

Nitrification Potential Rate of Different Sediment Types of the Ariake Sea Tidal Flat in Summer and Autumn

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Abstract: The purpose of this research was to investigate the nitrification potential in different sediment types in the Ariake Sea tidal flat. The Ariake Sea, a semi-closed shallow sea with macro-tidal and a large tidal flat, is located in the west part of Kyushu Island, Japan. Different kinds of sediment develop in the different parts of its tidal flat. We analyzed and classified the sediment textures, physico-chemical properties of sediment pore water, Ammonium-Oxidizing Bacteria (AOB) density, and determined their Nitrification Potential Rate (NPR). The sediment textures from intertidal zone were classified to be silty clay loam, sandy loam and loamy sand, and sediment from a sub-tidal zone to be sandy loam for 6 cm upper most sediment, and loamy sand for 6-9 cm sediment depth. The highest NPR was found in silty clay loam sediment (12.072-59.102 nmol N/g Dry Weight Sediment (DWS)/h) followed by sandy loam (7.29-21.056 nmol N/g DWS/h), and the other sediment textures. The high AOB densities, 2.278×10^3 - 7.171×10^4 and 1.429×10^3 - 7.171×10^4 cells/g sediment, were also found in silty clay loam sediment and sandy loam sediment, respectively. Correlations between pore water $\text{NH}_4\text{-N}$ concentration and NPR were found at sediment depths of 3-6 cm ($r = 0.381$; $\alpha = 0.05$) and 6-9 cm ($r = 0.786$; $\alpha = 0.05$). In these depths, the correlation also exhibited for AOB density and NPR ($r = 0.595$, $\alpha = 0.05$ for 3-6 cm and $r = 0.679$, $\alpha = 0.05$ for 6-9 cm depths). However, these two correlations was not approved for 3 cm upper most sediment. It is the first report on nitrification potential in the different kinds of sediment of the Ariake Sea which improve our understanding of the nitrification in this area.

Key words: Ammonium oxidizing bacteria, nitrification, sediment, texture, The Ariake Sea, tidal flat

INTRODUCTION

The Ariake Sea located in the west part of Kyushu island, Japan, is a semi-closed shallow sea with macro-tidal and several well-mixed estuaries. This sea covers 1,700 km² a long the inner bay with 96 km of the bay axis and 18 km of the average width. This sea is characterized by a macro tidal range (3-6 m) which is the largest tidal range in Japan (Kato and Seguchi, 2001; Hiramatsu *et al.*, 2005; Tsutsumi, 2006). A vast tidal flat area (approximately 20,000 ha) (Tsutsumi, 2006) that covers almost 40% of the total tidal flat in Japan develops in this sea. Kato and Seguchi (2001) have reported the distribution process of around 440,000 ton sediment per annum in the Ariake Sea. The sedimentation process which is affected by oceanographic conditions allows the coarse sediment formation in the eastern parts, and mud (silt and clay) sediment formation in the western shoreline of this area. This sedimentation process establishes the

vast tidal flat formation with the different kind of sediment textures in different parts of the Ariake Sea.

The estuaries system with huge tidal flat and a macro-tidal range create a unique and high productive ecosystem in the Ariake Sea. Sea lavers (*Porphyra* spp.) production that contributes 40% of the total Japanese sea lavers production (Yanagi and Abe, 2005), is a main fisheries product of the Ariake Sea. Two inorganic nitrogen fractions, NO_3^- and NH_4^+ , are the most important nutrient for production of *Porphyra* (Hafting, 1999). These two inorganic nitrogen fractions are up taken in six *Porphyra* species at similar rates (Kraemer *et al.*, 2004). Nitrogen uptake in *P. yezoensis* is also affected by the frequency and duration of immersion and exposure (Kim *et al.*, 2008a, b), which occur naturally in the high tidal range area as found in the Ariake Sea.

Nitrification, a microbial aerobic autotrophic oxidation of ammonia, is one of the ammonia removal processes in environment and one of the prominent

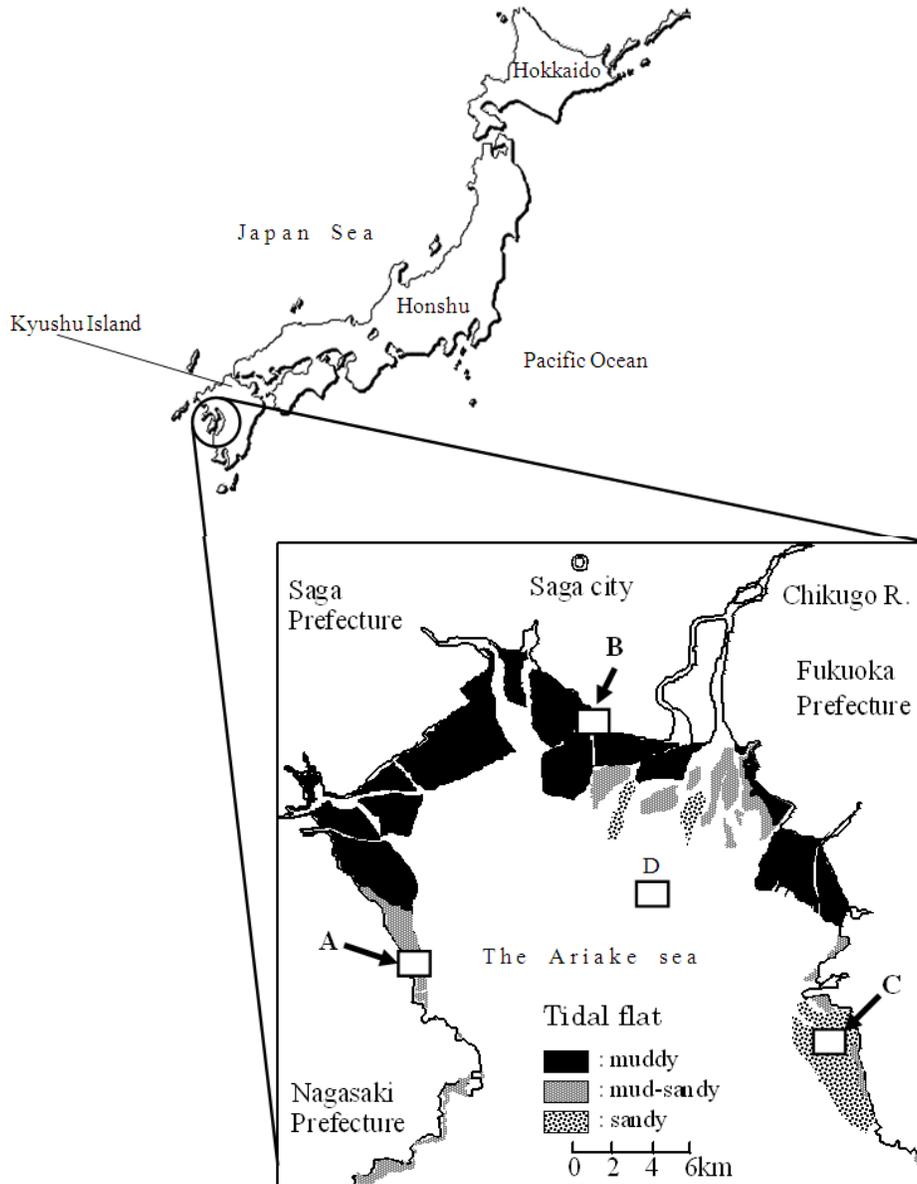


Fig.1: The Ariake sea and distribution of its sediment types (Koga *et al.*, 2009). The sampling points are indicated by open squares. A, Tara; B, Higashi Yoka; C, Nagasu; D, Saga University Observation Station

biochemical processes in the global nitrogen (N) cycle and in individual ecosystems. Galloway *et al.* (2004) have estimated that approximately 30% of global fixed-N loss occurs in the sediments of estuarine and continental-shelf. Coupled nitrification and denitrification in the estuary ecosystem also play critical removal processes of approximately 10 to 80% of anthropogenic N pollution (Seitzinger, 1988). Furthermore, approximately 6-70% of the N_2 generated from denitrification process was produced from nitrogenous oxides (nitrate and nitrite)

derived from nitrification (Nishio *et al.*, 1983). Despite the importance of nitrogen for high productivity of the Ariake Sea, nitrification as one of the biochemical processes in the nitrogen cycle has not been studied *hit herto*. As different sediment textures develop in the different parts of the Ariake Sea, we classified the sediment textures and investigated their Nitrification Potential (NP) rate. We also determined the microbiological and physico-chemical properties of the sediments.

MATERIALS AND METHODS

Sediment sampling: Sediment samples used in this study were collected in Tara (A), Higashi Yoka (B), and Nagasu (C) in the intertidal zone at low tide (Fig. 1) in June to October 2009. To compare the intertidal sediment samples, a sampling in sub-tidal zone closed to the Observation Tower Station (OTS) of Saga University (D) was conducted in summer. The sediment from B was collected by mean of an acrylic core with the inner diameter of 85 mm. Since sediment in A and C is sandy and also contains coarse sand, the sediment was collected by an acrylic core with diameter 50 mm. By this smaller core diameter, the sediments were easier to be collected. At sampling point D, sediment was collected by mean of a bottom core sampler (Rigoshia & Co. Ltd., Saitama, Japan) with 50 mm of inner core diameter. The cores were transported to the laboratory in a cool box. In the laboratory, the sediments were sectioned in three cm of depth intervals, and used for experiments.

Pore water geochemistry: The sediment sample in each depth interval was placed into a polypropylene centrifuge tube and tightly capped. After centrifugation at 4,000 g for 15 min, the supernatant was collected, filtered through 0.45 μm -pore-size cellulose ester filter (Advantec, Toyo Roshi Kaisha, Tokyo, Japan), and frozen immediately until analysis. $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_2+\text{NO}_3\text{-N}$, PO_4 , Total Nitrogen (TN) and Total Phosphate (TP) were analyzed by an automated water analyzer (Water auto-analyzer, swAAT, BLTEC, Tokyo, Japan). $\text{NH}_4\text{-N}$ concentration was determined by the method of the alkali phenol-hypochlorite reaction detected photometrically at 630 nm. $\text{NO}_2\text{-N}$ concentration was analyzed by diazotizing with sulfanilamide and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride to form a highly colored azo dye and detected photometrically at 550 nm. $\text{NO}_3\text{-N}$ was determined by the same method for $\text{NO}_2\text{-N}$ after NO_3 was reduced by the cadmium reduction process. PO_4 was determined by the ascorbic acid method at 800 nm. TN and TP concentrations were measured by peroxodisulfate oxidation (Ebina *et al.*, 1983).

Density and texture of sediments: Density of sediment in each depth interval was investigated by pycnometer. Texture of the sediments was tested by the combination of sieving and sedimentation method as described by Kettler *et al.* (2001). Around 30 g dry weight of sediment sample was placed in beaker glass, and added with 50 mL deionized water and 5 mL of 30% H_2O_2 and stirred well. The sediment mixture was incubated at 80°C for 10 min. Another 5 mL of H_2O_2 was added and the mixture was incubated at the same temperature for 10 min. After cooling down, the mixture was transferred to a bottle and added with 10 mL of 50 g/L sodium hexametaphosphate

solution. Then the bottle was shaken rigorously. The mixture was sieved through 2 mm and 75 μm to obtain coarse sand and sand fractions, respectively. The fraction that passed through 75 μm sieve was determined for silt and clay fractions by the sedimentation process at a constant temperature of 20°C. The sedimentation time was calculated by Stokes law. The textures sediments were classified based on triangle soil texture classification using the soil texture calculator (Natura Resources Conservation Service, USDA, <http://soils.usdagov/technical/aids/investigations/texture/>).

Density of Ammonium-Oxidizing Bacteria (AOB) in sediment samples: Density of ammonium-oxidizing bacteria was determined by the Most Probable Number (MPN) method in 1.5 mL sterile microtubes. The microtubes were filled with 900 μL sterile medium for ammonia-oxidizing bacteria as described by Cote and Ghena (1994). The medium was composed with $(\text{NH}_4)_2\text{SO}_4$, 1.32 g/L; KH_2PO_4 , 20 mg/L; $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 0.1 g/L; $\text{FeCl}_2\cdot 6\text{H}_2\text{O}$, 0.014 g/L; $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$, 0.18 g/L; $\text{Na}_2\text{MO}_4\cdot 2\text{H}_2\text{O}$, 100 $\mu\text{g/L}$; EDTA, 1.0 mg/L, phenol red, 0.002 g/L, dissolved in 70% artificial sea water (Tetramarin Salt Pro, USA). The medium was adjusted to pH 8 with Na_2CO_3 . One gram of mud was suspended with 9 mL sterile ASW, and 100 μL of the suspension was used to inoculate the microtube in triplicates, and serially tenfold diluted. Incubation was carried out at 25°C for 20 days. The tubes which exhibited the color change from red to yellow due to acid production were tested by adding three drops of a nitrite color reagent (sulfanilamide, 10g/L; *n*-(1-naphthyl)-ethylenediamine 2 HCl, 0.50 g/L; concentrated HCl, 100 mL/L). Tubes that exhibited a red color after addition of the reagent were scored positive for nitrite. The bacterial density was calculated by the MPN formula in Visual Basic program (Koch, 1994).

Nitrification Potential Rate (NPR): NPR is an index of the abundance and activity of nitrifying bacteria, which indicates the ability of a unit of sediment to oxidize $\text{NH}_4\text{-N}$ (when $\text{NH}_4\text{-N}$ and O_2 are not limiting) (Mayer *et al.*, 1995). NPR was determined by the method described previously (Mayer *et al.*, 1995; Bianchi *et al.*, 1997; Welsh and Castadelli, 2004; Dollhopf *et al.*, 2005). Core samples were divided into three sections, 0-3, 3-6 and 6-9 cm of sediment depth intervals. The samples were homogenized and slurried (20.0 g of wet sediment/200 mL of sterile artificial seawater) and placed into 300 mL Erlenmeyer. Duplicate flasks from each depth were amended with ammonium ($(\text{NH}_4)_2\text{SO}_4$; 500 μM) (Wako Pure Chemical Industries Ltd., Osaka, Japan) and sodium chlorate (KClO_3 ; 10 mM) (Wako Pure Chemical Industries Ltd.). Control flasks contained ammonium, sodium chlorate, and allylthiourea (ATU; 20 mg/L)

(Sigma-Aldrich, St. Louis MO, USA) (Belser and Mays, 1980; Dollhopf *et al.*, 2005). Flasks were capped with aluminum foil and incubated in the dark at 25°C with constant stirring with a magnetic stirrer at 100 rpm for 96 h. Samples were collected at intervals over the incubation time. NO₂-N was determined in interstitial waters of the slurry samples after filtering through 0.45 µm cellulose ester membrane filter (Advantec, Toyo Roshi Kaisha, Japan). Since sodium chlorate is a specific inhibitor of nitrification, which blocks the oxidation of NO₂-N to NO₃-N (Belser and Mays, 1980), in the presence of 10 mM chlorate, NPR can be determined as the linear accumulation of nitrite with time (Welsh and Castadelli, 2004; Dollhopf *et al.*, 2005). The linear regression analyses were done after the NO₂ concentrations in all treatments were corrected by subtracting its concentration in the ATU control flash.

RESULTS

The density of sediments varied among the station, but the densities were relatively similar among the different depth in the same location. Sediment density in

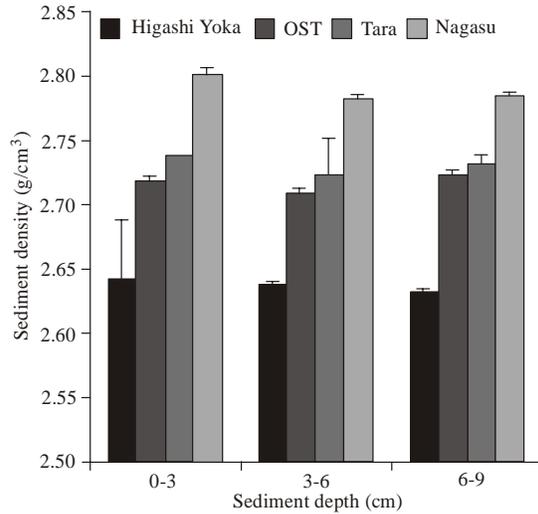


Fig. 2: Density of sediment collected from each sampling point. Sampling points A, Tara; B, Higashi Yoka; C, Nagasu; D, observation tower station (OTS). Bars indicate standard error

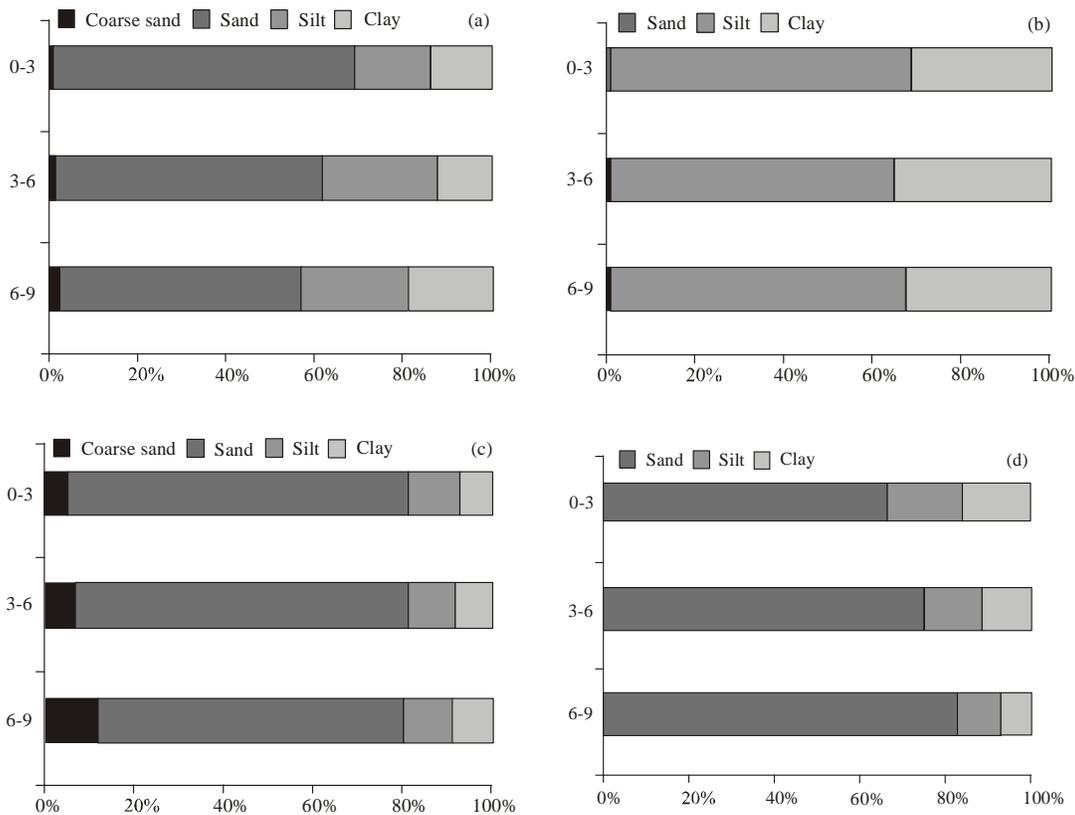


Fig. 3: Texture of sediments collected from each sampling point. Sampling points A, Tara; B, Higashi Yoka; C, Nagasu; D, Observation Tower Station (OTS)

Table 1: Physico-chemical properties of pore water sediments and density of ammonium oxidizing bacteria

Sediment depth	Concentration (mg/L)					Total P	Salinity (ppt)	AOB (cells/g)
	PO ₄	NH ₄ -N	NO ₂ -N	NO ₃ -N	Total N			
A sampling point (Tara)								
0-3 cm								
Average	89.752	2005.788	5.490	3.784	2056.633	86.446	25.333	2.94×10 ⁴
Range	15.395-133.170	618.406-3152.25	4.280-7.078	3.348-4.497	360.364-2936.123	33.815-120.966	16-34	7.27×10 ³ -7.17×10 ⁴
3-6 cm								
Average	107.761	2553.534	6.001	2.410	1534.109	80.846	26.7	1.50×10 ⁴
Range	41.805-157.296	2066.886-3070.005	3.038-9.903	1.327-4.3111	97.743-3606.068	23.198-183.951	16-34	1.43×10 ³ -2.30×10 ⁴
6-9 cm								
Average	183.715	3151.540	12.492	7.894	1872.656	228.208	28	282.13×10 ⁴
Range	114.388-313.744	3011.939-3236.417	8.1498-18.772	2.729-13.26	6151.208-4593.44	151.536-326.809	23-34	1.43×10 ³ -3.62×10 ⁴
B sampling point (Higashi Yoka)								
0-3 cm								
Average	103.699	2924.519	6.369	11.826	1882.750	205.619	18	1.93×10 ⁴
Range	54.114-153.284	1732.302-4116.736	5.834-6.903	3.367-20.285	1083.502-2681.997	127.139-284.098	16-20	2.28×10 ³ -3.63×10 ⁴
3-6 cm								
Average	404.083	5411.809	7.435	15.544	1207.412	908.971	21	5.80×10 ³
Range	332.844-475.321	3214.882-7608.736	3.714-11.157	4.615-26.472	1189.728-1225.096	304.096-1513.845	20-22	2.28×10 ³ -9.33×10 ³
6-9 cm								
Average	715.898	7436.086	4.143	2.549	1935.015	805.979	22	3.70×10 ⁴
Range	597.424-834.373	5303.052-9569.12	1.880-6.406	0.337-4.761	1935.015-1935.015	805.979-805.979	20-24	2.28×10 ³ -7.17×10 ⁴
C sampling point (Nagasu)								
0-3 cm								
Average	154.441	3166.726	5.514	6.407	1849.690	191.159	34	8.30×10 ³
Range	119.785-189.096	2740.908-3592.545	3.318-7.711	4.723-8.091	775.957-2923.423	166.88-215.437	32-36	7.27×10 ³ -9.33×10 ³
3-6 cm								
Average	242.402	3317.709	4.984	1.024	3865.663	79.326	33.5	1.25×10 ³
Range	201.610-283.193	1881.239-4754.178	3.189-6.779	0.988-1.0596	575.582-7155.743	35.714-122.937	32-35	2.31×10 ² -2.28×10 ³
6-9 cm								
Average	216.733	1243.068	18.163	2.801	2607.166	176.442	33	2.31×10 ²
D sampling point (OTS)								
0-3 cm								
Average	201.404	1404.979	3.123	1.943	ND	173.477	28	2.28×10 ³
3-6 cm								
Average	416.869	1229.333	7.263	8.021	178.378	421.060	25	2.31×10 ²
6-9 cm								
Average	499.806	1808.776	6.831	9.861	479.329	517.547	30	2.31×10 ²

ND: no data

sampling points A, B, C and D were 2.721-2.733, 2.632-2.642, 2.779-2.783 and 2.707-2.723 g/cm³, respectively (Fig. 2). The lowest sediment density was found in B as the sediment was predominated by mud fraction. In contrast, high sand fraction was found in C with the highest sediment density. These findings were in accordance with the results of texture analysis. Sediment in B contained 63.9-67.6% of silt and 31.8-35.9% of clay (Fig. 3). The remaining fraction was sand fraction. The sediment in this sampling site was classified to be silty clay loam. In contrast sediments in A and C were predominated by sand fraction, which were 54.9-68.5% in the former location and 68.7-76.4% in the later location. Silt and clay fractions in A ranged from 16.8-26.2 and 12.6-19.1%, respectively. The percentages of these two fractions in C were 10.7-11.5 and 7.8-9.0%, respectively. Sediment in A and C were classified to be sandy loam and

loamy sand, respectively. Sediment in D contained sand, silt and clay fractions in the range of 66.6-82.7, 9.9-13.5 and 7.4-15.7%, respectively. In this site, the percentage of sand fraction was lower in the deeper sediment, but the percentage of clay and silt fractions increased in the deeper sediment. Interestingly, sediments in this location could be classified to be sandy loam for the depths of 0-3 and 3-6 cm and loamy sand for sediment depth of 6-9 cm.

Pore water sediment geochemistry in A showed that PO₄, NH₄-N, NO₂-N, NO₃-N, total P and salinity exhibited higher concentration by increasing sediment depth (Table 1). However, this tendency did not find for total N and AOB density. D sediment also did not show such a tendency. In contrast, AOB density in this sediment was much higher in 0-3 cm sediment depth than the deeper sediment. In B sediment, PO₄ and NH₄-N and salinity tended to be higher by increasing the sediment depth. The

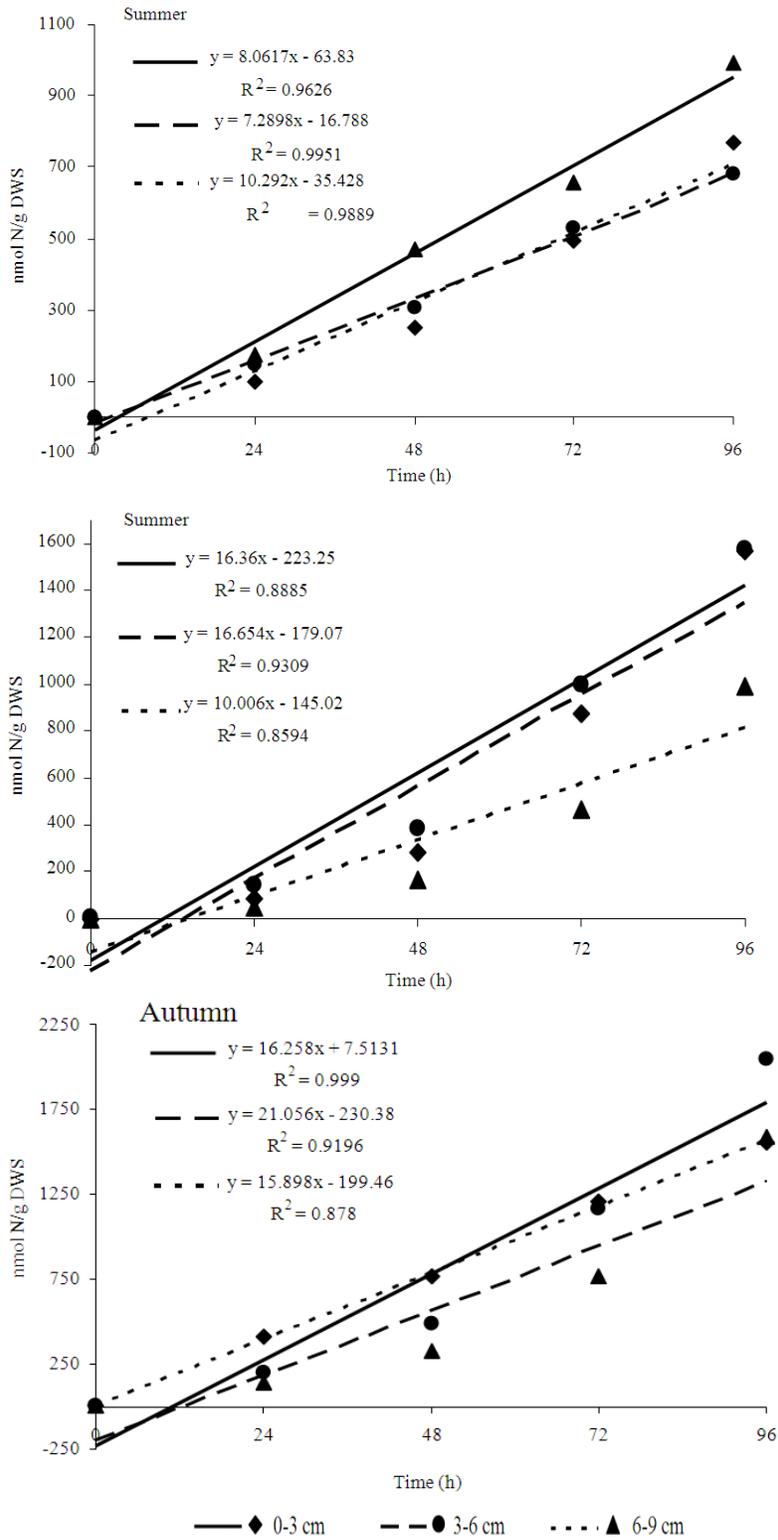


Fig. 4: NPR of sediment collected from A sampling point (Tara) in summer and autumn. Each experiment was done in duplicates

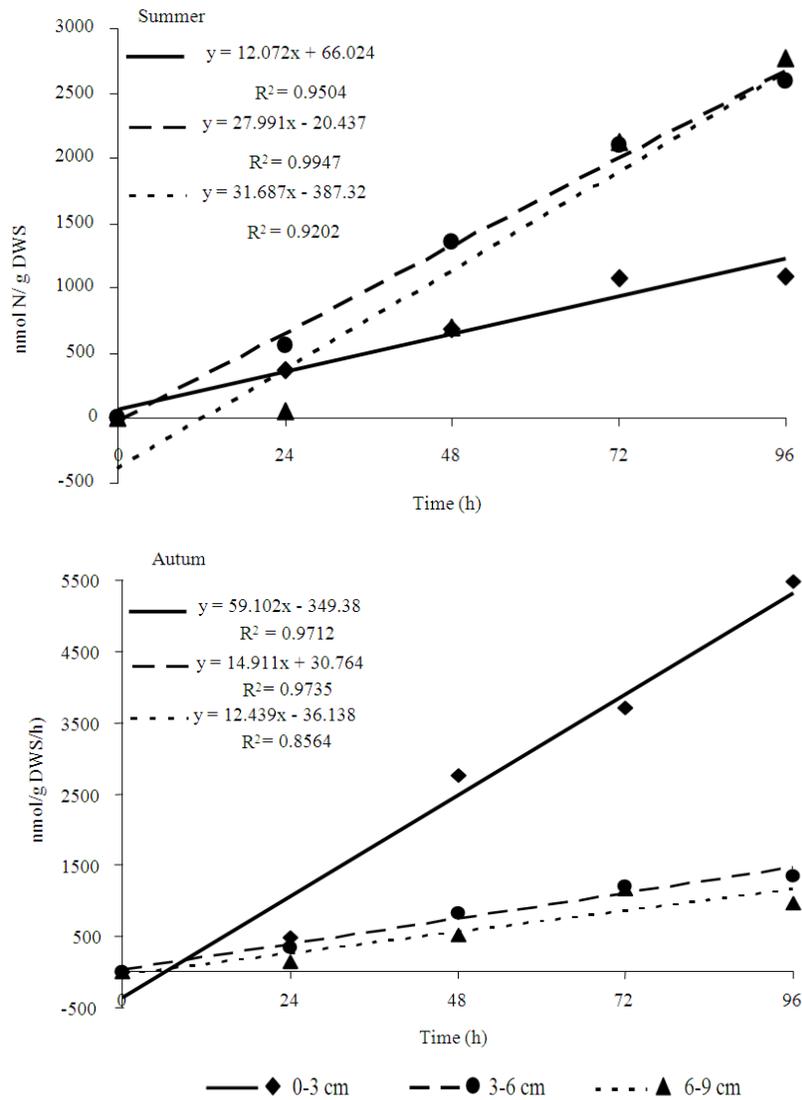


Fig. 5: NPR of sediment collected from B sampling point (Higashi Yoka) in summer and autumn. Each experiment was done in duplicates

density of AOB was much higher in the depth of 6-9 cm than in the depths of 0-3 and 3-6 cm. PO_4 , NH_4 -N, NO_2 -N and TP in A exhibited higher concentration in deeper sediment depth. NH_4 -N was predominant inorganic N in the sediment pore water collected from all sampling sites. Among the sampling point, concentration of ammonia was higher in the sediment collected from A and B than that of collected from two other sampling sites. NO_2 -N concentration exhibit relatively similar concentration in the sediment collected from these four sampling sites. However, concentration of NO_3 -N in sediment collected from C was much lower than that of collected from other three sampling sites. Sediments collected from A and B showed high AOB density in the range of 1.43×10^3 -

7.17×10^4 and 2.28×10^3 - 7.17×10^4 cells/g wet sediment, respectively. In contrast, the densities were much lower in sediment collected from C ranging from 2.31×10^2 to 9.33×10^3 cells/g wet sediment, and in sediment collected from D ranging from 2.31×10^2 to 2.31×10^3 cells/g wet sediment (Table 1).

In this study, nitrification was determined by measuring the accumulation of NO_2 -N after addition of sodium chlorate, an oxidation inhibitor of NO_2 -N to NO_3 -N. NPR measures the nitrification rate without limitation of ammonium and oxygen by amending ammonium sulfate and stirring during incubation. The accumulation of NO_2 -N production showed high correlations with incubation time showing coefficient correlations ranging

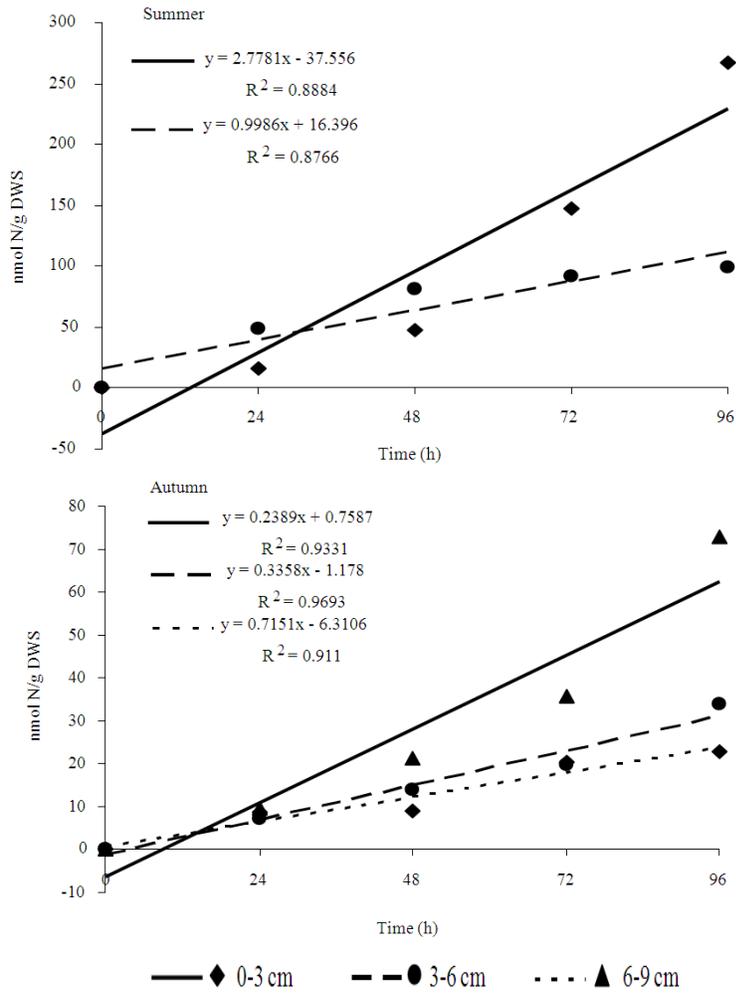


Fig. 6: NPR of sediment collected from C sampling point (Nagasu) in summer and autumn. Each experiment was done in duplicates

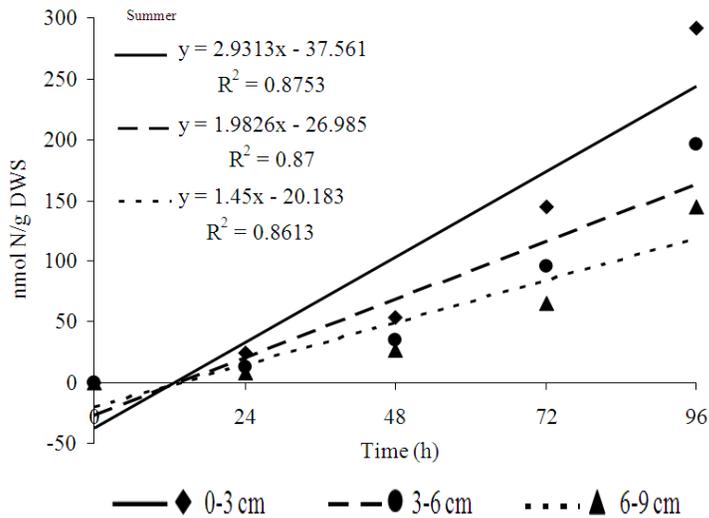


Fig. 7: NPR of sediment collected from D sampling point (OTS) in summer. Each experiment was done in duplicates

from 0.8594-0.999, 0.8564-0.9947, 0.8766-0.9693 and 0.87-0.8753 in sampling points A, B, C and D, respectively (Fig. 4-7). During 96 h incubation, the accumulation of $\text{NO}_2\text{-N}$ productions were in the range of 683.3706-2045.073, 976.7398-5484.424, 22.88425-267.4673, 145.2025-291.977 nmol N/g Dry Weight Sediment (DWS) for sediments from A, B, C and D, respectively.

PNRs varied significantly among four different sediments in the range of 7.29-21.056 nmol N/g DWS/h in A (Fig. 4), 12.072-59.102 nmol N/g DWS/h in B (Fig. 5), 0.239-2.778 nmol N/g DWS/h in C (Fig. 6) and 1.45-2.931 nmol N/g DWS/h in D (Fig. 7). The results indicated that high NPRs were found in B and A. In contrast, NPRs in two other locations were very low. The effect of sediment depth on NPR was not clearly exhibited in all sampling sites. A sediment exhibited relatively similar NPRs among sediment depth in the range of 8.0617-16.36, 7.2898-21.056 and 10.006-15.898 nmol N/g DWS/h for 0-3, 3-6 and 6-9 cm of sediment depths respectively. The higher NPR was found in deeper sediment in D and in B in summer 2009, but the contradiction pattern was observed when the sample was taken in the autumn 2009 in B. NPRs in this sampling site at 0-3, 3-6 and 6-9 cm ranged from 12.072 to 59.102, 14.911 to 27.991 and 12.439 to 31.687 nmol N/g DWS/h, respectively. A specific relationship between sediment depth and NPR also was not indicated by sediment collected in C. In this sampling site, NPRs was low in the range of 0.2389- 2.7781, 0.3358- 0.9986 and 0.7151 nmol N/g DWS/h at 0-3, 3-6 and 6-9 cm of sediment depths, respectively.

DISCUSSION

Nitrification, a microbial aerobic autotrophic oxidation of ammonia, is one of the ammonia removal processes in environment. Recently, however, anaerobic nitrification has been found as a new mechanism of nitrification (Mortimer *et al.*, 2004; Bartlett *et al.*, 2008). Other ammonia removal processes are anaerobic ammonia oxidation to dinitrogen gas (Mulder *et al.*, 1995; Bock *et al.*, 1995; Schmidt *et al.*, 2002; Sabumon, 2007; Engstrom *et al.*, 2005; Thamdrup and Dalsgaard, 2002) and assimilation (Lam *et al.*, 2008) into organic matters. Although both heterotrophic and autotrophic/chemolithotrophic ammonium-oxidizing bacteria oxidize the substrate in aerobic environment, the former one has much oxidation rate than the later one (Focht and Verstraete, 1977). Therefore, in this study we focused on potential nitrification by chemolithotrophic ammonium-oxidizing bacteria in aerobic condition.

Nitrification in different kind of sea sediment textures is little known, and has not been studied in the Ariake Sea sediment *hit herto*. Jensen *et al.* (1996) and Henriksen

et al. (1981) have investigated nitrification in different types of sea sediment, but have not described texture of the sediment. Laima *et al.* (1999) have reported nitrification potential in mudflats with ridge and runnel structures, but also have not indicated the texture of the sediment. In this study, we firstly investigated and classified the texture of the Ariake Sea sediment, analyzed the sediment geochemistry and microbiological properties, and evaluated NPR of the sediments.

Koga *et al.* (2009) have reported that three types of sediment, muddy, mud-sandy and sandy sediments, are found in the tidal flats of the interior parts of the Ariake Sea (Fig. 1). By analyzing the density and texture, we reclassified the muddy, mud-sandy and sandy sediments from the intertidal zone to be silty clay loam, sandy loam and loamy sand, respectively (Fig. 3). Interestingly, the sediments in a sub-tidal zone, sampling point D, have different textures in different sediment depth. In this location, 6 cm upper most sediment was sandy loam sediment and 6-9 cm depth sediment was loamy sand. The distribution of sediment with different texture in the Ariake Sea sediment is affected by the oceanographic condition, especially current and wave. Kato and Seguchi (2001) have estimated that the sediment transported by several rivers to this sea is around 440,000 ton per annum. The coarse sediment settles in the eastern parts of the bay, but the fine sediment develops the Ariake clay formation and mud tidal flat along the western shoreline of this area.

Concentration of inorganic nitrogen fractions in pore water of four sediment types did not exhibit significant variation. In these sediments, $\text{NH}_4\text{-N}$ dominated the inorganic nitrogen fractions. The highest $\text{NH}_4\text{-N}$ was found in B (silty clay loam sediment). This tendency is in accordance with the ammonium concentration reported by Koga *et al.* (2009) and Koriyama *et al.* (2009). These authors have reported that $\text{NH}_4\text{-N}$ in pore water of the Ariake Sea sediment is relatively high in summer and drastically decreases in winter. The depletion of $\text{NH}_4\text{-N}$ concentration in winter may be caused by low activity of ammonification at a low temperature and high uptake of this inorganic N by *Phycohyra* spp. which is cultured intensively in winter.

Nitrification potential can be used to estimate actual *in situ* nitrification when the temperature and depth of oxygen penetration are known (Henriksen *et al.*, 1981). The authors verified that estimation of actual nitrification rate using nitrification potential is a reliable method. As nitrification in the Ariake Sea sediment has not been studied yet, therefore, we firstly estimated its NPR. Generally, nitrification potential in silty clay loam sediment was higher than that of the other three types sediment, with an exception at 0-3 cm sediment depth in summer where the highest NPR was found in sandy loam sediment collected from A. In autumn, NPR in the depth of 0-3 cm was found to be highest in silty clay loam

sediment, but in deeper sediment, the highest rate was found in sandy loam sediment. This low NPR in the deeper sediment of silty clay may be caused by the decrease of bioturbation activity which makes the decrease of oxygen penetration to the deeper sediment. In summer, high population and activity of burrowing organisms such as mud skipper (Japanese: *mutugoro*) (*Boleophthalmus pectinirostris*), worm and several species of crabs make a significant bioturbation in the silty clay loam sediment (Isnansetyo *et al.*, 2011). Such organisms are not found in the other sediment textures.

Although sediment in D at 0-6 depth has the same sediment texture with A, the NPR in D was far lower than that of in A. This result indicated that other factors rather than sediment texture also affected NPR significantly. D sediment was taken from sub-tidal zone, but three other sediments were taken from intertidal zone. The comparison of our NPR data in specific sediment texture to published NPR is somewhat difficult as marine sediment texture is specific in each location affected by sediment inflow and oceanographic condition. Caffrey *et al.* (2003) have found that potential nitrification varies spatially and temporary in mud sediment in Elkhorn Slough, a small estuary in central California with the highest rate around 550 nmol NO₂/cm³/h. Nitrification in the sediment (mixture of fine sand and mud) collected from Tama Estuary, Japan increases from 246 nmol N/m²/h in February to 716 nmol N/m²/h in May (Usui *et al.*, 2001).

Publication on the comparison of nitrification rate in different marine sediment textures is limited. Jensen *et al.* (1996) have studied three types of sediment, coarse sand, fine sand and muddy sand in intertidal Konigshafen, Island of Sylt, Germany. The authors have found different seasonal pattern in the three sediments. The annual mean nitrification is higher in the muddy sediment than in the two other sediments. In contrast, Henriksen *et al.* (1981) have not found any different nitrification rate in sandy and muddy sediments. In our study, silty clay loam sediment generally had higher NPR than sandy loam and loamy sand. The nitrification potential of sediment is also affected by hydrologic structure as reported by Laima *et al.* (1999) who have determined that nitrification potential in intertidal mudflat with ridge structure is higher than that of runnel structure. Miranda *et al.* (2008) have estimated NPR in Kochi backwaters sediment to be 40 nmol N/g wet weight/h, but have not determined the texture of the sediment.

Mayer *et al.* (1995) and Miranda *et al.* (2008) have reported that *in situ* NH₄-N concentration correlates positively to NPR. However, we found that NH₄-N concentration was not likely to be a key factor for the difference of NPR in difference type of sediment as this average concentration in pore water was relative high

even in C and D which exhibited low NPR. Spearman rank correlation analysis indicated a low correlation between *in situ* pore water NH₄-N concentration and NPR ($r = 0.208$, $\alpha = 0.05$). Low correlations also were found when the analysis conducted for each sampling site. However, by grouping the data in different depths, relatively high correlations were found in the sediment depths of 3-6 cm ($r = 0.381$; $\alpha = 0.05$) and 6-9 cm ($r = 0.786$; $\alpha = 0.05$). This result reveals that nitrification in the upper most layer of the Ariake Sea sediment are significantly affected by other environmental conditions rather than pore water NH₄-N concentration. Caffrey *et al.* (2003) have concluded that NPRs are positively correlation with pore water NH₄-N concentration, but are negligible in the presence of high NH₄-N under low oxygen availability and/or hypersaline conditions. Insignificant effect of ammonium concentration on nitrification has also been reported by Miranda *et al.* (2008) in Kochi estuary during a non-monsoon season. The finding in this study reveals that high NH₄-N concentrations under low AOB density do not improve potential nitrification, implying the AOB density is a more dominant factor affecting nitrification than *in situ* pore water NH₄-N concentration.

Nitrification is microbiological oxidation of ammonia to nitrite and subsequently to nitrate, that involves Ammonium-Oxidizing Bacteria (AOB) and Nitrite-Oxidizing Bacteria (NOB). Ammonia oxidation is often the rate-limiting step of nitrification in a wide variety of environments, and therefore, critical to nitrogen removal and global N cycling (Kowalchuk and Stephen, 2001; Choi and Hu, 2008). Low NPR in C and D might be caused by the low of AOB, in the range of 2.3×10^2 - 9.3×10^3 and 2.3×10^2 - 2.3×10^3 cells/g sediment, respectively. In A and B that exhibited high NPR, higher AOB densities were found ranging from 2.3×10^3 to 7.2×10^4 and 1.4×10^3 to 7.2×10^4 cells/g sediment, respectively. By including all data, Spearman rank correlation analysis indicated fairly correlation between AOB density and NPR ($r = 0.383$, $\alpha = 0.05$). However, by classifying the data in different sediment depths, higher correlations were found in the depth of 3-6 cm ($r = 0.595$, $\alpha = 0.05$) and 6-9 cm ($r = 0.679$, $\alpha = 0.05$). This result reveals that the density of AOB and variation of pore water ammonium concentration are likely to be predominant factors in nitrification rate.

By using the MPN method, our results indicated that AOB densities in the Ariake Sea sediments were relatively lower than the density determined by ammonia monooxygenase (*amoA*) gene copy numbers in salt marsh sediments, which ranges from 5.6×10^4 to 1.3×10^6 /g of wet sediment (Dollhopf *et al.*, 2005). The limiting detection of MPN method may cause the fair correlation between AOB density and NPR. Therefore, AOB density in the Ariake sea should be confirmed by molecular methods as

culture-dependent methods usually give the density at several orders of magnitude lower than that of detected by quantitative PCR (qPCR) (Hoefel *et al.*, 2005) or other molecular techniques. In addition, the diversity of AOB in the Ariake sea has not been studied yet *hit herto*. Although O'Mullan and Ward (2005) have concluded that the diversity of AOB does not relate to nitrification rate, the diversity of AOB in this sea with a unique ecological condition and several types of sediment is necessary to be investigated in the next study. Fair correlation between AOB and NPR may be also caused by the involvement of either AOB or Ammonia-Oxidizing Archaea (AOA) in nitrification process in the Ariake Sea. AOA recently have been found in marine systems (Francis *et al.*, 2005; Beman and Francis, 2006).

In this study, we detected AOB in 0-9 cm depth of all sediment types implying AOB are able to tolerate and actively grow under a very low oxygen condition (Caffrey *et al.*, 2003). Although AOB oxidizes ammonium in oxic condition, these bacteria have been found in anoxic layer of marine sediment (Hansen *et al.*, 1981; Henriksen *et al.*, 1981; Freitag and Prosser, 2003; Kim *et al.*, 2006; Molina *et al.*, 2007). Recently, besides AOB, Ammonia-Oxidizing Archaea (AOA) has been discovered either in marine water and sediment by molecular techniques (Francis *et al.*, 2005; Beman and Francis, 2006). Caffrey *et al.* (2007) have found that potential nitrifications in estuarine sediments with differing histories of hypoxia do not correlate to abundance of AOB, but correlate positively to abundance of Ammonia-Oxidizing Archaea (AOA). The similar results have been reported by Lam *et al.* (2008) in the Endeavour hydrothermal plume. These reports suggest that AOA may play a significant role in nitrogen cycling especially in ammonia oxidation. Therefore, the diversity and density of AOA in the Ariake sea sediment are also necessary to be investigated in the next study for more understanding of the nitrification process.

In summary, we classify the Ariake sea sediments to be silty clay loam, sandy loam and loamy sand. High NPRs were found in the silty clay loam and sandy loam sediments collected from intertidal zone. However, sandy loam sediment collected from sub-tidal sediment has low NPR comparable to that of loamy sand collected from intertidal zone. NPRs positively correlate to density of AOB in 3-9 cm sediment depth, but the tendency is not found in 3 cm upper most layer of sediment. These correlation patterns are also found for *in situ* pore water NH₄-N concentrations and NPRs, which have higher correlation in the deeper sediment. This report represents the first study on nitrification potential of the different kind of textures of the Ariake Sea sediment, and on its textures analysis and classification. Results of this study significantly improve the understanding of nitrogen biogeochemical process especially nitrification, which has been little studied in this area.

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