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Analysis of phosphate solubilizing bacteria from mangrove sediments and their relation to the presence of waterbirds

DEVI N. CHOESIN♥, RESTU UTARI DEWINA
School of Life Sciences and Technology, Institut Teknologi Bandung. Jl. Ganesha 10, Bandung 40132, West Java, Indonesia.
Tel./fax.: +62-022-2511575, ♥email: devi@itb.ac.id

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Abstract. *Choesin DN, Dewina RU. 2025. Analysis of phosphate solubilizing bacteria from mangrove sediments and their relation to the presence of waterbirds. Biodiversitas 26: 2182-2188.* Phosphate Solubilizing Bacteria (PSB) play a key role in the functioning of ecosystems by making insoluble phosphorus in the soil available to plants. In the mangrove ecosystem, waterbirds may also act as a phosphate source through their excretion of guano (bird droppings). The island of Pulau Rambut in Indonesia is an avian wildlife sanctuary with an uneven distribution of waterbirds in its mangrove areas. This variation in waterbird abundance is expected to affect the mangrove ecosystem and its PSB community structure. The objectives of this study were to describe the PSB community structure in mangrove sediments and relate them to the presence of waterbirds as a source of available phosphate. Five PSB species each were found in bird nesting and non-nesting sites with a Sorensen similarity of 71.4%. Molecular analysis by 16s RNA identified the predominant PSB species as *Salinicola tamaricis*. PSB diversity was positively correlated with soil organic carbon and negatively correlated with soil salinity, available phosphate, and total phosphate in soil ($p<0.05$). Total and available phosphate were higher in the bird nesting site, while PSB diversity and abundance were lower. This may be attributed to the availability of other nutrients, specifically organic carbon.

Keywords: Mangrove, phosphate, Rambut Island, *Salinicola tamaricis*, solubilizing bacteria

INTRODUCTION

Mangrove forests are coastal wetland ecosystems that provide many important ecosystem services, including coastal protection; nursery and habitat for fish and other biota; food and livelihood for local communities; cultural and tourism services; and regulation of carbon and nutrient cycles (Getzner and Islam 2020; Dabalà et al. 2023; FAO 2023). An important component in maintaining a mangrove ecosystem's function is the microbial community. Microbes play a key role in the cycling of nutrients in mangrove ecosystems, i.e., through the process of decomposing organic compounds into inorganic forms that can be readily used by plants and other organisms. An important microbial group in this case is the phosphate solubilizing bacteria (PSB) (Fatimah et al. 2023; Pan and Cai 2023; Damo et al. 2024; de Carvalho et al. 2024). PSB can convert insoluble forms of phosphorus into soluble forms, thereby improving their absorption and use by plants (Yu et al. 2022; Pang et al. 2024).

Phosphorus (P) in the form of phosphate (also commonly abbreviated as P) is an essential macronutrient required for the growth and development of living organisms (Tian et al. 2021). Sources of P in soil include inorganic P derived from mineral rocks (in the form of apatite, hydroxyapatite, and oxyapatite) and organic P derived from organic matter (Prasad and Chakraborthy 2019). Phosphate is typically a large molecule that cannot be directly used by the cell; therefore, PSB is a key factor in converting it into dissolved phosphate ions (Pi, HPO_4^{2-}, $H_2PO_4^-$) or simpler organic molecules that can be processed by the cell. The term 'available P' is used to refer to the P element in these forms. The available P content of the soil is only 0.1% of the total P in the soil, thus, available P is a limiting factor for mangrove forest growth (Ingle and Padole 2017). Apart from PSB, another source of available P in the mangrove ecosystem is birds, or rather bird droppings (guano). The entry of bird droppings can alter microbial growth, community composition, and interactions within the ecosystem (Justel-Diez et al. 2023) while increasing the nutrient content of N, K, and especially P (Tomassen et al. 2005; Plotnikov and Sinyavskiy 2020; Luneva et al. 2022).

Pulau Rambut Wildlife Reserve is a 90-hectare island off the northern coast of Java Island in Indonesia. The site was designated as a Ramsar wetland site of international importance in 2011 (https://rsis.ramsar.org/ris/1987) because of the variety of wetland habitat types found in the area, e.g., coral reefs, intertidal flats, mangrove forests, lagoons, and seasonal freshwater marshes. The site is part of a chain of wetlands along the East Asian-Australasian Flyway and becomes an important transit station for waterbirds, especially from October to December, when they migrate from the northern hemisphere to Australia. Waterbird abundance in the mangrove forests of Rambut Island can reach thousands during the migration season. During breeding season, which usually starts in November and lasts until April or May, thousands of waterbirds from 15 species have been recorded to breed on this small island (Mardiastuti 2022). However, these birds are not evenly distributed across the island (Melinda et al. 2016; Firdausy et al. 2020). Spatial variation in bird abundance is thought to affect the mangrove ecosystem and the PSB community structure within it. Therefore, the objectives of this study were to describe the PSB community structure in mangrove sediments and link them to the presence of waterbirds as a source of available P in the mangrove areas of Rambut Island.

This study is important because PSB can promote mangrove growth and increase the water absorption capacity of mangroves (Fatimah et al. 2023; Pang et al. 2024). An understanding of the relationship between available P and PSB community structure and its effect on the mangrove ecosystem can contribute insight into effective mangrove conservation. It is hypothesized that there is a difference between PSB community structure (species richness, abundance, and diversity) in sediments of mangrove areas with high bird abundance compared to areas where birds are absent or rarely found.

MATERIALS AND METHODS

Study area

Pulau Rambut Wildlife Reserve is located in the special administrative capital region of Jakarta, Indonesia, at coordinates 05°58'S, 106°41'E (Figure 1). Data collection for this study was conducted in two areas of the island, i.e., the northern mangrove area (5°58'20.63"S, 106°41'30.18"E) and the western mangrove area (5°58'30.56"S, 106°41'23.91"E). The northern area is known as a bird nesting site and will hereafter be referred to as BNS (bird nesting site), while the western area is not a bird nesting site and will be referred to as NNS (non-nesting site). The general conditions and comparison between the two sites can be seen in Figure 2. Sample processing was carried out at the Microbiology Laboratory of the Research and Innovation Building, Institut Teknologi Bandung. Field data collection, followed by laboratory analysis, was conducted in March and July 2018.

Field data collection on vegetation, sediment, and waterbirds

Vegetation analysis was conducted in both BNS and NNS mangrove sites. At each site, three plots measuring 20x20m were used to collect data on tree species and abundance. Sediments were sampled by purposive random sampling at three points in each plot. These sediment samples were taken from a depth of 0-10 cm aseptically using a ceramic spatula, then stored at 4°C and brought to the laboratory for further processing. Abiotic factors in the form of microclimate (temperature, humidity, light intensity) and edaphic parameters (soil temperature, moisture, pH, salinity) were also measured in both BNS and NNS mangrove sites. To acquire a general description of the waterbird community, bird watching was conducted from 6 to 8 AM and from 3 to 5 PM for two consecutive days. Birds were observed from the bird observation tower located at 5°58'25.29"S, 106°41'31.17"E using the method of counting colonially nesting species (Wetlands International 2010, 2018; Prosser et al. 2023).

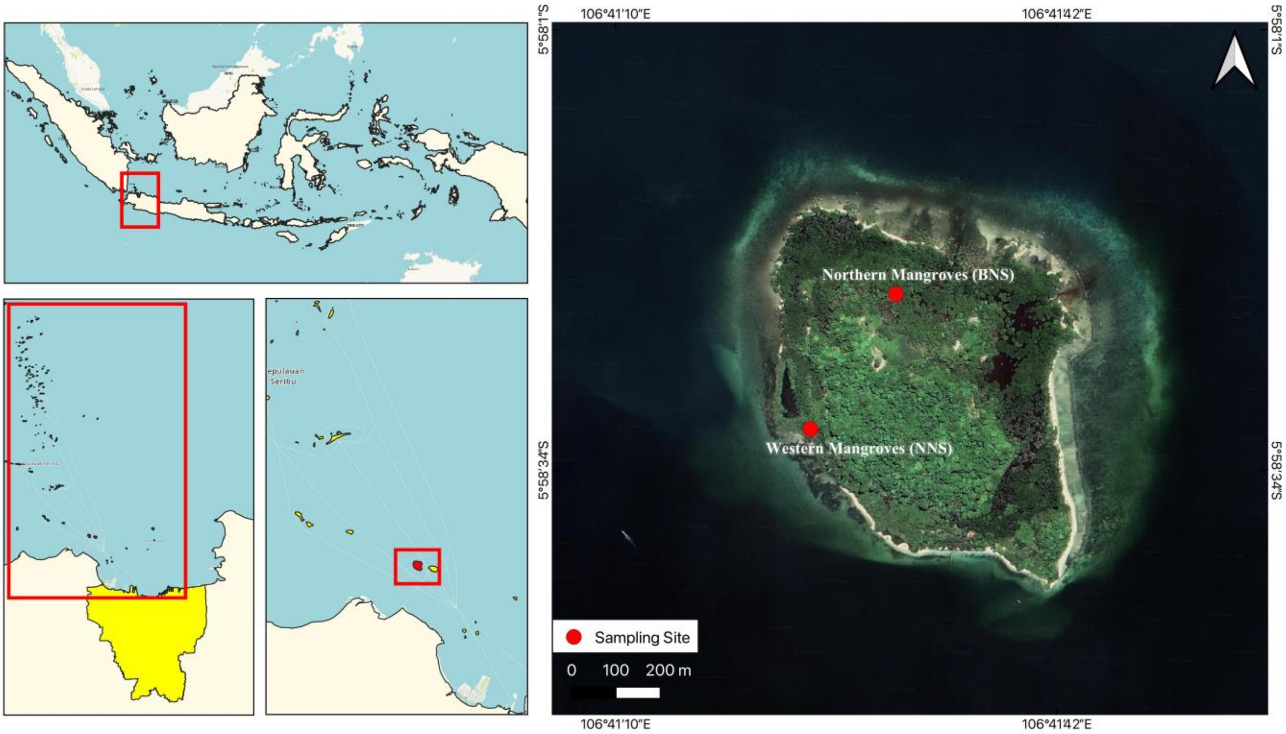

Figure 1. Map of Rambut Island and location of the two sampling sites described in this study: the northern mangroves (BNS: bird nesting site) and western mangroves (NNS: non-nesting site). Inset maps show the island's relative position to Indonesia and Southeast Asia; Java Island; and the Kepulauan Seribu Islands in the administrative capital region of Jakarta, Indonesia

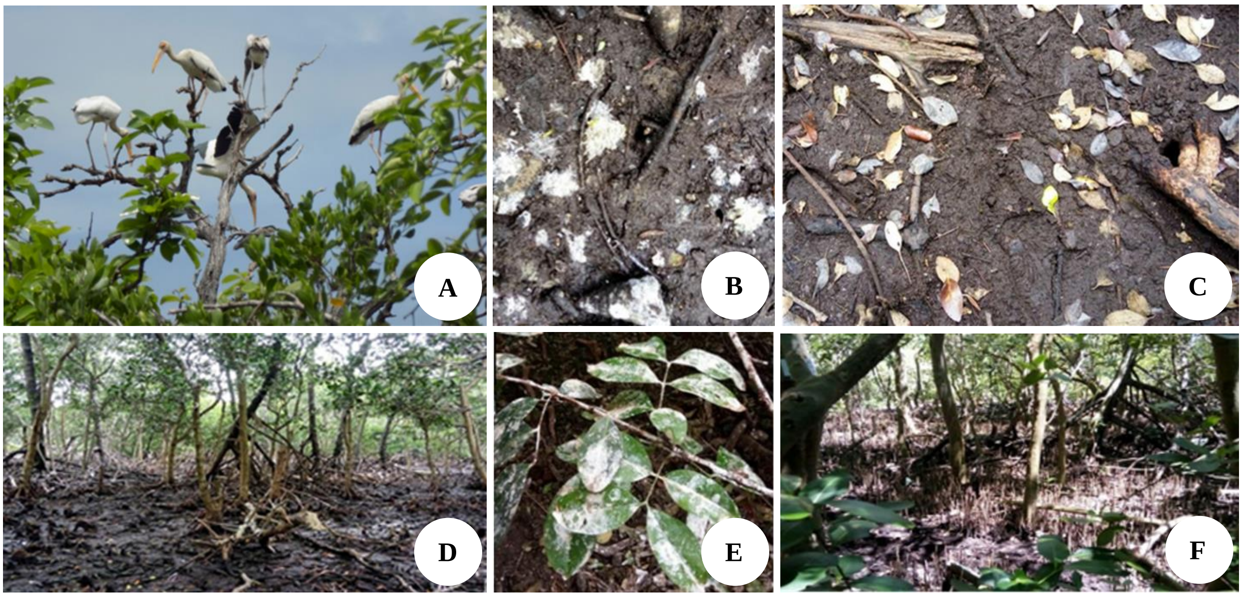

Figure 2. General conditions of the northern bird nesting site (BNS) and western non-nesting site (NNS) in Rambut Island. A. bird presence on the upper branches of the BNS canopy; B. tree density and canopy cover in BNS; C and D. abundant bird droppings on the forest floor and plant leaves and branches in BNS; E. absence of bird droppings on the forest floor of NNS; F. tree density and canopy cover in NNS

Isolation of phosphate solubilizing bacteria (PSB)

PSB was isolated from mangrove sediments collected from both BNS and NNS sites. 1 g of soil sample was dissolved using sterile sea water solution (salinity 30 ppt) with multilevel dilution. The sample was inoculated on NBRIP (National Botanical Research Institute's phosphate growth) selective medium, with the composition (in 1 L medium) as follows: 10 g glucose; 5 g $Ca_3(\text{-}PO_4)_2$; 5 g $MgCl_2\text{-}6H_2O$; 0.25 g $MgSO_4\text{-}7H_2O$; 0.2 g KCl and 0.1 g $(NH_4)_2SO_4$; at pH-7.0, and dilutions of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. Inoculation was performed by the spreading method in duplicates. Colonies that formed a clear zone (halozone) were selected as PSB and their abundance was calculated on the third day. Afterward, isolations were made of pure PSB culture, and their ability to solubilize phosphate was tested using the medium NBRIP-bromothymol blue broth medium. Bacteria capable of dissolving phosphate will change the pH of the medium, as indicated by the color change of the medium to yellow (Behera et al. 2017).

Identification of phosphate solubilizing bacteria (PSB)

PSB was identified based on morphology, Gram staining, and halozone size. The most abundant bacteria found from this analysis was further identified molecularly using 16S rRNA. The stages of identification which included DNA extraction, polymerase chain reaction (PCR), sequencing, and assembling, were carried out by Macrogen, South Korea. The obtained gene sequence data were edited to obtain a comparison sequence using the BioEdit application, and then the editable sequence data were uploaded to the NCBI online Basic Local Alignment Search Tool (BLAST) application to determine bacterial species. Afterward, a phylogenetic tree was made using the Mega 7.0 application, by applying the neighbor-joining DNA distance algorithm with 1000 bootstrap times (Behera et al. 2017).

Measurement of sediment nutrient content

Sediments were also analyzed for nutrients in the form of total phosphate, available phosphate, and organic carbon. Total phosphate was measured using the 25% HCl extractor method and spectrophotometry (Anschutz and Deborde 2016); available phosphate was measured using the Olsen method (IRRI 2011; FAO 2021); while organic carbon was measured using the Kurmies method (Batjes 2014; Kogut et al. 2023). Analysis was carried out by the Plant and Vegetable Crops Research Institute (*Balai Penelitian Tanaman dan Sayuran*) in Lembang, West Bandung Regency, West Java, Indonesia.

Data analysis

Vegetation data collected from the field and PSB data obtained from laboratory analysis were analyzed to compare BNS and NNS sites. Species richness and abundance data were used to calculate the Shannon-Wiener diversity index (Formula 1) (Sher and Molles 2022) and Sorensen similarity index (Formula 2) (Hao et al. 2019; Oluyinka-Christopher 2020) as follows:

$$H' = -\sum (p_i \log_e p_i) \qquad (1)$$

Where:
H' : Shannon-Wiener diversity index
p_i : Proportion of the ith species
$\log_e p_i$: Natural logarithm of p_i
$\sum$: Sum total for all species in the community (from species i=1 to s)

$$C_s = 2a/(2a+b+c) \qquad (2)$$

Where:
C_s: Sorensen similarity index
a: the number of species common to both communities
b: the number of species in community B, but not A
c: the number of species in community A, but not B

Environmental factors were compared between sites using paired t-tests. PSB diversity was tested for correlation with biotic and abiotic factors using Pearson correlation. Test results with values greater than 0.90 or smaller than -0.99 with significant P value ($P<0.05$) are considered factors that are highly correlated with PSB diversity.

RESULTS AND DISCUSSION

Mangrove vegetation and abiotic environment

Vegetation analysis revealed a clear difference between BNS and NNS in terms of plant tree species richness and abundance or density (Table 1). The mangrove community in BNS was characterized by only two main species, i.e., *Ceriops tagal* and *Rhizophora mucronata*; while the community in NNS consisted of seven species, dominated by *Avicennia officinalis*. The Shannon-Wiener diversity index for mangrove vegetation in BNS (0.654) was lower than in NNS (0.823). A low similarity index between communities (0.444 or 44.4%) further confirms the difference between the northern (BNS) and western (NNS) mangrove areas, which could be indicative of a difference in general tree architecture and canopy structure, with possible consequences to bird preference for nesting.

The abiotic environmental factors in BNS and NNS are compared in Table 2. There was a significant difference between the sites in terms of temperature, humidity, and light intensity. This could be attributed to the observation that mangroves in BNS tended to be more shaded due to a denser canopy (Figure 2). Edaphic conditions in the two sites were generally similar, except for a significantly higher salinity in BNS compared to NNS. This difference is thought to be caused by microsite features in which there were depressions in the NNS forest floor that collected rainwater, thereby likely reducing soil salinity.

Waterbird community

The relative abundance of waterbirds observed in the northern mangrove forests (i.e., BNS) is presented in Figure 3. Milky storks (*Mycteria cinerea*; Ciconiidae) and purple herons (*Ardea purpurea*; Ardeidae) were recorded as the most abundant species. Other species that were recorded include *Anhinga melanogaster* (Anhingidae); *Ardea cinerea* (Ardeidae); *Ardea sumatrana* (Ardeidae); *Egretta alba* (Ardeidae); *Nycticorax nycticorax* (Ardeidae); and *Phalacrocorax sulcirostris* (Phalacrocoracidae). The species observed in this study have also been reported in other studies conducted on Rambut Island (e.g., Mardiastuti 2022). The Pulau Rambut Wildlife Sanctuary is known to support three internationally threatened bird species, especially the vulnerable milky stork (*M. cinerea*), with one of the largest breeding colonies of this species in Indonesia (https://rsis.ramsar.org/ris/1987).

At the time of data collection, several birds in BNS were seen brooding their eggs or caring for their chicks, whereas no nesting birds were found in NNS. Birds in NNS generally only flew over the sampling plots or perched for a very short while. These results confirm the uneven distribution of waterbirds on Rambut Island and justify the comparison being made between the two sampling sites.

Table 1. Comparison of mangrove species and respective abundance and density in BNS (bird nesting site) and NNS (non-nesting site) in Rambut Island

Mangrove species	**Abundance (individuals)**		**Density (ind ha^{-2})**	
	BNS	**NNS**	**BNS**	**NNS**
Avicennia officinalis	0	138	0	1150
Bruguiera gymnorrhiza	0	3	0	25
Ceriops tagal	106	3	883	25
Rhizophora apiculata	0	9	0	75
Rhizophora mucronata	60	22	500	183
Rhizophora stylosa	0	1	0	8
Xylocarpus moluccensis	0	2	0	17

Table 2. Comparison between environmental conditions in the northern (BNS: bird nesting site) and western (NNS: non-nesting site) mangrove areas. Microclimatic measurements were conducted between 10 AM and 1 PM. Different superscripts within the same row indicate significant differences at $p<0.05$

Abiotic environmental factor	**BNS**	**NNS**
Microclimatic factors		
Temperature (°C)	33.3 ± 0.9^a	31.4 ± 0.4^b
Humidity (%)	100.0 ± 0.0^a	80.1 ± 6.6^b
Light intensity (lux)	$2,321 \pm 928^a$	$5,071 \pm 586^b$
Edaphic factors		
Soil temperature (°C)	26.7 ± 0.0^a	26.7 ± 0.0^a
Soil pH	6.9 ± 0.1^a	6.9 ± 0.1^a
Soil moisture (%)	83.0 ± 33.2^a	85.6 ± 14.2^a
Salinity (ppt)	30.7 ± 11.1^a	16.4 ± 3.4^b

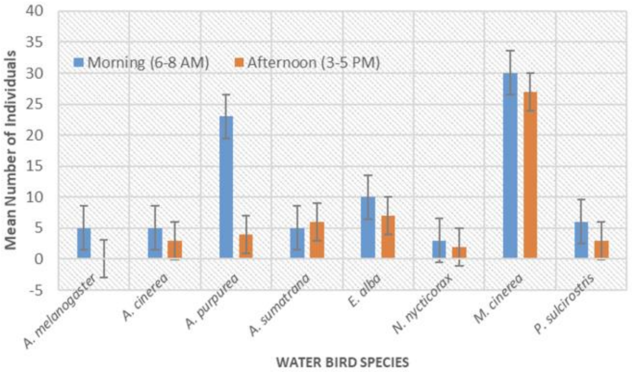

Figure 3. The abundance (mean number of individuals) of waterbird species recorded in the northern mangrove area of Rambut Island

Phosphate Solubilizing Bacteria (PSB)

Results from bacterial isolation and identification in the laboratory found overall seven isolates as PSB. For practicality before conducting molecular identification of the most abundant PSB, isolates were first designated as Sp.1 to Sp.7. Five PSB species were found in both BNS and NNS; however, the species composition differed between sites (Figure 4). Among the species, Sp.3 and Sp.5 were not found in BNS, while Sp.6 and Sp.7 were not found in NNS.

The two sites exhibited a Sorensen similarity index of 0.714 (71.4%), which indicates a relatively high species similarity between the two sites. PSB abundance in NNS tended to be higher than in BNS (Figure 4). Based on the Shannon-Wiener diversity index, PSB diversity in BNS (1.11) was lower than in NNS (1.23). As species richness in the two sites were the same (i.e., five species), it can be concluded that the difference in diversity between sites was a result of a difference in PSB abundance.

PSB diversity was tested for correlation with biotic and abiotic factors using the Pearson Correlation statistical test. Several factors were shown to be significantly correlated (<0.05) with PSB diversity, i.e., positively correlated with organic C content (0.99); and negatively correlated with salinity (-0.92), available P (-0.91) and total P (-0.99). The higher the content of organic C, the higher the source of nutrients for the growth of microorganisms, thus high organic C content will support the growth of microorganisms in the soil (Black and Black 2018). Meanwhile, the higher the salinity, PSB diversity becomes lower; this is due to the high salt concentration in the soil affecting the presence of water and nutrients, thus inhibiting the absorption of nutrients by microbes, and decreasing microbial growth and biomass (Canforra et al. 2014).

Analysis using 16S rRNA identified the PSB species with the highest abundance (i.e., Sp.2) as *Salinicola tamaricis*, with 100 percent similarity. The phylogenetic tree of the identification results is shown in Figure 5. These bacteria are Gram-negative bacteria in the form of bacilli and are halophiles, i.e., they can live in environments with high salinity and grow optimally at 35 ppt salinity (Black and Black 2018). *S. tamaricis* is an endophytic bacterium that can tolerate environments containing heavy metals (Zhao et al. 2017). Endophytic bacteria are bacteria that are found in plant tissue and are commonly found in soil. There is little information regarding the interaction of *S. tamaricis* with mangrove plants, but in general, endophytic bacteria can provide benefits to plants, including suppressing the growth of pathogenic bacteria, assisting nutrient absorption in the rhizosphere, and acting as a growth promoter (Castro et al. 2018).

Sediment nutrient content

Measurements for available P and total P were significantly higher in BNS compared to NNS (Table 3). This supports the assumption that the presence of waterbirds acts as a source of P for the mangrove ecosystem. A Pearson correlation test revealed that available P and total P were negatively correlated with PSB diversity (-0.99 and -1.00, respectively), i.e., the higher the sediment P content, the lower the PSB abundance. Lower PSB abundance in BNS compared to NNS (Figure 4) is in line with the fact that organic C content was also lower in BNS (Table 3).

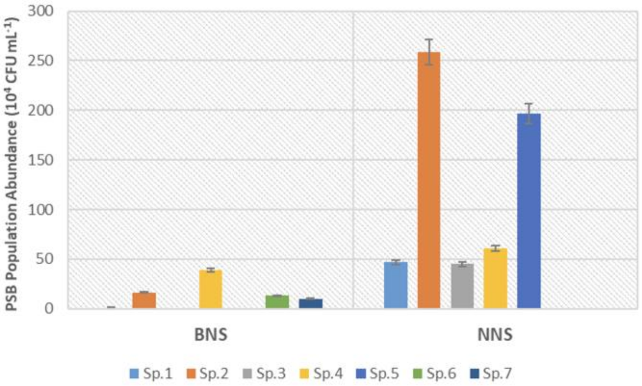

Figure 4. Abundances of different species of phosphate solubilizing bacteria (PSB) in two sampling sites in Rambut Island. BNS: bird nesting site; NNS: non-nesting site

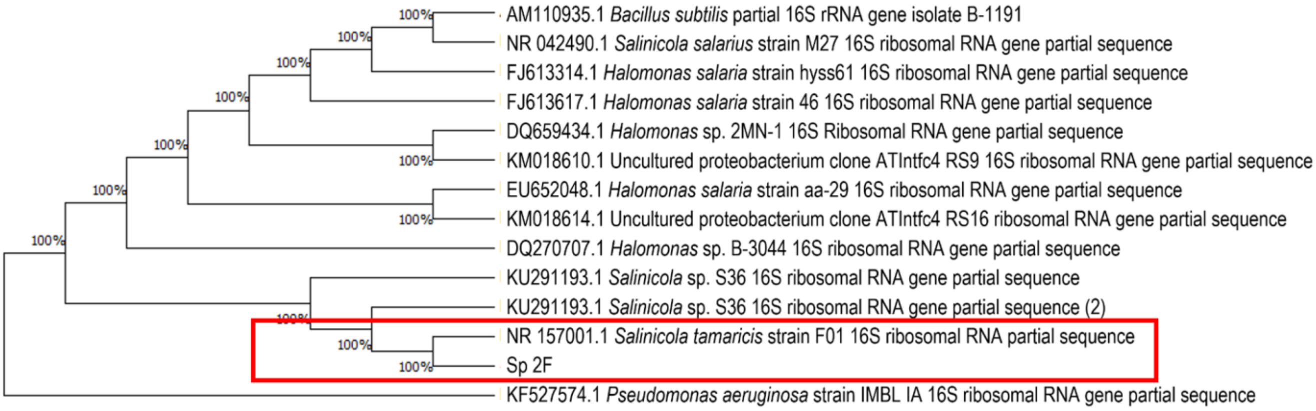

Figure 5. Phylogenetic tree of species identification results for the most abundant PSB found in this study (Sp.2)

Table 3. Comparison between sediment nutrient content in the northern (BNS: bird nesting site) and western (NNS: non-nesting site) mangrove areas. Different superscripts within the same row indicate significant differences at $p<0.05$

Nutrient content	BNS	NNS
Available phosphate (ppm)	930.4 ± 291.4^a	162.5 ± 77.8^b
Total phosphate (ppm)	$1,360.3 \pm 0.0^a$	156.4 ± 0.0^b
Organic carbon (%) *March sampling	6.00 ± 0.13^a	12.50 ± 0.46^b
Organic carbon (%) *July sampling	13.91	17.62

Soil organic C content is affected by the presence of P. Studies in several types of ecosystems have found that the addition of P can change the chemical structure of organic matter, making it easier for microbes to carry out the carbon decomposition process (Wang et al. 2021; Chen et al. 2024; Xia et al. 2024). Jindo et al. (2023) present a review of the complex interrelationship between soil organic matter and phytoavailable P in soils and discuss the many abiotic and biotic mechanisms involved. Biotic mechanisms include the solubilization of inorganic P mediated by microbes through the processes of acidification, chelation, and enzymatic hydrolysis (Jindo et al. 2023). By increasing the rate of decomposition, P addition to the soil promotes the release of CO_2 into the atmosphere, contributing to decreased carbon deposits in the soil (Castro et al. 2018; Wang et al. 2021; Chen et al. 2024; Xia et al. 2024). Therefore, the lower organic C content in BNS sediment may be attributed to a higher decomposition rate due to higher P content.

In the case of BNS sediments, the rationale that P addition affects soil organic C content is supported by data that show a temporal difference in organic C levels (Table 3). During the bird migration and breeding season, which typically ends in April or May, P may be more readily available in the sediments due to bird nesting activities, potentially altering microbial processes, and increasing decomposition rates. Conversely, when birds are no longer nesting in the BNS mangroves, the organic C content appears to increase, likely because the effects of P addition are reduced, and microbial decomposition slows down. This pattern of increasing organic C content post-migration season in BNS sediments is comparable to organic C levels observed in NNS, where P availability and its effects on decomposition are presumably lower. This temporal shift in organic C content is consistent with findings from similar ecosystems where nutrient fluctuations drive changes in decomposition and carbon cycling (Castro et al. 2018).

Further investigation is required to understand the specific contribution of PSB relative to the total microbial community present in the soil. In conditions where P content is low, such as in NNS, P may be a limiting factor for the growth of microorganisms. However, this condition could be an advantage for PSB because PSB can make use of organic P (Behera et al. 2017). In general, the differences in nutrient condition between BNS and NNS sediments are thought to have caused the difference in PSB abundance in the two sites.

In conclusion, analysis of field data confirmed significant differences between the two sites on Rambut Island in terms of mangrove community structure, microclimate, and waterbird abundance. Available and total P in mangrove sediments were significantly higher in the bird nesting site, thus supporting the assumption that waterbirds act as a significant source of P for the mangrove ecosystem. As hypothesized, the PSB community structure differed between sites. This study found that PSB diversity and abundance were lower in the bird nesting site. This may be attributed to the availability of other nutrients, specifically organic C. Besides nutrient availability and microbial competition, other possible explanations could be related to environmental conditions, seasonal or temporal variability, and dynamics in organic matter influx. The relationship among waterbird presence, P availability, and PSB abundance is likely complex, involving multiple ecological interactions and environmental factors that affect microbial community dynamics in mangrove sediments. Further studies are required to elucidate the precise mechanisms at play.

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