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Potential for microbial denitrification coupled with methanol oxidation found in abundant MAGs in Antarctic Peninsula sediments

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Abstract

Denitrification accounts for a substantial nitrogen loss from environmental systems, shifting microbial composition and impacting other biogeochemical cycles. In Antarctica, rising temperatures cause increased organic matter deposition in marine sediments, which can significantly alter microbially mediated denitrification. To examine the genetic potential of microorganisms driving N-cycling in these sediments, benthic sediment cores were collected at two sites in the Weddell Sea, Antarctica. DNA was extracted from multiple depths at each site, resulting in the reconstruction of 75 high-quality metagenome-assembled genomes (MAGs). Forty-seven of these MAGs contained reductases involved in denitrification. MAGs belonging to the genus *Methyloceanibacter* were the most abundant MAGs at both sites and all depths, except depth 3–6 cmbsf at one site, where they were not identified. The abundance of these *Methyloceanibacter* MAGs suggests the potential for nitrate-driven methanol oxidation at both sites. MAGs belonging to *Beggiatoaceae* and *Sedimenticolaceae* were found to have the genetic potential to produce intermediates in denitrification and the complete pathway for dissimilatory nitrate reduction to ammonia. MAGs within Acidimicrobiia and Dadabacteria had the potential to complete the final denitrification step. Based on MAGs, Antarctic peninsula sediment communities have the potential for complete denitrification and dissimilatory nitrate reduction to ammonia via a consortium.

Keywords: Acidimicrobiia; *Beggiatoaceae*; metagenomics; *Methyloceanibacter*; nitrogen cycling; *Sedimenticolaceae*

Introduction

Marine sediments host metabolically active microbial consortia that drives the development of predictable biogeochemical zonation patterns. Nitrate is the preferred electron acceptor once oxygen is depleted (Cole 1996, Jørgensen 2019). Denitrification, driven by a consortium of diverse microbial taxa, results in the sequential reduction of nitrate (NO_3^-) and nitrite (NO_2^-) to nitrogen gas (N_2), thereby returning molecular nitrogen to the atmosphere (Tiedje 1988). Denitrification requires a suite of denitrifying genes that encode enzymes such as nitrate reductases (*narG* and *napA*), nitrite reductases (*nirS* and *nirK*), nitric oxide reductase (*norB*), and nitrous oxide reductase (*nosZ*), which collectively convert nitrate to molecular nitrogen via intermediate nitrogen oxides (Zumft 1997). Denitrification in continental shelf sediments causes a substantial loss of biologically available nitrogen, and the presence of denitrifiers tends to be associated with denitrification (Seitzinger and Giblin 1996, Zumft 1997). Facultative anaerobes largely carry out this process in environments that routinely experience anoxia, such as marine sediments (Tiedje 1988). Denitrification has also been shown to occur in aerobic environments (Yang et al. 2020), increasing its potential shift in microbial communities and impacting the ecosystem.

Many studies of Antarctic marine sediments have focused on the taxonomy and diversity of microbial communities rather than genetic potential for denitrification (Bowman et al. 2003, Carr et al. 2013, Learman et al. 2016, Li et al. 2020, Wunder et al. 2021). One common taxon found in Southern Ocean sediments has been Proteobacteria, with some members from the genus *Pseudomonas* known for their role in denitrification (Wilkins et al. 2013, Alcántara-Hernández et al. 2014, Choi et al. 2016, Dutta et al. 2023a). Two additional members of the denitrifying community found in Antarctica are within the class Gammaproteobacteria: *Sedimenticolaceae* and *Beggiatoaceae* (Teske and Salmon 2013, Arora-Williams et al. 2022). *Beggiatoaceae* are filamentous bacteria found in microbial mats that have been previously observed in Antarctic lakes (MacGregor et al. 2013, Alcántara-Hernández et al. 2014). *Sedimenticolaceae* are found in various habitats, such as salt marshes, estuaries, and terrestrial mud volcanoes (Flood et al. 2015, Arora-Williams et al. 2022, Slobodkina et al. 2023). They are also identified as endosymbionts of marine invertebrates like mollusks (Lim et al. 2019). Both families exhibit chemolithoautotrophic capabilities, using hydrogen sulfide as an electron donor and nitrate as an electron acceptor (MacGregor et al. 2013, Slobodkina et al. 2023).

Few studies have directly examined the genetic potential of microbial communities to reduce nitrogen compounds in Antarctic marine sediments (Choi et al. 2016, Garber et al. 2021). The genetic potential for microbial denitrification has been extensively docu-

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mented in Antarctic lakes, streams, seawater, and soils, demonstrating its prevalence within polar environments (Alcántara-Hernández et al. 2014, Ortiz et al. 2020, Cabezas et al. 2022, Dutta et al. 2023b). In the current study, metagenomic analyses demonstrated a bacterial consortium may be responsible for denitrification in continental shelf sediments near the Antarctic Peninsula. Members of the genus *Methyloceanibacter* were documented to have the genetic potential to oxidize methanol while catalyzing nitrate reduction. Members of Acidimicrobiia and Dadabacteria participate in nitrous oxide reduction, and members within *Sedimenticolaceae* and *Beggiatoaceae* may be responsible for completing nitrite and nitric oxide reduction. Understanding microbial drivers in denitrification is essential for deciphering nitrogen cycling dynamics and predicting ecosystem responses to environmental change.

Materials and methods

Sample collection

Triplicate 12-cm sediment cores from two locations were sampled in the Weddell Sea, Antarctica, in November of 2020, from the *RV/IB Nathaniel B. Palmer* (NBP 20–10), using the ship's Megacorer: site 2020.AP.045 (63.375° S, 53.546° W) and site 2020.AP.058 (−63.4198° S, 53.553° W). On deck, the cores were sectioned into four depth intervals (0–3, 3–6, 6–9, and 9–12 cmbsf), transferred into Whirl-Pak bags, and immediately frozen at −80°C. Samples were then shipped to Central Michigan University for processing.

Chemical analysis

Frozen, unprocessed samples were submitted directly to two commercial facilities for chemical analysis. Total organic carbon (TOC), total nitrogen concentration, and stable isotope (δ ^{13}C and δ 15 N) analyses were done at EcoCore Analytic Services at Colorado State University (Fort Collins, CO, USA). Grain size analysis and bulk sediment concentrations of nitrate, nitrite, sulfate, and ammonia were collected at Trace Analytical Laboratories (Muskegon, MI, USA). Chemical and oceanographic data for this project have been submitted to the United States Antarctic Program Data Center (https://www.usap-dc.org/view/project/p0010235).

DNA extractions and sequencing

DNA from the sediments was extracted from each depth sample in triplicate using the Qiagen DNeasy PowerSoil Pro kit (Valencia, CA, USA) following the manufacturer's recommendations. The resulting extractions were then pooled and concentrated using a Zymo Clean & Concentrate Kit (Irvine, CA, USA) and quantified using a Qubit 2.0 fluorometer. The DNA samples were then sent to Michigan State University's Research Technology Support Facility (RTSF) Genomics Core (Lansing, MI, USA) for sequencing using Illumina NovaSeq 6000 (150 paired-end reads). Raw DNA sequencing reads are publicly available at NCBI GenBank: BioProject database ID PRJNA880629.

Bioinformatics

FastQC was used to check the quality of the raw DNA sequencing reads, and Trimmomatic v0.39 was used to trim the reads (Andrews 2010, Bolger et al. 2014). Reads were then assembled into metagenomes using Megahit v1.2.9, and Quast v5.0.0 was used to assess the quality of the assemblies (Gurevich et al. 2013, Li et al. 2015). Metagenomes were annotated using the DOE Joint Genome Institute's Integrated Microbial Genomes platform (Chen et al. 2017). BWA v0.7.17 was used to map reads to the assemblies to generate coverage data used for binning of metagenomes into metagenome-assembled genomes (MAGs) using Metabat2 with default parameters (Li and Durbin 2009, Kang et al. 2019). CheckM v1.1.3 was used to check the contamination and completeness of the MAGs (Parks et al. 2015). GTDB-Tk v1.3.0 was used for the taxonomic assignment of the MAGs with associated average nucleotide identity (ANI) values (Chaumeil et al. 2020). High-quality MAGs (>90% completion, <5% contamination) were annotated using Prokka v1.14.6 (Seemann 2014). Further, high-quality MAGs were annotated using LithoGenie within MagicLamp, which queries against a set of specifically curated HMMs designed to target lithotrophic metabolisms (Garber et al. 2020). Additionally, high-quality MAGs were annotated using Anvi'o (v7) with KEGG orthologs modules using the "anvi_estimate_metabolism" command (Eren et al. 2021, Veseli et al. 2023).

Normalized abundances of MAGs were calculated using a previously described protocol (Savoie et al. 2021) with some modifications. First, raw reads were quality filtered to remove noise with illumina utils v2.6 (Eren et al. 2013, 2021). High-quality MAGs were concatenated separately for each site and depth, and raw reads were competitively mapped back with bowtie2 v2.5.1 (Langmead and Salzberg 2012), resulting in sam files being converted to bam files with SAMtools v1.19.2 (Li et al. 2009). To remove low-quality read mappings, coverM v0.7.0 was used with a minimum identity of 95% and minimum read alignment of 75% (GitHub 2024). A count table was created to obtain read counts for each site and depth using the get_count_table.py script (edamame-course/Metagenome 2023). To get a normalized abundance of high-quality MAGs, the number of reads mapped back to each MAG was transformed to reads per kilobase pair million (RPKM).

Results

Geochemical analysis

The two sampling sites examined in this study were selected based on organic matter levels. Site 2020.AP.045 was at a water depth of 379 m, while site 2020.AP.058 had a depth of 639 m. Sample 2020.AP.045 had relatively low TOC, ranging (0.2%–0.37%) and total nitrogen content ranging (0.03%–0.04%) for 0–3, 3–6, 6–9, and 9–12 cmbsf. Nitrate values for site 2020.AP.045 were negligible (<1.0 mg/kg dry) for all depths. Sample 2020.AP.058 had relatively higher TOC ranging (1.47%–1.55%) and nitrogen content ranging (0.21%–0.23%) content for 0–3, 3–6, 6–9, and 9–12 cmbsf. Nitrate values (mg/kg dry) for site 2020.AP.058 consisted of 2.1 for 0–3 cmbsf and <1.3 for all other depths. TOC concentrations were relatively consistent within each site; however, in 2020.AP.045.6 (6–9 cmbsf) had a higher corresponding C/N ratio (14:4, range 5.8–14.4). Sulfate concentrations (mg/kg dry) were higher in samples from 2020.AP.058, ranging (4200–4900), relative to 2020.AP.045, ranging (750–1100), and at both sites, sulfate concentrations decreased with depth (Table S1). Additionally, δ^{13}C (TOC) values were consistently lower in 2020.AP.045, where they ranged from –23.85‰ to –24.18‰, than in 2020.AP.058, where they ranged from –22.18‰ to –22.66‰ (Table S1). Grain size analyses for site 2020.AP.045 documented a higher percentage of total sand, and a lower percentage of total fines (% fine silt and fine clay), while site 2020.AP.058 documented a higher percentage of contained a lower % of total fines (% fine silt and fine clay), and a lower percentage of total sand. Visual observation of the sites documented vastly different sediments at both sites. In site

2020.AP.058, the sediments were black, while in site 2020.AP.045, the sediments were sandy (green and brown) with burrows noted.

MAG description

DNA was sequenced on NovaSeq 6000 S4, using a TruSeq library prep, resulting in over 1.03 billion reads for all depths in 2020.AP.045 and over 1.29 billion in 2020.AP.058. The number of contigs from metagenome assemblies for the four depths in 2020.AP.045 ranged between 3 120 099 and 4 469 435, with GC content ranging from 52.79% to 55.93%. N50 values ranged from 937 to 996 for 2020.AP.045 (Table S2). The number of contigs from metagenome assemblies from the four depths in 2020.AP.058 ranged between 3 686 312 and 4 400 637. The largest contigs for each metagenome assembly ranged between 146 299 and 297 828 bp. The GC content ranged between 51.53% and 52.54%. N50 values ranged from 938 to 1042 for 2020.AP.058 (Table S2). The total length of all assembled metagenomes ranged from 3 036 785 586 to 4 364 310 132 bp. Binning of metagenomes for each site and within-site sample depth resulted in the construction of 75 high-quality MAGs.

Normalized abundance of MAGs

MAGs A107, C1, D57, E63, F96, G107, and H55 were identified down to the genus *Methyloceanibacter* and found at both sites, all depths, and also had the highest normalized abundances with RPKMs of 200, 229, 145, 232, 242, 136, and 183, respectively. At site 45, MAG B38, from the family *Methylloligellaceae,* was documented with the highest normalized abundance of 220 RPKMs (Figs 1 and 2). Acidimicrobiia MAGs were found in the 0–3, 6–9, and 9–12 cm depths for both sites (Figs 1 and 2). These Acidimicrobiia MAGs, A71, C62, C65, D16, G51, and H162, were also relatively abundant as they had RPKMs of 71, 12, 36, 16, 51, and 58, respectively (Figs 1 and 2).

Ammonia and methane oxidizing MAGs

In-depth analyses of MAGs documented high-quality MAGs with the potential to oxidize ammonia and methane at only site 2020.AP.045 (Fig. 1). MAGs A46 (*amoB*), B44 (*amoA,B*), and C31(*amoC*), all identified from the family *Nitrosomonadaceae*, contained ammonia/methane monooxygenases. MAG B44 was the only high-quality MAG in this study demonstrated to possess both the *pmoA/amoA* and *pmoB/amoB* genes. Additionally, MAGs A46, B44, and C1 contain urea hydrolysis genes *ureABC*, again only noted at site 2020.AP.045.

Denitrification potential MAGs

Of the 75 high-quality MAGs, 53 contained genes related to denitrification. At each site, three to seven MAGs were obtained for depths 0–3, 3–6, and 6–9 cmbsf. The highest number of MAGs were obtained for depths of 9–12 cmbsf for both sites, with 12 MAGs in 2020.AP.045 and 8 MAGs in 2020.AP.058. While oxygen concentrations were not measured, putative oxygen reductases were found in 25 of the 31 MAGs in 2020.AP.045 (Fig. 1) and 20 of the 22 MAGs in 2020.AP.058 (Fig. 2).

Methyloceanibacter MAGs (A107, C1, D57, E63, F96, G107, and H55) were the most abundant MAGs at both sites and contained essential nitrate reductase genes, *narGH*, responsible for catalyzing the conversion of nitrate to nitrite. Additionally, *Methyloceanibacter* MAGs A107, C1, D57, E63, F96, G107, and H55 all possess *xoxF*, a methanol dehydrogenase that methylotrophs use to oxidize methanol into formaldehyde (Le et al. 2021). Further, these key MAGs all contain a complete or near complete (>88%) serine formaldehyde assimilation pathway (Fig. 3), which utilizes CO_2 along with formaldehyde to form cellular biomass and convert to acetyl-CoA (Singh et al. 2022).

In sample 2020.AP.045, 31 of the resulting 43 high-quality MAGs possessed genes indicating the potential to carry out one or more steps involved in denitrification. Twenty-two of these MAGs contained putative genes to reduce nitrate (*narGH/napAB*). Nitrate reductases and oxygen reductases were found throughout all four depths. Genes for nitrite reduction to nitric oxide via *nirS/nirK* were present in eight MAGs in 2020.AP.045, although they were not detected in 2020.AP.045.12 (9–12 cmbsf). Genes for conversion of nitric oxide to nitrous oxide (*norBC*) were identified in MAG D114 in 2020.AP.045.12 (9–12 cmbsf), which was classified into the Family *Beggiatoaceae* (Table S3). Putative genes for nitrous oxide reduction to nitrogen gas (*nosDZ*) were found throughout depths in four MAGs, although *nosDZ* was not detected in 2020.AP.045.6 (3–6 cmbsf). Additionally, the putative genes involved in dissimilatory nitrate reduction to ammonia (DNRA) were found in two high-quality MAGs in 2020.AP.045.12 (9–12 cmbsf). One MAG, MAG D114, contained *nirBD*, which reduces nitrite to ammonia. This MAG also contained *napAB*, *narG*, and *nasA* (Fig. 1). MAG D114 also contained complete KEGG thiosulfate oxidation pathways (*soxABCDXYZ*), the reverse dissimilatory sulfate reduction pathway to oxidize sulfide to sulfate (*aprAB*, *sat*, and *dsrAB*), Embden–Meyerhof, and 3C glycolysis pathways. Also detected in this MAG was the genetic potential for carbon fixation with a near-complete Calvin–Benson cycle, with a complete reductive pentose phosphate cycle. The second MAG, MAG D49, had the genetic potential for DNRA, and *nrfH*, and was taxonomically related to *Desulfobacteria* (Fig. 1). MAG D49 also contained complete pathways for glycolysis, pentose phosphate, and dissimilatory sulfate reduction.

At the second site (2020.AP.058), 21 out of 30 high-quality MAGs had putative genes for the denitrification pathway. The potential to reduce nitrate to nitrite (*narGH/napAB*) was found in 16 of these MAGs. Nitrate reductases and oxygen reductases were found throughout all four depths. Nine MAGs contained the potential for nitrite reduction to nitric oxide (*nirS/nirK*) throughout all four depths. Two MAGs were found to possess the potential to reduce nitric oxide to nitrous oxide (*norBC*) in 2020.AP.058.9 and 2020.AP.058.12. The MAGs that contained *norBC* in 2020.AP.058 were classified as Gammaproteobacteria, with one, MAG G35, further classified as the family *Sedimenticolaceae* (Table S4). MAG G35 also contained *narGH*, the only *nirBD* genes detected in 2020.AP.058, and *nirS*. While absent at surface samples (0–3 cmbsf), *nosDZ* was detected in five MAGs throughout the depths. In addition to denitrification, MAG G35 had the genetic potential for DNRA (*nirBD*). MAG G35 contains complete pathways for glycolysis and the nonoxidative pentose phosphate pathway.

Nine MAGs harbored *nosDZ,* with the majority being taxonomically identified as Acidimicrobiia and the phylum Dadabacteria. Two of these nine MAGs were classified into the family *Cyclobacteriaceae* and *Sedimenticolaceae*, while the others were classified into the class Acidimicrobiia and the phylum Dadabacteria (Table S4). Three of the Acidimicrobiia MAGs, MAG A71, MAG D16, and MAG H162, were similar in ANI with values of 0.885114, 0.855625, and 0.88561, respectively. They were most closely related to the species UBA5794 sp002418265. MAG C62, another member of Acidimicrobiia, had an ANI of 0.79054 and was closely related to the species UBA6912 sp002450985. These four Acidimicrobiia MAGs harbored carbon monoxide dehydrogenases (*coxLS*) and a complete pathway for glycolysis. MAG C62 and H162 contain the pentose phosphate pathway, and MAGs A71 and D16 contain the nonoxidative phosphate pathway. MAG H162 also has the Entner–Doudoroff

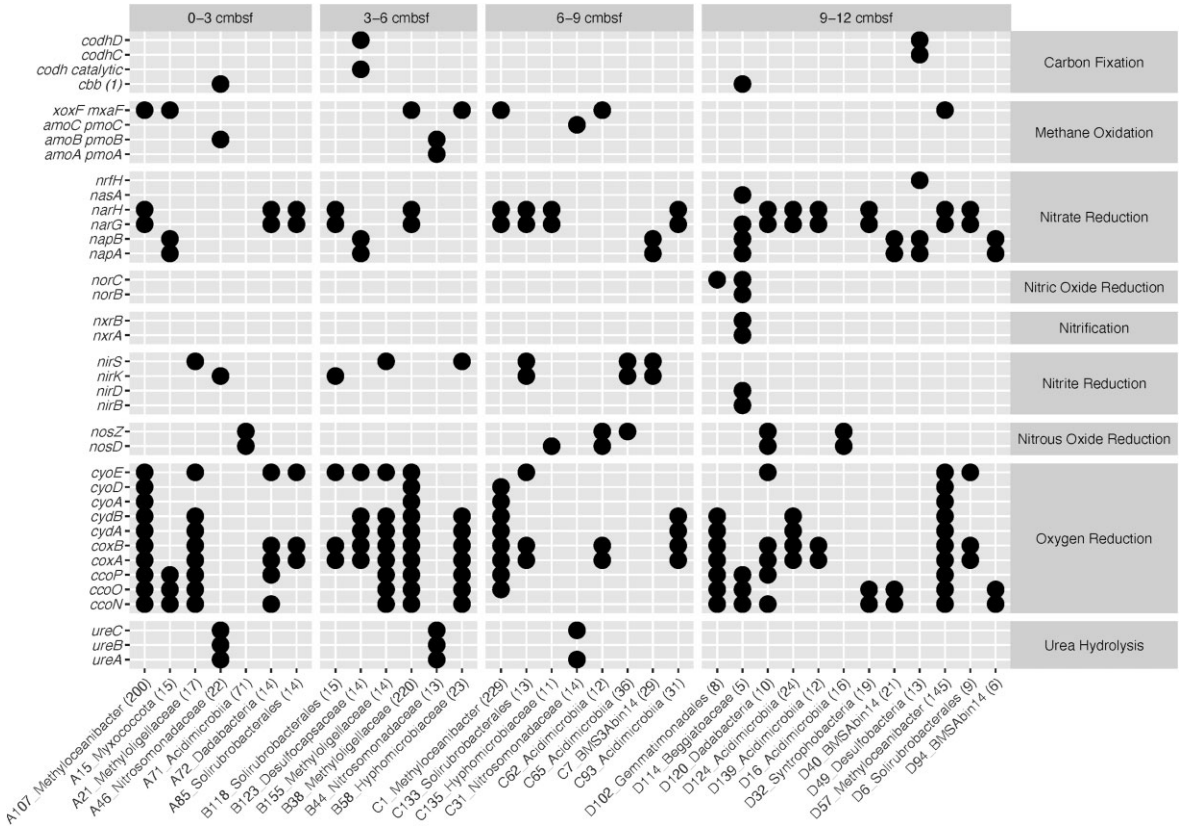

Figure 1. Dot plot of high-quality MAGs containing genes for denitrification, dissimilatory nitrate reduction to ammonia (DNRA), nitrification, oxygen reduction, methane oxidation, urea hydrolysis, and carbon fixation sequenced from sediments collected at the site 2020.AP.045 (Weddell Sea, Antarctica, November 2020). A dot indicates the presence of the gene within the MAG. Normalized abundances (RPKM) of each MAG are in parentheses. RPKM = number of reads mapped to MAG/(genome length/1000 × total number of reads/1 000 000).

pathway. MAG C62 additionally featured cytochrome C oxidases (*coxAB*).

Three Dadabacteria MAGs harbored *nosDZ*. These three MAGs, MAG D120, MAG F1, and MAG G46, belonged to the genus UBA2774 with ANI values of 0.825982, 0.826054, and 0.826675, respectively. These Dadabacteria MAGs also contained *cyoE*, *coxAB*, and *ccoNP* (Figs 1 and 2). MAG D120, G46, and F1 contain complete pathways for glycolysis and the pentose phosphate pathway; only D120 and G46 contain the Entner–Doudoroff pathway.

Sulfate reduction potential in MAGs

Eight high-quality MAGs with genetic potential for dissimilatory sulfate reduction were identified at both sites (*aprAB*, *sat*, and *dsrAB*). In sample 2020.AP.045 MAGs, sulfate reducing MAGs were identified in the 6–9 (C7) and 9–12 (D32, D40, D49, D102, and D123) cmbsf depths (Table S5). In sample 2020.AP.058, sulfate-reducing MAGs were identified in the 3–6 (F140) and 9–12 (H130) cmbsf depths (Table S5).

Discussion

Ammonia/methane-oxidizing bacteria

Methane is a potent greenhouse gas, and the Southern Ocean surrounding Antarctica is a known methane sink (Thurber et al. 2020). Both ammonia-oxidizing bacteria (AOB) and methane-oxidizing bacteria are known for their ability to assimilate ammonia and C1 compounds, utilizing ammonia and methane monooxygenases (Zheng et al. 2014). The Southern Ocean surrounding Antarctica is estimated to host as much as a quarter of the world's methane (Thurber et al. 2020). The first step in methane oxidation is the conversion of methane into methanol via *amoABC/pmoABC* (Hogendoorn et al. 2021). This study documented several *Nitrosomonadaceae* MAGs, A46, B44, and C41, with *amoABC/pmoABC* and *ureABC* (Fig. 1). The presence of *amo/pmo* genes combined with the ureases (*ureABC*) in these MAGs suggests they can use both methane and ammonia for growth, identifying them as critical members of the global nitrogen and carbon cycles. Zheng et al. (2014) noted that AOB will oxidize methane over ammonia when urea is present. The presence of *ureABC* in AOBs could suggest that methane could be a preferred carbon source oxidized by these MAGs. Additionally, urease genes in AOBs suggest the potential to hydrolyze urea to ammonia and CO_2 (Koper et al. 2004), allowing the microbes to utilize urea instead of ammonia as an energy source (Chiriac et al. 2023). These findings suggest *Nitrosomonadaceae* MAGs have specialized traits enabling them to utilize urea when N levels are low, a necessary adaptation for an oligotrophic environment, suggesting they could play a significant role in the sediments' carbon and nitrogen cycling.

Methyloceanibacter MAGs dominate sediments

Methyloceanibacter MAGs were abundant in sediments at both sites. *Methyloceanibacter* are facultative methylotrophs that oxidize methanol for energy while using nitrate as a nitrogen source (Takeuchi et al. 2013). Methanol is ubiquitous in anoxic marine sediments, with many microorganisms capable of using this abundant C1 compound for energy (Fischer et al. 2021). C1 com-

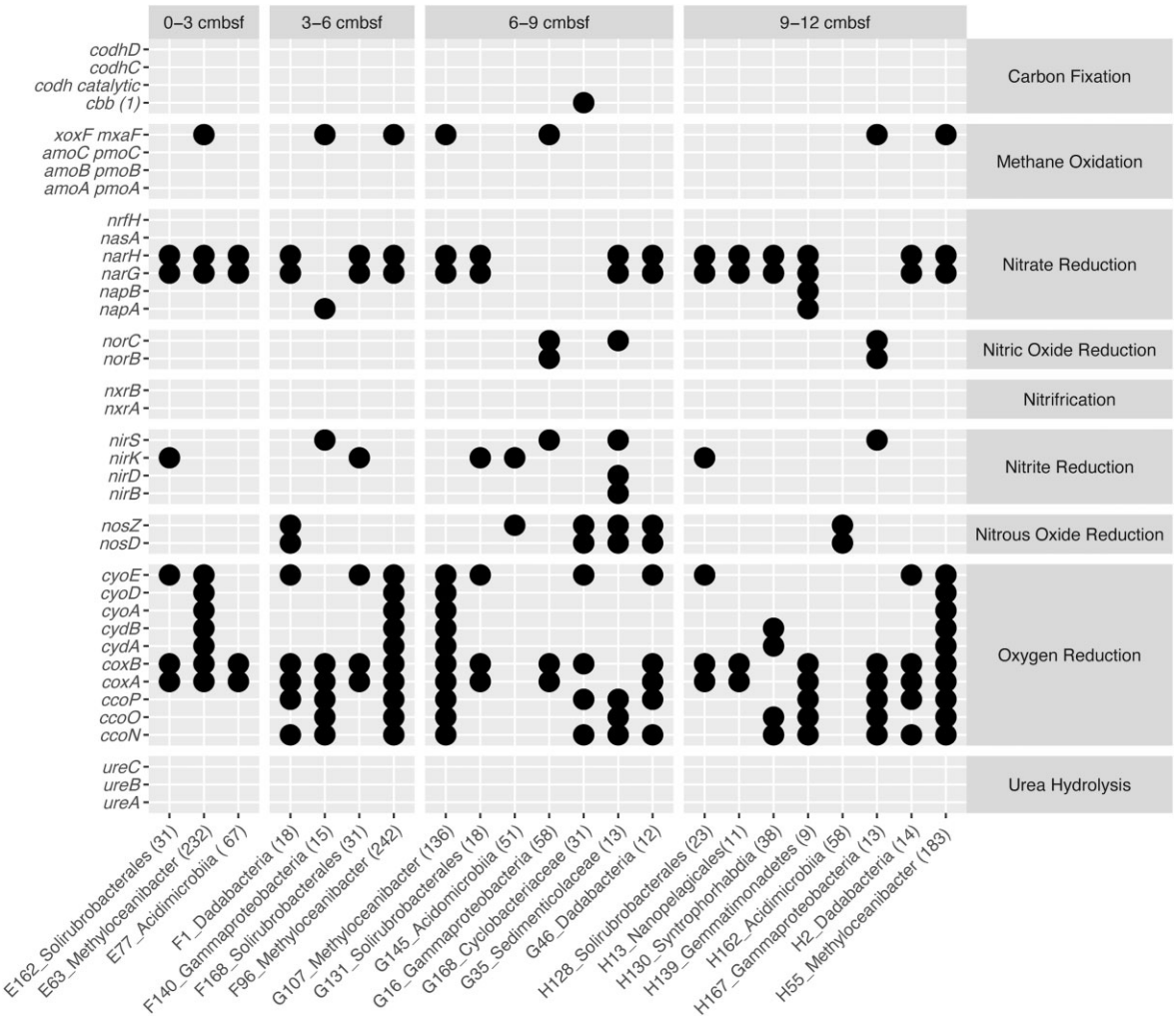

Figure 2. Dot plot of high-quality MAGs containing genes for denitrification, dissimilatory nitrate reduction to ammonia (DNRA), nitrification, oxygen reduction, methane oxidation, urea hydrolysis, and carbon fixation sequenced from sediments collected at the site 2020.AP.058 (Weddell Sea, Antarctica, November 2020). A dot indicates the presence of the gene within the MAG. Normalized abundances (RPKM) of each MAG are in parentheses. RPKM = number of reads mapped to MAG/(genome length/1000 × total number of reads/1 000 000).

pound oxidation can be coupled with nitrate reduction at the oxic–anoxic interphase in sediments (Fischer et al. 2021). In the current study, *Methyloceanibacter* MAGs (A107, C1, D57, E63, F96, G107, and H55) contain *xoxF* and the complete serine formaldehyde assimilation pathways. The *xoxF* gene is responsible for oxidizing methanol into formaldehyde. The presence of the nitrate reduction genes (*narGH*) suggests the *Methyloceanibacter* MAGs have the genetic potential to use nitrate as the final electron acceptor, possibly while oxidizing methanol into formaldehyde. These key *Methyloceanibacter* MAGs also contain formaldehyde assimilation pathways, incorporating formaldehyde into biomass through the serine pathway, thus converting it into acetyl-CoA, implicating their essential role in carbon cycling. The abundance of *Methyloceanibacter* MAGs at both sites suggests that methanol oxidation could be an important reaction in this habitat.

Nitrous oxide reduction by Acidimicrobiia and Dadabacteria

Normalized abundances of MAGs suggest the class Acidimicrobiia plays a vital role in the final step of denitrification within these Antarctic sediments. Acidimicrobiia MAGs were the second most abundant MAGs at both sites and all depths, except for the 3–6 cmbsf, where they were not located. Acidimicrobiia MAGs and Dadabacteria MAGs harbored *nosDZ* genes, which are responsible for reducing nitrous oxide to nitrogen gas (Zumft 1997, Scala and Kerkhof 1999, Sanford et al. 2012). Dadabacteria is a relatively newly described phylum found within diverse environments, from terrestrial hot springs to pelagic marine systems (Graham and Tully 2021). Although first described in 2014 and later denoted Candidatus Dadabacteria in 2016, the metabolic capabilities of this phylum remain poorly understood (Wang et al. 2014, Hug et al. 2016). Dadabacteria MAGs in this study contained complete pentose phosphate and glycolysis pathways, and D120 and G46 also contained the Entner–Doudoroff pathway, suggesting the genetic potential for these MAGs to oxidize carbon as an electron donor. One study found several Dadabacteria clades possessing the Entner–Doudoroff pathway, suggesting these microbes can recover phosphate for cellular processes from organic matter (Graham and Tully 2021). Previous studies found genetic potential for denitrification in Dadabacteria, specifically nitrous oxide reduction (Hug et al. 2016, Garber et al. 2021, Graham and Tully 2021). While Hug et al. (2016) documented the potential for nitrite reduction to ammonia via *nirBD* or nitric oxide via *nirK*, the MAGs documented in this study did not contain these genes. All three MAGs

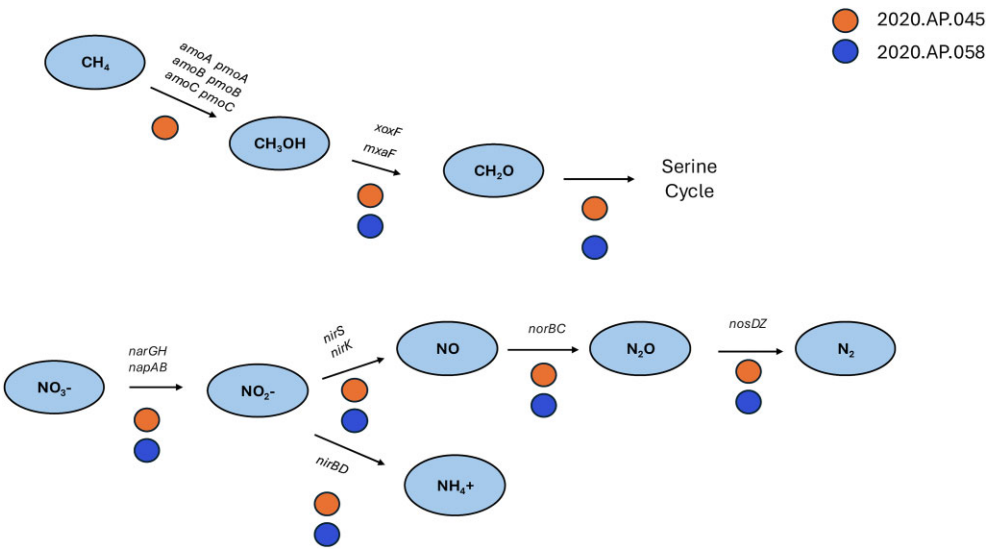

Figure 3. Methane oxidation to serine formaldehyde assimilation pathway. Methane oxidation to methanol was in MAGs A46, B44, and C31. Methanol oxidation to formaldehyde was in MAGs A107, A15, B38, B58, C1, C62, D57, E63, F140, F96, G107, G16, H167, and H55. The serine formaldehyde assimilation pathway was in MAGs A46, A107, B38, B44, B58, C1, C31, D57, E63, F96, G107, and H55. Nitrate to nitrite was in MAGs A107, A15, A72, A85, B118, B123, B38, C1, C7, C133, C135, C93, D6, D32, D40, D49, D57, D94, D114, D120, D124, D139, E63, D77, D162, F1, F96, F140, F168, G34, G46, G107, G131, H2, H13, H55, H128, H130, H139, and H162. Nitrite to nitric oxide was in MAGs A21, A46, B118, B155, B58, C7, C65, C133, E162, F140, F168, G16, G35, and H167. Nitric oxide reduction was in MAGs D102, D114, G16, G35, and H167. Nitrous oxide reduction was in MAGs A71, C62, C65, C135, D16, D120, F1, G35, G46, G145, G168, and H162. Nitrite reduction to ammonia was in MAGs D114 and G35.

contained terminal oxidases (*coxAB* and *ccoNP*). These genes, coupled with *nosDZ*, support a facultative anaerobic metabolism with the ability to utilize nitrous oxide as a final electron acceptor in the absence of oxygen (Zumft 1997, Dutta et al. 2023a). Denitrifying bacteria are often facultative anaerobes and can exist within an environment that routinely may experience anoxic conditions (Lam and Kuypers 2011). Overall, the data in the current study also supports a facultatively anaerobic lifestyle for these MAGs.

Four out of ten Acidimicrobiia MAGs found in this study contained *nosDZ*. Bertagnolli et al. (2020) found that members of Acidimicrobiia contained *nosDZ*, indicating they may play a prevalent role in nitrous oxide reduction. Within MAG C62, genes for oxygen reduction (*coxAB*) and nitrous oxide reduction (*nosDZ*) were documented, indicating a facultatively anaerobic lifestyle. Taxa within this class are known to be facultative anaerobes carrying out nitrate reduction (Ghai et al. 2014). However, *coxAB* was not found in the other three MAGs. The absence of these genes within the bins could result from their incompleteness or underrepresentation in the samples. Additionally, all four of these MAGs contained the potential to utilize carbon monoxide as an electron source via *coxLS*, which is consistent with other studies (Ghai et al. 2014, Mizuno et al. 2015). Acidimicrobiia MAGs were suggested to have the genetic potential to fix carbon via the rTCA pathway (Chiriac et al. 2023). Near complete rTCA pathways in this study also indicate the ability to fix carbon. The carbon metabolism in Acidimicrobiia is distinguished by the presence of glycolysis pathways and the nonoxidative branch of the pentose phosphate pathway (Chiriac et al. 2023), coinciding with the present study's findings.

Beggiatoaceae MAG

Beggiatoaceae are ubiquitous in marine, freshwater, and brackish environments (Teske and Salman 2013), with studies noting *Beggiatoaceae* can perform denitrification (MacGregor et al. 2013, Schutte et al. 2018). At site 2020.AP.045, MAG D114 was related to *Beggiatoaceae* and had the genetic potential for partial denitrification. Additionally, this was the only MAG in the study containing the *nxrAB* genes, which convert nitrite to nitrate in the nitrification process, suggesting this MAG may have the potential to make and store nitrate.

Beggiatoa MAG D114 may also connect aspects of the sulfur cycle to the nitrogen cycle. MAG D114 has the genetic potential to couple denitrification to sulfide and thiosulfate oxidation. Other studies have shown that *Beggiatoa* can oxidize sulfide and thiosulfate while utilizing nitrate as an electron acceptor (Kamp et al. 2006, Schwedt et al. 2012). The sulfate in this habitat must be reduced to support the oxidation of reduced sulfur compounds. Several MAGs found in this study have the genetic potential to reduce sulfate. Further, previous studies have also documented sulfate-reducing bacteria as members of the Antarctic marine sediment community (Bowman and McCuaig 2003, Garber et al. 2021).

The presence of *napAB/narG* with *norBC* in MAG D114 implies that this MAG could reduce nitrate to nitrite and nitric oxide to nitrous oxide, therefore playing a prominent role in the denitrification process within these sediments. While this *Beggiatoaceae* MAG had the genetic potential for partial denitrification, *nosDZ* or *nirS/nirK* were not found. The lack of presence of these genes in this MAG could result from an incomplete assembly, insufficient DNA sequencing effort, or the absence of the gene in the organism corresponding to the MAG. Further, Cabezas et al. (2022) have suggested that polar environments may house undefined novel genes that function in denitrification. Thus, the data used might be incomplete. Yet, our findings are consistent with Schutte et al. (2018), as two of the six publicly available MAGs their study examined lacked *nirS*, and three of their MAGs lacked *nosDZ*. In addition to denitrification, *nirBD* in MAG D114 suggests that it had the genetic potential to carry out DNRA, a function that other studies have found (Vargas and Strohl 1985, Schutte et al. 2018). While *nirBD* can indicate both assimilatory nitrate reduction and DNRA, the presence of *nasACDE* in this MAG suggests the genetic potential for assimilatory nitrate reduction (McAllister et al. 2021, Hu et al. 2022). Additionally, MAG D114 has the genetic potential

to fix carbon via the Calvin–Benson cycle. The presence of both assimilatory and dissimilatory nitrate reduction suggests this organism may utilize nitrate for biomass and energy, depending on the availability of O_2.

The *Beggiatoaceae* MAG (D114) found in this study could also be autotrophic. This MAG contained a gene annotated as Rubisco form I, which catalyzes the first step in CO_2 fixation. Other studies have also found members within this family to be autotrophs (Nelson and Jannasch 1983, Nelson et al. 1986, MacGregor et al. 2013). Since the Southern Ocean is an oligotrophic environment, especially in winter, bacterial autotrophy may be essential to support primary production (Dutta et al. 2023a). A previous study documented autotrophic bacteria were enriched in sediments that contained lower chlorophyll *a* and lower organic matter levels than in sediments with high chlorophyll *a* and organic matter levels (Currie et al. 2021), supporting the importance of autotrophy in low nutrient environments.

Sedimenticolaceae MAG

In 2020.AP.058, MAG G35, identified as a member of the family *Sedimenticolaceae*, contained genes for partial denitrification. Other *Sedimenticola* MAGs lack the genetic capacity for the concluding step of denitrification (Vavourakis et al. 2019, Arora-Williams et al. 2022). However, Slobodkina et al. (2023) reported that an isolate from the *Sedimenticola* genus was proficient in nitrous oxide reduction. Thus, the presence of *nosDZ* in MAG G35 suggests the potential for nitrous oxide reduction. Within MAG G35 from this study, *nirBD* was detected, indicating the genetic potential for nitrite reduction to ammonia. Previous studies indicate that two MAGs identified as *Sedimenticola* simultaneously expressed genes for ammonification (*nirABD*) and denitrification (*nirK/nirS* and *norBC*) (Arora-Williams et al. 2022). Thus, both processes may occur within the shelf sediments of Antarctica due to this MAG. This study identified that *Sedimenticolaceae* MAG has the genetic potential to utilize glucose as an energy source.

Nitrite reduction to nitric oxide

Seventeen MAGs were found to contain nitrite reductases (*nirS/nirK*). These MAGs were classified into two phyla: Proteobacteria and Actinobacteria. As previously noted, some members of the diverse Proteobacteria phylum are documented as denitrifiers. Six of the eight MAGs classified into the phylum Actinobacteria were further classified as Solirubrobacterales, which have been reported in environments with low organic carbon, such as Antarctic soils (Chong et al. 2012). The phylum Actinobacteria encompasses diverse potential metabolic capabilities, including nitrogen cycling (Ghai et al. 2014). These data further suggest that MAGs from Proteobacteria and Actinobacteria, particularly the order Solirubrobacterales, are prominent contributors to nitrite reduction to nitric oxide in Antarctic sediments.

Nitrate reduction to nitrite

Nitrate reduction via *napAB* and *narGH* was ubiquitous throughout both sites and all depths, while our most abundant MAGs at both sites contained *narGH*. This is consistent with other findings, as nitrate is preferentially used as an electron acceptor once oxygen is diminished (Lam and Kuypers 2011). Oxygen reductases were found throughout all depths at both sites, further indicating the facultative–anaerobic nature of these sediment communities (Rysgaard et al. 2004, Hartnett et al. 2008). Moreover, bioturbation, the mixing of sediments by macrofauna, likely introduces oxygen deeper into the sediments, enabling aerobic respiration at greater depths (Jochum et al. 2017). Compared to *narGH*, *napAB* has a higher affinity for nitrate, allowing those organisms with *napAB* to utilize nitrate at lower environmental concentrations (Wang et al. 1999). This may be reflected in our data as sediments showed slight variation in nitrate levels between sites, with 2020.AP.045 having lower nitrate levels and more MAGs containing *napAB* than 2020.AP.058.

Community-level denitrification and ecosystem function

Both collection sites sampled in this study demonstrated the genetic potential for complete denitrification via a consortium (Fig. 3). *Methyloceanibacter* MAGs were most abundant at both sites, with the genetic potential to oxidize methanol to acetyl-CoA while utilizing nitrate as a terminal electron acceptor. Many other community members in this study, such as those classified as Solirubrobacterales, also had the genetic potential to initiate the first step of nitrate reduction, in addition to members within Dadabacteria documented with the genetic potential to carry out the final step of nitrous oxide reduction. Acidimicrobiia, identified with a high normalized abundance at both sites, had the genetic potential to carry out the final step of nitrous oxide reduction. Further, several MAGs with members like MAG D114 and MAG G35 had the genetic potential to carry out most steps within the sequential process. Most denitrifying microbes perform only some of the steps for denitrification, requiring a consortium of bacteria to carry out the complete process (Zumft 1997).

Partial and complete denitrification have important implications for the environment. This study was able to document a diverse consortium associated with crucial biogeochemical cycling within these Antarctic sediments. Methylotrophic bacteria were reported to have the potential to initiate nitrate-driven methanol oxidation. Partial denitrification may result in the accumulation of intermediate nitrous oxide, a greenhouse gas (Betlach and Tiedje 1981). Further, completing this process may remove nitrous oxide and biologically available N from the ecosystem. While denitrification results in a net loss of N from the system, a competing pathway, DNRA, retains nitrogen (Giblin et al. 2013). Yet, DNRA has been shown to produce nitrous oxide as a byproduct, adding to the accumulation of this greenhouse gas (Giblin et al. 2013, Cabezas et al. 2022). While denitrification rates in Antarctic sediments are not as high as in Arctic continental shelf sediments, the size of the Antarctic shelf makes it an essential portion of the global nitrogen budget (Hartnett et al. 2008). Changing biogeochemical cycles such as these due to increased temperature and nutrient input from melting ice shelves may exacerbate climate change from increased microbial activity (Sahade et al. 2015).

Antarctica immensely influences the global climate and is the most rapidly warming region, particularly within the Western Antarctic Peninsula (Meredith and King 2005, Bromwich et al. 2013). The melting of ice sheets and ice shelves from warming added otherwise unavailable nutrients to the surrounding benthic marine sediments, stimulating the growth of microbial communities (Sahade et al. 2015). Nutrients and organic matter sinking to the seafloor with little degradation provide a year-long food bank that supports microbial metabolism and benthic food webs (Mincks et al. 2005). Sediments, characterized by higher sedimentation rates, tend to harbor higher microbial densities and are likely to affect the amount of labile organic matter supplied to the seafloor, and thus affect denitrification by these communities (Carr et al. 2013, Lohrer et al. 2013, Sahade et al. 2015). The alteration in denitrification can further reduce nutrient availability for

primary producers in the benthos. The altering of microbial communities can significantly alter important biogeochemical cycles as microbes play a crucial role in the cycling of these nutrients, thus providing the foundation of the food web and supporting life in the benthic and pelagic ecosystems (Mincks et al. 2005, Sahade et al. 2015).

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Supplementary data

Supplementary data are available at *FEMSLE* online.

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