Soil Nitrogen Stock of Primary and Restored Mangrove Forests in Ranong Biosphere Reserve, Thailand

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ABSTRACT

Mangrove forests play an important role in nutrient cycling, particularly due to their high rates of nitrogen enrichment. However, few studies have investigated the soil nitrogen stock in mangrove forests. This study was conducted at mangrove sites in the Ranong Biosphere Reserve in Thailand that represented three different conditions: primary, reforested, and natural regeneration. The soil nitrogen stock was estimated at four depth layers (0-10, 10.1-15, 15.1-30 and 30.1-50 cm). The results showed that the primary mangrove site had significantly higher soil nitrogen stock (mean±standard deviation; 10.38±1.29 t $N \cdot ha^{-1}$) due to the greater time for nitrogen accumulation in the soil, followed by the reforested site (6.02±0.69 t $N \cdot ha^{-1}$) and the natural regeneration site (5.16±0.37 t $N \cdot ha^{-1}$). The soil nitrogen content was highest in the surface soil (0-10 cm) and gradually decreased with increasing depth. Nitrogen accumulation rates were significantly higher for the primary mangrove site (8.16±0.39 g $N \cdot m^{-2} \cdot year^{-1}$), followed by the reforested site (2.56±0.07 g $N \cdot m^{-2} \cdot year^{-1}$) and the natural regeneration site (1.07±0.07 g $N \cdot m^{-2} \cdot year^{-1}$). Soil texture (clay and silt), bulk density, and total nitrogen content were significantly correlated to total nitrogen stock. These results demonstrate that conservation and restoration programs for mangrove forests are effective practices that help ensure this vital ecosystem continues to function as a long-term nitrogen sink and nitrogen cycling site for the coastal system as a whole.

Keywords: Mangrove forest, Nitrogen stock, Soil nitrogen, Thailand, Tropics

INTRODUCTION

Mangrove forests provide vital functions and services for regulating coastal nutrient cycling, filtering, and reducing pollution between marine and terrestrial environments (Spaninks and Beukering, 1997; UNEP, 2006; Kumar, 2010; Brander *et al*., 2012). Nitrogen is an essential nutrient for mangrove plant growth and an indicator of soil quality. Most mangrove nitrogen is obtained from the decomposition of primary plants (litter, downed wood, prop roots, pneumatophores, dead roots) and is mainly stored in the soil (Alongi, 2020). However, the nitrogen dynamics in mangroves are diverse, being governed by both abiotic and biotic variables, such as soil characteristics, climatic conditions, and plant communities. Recently, there has been increased interest in the role of mangrove nitrogen as a source of greenhouse gas emissions, especially for nitrous oxide (N_2O), due to it occurring commonly in anoxic conditions (Shiau and Chiu, 2020).

The establishment of pioneer mangrove forests over the past decades has led to long-term nitrogen storage in the soil. Deforestation causes nitrogen loss and affects soil fertility and nutrient supply (Adame *et al*., 2018). In contrast, restoration leads to nitrogen improvement in the mangrove ecosystem (Guo *et al*., 2018; Xu *et al*., 2021). Other studies demonstrated that the nitrogen content

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was variable due to the soil properties in different mangrove types. Typically, nitrogen content in mangrove soils decreases with an increased soil depth, due to more intense addition of nitrogen in the surface soil (Howe *et al*., 2009; Saintilan *et al*., 2013; Bulmer *et al*., 2016). Some studies reported that soil nitrogen stocks differed by soil texture (silt, clay, sand), soil bulk density, tree species, density, and forest development stage (Bulmer *et al*., 2016; Adame *et al*., 2018; Asanopoulos *et al*., 2021). The enhanced nitrogen stock varied with forest age or time after restoration (Adame *et al*., 2018). Soil nitrogen stocks have also been found to vary spatially, such as with distance from the seaward edge of the forest (Ellis *et al*., 2004; Yang *et al*., 2013), and with soil depth (Bulmer *et al*., 2016; Guo *et al*., 2018). Consequently, better understanding of soil nitrogen and its influencing factors, and of soil nitrogen stock in mangroves should enable more appropriate mangrove management.

A few localized studies have addressed nitrogen stock and accumulation in Thailand. We hypothesized that nitrogen stock would vary with soil conditions in three mangrove sites, being the highest in the primary mangrove forest, followed by reforested and natural regeneration sites. Thus, the objectives of the present study were to quantify the potential nitrogen stock based on vertical soil depth in three mangrove sites, and to investigate the relationship between nitrogen stock and soil characteristics (soil depth, bulk density, %N, sand, silt, and clay).

MATERIALS AND METHODS

Study site

The study was carried out in the Ranong Biosphere Reserve (RBR), Klong Ngao Estuary in Ranong Province, southern Thailand. The estuary lies along the Andaman Sea coast, where the tide regime is semi-diurnal. The climate in Ranong Province is tropical monsoon, with average annual rainfall of 4,200 mm and mean air temperature of 27.6 °C (Macintosh *et al*., 2002).

The three sampling sites in this study were in different areas of RBR (Figure 1), each with a different history of management. The primary mangrove site (PM) was in an undisturbed area containing several large mature trees, and located at the seaward edge of the RBR. The reforestation site (SM-R) was situated on land in the Ranong Mangrove Research Center, 50 m from Klong Ngao estuary and close to the local community zone. The natural regeneration site (SM-N) was located in a developing area and was composed of smaller trees with low growth rate. The reforestation site and the natural regeneration site had been disturbed in past decades by logging activity.

The mangrove species were different at the three study sites. The dominant species of the primary mangrove site and the reforestation site was *Rhizophora apiculata*, while the natural regeneration site was dominated by *Aegiceras corniculatum* and other mangrove species (*Rhizophora mucronata, Sonneratia alba*) (Kitpakornsanti *et al*., 2022).

Sampling plot design, soil sampling and analysis

For sampling we established a square plot (100×100 m) of 1 ha at each mangrove site, with four sampling points (10×10 m) randomly placed about 20 m apart within each plot. The soil sampling was performed in December 2019 during low tide. Soil samples with three replicates were collected to a depth of 50 cm and divided into four soil core sections (0-10, 10.1-15, 15.1-30, and 30.1-50 cm). In the laboratory, soil samples were dried for at least 48 h and then passed through a sieve to remove root debris before physiochemical analysis for soil grain size, total nitrogen, and bulk density. The soil texture was analyzed using the hydrometer method, with sodium hexametaphosphate as a dispersant (Gee and Bauder, 1986). The total nitrogen content (%N) in soil was determined using the Kjeldahl procedure (Bremner and Mulvaney, 1982). The bulk density was calculated based on the ratio of total oven-dried weight (g) and original volume (cm^3).

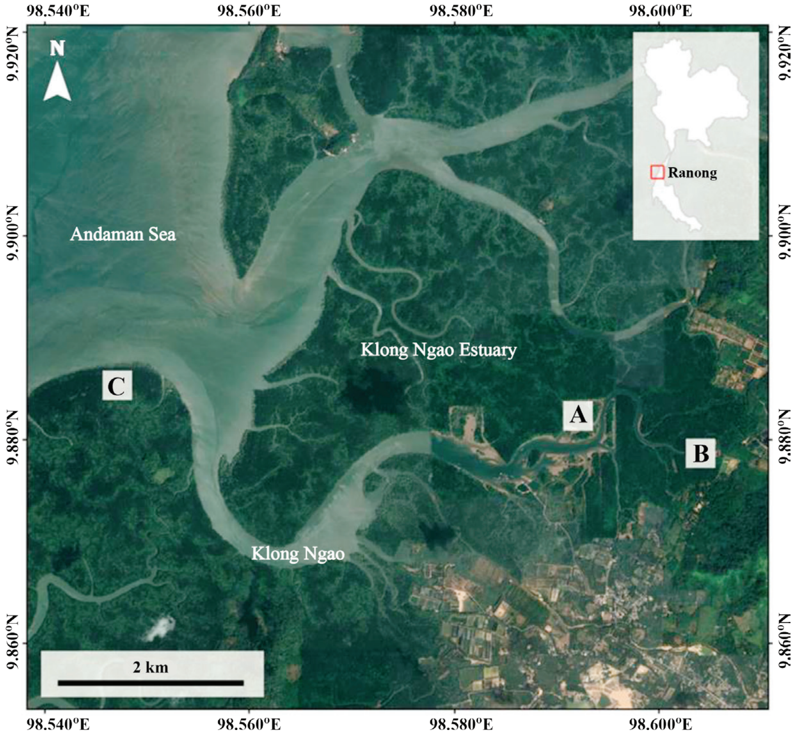

Figure 1. Location of (A) natural regeneration site (SM-N), (B) reforestation site (SM-R), and (C) primary mangrove site (PM) within Ranong Biosphere Reserve, Ranong Province, Thailand.

Calculation of total soil nitrogen stock and nitrogen accumulation rate

The total soil nitrogen stock (t N·ha^{-1}) was estimated by multiplying the soil nitrogen content by the bulk density and the soil depth interval, and then summing the soil nitrogen stock in each soil interval up to 50 cm, as shown in Equation 1 (Asanopoulos *et al*., 2021).

$$\text{Total soil nitrogen stock} = \sum_{i=1}^{n} \times \%N_i \times BD_i \times D_i \times (1\text{-}Pg) \qquad (1)$$

where n is the soil layer, $\%N_i$ is the nitrogen content, BDi is the bulk density (g·cm^{-3}), D_i is the thickness of the soil layer (cm), and Pg is the fraction occupied by gravel fragments >2 mm, (it was assumed Pg = 0 in this study).

The nitrogen accumulation rate (g N·m^{-2} ·year^{-1}) was calculated based on the soil accumulation rate (g soil·m^{-2}·year^{-1}) and the N fraction (g N·g^{-1} soil), as shown in Equation 2. The soil accumulation rate was obtained from Pengthamkeerati *et al*. (2021) and the N fraction was adopted from the 0-5 cm soil layer for these same mangrove sites.

$$\text{Nitrogen accumulation rate} = \text{Soil accumulation rate} \times \text{N fraction} \qquad (2)$$

Statistical analysis

A one-way analysis of variance was used to evaluate significant differences between mangrove sites and soil depths at the 95 % confidence level. Pearson correlation coefficients (r) determined the relationship between the soil bulk density, nitrogen content, nitrogen stock, and soil texture (clay, silt, and sand). All statistical analyses were performed using the SPSS version 21.0 software.

RESULTS

Soil characteristics

The soil bulk density and the amounts of total nitrogen, nitrogen density, clay, silt, and sand at three mangrove sites for the four soil intervals (0-10, 10.1-15, 15.1-30, and 30.1-50 cm) are shown in Table 1 and Figure 2. The results clearly show significant differences among the mangrove sites ($p<0.001$-0.014) in the sand, silt, and clay fractions (Table 1), but not among depth intervals ($p>0.05$).

The soil texture at the natural regeneration site had average (±standard deviation) values of 59.31±2.60, 37.43±1.78, and 3.26±4.01 % for the clay, silt, and sand fractions, respectively. The texture was mainly clay at each soil depth. For the reforested site, the soil was composed of 59.31±3.07, 37.43±5.58, and 3.26±4.28 % of clay, silt, and sand, respectively. The texture of the surface soil (0-10 and 10.1-15 cm) was silty clay, while the subsoil (15.1-30 and 30.1-50 cm) was clay. At the primary mangrove site, the soil texture had average values of 30.44±7.97, 22.16±4.17, and 47.41±10.81 % for the clay, silt, and sand fractions, respectively. The topsoil layer (0-10 cm) was clay loam, whereas at the depth ranges of 10.1-15, 15.1-30 and 30.1-50 cm, the soils were sandy clay loam. The reforested site and the natural regeneration site seemed to have greater fractions of clay and silt than the primary mangrove site, but less sand (Figure 2). These results indicated that the reforested site and the natural regeneration site had slightly finer-textured soils than the primary mangrove site.

The bulk density values were significantly different among the mangrove sites, while bulk density showed a slightly increasing trend with soil depth that was not significant (Table 1 and Figure 2). The primary mangrove site had the lowest mean bulk density (0.55±0.17 $g \cdot cm^{-3}$), followed by the reforested site (0.77±0.19 $g \cdot cm^{-3}$) and the natural regeneration site (0.81±0.19 $g \cdot cm^{-3}$).

Total soil nitrogen stock and accumulation

The results showed that soil nitrogen content and nitrogen density varied among the three mangrove sites (Table 1 and Figure 2). The primary mangrove site had a higher average nitrogen content (0.38 %) compared to the reforested site (0.16 %) and the natural regeneration site (0.15 %). The same pattern was found for soil nitrogen density, in the descending order of primary mangrove site (2.11 mg $N \cdot cm^{-3}$), reforested site (1.25 mg $N \cdot cm^{-3}$), and

Table 1. Analysis of variance of selected soil characteristics at soil depths of 0-10, 10.1-15, 15.1-30, and 30.1-50 cm in mangroves at Ranong Biosphere Reserve, Ranong Province, Thailand.

Soil variable	Factor	N	F value	p value	Significance
Bulk density	Site	3	41.770	<0.001	**
	Soil depth	4	0.161	0.855	NS
N content	Site	3	17.290	0.003	*
	Soil depth	4	0.444	0.660	NS
N density	Site	3	9.373	0.014	*
	Soil depth	4	0.817	0.486	NS
Sand	Site	3	86.949	<0.001	**
	Soil depth	4	0.025	0.976	NS
Silt	Site	3	60.963	<0.001	**
	Soil depth	4	0.020	0.980	NS
Clay	Site	3	22.393	0.002	*
	Soil depth	4	0.032	0.969	NS

Note: *significant difference at $p<0.05$; **significant difference at $p<0.001$

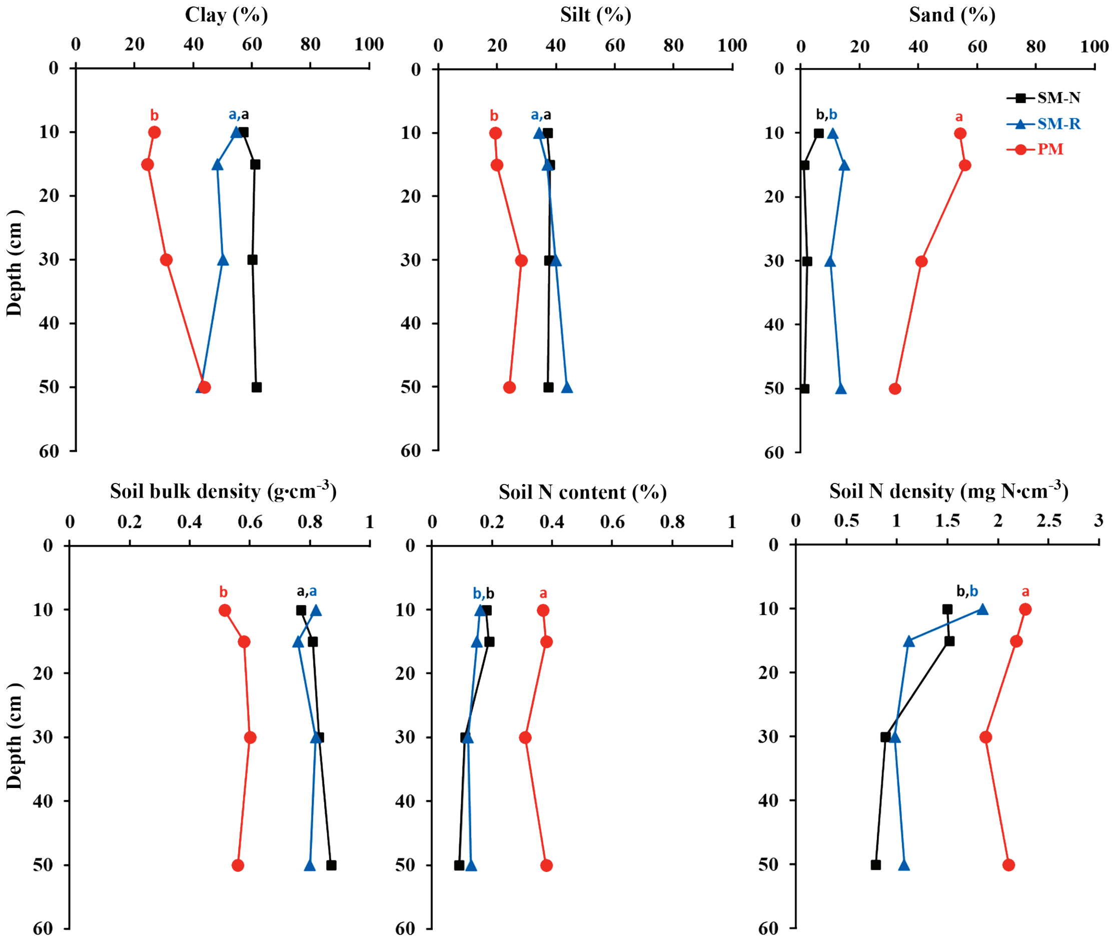

Figure 2. Values for clay, silt, sand, soil bulk density, soil nitrogen content, and soil nitrogen density at three mangrove sites in Ranong Biosphere Reserve, Ranong Province, Thailand. SM-N: natural regeneration site; SM-R: reforested site; PM: primary mangrove site. Different lowercase letters indicate significant ($p<0.05$) differences among mangrove sites across all soil depths.

natural regeneration site (1.17 mg N·cm^{-3}). Table 2 shows that the primary mangrove site had the significantly ($p<0.001$) highest total soil nitrogen stock (10.38 t N·ha^{-1}), followed by the reforested site (6.02 t N·ha^{-1}) and the natural regeneration site (5.16 t N·ha^{-1}).

The nitrogen accumulation rate was significantly ($p<0.05$) the highest for the primary mangrove site (8.16 g N·m^{-2}·year^{-1}). The reforested site (2.56 g N·m^{-2}·year^{-1}) and the natural regeneration site (1.07 g N·m^{-2}·year^{-1}) had similar nitrogen accumulation rates, but they were still significantly different (Table 2).

Correlation between soil nitrogen stock and soil variables

The correlation coefficients between the soil nitrogen stock, soil bulk density, soil nitrogen content, and soil texture parameters are shown in Table 3. The soil nitrogen stock had a positive correlation with bulk density, soil nitrogen content, and the clay and silt fractions at $p<0.001$ or $p<0.05$. The soil nitrogen content was significantly ($p<0.001$) negatively correlated with the soil bulk density and clay fraction.

Table 2. Soil nitrogen stock for soil depth intervals to 50 cm at three mangrove sites in Ranong Biosphere Reserve, Ranong Province, Thailand.

Forest type	N stock (t N·ha^{-1}) Soil depth (cm)				Total N stock (t N·ha^{-1})	N accumulation rate (g N·m^{-2}·year^{-1})
	0-10	10.1-15	15.1-30	30.1-50		
SM-N	1.50	0.76	1.33	1.58	5.16±0.37^{c}	1.07±0.07^{c}
SM-R	1.85	0.56	1.47	2.14	6.02±0.69^{b}	2.56±0.07^{b}
PM	2.27	1.09	2.81	4.21	10.38±1.29^{a}	8.16±0.39^{a}

Note: Means±SD in a column with different lowercase superscripts indicate significant differences ($p<0.05$) between mangrove sites; SM-N = natural regeneration site; SM-R = reforested site; PM = primary mangrove site.

Table 3. Pearson correlation coefficients (r) between soil variable and nitrogen stock in Ranong Biosphere Reserve, Ranong Province, Thailand.

Soil variable	Bulk density	Total N content	Total N stock
Bulk density	1	-	-
Total N content	-0.91**	1	-
Total N stock	0.64*	0.57*	1
Clay (%)	0.79**	-0.67**	0.53*
Silt (%)	0.45	0.42	0.62*
Sand (%)	-0.70	0.23	0.20

Note: *significant difference at $p<0.05$; **significant difference at $p<0.001$

DISCUSSION

Our soil samples showed considerable variation in the total nitrogen stock in the three mangrove sites in the RBR. Differences between the sites were largely driven by forest age and the resulting increases in tree and root densities, canopy, downed wood, and litter, which reflect a nutrient-rich soil. The soil nitrogen content was higher for the primary mangrove site compared to the natural regeneration and reforested sites. It was observed that the dense canopy at this site was composed of large mangrove species (*Rhizophora* sp.), downed wood, and large nutrient elements in a darker soil. These results were consistent with Wang *et al*. (2009), who showed that the vegetation and biomass of mangroves were correlated with the soil nitrogen and carbon; thus, a mangrove forest with a higher canopy had greater litter deposition, dead belowground roots, and higher accumulation of soil nitrogen and carbon content (Xin *et al*., 2016).

Additionally, the nutrient content in soil reflects the total nitrogen budget. The results showed the nitrogen pool was fundamentally different in the primary mangrove compared to the two restored forest sites. At the same time, the natural regeneration and reforested sites had similar ranges in total soil nitrogen stock in this study. Nitrogen is stored in the soil, accounting for 96 % of the total nitrogen in mangrove forests (Alongi, 2020). The study of Adame *et al*. (2018) revealed that the total nitrogen stock increased with forest age, with the nitrogen stock of a forest aged 40 years (after mangrove clearing) being 15 % lower than a forest aged 70 years. Primary productivity could accumulate as nitrogen nutrients on the mangrove floor through time. Litter production varies with mangrove tree species and tree density at each site (Srisunont *et al*., 2017). The nitrogen concentrations differ with each component of the stand, including leaves (1.61 %), stems (0.37 %), branches (0.31 %), roots (0.49 %), and litter (0.69 %), which together make up the nitrogen content and stock in the soil. The root biomass contains 0.45-0.75 % nitrogen, and accounts for 0.08-0.3 t N·ha^{-1} (Alongi *et al*., 2003). Leaf litter is the most easily decomposed by microorganisms to release its nitrogen-containing nutrients, whereas branches are hardest to break down (Alongi, 2020). The decay rate may vary due to the climate, hydrothermal conditions, vegetation species, and chemicals in the biomass (e.g., lignin).

Invasions of exotic tree species have been associated with the nitrogen stock in mangrove forests (Sampaio *et al*., 2021). A lower nitrogen stock was observed at a mangrove site with invasive species compared to a non-invaded site, and the leaf nitrogen concentration of the invasive species (*Hymenachne amplexicaulis* (*Rudge*) *Nees*) was determined to affect the total nitrogen stock. However, sites in the present study were mainly composed of native mangrove species such as *Rhizophora* sp. that grow locally. *Aegicera corniculatum* is commonly found in the muddy zone, and it was dominant in the natural regeneration site in this study. Thus, the tree species present at the study sites were likely determined by soil conditions, including nitrogen stock.

Soil nitrogen content and total nitrogen stock have been shown to increase with distance from the seaward edge of mangrove forests (Yang *et al*., 2013; Bulmer *et al*., 2016). The results of the present study indicated that soil bulk density was correlated to the soil particles (clay) present. The soil texture of the primary site mainly consisted of sand particles, resulting from the geomorphology of the site. The seaward location of the primary mangrove site may have been prone to sand accumulation by tidal flushing. Because it contained mature mangrove trees, the primary mangrove site also had a greater amount of soil organic matter than the restored mangrove sites (Kitpakornsanti *et al*., 2022). Despite the reforested and natural regeneration sites having finer-textured soils than the primary mangrove site, their soil bulk density and total soil nitrogen content were higher, possibly due to the lower levels of soil organic matter. These factors and their interaction resulted in a lower bulk density, higher soil porosity, higher total soil nitrogen and, consequently, higher total soil nitrogen stock in the primary mangrove site compared to the reforested and natural regeneration sites.

The total soil nitrogen stock increased with increasing depth, but not significantly so. The nitrogen stock in the topsoil (0-15 cm) was approximately 39 % of the total soil nitrogen stock (0-50 cm) at all three mangrove sites. In comparison, the total nitrogen stock of the subsoil layers (0-10, 10.1-15, and 15.1-30 cm) represented 59-69 % of the total nitrogen stock across the three mangrove sites. This indicated that the nitrogen nutrient content was primarily stored in surface soil and tended to decrease in the deeper soil layers (Sheikh *et al*., 2009; Ngaba *et al*., 2020), due to the litter and plant residue inputs from mangrove vegetation. The primary mangrove site and the restored forest site had nitrogen accumulation on the surface layer that reflected soil and biomass deposition with time.

The higher soil nitrogen stock in the primary mangrove site was due to the development of numerous breathing roots, leading to greater soil nitrogen accumulation. The nitrogen accumulation rates measured in this study were similar to the short-term N accumulation rates in Gulf mangroves on the coast of Saudi Arabia (Saderne *et al*., 2020), but lower than for some estuarine mangroves, such as the urbanized San Juan Bay Estuary in Puerto Rico, United States (Wigand *et al*., 2021), which had high accumulation rates due to anthropogenic activities such as wastewater inputs.

Tidal activity and tropical storms are natural forces that transport soil particles and nitrogen from allochthonous sources or adjacent areas (Adame *et al*., 2018), and their effects may be reflected in the surface soil accretion and nitrogen stock we found at each mangrove site. Similar results were reported by Srisunont *et al*. (2017), who found that soil nitrogen accumulation in the Klong Khone mangrove forest, Samut Songkhram Province, Thailand, increased with time. In addition, Wigand *et al*. (2021) showed that soil nitrogen accumulation contributed to the long-term sink of nitrogen in mangrove forests.

Although there was a lack of data indicating the nitrogen source in the present study, the C/N ratio of the surface soil (0-10 cm) had moderate values at three mangrove sites investigated by Pengthamkeerati *et al*. (2021): a natural regeneration site (15.89), a reforested site (27.05), and a primary undisturbed site (22.13). These C/N ratios were in the range typical of tropical mangroves (7-27; Bouillon *et al*., 2003), and suggested the nitrogen level was in an appropriate range for the mangrove ecosystem and that the organic matter in the mangrove forest was derived from internal nitrogen sources. However, external drivers, such as rainfall, wind, temperature, and storms, may also flush nitrogen from other areas for accumulation in the mangrove estuarine system (Wigand *et al*., 2021).

In comparison to mangrove environments, the total nitrogen stock to a depth of 1 m in tropical terrestrial forests was reported to be 22.33 Mg N·ha^{-1} (Alongi, 2020), where the main contribution was from soil components that accounted for more than 91-96 %. Table 4 shows the nitrogen stock observed in this study compared to several other mangrove forests, and demonstrates that the nitrogen stock varied with forest age, soil depth, and mangrove species. The total nitrogen stock of our undisturbed primary mangrove site was similar to that of a 70-year-old mangrove forest in Malaysia (Adame *et al*., 2018) and a 50-year-old mangrove forest in New Zealand at 1 m depth (Bulmer *et al*., 2016), and reflected the good health of this site in RBR. The nitrogen stock of the reforested and natural regeneration sites was higher than for mangrove forest in Fujian, China after two years of restoration (Guo *et al*., 2018), but lower than for a forest in Malaysia that had been replanted 30 years before (Adame *et al*., 2018). Such comparisons in total nitrogen stock are likely confounded by other environmental factors at each location. However, the soil nitrogen stock levels of the three mangrove forests in the present study were lower than the mean global value (50 t N·ha^{-1}) (Alongi, 2020).

Table 4. Soil nitrogen stock in different mangrove forests.

Country	Mangrove type	Forest age (years)	Dominant species	N stock (t N·ha^{-1})	Reference
Thailand, Ranong	Natural regeneration	26	*A. corniculatum*	5.16[a]	This study
	Reforested	28	*R. apiculate*	6.02[a]	This study
	Primary undisturbed	>100	*R. apiculate*	10.38[a]	This study
China, Fujian	Restored	2	*S. alterniflora*	1.37-2.25[b]	Guo *et al*. (2018)
New Zealand	Natural	>50	*A. marina*	17.7[b]	Bulmer *et al*. (2016)
		>10	*A. marina*	13.0[b]	Bulmer *et al*. (2016)
Malaysia, Matang	Reforested	5	*R. apiculate*	8.88[b]	Adame *et al*. (2018)
	Reforested	30	*R. apiculate*	9.48[b]	Adame *et al*. (2018)
	Pioneer	70	*Mixed species*	11.03[b]	Adame *et al*. (2018)
Puerto Rico, United States	Native	≈ 90	*A. germinans, L. racemosa* and *R. mangle*	7.1[b]	Wigand *et al*. (2021)
Southeastern Brazil	Native	-	*L. racemosa, R. mangle*	3.2[c]	Sampaio *et al*. (2021)
Northern Brazil, Potengi	Restored	-	*R. mangle*	4.62[b]	Ramos e Silva *et al*. (2007)
Nigeria, the Great Kwa River	Restored	-	*Nypa fruticans Wurmb* *R. racemose*	18.55[a]	Ononyume *et al*. (2022)

Note: [a] based on soil depth of 50 cm; [b] based on soil depth of 1 m; [c] based on soil depth of 20 cm; *Avicennia marina* (*A. marina*); *Sporobolus alterniflora* (*S. alterniflora*); *Rhizophora mangle* (*R. mangle*); *Laguncularia racemosa* (*L. racemosa*); *Rhizophora racemosa Meyer* (*R. racemosa*)

CONCLUSION

The soil nitrogen stocks differed among the three mangrove sites studied in the RBR. The primary mangrove site had the highest total nitrogen stock and nitrogen accumulation rate, due to its greater age. The restoration activity in the two other sites (natural regeneration and reforestation) resulted in similar soil nitrogen stock levels and accumulation rates. These measurements demonstrated that the past efforts at recovering degraded mangrove forests have been effective, and that nitrogen content from primary productivity has accumulated over time as leaf litter and dead woody material deposited on the mangrove floor. The nitrogen content in the soil tended to decrease with depth, but not significantly so. The soil bulk density and grain size for each site were controlling factors for the total nitrogen stock. These results suggest that mangrove conservation and a continuing restoration program could enhance the soil nitrogen stock and reduce nitrogen loss from the coastal zone. Consequently, suitable management practices might be a promising strategy for long-term nitrogen sequestration in mangrove forests.

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