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Earthworm-bioturbated soil and crab-bioturbated soil: comparative physicochemical and microbial properties

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Abstract

Wetlands are rich in bioturbating animals, whose activities modify the physicochemical and nutrient states of their habitat soils. Although bioturbations by earthworms and crabs have been investigated separately, a comparative study of their impact on soil quality has yet to be reported. We compared the microbial and physicochemical properties of earthworm- and crab-bioturbated soils from the same wetland habitat. Soils separately bioturbated by earthworms and crabs were sampled within randomly placed 1 m^2 quadrats and analysed for microbial and physicochemical properties using standard procedures, with unbioturbated (undisturbed) soil from the same area serving as the control. Bioturbated and unbioturbated soils exhibited significant differences ($p < 0.05$) in all measured parameters, with unbioturbated soil showing higher proportions of sand and silt, but lower biochemical and microbial activities. Crab-bioturbated soil had significantly higher ($p < 0.01$) moisture and water holding capacity, relative to earthworm-bioturbated soil. However, earthworm-bioturbated soil recorded significantly higher ($p < 0.01$) nitrogen ($0.45 \pm 0.02\%$), organic carbon ($1.26 \pm 0.02\%$), and total organic matter ($2.18 \pm 0.04\%$). Additionally, earthworm-bioturbated soil had significantly higher total bacteria, fungi, and actinomycetes counts of $129.33 \pm 18.15 \times 10^4$ CFU/g, $46.22 \pm 6.04 \times 10^4$ CFU/g, and $56.22 \pm 7.61 \times 10^4$ CFU/g, respectively. These results imply that both earthworms and crabs positively influence soil quality, but earthworm activities have a greater positive biochemical and microbial effects. Nevertheless, efforts should be made towards conserving the populations of wetland earthworms and crabs, as their contributions are complementary to soil enrichment.

Key words: *bioturbation; organic carbon; soil carbon; soil nitrogen; wetlands*.

1. Introduction

Bioturbation is the alteration, remixing, turning over, or reworking of sediments or soil structure by living organisms. Reworking and mixing of sediments and soil layers by plants and animals leads to increased oxygen and nutrient flow, particle movement, and water penetration, all of which constitute a powerful driver of terrestrial productivity and biodiversity (Meysman, Meddelburg, & Heip, 2006). Several burrowing and non-burrowing vertebrate and invertebrate animals, including earthworms, crabs, rodents, birds, ants, termites, antelopes, elephants play varied roles in this process. Plants are also known to contribute significantly to remixing and turning of soils. Tree root penetration and uprooting facilitate mineral weathering and penetration of soil organic matter (Algeo, & Scheckler, 1998; Sarker, Masud-Ul-Alam, Hossain, Rahman Chowdhury, &

Sharifuzzaman, 2020). The death and decay of tree roots serve as sources of organic matter to the soil.

Wetlands are rich in bioturbating animals, whose activities modify the physicochemical and nutrient qualities of their soils. Much attention has been paid to burrowing crabs as a major group of bioturbators in wetlands, where they promote or influence carbon transformation, nutrients cycling, and sediments remixing, among other ecosystem functions (Alberti et al., 2015; Xie et al., 2020). Burrowing crabs are recognised as one of the most essential macroinvertebrates in many wetlands, where they are often present in large numbers (Emmerson, 1994; Wang et al., 2010; Otero et al., 2020). Bioturbating activities of crabs in wetlands, including burrowing and feeding, can impact the physicochemical properties of wetland soils by: (1) breaking, transporting, and remixing sediments; (2) reducing wetland anoxia by increasing soil oxygenation; (3) reducing the hardness or compactness of soil; and (4) increasing decomposition rate of organic matter and debris (Botto, Valiela, Iribarne, Martinetto, & Alberti, 2005; Fanjul, Grela, & Iribarne, 2007; Wang et al., 2010). Crab burrows may change the air-water fluxes, increase the soil oxygen content, and oxidise the soil around the burrows (Nielsen, Kristensen & Macintosh, 2003). All these potentially influence plant growth and productivity.

Similarly, the positive impact of bioturbating activities of earthworms on soil health and nutrient qualities, plant growth and crop yield are globally recognised. Some bioturbating activities of earthworms that directly affect soil quality are burrowing movements, feeding and gut-grinding actions, tearing and breaking down of soil particles and plant debris into smaller units. Earthworm burrows serve as pathways for water and particle movements, nutrient flow, and soil aeration. The grinding of soil and organic matter in the gut of earthworms results in increased surface area for biological actions (Owa, Oyenusi, Joda, Morafa & Yeye, 2003; Dada et al., 2021). In addition, earthworms indirectly influence soil quality by facilitating the mineralisation and decomposition of soil organic matter through the activities of microorganisms and enzymes, which they release from their gut (Owa, Olowoparija, Aladesida & Dedeke, 2013; Dada et al., 2021). Although, more attention has been paid to the activities of earthworms in friable soil, some wetland or semiaquatic earthworms also contribute immensely to the productivity of their respective wetland habitats through their bioturbating activities, enzyme secretion, and vermicomposting actions (Owa et al., 2003).

Bioturbation by earthworms and crabs have been separately documented, however, studies have not investigated their comparative contributions to wetland soil quality. This study, therefore, aimed to compare the physicochemical qualities and microbial activities of earthworm- and crab-bioturbated soils in a wetland habitat.

2. Objectives

The objective of this study was to compare the physicochemical qualities and microbial activities of earthworm- and crab-bioturbated soils in a wetland habitat.

3. Materials and methods

3.1 Sampling location

The location of this study was the main campus of the University of Lagos, Nigeria. Bioturbated soil samples were collected from a wetland habitat within the campus. Crabs and earthworms are richly present in the wetland. The prevalent crab species in the sampling location of the wetland is *Cardiosoma armatum*. The crabs are always seen actively digging, burrowing and foraging throughout the day, especially in the early and late hours of the day, or after a rainfall (Figure 1). The predominant earthworm species in the sampling location of the wetland is *Alma millsoni*. These earthworms are endogeic (soil dwelling) species, which do not usually appear on soil surface, but their presence is always noticeable from their characteristic mouldy castings in the marsh (Figure 2).

Figure 1 Cross-section of the sampling location, showing foraging crabs

Figure 2 Cross-section of the sampling location, zoomed in to show mouldy casts of *Alma millsoni*

Figure 3 Cross-section of the sampling location, zoomed in to show dug-out soil by crab

3.2 Collection of soil samples

Soils separately bioturbated by earthworms (vermicasts) (Figure 2) and crabs (dug out soil) (Figure 3) were collected within three randomly placed 1 m^2 quadrats. Unbioturbated (undisturbed) soil samples were also collected from within the same proximity and used as control. Hand trowel was used to carefully scoop each soil sample into a polythene bag. The soil samples were taken to the laboratory, stored at < 4^o C, and analysed within 24 hours of collection.

3.3 Physicochemical analysis of soil samples

Earthworm-bioturbated, crab-bioturbated and unbioturbated (undisturbed) soil samples were analysed for physicochemical parameters, namely, soil particle size, pH, moisture, water holding capacity, electrical conductivity, total dissolved solid, cation exchange capacity, phosphorus, nitrogen, organic carbon, and total organic matter, using standard procedures. Soil particle size was determined by the hydrometer method as described by Gavlak, Horneck and Miller (2005). Electrical conductivity and pH were measured using the procedures described by Chaudhari, Ahire, Chkravarty and Maity (2014). Soil moisture content was measured using the procedure of the American Standard of Testing Method (2019). Water holding capacity was determined by the 'Droplet Counting Method' as described by Brischke and Wegener (2019). Phosphorus content was determined using the procedure described in Doolittle (2014). Soil nitrogen was evaluated using Kjeldahl method, as adapted by Jackson (1959). Soil organic carbon was determined by the Walkley-Black method (Walkley & Black, 1934). Total organic matter was estimated by the method of Chopra and Kanwar (1976).

3.4 Microbial counts in soil samples

Microbial communities (total bacteria, total fungi and total actinomycetes) were enumerated following the standard pour plate technique as described by Collins, Patricia, and Grange (1989). Ten grams (10 g) of soil sample was weighed with a sterile spatula using a chemical balance. The sample was introduced into a sterile pestle and mortal and then crushed. The sample was aseptically poured into a ninety millilitres (90 ml) bottle of sterile distilled water and properly mixed. One millilitre (1 ml) portion of the dilution was aseptically pipetted with a sterile pipette and introduced into 9 ml of sterile water. This was serially diluted up to the required 10^{-5} dilutions.

Disposable Petri-dishes were set out and labelled accordingly, while inoculation was carried out using the 'standard pour-plate method'. From the 10^{-4} and 10^{-5} dilutions, aliquot (1.0 ml) of inoculums was aseptically pipetted and inoculated into sterile Petri dish, while Nutrient agar, potato dextrose agar and starch casein agar were poured into the inoculums respectively, and rocked clockwise and anticlockwise, for even distribution of the inoculums. The plates were allowed to set properly. Nutrient agar plates were incubated aerobically at 37 ± 2˚ C for 24 hours, three to five (3-5) days. Potato dextrose agar plates for fungi were incubated at room temperature in an incubator set at 28 ± 2˚ C, for 3-5 days. Starch casein agar plates for actinomycetes were incubated aerobically at 27 °C, up to 7-10 days. At the end of the incubation period, the colony observed on the culture plates were counted using coulter colony counter. The colony or viable count per gram/ml was calculated by multiplying the average number of colonies per countable plate, by the reciprocal of the dilution, and reported as Colony Forming Units/g (CFU/g).

3.5 Statistical analysis of data

The data resulting from the laboratory analysis of soil samples were subjected to descriptive analysis using Analysis of Variance (ANOVA). Mean differences were separated using Duncan Multiple Range Test at 5% level of significance ($p < 0.05$). All statistical analyses were performed with IBM SPSS (version 26).

4. Results and Discussion

4.1 Texture of soil samples

Relative to earthworm- and crab-bioturbated soils, unbioturbated soil had the highest and significant ($p < 0.01$) sand and silt compositions of 42.73 ± 2.10% and 23.95 ± 1.75%, respectively (Figure 4). Conversely, bioturbated soil samples recorded relatively higher clay composition, with crab-bioturbated soil recording the highest (56.37 ± 0.58%). The differences in texture composition (sand, clay, silt) between earthworm- and crab-bioturbated soils were not significant (p < 0.05).

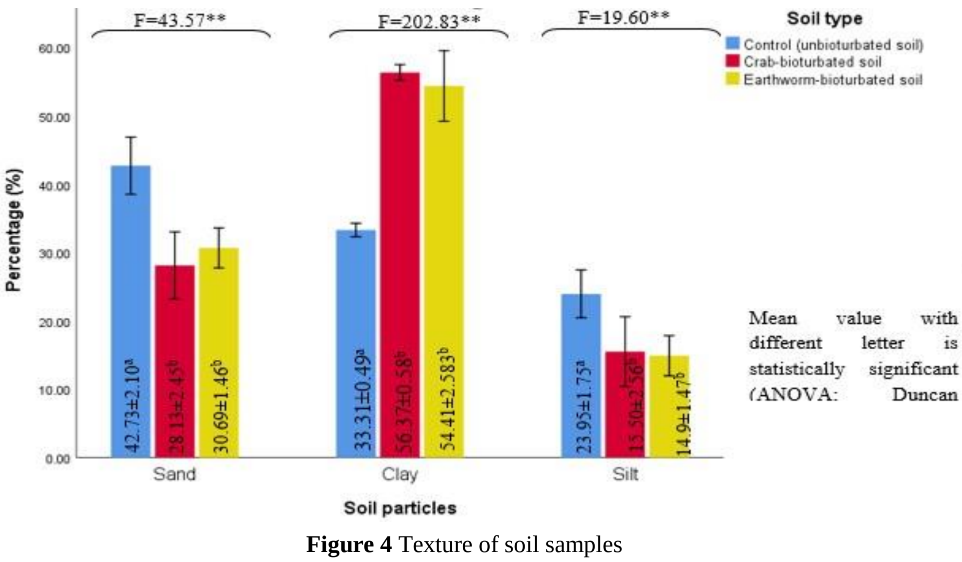

Figure 4 Texture of soil samples

4.2 Physicochemical properties of soil samples

Unbioturbated soil had the highest and significant ($p < 0.01$) pH (7.81 ± 0.05), electrical conductivity (1.86 ± 0.06 mS/cm), and cation exchange capacity (35.55 ± 0.79 mmol/kg). The pH of crab- and earthworm-bioturbated soils were 7.74 ± 0.03 and 6.85 ± 0.06, respectively. Crab-bioturabted soil had the highest and significant ($p < 0.01$) percentage moisture (50.48 ± 0.66%) and water holding capacity (53.67 ± 2.16%). However, earthworm-bioturbated soil recorded the highest percentage nitrogen (0.45 ± 0.02%), organic carbon (1.26 ± 0.02%), and total organic matter (2.18 ± 0.04%), while unbioturbated soil recorded the least (Table 1)

Table 1 Physicochemical properties of soil samples

Physicochemical parameters	Soil type			
	Unbioturbated	Crab-bioturbated	Earthworm-bioturbated	F
pH	7.81±0.05[a]	7.74±0.03[b]	6.85±0.06[c]	1136.04**
Moisture (%)	29.22±0.56[a]	50.48±0.66[b]	39.16±0.43[c]	2192.92**
Water holding capacity (%)	42.42±1.39[a]	53.67±2.16[b]	48.25±2.04[c]	52.84**
Electrical conductivity (mS/cm)	1.86±0.06[a]	0.93±0.32[b]	1.07±0.03[c]	1139.12**
Cation exchange capacity (cmolkg^{-1})	35.55±0.79[a]	25.95±2.22[b]	25.44±0.14[b]	104.80**
Phosphorus (mg/kg)	78.05±1.82[a]	87.01±2.20[b]	126.44±9.04[c]	132.84**
Nitrogen (%)	0.08±0.00[a]	0.09±0.00[a]	0.45±0.02[b]	2883.87**
Organic carbon (%)	0.65±0.02[a]	0.78±0.02[b]	1.26±0.02[c]	1379.90**
Total organic matter (%)	1.13±0.04[a]	1.35±0.03[b]	2.18±0.04[c]	1386.63**

NOTE: Each value is the mean of three replicates ± standard deviation. Different letter is statistically significant (ANOVA; Duncan multiple range test, $**P < 0.01$, $*P < 0.05$)

4.3 Microbial counts in bioturbated and unbioturbated soil samples

Earthworm-bioturbated soil had the highest and significant ($p < 0.01$) total bacteria, total fungi, and total actinomycetes counts of 129.33 ± 18.15x10^4 CFU/g, 46.22 ± 6.04x10^4 CFU/g and 56.22 ± 7.61x10^4 CFU/g, respectively. Microbial counts were least in unbioturbated soil samples (Figure 5).

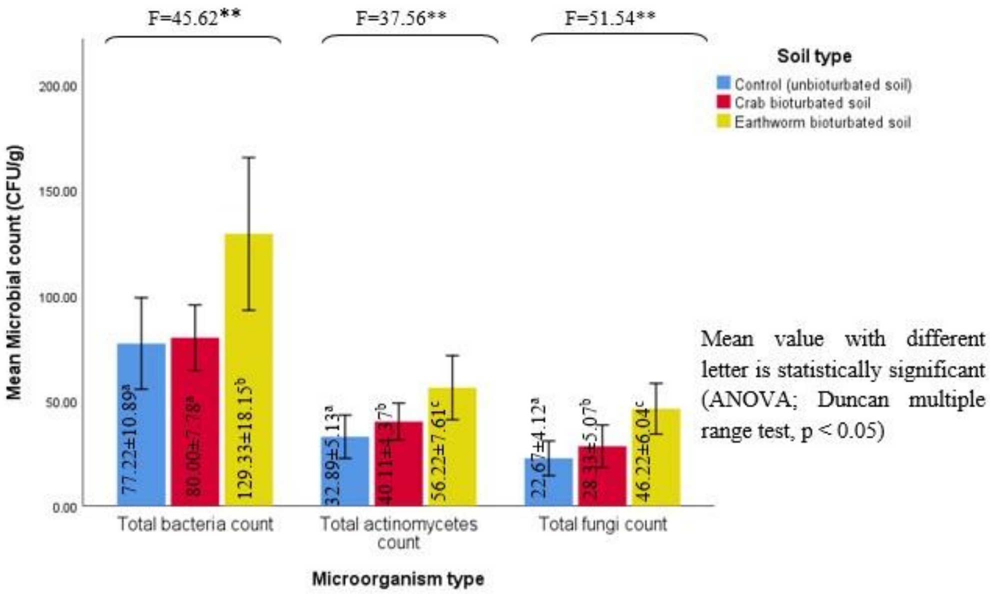

Figure 5 Microbial counts in bioturbated and unbioturbated soil samples

The observed improvement in the quality of bioturbated soils, relative to the unbioturbated control, agrees with Vidal et al. (2019) and Xie et al. (2019) who reported similar results for crab-bioturbated soil and earthworm-bioturbated soil, respectively. However, our results showed that earthworm-bioturbated soil had better biochemical and microbial properties, relative to crab-bioturbated soil. Despite the lack of similar or comparable past studies, the observed differences in the properties of bioturbated soil types can be related to the differences in structure, function, feeding, and bioturbating behaviours of earthworms and crabs. Bioturbation by crabs is exclusively exogenous; they turn soil over by transporting soil particles as they move across different layers, mixing the particles and soil constituents with their body and claws (Oyedele, Schjønning, & Amusan, 2006; Wang et al., 2010). Contrastingly, earthworm bioturbation is mostly endogenous; they ingest soil, mixing particles, minerals, and organic constituents with intestinal mucus, enzymes, and bacteria, and egest them as casts (Vidal et al., 2019). It is therefore expected that earthworm-bioturbated and crab-bioturbated soils showed significant differences in microbial mass and other parameters.

Despite earthworm-bioturbated soil and crab-bioturbated soil showing significant differences in biochemical and microbial properties, the differences in their soil textures (particle sizes of sand, clay, silt) were insignificant. This implies that the bioturbation habits of earthworms and crabs, though different, result in similar soil particle distribution. The increased water-holding capacity and moisture content recorded by crab-bioturbated soil, despite having lower organic matter, may be explained by its marginally higher clay and silt content, which could have created a relatively larger surface area for water adhesion, resulting in greater water and moisture retention (Voroney, 2007; Nath, 2014).

The earthworm-bioturbated soil sampled in this study is associated with *A. millsoni*, an endogeic species that feeds and burrows within the organic matter-rich mineral layers of soil. This partly explains why earthworm-bioturbated soil was richer in organic matter, relative to crab-bioturbated soil. The cutaneous mucus secreted by earthworms is also a potential contributor to increased soil organic matter. The higher soil organic matter recorded by the earthworm-bioturbated soil may also be related to the physical bioturbation actions of earthworms, which inherently promote the stabilisation and retention of soil organic carbon and soil organic matter in their casts (Angst et al., 2017).

It has been established that the gut of earthworms is host to several enzymes and a diverse population of microorganisms (Owa et al., 2013). Therefore, it is reasonable to suggest that the relatively higher microbial counts in earthworm-bioturbated soil are largely due to the passage of the soil through the gut of earthworms, before being egested as castings. Microorganisms facilitate the degradation (stabilisation/mineralisation) of soil organic matter into simpler compounds and minerals, such as phosphorus and nitrogen (Kiyasudeen, Ibrahim, Quail, & Ismail, 2016). This was seen in the higher phosphorus and nitrogen contents of earthworm-bioturbated soil, relative to crab-bioturbated soil, in the present study. Additionally, the higher electrical conductivity observed in earthworm-bioturbated soil agrees with the findings of Othaman, Isa, Ismail, Ahmad, and Hui (2020), who reported a positive relationship between electrical conductivity and soil minerals (nitrogen, phosphorus, potassium).

The improved quality recorded for the two types of bioturbated soil, relative to unbioturbated soil, is an indication that the presence and activities of both earthworms and crabs contribute significantly to wetland soil enrichment. However, the higher organic matter, total organic carbon, and microbial mass recorded for earthworm-bioturbated soil implies that earthworms have a greater impact on nutrient and microbial soil contents.

5. Conclusion

Earthworm bioturbated, crab-bioturbated, and unbioturbated soils were collected from a wetland habitat and analysed for microbial and physicochemical properties, for the purpose of comparative appraisal. Bioturbated soils generally recorded improved quality, relative to unbioturbated soil. However, earthworm-bioturbated soil had higher nitrogen, organic carbon, total organic matter, and microbial mass, relative to crab-bioturbated soil. Thus, we conclude that both earthworms and crabs positively influence wetland soil quality, but earthworms have a greater impact on nutrient and microbial soil contents. Nevertheless, there is the need to pay more research attention to crab-bioturbated soil in wetland environments.

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