Characteristics of Water Quality and Nitrogen-Associated Bacterial Functions in Mekong Delta Mangroves

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Abstract

Rapid changes, such as urbanization and artificial field expansion, are occurring in mangrove regions worldwide. To assess the characteristics of nitrogen-related processes in mangrove forests in the Mekong delta in relation to conditions in the surrounding environment, we measured water quality: inorganic nitrogen, inorganic phosphate and heavy metals; and nitrogen-associated bacterial activities: nitrogen-fixing activity and nitrogen-consuming activity. The water quality results suggest that key changes in Mekong delta mangroves include a high input of ammonium, phosphorus and heavy metals from cities and aquaculture farms upstream. The current value of mangrove soil bacterial nitrogen fixation in the Mekong delta, 0.87-24.57 mg N m^{-2} d^{-1}, has dropped to within the previously reported order of other mangrove regions. We determined that the addition of ammonium had reduced soil nitrogen fixation to less than half that of the control treatment. The addition of aquaculture pond water also suppressed soil nitrogen fixation. We postulated that if drainage from the artificial systems upstream continuously supplies the mangroves downstream, part of the mangrove soil nitrogen will shift from atmospherically-derived nitrogen to anthropogenically-derived nitrogen. If the nitrogen input from the upstream region exceeds the nitrogen-consuming capacity of the mangrove forest, effects on plant growth due to excessive ammonium and eutrophication of the surrounding ocean will be a concern.

Key words: heavy metals, mangrove, nitrogen, nitrogen fixation, phosphorus, water characteristics

1. Introduction

Mangrove plants form highly productive ecosystems on coastlines of tropical and subtropical regions. These ecosystems support large plant and animal communities, many of which are economically and ecologically important (Bunt, 1992). Despite their importance in ecosystems, mangrove areas have been continuously declining around the world (Spalding *et al.*, 2010), and the Mekong delta is no exception (Hong, 2004). In the past few decades, many mangrove forests in the Mekong delta have been converted to fields for aquaculture and agriculture. Furthermore, expanding urbanization in neighboring areas typically brings environmental change to mangrove ecosystems. The effects of these human-induced impacts on mangrove ecosystems have become a serious concern. The disappearance of habitats because of large-scale deforestation affects the animals living in the forest, and also fish and shellfish in neighboring water bodies. In addition to these obvious changes, subtler changes should also be discussed. For example, qualitative changes to the remaining mangrove forests caused by the intrusion of nutrient loadings and other chemicals from sewage, aquaculture and agriculture have not been fully studied. To assess some of these changes, this study focused on one key element, nitrogen. Nitrogen is one of the most important elements for plant growth, and thus the nitrogen level in the rooting zone of the soil reflects the conditions for plants. The coastline, the habitat for mangrove plants, is generally a nitrogen-exporting region. Large amounts of organic matter containing some nitrogen are continuously lost from the mangrove forest to the ocean by tidal processes (Boto & Robertson, 1990). Boto & Bunt (1981) calculated the amount of nitrogen exported via tidal activity from a large mangrove forest in northern Australia, and found that approximately 13% of the average annual forest net primary production nitrogen requirement was lost in this manner. Therefore, to maintain mangrove plant growth, the tidal nitrogen loss needs to be balanced by nitrogen inflows. There are two main nitrogen inflow pathways: (i) imports from the freshwater or terrestrial surroundings, and (ii) bacterial nitrogen fixation. The supplied nitrogen is mainly consumed by (a) plant growth; (b) bacterial assimilation; and (c) bacterial nitrification–denitrification in mangrove forests. To assess the characteristics of nitrogen-related processes in the Mekong delta mangrove forests in relation to the conditions of the surrounding environment, we measured water quality and nitrogen-associated bacterial activities.

2. Study Area and Methods

2.1 Sampling sites

Water quality and bacterial activities were measured in the following mangrove ecosystems of the Mekong delta in Vietnam: Ho Chi Minh, Soc Trang and Ca Mau (Fig. 1). The Can Gio mangrove forest is a 30,000 ha second-growth forest located 20-30 km downstream from the center of Ho Chi Minh City. The species planted in the forest was *Rhizophora apiculata*. The current forest thus mainly comprises *R. apiculata*, while there are naturally occurring species such as *Avicennia* spp. and *Sonneratia* spp. Because of its ecological importance, this forest has been acknowledged as a UNESCO Biosphere Reserve since 2000. Although the forest is not currently a candidate for large-scale deforestation followed by conversion to fields, it is surrounded by many paddy fields and aquaculture ponds. Furthermore, the growth of Ho Chi Minh City has been significant in the past few decades, as it has become highly populated and its industrial area has expanded.

The Soc Trang mangrove forest is located on a sandbank, Cu Lao Dung, formed in the estuary of the main stream of the Mekong River. Here, the afforested mangrove, *Sonneratia caseolaris* is distributed from the fringe of the coastline to about 1 km inland. Behind the mangrove forest, many paddy fields, aquaculture ponds and houses have been constructed, and shrimp farming is the main form of aquaculture. There are two types of cultivation: (i) separate shrimp ponds and mangrove forest farms, and (ii) mixed farms where the shrimp ponds consist of a series of long channels dug through the mangrove forest. The separated farms are currently dominant in Cu Lao Dung. Drainage from these artificial systems runs into man-made canals and finally out to the ocean through the mangrove forest.

The Ca Mau peninsula of the lower Mekong delta has 51,000-52,000 ha second-growth mangrove forest. Mangrove plants, mainly *R. apiculata*, are distributed from the fringe of the coastline to about 1 km inland. The center of Ca Mau is located 40-50 km inland from the coastline. Ca Mau has grown into one of the biggest commercial centers in the Mekong delta. The population has increased year by year, and many aquaculture ponds have been constructed. Shrimp farming is the main form of commercial aquaculture, and mixed-type farms are currently dominant in Ca Mau Province.

Sampling stations (n = 32) were located in these mangrove ecosystems (Table 1), and categorized into the following six groups: (i) canals in urban areas, (ii) rivers, (iii) canals through aquaculture farms, (iv) separate-type shrimp farms, (v) mixed-type shrimp farms, and (vi) mangroves.

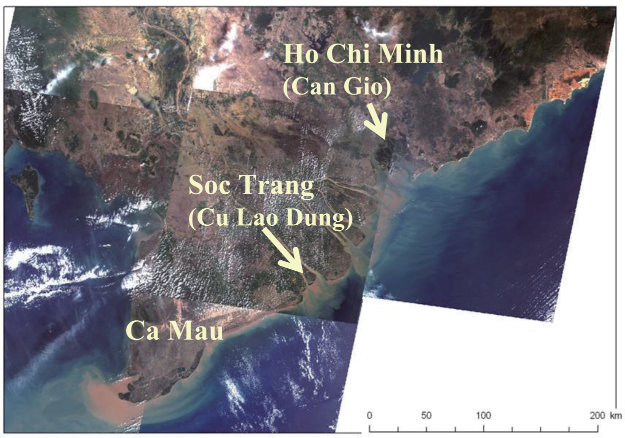

Fig. 1 Aerial photograph of field sites in the Mekong delta.

2.2 Water sampling procedure

Water from the canal or river was collected in a 1-liter polypropylene bottle and 30 ml of the water was immediately filtered through a 0.2-μm filter (DIDMIC-25cs, cellulose acetate, ADVANTEC, Tokyo, Japan) with a plastic syringe (ss-30ESZ, TERUMO, Tokyo, Japan) to remove bacteria. The sampling procedure was repeated three times at each sampling station during the lowest tidal conditions in September 2009.

In the middle of the forest, soil pore water was collected with a water-sampling tube rather than using the sampling procedure described above. The water-sampling tube comprised three parts: (a) an unglazed

Table 1 A list of the sampling stations in the Mekong delta.

Station	Category	Location	
Can Gio			
1	Urban area	10°47′36.08″	106°41′51.01″
2	Urban area	10°44′13.05″	106°42′11.03″
3	Urban area	10°46′29.03″	106°42′25.03″
4	River	10°39′58.05″	106°46′30.03″
5	River	10°35′03.07″	106°49′32.02″
6	River	10°31′23.00″	106°52′09.01″
7	River	10°23′38.02″	106°55′14.00″
8	Mangrove forest	10°31′19.64″	106°51′31.78″
Soc Trang			
9	River	09°29′36.44″	106°13′57.42″
10	River	09°29′26.05″	106°14′16.32″
11	Shrimp pond (S)	09°30′33.98″	106°13′53.94″
12	Shrimp pond (S)	09°30′29.99″	106°14′07.13″
13	Shrimp pond (S)	09°31′30.55″	106°13′18.96″
14	Canal in shrimp farm (S)	09°30′28.77″	106°13′57.86″
15	Canal in shrimp farm (S)	09°30′22.90″	106°14′07.11″
16	Canal in shrimp farm (S)	09°30′14.06″	106°14′15.05″
17	Mangrove forest	09°29′34.44″	106°14′16.91″
18	Mangrove forest	09°30′01.98″	106°14′14.11″
Ca Mau			
19	Urban area	09°10′49.09″	105°08′58.04″
20	Urban area	09°10′39.00″	105°08′55.00″
21	Shrimp pond (S)	08°53′20.09″	105°18′51.02″
22	Shrimp pond (S)	08°53′18.84″	105°18′47.19″
23	Shrimp pond (S)	08°53′19.27″	105°18′45.17″
24	Canal in shrimp farm (S)	08°53′21.14″	105°18′42.25″
25	Mangrove forest	08°53′26.61″	105°18′46.79″
26	Shrimp pond (M)	08°39′30.03″	105°04′02.06″
27	Shrimp pond (M)	08°39′28.07″	105°04′04.82″
28	Shrimp pond (M)	08°39′10.99″	105°03′43.94″
29	Shrimp pond (M)	08°39′28.03″	105°04′01.03″
30	Mangrove forest (M)	08°39′26.77″	105°03′59.81″
31	Mangrove forest (M)	08°39′09.68″	105°03′47.13″
32	Mangrove forest (M)	08°39′19.15″	105°04′14.06″

(S) Separate-type shrimp farm; (M) Mixed-type shrimp farm tube (2 mm diameter, 10 cm length), (b) a teflon-lined tube (2 mm diameter, 30 cm length), and (c) a hollow needle. The unglazed tube was buried in the sediment to depth of 10-20 cm. To collect the soil pore water, the needle was inserted into the silicon lid of a vacuum-sealed glass vessel, and soil pore water was drawn into the vessel through the teflon-lined tube. The first 2 ml of water were discarded and the next 30 ml were filtered through the 0.2-µm filter as described above. The samples were held below 5°C in a cooler bag prior to chemical analysis.

2.3 Chemical analysis of water

The dissolved inorganic nitrogen (NO_2^-, NO_3^- and NH_4^+) and phosphate concentrations in the water samples were measured with an auto-analyzer (AACS-II, BRAN+LUBBE, Norderstedt, Germany). Dissolved inorganic heavy metal concentrations (As, Pb, Cd, Cr, Cu, Zn, Ca, Fe, Mn, Mo, Sr and Ti) were measured by an inductively coupled plasma atomic emission spectrometer (ICP-JA, Nippon Jarrell-Ash, Kyoto, Japan) after diluting the samples in 1 N nitric acid.

2.4 Bacterial activity

The potential activity of soil nitrogen-consuming bacteria was determined using the following procedure. A 49-ml soil core collected at a soil depth of 1–6 cm was transferred to a 200 ml teflon flask together with 120 ml of the surrounding water. The solution was adjusted to 100 µM ammonium by adding ammonium chloride, and shaken in the dark at 28°C. After 0.3, 3 and 6 h from the start of the incubation, 30 ml of the solution from each flask was filtered through the 0.2-µm filter. The concentration of inorganic nitrogen in the solution was determined using the auto-analyzer. The potential activity of the bacteria consuming the inorganic nitrogen was determined by the decrease in inorganic nitrogen in the samples with time, using a linear regression analysis.

Soil nitrogen fixation was indirectly measured using the acetylene reduction technique (Hardy *et al.*, 1986). A 5 ml soil core (1 cm in diameter, 5 cm in height) from a 1-6 cm soil depth collected within the mangrove forest was transferred to a 50 ml glass vial with 5 ml of the surrounding water. Fifteen vials were prepared for each forest. The vials were purged with argon to provide an anoxic atmosphere, and sealed with teflon–silicon septa. For five of the vials, 4 ml of the gas phase were evacuated and replaced with acetylene via the Teflon–silicon septa. Five other vials were prepared without acetylene as blank test. The remaining five vials were used to assess eutrophication, and were incubated with added ammonium. The vials were shaken in the dark at 28°C, and 0.2 ml of the gas phase was sampled at 0.3, 5 and 10 h after the acetylene was introduced. Ethylene concentrations in the gas samples were determined with a gas chromatograph equipped with a Porapak N column and FID detector (GC-4000, GL Science Inc., Tokyo, Japan). The activity of the nitrogen-fixing enzyme nitrogenase was determined from the accumulation of ethylene in the vials with time, using a linear regression analysi.

3. Results and Discussion

3.1 Water characteristics

3.1.1 Inorganic nitrogen and inorganic phosphate

Very high concentrations of inorganic nitrogen were detected in the urban regions, Stations 1-3, 19 and 20 (Fig. 2). Most of the dissolved inorganic nitrogen in the water was present as ammonium, NH_4^+. This suggests that nitrogen in the urban system is mainly draining out as ammonium, and/or that the bacterial metabolic processes in these canals are mainly anaerobic, which convert dissolved nitrogen to the reduced ammonium form. In the downstream river waters (Stations 4-7, 9 and 10), total inorganic nitrogen concentration was decreased. Most of the dissolved inorganic nitrogen was present as nitrite and nitrate, probably because of bacterial nitrification processes in the aerobically stirred stream. In the separate-type shrimp ponds (Stations 11-13 and 21-23), total inorganic nitrogen concentration in the pond water was comparatively low and mainly present in the form of ammonium. However, in the canals receiving drainage from the ponds (Stations 14-16 and 24), a comparatively higher total inorganic nitrogen level was detected. While most of it was in the form of ammonium, one-third appeared as nitrite and nitrate. In the mixed-type shrimp farm systems (Stations 30-32), the total inorganic nitrogen concentration in the water was extremely high and most of it appeared as ammonium.

The phosphate concentration was remarkably high in the urban regions (Fig. 3). The phosphate concentration in water was lower in the downstream river. The separate-type shrimp farm systems showed high levels of dissolved phosphate, both in pond waters and in the canals receiving drainage from the ponds. Levels of dissolved phosphate in the mixed-type shrimp farm systems remained at a low level. The phosphate levels in the two

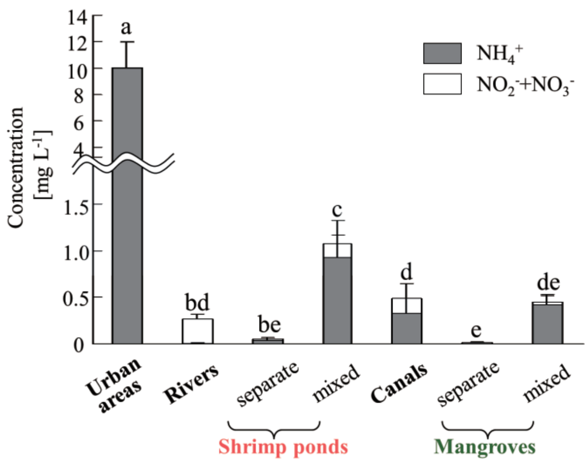

Fig. 2 Inorganic nitrogen dissolved in water in the Mekong delta. Results are expressed as mean ± standard error (n = 3–6). Different letters indicate significant differences ($p < 0.05$).

types of shrimp farm systems contrasted with the levels of inorganic nitrogen (total inorganic nitrogen was high in the mixed-type systems and low in the separate-type systems) (Fig. 4).

Most of the mangroves (Stations 8, 17, 18 and 25) were categorized as low-nutrient areas (Fig. 4). Concentrations of dissolved inorganic nitrogen and phosphate in tropical mangrove forests are generally reported to be low (within the µM range) because of the rapid and efficient uptake of dissolved materials by bacteria (Alongi *et al*., 1992). An exception was the mangroves in mixed-type shrimp farms (Station 30), which fell within the category of mixed-type shrimp ponds (high levels of inorganic nitrogen while phosphate levels were low).

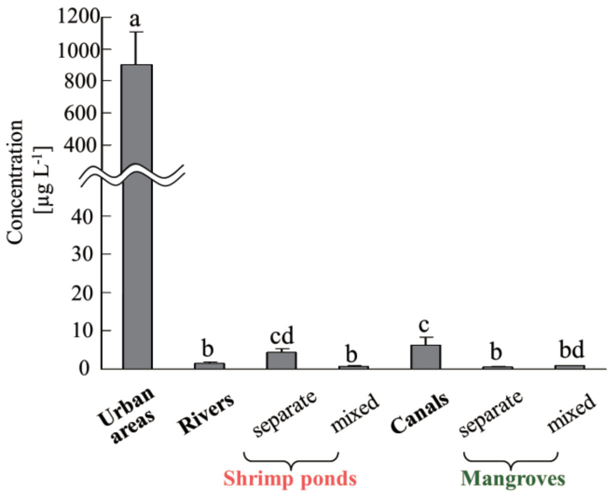

Fig. 3 Inorganic phosphate dissolved in water in the Mekong delta. Results are expressed as mean ± standard error (n = 3–6). Different letters indicate significant differences ($p < 0.05$).

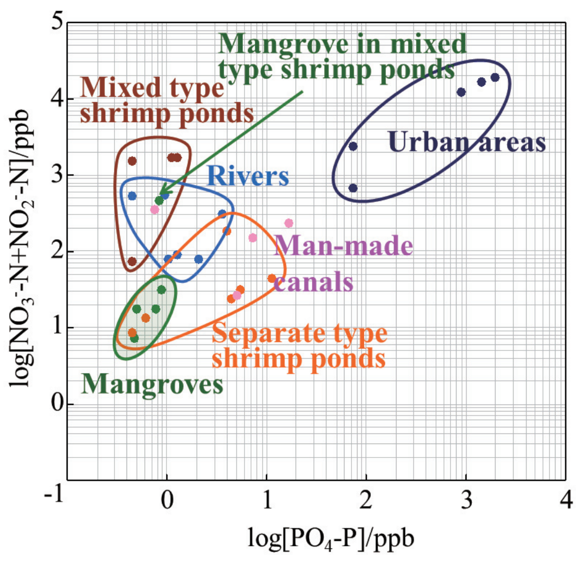

Fig. 4 Water characteristics of mangrove ecosystems in the Mekong delta.

3.1.2 Heavy metals

Of the metals analyzed, comparatively high levels of arsenic, cadmium and lead were detected; mean values exceeded the World Health Organization (WHO) standard for drinking water in some categories (Fig. 5). High levels of arsenic and cadmium were detected in the shrimp farm systems and the canals receiving drainage from the separate-type ponds. This observation suggests that the shrimp farm system may be a source of the two metals in drainage waters. Although the concentration levels were not as high, titanium showed a similar pattern to that of arsenic and cadmium. High levels of lead were detected in the urban regions and their river waters; mean values exceeded the WHO standard for drinking water in these two categories. In addition to lead, the iron concentration was higher in the urban regions compared with the other categories. One interpretation of this could be that the urban system may be a source of lead and iron. In mangrove soil pore water of these regions, higher concentrations of lead, manganese, chromium, zinc and molybdenum were detected compared with those of the other stations. These elements might accumulate in the soil through the ability of soil particles to adsorb metal ions, and the bacterial mineralization process allows them to become soluble. Relatively high levels of calcium and strontium in the shrimp farm systems may be attributed to the influence of seawater, because this generally contains comparatively high levels of these two elements compared with freshwater. Furthermore, separate-type shrimp ponds need the addition of $CaCO_3$ in order to regulate the pH conditions of the water, because these types of ponds tend to become acidic due to oxidation of pyrite. This may be a reason for the higher concentration of calcium in separate-type ponds than in mixed-type ponds.

3.1.3 Bacterial activity

On a dry-weight basis, the potential activity of inorganic nitrogen-consuming bacteria was considerably higher in root materials than in soil, probably because of the high nitrogen requirements of root cells (Fig. 6). The background soil nitrogen-consuming activity was calculated as 1.5-3.9 g N m^{-2} d^{-1} (Table 2). This value can only be applied to the top 1-6 cm of soil and therefore the rate of inorganic nitrogen consumption over the entire mangrove forest, including deeper soil, must be higher than this. There may be an effective nitrogen-exporting process in the mangrove root zone; that is, a nitrification–denitrification process. The first step in this process is the oxidation of ammonium nitrogen in soil pore water to form nitrite-N. The nitrite-N is reduced in the anoxic zone to form gaseous-N, which is then lost to the atmosphere. This process is accelerated when oxic and anoxic conditions co-exist, as is the case in the mangrove root zone. Most mangrove species develop aerial root systems to transport oxygen through internal lacunae (gas-space continua) to their root tips, which are buried in hypoxic sediments. A portion of this oxygen diffuses toward the rhizosphere via the root surface, and forms a thin oxida-

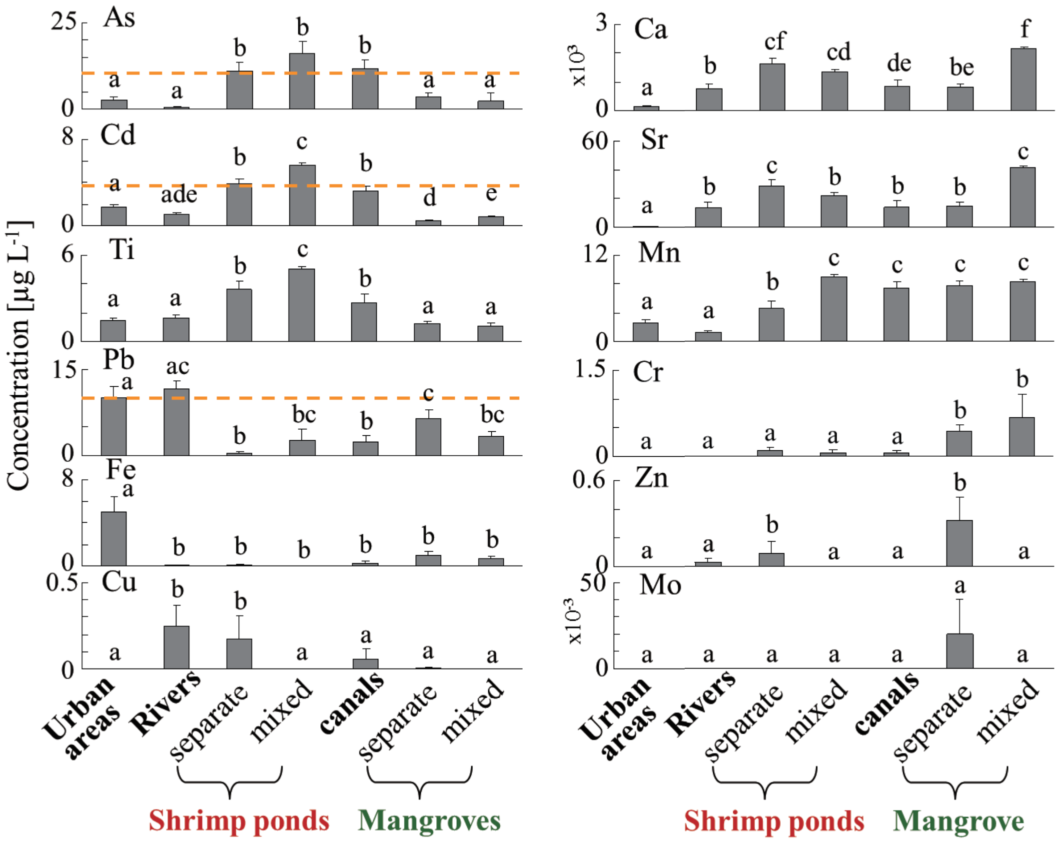

Fig. 5 Dissolved metal concentrations in the Mekong delta. Orange lines indicate the standard for drinking water set by the World Health Organization. Results are expressed as mean ± standard error (n = 3–6). Letters indicate significant differences ($p < 0.05$).

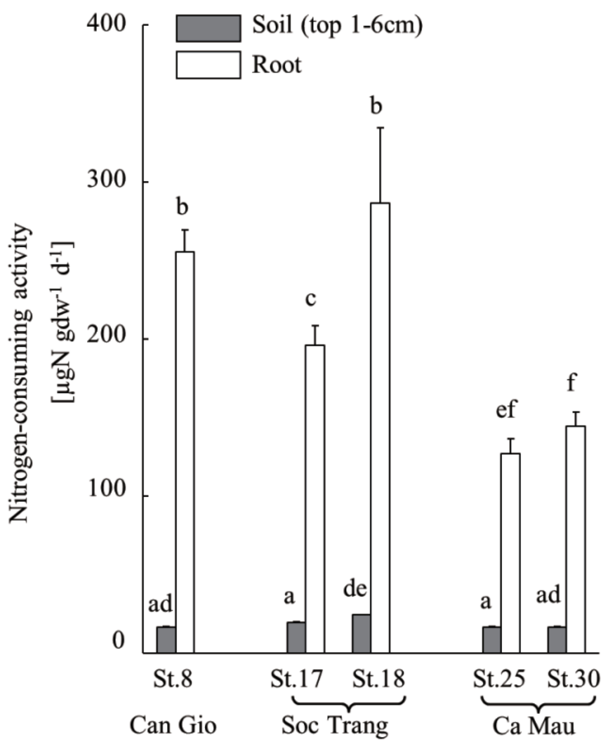

Fig. 6 Inorganic nitrogen-consuming bacterial activity by soil and roots in Mekong delta mangrove forests. Results are expressed as mean ± standard error (n = 5). Different letters indicate significant differences ($p < 0.05$).

Table 2 Background upper-soil inorganic-nitrogen-consuming activity in mangrove soils.

Station	N-consuming potential [gN area m^{-2} day^{-1}]
Can Gio	
8	3.5 ± 0.2
Soc Trang	
17	2.2 ± 0.4
18	1.5 ± 0.5
Ca Mau	
25	3.9 ± 0.5
30	3.6 ± 0.3

Mean ± SD

tive layer. It has been reported that the soil redox potential is higher in the mangrove rhizosphere than in the non-rhizosphere (Thibodeau & Nickerson, 1986; McKee *et al*., 1988; McKee, 1993). It is likely that nitrification would occur in this oxic zone.

The potential of nitrogen-fixing activity was significantly higher in root materials than in soil ($P < 0.05$, Fig. 7). This can be interpreted to mean that nitrogen-fixing bacteria are more abundant in the mangrove rhizosphere than in bulk soil or in the bacteria colonizing root materials. Detection of nitrogen-fixing activity in mangrove root materials (Inoue *et al*., 2011a) has been reported in previous studies. Inoue *et al*. (2011b) reported that nitrogen content (C:N) was significantly higher in the mangrove root zone than 10 cm away from the root zone. The high levels of nitrogen may be due to dead root

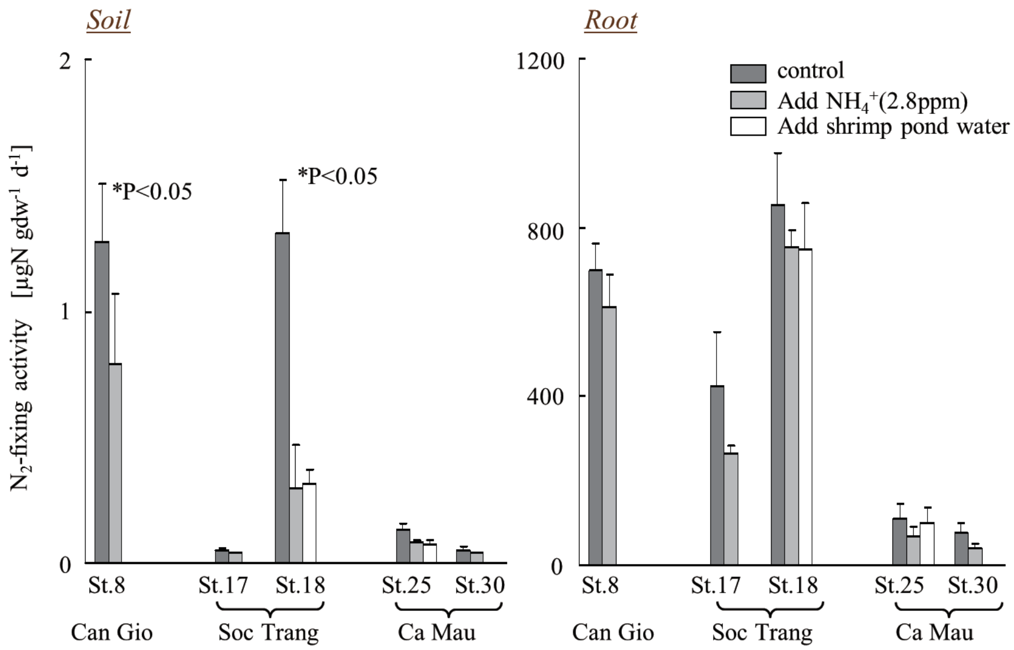

Fig. 7 Nitrogen-fixing activity by soil and roots under control, ammonium fertilization (2.8 ppm NH_4^+) and shrimp-pond-water-added conditions, respectively, in the Mekong delta. Results are expressed as mean ± standard error (n = 5).

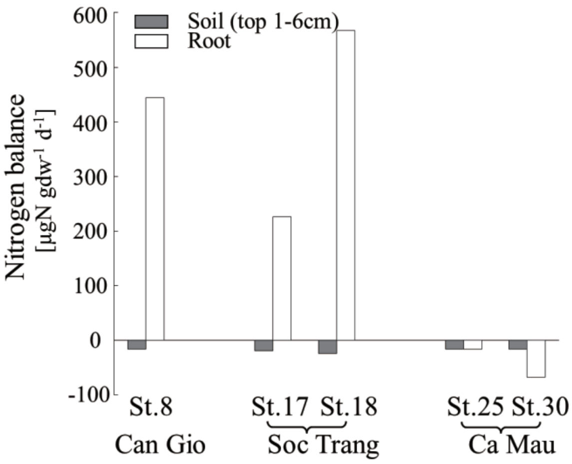

Fig. 8 Balance between nitrogen consumption and fixation.

detritus, which is abundant in the root zone, or to high levels of bacterial nitrogen immobilization in the root zone.

In Can Gio and Soc Trang mangroves, the balance between nitrogen consumption and fixation was negative in the top 1-6 cm of mangrove soils, while it was substantially positive in root materials (Fig. 8), indicating that nitrogen input to mangrove roots was guaranteed by nitrogen fixation itself. In contrast, the nitrogen balance was negative in both soil and root materials in the Ca Mau mangroves.

3.1.4 Effect of ammonium input on nitrogen fixation

The water quality results suggest that one of the key changes occurring around the mangrove forests is high nitrogen input from the urban areas upstream. Apart from the mangroves in the Ca Mau region, the addition of ammonium (2.8 ppm NH_4^+) reduced the potential of mangrove soil nitrogen-fixing activity to less than half of the control treatment (Fig. 7). Ammonium is relatively important in regulating nitrogen fixation in aquatic environments, because of the repression of nitrogenase synthesis and rapid reversible inhibition of nitrogenase activity (Capone, 1988). In contrast to the soil, the potential nitrogenase activity of root material was not reduced by the addition of NH_4^+ (Fig. 7). This suggests that the composition of nitrogen-fixing bacteria differs between the bulk soil and the rhizosphere (or in root materials), and the bacteria associated with root materials are insensitive to NH_4^+. Some sulfate-reducing bacteria, such as those in the genus *Desulfovibrio*, are known to be insensitive to NH_4^+ (Welsh *et al.*, 1997). In Ca Mau mangroves, the addition of ammonia did not reduce the potential nitrogenase activity of either soil or root material. At Stations 30-32, the ammonium concentration in soil pore water was already high and thus the potential nitrogenase activity might have already been suppressed.

Compared with the previously reported values (Potts, 1979; Hicks & Silvester, 1985; Boto & Robertson, 1990; Woitchik *et al.*, 1997; Kristensen *et al.*, 1998; Alongi *et al.*, 2000; Sjoling *et al.*, 2005), the value of bacterial nitrogen-fixing activity in the Can Gio forest (Station 7) and that in one of the Soc Trang forests (Station 18) analyzed in this study was not remarkably low compared with that in other forests. The values in the Ca Mau region (Stations 25 and 30) and in the other Soc Trang forest (Station 17) were comparatively low (Fig. 9). The Soc Trang forest, Station 17, is located on the seaward fringe, and thus the low bacterial nitrogen-fixing activity might be attributed to its low soil organic matter (0.9%), because the oxidation of organic matter is essential for

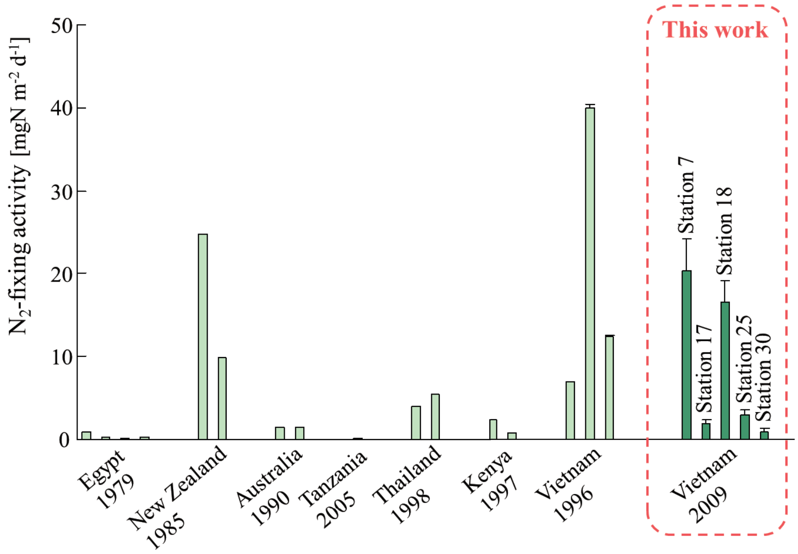

Fig. 9 Nitrogen-fixing activity of soil analyzed in mangrove forests. Egypt 1979 (Potts, 1979); New Zealand 1985 (Hicks & Silvester, 1985); Australia 1990 (Boto & Robertson, 1990); Tanzania 2005 (Sjoling *et al*., 2005); Thailand 1998 (Kristensen *et al*., 1998); Kenya 1997 (Woitchik *et al*., 1997) and Vietnam 1996 (Alongi *et al*., 2000).

nitrogen fixation. Alongi *et al*. (2000) analyzed soil nitrogenase activity in three mangrove forests in the Ca Mau region in 1996 (the 1996 Vietnam values in Fig. 9). Compared with the values in 1996 and those obtained in this study, the bacterial nitrogen-fixing activity in the Ca Mau region seems to have fallen remarkably in these thirteen years. Because the three mangrove forests in Alongi *et al*. (2000) are not the same forests studied here, some conditions such as forest age and surrounding circumstances were not the same, and thus the results from the two studies cannot simply be compared. However, there is the possibility that expansion of mixed-type shrimp farm areas have led to a reduction in the activity of nitrogen-fixing bacteria. A high level of ammonium drainage from the upstream regions in this study would affect the nitrogen balance in downstream mangrove ecosystems. Consequently, part of the mangrove soil nitrogen would shift from atmospherically derived N to anthropogenically derived N. If the nitrogen input from the artificial systems exceeded the nitrogen-consuming capacity of the mangrove forest, effects on plant growth due to excessive ammonium and eutrophication of the surrounding ocean would occur. Finally, addition of the shrimp pond water also reduced potential nitrogenase activity in the soil in the Soc Trang forest (Station 18) to less than half that of the control treatment (Fig. 7). The water characteristics results indicate that the shrimp pond water contained comparatively high levels of arsenic, cadmium, calcium, strontium and titanium (Fig. 5). The effect of heavy metals on nitrogen-associated bacteria in mangrove forests needs to be examined.

Acknowledgments

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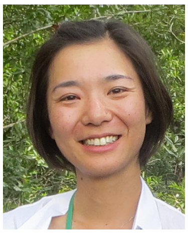

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