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# Bacteria to Fungi Ratio and Organic Carbon in No-till Ultisols after Applications of Corn Residues and Poultry Manure

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author BEU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AOB managed the literature searches, and wrote the second draft of the manuscript. Both authors read and approved the final manuscript.*

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## ABSTRACT

No-till system may greatly increase soil organic carbon (SOC), and modify soil structure and water retention properties in the plough layer. Additions of organic materials of contrasting C and N ratio in no-till system require better understanding because they could cause a shift in the bacterial to fungi ratio. We studied the effects of short-term additions of 10 t ha $^{-1}$ dry corn residues ($CNS_{10}$) and 10 t ha $^{-1}$ poultry manure ($PM_{10}$) on bacteria-fungi ratio and other properties in a no-till ultisols. Results showed that $PM_{10}$ improved SOC content of soil by 49.7% while $CNS_{10}$ added 14.2% of SOC to the soil. There were significant ($p < 0.05$) increases in total N and available P in $PM_{10}$ soils. Total N ranged from 4.8 g kg $^{-1}$ in the control to 8.1 g kg $^{-1}$ in $PM_{10}$, while available P increased by 117% compared with the control. Application of 10 t ha $^{-1}$ poultry manure caused a major shift in microbial community towards greater bacterial population, whereas, dry corn residues encouraged significant shift towards greater fungi population. Relationships showed highly significant positive correlation between bacteria and clay content ($r = 0.714$, $p = 0.01$), and negative correlation with sand. Result also showed that fungal population increased with increasing sand content. These results indicate that addition of 10 t ha $^{-1}$ of poultry manure in a no-till management leads to significant increases in bacterial population, SOC, total N and available P. This method can therefore be recommended to farmers where reserve C and N as well as increased mineralization are needed.

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## 1. INTRODUCTION

The management of soils by reduced or no-tillage (NT) is many centuries old, with examples dating from the Egyptian civilizations. In modern agriculture, benefits of NT systems have been stressed since the 1940s [1-2]. No-till farming eliminates pre-plant tillage operations with the current year's crop planted directly into residues left on the surface from the previous cropping season [3]. Thus, no-till has been promoted as a soil management practice that improves long-term agricultural productivity and profitability through reduced input costs, increased biodiversity and soil organic matter (SOM).

Reduced soil disturbance common to long-term no-till system resulted in changes in SOM, aggregate size distribution and water storage capacity [4]. Additions of organic substrate could further alter various factors affecting diversity and composition of micro-organisms. As annual ploughing leads to soil organic carbon (SOC) oxidation, adoption of NT management system can lead to progressive restoration of SOC in agricultural soils [5,6,7]. However, the extent in which NT encourages certain microbial species over others has recently been challenged as possibly being overly based on speculations [8].

In tropical ecosystems, factors that contribute to SOC accrual in NT systems include better water retention, moderation of soil temperature by crop residues left on the land surface, protection against soil erosion, improved soil structure, and physical protection of organic C within soil aggregates [9] and evolution of fungi-dominated microbial communities stimulated by applications of suitable substrate [10]. Published literature is not clear on short-term effects of additions of crop residues and organic manure on soil microbial diversities and SOC stock in NT sites although short-term and long-term NT sites can be used to quantitatively estimate the temporal variations in bacteria-fungi population and C storage in soils [11]. Thus, adequate information relating to bacterial and fungal populations as well as soil organic carbon stock under NT land management will benefit soil scientists and other land users.

Previous researchers demonstrated that no-till system affected soil organic carbon (SOC) content and global C balance [12]. It was also reported that conventional plow-based tillage disrupted soil structure and aggregates [13,14] leading to drastic changes in soil environment (i.e. temperature, moisture, and oxygen), decreas in overall biological activity [15], exacerbating $CO_2$ emissions [16], and depletion of SOC [17].

Since each kind of vegetation (natural or cropped) may provide particular substrates, which influence population of bacteria over fungi and vice versa, NT systems have been adopted to minimize risks of soil degradation and to sustain the productivity of agro-ecosystems [18]. Lal [19] emphasized the positive relationship between yield and SOC content, particularly in highly C-depleted soils of coarse texture, and reported that adopting NT not only stored atmospheric $CO_2$ in soils for off-setting anthropogenic emissions of $CO_2$, but also restored SOC pool depleted by the conversion of native vegetation (NV) into agro-ecosystems.

In South and Central Cerrado of Brazil, conversion to no-till has been driven by the adverse environmental impacts, particularly soil degradation by erosion, and high production costs of the traditional plow-based mechanized crop production [20]. Their studies showed that NT farming systems have the potential to increase crop production with a lower footprint on the environment. USDA [21] also reported increase in population and diversity of micro-organisms in soils due to cultivation and application of manure.

However, these studies did not consider bacteria-fungi ratio as affected by short-term additions of crop residues and organic substrates in no-till managed soil. Such study will provide an opportunity to access the conversion of C from crop residues into a stable soil organic pool and thus determine the effect of such management practices on bacterial-fungal ratio as index of soil sustainability. The objectives of this study were: (1) to assess the effects of additions of corn residues and poultry manure on soil organic carbon and bacteria-fungi ratio under no-till management in the humid tropics of southern Nigeria, (2) evaluate SOC stock and bacterial-fungal populations as estimates of benefits of supplementing poultry manure and corn residues through comparison with the adjacent native soils, and (3) determine the relationships between soil particle-size fractions (sand, silt, and clay) and distribution of bacteria and fungi in the soil.

## 2. MATERIALS AND METHODS

### 2.1 Site Description and Experiment Layout

The study was conducted at the University of Port Harcourt, Teaching and Research Farm (Lat 4°45' N and Long 6°15' E) in the rain forest zone of southern Nigeria. Total annual rainfall in the area is usually high, (about 2400 mm during peak periods in July and September). Mean monthly temperature ranges from 22° to 32°C, with minimum and maximum relative humidity of 35% and 78% respectively [22]. The soil is derived from the coastal plain sands and classified as Arenic acrisol [23], dominated by low activity clays. The soil is inherently moderately well-drained, with low base saturation, pH below 5.0 and C: N ratio of about 24. Soil organic matter content at 0-15 cm is less than 1.0% in most areas.

The experiment was laid out in a randomized complete block design (RCBD), consisting of three treatments in five replications viz: (1) control plot without poultry manure and corn residues (C), (2) 10 t $ha^{-1}$ poultry manure ($PM_{10}$), and (3) 10 t $ha^{-1}$ dry corn residues ($CNS_{10}$). The field was demarcated into 15 plots, each measuring 5 m x 10 m.

### 2.2 Application of Treatments and Soil Sampling

The site was cleared during the early rains and allowed for 14 days before application the poultry manure and dry corn residues. Poultry manure was obtained from the Poultry House of the University of Port Harcourt and the dry corn residues from the previous cropping season. The poultry manure and corn residues were applied and homogenized into the soil. Soil samples were collected at 0-30 cm depth at the beginning and also at the end of the experiment, when the applied materials had decomposed freely at the top 0-30 cm soil. Five soil samples were collected in each plot, making a total of 75 composite soil samples. The soil samples were air-dried and sieved through 2 mm sieve and stored in plastic containers for laboratory analysis of total fungi and bacteria, total organic carbon, total nitrogen, available phosphorus, pH and particle-size distribution.

### 2.3 Laboratory Analyses

#### 2.3.1 Determination of particle-size distribution and chemical properties

Particle size-distribution was determined by the method of Gee et al. [24] after dispersion with sodium hexa-metaphosphate. Total organic carbon was determined by the wet oxidation dichromate method with $H_2SO_4$-$K_2Cr_2O_7$ followed by residual titration with 1 N HCl [25]. Total N was determined by the modified macro Kjeldahl method [26]. Available phosphorus was measured by the Bray II soil extracting procedure [27]. Soil pH in water was measured with glass electrode using a 1:2.5 soil/water aqueous solution [27]. All determinations were in duplicates.

#### 2.3.2 Determination of bacterial and fungal populations

The total bacterial counts were determined by the serial dilution, and nutrient agar plate dilution techniques. In this method, 1 g of soil samples were prepared by 10 fold serial dilutions to obtain a $10^{-5}$ dilution which was spread on the surface of nutrient agar in triplicates. The samples were incubated for 48 hours at room temperature and the bacteria were identified using Gram stain method [28]. The bacterial isolates obtained from the samples as total counts were: *Bacillus aureus*, *Staphylococcus aureus*, *Pseudomonas* sp, *Streptococcus faecalis*, *Salmonella enterocoloris*, *Esherichia coli* and *Shigella dysenteria*. The total fungal count was determined using the serial dilution and spread plate technique on potato dextrose agar [29]. Soil samples were prepared by 10-fold serial dilutions with 1 gram of soil sample and then 10-5 dilution was spread on the plates in triplicates. The soil samples were incubated for 48 hours and the isolated colonies were counted and identified using lacto-phenol blue and direct microscopic view according to Monica [29]. Fungi isolates that constituted the total colony forming units (cfus) found in the soils were: *Aspergillus niger*, *Mucor* sp., *Yeast*, *Penicillium* sp, and *Rhizopus* sp.

#### 2.3.3 Data analysis

Analysis of variance (ANOVA) was used followed by multiple comparisons on the effects of applications of corn stalk and poultry manure on the measured parameters using the SAS software [30]. Means were separated according to the least significant difference using Fisher's protected test [31] at 5% probability. We used correlation analysis to determine the relationships between soil particle-size fractions and fungi and bacteria populations. Significant correlation coefficient was tested at 5% probability. T-test of paired comparison was used to determine the effects of corn residues and poultry manure on fungi-bacteria ratio in the soil.

## 3. RESULTS AND DISCUSSION

### 3.1 Soil Organic Carbon, Nitrogen, pH and Available Phosphorus

Soil organic carbon (SOC) was 21.1 g kg $^{-1}$ and 16.1 g kg $^{-1}$ in $PM_{10}$ and $CNS_{10}$ soils respectively (Table 2). These values represent 49.7% and 14.2% increases due to $PM_{10}$ and $CNS_{10}$ treatments respectively, compared to the initial SOC content before the experiment (Table 1). At 12 weeks after applications of poultry manure ($PM_{10}$) and dry corn residues ($CNS_{10}$), SOC was 31% higher in $PM_{10}$ than $CNS_{10}$ soils. Also, there was a slight decrease in SOC in the control plots compared to the initial value before the experiment. This is consistent with previous studies of [32,33] who found that cultivation reduced soil organic C content, and some structural and hydraulic properties.

On the other hand, significant increase in SOC within 12 weeks in $PM_{10}$ soils than $CNS_{10}$ is indication that the short-term period of application of poultry manure (PM) increased rate of mineralization of C, whereas corn residues (CNS) decreased the rate of mineralization of C in the short-term, as reported by Six et al. [11].

The trend was similar for total N and available P, being significantly higher in $PM_{10}$ than $CNS_{10}$. Such increases would benefit soil fertility, leading to improved crop yield. The C: N ratio was significantly higher in $CNS_{10}$ soils (3.92) than in $PM_{10}$ (2.6), indicating that dry corn residues have greater carbon which could led to decreased rate of decomposition and mineralization [15].

Soil pH and particle-size distribution showed no significant changes due to applications of poultry manure and dry corn residues although pH was lower in the $PM_{10}$ soil. The non-significant ($p > 0.05$) effects of poultry manure and dry corn residues on particle-size distribution further confirmed existing report that land management practices does not usually alter the soil textural class, rather dominance of the parent material [34].

**Table 1. Properties of the soil before applications of corn stalk and poultry manure**

| Soil properties | Values |
|---|---|
| Sand (2000 µm) (g kg $^{-1}$) | 716 |
| Silt (20-200 µm) (g kg $^{-1}$) | 197 |
| Clay (< 20 µm) (g kg $^{-1}$) | 87.0 |
| Texture | Sandy clay loam |
| pH ($H_2O$) | 4.86 |
| Total N (g kg $^{-1}$) | 1.1 |
| Total organic C (g kg $^{-1}$) | 14.1 |
| C: N ratio | 12.8 |
| Available P (mg kg $^{-1}$) | 6.1 |
| Bacteria (cfus g $^{-1}$) | $3.2 \times 10^5$ |
| Fungi (cfus g $^{-1}$) | $1.7 \times 10^5$ |

### 3.2 Bacteria to Fungi Ratio

The application of poultry manure and dry corn residues created offset in the population and composition of microbial communities. Bacterial and fungal growth were sensitive to application of organic material as evidence in changes in their populations (Table 3). Bacteria responded more to poultry manure than fungi. At 12 weeks after application of treatments, bacterial population was significantly ($p = 0.05$) higher in $PM_{10}$ soils, whereas, fungi dominated in $CNS_{10}$ soils. Bacterial population was in the order of PM10 > CNS10 > Control, while fungal population was in the order of Control > $CNS_{10}$ > $PM_{10}$. Bacteria-fungi ratio ranged between 3.8 in PM10 soils and 0.72 in the control, indicating that

**Table 2. Effects of corn residues and poultry manure application on chemical properties of the soil after 12 weeks**

| Soil | Sand (g kg $^{-1}$) | Silt (g kg $^{-1}$) | Clay (g kg $^{-1}$) | pH ($H_2O$) | TOC (g kg $^{-1}$) | TN (g kg $^{-1}$) | C:N ratio | Avail. P (mg kg $^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| $CNS_{10}$ | 714 | 183 | 103 | 5.3 | 16.1 | 4.1 | 3.92 | 30.6 |
| $PM_{10}$ | 697 | 176 | 127 | 4.6 | 21.1 | 8.1 | 2.60 | 49.4 |
| C (control) | 715 | 198 | 109 | 5.2 | 12.1 | 4.8 | 2.52 | 22.8 |
| LSD (0.05) | NS | NS | NS | NS | 2.11 | 1.02 | 0.44 | 16.41 |

*TOC- Total organic carbon, TN- Total nitrogen* poultry manure stimulated proliferation of bacteria than fungi in the soil. Similarly, reduction of organic matter due to cultivation in the control plot led to significant reduction in both bacterial and fungal populations. This pattern of bacterial to fungal populations showed that applications of organic material increased microbial biomass in no-till soils at the top 0-15 cm layer [15,35]. The positive shift in fungal population due to application of dry corn residues can be attributed to the high lignin content usually associated with organic materials such as the dry corn residues, wood shavings and rice husk. This is consistent with widely held knowledge that fungi usually dominate organic material with high lignin content [36,37,38,39]. There is tendency that the slight increase in soil pH found in the $PM_{10}$ soils may enhance availability of certain nutrient elements that stimulated bacterial population.

Test of paired comparison of some soil properties as affected by the dry corn residues and poultry manure (Table 4), showed that application of 10 t $ha^{-1}$ poultry manure significantly ($p < 0.01$) increased bacterial population, whereas, the dry corn residues had significant ($p < 0.05$) effect on fungal population within the 12 weeks of experiment. Soil organic carbon and total N were also significantly ($p < 0.05$) higher in $PM_{10}$ than $CNS_{10}$ soils.

### 3.3 Relationships between Soil Particle Size Fractions, pH and Bacteria and Fungi

In Table 5, bacteria showed highly significant ($p < 0.01$, $r = 0.714$) positive correlation with clay content and negative correlation with sand content (Table 5). On the other hand, increased in sand content of the soil led to increase in fungal population and significant ($p < 0.05$, $r = 0.702$) negative correlation with clay and silt content. Increasing sand content led to decreasing population of bacteria in the soil. There is indication that in soil where bacteria number and organic matter decomposition are limiting, increasing clay content of the soil would be a useful management option. The positive correlation between clay-size fractions and bacteria could be attributable to the abundance of negatively charged surfaces usually associated with silt and clay-size fractions with large surface area for the bacteria. This is consistent with earlier reports that bacteria and cation exchange capacity of soils are usually much higher on clay and organic colloids [40,41].

Soil pH correlated positively with bacteria ($p < 0.05$, $r = 0.581$) and negatively with fungi ($p < 0.05$, $r = 0.611$) (Table 5). This indicates that bacterial population increased as the soil pH increased within a range of about 4.6 – 5.3 (Table 2), while fungal population increased as acidity increased. Relating soil pH to microbial biomass [36,39] demonstrated that microbial biomass increased as pH increased to about 6.2. Maintenance of soil fertility implied that soil pH is managed to a threshold value for increase in microbial biomass activity. Short-term application of 10 t $ha^{-1}$ of poultry raised the soil pH to a range suitable for increased bacterial population, leading consequently to the improvement of soil fertility.

**Table 3. Effect of corn residues and poultry manure application on bacterial and fungal populations after 12 weeks**

| Soil | Bacteria | Fungi | Bacteria: Fungi ratio |
|---|---|---|---|
| $CNS_{10}$ | $5.3 \times 10^5$ | $3.8 \times 10^5$ | 1.4 |
| $PM_{10}$ | $7.6 \times 10^5$ | $2.0 \times 10^5$ | 3.8 |
| C (control) | $4.3 \times 10^5$ | $6.0 \times 10^5$ | 0.72 |
| LSD (0.05) | 1710.38 | 1901.15 | |

**Table 4. Comparisons of bacteria, fungi and some properties of the soil under corn stalk and poultry manure (N = 45)**

| Soil properties | $CNS_{10}$ | $PM_{10}$ | t-values |
|---|---|---|---|
| Bacteria (cfus $g^{-1}$) | $5.3 \times 10^5$ | $7.6 \times 10^5$ | 4.11** |
| Fungi (cfus $g^{-1}$) | $3.8 \times 10^5$ | $2.0 \times 10^5$ | 3.85* |
| Organic C (g $kg^{-1}$) | 15.5 | 17.1 | 2.99* |
| Total N (g $kg^{-1}$) | 5.1 | 8.1 | 3.41* |
| C:N ratio | 5.3 | 4.6 | 0.59* |
| pH ($H_2O$) | 5.3 | 4.6 | 0.41ns |

*ns: Non-significant at p > 0.05, * significant at p < 0.05, ** significant at p < 0.01*

**Table 5. Correlation coefficient (R) between bacteria and fungi and soil properties (N = 45)**

| Soil parameters | Sand (g kg $^{-1}$) | Silt (g kg $^{-1}$) | Clay (g kg $^{-1}$) | pH ($H_2O$) |
|---|---|---|---|---|
| Bacteria (cfus g $^{-1}$) | - 0.648* | 0.614* | 0.714** | 0.581* |
| Fungi (cfus g $^{-1}$) | 0.702** | - 0.622* | - 0.607* | - 0.611* |

** significant at p < 0.05, ** significant at p < 0.01*

## 4. CONCLUSION

Based on present study, application of 10 t ha $^{-1}$ of poultry manure increased soil organic C, total N and bacterial population. At 12 weeks after application of treatments, fungal population was significantly higher in $CNS_{10}$ soils, while $PM_{10}$ increased bacterial population in the soil. There was positive correlation between bacteria population and clay-size fractions of the soil whereas fungi showed positive correlation with sand content. Application of 10 t ha $^{-1}$ of poultry manure demonstrated superior positive effects on bacterial population, total N, organic C while dry corn residues tended to increase C:N ratio and fungal population.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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