

Effects of postharvest *Irvingia* fruit wastes on soil microbial diversity and physico-chemical properties

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ABSTRACT

The effects of *Irvingia* fruit wastes on soil microbial diversity and physico-chemical properties were assessed through metagenomics and chemical analyses. Topsoil was thoroughly homogenized and separated into three different groups (Treatments 1, 2 and 3 soils respectively). Treatment 1 soil samples were assessed without any further processing. Treatment 2 soil samples were incubated for 14 days at ambient temperature and assessed whilst Treatment 3 soil samples were amended with *Irvingia* fruit wastes and incubated for 14 days at same temperature. Results showed that microbial diversity, total sequence reads, Operational Taxonomic Units (OTUs) and soil physico-chemical properties were influenced by *Irvingia* fruit wastes. Bacterial diversity for Treatments 1, 2 and 3 soils were 0.90, 0.28 and 0.93, respectively whilst bacterial total sequence reads and OTUs amplified from Treatment 1, 2 and 3 soils were 3,040 (82 OTUs), 23,544 (87 OTUs) and 16 (10 OTUs), respectively. Fungal diversity for same soil samples were 0.95, 0.80 and 0.33, respectively. Fungal total sequence reads and OTUs were 4,394 (50 OTUs), 55 (13 OTUs) and 27713 (39 OTUs), respectively. Bacterial OTUs amplified from Treatment 3 soil belonged to *Streptococcus pneumoniae* (25%), *Novosphingobium aquiterrae* (12.50%), *Shewanella haliotis* (12.50%), *Acinetobacter iwoffii* (12.50%), *Pseudomonas syringae* (6.25%), *Delftia tsuruhatensis* (6.25%), *Sulfobacillus* species (6.25%), *Nocardioides* species (6.25%), *Pseudonocardia* species (6.25%) and *Clostridium* species (6.25%). Predominant fungal OTUs from same soil were *Issatchenkia hanoiensis* (81.42%), *Pichia* species (6.28%), *Coniochaeta fasciculate* (5.61%) and *Candida diversa* (2.14%). *Irvingia* fruit wastes triggered an increase in soil pH, total nitrogen, total organic carbon, sulphate, phosphate, chlorine, calcium and sodium but not potassium. *Irvingia* fruit wastes would potentially, positively impact agriculture and bioremediation of oil polluted soils.

Keywords: Metagenomics, operational taxonomic units, 16S rRNA gene, internal transcribed spacer region, *Irvingia*, bacteria, fungi, soil health and fertility, organic amendment.

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INTRODUCTION

Irvingia species are multipurpose hardwood trees native to the humid lowland forests of tropical Africa in Angola, Cameroon, Central African Republic, Congo, Equatorial Guinea, Gabon, Nigeria and Senegal (Lowe et al., 2000; Atangana et al., 2002). They have mango-like ellipsoidal fruits measuring 4 to 7 cm long with the pericarp differentiated into exocarp which forms the peel, and mesocarp, the fleshy pulp and the endocarp which is the hardstone enclosing the kernel (Harris, 1996; Etebu,

2013). They are highly sought after in West Africa for their food and commercial value (Ladipo et al., 1996). Although bush mango is recently being domesticated, less than 10 percent of the total annual harvest of fruits is obtained from planted trees while the rest are collected from the natural forests.

Although, *Irvingia* species are widely recognized and exploited, the interest of most local harvesters is the kernel or seed. The kernel or seed is considered to be the

most valuable component of the fruit because it has the most known uses. Thus, once the seed is extracted, the fleshy pericarp and other parts, which constitute over 80% of the fruit, are discarded as waste and left to rot in dumps, water bodies, pits or nearby bushes (Etebu, 2012, 2013). Postharvest *Irvingia* fruits decay within few days after harvest, and severity of decay increases with time after harvest (Etebu, 2012, 2013; Etebu and Tungbulu, 2016; Etebu et al., 2016; Etebu and Oku, 2017). Interestingly, extracts from postharvest *Irvingia* fruits undergoing decay have been reported to potentially inhibit bacterial growth (Etebu and Tungbulu, 2015). Similarly, a recently published article on the metagenomics of bacterial community associated with postharvest *Irvingia* fruits showed a decrease in number of bacterial taxonomic Phyla and Classes as postharvest period increased (Etebu et al., 2018), whilst preferentially accommodating the growth of specific Genera of bacteria. These findings apparently suggest that extracts of postharvest *Irvingia* fruit would significantly influence soil microbial structure and diversity. It is therefore imperative to study the potential influence postharvest *Irvingia* species fruit wastes would have on the microbial diversity and structure of agricultural soils whereon they are dumped.

The significance of biodiversity has been appreciated since the 1950s (Hutchinson, 1959; MacArthur, 1957; MacArthur and MacArthur, 1961), and has continued to attain international recognition. Biodiversity promotes health of agricultural soils; ensuring the formation of ecologically stable and resilient soils (Walker, 1992; Tilman and Downing, 1994; Tilman et al., 1996; Norberg et al., 2001; Etebu, 2015). Microbial communities, particularly bacteria and fungi constitute an essential biological component of soil ecosystems, and these are often influenced by agricultural inputs (Girvan et al., 2004; Hu et al., 2011). Soil fungal population, for example, has been reported to be favourably influenced by organic farming systems (Drinkwater et al., 1995; Girvan et al., 2004), but such works have not been extended to agricultural soils amended with postharvest *Irvingia* fruit wastes. An understanding of the microbial structure and diversity of soil amended with *Irvingia* fruit wastes, as organic supplement, is required to predict the potential impact the fruit waste would have on agricultural soils across the Niger Delta region of Nigeria, where the fruits are harvested.

However, our knowledge about bacteria in natural environments is limited, and studying microbial diversity in nature has been an uphill task for several reasons. Firstly, soil microbial community is diverse, and heterogeneous (Singh et al., 2004). Secondly, microorganisms exist in high numbers in natural ecosystems and there are several thousands of microbial species that have not yet been described (Fakruddin and Mannan, 2013). For example, an estimated 1.5 million fungal species are considered to inhabit natural ecosystems, but only 5 to 10% have been described formally (Hawksworth, 2001; Wang et al., 2008). Similarly, prior to the advent of molecular biological

techniques bacterial community studies had always relied on culture-dependent assays. These assays are substantially superficial and could not be unreservedly trusted (Kellenberger, 2001; Price et al., 2009) because most bacteria ($\geq 99\%$) cannot be cultured (Hugenholtz et al., 1998a; Torsvik et al., 1990; Dokić et al., 2010).

In contrast, the application of metagenomics in the assessment of microorganisms in their natural environments, without recourse to culture of individual species, have greatly enhanced the study of microbial communities (Pinto et al., 2014). Although metagenomics of bacterial community associated with postharvest *Irvingia* fruits have recently been studied (Etebu et al., 2018); the approach is yet to be extended to study the microbial community structure and diversity of soil amended with postharvest *Irvingia* fruit waste.

Hence in this research, the effects of postharvest *Irvingia* fruit waste on soil microbial diversity and physico-chemical properties were studied through metagenomics and standard chemical analytical procedures. Findings from this work would broaden our knowledge on the potential impact of postharvest *Irvingia* fruit wastes on agricultural soils. Also, findings would enable researchers harness such potential impacts to the benefit of locals and communities where these fruits are harvested.

MATERIALS AND METHODS

About 30 kg of topsoil was thoroughly homogenized. Thereafter, 100 g was weighed out in triplicates as control (Treatment 1 soil) and refrigerated until needed for analyses for its soil microbial and physico-chemical properties using metagenomics and standard procedures. Of the remaining bulk soil, 3 kg was weighed into three separate Nursery bags (replicates) and incubated in the open field under ambient temperature for 14 days (Treatment 2 soil). Similarly, another 3 kg of soil each was separately weighed into another set of three nursery bags (replicates). This set of nursery bags containing 3 kg of topsoil were separately amended with 1 kg each of fresh *Irvingia* fruit wastes and incubated in the open field under ambient temperature for 14 days while the fruits decayed (Treatment 3 soil).

Experimental treatments were therefore designated Treatment 1, 2 and 3 soils respectively. Treatment 1 soil represented the soil in its original form prior to experimentation because the soil samples were stored away in a refrigerator immediately after homogenization; the effect of homogenization would not have had an effect on the microbial and physico-chemical properties. Treatment 2 soils on the other hand were incubated for 14 days at ambient temperature after homogenizing, whilst Treatment 3 soils were treated same way as Treatment 2 soils except that they were first amended with postharvest *Irvingia* fruit wastes prior to incubation for 14 days under ambient temperature.

On the 14th day, 100 g of soil was taken separately from all three replicates of Treatments 1 and 2 soils (soils not amended with *Irvingia* fruit wastes) and Treatment 3 soil (soils amended with *Irvingia* fruit wastes) and were analyzed for their soil microbial diversity and physico-chemical properties using standard procedures as described below.

DNA extraction and metagenomic analysis

Ten grams of soil each from all three replicates of each Treatment soil samples were separately, thoroughly mixed to form composite

soil samples. Thereafter, genomic DNA from Treatment 1, 2 and 3 soils were separately extracted and purified using ZR Fungal/Bacterial DNA MiniPrep™50 Preps. Model D6005 (Zymo Research, California, USA) according to the Manufacturer's protocol. Briefly, 0.5 g of soil sample was suspended in 200 µl of deionized sterile water and transferred into a ZR Bashing Bead™ Lysis Tube. Exactly 750 µl Lysis solution was added and microbial cells were lysed by bead beating for 5 min at maximum speed, and centrifuged at 10,000 × g for 1 min (Zymo Research, California, USA). Thereafter 400 µl of the supernatant containing nucleic acids was transferred into a Zymo-Spin™ IV Spin Filter collection tube and centrifuged at 7,000 × g for 1 min. The filtrate was then mixed with 1.2 ml of DNA binding buffer and 800 µl of the mixture was thereafter transferred into a Zymo-Spin™ IIC column in a collection tube and centrifuge at 10,000 × g for 1 min. The flow through was discarded from the collection tube and the process was repeated. DNA was thereafter washed with 200 µl DNA pre-wash buffer and centrifuge at 10,000 × g for another 1 min. The DNA was finally washed with 500 µl of DNA Wash Buffer into the Zymo-Spin™ IIC column and centrifuged at 10,000 × g for 1 min and eluted with 100 µl of DNA Elution Buffer into a clean and sterile 1.5 ml Eppendorf tube by centrifugation at 10,000 × g for 30 s.

Genomic DNA samples were thereafter sent to Inqaba Biotechnology Industries, a commercial NGS service provider in Pretoria, South Africa for further metagenomic procedures. Briefly, bacterial 16S rRNA partial gene sequences were targeted and amplified through Polymerase chain reaction (PCR) using primers 341F (5'-CCT ACG GGN GGC WGC AG-3') and 785R (5'-GAC TAC HVG GGT ATC TAA TCC-3') respectively as described by Klindworth et al (2013), whilst fungal Primers ITS1F (CTT GGT CAT TTA GAG GAA GTA A) described by Gardes and Bruns (1993) and ITS4 (TCC TCC GCT TAT TGA TAT GC) described by White et al. (1990) were used to amplify the Internal Transcribed Spacer (ITS) region of the ribosomal RNA gene sequences of fungi in a separate PCR run. Resulting amplicons were gel purified, end repaired and illumina specific adapter sequence were ligated to each amplicon. Following quantification, the samples were individually indexed, and another bead based purification step was performed. Amplicons were then bead sequenced on illumina's MiSeq platform, using a MiSeq v3 (600 cycle) kit. 20 Mb of data (2 × 300 bp long paired end reads) were produced for each sample. The BLAST-based data analysis was performed using an Inqaba in-house developed data analysis pipeline.

Simpson's diversity

Simpson's Diversity Index (SDI) used as measure of the diversity of bacteria and fungi was calculated from the formula

$$SDI = 1 - \frac{\sum n(n-1)}{N(N-1)}$$

Where SDI = Simpson's diversity index, n = number of the sequence reads of individual OTUs, and N = total number of sequences of all OTUs (adapted from Fowler et al., 2005).

Soil physiochemical and data analyses

Soil samples of all three replicates for each of the Treatment soils (1, 2 and 3) were separately analyzed for physico-chemical properties at the Niger Delta University Central Science Laboratory following standard test procedures. Data was managed in Excel Microsoft software and subjected to Analysis of Variance (ANOVA) using Generalized Linear Model of SPSS version 16.0 Statistical software.

Mean physico-chemical data were further subjected to Tukey's mean separation test and comparison of physico-chemical properties was made with respect to the different Treatments 1, 2 and 3 soils.

RESULTS AND DISCUSSION

Effect of postharvest *Irvingia* fruit wastes on soil microbial structure and diversity

Postharvest *Irvingia* fruit wastes influenced the structure, species richness and diversity of bacterial and fungal communities of soil (Table 1). Whilst the number of bacterial Phyla, Classes, Genera and Species were comparable in soils not amended with postharvest *Irvingia* fruit wastes (Treatment 1 and 2 soils), soil amended with postharvest *Irvingia* fruit wastes (Treatment 3 soil) revealed a clear reduction in bacterial 16S rRNA partial gene sequence read numbers across all taxonomic levels (Table 1). In particular, the number of bacterial Phyla, Classes, Genera and Operational Taxonomic Units (OTUs representing species) obtained from Treatment 1 soil were 12, 19, 62 and 82, respectively while same taxonomic groups of Treatment 2 soil totaled 11, 19, 64 and 87, respectively. Conversely, soil amended with *Irvingia* fruit waste had a lesser number of bacterial Phyla (3), Classes (6), Genera (10) and Species (10) (Table 1). Total number of bacterial sequence reads for Treatment 1 soil was 3040, Treatment 2 soil 23,544 and Treatment 3 soil was 16. This showed that thorough homogenization and subsequent incubation of soil at ambient temperature would lead to proliferation of bacterial population. This was evident from the clear difference in the number of bacterial sequence reads of Treatments 1 and 2 soils. On the other hand, amending soil with the fruit waste prior to incubation at ambient temperature (Treatment 3 soil) drastically reduced the bacterial population (Table 1). A relatively recent study showed that decaying postharvest *Irvingia* fruits potentially inhibited bacterial growth (Etebu and Tungbulu, 2015). Also, Etebu and associates (2018) showed a decrease in number of bacterial taxonomic Phyla and Classes associated with *Irvingia* fruit wastes as postharvest period increased, whilst apparently enhancing the growth of specific genera of bacteria.

However, whilst soil amended with postharvest *Irvingia* fruit (Treatment 3 soil) had a lower number of bacterial sequence reads across all bacterial taxonomic groupings, compared to (Treatment 2 soil), the reverse was the case regarding fungal structure and diversity. Findings showed an increase in soil fungal numbers across all taxonomic levels in Treatment 3 soil compared to Treatment 2 soil (Table 1). Number of fungal Division (Phyla), Classes, Genera and OTUs obtained from Treatment 1 soil were 2, 9, 38 and 50, respectively. Those of Treatment 2 soils were 1, 4, 12 and 13, respectively while soil amended with *Irvingia* fruit waste (Treatment 3 soil) had 3 fungal Divisions (Phyla), 10 Classes, 23 Genera and 39 OTUs (species) (Table 1). The total numbers of fungal sequence

Table 1. Effect of postharvest *Irvingia* fruit wastes on soil microbial taxonomic groups, number and diversity.

Parameter/taxonomic groups	Treatment 1	Treatment 2	Treatment 3
Bacteria			
Number of bacterial phyla	12	11	3
Number of bacterial classes	19	19	6
Number of bacterial genera	62	64	10
Operational taxonomic units (species)	82	87	10
Total number of bacterial reads	3040	23544	16
Fungi			
Number of fungal division	2	1	3
Number of fungal classes	9	4	10
Number of fungal genera	38	12	23
Operational taxonomic units (species)	50	13	39
Total number of fungal reads	4394	55	27713
Simpson's diversity indices			
Bacterial diversity index	0.90	0.28	0.93
Fungal diversity index	0.91	0.80	0.33

Key:

Treatment 1: Topsoil at the onset of experiment;

Treatment 2: Topsoil incubated for 14 days at ambient temperature

Treatment 3: Topsoil amended with *Irvingia* fruit wastes and incubated for 14 days at ambient temperature.

reads were also very varied for soils treated differently. Treatment 1 soil had 4,394 fungal sequence reads; Treatment 2 soil had 55 reads whilst Treatment 3 soil had 27,713 reads. In contrast to bacterial population, Treatment 2 soil which was homogenized and subsequently incubated without *Irvingia* fruit waste amendment had a relatively low fungal population, while Treatment 3 soil which was amended with *Irvingia* fruit waste and subsequently incubated witnessed an exponential increase in fungal population (Table 1).

Several works have shown that organic and inorganic amendments significantly affect soil microorganisms (Hu et al., 2011), and this in turn impact plant growth by enhancing nutrient turnover and suppressing plant disease incidence and/or severity (Zhang et al., 2012). Findings from this work showed that amendment of *Irvingia* species fruit waste did not only affect soil bacterial and fungal population but it also influenced the structure for both groups of microorganisms (Table 2). Whilst bacterial 16S ribosomal RNA partial gene sequences belonging to *Acidobacteria*, *Bacteroidetes*, *Chlamydiae*, *Chloroflexi*, *Gemmatimonadetes*, *Nitrospirae* and *Planctomycetes* were amplified from the Treatment 1 and 2 soils, same sequences were not obtained from Treatment 3 soil amended with the fruit waste (Table 2). Most of these bacterial Phyla are known to comprise members that are ubiquitous and diverse; inhabiting very diverse environments including humans, animals, fresh and marine water, dairy products, decaying plant

materials, soil etc (Björnsson et al., 2002; Hugenholtz et al., 1998b; Naether et al., 2012; Thomas et al., 2011); some members are able to degrade plant materials and high molecular weight organic polymers. *Bacteroidetes* for example are known for their versatility in the degradation of complex organic matter, especially in the form of polysaccharides and proteins (Church, 2008). Several earlier works have also shown an upsurge in *Bacteroidetes* population following input of organic matter to the environment (Thomas et al., 2011). Ribosomal RNA partial gene sequences of *Bacteroidetes* were therefore expected to be more abundant in Treatment 3 soil which was amended with postharvest *Irvingia* fruit wastes than its un-amended counterparts (Treatments 1 and 2 soils). However, whilst sequences belonging to members of *Bacteroidetes* were amplified from Treatment 1 and 2 soils, not a single 16S rRNA partial gene sequence belonging to any member of *Bacteroidetes* was amplified from Treatment 3 soil. A very recent work on bacteria associated with postharvest *Irvingia* fruit wastes showed the presence of members of this bacterial Phylum irrespective of the stage of decay; albeit in relatively low proportions (Etebu et al., 2018). Studying the effect of postharvest *Irvingia* fruit wastes on members of *Bacteroidetes* would therefore make an interesting investigation.

Bacterial 16S rRNA partial gene sequences amplified from soil amended with *Irvingia* fruit wastes belonged to members of only three Phyla and six bacterial Classes;

Table 2. Relative percentage occurrence of bacterial Phyla and Classes in agricultural soils amended and un-amended with postharvest *Irvingia* fruit wastes.

Bacterial phylum/class	Treatment 1	Treatment 2	Treatment 3	Mean
Phylum Actinobacteria				
<i>Actinobacteria</i>	29.28	9.89	12.50	17.22
<i>Thermoleophilia</i>	0.03	0.14	-	0.06
Phylum Bacteroidetes				
<i>Sphingobacteria</i>	0.07	1.00	-	0.35
<i>Flavobacteriia</i>	-	0.01	-	0.00
Phylum Chlamydiae				
Chlamydiae	0.20	0.01	-	0.07
Phylum Chloroflexi				
<i>Chloroflexia</i>	14.41	0.11	-	4.84
<i>Thermomicrobia</i>	0.10	0.01	-	0.04
<i>Anaerolineae</i>	0.03	-	-	0.01
<i>Ktedonobacteria</i>	-	0.004	-	0.00
Phylum Cyanobacteria				
<i>Cyanophyceae</i>	0.23	-	-	0.08
Phylum Deinococcus-Thermus				
<i>Deinococci</i>	-	0.76	-	0.25
Phylum Firmicutes				
<i>Bacilli</i>	16.84	0.33	31.25	16.14
<i>Clostridia</i>	0.23	0.03	6.25	2.17
Phylum Gemmatimonadetes				
<i>Gemmatimonadetes</i>	0.13	0.06	-	0.06
Phylum Nitrospirae				
<i>Nitrospira</i>	0.07	0.02	-	0.03
Phylum Planctomycetes				
<i>Planctomycetes</i>	6.51	0.02	-	2.18
Phylum Proteobacteria				
<i>Alphaproteobacteria</i>	5.03	86.39	12.50	34.64
<i>Gammaproteobacteria</i>	6.94	0.66	31.25	12.95
<i>Betaproteobacteria</i>	2.40	0.13	6.25	2.93
<i>Deltaproteobacteria</i>	0.03	-	-	0.01
<i>Epsilonproteobacteria</i>	-	0.004	-	0.00
Phylum Verrucomicrobia				
<i>Spartobacteria</i>	0.03	-	-	0.01

Key:

Treatment 1: Topsoil at the onset of experiment; Treatment 2: Topsoil incubated for 14 days at ambient temperature; Treatment 3: Topsoil amended with *Irvingia* fruit wastes and incubated for 14 days at ambient temperature.

these were *Actinobacteria* (*Actinobacteria*); *Firmicutes* (*Bacilli* and *Clostridia*) and *Proteobacteria* (*Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*) (Table 2). Results also showed that only 10 OTUs (species) were amplified from soil amended with the fruit waste (Table 1); indicating that

Irvingia fruit extracts modulate soil properties in ways that allow only a few set of bacteria to grow, albeit at a seemingly slow rate going by the total number of reads. Although findings of this work showed only a handful of bacterial OTUs amplified from soil amended with *Irvingia* fruit wastes, it is important to reiterate that some bacterial species apparently thrived within the period of experimentation, and such bacteria were *Nocardioides* sp., *Pseudonocardia* sp., *Novosphingobium aquiterrae*, *Streptococcus pneumoniae*, *Sulfobacillus* sp., *Delftia tsuruhatensis*, *Clostridium* sp., *Shewanella haliotis*, *Acinetobacter iwoffii* and *Pseudomonas syringae* (Table 3).

Novosphingobium aquiterrae is a Gram-negative, strictly aerobic, non-motile, non-spore-forming, yellow and rod-shaped bacterium, first isolated from ground water at Daejeon in Korea, and described by Lee et al. (2014). It belongs to the Family *Sphingomonadaceae*, belonging to the Class *Alphaproteobacteria*, Phylum *Proteobacteria*. Very little has been reported in scientific literature regarding this bacterium, and this is probably the first time, and work wherein it is reported in Nigeria.

Four out of the 16 bacterial 16S rRNA gene sequences amplified from soil amended with postharvest *Irvingia* fruit waste belonged to *Streptococcus pneumoniae* but none of such were obtained from the other soils not amended with the fruit wastes (Table 3). This suggests that the topsoil used in this work, in its original un-amended form had no *Streptococcus pneumoniae*. Also, there has been no indication in earlier works that this bacterium is associated with postharvest *Irvingia* fruit wastes (Etebu and Tungbulu, 2015, Etebu, 2012, 2013; Etebu et al., 2018). It is therefore difficult to unreservedly explain the source of this bacterium in Treatment 3 soil. From the ongoing, it would be convenient therefore to opine that the presence of *S. pneumoniae* was most likely accidental. This position notwithstanding, it is recommended that postharvest *Irvingia* fruit wastes are properly processed to eliminate potential human pathogens, especially if the fruits are intended for use in agriculture or human consumption. This is important because *S. pneumoniae* is the leading cause of pneumonia and meningitis in children and older adults (Tzanakaki and Mastrantonio, 2007). Although there are significant advances in medical care, current estimates of mortality linked to pneumococcal meningitis are still appreciably alarming.

A single 16S rRNA sequence read which represented 6.25% of total bacterial in Treatment 3 soil was identified as belonging to *Nocardioides* species (Table 3). The generic name *Nocardioides* was first presented by Prauser in a paper presented at a symposium on Nocardia during the 10th International Association of Microbiological Societies Congress held at Mexico City in 1970 (Prauser, 1976). Members of this Genus belong to the Order *Actinomycetales*, and many of them are known to inhabit soil (Štursová et al., 2012; Yeager et al., 2017). Whilst a good number of *Actinomycetales* are generally known for

production of antibiotics, a few species of *Nocardioides* have been reported to produce antibiotics (Matson and Bush, 1989; El-Rufai et al., 2011). These observations are very instructive and interesting, given the fact that very few bacterial OTUs sequences were amplified from soil amended with *Irvingia* fruit wastes. *Nocardioides* species whose 16S rRNA partial gene sequence was amplified from Treatment 3 soils could be antibody producing type, which probably depended on postharvest *Irvingia* fruit wastes as a substrate for growth, and in the process produced antimicrobial substances inimical to the growth of other bacteria. The potential production of antimicrobials by microbes, whilst feeding on *Irvingia* fruit waste extract, has often been suggested (Etebu and Tungbulu, 2015; Etebu et al., 2018).

Similar to *Nocardioides* species, one sequence read amounting to 6.25% of total bacterial sequence reads amplified from Treatment 3 soil was identified as belonging to *Pseudomonas syringae* (Table 3). *Pseudomonas syringae* is an ubiquitous bacterial species associated with plant (Morris et al., 2013; Tekoriené, 2008); comprises several strains involved in various crop diseases (Lamichhane et al., 2015). Some of the strains have been implicated to be soil borne (van Overbeek et al., 2010; Monteil et al., 2013). It would be worthwhile to ensure that postharvest *Irvingia* fruit wastes are not applied as organic manure to crops susceptible to *Pseudomonas syringae*.

Also, 6.25% of total bacterial sequence reads amplified from Treatment 3 soil was identified as belonging to *Sulfobacillus* species (Table 3). Members of this Genus are Sulphur-oxidizing bacteria (SOB), living in different soil environments. Most of them have been studied for their importance in agriculture, and other scientific fields. In particular, heterotrophic Sulphur-oxidizing bacteria are able to degrade toxic organic matter (Johnson, 2008). In addition, soil SOB play very significant roles in plant growth and yield by increasing the availability of Sulphate for plants absorption (Smith et al., 2000).

Clostridium species are also ubiquitous; Gram positive anaerobes spread across different environments. They utilize a wide range of natural organic compounds for growth, and in the process produce acids, alcohols, carbon dioxide and hydrogen (Almarsdottir et al., 2010). Soil inhabiting *Clostridium* has been reported to be influenced by geographical locality, pH, soil type, and other members of the microbial community (Gamboa et al., 2005). Similar to several other OTUs amplified from Treatment 3 soil, only 1 (6.25%) OTU belonging to *Clostridium* species was amplified from soil amended with *Irvingia* fruit waste (Table 3).

Two 16S rRNA sequences amounting to 12.50% of total bacterial sequence reads amplified from Treatment 3 soil was identified as belonging to *Acinetobacter iwoffii* (Table 3). Bacteria belonging to the Genus *Acinetobacter* have received enormous recognition and attention because of their varying and numerous significance as human

Table 3: Relative percentage occurrence of bacterial OTUs (species) sequences recovered from soils amended with postharvest *Irvingia* fruit wastes compared to their un-amended counterparts.

Operational Taxonomic Units (species)	Relative percentage number of reads			
	Treatment 1	Treatment 2	Treatment 3	Mean
Class: Actinobacteria				
<i>Nocardioides</i> species	2.07	0.00	6.25	2.78
<i>Pseudonocardia</i> species	-	0.01	6.25	2.09
Class: Alphaproteobacteria				
<i>Novosphingobium aquiterrae</i>	-	-	12.50	4.17
Class: Bacilli				
<i>Streptococcus pneumoniae</i>	-	-	25.00	8.33
<i>Sulfobacillus</i> species	0.30	-	6.25	2.08
Class: Betaproteobacteria				
<i>Delftia tsuruhatensis</i>	-	-	6.25	2.08
Class: Clostridia				
<i>Clostridium</i> species	0.10	0.01	6.25	2.12
Class: Gammaproteobacteria				
<i>Shewanella haliotis</i>	-	0.04	12.50	4.18
<i>Acinetobacter iwoffii</i>	-	-	12.50	4.17
<i>Pseudomonas syringae</i>	-	-	6.25	2.08

Key:

Treatment 1: Topsoil at the onset of experiment;

Treatment 2: Topsoil incubated for 14 days at ambient temperature

Treatment 3: Topsoil amended with *Irvingia* fruit wastes and incubated for 14 days at ambient temperature.

pathogens (Pantophlet et al., 2002) and potential roles in mitigating environmental challenges (Wagner et al., 1994). Findings from earlier works indicate that members of *Acinetobacter* facilitate the removal of biological phosphorus from waste water treatment (Wagner et al., 1994). Furthermore, many environmental isolates of *Acinetobacter* are able to degrade hydrocarbon (Das and Chandran, 2011), making them a focal point of research interest for soil bioremediation.

Similar to *Acinetobacter iwoffii*, two bacterial sequences amounting to 12.50% of total bacterial sequence reads amplified from Treatment 3 soil were identified as belonging to *Shewanella haliotis* (Table 3). The Genus *Shewanella* is a member of the Class *Gammaproteobacteria* (Anzai et al., 2000) and comprises a group of Gram-negative, motile, rod-shaped, oxidase positive, non-fermentative and facultative anaerobic bacteria (Bowman, 2005). They are typically found and isolated from aquatic and marine environments. *S. haliotis*, in particular, has been reported to be a heterotypic synonym of *Shewanella algae* (Szeinbaum et al., 2018), isolated from a marine snail. To the best of our knowledge, there is no report of this bacterium inhabiting soil in Nigeria. This work therefore is potentially the first

report wherein its 16S rRNA partial gene sequence was amplified from soil bacterial community.

Findings from this work further showed that the effect of postharvest *Irvingia* fruit wastes on the structure and diversity of soil fungi were largely dissimilar to what obtained with soil bacteria (Table 1). Whilst Treatment 2 soil harbored more bacterial Phyla than soil amended with *Irvingia* fruit waste (Treatment 3 soil), there were more fungal taxonomic Divisions (equivalent to bacterial Phyla) in the latter soil than the former. Treatment 2 Soil incubated for 14 days without *Irvingia* fruit amendment harbored only members of *Ascomycota* fungal division while fungal OTUs recovered from soil amended with *Irvingia* species fruit waste (Treatment 3 soil) spanned across three different fungal divisions viz, *Ascomycota*, *Basidiomycota* and *Glomeromycota* (Table 4). Comparison of Treatments 2 and 3 soils showed that OTUs belonging to *Tremellomycetes*, *Agaricomycetes*, *Pucciniomycetes* and *Glomeromycetes* fungal Class subdivisions were amplified from Treatment 3 soil but not from Treatment 2 soil (Table 4). Both soil samples were the same in all respect except for the addition of postharvest *Irvingia* fruit waste which was applied only to Treatment 3 soil samples. This clearly indicates that

Table 4: Relative percentage occurrence of fungal Divisions and Classes in agricultural soils amended and un-amended with postharvest *Irvingia* fruit wastes.

Fungal division/class	Treatment 1	Treatment 2	Treatment 3	Mean
Division: Ascomycota				
Saccharomycetes	4.26	61.82	93.80	53.29
Sordariomycetes	42.90	18.18	5.61	22.23
Dothideomycetes	19.37	12.73	0.06	10.72
Eurotiomycetes	30.41	7.27	0.03	12.57
Chaethyriomycetes	-	-	0.004	0.001
Ascomycetes	0.43	-	-	0.14
Orbiliomycetes	0.25	-	-	0.08
Division: Basidiomycota				
Tremellomycetes	1.14	-	0.46	0.53
Agaricomycetes	1.21	-	0.01	0.40
Pucciniomycetes	-	-	0.01	0.002
Cystobasidiomycetes	-	-	0.004	0.001
Exobasidiomycetes	0.05	-	-	0.02
Division: Glomeromycota				
Glomeromycetes	-	-	-	0.002

Key:

Treatment 1: Topsoil at the onset of experiment;

Treatment 2: Topsoil incubated for 14 days at ambient temperature

Treatment 3: Topsoil amended with *Irvingia* fruit wastes and incubated for 14 days at ambient temperature.

Irvingia fruit extract would selectively enhance the proliferation of, at least, some members of these fungal Class subdivisions. Findings further showed that the overall majority of fungal OTUs amplified from all three Treatments belonged to members of the Class *Saccharomycetes* (Table 4). This was particularly obvious for Treatment 3 soil amended with the fruit waste, wherein 93.80% of amplified ITS sequences belonged to members of the fungal Class *Saccharomycetes*. Probing further, results showed that majority of its members belonged to the Genus *Issatchenkia* (Table 5). Out of the 27,713 sequence reads recovered from soil treated with the fruit waste (Treatment 3 soil), 81.42% amounting to 22,564 belonged to members of this fungal Genus. Interestingly, all the fungal ITS sequences assigned to the genus *Issatchenkia* were actually a single OTU which represented a single fungal species, *Issatchenkia hanoiensis* (Table 5). This clearly showed that extracts of postharvest *Irvingia* fruits would create a preferentially conducive medium or environment for the growth and proliferation of *I. hanoiensis* in soil. *I. hanoiensis* is an ascogenous yeast fungi and was first isolated from a fruit borer insect *Conopomorpha cramerella* Snellen (Thanh et al., 2003). There were two other Genera of the fungal Class *Saccharomycetes* that were amplified from Treatment 3 soil with relatively high percentage occurrence. These were *Pichia* (7.66%) and *Candida* (3.90%). Organic farming has been shown to enhance the

proliferation of soil fungal population (Drinkwater et al., 1995; Girvan et al., 2004; Swer et al., 2011).

Apart from *I. hanoiensis* which had the highest relative proportion of abundance (81.42%) in Treatment 3 soil, three other OTUs (species) identified as *Pichia manshurica* (6.28%), *Coniochaeta fasciculata* (5.61) and *Candida diversa* (2.14%) also occurred in appreciable proportions ($\geq 2\%$) in the same soil (Table 5). Three of these fungi, *I. hanoiensis*, *P. manshurica* and *Candida diversa* belong to the fungal Order *Saccharomycetales* of the Class *Saccharomycetes*. Members of the Order *Saccharomycetales* constitute yeasts whose major and well known characteristics is the ability to ferment sugars for the production of ethanol (Carlson, 1987; Schneiter, 2004). Whether or not these fungi ferment postharvest *Irvingia* fruit wastes to produce ethanol in soil is yet to be studied. Nevertheless, it is pertinent to note that about 15% of the fresh weight of postharvest *Irvingia* fruit wastes have been severally shown to be Carbohydrate (Etebu and Tungbulu, 2016; Etebu et al 2016; Etebu and Oku, 2017). The obvious abundance of *I. hanoiensis* in postharvest *Irvingia* fruit wastes (Etebu et al., 2018) therefore portends that the fungus has an affinity to carbohydrate and would potentially play significant role in its degradation and utilization.

Apart from microbial structure and population, results also clearly showed that postharvest *Irvingia* fruit wastes had varying impacts on the diversity of bacterial and

Table 5. Relative percentage occurrence of fungal OTUs (species) obtained from soil amended with *Irvingia* fruit wastes compared to their un-amended counterparts.

Fungal class	Operational taxonomic unit (species)	Relative percentage of sequence reads			
		Treatment 1	Treatment 2	Treatment 3	Mean
Agaricomycetes	<i>Russula</i> species	-	-	0.007	0.002
Chaetothyriomycetes	<i>Exophiala</i> species	-	-	0.004	0.001
Cystobasidiomycetes	<i>Occultifur</i> species	-	-	0.004	0.001
Dothideomycetes	<i>Aureobasidium</i> species	-	-	0.043	0.014
	<i>Cladosporium</i> species	4.142	5.455	0.022	3.206
Eurotiomycetes	<i>Hamigera avellanea</i>	9.558	-	0.014	3.191
	<i>Aspergillus penicillioides</i>	0.091	-	0.007	0.033
	<i>Talaromyces pinophilus</i>	0.956	-	0.004	0.320
Glomeromycetes	<i>Glomus</i> species	-	-	0.007	0.002
Pucciniomycetes	<i>Rhodotorula glutinis</i>	-	-	0.004	0.001
	<i>Rhodotorula</i> species	-	-	0.004	0.001
Saccharomycetes	<i>Issatchenkia hanoiensis</i>	0.933	41.818	81.420	41.391
	<i>Pichia</i> species	0.319	-	6.282	2.200
	<i>Candida diversa</i>	0.023	-	2.136	0.720
	<i>Candida intermedia</i>	-	-	0.963	0.321
	<i>Pichia terricola</i>	-	-	0.891	0.297
	<i>Candida</i> species	1.843	-	0.696	0.847
	<i>Pichia hampshirensis</i>	-	-	0.476	0.159
	<i>Dekkera</i> species	-	-	0.292	0.097
	<i>Saturnispora diversa</i>	-	-	0.224	0.075
	<i>Galactomyces candidum</i>	-	5.455	0.130	1.861
	<i>Myxozyma</i> species	-	-	0.087	0.029
	<i>Candida incommunis</i>	-	-	0.069	0.023
	<i>Wickerhamiella</i> species	-	-	0.043	0.014
	<i>Candida tropicalis</i>	0.023	-	0.025	0.016
	<i>Dekkera bruxellensis</i>	-	-	0.025	0.008
	<i>Hanseniaspora opuntiae</i>	-	-	0.022	0.007
	<i>Pichia occidentalis</i>	-	-	0.007	0.002
<i>Candida bentonensis</i>	-	-	0.004	0.001	
<i>Candida boidinii</i>	-	-	0.004	0.001	
<i>Galactomyces</i> species	0.797	9.091	0.004	3.297	
<i>Pichia kluyveri</i>	-	-	0.004	0.001	
Sordariomycetes	<i>Coniochaeta fasciculata</i>	-	1.818	5.615	2.478
	<i>Tremella fuciformis</i>	-	-	0.253	0.084
Tremellomycetes	<i>Cryptococcus</i> species	-	-	0.177	0.059
	<i>Cryptococcus laurentii</i>	-	-	0.018	0.006
	<i>Trichosporon asahii</i>	-	-	0.004	0.001
	<i>Cryptococcus flavescens</i>	-	-	0.004	0.001
	<i>Cryptococcus humicola</i>	-	-	0.004	0.001

Key: Treatment 1: Topsoil at the onset of experiment; Treatment 2: Topsoil incubated for 14 days at ambient temperature; Treatment 3: Topsoil amended with *Irvingia* fruit wastes and incubated for 14 days at ambient temperature

fungal communities in soil (Table 1). Results showed that bacterial diversity indices for Treatments 1, 2 and 3 soils were 0.90, 0.28 and 0.93, respectively whilst fungal diversity indices for same Treatment soils were 0.95, 0.80

and 0.33, respectively (Table 1). Microbial diversity, often synonymously referred to as biological diversity occurs at three levels: within species (genetic), species number (species) and community (ecological) diversity (Harpole, 2010). The term species diversity consists of two components which are species richness and evenness. Species richness simply means the total number of species (Fakruddin and Mannan, 2013). The term Operational Taxonomic Units was often interchangeably used to represent this phenomenon in this work. The total numbers of bacterial 16S rRNA partial gene sequence reads were as follows: Treatment 1 soil (3,040), Treatment 2 soil (23,544) and Treatment 3 soil (16). This indicated a drastic reduction in soil bacterial population occasioned by postharvest *Irvingia* fruits wastes. A similar trend was observed with respect to bacterial species richness (OTUs). The total number of bacterial species for treatment 1, 2 and 3 soils were 82, 87 and 10, respectively. These findings clearly showed that postharvest *Irvingia* fruit wastes did not only influence the reduction of bacterial population in soil after 14 days of incubation but also reduced the number of bacterial species. However, notwithstanding the abysmal decrease in bacterial species richness in soil amended with the fruit waste (Treatment 3 soil) compared to its un-amended counterpart (Treatment 2 soil), the bacterial diversity of the former Treatment soil was much higher than the latter (Table 1). This is because the few bacterial OTUs (species) obtained from Treatment 3 soil were relatively more evenly distributed than those amplified from Treatment 2 soil. This proves the relevance of the interplay between species richness and evenness in the determination of biological diversity, and this was further exemplified by the findings of this present work regarding fungal diversity. Whilst the total fungal ITS sequence read counts for Treatment 1 soil was 4,394, Treatment 2 soil (55) and Treatment 3 soils (27,713), the total number of species represented by number of OTUs were 50, 13 and 39, respectively. Meanwhile, in contrast to bacterial diversity, fungal diversities were observed to be 0.91 (Treatment 1 soil), 0.80 (Treatment 2 soil) and 0.33 (Treatment 3 soil). Notwithstanding the relatively higher number of OTUs (species) and fungal read counts in Treatment 3 soils, as compared to Treatment 2 soils. Soil amended with *Irvingia* fruit wastes (Treatment 3 soil) had a lower fungal diversity because the sequence reads were hugely disproportionately distributed among the different fungal Operational Taxonomic Units (Species) (Table 5).

The stability of a biological community is dependent on its biological diversity (Yannarell and Triplett, 2005). A biological community that has attained a high degree of diversity is generally known to be stable, and often resilient. The relationship between biological diversity, resilience and plant disease suppressiveness has been described by several authors in ecological cycles (Tilman and Downing, 1994; Tilman et al., 1996; Etebu 2015). It has been shown that biological diversity enhances the

efficiency and stability of some functions of the ecosystems (Tilman and Downing 1994; Tilman et al., 1996). The role of biodiversity in the stability of functions within an ecosystem is related to the diversity of functional groups in it, and the species diversity within these groups (Norberg et al., 2001; Walker, 1992). The diversity would change when the community stability collapses following the presence of some kind of stress introduced to the community. Diversity is therefore used to monitor successions and effect of perturbations (Yannarell and Triplett, 2005). Results from this present work showed that homogenization and incubating soil under ambient temperature for 14 days clearly reduced its bacterial diversity (Table 1). Whilst *Irvingia* fruit wastes improved soil bacterial diversity, it greatly reduced the soil fungal diversity. It would be interesting to assess the effect of postharvest *Irvingia* fruit waste on soil microbial diversity for a much longer period of time. This would enable one assess how long it would take soils to regain or improve on their microbial species richness and diversity following amendment with postharvest *Irvingia* fruit wastes.

Effect of *Irvingia* fruit wastes on soil physico-chemical properties

Irvingia species fruit wastes significantly influenced the physico-chemical properties of agricultural soil (Table 6). Physico-chemical properties assessed in this work include pH, nitrogen, sulphur, phosphorus, calcium, magnesium, sodium, potassium, carbon/nitrogen ratio, chlorine and total organic carbon. Postharvest *Irvingia* fruit wastes caused a significant ($P \leq 0.05$) increase in pH, total nitrogen, sulphur, phosphorus, calcium, magnesium, sodium and chlorine whilst causing a significant ($P \leq 0.05$) decrease in carbon/nitrogen ratio (Table 6).

In particular, Treatment 1 soil which represented soil in its original form prior to fruit amendment and incubation was observed to have a mean pH of 6.40. This value significantly ($P \leq 0.05$) increased to 6.86 when the soil was incubated in nursery bag for 14 days without any form of organic amendment. The pH value increased further to 7.34 for soil amended with postharvest *Irvingia* fruit wastes and incubated in nursery bags for 14 days at atmospheric room temperature.

Generally, solutions with pH of less than 7 are said to be acidic whilst those above 7.00 are said to be alkaline (Slessarev et al., 2016). However, a recent categorization described soils with pH of 6.5 to 7.5 as neutral; those with a pH of over 7.5 are considered alkaline, while those with a pH of less than 6.5 are categorized as acidic, and soils with pH less than 5.5 are considered strongly acidic (Queensland Department of Environment and Heritage Protection 2016). Acidity and alkalinity of soils are very important chemical properties in soil assessment because the nature of a soil and the roles soil play regarding availability of nutrients, and the capacity of plants to

Table 6. Comparison of physico-chemical content of agricultural soils amended and un-amended with postharvest *Irvingia* fruit wastes.

Parameter	Treatment 1	Treatment 2	Treatment 3
pH	6.40 ^a	6.86 ^b	7.34 ^c
TKN	3.75 ^a	4.04 ^a	4.74 ^c
NO ₃	3.69 ^b	3.13 ^a	4.27 ^c
TOC	9.44 ^b	9.05 ^a	9.89 ^c
C/N	2.52 ^c	2.24 ^b	2.09 ^a
SO ₄	2.63 ^a	3.09 ^a	3.95 ^b
PO ₄	0.79 ^a	0.71 ^a	1.53 ^b
Cl ⁻	4.62 ^b	4.18 ^a	5.06 ^c
Ca	4.26 ^b	4.03 ^a	4.77 ^c
Mg	1.77 ^b	1.38 ^a	1.94 ^b
Na	3.17 ^b	2.72 ^a	3.80 ^c
K	1.22 ^a	1.14 ^a	1.38 ^a

Key:

Treatment 1: Topsoil at the onset of experiment;

Treatment 2: Topsoil incubated for 14 days at ambient temperature

Treatment 3: Topsoil amended with *Irvingia* fruit wastes and incubated for 14 days at ambient temperature

*Different letters denote significant difference at $P \leq 0.05$

** All physico-chemical parameters, except pH and C/N ratio are measured in mg kg⁻¹Soil.

absorb and hold materials is dependent on its pH (Queensland Department of Environment and Heritage Protection 2016; Slessarev et al., 2016).

Increase in soil pH has been shown to enhance C mineralization in acidic soils (Khalil et al., 2005) whilst application of organic residues in soil produce more mineral N in the form of NO₃ under neutral and slightly alkaline conditions (Fu et al., 1987). However, 5.5 to 7.5 has been reported as the optimum pH range for most plants, and many plants have adapted to thrive at pH values outside this range (Slessarev et al., 2016). This indicates that all soil treatments in this present work would sufficiently accommodate plant growth with respect to their mean pH values (Table 6). However, the significant increase in soil pH following amendment with *Irvingia* fruit wastes is a clear indication that *Irvingia* fruit wastes may serve to ameliorate acidic soils making them more alkaline, which would in turn enhance C mineralization.

Nitrogen in this work was measured as Total Kjeldahl Nitrogen (TKN) and Nitrate. *Irvingia* fruit wastes were observed to significantly impact on the Nitrogen content of agricultural soil, be it TKN or Nitrate (Table 6). Similar to pH, findings from this work showed that taking soils from their natural environment and incubating same in nursery bags for 14 days significantly increased TKN whether or not the soil was amended with *Irvingia* fruit waste. Whilst TKN of the soil at the outset of experimentation was 3.75 mg kg⁻¹soil, incubating same in nursery bags for additional 14 days significantly ($P \leq 0.05$) increased TKN to 4.04 mg kg⁻¹soil. This value increased significantly yet the more, up to 4.74 mg kg⁻¹soil, when the soil was incubated in nursery bags for 14 days following amendment with *Irvingia* fruit

wastes.

Total Kjeldahl Nitrogen is the total concentration of organic nitrogen and ammonia, and this parameter is often required to provide a means of monitoring plant operations. Whilst soil amended with the fruit wastes significantly increased in Nitrate content after 14 days of incubation, the content decreased significantly when the soil was stored without any organic amendment. Mean Nitrate content of Treatments 1, 2 and 3 soils were 3.69, 3.13 and 4.27 mg kg⁻¹soil, respectively (Table 6).

Nitrogen plays very vital and fundamental roles in the plant life cycle and crop yield. It is required for chlorophyll production and other plant cell components such as proteins and nucleic acids (Sinfield et al., 2010; Hoffland et al., 2000). Plants obtain nitrogen from the environment, majorly from soil, in the form of ammonium (NH₄⁺) and nitrate (NO₃⁻) (Gastal and Lemaire, 2002) but nitrogen is in short supply in the soil (Vigneau et al., 2011), and this has often led to the heightened quest for N fertilizers by farmers who are desperate to increase their crop yields (Muñoz-Huerta et al., 2013).

The importance of nitrogen notwithstanding, it is important to note that excessive fertilization with nitrogen is counter-productive. Excessive N-fertilization often leads to reduction in plant productivity (Rubio-Covarrubias et al., 2009) because plants are not able to absorb the excess of N-fertilizer (Zebarth et al., 2009). However, whilst not advocating for excessive N fertilization, findings from this work showed that postharvest *Irvingia* fruit waste could be used to amend soil as manure to enhance the nitrogen content often limited in most agricultural soils.

Similar to nitrate, the mean Total Organic Carbon

content of agricultural soil used in this work decreased significantly ($P \leq 0.05$) from 9.44 to 9.05 mg kg⁻¹ soil after 14 days of incubation soil without amendment with the fruit wastes. However, amending the soil with *Irvingia* fruit waste significantly ($P \leq 0.05$) increased the mean Total Organic Carbon content to 9.89 mg Kg⁻¹ soil after 14 days of incubation (Table 6). Organic matter comprises only a small fraction of the total mass of most soils, but this important and ever changing soil component does not only wield overwhelming influence on many soil physical, chemical, and biological properties (Brady and Weil, 1999) but also plays a central role in the global Carbon balance, thought to be the major factor affecting global warming, or the greenhouse effect (Heczko et al., 2011). Agricultural practices that increase the Total Organic Carbon is often desired, and one of the means of achieving this is to increase organic matter inputs to the soil (Goyal et al., 1999; Follett, 2001). Production of greater biomass, improved retention of crop residues near the soil surface are some of the ways through which increased soil Organic Carbon is achieved (Lal, 1998; Reicosky and Allmaras, 2003). Findings from this work showed that amending soils with *Irvingia* fruit wastes would be a cost effective means of enhancing Total Organic Carbon content of agricultural soils in the Niger Delta where the fruits wastes are discarded indiscriminately after extraction of the often desired kernels.

Soil organic carbon and nitrogen are often used as indices of soil quality and assessment of sustainable land use management. Tied to these indices is the soil C/N ratio critical for monitoring soil quality and for assessing the carbon and nitrogen nutrition balance of soils (Zhang et al., 2011). Amongst other factors, land use management is known to significantly influence the soil organic carbon and nitrogen content, and by extension the carbon/nitrogen ratio. Carbon/nitrogen ratios observed in this work were Treatment 1 soil (2.52:1), Treatment 2 soil (2.24:1) and Treatment 3 soil (2.09:1) (Table 6). Reports had shown that organic residues having low C/N ratios (< 25:1) indicate N mineralization while those with wide C/N ratios (> 25:1) mostly cause N immobilization during decomposition (Myers et al., 1994; Mary et al., 1996). Findings from this present work showed that soil amended with postharvest *Irvingia* fruit waste significantly reduced the C/N ratio when compared to soil without amendment of the fruit wastes (Table 6), and would therefore enhance N mineralization. Nitrogen (N) is often present in the environment in forms that are unavailable for plant uptake. Nitrogen occur around the plant root zone either as a gas (N₂) or as organic matter (including plant and microbial proteins and amino acids) (Watts and Torbert, 2012; Deenik, 2016). These different forms of Nitrogen contained in soil organic matter are usually converted into inorganic forms, ammonium (NH₄⁺) and nitrate (NO₃⁻) for plants to absorb and utilize for their growth and other metabolic processes. The conversion of nitrogen in soil organic matter to inorganic forms, useable by plants, is

mediated by soil microorganisms, and the process is called N mineralization (Deenik, 2016).

It was not surprising to observe a significantly lower C:N ratio, and therefore a relatively greater N mineralization capacity, in soil amended with *Irvingia* fruit waste compared to its un-amended counterparts. This is because N mineralization is largely dependent on the capacity of soil microorganisms to degrade complex soil organic matter. Metagenomic results obtained from this work showed that 16S rRNA partial gene sequences of 10 bacterial species, which included *Nocardioidea* sp., *Pseudonocardia* sp., *Novosphingobium aquiterrae*, *S. pneumoniae*, *Sulfobacillus* sp., *Delftia tsuruhatensis*, *Clostridium* sp., *Shewanella haliotis*, *Acinetobacter iwoffii* and *Pseudomonas syringae* were amplified from soil amended with postharvest *Irvingia* fruit waste (Table 3). Majority of these bacterial species are known for their versatility in degrading complex soil organic matter (Johnson, 2008; Štursová et al., 2012; Yeager et al., 2017). Also, two out of the three predominant fungal species whose ITS sequences were amplified from same soil Treatment belong to the Order *Saccharomycetales* which are known to degrade complex polysaccharides. Amending agricultural soils with postharvest *Irvingia* fruit wastes would therefore significantly enhance mineralization of N immobilized in soil organic matter.

However, the N supplying capacity of certain soils is reported to depend mostly on the indigenous soil organic matter, the addition of organic residues, and the various soil environmental factors (Fu et al., 1987). Workers have advocated for the improvement of soil organic carbon to increase the steady growth of soil C:N ratio (Ge et al., 2013).

As stated earlier on, findings from this work further showed that soil amended with postharvest *Irvingia* fruit wastes also significantly increased sulphate, phosphate, chlorine, calcium and sodium contents compared to its un-amended counterpart (Table 6).

Sulphate containing compounds such as aluminum sulphate is often used to acidify soil because acidified soils are known to suppress many plant root diseases (Fichtner, 2003). Although the mechanism of suppression is not known, the determinant factor is reportedly the lowering of the pH in soil. Findings from this work showed an increase in sulphate content in soil amended with *Irvingia* fruit wastes which did not lead to a corresponding increase in acidity (low pH) but on the contrary, postharvest *Irvingia* fruits wastes was observed to increase pH (alkalinity). This shows that increase in sulphate in soil would require other factors to result to acidification of soil.

Aside suppressing root diseases through acidification of soil, sulphur is needed in agriculture for crop production but its deficiency has become a huge challenge in recent times resulting to decreased crop yield and quality (McGrath et al., 1996). As a result, appropriate application of fertilizers has been identified as a remedy. The

significant increase in sulphate content in soil amended with postharvest *Irvingia* fruit wastes suggests that the fruit wastes could be used to enhance the sulphur status of soils, and still maintain the optimum pH range for plant growth.

Findings further showed a significant increase in phosphate contents in soil amended with postharvest *Irvingia* fruit wastes compared to soils not amended with the fruit wastes (Table 6). This again, is an indication that the fruit wastes could be used to improve plant growth and yield because phosphorus is second only to nitrogen in the order of important macro-nutrient required by plants (Saber et al., 2005; Xiao et al., 2011). Plants utilize phosphorus for several important functions; some of which include root development, flowering and fruiting, nitrogen fixation in legumes and resistance against plant diseases (Walpolo and Yoon, 2012). Phosphorus exists in small concentrations in soil solution, and plants absorb this all important element majorly in its phosphate forms (Mahidi et al., 2011). Although most agricultural soils are known to have a large reservoir of phosphorus, it is regarded as a limiting plant nutrient in soil because inorganic phosphate applied as fertilizers gets rapidly immobilized soon after application (Toro, 2007; Guiñazú, et al., 2010), rendering the element unavailable to plants. The means of immobilization is usually dependent on the soil conditions. For example free oxides and hydroxides of aluminium and Iron are said to fix phosphorus in acidic soils whilst calcium does the same in alkaline soils (Toro, 2007). It has been reported in some quarters that there is enough accumulated phosphate occurring in soil to sustain maximum crop yield for the next 100 years (Walpolo and Yoon, 2012). This group of researchers advocates seeking ways to solubilize immobile phosphorus in soil as against continual application of phosphate fertilizers, majority of which would get immobilized and add to the already super abundant deposit of the element (Khan et al., 2007). To this end, Researchers have turned to search for microbes that are able to solubilize immobile phosphorus (Khan et al., 2010). Interestingly, a diverse group of soil microorganisms (fungi and bacteria), collectively termed phosphate solubilizing microorganisms (PSMS) are known to solubilize insoluble phosphorus rendering it available to plants for absorption (Tripura et al., 2005). Some bacterial genera belonging to this group of microbes include *Bacillus*, *Pseudomonas* (Wani et al., 2007), *Rhizobium*, *Enterobacter* (Wakelin et al., 2004), *Rhodococcus*, *Arthrobacter*, *Serratia*, *Chryseobacterium*, *Gordonia*, *Delftia*, *Phyllobacterium* (Chen et al., 2006), *Azobacter*, *Pantoea* and *Klebsiella* (Chung et al., 2005). Although soil amended with postharvest *Irvingia* fruit wastes in this present work had a significantly lower number of bacteria, it is interesting to note that some of the 16S rRNA partial gene sequences amplified from Treatment 3 soil belonged to these genera of phosphate solubilizing microorganisms. This indicates that postharvest *Irvingia* fruit wastes would potentially enhance

the growth of PSMS which would solubilize phosphate in soil.

Results from this work further showed that application of postharvest *Irvingia* fruit wastes significantly ($P \leq 0.05$) led to an increase in the calcium content of soil (Table 6). This is very interesting because calcium (Ca) is an important plant macronutrient that plays key structural and signalling functions. Among other roles, calcium ions (Ca^{2+}) stabilizes and strengthens cell walls and membranes, and also serves as a secondary messenger for a multitude of signals (White and Broadley, 2003; McAinsh and Pittman, 2009; Dodd et al., 2010). However, the effectiveness of calcium in the delivery of these important functions has been linked to both the absolute amount of calcium, and on the ratio of calcium to other elements. For example, better fruit storage and less susceptibility to bitter pit in apples is achieved in the face of a lower N/Ca (nitrogen to calcium), K/Ca (potassium to calcium), or Mg/Ca (magnesium to calcium) ratios (Casero et al. 2009; Guerra and Casquero 2010). Consequently, calcium fertilizers are reportedly used from early fruit development stage to harvest to increase fruit calcium content (Conway et al. 2002; Peryea et al. 2007; Wójcik et al. 2009). The significant ($P \leq 0.05$) increase in calcium content of soil following application of postharvest *Irvingia* fruit wastes in this work (Table 6) indicates that *Irvingia* fruit wastes could be useful in agricultural practices that require organic calcium. It would be worthwhile to investigate the effect of soils amended with these fruits wastes on the overall health of crop plants and the postharvest quality of crop produce.

Treatment 3 soil amended with postharvest *Irvingia* fruit wastes was observed to have a significantly ($P \leq 0.05$) higher content of sodium (Na) than un-amended soil (Table 6). Excessive sodium in plant growth media is generally considered detrimental to plant because it negatively affects the availability of water to the plant. However, research have shown that toxicity of sodium, or rather the response of plants to salinity occasioned by sodium is dependent on, among other factors, the source of Sodium ion and the availability of other minerals. For example, sodium sulphate is reportedly less toxic than sodium chloride to the growth of wheat provided, potassium and calcium are available in the growth medium (Zaman, et al., 2002). Hence application of Ca-containing compounds considerably alleviates Na-dependent cell damage in plants (Rengel, 1992; Shabala et al., 2006). Calcium is said to ameliorate sodium toxicity in plants by reducing Na uptake and improving K nutrition of plants (Shabala et al. 2006). It therefore follows that plants would do well on soil amended with postharvest *Irvingia* fruit wastes, notwithstanding the relatively high sodium content.

In contrast to C/N ratio, soil amended with postharvest *Irvingia* fruit wastes had no significant ($P \leq 0.05$) differential effect on potassium (K) content of the soil compared to its un-amended counterpart.

Conclusion

The fleshy pericarp and associated parts of postharvest *Irvingia* fruit are often treated as wastes by majority of locals who harvest the fruits solely for its kernel. Findings from this present work showed that postharvest *Irvingia* fruits wastes on the overall significantly, positively impacts soils on which they are left to rot, and would potentially be useful in the agro-allied industry and bioremediation of crude oil polluted soils.

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