Nig. J. Biotech. Vol. 41(2): 45-53 (Dec. 2024)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and https://bsn.org.ng
DOI: https://dx.doi.org/10.4314/njb.v41i2.4

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Potential of anthill soils and their bacteria as a viable source of soil amendment, biofertilizer, and biocontrol

[1]B. J. Enagbonma and [2]*E. E. Imade

[1]Department of Environmental Management and Toxicology; and [2]Department of Microbiology,
Faculty of Life Sciences, University of Benin, P.M.B. 1154, Benin City, Nigeria
*Corresponding author's email: emmanuel.imade@uniben.edu; Tel: +2347734248047

Abstract

The escalating costs of synthetic fertilizers and their harmful environmental impacts have sparked a growing interest in exploring sustainable alternatives for crop cultivation. This investigation explored the potential of soil from anthills as a nutrient-rich resource for use in soil amendment, biological fertilizers, and biocontrol applications. Anthill soils and nearby areas were examined for their nutrient content and bacterial diversity. The microbial isolates were also examined for plant growth-promoting (PGP) attributes by employing standard analytical procedures and cultural approaches. To test for the antagonistic properties of the screened microbial isolates against *Fusarium oxysporum*, the diffusible substance method was used. The results revealed that anthill soils possess higher total bacterial counts and superior physicochemical properties compared to adjacent soils, except for silt, sand, and pH levels. The study identified beneficial bacteria from genera such as *Bacillus* and *Pseudomonas*, which exhibited plant growth-promoting properties and antagonistic activity against soil *Fusarium oxysporum.* These findings suggest that anthill soils are nutrient-rich and harbour beneficial bacteria that can promote plant growth and suppress soil pathogens.

Keywords: bioengineering; chemical fertilizer, food sustainability; soil assessment; soil microbial community

Introduction

In recent years, the agricultural sector has witnessed an increasing demand for sustainable and eco-friendly practices due to the negative impacts of conventional farming (Amoo et al. 2021). The extensive utilization of synthetic pesticides and fertilizers, while effective in boosting crop yields, has led to significant environmental degradation, including soil depletion, water contamination, and the decline of biodiversity (Enagbonma et al. 2024). These challenges have driven researchers to explore alternative methods of soil management and crop production that are both economically viable and environmentally sustainable (Enagbonma and Babalola 2019). One such promising approach involves the use of naturally occurring biological resources, particularly anthill soils, which have shown considerable potential in enhancing soil fertility, promoting plant growth, and controlling plant pathogens (Enagbonma and Babalola 2023).

Anthills are complex structures built by social insects such as ants and termites and these structures are not only architectural marvels but also serve as nutrient-rich environments that support diverse microbial communities (Fernandez-Bou et al. 2019). Soil ants are essential in decomposing organic matter and recycling nutrients into the soil, thereby aiding in soil formation and enhancing its fertility (Santamaría et al. 2020). As a result, anthill soils are often found to contain potassium, phosphorus, and nitrogen, which are critical nutrients for plant development (Urbańczyk and Szulc 2023). Moreover, the unique physicochemical properties of these soils, coupled with the diverse microbial populations they could harbour, make them a potential resource for sustainable agriculture (Delgado-Baquerizo et al. 2019).

The idea of utilizing anthill soils as a natural soil amendment has gained traction due to their reported ability to enhance soil structure, boost nutrient availability, and stimulate beneficial microbial activity (Chisanga et al. 2020). Soil amendments are substances incorporated into the soil to enhance its chemical and physical properties, thereby improving its capacity to support plant growth (Campos et al. 2020). In the case of anthill soils, the information on the presence of beneficial bacteria, including those with plant growth-promoting (PGP) capabilities is still handy, and this can contribute immensely in fostering a healthy soil microbiome (Enagbonma and Babalola 2020). These bacteria could have the capacity to fix atmospheric nitrogen, solubilize phosphate, and produce growth hormones, all of which contribute to improved plant health and increased crop yields (Enagbonma and Momoh 2024). Additionally, certain bacterial isolates found in anthill soils could exhibit antagonistic properties against soil-borne pathogens, offering a natural form of biocontrol.

Building on previous research that has shown anthill soil to have exceptional nutrient properties (Hidalgo et al. 2021; Ezeaku et al. 2015), this study aims to examine the soil nutrient properties of anthills, along with the contribution of resident microbes to nutrient cycling and the mitigation of disease causing organisms in plants. This investigation aimed to confirm three key hypotheses: i. anthill soil has a higher nutrient content compared to surrounding soil; ii. the bacterial communities in anthill soil are distinct from those in adjacent soil; and iii. bacteria in anthill soil exhibit enhanced capabilities for nutrient cycling and pathogen suppression. This study sought to further understand the unique properties of anthill soil, which previous studies have attributed to factors such as improved temperature regulation, near-neutral pH, increased organic matter, and enhanced aeration (Kristiansen and Amelung 2001; Frouz and Jilková 2008).

Materials and methods

Soil collection and study locations

Soils (50 g) were mined from eight anthills, with two sets of four anthills each located in Ekosodin (A1a-d) (Lat. 6^0 23^1 42^{11} North, Long. 5^0 36^1 49^{11} East) and Ugbowo (A2a-d) (Lat. 6^0 23^1 45^{11} North, Long. 5^0 36^1 54^{11} East), both in Benin City, Nigeria. Each anthill was approximately 2 meters apart, and soils were mined from up to the bottom of the anthill (0-15 cm depth), where ant activity was evident (Chisanga et al. 2020). For comparison, adjacent soil samples (S1a-d and S2a-d) were collected 10 meters away from each anthill, at the same depth. This distance was chosen to ensure the adjacent soil was unaffected by anthill activities. Soil samples were stored in containers with ice packs during transport to the laboratory for further analysis of bioagents and physicochemical properties. The 0–15 cm soil depth was selected, as this zone typically exhibits the highest microbial activity (Enagbonma et al. 2022).

Soil Parameter assessment

Pre-processed soil samples (20g) were analysed for physical and chemical properties. Available phosphorus was measured spectrophotometrically (Mussa et al. 2009), and organic carbon was determined following the technique outlined by Wakung'oli et al. (2020). Exchangeable magnesium and calcium levels were measured using atomic absorption spectrophotometry (Imbrea et al. 2016). potassium was evaluated using flame photometry (Banerjee and Prasad 2020). Nitrogen content was determined through the Kjeldahl method (Goyal et al. 2022). The pH level was evaluated via a pH-meter with a 1:2.5 soil-to-water ratio (Enagbonma and Solomon 2024). The particle size distribution was assessed using a hydrometer technique (Ashworth et al. 2001).

Bacterial isolation and characterization

A serial dilution series was prepared from 1g of soil, progressing up to the fifth dilution. Subsequently, aliquots were transferred to various sterile agar plates as previously described by Lahsini et al. (2022), then the inoculated plates were subsequently incubated for 24 hours at 37°C. Thereafter, distinct colonies were picked depending on their morphological features and pure isolates were obtained via subculturing. The bacterial isolates were characterized using a combination of biochemical and morphological tests (ALKahtani et al. 2020; Okoduwa et al. 2022).

Assessment of microbial isolates for PGP attributes Phosphate solubilization test

A spot inoculation of the bacterial isolate was made at the middle of a sterile molten Pikovskaya agar plate, which was then incubated at 30°C for a period of 72 hours. Subsequently, the diameters of the phosphate solubilization zones surrounding the colonies were measured and recorded. The solubilization-index was calculated with the formular previously used by Wasoontharawat

(2017) by using the colony diameter to divide the total halo diameter.

Test for ammonia production

Bacterial cultures in their exponential growth phase were introduced into 10ml of nutrient broth and subjected to incubation at 30°C for 48 hours, with constant agitation provided by a rotator shaker. Following incubation, 0.5ml of Nessler's reagent was added into the individual tube. The appearance of a brown and yellow coloration signified a positive result for the production of ammonia (Adebajo et al. 2021).

Antifungal effects of PGP bacteria against Fusarium oxysporum

The antifungal activity of bacterial strains was evaluated against *Fusarium oxysporum* using a dual culture assay. *F oxysporum* was cultivated on Sabouraud dextrose agar plates, and a 7mm disc was excised from the actively growing culture and placed at the plate's centre. A 24-hour-old bacterial culture was streaked approximately 2.5cm away from the fungus. Following incubation at 28°C for 5 days, the plates were examined for zones of inhibition surrounding the bacterial colonies. The percentage inhibition of fungal growth was measured following a modified formula from Adebajo et al. (2021).

Statistical analysis

To ensure accuracy and reliability, all tests were performed in triplicates. The means of resulting data were then subjected to statistical analysis using a combination of software tools, including SPSS Statistics v21, PAST v2.17c, and Microsoft Excel v2010. This involved conducting ANOVA and using descriptive statistics to analyse the dataset and draw meaningful conclusions.

Results

Examination of soil physical and chemical properties

An examination of soil physicochemical characteristics (Table 1) indicated that anthill soils possessed elevated levels of essential nutrients, including OC, P, K, OM, Ca, Mg, TKM, and clay (with the notable exceptions of S2b and S2d), when compared to neighbouring soils. Conversely, adjacent soil samples displayed increased proportions of sand, silt, and pH levels, distinguishing them from anthill soils.

Table 1: Soil nutrient values from both soil samples

Site	pH	OC (%)	OM (%)	TKN (%)	P (mg/L)	Ca (mg/L)	K (mg/L)	Mg (mg/L)	Sand (%)	Silt (%)	Clay (%)
A1a	6.1	1.74	3	2.16	23.15	0.35	6.32	0.97	94	1.72	4.28
A1b	6	1.82	3.14	1.78	48.78	0.32	6.12	0.88	92	1.74	6.26
A1c	5.2	1.72	2.97	1.56	22.63	0.27	5.63	0.97	95	1.2	3.8
A1d	6.1	1.82	3.14	2.38	48.78	0.32	6.12	0.94	93	1.24	2.76
S1a	6.6	1.72	2.97	1.22	22.63	0.27	5.63	0.95	96	2.75	1.25
S1b	6.4	1.16	2	1.14	20.27	0.25	5.48	0.78	93	2.14	4.86
S1c	6.6	1.37	2.36	1.22	28.17	0.22	4.36	0.82	96	2.75	1.25
S1d	6.4	1.28	2.21	1.78	31.46	0.18	5.27	0.78	96	2.14	4.86
A2a	5.9	0.65	2.53	2.13	36.54	0.28	5.18	0.93	94	1.25	3.37
A2b	6.3	0.87	2.5	2.36	43.11	0.37	5.23	0.82	91	1.85	6.73
A2c	6.4	0.92	2.53	2.13	36.54	0.28	5.18	0.93	93	1.25	5.68
A2d	6.3	0.83	2.5	2.36	43.11	0.37	5.23	0.93	92	1.48	3.52
S2a	6.8	1.47	1.21	1.28	18.63	0.12	3.11	0. 74	95	1.63	4.75
S2b	6.5	1.45	1.5	1.94	9.54	0.18	3.28	0.79	92	2.27	6.15

S2c	6.8	1.47	1.59	1.22	14.93	0.13	2.87	0.78	94	1.35	4.75
S2d	6.6	1.45	1.43	1.17	17.62	0.14	2.93	0.82	95	1.85	6.15

A1 and A2 stand for anthill from Ekosodin and Ugbowo respectively, S1 and S2 stand for adjacent soils from Ekosodin and Ugbowo respectively; a, b, c, and d represent the four replicates of each soil sample.

Total bacterial count and occurrence

A comparative analysis of bacterial populations revealed that anthill soils in A1 and A2 (Ekosodin) harbored significantly higher total bacterial counts (log10 cfu/g) than adjacent soils (S1 and S2, Ugbowo), with the notable exception of sample A2a (Fig. 1). The anthill soils exhibited mean total bacterial counts of 6.67 ± 0.07 (A1) and 6.42 ± 0.05 (A2), surpassing those of adjacent soils, which had mean values of 6.32 ± 0.09 (S1) and 6.39 ± 0.04 (S2). Furthermore, a diverse array of bacterial isolates, including *Corynebacterium* sp., *Klebsiella* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Citrobacter* sp., *Micrococcus* sp., *Shigella* sp., and *Bacillus* sp., were present in A1, A2, S1, and S2 (Fig. 2). In contrast, *Salmonella* sp., *Serratia* sp., and *Enterobacter* sp. were more prevalent in the anthill soil samples.

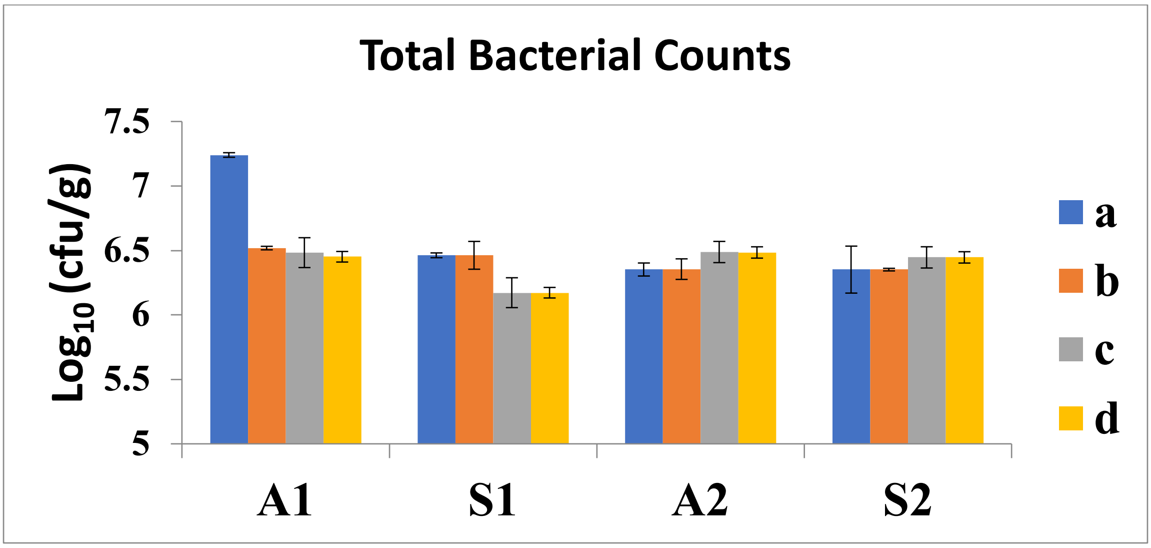

Fig. 1: Enumeration of the bacteria present in all soil samples

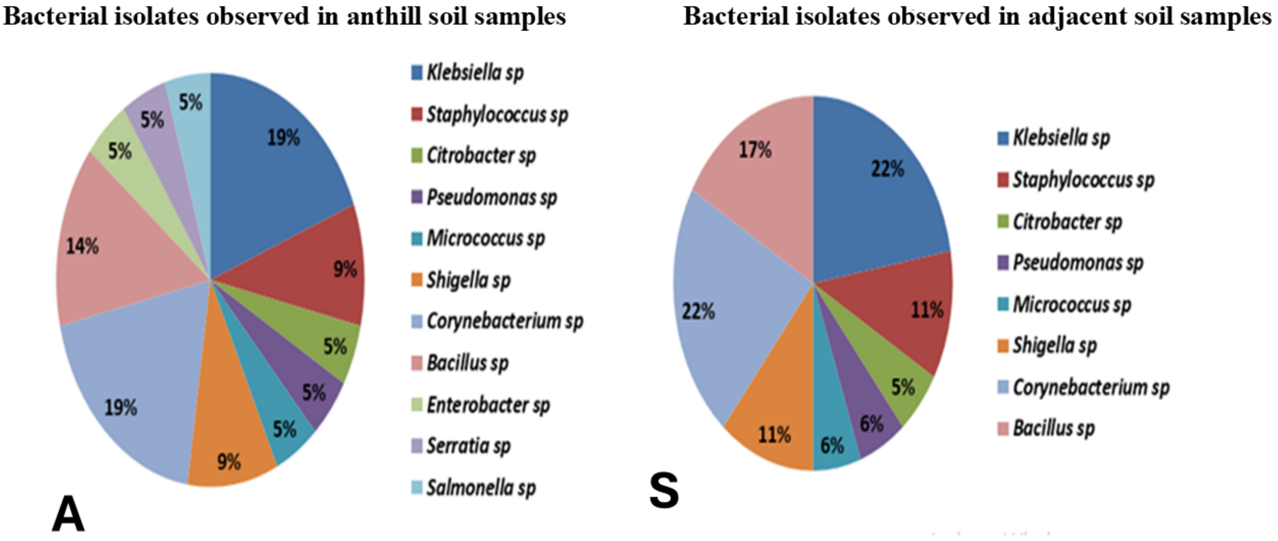

Fig. 2: Relative abundance (%) of bacterial isolates from the soil samples

Key: A = anthill soil samples S = adjacent soil samples

Plant growth-promoting activities

In the anthill soil samples, only two bacterial isolates, *Bacillus* sp. and *Pseudomonas* sp., demonstrated the ability to solubilize phosphates and produce ammonia, as indicated by positive test results (Table 2 and 3).

Table 2: Phosphate-solubilizing bacteria in both soil samples

Isolate	***Enterobacter* sp.**	***Pseudomonas* sp.**	***Bacillus* sp.**	***Serratia* sp.**
A1	-	+	+	-
S1	+	+	+	+
A2	-	+	+	-
S2	+	+	+	+

KEY: + (Present/Positive) - (Absent/ Negative)

Table 3: Ammonia-producing bacteria in both soil samples

Isolate	***Enterobacter* sp.**	***Pseudomonas* sp.**	***Bacillus* sp.**	***Serratia* sp.**
A1	-	+	+	-
S1	+	+	+	+
A2	-	+	+	-
S2	+	+	+	+

KEY: + (Present/Positive) - (Absent/ Negative)

Antifungal activities of anthill soil bacteria against Fusarium oxysporum

The biological control function of the anthill soil was evidenced by the suppression of the radial growth of *Fusarium oxysporum*. A subset of bacterial isolates, including *Salmonella* sp., *Enterobacter* sp., *Pseudomonas* sp., *Serratia* sp., *Staphylococcus* sp., and *Bacillus* sp., exhibited antifungal properties, with pronounced inhibition zones observed against *Fusarium oxysporum*. Notably, *Enterobacter* sp., *Serratia* sp., *Pseudomonas* sp., and *Bacillus* sp., displayed considerable clearance zones, measuring 17.00 ± 1.33, 17.00 ± 1.33, 20.00 ± 1.75, and 22.00 ± 1.50, correspondingly (Fig. 3).

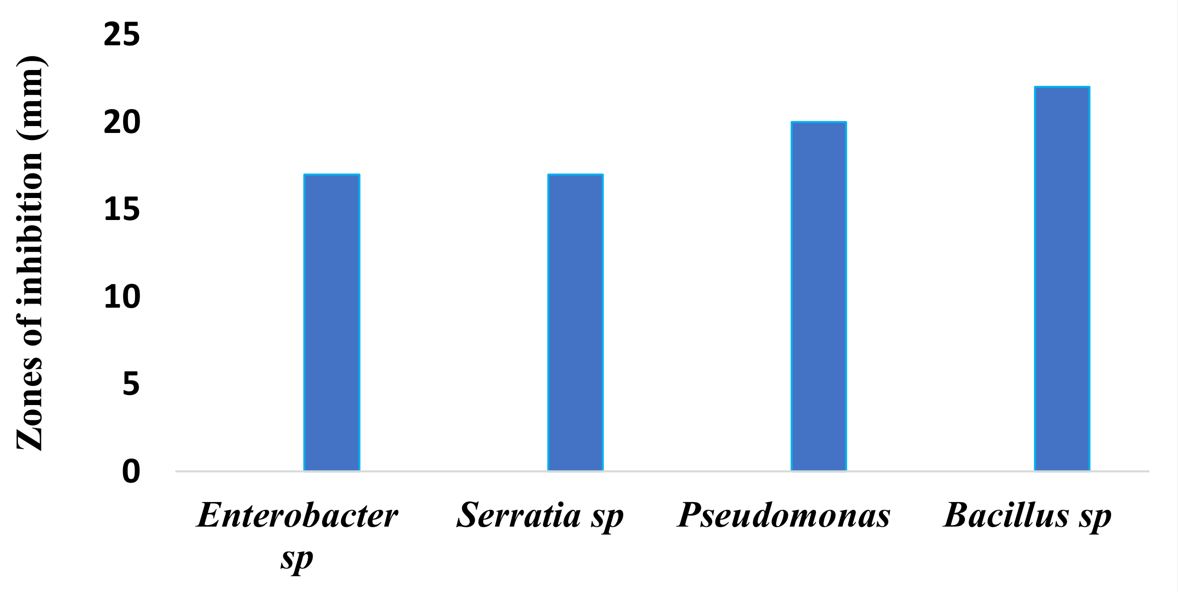

Fig. 3: Inhibition zones (mm) of some bacterial isolate from anthill soils against *Fusarium oxysporum*

Discussion

This study offers a comprehensive examination of the physicochemical properties of anthill soils compared to adjacent soils, the bacterial populations present, and the plant growth-promoting activities of the soil bacteria. The results reveal notable differences in soil characteristics, microbial diversity, and functional capabilities that underscore the potential of anthill soils as valuable ecological resources. Analysis indicates that anthill soils from Ekosodin (A1) and Ugbowo (A2) generally exhibit higher levels of essential nutrients, such as organic carbon (OC), phosphorus (P), potassium (K), organic matter (OM), calcium (Ca), magnesium (Mg), and clay content compared to adjacent soils (S1 and S2). This trend suggests that anthill formation may contribute to soil enrichment through the accumulation and concentration of nutrients (Babalola and Enagbonma 2024). Notably, samples S2b and S2d from Ugbowo are exceptions to this trend, exhibiting lower nutrient levels which could be attributed to specific local soil conditions or variations in anthill composition (Enagbonma et al. 2021). The increased proportions of sand and silt, along with higher pH levels, observed in adjacent soils compared to anthill soils, may reflect differences in soil texture and mineral composition between these soil types. Higher pH and sand content in adjacent soils could result from erosion or leaching processes that are less prevalent in anthill soils (Ezeaku et al. 2015).

The total bacterial counts in anthill soils (A1 and A2) were significantly higher than those in adjacent soils (S1 and S2), except for A2a. This finding aligns with previous studies suggesting that anthills provide a unique microhabitat that supports higher microbial diversity and abundance (Lenoir et al. 2001; Enagbonma et al. 2020). The presence of a wide range of bacterial isolates in both anthill and adjacent soils, including genera such as *Corynebacterium, Klebsiella,* and *Pseudomonas*, highlights the rich microbial community within these environments (Boots and Clipson 2013). However, the prevalence of certain bacteria such as *Salmonella, Serratia*, and *Enterobacter* in anthill soils may indicate a different microbial community structure influenced by soil management practices or environmental conditions (Chisanga et al. 2019). The differences in bacterial communities between soil types underscore the distinct ecological roles that anthill soils and their adjacent counterparts play (Enagbonma and Babalola 2022).

The ability of anthill soil bacteria to solubilize phosphates and produce ammonia is a critical factor in promoting plant growth (Wasoontharawat 2017). The study identifies *Bacillus* sp. and *Pseudomonas* sp. in anthill soils as capable of both phosphate solubilization and ammonia production, highlighting their potential as biofertilizers. This is contrasted with adjacent soils, where similar activities were observed across a broader range of bacterial isolates, including *Enterobacter* sp. and *Serratia* sp. The limited number of plant growth-promoting bacteria in anthill soils suggests a more specialized microbial community compared to adjacent soils. Despite this, the presence of *Bacillus* sp. and *Pseudomonas* sp. in anthill soils indicates that these bacterial strains could be leveraged for sustainable agricultural practices, particularly in nutrient-poor or degraded soils (Mohan and Radhakrishnan 2012).

The antifungal properties of bacteria from anthill soils against *Fusarium oxysporum* further illustrate the potential of these soils for biological control applications. The substantial inhibition zones produced by isolates such as *Pseudomonas* sp., *Bacillus* sp., and *Enterobacter* sp. suggest their effectiveness in suppressing fungal pathogens. This aligns with the observed higher bacterial counts in anthill soils, which may contribute to their enhanced biological control capabilities (Katun et al. 2020). The study highlights the ecological significance of anthill soils, demonstrating their enriched nutrient content, diverse bacterial populations, and functional capabilities, including plant growth promotion and pathogen suppression. These findings suggest that anthill soils could serve as valuable resources for sustainable agricultural practices and soil management strategies (Suryadi et al. 2014; Enagbonma and Babalola 2023). Future research should focus on understanding the mechanisms underlying the nutrient enrichment and microbial dynamics in anthill soils, as well as exploring their practical applications in enhancing soil fertility and plant health (Gupta and Buch 2019).

Conclusion

This study underscores the potential of anthill soil as an asset for sustainable agricultural practices. Our results reveal that anthill soil is nutrient-rich and supports a range of beneficial bacteria, including *Bacillus* sp., *Enterobacter* sp., *Pseudomonas aeruginosa*, and *Serratia* sp. These bacteria exhibit important plant growth-promoting functions, such as phosphate solubilization and ammonia production. Such characteristics can significantly improve soil fertility and help control plant diseases. Consequently, anthill soil presents a promising, eco-friendly alternative for soil enhancement, biofertilizer application, and biocontrol, contributing to healthier plant growth and reduced dependency on chemical fertilizers.

Acknowledgements

We are grateful to the Laboratory staff of the Department of Microbiology, University of Benin, for providing favourable Laboratory for this project.

Authors' contributions: Both authors designed and carried out the study.

Funding: The African-German Network of Excellence

Competing interests: The authors declared no conflict of interest.

References

Adebajo SO, Akintokun PO, Ezaka E, Ojo AE, Olannye DU, Ayodeji OD (2021) Use of termitarium soil as a viable source for biofertilizer and biocontrol. Bull. Natl. Res. Cent. 45:1-8

ALKahtani MD, Fouda A, Attia KA, Al-Otaibi F, Eid AM, Ewais EE-D, Hijri M, St-Arnaud M, Hassan SE-D, Khan N (2020) Isolation and characterization of plant growth promoting endophytic bacteria from desert plants and their application as bioinoculants for sustainable agriculture. Agronomy 10 (9):1325

Amoo AE, Enagbonma BJ, Ayangbenro AS, Babalola OO (2021) Biofertilizer: An eco-friendly approach for sustainable crop production. In: Food Security and Safety: African Perspectives. Springer, Cham, pp 647-669

Ashworth J, Keyes D, Kirk R, Lessard R (2001) Standard procedure in the hydrometer method for particle size analysis. Commun. Soil Sci. Plant Anal**.** 32 (5-6):633-642

Babalola OO, Enagbonma BJ (2024) Dataset of amplicon metagenomic assessment of barley rhizosphere bacteria under different fertilization regimes. Data in Brief 52:109920

Banerjee P, Prasad B (2020) Determination of concentration of total sodium and potassium in surface and ground water using a flame photometer. Appl. Water Sci. 10 (5):1-7

Boots B, Clipson N (2013) Linking ecosystem modification by the yellow meadow ant (Lasius flavus) to microbial assemblages in different soil environments. Eur. J. Soil Biol. 55:100-106

Campos P, Miller AZ, Knicker H, Costa-Pereira MF, Merino A, De la Rosa JM (2020) Chemical, physical and morphological properties of biochars produced from agricultural residues: Implications for their use as soil amendment. Waste Manag. 105:256-267

Chisanga K, Mbega E, Ndakidemi PA (2019) Socio-economic factors for anthill soil utilization by smallholder farmers in Zambia. Sustainability 11 (18):4849

Chisanga K, Mbega E, Ndakidemi PA (2020) Maize (*Zea mays*) response to anthill soil (Termitaria), manure and NPK fertilization rate under conventional and reduced tillage cropping systems. Sustainability 12 (3):928

Delgado-Baquerizo M, Eldridge DJ, Hamonts K, Singh BK (2019) Ant colonies promote the diversity of soil microbial communities. ISME J. 13 (4):1114-1118

Enagbonma B, Momoh P (2024) Profiling the phosphate solubilizing and nitrogen-fixing bacteria from the rhizosphere of *Talinum fruticosum* (L.) Juss. OJER 5 (1):14-23

Enagbonma B, Solomon B (2024) Assessment of plant pathogenic bacteria and fungi from *Talinium fruticosum* rhizosphere. OJER 5 (1):48-61

Enagbonma BJ, Ajilogba CF, Babalola OO (2020) Metagenomic profiling of bacterial diversity and community structure in termite mounds and surrounding soils. Arch. Microbiol. 202 (10):2697-2709

Enagbonma BJ, Amoo AE, Babalola OO (2021) Biopedturbation by termites affects respiration profiles of microbial communities from termite mound soils. J. Soil Sci. Plant Nutr. 21 (3):2115-2123

Enagbonma BJ, Babalola OO (2019) Environmental sustainability: A review of termite mound soil material and its bacteria. Sustainability 11 (14):3847

Enagbonma BJ, Babalola OO (2020) Unveiling plant-beneficial function as seen in bacteria genes from termite mound soil. J. Soil Sci. Plant Nutr. 20 (2):421-430

Enagbonma BJ, Babalola OO (2022) Metagenomics shows that termite activities influence the diversity and composition of soil invertebrates in termite mound soils. Appl. Environ. Soil Sci. 2022 (1):7111775

Enagbonma BJ, Babalola OO (2023) Metagenomics reveals the microbiome multifunctionalities of environmental importance from termite mound soils. Bioinformatics Biol. Insights. 17:11779322231184025

Enagbonma BJ, Fadiji AE, Babalola OO (2024) Anthropogenic fertilization influences a shift in barley rhizosphere microbial communities. PeerJ 12:e17303. doi:10.7717/peerj.17303

Enagbonma BJ, Sese-Owei E, Imade EE, Okoduwa IO, Joanne AA (2022) Potentials of Pisum sativum Extract and Cocos nucifera Water in Mitigating Lead Toxicity in Body Weight and Serum Lipid Profile of Rat Model: Trop. J. Nat. Prod. Res. 6 (2):213-218

Ezeaku P, Amanambu C, Ede I, Ene J, Okebalama C (2015) Yield parameter response of maize (Oba Super 2) to earthworm cast and anthill soil under greenhouse condition. Afr. J. Plant Sci. 9 (4):205-214

Fernandez-Bou AS, Dierick D, Swanson AC, Allen MF, Alvarado AGF, Artavia-León A, Carrasquillo-Quintana O, Lachman D, Oberbauer S, Pinto-Tomás AA (2019) The role of the ecosystem engineer, the leaf-cutter ant Atta cephalotes, on soil CO_2 dynamics in a wet tropical rainforest. J. Geophys. Res. Biogeosci. 124 (2):260-273

Frouz J, Jilková V (2008) The effect of ants on soil properties and processes (Hymenoptera: Formicidae). Myrmecol. News. 11 (11):191-199

Goyal K, Singh N, Jindal S, Kaur R, Goyal A, Awasthi R (2022) Kjeldahl method. Adv. Technol. Anal. Chem. 1 (1):105

Gupta V, Buch A (2019) *Pseudomonas* aeruginosa predominates as multifaceted rhizospheric bacteria with combined abilities of P-solubilization and biocontrol. J. Pure Appl. Microbiol. 13 (1):319-328

Hidalgo FJ, Canepuccia AD, Arcusa J, Fanjul E, Álvarez G, Iribarne OO (2021) Black fire ant mounds modify soil properties and enhanced plant growth in a salt marsh in Argentina. Estuarine Coast. Shelf Sci. 261:107534

Imbrea IM, Radulov I, Nicolin AL, Imbrea F (2016) Analysis of macroelements content of some medicinal and aromatic plants using flame atomic absorption spectrometry (FAAS). Rom. Biotechnol. Lett. 21 (4):11641

Katun A, Abdulmumin A, Yahaya M, Habeeb N, Bala A (2020) Anthill inhibiting bacteria, a promising source of bio efficacy. J Adv Med Pharm Sci 22:14-21

Kristiansen SM, Amelung W (2001) Abandoned anthills of Formica polyctena and soil heterogeneity in a temperate deciduous forest: morphology and organic matter composition. Eur. J. Soil Sci. 52 (3):355-363

Lahsini AI, Sallami A, Obtel M, Douira A, El Modafar C, Benkerroum N, Talbi C, Chakhchar A, Filali-Maltouf A (2022) Isolation and molecular identification of an indigenous abiotic stress-tolerant plant growth-promoting rhizobacteria from the rhizosphere of the olive tree in southern Morocco. Rhizosphere 23:100554

Lenoir L, Persson T, Bengtsson J (2001) Wood ant nests as potential hot spots for carbon and nitrogen mineralisation. Biol. Fertil. Soils. 34:235-240

Mohan V, Radhakrishnan A (2012) Screening of phosphate solubilizing bacterial isolates for the growth improvement of Tectona grandis Linn. Res. J. Microbiol. 7 (2):101-113

Mussa SAB, Elferjani HS, Haroun FA, Abdelnabi FF (2009) Determination of available nitrate, phosphate and sulfate in soil samples. Int. J. PharmTech Res. 1 (3):598-604

Okoduwa I, Enagbonma B, Imade E (2022) Effects of pharmaceutical effluents on soil microbiome and physicochemical parameters. J. Appl. Sci. Environ. Manag. 26 (8):1363-1367
Santamaría C, Lachaud J-P, Armbrecht I (2020) Effects of nest building by the dominant hunting ant, *Ectatomma* sp. 2 (*E. ruidum complex*), on Andean coffee plantations. Pedobiologia 79:150626
Suryadi Y, Susilowati D, Lestari P, Priyatno T, Samudra I, Hikmawati N, Mubarik N (2014) Characterization of bacterial isolates producing chitinase and glucanase for biocontrol of plant fungal pathogens.
Urbańczyk DA, Szulc W (2023) Effect of anthills on changes in the physico-chemical properties in sandy soil. Soil Sci. Annu. 74 (1)
Wakung'oli M, Amoo AE, Enagbonma BJ, Babalola OO (2020) Termite Societies Promote the Taxonomic and Functional Diversity of Archaeal Communities in Mound Soils. Biology 9 (6):136

Wasoontharawat M (2017) Isolation and screening phosphoric solubilizing bacteria from organic anthill fertilizer and phosphorus release capacity. SNRU J. Sci. Technol. 9 (2):454-464