JEST_2019

by Melki Melki

Submission date: 17-Sep-2019 05:42AM (UTC+0700)
Submission ID: 1174012108
File name: Melki_et_al_2019_JEST_108-116.pdf (750.75K)
Word count: 5063
Character count: 26812

Journal of Environmental Science and Technology

ISSN 1994-7887

science alert

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OPEN ACCESS Journal of Environmental Science and Technology

ISSN 1994-7887
DOI: 10.3923/jest.2019.108.116

Research Article
Seasonal Abundance and Community of Ammonia-oxidizing Bacteria in the Musi River Sediment, South Sumatra, Indonesia

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Abstract

Background and Objective: Human activities along the Musi river contribute to the waste inputs especially nitrogen which has a negative ecological impact. A micro-organism group that play a crucial role in the nitrogen cycle is Ammonia-Oxidizing Bacteria (AOB). **Materials and Methods:** In the present study, AOB community were analyzed during rainy and dry seasons based on PCR T-RFLP analysis of 16S rRNA and *amo*A genes. The amplified genes were digested with *Alu*I, *Bsu*FI and *Msp*I. **Results:** *Nitrosospira* and *Nitrosomonas* from class β-proteobacteria were the dominant abundances based on 16S rRNA and *amo*A genes were found from freshwater to brackish water but *Nitrosococcus* from class γ-proteobacteria was found only in brackish water. The AOB communities based on 16S rRNA and *amo*A genes in dry season were higher than in rainy season. The salinity, temperature, DO and nutrients contributed to the AOB community. Salinity was the most dominant factor affected the AOB community. **Conclusion:** Variability in salinity caused the spatial distribution to the AOB in the Musi river.

Key words: Abundance, AOB, community, physicochemical properties, season

Citation : Melki, Alim Isnansetyo, Jaka Widada and Murwantoko, 2019. Seasonal abundance and community of ammonia-oxidizing bacteria in the Musi river sediment, south Sumatra, Indonesia. J. Environ. Sci. Technol., 12: 108-116.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nitrification is the oxidation of ammonia to nitrate via nitrite. It is a fundamental process in the biological removal of nitrogen. The first step of nitrification is carried out by the Ammonia-Oxidizing Bacteria (AOB) that oxidizes ammonia to nitrite. These bacteria played an essential role in terrestrial and aquatic nitrogen cycles. The AOB species based on the 16S rRNA and the *amoA* marker molecule into two monophyletic groups (β-proteobacteria and γ-proteobacteria), β-proteobacteria consists of *Nitrosomonas* and *Nitrosospira* groups and γ-proteobacteria consists of *Nitrosococcus halophilus* and *Nitrosococcus oceani*[1,2].

The AOB is difficult to cultivate in the laboratory and the identification is traditionally based on a limited number of phenotypic characters. Molecular characterization has been particularly valuable for the analysis of ammonia oxidizer, they are different in phylogeny and physiological characters, leading to significant variations in the relative abundance and community structure between them under different environmental conditions. For example, on the analysis of the abundance and diversity of AOA and AOB in sediment from freshwater[3], on the diversity and abundance of AOA and AOB in sediment from estuary/seawater[4,5], on the diversity and abundance of AOB from seawater[6,7], the diversity of AOB from water column and/or sediment from freshwater and/to seawater[8-10], on the diversity of AOB from sediment from freshwater to seawater[11].

Terminal-restriction Fragment Length Polymorphism (T-RFLP) analysis is one of the most frequently used high-throughput fingerprinting technique to monitor changes in the structure and composition of microbial communities. Because of its relative simplicity, T-RFLP analysis has been applied to the analysis of AOB gene in aquatic systems[7,12-14]. The technique is used to amplify small subunit genes from total community DNA using Polymerase Chain Reaction (PCR) wherein one or both of the primers used are labeled with a fluorescent dye and then digested with restriction enzymes, in which the sizes and relative abundances of the fluorescently labeled TRFs are determined using an automated DNA sequencer[15].

Musi river is one of south Sumatra icons and is the longest river in Sumatra Island, Indonesia. Human activities related to agriculture, plantation, coal stockpile, harbor and water transportation as well as the industry are found along the Musi river that probably contribute to the waste inputs containing some chemical components and eutrophication. At the downstream of Musi river, industries are the major activities with their waste products that are discharged directly into Musi river[16]. The main purposes of this study were to analyze the abundance and community of AOB using PCR T-RFLP analysis of 16S rRNA and *amo*A genes in the sediment of tropical freshwater and brackish water and to determine the seasonal and spatial abundance of AOB.

MATERIALS AND METHODS

Research work were carried out in the Marine Bio-Ecology Laboratory, Department of Marine Science, Sriwijaya University, Inderalaya, Indonesia and Fish Disease Laboratory, Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta, Indonesia from March, 2016 to December, 2017.

Area study and sample collection: This study was conducted in 5 sites. Three sites were located at freshwater (Gandus, Palembang and Upang), whereas two (Sungsang and Tg Carat) were located on the brackish water. The description of field samplings and sample collection have been described in the previous publication[17].

Physicochemical properties: The temperature, salinity, dissolved oxygen and pH were measured in sampling sites using a Midas CTD+ (Multiparameter Profiler, Valeport Ltd., UK). The concentrations of ammonia, nitrite and nitrate in the sediments were measured by the spectrophotometric method[18].

DNA extraction: Sediment samples (<50 g) transported on ice were mixed by shaking, divided into 5 g aliquots within 5 h and frozen at -70°C until DNA was ready to be isolated. DNA was extracted according to the methods of Christman *et al.*[7] and Osborn *et al.*[19].

PCR amplification of 16S rRNA gene: Amplification of 16S rRNA genes was performed as specified by Jinsheng *et al.*[20] and Melki *et al.*[21] using primers 27F (5′ labelled with 56-FAM) and 1492R.

PCR amplification of *amo*A gene: Amplification of *amo*A genes was performed as specified by Rotthauwe *et al.*[22] using primers *amo*A-1F and *amo*A-2R, which forward primer fluorescently labeled with 56-FAM. Reaction mixtures in a total volume of 50 µL containing 24 µL My *Taq* HS DNA polymerase (Bioline), 2 µL of each primer (10 µM), 2 µL DNA template (10 ng mL^{-1}) and 20 µL nuclease-free water. Thermal cycling was carried out by an initial denaturation step at 95°C for

3 min followed by 35 cycles of 95°C for 15 sec, 55°C for 15 sec, 72°C for 10 sec, final extension at 72°C for 5 min and visualization by agarose gel electrophoresis. The PCR products were visualised on a 1.5% agarose gels (Promega, USA). The PCR products were visualized on a 1.5% agarose gels (Promega, USA).

T-RFLP analysis: The PCR products of each sample were digested with *Alu*I, *Bsu*RI and *Msp*I (Thermo Scientific), following the manufacturer's protocol: 10 µL product PCR, 1 µL Fast digest enzymes, 2 µL 10X Fast digest buffer, 17 µL nuclease-free water in a total volume of 30 µL and then incubated at 37°C for 5 min in water thermostat. The digested products of each sample were size-separated using ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The T-RFLP electropherograms were analyzed with Peak Scanner v1.0 software (Applied Biosystems). The TRFs with peak size between 50-500 bp and with a peak area ≥1% were only included in the analysis[23,24].

Phylogenetic assignments were performed by using a default database generated from MiCA (http://mica.ibest.uidaho.edu/) and AOB genes database search which matches were performed by using BioEdit Sequence Alignment Editor software, a program that the TRFs (Terminal Restriction Fragments) were generated from in silico digestions of AOB gene sequences and submitted to the National Center for Biotechnology Information (NCBI) GenBank database (https://www.ncbi.nlm.nih.gov).

Statistical analysis: To evaluate richness and evenness, the diversity statistics were calculated from each standardized and average enzyme profile of a sample. The calculation was obtained by using the number and height of peaks in each average profile as representations of the number and relative abundance of different phylotypes in a sample. Phylotype richness (S) was calculated as the total number of distinct TRF sizes in a profile. The Shannon-Weiner index (H') was calculated utilizing Eq. 1[25]:

$$H' = -\Sigma\, pi \ln pi \quad (1)$$

where, p was the proportion of an individual peak height relative to the sum of all peak heights. Evenness (E) was calculated from the Shannon-Weiner index function utilizing Eq. 2[25]:

$$E = \frac{H}{H_{max}} \quad (2)$$

where, H_{max} was calculating utilizing Eq. 3[25]:

$$H_{max} = \log_2(S) \quad (3)$$

The Principal Component Analysis (PCA) was performed in the XIstat 2016 software program to determine relationships between AOB community and physicochemical properties.

RESULTS

Physicochemical properties: The physicochemical properties in the bottom water of sampling sites have fluctuated during the rainy and dry seasons (Table 1). The temperature and salinity of bottom water were higher in the dry season than in the rainy season. However, dissolved oxygen and pH in the bottom water and the concentrations of sediment nutrients were higher in the rainy season than in the dry season.

AOB abundance: The results showed that AOB abundance in the sediment during rainy and dry seasons was indicated by *Nitrosomonas* and *Nitrosospira* from class β-proteobacteria in all sampling sites (freshwater and brackish water) but *Nitrosococcus* from class γ-proteobacteria only found in brackish water (Sungsang and Tg Carat sites). Figure 1a shows the AOB abundance based on the 16S rRNA gene in the rainy season exhibiting *Nitrosospira* (on average, 78%) in Gandus site was the highest from other species of AOB. Further, AOB abundance based on the 16S rRNA gene in the dry season (Fig. 1b) exhibiting *Nitrosomonas* (on average, 58%) in Sungsang site was the highest from other species of AOB.

The AOB abundance in the sediment based on *amo*A gene in the rainy season (Fig. 2a) detecting *Nitrosomonas* (on average, 64%) in Palembang site was the highest from other members of AOB. Furthermore, AOB abundance based on *amo*A gene in the dry season (Fig. 2b) exhibiting *Nitrosomonas* (on average, 53%) in Palembang site was the highest from the other species of AOB.

AOB community: The AOB community in the sediment based on the 16S rRNA gene in the dry season was higher than in the rainy season (Table 2). Phylotype richness of TRF-15 was the highest found in Gandus, Palembang and Tg Carat sites. The highest Shannon-Wiener index and the highest evenness were found in Palembang site (1.42 and 0.52), respectively.

Similarly, AOB community in the sediment based on *amo*A gene in the dry season was higher than the rainy season (Table 2). The highest phylotypes richness of TRF-14 was

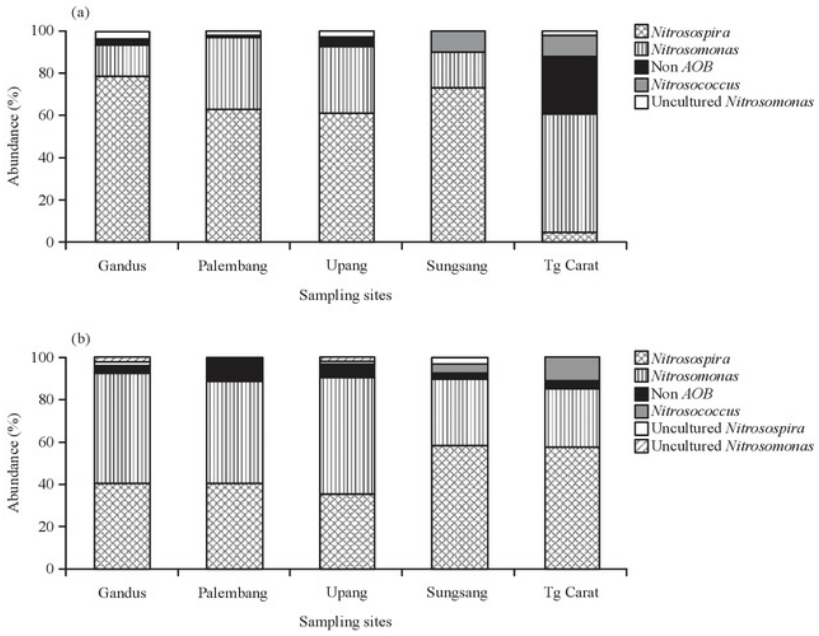

Fig. 1(a-b): AOB abundance based on T-RFLP analysis derived from (a) 16S rRNA gene in the rainy season and (b) 16S rRNA gene in the dry season

Freshwater sites: Gandus, Palembang, and Upang. Brackish water sites: Sungsang and Tg Carat

Table 1: Physicochemical properties in the bottom water and concentrations of sediment nutrient in the sampling sites

Sampling sites	Temperature (°C)	Salinity (ppt)	DO (mg L^{-1})	pH	NH_3 (mg L^{-1})	NO_2 (mg L^{-1})	NO_3 (mg L^{-1})
Gandus							
Rainy season	29.60	0	6.67	6.72	0.09	0.09	0.44
Dry season	31.34	0	6.06	6.72	0.76	0.65	1.54
Palembang							
Rainy season	29.56	0	4.12	5.77	0.05	0.08	2.01
Dry season	30.87	0	4.01	5.35	0.09	0.98	0.98
Upang							
Rainy season	29.58	0	5.12	5.55	0.76	0.07	1.66
Dry season	30.54	0	3.42	7.11	0.04	0.54	1.08
Sungsang							
Rainy season	29.63	9.04	6.06	7.59	0.43	0.06	0.87
Dry season	31.32	12.03	4.51	7.16	0.30	0.08	0.76
Tg Carat							
Rainy season	29.29	22.64	6.53	6.51	0.20	0.08	0.65
Dry season	31.82	23.38	5.23	6.54	0.37	0.43	0.54

Freshwater sites: Gandus, Palembang and Upang. Brackish water sites: Sungsang and Tg Carat

found in Gandus site. Furthermore, the highest Shannon-Wiener index and the highest evenness were found in Sungsang site (2.09 and 0.83), respectively.

Relationship between AOB community and physicochemical properties to sampling sites: The PCA based on the 16S rRNA gene in the rainy season revealed that the cumulative eigenvalues were 78.52% formed two groups of AOB. The first group in the Sungsang site showed the relation to pH, Shannon-Wiener index and evenness. On the other hand, the second group in the Gandus site showed the relation to temperature (Fig. 3a) and also formed two groups based on the 16S rRNA gene in the dry season (cumulative eigenvalues, 77.64%). The first group in the Tg Carat site showed the relation to the temperature, salinity, dissolved oxygen and concentration of nitrite. The second group in the Palembang

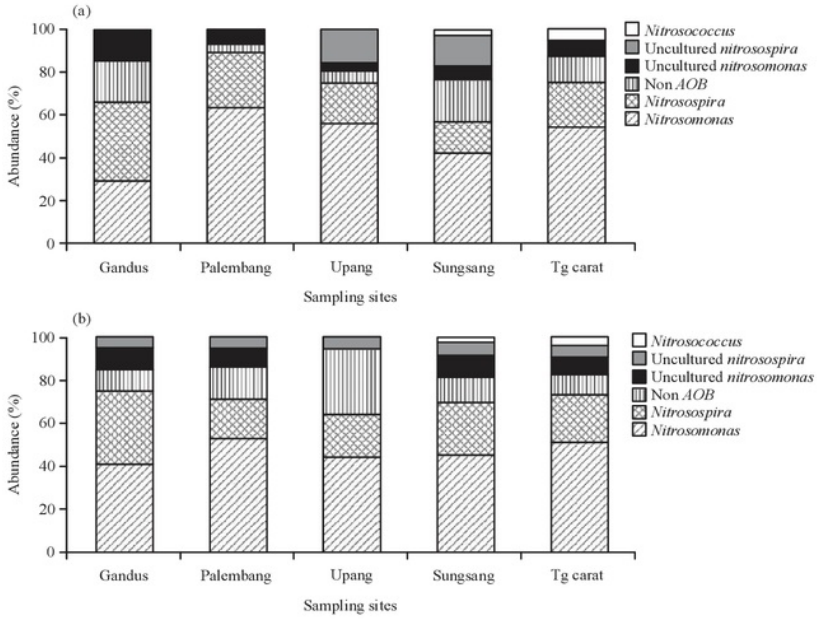

Fig. 2(a-b): AOB abundance based on T-RFLP analysis derived from (a) *amo*A gene in the rainy season and (b) *amo*A gene in the dry season

Freshwater sites: Gandus, Palembang, and Upang. Brackish water sites: Sungsang and Tg Carat

Table 2: The AOB community profiles based on T-RFLP analysis in sampling sites include Phylotype richness (S), Shannon-Weiner diversity index (H') and Evenness (E)

	16S rRNA gene			*amo*A gene		
Sampling sites	S	H'	E	S	H'	E
Gandus						
Rainy season	10	1.03	0.50	12	1.90	0.77
Dry season	15	1.27	0.47	14	2.05	0.79
Palembang						
Rainy season	10	0.78	0.29	12	1.71	0.69
Dry season	15	1.42	0.52	12	2.02	0.81
Upang						
Rainy season	10	0.68	0.29	13	1.78	0.70
Dry season	14	1.23	0.48	13	2.00	0.78
Sungsang						
Rainy season	10	1.10	0.51	13	1.82	0.72
Dry season	13	1.18	0.46	13	2.09	0.83
Tg Carat						
Rainy season	12	0.90	0.36	12	1.95	0.78
Dry season	15	1.37	0.51	12	2.02	0.82

Freshwater sites: Gandus, Palembang and Upang. Brackish water sites: Sungsang and Tg Carat

site showed the relation to the phylotypes richness, Shannon-Wiener index and evenness (Fig. 3b).

Furthermore, the cumulative eigenvalue based on *amo*A gene in the rainy season was 68.55% with only one group in the Tg Carat. This analysis also indicated the significant positive relation of AOB to salinity dissolved oxygen, Shannon-Wiener index and evenness (Fig. 4a). While formed two groups based on *amo*A gene in the dry season (cumulative eigenvalues, 76.25%). The first group in the Tg Carat site showed the relation to temperature, salinity, dissolved oxygen and concentration of nitrite. The second group in the Upang site showed the relation to pH and concentration of ammonia (Fig. 4b).

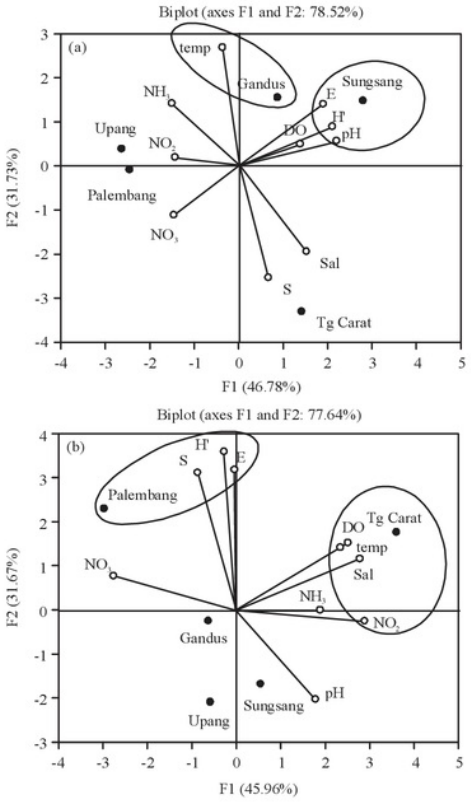

Fig. 3(a-b): PCA of relationship between AOB community and physicochemical properties to sampling sites derived from (a) 16S rRNA gene in the rainy season and (b) 16S rRNA gene in the dry season

Freshwater sites: Gandus, Palembang and Upang. Brackish water sites: Sungsang and Tg Carat. sal: Salinity. temp: temperature. DO: Dissolved oxygen. NH_3: Ammonia. NO_2: Nitrite. NO_3: Nitrate. S: Phylotype richness. H': Shannon-Wiener index. E: Evenness

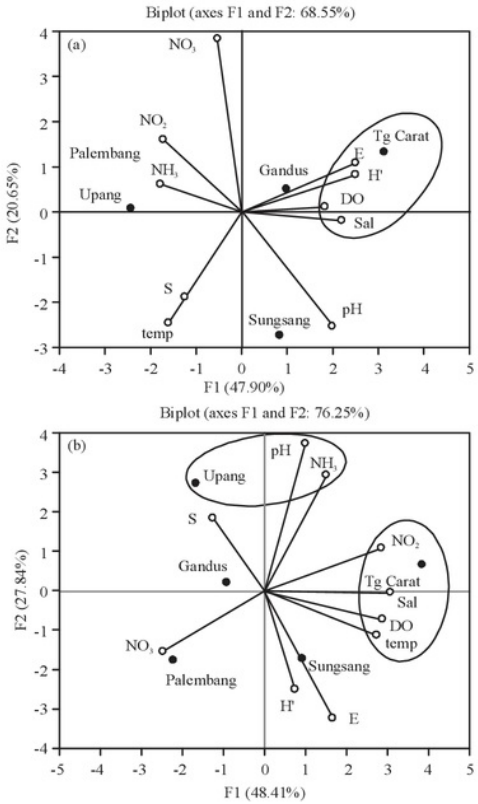

Fig. 4(a-b): PCA of relationship between AOB community and physicochemical properties to sampling sites derived from (a) *amo*A gene in the rainy season and (b) *amo*A gene in the dry season

Freshwater sites: Gandus, Palembang and Upang. Brackish water sites: Sungsang and Tg Carat. sal: Salinity. temp: Temperature. DO: Dissolved oxygen. NH_3: Ammonia. NO_2: Nitrite. NO_3: Nitrate. S: Phylotype richness. H': Shannon-Wiener index. E: Evenness

DISCUSSION

Nitrosospira and *Nitrosomonas* from class β-proteobacteria were dominant based on 16S rRNA and *amo*A genes in the sediment of all sampling sites. The previous publication reported that *Nitrosomonas, Nitrosospira* and *Nitrosococcus* were dominant in the water surface of Musi river, Indonesia[21]. β-proteobacteria was dominant in different environmental samples, such as water and sediment from freshwater or brackish/seawater suggesting the *Nitrosospira* and *Nitrosomonas* were nearly ubiquitous[8-11,26,27].

Nonetheless, the abundance of *Nitrosococcus* from class γ-proteobacteria was clearly found only in brackish water sites (Sungsang and Tg Carat). As noted by Campbell *et al.*[28], the members of the genus *Nitrosococcus* were marine aerobic AOB that belonged to the class γ-Proteobacteria order Chromatiales, *Nitrosococcus* species that were restricted to marine environments and salt lakes. In most cases, AOB belonged to two monophyletic lineages of β-Proteobacteria including genera *Nitrosomonas* and *Nitrosospira* and γ-Proteobacteria including species *Nitrosococcus oceani* and *N. halophilus*[1,2,29].

The community of AOB in dry season was higher than in rainy season. However, there was a negative correlation of

AOB community with soil ammonia concentrations in dry season which was lower than in rainy season. Probably, it was influenced by competition with phytoplankton and other microbes for ammonia uptake and light inhibition. Christman *et al.*[7] reported the similar result that the abundance of nitrification rates of β-proteobacterial and archaeal ammonia oxidizers were driven by the interactions between competition with phytoplankton for ammonium, fluxes of ammonium from sediments and light inhibition, in which all of these factors led to nitrification being seasonally uncoupled from primary production and explain the seasonal differences in abundance.

Using 16S rRNA and *amo*A genes as a molecular marker, these study found a similar result as reported by Sahan and Muyzer[4]. Virtually, all AOB isolates grew at an optimum temperature[30,31] below 30°C. Overall, these results showed that the majority of AOB could be found in and was very likely to adapt to the various concentrations and the availability of nutrients (ammonia, nitrite and nitrate). The ammonia as the primary energy source might promote the activity of ammonia-oxidizing prokaryotes, however, high concentrations of ammonia inhibit the activity of ammonia-oxidizing prokaryotes[32]. The previous publication also reported that high concentrations of ammonia partially inhibited the density and diversity activity of ammonia-oxidizing bacteria[17,21]. In general, nutrient concentrations were higher in rainy season than in dry season. It seemed that the impact of the higher concentration was caused by the land-use that eventually ended up in the river system due to runoff.

Finally, the results from PCA indicated that the effects of salinity, temperature, dissolved oxygen and nutrients contributed to AOB community. However, salinity significantly affected on AOB community. Similar result noted by Bernhard *et al.*[33] and Sahan and Muyzer[4], that with *amo*A analysis, the salinity was considered as the only stress factor that selected a narrow range of best-adapted AOB. Salinity played a major role in ammonia adsorption in sediments and has been suggested as a key parameter regulating AOB communities[33-37]. Nevertheless, this study and others studies suggested that the AOB community is complex, more studies are needed to obtain community structures in waters environment in order to further exploration through the relationships among environment, season and bacteria from water and sediment of Musi river.

CONCLUSION

This study shows the presence of AOB in sediments and has led to a better understanding of the dynamics of the AOB diversity under the seasonal and spatial conditions. The dominant groups of AOB identified were *Nitrosomonas* and *Nitrosospira* from class β-proteobacteria. *Nitrosococcus* from class γ-proteobacteria was found only in brackish water. The variability in salinity caused the spatial distribution and temperature was the primary seasonal factor affecting the community shift of the AOB.

SIGNIFICANCE STATEMENT

This study discovers the community of AOB in the sediment of tropical freshwater and brackish and relationship with the physicochemical properties. Salinity was factor affected the community shift of the AOB. This study will help the researchers to uncover the community of AOB in the tropical freshwater and brackish water during rainy and dry seasons that many researchers were not able to explore. The study may be helpful for water quality management in the Musi river.

ACKNOWLEDGMENT

This study would not have been possible without the financial support from Indonesian Ministry of Research, Technology and Higher Education (No. 102/SP2H/LT/ DRPM/ IV/2017).

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