Vietnam Journal of Marine Science and Technology; Vol. 20, No. 4; 2020: 437–445
DOI: https://doi.org/10.15625/1859-3097/15787
http://www.vjs.ac.vn/index.php/jmst

Density and nitrifying potential of indigenous bacterial community in mangrove and seagrass in the north of Vietnam

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Received: 23 June 2020; Accepted: 19 August 2020

Abstract
Density and nitrification potential of indigenous microorganism in mangroves (Tien Yen - Quang Ninh and Bang La - Hai Phong) and seagrass (Ha Coi, Dam Ha - Quang Ninh and Tam Giang - Thua Thien Hue) in the north of Vietnam were evaluated through 4 sampling times in the dry and rainy seasons in the years of 2017-2019. The analytical results showed that the average density of nitrifying bacteria was $4.6 \pm 1.8 \times 10^2$ MPN/ml, in which the density in mangroves tended to be higher than that in the seagrass beds ($P < 0.05$) in both the rainy and dry seasons. The average nitrifying rate was 2.7 ± 0.6 µgN/g wet soil/hour, in which the rate in mangroves tended to be higher than that in seagrass beds in the rainy season ($P < 0.05$). Nitrifying density and rate are not only correlated with substrate concentration but also with other environmental factors such as $P\text{-}PO_4$, BOD_5, total phosphate in sediment and total bacterial density.

Keywords: Nitrifying bacteria, density, speed, mangrove forest, seagrass beds, north of Vietnam.

Citation: Le Thanh Huyen, Dao Thi Anh Tuyet, Le Minh Hiep, Nguyen Tien Dat, Ha Thi Binh, Do Trung Sy, Do Manh Hao, 2020. Density and nitrifying potential of indigenous bacterial community in mangrove and seagrass in the north of Vietnam. *Vietnam Journal of Marine Science and Technology*, *20*(4), 437–445.

INTRODUCTION

The concentration of inorganic nitrogen substances in estuary and coastal areas has been increasing in recent years due to increasing discharge of wastewater from agriculture, aquaculture and residential areas from the domestic activities [1]. High concentrations of the pollutants lead to eutrophication in many estuarine areas and can even be directly toxic to organisms [2]. High concentrations of ammonium and nitrite can disrupt metabolic processes and inhibit oxygen transport in the body, thus greatly affecting the survival, growth and resistance of the organism [3, 4].

Nitrification is a two-stage oxidization process from ammonium to nitrate and plays an important role in the nitrogen cycle and natural ecosystems. In the first stage, ammonium is oxidized to nitrite by ammonium oxidizing bacteria, mainly bacteria species belonging to the genus Nitrosomonas. In the second stage, nitrite is oxidized to nitrate by nitrite oxidizing bacteria, mainly bacteria group belonging to the genus Nitrobacter [5]. Through nitrification, inorganic nitrogen contaminants are partially removed (self-cleaning process). Although there have been some studies evaluating the nitrification potential in some marine areas such as Kim et al., (1997) in Hiroshima Bay, Japan [5], Magalhães et al., (2005) in the Douro estuary, Portugal [2], but there are not any studies on evaluating the nitrifying potential in the mangrove and seagrass ecosystems. Therefore, the study on evaluating the density, self-cleaning potential of ammonium and nitrite pollutants and evaluating the effects of environmental factors on the density and self-cleaning potential of the indigenous microorganism in coastal areas in general, in mangroves and seagrass in particular has high scientific and practical significance. The research results will be the scientific basis for proposing solutions to improve self-cleaning capacity for coastal ecosystems.

MATERIALS AND METHODS

Site and sampling time

Samples were collected at five areas representing the mangrove ecosystems (Tien Yen - Quang Ninh and Bang La - Hai Phong) and seagrass ecosystems (Ha Coi, Dam Ha - Quang Ninh and Tam Giang - Thua Thien-Hue) along the northern coast of Vietnam (fig. 1). At each area, the samples were collected at 9 points on 3 different transects (3 points/transect) in 4 sampling times over a period of 3 years (2017–2019) in dry season (April) and rainy season (August).

Sampling methods

Bottom layer water samples were collected in Bathomet and stored in 1 liter sterilized glass bottles. Sediment samples were collected by a Van Veen grab sampler, then a sterile stainless steel spoon was used to collect surface layer of sediment into a nylon bag. The collected samples were stored in cold conditions (4^{o}C) and transferred to the laboratory for further treatment.

Environmental variables

Water sample

Concentration of total ammonium nitrogen (TAN) was measured by the modified indophenol method, NO_2^- + NO_3^- was measured by the cadmium reduction method. PO_4^{3-} was measured by ascorbic acid method. Spectrophotometer AP1101 (Apel, Japan) was used to read results [6].

Biological oxygen demand (BOD_5) was analyzed by the iodometric titration method according to TCVN 6001-2:2008 [7]. Chemical oxygen demand (COD) was analyzed by oxidation method with $K_2Cr_2O_7$ in acidic environment according to TCVN 6491-1999 [8].

Total bacteria density was determined by colony count method, the sample was cultured on heterotrophic medium at 37^{o}C for 24 hours [9].

Sediment sample

Total nitrogen (Nts) was analyzed according to TCVN 6643:2000 [10], total phosphorus (Pts) was analyzed by colorimetric method according to TCVN 8940:2011 [11].

Nitrifying bacteria

Density of nitrifying bacteria was analyzed by most possible number (MPN). Medium tubes containing diluted samples at different concentrations are grown on 120 rpm shaker at 30^{o}C. The presence of nitrifying bacteria was

checked with diphenilamine dye [9]. Bacterial density was calculated using Mac Crady statistics table [12].

Nitrifying potential

Nitrification rate was analyzed by the acetylene inhibition method as described by Kim et al., (1997) [5]. Each water sample is filtered through a 0.2 µm membrane and divided into 150 ml flask each. To prepare for the nitrification rate analysis experiment, NH_4Cl solution was added to the flasks to give the final concentration the increase by 0.1 mgN/l and 1.0 mgN/l from the original concentration of samples [2, 5].

0.3 g of wet soil was weighed into each 50 ml peni vials, a volumetric tube was used to measure 30 ml of the pre-made incubation water into the peni vials, which were covered with a rubber stopper and aluminum mount. Each experiment was repeated 3 times [2, 5].

The culture flask (prepared above) was divided into 2 groups: the positive group was supplemented with 20% acetylene gas (according to the volume ratio v/v), the negative group did not add acetylene gas. The experimental vials were grown on a shaker at 120 rpm at 30^oC for 4 hours. Nitrification rate is determined by the difference in TAN content between positive and negative groups. TAN concentration was analyzed by modified indophenol method as described by Aminot et al., (1996) [13].

Data analysis

Changes in environmental and microbiological indicators were spatially and temporally evaluated by T-test method. Correlation between studied factors was evaluated by correlation coefficient (Pearson, R) on Microsoft Excel 2010 software.

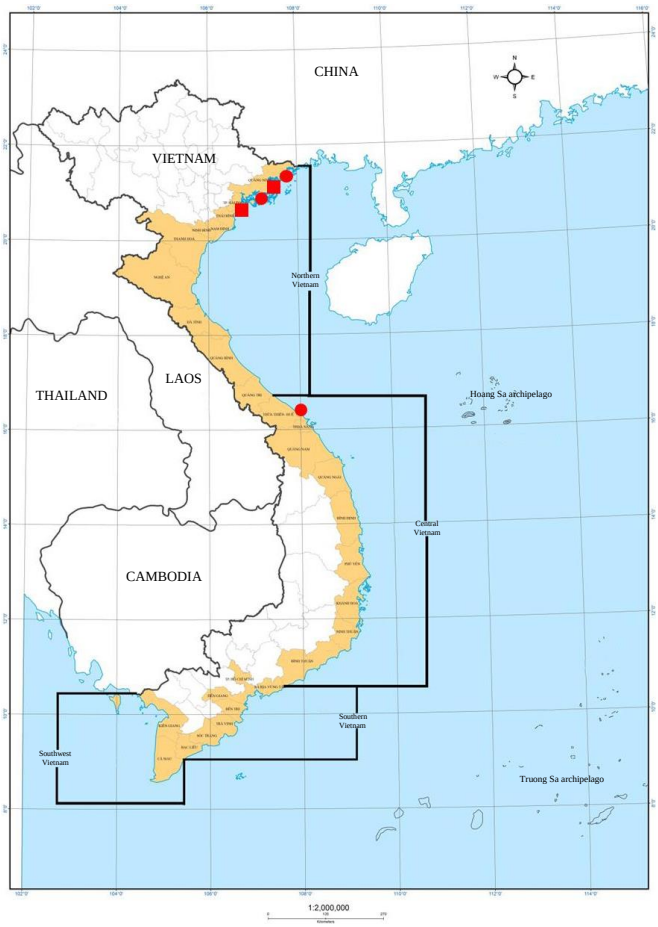

Figure 1. Diagram of the study sites, ■ mangrove forest, ● seagrass

RESULTS

Environmental quality of water

TAN ranged from 0–110 µgN/l, averaging 45.1 ± 28.9 µgN/l (fig. 2a). There was not significant difference between rainy and dry seasons and between mangrove and seagrass beds ($P < 0.05$).

Nitrite ranges from 0–30 µgN/l, with an average of 7.8 ± 8.2 µgN/l (fig. 2b). N-NO^{2-} has no seasonal variation, but there is variation according to the ecosystems, the concentration of N-NO^{2-} in the seagrass is higher than that in mangrove in both rainy and dry seasons ($P < 0.05$).

Nitrates ranged from 1.0 to 80.0 µgN/l, averaging 11.3 ± 8.2 µgN/l (fig. 2c). NO^{3-} varies by season and ecosystem, except in the rainy season, the NO^{3-} content between mangroves and seagrass does not differ significantly ($P < 0.05$). Seasonally, the concentration of NO^{3-} in the dry season is higher than that in the rainy season in both mangroves and seagrass. According to the ecosystem, the concentration of NO^{3-} in the seagrass is higher than that in the mangroves in the dry season.

Phosphates ranged from 10–50 µgP/l, averaging 24.8 ± 9.9 µgP/l (figure 2d). The P-PO_4^{3-} content fluctuated according to season and ecosystem ($P < 0.05$). Seasonally, the concentration of P-PO_4^{3-} in the rainy season was higher than that in the dry season. Meanwhile, according to the ecosystem, the P-PO_4^{3-} concentration in mangrove is higher than that in seagrass.

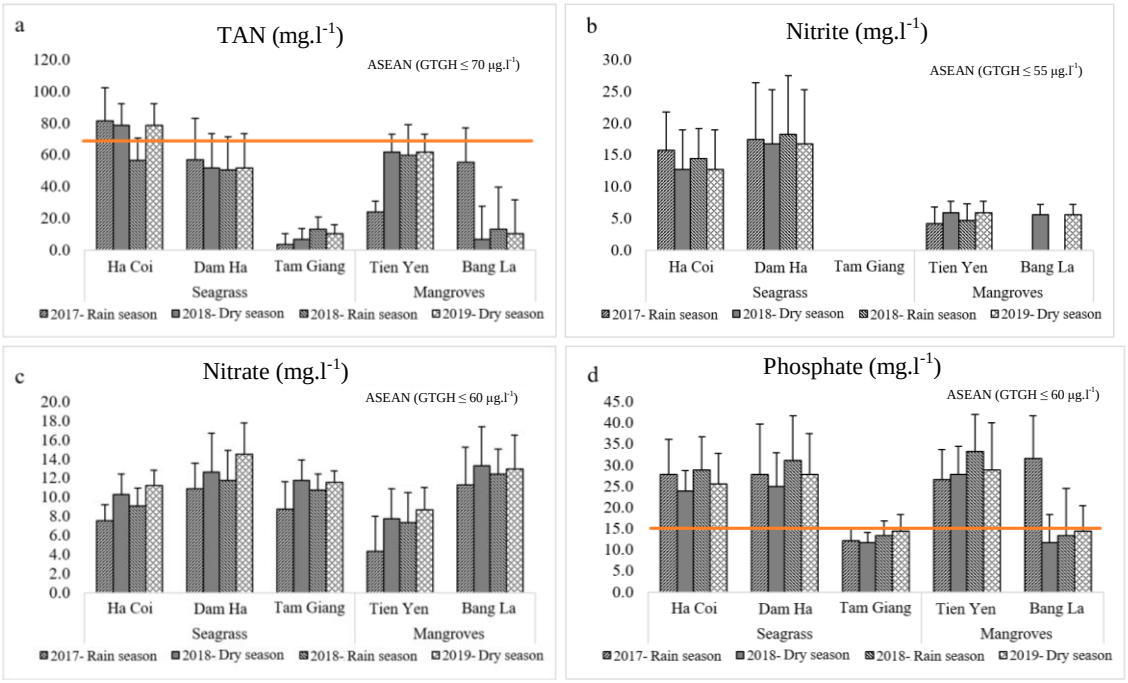

Figure 2. Water quality: (a): TAN, (b): Nitrite, (c): Nitrate, (d): Phosphate

BOD_5 ranged from 0.8 mg/l to 2.6 mg/l, with an average of 1.6 ± 0.4 mg/l (fig. 3a). BOD_5 in mangrove in rainy season is higher than that in dry season. In dry season, BOD_5 in mangrove is higher than that in seagrass ($P < 0.05$).

COD ranged from 0.3 mg/l to 7.2 mg/l, averaging 2.9 ± 1.3 mg/l (fig. 3b). COD fluctuated according to seasons and ecosystems, except in the dry season between mangrove and seagrass ($P < 0.05$). Seasonally, COD in rainy season in seagrass is higher than in mangrove. According to the ecosystem, the COD concentration in mangroves in the dry season is higher than that in the rainy season, but in seagrass, the COD in the rainy season is higher than that in the dry season.

Assessment of water quality according to the standard values specified in the standards for the purpose of aquaculture and aquatic conservation (figs. 2, 3) shows that the values of BOD_5 COD, nitrite, nitrate in all monitoring stations satisfy the standard values while TAN is higher than the standard limit values in Ha Coi seagrass and the average phosphate at most monitoring points is above the standard limit values. However, according to QCVN 10-MT:2015/BTNMT, TAN and phosphate are both lower than the standard limit values (100 µgN/l for TAN and 200 µgP/l for phosphate).

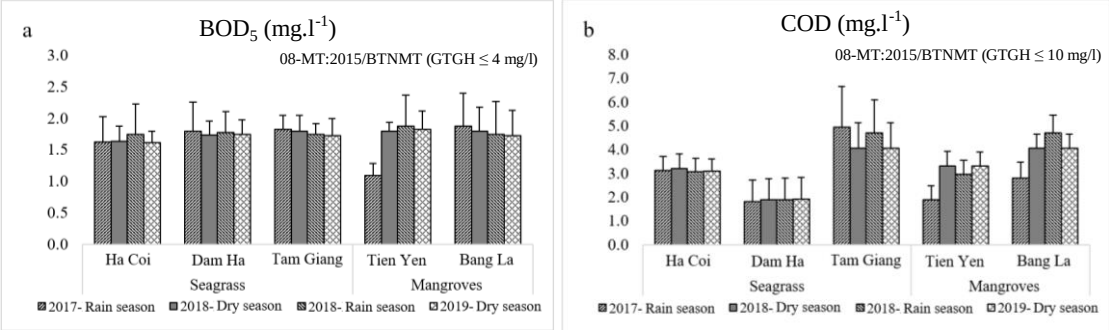

Figure 3. Environmental quality of water: (a): BOD_5, (b): COD

Total bacteria density

The total bacteria density ranged from 6.0 × 10^4 CFU/ml to 8.0 × 10^6 CFU/ml, averaging 1.4 ± 0.9 × 10^6 CFU/ml (fig. 4). In which, the average density of the total bacteria group in the mangrove is 1.9 ± 1.1 × 10^6 CFU/ml, the average density of the total bacteria in the seagrass is 1.0 ± 0.7 × 10^6 CFU/ml.

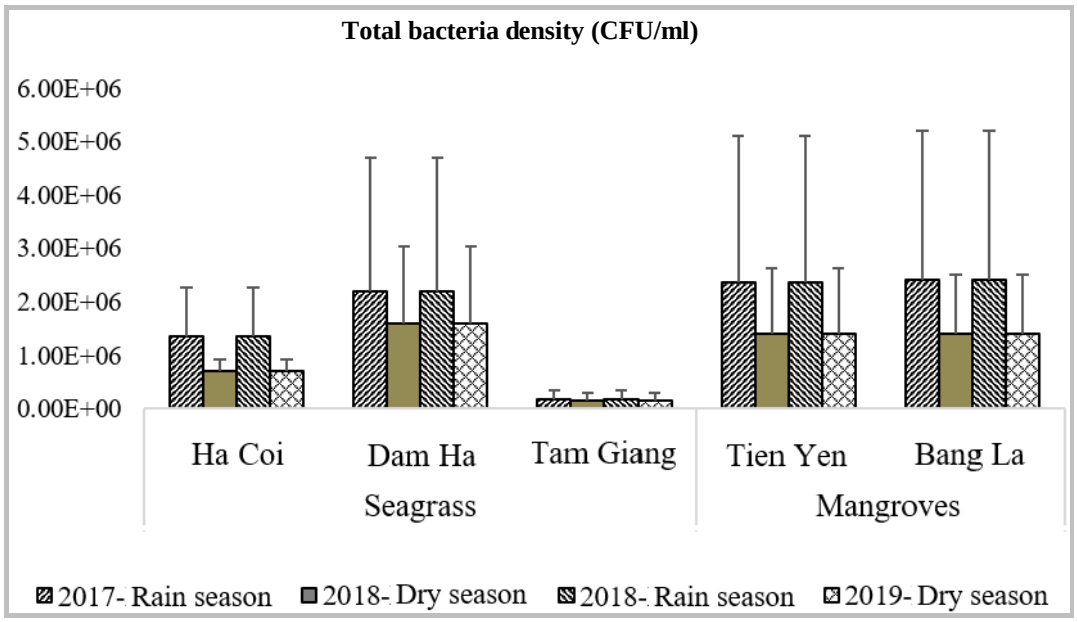

Figure 4. Total bacteria density

Over time, the total density of bacteria in the rainy season was higher than that in the dry season in both ecosystems ($P < 0.05$). According to the space, the total density of bacteria in the mangrove is higher than that in seagrass in both seasons ($P < 0.05$).

Environmental quality of sediment

Nts ranged from 0.4–1.8 g/dry soil kg, averaging 1.1 ± 0.4 g/dry soil kg (fig. 5a). Nts in mangrove has no difference between rainy season and dry season; but in seagrass Nts in dry season is greater than that in rainy season. According to the ecosystem, Nts in dry season in seagrass is higher than that in mangrove, meanwhile there is no difference between seagrass and mangrove in rainy season.

Pts ranged from 0.1–0.5 g/dry soil kg, averaging 0.25 ± 0.1 g/dry soil kg (fig. 5b). Pts value was not different between rainy and dry seasons and between two ecosystems ($P < 0.05$).

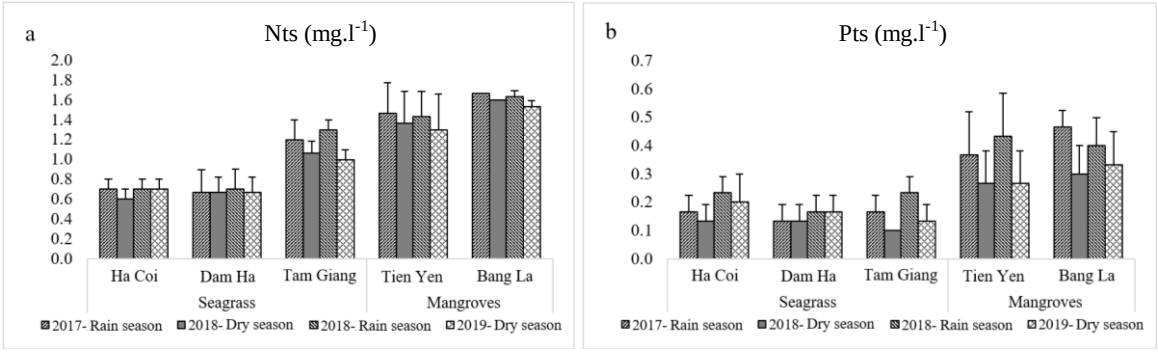

Figure 5. Environment quality of sediment. (a): Nts, (b): Pts

Nitrifying bacteria density

The density of nitrifying bacteria ranged from 0–1.5 × 10^3 MPN/ml, the average was 4.6 ± 1.8 × 10^2 MPN/ml (fig. 6). In which, the average density of nitrifying bacteria in mangroves is 5.5 ± 1.8 × 10^2 MPN/ml and in seagrass is 3.7 ± 1.9 × 10^2 MPN/ml.

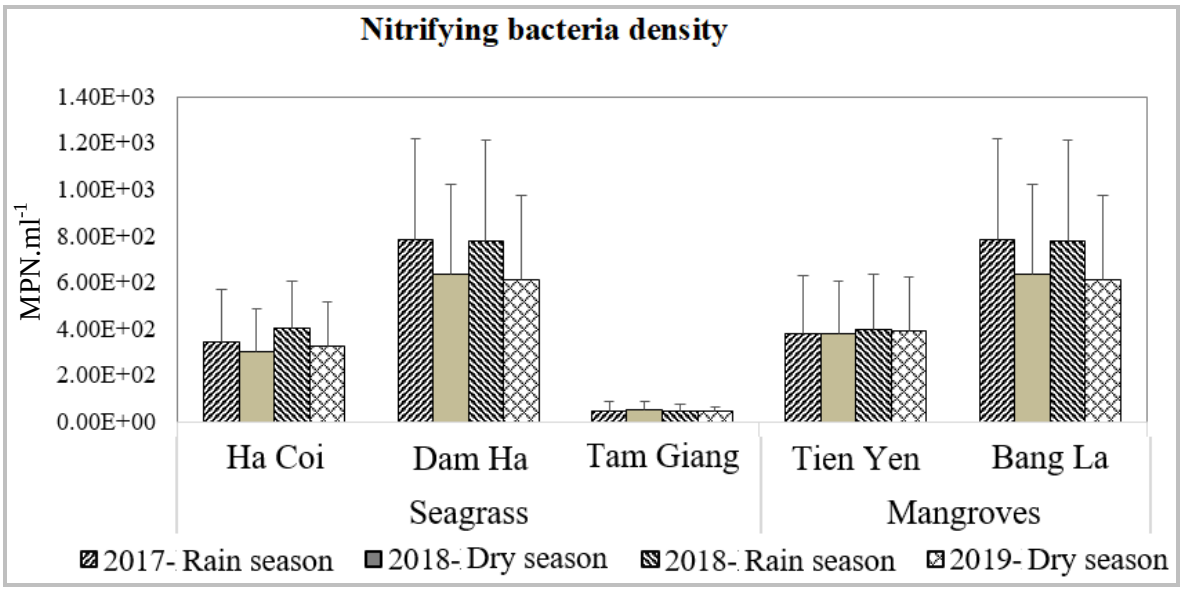

Figure 6. Density of nitrifying bacteria

According to time, the average density of nitrifying bacteria in seagrass in rainy seasons is higher than in dry season, but there is not significant difference in mangrove between dry and rainy seasons ($P < 0.05$). According to space, the average density of nitrifying bacteria in mangrove is higher than that in seagrass in both seasons ($P < 0.05$).

Nitrifying potential

Nitrification rates in the areas ranged from 0.6 µgN/g to 9.9 µgN/g wet soil/hour. Nitrification rate reached the lowest value in Tam Giang area and the highest value in Bang La area. The average rate during the study period was 2.7 ± 0.6 µgN/g wet soil/hour. In which, the average rate in the rainy season is 2.7 ± 0.7 µgN/g wet soil/hour, while that in the dry season is 2.5 ± 0.5 µgN/g wet soil/hour.

Over time, the average rate of nitrification between the rainy and dry seasons is not significantly different ($P < 0.05$) in both the mangroves and the seagrass. According to space, the average nitrification rate in mangrove is higher than that in the seagrass in the rainy season, but there is no significant difference in the dry season ($P < 0.05$).

The average nitrification rate at substrate concentration supplemented with 0.1 mg/l and 1.0 mg/l did not differ significantly ($P < 0.05$). The results of this study showed that the nitrification rate did not increase when substrate was added at a high concentration (fig. 7).

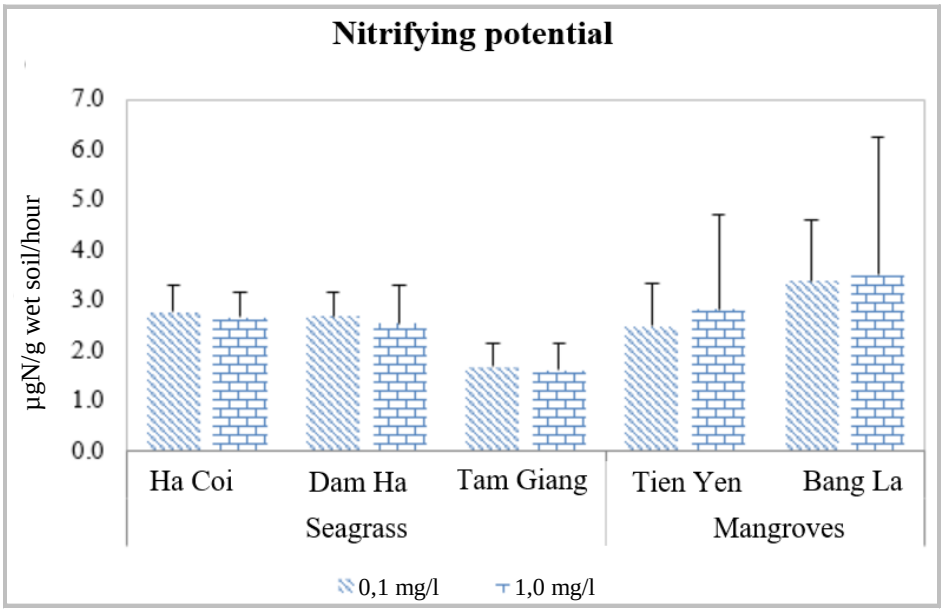

Figure 7. Nitrification potential of microorganisms in mangrove and seagrass

Correlation between environmental factors and nitrifying bacteria

Correlation between environmental factors and nitrifying bacteria is presented in table 1. From this table, it can be seen that the density of nitrifying bacteria in water has significant correlation with most environmental factors with the exception of Pts. Correlation coefficient between the density of nitrifying bacteria with $P\text{-}PO_4^{3-}$ is highest, followed by TAN, $N\text{-}NO_2^-$, BOD_5, Nts and the lowest is with $N\text{-}NO_3^-$.

Nitrification rate at supplemental substrate concentration of 0.1 mg/l was significantly correlated with most of the environmental factors with the exception of $N\text{-}NO_3^-$. In which, nitrification rate has the highest correlation with total bacteria, followed by TAN, $N\text{-}PO_4^{3-}$, Nts, density of nitrifying bacteria, rate of nitrification at additional substrate concentration of 1.0 mg/l, BOD_5, COD, Pts and the lowest with $N\text{-}NO_2^-$.

The nitrification rate at the supplemental substrate concentration of 1.0 mg/l was only significantly correlated with 6/12 survey parameters. Nitrification rate had no significant correlation with the total bacteria density, $N\text{-}NO_2$, $N\text{-}NO_3$, $P\text{-}PO_4$ and BOD_5 ($R < 0.1$; $n = 80$). Among the correlated parameters, the nitrification rate was most correlated with Pts, followed by Nts, the nitrification rate at the supplemental substrate concentration 0.1 mg/l,

COD, density of nitrifying bacteria and the lowest with TAN.

The results of this study showed that the nitrifying bacterial density and their rate related to substrate concentrations such as TAN, $N\text{-}NO_2^-$, Nts. Other factors can also stimulate the growth of nitrifying bacteria such as $P\text{-}PO_4^{3-}$, BOD_5 and total bacteria.

Table 1. Correlation coefficient between the density and activity of nitrifying bacteria and the environmental factors

No.	Parameter	Nitrifying bacteria density (MPN/ml)	Nitrification rate upon addition of 0.1 mgN/l	Nitrification rate upon addition of 1.0 mgN/l
1	Nts (mg/kg dry soil)	-0.20 (n = 80)	0.36 (n = 60)	0.33 (n = 60)
2	Pts (mg/ kg dry soil)		0.15 (n = 60)	0.46 (n = 60)
3	TAN (µgN/l)	0.54 (n = 180)	0.52 (n = 60)	0.13 (n = 60)
4	$N\text{-}NO_2$ (µgN/l)	0.41 (n = 180)	0.12 (n = 60)	
5	$N\text{-}NO_3$ (µgN/l)	0.11 (n = 180)		
6	$P\text{-}PO_4$ (µgP/l)	0.65 (n = 180)	0.51 (n = 60)	
7	BOD_5 (mg/l)	0.37 (n = 180)	0.21 (n = 60)	
8	COD (mg/l)	-0.18 (n = 180)	-0.20 (n = 60)	-0.17 (n = 60)
9	Total bacteria density (CFU/ml)	0.40 (n = 180)	0.54 (n = 60)	
10	Density of nitrifying bacteria (MPN/ml)	1.00 (n = 180)	0.29 (n = 60)	0.14 (n = 60)
11	Nitrification rate when 0.1 mgN/l is added	0.29 (n = 60)	1.00 (n = 60)	0.24 (n = 60)
12	Nitrification rate when 1.0 mgN/l is added	0.14 (n = 60)	0.24 (n = 60)	1.00 (n = 60)

CONCLUSION

The average density of nitrifying bacteria was $4.6 \pm 1.8 \times 10^2$ MPN/ml. In which, the average density of the bacteria in the mangrove ($5.5 \pm 1.8 \times 10^2$ MPN/ml) tended to be higher than in the seagrass ($3.7 \pm 1.9 \times 10^2$ MPN/ml) in both seasons ($P < 0.05$). The average nitrification rate was 2.7 ± 0.6 µgN/g wet soil/hour, and in the mangrove (3.3 ± 1.1 µgN/g wet soil/hour) it tended to be higher than in the seagrass (2.4 ± 0.3 µgN/g wet soil/hour) in the wet season ($P < 0.05$).

Nitrifying bacterial density and rate are not only correlated with substrate concentration factors but also with other environmental factors such as $P\text{-}PO_4$, BOD_5, Pts and total bacterial density.

Acknowledgments: This article is part of the research results of the Ph.D. thesis topic of PhD student Le Thanh Huyen and of sub-component task 5 under project 47, code VAST.DA47.12/16–19. The authors would like to express their sincere gratitude to the Vietnam Academy of Science and Technology (VAST) and their colleagues' collaboration at the Institute of Marine Resources and Environment (IMER).

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