

Original Research Article

Size, Abundance, and Shape Diversity of Black Mangrove (*Avicennia Germinans*)

Pneumatophores in a Deforested and Sand-Filled Forest at Eagle Island, Niger Delta

Nigeria

ABSTRACT

Pneumatophores are a major channel of oxygen circulation in mangrove forest. ... It is hypothesized that soil condition, canopy cover and stagnant pool of water can influence pneumatophore growth rate. Pneumatophore abundance, diversity in root types, microbial and chemical composition in sand-filled mangrove forest was studied. Six plots were established within a rectangular area measuring 4152.24m² in a random block design to investigate the effect of soil conditions on pneumatophore growth. A total of 9,586 pneumatophores were physically counted and differentiated into four types namely 1-branch, 2-branch, 3-branch, and 4-branch pneumatophores. The ANOVA results shows that there is significant difference in the abundance of pneumatophore types ($F_{3, 20} = 7.61, p < .001$). The most abundant pneumatophore type is the one branch pneumatophore ($n=4747$) while the least is the four branched pneumatophores. The stagnant pool site with silty and muddy soil has the most abundant pneumatophore growth whereas plots in the seashore site with sandy soil has the least abundant pneumatophores. In contrast, the seashore site has the highest diversity ($H=1.367$) while the stagnant pool has the lowest diversity ($H=0.956$). Metal concentration was higher in the stagnant pool site while microbial count is higher in the seashore site. The study shows that soil condition, canopy cover, stagnant pool plus tidal action influenced pneumatophore growth...

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Keywords: pneumatophore, branching, muddy soil, climate change, oxygen, black mangrove

INTRODUCTION

Mangroves are coastal halophytic plants that can survive in marine, freshwater, and estuarine environments[1]. They are aquatic and semi-aquatic because of their growth in the interface between the land and the sea. Mangroves are reported to be land forming by using their

adventitious roots to trap sediments[2], which metamorphose into muddy soil and later a hard land [3]. Mangroves can survive the difficult, turbulent and salty coastal terrain because of their unique root system that is used for respiration [4]and excretion of heavy metals [5] and salts [6]. The adventitious root of mangroves is called “breathing root” because it is used for gaseous exchange through the transmission of atmospheric oxygen in and out of the plant[7]. The breathing root thus, serves as the lungs of the mangrove trees[8, 9]. This is the reason they can survive when submerged in an aquatic environment or impacted by tidal currents.

The pneumatophore is part of the root system of the black mangroves (*Avicenniagerminans*), which are vertical finger-like projections that protrude from the forest ground beneath the trees[10]. The pneumatophore grows out of the soil into the air to facilitate atmospheric gaseous exchange[11]. The atmospheric oxygen complements the underwater oxygen for submerged roots. There are thousands of these pneumatophores at the base of black mangrove trees forming radial circumference around the forest canopy[12]. The pneumatophores also have the biological significance of regulating salinity exchange between the plant and the marine environment[6]. They act as a shutdown system that prevent the excessive intake of salt solutions into the plant, which helps the mangrove to survive high concentration of sodium chloride and prevent the osmotic collapse of its cell from excess salt crystals. The pneumatophores also serve as an erosion break [13], conduit pipe for the transmission of nutrient minerals and water into the plant and the excretion of salt crystals and other harmful waste substances from the plant. The pneumatophore is one of the organs of the mangroves that has made it to be successful as an aquatic plant. Pneumatophores also play other vital roles in the mangrove ecosystem by providing soil nutrients themselves through the decomposition of their death parts. They increase

the soil nutrients when their litter attract microbes that carry out the decomposition of the organic materials.

...

Pneumatophores are good spawning ground for fingerlings of fish (e.g., tilapia) and other crustaceans e.g., fiddler crabs that reside in the mangrove forest [14]. The finger like projections of the pneumatophores also traps other organisms during low tides, which serves as food for other organisms in the food chain. Crabs and other organisms feed on the soft inner part of the pneumatophores [15]. Because of the numerous physical, chemical, and biological roles played by the pneumatophores, it is thus, important to study the factors that affect their growth, distribution, abundance, and diversity in the deforested sand-filled area. The chemical composition of the pneumatophore is studied because they don't only serve as a sanctuary for spawning organisms, but also serve as a trap for harmful waste such as plastics, which decompose to produce harmful substances consumed by the fish, which is later transferred to human thereby leading to public health issues. The black mangrove was selected because it is the most dominant mangrove species in this study area and the only species with pneumatophores. Other mangroves have giant adventitious roots system that protrude from the ground and form long chains and knee joints but not like that of the black mangroves e.g., red mangroves (e.g., *Rhizophora* species).

The goal of this study, therefore, was to determine the pneumatophore abundance and types, and the chemical, and microbial composition of soil around the pneumatophore in the sand-filled area. The following questions were thus addressed: (1) Are there differences in pneumatophore abundance and diversity within plots. If so which part of the plot or site will have higher pneumatophore abundance and diversity, that is, the stagnant pool that is muddy or seashore that

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is sandy? (2) Are there differences in chemical and microbial composition of the soil at the stagnant pool and seashore sites of the deforested and sand filled mangrove forest? (3) Are there correlations between living (i.e., those still fresh and alive with root still intact) and dead (those whose underground parts have been detached from the soil and had shrunken and withered) or length versus weight, length versus branch and weight versus branch of pneumatophores?

MATERIAL AND METHODS

Description of study area

The study was conducted in a re-grown mangrove forest directly behind the back gate of Rivers State university (Figure 1). Several years ago, this area was covered by a luxuriant mangrove forest with some scattered nypa palm trees. But now part of the forest is gone because it was cut down for the establishment of a sand mine, which has also been abandoned. The area is currently in an early stage of succession by young mangrove seedlings with other non-mangrove plants such as grasses and aquatic weed. There are four mangrove species present in the area namely red (*Rhizophora racemosa*), black (*Avicennia germinans*), grey (*Acrosticum aureum*) and white (*Laguncularia racemosa*). However, the black mangrove is the most dominant species in this ecosystem [16]. During high tides the plant trap plastic and other types of waste within the sand filled area. The soil is light to dark brown in color and has low to medium plasticity because of the muddy nature of the soil and has a fine to coarse sand particles. The temperature is between 28 to 34 °C and the pH is from 6 to 7 while the salinity ranges from 14.5 to 16.2 psu. The muddy area is brownish soft and silty while the sandy area is whitish, coarse, and rough in texture. The area experiences a 6-hourly tidal cycle with water rushing into the sand-filled area during high tide and flowing back into the sea during the low tide.

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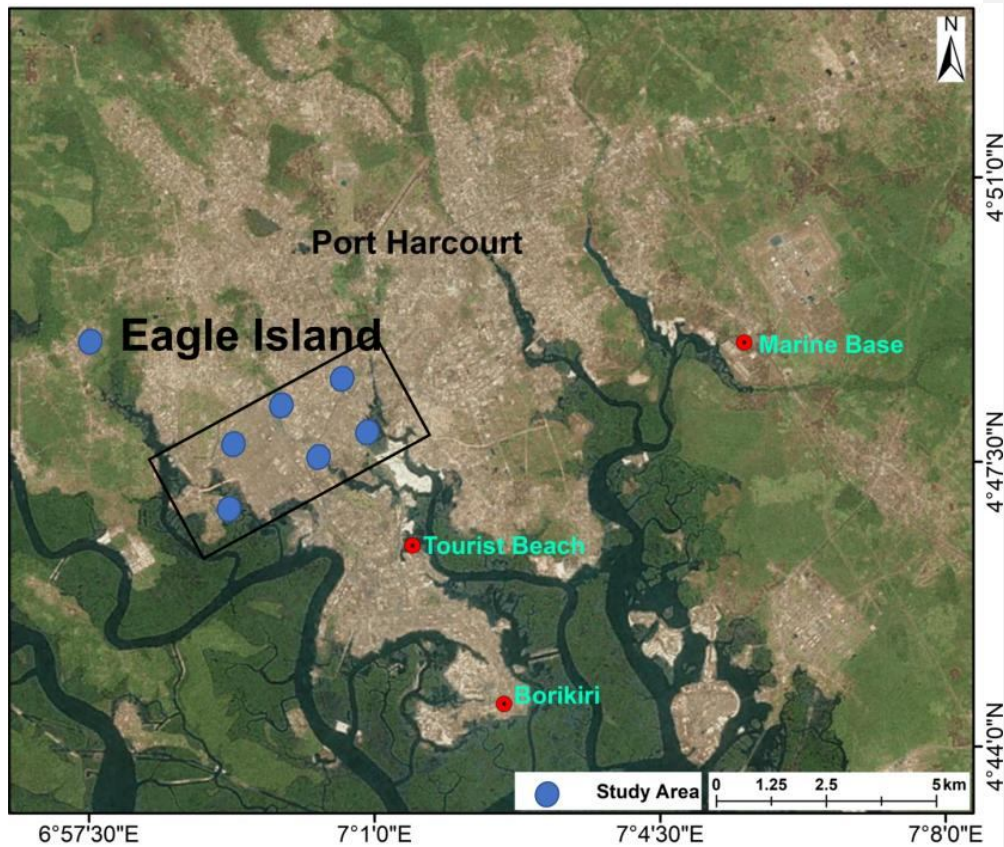


Figure 1. Map of study area at Eagle Island, Niger Delta, Nigeria (NB. rectangle on map not to scale).

Experimental design

The study used a random block design in an area measuring 94.78 m × 43.76 m (4147.577 m²), which was further sub divided into six plots (habitat types) (Figure2). Each plot was delineated equally using a standard tape measure at an accuracy of 0.1m. Two key areas of the plots are the seashore that is sandy and coarse and close to the river (Plots 1-3) and stagnant pool (Plots 4-6) that is muddy and silty and away from the river.

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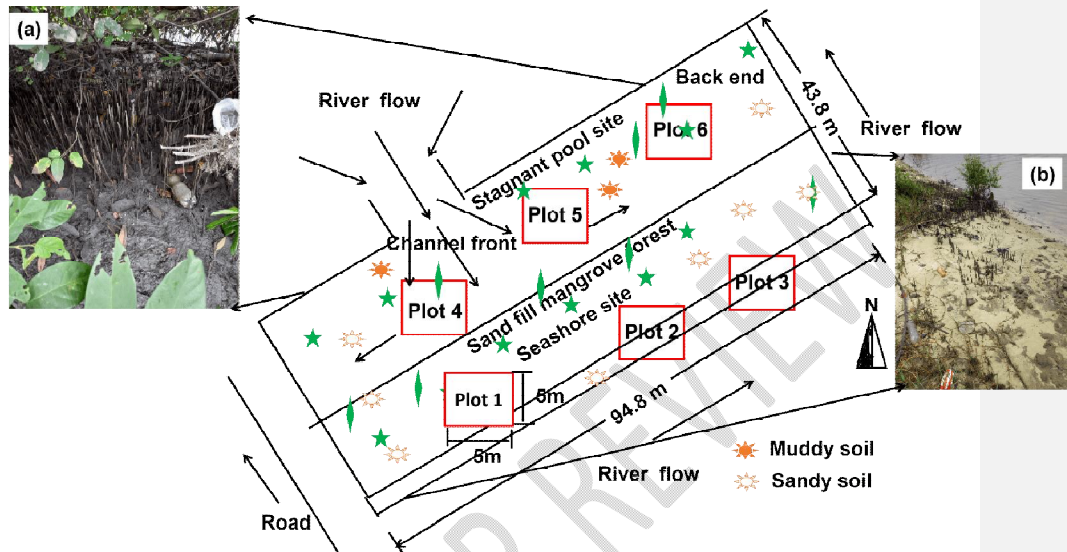


Figure 2. Experimental design of pneumatophore growth, abundance and diversity study carried out in a $94.78 \text{ m} \times 43.76 \text{ m}$ (4152.24 m^2) plot at Eagle Island, Niger delta. The picture indicates (a) the stagnant pool and (b) the seashore sites where the pneumatophores were studied. The drawing of the study area shows the points of sample collection.

Furthermore, the seashore site is always dry during the low tide and covered with water during the high tide while the stagnant pool is constantly wet because it is supplied by river water during high tide and rainwater. The wetness of the stagnant pool has caused the growth of algae (spirogyra) and the standing pool of water serves as a death trap for organisms ranging from insects, fish, and crabs.

In each plot all the pneumatophores that were visible were painstakingly counted physically to get the abundance using line transects measuring $5 \text{ m} \times 5 \text{ m}$ thrown across the ground in series.

Pictures and videos were also used to get a view of the number of pneumatophores. The pneumatophores were grouped according to the number of branches that was identified for the purpose of this study as follows: 1-branch, 2-branch, 3-branch, and 4-branch (Fig. 3).



Figure 3. The four branch types of pneumatophores identified at Eagle Island, Niger Delta, Nigeria.

They refer to the pneumatophores that have one, two, three and four branches growing between 5 cm of the sub surface and surface soil. These branches develop and remain underneath the soil with only one branch protruding from the soil, but when pulled out other attached branches becomes visible. The six plots were georeferenced with a Garmin GPS (USA) (Table 1).

Table 1. Soil characteristics in different plots at Eagle Island, Niger Delta, Nigeria

Plots	Coordinates	Elevation (m)	Moisture content (%)	Total organic content (TOC)	Pore water salinity (%)	Soil compaction (kg/cm ²)	pH	Temp (°C)
Plot 1	N04°47.289; E006°58.558	02.00	20.00	1.44±0.01	1.16±0.02	0.25±0.01	6.8±0.1	26.1±0.1
Plot 2	N04°47.305; E006°58.553	03.00	20.00	1.40±0.01	1.45±0.01	0.21±0.02	6.6±0.1	26.2±0.1
Plot 3	N04°47.315; E006°58.561	03.00	30.00	1.13±0.01	1.48±0.02	0.26±0.01	6.8±0.2	26.4±0.1
Plot 4	N04°47.296; E006°58.521	54.00	60.00	2.03±0.01	1.53±0.02	0.20±0.01	5.4±0.1	29.1±0.1
Plot 5	N04°47.299; E006°58.519	22.00	38.00	1.79±0.01	1.55±0.01	0.23±0.03	6.1±0.1	27.7±0.1
Plot 6	N04°47.293; E006°58.552	04.00	55.00	2.22±0.01	1.55±0.02	0.21±0.02	5.3±0.1	28.5±0.1

Pneumatophore growth and development

Pneumatophores are dominant in this study area because of the large population of black mangroves [16]. They grow in large or small populations underneath the black mangrove trees. They are made up of a soft dark outer and a light inner coat. The outer coat is slippery to the touch and can easily be pulled away. The pneumatophores prevent the growth of other plant species at ~1.8 m circumference around the black mangrove tree.

Pneumatophore abundance and diversity

All pneumatophores in sight were counted *in situ*, and their abundance recorded in a data sheet. The Shannon diversity index (H) was used to determine the pneumatophore types using diversity indices to calculate how different they are. It is the natural logarithm which considers low and high diversities based on abundances. High diversity index means high biodiversity while low diversity index means low biodiversity [17].

Soil sand pneumatophore samples collection

A hand-held soil augur was used to randomly collect soil samples from each plot 5 cm below the soil surface within each transect ($n=10$) in each plot. The samples were placed in a well-labelled polyethylene bags and sent to the laboratory for physico-chemical and microbial analysis. The seashore and stagnant pool sites were studied in detail to determine their influence on the growth and proliferation of the pneumatophore around the coastal mangrove ecosystem. The soil texture and composition were also studied. Ten pneumatophore samples from each plot were randomly pulled by hand, bagged, and sent to the laboratory for measurements of length (cm) and weight (g) in m at the level of accuracy of 0.1 m and 0.1 g respectively and physico-chemically analyzed.

Physico-chemical analysis

Nitrate, iron, cadmium, lead, zinc, copper, and total hydrocarbon content (THC) were determined using the method of [18] Aigberua & Tarawou, (2018) where aliquots of 0.25 g of air-dried sediment samples were weighed into a Teflon inset of a microwave digestion vessel, and 2 ml concentrated (90%) nitric acid (Sigma-Aldrich, Dorset, UK) were added. The metals were extracted using a microwave accelerated reaction system (MARS Xpress, CEM Corporation, Matthews, North Carolina) at 1500 W power (100%), ramped to 175 °C in 5.5 min, held for 4.5 min, and allowed to cool down for 1 h. The cool digest solution was filtered through the Whatman 42 filter paper and made up to 100 ml in a volumetric flask by adding de-ionized water. The detection limit for the metals analyzed was 0.001 mg/l.

Soil pH was determined with a Kelway soil tester while the soil compaction was determined with a pocket penetrometer. Soil temperature was determined with a digital dual sensor thermometer to a detection unit of $\pm 1^\circ\text{C}$. Salinity of the pore water soil was determined with a salinity meter

(OAKTON Salt 6 Acorn Series). The salinity meter probe was used to test standing water in dug out holes during low tide.

Total organic content (TOC) was determined using Walkey-Black titrimetric method (Table 1). The TOC was used to determine the nutrients in the soil. The TOC was determined because soil organic content influences soil texture and composition, which in turn influences mangrove growth [19].

Microbial analysis

Total heterotrophic bacteria (THB) and total heterotrophic fungi (THF) were analyzed from soils collected randomly from each plot. Firstly, the laboratory procedure for the THB was determined as follows: 1g of soil sample was weighed into 9ml sterile diluents (0.85% NaCl) under aseptic condition. It was then shaken vigorously to homogenize and serially diluted. Then 0.1ml aliquot of the inoculums was collected using a sterile pipette, inoculated on nutrient agar surface. The inoculums were spread evenly with a sterile rod. Plates were incubated at 37°C for 24 hours. Thereafter, colonies were counted to obtain colony forming unit (cfu) value per ml of the soil sample. Distinct colonies were picked and streaked on freshly prepared nutrient agar medium to obtain pure culture after 24 hours incubation at 37°C. The pure culture was gram stained for microscopic examination. Secondly, the laboratory procedure for the THF was determined as follows: 1g of soil sample was weighed into 9ml sterile diluents (0.85% NaCl) under aseptic condition. It was then shaken vigorously to homogenize and serially diluted. A 0.1ml aliquot was inoculated on Potato Dextrose Agar (PDA) acidified with 0.1% lactic acid to inhibit growth of bacteria and allowed for only the growth of fungi. Inoculated plates were incubated at ambient temperature for 3-5 days. Cultural characteristics of isolates were observed

and sub-cultured for purification. Microscopic examination was done using lacto phenol cotton blue stain with $\times 400$ magnifications.

Statistical analysis

A one-way analysis of variance (ANOVA) was conducted since there were multiple samples per block ($n = 60$) to test whether there was any significant difference in pneumatophore abundance within plots and habitat types (i.e., six plots) following the example of [20]. Similarly, an ANOVA was used to determine if there were any significant differences in metal concentration and microbial population between plots and sites. Logarithmic transformation of the data was performed to meet assumptions of normality and homoscedasticity [21]. Similarly, a post-hoc Tukey's HSD test was done to investigate pair-wise mean differences between groups. Pearson's product moment correlation was done to compare whether the H_0 of the correlation coefficient $r=0$. Regression analysis was also done to compare pneumatophore abundance and siltiness of the soil. All analyses were performed in *R* statistical environment, 3.0.1 [22].

RESULTS

Pneumatophore abundance

The ANOVA result shows that there is a significant difference in the abundance of pneumatophore ($F_{3, 20} = 7.61$, $p < .001$, Table 2. Figure 4). Similarly, there was significant difference between pneumatophore abundance and sites ($F_{1, 22} = 6.82$, $p < .02$, Table 2. Figure 3). However, there was no significant difference in the abundance of pneumatophore between plots ($F_{5, 18} = 1.33$, $p = 0.30$, Table 2. Fig. 4). A post hoc Tukey's test showed that 1-branch and 4-branch pneumatophores differed most significantly at $P < .05$ (Figure 3). Moreover 1-branch

pneumatophores were the most abundant ($n=4747$) followed by 2-branch ($n= 3620$) and 3-branch pneumatophores ($n=1099$) in all plots. In contrast the 4-branch pneumatophores were the least. Plots 4-6 in the stagnant pool site has the most abundant pneumatophore growth whereas plots 1-3 in the seashore site have the least abundant pneumatophores. In the overall plot 6 has the highest number of pneumatophores while plot 1 has the least number of pneumatophores (Table 2).

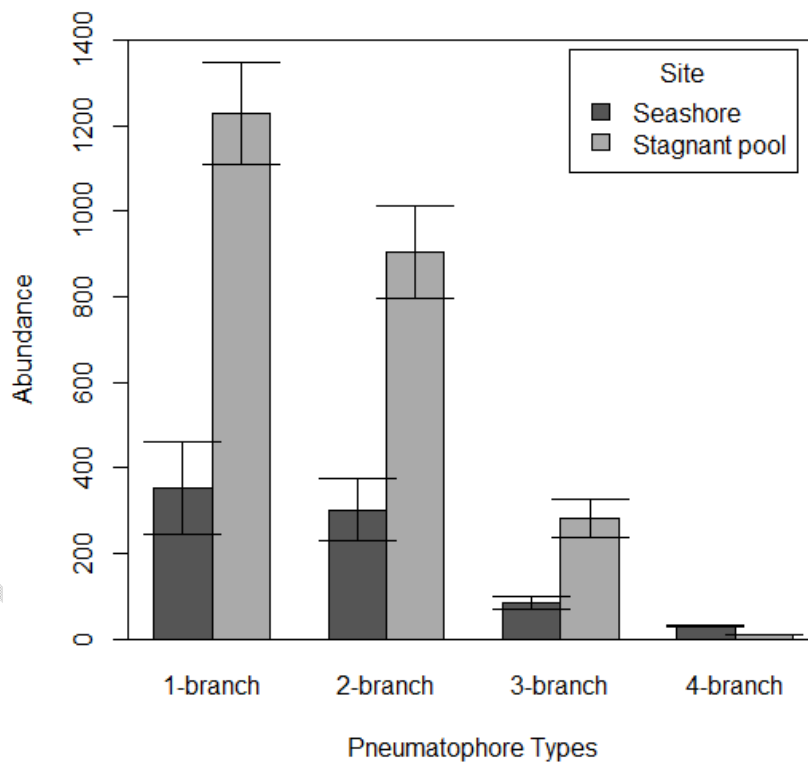


Figure 4. Mean values (\pm SE) of abundance of different types of pneumatophores of black mangroves (*A. germinans*) in different sites at Eagle Island, Niger Delta. It shows that stagnant pool area with muddy soil has a significantly higher pneumatophore abundance than the seashore area with sandy soil. There is also an increase in siltiness (muddy soil condition) from the seashore to the stagnant pool.

Pneumatophore diversity

The pneumatophore diversity was estimated from the pneumatophore abundance within each plot. The result (Table 2) indicates that plot 3, along the seashore has the highest diversity of shapes ($H=1.367$) followed by plot 2 ($H=1.190$) and plot 1 ($H=1.062$), which are all at the seashore and made of sandy soil.; while plot 5, at the stagnant pool made of muddy soil has the lowest diversity ($H=0.956$)

Table 2. Pneumatophore abundance and diversity indices in different plots at Eagle Island, Niger Delta, Nigeria. It shows that the seashore site (Plots 1–3) has higher biodiversity than the stagnant pool site (Plots 4–6).

Site	Plot	Pneumatophore type	Abundance	Proportion (Pi)	Ln (Pi)	Pi* Ln (Pi)	H
Seashore	Plot 1	1-branch	502	0.4883	-0.717	-0.350	-1.062
		2-branch	390	0.3794	-0.969	-0.368	
		3-branch	100	0.0973	-2.330	-0.227	
		4-branch	36	0.0350	-3.352	-0.117	
		Total	1028				
	Plot 2	1-branch	146	0.3842	-0.957	-0.368	-1.190
		2-branch	155	0.4079	-0.897	-0.366	
		3-branch	54	0.1421	-1.951	-0.277	
		4-branch	25	0.0658	-2.721	-0.179	
		Total	380				
	Plot 3	1-branch	412	0.4568	-0.784	-0.358	
		2-branch	362	0.4013	-0.913	-0.768	

	3-branch	98	0.1087	-2.219	-0.241			
	4-branch	30	0.0333	-3.402	-0.113			
	Total	902				-1.367		
Stagnant pool	Plot 4	1-branch	1440	0.5012	-0.691	-0.346		
		2-branch	1119	0.3895	-0.943	-0.367		
		3-branch	302	0.1051	-2.253	-0.237		
		4-branch	12	0.0042	-5.473	-0.023		
		Total	2873				-0.973	
		Plot 5	1-branch	1025	0.4971	-0.699	-0.348	
			2-branch	835	0.4050	-0.904	-0.366	
			3-branch	195	0.0946	-2.358	-0.223	
			4-branch	7	0.0034	-5.684	-0.019	
			Total	2062				-0.950
		Plot 6	1-branch	1220	0.5220	-0.650	-0.339	
			2-branch	759	0.3242	-1.126	-0.365	
			3-branch	350	0.1495	1.901	0.284	
		4-branch	10	0.0043	-5.449	-0.023		
		Total	2341				-1.011	
	Gross Total	9586						

Physico-chemical analysis

The ANOVA result showed that there is a significant difference between concentration of metals in the soil ($F_{3, 20} = 197.1, P < 0.001$, Table 3, Figure 5). But there was no significant difference in metal concentration between sites ($F_{1, 22} = 0.15, P > 0.05$, Table 3). However, there was higher Nitrate concentration in plots 1 (1.48 mg/kg) and 3 (1.50 mg/kg) (Table 3). Similarly, there was higher iron concentration in the seashore plots (1–3) than the stagnant pool plots (4–6).

Table 3. Soil metal composition of different study plots at Eagle Island, Niger Delta, Nigeria.

Seashore plots are 1–3 and stagnant pool plots are 4–6.

Metals (mg/kg)						
Metal	Plot 1	Plot 2	Plot 3	Plot 4	Plot 5	Plot 6
THC	1.22±0.01	0.01±0.00	0.20±0.01	0.40±0.01	2.00±0.01	5.50±1.10
Cadmium	0.21±0.02	0.001±0.00	0.12±0.00	0.001±0.00	0.001±0.00	0.001±0.00

Lead	0.36±0.02	4.63±1.20	0.001±0.00	1.05±0.01	0.46±0.00	1.62±0.1
Zinc	0.001±0.00	0.001±0.01	0.001±0.00	10.06±0.11	2.47±0.20	2.43±0.2
Iron	1631.48±3.22	888.93±7.34	2310.87±2.55	158.38±1.13	156.61±4.66	619.63±
Nitrate	1.48±0.10	1.24±0.01	1.50±0.01	1.42±0.01	1.30±0.01	1.24±0.01
Copper	2.01±0.20	0.85±0.01	2.02±0.01	0.21±0.00	0.10±0.00	1.33±0.00

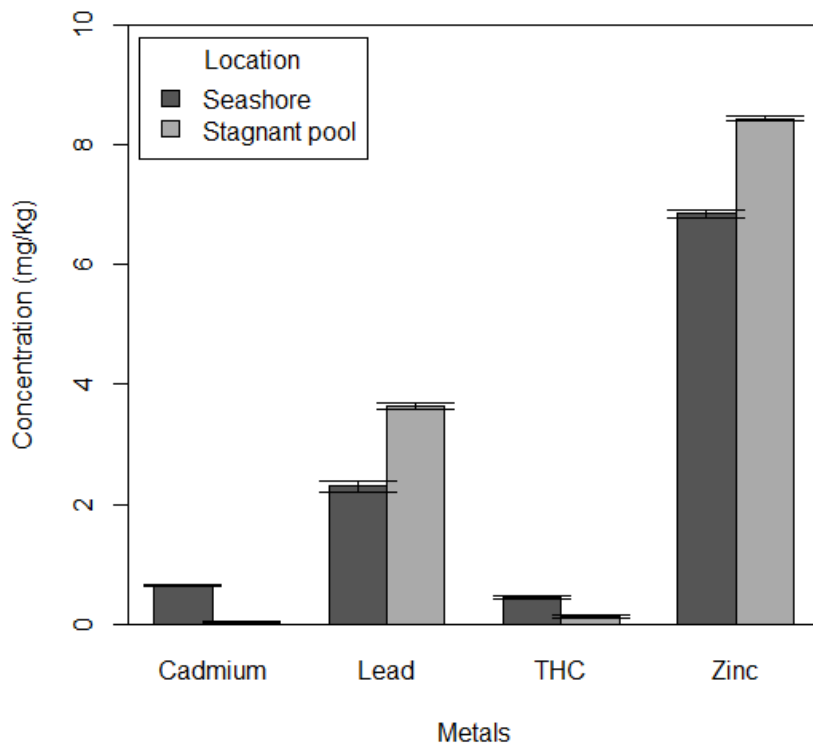


Figure 5. Physico-chemical analysis of soil at some locations at Eagle Island, Niger Delta, Nigeria. It shows that seashore has more Cd and THC while the stagnant pool has more concentration of Pb, and Zn.

Microbial analysis

The result indicates that there is significant difference between bacterial and fungal populations in the soil ($F_{1, 10} = 7.40$, $P=0.02$, Figure. 6). Similarly, there was significant difference in microbial population across plots ($F_{1, 10} = 5.50$, $P < 0.04$).

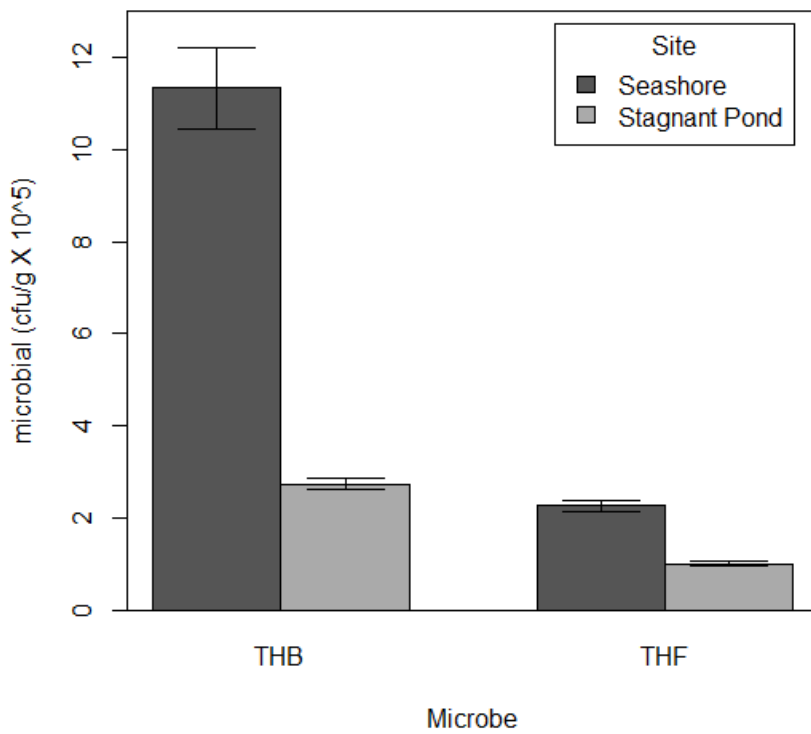


Figure 6. Microbial population at different sites at Eagle Island, Niger Delta, Nigeria. It shows that bacterial population is more dominant than the fungi population. Similarly, seashore site has more microbial population than the stagnant pool site.

Size of pneumatophores

The size of pneumatophore at different sites were recorded by measuring the length (Fig. 7) and weight. The ANOVA result shows that there is a significant difference in the length of the pneumatophores growing in the seashore and stagnant pool sites ($P_{1, 49} 35.7$, $P < 0.001$, Figure 7a). In contrast there is no significant difference in the weight of pneumatophore ($P_{1, 48} 0.02$, $P = 0.897$, Figure 7b),

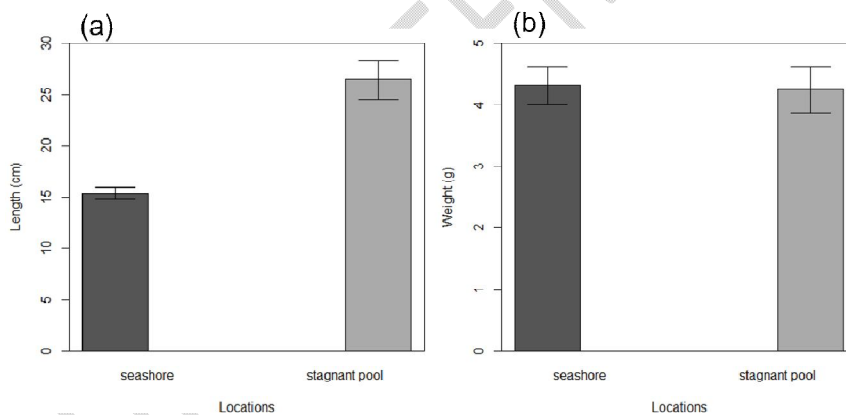


Figure 7. length and weight comparison of pneumatophores growing in different sites at Eagle Island, Niger Delta, Nigeria.

Correlation between living and dead pneumatophores and their length, branch, and weight

Furthermore, the correlation results indicate that there was little or no correlations in the number of dead vs. live ($t=0.033$, $df=37$, $p\text{-value}=0.974$, $cor=0.005402276$, Figure 8), the length vs. branch ($t= -0.43$, $df=48$, $p\text{-value}=0.664$, $cor=0.06289677$); and the weight vs. branch ($t= 0.108$, $df=48$, $p\text{-value}=0.914$, $cor=0.0156338$) of pneumatophores at both sites. However, there is slight correlation between length and weight of pneumatophores ($t=3.427$, $df=48$, $p\text{-value}=0.00126$, $cor=0.4433579$). This means the taller the pneumatophore the heavier it is.

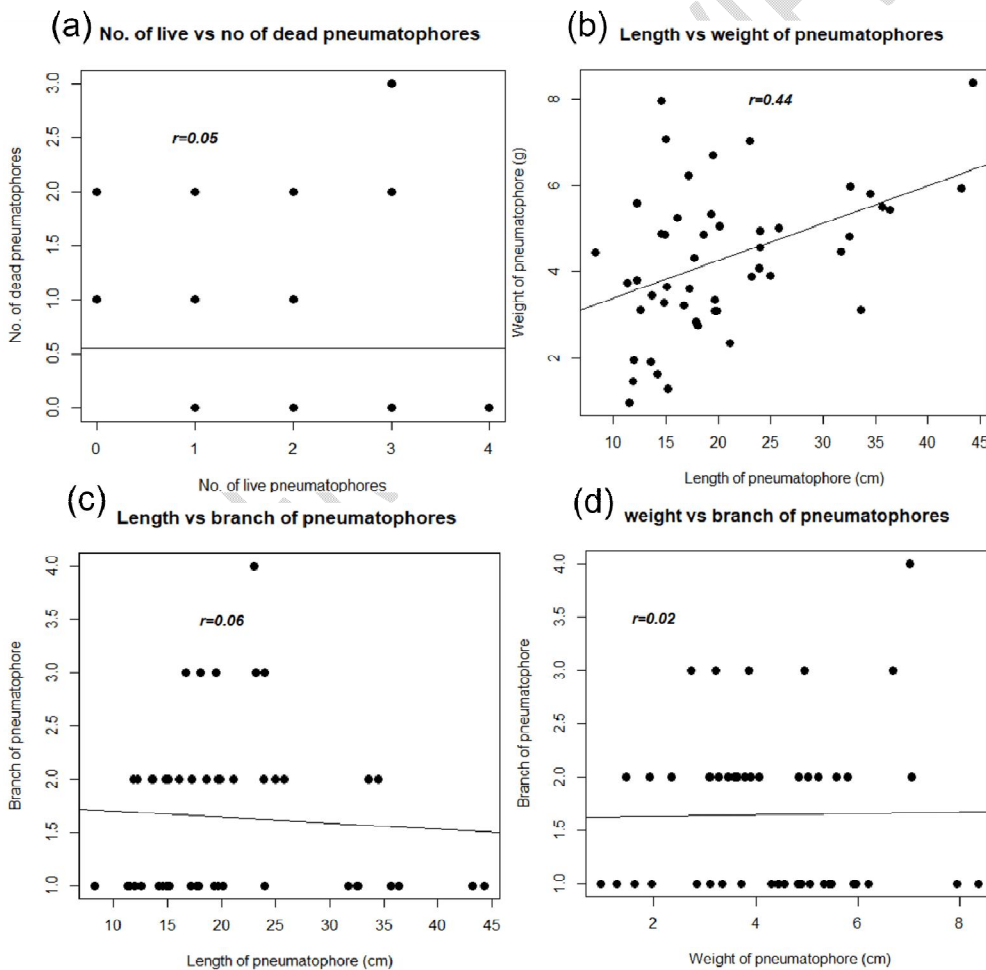


Figure 8. Regression analysis of (a) no. of dead vs, no of alive and (b) length versus weight, (c) length versus branch and (d) weight versus branch of pneumatophores at Eagle Island.

DISCUSSION

Pneumatophores play a unique role in oxygen circulation by acting as the breathing apparatus of mangrove forest globally [23, 24]. Pneumatophores don't only circulate oxygen in mangroves but also play a key role in climate change by helping to sequester carbon through the absorption of atmospheric carbon dioxide by the network of their root systems [25-27]. Therefore, this study is one of the first in the Niger Delta to report the abundance and to classify pneumatophores based on their branches. The result of this study shows that soil chemical and microbial composition influence the growth of pneumatophores (e.g., [28]). Areas with silt and muddy soils have taller (Figure 6a) and more abundant (Table 2) pneumatophores compared to areas with sandy soil (e.g., [29, 30]). Pneumatophores with one branch (i.e., 1-branch) is the most abundant and has high population in the stagnant pool (Figure 3). In contrast the sandy seashore site has more shaped diversity by having more pneumatophore types (1-branch, 2-branch, 3-branch, and 4-branch). Nonetheless, the length and weight of the pneumatophores have little or no correlation to the number of branches (Figure 7). However, the presence of more pneumatophore types is a survival tendency for the *A. germinans* tree to survive in a nutrient-depleted environment such as the seashore where the coast is constantly battered by harsh tidal currents. In other words, the more the branches of the pneumatophore the better it becomes in the utilization of oxygen in an anaerobic environment. On the other hand, the stagnant pool being a more stable environment supports more single branch pneumatophores (1-branch), which grow taller and transmit more oxygen into the mangrove environment and surrounding sediment [31]. Although,

the rich supply of pneumatophores attracts spawning organisms [32, 33], it also serves as a trap for plastic pollutants [34] that contaminates organisms (Figure 3) [35].

This study showed that three key factors influence the growth of pneumatophores in the deforested terrain, namely: stagnant pool of water, soil composition, and tree canopy cover. The stagnant pool has the largest population of pneumatophores because the standing water acts as a trap for organisms (Figure 2), which die and decompose to increase the total organic content of the soil (Table 1). In addition, when the sun heats the pond, chemical reactions do occur (i.e., hydration and hydrolysis), which erode the subsoil leading to an increase in the acidity and heavy metal concentration of the water (Figure 4). The acidic water breaks down many plant and animal materials that enter the stagnant pool [36]. The disintegrated organic matter increases the TOC, which in turn accelerates the growth of pneumatophores. In contrast, the seashore site is made of sandy soil that has little organic content mostly because of the “flushing action” of the tides that sweeps the surface clean of any plant and animal materials during high tide. Nevertheless, the high microbial action at the seashore site (Figure 5) does not translate to a high decomposition rate, leading to low pneumatophore growth. Furthermore, the presence of the black mangrove canopy cover at the stagnant pool sites influences the local climate, which regulates the temperature of the soil and water beneath [37]. The warming of the stagnant pool influences its chemistry and microbiology. Similarly, increased litterfall from nearby trees leads to the increase in the decomposition rate of the fallen leaves. The decomposed leaves form organic matter that increases the fertility of the soil leading to the growth of more pneumatophores.

The presence of a stagnant pool is also a strong factor that facilitates the growth of the pneumatophores by changing the chemical and microbial dynamics of the soil beneath the pool of water. The water helps to hydrolyze and liquify dead plant and animal matter and convert them

to manure for the micro-organisms (e.g., bacteria, and fungi) to act upon. Pneumatophores help the black mangroves to survive the harsh swampy environmental condition by acting as the channel for oxygen transmission into the plants. Their presence in the mangrove forest also contribute to climate stabilization through their action of carbon sequestration. Soil condition, forest canopy and stagnant pool facilitate the growth and development of pneumatophores. The more the population of the pneumatophores the better the environmental quality, which can lead to the proliferation of spawning aquatic organisms useful to humans. Therefore, the abundance and growth in length of the pneumatophores can be used as biological indicator to determine the level of pollution of the mangrove forest, which is significant in pollution studies, restoration ecology and fish population studies.

Comment [A7]: We recommend that the findings and statements in this paragraph can be compared with the results of similar studies.

CONCLUSION

Pneumatophores of the black mangroves (*Avicennia germinans*) play significant role in wetland ecology. Thus, this study reveals that pneumatophores occur in different branches, and the branch formation is influenced by stagnant pool of water, soil composition, microbial content, and tree canopy cover. Areas with stagnant pool of water, rich in nutrients, had more branches compared to the sandy areas with little or no nutrients. Therefore, this study shows that pneumatophores with many branches will have the ability to absorb more oxygen and sequester atmospheric carbon thereby helping to regulate the climate. Future studies will thus consider, sampling more regions and determine the carbon content within the pneumatophores and the soil.

REFERENCES

1. Spencer T, Möller I, Reef R. Mangrove systems and environments. *Journal: Treatise on Geomorphology*, 2022: 675-712.
2. Carlton JM. Land-building and stabilization by mangroves. *Environmental conservation*, 1974; 1(4), 285-294.
3. Krauss KW, Mckee KL, Lovelock CE, Cahoon DR, Saintilan N, Reef R, Chen L. How mangrove forests adjust to rising sea level. *New Phytologist*, 2014; 202(1), 19-34.
4. Pratolongo PD. Salt Marshes and Mangroves: Tidal Saline Wetlands Dominated by Vascular Plants. *Marine Biology: A Functional Approach to the Oceans and their Organisms*, 2022; 211.
5. Hilmi E, Dewi R, Sudiana E, Mahdiana A, Sari LK, Cahyo TN. The Clustering and Distribution of Heavymetal Accumulation and Translocation as an Ability of Mangrove Vegetation to Reduce Impact of Heavymetal (Hg, Cd and Zn) Pollution, 2022.
6. Munir N, Hasnain M, Roessner U, Abideen Z. Strategies in improving plant salinity resistance and use of salinity resistant plants for economic sustainability. *Critical Reviews in Environmental Science and Technology*. 2022; 18;52(12):2150-96.
7. Numbere AO. Mangrove species distribution and composition, adaptive strategies and ecosystem services in the Niger River Delta, Nigeria. *Mangrove ecosystem ecology and function*. 2018; 7;7:17.
8. Ke Y, Li J, Yuan W, Chen Y, Zhao B, Tang Z, Wu X, Zhang S, Tang Y. Mangrove Root-Inspired Carbon Nanotube Film for Micro-Direct Methanol Fuel Cells. *ACS Applied Materials & Interfaces*. 2022; 21;14(17):19897-906.

Comment [A8]: Yellow Highlight >>
Reference year is too long. You should use the last 10 years: 2012-2023

9. Hoogeveen SJ. *Mangrove dynamics in the Richmond River's estuary* (Master's thesis, University of Twente); 2020.
10. Eisenberg EA. Field Guide to the Southeast Coast and Gulf of Mexico. In *A Field Guide to the Southeast Coast and Gulf of Mexico*. Yale University Press; 2002.
11. Clough B. Continuing the journey amongst mangroves. *ISME mangrove educational book series*, 2013; (1), 86.
12. Ong JE, Gong WK. Structure, function and management of mangrove ecosystems. *ISME Mangrove educational book series*. 2013(2):81.
13. Stewart M, Fairfull S. *Mangroves*. Industries; NDOP, Ed.; NSW Government: Parramatta, Sydney. 2008.
14. Mandal B, Mukherjee A, Banerjee S. A review on the ichthyofaunal diversity in mangrove based estuary of Sundarbans. *Reviews in fish biology and fisheries*. 2013; 23:365-74.
15. Roy S. Seasonally and spatially coordinated strategy of detritus conservation and use in the world's largest mangrove ecosystem. In *Proceedings of the Zoological Society* 2011; 64(2): 63-71). Springer-Verlag.
16. Numbere AO. Natural seedling recruitment and regeneration in deforested and sand-filled Mangrove forest at Eagle Island, Niger Delta, Nigeria. *Ecology and Evolution*. 2021;11(7):3148-58.
17. Shannon CE, Wiener W. *The mathematical theory of communication*. Urbana, University of Illinois Press, 1949; 177 p.
18. Aigberua A, Tarawou T. Speciation and mobility of selected heavy metals in sediments of the nun river system, Bayelsa State, Nigeria. *Environ. Toxicol. Stud. J*. 2018;2(1).

19. Alongi D. The energetics of mangrove forests. Springer Science & Business Media; 2009.
20. Quinn GP, Keough MJ. *Experimental design and data analysis for biologists*. Cambridge university press, 2002.
21. Logan M. *Biostatistical design and analysis using R: a practical guide*, John Wiley and Sons, England, 2010.
22. R Development Core Team. R: A language and Environment for Statistical Computing. R Foundation for Statistical Computing, 2013.
23. Kathiresan K, Bingham BL. Biology of mangroves and mangrove ecosystems, 2001; 81-251.
24. Hogarth PJ. *The biology of mangroves and seagrasses*. Oxford University Press, 2015.
25. Zhang C, Zhang Y, Luo M, Tan J, Chen X, Tan F, Huang J. Massive methane emission from tree stems and pneumatophores in a subtropical mangrove wetland. *Plant and Soil*, 2022; 473(1), 489-505.
26. Romero-Uribe HM, López-Portillo J, Reverchon F, Hernández ME. Effect of degradation of a black mangrove forest on seasonal greenhouse gas emissions. *Environmental Science and Pollution Research*, 2022; 29(8), 11951-11965.
27. Ouyang X, Lai DY, Marchand C, Lee SY. Carbon storage and mineralization in coastal wetlands. In *Carbon Mineralization in Coastal Wetlands* (pp. 295-310). Elsevier, 2022.
28. Fusi M, Booth JM, Marasco R, Merlino G, Garcias-Bonet N, Barozzi A, Garuglieri E, Mbobo T, Diele K, Duarte CM, Daffonchio D. Bioturbation intensity modifies the sediment microbiome and biochemistry and supports plant growth in an arid mangrove system. *Microbiology Spectrum*. 2022;10(3):e01117-22.
29. Sarker S, Masud UI Alam M, Hossain MS, Rahman Chowdhury S, Sharifuzzaman SM. A review of bioturbation and sediment organic geochemistry in mangroves. *Geological Journal*, 2021; 56(5), 2439-2450.

30. Best ÜS, Van Der Wegen M, Dijkstra J, Reynolds J, Van Prooijen BC, Roelvink D. Wave attenuation potential, sediment properties and mangrove growth dynamics data over Guyana's intertidal mudflats: assessing the potential of mangrove restoration works. *Earth System Science Data*, 2022; 14(5), 2445-2462.
31. Krauss KW, Allen JA, Cahoon DR. Differential rates of vertical accretion and elevation change among aerial root types in Micronesian mangrove forests. *Estuarine, Coastal and Shelf Science*, 2003; 56(2), 251-259.
32. Wickramasinghe S, Wijayasinghe M, Sarathchandra C. Sri Lankan Mangroves: Biodiversity, Livelihoods, and Conservation. In *Mangroves: Biodiversity, Livelihoods and Conservation* (pp. 297-329). Springer, Singapore, 2022.
33. Deng H, Fu Q, Zhang Y, Li D, He J, Feng D, Zhao Y, Yu H, Ge C. Bacterial communities on polyethylene microplastics in mangrove ecosystems as a function of exposure sites: Compositions and ecological functions. *Journal of Environmental Chemical Engineering*. 2022;10(3):107924.
34. Cesarini G, Scalici M. Riparian vegetation as a trap for plastic litter. *Environmental Pollution*, 2022; 292, 118410.
35. Portz L, Manzolli RP, Villate-Daza DA, Fontán-Bouzas Á. Where does marine litter hide? The Providencia and Santa Catalina island problem, seaflower reserve (Colombia). *Science of the Total Environment*, 2022; 813, 151878.
36. Das, N, Mondal A, Mandal S. Polluted waters of the reclaimed islands of Indian Sundarban promote more greenhouse gas emissions from mangrove ecosystem. *Stochastic Environmental Research and Risk Assessment*, 2022; 36(5), 1277-1288.

37. Mariano H, Aguilos M, Dagoc FL, Sumalinab B, Amparado R. Abandoned Fishpond Reversal to Mangrove Forest: Will the Carbon Storage Potential Match the Natural Stand 30 Years after Reforestation?. *Forests*, 2022; 13(6), 847.

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