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Microbial Community Structure and Activity Changes in Response to the Development of Hypoxia in a Shallow Estuary

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Abstract.

We examined the effects of changing from oxic to anoxic conditions on microbial communities using both biogeochemical and molecular approaches in a semi-enclosed estuary (Jinhae Bay, Republic of Korea). Total microbial activity, represented by oxygen demand in the water column (WOD) or sediment (SOD), revealed that the respective microbial communities in the water and sediment responded differently to low dissolved-oxygen (DO) conditions. In the sediment, SOD and the total microbial abundance, as assessed by quantitative polymerase chain reaction (qPCR) analysis, decreased under low DO conditions, indicating that the microbial adaptation to anaerobic metabolism was not well established during hypoxia development. In the water column, however, neither the total abundance of microbes nor the WOD were affected by hypoxic conditions. Regardless of DO concentration, WOD showed a positive correlation with water temperature, implying that the aerobic metabolism was sustained even under hypoxic conditions, through the intermittent supply of oxygen. In addition to the spatially different responses of microorganisms, unique responses of specific groups were noted in sulfur (S) and nitrogen (N) cycling microbes. Sulfide-oxidizing prokaryotes (SOP) dominated in the water column, and no significant changes were evident in their abundance or diversity with hypoxia. However, in sediment, distinctive sulfate-reducing bacteria (SRB) were present at each sampling period during hypoxia development (a "SRB succession"), implying that each SRB group has varying sensitivity to DO and other electron acceptors. Our results illustrated similarities in composition and activity of N-cycling microbes between the seasonal hypoxia and permanent oxygen minimum zone (OMZ). Vertical profiles of dissolved inorganic nitrogen, including ammonium (NH_4^+) and nitrate (NO_3^-), and changes in archaeal abundance indicated that the NH_4^+-oxidizing archaea (AOA) varied spatially and temporally, depending on NH_4^+ and oxygen

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availability in the water column, under mature hypoxic conditions. The intriguing N dynamics recently discovered in the OMZ might also be important in the coastal hypoxic zone.

1 Introduction

Human influences on the coastal environment are increasing as a result of industrial and urban development and pollution (Nogales et al., 2011). Cultural eutrophication and the consequences of anthropogenic influences cause undesirable algal bloom and hypoxic water (Diaz and Rosenberg, 2009; Lee et al., 2017). The development of seasonal hypoxia has increased globally, and has negative economic and environmental consequences (Diaz and Rosenberg, 2008; Naqvi et al., 2010).

Microbes function in the biogeochemical cycling of essential elements, including carbon, nitrogen, phosphate, and sulfur; hence, the response of microbial communities to low–dissolved–oxygen (DO) conditions has implications for both coastal management and the global environment (Ward et al., 2011; Abell et al., 2011). However, detailed microbial ecology studies on microbial responses to oxygen depletion have been conducted mostly on permanent-oxygen-minimum zones (OMZ) of open ocean areas, such as the Eastern Tropical North Pacific (ETNP), Eastern South Pacific (ESP), Northern Indian Ocean, and Arabian Sea (Ulloa et al., 2012; Jensen et al., 2011). Interactions between microbial nitrogen-removal processes (anaerobic ammonium (NH_4^+) oxidation [anammox] and denitrification) and other nitrogen-related processes (e.g. dissimilatory nitrate (NO_3^-) reduction to NH_4^+ [DNRA] and nitrification) are well studied in these areas (Kalvelage et al., 2013; Lam et al., 2009; Jensen et al., 2011). The discovery of NH_4^+-oxidizing archaea (AOA) and their prominent roles in global nitrogen cycling in the OMZ have attracted great attention, because NH_4^+ oxidation (nitrification) is the main process producing

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61 NO_3^- which then cause available N loss through denitrification and anammox (Ward et al., 2011;
62 Hatzenpichler, 2012). In addition to nitrogen cycle studies, examination of the general
63 organization and factors controlling the microbial community in the OMZ are still lacking (Ulloa
64 et al., 2012). Pelagic prokaryotes in the OMZ do not seem fundamentally different from those
65 present in oxygen-rich waters (Wright et al., 2012).

66 The basic mechanisms of oxygen depletion are similar in the permanent OMZ of the
67 Open Ocean and seasonally hypoxic coastal areas. Both show a negative oxygen budget, despite
68 the existence of large differences in water depth (several thousand meters versus < 100 m) and
69 temporal scale (almost permanent versus seasonal). Comparison of the environments and biota of
70 these two oxygen-minimum areas are needed, but few studies related to seasonal hypoxia and
71 microbial ecology in coastal areas are available. Previous studies of coastal hypoxic areas reveal
72 that decreased DO concentrations do not decrease microbial activity in the water column
73 (Nogales et al., 2011; Crump et al., 2007). According to bacterial secondary production data,
74 bacterial activity remains unchanged or even increases as hypoxia matures (Bastviken et al.,
75 2001; Cole and Pace, 1995). However, bacterial production estimates based on leucine or
76 thymidine incorporation may not represent total remineralization activity; rather, it is an index of
77 the net growth of limited microbial groups (Tuominen, 1995). Further, changes in the microbial
78 community in the sediment may differ from those in the water column; more dramatic changes
79 are expected in the sediment because of the steep redox gradient and elevated H_2S accumulation
80 under hypoxic conditions (Gundersen and Jorgensen 1990). The hypoxic conditions also have
81 significant impacts on the biogeographical shift of sediment microbial community composition
82 depending on the salinity and sediment characteristics (Dang et al., 2008). Most microbial
83 sediment studies have focused on compositional changes, rather than adopting combined

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84 approaches that monitor microbial activity and molecular community changes (Ye et al., 2016).
85 Benthic and pelagic systems are coupled tightly in the shallow coastal environments where
86 seasonal hypoxia occurs (Rowe, 2001). Comprehensive studies incorporating both quantitative
87 (microbial activity) and qualitative (genetic diversity and composition) approaches are needed to
88 improve understanding of microbial responses to hypoxia (Crump et al., 2007).

89 Sulfur cycling microorganisms dominate anoxic metabolism in the ocean and are
90 involved in various oxidative or reductive processes (Jørgensen, 1982). Sulfur-oxidizing
91 prokaryotes (SOP) use diverse electron acceptors, such as O_2, NO_3^-, $Mn^{3+}/^{4+}$ and $Fe^{3+}$and
92 perform CO_2 fixation (Mattes et al., 2013). However, sulfate-reducing prokaryotes (SRP) use
93 sulfate as an electron acceptor and help degrade organic materials in low-oxygen zones
94 (Jørgensen, 1982; Jørgensen and Postgate, 1982; Bowles et al., 2014). Specific 16S ribosomal
95 RNA (rRNA) probes targeting specific genera of interest were developed to determine
96 abundance of sulfur cycling prokaryotes in marine sediment (Ravenschlag et al., 2000; Gittel et
97 al., 2008). This technique provides valuable genetic information, but the phylogenetic diversity
98 of sulfur cycling prokaryotes complicates their detection (Stahl et al., 2002). The use of
99 functional marker genes provides an alternative molecular approach. One such functional gene is
100 *dissimilatory sulfite reductase*, which is the key enzyme of sulfate reduction and is present in
101 genetically diverse sulfur cycling species, including Gram-positive and Gram-negative bacteria
102 (Wagner et al., 1998; Kondo et al., 2004). However, the primer sets (for example DSR1F&4R)
103 were restricted to SRP species, which is a major limitation, especially when the concomitant
104 detection of both SRP and SOP organisms is required (Wagner et al., 1998). Another functional
105 gene candidate is the *adenosine 5'-phosphosulfate* (*APS*) *reductase alpha subunit* gene (*aprA*).
106 APS reductase consisting of an alpha (aprA) and beta (aprB) subunit is involved in dissimilatory

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107 sulfate-reduction and converts APS into sulfite and adenosine monophosphate. PCR trials using
108 primers for the *aprA* gene amplified both SRP and SOP species successfully in estuarine
109 sediments and hydrothermal water, allowing detection of more diverse groups and improving
110 understanding of the community structures of sulfur cycling prokaryotes (Meyer and Kuever,
111 2007a; Meyer and Kuever, 2007b).

112 Jinhae Bay is a semi-enclosed bay on the south-eastern coast of South Korea. Massive
113 industrialization and urbanization, started in the 1960s, has caused serious water-quality
114 problems, including seasonal hypoxia every summer. Significant governmental efforts to reduce
115 hypoxia since the 1980s have achieved only limited success (Lee et al., 2017). The development
116 of seasonal hypoxia in coastal areas like Jinhae Bay may have a significant impact both directly
117 and indirectly on the structure of microbial communities. However, the structure of the microbial
118 community in Jinhae Bay under hypoxic conditions has not been studied in detail.

119 In this study, we investigated the dynamics of the microbial community with hypoxia
120 development in Jinhae Bay to examine the following hypotheses: (a) the influences of hypoxia
121 differ between water column and sediment (b) sulfur related microbes dominate microbial
122 community changes during hypoxia development (c) various nitrogen dynamics in the permanent
123 OMZ might also be important in coastal hypoxic zones like Jinhae Bay. Water-column
124 characteristics were monitored to evaluate environmental changes. Oxygen demands were
125 measured in both the water column (WOD) and sediment (SOD), to determine the correlation
126 between oxygen depletion and microbial activity. Total remineralization activity was measured
127 by membrane inlet mass spectrometer (MIMS) (An et al., 2001; Kana et al., 1994) to quantify
128 DO changes for WOD and SOD. Temporal dynamics of NO_3^- and NH_4^+ concentrations were
129 measured to examine the effects of hypoxia on nitrogen cycling microorganisms. Relative

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Discussion started: 13 February 2018

130 microbial abundance was measured using quantitative PCR (qPCR) with various primers,
131 including those for bacterial and archaeal 16S rRNA genes. We also examined changes in the
132 abundance and community structure of sulfur-cycling microbial groups using molecular
133 techniques.
134
135
136 **2 Materials and methods**
137 **2.1 Site description and sample collection**
138 Dangdong Bay is a shallow (~13 m mean depth), semi-enclosed inner bay of Jinhae Bay in the
139 southeastern part of the Korean Peninsula. The semi-diurnal, mean tidal range is 203 cm. Water
140 exchange with the open ocean is limited in Jinhae Bay because it is surrounded by many small
141 islands (Lee et al., 2017; Ministry of Oceans and Fisheries, 2015).
142 Vertical profiles of temperature and DO concentration were investigated weekly or
143 biweekly from January–November 2015 using a Hydrolab multiprobe (Hydrolab® 4a) at the
144 Dangdong Bay study site (Lee et al., 2017). Water samples were collected from the surface,
145 middle, and bottom layers using a 5 L Niskin water sampler, and sediment cores were collected
146 by scuba divers in five sampling periods (Fig. 1; S1(Solubility period), H2A, H2B, H3A, and
147 H3B (Hypoxia periods)). Water samples for NH_4^+ and NO_3^- measurement were filtered (25-mm
148 GF/F filters; Whatman International, Maidstone, Kent, UK) and the filtrates transferred into 50-
149 ml conical tubes and frozen until analysis. The concentrations of NH_4^+ and NO_3^- were
150 determined by standard methods using a spectrophotometer (Strickland and Parsons, 1972).
151 Water samples for WOD measurement were collected with Niskin bottles, and
152 transferred into four 20-ml bottles (Wheaton Industries, Inc., Millville, NJ, USA). $ZnCl_2$ (50%

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Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

153 [w/v]) was used to fix the samples in two bottles, and the other two were not fixed. DO
154 concentration was measured using a MIMS system after 24–48 hrs of dark incubation at the *in*
155 *situ* temperature (McCarthy et al., 2013).

156 For SOD measurement, eight intact sediment cores (internal diameter 8 cm; length 33
157 cm) were collected by scuba divers (An and Joye, 2001) and pre-incubated with the overlying
158 water for 12–24 h at *in situ* temperature to achieve equilibrium. After pre-incubation, the cores
159 were closed with rubber stoppers, and duplicate cores were sacrificed at 0, 1, 2, and 24 h for DO
160 measurement using MIMS. Oxygen concentration was quantified from the O_2: Ar ratio (Kana et
161 al., 1994; An et al., 2001). WOD (μmol O_2 l^{-1} h^{-1}) obtained from the DO concentration
162 difference between the fixed and unfixed samples. SOD (mmol m^{-2} d^{-1}) was calculated from the
163 temporal DO concentration change considering the sediment core surface area.
164
165 **2.2 DNA Extraction**
166 Water samples for DNA extraction were collected from three different depths (top, middle, and
167 bottom). Cells from 1 liter of each seawater sample were filtered through a polycarbonate filter
168 with a pore size of 0.22 μm (diameter: 25 mm; EMD Millipore Corp., Billerica, MA, USA),
169 transferred into 35-mm diameter sterile Petri dishes, and kept at −20°C until further processing in
170 the laboratory. The filter paper containing the cells was folded in half using sterilized tweezers,
171 and cut aseptically into two equal pieces. Each piece was inserted into a separate microcentrifuge
172 tube and used for DNA extraction. DNA was extracted using a QIAamp® DNA Mini Kit (Qiagen,
173 Hilden, Germany) according to the manufacturer's instructions, eluted with 100 μl of double-
174 distilled water, and kept at −80°C until use. DNA was extracted from 0.4–0.5 g sediment using a
175 FastDNA® SPIN KIT for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the

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Discussion started: 13 February 2018

manufacturer's protocol. DNA was eluted in a final volume of 50 µl of double-distilled water, and kept at −80°C until use. DNA quantity and quality were measured using a NanoDrop® 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.3 Polymerase chain reaction amplification

Each PCR was performed in a 20 µl reaction volume using F-Star Taq DNA Polymerase (BioFact Co., Ltd., Daejeon, South Korea) following the manufacturer's instructions. Six different primers, comprising AprA-1-FW (5′-TGG CAG ATC ATG ATY MAY GG-3′), AprA-5-RV (5′-GCG CCA ACY GGR CCR TA-3′), Bac340 Forward (5′-TCC TAC GGG AGG CAG CAG T-3′), Bac515 Reverse (5′-CGT ATT ACC GCG GCT GCT GGC AC-3′), Arc915 Forward (5′-AGG AAT TGG CGG GGG AGC AC-3′), and Arc1059 Reverse (5′-GCC ATG CAC CWC CTC T-3′), were used in this study (Meyer and Kuever, 2007b; Nadkarni et al., 2002; Takai and Horikoshi, 2000; Yu et al., 2005). The PCR mixtures contained 10 µl of F-Star Taq mix, 2 µl of template, 1 µl of each forward and reverse primer (final concentration: 200 nM), and 6 µl of double-distilled water, yielding a final volume of 20 µl. The PCR cycle consisted of a 4-min denaturation step at 94°C, followed by 40 cycles of 94°C for 1 min, 48°C for 1 min, and 72°C for 2 min, and a final elongation step of 72°C for 10 min.

2.4 Cloning and sequencing of *adenosine 5′-phosphosulfate reductase alpha subunit* genes

The amplified products were viewed on 1% agarose gels using electrophoresis, and then purified using a HiGene™ Gel & PCR Purification System (BioFact Co., Ltd., South Korea) following the manufacturer's protocol. The purified amplicons were ligated into the T-Blunt™ vector, and transformed into T-Blunt™ Chemically Competent *Escherichia coli* Cells using a T-Blunt™

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

PCR Cloning Kit according to the manufacturer's instructions (SolGent Co., Ltd., Daejeon, South Korea). Colony PCR amplifications were performed with M13 reverse (−20) and forward (−20) primer sets, and clones with DNA inserts of the expected size were sent to the Bioneer Sequencing Service Center for sequencing (Bioneer, Daejeon, South Korea). Vector sequences were removed and edited using BioEdit software (Hall, 1999).

2.5 Quantitative PCR analysis

Quantitative PCR measurements of bacterial and archaeal 16S rRNA and *aprA* genes were conducted in triplicate on a Chromo4™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc., Hercules, CA, USA). DNA standards for bacterial and archaeal 16S rRNA genes were made from pure cultures of *E. coli* and *Sulfolobus acidocaldarius*, respectively. PCR amplification of 16S rRNA gene was performed and the products were cloned into the T-Blunt™ vector, as described above. Plasmids containing an insert of the expected size were confirmed by sequence analysis, and purified further using a HiGene™ Plasmid Mini Prep Kit (BioFact Co., Ltd.) following the manufacturer's protocol. Initial gene copy numbers of the extracted plasmids were calculated from the DNA concentrations, the length of the cloned gene fragments, and the mean weight of a base pair (660 g/mole). Serial dilution of the plasmids was performed to adjust the concentrations from 1×10^1 to 1×10^9 copies/µl. For the *aprA* gene standards, one *aprA* clone obtained from this study was selected, purified, and prepared as described above for bacterial and archaeal DNA standards. The concentration of the *aprA* clone was adjusted to be from 1×10^1 to 1×10^7 copies/µl.

The qPCR assay was performed as described previously (Purcell et al., 2014). Briefly, qPCR was performed using SYBR® green TOPreal™ qPCR 2X PreMIX (Enzynomics, Daejeon,

South Korea), and a primer concentration of 80 nM for both the bacterial and archaeal 16S rRNA genes and 200 nM for the *aprA* gene. The qPCR amplification conditions were as follows: 95°C for 5 min, and 40 cycles of 95°C for 1 min and 60°C for 30 s. A 2-µl sample of DNA was used in each PCR reaction, which had a total volume of 20 µl. Melting curve analyses were conducted after each assay to check PCR specificity. Coefficients of determination (R^2) for standard curves ≥0.99 and qPCR efficiencies (E)≥80% were accepted.

2.6 Phylogenetic analysis

Nucleotide sequences were aligned and analyzed using the BioEdit program (Hall, 1999). After the vector sequences were removed, the nucleotide sequences were translated into amino-acid sequences, and clustered using ClustalW (Larkin et al., 2007) in BioEdit. The operational taxonomic units (OTUs) of the *aprA* gene were defined by a 90% or greater amino-acid sequence identity. Each *aprA* gene sequence was grouped into OTUs using the BLASTClust tool (http://www.toolkit.tuebingen.mpg.de/blastclust). A similarity search at the amino-acid level was performed against the NR database of the National Center for Biotechnology Information using BLASTP (http://blast.be-md.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic trees were calculated using the neighbor-joining method. A tree was drawn with a bootstrap analysis of 1,000 replicates using the MEGA program (Tamura et al., 2013). Nucleotide sequences of the *aprA* clones obtained in this study were deposited in GenBank under the accession numbers from KY223831 to KY223998.

3 Results

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

245 **3.1 Development of hypoxia in Jinhae Bay**

246 The bottom-water DO concentration in Jinhae Bay showed a typical bowl-shaped pattern (An's
247 bowl, Lee et al., 2017) with hypoxia occurring during the summer (Fig. 1). During the winter and
248 spring (January–March; Period S1), the water column was well mixed, and thermal stratification
249 was not observed (Figs. 1,2A). The water temperature difference between the surface and bottom
250 water increased in April, and caused thermal stratification and pycnocline formation (Fig. 2A).
251 The temperature difference between the surface and bottom water increased, and the pycnocline
252 was strengthened during the summer (late May to August; Period H2; Fig. 1) (Fig. 2A). Seasonal
253 salinity variation was low in Jinhae Bay, indicating limited fresh water inputs, and the influence
254 of salinity on pycnocline formation was not important (Lee et al., 2017). During the summer
255 (May 28–Aug 22; Period H3), hypoxic conditions occurred with low DO (16–92 μM O_2**;** Figs.
256 1,2B). The DO level recovered when the thermocline disappeared in September (Period H4, Fig.
257 1).

258

259 **3.2 Oxygen, NO_3^-, and NH_4^+ profile changes**

260 The DO concentration showed distinct vertical profiles between normoxic (S1, H2, H4, and S5)
261 and hypoxic (H3) conditions (Fig. 2A). Depth profiles for NO_3^- under normoxic conditions were
262 vertically homogenous, although a peak in surface water was observed in Period H2. During
263 hypoxia, however, the maximum concentration was observed in the middle and bottom water
264 (Fig. 2C). NH_4^+ showed similar temporal variations to NO_3^- (Fig. 2D). The depth profile was
265 vertically homogeneous during normoxia. Accumulation of NH_4^+ in the bottom water was clear,
266 with near detection limit concentrations in surface waters during hypoxia (Fig. 2D). In this
267 period, the peak depth of NO_3^- tended to be shallower (7–12 m) than that of NH_4^+ (>12 m; Fig.

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

2D).

3.3 Oxygen demand in the water column and sediment

WOD and SOD represent the rates of bacterial respiration and organic matter degradation. WOD varied from 0.1–0.5 μmol l^{-1} h^{-1} (Fig. 3A). Bottom WOD increased with water temperature (r^2 = 0.99). SOD ranged from 2.4–23.5 mmol m^{-2} d^{-1} (Fig. 3B). SOD tended to increase in early stages of hypoxia (H2A and H2B) and decreased with mature hypoxia (H3B; Fig. 3B). SOD was sensitive to the DO concentration of the bottom water and approached zero during hypoxia. Unlike WOD, SOD did not show a good correlation with water temperature.

3.4 Quantitative PCR analysis

qPCR for the bacterial and archaeal 16S rRNA and *aprA* genes in 20 samples revealed both spatial and temporal variations (Fig. 4**)**. Comparison of qPCR results from water samples showed that the abundance of bacterial 16S rRNA genes was the lowest in bottom water, regardless of the sampling date. However, the abundance of archaeal 16S rRNA genes were the highest in middle water samples, implying that bacterial and archaeal groups respond differently to spatial variations. Increased copy numbers of the *aprA* gene were detected in bottom water at most stages except S1, indicating that most sulfur cycling prokaryotes was present in the bottom water. The relative abundance was measured with the percentage of *aprA* genes in relation to total prokaryote 16S rRNA genes. The percentage of *aprA* to the total prokaryote 16S rRNA gene in surface and mid-depth waters ranged from 0.04%–0.84% versus 5.35%–11.09% in bottom waters, showing that the relative abundance of *aprA* increased significantly with depth (t-test, $p<0.01$). In sediment, higher copy numbers were observed for all genes, as expected. In addition,

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

291 the percentage of *aprA* to bacterial 16S rRNA gene ranged from 16.32%–44.76% in sediment
292 samples, showing that sulfur cycling prokaryotes were more important in sediments than in the
293 water column of Jinhae Bay.

294 In addition to spatial variations, changes in abundance associated with environmental
295 conditions, especially hypoxia development, were examined using qPCR. No significant trends
296 in the copy numbers of the three genes were observed in all water samples, except for a slight
297 increase in the abundance of the archaeal 16S rRNA gene in the middle water column layer with
298 hypoxia development (Fig. 4B). In addition, the abundance of the *aprA* gene relative to total
299 bacteria was variable with oxygen depletion. For examples, water samples collected in Period S1
300 ranged from 0.16–10.74%, whereas those water samples collected in Period H3B ranged from
301 0.09%–6.86%. However, in the sediment, clear correlations between oxygen depletion and
302 changes in microbial community abundance were observed. The total abundances of all three
303 genes decreased with hypoxia development (Fig. 4D). In addition, the relative abundances of the
304 *aprA* gene to total prokaryotes also significantly decreased from 14.9% to 5.2%.
305
306 **3.5 Sequence analysis of the *adenosine 5'-phosphosulfate reductase alpha subunit* gene**
307 PCR trials targeting a region of the *aprA* gene about 390 base pairs in size were successful in all
308 samples, and the products were used for further cloning experiments (data not shown). Cloning
309 and sequence analysis of the amplicons confirmed the detection of the *aprA* gene. From the 20
310 different environmental samples, comprising five sediment and 15 water samples, a total of 168
311 *aprA* clones were sequenced. Among them, 66 originated from various depths in the water
312 column, and 102 were obtained from the sediment. Using the 90% amino-acid identities, all 168
313 *aprA* sequences were grouped into 33 distinct OTUs. Comparison of the number of OTUs with

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

314 respect to the number of clones revealed that similar ratios (13–30%) of water and sediment
315 clones were grouped into OTUs. Nine OTUs among the 66 water-derived clones (13%) were
316 identified, whereas 31 OTUs among the 102 sediment-derived clones (30%) were detected. The
317 ratios were variable, ranging from 21–40%, when the OTU numbers with respect to the clone
318 numbers were compared in each layer, including the surface, middle, and bottom water and
319 sediment. The smallest ratio was observed in the top layer of the water clones. Only seven OTUs
320 were observed among the 32 sequenced clones (21%), indicating that most similar *aprA* clones
321 were obtained from the surface water.

322 Sequence analysis revealed that the most commonly detected group was OTU 13. About
323 29% of the *aprA* sequences (50/168) belonged to this group. Phylogenetic analysis indicated that
324 OTU 13 was affiliated to SOP Lineage I, which showed the highest amino-acid-sequence identity
325 (92%) to Gamma proteobacterium SCGC (Table 1). The second largest group, OTU 21,
326 consisting of 35 clones (20% of *aprA* clones), was also designated as SOP Lineage I. Although
327 two large OTUs belonged to the same functional group, most clones in OTU 13 were derived
328 from the water (45/50), especially the surface layer (24/42), whereas the clones in OTU 21
329 mainly originated from sediment (27/35). In addition, SOP Lineage I consisted of three other
330 OTUs: 4, 9, and 28 (Fig. 5). About 60% (101/168) of the clones analyzed in this study fell into
331 this group, implying that this is the most common functional group in Jinhae Bay. Comparison of
332 the sequence similarity revealed that about 10% (17/168) of the *aprA* clones showed similarity to
333 another sulfur-oxidizing functional group: SOP Lineage II. Most of these clones were grouped
334 into OTU 3 (94%, 16/17), and were mainly obtained from sediment (88%, 15/17). In addition to
335 OTU 3, OUT 29 was affiliated with the SOP Lineage II group and showed amino-acid-sequence
336 identities (67–97%) with various organisms, such as *Candidatus pelagibacter ubique*, *Olavius*

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

337 *algarvensis* gamma 1 endosymbiont isolates, and bacterial endosymbionts of *Lucinoma* aff.
338 *kazani*.

339 Nineteen *aprA* OTUs were designated Gram-positive sulfate-reducing bacteria (SRB)
340 and comprised about 14% of the total *aprA* clones (24/168; Table 1). Considering both clone and
341 OTU numbers, the highest OTU numbers were observed in this group. Nine-teen OTUs were
342 detected among 24 *aprA* clones, indicating a large range of genetic diversity. Most clones (23/24)
343 were derived from the sediment, in contrast to the SOP Lineage I group. A BLASTP search
344 showed various amino-acid identities, ranging from 58–97%, to known SRB species, including
345 *Desulfotomaculum kuznetsovii* (66–88%), *Desulfomonile tiedjei* (62–85%), and *Nitrospira*
346 bacteria (74–97%; Fig. 5). Seven *aprA* OTUs (2, 5, 6, 11, 16, 25 and 27) resembled other SRB
347 groups (Table 1). Six OTUs (2, 5, 6, 11, 16 and 27) were grouped as Deltaproteobacterial SRB
348 species, whereas the last OTU 25 was attributed to the thermophilic SRB group. Most clones
349 (80%, 21/26) of these seven OTUs originated from the sediment as observed for the Gram-
350 positive SRB group. One thermophilic OTU displayed the highest amino-acid identity (58–76%)
351 to *Thermodesulfovibrio islandicus*, whereas the other six OTUs revealed various amino-acid
352 identities (67–97%) to known SRB species, including *Desulfonatronovibrio hydrogenovorans*
353 (69–76%), *Desulfofustis glycolicus* (73–97%), and *Desulfobacter curvatus* (67–95%).

354 Occurrences of *aprA* gene were compared to examine the effects of hypoxia
355 development on the diversity of sulfur cycling microorganisms, Sulfur-oxidizing *aprA* sequences,
356 especially from Lineage I group, dominated all water samples (Fig. 6). About 83% (20/24) of the
357 clones originating from water samples collected in Period S1 were grouped into either sulfur-
358 oxidizing Lineages I or II, and similarly, all clones originating from water samples collected in
359 Period H2B were classified as the same functional SOP groups of S1 clones. In contrast to the

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

presence of similar functional groups in water samples, changes in *aprA* functional groups with hypoxia development were observed. In sediment collected in Period S1, the sulfur-oxidizing groups SOP I and II were dominant. A total of 82% of clones (19/23) belonged to these two groups. However, in sediment collected in Period H3B, *aprA* clones related to sulfur oxidizers decreased to 29% (7/24), whereas clones grouped as sulfate reducers increased to 70% (17/24; Fig. 6).

4 Discussions and conclusions

Microbial abundance and activity changes

Microbial communities are complex in nature, and their structures and functions change rapidly in response to various environmental factors (Fuhrman et al., 2015). However, the major factors affecting the structural dynamics of microbial communities differ depending on time and space (Dang and Chen, 2017). The main purposes of this study were to understand the microbial community responses to suboxic/anoxic conditions, such as 1) how the amount of microbial activity are affected (quantitative aspects) and 2) how the structure of the microbial community are changed (qualitative aspects). In our study, an overall decrease in bacteria and archaea in the sediment occurred with developing hypoxic conditions, indicating that oxygen depletion is a main driver of change in microbial communities. In addition to oxygen concentration, other environmental factors, such as temperature, had a direct impact on microbial communities. For example, SOD increased in the early stage of hypoxia (Period H2A and H2B, Fig. 3B) compared with Period S1, probably due to favorable water temperature. At these H2 stages, DO levels decreased but were high enough for aerobic respiration. However, at hypoxia stage H3B, despite

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

383 the highest water temperature, SOD was significantly decreased, suggesting that low oxygen
384 concentration is the major factor leading to changes in sediment microbial communities

385 Unlike SOD, WOD gradually increased from stages S1 to H3B, indicating that oxygen
386 depletion may not affect microbial communities in the water as significantly as those in the
387 sediment (Fig. 3A). Instead, positive correlations between WOD and temperature from stages S1
388 to H2B indicate that temperature affected microbial activities in the water more than oxygen
389 depletion. Likewise, Crump et al. (2007) also reported that microbial activity among water-
390 column bacteria was unaffected by oxic–anoxic transitions, although compositional and
391 functional group changes occurred under mature hypoxia (Crump et al., 2007; Nogales et al.,
392 2011). Interestingly, Crump et al. (2007) were aware of the importance of the benthic microbial
393 community in shallow coastal waters like Chesapeake Bay (USA); however, the clear decrease in
394 bacterial activity in the sediment with hypoxia development observed in this study was not
395 foreseen in their study. In addition to temporal effects on oxygen concentration, our WOD data
396 displayed an increase in bottom water and a decrease in both surface and middle water during
397 hypoxia (H3B), revealing that oxygen concentration may lead to spatial variations in microbial
398 communities of water column (Fig. 3A). In addition, the detection of a relatively high WOD in
399 the bottom water in Period H3B was quite unexpected because it is contradictory to the result
400 observed in the sediment at the same stage (Fig. 3). From our data, we conclude that microbial
401 aerobic respiration continues under hypoxic conditions in the water, but not in the sediment. The
402 flux of oxygen through eddy diffusion from the surface water column appeared to support
403 aerobic respiration, even though DO concentration was quite low at this stage (< 20% saturation,
404 Fig. 1). Substantial transport of oxygen into the hypoxic zone could occur during the Period H3,
405 although increase of DO was not observed due to the high oxygen consumption rates (Lee et al.,

406 2017).

407 Another interesting result was the positive correlation between microbial activity and
408 abundance. Many reports have shown a positive correlation between SOD and DO concentration;
409 but the mechanisms of the correlations are not clear (Rowe, 2001; Hetland and DiMarco, 2008;
410 Murrell and Lehrter, 2011). It is possible that the decreased SOD in low-DO conditions can be
411 attributed to either a reduction in the aerobic microbial community or a transition to less efficient
412 anaerobic metabolism pathways (Rowe 2001; An et al., 2001; Dang and Jiao 2014). However,
413 under mature hypoxic conditions, the microbial communities may have already been adapted to
414 anaerobic respirations using alternative electron acceptors such as $Mn^{3+/4+}$, Fe^{3+} and SO_4^{2-} (Dang
415 and Jiao, 2014). In such cases, the decrease of SOD may not necessarily mean the decreased
416 microbial respiration activity. In our study, bacteria abundances (qPCR) decreased with
417 decreasing microbial activity (SOD) and vice versa, revealing a positive correlation across
418 sampling site, even though the overall activity trend in the sediment was opposite to that in the
419 water (Fig. 3)**.** Considering both SOD and qPCR data together, we speculate that perhaps the
420 decreased SOD with hypoxia development is caused by a reduction in total microbial abundance
421 more than a switch to less efficient anaerobic metabolism. However, in water, both WOD and
422 microbial abundance correlated with temperature changes, but not to hypoxia development,
423 implying no significant effects of hypoxia on either microbial activity or abundance in water.

424

425 **Nitrogen-cycling microorganisms**

426 In addition to variations in the total microbial community, bacterial and archaeal groups
427 responded differently to hypoxia development (Fig. 4). Recently, Hewson et al. (2014) showed
428 increased archaeal activity occurred as hypoxia matured in Chesapeake Bay (Hewson et al.,

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

2014). They proposed that increased NH_4^+ availability under low DO conditions provided potential substrates for chemoautotrophic archaeal, such as AOA (mostly *Nitrofopumilus marinus*) and caused archaeal increases. Considering both the ubiquitous nature of AOA and our vertical nutrient profile data together, the variations in archaeal abundance in water column with hypoxia development may possibly be caused by AOA in the water column (Berg et al., 2015). During hypoxia, the NO_3^- profile peaked at the middle depth (7–12 m), whereas NH_4^+ concentrations were low at the middle depth, compared with the bottom water, implying active nitrification activity (Figs. 2C,D).

The temporal variation in archaea at each water depth indicates that AOA could be related to this pattern. During the early stage of hypoxia (H2A), archaea increased in surface water when enough NH_4^+ was present (Figs. 2D,3A). However, they decreased abruptly from H2B when NH_4^+ was exhausted, because the intensified stratification blocked the supply of abundant NH_4^+ from the bottom water (Figs. 2A,D). At this stage, nitrification activity could be limited by low NH_4^+ availability, despite the high DO concentration. Similarly, a decrease in archaea with hypoxia development was observed in the bottom water (Fig. 4C). However, the reason for this decrease may differ from that of the surface water. In the bottom water, we assumed that sufficient NH_4^+ was present, but not oxygen, because of the strong stratification and hypoxia (Figs. 2B,D). Reduced export of NH_4^+ can be expected with strong stratification (Lee et al., 2017). A high H_2S concentration at the bottom can also inhibit nitrification, although AOA seem to be less sensitive to H_2S toxicity than NH_4^+-oxidizing bacteria (AOB) (Joye and Hollibaugh, 1995; Berg et al., 2015). Nonetheless, the decrease in archaeal abundance in the bottom water may have resulted from decreased nitrification activity caused by low oxygen concentration with hypoxia, rather than a shortage of NH_4^+. Because the middle water has

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

452 frequent chances to develop favorable conditions for nitrification such as high availability of
453 both NH_4^+ and oxygen, relatively high nitrification activity is expected. Interestingly, in our
454 study, the overall proportion of archaea (2–30%) tended to be higher in the middle water than in
455 surface (0.1–2%) or bottom (2–50%) waters. Archaeal abundance gradually increased as the
456 hypoxia matured, possibly supporting our hypothesis of a close relationship between nitrification
457 activity and archaeal abundance in Jinhae Bay.

458 Our results demonstrate that seasonal hypoxia shows analogous microbial dynamics to
459 permanent OMZ's despite the size difference (15 m versus ~3000 m water depth) as
460 hypothesized. DO and DIN profiles observed in this study suggested that optimal conditions for
461 AOA activity could be formed in the middle depth water. Similarly, active transcripts (up to 20%
462 of all protein coding) of the *amoA* gene of AOA belonging to the phylum Thaumarchaeota
463 occurred in the upper boundary of the OMZ, where optimal conditions for nitrification (high DO
464 supply from surface water and high NH_4^+ supply from bottom water) existed (Stewart et al.,
465 2012). High abundance of archaeal *amoA* genes were evident in the upper boundary of almost all
466 OMZs including ETNP, ESP, Black Sea, Gulf of California, and Baltic Sea (Ulloa et al., 2012).
467 Unlike open oceans, however, the depth of optimal nitrification may quite vary as the pycnocline
468 weakens or strengthens depending on the tide and weather conditions in a shallow estuary like
469 Jinhae Bay (Lee et al., 2017). More detailed future studies, related to the various nitrogen
470 processes such as denitrification, anammox, and DNRA under hypoxic condition, are definitely
471 required in coastal regions because they are less studied compared to the permanent OMZ region
472 (Abell et al., 2011; Ward et al., 2013). Moreover, the studies related to the nutrient-replete
473 conditions and intensive interactions with sediment are also important to understand the complex
474 nitrogen dynamics in coastal areas (McCarthy et al., 2013).

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

475 The abundance of sediment bacteria decreased as hypoxia developed (Fig. 4D), which is
476 consistent with the total microbial abundance and SOD results (Fig. 3B). We believe that the
477 succession from aerobic to anaerobic metabolism was not established, and the aerobic bacteria
478 might be responsible for the decrease. In contrast to the bacteria, sediment archaea were less
479 affected by hypoxia and dominated in Periods H2A, H3A, and H3B as hypoxia developed (Fig.
480 4D). Swan et al. (2000) reported that archaea appeared to be more tolerant to low oxygen than
481 bacteria in sediment (Swan et al., 2010). In this study, the increasing archaeal abundance with
482 hypoxia development was observed in the middle water as well as in the sediment (Fig. 4B).
483 Although similar trends were observed in both middle water and sediment; the reasons for
484 increasing archaeal populations could differ. As discussed above, AOA seems to be responsible
485 for the archaea abundance variation in the middle water. Abell et al. (2011) reported lower DO
486 sensitivity of AOA compared to AOB (ammonium oxidizing bacteria) in an *amoA* transcription
487 study (Abell et al., 2011). Therefore, the AOA seems to be responsible for the increased archaeal
488 importance with middle water hypoxia development. In sediment, however, the oxygen
489 availability is very low and should limit AOA activity during hypoxia, so the increase of archaeal
490 importance in the hypoxia cannot be explained with AOA. A methanogenesis processing archaeal
491 group seems to be another explanation, considering the organic-rich conditions of Jinhae Bay
492 (Munson et al., 1997). Although methanogens are less competitive than SRP under anoxic
493 conditions, methanogenic archaea successfully co-occur in certain conditions if noncompetitive
494 substrates, including methanol, methylamine, and methionine are available (Munson et al., 1997).
495 More future studies addressing how the relative dominance of SRP vs. archaea occurs in Jinhae
496 Bay may be interesting.
497

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

Sulfur-cycling microorganisms

Sequence analysis indicated that large numbers of clones were SOPs; however, most of these SOP-related OTUs were not assigned clearly into specific SOP species or genera (Watanabe et al., 2013). Similar problems were faced in other studies, because only limited numbers of *aprA* sequence data are available and low bootstrap values were present in the SOP lineages. Although each OTU was not classified into a specific species, the most common one, OTU 13, was affiliated to the *aprA* Lineage I cluster, which includes bacterial species such as the chemolithoautotrophic betaproteobacterium *Thiobacillus thioparus* and the phototrophic gammaproteobacterium *Thiocapsa rosea*. The second most common OTU, 21, was also affiliated with the *aprA* Lineage I cluster, suggesting that the main groups of SOP present in Jinhae Bay belong to *aprA* Lineage I. Purcell et al. (2014) also showed that most sulfur oxidizers in the subglacial Lake Whillans (Antarctica) belonged to the *aprA* Lineage I (Purcell et al., 2014). However, all clones from this subglacial lake originated from the sediment, whereas our clones derived from both the water and the sediment. Most sulfur-oxidizer clones in the water column belonged to OTU 13, demonstrating a close relationship to *aprA* Lineage I. In contrast to the existence of one major group of sulfur oxidizers in water, at least three different groups, including OTUs 3, 4, and 21, were present in the sediment. These clones were affiliated to either *aprA* Lineages I or II. Our results suggest that more genetically diverse groups of sulfur oxidizers are present in the sediment of Jinhae Bay, although more comprehensive studies with enough clone library sizes might be helpful to sharpen our data.

Diverse sulfate-reducing *aprA* genes were identified in the current study and many clones were affiliated to the family *Desulfobacteraceae*. Reportedly, *Desulfobacteraceae* is one of the most abundant SRB groups in the subsea floor environment (Foti et al., 2007; Kjeldsen et

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

al., 2007; Kuever, 2014). In addition to the heterogenetic metabolism, members of the *Desulfobacteraceae* family can grow chemolithoautotrophically using hydrogen or by fermentation, iron reduction, or the disproportionation of inorganic sulfur compounds. Other *aprA* gene groups such as *Desulfotomaculum* and *Desulfobulbaceae* occurred in Jinhae Bay and can oxidize various organic substances using sulfur compounds as the electron acceptor.

Both qPCR and studies of genetic diversity demonstrated that *aprA*-related microorganisms were affected by hypoxia development, but they responded differently depending on sampling site and their functional groups. In the water column, SOPs were dominant, and no significant changes were observed in either their abundance or diversity (Fig. 6). However, in the sediment, more genetically diverse SRPs were detected with hypoxia development, suggesting that hypoxia causes favorable conditions for SRPs in the sediment, but not in the water. OTUs 13 and 21, belonging to SOP Lineage I, occurred frequently both in the water column and the sediment. However, sensitivity to oxygen concentration differs between these OTUs. When hypoxia matured in Period H3B, OTU 21 still appeared in the sediment, whereas OTU 13 was absent in both the sediment and the bottom water (although OTU 13 still appeared in the top and middle water, where oxygen levels remained high). Therefore, OTU 13 may represent a candidate instigator of the reduced *aprA* abundance evident in the qPCR analysis of sediment samples (Fig. 4D). Most sulfur reducers found increasingly during hypoxic stages were SRB of the class *Deltaproteobacteria* or Gram-positive SRB of the genus *Desulfotomaculum*. *Deltaproteobacteria*-SRB (D-SRB) groups were detected throughout the seasons, whereas different OTUs belonging to Gram-positive-SRB (G-SRB) mostly present in the sediment (except OTU 1 of top layer in stage S1) and showed distinctive occurrences in each stage (a “SRB succession”; Fig. 6D). The SRB succession implies that the various G-SRB OTUs

are sensitive to a small DO change. The number of OTUs during the SRB succession tended to increase as hypoxia matured (From H2B to H3B). At pre-hypoxia stage H2A, diverse and distinctive G-SRB OTUs were observed. This result was not expected because DO should be relatively high compared to the H3 states. Other environmental factors, including temperature, salinity, and organic compounds may be important for the SRB succession (Wright et al., 2012). The SRB succession might also be the results of interactions with the cryptic sulfur cycle (autotrophic denitrification), which could enhance the SRB activity in certain microenvironments (Canfield et al., 2010; Shao et al., 2010; Dang and Lovell, 2016). More detailed studies are certainly necessary to define and explain how SRB succession occurs, but our results demonstrate distinctive responses of sediment microbes to hypoxia in terms of microbial community structure.

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Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

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Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

Table 1: List of Jinhae Bay *adenosine 5'-phosphosulfate reductase alpha subunit* operational taxonomic units (OTUs) and their relatedness to known sulfur-cycle–related groups (T: top, M:middle, B: bottom layers of water and S: sediment).

OTU type (#)	Sample originated (#)	AA identity (%)	Highest match (accession number)	AA identity (%)	Highest cultured match (accession number)
Sulfur-oxidizing (SOP)- SOP lineage I					
4(14)	T(2), M(2), S(10)	88-99	Uncultured bacterium clone 1cm_60 (AKQ24881)	78-94	Gamma proteobacterium SCGC AAA240-C17 (ADX05650)
9(1)	S(1)	84-90	Uncultured prokaryote clone MC-3C 3.5-8cm-10 (AIW55966)	75-88	*Olavius algarvensis* gamma 3 endosymbiont (CAJ81241)
13(50)	T(24), M(11), B(10), S(5)	82-94	Uncultured prokaryote clone aprE9 (CBH30854)	80-92	Gamma proteobacterium SCGC AAA001-B15(WP_010507240)
21(35)	T(2), M(3), B(3), S(27)	85-95	Uncultured gamma proteobacterium clone bcs3.30 (CAT03609)	82-97	*Candidatus thiobios zoothamnicoli* strain calvi (ACC95127)
28(1)	B(1)	87-96	Uncultured gamma proteobacterium clone LS.Fer.APS.12 (CCC58236)	79-95	Bacterium symbiont of *Christineconcha regab* 225-V5 (AGR49140)
Sulfur-oxidizing prokaryote (SOP) *aprA* lineage II					
3(16)	T(1), M(1), S(14)	86-98	Uncultured gamma proteobacterium clone bcs 3.1 (FM879016)	73-92	Bacteria endosymbiont of *Lucinoma* aff. *kazani* clone L8 (AM236338)
29(1)	S(1)	83-98	Uncultured bacterium clone A49 (AFK76430)	67-92	*Olavius algarvensis* gamma 1 endosymbiont Isolate 5 (CAJ81240)
Gram-positive sulfate-reducing bacteria (SRB) and related *Deltaproteobacteria*					
1(1)	T(1)	78-89	Uncultured bacterium clone DdH6 (AFV48086)	66-88	*Desulfotomaculum kuznetsovii* DSM 6115 (AF418152)
7(2)	S(2)	75-92	Uncultured bacterium clone aspA70m14 (ADD84947)	60-92	*Gemmatimonas* sp. SG8_17 (KPJ93694)
8(1)	S(1)	73-90	Uncultured bacterium clone 28_13 (AGV76884)	63-81	*Desulfotomaculum kuznetsovii* DSM 6115 (AAL57419)
10(2)	S(2)	71-89	Uncultured bacterium clone 1cm_37 (AKQ24858)	62-89	*Gemmatimonas* sp. SG8_17 (KPJ93694)
12(1)	S(1)	72-93	Uncultured delta proteobacterium clone bcs 3.41 (CAT03614)	63-88	*Desulfomonile tiedjei* DSM 6799 (AAL57429)
14(1)	S(1)	70-91	*Gemmatimonas* sp. clone SG8_38_2 (KPK65885)	61-91	Delta proteobacterium SM-66-47 (CCC55932)
15(1)	S(1)	76-94	Uncultured bacterium clone 1cm_13 (AKQ24834)	66-88	*Desulfotomaculum geothermicum* DSM 3669 (AAL57382)
17(1)	S(1)	73-95	Uncultured bacterium clone A079 (ADW77112)	63-79	*Desulfotomaculum kuznetsovii* DSM 6115 (AAL57419)
18(1)	S(1)	71-88	Uncultured bacterium clone 1cm_13 (AKQ24834)	62-79	*Desulfotomaculum geothermicum* DSM 3669 (AAL57382)

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

Table 1 (Continued)

OTU type (#)	Sample originated (#)	AA identity (%)	Highest match (accession number)	AA identity (%)	Highest cultured match (accession number)
19(2)	S(2)	73-88	Uncultured delta proteobacterium clone bcs 3.41 (CAT03614)	62-85	*Desulfomonile tiedjei* DSM 6799 (AAL57429)
20(1)	S(1)	68-96	Uncultured bacterium clone Ha_3.5m_57 (BAO79242)	58-82	Delta proteobacterium SM-66-64 (CCC55931)
22(1)	S(1)	74-92	Uncultured bacterium clone 28_13 (AGV76884)	64-81	*Desulfotomaculum kuznetsovii* DSM 6115 (AAL57419)
23(1)	S(1)	73-86	Uncultured *Firmicutes* bacterium clone T8-*aprA*-22 (AFC36470)	63-82	*Desulfotomaculum kuznetsovii* DSM 6115 (AAL57419)
24(2)	S(2)	76-95	Uncultured sulfate-reducing bacterium (CAJ31201)	64-93	*Gemmatimonas* sp. SG8_17 (KPJ93694)
26(2)	S(2)	74-97	Uncultured bacterium clone C6507_*aprA*_7B101 (ACM47772)	74-97	*Nitrospira bacterium* SG8_3 (KPK16323)
30(1)	S(1)	70-96	Uncultured bacterium clone 89_APS30_EDS_640 (ADJ38098)	59-82	*Bacteroides* sp. SM23_62_1 (KPK79634)
31(1)	S(1)	72-84	Uncultured bacterium clone GoM_*AprA*_3 (CCB84471)	63-78	Delta proteobacterium SM-66-47 (CCC55932)
32(1)	S(1)	73-98	Uncultured bacterium clone SKCK0606_1H2_6 (BAQ03184)	65-84	*Desulforhabdus amnigena* DSM 10338 (AAL57406)
33(1)	S(1)	75-89	Uncultured bacterium clone 1cm_50 (AKQ24871)	64-86	Delta proteobacterium SM-66-47 (CCC55932)
Thermophilic sulfate reducing bacteria (SRB)					
25(1)	S(1)	63-81	Uncultured prokaryote clone CVA-*aprA*- 28 (CCG27943)	58-76	*Thermodesulfovibrio islandicus* DSM 12570 (AAL57380)
Deltaproteobacterial sulfate reducing bacteria (SRB)					
2(5)	T(1), M(2), S(2)	73-94	Uncultured bacterium clone PB70 (KF788937)	69-76	*Desulfonatronovibrio hydrogenovorans* DSM 9292 (AF418111)
5(5)	T(1), S(4)	89-99	Uncultured delta proteobacterium (EU265806)	78-95	*Desulfobulbus elongates* clone DSM 2908 (AF418146)
6(1)	S(1)	81-96	Uncultured bacterium clone BSCK0903_1H3_21 (BAQ03029)	76-95	*Nitrospira* bacterium SG8_3 (LJNR01000453)
11(8)	S(8)	86-97	Uncultured bacterium clone 1cm_42 (AKQ24863)	76-95	*Olavius algarvensis* delta 1 endosymbiont isolate 5 (CAJ81242)
16(5)	S(5)	83-99	Uncultured bacterium clone 1cm_1 (AKQ24822)	73-97	*Desulfofustis glycolicus* DSM 9705 (AAL57397)
27(1)	B(1)	77-95	Uncultured bacterium clone 1cm_17 (AKQ24838)	67-95	*Desulfobacter curvatus* DSM 3379 (AAL57374)

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

Figure 1: Temporal variations in surface and bottom oxygen saturation (%) and water temperature (°C) from January–December 2015 at each time period (S1, S5: solubility period, H2, H3, H4: hypoxia period). The arrows show the five sampling dates for the microbial study (S1, H2A, H2B, H3A, and H3B) in Jinhae Bay.

Figure 2: Vertical profiles of (A) temperature, (B) dissolved oxygen, (C) NO_3^-, and (D) NH_4^+ during normoxic (Jan, Apr, May, Nov) and hypoxic (Jun, Jul, Sep) periods at Jinhae Bay.

Figure 3: Copy numbers of prokaryotes (bacteria and archaea) during quantitative polymerase chain reaction analysis and water-column oxygen demand in the (A) top, middle, and bottom layers, and (B) sediment oxygen demand of Jinhae Bay. Each sample was collected across seasonal time periods from January to June. S1, S5: solubility period, H2, H3, H4: hypoxia period, See Fig. 1 for sample dates.

Figure 4: Copy numbers of bacterial, archaeal, and *adenosine 5'-phosphosulfate reductase alpha subunit* (*aprA*) genes in the (A) top, (B) middle, and (C) bottom layers of the water column or in the (D) sediment of Jinhae Bay. (BAC = bacterial 16S ribosomal RNA (rRNA), ARC = archaeal 16S rRNA and APR = *aprA* gene). See Fig. 1 for sample dates.

Figure 5: Phylogenetic analysis of *adenosine 5'-phosphosulfate reductase alpha subunit* (*aprA*) gene sequences obtained from Jinhae Bay. The *aprA* gene sequence from *Pyrobaculum aerophilum* (GenBank accession number: AAL64282) was used as an outgroup. Bootstrap values (1000 samples) are shown on the corresponding nodes.

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-64
Manuscript under review for journal Biogeosciences
Discussion started: 13 February 2018

Figure 6: Operational taxonomic units (OTUs) occurred at each period of hypoxia in the water column for (A) top, (B) middle, (C) bottom layers, and (D) surface sediment. Each OTU was classified as either a sulfide-oxidizing prokaryote or a sulfate-reducing prokaryote. See text for details.

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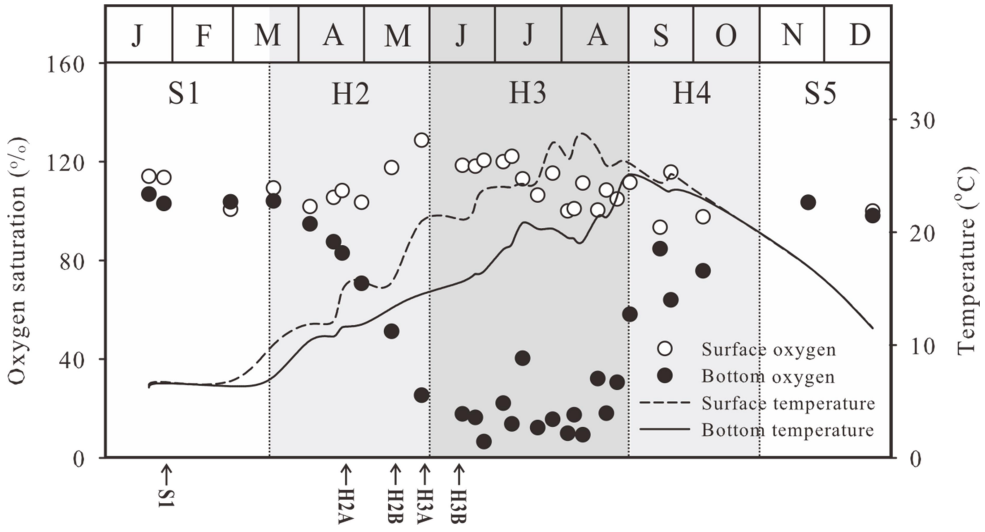

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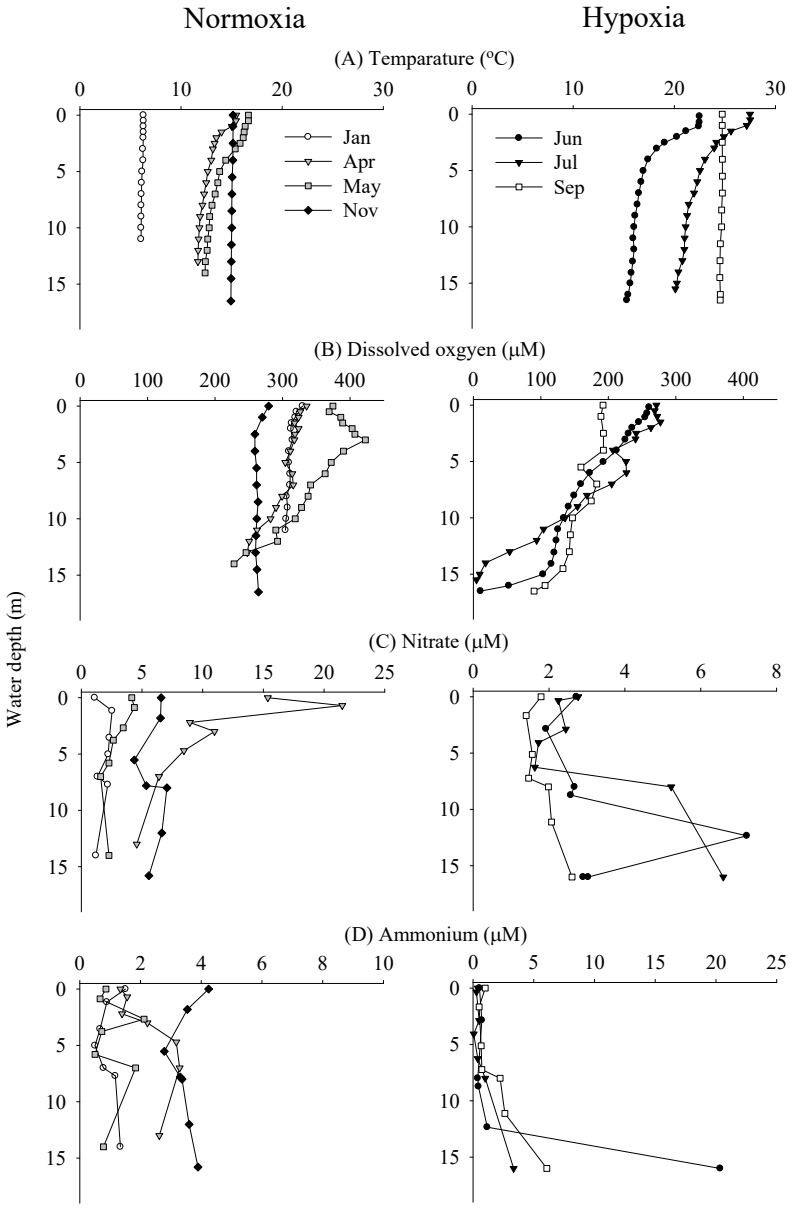

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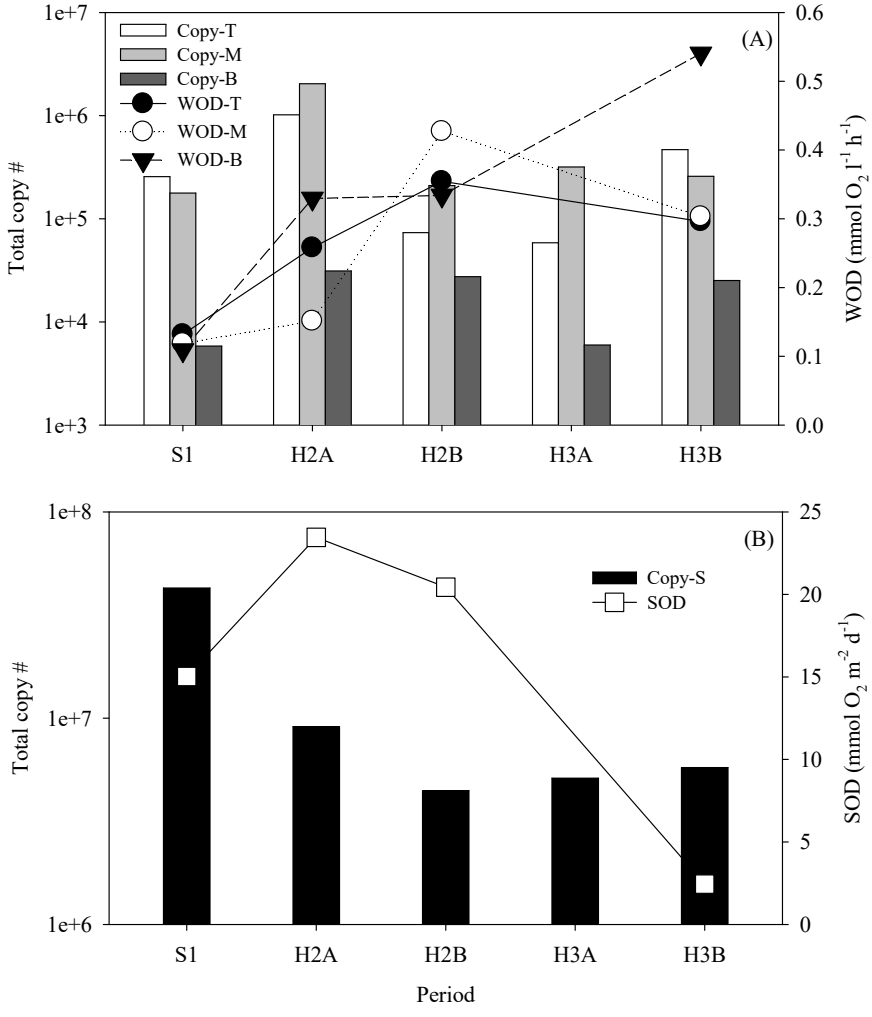

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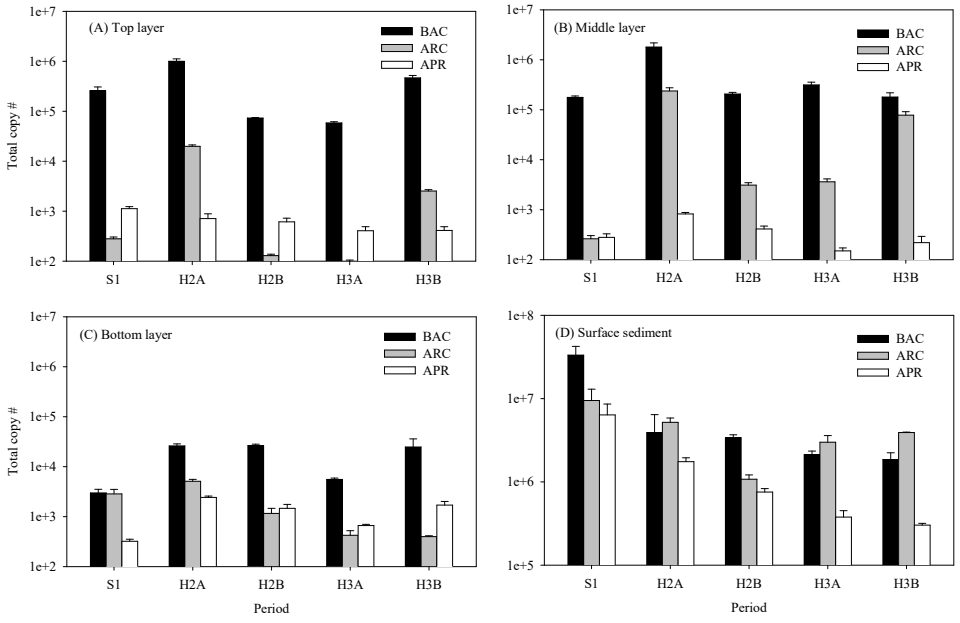

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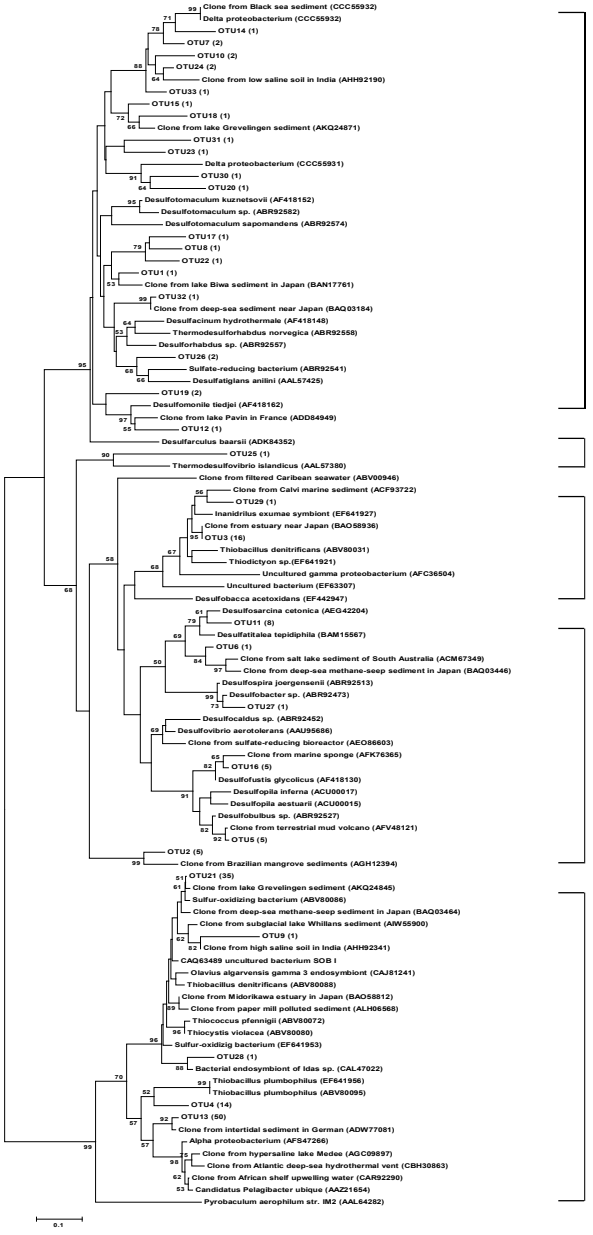

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