacities of these prokaryotes of transport metals could explain this pattern.

The seasonal dynamics of the contribution of prokaryotic groups to trace metal transporters as observed in the present study point to distinct capabilities in the acquisition of these elements. In combination with the temporal trends in the abundances of a given trace metal transporter, our findings provide insights to the potential roles of abundant prokaryotic groups in the cycling of trace metals during consecutive spring and summer phytoplankton blooms in the Southern Ocean. *Rhodobacteraceae* had a broad repertoire of trace metal transporters (Mn, Zn, Ni, W, Mo) a feature that likely reflects the large diversity of metabolic functions present in this prokaryotic group. Our data suggest a more restricted repertoire for *Flavobacteriaceae* (Zn) and *Nitrincolaceae* (Ni, W), which could point to a more specialized functional role possibly associated with requirements of a reduced set of trace metals. The temporal variability in the abundances of genes encoding for trace metal transporters provides insight on the bloom stages that are favorable for diverse metal-requiring pathways to occur. Our results, based on trace metal transporter capacities, represent a novel perspective on the functional role of prokaryotes in the biogeochemical cycling of elements in the ocean.

**Author contributions**

Yanhui Kong, Stephane Blain and Ingrid Obernosterer designed the research. Bioinformatic analyses were performed by Yanhui Kong and Rui Zhang. Yanhui Kong and Ingrid Obernosterer wrote the first draft of the manuscript. All authors contributed to editing the manuscript.

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**Competing interests**

The authors declare no competing interests.

**Data availability statement**

The data sets generated and analyzed during the current study are available in the European Nucleotide Archive (ENA) repository at https://www.ebi.ac.uk/ena under the project ID PRJEB56376.

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# Supplementary Information for

# "Seasonal dynamics in microbial trace metals transporters during phytoplankton blooms in the Southern Ocean"

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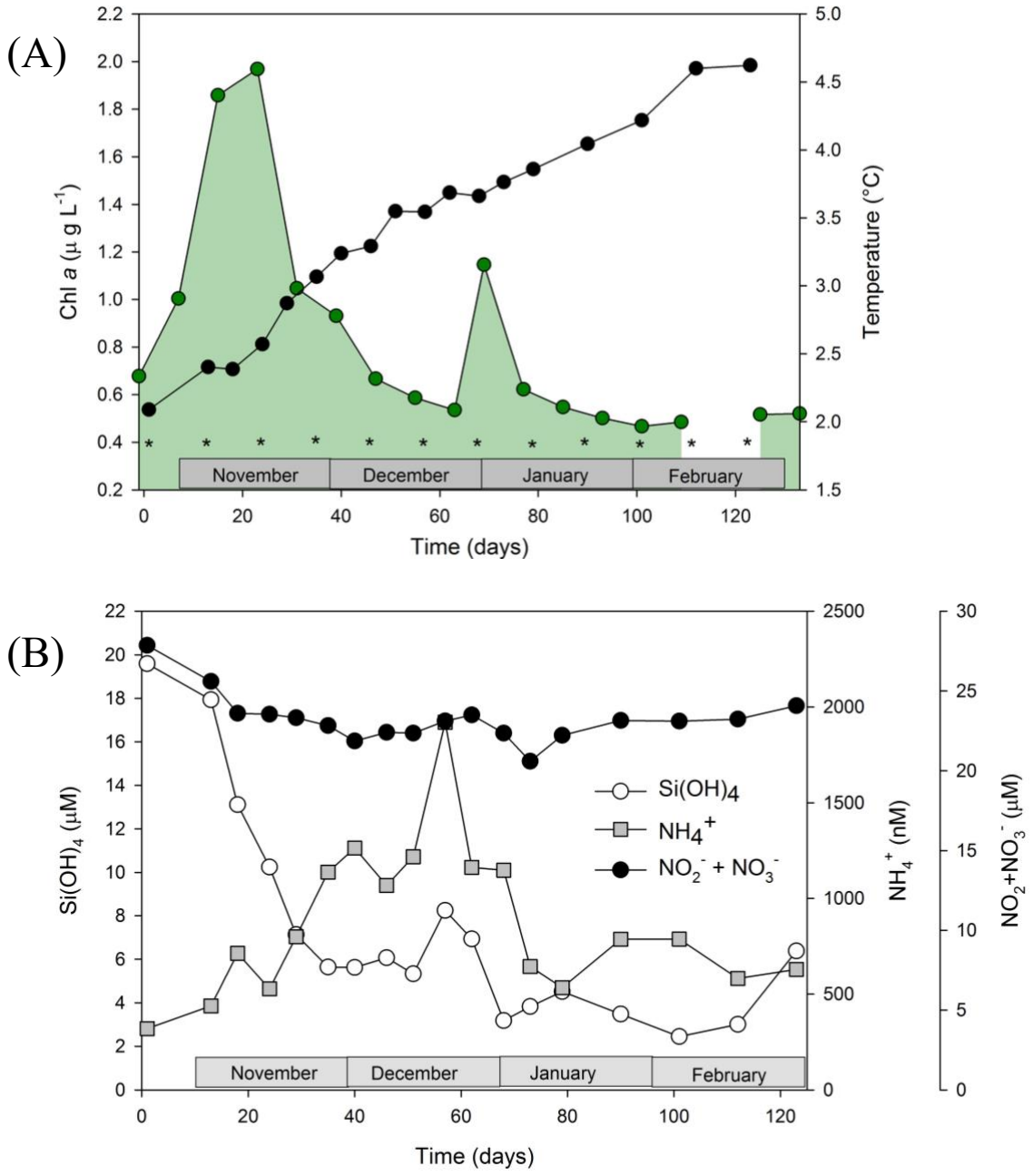
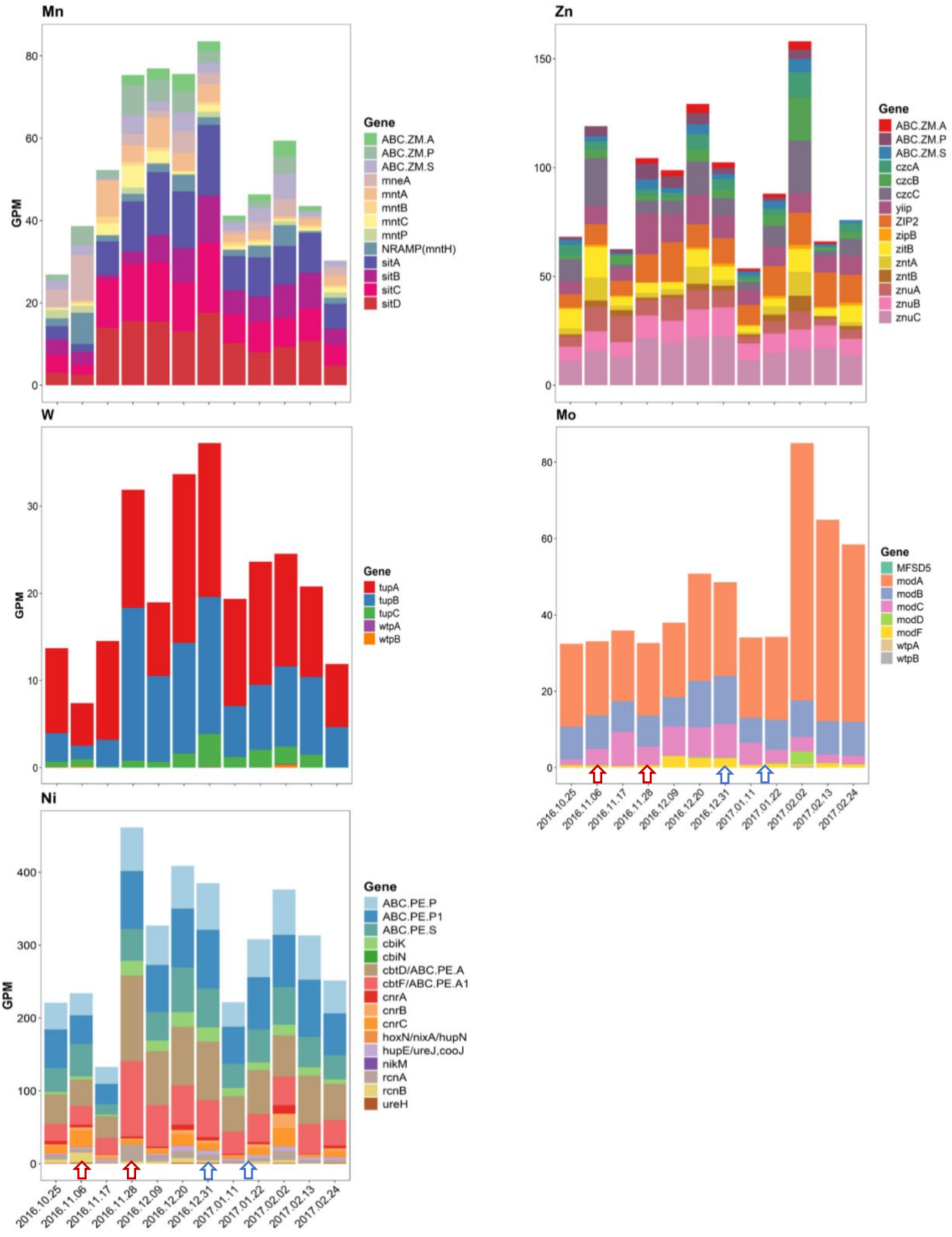



**Figure S1** Temporal changes of Chlorophyll a (green shaded area) and temperature (black circles) during the period of RAS deployment (**A**). Temporal changes of silicic acid ($Si(OH)_4$), ammonium ($NH_4^+$) and nitrite+nitrate ($NO_2^- + NO_3^-$) during the period of sampling (25 October 2016 to 24 February 2017) (**B**). Data are from Blain *et al.* (2021).


Mn
GPM
Gene
ABC.ZM.A
ABC.ZM.P
ABC.ZM.S
mneA
mntA
mntB
mntC
mntP
NRAMP(mntH)
sitA
sitB
sitC
sitD
Zn
Gene
ABC.ZM.A
ABC.ZM.P
ABC.ZM.S
czcA
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yiip
ZIP2
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modA
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Ni
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Gene
ABC.PE.P
ABC.PE.P1
ABC.PE.S
cbiK
cbiN
cbtD/ABC.PE.A
cbtF/ABC.PE.A1
cnrA
cnrB
cnrC
hoxN/nixA/hupN
hupE/ureJ,cooJ
nikM
rcnA
rcnB
ureH
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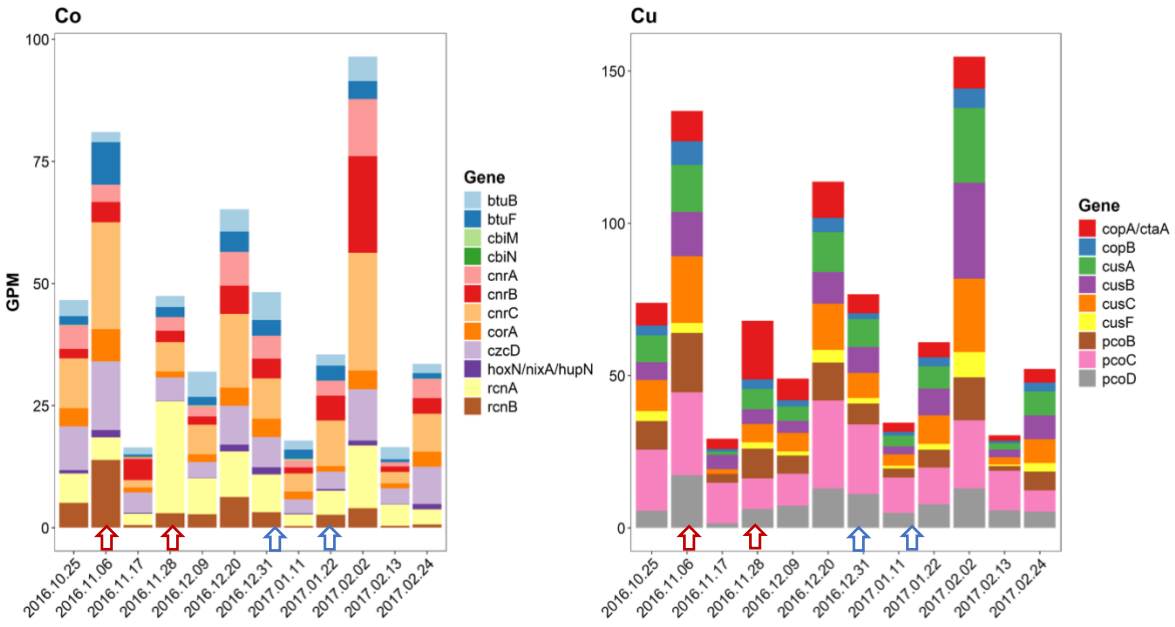


**Figure S2** Abundance of genes related to Mn, Zn, W, Mo, Ni, Co and Cu transport at the 12 time points. Gene abundance is represented by genes per kilobase million (GPM). Red arrows indicate the first spring phytoplankton bloom, and blue arrows indicate the second summer phytoplankton bloom.

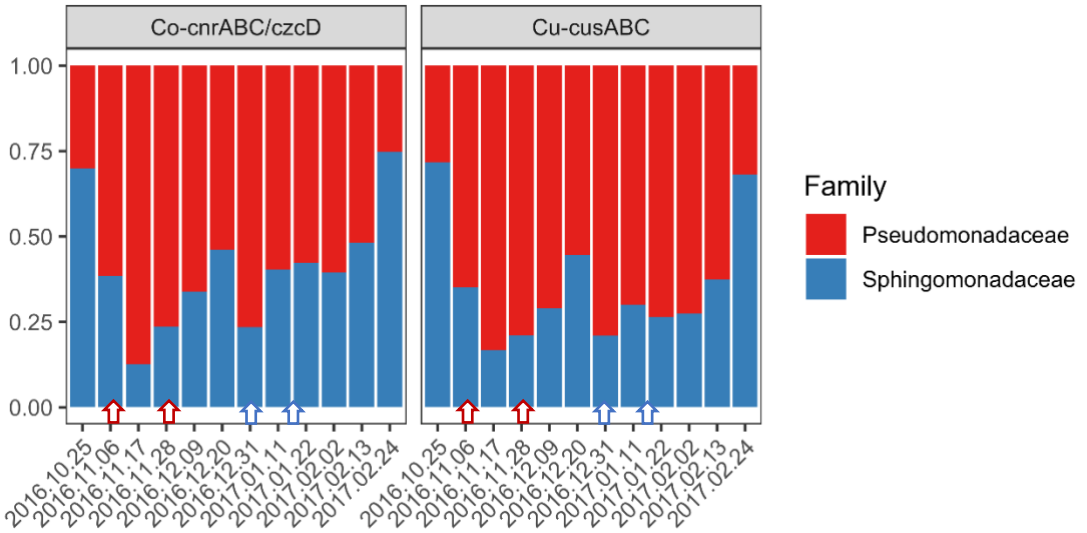


**Figure S3** Relative contribution of prokaryotic groups to specific genes associated with Co and Cu efflux transporters. Prokaryotic groups are based on the family level. Red arrows indicate the first spring phytoplankton bloom, and blue arrows indicate the second summer phytoplankton bloom.

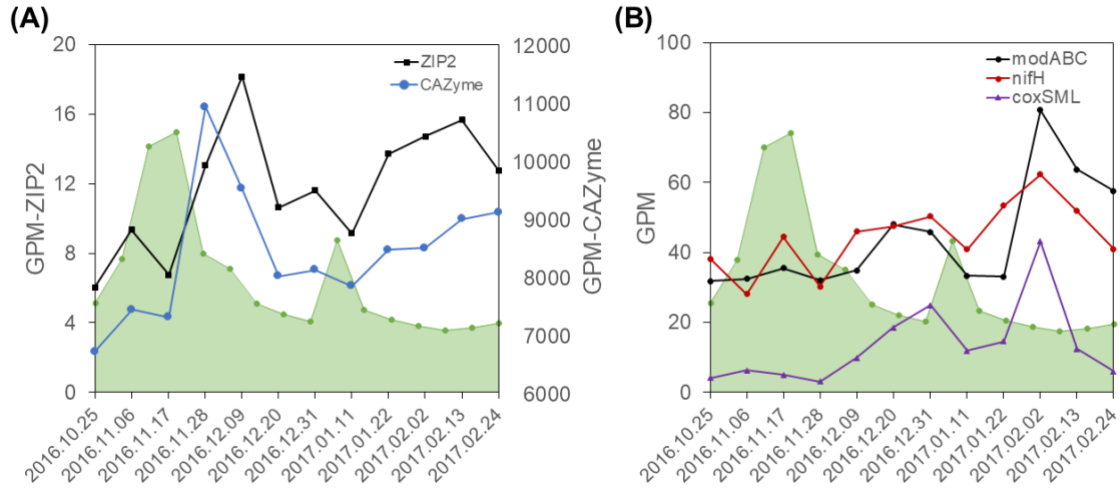


**Figure S4** Temporal changes of Chlorophyll a (green shaded area) and abundance of genes for Zn transporter (ZIP2) and carbohydrate-active enzymes (CAZymes) (**A**), Mo transporters (*modABC*), nitrogen fixation gene (*nifH*) and carbon monoxide dehydrogenase (*coxSML*) (**B**). Normalized gene abundances are given in genes per kilobase million (GPM).

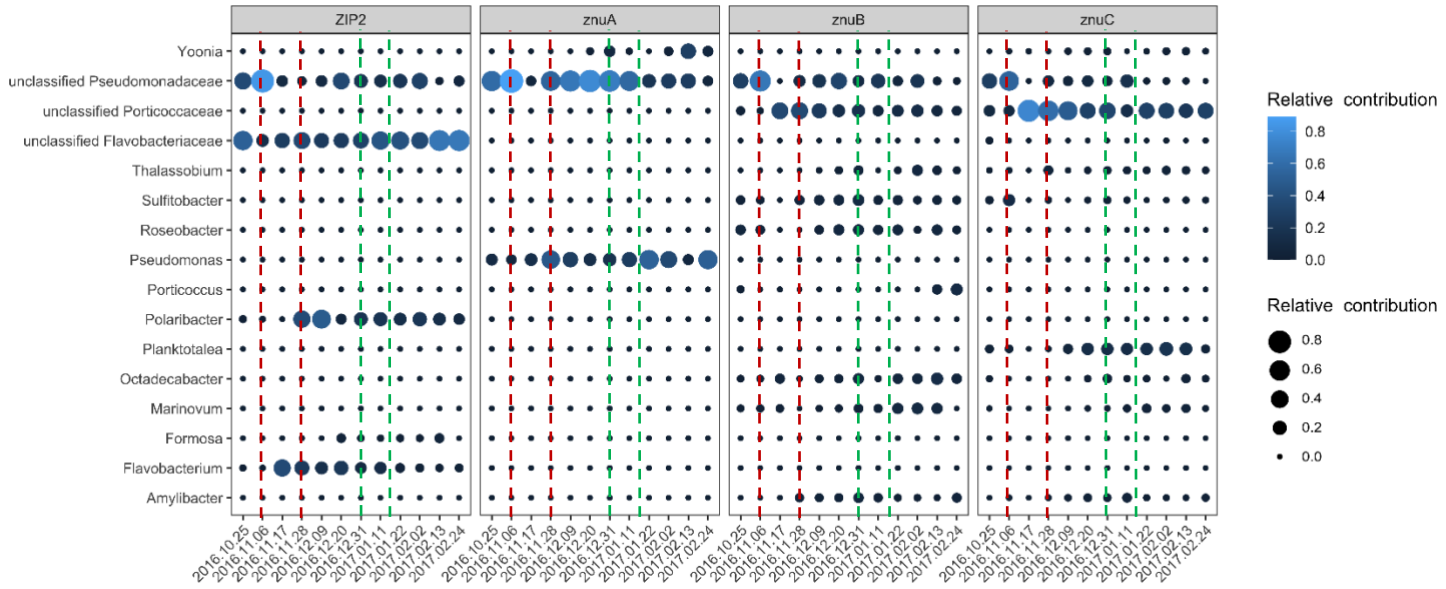


**Figure S5** Relative contribution of prokaryotic groups to specific genes associated with Zn transport. Prokaryotic groups are based on the genus level. The red lines represent the first spring phytoplankton bloom, and green lines represent the second summer phytoplankton bloom.

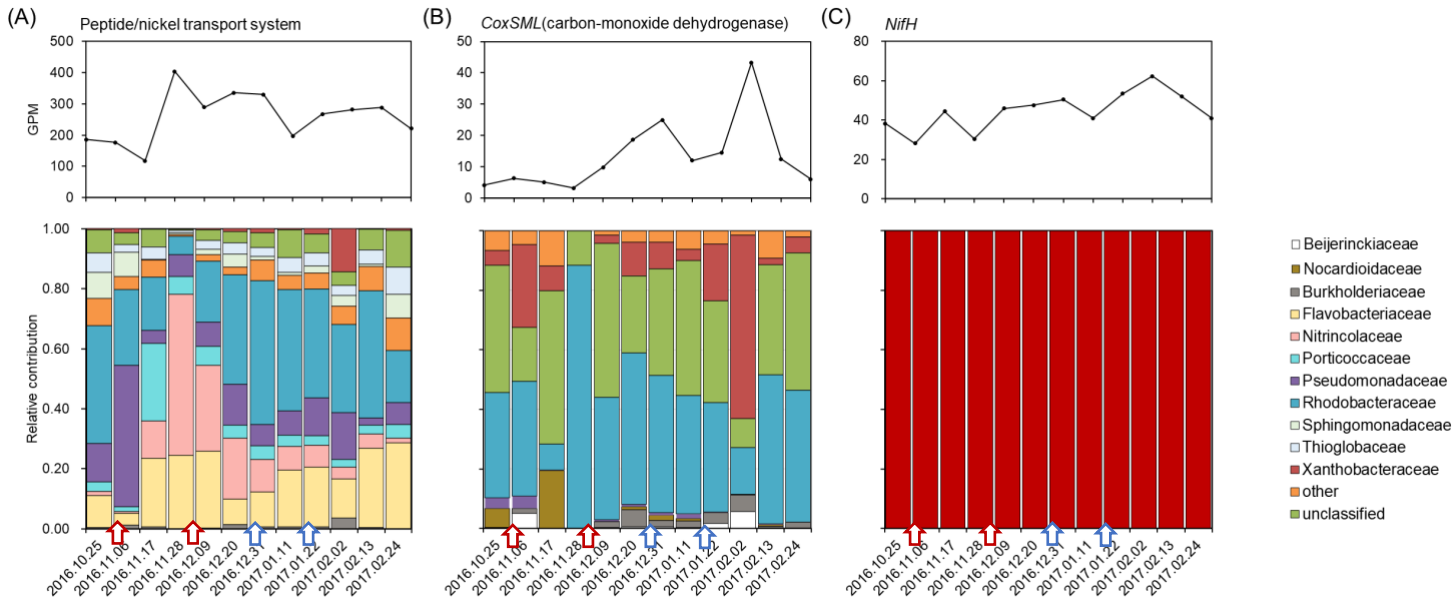


**Figure S6** Temporal changes of normalized gene abundance (GPM) and relative contribution of prokaryotic groups to peptide/nickel transport system (*ddp*, PE.P, PE.P1, PE.S) (**A**), carbon monoxide dehydrogenase (*coxSML*) (**B**) and nitrogen fixation gene (*nifH*) (**C**). Prokaryotic groups are based on the family level. Red arrows indicate the first spring phytoplankton bloom, and blue arrows indicate the second summer phytoplankton bloom.

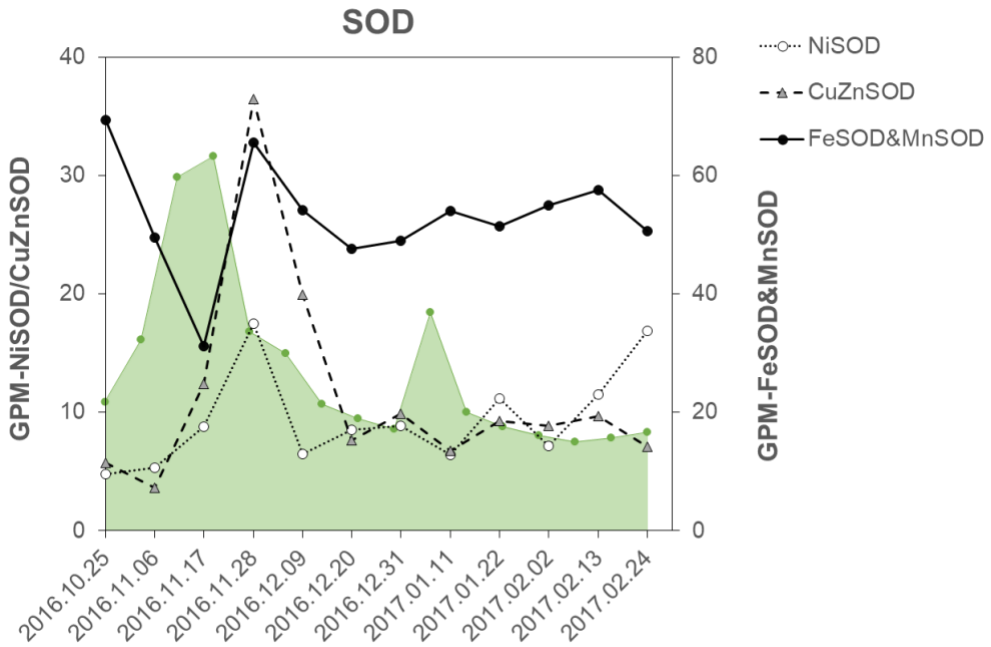


**Figure S7** Temporal changes of Chlorophyll a (green shaded area) and abundance of genes for different types of superoxide dismutases (SODs). Normalized gene abundances are given in genes per kilobase million (GPM).

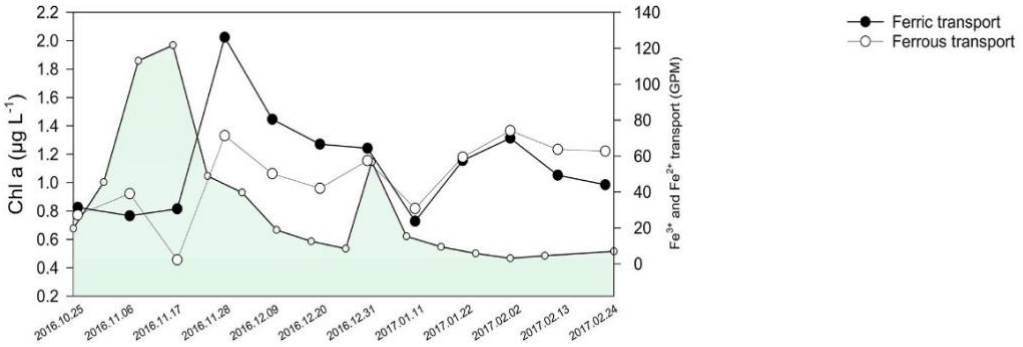


**Figure S8** Temporal changes of Chlorophyll *a* (green shaded area) and abundance of genes for $Fe^{3+}$ and $Fe^{2+}$ transporters Normalized gene abundances are given in genes per kilobase million (GPM). Data are from Zhang *et al*. (2023).

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