Isolation And Identification of Nitrogen Fixing Bacteria at The Rhizosphere of Maize Cultivated Within Three Area NDU Teaching and Research Farm, Ogobiri and Amasoma

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Abstract
This study presents a comparative analysis of soil physical properties across three selected locations—Ogobiri, Amassoma, and Niger Delta University (NDU)—in Bayelsa State, Nigeria, with the aim of assessing their implications for agricultural land use and soil management. Soil samples were collected at two depths (0–15 cm and 15–30 cm) and analyzed for texture, bulk density, porosity, and microbial characteristics. Results showed that soils across all sites were predominantly sandy loam or loam, with varying proportions of sand, silt, and clay. Amassoma soils recorded the highest porosity (75%) and lowest bulk density (0.65 g/cm³), indicating better soil aeration and potential for root penetration, while Ogobiri soils showed higher bulk density (1.05 g/cm³), potentially limiting plant root development. NDU recorded the highest sand content (81.2%) at surface level, indicating lower water-holding capacity but good drainage. Microbial analysis revealed a high concentration of colony-forming units (CFUs) across all sites, with Bacillus spp. (rod-shaped bacteria) dominating Ogobiri and NDU topsoils, while Cocci spp. (spherical bacteria) were prevalent in Amassoma soils. The presence of beneficial microbial communities and their abundance, especially in NDU and Ogobiri, suggests high microbial activity that could enhance soil fertility and nutrient cycling. The findings suggest that Amassoma soils, with higher porosity and loam texture, are most suitable for crop cultivation, particularly maize, due to favorable physical and microbial characteristics. Ogobiri and NDU soils, while still agriculturally viable, may require amendments or specific management practices to improve water retention and reduce compaction. This study underscores the importance of site-specific soil evaluation for optimized land use planning and sustainable agriculture in the Niger Delta region.

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops cultivated globally for food, feed, and industrial uses. In Nigeria, maize plays a vital role in ensuring food security and generating income for rural households (Adebayo et al., 2018). However, its production is often limited by low soil fertility, particularly nitrogen deficiency, which is a major constraint in tropical agriculture. Nitrogen is an essential macronutrient required for plant growth and development, as it is a fundamental component of amino acids, proteins, and nucleic acids (Raghunath *et al*., 2021). Biological nitrogen fixation (BNF) has emerged as a sustainable and eco-friendly approach to improving soil fertility. This natural process is facilitated by a group of specialized microorganisms known as nitrogen-fixing bacteria, which convert atmospheric nitrogen (N_2) into ammonia (NH_3), a form that plants can readily absorb (Kumar et al., 2020). Among these beneficial microbes, species from genera such as *Azotobacter*, *Azospirillum*, *Rhizobium*, and *Klebsiella* are

commonly associated with the rhizosphere — the narrow zone of soil surrounding plant roots (Muthukumarasamy *et al*., 2017). These bacteria not only fix nitrogen but also enhance plant growth through the production of phytohormones, siderophores, and other growth-promoting substances.

The maize rhizosphere presents a dynamic environment that supports a diverse population of nitrogen-fixing bacteria, influenced by factors such as soil type, plant genotype, and agricultural practices (Singh et al., 2019). Investigating the microbial communities in different maize-growing locations can offer insights into their potential for use as biofertilizers. In this context, the isolation and identification of nitrogen-fixing bacteria from the rhizosphere of maize cultivated in three specific areas — the Niger Delta University (NDU) Teaching and Research Farm, Ogobiri, and Amassoma — is of particular importance. These sites represent varied agro-ecological zones within the Niger Delta region and provide an opportunity to assess the diversity and distribution of nitrogen-fixing bacteria in maize cultivation systems. Understanding the types and capabilities of indigenous nitrogen-fixing bacteria can contribute significantly to the development of locally adapted biofertilizers that enhance maize productivity and reduce dependence on synthetic nitrogen fertilizers, which are often expensive and environmentally harmful (Deng *et al*., 2021). Therefore, this study aims to isolate and identify nitrogen-fixing bacteria associated with the rhizosphere of maize in the selected locations, thereby providing a scientific basis for sustainable agricultural practices in the region.

Materials and Methods

Study Area

The study was conducted in three maize cultivation sites located in Bayelsa State, Nigeria: Niger Delta University (NDU) Teaching and Research Farm, Ogobiri, and Amassoma. These locations lie within the humid tropical rainforest zone and are characterized by high annual rainfall, warm temperatures, and loamy to clayey soils. The coordinates of the sites are approximately:

- NDU Teaching and Research Farm: 4.9785° N, 6.1020° E
- Ogobiri: 4.9611° N, 6.1147° E
- Amassoma: 4.9606° N, 6.1097° E

Soil Sample Collection

Soil samples were collected from each location at two depths: 0–15 cm (topsoil) and 15–30 cm (subsoil). A soil auger was used to extract samples at randomly selected points within each site. Three replicates were collected per depth, and composite samples were prepared by mixing the replicates thoroughly in clean, labeled polythene bags. Samples were transported to the laboratory for further analysis.

Determination of Soil Physical Properties

The following physical properties were analyzed:

Soil Texture: The particle size distribution (% sand, silt, and clay) was determined using the hydrometer method (Gee & Or, 2002). The soil textural class was then determined using the USDA soil texture triangle.

Bulk Density: Bulk density was determined by the core method as described by Blake and Hartge (1986). Undisturbed soil cores were weighed, oven-dried at 105°C for 24 hours, and the bulk density was calculated as the ratio of dry weight to the volume of the soil core.

Total Porosity: Porosity was computed from bulk density values using the formula:

Porosity (%)=(1−Bulk DensityParticle Density)×100\text{Porosity (\%)} = \left(1 - \frac{\text{Bulk Density}}{\text{Particle Density}}\right) \times 100Porosity (%)=(1−Particle DensityBulk Density)×100
A particle density of 2.65 g/cm³ was assumed for mineral soils.

Microbial Analysis
Soil microbial populations were assessed using the serial dilution and pour plate method. One gram of each soil sample was suspended in 9 ml of sterile distilled water and serially diluted up to 10^{-6}. Aliquots (1 ml) from appropriate dilutions were plated on nutrient agar and incubated at 37°C for 24–48 hours. Colonies were counted and expressed as colony-forming units (CFU) per gram of soil.

Bacterial Identification
Distinct colonies were selected based on morphology and streaked onto fresh nutrient agar plates for purification. Gram staining and microscopic examination were used to identify the bacterial shape (rod or spherical). The genera of the isolates, primarily *Bacillus* and *Cocci*, were tentatively identified based on colony morphology, gram reaction, and literature comparison (Cheesbrough, 2006).

Data Analysis
Data collected were subjected to descriptive statistical analysis to determine the mean values of soil physical parameters and microbial counts across locations and depths. Comparative assessments were made between sites to determine the relative soil quality and potential for agricultural land use. Graphical illustrations (bulk density and porosity distribution) were generated using Microsoft Excel.

Results and Discussion
The colony forming units (CFUs) in Table 1 varied across the three sampled locations: Amassoma, Ogobiri, and Niger Delta University (NDU). Notably, NDU samples recorded the highest microbial activity, with CFU values reaching up to 284 in replicate NDU 2A. Ogobiri followed closely, with values such as 267 and 279 in 1B1 and 2B samples respectively. Amassoma soils generally had lower CFU values, especially in the 2B series. These results indicate higher microbial loads in NDU and Ogobiri, suggesting more biologically active soils. This observation aligns with the findings of Ibekwe *et al*. (2010), who reported higher microbial populations in soils with moderate-to-high porosity and favorable textural conditions. Furthermore, Araujo *et al.* (2008) emphasized that microbial abundance is influenced by soil aeration, organic matter content, and texture—factors likely favorable in NDU and Ogobiri soils. Contrastingly, Amassoma's lower microbial counts could result from higher clay content at depth, which may restrict air flow and microbial activity (Chaudhary *et al*., 2012). This supports the report by Njoku *et al*. (2015), which found clayey soils in southern Nigeria to have reduced microbial diversity and abundance.

Table 1: Colony forming of soil used for planting of maize

AMA 1A	OGO 1A	NDU 1A
A 71	A 86	A 96
B 98	B 157	B 124
C 120	C 200	C 158
AMA 1B1	OGO 1B1	NDU 1B1
A 79	A 162	A 172
B 78	B 211	B 148
C 118	C 267	C 158
AMA 2A	OGO 2A	NDU 2A
A 117	A 109	A 87
B 97	B 118	B 284
C 167	C 113	C 229
AMA 2B	OGO 2B	NDU 2B
A 2B 117	A 162	A 143
B 83	B 233	B 164
B 100	C 279	C 225

Table 2: Morphological Characteristics of Bacterial Isolates

Morphological analysis of isolates revealed that Ogobiri and the NDU topsoil harbored rod-shaped *Bacillus* spp., while Amassoma soils, along with NDU subsoil, had spherical *Cocci* spp. Furthermore, the bacterial load was higher in Ogobiri (2.6×10^6 CFU at 15–30 cm) and NDU subsoil (2.2×10^6 CFU), suggesting active microbial communities even at deeper layers. The dominance of *Bacillus* spp. in sandy and well-drained Ogobiri soils corresponds with the observations of Singh *et al*. (2011), who noted the preference of *Bacillus* for aerated soils due to their spore-forming abilities. Meanwhile, *Cocci* spp. found in the denser Amassoma subsoils may reflect their ability to survive in more compacted environments. This aligns with the report of Nannipieri *et al.* (2017), which explains how microbial morphology and diversity change with depth and physical conditions like bulk density and porosity. The presence of high CFU counts in subsoils challenges the conventional belief that microbial activity is limited to the surface (Venkateswarlu *et al*., 2013), indicating vertical microbial diversity in these soils.

Table 2: Morphological Characteristics of Bacterial

Isolates	Soil depth (cm)	Shape	Type of bacteria present	Colony Forming Unit (CFU)
Ogobiri	0-15	Rod	Bacillus spp	**8.6×10^5**
Ogobiri	15-30	Rod	Bacillus spp	**2.6×10^6**
Amassoma	0-15	Spherical	Cocci spp	**9.8×10^5**
Amassoma	15-30	Spherical	Cocci spp	**7.9×10^5**
NDU	0-15	Rod	Bacillus spp	**8.7×10^5**
NDU	15-30	Spherical	Cocci spp	**2.2×10^6**

Bulk Density Distribution in the Sampling Locations

Figure 1 illustrates the bulk density distribution across the locations. Amassoma exhibited the lowest bulk density (0.65 g/cm³) in the 0–15 cm layer, while Ogobiri had the highest (1.05 g/cm³) at the same depth. Lower bulk density is generally favorable for root growth, microbial activity,

and water infiltration (Hillel, 2004). The findings corroborate Lal (2015), who reported that loam soils, as found in Amassoma, often have lower bulk densities due to their balanced sand, silt, and clay content. On the other hand, Ogobiri's higher sand content and compaction likely contributed to its higher bulk density, a trait known to hinder root penetration and gas exchange (Brady & Weil, 2017).

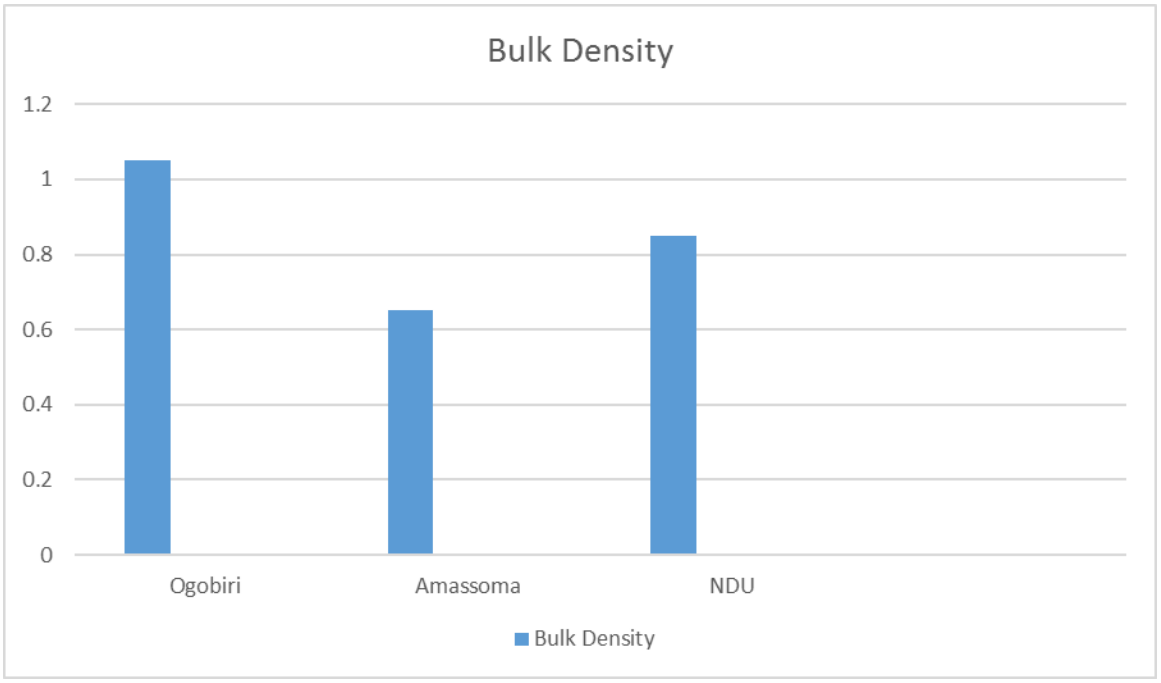

Fig 1: Bulk Density Determinations Distribution in the Sampling Locations

Porosity Percentage Distribution in the Sampling Locations

The inverse relationship between bulk density and porosity is evident in Figure 2. Amassoma's high porosity (75%) supports microbial activity and water retention, whereas Ogobiri's lower porosity (60%) may reduce microbial aeration and infiltration rates. This aligns with the work of Batey (2009), who emphasized that soil porosity above 50% is necessary for optimal microbial and root activities. The lower porosity in Ogobiri is consistent with its higher bulk density and coarser texture.

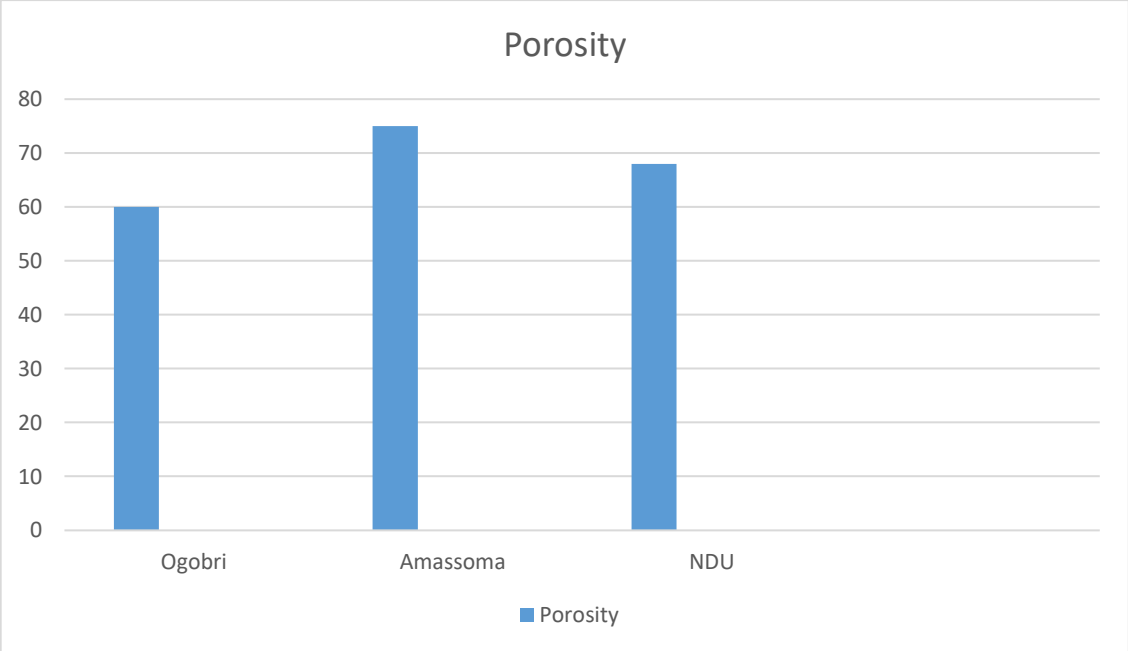

Fig 2: Porosity Percentage distribution in the Sampling Locations

Table 3: Soil Physical Properties

From Table 3, the textural classifications show that:

- Ogobiri soils are sandy loam with increasing sand content with depth.
- Amassoma soils are loam, with higher clay content in the subsoil (27.4%).
- NDU topsoil is loamy sand, transitioning to sandy loam at depth.

These textural variations significantly influence bulk density and porosity. Amassoma's loam texture explains its low bulk density and high porosity, conducive for root and microbial development. This is supported by the findings of Adamu and Tanimu (2020), who noted that loamy soils promote better water and nutrient retention than sandy soils. In contrast, NDU's loamy sand texture, while aerated, may require organic amendments to improve water retention. Ogobiri's moderate sand and silt combination yielded a balanced sandy loam but with higher compaction, possibly due to land use history or low organic matter input (Ogunwale et al., 2002).

Table 3: Soil Physical Properties

Depth	Location	% Sand	% Silt	% Clay	Textual Class	Bulk Density	Total Porosity (%)
0-15	Ogobiri	51.2	39.4	9.4	Sandy Loam	1.05	**60**
15-30	Ogobiri	59.2	27.4	13.4	Sandy Loam		
0-15	Amassoma	43.2	37.4	19.4	Loam	0.65	**75**
15-30	Amassoma	35.2	37.4	27.4	Loam		
0-15	NDU	81.2	5.4	13.4	Loamy Sand	0.85	**68**
15-30	NDU	51.8	27.4	21.4	Sandy Loam		

Conclusion

In summary, the physical and biological characteristics of the studied soils show spatial variation across the three locations. Amassoma soils emerged as most suitable for sustainable agriculture, given their low bulk density, high porosity, and favorable microbial populations. NDU soils, while sandy, hosted high bacterial loads, especially in subsoils, suggesting deeper-rooted crops may thrive. Ogobiri's relatively compacted soils may require management interventions to enhance structure and porosity. These results provide critical insights for site-specific soil fertility and land-use planning in Bayelsa State, in line with recommendations by FAO (2006) for soil management based on localized physical and biological assessments.

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