Potentials of anthill soils and its bacteria as a viable source of soil amendment, biofertilizer and biocontrol

*[1]Enagbonma Ben Jesuorsemwen; [2]Imade Edoghogho Emmanuel; [1]Iyase Nomamien; [1]Igbinadolor Imuwahen Josephine; [1]Efosa Erhunmwunse

[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. Email: benjamin.enagbonma@uniben.edu; Tel: +2349133248167

Running title: Anthill soils potentials

Abstract

High cost of synthetic fertilizers and the environmental degradation arising from prolong and excess use of chemical input for farming has led to the search of eco-friendly resources of cultivating crop. Thus this study examined the use of anthill soil as a viable source for soil amendment, biofertilizer and biocontrol. Here, soil samples collected from anthill and their adjacent soils were analysed for soil nutrients using standard analytic methods and subsequently examined for bacterial diversity as well as screening isolates for plant growth-promoting (PGP) activity via standard bacteriological, morphological and biochemical methods. Activities of the plant growth-promoting bacteria were carried out using antagonism by difusible substance method and antagonistic activity of cell-free culture filtrate of bacterial isolates against *Fusarium oxysporum*. Results reveal that the total bacterial count and most of the soil physicochemical properties were higher in anthill soils except for silt, sand and pH when related to the adjacent soils. Isolates belonging to the genera *Bacillus* sp., *Shigella* sp., *Micrococcus* sp., *Citrobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Klebsiella* sp., and *corynebacterium* sp. were seen in all soil samples. However, *Enterobacter* sp., *Serratia* sp., and *Salmonella* sp., predominated the adjacent soils. Only *Bacillus* sp. and *Pseudomonas* sp. were positive for phosphate solubilization assay and ammonia production test in anthill soils while three bacterial isolates had antagonistic activities against *Fusarium oxysporum*. The result showed that anthill soils are rich in nutrients and also contains some useful bacteria that are capable of promoting plant growth as well as suppressing plant soil pathogen.

Keywords: bio-engineer; food security; soil analysis; soil microbiome; synthetic fertilizer

Introduction

Soil nutrients in some farm area are inadequate and may not support soil health and plant growth (Amoo, Enagbonma, Ayangbenro, & Babalola, 2021). To augment for the deficiency, some nutrients are made unnaturally and applied to soil as chemical fertilizers (Xie, Wu, Tang, Zhang, & Chen, 2010). These synthetic fertilizers can only promote soil health and plant growth for short period of time (Kobua, Jou, & Wang, 2021). Many authors have reported that prolonged and excessive misuse of these chemical fertilizers have led to environmental degradation such as water pollution (Xu et al., 2020), poor friable soil and prevent plant root penetration because chemical fertilizers combine with clay to harden soil layer (Hati, Mandal, Misra, Ghosh, & Bandyopadhyay, 2006; Massah & Azadegan, 2016; Pahalvi, Rafiya, Rashid, Nisar, & Kamili, 2021). Furthermore, it also promote soil acidity which is lethal to soil microbes that would otherwise improve soil health , mitigate pests and diseases in plant and enhance plant growth in general (Ge, Zhu, & Jiang, 2018; Nguyen et al., 2018). To avoid the negative impact arising from the misuse of chemical fertilizers, soil ecologist have called for the use of eco-friendly materials like biofertilizers and biocontrol agents (Enagbonma & Babalola, 2019a; Imade & Babalola, 2021; Riaz et al., 2020). Biofertilizers and biocontrol materials are made up of live microbes that promote the supply of key nutrients to plants, control bioprocesses in soil that stimulate plant wellbeing and the manufacture of antibiotics that mitigate soil borne plant pathogens like fungi, bacteria, nematodes and viruses (Bajracharya, 2019; Pirttilä, Mohammad Parast Tabas, Baruah, & Koskimäki, 2021).

Soil ant's gut has been reported to house many microorganisms that can serve as a source of biofertilizers and biocontrol agents (Moreau, 2020), although our insight into the key eco-services provided by them is still not complete. Soil ants are strong ecological engineers as their activities during anthill construction have a considerable impact on soil morphology such as the formation of subsurface horizons, soil structures, soil aeration, and aggregation,

(Fernandez-Bou et al., 2019). Bioturbation by soil ants during anthill construction and the fact that anthill are constructed with a combination of partially digested diet, saliva, and faeces of ants lead to upward movement and relocation in organic and inorganic resources through the soil profile (Santamaría, Lachaud, & Armbrecht, 2020). This led to anthill soils been described to be better-off in soil nutrients and minerals than the neighbouring soils(Enagbonma, Imade, & Omoregbe, 2023). This may in turn have an effect on the microbial composition and structures in anthill soils (Delgado-Baquerizo, Eldridge, Hamonts, & Singh, 2019). Yet information on the abundance of soil microorganisms and their functional abilities in anthill soils are largely underexplored. In light of this, we chose to investigate the physical and chemical characteristics of the anthill soil as well as the ability of the microorganisms present to participate in the cycling of nutrients and the suppression of plant soil pathogens. In this research work, we planned to test the assumption that (1) the soil engineered by ants' activities is richer in soil nutrients than the adjacent soil (2) the bacterial isolates in anthill soil may be different from their adjacent soil (3) the bacteria in soil engineered by ants will show a positive respond to nutrient cycling and suppression of plant soil pathogens. These assumptions were founded on the notion that some research works have revealed the exceptional nutrients properties of anthill compartment when related with the adjacent environment. They include: improved temperature inside the nest, pH close to neutral, high amount of organic matter, and increased aeration (Chen et al., 2019; Wang et al., 2019).

Materials and methods

Study sites and soil sampling

Four soil samples of 50 g were collected from anthills (each of about 2 m apart) at 0 - 15 cm depth [that is from the top of the anthill to the bottom where ants activities had effect (Chisanga,

Mbega, & Ndakidemi, 2020) using soil coil from Ekosodin (A1a–d) and four soil samples from anthills (about 2 m apart) from Ugbowo (A2a–d). Both Ekosodin (Lat. 6^0 23^1 42^{11} North, Long.5^0 36^1 49^{11} East) and Ugbowo (Lat. 6^0 23^1 45^{11} North, Long. 5^0 36^1 54^{11} East) are in Benin City, Nigeria. For proper comparison, four samples of the adjacent soil from Ekosodin (S1a–d) and Ugbowo (S2a–d) were also collected at a depth of 0 – 15 cm. This depth (0 – 15 cm) was selected since the mainstream of microbial activity occurs within the 0 - 15 cm (Enagbonma & Babalola, 2022). The distance between the anthill and adjacent soils was separated by 10 m and the absence of anthills in these regions influenced the selection of the 10 m between the adjacent soil and the anthill. The soil samples were conserved for a short period in cooler boxes filled with ice blocks during sampling and afterward transported to the laboratory that same day for more isolation of bioagents and physicochemical analysis.

Soil properties analysis

Soil samples (20 g) that have been pre-processed to remove debris and solid wooden material were used for soil physical and chemical properties analysis. Soil pH in distilled water was measured using a pH-meter in a 1:2.5 soil: water ratio and total nitrogen was determined by the Kjeldhal method (Muwawa et al. 2010). Atomic absorption spectrophotometer (AAS) was employed in reading exchangeable calcium (Ca) and magnesium (Mg) present in the extracts obtained from 1M ammonium acetate at pH 7.0. The flame photometer was used in reading exchangeable potassium (K). Accessible phosphorus (P) was determined spectrophotometrically while organic carbon was determined using method previously described by Wakung'oli, Amoo, Enagbonma, and Babalola (2020).

Isolation and identification of isolates

Serial dilution was done on 1 g of the soil samples up to the fifth dilution. Thereafter, Aliquot was inoculated into sterile agar plate containing nutrient agar, MacConkey agar plates, eosin methylene blue agar and plate count agar based on the manufacturer's guidelines, and the

incubation of the inoculated plates were done. For further analysis to be done, discrete colonies were chosen based on their morphological characteristics and then subcultured to get pure cultures. Morphological and biochemical features were employed for characterizing bacterial isolates. We also carried out Gram staining, catalase test, methyl red test, indole test, Voges–Proskauer test, urease test, oxidase test, coagulase test, triple sugar iron test and citrate utilization test (Luo, Zhao, Wang, Raza, & Yin, 2022; Okoduwa, Enagbonma, & Imade, 2022).

Screening of anthill soil bacteria for plant growth-promoting properties

Phosphate solubilization test

The bacterial isolate was spot inoculated at the centre of the prepared sterile Pikovskaya agar plate and incubated for 72 h at 30 °C. The zones of phosphate solubilization formed around the colonies were recorded after 72 h. The solubilization index was determined by dividing the total diameter of the halo with the diameter of the colony (Wasoontharawat, 2017).

Ammonia production test

Freshly grown bacterial cultures were inoculated in 10 ml nutrient broth and incubated at 30°C for 48h in a rotator shaker. After incubation, 0.5 ml of Nessler's reagent was added to each tube. The development of a yellow to brown colour indicated a positive reaction for ammonia production (Adebajo et al., 2021).

Antagonistic activities of plant growth-promoting bacteria against *Fusarium oxysporum*

The antagonistic effect of diffusible compounds on the pathogenic fungus was evaluated in vitro by dual culture techniques. *Fusarium oxysporum* was grown on Sabouraud dextrose agar (SDA) plates, disc of 7 mm diameter was cut from the actively growing lawn and inoculated at the center of the Sabouraud dextrose agar plates, and 24-h-old culture of isolated bacterial strains was streaked about 2.5 cm away from *Fusarium oxysporum*. The plates were

incubated at 28 °C for 5 days, and the result was recorded by measuring the clear zones around the bacterial colony. Inhibition of fungal growth was calculated using the formula earlier used by Adebajo et al. (2021):

$$\frac{R1-R2}{R1} \times 100$$

Where R1 (a control value) represents the largest radial distance grown by the fungus in the direction of the antagonist.

R2 represents the distance on a line between the inoculation positions of the fungus and the bacteria.

Statistical analysis

All the analyses were done in triplicates. Analysis of variance and descriptive statistics were employed to examine the mean data gotten from the study using Statistical Package for the Social Sciences ® version 21, PAST version 2.17c and Microsoft Excel version 2010.

Results

Analysis of soil properties from anthill and adjacent soil samples

Evaluation of the soil physical and chemical properties (Table 1) showed higher values of K, Ca, TKM, Mg, OM, P, OC, and clay (except in S2b and S2d) in soils from anthill in relation to the adjacent soils. Conversely, the values of sand, silt and pH in adjacent soil samples were higher than those in the anthill soils.

147 Table 1: Soil properties assessment from both soil samples

	A1a	A1b	A1c	A1d	S1a	S1b	S1c	S1d	A2a	A2b	A2c	A2d	S2a	S2b	S2c	S2d
pH	6.1	6	5.2	6.1	6.6	6.4	6.6	6.4	5.9	6.3	6.4	6.3	6.8	6.5	6.8	6.6
OC (%)	1.74	1.82	1.72	1.82	1.72	1.16	1.37	1.28	0.65	0.87	0.92	0.83	1.47	1.45	1.47	1.45
OM (%)	3.00	3.14	2.97	3.14	2.97	2.00	2.36	2.21	2.53	2.5	2.53	2.5	1.21	1.5	1.59	1.43
TKN (%)	2.16	1.78	1.56	2.38	1.22	1.14	1.22	1.78	2.13	2.36	2.13	2.36	1.28	1.94	1.22	1.17
P (mg/L)	23.15	48.78	22.63	48.78	22.63	20.27	28.17	31.46	36.54	43.11	36.54	43.11	18.63	9.54	14.93	17.62
Ca (mg/L)	0.35	0.32	0.27	0.32	0.27	0.25	0.22	0.18	0.28	0.37	0.28	0.37	0.12	0.18	0.13	0.14
K (mg/L)	6.32	6.12	5.63	6.12	5.63	5.48	4.36	5.27	5.18	5.23	5.18	5.23	3.11	3.28	2.87	2.93
Mg (mg/L)	0.97	0.88	0.97	0.94	0.95	0.78	0.82	0.78	0.93	0.82	0.93	0.93	0. 74	0.79	0.78	0.82
Sand (%)	94	92	95	93	96	93	96	96	94	91	93	92	95	92	94	95
Silt (%)	1.72	1.74	1.2	1.24	2.75	2.14	2.75	2.14	1.25	1.85	1.25	1.48	1.63	2.27	1.35	1.85
Clay (%)	4.28	6.26	3.8	2.76	1.25	4.86	1.25	4.86	3.37	6.73	5.68	3.52	4.75	6.15	4.75	6.15

148 A1 anthill from Ekosodin, A2 anthill from Ugbowo, S1 adjacent soils from Ekosodin, and S2 adjacent soils from Ugbowo

149

Total bacterial count and occurrence

Overall, the total bacterial count (log10 (cfu/g)) in anthill soils in both locations (A1 and A2 (Ekosodin)) were higher than the total bacterial count obtained from the adjacent soils (S1 and S2 (Ugbowo)) except in A2a (Fig. 1). The mean total bacterial count in anthill soils were A1 = 6.67 ± 0.07 and A2 = 6.42 ± 0.05 while the mean total bacterial count in adjacent soils were S1 = 6.32 ± 0.09 and S2 = 6.39 ± 0.04 respectively. Isolates seen in all soil samples included *Bacillus* sp., *Shigella* sp., *Micrococcus* sp., *Citrobacter sp.*, *Pseudomonas* sp., *Staphylococcus* sp., *Klebsiella* sp., and *Corynebacterium* sp. were seen in all soil samples (Fig 2). However, *Enterobacter* sp., *Serratia* sp., and *Salmonella* sp. Predominated the adjacent soils.

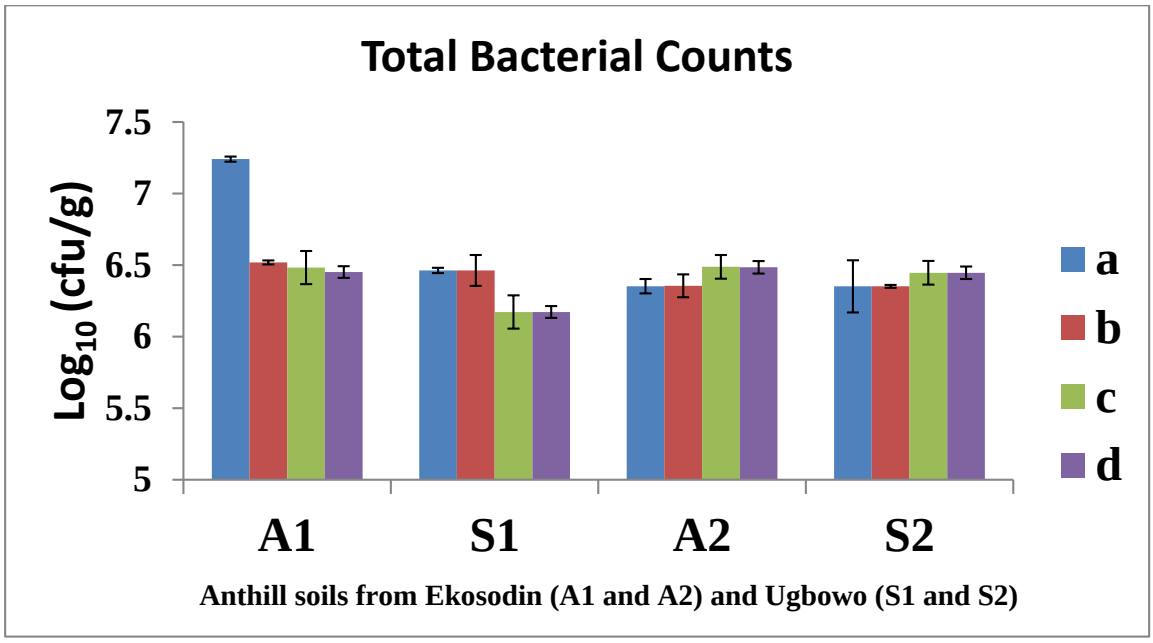


Fig. 1: Total bacterial count in all soil samples. A1a–d and A2a–d mean anthill soils from Ekosodin and Ugbowo, respectively, while S1a–d and S2a–d mean adjacent soils from Ekosodin and Ugbowo, respectively.

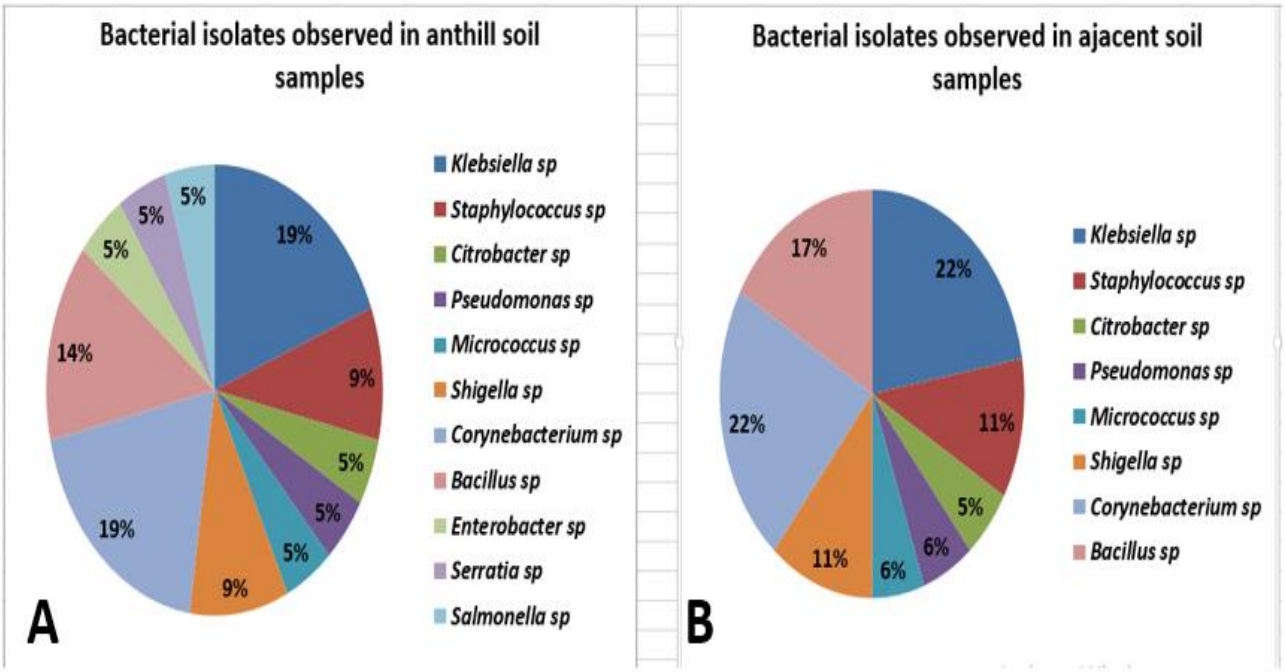


Fig. 2: Percentage occurrence of bacterial isolate from anthill and adjacent soil samples

Plant growth-promoting activities

Of the bacterial isolates, only *Bacillus* sp. and *Pseudomonas* sp. were positive for phosphate solubilization assay and ammonia production test in anthill soil samples (Table 2 and 3).

Table 2: Phosphate-solubilizing bacteria in anthill and adjacent soils

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

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

172

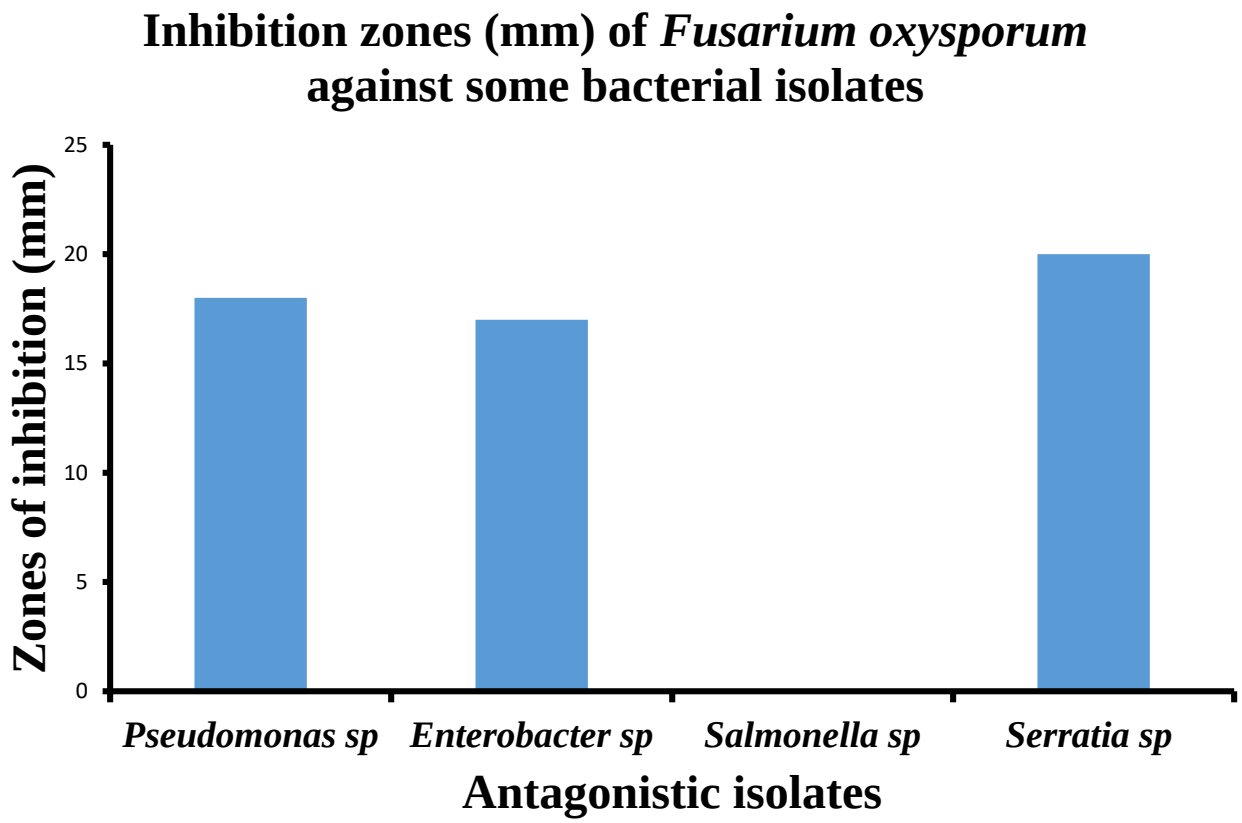
173 **Table 3: Ammonia-producing bacteria in anthill and adjacent soils**

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

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

175 **Antagonistic activities of anthill soil bacteria against *Fusarium oxysporum***

176 Anthill soil biocontrol activity was detected by reduced radial growth of the *Fusarium*
177 *oxysporum* (the test fungus). Three isolates displayed the potential to control *Fusarium*
178 *oxysporum*. The inhibition zones of *Fusarium oxysporum* against some bacteria from the anthill
179 soil were found to inhibit *Fusarium oxysporum*, some of these bacteria include *Bacillus* sp,
180 *Staphylococcus* sp, *Serratia* sp, *Enterobacter* sp, *Pseudomonas* sp, and *Salmonella* sp., for
181 instance, *Bacillus* sp, *Pseudomonas* sp, *Enterobacter* sp. and *Serratia* showed some zones of
182 clearance with values of 22.00 ± 1.50, 20.00 ± 1.75, 17.00 ± 1.33 and 17.00 ± 1.33 respectively
183 (Fig. 3).



184

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

187 **Discussion**

188 This research made extensive effort to assess the viability of anthill soil as a source for soil
189 amendment, biofertilizer and biocontrol. Results revealed that most macro and micro nutrients
190 were adequate in anthill soils except for silt, sand and pH when compared to the adjacent soils
191 (Table 1). Regarding texture class, the physical characteristics of the investigated soils were
192 classified into three groups: clay, sand and silt. Soil texture is vital as it impacts carbon storage
193 and nutrient supply for the soil bacteria, archaea and fungi (Chisanga et al., 2020).
194 Consequently the particle size distribution of soil essentially influences the activity of the
195 microbial communities (Fang & Achal, 2020). In this study, we found that the anthill soil had
196 higher content of clay in comparison with the control soil, which exhibited sandy

characteristics. Organic matter (which was higher in anthill soils compared to the adjacent soils) is the most popular natural fertilizer used in farming (Enagbonma & Babalola, 2019b). It is an abundant reservoir of carbon and plays an important role in maintaining the CO_2 balance in the environment. Golichenkov et al. (2019) reported that the long-term application of organic soil amendments helps to intensify the sequestration of carbon in the soil and increase food safety. This may answer why most farmers explained that anthill soil utilization has been beneficial to their crop production (Chisanga, Mbega, & Ndakidemi, 2019). The eco-friendly plant growth-promoting potential and disease control approaches are significant in growing crops were seen in this study. Microbial groups such as *Bacillus* sp., *Shigella* sp., *Micrococcus* sp., *Citrobacter* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Klebsiella* sp., *Corynebacterium* sp., *Enterobacter* sp., *Serratia* sp., and *Salmonella* sp. (Fig 2) endowed with nitrogen fixation, polysaccharide, phosphate and solubilization of potassium as well as production of indole acetic acid (IAA) from tryptophan were reported from anthill soil from this study (Kumari, Rastogi, Singh, & Rajput, 2022; Luo et al., 2022). The high total bacterial counts in anthill soils (Fig. 1) may be due to the high levels of organic matter in the soil, which may promote plant growth. The difference in the physicochemical parameters between the anthill soils and the adjacent soils could account for the presence of *Enterobacter* sp., *Serratia* sp., and *Salmonella* sp. found only in anthill soils.

The bacterial isolates (*Bacillus* sp. and *Pseudomonas* sp.) observed in this study showed higher affinity to solubilize phosphorus and ammonia. Wasoontharawat (2017) stated that anthill soils hold higher amount of phosphorus when likened to the adjacent soils owing to the occurrence of highly proficient phosphate-solubilizing bacteria. Ability of these phosphate solubilizing bacteria to solubilize inorganic and organic phosphorus is seen as important features for promoting soil fertility and their use as inoculants concomitantly can promote plant phosphorus uptake and increase crop yield (Mohan & Radhakrishnan, 2012).

The ability of the bacterial isolates with antibacterial and antifungal ability indicated that they produce extracellular cell wall-degrading enzymes such as chitinase and β-1,3-glucanase and antifungal compound (Kumari et al., 2022; Xiao, Liu, & Liao, 2009). *Pseudomonas* sp, *Enterobacter* sp, and *Serratia* sp. from anthill soils are the isolates that showed highest biocontrol activity against *Fusarium oxysporum*. The strains of these bacteria play a significant role in the biocontrol of fungal diseases because they produce different types of metabolites (volatile and diffusible) and may use multiple mode of action against fungal pathogens (Yang, 2019). The occurrence of these soil beneficial organisms and high nutrient content in anthill soil have been shown to enhance crop yield and thus can be used as biofertilizers. *Pseudomonas aeruginosa* solubilized phosphate, phosphorus and IAA which provide additional advantages for their ability to be used as biocontrol agents for agricultural management (Wasoontharawat, 2017).

Conclusion

The outcome from this study revealed that anthill soil contains high nutrients and some beneficial bacteria such as *Bacillus* sp., *Enterobacter* sp., *Serratia* sp. and *Pseudomonas aeruginosa*. These bacteria are capable of solubilizing phosphate and ammonia which are some of the potentials required of the organisms and soils to promote plant growth and suppress plant–soil pathogen. Hence, anthill soil could be embraced and encouraged as a sustainable source for soil amendment, biofertilizer and biocontrol.

Acknowledgements

We are grateful to the Laboratory Managements of the Department of Microbiology and Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, for providing conducive environment for this project.

Authors' contributions: BJE and EEI conceived and designed the study. IN, IIJ and EE carried out most of the laboratory work. BJE and EEI analysed and interpreted the data. BJE helped in writing the original draft. All authors read and approved the final manuscript.

Funding: The African-German Network of Excellence (AGNES), The Federal Ministry of 241 Education (BMBF) and The Alexander von Humboldt Foundation (AvH) supported research 242 done in this lab..

Availability of data and materials: The authors declare that all relevant data supporting the findings of this study are included in this article.

Declarations Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Competing interests: The authors have no conflict of interest to declare.

References

Adebajo, S. O., Akintokun, P. O., Ezaka, E., Ojo, A. E., Olannye, D. U., & Ayodeji, O. D. (2021). Use of termitarium soil as a viable source for biofertilizer and biocontrol. *Bulletin of the National Research Centre, 45*, 1-8.

Amoo, A. E., Enagbonma, B. J., Ayangbenro, A. S., & Babalola, O. O. (2021). Biofertilizer: An Eco-friendly Approach for Sustainable Crop Production. In *Food Security and Safety* (pp. 647-669). Chem: Springer.

Bajracharya, A. M. (2019). Plant growth promoting rhizobacteria (PGPR): Biofertiliser and Biocontrol agent-Review article. *Journal of Balkumari College, 8*, 42-45.

Chen, M. K., Wang, S. J., Chen, W. Q., Cao, R., Cao, Q. B., Wang, P., . . . Li, S. H. (2019). Effects of ant nesting on soil microbial biomass carbon and quotient in tropical forest of Xishuangbanna. *Journal of Applied Ecology, 30*(9), 2973-2982.

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

Chisanga, K., Mbega, E., & Ndakidemi, P. A. (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, D. J., Hamonts, K., & Singh, B. K. (2019). Ant colonies promote the diversity of soil microbial communities. *ISME Journal, 13*(4), 1114-1118.

Enagbonma, B. J., & Babalola, O. O. (2019a). Environmental sustainability: A review of termite mound soil material and its bacteria. *Sustainability, 11*(14), 3847.

Enagbonma, B. J., & Babalola, O. O. (2019b). Potentials of termite mound soil bacteria in ecosystem engineering for sustainable agriculture. *Annals of Microbiology, 69*, 211-219.

Enagbonma, B. J., & Babalola, O. O. (2022). Metagenomics Shows That Termite Activities Influence the Diversity and Composition of Soil Invertebrates in Termite Mound Soils. *Applied and Environmental Soil Science, 2022*.

Enagbonma, B. J., Imade, E. E., & Omoregbe, N. (2023). Assessment of Plant-Pathogenic Fungi in Anthill Soils. *African Scientist, 24*(3), 1595-6881.

Fang, C., & Achal, V. (2020). Physico-Chemical Aspects and Complete Bacterial Community Composition Analysis of Wasp Nests. *Sustainability, 12*(7), 2652.

Fernandez-Bou, A. S., Dierick, D., Swanson, A. C., Allen, M. F., Alvarado, A. G. F., Artavia-León, A., . . . Pinto-Tomás, A. A. (2019). The role of the ecosystem engineer, the leaf-cutter ant Atta cephalotes, on soil CO2 dynamics in a wet tropical rainforest. *Journal of Geophysical Research: Biogeosciences, 124*(2), 260-273.

Ge, S., Zhu, Z., & Jiang, Y. (2018). Long-term impact of fertilization on soil pH and fertility in an apple production system. *Journal of Soil Science Plant Nutrition, 18*(1), 282-293.

Golichenkov, M., Maksimova, I., Zakalyukina, Y. V., Dymova, A., Churilina, A., & Kiryushin, A. (2019). *Ants' nesting activity as a factor of changes in soil physical properties.* Paper presented at the IOP Conference Series: Earth and Environmental Science.

Hati, K., Mandal, K., Misra, A., Ghosh, P., & Bandyopadhyay, K. (2006). Effect of inorganic fertilizer and farmyard manure on soil physical properties, root distribution, and water-use efficiency of soybean in Vertisols of central India. *Bioresource Technology, 97*(16), 2182-2188.

Imade, E. E., & Babalola, O. O. (2021). Biotechnological utilization: the role of Zea mays rhizospheric bacteria in ecosystem sustainability. *Applied Microbiology and Biotechnology, 105*(11), 4487-4500. doi:10.1007/s00253-021-11351-6

Kobua, C. K., Jou, Y.-T., & Wang, Y.-M. (2021). Advantages of amending chemical fertilizer with plant-growth-promoting rhizobacteria under alternate wetting drying rice cultivation. *Agriculture, 11*(7), 605.

Kumari, P., Rastogi, N., Singh, H. B., & Rajput, R. S. (2022). Plant growth-promoting bacteria associated with the nests of the seed-harvester ant, *Trichomyrmex scabriceps*.

Luo, L., Zhao, C., Wang, E., Raza, A., & Yin, C. (2022). Bacillus amyloliquefaciens as an excellent agent for biofertilizer and biocontrol in agriculture: An overview for its mechanisms. *Microbiological Research*, 127016.

Massah, J., & Azadegan, B. (2016). Effect of chemical fertilizers on soil compaction and degradation. *Agricultural Mechanization in Asia, Africa, Latin America, 47*(1), 44-50.

Mohan, V., & Radhakrishnan, A. (2012). Screening of phosphate solubilizing bacterial isolates for the growth improvement of Tectona grandis Linn. *Research Journal of Microbiology, 7*(2), 101-113.

Moreau, C. S. (2020). Symbioses among ants and microbes. *Current Opinion in Insect Science, 39*, 1-5.

Nguyen, L. T., Osanai, Y., Lai, K., Anderson, I. C., Bange, M. P., Tissue, D. T., . . . Biochemistry. (2018). Responses of the soil microbial community to nitrogen fertilizer regimes and historical exposure to extreme weather events: flooding or prolonged-drought. *Soil Biology, 118*, 227-236.

Okoduwa, I., Enagbonma, B., & Imade, E. (2022). Effects of pharmaceutical effluents on soil microbiome and physicochemical parameters. *Journal of Applied Sciences and Environmental Management, 26*(8), 1363-1367.

Pahalvi, H. N., Rafiya, L., Rashid, S., Nisar, B., & Kamili, A. N. (2021). Chemical fertilizers and their impact on soil health. In *Microbiota and Biofertilizers, Vol 2* (pp. 1-20). Chem: Springer.

Pirttilä, A. M., Mohammad Parast Tabas, H., Baruah, N., & Koskimäki, J. J. (2021). Biofertilizers and biocontrol agents for agriculture: How to identify and develop new potent microbial strains and traits. *Microorganisms, 9*(4), 817.

Riaz, U., Mehdi, S. M., Iqbal, S., Khalid, H. I., Qadir, A. A., Anum, W., . . . Murtaza, G. (2020). Bio-fertilizers: eco-friendly approach for plant and soil environment. In *Bioremediation and Biotechnology* (pp. 189-213). Chem: Springer.

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.

Wakung'oli, M., Amoo, A. E., Enagbonma, B. J., & Babalola, O. O. (2020). Termite societies promote the taxonomic and functional diversity of archaeal communities in mound soils. *Biology, 9*(6), 136.

Wang, S., Li, J., Zhang, Z., Chen, M., Li, S., & Cao, R. (2019). Feeding-strategy effect of Pheidole ants on microbial carbon and physicochemical properties in tropical forest soils. *Applied Soil Ecology, 133*, 177-185.

345 Wasoontharawat, M. (2017). Isolation and screening phosphoric solubilizing bacteria from
346 organic anthill fertilizer and phosphorus release capacity. *SNRU Journal of Science and*
347 *Technology, 9*(2), 454-464.
348 Xiao, X., Liu, K., & Liao, Z. (2009). Disease-control effect and mechanism research of
349 biological organic fertilizer on tomato bacterial wilt. *Journal of Agro-Environment*
350 *Science, 28*(11), 2368-2373.
351 Xie, J., Wu, X., Tang, J., Zhang, J., & Chen, X. (2010). Chemical fertilizer reduction and soil
352 fertility maintenance in rice-fish coculture system. *Frontiers of Agriculture in China,*
353 *4*(4), 422-429. Retrieved from https://doi.org/10.1007/s11703-010-1049-z
354 Xu, R., Cai, Y., Wang, X., Li, C., Liu, Q., & Yang, Z. (2020). Agricultural nitrogen flow in a
355 reservoir watershed and its implications for water pollution mitigation. *Journal of*
356 *Cleaner Production, 267*, 122034.
357 Yang, W. (2019). Components of rhizospheric bacterial communities of barley and their
358 potential for plant growth promotion and biocontrol of Fusarium wilt of watermelon.
359 *Brazilian Journal of Microbiology, 50*(3), 749-757.

360