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RESEARCH ARTICLE

Metagenomics of Antarctic Marine Sediment Reveals Potential for Diverse Chemolithoautotrophy

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ABSTRACT The microbial biogeochemical processes occurring in marine sediment in Antarctica remain underexplored due to limited access. Further, these polar habitats are unique, as they are being exposed to significant changes in their climate. To explore how microbes drive biogeochemistry in these sediments, we performed a shotgun metagenomic survey of marine surficial sediment (0 to 3 cm of the seafloor) collected from 13 locations in western Antarctica and assembled 16 high-quality metagenome assembled genomes for focused interrogation of the lifestyles of some abundant lineages. We observe an abundance of genes from pathways for the utilization of reduced carbon, sulfur, and nitrogen sources. Although organotrophy is pervasive, nitrification and sulfide oxidation are the dominant lithotrophic pathways and likely fuel carbon fixation via the reverse tricarboxylic acid and Calvin cycles. Oxygen-dependent terminal oxidases are common, and genes for reduction of oxidized nitrogen are sporadically present in our samples. Our results suggest that the underlying benthic communities are well primed for the utilization of settling organic matter, which is consistent with findings from highly productive surface water. Despite the genetic potential for nitrate reduction, the net catabolic pathway in our samples remains aerobic respiration, likely coupled to the oxidation of sulfur and nitrogen imported from the highly productive Antarctic water column above.

IMPORTANCE The impacts of climate change in polar regions, like Antarctica, have the potential to alter numerous ecosystems and biogeochemical cycles. Increasing temperature and freshwater runoff from melting ice can have profound impacts on the cycling of organic and inorganic nutrients between the pelagic and benthic ecosystems. Within the benthos, sediment microbial communities play a critical role in carbon mineralization and the cycles of essential nutrients like nitrogen and sulfur. Metagenomic data collected from sediment samples from the continental shelf of western Antarctica help to examine this unique system and document the metagenomic potential for lithotrophic metabolisms and the cycles of both nitrogen and sulfur, which support not only benthic microbes but also life in the pelagic zone.

KEYWORDS Antarctica, chemolithotrophy, marine microbiology, metagenomics, marine sediment

Chemolithoautotrophic metabolisms play important roles in marine sediment ecosystems. For example, nitrification (oxidation of ammonia to nitrate) is considered an important process in global marine nitrogen cycles (1–4); however, little research has been done on ammonia oxidation in polar oceans (5, 6), especially for Antarctic benthic

Editor Hideyuki Tamaki, National Institute of Advanced Industrial Science and Technology

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The authors declare no conflict of interest.

Received 12 September 2021
Accepted 10 November 2021
Published 24 November 2021

microbial community nitrification. A greater understanding of chemolithoautotrophic metabolisms is needed, as the cycling of nitrogen and sulfur not only drives microbial communities but also supports life in higher trophic levels in the Southern Ocean (7). Importantly, the biogeochemical cycling of nitrogen- and sulfur-containing compounds impacts the flux of elements relevant to benthic-pelagic coupling in an area being impacted by climate change.

Geochemical evidence suggests that nitrification is significant in polar regions, and chemoautotrophy, supported by nitrification, has been suggested as an important contribution to prokaryotic primary production during the polar winter (5, 8–12). Nitrification has been documented in Antarctic benthic sediments (13). Further, Franco et al. (14) found alphaproteobacterial operational taxonomic units (OTUs) that were similar to bacteria known to play a role in the nitrogen cycle. Learman et al. (15) documented an OTU related to *Thaumarchaeota*, an ammonium-oxidizing archaeon (AOA) commonly found in the euphotic water column (16) and marine sediments (1); this OTU was detected in western Antarctica regions with relatively low organic carbon (15). These ecological studies point to potential key microbial participants in the nitrogen cycle. However, direct examination of the genetic/functional potential for nitrification with this system is lacking.

The cycling of sulfur is also important for both lithotrophy and organic matter mineralization (17). In highly productive continental margin marine sediments, the sulfur cycle is driven by anaerobic microorganisms carrying out dissimilatory sulfate reduction (DSR) (17, 18), which acts as an important pathway for organic matter decomposition (17). Through a series of intermediates, DSR ultimately results in the formation of reduced sulfur. Sulfur (and its reduced intermediates, including thiosulfate and elemental sulfur) is an important source of energy in surface marine sediment due to availability of reduced sulfur compounds and oxygen (19–21), while in deeper anoxic sediment, sulfate reduction is more important (17, 22). Sulfur oxidation in Antarctica has been documented in subglacial outflow (23), lakes (24), and sediments (25). Sulfur oxidizers have also been found in surface sediment cores obtained from the Antarctica continental shelf within the Mertz Glacier polynya (26). Similar to nitrification, the genetic/functional potential for sulfur cycling in Antarctica marine sediments has not, to our knowledge, been directly investigated.

Previous microbial studies of Antarctic sediments have focused on how community diversity was impacted by organic matter (14, 15, 27–31). Western Antarctica, compared to the Antarctica Peninsula, has been shown to have lower concentrations of sedimentary organic matter (15). We hypothesize that this environmental condition is conducive to a diversity of lithotrophic metabolisms. To this end, we examined both shotgun metagenomic and geochemical data from surficial sediment samples from the continental shelf of western Antarctica (Amundsen Sea, Bellingshausen Sea, and Ross Sea) to document the genetic/functional potential for chemolithoautotrophy within this ecosystem. Our results provide insights into how microbes cycle nitrogen and sulfur and drive essential biogeochemical cycles in the Southern Ocean.

RESULTS AND DISCUSSION

Nitrification in benthic sediments. In 12 of the 13 western Antarctica (WA) sites spanning from the Ross Sea to the Amundsen Sea (Fig. 1 and 2A), we detected genes for ammonia oxidation (*amoABC*), the first step of the conversion of ammonia to nitrite (5). The identified *amo* genes were derived from *Nitrosomonas* and *Nitrosospira* (Table S1). This is consistent with previous reports of *Nitrosomonas* and *Nitrosospira* lineages in Antarctic surface waters (10), metabolizing ammonia and releasing nitrite as a by-product (32–36). The nitrite-oxidizing *nxrAB* genes, used previously as markers for nitrite-oxidizing bacteria (37–39), were also detected, but in only nine of the 12 sites that contain *amo* genes, supporting full nitrification of ammonia to nitrate in those sites (Fig. 2A). Phylogenetic comparisons of *nxrAB* genes reveal a close relationship to *Nitrospina* (Table S1), which is a known nitrite oxidizer in Antarctic marine sediments (40, 41) and coastal surface waters (10). The presence of both *amoABC* and *nxrAB* in sediments replete with oxygen (Table S2) (oxygen

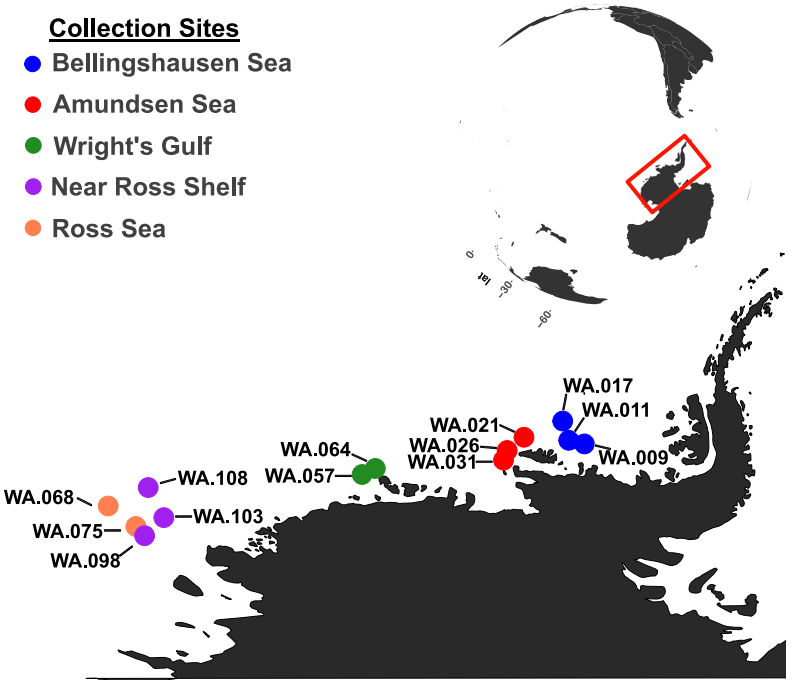

FIG 1 Map of Antarctica's western peninsula, showing the geographic locations from which the metagenomic samples were derived.

in the water above the sediments ranges from 4.1 to 6.2 mL/L) indicates that ammonia and nitrite are sources of energy in these communities.

Unlike genes for ammonia and nitrite oxidation, genes for reductive nitrogen processes (e.g., nitrate and nitrite reduction) are less common throughout the 13 sequenced sites. For example, dissimilatory nitrate reductase genes, *napAB*, are found in six of the 13 sites, while genes for nitrous oxide reduction, *nosDZ*, are found in only one of the Antarctica sites. Even though it is found in only five of the 13 Antarctica site, in WA.075, the nitric oxide reduction gene *norB* displays the greatest copy number (~60 reads per million [RPM], i.e., read coverage normalized to gene length and sequencing depth), as well as the greatest diversity (~0.8 genes per million [GPM], i.e., number of different assembled *norB* sequences normalized to the total number of genes predicted from each site assembly). The gene *nirS*, encoding a nitrite reductase, however, appears to be absent from that assembly, so the source of nitric oxide in that site remains unknown.

The most common pathways for nitrogen cycling, present in 12 of the 13 data sets, are those for oxidative reactions, converting ammonia to nitrate (i.e., nitrification). The absence of the full denitrification pathway in most assemblies could indicate that these sites were not sequenced deeply enough and the genes were not assembled or that the microbes responsible for the undetected parts of the denitrification pathway are part of the rare, or low-abundance, biosphere. In support of the latter possibility, WA.098, our most deeply sequenced site, appears to contain all required genes for the full nitrogen cycle. This sample, as well as four others, also contain *hzoA* and *hzsA* (Fig. 3), which are markers for anammox reactions (42, 43), allowing the direct conversion of ammonium to molecular nitrogen. Overall, during the southern summer, nitrification of reduced nitrogen sources, likely sourced from sinking detritus, is an important process that drives lithoautotrophy.

Sulfur cycling. In the benthic sediments along the western Antarctic Peninsula, genes encoding sulfate reduction are absent, with the exception of a single copy of a sulfate-reducing operon (*cys*) in site WA.098. Rather, the oxidative version of the dissimilatory sulfite reductase pathway (*rdsrAB*) was found in nearly all samples (Fig. 2B).

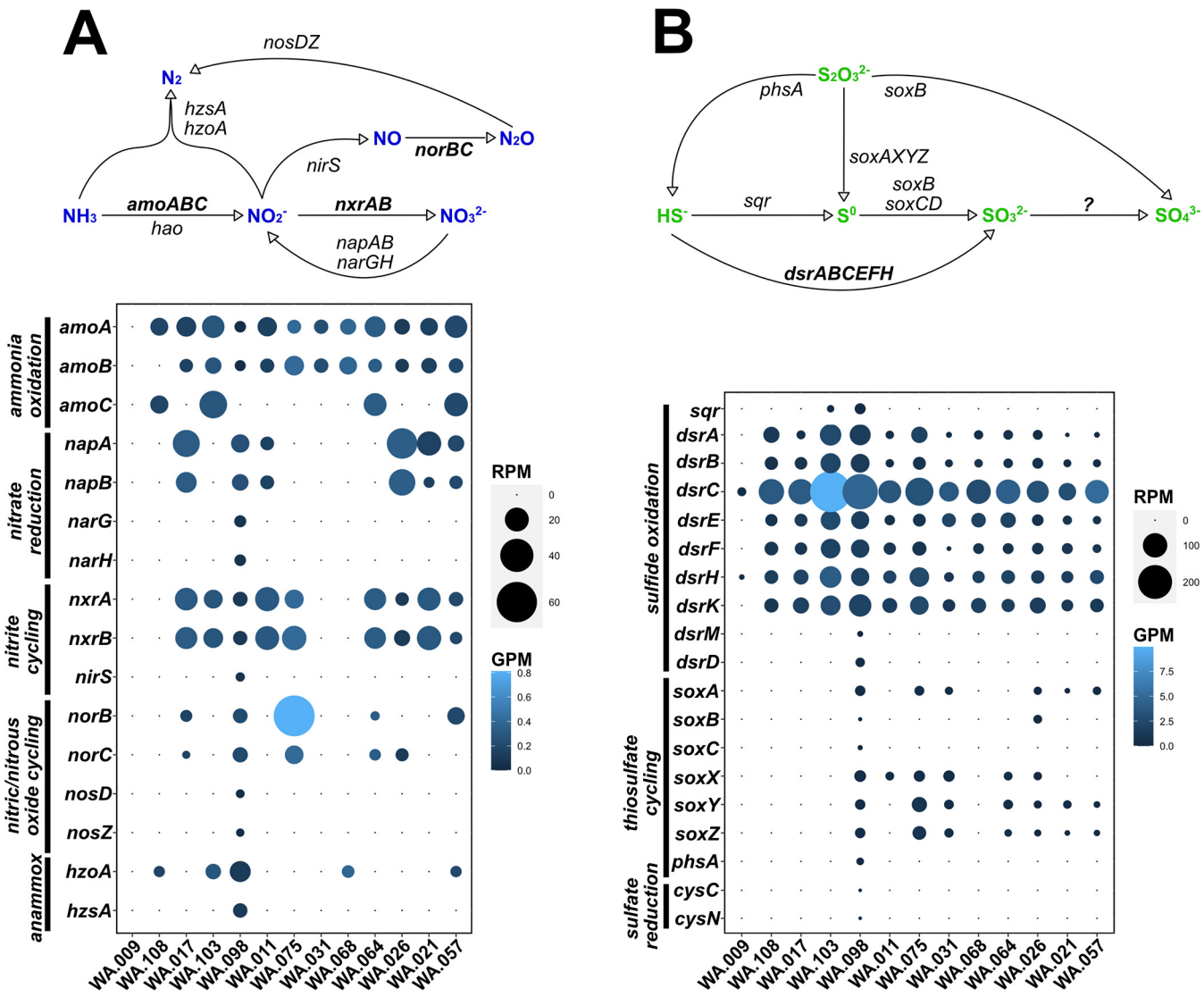

FIG 2 Dot plots and redox cycling of various nitrogen (A) and sulfur (B) compounds. The various elemental species are colored blue and green (for nitrogen and sulfur, respectively) to visually separate them from the names of genes attributed to each reaction. Genes that appear to be abundant or consistently present through all or most of the sites are shown in bold in the redox cycling schematics above each dot plot. The size of each dot represents reads per million (RPM), a measure of gene abundance based on gene mapping, normalized to the length of each gene and size of the data set. The color gradients denote genes per million (GPM), a measure of gene diversity based on the number of different gene homologs identified, normalized to the total number of genes predicted from each metagenome.

Phylogenetic analysis of recovered *dsr* genes revealed their close relationship with those of *Gammaproteobacteria*, specifically, *Acidithiobacillales* and *Thioflexothrix* (Table S1). The additional presence of *dsrEFH*, genes specific to sulfur oxidation (39), suggests that the DSR pathway in these samples operates in reverse (i.e., reverse DSR) (44–48). This would suggest that sulfate is likely produced in the sampled sediments.

In contrast to the ubiquity of sulfide oxidation genes, genes for thiosulfate cycling via the *sox* and *phsA* pathways were found in only some of the sites. We did not detect the *soxCD* genes, which are required for complete oxidation of thiosulfate to sulfate (44). Rather, the absence of *soxCD* suggests that elemental sulfur may accumulate intracellularly and be oxidized to sulfate via the reverse DSR pathway and sulfide-quinone oxidoreductase (SQR) (Fig. 2B) (49–52). Our observations are consistent with previous reports of the mutual exclusivity of *dsr* and *soxCD* (53–55) reported for individual organisms. Here, we report this trend at the community (metagenome) level, with *soxCD* missing from all surveyed sites. Our data show that, in Antarctica benthic

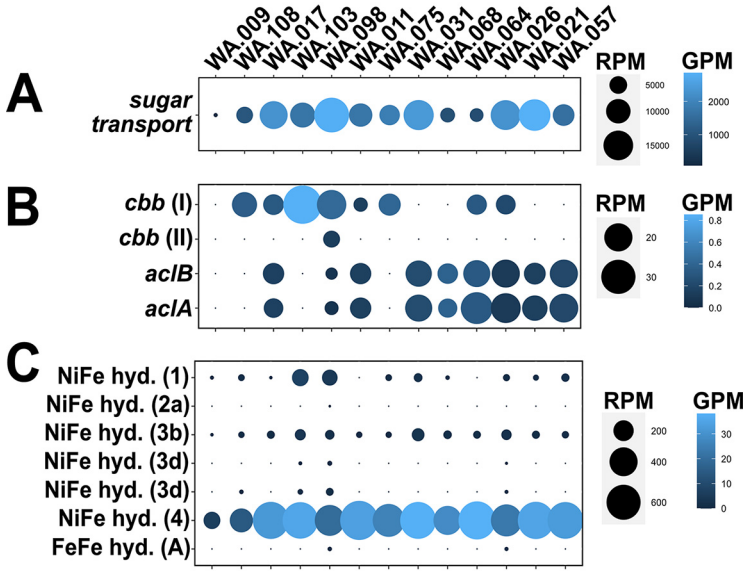

FIG 3 Dot plots for the sugar transporters (A), carbon fixation (B), and hydrogenases (hyd.) (C) identified in the metagenome assemblies. As in Fig. 2, the size of each dot represents reads per million (RPM), a measure of gene abundance based on gene mapping, normalized to the length of each gene and size of the data set. The color gradients denote genes per million (GPM), a measure of gene diversity based on the number of different gene homologs identified, normalized to the total number of genes predicted from each metagenome.

sediments, sulfur cycling is dominated by sulfide oxidation, which, in addition to ammonia oxidation, likely serves as a significant driver of lithoautotrophy.

Organotrophy in Antarctica benthic sediment. The genetic potential for organotrophy was also found in Antarctic sediments. Previous research suggested phytodetritus to be a large component of organic matter delivered to benthic sediments in coastal Antarctica (15). Indeed, we observe DNA sequences derived from cyanobacteria and eukaryotes, which we interpret as detrital factions (Fig. S1). All sites had comparable abundances of cyanobacterial contigs, with the exception of WA.009, which is consistent with the fact that this site had the lowest measured amount of total organic carbon (Table S1). Along with the presence of possible exogenous sources of carbon, we observed many genes coding for sugar transporters (Fig. 3A). These genes were more abundant (RPM and GPM), relative to other pathways (e.g., carbon fixation) (Fig. 3B). Using the CAZy database as a reference to search for complex carbohydrate metabolisms, we detected various genes, including those with carbohydrate-binding modules, as well as those encoding glycoside hydrolases and carbohydrate esterases (Fig. S2), all of which may be involved in chitin degradation. Complex organic matter, like chitin, is an important source of carbon and nitrogen in marine systems (56–60). Chitin is known to be sourced from crustaceans, such as krill (*Euphausia superba*) in Antarctica (61). Further, chitin-degrading (chitinolytic) bacteria have been documented in sediments from Antarctica (62, 63). Our identification of this genetic repertoire for the metabolism of complex carbon supports the fact that these benthic communities are primed to use detritus for energy and carbon.

The data also show hydrogen as an important energy source and by-product of the metabolic reactions occurring in these benthic sediments (Fig. 3C). By far, the most abundant hydrogenase detected is the hydrogen-evolving group 4 hydrogenase, which functions to relieve reducing equivalents generated by fermentation, conserving energy in the process (64, 65). Group 1 and 3b hydrogenases were also relatively ubiquitous among the sampled sediment. Group 3b hydrogenases are similar to group 4 in that they are coupled to fermentation, while group 1 hydrogenases allow the use of molecular hydrogen as an electron donor and source of energy (65). Indeed,

fermentation, likely an important process in these sediments, can also serve as a source of hydrogen (64), which could be used for energy by other microbes. The ubiquitous presence of oxygen-dependent terminal oxidases, however, suggests that oxygen was present in our samples, inhibiting the efficiency of microbial fermentation.

The ubiquity and relative abundance of genes diagnostic of organotrophy suggest that benthic communities in Antarctica have the genetic potential to remineralize carbon. However, we also observe genes for carbon fixation (Fig. 3B). The *cbbI* (RuBisCO form 1) gene was found at eight sites and appears to be most common at WA.103. Genes involved in the reverse tricarboxylic acid (rTCA) cycle, another mechanism for carbon fixation, were present in nine sites, including sites where *cbbI* genes were not detected (with the exception of WA.009). We did not detect any genes for light sensing in our metagenomic data (e.g., proteorhodopsin or chlorophyll for photoheterotrophy and photoautotrophy). This is again consistent with the sediment depths from which these samples were collected (water depth range, 412 to 765 m). Thus, our detection of genes for carbon fixation (Fig. 3B) supports a role for chemolithoautotrophy, with reduced nitrogen and sulfur (Fig. S2) as the predominant sources of energy.

Oxygen reduction. Even though the sediment deposition rates at our samples sites are high (~1 mm/year) (98), the surficial nature of our sediment samples, combined with the influence of bioturbation (13), implies that the bulk of the microbial constituents of the surveyed communities are exposed to oxic conditions. Accordingly, our detection of terminal oxidases (Fig. S3B) and relative dearth of genes diagnostic of anaerobic processes (e.g., nitrate and sulfate reduction) suggest that our sampled communities are poised to use oxygen as a final electron acceptor. Despite some signals suggesting the reduction of nitrate and, perhaps, iron (Fig. S3A), the net catabolic pathway in our samples remains aerobic respiration, likely coupled to organic matter oxidation.

Description of MAGs from the Ross Sea. We recovered a total of 61 metagenome-assembled genomes (MAGs) from our deeply sequenced sample (WA.098); of these MAGs, 16 had completion scores above 60% (Table S3), and this subset of higher-quality MAGs was analyzed in more detail. The 16 MAGs ranged in size from 1.2 to 4.6 Mb, had GC contents ranging from 33% to 56%, and had genome completion scores between 61 and 99% (Table S3). Gene density ranged from 0.68 to 1.12 genes per kb (Fig. S4; Table S3). Gene density seemed to correlate inversely with MAG genome size (corrected using estimated completion scores) (Fig. S4). This rough correlation supports the idea that smaller, more-streamlined genomes encode less nongenic sequences, compared with larger genomes (66).

Only one archaeal MAG (MAG 48) was recovered, and is most closely related to *Nitrosopumilus*, within the phylum *Thaumarchaeota* (Fig. 4A), although analysis using the SprayNPray software (67) reveals that this MAG is only about 75% similar (average amino acid identity) to *Nitrosopumilus* sequences available in NCBI (Table S4). This thaumarchaeal MAG had one of the smallest estimated genome sizes (1.29 Mb) and the highest gene density (~1.2 genes/kb), consistent with previous reports of streamlining in this lineage of *Archaea* (68). Thaumarchaea are generally considered to make a living by oxidizing ammonia (69), consistent with our broader metagenomic survey demonstrating ammonia oxidation as an important process in these Antarctica sediments. Although this MAG did not appear to harbor genes for ammonia oxidation, BLAST analysis revealed a three-gene operon, encoding hypothetical proteins, with remote homology to *amoABC*.

Two of the MAGs (MAG 44 and MAG 45) were most closely related to the family *Nitrosomonadaceae*. This lineage is known to play a role in nitrification (32, 70) and may be drivers of the biogeochemical cycling of nitrogen that we observe in the nonbinned metagenome assemblies. However, we did not detect any ammonia oxidation genes (*amo*) genes in these MAGs (Fig. 5). These two *Nitrosomonadaceae* MAGs are 97% and 98% complete; the high genome completeness makes it relatively unlikely that *amo* genes are missing due to chance, although it is possible that the genes, which are generally highly abundant in our samples, ended up on short unbinned contigs. Nonetheless,

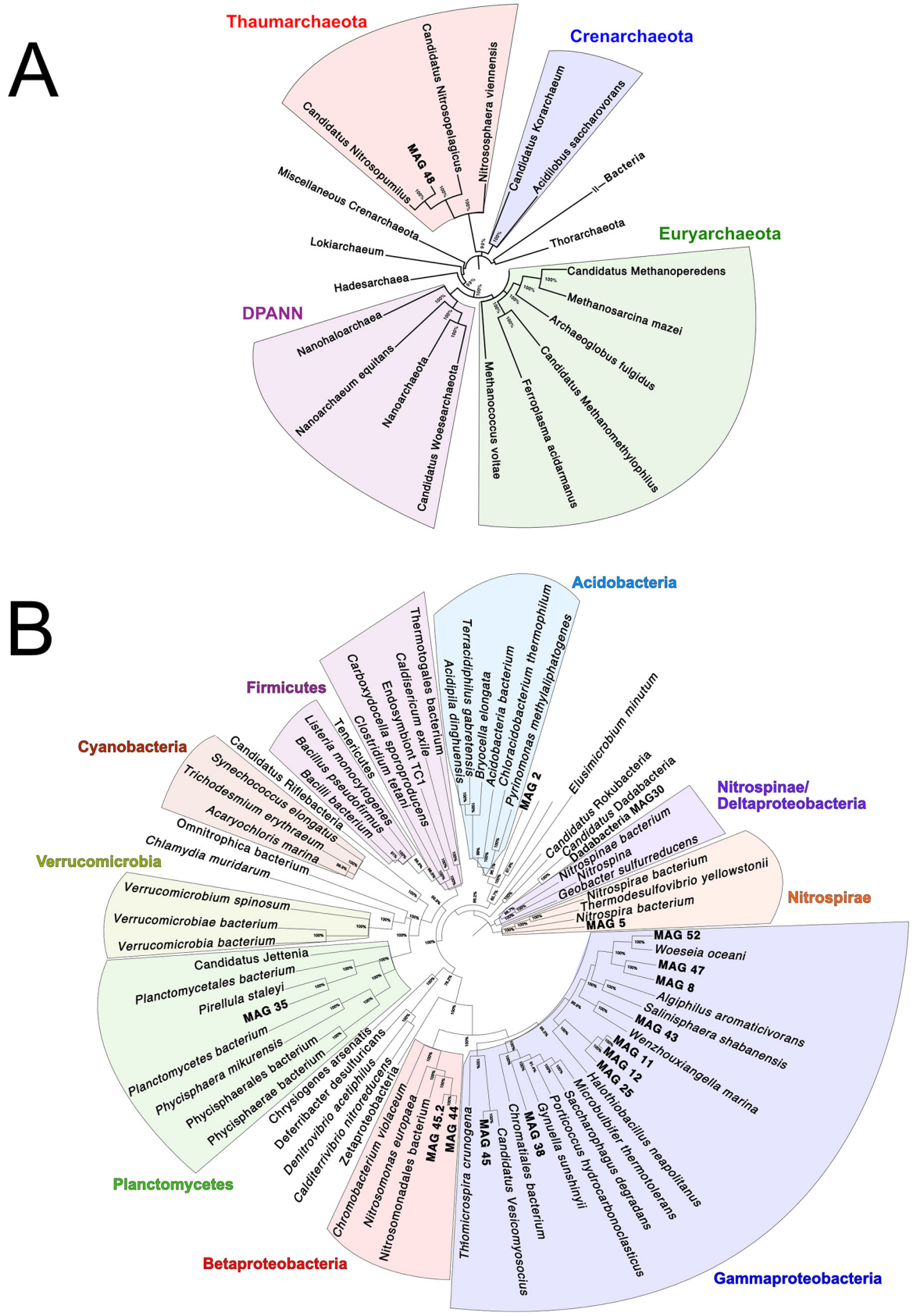

FIG 4 Phylogenomic trees demonstrating the taxonomic context for (A) one high-quality archaeal MAG and (B) 15 high-quality bacterial MAGs reconstructed from WA.098.

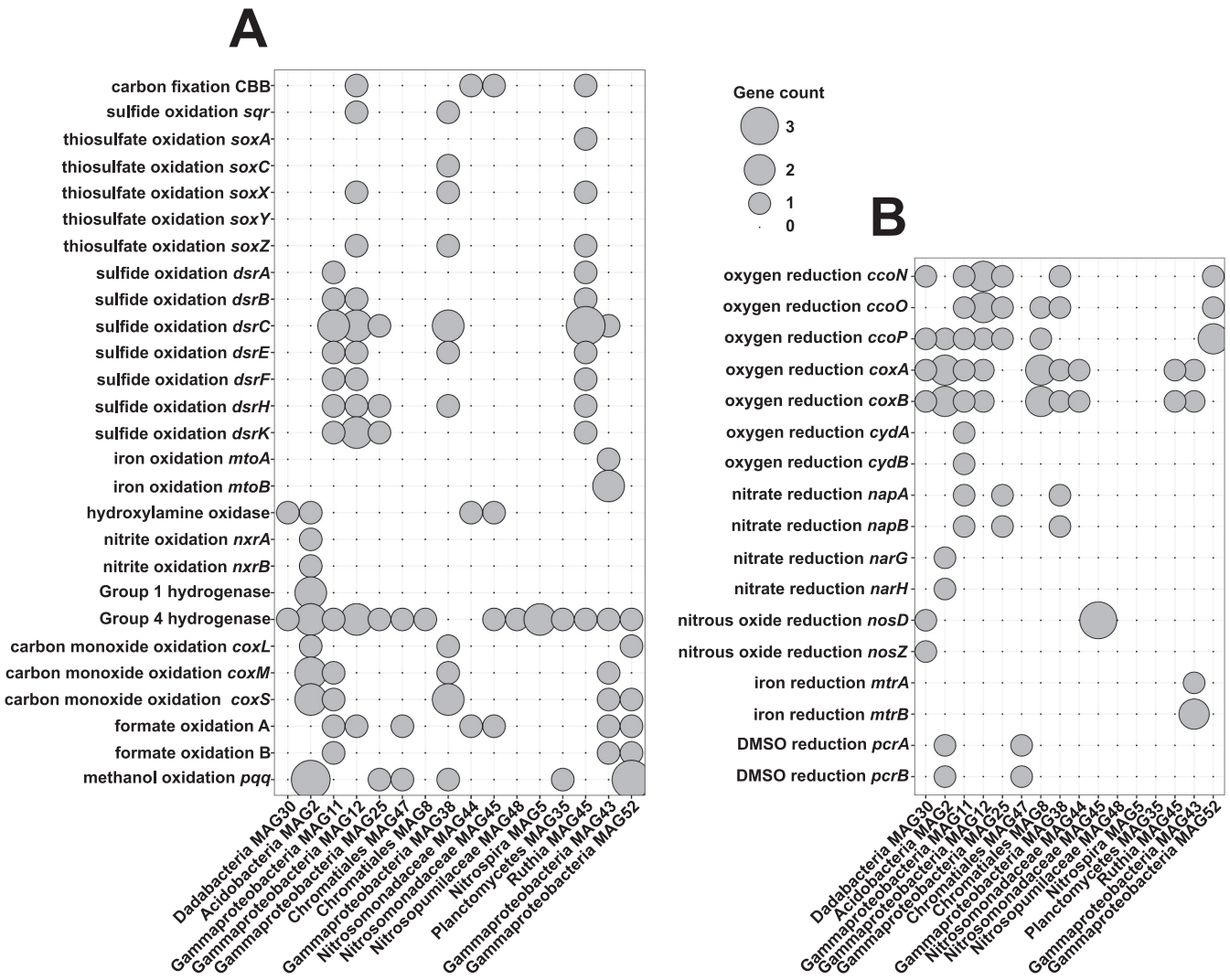

FIG 5 Dot plots summarizing (A) energy sources and (B) terminal electron acceptors utilized by the 16 high-quality MAGs from WA.098. DMSO, dimethyl sulfoxide.

both *Nitrosomonadaceae* MAGs harbor the hydroxylamine oxidase gene *hao*, allowing the oxidation of ammonium to nitrite. Oxidation of nitrite to nitrate could then be carried out by *Acidobacteria* MAG 2, which carries the nitrite oxidation genes *nxrAB*.

Four of the MAGs have the genetic potential to reduce nitrate via the dissimilatory nitrate reductase genes *napAB* and *narGH* (Fig. 5). Gammaproteobacteria MAG 11 has the genetic potential to reduce nitrite to nitric oxide. Although none of the 16 high-quality MAGs were found to carry genes for the reduction of nitric to nitrous oxide, the final step of denitrification (nitrous oxide reduction via *nosDZ*) is encoded by a MAG whose closest sequenced relative is part of the recently described phylum "*Candidatus* Dadabacteria." This phylum is part of the candidate phyla radiation and has only been documented in marine ecosystems in the past 5 years (71, 72). Taken together, our data allowed us to identify seven MAGs that are likely key players in denitrification and may play key roles impacting nitrogen cycling in Antarctica sediment.

At least four MAGs from WA.098 harbor genes for sulfur cycling (Fig. 5). Three MAGs encode most of the DSR pathway for dissimilatory sulfite reduction. The presence of *dsrEFH* in these MAGs is indicative of reverse DSR, where sulfide is oxidized to sulfite. Two of these MAGs (MAG 11 and MAG 12) are within the *Gammaproteobacteria* but could not be further taxonomically resolved. One of the sulfur-oxidizing MAGs (MAG 45) appears to be affiliated with the genus "*Candidatus* Ruthia," which consists of

chemoautotrophic sulfur-oxidizing symbionts (73), sharing ~86% average amino acid identity with the sequenced "*Candidatus* Ruthia endofausta" genome. Similar to the genome sequence of "*Candidatus* Ruthia magnifica," a symbiont of a hydrothermal vent clam (73, 74), the 99.3%-complete MAG 45 has a 1.3-Mbp genome, a relatively small size for bacteria (very similar in size to the recovered thaumarchaea-related MAG [MAG 48]), and a low GC content (37.3%). Both of these properties are hallmarks of endosymbionts having undergone genome reduction (75). The chemoautotrophic symbiont MAG 45 also has genes for thiosulfate oxidation (*soxZXA*), which provides additional energy for carbon fixation via the Calvin-Benson-Bassham (CBB) cycle.

The endosymbiont MAG 45 likely carries out carbon fixation inside a host, but three other MAGs, presumably free-living, were also found to encode the CBB carbon fixation pathway (Fig. 5). One of these MAGs (*Gammaproteobacteria* MAG 12) also has genes for sulfide and thiosulfate oxidation, via reverse DSR and *soxZX*, respectively. The other two MAGs that encode carbon fixation pathways (*Nitrosomonadaceae* MAG 44 and MAG 45) are affiliated with known ammonia oxidizers but, as mentioned above, do not appear to have genes for ammonia oxidation (Fig. 5).

This study was not able to assign any MAGs to a specific genus. For example, one bin clustered with *Pirellula staleyi* but shares only about 60% amino acid identity with closest homologs available in NCBI (and even less with the reference *P. staleyi* genome). Analysis using the SprayNPray (62) software reveals that in most of the 16 bins examined, the taxonomic affiliations of the top hits to each bin are from an unexpectedly high diversity of species, genera, and, in some cases, phyla (Fig. S5). Notably, differences in top taxonomic hits were found not only between contigs but also between different regions within individual contigs. These observations could be the result of chimeric assemblies (76) or, more likely, due to poor representation of the sequenced microorganisms in NCBI's nonredundant (nr) database. By comparing the average amino acid identities with the number of different taxonomic hits to each bin, we observe an inverse relationship between these two variables, where MAGs with higher amino acid identity to representative orthologs in NCBI have a significantly ($P = 1.254e{-}06$) lower variety of taxonomic hits (Fig. S6). Some of this taxonomic diversity is possibly the result of pervasive horizontal gene transfer events. For example, three of the MAGs that clustered within the class *Gammaproteobacteria* (*Gammaproteobacteria* MAG 11, MAG 12, and MAG 25) recruit hits from a wide variety of taxa, but mostly within the phylum *Proteobacteria*; however, some of the proteobacterial contigs also recruit hits to *Planctomycetes*, *Chloroflexi*, and *Firmicutes* genes. While it is possible that some of these discordant DIAMOND hits may represent horizontal gene transfer (HGT) events, it is unlikely that all of the observed taxonomic inconsistency is due to HGT alone.

Conclusions. Overall, our results reveal sedimentary communities that benefit from the input of reduced nitrogen, sulfur, and carbon, likely from the overlying water column. Genetic potential for lithotrophic metabolism was abundantly documented in sediments from the Ross to the Bellingshausen Sea. Most of the MAGs defined in the Ross Sea were not able to be placed into a specific genus, relative to what is known in published databases, which suggests that this ecosystem hosts organisms that are unique and novel. Nonetheless, these MAGs from Ross Sea, as well as the functional potential observed in all of our sequenced samples, show that these communities may play key roles in the pelagic-benthic biogeochemical cycling of important compounds in Southern Ocean waters off western Antarctica.

MATERIALS AND METHODS

Sampling details. Surface sediment samples from the continental shelf of western Antarctica (WA) were collected on the *RVIB Nathaniel B. Palmer* (December 2013 to February 2014) using a MC-800 multicorer (Ocean Instruments). Surface sediment from the top of the cores (approximately the top 3 cm) was aseptically transferred with a spatula into conical tubes and immediately frozen (−80°C). Samples were shipped frozen from the field after collection to the lab at Central Michigan University (CMU). The sampling locations, which include the Amundsen Sea, Bellingshausen Sea, and Ross Sea (Fig. 1), have low organic matter relative to the Antarctica Peninsula (15), and as the sediments were sampled in the austral summer, they were at the forefront of incoming carbon flux from the surface waters. A detailed account

of sampling locations and sediment nutrient data was published previously (15), and an abbreviated list can be found in Table S1.

DNA extraction and sequencing. DNA from sediment was extracted and cleaned as previously reported by Learman et al. (15). Briefly, DNA was extracted using a PowerSoil DNA extraction kit (MoBio) and concentrated using a DNA Clean & Concentrator kit (Zymo). Clean and concentrated DNA was quantified using a Qubit2.0 fluorometer (Life Technologies) and stored at −20°C. DNA for shotgun metagenomics was sequenced using an Illumina HiSeq 2500 instrument with paired-end 150-bp reads at Michigan State University's Research Technology Support Facility (RTSF) Genomics Core.

Assembly and binning. Raw reads were initially checked for quality with FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and trimmed with Trimmomatic v0.33 using default parameters (77). Reads were then assembled with metaSPAdes v3.15.2 (78). The sample from WA.098 was randomly chosen as a representative sample for the data set and was sequenced more deeply to support the assembly of metagenome-assembled genomes (MAGs) (Table S2). The resulting SPAdes assembly from WA.098 was then binned into MAGs with Metabat2 (79) using multiple parameters (superspecific, veryspecific, specific, sensitive, and verysensitive). The resulting MAGs were then consolidated using DASTool (80), and manually curated using Anvi'o v5 (81). The final completion and redundancy scores of the resulting MAGs were calculated using Anvi'o (Table S3).

Annotation. All assemblies were annotated using the DOE Joint Genome Institutes (JGI) Integrated Microbial Genomes (IMG) (82, 83) and Prodigal v/2.6.3 (84). IMG GOLD genome ID numbers can be found in Table S3 and S4. Shotgun metagenomic assemblies recovered 15,085 to 188,505 contigs of >1,000 bp (Table S2), resulting in 325,721 (WA.068) to 2,486,981 (WA.098) protein-coding genes (defined by IMG annotations) (Table S4). To further target lithotrophic metabolisms, site assemblies and MAGs were additionally annotated using MagicLamp (https://github.com/Arkadiy-Garber/MagicLamp). This software uses a set of publicly available HMMs, designed and compiled from Pfam and TIGRFAMS (85). We also used FeGenie to identify genes relevant to iron cycling (86). To target genes associated with recalcitrant carbon degradation, we used a set of KEGG Orthology identifiers published by Anantharaman et al. (85). Carbohydrate-active enzymes were identified using the CAZy database (87). Moreover, we used GhostKOALA (88) to generate KEGG Orthology identifiers for genes predicted from our data sets; KEGG-Decoder (89) was used to organize the KEGG Orthology annotation data into KEGG module pathways based on percent completion.

Phylogenetic placement. Using GToTree (90), we generated phylogenomic trees of high-quality WA.098 MAGs (completion > 60%; contamination < 8.7%). In addition to the MAGs collected herein, for taxonomic context, we included a phylogenetically broad set of genomes downloaded from RefSeq (91). To assess the evolutionary placement of the archaeal MAG, we used the *Archaea*-specific single-copy gene (SCG) set that is available within the GToTree package (90). For the rest of the MAGs that were within the domain *Bacteria*, we used the *Bacteria*-specific SCG set. Trees were visualized using FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Taxonomic assignment to MAGs was done using GTDB-Tk, which uses a combination of metrics, including the average nucleotide identity to reference genomes in the NCBI Assembly database, placement in the GTDB reference tree, and the relative evolutionary divergence (92, 93).

To better understand the taxonomic relationship of the high-quality MAGs from WA.098 to previously sequenced organism, we used SprayNPray (https://github.com/Arkadiy-Garber/SprayNPray), which queries, using DIAMOND (94), the genes from each MAG against a reference database (NCBI nonredundant proteins [nr]). DIAMOND results are then parsed and visually inspected for downstream processing (Table S3).

Finally, we examined the phylogenetic placement of the *dsr*, *amo*, and *cyc2* genes as a proxy for determining function. Using BLAST (95), we identified homologs to select genes in RefSeq (91), with alignments generated with Muscle (96). Subsequently, phylogenetic trees were then generated using RAxML (substitution matrix = PROTCATBLOSUM62) (97) and visualized with FigTree.

Data availability. Raw sequencing reads were deposited in the NCBI Sequence Read Archive (SRA) under the project number PRJNA573088.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

FIG S1, EPS file, 0.3 MB.
FIG S2, EPS file, 0.7 MB.
FIG S3, EPS file, 0.9 MB.
FIG S4, EPS file, 0.1 MB.
FIG S5, EPS file, 0.9 MB.
FIG S6, EPS file, 0.1 MB.
TABLE S1, DOCX file, 0.03 MB.
TABLE S2, DOCX file, 0.03 MB.
TABLE S3, DOCX file, 0.03 MB.
TABLE S4, DOCX file, 0.03 MB.

ACKNOWLEDGMENTS

Support for this project was provided by the National Science Foundation's Antarctic Program: A.R.M. (CMU award 1043670) and K.M.H. (AU award 1043745) and

from Central Michigan University Faculty Research and Creative Endeavors (FRCE) Committee and College of Science and Engineering.

Thanks go to the crew and scientific staff onboard the *RVIB Nathaniel B. Palmer* for assistance with sample collection. Thanks go to Michael Pavia for help in data analysis and to Sean McAllister for insightful discussions regarding analysis of these metagenomes.

REFERENCES

1. Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB. 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. Proc Natl Acad Sci U S A 102:14683–14688. https://doi.org/10.1073/pnas.0506625102.
2. Casciotti KL, Sigman DM, Ward BB. 2003. Linking diversity and stable isotope fractionation in ammonia-oxidizing bacteria. Geomicrobiol J 20:335–353. https://doi.org/10.1080/01490450303895.
3. Hatzenpichler R. 2012. Diversity, physiology, and niche differentiation of ammonia-oxidizing archaea. Appl Environ Microbiol 78:7501–7510. https://doi.org/10.1128/AEM.01960-12.
4. Stahl DA, de la Torre JR. 2012. Physiology and diversity of ammonia-oxidizing archaea. Annu Rev Microbiol 66:83–101. https://doi.org/10.1146/annurev-micro-092611-150128.
5. Kalanetra KM, Bano N, Hollibaugh JT. 2009. Ammonia-oxidizing Archaea in the Arctic Ocean and Antarctic coastal waters. Environ Microbiol 11:2434–2445. https://doi.org/10.1111/j.1462-2920.2009.01974.x.
6. Yool A, Martin AP, Fernandez C, Clark DR. 2007. The significance of nitrification for oceanic new production. Nature 447:999–1002. https://doi.org/10.1038/nature05885.
7. Cavicchioli R. 2015. Microbial ecology of Antarctic aquatic systems. Nat Rev Microbiol 13:691–706. https://doi.org/10.1038/nrmicro3549.
8. Tolar BB, Ross MJ, Wallsgrove NJ, Liu Q, Aluwihare LI, Popp BN, Hollibaugh JT. 2016. Contribution of ammonia oxidation to chemoautotrophy in Antarctic coastal waters. ISME J 10:2605–2619. https://doi.org/10.1038/ismej.2016.61.
9. Manganelli M, Malfatti F, Samo TJ, Mitchell BG, Wang H, Azam F. 2009. Major role of microbes in carbon fluxes during Austral winter in the Southern Drake Passage. PLoS One 4:e6941. https://doi.org/10.1371/journal.pone.0006941.
10. Williams TJ, Long E, Evans F, DeMaere MZ, Lauro FM, Raftery MJ, Ducklow H, Grzymski JJ, Murray AE, Cavicchioli R. 2012. A metaproteomic assessment of winter and summer bacterioplankton from Antarctic Peninsula coastal surface waters. ISME J 6:1883–1900. https://doi.org/10.1038/ismej.2012.28.
11. Grzymski JJ, Riesenfeld CS, Williams TJ, Dussaq AM, Ducklow H, Erickson M, Cavicchioli R, Murray AE. 2012. A metagenomic assessment of winter and summer bacterioplankton from Antarctica Peninsula coastal surface waters. ISME J 6:1901–1915. https://doi.org/10.1038/ismej.2012.31.
12. Smart SM, Fawcett SE, Thomalla SJ, Weigand MA, Reason CJC, Sigman DM. 2015. Isotopic evidence for nitrification in the Antarctic winter mixed layer. Global Biogeochem Cycles 29:427–445. https://doi.org/10.1002/2014GB005013.
13. Nedwell DB, Walker TR. 1995. Sediment-water fluxes of nutrients in an Antarctic coastal environment—influence of bioturbation. Polar Biol 15:57–64. https://doi.org/10.1007/BF00236125.
14. Franco DC, Signori CN, Duarte RTD, Nakayama CR, Campos LS, Pellizari VH. 2017. High prevalence of Gammaproteobacteria in the sediments of Admiralty Bay and North Bransfield Basin, Northwestern Antarctic Peninsula. Front Microbiol 8:153. https://doi.org/10.3389/fmicb.2017.00153.
15. Learman DR, Henson MW, Thrash JC, Temperton B, Brannock PM, Santos SR, Mahon AR, Halanych KM. 2016. Biogeochemical and microbial variation across 5500 km of Antarctic surface sediment implicates organic matter as a driver of benthic community structure. Front Microbiol 7:284. https://doi.org/10.3389/fmicb.2016.00284.
16. Wuchter C, Abbas B, Coolen MJL, Herfort L, van Bleijswijk J, Timmers P, Strous M, Teira E, Herndl GJ, Middelburg JJ, Schouten S, Damste JSS. 2006. Archaeal nitrification in the ocean. Proc Natl Acad Sci U S A 103:12317–12322. https://doi.org/10.1073/pnas.0600756103.
17. Jørgensen BB, Findlay AJ, Pellerin A. 2019. The biogeochemical sulfur cycle of marine sediments. Front Microbiol 10:849. https://doi.org/10.3389/fmicb.2019.00849.
18. Jørgensen BB, Kasten S. 2006. Sulfur cycling and methane oxidation, p 271–309. *In* Schulz HD, Zabel M (ed), Marine geochemistry. Springer, New York, NY.
19. Canfield DE. 1989. Reactive iron in marine sediments. Geochim Cosmochim Acta 53:619–632. https://doi.org/10.1016/0016-7037(89)90005-7.
20. Thamdrup B, Fossing H, Jorgensen BB. 1994. Manganese, iron, and sulfur cycling in a coastal marine sediment, Aarhus Bay, Denmark. Geochim Cosmochim Acta 58:5115–5129. https://doi.org/10.1016/0016-7037(94)90298-4.
21. Thamdrup B, Finster K, Fossing H, Hansen JW, Jorgensen BB. 1994. Thiosulfate and sulfite distributions in porewater of marine sediments related to manganese, iron, and sulfur geochemistry. Geochim Cosmochim Acta 58:67–73. https://doi.org/10.1016/0016-7037(94)90446-4.
22. Jørgensen BB. 1982. Mineralization of organic matter in the sea bed—the role of sulfate reduction. Nature 296:643–645. https://doi.org/10.1038/296643a0.
23. Mikucki JA, Priscu JC. 2007. Bacterial diversity associated with blood falls, a subglacial outflow from the Taylor Glacier, Antarctica. Appl Environ Microbiol 73:4029–4039. https://doi.org/10.1128/AEM.01396-06.
24. Achberger AM, Christner BC, Michaud AB, Priscu JC, Skidmore ML, Vick-Majors TJ, Team WS, WISSARD Science Team. 2016. Microbial community structure of Subglacial Lake Whillans, West Antarctica. Front Microbiol 7:1457.
25. Purcell AM, Mikucki JA, Achberger AM, Alekhina IA, Barbante C, Christner BC, Ghosh D, Michaud AB, Mitchell AC, Priscu JC, Scherer R, Skidmore ML, Vick-Majors TJ, The Wissard Science Team. 2014. Microbial sulfur transformations in sediments from Subglacial Lake Whillans. Front Microbiol 5:594. https://doi.org/10.3389/fmicb.2014.00594.
26. Bowman JP, McCuaig RD. 2003. Biodiversity, community structural shifts, and biogeography of prokaryotes within Antarctic continental shelf sediment. Appl Environ Microbiol 69:2463–2483. https://doi.org/10.1128/AEM.69.5.2463-2483.2003.
27. Mincks SL, Smith CR, DeMaster DJ. 2005. Persistence of labile organic matter and microbial biomass in Antarctic shelf sediments: evidence of a sediment 'food bank'. Mar Ecol Prog Ser 300:3–19. https://doi.org/10.3354/meps300003.
28. Jamieson RE, Heywood JL, Rogers AD, Billett DSM, Pearce DA. 2013. Bacterial biodiversity in deep-sea sediments from two regions of contrasting surface water productivity near the Crozet Islands, Southern Ocean. Deep Sea Res 1 Oceanogr Res Papers 75:67–77. https://doi.org/10.1016/j.dsr.2012.12.012.
29. Carr SA, Vogel SW, Dunbar RB, Brandes J, Spear JR, Levy R, Naish TR, Powell RD, Wakeham SG, Mandernack KW. 2013. Bacterial abundance and composition in marine sediments beneath the Ross Ice Shelf, Antarctica. Geobiology 11:377–395. https://doi.org/10.1111/gbi.12042.
30. Ruff SE, Probandt D, Zinkann AC, Iversen MH, Klaas C, Wurzberg L, Krombholz N, Wolf-Gladrow D, Amann R, Knittel K. 2014. Indications for algae-degrading benthic microbial communities in deep-sea sediments along the Antarctic Polar Front. Deep Sea Res 2 Top Stud Oceanogr 108:6–16. https://doi.org/10.1016/j.dsr2.2014.05.011.
31. Signori CN, Thomas F, Enrich-Prast A, Pollery RC, Sievert SM. 2014. Microbial diversity and community structure across environmental gradients in Bransfield Strait, Western Antarctic Peninsula. Front Microbiol 5:647. https://doi.org/10.3389/fmicb.2014.00647.
32. Chain P, Lamerdin J, Larimer F, Regala W, Lao V, Land M, Hauser L, Hooper A, Klotz M, Norton J, Sayavedra-Soto L, Arciero D, Hommes N, Whittaker M, Arp D. 2003. Complete genome sequence of the ammonia-oxidizing bacterium and obligate chemolithoautotroph Nitrosomonas europaea. J Bacteriology 185:2759–6496. https://doi.org/10.1128/JB.185.9.2759-2773.2003.
33. Arp DJ, Sayavedra-Soto LA, Hommes NG. 2002. Molecular biology and biochemistry of ammonia oxidation by Nitrosomonas europaea. Arch Microbiol 178:250–255. https://doi.org/10.1007/s00203-002-0452-0.

34. Shaw LJ, Nicol GW, Smith Z, Fear J, Prosser JI, Baggs EM. 2006. Nitrosospira spp. can produce nitrous oxide via a nitrifier denitrification pathway. Environ Microbiol 8:214–222. https://doi.org/10.1111/j.1462-2920.2005.00882.x.
35. Sayavedra-Soto LA, Hommes NG, Alzerreca JJ, Arp DJ, Norton JM, Klotz MG. 1998. Transcription of the amoC, amoA and amoB genes in Nitrosomonas europaea and Nitrosospira sp, NpAV. FEMS Microbiol Lett 167:81–88. https://doi.org/10.1111/j.1574-6968.1998.tb13211.x.
36. Voytek MA, Ward BB. 1995. Detection of ammonium-oxidizing bacteria of the beta-subclass of the class Proteobacteria in aquatic samples with the PCR. Appl Environ Microbiol 61:1444–1450. https://doi.org/10.1128/aem.61.4.1444-1450.1995.
37. Vanparys B, Spieck E, Heylen K, Wittebolle L, Geets J, Boon N, De Vos P. 2007. The phylogeny of the genus Nitrobacter based on comparative rep-PCR, 16S rRNA and nitrite oxidoreductase gene sequence analysis. Syst Appl Microbiol 30:297–308. https://doi.org/10.1016/j.syapm.2006.11.006.
38. Poly F, Wertz S, Brothier E, Degrange V. 2008. First exploration of Nitrobacter diversity in soils by a PCR cloning-sequencing approach targeting functional gene nxrA. FEMS Microbiol Ecol 63:132–140. https://doi.org/10.1111/j.1574-6941.2007.00404.x.
39. Pester M, Maixner F, Berry D, Rattei T, Koch H, Lucker S, Nowka B, Richter A, Spieck E, Lebedeva E, Loy A, Wagner M, Daims H. 2014. NxrB encoding the beta subunit of nitrite oxidoreductase as functional and phylogenetic marker for nitrite-oxidizing Nitrospira. Environ Microbiol 16:3055–3071. https://doi.org/10.1111/1462-2920.12300.
40. Zhao R, Hannisdal B, Mogollon JM, Jorgensen SL. 2019. Nitrifier abundance and diversity peak at deep redox transition zones. Sci Rep 9:8633. https://doi.org/10.1038/s41598-019-44585-6.
41. Rani S, Koh HW, Rhee SK, Fujitani H, Park SJ. 2017. Detection and diversity of the nitrite oxidoreductase alpha subunit (nxrA) gene of Nitrospina in marine sediments. Microb Ecol 73:111–122. https://doi.org/10.1007/s00248-016-0897-3.
42. Harhangi HR, Le Roy M, van Alen T, Hu BL, Groen J, Kartal B, Tringe SG, Quan ZX, Jetten MSM, Op den Camp HJM. 2012. Hydrazine synthase, a unique phylomarker with which to study the presence and biodiversity of anammox bacteria. Appl Environ Microbiol 78:752–758. https://doi.org/10.1128/AEM.07113-11.
43. Hirsch MD, Long ZT, Song B. 2011. Anammox bacterial diversity in various aquatic ecosystems based on the detection of hydrazine oxidase genes (hzoA/hzoB). Microb Ecol 61:264–276. https://doi.org/10.1007/s00248-010-9743-1.
44. Ghosh W, Dam B. 2009. Biochemistry and molecular biology of lithotrophic sulfur oxidation by taxonomically and ecologically diverse bacteria and archaea. FEMS Microbiol Rev 33:999–1043. https://doi.org/10.1111/j.1574-6976.2009.00187.x.
45. Stahl DA, Loy A, Wagner M. 2007. Molecular strategies for studies of natural populations of sulphate-reducing microorganisms, p 39–116. *In* Barton LL, Hamilton WA (ed), Sulphate-reducing bacteria: environmental and engineered systems. Cambridge University Press, Cambridge, United Kingdom.
46. Loy A, Duller S, Wagner M. 2008. Evolution and ecology of microbes dissimilating sulfur compounds: insights from siroheme sulfite reductases., p 46–59. *In* Dahl C, Friedrich CG (ed), Microbial sulfur metabolism. Springer, Berlin, Germany.
47. Loy A, Duller S, Baranyi C, Mussmann M, Ott J, Sharon I, Beja O, Le Paslier D, Dahl C, Wagner M. 2009. Reverse dissimilatory sulfite reductase as phylogenetic marker for a subgroup of sulfur-oxidizing prokaryotes. Environ Microbiol 11:289–299. https://doi.org/10.1111/j.1462-2920.2008.01760.x.
48. Zverlov V, Klein M, Lucker S, Friedrich MW, Kellermann J, Stahl DA, Loy A, Wagner M. 2005. Lateral gene transfer of dissimilatory (bi)sulfite reductase revisited. J Bacteriol 187:2203–2208. https://doi.org/10.1128/JB.187.6.2203-2208.2005.
49. Griesbeck C, Günter H, Schütz M. 2000. Biological sulfide-oxidation: sulfide-quinone reductase (SQR), the primary reaction, p 179–203. *In* Pandalai SG (ed), Recent research developments in microbiology, vol 4. Research Signpost, Trivadrum, India.
50. Dahl C (ed). 2008. Inorganic sulfur compounds as electron donors in purple sulfur bacteria. Springer, Dordrecht, The Netherlands.
51. Grimm F, Franz B, Dahl C (ed). 2008. Thiosulfate and sulfur oxidation in purple sulfur bacteria. Springer, Berlin, Germany.
52. Anantharaman K, Breier JA, Sheik CS, Dick GJ. 2013. Evidence for hydrogen oxidation and metabolic plasticity in widespread deep-sea sulfur-oxidizing bacteria. Proc Natl Acad Sci U S A 110:330–335. https://doi.org/10.1073/pnas.1215340110.
53. Gregersen LH, Bryant DA, Frigaard NU. 2011. Mechanisms and evolution of oxidative sulfur metabolism in green sulfur bacteria. Front Microbiol 2:116. https://doi.org/10.3389/fmicb.2011.00116.
54. Frigaard N-U, Bryant DA. 2008. Genomic insights into the sulfur metabolism of phototrophic green sulfur bacteria, p 60–76. *In* Hell R, Dahl C, Knaff D, Leustek T (ed), Sulfur metabolism in phototrophic organisms. Springer, New York, NY.
55. Eisen JA, Nelson KE, Paulsen IT, Heidelberg JF, Wu M, Dodson RJ, Deboy R, Gwinn ML, Nelson WC, Haft DH, Hickey EK, Peterson JD, Durkin AS, Kolonay JL, Yang F, Holt I, Umayam LA, Mason T, Brenner M, Shea TP, Parksey D, Nierman WC, Feldblyum TV, Hansen CL, Craven MB, Radune D, Vamathevan J, Khouri H, White O, Gruber TM, Ketchum KA, Venter JC, Tettelin H, Bryant DA, Fraser CM. 2002. The complete genome sequence of Chlorobium tepidum TLS, a photosynthetic, anaerobic, green-sulfur bacterium. Proc Natl Acad Sci U S A 99:9509–9514. https://doi.org/10.1073/pnas.132181499.
56. Veliz EA, Martinez-Hidalgo P, Hirsch AM. 2017. Chitinase-producing bacteria and their role in biocontrol. Aims Microbiol 3:689–705. https://doi.org/10.3934/microbiol.2017.3.689.
57. Beier S, Bertilsson S. 2013. Bacterial chitin degradation-mechanisms and ecophysiological strategies. Front Microbiol 4:149. https://doi.org/10.3389/fmicb.2013.00149.
58. Bai YN, Eijsink VGH, Kielak AM, van Veen JA, de Boer W. 2016. Genomic comparison of chitinolytic enzyme systems from terrestrial and aquatic bacteria. Environ Microbiol 18:38–49. https://doi.org/10.1111/1462-2920.12545.
59. Corno G, Salka I, Pohlmann K, Hall AR, Grossart HP. 2015. Interspecific interactions drive chitin and cellulose degradation by aquatic microorganisms. Aquat Microb Ecol 76:27–37. https://doi.org/10.3354/ame01765.
60. Gooday GW. 1990. Physiology of microbial degradation of chitin and chitosan. Biodegradation 1:177–190. https://doi.org/10.1007/BF00058835.
61. Anderson CG, DePablo N, Romo CR. 1978. Antarctic krill (Euphausia superba Dana) as a source of chitin and chitosan, p 5–10. *In* Muzzarellia RAA, Pariser ER (ed), Proceedings of the First International Conference on Chitin/Chitosan. MIT, Cambridge, MA.
62. Herwig RP, Pellerin NB, Irgens RL, Maki JS, Staley JT. 1988. Chitinolytic bacteria and chitin mineralization in the marine waters and sediments along the Antarctic Peninsula. FEMS Microbiol Ecol 53:101–111. https://doi.org/10.1111/j.1574-6968.1988.tb02653.x.
63. Xiao X, Yin XB, Lin H, Sun LG, You ZY, Wang P, Wang FP. 2005. Chitinase genes in lake sediments of Ardley Island, Antarctica. Appl Environ Microbiol 71:7904–7909. https://doi.org/10.1128/AEM.71.12.7904-7909.2005.
64. Kraemer JT, Bagley DM. 2007. Improving the yield from fermentative hydrogen production. Biotechnol Lett 29:685–695. https://doi.org/10.1007/s10529-006-9299-9.
65. Vignais PM, Billoud B. 2007. Occurrence, classification, and biological function of hydrogenases: an overview. Chem Rev 107:4206–4272. https://doi.org/10.1021/cr050196r.
66. Giovannoni SJ, Cameron Thrash J, Temperton B. 2014. Implications of streamlining theory for microbial ecology. ISME J 8:1553–1565. https://doi.org/10.1038/ismej.2014.60.
67. Garber A, Armbruster CR, Lee SE, Cooper VS, Bomberger JM, McAllister SM. 2021. SprayNPray: user-friendly taxonomic profiling of genome and metagenome contigs. bioRxiv https://doi.org/10.1101/2021.07.17.452725.
68. Aylward FO, Santoro AE. 2020. Heterotrophic thaumarchaea with small genomes are widespread in the dark ocean. mSystems 5:e00415-20. https://doi.org/10.1128/mSystems.00415-20.
69. Walker CB, de la Torre JR, Klotz MG, Urakawa H, Pinel N, Arp DJ, Brochier-Armanet C, Chain PSG, Chan PP, Gollabgir A, Hemp J, Hugler M, Karr EA, Konneke M, Shin M, Lawton TJ, Lowe T, Martens-Habbena W, Sayavedra-Soto LA, Lang D, Sievert SM, Rosenzweig AC, Manning G, Stahl DA. 2010. Nitrosopumilus maritimus genome reveals unique mechanisms for nitrification and autotrophy in globally distributed marine crenarchaea. Proc Natl Acad Sci U S A 107:8818–8823. https://doi.org/10.1073/pnas.0913533107.
70. Purkhold U, Pommerening-Roser A, Juretschko S, Schmid MC, Koops HP, Wagner M. 2000. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoA sequence analysis: implications for molecular diversity surveys. Appl Environ Microbiol 66:5368–5382. https://doi.org/10.1128/AEM.66.12.5368-5382.2000.
71. Rasigraf O, Schmitt J, Jetten MSM, Luke C. 2017. Metagenomic potential for and diversity of N-cycle driving microorganisms in the Bothnian Sea sediment. Microbiologyopen 6:e00475. https://doi.org/10.1002/mbo3.475.

72. Tully BJ, Graham ED, Heidelberg JF. 2018. The reconstruction of 2,631 draft metagenome-assembled genomes from the global oceans. Sci Data 5:170203. https://doi.org/10.1038/sdata.2017.203.
73. Roeselers G, Newton IL, Woyke T, Auchtung TA, Dilly GF, Dutton RJ, Fisher MC, Fontanez KM, Lau E, Stewart FJ, Richardson PM, Barry KW, Saunders E, Detter JC, Wu D, Eisen JA, Cavanaugh CM. 2010. Complete genome sequence of Candidatus Ruthia magnifica. Stand Genomic Sci 3:163–173. https://doi.org/10.4056/sigs.1103048.
74. Newton IL, Woyke T, Auchtung TA, Dilly GF, Dutton RJ, Fisher MC, Fontanez KM, Lau E, Stewart FJ, Richardson PM, Barry KW, Saunders E, Detter JC, Wu D, Eisen JA, Cavanaugh CM. 2007. The Calyptogena magnifica chemoautotrophic symbiont genome. Science 315:998–1000. https://doi.org/10.1126/science.1138438.
75. McCutcheon JP, Moran NA. 2011. Extreme genome reduction in symbiotic bacteria. Nat Rev Microbiol 10:13–26. https://doi.org/10.1038/nrmicro2670.
76. Ayling M, Clark MD, Leggett RM. 2020. New approaches for metagenome assembly with short reads. Brief Bioinform 21:584–594. https://doi.org/10.1093/bib/bbz020.
77. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.
78. Li DH, Luo RB, Liu CM, Leung CM, Ting HF, Sadakane K, Yamashita H, Lam TW. 2016. MEGAHIT v1.0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods 102:3–11. https://doi.org/10.1016/j.ymeth.2016.02.020.
79. Kang DWD, Li F, Kirton E, Thomas A, Egan R, An H, Wang Z. 2019. MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. Peerj 7:e7359. https://doi.org/10.7717/peerj.7359.
80. Sieber CMK, Probst AJ, Sharrar A, Thomas BC, Hess M, Tringe SG, Banfield JF. 2018. Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy. Nat Microbiol 3:836–843. https://doi.org/10.1038/s41564-018-0171-1.
81. Eren AM, Esen OC, Quince C, Vineis JH, Morrison HG, Sogin ML, Delmont TO. 2015. Anvi'o: an advanced analysis and visualization platform for 'omics data. Peerj 3:e1319. https://doi.org/10.7717/peerj.1319.
82. Chen IMA, Markowitz VM, Chu K, Palaniappan K, Szeto E, Pillay M, Ratner A, Huang JH, Andersen E, Huntemann M, Varghese N, Hadjithomas M, Tennessen K, Nielsen T, Ivanova NN, Kyrpides NC. 2017. IMG/M: integrated genome and metagenome comparative data analysis system. Nucleic Acids Res 45:D507–D516. https://doi.org/10.1093/nar/gkw929.
83. Markowitz VM, Chen I-MA, Palaniappan K, Chu K, Szeto E, Grechkin Y, Ratner A, Jacob B, Huang J, Williams P, Huntemann M, Anderson I, Mavromatis K, Ivanova NN, Kyrpides NC. 2012. IMG: the integrated microbial genomes database and comparative analysis system. Nucleic Acids Res 40:D115–D122. https://doi.org/10.1093/nar/gkr1044.
84. Hyatt D, Chen GL, Locascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11:119. https://doi.org/10.1186/1471-2105-11-119.
85. Anantharaman K, Brown CT, Hug LA, Sharon I, Castelle CJ, Probst AJ, Thomas BC, Singh A, Wilkins MJ, Karaoz U, Brodie EL, Williams KH, Hubbard SS, Banfield JF. 2016. Thousands of microbial genomes shed light on interconnected biogeochemical processes in an aquifer system. Nat Commun 7:13219. https://doi.org/10.1038/ncomms13219.
86. Garber AI, Nealson KH, Okamoto A, McAllister SM, Chan CRS, Barco RA, Merino N. 2020. FeGenie: a comprehensive tool for the identification of iron genes and iron gene neighborhoods in genome and metagenome assemblies. Front Microbiol 11:37. https://doi.org/10.3389/fmicb.2020.00037.
87. Lombard V, Ramulu HG, Drula E, Coutinho PM, Henrissat B. 2014. The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42:D490–D495. https://doi.org/10.1093/nar/gkt1178.
88. Kanehisa M, Sato Y, Morishima K. 2016. BlastKOALA and GhostKOALA: KEGG tools for functional characterization of genome and metagenome sequences. J Mol Biol 428:726–731. https://doi.org/10.1016/j.jmb.2015.11.006.
89. Graham ED, Heidelberg JF, Tully BJ. 2018. Potential for primary productivity in a globally-distributed bacterial phototroph. ISME J 12:1861–1866. https://doi.org/10.1038/s41396-018-0091-3.
90. Lee MD. 2019. GToTree: a user-friendly workflow for phylogenomics. Bioinformatics 35:4162–4164. https://doi.org/10.1093/bioinformatics/btz188.
91. O'Leary NA, Wright MW, Brister JR, Ciufo S, McVeigh DHR, Rajput B, Robbertse B, Smith-White B, Ako-Adjei D, Astashyn A, Badretdin A, Bao YM, Blinkova O, Brover V, Chetvernin V, Choi J, Cox E, Ermolaeva O, Farrell CM, Goldfarb T, Gupta T, Haft D, Hatcher E, Hlavina W, Joardar VS, Kodali VK, Li WJ, Maglott D, Masterson P, McGarvey KM, Murphy MR, O'Neill K, Pujar S, Rangwala SH, Rausch D, Riddick LD, Schoch C, Shkeda A, Storz SS, Sun HZ, Thibaud-Nissen F, Tolstoy I, Tully RE, Vatsan AR, Wallin C, Webb D, Wu W, Landrum MJ, Kimchi A, Tatusova T, et al. 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 44:D733–D745. https://doi.org/10.1093/nar/gkv1189.
92. Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. 2019. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics 36:1925–1927. https://doi.org/10.1093/bioinformatics/btz848.
93. Parks DH, Chuvochina M, Waite DW, Rinke C, Skarshewski A, Chaumeil PA, Hugenholtz P. 2018. A standardized bacterial taxonomy based on genome phylogeny substantially revises the tree of life. Nat Biotechnol 36:996–1004. https://doi.org/10.1038/nbt.4229.
94. Buchfink B, Xie C, Huson DH. 2015. Fast and sensitive protein alignment using DIAMOND. Nat Methods 12:59–60. https://doi.org/10.1038/nmeth.3176.
95. Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. https://doi.org/10.1093/nar/25.17.3389.
96. Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340.
97. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033.
98. Masque P, Isla E, Sanchez-Cabeza JA, Palanques A, Bruach JM, Puig P, Guillen J. 2002. Sediment accumulation rates and carbon fluxes to bottom sediments at the Western Bransfield Strait (Antarctica). Deep Sea Res 2 Top Stud Oceanogr 49:921–933.