INDIGENOUS BACTERIAL DIVERSITIES IN TWO AGRICULTURAL SOILS AS INFLUENCED BY CHEMICAL AND ORGANIC HERBICIDES

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DEDICATION

I dedicate this study to God Almighty for the His kindness and unmerited favour which He granted me throughout the course of this research study up to its completion

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ABSTRACT

Agricultural soils as habitats for beneficial soil bacteria are usually invaded by weeds, an occurrence which has detrimental effects on crops. Chemical herbicides mostly employed for weed control adversely affect soil bacteria. Previous studies have reported chemical herbicides' effects on soil bacterial diversity suppressing microbial growth, however, information on soil types, and organic herbicides that could be an alternative is limited. Therefore, this study was designed to investigate the effects of chemical and organic herbicides on bacterial diversity in Alfisol and Inceptisol.

Soils (Alfisol and Inceptisol) were collected from research farms at University of Ibadan, Institute of Agricultural Research and Training, National Horticultural Research Institute, and International Institute of Tropical Agriculture. In a screen house experiment using a factorial arrangement in a completely randomised design, Atrazine and S-Metolachlor (AS-M), Isopropyl Amine (IA), and Acetic Acid (AA) herbicides were applied as pre-, post- and organic herbicides, respectively on the soils. In the field experiments, AS-M, IA and AA herbicides were also applied to the Alfisol and Inceptisol. All herbicide-treated soils were sampled at 4 and 8 weeks and bulked to determine physico-chemical parameters and herbicides persistency of the treated and untreated soils using standard methods. The effect on bacterial diversity in herbicide treated Alfisol and Inceptisol were determined by DNA extraction from the soil samples using 16S rRNA amplicons sequenced on illumina miseq. Data were analysed using descriptive statistics, QIIME 2, and ANOVA at $\alpha_{0.05}$.

Untreated Alfisol pH (6.20±0.12), organic carbon (3.90±0.04g/kg) and clay content (112.20±0.55g/kg) were significantly higher than Inceptisol pH (5.50±0.15), organic carbon (1.90±0.01g/kg) and clay content (103.90±0.81g/kg). At week 4, pH and organic carbon were significantly higher in Alfisol (6.30±0.21: 0.40±0.06g/kg) than Inceptisol (5.40±0.21; 0.10±0.06g/kg). Herbicides' persistency was higher in Alfisol than Inceptisol. Intermediate products (acetamide) in AA-treated soil (Alfisol: 30.2%; Inceptisol: 25.0%) < 2-amino-3-4-dihydro-4-4-dimethyl-6-pyrimidinone (Alfisol: 48.7%; Inceptisol: 35.3%) in IA < atrazine (Alfisol: 50.9%; Inceptisol: 37.1%) in AS-M at week 4, with a similar trend Alfisol: 22.1%, Inceptisol: 19.2%; Alfisol: 42.6%, Inceptisol: 33.2%; Alfisol: 43.9%, Inceptisol: 35.1% at week 8. Alfisol had significantly higher bacteria taxa of 79% than 21% in Inceptisol. *Methylomicrobium, Saccharopolyspora, Domibacillus, Blatococcus, Fuctibacillus, Limnobacter*, *Sneathiella, Nocardiopsis, Aquisphaer*a, and *Stenotrophomonas* were found in Alfisol and Inceptisol. However, *Limnobacter* and *Methylomicrobium; Sneathiella* and *Nocardiopsis; Aquisphaer*a and *Saccharopolyspora;* were the bacterial genera that survived in AS-M, IA and AA treated-soils, respectively. Significant effect in relative abundance of bacterial genera *Chthoniobacter* (Alfisol: 0.5%, 0.4%, 0.3%, 0.6%: Inceptisol: 0.3%, 0.2%, 0.3%, 0.5%) and *Pedosphaera* (Alfisol: 0.6%, 0.6%, 0.5%, 0.9%, Inceptisol: 0.7%, 0.5%, 0.5%, 0.9%) were observed at week 4, *Chthoniobacter* (Alfisol: 0.5%, 0.4%, 0.4%, 0.6%: Inceptisol: 0.3%, 0.3%, 0.4%, 0.5%) and *Pedosphaera* (Alfisol: 0.6%, 0.8%, 0.5%, 0.9%, Inceptisol: 0.3%, 1.2%, 0.9%, 1.3%) at week 8 in untreated and soil treated with AS-M, IA and AA, respectively. Alpha and beta diversity indicated significant differences in the relative abundance of bacteria between Alfisol and Inceptisol.

The relative abundance of bacteria diversity in Alfisol and Inceptisol were reduced by chemicals but increased by organic herbicides.

Keywords: Soil bacterial community, Chemical and organic herbicides, Alfisol and Inceptisol, Herbicide persistence.

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Agricultural production is known globally to be negatively impacted by weeds (Oerke, 2005; MacLaren *et al.,* 2020; Kubiak *et al.,* 2022). There are vast distribution of weeds mostly in agricultural lands which brings about significant loss in yield of crops annually majorly due to its competition in the soil and with the attending high cost of its control (Barroso *et al.*, 2010 ; Aurelio and Giovanni, 2020). There have been reports on the inability of the farmers to control weeds early during the growing season of crops, thereby leading to significant reduction in yields (Abouziena and Haggag, 2016). There is the complexity of weed seed bank resulting in annual growth from previous years without new seed production (Brainard and Bellinder, 2004). This gives it a comparative advantage over the crop, subsequently leading to low crop yield.

Mechanized tillage, had been previously used for weed control, and this allowed efficient weed control and favourable crop-growing conditions. Weed seed growth and production which is associated with crops in a mixed cropping farm had been controlled by crop rotation practice due to their different planting and harvest dates. This helps to obstruct weed establishment and also reduce their allelopathic effect (Virginia *et al.*, 2015; Fang and Zhihui, 2016). Weeds had also been controlled by some farmers with the use of cover crops which can easily smother weed crops due to their ability to grow fast and stand thick to create overshadowing effect (Adewale, *et al.*, 2018).

Community of microbes can be found in diverse environmental niches including agricultural soils where weed growth competes with crops (Ley *et al.*, 2006). Interactive microbial community in agricultural soil deposit key nutrients which are vital for plant growth, they also share functional gene through horizontal gene transfer. Various metabolites are being produced by these microbes including certain signalling molecules for sharing and communication and they join forces most of the times to fight against

pathogens, hence they are important to the health of their environment (Foxman and Martin, 2015).

Soil microbial population also aid greatly the health and stability of the ecosystem through their ability to recycle nutrient (which influences the soil's nutritional profile and crop productivity), sequestration of carbon as well as environmental restoration (Bender *et al*. 2016; Dubey *et al*. 2018; Malla *et al.* 2018). Contrarily, environmental imbalance usually caused through human actions which includes chemical applications made without proper control in agricultural soil management (in terms of weed control) has negative impact on the properties that make up the soil. This may trigger many changes in the nature and make-up of the soil microbial population and diversity (Fierer *et al*., 2012; Zhang *et al*., 2016).

Herbicides are group of chemicals and biological substances that are sprayed on purpose into the soil to control weeds. Scientists have reported that different types of herbicides are largely applied in the environment yearly worldwide (Kem, 2003; Pimentel, 1995). Increasing the availability and use of pesticides in agricultural production through policies were considered and introduced by the Russian government between 1990-2000 when their farmers experienced serious reduction in wheat and cereal production (Zakharenko, 2004). Effective weed control was achieved in the mid-1970s in Brazil, with the use of glyphosate (Bolliger *et al.,* 2006). This substituted for labour in hand weeding, ploughing and harrowing in maize farms (Ribeiro *et al*., 2007).

Previous studies reported low wheat yield and serious reduction in its production which was caused by difficulty in weed control (Banga *et al.,* 2003), high cost and scarcity of labour as well as lack of effective means of weed control in Pakistan and India. This situation resulted to use of herbicide (Ashiq *et al.,* 2006). Herbicide application generally, has recorded high grain yield improving as a result of efficient weed control (Khan *et al*., 2005). Herbicide application as a method of weed control had been reported to positively influence agricultural activities in different ways including reduction in operation time (Takeshita and Noritake, 2001). An increase of up to 20% in maize yield was reported in the United State due to increased acceptance and the use of herbicides. Grain production in Western Canada was also realised through the use of herbicides since it made minimum-tillage and zero-tillage farming techniques more widely used (Holm and Johnson, 2010).

In Africa, large quantity of annual loss in rice production had been recorded as a result of weed infestation (Olµmide *et al.*, 2020). Weed infestation resulted in serious decrease in maize yield in a farm in Nigeria. In contrast, increase in maize yield was observed when weed was effectively controlled with herbicide compared to the hand weeding practiced by farmers in Kenya (Muthamia *et al*., 2001). Since their introduction in the early 1950s for the cultivation of cocoa, chemical herbicides have become more widely used in Nigeria. According to Asogwa and Dongo (2009), Nigeria uses between 125,000 and 130,000 metric tonnes of herbicides annually, which causes a considerable accumulation of herbicide residues in the environment's soil. (Mohammed, 2009). With respect to various use of herbicides, about 80% of the them are utilized in agriculture, with the remaining portion going toward controlling pests related to structures and public health (Grube *et al*., 2011).

It is vital to note that the properties of herbicide transformation and how they behave in soil environments with regard to persistence are controlled by their ability to possess different chemical components and herbicides structural properties, their level of adsorption-desorption haviour, their ability to undergo different reactions catalysed by microbes to generate either simple or complex products (Singh *et al.*, 2006; Purnomo *et al*., 2011; Wang *et al*., 2013). Nevertheless, most herbicides used in soil are essentially subjected to biotic or abiotic degradation processes, which are made easier by specific environmental elements, soil types, the chemical components of the herbicide, and its preference for specific modification techniques. (Singh *et al.*, 2006; Benoit *et al*., 2008).

1.2 Statement of the Problem

Out of about 2.3 billion kg of different chemical herbicides that are being introduced globally into the environment annually for weed control (Carriger *et al*., 2006), most of the herbicide’s residue accumulates especially on agricultural soil and had resulted in significant environmental damage, most importantly, is their lethal effect on beneficial microorganisms in the soil (Jacobsen and Hjelmsø, 2014). These ecological alterations have been reported to initiate series of indirect effects such as destruction or decrease in specific microbial richness by herbicides. These processes have led to striking change in microbial structure (Lupwayi, 2009) which created disturbance in the flow of normal microbial functions (Widenfalk *et al*., 2004).

It is evident, that some experimental investigations have shown that a few microbial populations may develop the ability to biodegrade herbicide under different physiological conditions, which can serve as their carbon source, depending on their level of exposure to such chemicals (Wu *et al.,* 2014; Mohammed *et al*., 2019). Nevertheless, chemical herbicides are considered xenobiotics and most of them are recalcitrant and resistant to biodegradation. They are potentially toxic substances to both terrestrial and aquatic habitats. These ecosystems serve as the ultimate sinks for contaminants, which either enable them to persist or degrade into simpler forms. (Adriano, 2003).

1.3 Aim and Objectives of the Study

Thus, the main aim of this research is to examine how organic, pre- and post-emergence chemical herbicides affect the native soil microbial communities with regard to their stability and ecological roles.

The specific objectives of this study were to:

1. investigate the persistence of organic herbicides and two commonly used chemical herbicides under the pre- and post-emergence types in Alfisol and Inceptisol for a twelve-week period;

2. explore the relationship between the bacterial population in the soil for a period of 12 weeks and the level of biodegradability in pre- and post-emergence chemical and organic herbicides;

3. determine the variations in population and diversity level of bacterial community present in both Alfisol and Inceptisol soils and

4 examine the impact of herbicides application on the bacterial community changes in both Alfisol and Inceptisol soils.

1.4 Justification for the Study

Due to the lethal effect of chemical herbicides on soil microbes, there is a need for alternative herbicide in form of bio-herbicide which is eco-friendly since prior studies have concentrated more on the persistence level and effect of chemical herbicides on soil microbes. It is important to mention that previous studies had reported that organic agriculture promotes the use of organic herbicides compared to that of synthetic pesticides,

because it aims at preserving soil fertility (Seufert and Ramankutty, 2017; Smith *et al.*, 2020) however, there is dearth of information on the persistence level and effect of bioherbicide on microbial population on soils as well as information on the persistence level and effect of herbicides on microbial population of Alfisol and Inceptisol.

Additionally, it has been discovered that in the past two decades, researches involved in identification of microbial communities (which includes identification of the effect of chemicals introduced to the soil such as herbicides for weed control, on microbial communities) had been able to reveal sub-optimal information on the microbial communities, because of the focus on the -techniques such as; the use of artificial media for isolation of culturable microbes (which are able to allow the growth of less than 1 % of the microbial population but do not take care of the 99 % microbial population that are yet to be cultured), phospholipid fatty acid (PLFA) whose signature molecules cannot be connected to a specific group or species of microorganisms (hence this method cannot be utilized to research the soil microbiome down to the genus or species level), PCR-Denaturing Gradient Gel Electrophoresis (DGGE) technology, which allows possibility of numerous bands from the same bacterial species while single bands may represent multiple species resulting to wrong interpretation of microbial community structure) and Sanger sequencing (which have the ability to sequence individual microbes but not at community level). All these techniques have different limitations as mentioned above and this reduces their capacity to offer comprehensive details on the variety and number of members of the community of microbes, in addition to their potential activities.

Nevertheless, Since the 2000s, research focus has shifted towards investigating and documenting the soil microbiome's richness and evenness as well as their functional diversity through 'omics' studies like metagenomics, metatranscriptomics, metabolomics and metaproteomics which are high throughput techniques (Next Generation Sequencing NGS) and they provides broader spectrum to the potential and current roles that microbial communities may have (Aguiar-Pulido *et al.*, 2016 ; Hema *et al.*, 2020). With respect to metagenomics, it enables the study of microbes at their community level and can allow utilization of specific biomarkers such as 16S rRNA and ITS (Internal Transcribed Spacer) to investigate particular types of microorganisms at their community level, such as bacteria and fungi respectively. Metagenomics study using 16S rRNA gives a deep insight into

which and which bacterial community and diversity are present in a particular habitat as well as give an idea of their potential functions.

This research therefore, adopted the 16S rRNA metagenomics study approach in investigating the variations in soil bacterial population of Alfisol and Inceptisol agricultural soil orders so as to come up with more evidence-based information of their bacterial community and diversity as well as a clear information on the effect of both chemical herbicide and bio-herbicides on the bacterial population within this soil orders as well as their diversity.

1.5 Scope of the Study

The field part of this study was conducted at six locations within the research farms of the following institutes- Department of Agronomy, University of Ibadan, Institute of Agricultural Research and Training, Moore plantation (IAR&T); National Horticultural Research Institute (NIHORT); and International Institute of Tropical Agriculture (IITA) which are the make-up of the Alfisol and Inceptisol sites of this study. Whereas soil samples used for the screenhouse part of this study were all sampled at a depth of 0-15cm from all these locations to make up the Alfisol and Inceptisol types of the soil used for this study. Therefore, the two soil types were exposed to chemical and organic herbicides and were used to determine, the persistence and biodegradation level of chemical and organic herbicide on Alfisol and Inceptisol as well as the impact of chemical and organic herbicide on the bacterial population and diverties on Alfisol and Inceptisol This research is different from the previous research that focused more on effect of chemical herbicide on microbes.

CHAPTER 2

LITERATURE REVIEW

2.1 History of Herbicides Application in Agriculture

In agriculture, varieties of synthetic organic or inorganic substances known as pesticides are utilize to shield agricultural produce from different kinds of pests and pathogens (Yang *et al.*, 2007). According to earlier research, pesticides are normally classified according to the kinds of organisms they are meant to control. These consist of soil fumigants, nematicides, fungicides, insecticides, and herbicides (Imfeld and Vuillemier, 2012). However, the main groups of these pesticides can be further divided into subgroups based on their structural (organochlorine and organophosphorus, for example) and biochemical (choline esterase inhibitors, for instance) targets. Although Australia has employed over 8000 pesticides for agricultural reasons, Zhang *et al.* (2011) indicated that approximately 4.6 million tons of chemical pesticides are applied in ecosystem annually (Immig, 2010). According to some studies, the predicted global population of 6.8 billion at present which is likely to geometrically rise upto 9.1 billion by 2050, in addition to the availability of few crop areas, will eventually accelerate the use of pesticides to boast crop yield and maintain food security (Alexandratos and Bruinsma, 2012).

Herbicides accounts for approximately 80% of all used pesticides in agriculture (Grace communication, 2018). However, many modern chemicals which are used for agricultural purposes are specifically formulated in a way that they can decompose in a short while after application. This will enable crop growth on same land in future season without being affected by this herbicide. Herbicides can be classified and used as non-selective, systemic herbicides (non-selective, systemic herbicide tries to kill most plants as they are easily taken in by the leaves or roots and move to other sections of the plant) eg. glyphosate and non-selective, contact herbicide (non-selective, contact herbicide tries to kill most plants especially only at the part where the plants have contact with the chemical) eg. paraquat. The natural amino acid glycine, which possesses several dissociable hydrogens of the phosphate group, is identical to glyphosate. It can effectively kill a large number and variety

of plants when used for agricultural, horticultural and silvicultural purposes (Shipitalo, 2008).

Paraquat (1,1-dimethyl-4,4-bipyridyliμmdichloride), a non-selective, contact, broad-spectrum herbicide, has been globally and locally used for many decades (Tomlin, 2003). Paraquat has been sold to many countries where they are used on large and small farms, paddies, and for weed control in non-agricultural lands. Paraquat is sold under different brand names such Gramoxone, Paraforce, Bushfire etc. Dicamba and atrazine are examples of selective systemic herbicides. Dicamba is used as pre-emergence (Pre-emergent herbicides inhibits seed- weed germination by pausing the activation of a key enzyme) and post-emergence *(*post-emergence herbicides are often used to remove weeds) broadleaf weed control in maize and other crops. In order to control weeds in range land and non-crop areas (fence rows, highways, and waste), dicamba can be used in conjunction with other herbicides.

Dicamba controls weeds by increasing plant growth rate. Therefore, at sufficient concentrations, the plant grows more than its nutrient supplies, and dies. Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-s-triazine) an organic compound consisting of a striazine-ring is a widely used herbicide. It is one of the herbicides that is most frequently used worldwide (Duhigg, 2009) despite its exclusion from re-registration process in European Union. Its use is associated with cancer and birth defects when humans consume drinking water which has been contaminated by atrazine as a herbicide. Atrazine is the herbicide used in conservation tillage systems most frequently and extensively (Ackerman, 2007; Fawcett, 2008).

Herbicides can also be classified based on time of application which includes preplant, pre-emergence and post-emergence. Pre-plant herbicides can be non-selective herbicides applied to soil before planting which are mostly incorporated into the soil to prevent dissipation through photodecomposition. Herbicides applied before the weed seedlings and crop emerge through the soil surface are called pre-emergence herbicides. Dithiopyr and Pendimethalin are examples of pre-emergence herbicides. Herbicides disrupt the cell division of the emerging seedling, killing weeds as they grow through the herbicide-treated zone, but they do not stop weeds from germinating (Dan *et al*. (2011). Post-emergence

herbicides are used to eliminate weed seedlings after emergence and needs to be applied multiple times for adequate control. Post-emergence herbicides can be root absorbed, selective or non-selective, contact or systemic. 2,4-Dichlorophenooxyacetic acid (usually called 2,4-D) is an example of a selective, systemic, foliar absorbed post-emergence broad-leaf herbicide. Studies have also shown that diphenyl ether herbicides provide post-emergence broadleaf weed control in soybeans (Cobb, 2000).

2.2 Functional Roles and Economic Importance of Herbicides in Agriculture Globally

Soil, in its dynamic nature, provides habitat for more than 60 % of microbes which are the most numerous, diversified, and abundant group of organisms that inhabit soil (Bar-On *et al*. 2018), with distinct and critical functions they perform within the terrestrial ecosystem (Vibha and Neelam 2012; Muller *et al.*, 2016). Functioning of communities of microbes in the soil is crucial for its fertility and the high yield of produce from agriculture (Buckley and Schmidt, 2001). Microorganisms also serve as the linkage provider with respect to ecosystem functioning and environment due to their ability to sustain biogeochemical processes (Pete *et al*., 2015).

However, some agricultural management techniques, such as weed control, have an impact on various characteristics of the soil and this produce considerable modifications in structure of microbes and their populations (Fierer *et al*., 2012; Zhang *et al*., 2016). Previous reports confirmed there had been difficulty on the part of the farmers to control weed early during the crop growth season, thereby leading to significant reduction of yields (Abouziena and Haggag, 2016). The ability of a single weed plant to multiply fast due to presence of high seed content, makes it difficult for such weed plant to be controlled; for instance, Redroot Pigweed (*Amaranthus retroflexus*) produces 200,000 weed seed/plant (Brainard and Bellinder, 2004). This gives it a comparative advantage over the crop, subsequently leading to low crop yield.

Mechanized tillage, had been previously used for weed control, which created favourable circumstances for crop growth. Weed seed growth and production which is associated to crops in a mixed cropping farm had been controlled by crop rotation practice due to their different planting and harvest date. This helps to obstruct weed establishment and also

reduce their allopathic effect (Virginia *et al.,* 2015; Fang and Zhihui, 2016). Weeds had also been controlled by some farmers with the use of cover crops which can easily smother weed crops due to their ability to grow fast and stand thick to create overshadowing effect (Adewale, *et al.,* 2018). However, It has been discovered that, arable farmers had gone into alternative weed control which involves utilization of certain chemicals (Yue *et al*., 2020; Ustuner *et al.,* 2020).

Rules and regulations that encourage the accessibility and use of herbicides in agricultural production were considered and introduced by the Russian government between 1990-2000 when their farmers experienced serious reduction in wheat and cereal production (Zakharenko, 2004). Effective weed control was achieved in the mid-1970s in Brazil, with the use of glyphosate (Bolliger *et al.,* 2006). This substituted for laborious process in hand weeding, ploughing and harrowing in maize farms (Ribeiro *et al*., 2007). Previous studies reported low wheat yield and serious reduction in its production which was caused by difficulty in weed control (Banga *et al.,* 2003), high cost and scarcity of labour as well as lack of effective means of weed control in Pakistan and India. This situation resulted to their adoption of use of herbicide (Ashiq *et al.,* 2006).

Herbicide application generally, has recorded highly effective weed management which resulted to high grain yield (Khan *et al*., 2005). A significant amount of annual loss in rice production in Africa has been linked to weed infestation. (Olumide *et al.,* 2020). Weed infestation has also been reported to cause serious reduction in maize in a farm in Nigeria. However, increase in maize yield was observed when weed was controlled with herbicide compared to the farmers practice of hand weeding in Kenya (Muthamia *et al*., 2001). Herbicide application as a method of weed control had been reported to positively influence agricultural activities in different ways like; reduction in operation time experienced in Japan when 97% herbicides was used for weed management (Takeshita and Noritake, 2001). An increase of up to 20% in maize yield was reported in the United State due to increased acceptance and use of herbicides. Grain production in western Canada was also transformed by the use of herbicides as it encouraged the use conservation tillage by many farmers (Holm and Johnson, 2010).

2.3 Fate of Different Chemical Herbicides in the Environment

Application of herbicides in agriculture shows the soil to be the basic receptacle of these chemicals (Fabio *et al*., 2018). With respect to the availability of herbicides on soils, there is the tendency for major interactions between these herbicides and the soil as a receptacle. Experimental evidence has illuminated the three main routes of herbicides interaction with the soil environment. These are chemical, biological and physical. Chemical routes expressing soil-herbicide interaction include; Photodecomposition (Taking into account the chemistry of soil) and adsorption (Chen *et al.,* 2010 ; Ghafoor *et al.* 2011). Biologically, microbes which are the most influential soil flora greatly induce herbicide biotransformation within the soil environment (Idziak and Woźnica 2008). Third route involves the physical processes such as lixiviation (by water), soil desorption, volatilisation and erosion. The aforementioned methods rely on soil characteristics such texture, colloid type, soil pH, temperature, and moisture. (Spadotto and Hornsby 2003). The chemistry of herbicides also plays a key function with respect to molecular structure, ionisation, polarisation etc (Sparks and Swift, 2002).

With respect to adsorption, soil colloidal surfaces (Organic matter and clay) have the ability to attract molecules, ions (and other particles like herbicide) and they are drawn to the soil colloids through a means generally known as sorption (Passos *et al.*, 2013; Prado *et al.*, 2014). Basically, sorption of soil is a physical as well as reversible chemical reaction which could be regulated by forces like Vander waal strength, hydrogen bond, hydrophobic bind etc (Vivian *et al*., 2007). Studies have shown that as a reversible chemical process, soil sorption could always facilitate maintenance of balance on those pesticides accumulated in soil water and those that have been adsorbed on colloidal surfaces (Rojas *et al*., 2015). However, empirical evident have shown that sometimes soil sorption could result to irreversible chemical process when herbicide and soil colloidal surfaces interact. This usually generates complex stable molecules.

Significant evidence suggests that soil characteristics like pH, type of soil colloid, soil moisture and temperature as well as the molecular properties of herbicides solely control the level and intensity of processes that regulates sorption and desorption phenomena (Stipièeviæ *et al.*, 2014 Bontempo *et al*., 2016). Larger colloidal surfaces facilitate higher adsorption of herbicides. Herbicide sorption is also increased when the soil has less

moisture. This process discourages competition with H^+ concentration which could occur when the soil moisture is high (Blasioli *et al.* 2011). Experimental evidence with respect to the dynamic nature of the soil has shown that the complex nature of soil as regards its biological and chemical component bring up barriers which hinder clarity of the interdependent relationships and interaction among several components that participates in the herbicide's sorption processes in the soil, this impairs the process's ability to move freely (Yang *et al*.2000; Ye *et al.* 2001).

Certain kinds of movements are expected when herbicides are applied in the soil, the pre-emergence herbicides are required to make a soil surface incorporation so as to be taken up by weed seedling roots for maximum weed control. However, studies have shown that these herbicides can move in all directions and can as well move in gaseous phase when exposed to intense wind at a particular period of the year (Rojas *et al.*, 2015). It could also move to a vertical route which is majorly referred to as herbicide lixiviation (Vonberg *et al*. 2014).

Many projects had been completed in the last two decades to show that factors related to herbicides molecular properties (like water solubility, ionization capacity, molecular size and weight and so on), herbicides application methods including the edaphoclimatic factor such as rainfall and temperature, soil type, relief etc, all together influences herbicide lixiviation. This is a determinant factor for herbicide persistence in ecosystem (Wu *et al*. 2015; Kaur *et al.* 2017; Yang *et al*. 2018). Few populations of microbes as well as their absence in the lower horizon of the soil influences persistency of herbicides in such environment through lixiviation (Jablonowski *et al*. 2011).

Pesticides can enter water bodies in a variety of ways, such as unintentional spills, chemical waste from industries, groundwater runoff and transfer coming from pesticide-treated soils, rinsing pesticide sprayers after use, floating into water bodies, streams, lakes, as well as river water, aerial application for managing pests that hinder the absorption of water, and the discharge from rain or irrigation systems (Singh and Mandal, 2013). Due to their effect on human communities and other biological communities, pesticide residues in water have therefore given rise to serious concerns. Contrarily, a number of elements, including aerosol slide, vaporization from treated areas, and airborne application of pesticides might influence high accumulation of chemical pesticides in the atmosphere. The surface the pesticide lands

on, the amount of time during which it was applied, the surrounding temperature, humidity, direction of the wind, and component vapor pressure are all considered to have an effect on the rate of volatilization (Taylor *et al*., 2020).

2.4 Influence of Physical and Chemical Factors on Microbial Community Balance in Soils

Research has shown that various soil types influence microbial communities differently with respect to their inherent quality such as soil moisture, temperature, pH, organic and mineral colloids and other environmental conditions (Jon and Jackie, 2015 ; Georges *et al*., 2019) as well as the type of vegetation found within such soil type over time (He *et al.* 2019; Khondoker *et al.* 2020). This may happen because the total number of the microorganisms around the plant root system is impacted by the root exudates from this plant.

In line with the impact of soil components on microbial richness and composition, adaptation of higher population of fungi on low soil pH compared to bacteria had been observed (Kluber *et al*., 2012). Additional investigation observed that when arable soils were analysed for bacterial community and diversity, that acid loving microbes (acidophilic bacteria) were found as the dominant microbes (Rousk *et al.* 2010; Batram *et al*. 2014). Additionally, it has been noted that the pH of a particular soil type during a certain season affected any bacterial species that was prevalent at that time. (Rousk *et al*. 2010; Shen *et al.* 2013; Batram *et al*. 2014; Liu *et al.* 2014).

Other researchers indicated that the source and quantity of humus added to specific soils could affect biodiversity and evenness of microbes in those soils (Fu *et al.* 2015). Additionally, reports had shown that humus is the primary cause of aggregate stability, which is a sign of improvement in the performance of diverse soil microbes. (Theuerl *et al.*, 2010; Chen *et al*. 2015). There have been more research confirmations that elevated level of carbon content influences higher microbial population and reduction in nutritional stress compared to soil with lower organic carbon content (Kallenbach and Grandy 2011; Luo *et al.* 2015).

Empirical evidence has illuminated that microbial richness and evenness are impacted by variations in soil moisture and temperature (Prescott *et al.*,2000; Prescott *et al.*,2004; Hackl *et al.*, 2005; Carletti *et al.*, 2009). According to Hackl *et al.* (2005), Comparatively to non-

zonal areas where soil moisture was the influencing element for the microbial community organization and makeup, zonal forests experienced a stronger impact from mean annual temperature on the microbial community. Research carried out on microtopographic position also confirmed that microbial structure can be influenced by the influence of moisture and temperature within the arctic and alpine environments (Dennis *et al*., 2012; Janet and Kirsten, 2020).

In the terrestrial ecosystem, abiotic elements like precipitation. significantly influence the quantity, community makeup and possible activities of the soil microbiome which are in charge of decay and the mineralization of nutrients (Kaoping, *et al*., 2016; Anamika *et al.*, 2019). Most alteration reported on soil microbiota population were facilitated by changes in precipitation regime which resulted to a different rearrangement in community composition (de Nijs *et al.*, 2018). Soil moisture can mostly disrupt the flow of activities in a microbial community but not necessarily affect their population (Julia *et al*., 2019). Soil microbiomes have various adaptation mechanisms to varying moisture levels such as flooding, or drying and rewetting (Leizeaga, 2015).

By default, reduction in soil moisture will bring about reduction in soil microbial population or otherwise attract only drought tolerant microbes to inhabit such ecosystem since such microbes have the ability to adapt to unfavourable conditions where there is little or no moisture which causes drastic reduction in available nutrient (Stefano *et al*., 2012). Soil moisture reduction could probably result to drought if there is no rainfall for a long time hence microbial growth and their community structure may be affected as well as microbial functions (Lucia, *et al*., 2016). Other scientists have reported that sometimes total microbial community structure might not be affected by drought especially if such microbial community are resistant to drought while some group of microbes are partly influenced by drought (Christine *et al*., 2010).

2.5 Differential Soil Reactions and Possible Diversity of Microbes

According to Keenan and Williams (2018), the most significant land absorber for organic carbon (C) is found in Alfisol soils, containing almost two times the amount of organic carbon as the surrounding environment. These soils often grow under forest vegetation. In general, soil organic matter (SOM) and vegetation main products from above- and below-ground litter serve as the bacteria' major sources of carbon. As it is regulated by intricate interactions with plants and is a role mediated by net basic output, plant C distribution, root zone activity, and the degree of litter substrate quality (Butler *et al*., 2004), it typically has an impact on composition of microbes (Yuhua *et al*., 2021).

Studies have demonstrated that excluding roots and litter from wooded habitats reduces heterotrophic soil CO_2 outflow although there is insignificant effect of these changes with respect to alteration in biomass of soil microbiota within mineral soil (Nadelhoffer *et al.*, 2004; Sulzman *et al.*, 2005). However, with increasing depth, modifications to the microbial structure that composes mineral-rich soils of prairies was discovered, this was linked to variations in the amount of root nutrients as observed in the profile (Fierer *et al.*, 2003). In organic strata of forest soils, but not mineral horizons, root exclusion was observed to reduce the fungi population and modify the nature of bacterial ecosystems.

Inceptisol soils on the other hand are in the early stage of soil profile formation. The variations in layers are just at the initial appearance state. Some colour modifications were becoming obvious at this point between the developing layers of soil profile, and the initial development of a B horizon may be found to be accumulated with small amounts of clay, salts, and organic material. Inceptisol are usually present globally, but they are predominantly found in mountainous regions. Generally, the natural output of these soils has very broad variations, and this is influenced majorly by the degree of clay and organic matter content present as well as other edaphic (plant-related) factors. Due to their low soil water contents, Inceptisol, which are common in hilly areas and are mostly at the beginning stage of soil profile formation, have been observed to cause decrease in soil microbial richness and evenness (Archana *et al*., 2015), an effect observed to be induced by changes in soil nutrient and water utilization efficiency (Jingjie *et al*., 2020).

2.6 Interaction between Herbicides and Microbial Community in the Soil

2.6.1 Herbicides Impact on Indigenous Microbial Community

With respect to soil health regulation and maintenance, experimental evidence has shown that the array and microbial population of soil play significant roles through their abilities to recycle nutrient, sequester carbon as well as induce ecosystem restoration (Garcia- Garcia 2016; Malla *et al*. 2018). However, report has shown that arable farmers in a way to control weed (for better management and productivity of crops) employ various method including chemical herbicides utilization (Delorenzo *et al*., 2001). Soil microbial structure and biodiversity has been threatened by agricultural practices like use of herbicide for management of weed. It has been identified that only about 0.1% gets to the target organisms while large quantities of these chemical herbicides are been introduced into the ecosystem annually (Carriger *et al*., 2006; Mariane *et al.*,2020).

The remaining accumulation of pesticides has resulted in high level of environmental damage and most importantly, is their lethal effect on beneficial microorganisms in the soil (Ephantus *et al.* 2017). This might result in modifications to the various functions of soil microorganisms, diversity, and/or genetic makeup, as well as changes to soil nutrient levels (Makova *et al*., 2011). Herbicide degrees of toxicity determine their extent of impact and hazard on soil microbial community. It has also been reported that factors like adsorption, desorption, leaching, run-off, degradability, volatilization, persistence, uptake by plant etc can influence their bioavailability level in the soil (Jacobsen and Hjelmsø, 2014). Accumulated pesticides used by farmers around the world have been found to have detrimental impact on the structure and microbiological population of soil (Alvarez-martin, 2016; Mariane *et al.*,2020). This in turn cause fluctuation on nutrient status of the soil since enzyme activities such as nitrate reductase, oxidoreductases, dehydrogenase as well as biological nitrogen fixation and their associated biotransformation activities are also being affected (Monkiedje and Spiteller 2005).

Wang *et al*. (2006) also reported that intensive herbicide application in contemporary agriculture negatively influence the diverse range of functions for the soil microorganisms as well as reduction in microbial carbon biomass. Application of herbicides for weed control interfered with microbial metabolism with respect to carbon substate utilization and

nitrification process as earlier reported (Jacobsen and Hjelmso, 2014). Recent investigation on effect of chlorsulfuron and sulfosulfuron herbicides on soil microbes, their community as well as activities reported that both herbicides can negatively modify microbial enzyme activity, such as cellobiohydrolase, arylsulphatase, dehydrogenase, phosphatase, and FDA hydrolase. However, the microbial composition was not highly affected (Juraj *et al*., 2020).

Research had shown that pesticide such as organochlorine compounds caused a positive alteration in microbial community structure of easy to culture heterotrophs during a lab-based experiment (Awadehesh *et al.*, 2014; Satya *et al*, 2019). A rise in population of species such as *Vanovorax* and *Burkholderia* were also discovered in a field-base study when same organochlorine compounds were also used (Humphries *et al*., 2005). Previous studies discovered that applying herbicide over an elongated time on arable farms resulted to loss of microbial diversity. Repeated application of more than one pesticide with different chemical compositions can alter microbial composition and diversity (Goulson, 2013; Rahman *et al.*, 2020). Scientific evidence has demonstrated that some pesticides' persistence may change their function in addition to facilitating reduction in diversity of soil microbes (Fang *et al*., 2009). Cycon and Piotrowska, (2015) also reported alteration of microbial community structure of those that oxidizes ammonia. This negatively affected rate of nitrification process in soil.

Certain bacteria can be prevented from growing when they have contact with pesticides through disrupting their enzyme function. According to Hussain *et al.* (2009), for instance organophosphate pesticide, can block nitrogenase activity, which can limit the level of nitrogen available to plants and lower agricultural production. Researchers have found that the fungicides chlorothalonil and dinitrophenyl disturb the bacterial-dependent processes of nitrification and denitrification (Lang and Cai, 2009). According to Udochukwu *et al.* (2018), pesticides can hinder the soil bacteria responsible for turning ammonia into nitrite. Additionally, studies have demonstrated that the non-selective herbicide glyphosate prevents soil bacteria that fix nitrogen from growing and functioning (Marcos *et al.*, 2020).

Herbicides like trifluralin and oryzalin have also been found to significantly harm fungi in soil by preventing the formation of symbiotic mycorrhizal fungi, that helps to facilitate uptake of nutrients (Franci and Katarina, 2016; Hage-Ahmed *et al.*, 2019). Schreck *et al.*

(2008) revealed that pesticides and/or fungicides have neurotoxic impacts on earthworms. Earthworms play great role on ecosystem since they serve as bio-markers of soil pollution and as tools for investigating soil contamination. Once they become impacted by pesticides for a while, they typically suffer physiological damage. According to Casabé *et al*. (2007), glyphosate and chlorpyrifos harm earthworms' ability to feed and survive by disrupting their DNA at the cellular level. The xenobiotic features of pesticides have encouraged the persistence of pesticides in soil environment which eventually gets into the food chains (Arias-Estevez *et al*., 2008).

It has been documented that herbicides are exogenous to soil component pools hence their influence on the behaviour and catalytic efficiency of soil enzymes (Riah *et al*., 2014), which adds to the biological activity of soil-plant ecosystem in a variety of conditions. Additionally, there have been reports on the chrematistics and measurable modifications in enzyme functionality of soil bacteria after herbicide application (Sebiomo *et al*., 2011, Xia *et al.,* 2011). Consequently, the interaction between soil microorganisms and herbicides frequently resulted to inhibitions in microbial activities that would contribute to soil fertility. Scientists have elucidated that microorganisms can be considered as an indicator of soil contaminant since they could easily response to them, hence, maintaining the fertility and quality of the soil is vital, as well as soil microbial population (Huera-Lucero *et al*.,2020; Alaa *et al*., 2020).

Soil enzyme activities could give great insight to status of the microbial environment. The population and variety of microbes can function as markers of soil productivity (Yi *et al*., 2019).. Hubert *et al*. (2005) had reported a modification in bacterial community's diversity which resulted due to the co-metabolism of the organochlorine TCE (trichloroethylene) and toluene. After the use of toluene and TCE for incubation, culturable heterotrophs doubled in population compared to the population growth seen during incubation with TCE alone in this experiment. But it was shown that the microbial ecology's genetic makeup was altered by TCE (and phenol) through a rise in *Burkholderia* and *Variovorax* species in TCE-contaminated wastewater.

Exogenous endosulfan treatment enhanced bacterial biomass but decreased fungal biomass to 50% of the bacterial biomass, according to Xia *et al.* (2011). Nevertheless, it turned out

that nitrification of soil bacteria was extremely responsive to the incorporation of fenamiphos, making it obvious that the health of the soil was at stake. This contrasts with another study on the impact of this same pesticide on the metabolic processes of certain enzymes, which showed no effect on these enzymes in Australian soils (Cáceres *et al.*, 2008). Zhang *et al.* (2006) identified decrease in the variety of soil microbes as a result of use of pesticides such methyl parathion for an elongated time. Repeated use of number of pesticides with various chemical characteristics was found to have unpredictable effects on soil microbial populations when it was studied by a team of researchers (Wang *et al*., 2007).

Previous studies observed that persistence of chlorpyrifos negatively affected stability and efficiency of the soil microbial structure (Supreeth *et al.*, 2016). The primary indicator of microbial activity, such as microbial biomass, and other ecological processes have been shown to be adversely affected by the smallest possible concentration of chlorpyrifos (CP) used for agriculture, 10 mg/kg (Vischetti *et al.*, 2008). Over the course of the investigation, there was a discovery made that adding CP to an agricultural loamy soil at concentrations ranging from 10 to 300 g/g significantly reduced aerobic bacteria responsible for fixing nitrogen (Azotobacter sp.). Overall active fungal and denitrification bacterial population weren't impacted by this dosage, though (Martinez-Toledo *et al*., 1992). According to several research on the usage of CP at various concentrations, the microbial community and their makeup, microbial abundance, and their functional activities in their communities have all suffered significantly (Fang *et al.* 2009; Chen *et al.*, 2014).

Imidacloprid (IC) application at field rates was shown to have little to no impact on the mix and operation of soil microorganisms (Cycon and Piotrowska-Seget, 2015a; Cycon and Piotrowska-Seget, 2015b). However, at concentrations greater than field rates, vital modifications in community makeup were induced (Qingming *et al.*, 2015). According to Goulson (2013), frequent applications of IC in soils resulted to increase in the level of accumulation that eventually changed the behaviour of soil bacteria. The community structure of ammonia-oxidizing bacteria may also be altered by IC, which could exert an adverse effect on the global N-cycle, soil quality, and soil oxidation rates (Cycon and Piotrowska-Seget, 2015b). Additionally, there were noticeable little or non-impact on certain soil enzyme activity, when IC was applied in the maintenance of turfgrass and peanut fields (Ingram *et al*., 2005).

The community structure of ammonia-oxidizing bacteria may also be altered by IC, which could consequently affect the soil nitrification rates, the world-wide N-cycle, and soil characteristics (Cycon and Piotrowska-Seget, 2015b). The population mixture of fungi and actinomycetes groups, however, were negatively impacted in a different study when IC was applied (Singh and Singh, 2005). Xenobiotics negatively altered the overall number and functionality of soil bacteria. External variables including the soil's makeup the toxicity, accumulation, and long-term persistence of the applied xenobiotics, as well as their bioavailability to soil microorganisms, may, however, mitigate the effect (Hussain *et al.*, 2009).

2.6.2 Effects of Pesticides on the Manufacture of Lipids, Sterol, and Other Membrane Components of Microorganisms

Organism's cell membrane which typically isolates it from the outside of its environment, is known as a selectively permeable cell wall. In all live cells, it performs a variety of biological tasks, including as regulating cell water potentials, participating in signal transmission, giving the cell its shape, and preventing the passage of big molecules (Doralicia *et al*., 2019). Studies have revealed that some fungicides, such as dicloran (2,6-dichloro-4-nitroaniline), are phototoxic and can harm the membrane of microorganisms in their community, changing their structure and function (Chao *et al.*, 2011). De Ouveira (2009) found fungicides also to negatively impacts certain groups soil microbes. For example, dicloran has the potential to cause *Salmonella typhimurium* to mutate by disrupting hydrophobic connections within its membrane.

2.6.3 Effects of Pesticides on Amino Acids and Protein Synthesis in Microorganisms

According to reports (Ebimieowei and Ibemologi, 2016; Raquel *et al.*, 2020), fungicides have the ability to disrupt the synthesis of protein, which is the most crucial component of all living things and serves a variety of crucial biological functions including sending signals between cells, forming the cytoskeleton, and catalysing biochemical reactions in the system. Research utilizing a mutant strain of *Thermus thermophilus* verified earlier studies' findings that the alteration of streptomycin on bacteria may be responsible for the wrong reading of the genetic material responsible for synthesizing protein molecules (Hasan *et al.*, 2013).

Additional research clarified that in addition to being hazardous to fungus and bacteria, certain antibiotics (including streptomycin) may also be harmful to eukaryotes (Axel, 2020).

2.6.4 Effects of Pesticides on Microbial Respiration

According to prior research (Chao *et al*., 2011), the mechanism process of several fungicides widely used in agriculture can greatly suppress microbial respiratory activity. But the majority of these fungicides target the cytochrome bc1, succinate-dehydrogenase (Complex II), NADH oxidoreductase (Complex I), and oxidative phosphorylation uncoupler enzymes. However, only a small number of fungicides have been discovered to block the Complex I system in fungus mitochondria (Mike *et al*., 2016). 5-Chloro-N-[4-(difluoromethoxy)phenyl]-Diflmetorim (RS) propyl according to reports, propyl suppresses NADH oxidoreductase activity, which causes fungal mortality. It is a component of the fungicide -6-methylpyrimidin-4-ylamine, which helps prevent mildew in powder form and rust on decorative trees (Sajad *et al.*, 2017).

2.6.5 Effects of Pesticides on Mitosis and Cell Division of Microorganisms

Previous studies on mitosis and cell division revealed that fungicides such as the methyl benzimidazole carbamate (MBC) can impact and inhibit the polymerization of tubulin into microtubules (McCarroll, *et al.*, 2002). It was also discovered that these MBC fungicides also inhibited the proliferation in microbes hence suppressed their dynamic instability through their binding effects on β-tubulin in microtubules (Koo *et al*., 2009). The cytoskeletal polymers known as microtubules in eukaryotic cells are crucial for a number of cellular activities. However, when MBC fungicides are used as pest control, the cells may experience chromosome loss in the target cells, suppression of constructed spindle microtubules, disruption of chromosomal synchronization at the metaphase plate and chromatid degradation triggered by microtubule-kinetochore interactions (Rathinasamy and Panda, 2006).

This also leads to the list of potential negative effects on other microorganisms that follows. For instance, prior research has demonstrated that the MBC fungicides Benomyl and carbendazim, which are both often used in crop cultivation, suppress the mitotic division of fungi. The beneficial arbuscular mycorrhiza fungus (AMF), on the other hand, has been alleged to be impacted by MBC fungicides, despite the fact that the direct effects of these

chemicals on soil bacteria have not yet been reported. The microbially-driven process of nitrification in soil has been connected by some studies to the lowering of these fungicides (Chen *et al.*, 2001).

2.6.6 Effects of Pesticides on Microbial Nucleic Acids Synthesis

Nucleic acid synthesis has been seen to be impacted by the fungicide. It does this by reducing the functionality of RNA polymerase I, preventing rRNA from manufacturing within the scope of uridine transcription (i.e., preventing incorporation of uridine into the RNA chain), and interfering with the production of nucleic acids (Carvalho *et al*., 2019). Since there have been reports of this fungicide's negative effects on bacteria related to N cycling, fungicides in the PA group should be handled carefully (Monkiedje and Spiteller, 2005). Adenosine-deaminase is inhibited by fungicides containing hydroxy pyrimidines, as well.

2.7 Biodiversity of Bacteria Degrading Pesticides in Agricultural Soils

Inherently toxic chemicals like herbicides can easily be broken down by soil microorganisms. These organisms are greatly beneficial to modern agriculture since they can reduce the persistence level of these chemicals which could cause harm to human and environmental health. Previous studies have shown that chemicals which were previously recalcitrant to biodegradation can now be degraded by already adapted and proliferated soil microorganisms (Tinatin *et al.*, 2018; Balendu *et al*., 2020). Novel chemical structures of these pesticides which are applied in agricultural soils mostly serve as nutritional desert and carbon sources to support growth and multiplication of dominant microbial occupants (Johannes *et al.*, 2020). Biodegradative bacteria and their adaptative mechanism are of significant scientific interest (Sandhya *et al*., 2021). Research has shown that inoculation of bacteria possessing biodegradative properties which could aid the decontamination of contaminated soils has encouraged bioremediation processes (Tinatin *et al.*,2018; Maqshoof *et al.*, 2018).

2.8 Biodegradation (Transformation) of Xenobiotics (Pesticides) by Soil Microbial Communities

One of the strategies primarily used by microorganisms for survival is the biodegradation of xenobiotic chemicals which is a natural process of removing such chemicals from the

environment (Nikita *et al.*, 2020). There have been reports of xenobiotic chemical biodegradation or bioremediation in the past (Liang *et al.*, 2020; Shalini *et al.*, 2020; Pankaj *et al.*, 2020). In spite of the fact that most microbes can detoxify (i.e., mineralize, convert, or immobilize toxins), previous research found that bacteria especially play a critical part in the biogeochemical processes so as to sustain the biosphere (Lei *et al*., 2020). Additionally, it has been noted that bacteria can easily adapt even in harsh environments where the growth of other living things is prohibited, because of their capacity for horizontal gene transfer, rapid growth, and metabolic versatility (Rebecca and Alan, 2019). It has been demonstrated that bacteria are preferable for the biodegradation of xenobiotics because they manufacture effective enzymes which catalyses the degradation of xenobiotics (Xue *et al.*, 2020; Jie *et al.*, 2020).

Biological degradation of xenobiotics can be loosely split into respiratory and anaerobic processes to break down and utilise various dangerous compounds, according to the most complex catalytic mechanisms and metabolic systems of numerous bacteria (Arora *et al.*, 2010; Manoj *et al.*, 2017). When microorganisms degrade organic compounds for the sake of deriving energy required for growth, the process is referred to as growth-linked degradation. However, some organic compounds, such as organochlorine, are extremely persistent and resistant to biodegradation because they contain a highly electronegative halogen group that gives the molecule stability. Co-metabolism is a process that can primarily convert certain kinds of chemical molecules. In this process, microorganisms convert a chemical but are unable to use the energy generated by the process since a growth substrate is required (Kassotaki *et al*., 2016).

2.9 Biodegradation of Organic Pollutants

Previous studies have discovered that microorganisms are useful in biodegradation of persistent organic pollutant like xenobiotics which could be their carbon source for growth and development. These compounds are also common environmental pollutants which have toxic, genotoxic, mutagenic (Enrica *et al*., 2020; Marcos *et al.*, 2020) and carcinogenic properties. Earlier studies have shown that use of several bacterial strains for biodegradation showed higher and faster biodegradation rate contrasted with a single bacterial strain (Hana *et al.*, 2018).

Pesticides such as CP can be enormously transformed by applying Streptomycetes strains for their degradation processes using agar mediμm with a stable pH to improve its degradation efficiency (Pankaj *et al.*, 2020; Anμm and Azra, 2020). Bacteria from the genera *Flavobacterium* and *Alcaligenes*, as well as strains from the genera *Pseudomona*s and *Alcaligenes*, were found to break down carbofuran and methomyl respectively in previous studies using 16S rDNA sequence analysis (Mbogo *et al.*, 2012).

Organochlorine pesticide elimination out of soil is facilitated by microbial treatments in ideal environmental circumstances. Potassium humate, however, is added, and this offers better results because it increases the number of microorganisms present (Soromotin *et al.*, 2012; Abdeen, 2020). Permethrin and Cypermethrin can effectively be biodegraded by strain of *Pseudomonas putida* and *Pseudomonas mendocina*. These bacterial strains can achieve high level of biodegradation of these pollutants within a short while (Mendoza *et al*., 2011; Pankaj *et al.*, 2021).

Deltamethrin has also been successfully broken down by 3-phenoxybenzaldehyde as the primary decomposition component of the an actinomycete species, which was cultured from activated sludge. This has given bioremediation of pesticide-related environmental pollution an efficient tool (Chen *et al.*,2011; Mohammad *et al*., 2020). Research has shown that use of glucose as substitute carbon sources, can increase microbial growth and concentration, which in turn facilitates a greater rate of diazinon breakdown (Briceno *et al*., 2016; Pourbabaee *et al.*, 2018). Additionally, employing bacterial strains identified via the enrichment approach, large concentrations of profenofos have been observed to breakdown in 90 hours (Ortiz-Hernandez and Sanchez-Salinas,2010). It was also discovered that organophosphate pesticides can be efficiently degraded by a bacterial consortiμm isolated from agricultural soil (Góngora-Echeverría, 2020).

Since organophosphate provides energy and phosphate for lactic acid bacteria, they can biodegrade organophosphorus pesticides through fermentation (Kye *et al*., 2009; Ying-Hua *et al.*, 2014). Furthermore, the lactic acid bacteria's breakdown of organophosphates was expedited and increased by the addition of Na succinate at higher levels because they utilise phosphorus and as carbon sources (Baodan *et al*., 2021). Malathion can be broken down by the bacterium *Pseudomonas stutzerion* in minimal salt environments. However, the addition

of glucose and yeast increased bacterial growth by 105 times, which aided in the 30-day breakdown of more than 99% of the pesticide malathion (Briceño *et al.,* 2016; Vaishali *et al.,* 2020). For the microbial breakdown of organophosphate insecticides, strains like *Bacillus, Actinobacteria,* and *L-Proteobacteria* have been utilized (Sabdono *et al.,* 2008). Esbiothrin was greatly reduced on magnetic polyurethane by immobilized *Acinetobacter* (Ha *et al.,* 2009). The breakdown of organophosphate pesticide utilizing encapsulated bacteria on Ca-alginate gel beads has also been documented in earlier investigations (Zeinat *et al.,* 2008).

According to C'aceres *et al.* (2008), fenamiphos was broken down into many resilient, harmless components by cyanobacteria and blue green algae using a cultured method. Dichlorodiphenyltrichloroethane (DDT) and endosulfan were effectively broken down by the *Stenotrophomonas maltophilia* bacterial strain, whose growth was promoted by the use of green coffee beans in a medium supplemented with glucose (Barrag'an-Huerta *et al*., 2007). *Pseudomonas* species can degrade atrazine through two steps (Wyss *et al.,* 2006). Research has shown that microscopic organisms (3 bacterial strains by hydrolysis technique) were able to breakdown mefenacet and several other amide insecticides, including propanil and metolachlor (Harada *et al*., 2006).

According to Harada *et al.* (2006) the fermentation process is an efficient way for *Rhodobacter sphaeroides* to break down pesticides such as Organophosphate pesticides (Ops), chlorinated pesticides, herbicides, and fungicides. Compared to its R-enantiomers, the S-enantiomer of methylaxyl requires screened bacteria to degrade at a relatively high rate (Shengwen *et al*., 2006). By completely different means than photocatalytic degradation, considerable methyl parathion breakdown by *Vibrio* and *Shewanella* bacteria is accomplished (Liu *et al.,* 2006). Pesticides like triazophos, chlorpyrifos, and phoxim can all be effectively broken down by photosynthetic bacteria. (Zhang and Bennet, 2005). For instance, *Ochrobactrm* can metabolize triazophos up to 95% in crops and readily oxidize it into its acidic form (Shunpen and Shen, 2005). When combined with the application of sugar solution and aerobic-anaerobic decomposition, chlorinated pesticides can be biodegraded effectively (Li and Rutherford, 2005).

Immobilized bacteria can break down different pesticides including herbicides, fungicides and carbamates at varying flow rates (Baoyu *et al*., 2020; Tong *et al*., 2020). Anaerobic microbes degrade aldrin (an organochlorine insecticide) utilizing extracted yeast as their carbon source (Guohui, 2004). Bacterial strains like *Azospirillµm and Pseudomonas* as well as mesophilic bacteria anaerobically degraded ethion (OPs) (Zhang *et al*., 2007).

According to previous investigations, a consortium of bacterial strains, including *Bacillus* sp. and *Chryseobacter joostei*, utilised the pesticides in their co-metabolic pathways to break down certain group of pesticides (Foster *et al*., 2004). Through sensitive temperature and pH variations of psychrotrophic bacterium can degrade me-parathion (Kumar *et al.,* 2019). *Micrococcus and Pseudomonas*, two of the six taxa that could break down organochloride insecticides, were shown to be more active than the others (Kumar *et al.,* 2019; Mohammad *et al*., 2020). Some pesticides can effectively be degraded by immobilized microorganisms like *Escherichia coli* (Ahankoub *et al.,* 2020). Pesticides found in soil and plant systems may be biologically degraded by certain species of bacteria (Prabha, *et al.,* 2017).

2.10 Microbial Enzymes in Biodegradation

Biologically generated catalysts called microbial enzymes are frequently employed to improve substrate breakdown into products by creating favourable circumstances that reduce the reaction's activation energy. An enzyme is made up of at least one polypeptide component and can be either a protein or a glycoprotein. Bacteria are crucial to the breakdown of organic contaminants by employing extracellular enzymes (Chengalroyen and Dabbs, 2013; Nazia *et al*., 2017). Given that pollutants with molecular masses less than 600 daltons have the potential to pass through cell pores, this is a significant step toward the destruction and usage of organic polymers with large molecular weight. The breakdown of chemical linkages present in the toxic molecules are enhanced by hydrolytic enzymes since they are generally involved in degradation of pollutants and reduction of their toxicity.

2.10.1 Microbial Oxidoreductases

Numerous fungus and bacteria were successfully identified to use oxidoreductases to catalyse the breakdown of hazardous organic chemicals (pesticides) through oxidative coupling in previous research (Varga, *et al.,* 2019). Oxidoreductases also catalyse the humification of a number of phenolic compounds formed during the breakdown of lignin in

soil sediments (Deepti and Neeraj, 2020). Similar to this, oxidoreductases use polymerization, co-polymerization to catalyse the breakdown of hazardous xenobiotics such phenolics or anilinic compounds (Nazia *et al*., 2017).

According to reports, microbial enzymes are utilized to decolourize and break down azo dyes (Chengalroyen and Dabbs, 2013). Toxins in the soil were degraded by a group of ligninolytic enzymes secreted by fungal mycelium, including laccase, lignin peroxidase, and manganese peroxidase (Tayssir *et al.*, 2017). It has been observed that some fungal species, and white rot fungus in particular, effectively remove chlorinated phenolic chemicals from contaminated environments (Ursula, 2015). Due to their larger surface area than bacteria, filamentous fungi can reach soil contaminants more effectively (Jorge *et al.*, 2020).

2.10.2 Microbial Oxygenases

Oxygenase have been the subject of the most in-depth research in bioremediation, and they can be classified as monooxygenases or dioxygenases depending on the amount of oxygen atoms needed for oxygen supply (Arora *et al.*, 2009). It is a member to the oxidoreductase family of enzymes. It utilizes Flavin Adenine Dinucleotide/Nicotinamide Adenine Dinucleotide + Hydrogen/ Nicotinamide Adenine Dinucleotide Phosphate + Hydrogen (FAD/NADH/NADPH) as a 2 co-substrate while oxidizing lowered carbohydrates via exchanging oxygen from molecular oxygen. These enzymes are vital for the metabolism of organic molecules because they increase the reactivity or water solubility of aromatic compounds or cleave the aromatic ring. Usually, the oxygenase enzyme helps to cleave aromatic rings by introducing O atoms into the 2-organic molecule. Halogenated chemicals, such as those used widely in pesticides, fungicides, heat transfer fluids, herbicides, hydraulic fluids, and plasticizers, are also environmental contaminants. Such contaminants can be degraded by certain oxygenases. However, it is also known that oxygenases can catalyze the dehalogenation of halogenated ethylenes, ethanes and methanes in conjunction with various polymorphic enzymes.

2.10.3 Monooxygenases

Monooxygenases can catalyse a large number of processes, such as the dehalogenation, desulfurization, biotransformation, hydroxylation, ammonification, denitrification, as well as breaking down of various aromatic and aliphatic molecules. Such oxygenase abilities had

been used for significant applications, like the biodegradation as well as biotransformation of aromatic chemicals (Arora *et al.*, 2010). Arora *et al.* (2009) asserts that oxygenases are crucial for the destruction of xenobiotics.

2.10.4 Microbial Dioxygenases

Dioxygenases has been reported to catalyse the oxygenation of diverse substrates and they are multicomponent enzyme systems which incorporate molecular oxygen into substrates. Dioxygenases are known to catalyse the oxidation of aromatic chemicals, which has been demonstrated in earlier research to have uses in environmental clean-up. Each alpha subunit of naphthalene dioxygenase has a Rieske (2Fe-2S) complex and monomer iron, according to the structure of the crystal of the enzyme. (Dua *et al*., 2002).

2.11 Microbiomes Studies with Respect to Herbicide Impact and Experimental Application

Scientists had used a variety of experimental techniques to examine how herbicides affected soil microbiomes. Jadwiga *et al*. (2016) used screenhouse-base study to analyse the response of microorganisms and enzymes when Pethoxamid and Terbuthylazine which are mixture of herbicides were used for weed control. However, he discovered negative effect of these herbicides on Azotobacter, Oligotrophic sporulating bacteria, actinomycetes and fungi. Soil microbial populations were subjected to the impact of the herbicide glyphosate during its application to several agricultural ecosystems as observed in a field investigation. Nevertheless, it was reported that glyphosate herbicides caused zero impact on microbial communities (Ryan *et al*., 2020).

Herbicide application influenced soil microbial biomass carbon negatively when studies were conducted using a field condition to assess how Imazethapyr and flumioxazin affect the biomass of microbes and enzymatic activities in soil (Mariane *et al*., 2020). Chronic herbicide use on field farm soil was found to result in a deleterious impact on the population of soil microbes (Tudararo-Aherobo, 2020). Research evidence on microbial communities under field-base study which were exposed to herbicide application on agrosoddy-podzodic soil and were assessed using Next Generation Sequencing (NGS)revealed a negative reaction to the shift in the relative distribution of the Actinobacteria and antibiotic-resistant bacteria phyla (Astaykina, 2020).

A mesocosm, -based study was used to simulate field condition so as to assay the influence of accumulated atrazine herbicide on microbial diversity in agricultural soil. However, it was discovered through metagenomics analysis that bacteria of the genera *Achromobacter, Xanthomonas, Stenotrophomonas* and *Cupriavidus* utilised atrazine herbicides as their source of carbon hence an increase in their population amongst the microbial community (Pooja *et al.,* 2020). The impact of herbicides on the microbial population and dehydrogenase activity was assessed in a laboratory-based investigation. It was reported that when herbicide was applied, its level of toxicity decreased both the microbial population and the dehydrogenase roles of the microbes (Sebiomo *et al.*, 2011). Under field-based study, soil microbial community, activities and structure were exposed to Chlorotoluron and flufenacet herbicides which were used for weed control. It was discovered that microbial population was reduced over time with significant modification on the microbial structure. Dehydrogenase activity also reduced over time.

Long-term glyphosate treatment influenced negative modification on species diversity of cultivable fungus as well as changes in molecular makeup of the fungi that live in soil (Vázquez *et al*., 2021). Some of the herbicides (eg metribuzin) displayed a negative effect on the microbes, some herbicides positively affected the microbes as they provided carbon and energy to them (e.g., clodinafop) while sulphonylurea herbicides a neutral effect (Aabid *et al.,* 2014). A microcosm based-study which was subjected to factorial experiment was carried out to access the influence of pesticides on microbial population. According to reports, pesticide application was found to significantly decrease the activity and concentrations of soil fungi, bacteria, and actinomycetes (AL-Ani *et al.,* 2019).

CHAPTER 3

MATERIALS AND METHODS

3.1 Collection of Samples

Pre-emergence herbicide, PrimextraGold (with active ingredient; Atrazine + S-metolachlor) post-emergence herbicide, Imazapyr (with active ingredient Isopropyl amine) and organic (Vinegar) herbicide, also known as weed care (with active ingredient Acetic acid) were all purchased from an agrochemical store and organic farming store respectively.

Soil samples (Alfisol and Inceptisol soils) were randomly collected from six locations within the research farms of the following institutes- Department of Agronomy, University of Ibadan, Institute of Agricultural Research and Training, Moore plantation (IAR&T); National Horticultural Research Institute (NIHORT); and International Institute of Tropical Agriculture (IITA) where the field experiment layout was done at a depth of 0-15cm. However, it is important to note that Department of Agronomy, University of Ibadan and Institute of Agricultural Research and Training, Moore plantation (IAR&T) have locations within their research farms which have both Alfisol and Inceptisol classifications and this completed the soil sample sites as 3 locations for Alfisol and 3 locations for Inceptisol.

Soil samples collected were bulked to form composite soil respectively and were used for screenhouse study. Part of the soils were sterilized using autoclave at 121^{o}C for I hr and this sterilization process was done for 3 consecutive days (Pose-Juan *et al*.,2017). The sterilized soils were used as control to check for the level and cause of degradation of PrimextraGold (pre-emergence herbicide), Imazapyr (postemergence herbicide) as well as weed care (organic herbicide) for same microcosm experiment.

3.2 Experimental Design/ Setup

Ten kilogrammes (10 kg) portion of the soil (both sterile and non-sterile soil) was introduced into pots (10 litre bucket) which was perforated basally to facilitate draining of excess water. Hence microcosm experiment was set up to involve a 2×2×4 factorial in a completely randomized design which was replicated 3 times (2 represents non-sterilized Alfisol and

Inceptisol x 2 represents sterilized Alfisol and Inceptisol x 4 represents herbicide treatment+control). The factors considered were (i) sterilization of soil (sterilized and non-sterilized soil) (ii) type of soils used (Alfisol and Inceptisol) (iii) herbicide treatment (treatment with PrimextraGold, Imazapyr and Organic (vinegar/weed care) herbicides). All the microcosm experiments were set up in screenhouse of the Soil Microbiology unit of International Institute of Tropical Agriculture (IITA) Ibadan which is located at latitudes 7°24′ 7.0632″ N and longitudes 3°55′ 2.3268″ E.

For this microcosm experiment, weeds were allowed to grow on the unsterilized soil after which they were treated with chemical (Imazapyr) and biological (organic weed care) post-emergence herbicides while pre-emergence herbicide was applied on non-sterilized soil before weed growth. Herbicides were not applied on all controlled experiment. All sterilized soils received pre and post emergence herbicides as well as the organic herbicides except for control experiments (Table 3.1).

3.2.1 Field Layout

This study was conducted in Ibadan, Nigeria (7°24′ 7.0632″ N, 3°55′ 2.3268″ E) from September to November 2019 at the research farms within the following institutes- Department of Agronomy, University of Ibadan, Institute of Agricultural Research and Training, Moore plantation (IAR&T); National Horticultural Research Institute (NIHORT); and International Institute of Tropical Agriculture (IITA). Factorial arrangement on a Randomized Complete Block Design (RCBD) was used at each location to map out 2 m^2 x 2 m^2 subplots on 11 m^2 x 8 m^2 plots for the field experiment (Fig 3.1).

The experimental setup was replicated 3 times for each treatment to form 72 plots (2 soil types x 4 herbicide treatments + control x 3 locations x 3 replication). The chemical and organic herbicides used for this study were applied at the following rate of application: PrimextraGold herbicide (PMG) was applied at 2.64 kg ai Atrazine + S-metolachlor/ha (i.e. 0.019 kg/72 m^2), before weed growth at all plots where it was required. Imazapyr herbicide (IM) was applied at 0.3 kg ai Isopropyl amine (0.0022 kg/72 m^2) within plots with the presence of weeds as required, while organic herbicide (ORGH) was applied at 1.26 kg ai acetic acid (0.0092kg/72m^2) in another set of plots with weeds as required.

Table 3.1: Experimental Structure

Soil types	H0	H1	H2	H1H2
S0.1	S0.1H0	S0.1H1	S0.1H2	S0.1H1H2
S0.2	S0.2H0	S0.2H1	S0.2H2	S0.2H1H2
S1	S1H0	S1H1	S1H2	S1H1H2
S2	S2H0	S2H1	S2H2	S2H1H2

LEGEND:

S0.1: Sterilized soil type 1(Alfisol)
S0.2: Sterilized soil type 2 (Inceptisol)
S1: Unsterilized soil type 1
S2: Unsterilized soil type 2
H0: No herbicide treatment
H1: Herbicide type 1 (preemergence H: Primextragold ai Atrazine + S-metolarchlor)
H2: Herbicide type 2 (postemergence H (Imazapyr ai Isopropyl amine)
H1H2: Herbicide 3 (weed care organic herbicide ai Acetic acid)
S0.1H0: Sterilized Alfisol without herbicide
S0.2H0: Sterilized Inceptisol without herbicide
S1H0: Unsterilized Alfisol without herbicide
S2H0: Unsterilized Inceptisol without herbicide
S0.1H1: Sterilized Alfisol with preemergence H
S0.1H2: Sterilized Alfisol with postemergence H
S0.2H1: Sterilized Inceptisol with preemergence H
S0.2H2: Sterilized Inceptisol with postemergence H
S1H1: Unsterilized Alfisol with preemergence H
S1H2: Unsterilized Alfisol with postemergence H
S2H1: Unsterilized Inceptisol with preemergence H
S2H2: Unsterilized Inceptisol with postemergence H
S0.1H1H2: Sterilized Alfisol with organic herbicide
S0.2H1H2: Sterilized Inceptisol with organic herbicide
S1H1H2: Unsterilized Alfisol with organic herbicide
S2H1H2: Unsterilized Inceptisol with organic herbicide

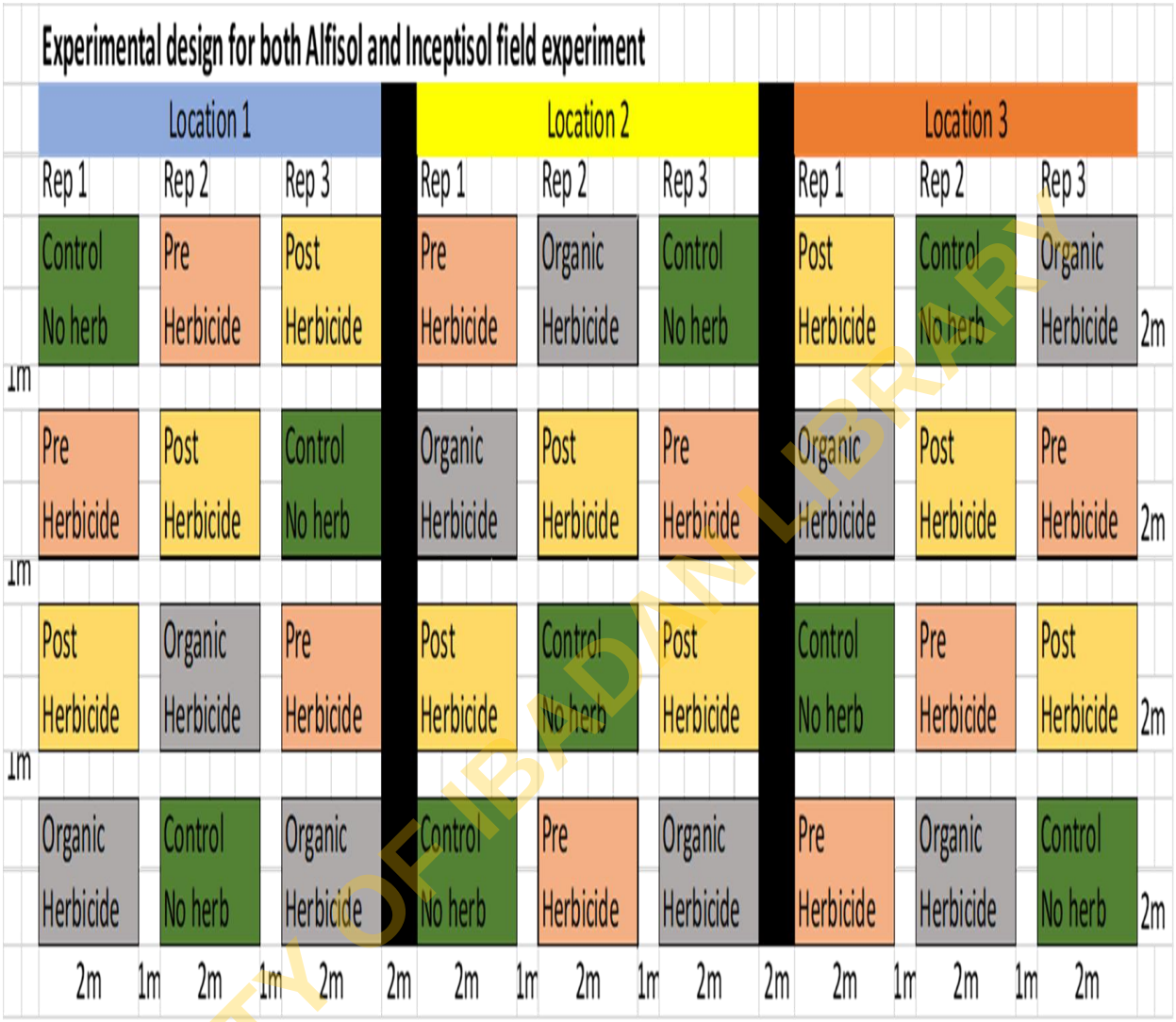

Fig 3.1: Experimental design for both Alfisol and Inceptisol field experiment

Both the chemical and organic herbicides were delivered into 2.16 litres of water at each time in a knapsack tank respectively where they were mixed before spraying. Herbicides were not applied on some set of plots which served as control experiment plots. All herbicide treatments were applied in the morning.

3.3 Soil Sampling Procedure

Soil auger was used to collect a total of 360 field cores (depth 15cm, diameter 3cm), approximately 200 g each). All samples were transferred to the -20℃ freezer before genomic DNA extraction. Soil samples for Gas Chromatograph Mass Spectrometry (GC-MS) were collected from microcosm set up, while soil samples for DNA extraction for 16S rRNA biomarker and Next Generation (NGS) Sequencing, were all collected from field lay out.

3. 3.1 Physical and Chemical Analyses of Soil

A subset of the soil samples (n=36 samples each from Alfisol and Inceptisol, were randomly collected from 0, 4 and 8 weeks) and were used for physical and chemical soil analysis.

Soil particle size analysis was done using the hydrometer method according to Bouyoucos (1962) and Agbenin (1995); Fifty (50) grams of air-dried soil was sieved into a 250 mL conical flask. 100 mL of 5% Calgon solution was poured into the sample, flask was capped, and rotated for several minutes until solution and soil were well mixed. Mixture was allowed to sit overnight (a minimum of 12 hours) to allow the solution to effectively disperse the soil separates (sand, silt, clay). Soil-Calgon mixture was transferred from flask to electric mixer cup and a water bottle was used to completely rinse all material from the flask into the mixing cup. Mixing cup was filled with water to about 3 inches from the top. Mixing cup was attached to mixer and stirred for 3 minutes. Mixing cup was slowly removed and lowered so that the mixer propeller is just above water level. Mixing cup of soil, Calgon, and water was emptied into 1000 mL graduated cylinder.

Remaining residue were completely washed out of the mixing cup with a water bottle into the graduated cylinder and the cylinder was filled to 1000 mL mark. The graduated cylinder content was gently mix so that a uniform soil suspension was obtained (at least 30 seconds). The uniform mixture was placed on a white surface to settle for 40 seconds. After 40 seconds has elapsed hydrometer was inserted into the graduated cylinder with the soil-Calgon

mixture and the 40-second hydrometer reading was taken and recorded. Thermometer was also placed into soil water-Calgon solution to read temperature. After 2 hours have elapsed, another hydrometer reading was taken from soil solution and was recorded for the 2-hour hydrometer reading. Thermometer was place into soil water-Calgon solution and temperature after 2 hours was taken. The percentages of soil fractions were calculated using the following equations:

$$\%Clay = (calibrated\ 2hours\ reading) \times (100/sample\ weight) \quad (1)$$

$$\%silt = (calibrated\ 40\ second\ reading) \times (100/sample\ weight) \quad (2)$$

$$\%sand = 100 - (\%silt + \%clay) \quad (3)$$

Organic carbon content was estimated with potassium dichromate method (Walkey and Black, 1934); One (1) g of air-dried soil was run through 0.5 mm sieve and was measured into a 250 mL conical flask. Ten (10) mL of 0.167 M of $K_2Cr_2O_7$ was poured in the soil inside the flask and was vigorously agitated to evenly distribute the soil in the solution. Subsequently, 20 mL of concentrated H_2SO_4 was carefully but quickly added into the suspension and was swiftly revolved gently then more vigorously for a total of 1 minute, until soil and reagents were mixed. In order to reduce heat loss, the glass flask was kept in a combustion chamber for thirty minutes on an evacuated surface. Seventy (70) mL of distilled water and also three drops of o-phenanthroline indicator were added to the flask. A 0.5 M $FeSO_4$ solution was used to titrate the mixture. The solution first took on a greenish tinge as it got near to the finish point, and then it developed a dark green. At this end point, the ferrous sulphate heptahydrate in the burette was incorporated in droplets till the colour changed completely to maroon red colour. Blank was determined using same steps used for samples, but without soil, to standardize the $K_2Cr_2O_7$. Percent (%) Organic Carbon was computed using

$$\frac{(B-S)\times M\ of\ FeSO_4 \times 12 \times 100}{Gram\ of\ Soil\ used \times 4000} \quad (4)$$

Soil pH was evaluated with the pH meter in soil solution ratio of 1:1 according to (Thomas, 1966); soil samples were air-dried and passed through 2 mm sieve to remove the coarser soil fraction and 10 g of the air-dried and sieved soil samples were weighed into glass beaker. Ten (10) mL of distilled water was added into the soil in the beaker and was

thoroughly mixed for 5 minutes and it was allowed to stand for 1 hour. The glass electrode on the pH meter was calibrated for acidity, neutrality and alkalinity using buffer 4, 7 and 9. The glass electrode was rinsed with distilled water and blotted dry with clean paper towel. With the meter on, the glass electrode was placed in the partially settled sample suspension to be measured and the pH reading was taken and recorded.

Available phosphorus was determined using Bray 1 extraction solution (Murphy and Riley, 1972); soil sample was air-dried and was passed through 2mm sieve. One (1) g of air-dried soil was weighed into a centrifuge tube and 7 mL Bray extracting solution was dispensed into the soil. The tubes were stoppered and they were vigorously shaken for 5 minutes using orbital shaker machine. The tubes were transferred to the centrifuge and spined at 6 000 rpm for 5 minutes. Supernatant of 0.50 mL plus 2.0 mL of murphy and riley as well as ascorbic acid each was added into a colorimeter tube. They were mixed and allowed to stand for 30 minutes. A set of reference standards were prepared using 5 ml of KH_2PO_4 from stock solution which was topped to mark in a 250 ml volumetric flask. Both the absorbance of the standards prepared as well as color development in aliquot were measured and recorded using spectrophotometry at wavelength of 882 nm. A graph was prepared from the standards data to plot phosphorus concentration against absorbance. Slope was derived from the graph was used to determine the phosphorus concentration in the sample solutions using the formular:

$$ABS \times Slope \times EF \times CDF \quad (5)$$

(where ABS= Absorbance, EF = extracting factor, DF = color developing factor).

Extraction of soil sample (2 g soil) with the use of one normal ammonium acetate (1N NH40Ac: pH=7) for Exchangeable bases determination according to Okalebo *et al.* (1993). This was done as follows, 2 grammes of soil with low moisture content was weighed and poured in 50 mL tubes and 20 mL of ammonium acetate extraction solution was added to each tube. The Falcon tubes were then placed on reciprocating shaker and was shaken on slowly for one hour. Aliquot was filtered using Whatman filter. Estimated exchangeable potassium (K) and sodium (Na) were measured using flame photometer while the calcium (Ca) and magnesium (Mg) were measured using Atomic Assumption Spectrophotometer (AAS).

The exchangeable acidity which measures the Aluminium (Al) and Hydrogen (H) concentration of the soil was determined using 1N KCl extraction procedure according to Mclean (1965). This approach utilizes titrimetric method (which is a standard method), according to the routine methodology adapted from McLean, (1965). Primarily, the exchangeable acidity (Al_3+ + H+ titre values) were determined by titration of 25 mL KCl extract with 0.025 mol L-1 NaOH, using 1 g L-1 phenolphthalein as an indicator (titration end point was from colourless to pink). Then, the concentration of Al3+ was obtained by back-titration of the same KCl extract, previously used, after the acidification with a drop of HCl and addition of 40 g L-1 NaF, with 0.025 mol L-1 HCl (titration changes from pink to colourless).

Heavy metal in soil was determined as follows; Each 1 g of soil sample was moistened with a few drops of water in each beaker where soil samples were weighed into. Then a mixture of 7 mL of HF, 3 mL of HNO_3, and 1 mL of $HClO_4$ was added to each beaker, and the sample swirled until completely wetted. The beakers were then covered with lids and kept overnight for digestion after adding 1 mL of each 10 µg/mL Rhodium Internal Standard. The next day, the beakers were heated on a hot plate at 200 ^oC for about 1 hour. The lids were removed, and the contents evaporated to near dryness. The evaporation process was repeated three times after adding a mixture of 3 mL HF and 1 mL $HClO_4$ in each case. The contents were dissolved using 10 mL of 1:1 HNO_3, then brought to 250-mL volume with distilled water, and was stored in polythene bottles. In all cases, clear solutions were obtained. Heavy metals such as Zinc (Zn), Copper (Cu), Manganese (Mn) and Iron (Fe) were determined using inductively coupled plasma optical emission spectrometry (ICP-OES) (PerkinElmer Optima 5300 DV).

The total Nitrogen content of soil samples was determined after digestion of the samples with concentrated H_2SO_4 in the presence of Kjeldahl catalyst using Kjeldahl digestion and distillation apparatus (Bremner, 1965); The procedure was done as follows; One (1) g of the homogeneous air- dried soil sample which was passed through 0.5 mm sieve was weighed into digestion tubes and a Kjeldahl tablets was also added as a catalyst. Twenty (20) ml 98 % Sulfuric acid was gently added into the soil in the digestion flask. Blank was also prepared in another digestion tube by adding every other material except soil. The digestion tubes and mixture were placed on the digestion unit and were fixed on a heating block.

The mixtures were heated at a temperature of 350 ^{o}C till white vapours appeared. For almost 180 minutes, the temperature was maintained. The digestion process came to a conclusion when the samples were entirely creamy in colour. After allowing the samples to get to ambient temperature, 10 millilitres of water were carefully introduced. Subsequently, the substances contained within the glass tube were moved to the distillate apparatus. In the distillation unit, samples were already digested with Sulfuric acid 98%. The sample was mixed with fifty millilitres of a 50% NaOH solution to adjust the pH and change NH_4^+ to NH_3 gas.

To extract the generated NH_3, a flow of dissolved water was introduced into the sample. A 50 ml boric acid solution (4%), containing 6–7 drops of Tashiro's indicator, was used to collect NH_3. The colour of NH_3 changed from red violet to green (pH 4.4-5.8) when it reacted with Boric acid solution because the indicator shifted from a solution with acid to an alkaline medium. The Boric acid solution held roughly 150 millilitres of condensate for approximately five minutes. The green-coloured solution was titrated with 0.25 mol/l of HCl till it turned into somewhat violet colour. Considering the HCl's quantity and concentration needed, the percent (%) nitrogen in the soil samples were calculated using; % Nitrogen = (ml standard acid for sample - ml standard acid for blank) x N of acid x 1.4007/ weight of sample in grams.

3.4 Procedure for Extraction of Soil Samples and Gas Chromatography-Mass Spectrometry Analysis

3.4.1 Extraction of Soil Samples

Samples were extracted using modified methanol-chloroform-water extraction method at ratio 1:1:0.9 (Axelsson and Gentili, 2014). Samples were homogenized twice in 1.5ml cold methanol (-30^{o}C), 0.75 ml cold chloroform (-30^{o}C), and 0.6 ml cold water (4 ^{o}C) to reach the first ratio of 1: 0.5: 0.3, then another 0.75ml cold chloroform was added and mixed to form the second ratio 1:1:0.3. Furthermore, 0.75ml cold water was added to get the final ratio of 1:1:0.9. Samples were then vortexed and centrifuged at a maximum speed of 10,000 × g for 10 minutes at room temperature. The upper phase (which was made up of methanol and water) were separated from the lower phase (the chloroform) using a glass syringe which was transferred to a new vial independently. The sample fractions were stored in - 80 ^{o}C for further analysis**.**

3.4.2 Gas Chromatography-Mass Spectrometry Analysis

Soil sample extracts were treated with methanol solution for 30 min at 0^{o}C while stirring. The mixture was evaporated with N_2 stream. A GCMS- QP 2010SE instrument (Shimaadzu, Kyoto, Japan) equipped with a monopolar capillary column MDV-5 (30 m by 0.25 mm inner diameter, film thickness 0.25 µm Supelco; Belleforte, pa) was used for the analysis as recommended by the manufacturer at 0, 4, 8, 12 weeks with herbicides treated sterile and unsterile soils (Alfisols and Inceptisol soils) extracts. GC oven was held at 140 ^{o}C for one minute, then raised to 280 ^{o}C at 4 ^{o}C every five minutes while the injector temperature was left at 250 ^{o}C. Helium was employed as the carrier gas at a flow rate of 6.4 ml/min. The identification of the compound was based on 90 % similarity between the MS spectra of unknown and reference standard (e.g., PrimextraGold, Imazapyr and Organic (Vinegar) weed care herbicides) in an MS spectra library. The compound reference standard was tested by a GCMS method to compare their retention times and MS spectra with those of the metabolites present in the extracts or fractions.

3.5 Determination of the Variations in Population and Diversity Level of Bacteria using 16S rRNA Amplicon on Illµmina Miseq Sequencer

3.5.1 DNA Extraction

The E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Inc.; Norcross, GA) was used to extract the genomic DNA from 1 g of soil. With very minor modifications, the DNA of soil bacteria were extracted by following the producer's directions, and the samples were then eluted in a final volume of 70µl. For a more thorough approach, Prior to being placed in a disruptor tube, 1g of soil sample was crushed in a sterilized porcelain mortar and pestle. The soil samples were put into a disruptor tube together with 725 µl of (SLX-Mlus) buffer, and the samples were disrupted on a gene disruptor machine for 8 minutes at maximum speed to lyse the samples. Transferring the tubes to the centrifuge allowed the mixture to be agitated at 500 x g for 5 seconds, clearing any liquid from the lid. Later, 72 µL of (DS) buffer were added, and the mixture was vortexed for 4 minutes for thoroughly combination of the mixture. After that, the mixture was incubated for 10 minutes at 70°C.

During the period of incubation, tubes containing the mixture were briefly vortexed once. Centrifugation was carried out at room temperature for 5 minutes at 10,000 x g. Supernatant from the combination was transferred into a new 1.5 mL microcentrifuge tube along with

400 µL of supernatant, 135 µL of chilled (P2) buffer, and vortexed for one minute to properly combine the mixture. Following a 5-minute period on ice, it received a 1-minute 20,000- x g centrifugation. A new 1.5 ml micro-centrifuge tube was used to transfer the clear supernatant from the mixture, and 200 µL of (cHTR) reagent was also added to the mixture in the new 1.5 ml tube. After fully mixing it with a vortex, the mixture was let to stand at room temperature for three minutes. The mixture was centrifuged for one minute at a maximum speed of 20,000 x g. In order to obtain a clearer supernatant, 500 µL of the supernatant was transferred to a fresh 1.5 mL microcentrifuge tube, and 200 µL of the (cHTR) reagent was added twice, thoroughly mixed, and left to sit at room temperature for 3 minutes. Centrifugation was then performed as before, at the same speed and time.

A new 1.5 mL microcentrifuge tube was used for the cleared supernatant, and an equal volume of (XP1) buffer was added to it. To fully combine the mixture, it was vortexed for one minute. left to rest for three minutes at room temperature. While up to 700 µL of sample from the mixture was transferred to the HiBind® DNA Mini Column, it was inserted into a 2 mL collection tube. At room temperature, the mixture was centrifuged at 20,000 x g for one minute. The collection tube was used again after the filtrate was discarded. The HiBind® DNA Mini Column was placed into the collection tube and 500 µL of (HBC) buffer was added (Note: (HBC) Buffer was diluted with 100% isopropanol before usage.) to sit at room temperature for 3 minutes. To get rid of extra (HBC) buffer, it was centrifuged at 20,000 x g for 1 minute, after which the filtrate and collection tube were discarded. A fresh 2 mL collection tube was used to transfer HiBind® DNA Mini Column, and 700 µL of DNA Wash Buffer was added (Note: Deoxyribonucleic acid (DNA Wash) buffer was diluted with 100% ethanol prior to use).

The collection tube was reused after the filtrate had been centrifuged at 20,000 x g for one minute. The second addition of deoxyribonucleic acid (DNA Wash) buffer was followed by the same speed and length of centrifugation. Empty HiBind® DNA Mini Column was centrifuged for 4 minutes at room temperature at maximum speed of 20,000 x g. A clean 1.5 mL microcentrifuge tube was used to transfer HiBind® DNA Mini Column. The center of the HiBind® DNA Mini Column was saturated with 100 L of 70°C (elution) buffer, which was then left to stand at room temperature for two minutes. Then, it was centrifuged for 1 minute at its maximum speed of 20,000 x g. The same HiBind® DNA Mini Column

that was used during the procedure was filled with the filtrate from the collecting tube. After another two minutes at ambient temperature, it was centrifuged for two minutes at a maximum speed of 20,000 x g. Prior to library preparation and amplicon sequencing, eluted DNA was kept at -20°.

3.5.2 Preparation of Standards for Calibrating and Reading of Standards and Samples Using Qubit® Fluorometer

For sample DNA quality and concentration, extracted DNA was measured using Nanodrop and a QubitTM dsDNA BR Assay Kit (Thermo Fisher; Waltham, MA). Requisite number of 0.5-mL tubes for standards and samples were set up. Two standards are necessary for the Qubit® dsDNA BR Assay. To prevent ink from interfering with the sample read, only the tube lids for the samples and standards were properly labelled. The Qubit® Fluorometer was calibrated in accordance with its standard procedure, which calls for the standards to be placed into the instrument in the correct order. The Qubit® dsDNA BR Reagent was diluted 1:200 in Qubit® (dsDNA BR) buffer to create the Qubit® working solution. Each time the Qubit® working solution was made, a clean plastic tube was used instead of a glass container. (Note: Each standard tube requires 190 µL of Qubit® working solution, while each sample tube requires between 180 and 199 µL.

The total volume in each tube was 200 µL. There was enough Qubit® working solution made to handle all standards and samples. Each of the tubes used for standards received 190 µL of Qubit® working solution, which was then combined with 10 µL of Qubit® standard in the corresponding tubes of the working solution by vortexing for close to 2-3 seconds. Each sample was added to the appropriate volume of Qubit® working solution in the assay tubes, and the mixture was vortexed for two to three seconds. Each tube's final volume was 200 µL. Following a 2-minute incubation period at room temperature for all tubes, readings for standards and samples were taken in accordance with the instruction's recommended protocol. In order to prepare for later applications, read samples were used for 16S rRNA PCR amplification.

3.5.3 PCR Amplification of the 16S rRNA V4 Region

The 16S Amplicon PCR Forward Primer = 5' [515F-GTGCCAGCMGCCGGTAA and 16S Amplicon PCR Reverse Primer = 5' 806R-GGACTACHVGGGTWTCTAAT with barcodes were used to perform PCR amplification of the 16S V4 region (Caporaso *et al*., 2012). The 5ng/µl microbial DNA in 2.5µl was subjected to PCR using 12.5 µl of 2x KAPA HiFi HotStart Ready Mix from Ilumina in San Diego, California, and 5µl (1 µM) each of the 515F and 806R primers. The amplification's cycle parameters included a 3-minute initiation step at 95°C, followed by 25 cycles of denaturing at 95°C for 30 seconds, annealing at 55°C for 30 seconds, enlongations at 72°C for 30 seconds and a final elongation step at 72°C for 5 minutes (Klindworth, *et al.,* 2012). This amplification reaction was performed in a thermal cycler (Bio-Rad, MyCycler, at the Kansas State University Integrated Genomics Facility, USA (Fig 3.2).

3.5.4 Library Quantification, Normalization, and Pooling

Fluorometric quantification utilizing dsDNA-binding dyes was used to quantify illumina libraries according to manufacturer's instruction. Based on the size of DNA amplicons as measured by an Agilent Technologies 2100 Bioanalyzer trace, the concentration of DNA was calculated in nM as follows: (concentration in ng/µl)/ (660 g/mol average library size) 106 = concentration in nM. Concentrated final library was diluted using 10 nM Tris pH 8.5 to 4 nM. Diluted DNA of 5 µl by volume was aliquoted from each library and were mixed for pooling libraries with unique indices. Up to 96 libraries were pooled for one MiSeq run for 16S rRNA sequencing (Figure.3.2).

3.5.5 Metabarcoding and Analyses

The acquired bacterial sequences passed quality control (QC) and trimming. Processing the sequence data and profiling the soil bacterial populations were done using QIIME 2 (v. 2019.7) (Caporaso *et al*., 2010). The primer sequences were removed using the QIIME 2 plugin cutadapt (Martin, 2011), and reads without a primer were deleted. Furthermore, DADA2 was employed for quality control using the same parameters in various runs, and the readings were shortened to a length where the 25th percentile of the reads had a quality score below 15. For the taxonomic assignment of bacteria, the Silva database (v. 132) pre-trained classifier provided by QIIME 2 was used (Quast *et al*., 2013).

The species diversity in each sample was estimated and displayed (Consortium and The Human Microbiome Project Consortium 2012). Using a rarefield dataset, observed richness (Obs), Shannon's diversity (H'), and Faith's PD were all estimated. Similar to the -diversity analysis, the community dissimilarity across the various ecotypes was compared using UniFrac and Bray Curtis distances, and the distance matrices were visualized using non-metric multidimensional scaling (NMDS). ANOVA in R and the Tukey's post-hoc test (p 0.05) was used to assess differences in the relative abundances of bacterial phyla among the treatments (R Core Team, 2015). To identify the community members who contributed most to the variations in the treatments, indicator taxon analyses were carried out.

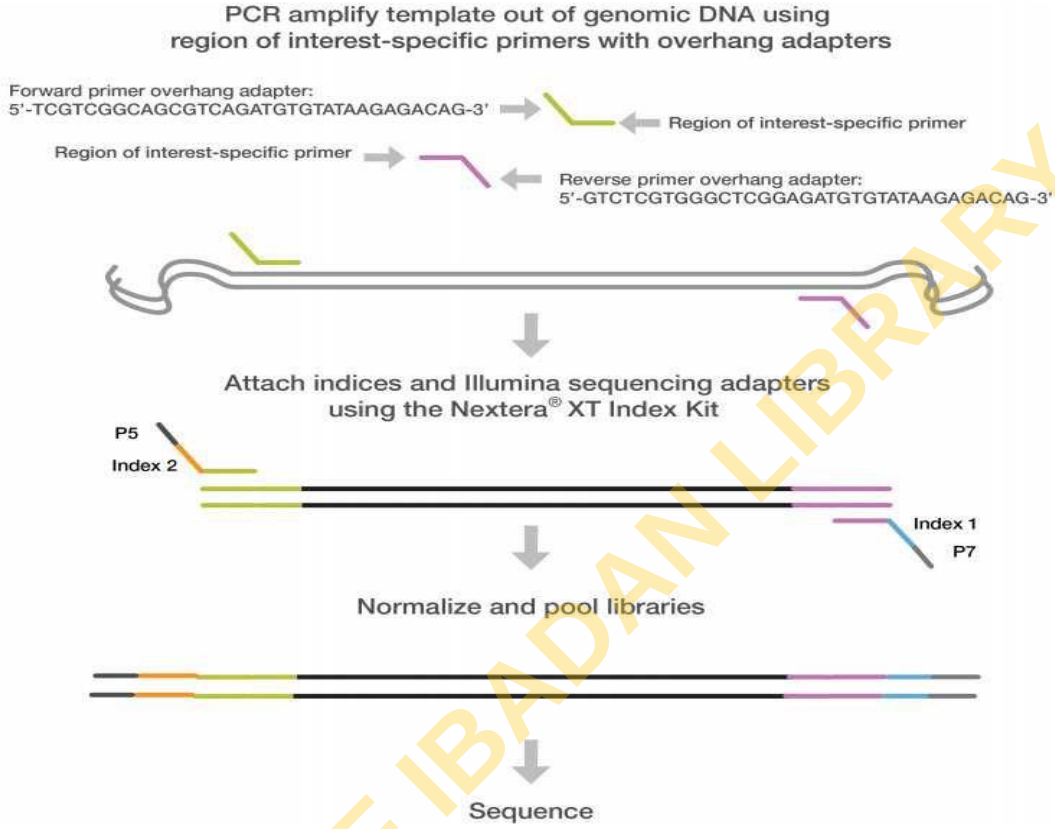

Fig. 3.2: PCR Amplification of the 16S rRNA V4 Region Workflow

(User-defined forward and reverse primers that are complementary upstream and downstream of the region of interest as designed with overhang adapters, and used to amplify templates from genomic DNA. A subsequent limited-cycle amplification step is performed to add multiplexing indices and Illµmina sequencing adapters. Libraries are normalized and pooled, and sequenced on the HiSeq system using v4 reagents)

CHAPTER 4

RESULTS

4.1 Physical and Chemical Properties of Alfisol and Inceptisol Before and After Herbicide Treatment Applications

The physical and chemical parameters of the herbicide treated and non-herbicide treated soil samples from Alfisol and Inceptisol were presented in Table 4.1, 4.2 and 4.3 respectively. Significant differences were observed in the physical and chemical properties of Alfisol and Inceptisol before the herbicide treatments, but no significant change was noticed after herbicide application on the physical and chemical properties in both soil types. Soil pH in Alfisol was observed to be 6.2, while that of Inceptisol was 5.5, Soil organic carbon in Alfisol was observed to be 3.9 g/kg, while 1.9 g/kg of organic carbon was found in Inceptisol. With respect to Clay particle in Alfisol 112.2 g/kg was observed as the result obtained, while 103.9 g/kg was the clay particle value found in Inceptisol.

For total nitrogen in Alfisol, it was observed to be 0.7 g/kg, while Inceptisol had 0.6 g/kg as its total nitrogen value. Potassium content as a chemical property of Alfisol was observed as 0.5 Cmol/kg, while Inceptisol had 0.3 Cmol/kg as its potassium content value. Calcium in Alfisol was also observed as 1.7 Cmol/kg, while Inceptisol was found to be 4.8 Cmol/kg. All these soil properties were observed to be significantly higher in Alfisol at $p < 0.05$ compared to Inceptisol, except for calcium before herbicide treatment, while higher numerical value was observed for phosphorus in Alfisol 25.9 mg/kg, compared to Inceptisol 20.8 mg/kg, (Table 4.1). In terms of heavy metals, while there was no significant difference observed in magnesium between the two pre-treated soil types, zinc in Alfisol was observed to be 150.4 mg/kg, while Inceptisol gave 2.6 mg/kg, manganese in Alfisol was 0.7 mg/kg, while in Inceptisol it was observed to be 175.2 mg/kg, and iron in Alfisol was 3.6 mg/kg, while Inceptisol was found to be 5.3 mg/kg.

Table 4.1: Chemical Properties of Alfisol and Inceptisol Before and After Herbicide Application

Treatments	pH(H_2O) 1:1	OC	N	Bray P	Ca	Mg	K	Na	Zn	Cu	Mn	Fe
		g/kg		(mg/kg)		(Cmol/kg)				(mg/kg)		
Soil type												
Alfisol	6.2a	3.9a	0.7a	25.9	17b	0.8	0.5a	0.1	150.4a	100.2	0.7b	3.6b
Inceptisol	5.5b	1.9b	0.6b	20.8	48a	0.6	0.3b	0.1	2.6b	98.5	175.2a	5.3a
				ns		ns		ns		ns		
Week												
0	5.7b	2.7	0.6b	40.4a	42a	0.9	0.6a	0.1	76.6	103.2	70.1	4.5
4	5.8b	2.9	0.6b	17.2b	25b	0.6	0.4b	0.1	77.9	99.1	128.1	4.9
8	6.1a	3.1	0.8a	12.3b	29b	0.6	0.3b	0.1	74.9	95.8	65.5	3.9
		ns				ns		ns	ns	ns	ns	ns
Herbicides												
Control	6.1	2.8	0.6	19.7	32	0.6	0.4	0.1	74.6	97.7	68.3	4.7
IM	5.8	3.2	0.7	24.2	31	0.7	0.5	0.1	79.7	100.8	71.7	4.7
ORGH	5.8	2.8	0.6	16.6	30	0.6	0.4	0.1	76.5	98.0	142.7	4.2
PMG	5.8	2.8	0.7	32.8	36	0.9	0.5	0.1	75.1	101.0	69.0	4.1
	Ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S*W	*	*	*	ns	*	ns	ns	ns	ns	ns	ns	ns
S*H	Ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	*
W*H	Ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
S*W*H	Ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Means with same letter (s) in a column are not significantly different at 5 % level of probability by Duncan Multiple Range Test (DMRT),: significant at P=0,05. ns= not significant IM = Imazapyr, ORG.H = Organic (Vinegar) herbicide, PMG= PrimextraGold; S * W = interaction between soil and weeks, S * H = interaction between Soil and Herbicides, W * H = interaction between Weeks and Herbicides, S * W * H = interaction between Soil, Weeks and Herbicides.

Table 4.2: Physical Properties of Alfisol and Inceptisol Before and After Herbicide Application Contd

Treatments	Sand	Clay (g/kg/)	Silt
Soil type			
Alfisol	822.2	112.2a	65.6
Inceptisol	828.9	103.9b	67.5
	Ns		ns
Weeks			
0	845.0a	94.2b	60.8b
4	839.2a	102.5b	58.8b
8	792.5b	127.5a	80.0a
Herbicides			
Control	823.3	104.4	72.2
IM	825.6	107.8	66.7
ORGHs	828.9	108.9	62.2
PMG	82.44	111.1	65.0
	Ns	Ns	ns
S*W	*	*	*
S*H	Ns	Ns	ns
W*H	Ns	Ns	ns
S*W*H	Ns	Ns	ns

Means with same letter (s) in a column are not significantly different at 5 % level of probability by Duncan Multiple Range Test (DMRT), ns: not significant*: significant at P=0.05

IM = Imazapyr, ORG.H = Organic (Vinegar) herbicide, PMG= PrimextraGold; S * W = interaction between soil and weeks, S * H = interaction between Soil and Herbicides, W * H = interaction between Weeks and Herbicides, S * W * H = interaction between Soil, Weeks and Herbicides

Table 4.3: Physical and Chemical properties of Alfisol and Inceptisol as Interacted with Different Weeks

Soil type	Weeks	pH (H_2O)	OC	N	Sand	Clay	Silt
			(g/kg)			(g/kg)	
Alfisol	0	6.2a	0.4a	0.8a	823c	110b	67b
	4	6.3a	0.4a	0.7a	828bc	112b	60b
	8	6.2a	0.4a	0.7a	815c	115b	70b
Inceptisol	0	5.1b	0.2c	0.5b	867a	78d	55b
	4	5.4b	0.1c	0.5b	850ab	93c	58b
	8	6.0a	0.3b	0.8a	770d	140a	90b

Means with same letter (s) in a column are not significantly different at 5 % level of probability by Duncan Multiple Range Test (DMRT)
OC = Organic carbon, N= Nitrogen

The concentration of zinc in Afisol was significantly higher compared to that of Inceptisol while the concentration of manganese and Iron were noticed to be significantly higher in Inceptisol compared to Alfisol at $p < 0.05$ (Table 4.1).

While there was no significant difference in all the physical and chemical parameters for interaction between Alfisol and Inceptisol with herbicide application, it was observed that there was significant difference in same parameters as impacted by interaction between weeks and the two soil types used for this study. Interaction between week 0 and Alfisol recorded the following values for soil pH, organic carbon and total nitrogen respectively; 6.2, 0.4 g/kg and 0.8 g/kg, while the interaction between week 0 and Inceptisol for the same parameters recorded these values 5.1, 0.2 g/kg and 0.5g/kg respectively. Interaction between week 4 and Alfisol as regards to soil pH, organic carbon and total nitrogen recorded; 6.3, 0.4 g/kg, 0.7 g/kg respectively while the values found when Inceptisol interacted with week 4 for the same parameters includes 5.4, 0.1 g/kg and 0.5 g/kg respectively. However, it was also observed that these parameters as seen in both week 0 and 4 were significantly higher in Alfisol at $p \leq 0.05$ compared to Inceptisol (Table 4.2).

Interaction between week 8 and Alfisol for soil pH, organic carbon and total nitrogen recorded 6.2, 0.4 g/kg, 0.7 g/kg respectively while interaction between week 8 and Inceptisol for the same soil parameters recorded 6.0, 0.3 g/kg and 0.8 g/kg as their values. However, it was observed that the interaction between week 8 and Alfisol only influenced higher significant difference at $p < 0.05$ in soil organic carbon but not in soil pH and total nitrogen compared to the interaction between week 8 and Inceptisol. On the other hand, it was observed that there were no significant differences in the interactions between soil types and weeks with respect to available phosphorus, calcium and potassium. However, higher significant differences were observed when Alfisol interacted with week 0 and 4 in clay particles 110 g/kg and 112 g/kg respectively compared to clay particle values obtained when Inceptisol interacted with week 0 and 4 respectively 78 g/kg and 93 g/kg at $p \leq 0.05$. Nevertheless, it was observed that interaction between Inceptisol and week 8 gave values such as 140 g/kg clay particles while interaction between Alfisol and week 8 had values of clay particles as 115 g/kg, hence, interaction between Inceptisol and week 8 were significantly higher at $p < 0.05$ compared to when Alfisol interacted with week 8 (Table 4.3).

4.2 Persistency of Herbicide Metabolites at 0 Week After Herbicide Application in Non-sterilized Alfisol and Inceptisol

Fig. 4.1 shows percent (%) peak area as the indicators of metabolites persistency observed across Alfisol and Inceptisol at 0 week in non-sterilized condition. At week 0, while application of PrimextraGold herbicide revealed an interesting outcome where its metabolite metolachlor was the most persistent with 84.94 % peak area in Alfisol, similar chemical metabolite was also found in Inceptisol with a reduced % peak area compared to that observed in Alfisol (52.55 % peak area). Pyrrolidinone compounds such as 2-Amino-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one which was the metabolite formed under the application of Imazapyr herbicide in both Alfisol and Inceptisol, was more persistent in Alfisol (61 % peak area) compared to its persistency level in Inceptisol (50.15 % peak area). Furthermore, application of organic herbicide to both soil types also resulted in metabolites formed being more persistent in Alfisol compared to Inceptisol. For instance, Acetamide, was more persistent in Alfisol, having 35.13% peak area, while Acetamide formed under application of the same ORGH in Inceptisol had 28.0 % peak area.

4.3 Persistency of Herbicides Metabolites at 4 Week After Herbicide Application in Non-sterilized Alfisol and Inceptisol

Various metabolites % peak area indexes observed across non-sterilized Alfisol and Inceptisol at 4 weeks were also presented in Fig.4.1. For all the herbicides applied, metabolites release from Imazapyr such as 2-Amino-3-carboxymethy-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one and that of Primextragold such as metolachlor were more highly persistent in Alfisol (48.71 % peak area; 50.97 % peak area) compared to Inceptisol (35.31 % peak area; 37.05 % peak area). This indicates that Alfisols may be more susceptible to metabolite toxicity compared Inceptisols. Metabolites formed under organic herbicide application (acetamide) was also more persistent in Alfisol (30.18 % peak area) compared to Inceptisol (25.0 % peak area), although its persistency level was lower than those of the metabolites formed under the application of chemical herbicides.

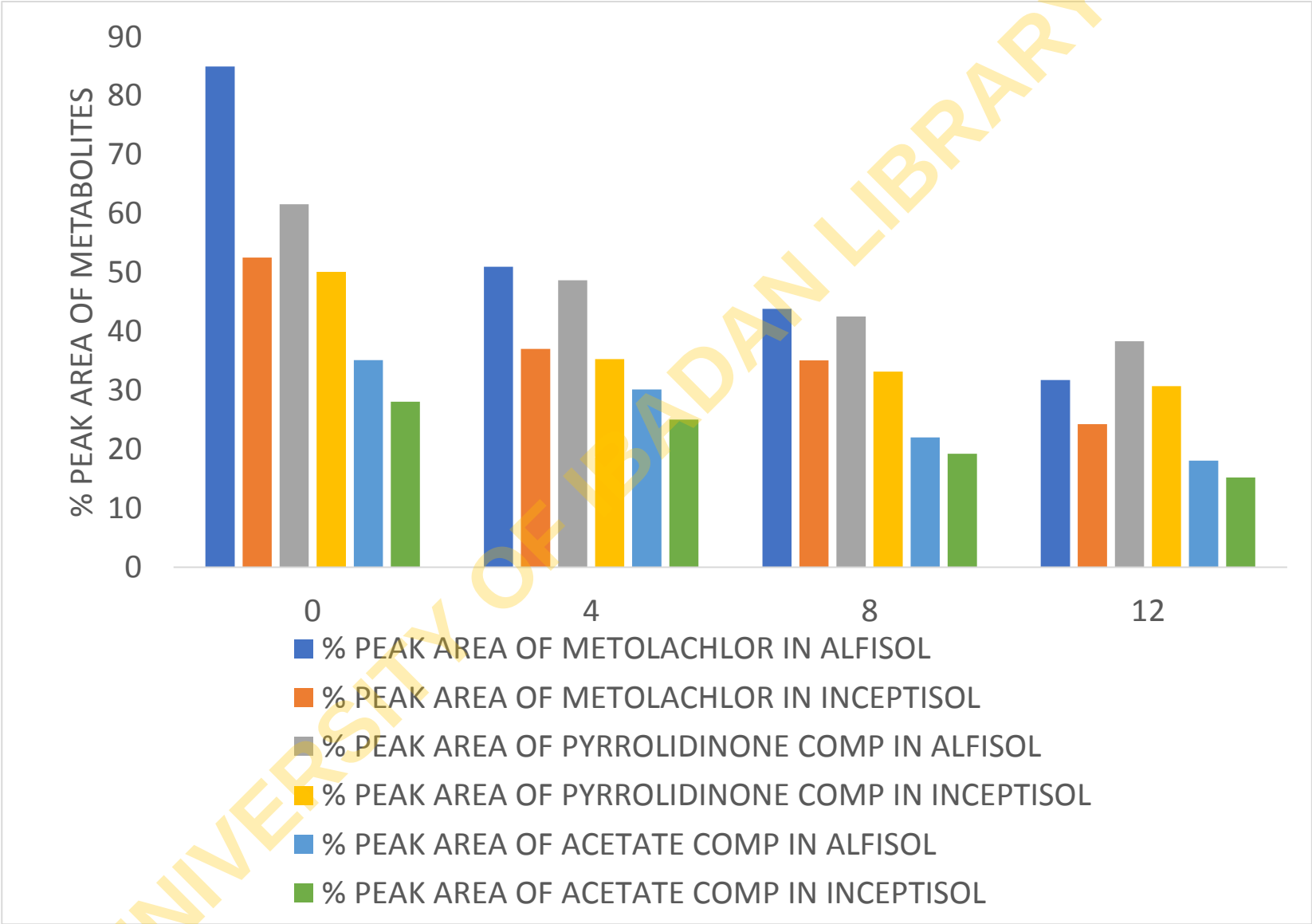

Fig. 4.1: Persistency of Herbicide Metabolites at 0-12 Weeks After Herbicide Application in Non-sterilized Alfisol and Inceptisol

4.4 Persistency of Herbicides Metabolites at 8 Week After Herbicide Application in Non-sterilized Alfisol and Inceptisol

Fig 4.1 similarly shows % peak area as the indices of persistence of various metabolites observed in Alfisol and Inceptisol under non-sterilized soil condition at 8 weeks. It was clearly observed that the persistence level of metabolite varieties differed between the two soil types under consideration for each herbicide treatment. However, in both the Alfisols and Inceptisols similar trend was observed where metabolites formed under Imazapyr (2-Pyrrolidinone,1-methyl) and PrimextraGold (Atrazine) herbicide applications were more persistent in Alfisol (42.55 % peak area; 43.85 % peak area) compared to Inceptisol (33.20 % peak area; 35.09 % peak area). Organic herbicide metabolite (acetamide) however, was less persistent in both Alfisol and Inceptisol (22.08 % peak area; 19.2 % peak area) respectively compared to the chemical herbicide metabolites.

4.5 Persistency of Herbicides Metabolites at 12 Week After Herbicide Application in Non-sterilized Alfisol and Inceptisol

Results shown in Fig 4.1 is not in any way different from the others in terms of metabolites concentration differences across the treatments in both Alfisol and Inceptisol. For example, while 2-Amino-3,4-dihydro-4-4-dimethyl-6-pyrimidinone (38.35 % peak area), Atrazine (31.75 % peak area) and Acetamide (18.11 % peak area) were the most persistent in Alfisols treated with IM, PMG and ORGH respectively, 2-Amino-3,4-dihydro-4-4-dimethyl-6-pyrimidinone (30.75 % peak area), Atrazine (24.29 % peak area) and Acetamide (15.20 % peak area) in Inceptisols, had lower persistence at 12 weeks after application. From the result obtained it was observed that these various metabolites formed from application of IM, PMG and ORGH were more persistent in Alfisol compared to Inceptisol. However, ORGH herbicide metabolites persistence level was observed to be lower compared to those of the chemical herbicides in both Alfisol and Inceptisol.

4.6 Biodegradation Level of Metabolites in Alfisol at 4 Weeks After Herbicide Application

In Fig. 4.2, It was observed that biodegradation level of metabolites formed after Imazapyr and PrimexraGold herbicides were applied in non-sterilized Alfisol at week 4 were low compared to biodegradation level of their counterpart metabolites formed in sterilized Alfisol.

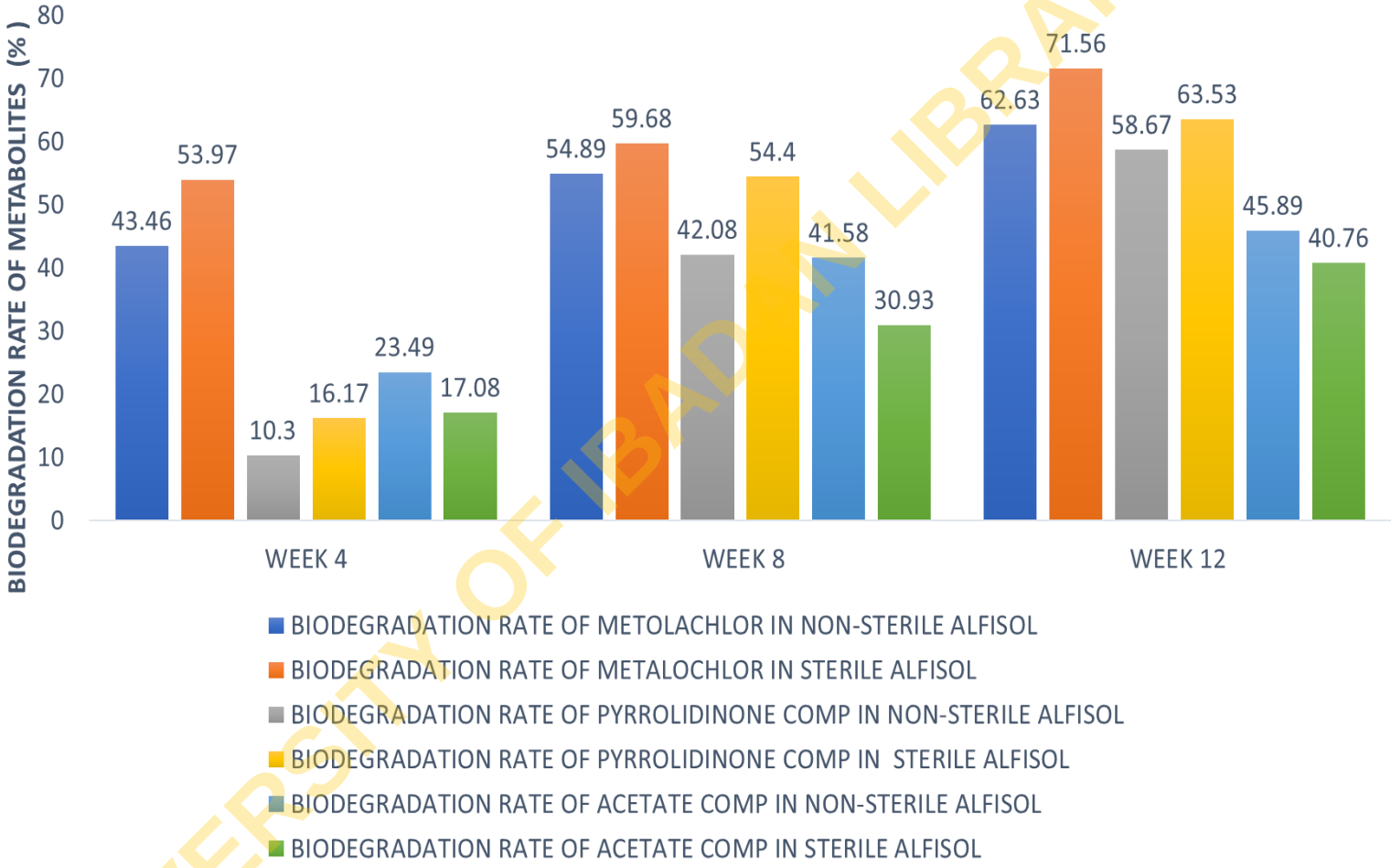

Fig 4.2: **Biodegradation level of metabolites in Alfisol at 0-12 weeks after herbicide application**

Example; 2-Amino-3-carboxymethy-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one which was the metabolite formed after Imazapyr herbicide application in non-sterilized Alfisol had low biodegradation level of 10.30 % compared to 16.17% in sterilized Alfisol, Metolachlor which was the metabolite formed after application of primextraGold herbicide in non-sterilized Alfisol also had low biodegradation level of 43.46 % in non-sterilized Alfisol compared to 53.97 % in sterile Alfisol. However, Biodegradation level of metabolite formed on non-sterilized Alfisol after application of organic herbicide (Acetamide) was higher (23.49 %) compared to degradation level of the same metabolite on sterile Alfisol (17.08 %). In overall, organic herbicide metabolites biodegradation level was observed to be higher compared to the chemical herbicide metabolites.

4.7 Biodegradation Level of Metabolites in Alfisol at 8 and 12 Weeks After Herbicide Application

Fig. 4.2 also relates the result for biodegradation level of metabolites formed after application of Imazapyr, primextraGold and organic herbicides on non-sterilized and sterilized Alfisol at week 8 and 12 which followed similar trend as in week 4.

4.8 Biodegradation Level of Metabolites in Inceptisol at 4, 8 and 12 Weeks After Herbicide Application

Results in Fig. 4.3 showed variations in biodegradation level of metabolites formed due to transformation of Imazapyr, PrimextraGold and Organic herbicides applied in non-sterilized and sterilized Inceptisol. Observations made at week 4 showed that the pyrrolidinone compounds which were the metabolites formed after Imazapyr application in non-sterilized and sterilized Inceptisol consistently had low biodegradation level of 8.94 % in non-sterilized Inceptisol compared to 29.49 % in sterilized Inceptisol, 9.46 % in non-sterilized Inceptisol compared to 31.62 % in sterilized Inceptisol, 31.72 % in non-sterilized Inceptisol compared to 34.75 % in sterilized Inceptisol respectively at weeks 4, 8 and 12. Atrazine which was metabolite formed after application of PrimextraGold in both non-sterilized and sterilized Inceptisol had high biodegradation level at week 4 (61.96 %) and 8 (68.17 %) compared to the degradation level of the same metabolite in sterilized Inceptisol (41.98 %) and (56.49 %) respectively.

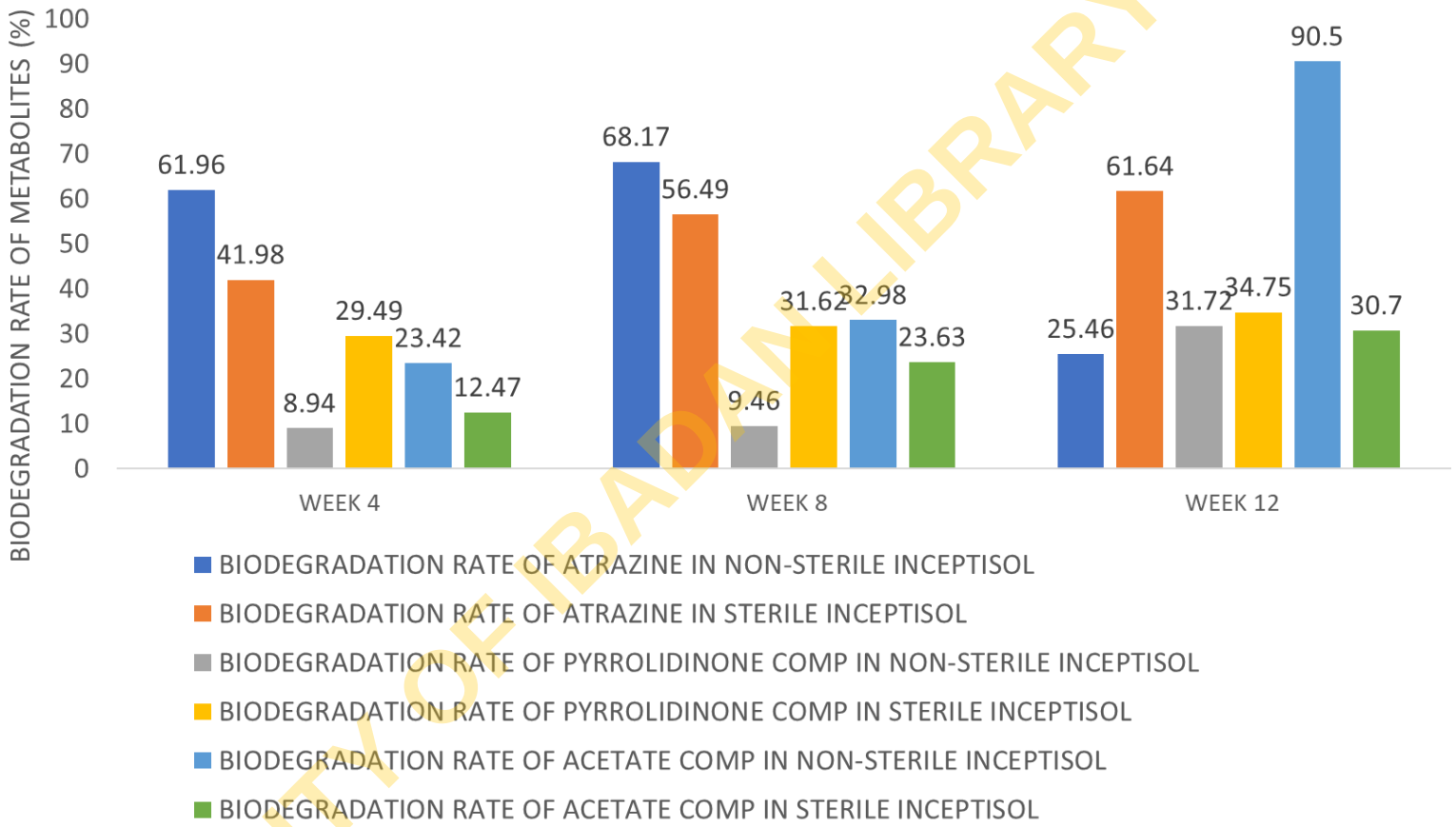

Fig 4.3**: Biodegradation level of metabolites in Inceptisol at 0-12 weeks after herbicide application**

However, Atrazine biodegradation level was observed to be low in non-sterilized Inceptisol at week 12 (25.46 %) compared to weeks 4 (61.96 %) and 8 (68.17 %). It was also observed that biodegradation level of metabolite formed after application of organic herbicide was high in non-sterilized Inceptisol (23.42 %; 32.98 %; and 90.5%) compare to sterile Inceptisol (12.47%; 23.63 % and 30.7 %) at weeks 4, 8 and 12 respectively. Nevertheless, it was generally observed that biodegradation level of metabolites formed under the application of organic herbicide was higher compared to the biodegradation level of metabolites formed under the application of both Imazapyr and primextraGold which are chemical herbicides.

4.9 Bacterial Diversity Difference between Locations, Soil type and Time with the exception of Herbicide

Alpha diversity of the bacterial communities was examined by calculating the Observed features (a measure that accounts for the number of different species within a sample), the Shannon index (a measure that accounts for both richness and evenness), and Faith's PD (a measure that incorporates phylogenetic differences between species) for Soil types, Locations, Treatments and Weeks and their various components.

Regardless of the metric tested, it was observed that there were significant differences in the Observed features (P- adjusted <0.0 5), Shannon (P- adjusted <0.05) and Faith's PD (P-adjusted <0.05) for alpha diversity with respect to location, soil type, and weeks, however, no significant difference was found in alpha diversity with respect to treatment (P- adjusted <0.05) (Tables 4.4, 4.5, 4.6, 4.7) as well as (Figures 4.4, 4.5, 4.6 and 4.7). In summary, this finding suggests that soil type, location, and weeks have significant influence on changes that occurred in alpha bacteria diversity within these variables investigated except for treatment which has no significant influence in the changes that occur within the bacteria diversity.

Table 4.4: Kruskal Wallis Test using Observed Feature, Faith's PD and Shannon Metrics to show Alpha Diversity in Soil Types

p-value for all groups	**GP 1**	**GP 2**	P-Value	P-adjusted	P value for all groups	**GP 1**	**GP2**	p-value	P-adjusted	P value for all groups	**GP 1**	**GP 2**	p-value	P-adjusted	H-value
Observed feature					Faith's PD					Shannon					
2.5538e-21	**ALF**	**INC**	2.5538e-21	2.5538e-21	1.73644e-16	**ALF**	**INC**	1.7364e-16	1.7364e-16	1.5367e-7	**ALF**	**INC**	1.5367e-07	1.5367e-07	89.8618

Legend: ALF = Alfisol, INC = Inceptisol, GP 1= Group 1, GP 2= Group 2

Table 4.5: Kruskal Wallis Test using Observed Feature, Faith's PD and Shannon Metrics to show Alpha Diversity in Different Locations

P value for all groups Observed features	Grp 1	Grp 2	P-Value	P-adjustd	Pvalue for all grps Faith's PD	Grp 1	Grp 2	p-value	P-adjusted	Group 1	Group 2	P value for all groups Shannon	p-value	P-adjusted	H-value
9.2737e-12	FIITA	F_MP	7.924e-04	9.5093e-04	7.10539e-13	F_IITA	FIAR&T	5.4668e-05	8.2002e-05	F_IITA	FIAR&T	0.0003	0.0509	0.0725	11.2588
		F_NIHORT	4.9985e-11	2.9991e-10			FNIHORT	5.9857e-12	3.5914e-11		FNIHORT		0.0001	0.0009	43.1777
		F_UI	2.1687e-05	4.3374e-05			FUI	5.8127e-06	1.1625e-05		FUI		0.0298	0.0596	18.0350
	FIAR&T	F_NIHORT	5.2125e-08	1.5637e-07		FIAR&T	FNIHORT	1.3107e-08	3.9323e-08	F_MP	FNIHORT		0.0022	0.0066	29.6360
		F_UI	8.5874e-02	8.5874e-02			FUI	1.4355e-01	1.4355e-01		FUI		0.3877	0.3877	2.9500
	FNIHORT	F_UI	6.1133e-05	9.1699e-05		F_NIHORT	FUI	1.0699e-04	1.2839e-04	FNIHORT	FUI		0.0604	0.0725	16.0672

Legend: F-IITA = Field sample from International Institute for Tropical Agriculture, F- IAR&T = Field sample from Institute of Agricultural Research and Training, Moore plantation, F- NIHORT = Field sample from National Horticultural Research Institute and F- UI = Field sample from University of Ibadan (UI)}, Treatment;

Table 4.6: Kruskal Wallis Test using Observed Feature, Faith's PD and Shannon Metrics to show Alpha Diversity at Different Time Points

p-value for all groups Observed Feature	GP 1	GP 2	P-Value	P-adjusted	p-value for all groups Faith's PD	G P1	G P2	p-value for all groups	P-adjusted	p-value for all groups Shannon	G P1	GP2	p-value	P-adjusted	H-value
0.0005	0	2	0.0296	0.0592	0.0002	0	2	0.0108	0.0282	0.0003	0	2	0.0222	0.0444	4.7300
		4	0.3686	0.4551			4	0.0117	0.0282			4	0.0022	0.0074	0.8081
		6	0.0219	0.0549			6	0.1218	0.1740			6	0.8937	0.8937	5.2476
		8	0.4096	0.4551			8	0.2966	0.3296			8	0.0003	0.0034	0.6797
	2	4	0.1687	0.2811		2	4	0.8937	0.8937		2	4	0.4879	0.5421	1.8943
		6	0.0000	0.0004			6	0.0002	0.0011			6	0.0593	0.0989	16.6612
		8	0.2490	0.3558			8	0.1081	0.1740			8	0.0767	0.1097	16.6612
	4	6	0.0025	0.0103		4	6	0.0002	0.0011		4	6	0.0152	0.0381	9.1232
		8	0.8791	0.8791			8	0.1410	0.1763			8	0.2500	0.3126	0.0231
	6	8	0.0031	0.0103		6	8	0.0141	0.0282		6	8	0.0010	0.0053	8.7387

Legend: GP 1= Group 1, GP 2= Group 2

Table 4.7: Kruskal Wallis Test using Observed Feature, Faith's PD and Shannon Metrics to show Alpha Diversity in Herbicide Treatment

p-value for all groups Observed features	GP 1	GP2	P-Value	P-adjusted	P value for all groups Faith's PD	GP 1	GP2	p-value	P-adjusted	P value for all groups Shannon	GP1	Group 2	p-value	P-adjusted	H-value
0.8777	CT	IM	0.5918	9.5093e-04	0.7616	CT	IM	0.3717	0.9240	0.2031	CT	IM	0.0724	0.2318	0.2875
		ORGH	0.4618	0.9833			ORGH	0.5151	0.9240			ORGH	0.1211	0.2422	0.5413
		PMG	0.4763	0.9833			PMG	0.3390	0.9240			PMG	0.0772	0.2318	0.5072
	IM	ORGH	0.8844	0.9833		IM	ORGH	0.7700	0.9240		IM	ORGH	0.6406	0.7737	0.0211
		PMG	0.9814	0.9833			PMG	0.9550	0.9550			PMG	0.9523	0.9523	0.0005
	ORGH	PMG	0.9833	0.9833		ORGH	PMG	0.7586	0.9240		ORGH	PMG	0.6448	0.7737	0.0004

Legend: CT= Control, IM =Imazapyr, PMG = Primextragold, ORGH= Organic (Vinegar) Herbicide

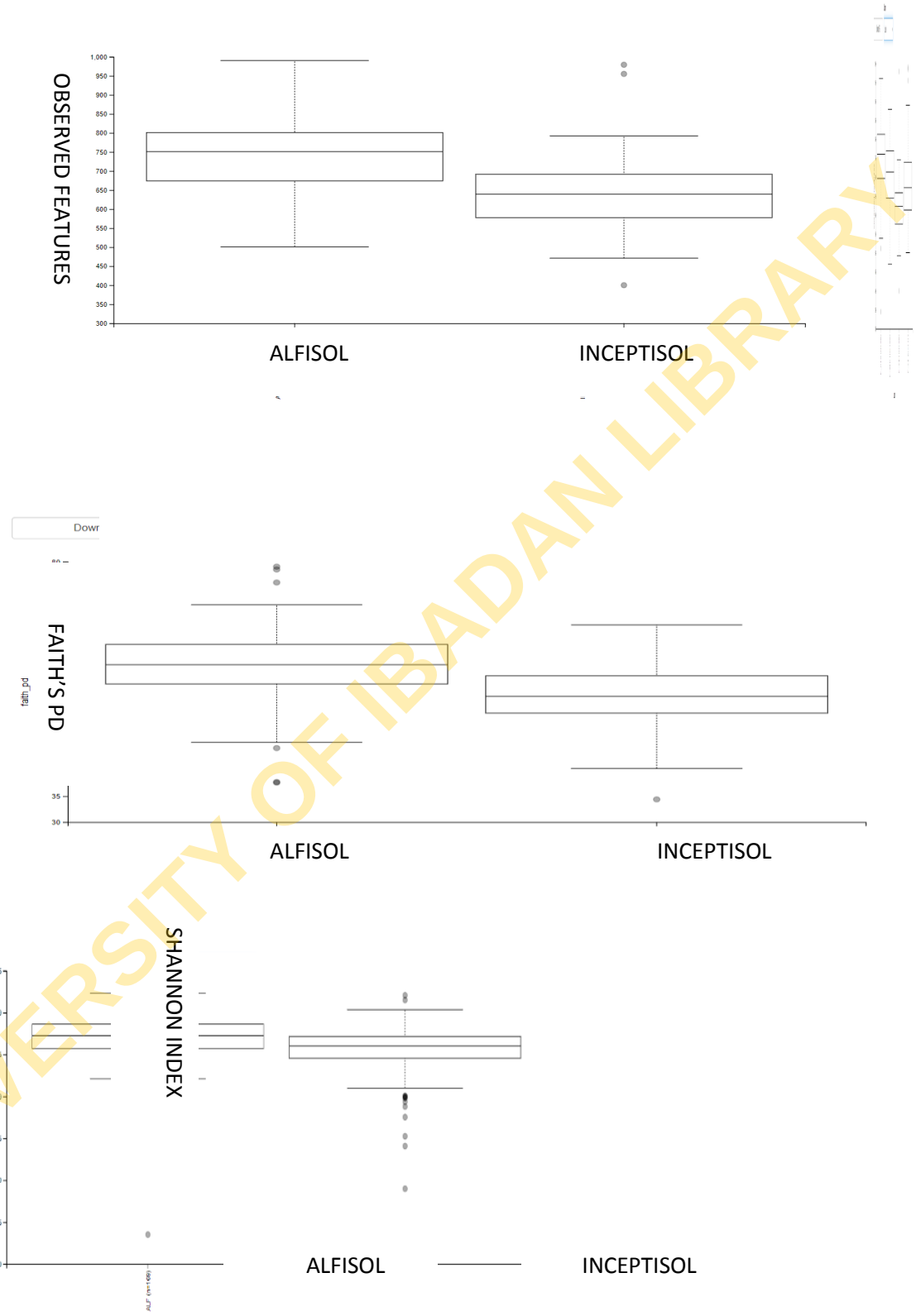

Fig 4.4: Boxplot Showing Observed Feature, Faith's PD and Shannon Metrics of Alpha Diversity for Soil

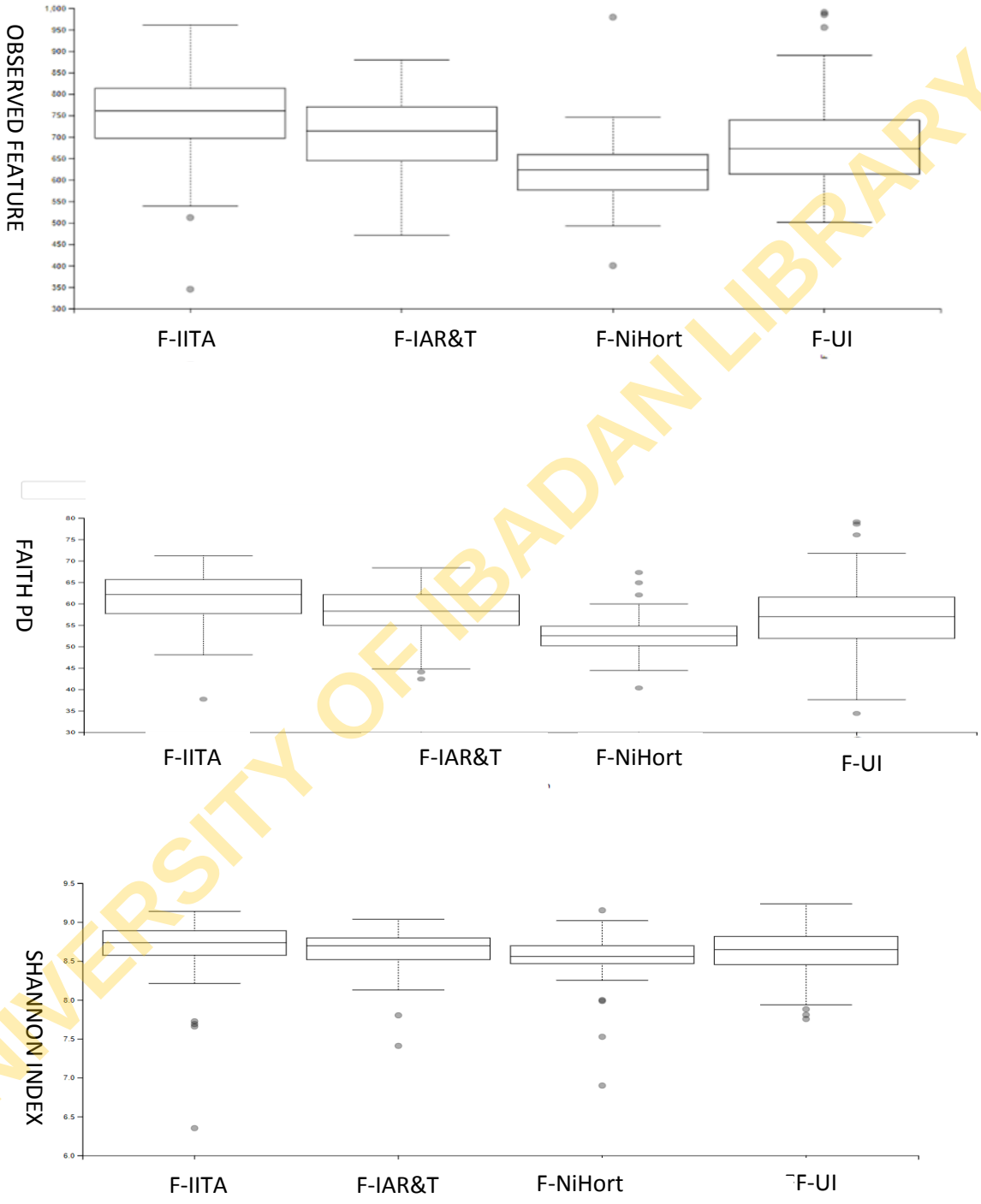

Fig4.5: Boxplot Showing Observed Feature, Faith's PD and Shannon Metrics for Alpha Diversity for Different Locations

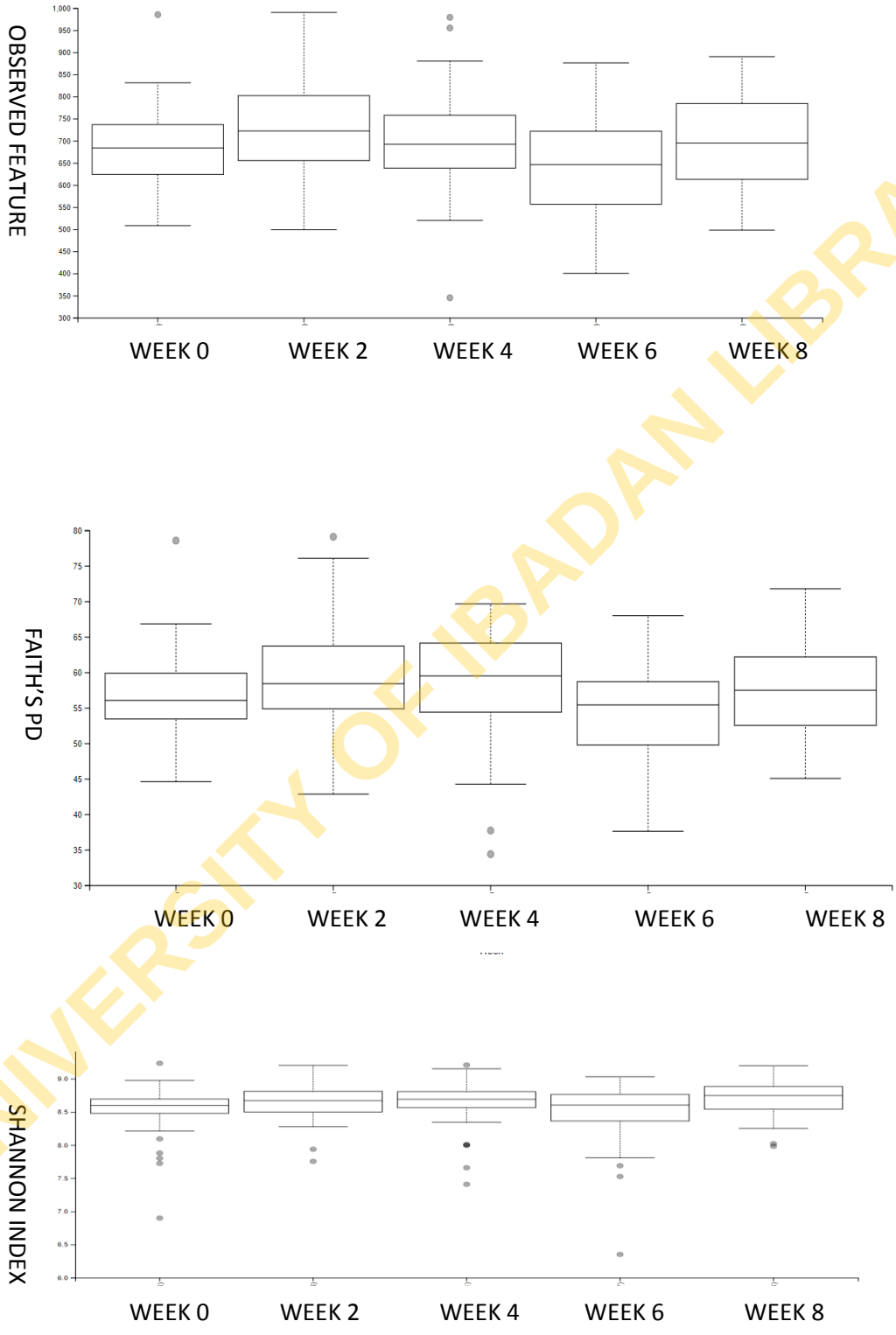

Fig4.6: Boxplot Showing Observed Feature, Faith's PD and Shannon Metrics for Alpha Diversity of Weeks

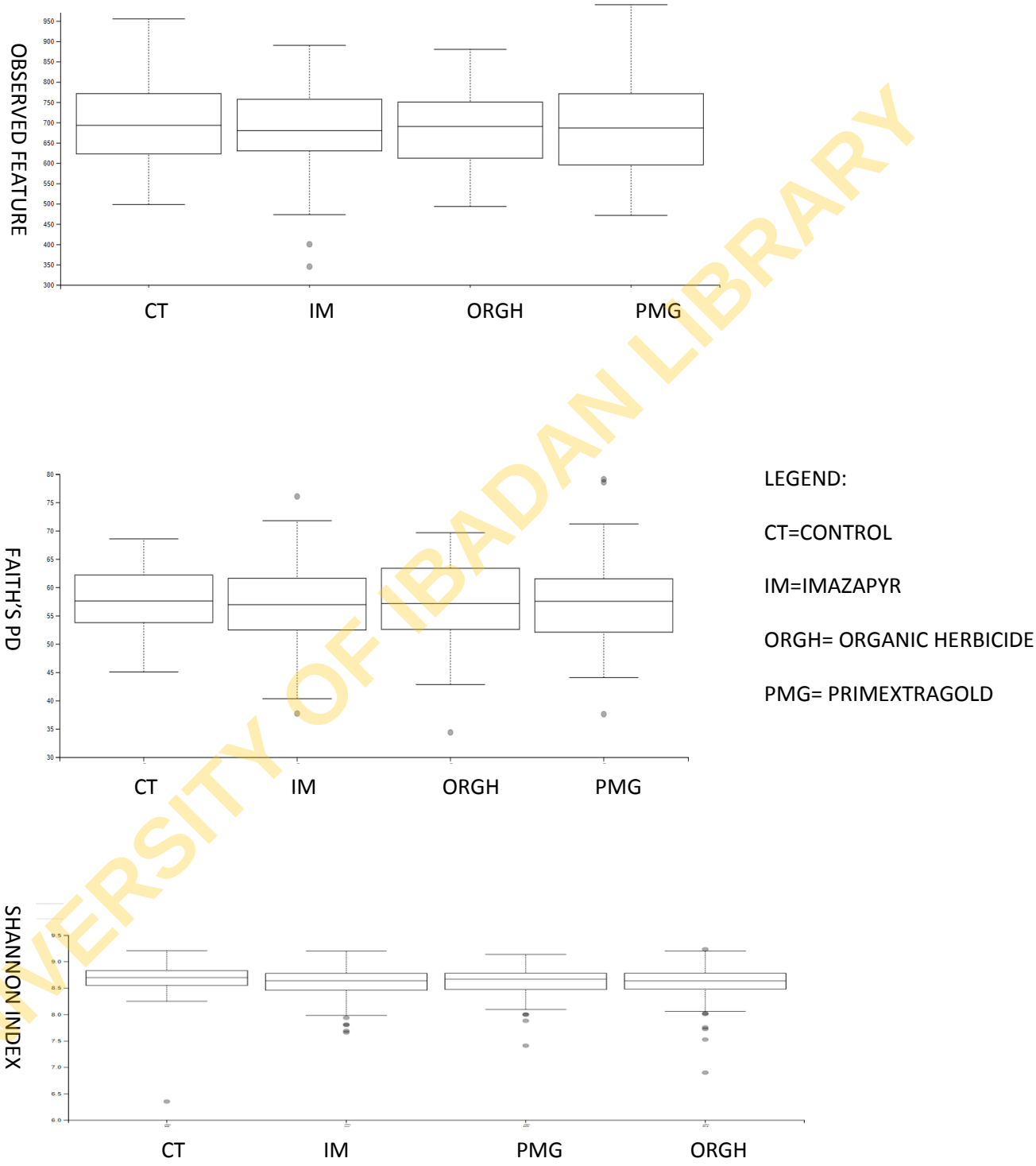

Fig 4.7: Boxplot Showing Observed Feature Faith's PD and Shannon Metrics for Alpha Diversity of Treatments

With respect to bacterial diversity within and among Alfisol and Inceptisol, there was significant difference (p-adjusted < 0.05) found at α diversity measures (observed features index, Faith's PD and Shannon index) of the soil types (Table 4.4). More interestingly, it was observed that Alfisol had higher number of different species (Observed Feature), higher number of shared ancestors (Faith PD) and higher number of different species as well as their relative abundance (Shannon index) compared to Inceptisol which was highly significant at P < 0.05 (Fig.4.4). From the results, it was observed generally that there was significant difference within and among bacterial diversity of locations such as; IITA and IAR&T, IITA and NIHORT and also IITA and UI; IAR&T and NIHORT, IAR&T and UI as well as NIHORT and UI. However, it was observed that locations such as IITA and NIHORT, IITA and UI as well as IAR&T and NIHORT showed significant differences in number of different species (observed features) p-adjusted < 0.05 and number of shared ancestors (Faith's phylogenetic diversity) p-adjusted < 0.05 (Table 4.5).

Locations such as IITA and IAR&T, IAR&T and UI as well as NIHORT and UI also showed significant difference in number of different species (p-adjusted < 0.05) and number of shared ancestors (p-adjusted < 0.05) but had no significant difference in number of different species and their relative abundance (p-adjusted < 0.05). Furthermore, locations such as IITA had higher number of different species and higher number of shared ancestors compared to IAR&T, NIHORT and UI and was highly significant at ($P \leq 0.05$), IITA also had higher number of different species and their relative abundance compared to IAR&T and NIHORT but not significantly different from Shannon index in UI.

Location such as IAR&T had higher number of different species and higher number of shared ancestors compared to NIHORT and UI, however, the number of different species and their relative abundance was higher in IAR&T compared to NIHORT but not significantly different from that of UI, nevertheless, all were highly significant at (P < 0.05). Conversely, the number of different species (Observed Features), and number of shared ancestors (Faith PD) observed in NIHORT location were found to be lower compared to those found in UI and they were significantly lower at (P < 0.05). Nevertheless, the number of different species and their relative abundance in NIHORT were not significantly different

from those present in UI. (Fig. 4.5). As regards to how weeks impacted bacterial diversity, it was observed that there was significant difference within and among α diversity within the soil types as influenced by different experimental time points (Table 4.6). For instance, comparisons of different time points such as weeks 0 and 2 as well as week 4 and 6 showed significant difference (p-adjusted < 0.05) for all α diversity measures such as number of different species (Observed features), number of shared ancestors (Faith's PD), including number of different species and their relative abundance (Shannon index). There was no significant difference observed in α diversity measures (Observed features, Faith's PD and Shannon index) respectively in comparisons such as weeks 2 and 4, weeks 2 and 8, weeks 4 and 8. However, significant difference was observed in number of different species, number of shared ancestors and number of different species and their relative abundance for comparisons such as weeks 0 and 2, weeks 2 and 6, weeks 4 and 6, as well as weeks 6 and 8. In addition, there was a contrast observed in α diversity measures for comparisons such as found in weeks 0 and 4, weeks 0 and 6, as well as weeks 0 and 8. For instance for comparison between weeks 0 and 4, number of different species and their relative abundance (Shannon index) as well as number of shared ancestors (Faith's phylogenetic diversity) increased at week 4 compared to week (Fig. 4.6).

With respect to comparison between week 0 and 6, number of different species was found to be (Observed feature) slightly increased at week 6 compared to week 0 (Fig 4.6). For comparison between week 0 and 8, it was noticed that number of different species and their relative abundance (Shannon index diversity measure) widely increased at week 8 compared to week 0 and it was highly significant at ($P \leq 0.05$) (Fig 4.6). There was no significant difference observed in bacterial diversity between and among all treatments (CT, IM, PMG and ORGH) applied in both Alfisol and Inceptisol (Table 4.7).

4.10. Bacterial Community Composition under Herbicide Application as Revealed by Non-metrics Multidimensional Scaling (NMDS)

Generally, most of the bacterial community compositions within Alfisol were similar to those within Inceptisol as shown in (Fig. 4.8), as this was well represented in their nature of cluster.

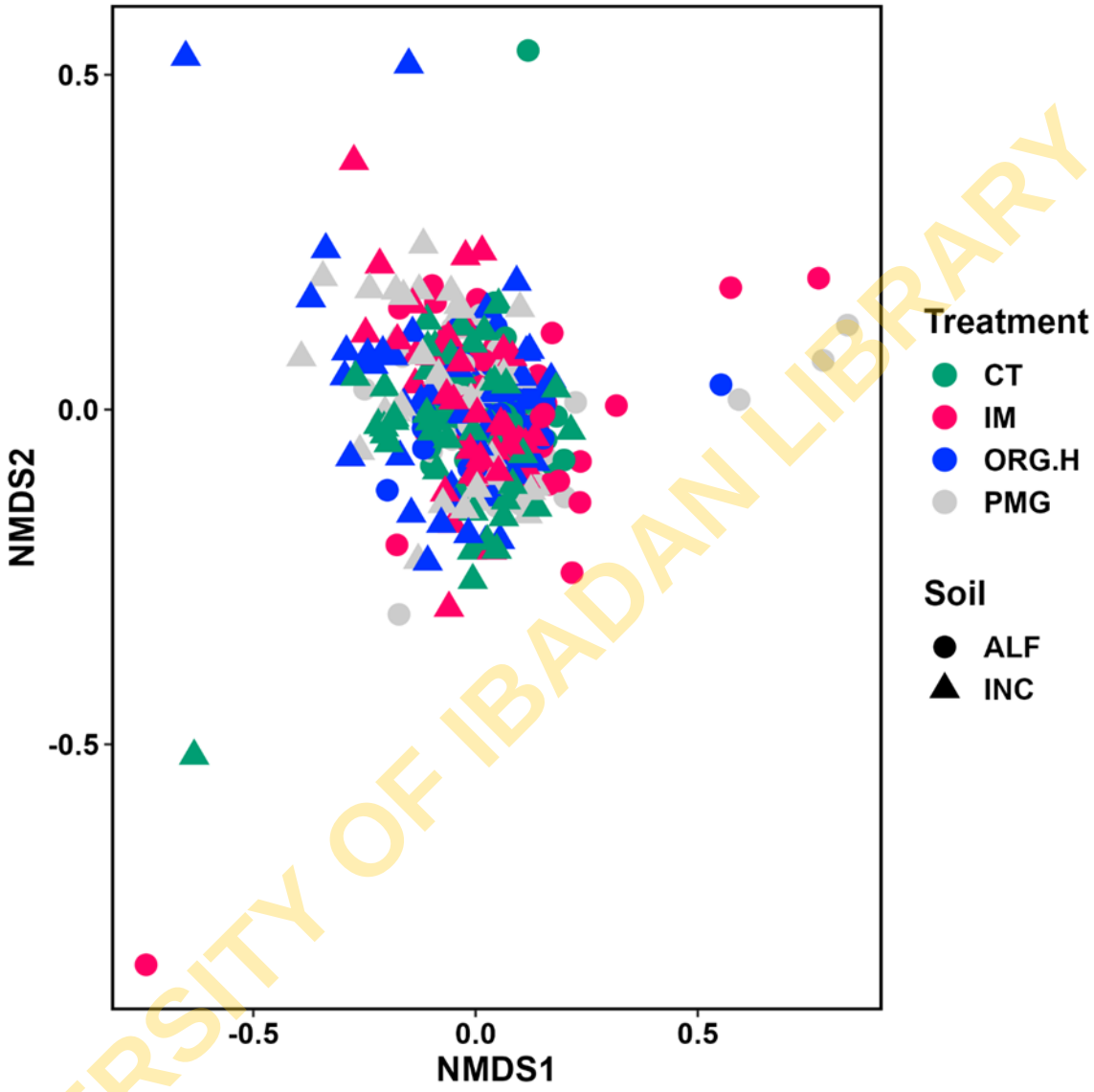

Fig 4.8: Non-metrics Multidimensional Scaling (NMDS) Showing Bacterial Community Compositions within Alfisol and Inceptisol Treated and Non-treated Soils

Legend: ALF = Alfisol, INC = Inceptisol, CT = Control, IM= Imazapyr, ORGH = Organic (Vinegar) herbicide, PMG = PrimextraGold

Furthermore, some of the bacterial community composition in Alfisol impacted by herbicides such as organic herbicide and Imazapyr were also similar to those bacterial community compositions in Inceptisol impacted by the same set of herbicide treatment.

However, certain bacterial community compositions found in Inceptisol which were impacted by primextraGold herbicide, Imazapyr and organic herbicides as well as soil with no herbicide treatment (CT) had dissimilar bacteria community composition as those found in Alfisol. Similar result was also observed in Alfisol impacted by the herbicide treatments mentioned above compared to the Inceptisol bacterial community. This is shown in the distance apart found between these bacterial communities in Alfisol and Inceptisol.

4.11 Beta Diversity Result as Influenced by Location, Treatment, Week, Soil and their Interactions

About 11 % of the variation observed between the bacterial community composition of Alfisol and Inceptisol were influenced by location (Table 4.8). This indicated that location significantly impacted these bacterial community compositions (P= 0.001, F= 13.6601). It was also observed that 1 % variation in bacterial community composition which occurred between Alfisol and Inceptisol was caused by herbicide treatment and this could be said that treatment had significantly influenced this variation (P= 0.001, F= 2.6696). Further variation in bacterial community composition of about 6 % between Alfisol and Inceptisol was influenced by length of time (weeks) after herbicide application as well as duration of the experiment. This showed that length of time (weeks) also had significant effect on the variations in bacteria community compositions between the two soil types (P= 0.001, F= 28.8668).

Interaction between location and soil resulted in about 1 % variation between Alfisol and Inceptisol bacteria community compositions and their interactions significantly influenced the variations in these bacteria communities (P = 0.001, F = 24.5863). This permanova test result also revealed a significant effect (P = 0.007, F = 1.743) due to interaction between location and week.

Table 4.8: Assessment of Bacteria Community Composition Variation in Field Alfisol and Inceptisol Samples as Influenced by Location, Treatments, Time and their Interactions using Permanova – Statistically Significant Test

Source of Variation	Df	Sumof squares	R^2	F- value	Pr (> F)	Significant level
Location	4	2.4372	0.11707	13.6601	0.001	***
Treatment	3	0.3572	0.01716	2.6696	0.001	***
Week	1	1.2876	0.06185	28.8668	0.001	***
Location: Soil	2	2.1933	0.10536	24.5863	0.001	***
Location: Treatment	9	0.4571	0.02196	1.1388	0.133	Ns
Location: Week	3	0.2330	0.01119	1.7413	0.007	**
Treatment: Week	3	0.2132	0.01024	1.5932	0.023	*
Location:Treatment:Week	9	0.3029	0.01455	0.7545	0.972	Ns
Residual	299	13.3365	0.64063			
Total	333	20.8180	1.00000			

The interaction was observed to have caused 1 % variation in bacteria community compositions between Alfisol and Inceptisol. Interaction between treatment and week also resulted in 1 % variation in bacterial community composition and this interaction also has a significant influence (P = 0.023, F = 1.5932) on Alfisol and Inceptisol bacterial community compositions. Other variables such as location and treatment interaction as well as location, treatment and time interaction did not significantly influence the bacterial community composition of the two soil types used for the study.

Permanova pairwise comparison of the variation in bacterial community makeup between Alfisol and Inceptisol indicated that the dissimilarity in their bacterial community composition and population was significant (Table 4.9). Significant variations were observed between the bacterial community compositions and population in Alfisol and Inceptisol with respect to the effect of application of herbicide treatments such as Imazapyr in comparison to Organic herbicide. The result also indicated significant variations between the bacteria community compositions and population of the two soils used for this experiment when application of Imazapyr herbicide and its impact was compared pairwisely with non- application of herbicide (ie control) in soil (CT). Similar result was observed when pairwise comparison was carried out between application and impact of primextragold and no herbicide application (CT) on the bacterial community compositions and population of Alfisol and Inceptisol.

Application and effect of IM on bacterial community compositions and population recorded no significant variations compared to that of PMG between Alfisol and Inceptisol. Similar trend was observed on bacterial community compositions and population when PMG application and its effect was compared with that of ORGH and when ORGH herbicide application and its effect was compared with CT between Alfisol and Inceptisol respectively (Table 4.10).

Changes that occurred between bacterial community compositions and population of Alfsol and Inceptisol when chemical and organic herbicides were applied as a result of length of time after application (weeks), were analysed using permanova pairwise adonis test (Table 4.21).

Table 4.9: Assessment of Variation in Bacterial Community Composition between Alfisol and Inceptisol using Permanova Pairwise Comparison

Source of Variation	Df	Sumof squares	F.Model	R^2	P-value	p.adjusted	Significant level
Alfisol vs Inceptisol	1	0.9779532	16.36492	0.04697638	0.001	0.001	***

Table 4.10: Assessment of the variation in Bacterial Community Composition between Alfisol and Inceptisol Samples as Influenced by Treatments using Permanova Pairwise Comparison

Pairs	Df	Sumof squares	F.Model	R^2	P-Value	P-adjusted	Significant level
IM vs PMG	1	0.07273531	1.1571539	0.007049062	0.269	1.000	Ns
IM vs ORGH	1	0.18403930	2.9951049	0.017723028	0.002	0.012	*
IM vs CT	1	0.16527064	2.6443684	0.015773679	0.003	0.018	*
PMG vs ORGH	1	0.09884409	1.6068284	0.009644433	0.080	0.480	ns
PMG vs CT	1	0.12958486	2.0708971	0.012469958	0.035	0.210	*
ORGH vs CT	1	0.06064150	0.9912401	0.005900546	0.436	1.000	Ns

Legend: CT= Control, IM =Imazapyr, PMG = Primextragold, ORGH= Organic (Vinegar) herbicide

Table 4.11: Assessment of the Variation in Bacterial Community Composition between Alfisol and Inceptisol samples as Influenced by Time using Permanova Pairwise Comparison

Pairs	Df	Sumof squares	F.Model	R^2	P-Value	P-adjusted	Significant level
0 vs 2	1	0.08880253	1.720399	0.01258334	0.045	0.45	Ns
0 vs 4	1	0.36634362	6.995015	0.05181684	0.001	0.01	*
0 vs 6	1	0.67875864	10.441620	0.07330456	0.001	0.01	*
0 vs 8	1	0.80585783	15.079215	0.10688474	0.001	0.01	*
2 vs 4	1	0.33977710	6.469670	0.04573185	0.001	0.01	*
2 vs 6	1	0.72270423	11.202795	0.07458447	0.001	0.01	*
2 vs 8	1	0.78241309	14.615284	0.09900928	0.001	0.01	*
4 vs 6	1	0.15599859	2.366245	0.01761041	0.009	0.09	Ns
4 vs 8	1	0.15758427	2.896395	0.02247072	0.003	0.03	Ns
6 vs 8	1	0.15387457	2.290762	0.01731611	0.011	0.11	Ns

0 vs 2 weeks ……6 vs 8 weeks

Significant variations in bacterial community composition and population between Alfisol and Inceptisol were observed when chemical and biological herbicides such as IM, PMG and ORGH respectively were applied and their effects were compared pairwisely at 0 and 4 weeks, 0 and 6 weeks, 0 and 8 weeks, 2 and 4 weeks, 2 and 6 weeks as well as at 2 and 8 weeks. Other sampling times after herbicide application such as 0 and 2 weeks, 4 and 6 weeks, 4 and 8 weeks, 6 and 8 weeks did not cause significant variation in bacteria community composition and population of these two soil types used for this study.

4.12 Indicator Taxa of Bacteria Genera in Alfisol and Inceptisol Associated with the Soil Types, Location, Treatment and Time

Indicator taxa analysis was carried out to assess the core bacterial population which dominated the variables evaluated in this study. It was observed that 310 indicator taxa were the core bacterial populations which dominated either soil types, locations and combination, herbicide treatments and their combinations as well as weeks and their combination within the experimental period. However, 107 indicator taxa were the number of core bacteria population associated with the soil types and it was discovered that more indicator taxa were predominating Alfisol (85) compared to Inceptisol which were predominated by less (22) indicator taxa (Fig.4.9). It was observed that among the indicator taxa associated with Alfisol, *Paenarthrobacter,Isosphaera,Methylobacter,Methylomicrobium,P3OB42,Pseudogacillibacillus,Chryseobacterium, Agromyce,Cellulomonas*, and *Stenotrophomonas,* were the core genera of this soil type which had higher relative abundance compared to other genera (Table 4.12). On the hand, it was discovered that the indicator taxa *Domibacillus, Blastococcus, Hymenobacter, Fuctibacillus, Alkanindiges* and *Terrabacter* were the core genera within Inceptisol with higher relative abundance compared to other genera within their group (Table 4.13). Among the 141 indicator taxa which were the core bacteria populations associated with locations, 68 indicator taxa predominated single locations, whereas 73 taxa predominated a combination of either two or three locations. For the single locations, UI was predominated by more indicator taxa (35) compared to other locations such as IITA, IAR&T and NIHORT (Table 4.14, 4.15, 4.16 and 4.17). For the three location combinations, it was observed that IITA, IAR&T and UI location combinations were

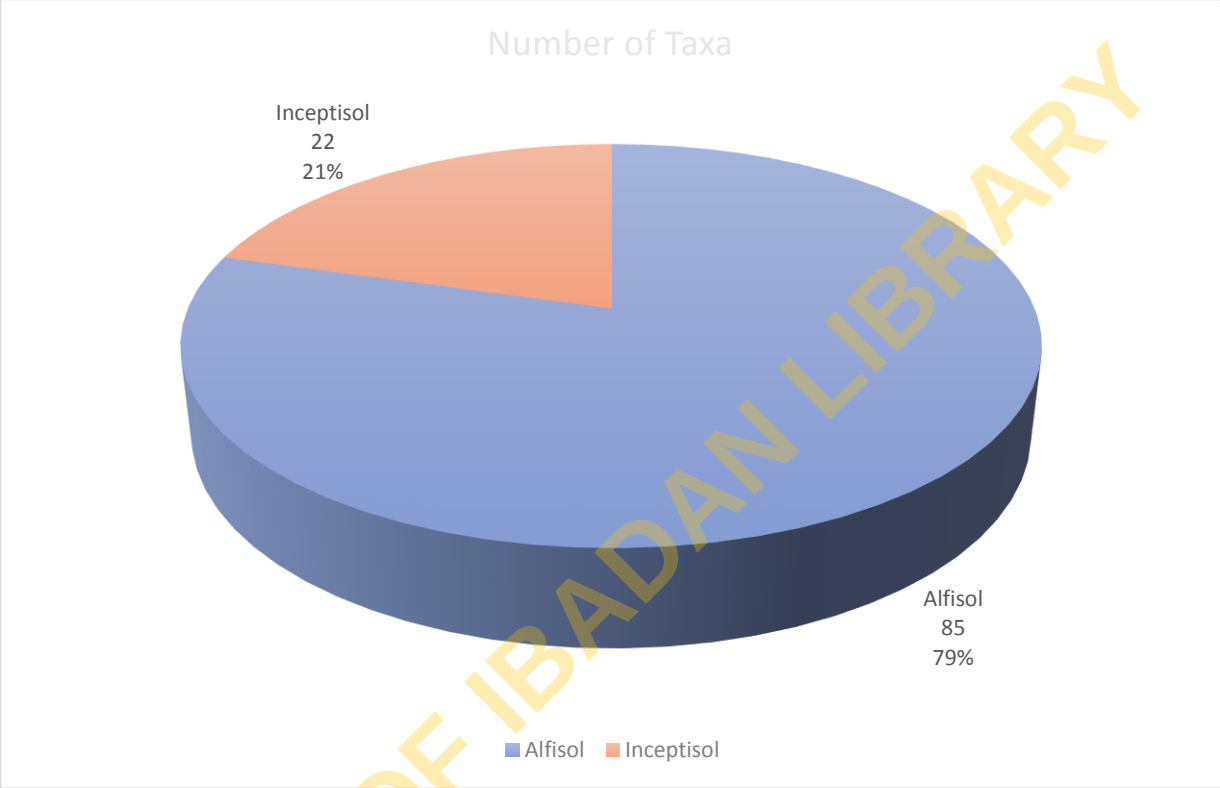

Fig 4.9: Number of Taxa Associated with Alfisol and Inceptisol

Table 4.12: Eighty-five (85) Indicator Taxa Tssociated with Alfisol and their Relative Abundant Values

S/N	Alfisol Genera	Relative Abundance values	Significant Difference
1	***Paenarthrobacter***	0.216%	***
2	***Isosphaera***	0.186%	*
3	***Methylobacter***	0.180%	*
4	***Methylomicrobium***	0.102%	*
5	***Chryseobacterium***	0.097%	***
6	***P3OB-42***	0.094%	*
7	***Pseudogracilibacillus***	0.082%	***
8	***Agromyces***	0.074%	***
9	***Cellulomonas***	0.069%	***
10	***Stenotrophomonas***	0.067%	***
11	*Hamadaea*	0.058%	***
12	*Flavihumibacter*	0.053%	***
13	*Roseomonas*	0.052%	***
14	*Romboutsia*	0.052%	***
15	*Alcaligenes*	0.052%	***
16	*Tissierella*	0.052%	***
17	*Solitalea*	0.049%	***
18	*Limnochordaceae*	0.047%	***
19	*Hydrogenispora*	0.046%	***
20	*Clostridium_sensu_stricto_13*	0.044%	**
21	*Delftia*	0.044%	***
22	*Dactylosporangium*	0.043%	**
23	*Skermanella*	0.041%	***
24	*EC3*	0.040%	***
25	*Melghirimyces*	0.040%	***
26	*Saccharomonospora*	0.040%	**
27	*Turicibacter*	0.038%	**
28	*Nocardiopsis*	0.033%	***
29	*Salipaludibacillus*	0.031%	***
30	*Dinghuibacter*	0.028%	***
31	*Amaricoccus*	0.025%	***
32	*Thermoactinomyces*	0.025%	*
33	*Actinopolymorpha*	0.024%	***
34	*Pseudorhodoplanes*	0.024%	**
35	*Actinomycetospora*	0.022%	***

Table 4.21 contd.

36	*Brevibacterium*	0.022%	*
37	*Longispora*	0.021%	*
38	*Nannocystaceae*	0.020%	**
39	*Virgibacillus*	0.019%	*
40	*Kurthia*	0.017%	***
41	*PeM15*	0.016%	***
42	*Isoptericola*	0.016%	***
43	*Brachybacterium*	0.016%	*
44	*Promicromonospora*	0.016%	*
45	*Corynebacterium*	0.015%	*
46	*Woeseia*	0.015%	**
47	*Defluviitalea*	0.015%	*
48	*Sphingobacterium*	0.014%	**
49	*Arboricoccus*	0.014%	***
50	*Nakamurella*	0.014%	**
51	*Cerasibacillus*	0.014%	***
52	*Thermobifida*	0.014%	*
53	*Minicystis*	0.013%	*
54	*Truepera*	0.013%	*
55	*Providencia*	0.011%	**
56	*Paenalcaligenes*	0.011%	*
57	*Dysgonomonas*	0.010%	**
58	*MBA03*	0.010%	**
59	*Ruania*	0.010%	*
60	*Ga0074140*	0.009%	*
61	*GOUTA6*	0.009%	*
62	*IMCC26207*	0.009%	**
63	*Planifilum*	0.009%	**
64	*PB19*	0.008%	***
65	*Myroides*	0.008%	**
66	*ZOR0006*	0.007%	***
67	*Methylocaldum*	0.007%	*
68	*Phormidium_IAM_M-71*	0.006%	**
69	*Nitrosomonas*	0.006%	**
70	*Acidipila*	0.006%	*
71	*Aeromonas*	0.006%	*
72	*Herbidospora*	0.006%	**
73	*Desulfohalotomaculum*	0.005%	**

Table 4.21: contd.

74	*Modestobacter*	0.004%	*
75	*Paenochrobactrum*	0.004%	**
76	*Actinocorallia*	0.004%	*
77	*Ornithinicoccus*	0.004%	*
78	*Limnobacter*	0.004%	*
79	*Turneriella*	0.003%	*
80	*Microgenomatia*	0.003%	*
81	*Novibacillus*	0.002%	*
82	*Desulfitobacterium*	0.002%	*
83	*Treponema*	0.002%	*
84	*Sporomusa*	0.001%	***
85	*Syntrophaceticus*	0.001%	*

Table 4.13: Twenty-two (22) Indicator Taxa Associated with Inceptisol and their Relative Abundance Values

S/N	Inceptisol Genera	Relative Abundance values	Significant Difference
1	***Domibacillus***	0.236%	***
2	***Blastococcus***	0.122%	***
3	***Hymenobacter***	0.108%	*
4	***Fictibacillus***	0.100%	***
5	***Alkanindiges***	0.055%	***
6	***Terrabacter***	0.045%	***
7	*Parviterribacter*	0.035%	***
8	*Actinoalloteichus*	0.034%	*
9	*oc32*	0.023%	***
10	*Alicyclobacillus*	0.018%	***
11	*Dyella*	0.018%	*
12	*Acidicaldus*	0.012%	**
13	*Sporichthya*	0.011%	***
14	*Azospira*	0.009%	**
15	*Kutzneria*	0.008%	*
16	*Gordonia*	0.007%	**
17	*Granulicella*	0.007%	**
18	*Carboxydothermus*	0.002%	***
19	*Seinonella*	0.002%	*
20	*Leptospirillum*	0.002%	*
21	*Asticcacaulis*	0.001%	**
22	*EV818SWSAP88*	0.001%	*

Table 4.14: Thirty-five (35) Indicator Taxa Associated with UI location and their Relative Abundant Values

S/N	UI Genera	Relative Abundance values	Significant Difference
1	*Methylomicrobium*	0.321%	*
2	*Lysinibacillus*	0.280%	***
3	*Pseudogracilibacillus*	0.251%	***
4	*Oceanobacillus*	0.198%	**
5	*Romboutsia*	0.147%	***
6	*Ellin6055*	0.136%	**
7	*Limnochordaceae*	0.134%	**
8	*Saccharomonospora*	0.125%	**
9	*Melghirimyces*	0.124%	***
10	*Turicibacter*	0.119%	***
11	*Isosphaera*	0.109%	**
12	*Nocardiopsis*	0.092%	**
13	*Thermoactinomyces*	0.076%	***
14	*Solibacillus*	0.070%	***
15	*Brevibacterium*	0.069%	*
16	*Virgibacillus*	0.061%	*
17	*Pseudorhodoplanes*	0.060%	*
18	*Brachybacterium*	0.050%	**
19	*Corynebacterium*	0.046%	**
20	*Thermobifida*	0.044%	*
21	*Woeseia*	0.043%	*
22	*Haloactinopolyspora*	0.043%	*
23	*Cerasibacillus*	0.040%	**
24	*Truepera*	0.039%	*
25	*Geminicoccus*	0.031%	**
26	*Ruania*	0.031%	*
27	*MBA03*	0.029%	**
28	*Stackebrandtia*	0.026%	*
29	*IMCC26207*	0.025%	*
30	*Qipengyuania*	0.017%	**
31	*Glycomyces*	0.017%	*
32	*Lautropia*	0.015%	*
33	*Sporosarcina*	0.014%	**
34	*Ornithinicoccus*	0.011%	*
35	*Granulicella*	0.000%	*

Legend, UI Location = University of Ibadan

Table 4.15: Seventeen (17) Indicator Taxa Associated with IITA Location and their Relative Abundance Values

S/N	IITA Location Genera	Relative Abundance values	Significant Difference
1	*Phycisphaera*	1.574%	**
2	*Cytophaga*	0.258%	***
3	*Alcaligenes*	0.137%	***
4	*Clostridium_sensu_stricto_13*	0.094%	***
5	*Delftia*	0.083%	***
6	*Sulfurifustis*	0.071%	***
7	*Dinghuibacter*	0.048%	***
8	*Actinomycetospora*	0.042%	*
9	*Thermincola*	0.039%	***
10	*Paenalcaligenes*	0.032%	***
11	*Providencia*	0.026%	***
12	*Tellurimicrobium*	0.023%	**
13	*DEV114*	0.021%	**
14	*CENA33*	0.017%	***
15	*Microcoleus_PCC.7113*	0.014%	***
16	*Paenochrobactrum*	0.012%	***
17	*Geothrix*	0.008%	***

Legend: IITA Location = International Institute for Tropical Agriculture

Table 4.16: Seven (7) Indicator Taxa Associated with IAR&T Location and their Relative Abundance Values

S/N	IAR&T Location Genera	Relative Abundance values	Significant Difference
1	*Isoptericola*	0.043%	***
2	*GOUTA6*	0.026%	***
3	*Ga0074140*	0.021%	**
4	*Phycicoccus*	0.020%	**
5	*Herbidospora*	0.015%	**
6	*Phyllobacterium*	0.014%	*
7	*Actinoalloteichus*	0.003%	**

Legend: IAR&T Location = Institute of Agricultural Research and Training, Moore plantation

Table 4.17: Eight (8) indicator Taxa Associated with NIHORT Location and their Relative Abundance Values

S/N	NIHORT Location Genera	Relative Abundance values	Significant Difference
1	*Fictibacillus*	0.249%	***
2	*Roseisolibacter*	0.161%	***
3	*AKAU4049*	0.071%	***
4	*oc32*	0.050%	***
5	*FFCH5858*	0.033%	***
6	*Azospira*	0.025%	***
7	*Paludibacterium*	0.015%	**
8	*C86*	0.005%	**

Legend: NIHORT Location = National Horticultural Research Institute

Table 4.18: Fourteen (14) Indicator Taxa Associated with IITA, IAR&T and UI Locations and their Relative Abundance Values

S/N	IAR&T, IITA and UI Location Genera	IAR&T	IITA	UI	Significant Difference
1	*Xanthobacteraceae*	2.352%	2.102%	1.539%	***
2	*Rhodanobacter*	0.176%	0.148%	0.034%	***
3	*Paenarthrobacter*	0.167%	0.148%	0.343%	**
4	*Jatrophihabitans*	0.123%	0.099%	0.118%	***
5	*Actinoplanes*	0.090%	0.149%	0.086%	**
6	*Amycolatopsis*	0.089%	0.113%	0.039%	***
7	*Kitasatospora*	0.085%	0.076%	0.033%	***
8	*Brevibacillus*	0.049%	0.153%	0.152%	***
9	*Roseiarcus*	0.049%	0.012%	0.004%	*
10	*Actinoallomurus*	0.043%	0.078%	0.060%	***
11	*Chryseobacterium*	0.033%	0.130%	0.131%	*
12	*Crossiella*	0.021%	0.079%	0.149%	***
13	*Angustibacter*	0.020%	0.144%	0.100%	***
14	*PeM15*	0.015%	0.017%	0.017%	**

Legend: IAR&T Location = Institute of Agricultural Research and Training, Moore plantation, IITA Location = International Institute for Tropical Agriculture and UI Location = University of Ibadan

Table 4.19: Twelve (12) Indicator Taxa Associated with IAR&T, NH and UI Locations and their Relative Abundance Values

S/N	IAR&T, NH and UI Location Genera	IAR&T	NIHORT	UI	Significant Difference
1	*Vicinamibacter*	2.752%	6.824%	2.984%	***
2	*Frankiales*	1.049%	0.204%	0.628%	*
3	*Blastococcus*	0.299%	0.043%	0.050%	*
4	*Ramlibacter*	0.212%	0.216%	0.220%	***
5	*Virgisporangium*	0.111%	0.086%	0.031%	***
6	*Hyphomicrobium*	0.082%	0.174%	0.275%	***
7	*PLTA13*	0.048%	0.443%	0.054%	***
8	*Rhodomicrobium*	0.045%	0.031%	0.020%	**
9	*Stenotrophobacter*	0.026%	0.008%	0.019%	*
10	*Hirschia*	0.009%	0.038%	0.034%	***
11	*R7C24*	0.009%	0.015%	0.045%	**
12	*Bosea*	0.007%	0.024%	0.021%	*

,Legend: IAR&T Location = Institute of Agricultural Research and Training, Moore plantation, NIHORT Location = National Horticultural Research Institute and UI Location = University of Ibadan

Table 4.20: Nine (9) Indicator Taxa Associated with IITA, IAR&T and NH Locations and their Relative Abundance Values

S/N	IITA, IAR&T and NIHORT Location Genera	IITA	IAR&T	NIHORT	Significant Difference
1	*Luedemannella*	0.444%	0.354%	0.306%	***
2	*Flavisolibacter*	0.135%	0.234%	0.155%	***
3	*Flavihumibacter*	0.105%	0.019%	0.040%	**
4	*Aridibacter*	0.070%	0.137%	0.076%	**
5	*Hydrogenispo*	0.049%	0.012%	0.013%	**
6	*vadinHA49*	0.020%	0.014%	0.008%	***
7	*Koribacter*	0.013%	0.013%	0.017%	**
8	*Lechevalieria*	0.008%	0.047%	0.034%	**
9	*Anaerolinea*	0.000%	0.001%	0.001%	*

Legend: IITA Location = International Institute for Tropical Agriculture, IAR&T Location = Institute of Agricultural Research and Training, Moore plantation and NIHORT Location = National Horticultural Research Institute

Table 4.21: Four (4) Indicator Taxa Associated with IITA, NIHORT and UI Locations and their Relative Abundance Values

S/N	IITA, NIHORT and UI Location Genera	IITA	NIHORT	UI	Significant Difference
1	*Novosphingobium*	0.033%	0.067%	0.049%	**
2	*Gammaproteobacteria*	0.012%	0.032%	0.005%	**
3	*Dyadobacter*	0.002%	0.002%	0.011%	*
4	*Pseudoxanthomonas*	0.000%	0.012%	0.003%	*

Legend: IITA Location = International Institute for Tropical Agriculture, NIHORT Location = National Horticultural Research Institute and UI Location = University of Ibadan

predominated by more taxa (14 genera) compared to other three location combinations (Table 4.18, 4.19,4.20 and 4.21). Among the indicator taxa predominating the IITA, IAR&T and UI as part of the three locations combination, the relative abundance of the genera *Crossiella, Chryseobacterium* and P*aenarthrobacter* were higher in UI compared to IITA and IAR&T (Table 4.18).

It was noticed that IITA and IAR&T had higher number of genera (10) compared to other groups in this category for the two location combinations (Table 4.22, 4. 23, 4.24, 4.25 and 4.26). It was also observed, that *Cohnella, and Pedosphaera* were the indicator taxa predominating IITA and IAR&T; however, *Pedosphaera* had higher relative abundance across the locations within the shared group.

It was observed that 32 genera predominated the herbicide treatments as well as control for core genera associated with the various herbicides applied. It is important to mention that most of the genera associated with these herbicide treatments were found to be more dominant in Alfisol compared to Inceptisol. *Methylomicrobium Desulfohalotomaculum, Desulfovobrio, MBA03, Defluviitalea, Desulfitobacterium* and *Limnobacter* predominated PMG herbicide, however, it was observed that the relative abundance of *Methylomicrobium* was higher compared to other genera within this group (Table 4.27). *Nocardiopsis, Rhizocola* and *Sneathiella* were identified to be among the genera predominating IM, with higher relative abundance in *Nocardiopsis* compared to other genera in both Alfisol and Inceptisol but especially in Alfisol. All were significantly different at $P<0.05$ (Table 4.28).

For shared taxa, it was observed that some indicator taxa had preference for two herbicides, for instance, Nannocystis was found predominant in CT and IM however, the relative abundance of Nannocystis was higher in IM (0.035 %) compared to CT (0.020 %) especially in Alfisol where there was high concentration of organic matter as soil property compared to Inceptisol (Table 4.29). *Panaerthrobacter, Melghirimyces, Oxalophgus, Thermobifida, Saccharomonospora, Qupengyyuania* and *Brachybacterium* were observed as the indicator taxa predominating IM and PMG. It was nevertheless observed that among these genera, the relative abundance of *Panaerthrobacter* (0.228 % and 0.552 %) and *Melghirimyces* (0.052 % and 0.094 %) were higher in IM and PMG herbicides respectively compared to control (0.040 %) especially in Alfisol (Table 4.30).

Table 4.22: Ten (10) Indicator Taxa Associated with IITA and IAR&T Locations and their Relative Abundance Values

S/N	IITA and IAR&T Location Genera	IITA	IAR&T	Significant Difference
1	***Pedosphaera***	**0.707%**	**0.503%**	***
2	*Cohnella*	0.259%	0.298%	***
3	*Dactylosporangium*	0.062%	0.056%	***
4	*Mucilaginibacter*	0.059%	0.101%	***
5	*EC3*	0.055%	0.047%	***
6	*Armatimonadales*	0.019%	0.009%	***
7	*Kurthia*	0.013%	0.037%	*
8	*ZOR0006*	0.008%	0.013%	***
9	*Telmatospirillum*	0.002%	0.001%	*
10	*Sporomusa*	0.000%	0.003%	**

Legend: IITA Location = International Institute for Tropical Agriculture and IAR&T = Institute of Agricultural Research and Training, Moore plantation

Table 4.23: Six (6) Indicator Taxa Associated with NIHORT and UI Locations and their Relative Abundance Values

S/N	NIHORT and UI Location Genera	NIHORT	UI	Significant Difference
1	*Ensifer*	0.193%	0.017%	***
2	*Planctomicrobium*	0.021%	0.008%	***
3	*Dyella*	0.017%	0.026%	*
4	*Nannocystis*	0.008%	0.012%	***
5	*Acidicaldus*	0.008%	0.027%	*
6	*Microgenomatia*	0.002%	0.001%	*

Legend: NIHORT Location = National Horticultural Research Institute and UI Location = University of Ibadan

Table 4.24: Nine (9) Indicator Taxa Associated with IITA and UI Locations and their Relative Abundance Values

S/N	IITA and UI Location Genera	IITA	UI	Significant Difference
1	*Actinomadura*	0.071%	0.218%	***
2	*Stenotrophomonas*	0.055%	0.126%	**
3	*Skermanella*	0.054%	0.070%	***
4	*Microbispora*	0.039%	0.019%	*
5	*Mucilaginibacter*	0.028%	0.008%	**
6	*Ralstonia*	0.024%	0.005%	*
7	*Longispora*	0.014%	0.022%	*
8	*Arboricoccus*	0.012%	0.022%	*
9	*Smaragdicoccus*	0.008%	0.009%	*

Legend: IITA Location = International Institute for Tropical Agriculture, and UI Location = University of Ibadan

Table 4.25: Five (5) Indicator Taxa Associated with IAR&T and NIHORT Locations and their Relative Abundance Values

S/N	IAR&T and NIHORT Location Genera	IAR&T	NIHORT	Significant Difference
1	*Domibacillus*	0.518%	0.180%	***
2	*Terrabacter*	0.070%	0.068%	***
3	*Parviterribacter*	0.059%	0.043%	***
4	*Nitrosotaleaceae*	0.059%	0.046%	**
5	*Geobacter*	0.002%	0.007%	*

Legend: IAR&T Location = Institute of Agricultural Research and Training, Moore plantation and NIHORT Location = National Horticultural Research Institute

Table 4.26: Four (4) Indicator Taxa Associated with IAR&T and UI Locations and their Relative Abundance Values

S/N	IAR&T and UI Location Genera	IAR&T	UI	Significant Difference
1	*Cellulomonas*	0.111%	0.091%	**
2	*Amaricoccus*	0.034%	0.042%	**
3	*Dokdonella*	0.024%	0.047%	*
4	*Cellulosimicrobium*	0.008%	0.020%	**

Legend, IAR&T Location = Institute of Agricultural Research and Training, Moore plantation and UI Location = University of Ibadan

Table 4.27: Nine (9) Indicator Taxa Associated with Primextragold Herbicide (PMG) in Alfisol and Inceptisol and their Relative Abundance Values

S/N	PMG genera	ALF PMG	INC PMG	Significant Difference
1	***Methylomicrobium***	**0.324%**	0.000%	*
2	*MBA03*	0.029%	0.000%	*
3	*Methylocaldum*	0.026%	0.000%	**
4	*Limnobacter*	0.011%	0.000%	*
5	*Bacillaceae*	0.011%	0.000%	*
6	*Methanoculleus*	0.007%	0.000%	*
7	*Desulfovibrio*	0.006%	0.000%	**
8	*Desulfitobacterium*	0.006%	0.000%	*
9	*Desulfohalotomaculum*	0.000%	0.000%	*

Legend: ALF = Alfisol, INC = Inceptisol, PMG = PrimextraGold

Table 4.28: Three (3) Indicator Taxa Associated with Imazapyr Herbicide (IM) in Alfisol and Inceptisol and their Relative Abundance Values

S/N	IM genera	ALF IM	INC IM	Significant Difference
1	***Nocardiopsis***	**0.087%**	0.002%	*
2	*Rhizocola*	0.005%	0.000%	*
3	*Sneathiella*	0.005%	0.000%	*

Legend: ALF = Alfisol, INC = Inceptisol, IM= Imazapyr

Table 4.29: An Indicator Taxon Associated with IM and CT in Alfisol and Inceptisol and its Relative Abundance Values

	IM and CT genus	ALF IM	INC IM	ALF CT	INC CT	Significant Difference
1	*Nannocystis*	**0.035%**	0.008%	0.020%	0.008%	*

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, CT = Control

Table 4.30: Ten (10) Indicator Taxa Associated with IM and PMG in Alfisol and Inceptisol and their Relative Abundance Values

S/N	IM and **PMG** genera	ALF IM	INC IM	ALF PMG	INC PMG	ALF CT	INC CT	Significant Difference
1	***Paenarthrobacter***	**0.228%**	0.007%	**0.552%**	0.005%	**0.216%**	0.004%	***
2	*Turicibacter*	0.116%	0.011%	0.035%	0.000%	0.038%	0.004%	**
3	***Melghirimyces***	**0.052%**	0.000%	**0.094%**	0.006%	**0.040%**	0.000%	**
4	*Saccharomonospora*	0.049%	0.026%	0.041%	0.076%	0.037%	0.047%	*
6	*Thermobifida*	0.025%	0.000%	0.030%	0.009%	0.014%	0.002%	*
7	*Brachybacterium*	0.024%	0.000%	0.040%	0.000%	0.016%	0.000%	*
8	*M55.D21*	0.015%	0.000%	0.000%	0.000%	0.015%	0.000%	*
9	*Qipengyuania*	0.008%	0.005%	0.016%	0.000%	0.007%	0.001%	*
10	*Oxalophagus*	0.003%	0.017%	0.008%	0.011%	0.003%	0.008%	*

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG= PrimextraGold, CT = Control

Solibacillus was discovered to be the taxon predominating combination of CT, IM and PMG treatments. However, the relative abundance of *Solibacillus* was lower in PMG (0.019 %) compared to CT (0.023 %) in Alfisol while no difference was observed in Inceptisol (Table 4.31). *Aquisphaera* and Lechevalieria were the indicator taxa associated with combination of CT, ORGH and PMG. However, it was observed that the relative abundance of *Aquisphaer*a was higher in ORGH (0.223 % and 0.312%) compared to CT (0.186% and 0.218%) and PMG (0.201% and 0.196%) in both Alfisol and Inceptisol (Table 4.32). The genera *Saccharopolyspora*, *Salipaludibacillus, Candidatus Ntrosotalea, Group_1.1c* and *Nitrosotaleaceae* which were the indicator taxa observed predominating IM, ORGH and PMG herbicides were found to be dominant in both Alfisol and Inceptisol.

Result from this study showed that the relative abundance of *Candidatus Ntrosotalea* (0.201%; 0.063%), *Saccharopolyspora* (0.049%; 0.026%), *Salipaludibacillus (*0.043%; 0.023%*),* and *Nitrosotaleaceae* (0.033%; 0.002%*)* were higher in IM herbicides within Alfisol compared to IM within Inceptisol but not higher than ORGH and PMG within Inceptisol (Table 4.33). Relative abundance of *Salipaludibacillus* was found to be lower in PMG herbicide within Inceptisol (0.006%) compared to other genera. Relative abundance of *Saccharopolyspora* was observed to be higher in ORGH herbicide (0.048%) compared to PMG (0.041%) in Alfisol whereas the relative abundance of the same genus was also higher in ORGH herbicide (0.71%) compared to IM herbicide (0.026%) in Inceptisol and all were significantly different at ($P< 0.05$).

Furthermore, the indicator taxa within the two soil types which were associated with the three herbicides as influenced by time (experimental duration) were investigated. It was observed that the indicator taxa were mostly dominant in Alfisol compared to Inceptisol and they had different preferences for the varying herbicides applied on the two soil types across the weeks. *DSSD61*, *Azospirillum, Paenochrobactrum* and *Tundrisphaera* were the indicator taxa identified to be strongly associated with week two (Table 4.34). Nevertheless, it was observed that the relative abundance of *Paenochrobactrum* was more dominant at week 2 after application of IM (0.024%) and PMG (0.020%) in Alfisol compared to ORGH (0.000%) and control (0.000%).

Table 4.31: An Indicator Taxon Associated with CT, IM and PMG in Alfisol and Inceptisol and their Relative Abundance Values

S/N	CT, IM and PMG genus	ALF CT	INC CT	ALF IM	INC IM	ALF PMG	INC PMG	Significant Difference
1	*Solibacillus*	0.023%	0.032%	**0.068%**	0.033%	0.019%	0.038%	*

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr PMG = PrimextraGold, CT = Control

Table 4.32: Two (2) Indicator Taxa Associated with CT, ORGH and PMG in Alfisol and Inceptisol and their Relative Abundance Values

S/N	CT, ORGH and PMG genera	ALF CT	INC CT	ALF ORGH	INC ORGH	ALF PMG	INC PMG	Significant Difference
1	*Aquisphaera*	0.186%	0.218%	0.223%	**0.312%**	0.201%	**0.196%**	***
3	*Lechevalieria*	0.025%	0.030%	0.013%	0.024%	0.035%	0.043%	*

Legend: ALF = Alfisol, INC = Inceptisol, PMG = PrimextraGold, ORGH= Organic (Vinegar)herbicide, CT = Control

Table 4.33: Five (5) Indicator Taxa Associated with IM, ORGH and PMG in Alfisol and Inceptisol and their Relative Abundance Values

S/N	IM, ORGH and PMG **genera**	ALF CT	INC CT	ALF IM	INC IM	ALF ORGH	INC ORGH	ALF PMG	INC PMG	Significant Difference
1	*Candidatus_Nitrosotalea*	0.117%	0.140%	0.201%	0.063%	0.099%	0.066%	0.091%	0.411%	**
2	*Saccharopolyspora*	0.037%	0.047%	0.049%	0.026%	0.048%	0.071%	0.041%	0.076%	*
3	*Salipaludibacillus*	0.031%	0.010%	0.043%	0.023%	0.027%	0.010%	0.035%	0.006%	*
4	*Nitrosotaleaceae*	0.021%	0.015%	0.033%	0.002%	0.011%	0.010%	0.035%	0.042%	*
5	*Group_1.1c*	0.010%	0.027%	0.008%	0.021%	0.007%	0.023%	0.021%	0.052%	*

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr PMG = PrimextraGold, ORGH= Organic (Vinegar) herbicide, CT = Control

Table 4.34: Indicator Taxa Associated with Week 2 as Impacted by IM, PMG and ORGH in both Alfisol and Inceptisol

S/N	Indicator Taxa associated with week 2	ALF CT	ALF IM	ALF PMG	ALF ORGH	INC CT	INC IM	INC PMG	INC ORGH
1	*Paenochrobactrum*	0.004%	0.024%	0.020%	0.000%	0.000%	0.000%	0.000%	0.000%
2	*DSSD61*	0.002%	0.005%	0.019%	0.000%	0.000%	0.000%	0.000%	0.000%
3	*Azospirillum*	0.001%	0.000%	0.008%	0.008%	0.001%	0.000%	0.010%	0.000%
4	*Tundrisphaera*	0.001%	0.000%	0.009%	0.000%	0.001%	0.011%	0.000%	0.000%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar) herbicide, CT= Control

The indicator taxa strongly associated with week 4 were *YC-ZSS-LKJ147* and *Methylophilus*, however, it was observed that the relative abundance of *YC-ZSS-LKJ147* (0.007%) was higher in ORGH herbicide within Alfisol compared to other herbicide treatments as well as control (0.003%) but no significant difference was observed in Inceptisol when ORGH herbicide was applied compared to control (Table 4.35).

Thermaerobacter was the indicator taxon associated with week 6, however, its relative abundance was found to be very low in both Alfisol and Inceptisol in addition, the relative abundance of this indicator taxon was observed to experience highly negative shift as influenced by the condition of the soil when IM (0.000%), PMG (0.000%) and ORGH (0.000%) herbicides were applied in both soils (Table 4.36). Relative abundance of *Morganella* was observed to be higher in IM (0.054%) and PMG (0.023%) herbicides at week 8 within Alfisol compared to CT (0.011%). A reversed trend was observed in Inceptisol compared to Alfisol (Table 4.37).

Among the indicator taxa associated with week 0 and 2, the relative abundance of *Delftia* was higher in all the applied herbicide treatments – IM (0.120), PMG (0.131%) and ORGH (0.128) at week 2 compared to -IM (0.035%), PMG (0.047%) and ORGH (0.065%) at week 0 in Alfisol.Relative abundance of *Flavitalea* was lower in IM herbicide for both week 0 (0.013%) and 2 (0.009%) in Alfisol, which was different from the result observed in Inceptisol. Nevertheless, the relative abundance of *Flavitalea* was found to be higher in ORGH herbicide for week 0 (0.025%; 0.043%) and 2 (0.018%; 0.086%) in both Alfisol and Inceptisol respectively (Table 4.38).

Relative abundance of *Nitrosomonas*, an indicator taxon associated with weeks 0 and 4, was observed to be lower in PMG (0.000%) and ORGH (0.000%) herbicides at week 4 compared to PMG (0.014%) and ORGH (0.017%) at week 0 in Alfisol where it was observed to be highly dominant (Table 4.39). However, the relative abundance of *Nitrosomonas* was found to be higher in ORGH herbicide in Inceptisol at week 4 (0.004%) compared to week 0 (0.000%). *Turneriella* was the indicator taxon identified at week 2 and 4, however, it was observed that its relative abundance was lower in PMG (0.000%), IM (0.002%) and ORGH (0.000%) herbicides at week 2 however, it was observed to be highest in relative abundance

Table 4.35: Indicator Taxa Associated with Week 4 as Impacted by ORGH in both Alfisol and Inceptisol

S/N	Indicator Taxa associated with week4	ALF CT	ALF IM	ALF PMG	ALF ORGH	INC CT	INC IM	INC PMG	INC ORGH
1	*Methylophilus*	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
2	*YC-ZSS-LKJ147*	0.003%	0.000%	0.004%	0.007%	0.008%	0.008%	0.004%	0.007%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Isopropyl amine, PMG = PrimextraGold, ORGH= Organic (Vinegar)herbicide, CT = Control

Table 4.36: Indicator Taxa Associated with Week 6 as Impacted by all Herbicides Applied in both Alfisol and Inceptisol

S/N	Indicator Taxon associated with week 6	ALF CT	ALF IM	ALF PMG	ALF ORGH	INC CT	INC IM	INC PMG	INC ORGH
1	*Thermaerobacter*	0.001%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar)herbicide, CT = Control

Table 4.37: Indicator Taxa Associated with Week 8 as impacted by all herbicides applied in both Alfisol and Inceptisol

S/N	Indicator Taxa associated with week 8	ALF CT	ALF IM	ALF PMG	ALF ORGH	INC CT	INC IM	INC PMG	INC ORGH
1	*Morganella*	0.011%	0.054%	0.023%	0.000%	0.000%	0.000%	0.000%	0.000%
2	*Pseudaminobacter*	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
3	*Defluviicoccus*	0.001%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
4	*Catenuloplanes*	0.001%	0.000%	0.000%	0.000%	0.003%	0.000%	0.000%	0.000%
5	*Rhodovastum*	0.000%	0.000%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar)herbicide, CT = Control

Table 4.38: Indicator Taxa Associated with Week 0 and 2 as Impacted by all Herbicides Applied in both ALF and INC

	WK0								WK2							
	ALF	IM	PMG	ORGH	INC	ORGH	PMG	ORGH	ALF	IM	PMG	ORGH	INC	IM	PMG	ORGH
Delftia	0.044%	0.035%	0.047%	0.065%	0.003%	0.000%	0.000%	0.000%	0.044%	0.120%	0.131%	0.128%	0.003%	0.000%	0.053%	0.000%
Ahniella	0.021%	0.063%	0.012%	0.027%	0.011%	0.000%	0.011%	0.027%	0.021%	0.014%	0.040%	0.037%	0.011%	0.035%	0.009%	0.043%
Bosea	0.024%	0.082%	0.015%	0.045%	0.018%	0.000%	0.048%	0.070%	0.024%	0.035%	0.025%	0.026%	0.018%	0.008%	0.039%	0.057%
Dinghuibacter	0.028%	0.036%	0.095%	0.031%	0.008%	0.025%	0.000%	0.005%	0.028%	0.051%	0.013%	0.045%	0.008%	0.000%	0.060%	0.000%
Flavitalea	0.015%	0.013%	0.015%	0.025%	0.017%	0.019%	0.031%	0.043%	0.015%	0.009%	0.033%	0.018%	0.017%	0.022%	0.028%	0.086%
Rubellimicrobium	0.006%	0.004%	0.005%	0.012%	0.007%	0.000%	0.009%	0.024%	0.006%	0.015%	0.023%	0.011%	0.007%	0.020%	0.024%	0.000%
Minicystis	0.013%	0.070%	0.000%	0.000%	0.004%	0.000%	0.000%	0.000%	0.013%	0.036%	0.047%	0.067%	0.004%	0.000%	0.000%	0.000%
Providencia	0.011%	0.000%	0.056%	0.015%	0.000%	0.000%	0.000%	0.000%	0.011%	0.054%	0.023%	0.000%	0.000%	0.000%	0.000%	0.000%
Aeromonas	0.006%	0.018%	0.019%	0.007%	0.001%	0.014%	0.000%	0.000%	0.006%	0.021%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%
Comamonas	0.005%	0.000%	0.000%	0.013%	0.004%	0.000%	0.051%	0.000%	0.005%	0.012%	0.008%	0.000%	0.004%	0.000%	0.025%	0.026%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar)herbicide, CT = Control

Table 4.39: Indicator Taxa Associated with Week 0 and 4 as Impacted by all Herbicides Applied in both ALF and INC

	WK0								WK4							
	ALF	IM	PMG	ORGH	INC	IM	PMG	ORGH	ALF	IM	PMG	ORGH	INC	IM	PMG	ORGH
Nitrosomonas	0.006%	0.002%	0.014%	0.017%	0.001%	0.000%	0.000%	0.000%	0.006%	0.003%	0.000%	0.000%	0.001%	0.000%	0.000%	0.004%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr PMG = PrimextraGold, ORGH= Organic (Vinegar)herbicide, CT = Control

Table 4.40: Indicator Taxa Associated with Week 2 and 4 as Impacted by all Herbicides Applied in both ALF and INC

	WK2								WK4							
	ALF	IM	PMG	ORGH	INC	IM	PMG	ORGH	ALF	IM	PMG	ORGH	INC	IM	PMG	ORGH
Turneriella	0.003%	0.002%	0.000%	0.000%	0.001%	0.000%	0.000%	0.000%	0.003%	0.007%	0.006%	0.008%	0.001%	0.000%	0.000%	0.000%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar) herbicide, CT = Control

in ORGH (0.008%) herbicides at week 4 compared to PMG (0.006%) and IM. (0.007%) in Alfisol where it mostly dominated (Table 4.40).

Cutibacterium was the indicator taxon associated with week 2 and 8. It was observed that the relative abundance of *Cutibacterium* was higher in both IM (0.007%) and ORGH (0.007%) herbicides after application of all the herbicide treatments Alfisol compared Inceptisol at week 2. Conversely, the relative abundance of *Cutibacterium* was observed to be higher in PMG (0.017%) herbicide within Inceptisol compared to PMG (0.005%) within Alfisol at week 8 (Table 4.41). SM1A02, the indicator taxon associated with weeks 4 and 8 was observed to be higher in relative abundance in IM herbicide at week 4, in Alfisol (0.102%) and Inceptisol (0.090%), however, its relative abundance later dropped at week 8 (0.099%; 0.082%) in both soils (Table 4.42). Among the indicator taxa which were strongly associated with weeks 6 and 8, the relative abundance of *Enhydrobacter* (0.009%) and *Alkanindiges* (0.0000%) were lower in IM herbicide at week 6 in Alfisol. Relative abundance of *Enhydrobacter was* higher in ORGH (0.059%) and PMG (0.064%) herbicides in Alfisol.

Conversely, among the IM, PMG and ORGH herbicides applied, the relative abundance of *Enhydrobacter* was higher in ORGH (0.122%) herbicide compared to others whereas the relative abundance of *Alkanindiges* was observed to be high within IM (0.238%), PMG (0.164%) and ORGH (0.152%) herbicides at week 6, in Inceptisol. At week 8, the relative abundance of *Enhydrobacter* was higher in ORGH (0.045%) herbicides in Afisol compared to others (Table 4.43). They were all significantly different at ($P<0.05$).

4.13: Relative Abundance of Different Genera Found in Alfisol and Inceptisol as Influenced by Applied Chemical and Biological Herbicides After Different Time Point

Relative abundance of the genus *Candidatus udaeobacter,* belonging to the phylum *Verrucomicrobiot*a at week 2 after Imazapyr (IM) with active ingredient isopropyl amine and Primextragold (PMG) with active ingredient Atrazine + S metolachlor herbicide application was observed to decrease from 6.329 % (CT) to 5.111 % and 5.796 % respectively compared to Organic Herbicide (ORGH) with active ingredient acetic acid 6.351 % in Alfisol. The result was significantly different at $P < 0.05$ (according to ANOVA in R followed by Tukey's post-hoc test).

Table 4.41: Indicator Taxa Associated with Week 2 and 8 as Impacted by all Herbicides Applied in both Alfisol and Inceptisol

	WK2								WK8							
	ALF	IM	PMG	ORGH	INC	IM	PMG	ORGH	ALF	IM	PMG	ORGH	INC	IM	PMG	ORGH
Cutibacterium	0.003%	0.007%	0.005%	0.007%	0.002%	0.000%	0.000%	0.000%	0.003%	0.007%	0.005%	0.007%	0.002%	0.000%	0.017%	0.007%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar)herbicide, CT = Control

Table 4.42: Indicator Taxa Associated with Week 4 and 8 as Impacted by all Herbicides Applied in both Alfisol and Inceptisol

WK4									WK8							
	ALF	IM	PMG	ORGH	INC	IM	PMG	ORGH	ALF	IM	PMG	ORGH	INC	IM	PMG	ORGH
SM1A02	0.077%	0.102%	0.072%	0.088%	0.069%	0.090%	0.055%	0.094%	0.077%	0.099%	0.087%	0.080%	0.069%	0.082%	0.043%	0.032%

Legend: ALF = Alfisol, INC = Inceptisol, IM= Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar)herbicide, CT = Control

Table 4.43: Indicator Taxa Associated with Week 6 and 8 as Impacted by all Herbicides Applied in both Alfisol and Inceptisol

	WK6								WK8							
	ALF	IM	PMG	ORGH	INC	IM	PMG	ORGH	ALF	IM	PMG	ORGH	INC	IM	PMG	ORGH
Enhydrobacter	0.012%	0.009%	0.064%	0.059%	0.032%	0.111%	0.063%	0.122%	0.012%	0.004%	0.010%	0.045%	0.032%	0.038%	0.020%	0.006%
Alkanindiges	0.019%	0.000%	0.150%	0.071%	0.055%	0.238%	0.164%	0.152%	0.019%	0.006%	0.039%	0.000%	0.055%	0.060%	0.095%	0.030%
Phycicoccus	0.008%	0.010%	0.060%	0.023%	0.000%	0.000%	0.000%	0.000%	0.008%	0.013%	0.030%	0.012%	0.000%	0.000%	0.000%	0.000%
Verrucomicrobium	0.001%	0.004%	0.000%	0.006%	0.001%	0.000%	0.000%	0.025%	0.001%	0.004%	0.000%	0.009%	0.001%	0.000%	0.000%	0.000%

Legend: ALF = Alfisol, INC = Inceptisol, IM= Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar) herbicide, CT = Control

Pedosphaera and *Chthoniobacter* belonging to the same phylum also reduced in their relative abundance when IM and PMG herbicide were applied in Alfisol; from 0.678% (CT) to 0.569% (IM); 0.663% (PMG) and from 0.485% (CT) to 0.390% (IM) and 0.401% (PMG) after week 4 respectively while ORGH increased their relative abundance compared to others (0.964% and 0.571%) respectively. Decrease in relative abundance of the genus *AdurBin063-1* was consistently observed from (CT) 0.473 % to 0.230 % and 0.121 % at week 2 to 4 after application of IM herbicide respectively in Alfisol. Concomitantly, application of IM herbicide decreased the relative abundance of the genera *Candidatus xiphinematobacter* and *Luteolibacter* as observed at week 4 (0.107 %, 0.012 %) compared to week 2 (0.230 %, 0.123 %) respectively in Alfisol. (Table 4.44).

It is also imperative to mention that in Inceptisol, application of IM and PMG resulted to decrease in the relative abundance of the genera *Pedosphaera* from 0.691 % to 0.330 %; 0.280 % at week 2 and 0.512 %; 0.501% at week 4 but increased after ORGH (0.889%) was applied at week 4. Similarly, Chthoniobacter reduced from 0.339% (CT) to 0.344% (IM) and 0.261% (PMG) but increase after ORGH (0.523%) was applied at week 4. Relative abundance of the genus *Ellin516* also decreased in Inceptisol at week 2 after IM and PMG herbicide application from 0.219 % (CT) to 0.123 % and 0.180 % respectively.

Subsequently, consistent reduction in the relative abundance of the genus *Candidatus xiphinematobacter* from 0.215 % (CT) to 0.189 % and 0.166 % respectively were observed at weeks 2 and 4 after IM herbicide application in Inceptisol. Nevertheless, all were significantly different at $P<0.05$ (Table 4.44). Furthermore, relative abundance of the genus *Edaphobaculum* belonging to the phylum *Bacteroidota* decreased after application of PMG at week 2 and IM at week 4 from 0.214 % to 0.148 % and 0.146 % respectively. Relative abundance of the genus Puia decreased consistently as observed from week 2 to 4 after PMG application (0.079 % and 0.070 %) respectively compared to CT (0.108 %) whereas, PMG herbicide application significantly increased relative abundance of the genus *Edaphobaculum* which also belong to *Bacteroidota* at week 4 from (CT) 0.214 % to (PMG) 0.308 % after its reduction at week 2.

Table 4.44: Effects of Herbicides Treatment as Influenced by Weeks 2 and 4 on Relative Abundance of *Verrucomicrobiota* and *Proteobacteria* at Genus Level

P-Verrucomicrobiota;	**ALF TL AV**	**WK2 IM AV**	**WK2 PMG AV**	**WK2 ORGH AV**	**WK4 IM AV**	**WK4 PMG AV**	**WK4 ORGH AV**	**INC AV TL**	**WK2 IM AV**	**WK2 PMG AV**	**WK2 ORGH AV**	**WK4 IM AV**	**WK4 PMG AV**	**WK4 ORGH AV**
Candidatus_Udaeobacter	6.329%	5.111%	5.796%	6.351%	6.176%	6.275%	6.354%	6.768%	6.980%	5.916%	5.887%	7.253%	7.487%	8.034%
ADurb.Bin063-1	0.473%	0.230%	0.384%	0.566%	0.121%	0.452%	0.628%	0.618%	0.336%	0.416%	0.383%	0.666%	0.677%	0.775%
Candidatus_Xiphinematobacter	0.191%	0.230%	0.178%	0.219%	0.107%	0.179%	0.157%	0.215%	0.189%	0.309%	0.167%	0.166%	0.218%	0.200%
Chthoniobacter	0.485%	0.465%	0.451%	0.545%	0.390%	0.401%	0.571%	0.339%	0.445%	0.460%	0.361%	0.344%	0.261%	0.523%
Ellin516	0.242%	0.116%	0.184%	0.209%	0.310%	0.264%	0.477%	0.219%	0.123%	0.180%	0.222%	0.212%	0.292%	0.343%
Opitutus	0.090%	0.040%	0.097%	0.113%	0.145%	0.068%	0.072%	0.094%	0.042%	0.068%	0.060%	0.042%	0.085%	0.155%
Pedosphaera	0.678%	0.407%	0.329%	0.419%	0.569%	0.663%	0.964%	0.691%	0.330%	0.280%	0.277%	0.512%	0.501%	0.889%
LD29	0.052%	0.021%	0.041%	0.076%	0.022%	0.068%	0.023%	0.038%	0.024%	0.014%	0.050%	0.053%	0.064%	0.043%
S-BQ2-57_soil_group	0.041%	0.028%	0.050%	0.060%	0.029%	0.043%	0.073%	0.058%	0.050%	0.023%	0.045%	0.050%	0.046%	0.096%
Luteolibacter	0.055%	0.123%	0.047%	0.075%	0.012%	0.044%	0.042%	0.036%	0.069%	0.018%	0.049%	0.031%	0.022%	0.006%
p__Proteobacteria														
Acinetobacter	3.006%	2.014%	2.098%	2.906%	2.565%	1.824%	1.993%	2.291%	1.155%	1.125%	2.484%	1.088%	0.980%	2.693%
Sphingomonas	2.531%	2.291%	2.503%	3.779%	2.182%	2.264%	2905%	2.734%	2.733%	3.722%	4.950%	2.866%	2.934%	5.150%
Bradyrhizobium	2.008%	1.781%	1.885%	2.183%	1.907%	1.976%	1.983%	1.995%	2.024%	2.332%	2.013%	1.991%	2.055%	1.941%
mle1-7	0.832%	0.713%	0.761%	0.786%	1.141%	0.877%	0.774%	1.064%	1.286%	1.038%	1.179%	1.254%	1.398%	0.933%
Microvirga	1.187%	1.719%	1.211%	1.389%	1.412%	1.160%	0.992%	1.332%	1.823%	1.839%	2.053%	1.748%	1.488%	0.945%
Ellin6067	0.892%	1.109%	0.897%	1.134%	1.147%	0.813%	0.881%	0.768%	0.763%	0.903%	0.761%	0.966%	0.712%	0.822%
Pedomicrobium	1.073%	1.131%	1.011%	1.014%	1.376%	1.092%	0.843%	0.948%	1.151%	0.838%	0.940%	0.860%	0.995%	0.826%
Pseudomonas	0.866%	1.353%	0.989%	1.556%	0.329%	0.393%	0.420%	1.035%	0.571%	0.692%	0.863%	0.318%	0.388%	0.569%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar), herbicide CT = Control

The genus *Chryseobacterium* was also observed to consistently decrease in their relative abundance of from 0.097% to 0.062% and 0.033% at week 2 to 4 respectively after application of PMG herbicide in Alfisol (Table 4.45). Similar result observed in Alfisol with respect to decrease in the relative abundance of the genus *Puia* at week 4 after application of PMG from (CT) 0.132 % to 0.104 % was also observed in Inceptisol. Relative abundance of the genus *Edaphobaculum* decreased at week 4 (0.067 %) in Inceptisol after application of PMG herbicide compared to week 2 (0.212%) which was different from the result observed in Alfisol. All were significantly different at $P<0.05$ (Table 4.45).

Application of ORGH herbicide increased the relative abundance of the genus *Candidatus udaeobacter* belonging to the phylum *Verrucomicrobiota* at week 4 (6.354%) and week 6 (8.995%) compared to the relative abundance of the same genus when IM and PMG herbicides were applied in Alfisol respectively at week 4 (6.176 %, and 6.275 %) and week 6 (7.902% and 8.099%). Similar trend was observed in the relative abundance of the genus, *Pedosphaera* at week 4 to 6 after the same herbicides were applied in Alfisol. They are all significantly different at ($P<0.05$).

Concomitantly, the genus *AdurBin063-1* was observed to increase in relative abundance after application of IM at week 6 (0.433%) compared to weeks 2 (0.230%) and 4 (0.121%) in Alfisol. Relative abundance of the genera *Candidatus xiphinematobacter* and *Luteolibacter* were observed to maintained their decreasing trend as seen in week 2 (0.230 %,0.123 %) to week 4 (0.107%, 0.012 %) up to 6 (0.166%, 0.007 %) respectively after application of IM herbicide in Alfisol (Table 4.45 and 4.46).

Table 4.45: Effects of Herbicides Treatment as Influenced by Weeks 2 and 4 on Relative Abundance of *Verrucomicrobiota* and *Bacteroidota* at Genus Level

P-Verrucomicrobiota;	**ALF TL AV**	**WK2 IM AV**	**WK2 PMG AV**	**WK2 ORGH AV**	**WK4 IM AV**	**WK4 PMG AV**	**WK4 ORGH AV**	**INC AV TL**	**WK2 IM AV**	**WK2 PMG AV**	**WK2 ORGH AV**	**WK4 IM AV**	**WK4 PMG AV**	**WK4 ORGH AV**
Candidatus_Udaeobacter	6.329%	5.111%	5.796%	6.351%	6.176%	6.275%	6.354%	6.768%	6.980%	5.916%	5.887%	7.253%	7.487%	8.034%
ADurb.Bin063-1	0.473%	0.230%	0.384%	0.566%	0.121%	0.452%	0.628%	0.618%	0.336%	0.416%	0.383%	0.666%	0.677%	0.775%
Candidatus_Xiphinematobacter	0.191%	0.230%	0.178%	0.219%	0.107%	0.179%	0.157%	0.215%	0.189%	0.309%	0.167%	0.166%	0.252%	0.200%
Chthoniobacter	0.485%	0.465%	0.451%	0.545%	0.390%	0.401%	0.571%	0.339%	0.445%	0.460%	0.361%	0.344%	0.261%	0.523%
Ellin516	0.242%	0.116%	0.184%	0.209%	0.310%	0.264%	0.477%	0.219%	0.123%	0.180%	0.222%	0.212%	0.292%	0.343%
Opitutus	0.090%	0.040%	0.097%	0.113%	0.145%	0.068%	0.072%	0.094%	0.042%	0.068%	0.060%	0.042%	0.085%	0.155%
Pedosphaera	0.678%	0.407%	0.329%	0.419%	0.569%	0.663%	0.964%	0.691%	0.330%	0.280%	0.277%	0.512%	0.501%	0.889%
LD29	0.052%	0.021%	0.041%	0.076%	0.022%	0.068%	0.023%	0.038%	0.024%	0.014%	0.050%	0.053%	0.064%	0.043%
S-BQ2-57_soil_group	0.041%	0.028%	0.050%	0.060%	0.029%	0.043%	0.073%	0.058%	0.050%	0.023%	0.045%	0.050%	0.046%	0.096%
Luteolibacter	0.055%	0.123%	0.047%	0.075%	0.012%	0.044%	0.042%	0.036%	0.069%	0.018%	0.049%	0.031%	0.022%	0.006%
p__Bacteroidota														
Flavobacterium	0.718%	0.772%	0.624%	0.849%	0.530%	0.742%	0.754%	0.389%	0.508%	0.686%	0.315%	0.317%	0.319%	0.311%
Terrimonas	0.242%	0.218%	0.400%	0.192%	0.318%	0.277%	0.314%	0.251%	0.339%	0.504%	0.303%	0.279%	0.243%	0.233%
Adhaeribacter	0.177%	0.150%	0.157%	0.194%	0.167%	0.207%	0.279%	0.108%	0.113%	0.188%	0.076%	0.122%	0.112%	0.136%
Puia	0.108%	0.110%	0.079%	0.110%	0.102%	0.070%	0.054%	0.132%	0.080%	0.188%	0.128%	0.140%	0.104%	0.262%
Chryseolinea	0.160%	0.322%	0.255%	0.171%	0.154%	0.096%	0.174%	0.092%	0.126%	0.106%	0.145%	0.098%	0.107%	0.045%
AKYH767	0.304%	0.390%	0.287%	0.227%	0.240%	0.308%	0.357%	0.227%	0.293%	0.274%	0.161%	0.204%	0.285%	0.192%
Flavisolibacter	0.148%	0.116%	0.206%	0.181%	0.143%	0.153%	0.241%	0.171%	0.242%	0.352%	0.229%	0.237%	0.174%	0.214%
Edaphobaculum	0.214%	0.210%	0.148%	0.157%	0.146%	0.308%	0.263%	0.123%	0.066%	0.212%	0.061%	0.141%	0.067%	0.150%
Parafilimonas	0.073%	0.070%	0.070%	0.091%	0.075%	0.096%	0.060%	0.068%	0.099%	0.060%	0.139%	0.122%	0.058%	0.046%
Chryseobacterium	0.097%	0.096%	0.062%	0.530%	0.000%	0.033%	0.081%	0.023%	0.026%	0.070%	0.013%	0.007%	0.000%	0.000%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar), herbicide CT = Control

Table 4.46: Effects of Herbicides Treatment as Influenced by Weeks 2 and 6 on Relative Abundance of *Verrucomicrobiota* and *Actinobacteriota* at Genus Level

P-Verrucomicrobiota;	**ALF TL AV**	**WK2 IM AV**	**WK2 PMG AV**	**WK2 ORGH AV**	**WK6 IM AV**	**WK6 PMG AV**	**WK6 ORGH AV**	**INC TL AV**	**WK2 IM AV**	**WK2 PMG AV**	**WK2 ORGH AV**	**WK6 IM AV**	**WK6 PMG AV**	**WK6 ORGH AV**
Candidatus_Udaeobacte	6.329%	5.111%	5.796%	6.351%	7.902%	8.099%	8.995%	6.768%	6.980%	5.916%	5.047%	7.120%	7.742%	8.985%
ADurb.Bin063-1	0.473%	0.230%	0.384%	0.566%	0.433%	0.486%	0.445%	0.618%	0.336%	0.416%	0.383%	0.271%	0.962%	0.890%
Candidatus_Xiphinematobacter	0.191%	0.230%	0.178%	0.219%	0.166%	0.168%	0.196%	0.215%	0.189%	0.309%	0.167%	0.200%	0.213%	0.284%
Chthoniobacter	0.485%	0.465%	0.451%	0.545%	0.456%	0.272%	0.374%	0.339%	0.445%	0.460%	0.361%	0.182%	0.135%	0.542%
Ellin516	0.242%	0.116%	0.184%	0.209%	0.287%	0.247%	0.263%	0.219%	0.123%	0.180%	0.222%	0.150%	0.271%	0.301%
Opitutus	0.090%	0.040%	0.097%	0.113%	0.017%	0.049%	0.101%	0.094%	0.042%	0.068%	0.060%	0.102%	0.067%	0.140%
Pedosphaera	0.678%	0.407%	0.329%	0.419%	0.648%	0.608%	0.737%	0.691%	0.330%	0.280%	0.277%	0.793%	0.856%	1.051%
LD29	0.052%	0.021%	0.041%	0.076%	0.093%	0.079%	0.086%	0.038%	0.024%	0.014%	0.050%	0.087%	0.000%	0.030%
S-BQ2-57_soil_group	0.041%	0.028%	0.050%	0.060%	0.065%	0.046%	0.056%	0.058%	0.050%	0.023%	0.045%	0.086%	0.011%	0.077%
Luteolibacter	0.055%	0.123%	0.047%	0.075%	0.007%	0.047%	0.044%	0.036%	0.069%	0.018%	0.049%	0.013%	0.014%	0.011%
p__Actinobacteriota														
Solirubrobacter	1.233%	1.335%	1.217%	1.606%	1.260%	1.068%	0.919%	0.992%	1.112%	0.984%	1.302%	1.078%	1.049%	0.776%
Mycobacterium	1.289%	1.309%	1.210%	1.468%	1.292%	1.395%	1.323%	1.263%	1.390%	1.702%	1.878%	1.067%	1.267%	0.977%
Streptomyces	1.195%	1.128%	1.142%	1.290%	0.885%	1.168%	1.102%	1.351%	1.110%	1.478%	1.707%	1.141%	1.219%	1.586%
Nocardioides	1.637%	1.260%	1.254%	1.523%	1.596%	1.832%	1.734%	1.247%	1.347%	1.342%	2.074%	1.690%	1.110%	1.086%
MB-A2-108	1.547%	1.191%	1.118%	1.428%	1.816%	1.709%	1.894%	2.059%	1.891%	1.638%	1.636%	2.135%	2.219%	1.912%
IMCC26256	1.724%	1.465%	1.458%	1.679%	1.889%	1.820%	2.140%	1.801%	2.032%	1.922%	1.929%	1.660%	1.634%	1.737%
Acidothermus	0.478%	0.398%	0.463%	0.896%	0.344%	0.528%	0.535%	0.603%	0.385%	0.407%	0.599%	0.592%	0.709%	0.913%
Microlunatus	0.502%	0.674%	0.255%	0.546%	0.336%	0.414%	0.455%	0.294%	0.471%	0.304%	0.385%	0.293%	0.105%	0.242%
Ilumatobacter	0.205%	0.218%	0.232%	0.089%	0.247%	0.211%	0.148%	0.149%	0.178%	0.251%	0.188%	0.142%	0.062%	0.076%
Luedemannella	0.324%	0.259%	0.249%	0.334%	0.460%	0.187%	0.336%	0.161%	0.072%	0.263%	0.000%	0.362%	0.043%	0.192%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar), herbicide CT = Control

Application of IM resulted to decrease in the relative abundance of the genus *Ellin516* of the phylum *Verrucomicrobiota* from 0.219% to 0.150% as observed at week 6 in Inceptisol. Similarly, relative abundance of the genus *Luteolibacter* was observed to decrease after IM application at week 6 (0.013 %) compared to week 2 (0.069%) in Inceptisol. Interestingly, the genus *Pedosphaera* increased progressively in its relative abundance from week 4 (0.889%) to 6 (1.051%) after application of ORGH compared to week 2 (0.277%). (Table 4.45 and 4.46).

In Alfisol, the relative abundance of the genus *Streptomyces* which belong to the phylum *Actinobacteria* was observed to decrease at week 6 (0.885 %) after IM application compared to week 2 (1.128 %) as well as control (1.195 %). However, the genus *Microlunatus* of the same phylum decreased in its relative abundance at week 2 (0.255%) and week 6 (0.414 %) after application of PMG compared to ORGH (0.546%; 0.455%). The same genus also decreased in its relative abundance at week 6 (0.336 %) after application of IM compared to ORGH (0.455%). Interestingly, application of ORGH was also observed to facilitate increase in the relative abundance of genus *Acidothermus* at week 2 (0.896%) compared to control (0.478%). Relative abundance of the genus Ilumatobacter increased at week 6 (0.148 %) compared to week 2 (0.089 %) after application of the ORGH herbicides (Table 4.46). In Inceptisol, application of IM reduced the relative abundance of the genus *Acidothermus* at week 2 (0.385 %) compared to control (0.603%). Conversely, relative abundance of the genera *Ilumatobacter* decreased after application of PMG at week 6 (0.062 %,) compared to control CT (0.149 %) Table 4.46.

Candidatus udaeobacter which belong to the phylum *Verrucomicrobiota* consistently maintained a stable increase in their relative abundance from week 2 (6.351 %) to week 8 (6.692 %) after ORGH herbicides application in Alfisol respectively. For the genus *AdurBin063-1*, its relative abundance was also observed to increase after ORGH herbicide application at week 8 (0.763%) compared to week 2 (0.566 %) and control (0.473 %). Relative abundance of *Pedosphaer*a also reduced at week 8 after application of IM (0.548%) and PMG (0.819%) compared to ORGH (0.928%). Relative abundance of the genus *Luteolibacter* also decreased after IM herbicide application, at week 8 (0.054 %,) compared to week 2 (0.123 %) in Alfisol (Table 4.47).

Table 4.47: Effects of Herbicides Treatment as Influenced by Weeks 2 and 8 on Relative Abundance of *Verrucomicrobiota* and *Proteobacteria* at Genus Level

P-Verrucomicrobiota;	ALF TL AV	WK2 IM AV	WK2 PMG AV	WK2 ORGH AV	WK8 IM AV	WK 8 PMG AV	WK8 ORGH AV	INC TL AV	WK2 IM AV	WK2 PMG AV	WK2 ORGH AV	WK8 IM AV	WK 8 PMG AV	WK8 ORGH AV
Candidatus_Udaeobacte	6.329%	5.111%	5.796%	6.351%	7.165%	8.122%	6.692%	6.768%	6.980%	5.916%	5.047%	7.418%	8.638%	6.970%
ADurb.Bin063-1	0.473%	0.230%	0.384%	0.566%	0.456%	0.756%	0.763%	0.618%	0.336%	0.416%	0.383%	0.527%	0.982%	0.797%
Candidatus_Xiphinematobacter	0.191%	0.230%	0.178%	0.219%	0.160%	0.244%	0.211%	0.215%	0.189%	0.309%	0.167%	0.265%	0.220%	0.222%
Chthoniobacter	0.485%	0.465%	0.451%	0.545%	0.459%	0.464%	0.647%	0.339%	0.445%	0.460%	0.361%	0.377%	0.274%	0.453%
Ellin516	0.242%	0.116%	0.184%	0.209%	0.214%	0.252%	0.222%	0.219%	0.123%	0.180%	0.222%	0.077%	0.200%	0.220%
Opitutus	0.090%	0.040%	0.097%	0.113%	0.066%	0.067%	0.141%	0.094%	0.042%	0.068%	0.060%	0.065%	0.100%	0.084%
Pedosphaera	0.678%	0.407%	0.329%	0.419%	0.548%	0.819%	0.928%	0.691%	0.330%	0.280%	0.277%	0.968%	1.266%	1.341%
LD29	0.052%	0.021%	0.041%	0.076%	0.053%	0.026%	0.067%	0.038%	0.024%	0.014%	0.050%	0.000%	0.065%	0.016%
S-BQ2-57_soil_group	0.041%	0.028%	0.050%	0.060%	0.038%	0.040%	0.050%	0.058%	0.050%	0.023%	0.045%	0.066%	0.093%	0.091%
Luteolibacter	0.055%	0.123%	0.047%	0.075%	0.054%	0.025%	0.047%	0.036%	0.069%	0.018%	0.049%	0.027%	0.018%	0.000%
p__Proteobacteria														
Acinetobacter	3.006%	2.014%	2.098%	1.906%	2.354%	0.858%	1.030%	2.291%	4.155%	3.125%	0.584%	1.292%	0.674%	1.268%
Methyloligellaceae	1.469%	1.140%	1.168%	0.922%	1.821%	1.748%	1.851%	1.409%	1.454%	1.346%	1.424%	1.740%	1.302%	0.961%
Sphingomonas	2.531%	2.291%	3.503%	3.139%	2.049%	2.131%	1.641%	2.734%	2.733%	3.722%	4.950%	2.017%	1.715%	1.180%
Bradyrhizobium	2.008%	1.781%	1.885%	2.183%	1.633%	1.615%	1.925%	1.995%	2.024%	2.332%	2.013%	1.542%	1.523%	1.833%
Steroidobacter	0.636%	0.839%	0.692%	0.480%	0.756%	0.582%	0.505%	0.478%	0.570%	0.521%	0.567%	0.489%	0.269%	0.308%
mle1-7	0.832%	0.713%	0.761%	0.786%	0.827%	0.888%	0.834%	1.064%	1.286%	1.038%	1.179%	1.429%	0.961%	0.677%
Microvirga	1.187%	1.719%	1.211%	1.389%	1.071%	0.999%	0.713%	1.332%	1.823%	1.839%	2.053%	1.346%	0.621%	0.597%
Ellin6067	0.892%	1.109%	0.897%	1.134%	0.568%	0.583%	0.765%	0.768%	0.763%	0.903%	0.761%	0.679%	0.636%	0.857%
Pedomicrobium	1.073%	1.131%	1.011%	1.014%	1.094%	1.034%	0.864%	0.948%	1.151%	0.838%	0.940%	1.012%	1.010%	0.599%
Pseudomonas	0.866%	1.353%	0.989%	1.556%	0.425%	0.334%	0.293%	1.035%	0.571%	0.692%	0.863%	0.431%	0.392%	0.404%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar), herbicide CT = Control

Relative abundance of the genera *Candidatus udaeobacter* was observed to increase after application of IM and PMG at week 8 (7.418 %, 8.638%) respectively compared to week 2 (6.980 %, 5.916 %) in Inceptisol. Relative abundance of *Pedosphaera* also reduced at week 8 after application of IM (0.968%) and PMG (1.266%) compared to ORGH (1.341%) in Inceptisol. They were all significantly different at ($P<0.05$). It is pertinent to note that the genus *Acinetobacter* which belong to the phylum *Proteobacteria* decreased in its relative abundance at week 2 (2.098 %) and 8 (0.858 %) after PMG application compared to control (3.006 %) in Alfisol (Table 4.47). Conversely, relative abundance of the genus *Ellin6067* increased at week 2 (1.109 %) after IM herbicide application compared to control (0.892 %) but decreased at week 8 (0.568 %) compared to week 2 in Alfisol. The genus *Pseudomonas* decreased in its relative abundance after application of PMG (0.989 %) compared to ORGH (1.556%) at week 2 in Alfisol. On the other hand, relative abundance of the genus *Acinetobacter* increased after IM and PMG application at week 2 (4.155 % and 3.125 %) compared to control (2.291 %) but decreased geometrically at week 8 (1.292 % and 0.674 %) compared to week 2 in Inceptisol. Relative abundance of the genus *Ellin6067* also decreased after IM and PMG application at week 8 (0.679 % and 0.636 %) compared to control (0.768 %) whereas relative abundance of the genus *Pseudomonas* decreased consistently at weeks 2 (0.571 and %,0.692 %) and 8 (0.431 %, and 0.392%) after IM and PMG application respectively compared to control (1.035 %) in Inceptisol. All were significantly different at $P<0.05$ (Table 4.47).

It was observed that the relative abundance of the genera *Acinetobacter* and *Sphingomonas* belonging to the phylum *Proteobacteria* decreased after IM application at week 4 (2.565 % and 2.182 %) compared to control (3.006 % and 2.531 %). Relative abundance of the genus *Bradyrhizobium* consistently decreased from week 4 (1.907 %) to 6 (1.655 %) after application of IM herbicide compared to ORGH at week 4 (1.983%) and week 6 (2.042 %) whereas relative abundance of the genus *Pseudomonas* decreased from week 4 (0.393 %) to 6 (0.327 %) after PMG herbicide application compared to ORGH (0.420%; 0.407 %) in Alfisol (Table 4.58). In Inceptisol, the genus *Acinetobacter* decrease in its relative abundance after PMG herbicides application at week 4 (0.980 %) compared to control (2.693 %). The genus *Sphingomonas* also reduced in its relative abundance at weeks 4 (2.866 %) after IM herbicide application compared to ORGH herbicide 5.150 % (Table 4.48).

Table 4.48: Effects of Herbicides Treatment as Influenced by Weeks 4 and 6 on Relative Abundance of *Proteobacteria* and *Myxococcota* at Genus Level

p__Proteobacteria	ALF TL AV	WK4 IM AV	WK4 PMG AV	WK4 ORGH AV	WK6 IM AV	WK6 PMG AV	WK6 ORGH AV	INC TL AV	WK4 IM AV	WK4 PMG AV	WK4 ORGH AV	WK6 IM AV	WK6 PMG AV	WK6 ORGH AV
Acinetobacter	3.006%	2.565%	1.824%	1.563%	5.187%	1.036%	2.082%	2.291%	1.088%	0.980%	2.693%	1.121%	8.124%	1.132%
Methyloligellaceae	1.469%	1.642%	1.509%	1.325%	1.621%	1.526%	1.799%	1.409%	1.737%	1.536%	1.354%	1.463%	1.171%	1.345%
Sphingomonas	2.531%	2.182%	2.264%	1.905%	5.007%	4.738%	1.968%	2.734%	2.866%	2.934%	5.150%	4.909%	1.846%	1.546%
Bradyrhizobium	2.008%	1.907%	1.976%	1.983%	1.655%	1.809%	2.042%	1.995%	1.991%	2.055%	1.941%	1.887%	2.011%	2.017%
Steroidobacter	0.636%	1.058%	0.598%	0.586%	0.578%	0.563%	0.485%	0.478%	0.558%	0.495%	0.322%	0.541%	0.279%	0.393%
mle1-7	0.832%	1.141%	0.877%	0.774%	0.690%	0.806%	0.807%	1.064%	1.254%	1.398%	0.933%	1.185%	1.091%	0.699%
Microvirga	1.187%	1.412%	1.160%	0.992%	0.982%	0.823%	0.931%	1.332%	1.748%	1.488%	0.945%	1.560%	0.403%	0.799%
Ellin6067	0.892%	1.147%	0.813%	0.881%	0.694%	0.715%	0.994%	0.768%	0.966%	0.712%	0.822%	0.420%	0.870%	0.908%
Pedomicrobium	1.073%	1.376%	1.092%	0.843%	0.955%	1.185%	1.037%	0.948%	0.860%	0.995%	0.826%	1.089%	0.797%	0.840%
Pseudomonas	0.866%	0.329%	0.393%	0.420%	3.154%	0.327%	0.407%	1.035%	0.318%	0.388%	0.269%	0.393%	0.936%	0.842%
p__Myxococcota														
bacteriap25	1.843%	2.183%	2.215%	1.924%	1.920%	2.022%	1.680%	1.586%	1.709%	1.631%	1.553%	1.649%	1.276%	1.592%
Haliangium	1.510%	1.599%	1.181%	1.226%	1.278%	1.079%	1.504%	1.250%	1.287%	1.170%	1.136%	0.920%	1.254%	1.229%
Anaeromyxobacter	0.656%	0.620%	0.631%	0.759%	0.481%	0.677%	0.761%	0.574%	0.592%	0.862%	0.551%	0.455%	1.102%	0.840%
Phaselicystis	0.189%	0.296%	0.253%	0.216%	0.105%	0.185%	0.217%	0.189%	0.172%	0.196%	0.192%	0.102%	0.171%	0.195%
Polyangium	0.076%	0.055%	0.115%	0.045%	0.034%	0.072%	0.117%	0.065%	0.256%	0.036%	0.000%	0.033%	0.000%	0.000%
Pajaroellobacter	0.328%	0.222%	0.129%	0.435%	0.288%	0.250%	0.397%	0.234%	0.262%	0.267%	0.202%	0.221%	0.273%	0.290%
Sorangium	0.068%	0.095%	0.077%	0.016%	0.071%	0.040%	0.031%	0.035%	0.030%	0.038%	0.038%	0.024%	0.000%	0.082%
BIrii41	0.268%	0.318%	0.200%	0.280%	0.138%	0.234%	0.225%	0.165%	0.095%	0.173%	0.233%	0.056%	0.148%	0.266%
Blfdi19	0.087%	0.045%	0.090%	0.129%	0.053%	0.059%	0.080%	0.097%	0.068%	0.092%	0.052%	0.078%	0.117%	0.078%
mle1-27	0.059%	0.058%	0.110%	0.044%	0.032%	0.034%	0.048%	0.022%	0.026%	0.019%	0.015%	0.024%	0.033%	0.024%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar), herbicide CT = Control

Most interestingly, the relative abundance of the genera *Polyangium and Mle1-27* belonging to the phylum *Myxococcota* increased at week 4 (0.115 % and 0.110 %) after PMG application compared to control (0.076% and 0.059%) with a sharp decrease in their relative abundance observed at week 6 (0.034 % and 0.032 %) compared to week 4 in Alfisol. Nevertheless, relative abundance of the genus *Polyangium* increased at week 4 (0.256 %) after IM application compared to control (0.065 %) but a rapid decrease was observed at week 6 (0.033 %) after application of the same herbicide treatment in Inceptisol. Conversely, the genus *Polyangium* decreased in its relative abundance at week 4 (0.036 % and 0.000 %) and 6(0.000 % and 0.000 %) after PMG and ORGH herbicides application respectively compared to control (0.065%) in Inceptisol (Table 4.48).

Increase in relative abundance of the genus *Bacteriap25* which belong to the phylum *Myxococcota* was observed after IM and PMG herbicides application at week 4 (2.183 % and 2.215 %) compared to control (1.843 %), however, this change in relative abundance of the genus *Bacteriap25* later dropped at week 8 (1.738 %and 1.981 %) compared to week 4 in Alfisol. Interestingly, the genera *Haliangium*, *Polyangium* and *Mle 1-27* also belonging to the phylum *Myxococcota* decrease in their relative abundance at week 8 (0.986 %, 0.072 % and 0.036 %) after PMG herbicide application compared to week 4 (1.181 %, 0.115 % and 0.110 %) respectively, whereas, the genus *Pajaroellobacte*r increased in its relative abundance at week 8 (0.399 %) after PMG herbicide application compared to compared to week 4 (0.129 %) in Alfisol (Table 4.59). In Inceptisol, the relative abundance of the genus *Bacteriap25* increased at week 8 (2.108 %) after IM herbicide application compared to week 4 (1.709%) as well as control (1.586 %). Conversely, relative abundance of the genus *Haliangium* decreased after IM and ORGH herbicides application at week 8 (1.034 % and 1.007 %) compared to control (1.250 %) Table 4.49.

Relative abundance of the genus *LWQ8* which belong to the phylum *Patescibacteria* decreased at week 4 (0.046 %) and 8 (0.043 %) after PMG application in Alfisol. It was also observed that the relative abundance of the genera *TM7a* and *TM7* belonging to the phylum *Patescibacteria* decreased at week 4 (0.000 % and 0.008 %) after IM application (Table 4.49). In Inceptisol, relative abundance of *LWQ8* decreased at week 8 (0.040%, 0.042 %

and 0.027 %) after IM, PMG and ORGH herbicides application compared to week 4 (0.134 %,0. 107 % and 0.180 %) as well as control (0.105 %). Relative abundance of *TM7a* and *TM7* also decreased at week 8 after PMG (0.000 % and 0.012 %) and ORGH (0.005 % and 0.000 %) herbicide application compared to control in Inceptisol (Table 4.49). They were all significantly different at ($P<0.05$).

It was Conspicuously observed that the relative abundance of the genera *Acinetobacter, Sphingomonas* and *Pseudomonas* belonging to the phylum Proteobacteria decreases at week 8 (2.354 %,2.049 % and 0.425 %) after IM herbicide application compared to control (3.006 %, 2.531 % and 0.866 %). Concurrently, at week 6 (1.036 % and 0.327 %) and 8 (0.858% and 0.334%) after PMG herbicide application, the relative abundance of the genera *Acinetobacter* and *Pseudomonas* consistently decreased descendingly compared to control (3.006 % and 0.866 %) in Alfisol (Table 4.50).

Concomitantly, relative abundance of *Acinetobacter* decreased at week 8 (0.674 %) after PMG herbicide application in Inceptisol whereas, the relative abundance of the genus *Pseudomonas* decreased consistently from week 6 (0.393 %; 0.936 %) to 8 (0.431 %; 0.392 %) respectively after IM and PMG herbicide application compared to control (1.035 %). It was also interesting to observe that the relative abundance of the genus *Ammoniphilus,* belonging to the phylum *Firmicutes* decreased at week 6 (0.388 %) after IM herbicide application compared to control (0.812 %) in Alfisol. Similar trend was also observed in the relative abundance of the genera *Lysinibacillus, Turicibacter* and *Melghirimyces* also belonging to the phylum Firmicutes, when IM herbicide was applied.

Relative abundance of the genera *Pseudogracibacillus* and *Turicibacter* also decreased at week 8 (0.019 % and 0.008 %) after PMG herbicide application respectively, compared to control (0.082 % and 0.038 %) in Alfisol (Table 4.60). Conversely, relative abundance of the genus *Turicibacteria* was observed to increase at week 8 (0.022 % and 0.011 %) after IM and PMG herbicide application, compared to week 6 (0.000 % and 0.000 %) and control (0.004 %) in Inceptisol. (Table 4.50). They were all significantly different at ($P<0.05$).

Table 4.49: Effects of Herbicides Treatment as Influenced by Weeks 4 and 8 on Relative Abundance of *Myxococcota* and *Patescibacteria* at Genus Level

p__Myxococcota	ALF TL AV	WK4 IM AV	WK4 PMG AV	WK4 ORGH AV	WK8 IM AV	WK 8 PMG AV	WK8 ORGH AV	INC TL AV	WK4 IM AV	WK4 PMG AV	WK4 ORGH AV	WK8 IM AV	WK 8 PMG AV	WK8 ORGH AV
bacteriap25	1.843%	2.183%	2.215%	1.924%	1.738%	1.981%	1.733%	1.586%	1.709%	1.631%	1.553%	2.108%	1.889%	1.769%
Haliangium	1.510%	1.599%	1.181%	1.226%	1.310%	0.986%	1.621%	1.250%	1.287%	1.170%	1.136%	1.034%	1.672%	1.007%
Anaeromyxobacter	0.656%	0.620%	0.631%	0.759%	0.596%	0.713%	0.948%	0.574%	0.592%	0.862%	0.551%	0.462%	0.966%	0.793%
Phaselicystis	0.189%	0.296%	0.253%	0.216%	0.163%	0.211%	0.222%	0.189%	0.172%	0.196%	0.192%	0.184%	0.259%	0.171%
Polyangium	0.076%	0.055%	0.115%	0.045%	0.085%	0.072%	0.067%	0.065%	0.256%	0.036%	0.000%	0.000%	0.066%	0.000%
Pajaroellobacter	0.328%	0.222%	0.129%	0.435%	0.138%	0.399%	0.311%	0.234%	0.262%	0.267%	0.202%	0.222%	0.278%	0.166%
Sorangium	0.068%	0.095%	0.077%	0.016%	0.032%	0.042%	0.000%	0.035%	0.030%	0.038%	0.038%	0.000%	0.052%	0.000%
BIrii41	0.268%	0.318%	0.200%	0.280%	0.223%	0.185%	0.305%	0.165%	0.095%	0.173%	0.233%	0.223%	0.172%	0.195%
Blfdi19	0.087%	0.045%	0.090%	0.129%	0.119%	0.093%	0.070%	0.097%	0.068%	0.092%	0.052%	0.092%	0.047%	0.046%
mle1-27	0.059%	0.058%	0.110%	0.044%	0.072%	0.036%	0.042%	0.022%	0.026%	0.019%	0.015%	0.005%	0.013%	0.030%
p__Patescibacteria														
LWQ8	0.142%	0.105%	0.046%	0.143%	0.168%	0.043%	0.062%	0.105%	0.134%	0.107%	0.180%	0.040%	0.042%	0.027%
TM7a	0.026%	0.000%	0.062%	0.025%	0.017%	0.000%	0.000%	0.033%	0.015%	0.029%	0.046%	0.064%	0.000%	0.005%
Saccharimonadales	0.135%	0.168%	0.063%	0.139%	0.170%	0.060%	0.066%	0.152%	0.166%	0.166%	0.122%	0.100%	0.099%	0.057%
WWH38	0.081%	0.036%	0.034%	0.099%	0.100%	0.032%	0.068%	0.147%	0.071%	0.217%	0.210%	0.155%	0.214%	0.067%
TM7	0.050%	0.008%	0.066%	0.056%	0.033%	0.053%	0.053%	0.025%	0.019%	0.045%	0.033%	0.014%	0.012%	0.000%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar), herbicide CT = Control

Table 4.50: Effects of Herbicides Treatment as Influenced by Weeks 6 and 8 on Relative Abundance of *Proteobacteria* and *Firmicutes* at Genus Level

p__Proteobacteria	**ALF TL AV**	**WK6 IM AV**	**WK6 PMG AV**	**WK6 ORGH AV**	**WK8 IM AV**	**WK 8 PMG AV**	**WK8 ORGH AV**	**INC TL AV**	**WK6 IM AV**	**WK6 PMG AV**	**WK6 ORGH AV**	**WK8 IM AV**	**WK 8 PMG AV**	**WK8 ORGH AV**
Acinetobacter	3.006%	5.187%	1.036%	2.082%	2.354%	0.858%	1.030%	2.291%	1.121%	8.124%	1.132%	1.292%	0.674%	1.268%
Methyloligellaceae	1.469%	1.621%	1.526%	1.799%	1.821%	1.748%	1.851%	1.409%	1.463%	1.171%	1.345%	1.740%	1.302%	0.961%
Sphingomonas	2.531%	5.007%	4.738%	1.968%	2.049%	2.131%	1.641%	2.734%	4.909%	1.846%	1.546%	2.017%	1.715%	1.180%
Bradyrhizobium	2.008%	1.655%	1.809%	2.042%	1.633%	1.615%	1.925%	1.995%	1.887%	2.011%	2.017%	1.542%	1.523%	1.833%
Steroidobacter	0.636%	0.578%	0.563%	0.485%	0.756%	0.582%	0.505%	0.478%	0.541%	0.279%	0.393%	0.489%	0.269%	0.308%
mle1-7	0.832%	0.690%	0.806%	0.807%	0.827%	0.888%	0.834%	1.064%	1.185%	1.091%	0.699%	1.429%	0.961%	0.677%
Microvirga	1.187%	0.982%	0.823%	0.931%	1.071%	0.999%	0.713%	1.332%	1.560%	0.403%	0.799%	1.346%	0.621%	0.597%
Ellin6067	0.892%	0.694%	0.715%	0.994%	0.568%	0.583%	0.765%	0.768%	0.420%	0.870%	0.908%	0.679%	0.636%	0.857%
Pedomicrobium	1.073%	0.955%	1.185%	1.037%	1.094%	1.034%	0.864%	0.948%	1.089%	0.797%	0.840%	1.012%	1.010%	0.599%
Pseudomonas	0.866%	3.154%	0.327%	0.407%	0.425%	0.334%	0.293%	1.035%	0.393%	0.936%	0.842%	0.431%	0.392%	0.404%
p__Firmicutes														
Tumebacillus	1.437%	1.060%	1.115%	1.315%	1.502%	1.260%	1.126%	2.136%	2.100%	1.760%	1.069%	1.951%	1.561%	1.907%
Ammoniphilus	0.812%	0.388%	0.762%	0.826%	1.227%	1.179%	0.656%	1.623%	1.637%	1.547%	0.966%	1.622%	1.741%	1.264%
Paenibacillus	1.120%	1.105%	1.086%	1.425%	1.685%	1.392%	1.447%	1.040%	1.113%	1.052%	0.509%	0.778%	0.708%	0.808%
Cohnella	0.212%	0.172%	0.183%	0.143%	0.206%	0.105%	0.159%	0.100%	0.147%	0.064%	0.051%	0.083%	0.074%	0.000%
Lysinibacillus	0.104%	0.056%	0.053%	0.074%	0.144%	0.124%	0.000%	0.309%	0.562%	0.241%	0.097%	0.440%	0.095%	0.221%
Brevibacillus	0.117%	0.193%	0.237%	0.258%	0.108%	0.064%	0.070%	0.106%	0.220%	0.380%	0.058%	0.000%	0.047%	0.159%
Pseudogracilibacillus	0.082%	0.082%	0.158%	0.000%	0.152%	0.019%	0.000%	0.001%	0.000%	0.000%	0.000%	0.000%	0.021%	0.000%
Turicibacter	0.038%	0.006%	0.021%	0.000%	0.266%	0.008%	0.000%	0.004%	0.000%	0.000%	0.000%	0.022%	0.011%	0.000%
Melghirimyces	0.040%	0.000%	0.041%	0.008%	0.104%	0.036%	0.003%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%	0.000%
_Aneurinibacillus	0.022%	0.022%	0.028%	0.018%	0.009%	0.006%	0.026%	0.038%	0.015%	0.034%	0.000%	0.057%	0.050%	0.035%

Legend: ALF = Alfisol, INC = Inceptisol, IM = Imazapyr, PMG = PrimextraGold, ORGH= Organic (Vinegar), herbicide CT = Control

CHAPTER 5

DISCUSSION

5.1 Physical and Chemical Properties of Alfisol and Inceptisol Before and After Herbicide Treatment

The physical and chemical properties of soil samples obtained from Alfisol wre significantly higher compared to those obtained from Inceptisol as observed in this study. Most importantly were the moderate level of soil pH, higher concentration of organic matter and clay particles as well as total nitrogen which influenced high population of microbial community in Alfisol compared to Inceptisol as observed in this study. This result corroborates the previous investigations where, microbial community composition was highly correlated to physical and chemical parameters (Keshri *et al.*, 2015). Soil organic matter (SOM) has been reported as an important component and one of the vital determinants of soil health due to varying roles it plays (Albarrán *et al*., 2004; Lehmann *et al.*, 2011; Horák *et al*., 2020). It revealed how sensitive microbial community composition are to the soil organic matter indices such as soil organic carbon, organic nitrogen and readily mineralizable C and N (Allison and Martiny, 2008; Liu . *et al*., 2022).

In addition, soil pH as part of chemical properties of the soil has been identified and considered as part of the factors that determines the level of soil health as impacted by land use change and agricultural practices (Gil *et al*., 2009). Parent material, vegetation and climate influence, soil types and their pH levels. have the ability to impact shift in soil chemical and biological activities (Dalal and Moloney, 2000). Furthermore, the impact of soil texture on microbial populations in soils has received much attention from previous researchers and their findings showed that clay particles influenced the highest bacterial populations as well as promote various soil biological processes (Jarvis, 2007; Hamarashid *et al.*, 2010).

5.2 Metabolites Persistency in Non-sterile Alfisol as Impacted by Organic and Chemical Herbicide Application at Different Weeks

High persistent level of metabolite from most of the herbicides used for this study observed across the weeks in Alfisol compared to Inceptisol could be due to the presence of high concentration of organic matter and clay soil particles as well as moderate level of soil pH in Alfisol as observed from this study compared to Inceptisol. This result is in agreement with previous studies who reported the ability of applied soil organic amendments and clay particle to influence as well as increase the persistency of herbicides by improving the sorption process (Barker and Bryson 2002; Wanner *et al.* 2005; Glaspie *et al*., 2021). There has also been report on the influence of soil organic matter on herbicide adsorption and binding in soil which as well increases its persistency (Wauchope *et al.,* 2002, Monaco *et al.,* 2002, Weber *et al*., 2007). Other researchers had reported that soil organic matter (SOM), among other soil components, is a very vital component in the soil which mediates the retention of herbicides, and as such facilitates decrease in the soil profile movement of the applied herbicides It also influence the transformation of these herbicides into different metabolites within the soil and this encourages high persistence level of this metabolites **(**López-Piñeiro *et al.,* 2013**;** Bonfleur *et al.,* 2015**;** Tejada and Benítez, 2017**).**

Other discoveries made on soil organic matter which determines the extent of its role with respect to adsorption of herbicide includes its variation in functional group type and abundance, and this depends on the origin of the SOM, soil pH, climate, as well as the microbial community (Benoit *et al*.,2008, Walker and Austin, 2003; Glaspie *et al.*,2021). Previous studies also reported that soil organic matter is not the only soil property that can sorb many herbicides, however, soil clay particles, were also observed to have the ability for herbicide sorption due to their net negative charge, and had been reported to be preferred for sorption by certain herbicides (Monaco *et al.,* 2002; Glaspie *et al*.,2021). Other factors which can determine the persistence level of herbicides in soil include the cation exchange capacity (CEC) of the soil which is a measure of the quantity of adsorptive sites present in a soil specifically on the clay and organic matter components. Previous studies had reported that the organic matter content have higher adsorptivity site compared to clay content due to their smaller size and larger surface area. Higher CEC facilitates more herbicide bound

to soil colloids while less becomes available in the soil solution.(Li *et al*., 2016 ; Luo *et al.,* 2020).

Soil pH, which is a measure of the availability of hydrogen ions (H^+) in a solution have a great impact on herbicides adsorption on soil. This is because many herbicides incorporate hydrogen ions into their molecular structure which is the ion majorly found in soil solution when soil pH decreases (Hyun *et al.,* 2003). For instance, atrazine could take up hydrogen ions from the soil solution when soil pH is tending towards acidity causing the atrazine to become positively charged. At this point atrazine herbicide molecule becomes highly attracted to the negatively charged soil colloids because of the positive charge it has acquire. However, atrazine maintains a neutral charge mostly at pH 7 thus the herbicide is at this point less tightly adsorbed but more available to plants. Soil moisture is also a major determinant of herbicide persistency and performance.

5.3 Biodegradation Level of Metabolites in Alfisol and Inceptisol at Different Weeks After Herbicide Application

Generally, it was expected that herbicide biodegradation level will be high in non-sterile Alfisol due to high population and diversity of bacteria genera present in Alfisol as observed in this study. However, it is also important to mention that the low biodegradation level observed in metabolites such as pyrrolidinone compounds and metolachlor formed after the application of Imazapyr and primextragold respectively could be as a result of the inability of these bacteria genera to catalyze and utilize these metabolites as their source of carbon. Hence these bacteria genera could have catalyzed a conjugation reaction which may have impacted the structure of these metabolites transforming them into complex as well as difficult to biodegrade metabolites. This is in line with previous findings who reported that sudden modification in herbicide metabolite's structure may cause an obvious impact on the susceptibility to their biotransformation process (Pal *et al*., 2006; Hussain *et al.,* 2015).

More evidently, organic herbicide metabolites highly biodegraded in non-sterile Alfisol compared to chemical herbicide metabolite. The high biodegradation level of Atrazine metabolites formed after application of primetraGold in non-sterile Inceptisol at week 4 and 8 compared to week 12 could be that the bacteria genera present at week 4 and 8 were able to biodegrade and utilize this metabolite as their source of carbon, while the low

biodegradation level of this metabolite as observed at week 12 could be that this intermediate product of primextragold was toxic to bacteria genera present at week 12 and this could have resulted in low biodegradation level of Atrazine. This result is in corroboration with the findings of Chen *et al.* (2021) who reported that Atrazine could reduce the relative abundance of certain soil microbes which do not have the capacity to withstand its level of toxicity. It is also in line with the findings of Kaiser *et al*. (2016) who reported that herbicide of the triazine group has the ability to be toxic to soil and its microbial community when applied over a long period of time due to it persistence characteristics as well as residual impact in soil. Hazardous effect from atrazine residues and its related metabolites to the soil environment had been reported (Chamberlain, 2011). High persistence of Atrazine in soil with pH range of 5.5 to 9.0 had been reported and this falls between the pH range of Inceptisol used for this study. Atrazine volatilization potential limitations as well as its little or moderate susceptibility to aerobic biodegradation in soil has been observed (Liu, 2014).

Concomitantly, low biodegradation level of pyrrolidinone compounds which were metabolites formed after application of Imazapyr in non-sterile Inceptisol as observed consistently from weeks 4 to 12 could be due to the fact that the low pH status as observed in Inceptisol facilitated binding of pyrrolidinone compounds on Inceptisol particle making them not available to microbial degradation. This is in confirmation with the investigation of Saheem *et al.* (2022), who reported that Imazapyr herbicide can easily bind on soil with low pH making them unavailable to biodegradation. It is however, very pertinent to state that metabolites formed after application of organic herbicide in this study had higher biodegradation level in non-sterile Alfisol and Inceptisol compared to the chemical herbicide's metabolites. Most of the metabolite degradation observed in sterile Alfisol and Inceptisol could had been facilitated by chemical hydrolysis of these metabolites by water as well as volatilization.

Different chemical components and herbicides structural properties, their level of adsorption-desorption behaviour, their ability to undergo conjugative reactions catalysed by microbes to form complex structures are the determinant factor for herbicide transformation and their fate in soil environment with respect to persistency as well as biodegradation level (Singh *et al.,* 2006; Purnomo *et al*., 2011; Wang *et al*., 2013). It is imperative to remember

that most herbicides applied in the soil are basically exposed to either biotic (microbial) degradation or abiotic (photodegradation and chemical) degradation pathways, which are influenced by the certain environmental conditions, soil types, herbicide chemical structure, and its affinity to certain transformation processes (Bontempo *et al*., 2016).

5.4 Alpha and Beta Bacterial Diversity within and between Locations, Soil Type, Time, as well as Herbicide Treatments

It was observed from this study that location, soil type, and weeks had significant influence as shown from alpha bacteria diversity result (ie differences in bacteria diversity within location, soil type, and weeks), while herbicide treatments had no significant impact on the alpha bacteria diversity. Conversely, location, soil type, weeks, herbicides, interaction between location and soil, interaction between location and week as well as the interaction between herbicide treatment and week had significant influence on bacterial community composition between Alfisol and Inceptisol.

The significant variations observed in both alpha bacteria diversity and beta bacterial community composition within Alfisol and Inceptisol of this study could be due to the impact of varying abiotic factors like rain and temperature as influence by the different sampling locations. It could also be from the variations in the physical and chemical properties of the two soil types (for instance, it was observed from this study that soil properties like total nitrogen, organic carbon, soil pH and clay particles were all significantly higher in Alfisol compared to Inceptisol. Influence of time with respect to seasonal variations could also be a contributing factor (for instance part of the sampling time was towards dry season). This findings corroborates with some studies on the effect of properties of different soil type, time with respect to seasonal variations and fluctuations, various locations with different vegetations as well as effect of herbicides and their influence on soil microbial population and diversity (Rasche *et al*., 2010; Li *et al*., 2015; Jon and Jackie, 2015 ; Wu *et al*. 2016; Georges *et al*., 2019; He *et al.* 2019; Khondoker *et al.* 2020; Ruan *et al*., 2020;).

5.5 Non-metrics Multidimensional Scaling (NMDS)

The findings from this study according to non-metrics multidimensional scaling (NMDS) showed that herbicide treatment had a significantly reduced impact on the diversity of

bacteria present in both Alfisol and Inceptisol, It also revealed that some bacteria species preent in Alfisol were also present in Inceptisol. However, there were significant variation in bacteria diversity found within Alfisol and Inceptisol as well as between them. This could be due to variations in concentrations of total nitrogen, organic carbon, soil pH and clay particles observed between Alfisol and Inceptisol which were used in this study. Organic matter and Soil pH played vital role in bacterial diversity variation found both within and between Alfisol and Inceptisol. This finding was in line with previous investigations which reported how the different soil properties from various soil type, as influenced by different types of vegetations, parent materials as well as other biotic and abiotic factors influenced soil microbial population and diversity (Sui *et al.* 2021)

5.6 Indicator Taxa

Highly diverse soil microbial communities which play key role in the functioning of agricultural soil ecosystems such as mineral recycling, water regulation and degradation of harmful molecules had been observed (Bar-On *et al*. 2018; Muller *et al*., 2016). However, the impact of chemical and biological herbicides on these microbial diversity as well as taxonomic groups remain poorly understood. Assessing the impact of herbicides used in weed management practices on soil microbiomes is an important assay for sustainability of agricultural practices. The present study focused on a detailed comparison of the impacts of chemical and biological herbicides on soil bacterial composition of Alfisol and Inceptisol.

It was observed that 310 indicator taxa were the core bacterial population that dominated either of the soil types, locations and their combinations, herbicide treatments and their combinations as well as weeks/duration of herbicide application and their combination within the experimental period. However, 107 indicator taxa were the number of core bacterial population associated with the soil types, also more indicator taxa were observed (85) predominating Alfisol compared to Inceptisol which were predominated by lesser (22) indicator taxa. More indicator taxa observed predominating Alfisol could be due to the higher soil pH, organic carbon, clay particle and total nitrogen observed in Alfisol compared to Inceptisol. This result is in line with previous findings which reported that soil characteristics can influence increase in microbial population and diversity (Rousk *et al.,* 2010; Koyama *et al*., 2014, Zhang *et al.,* 2014; Wenbing *et al.,*2019).

It has been reported that, *Paenarthrobacter, Isosphaera, Methylobacter, Methylomicrobium, P3OB42,Pseudogracillibacillus,Chryseobacterium, Agromyce, Cellulomonas*, and *Stenotrophomonas,* which were among the core genera in Alfisol of this study with high relative abundance possess genetic element for biodegradation and metabolism of herbicides in agricultural soils as well as possess the capacity to degrade oil fruit (organic material) bunch (Deutch *et al.*, 2018 ; Shany *et al*., 2020 ; Chen *et al*., 2021). Previous findings also reported that most of the genera (*Terrabacter and Blastococcus Domibacillus, Fuctibacillus)* found predominating Inceptisol of this study can either survive harsh environmental condition like heavy metals accumulated environment as well as possess the capacity to metabolize xenobiotic materials like petroleum hydrocarbon and polycyclic aromatic hydrocarbon (PAHs) as their carbon and energy source (Essoussi *et al*., 2010; Chouaia *et al*., 2012**;** Bararunyeretse *et al*., 2019; Liu *et al.*, 2022). This showed that most of the genera found in both Alfisol and Inceptisol are beneficial as they could serve as biodegraders.

It was noticed that among the 141 indicator taxa which were the core bacterial population associated with locations, 68 indicator taxa predominated single location, whereas 73 taxa predominated a combination of either two or three locations. *Crossiella, Jatrophihabitans* and *Paenarthrobacter,* predominating three location combination in this study have a wide range of functions such as the ability to biodegrade soil pollutants like herbicides as well as maintain biological soil quality (Shany *et al* 2020). For the two-location combination, *Cohnella*, *and Pedosphaera* have the ability to improve soil quality through N_2 fixation as well as improve soil fertility and the plant health (Jian *et al*., 2020; Liu *et al.,* 2022).

Among *Methylomicrobium, Desulfohalotomaculum, Desulfovobrio, MBA03, Defluviitalea, Desulfitobacterium* and *Limnobacter* which were the core bacteria that predominated PMG herbicide, PMG herbicide application significantly increased the relative abundance of *Methylomicrobium* compared to other genera within this group. *Methylomicrobium* is a methanothrophic microbe which possess particulate methane monooxygenase methylloenzyme that facilitates the degradation of xenobiotics (Rayu, 2016) hence its ability to biodegrade primextragold a pre-emergence herbicide used in this study. This was observed in the increase in the relative abundance of Methylomicrobium as impacted by application of PMG herbicide.

On the other hand, *Nocardiopsis, Rhizocola* and *Sneathiella* were found predominating IM, and application of IM herbicide significantly increased the relative abundance of *Nocardiopsis* compared to other genera in both Alfisol and Inceptisol especially in Alfisol. This could be that *Nocardiopsis* possesses the catalyzing ability to metabolize IM herbicide as its source of carbon compared to other genera predominating IM herbicide. This result is in agreement with the findings of Pravin *et al.* (2018) who worked on degradation of organophosphate and organochlorine pesticides in liquid using *Nocardiopsis*. Previous studies have also reported the ability of the genus *Sneathiella* in the degradation of naphthalene and fluorene (Kappell *et al.,* 2014)

For shared taxa, *Nannocystis* was predominantly found in CT and IM, however, the relative abundance of *Nannocystis* was higher in IM compared to CT especially in Alfisol where there is significantly higher organic matter as the soil property compared to Inceptisol. Organic matter in Alfisol has the capacity to actively adsorbed the herbicide applied, which the microbe utilizes as their source carbon hence a resultant increase in their relative abundance (Bonfleur *et al.,* 2015).

Among *Panaerthrobacter, Melghirimyces, Oxalophgus, Thermobifida, Saccharomonospora, Qupengyyuania* and *Brachybacterium* genera which majorly predominated IM and PMG herbicides, it was observed that both herbicides significantly increased the relative abundance of *Panaerthrobacter*, and *Melghirimyces* compared to control especially in Alfisol. Previous studies had reported that *Panaerthrobacter* can degrade pesticides as their source of carbon and energy (Shany *et al* 2020), and this corresponds with the ability of *Panaerthrobacter,* to metabolize IM and PMG herbicides as observed in this study, resulting in increase in their relative abundance. *Melghirimyces is an* aerobic, halophilic, gram-positive bacterium (Addou *et al.,* 2013), and *Melghirimyces* may have the potential to degrade herbicide since halophilic microbes can degrade aromatic compounds (Mainka *et al.,* 2021). This may be the reason for the increase in relative abundance of *Melghirimyces* as observed in this study.

Application of IM significantly increased relative abundance of *Solibacillus* compared to PMG and CT in Alfisol while no significant difference was observed in Inceptisol after application of IM. *Solibacillus* is a gram positive, spore-forming bacteria (Krishnamurthi *et*

al., 2009). As a spore forming bacteria, *Solibacillus* could be potentially capable of biodegrading herbicides since previous studies reported that some spore-forming bacteria of the genus *Bacillus* have the ability to degrade pesticides (Gangola *et al.,* 2018; Jiang *et al*., 2019). While *Aquisphaer*a and *Lechevalieria* predominates CT, ORGH and PMG, application of ORGH increased the relative abundance of *Aquisphaer*a compared to CT in both Alfisol and Inceptisol.

Conversely, increase in relative abundance of *Aquisphaera* was observed in Alfisol while a slight decrease in relative abundance of same taxon was discovered in Inceptisol after PMG application. Low pH and organic matter identified in Inceptisol in the course of this study could affect the availability of PMG in Inceptisol, hence while pH negatively impacts the population of *Aquisphaera*, low organic matter affects the availability of PMG which the microbes could have utilized as their source of carbon (Liu *et al*., 2022). Soil organic matter (SOM) has been reported to be an important facilitator in the soil for herbicides retention after application during weed management. This mostly discourages soil profile mobility of these herbicides and has the potential to cause herbicide persistence within soil environment which could either be toxic to certain microbes in the soil while others utilize it as their source of carbon (López-Piñeiro *et al*., 2013; Bonfleur *et al*., 2015; Tejada and Benítez, 2017). There is however, dearth of information on the impact of organic herbicide on bacteria hence the mechanisms of metabolism of ORGH by *Aquisphaera* are yet to be understood.

Among *Saccharopolyspora*, *Salipaludibacillus, Candidatus Ntrosotalea, Group_1.1c* and *Nitrosotaleaceae* genera which predominates IM, ORGH and PMG herbicides in both Alfisol and Inceptisol, application of IM herbicides significantly increased the relative abundance of *Candidatus nitrosotalea, Saccharopolyspora*, *Salipaludibacillus,* and *Nitrosotaleaceae* in Alfisol compared to Inceptisol, however, application of PMG herbicide reduced the relative abundance of *Salipaludibacillus* in Inceptisol compared to other genera whereas application of ORGH herbicide increased the relative abundance of *Saccharopolyspora*, compared to PMG and IM in both Alfisol and Inceptisol respectively. The differences in relative abundance of the genera observed in both Alfisol and Inceptisol when IM, ORGH and PMG herbicides were applied, may be influenced by differences in the characteristics of the two soil types used in this study.

Typically, soil pH, % O.C as well as clay particles of Alfisol were observed to be significantly higher compared to that of Inceptisol. This may have influenced the binding and persistence of herbicide in Alfisol which served as source of carbon more to the genera *Salipaludibacillus, Nitrosotaleaceae* and *Candidatus Ntrosotalea* and which could have facilitated the reduction in relative abundance in some of the genera by PMG and IM compared to ORGH herbicides. This supports previous findings with respect to the role of soil organic matter (SOM) and clay particles of soil in herbicide binding (Weber *et al.,* 2007; Đurović *et al*., 2009; Takeshita *et al.,*2019). However, by description, studies have reported that *Salipaludibacillus* is a facultative anaerobic, moderately halophilic and alkaliphilic, growing over a wide range of NaCl concentrations and pH of 7.5 (Amoozegar *et al*., 2018*)*. *Candidatus nitrosotalea* and *Nitrosotaleaceae* has been reported *as* autotrophic ammonia oxidizers that do not grow at neutral pH except for acidic environment (Zhao *et al*., 2022) while *Saccharopolyspora* is an aerobic, Gram-positive, non-acid fast bacterium actinomycete and their growth occurs from pH 6.0 to 8.0 and they can degrade gelatin and starch (Zi-Wen *et al*., 2018).

The indicator taxa within the two soil types which were associated with the three herbicides as influenced by time points were considered. Indicator taxa were mostly dominant in Alfisol compared to Inceptisol and they had differential preferences to the various herbicides applied on the two soil types across the weeks. *DSSD61*, *Azospirillum, Paenochrobactrum* and *Tundrisphaera* were the indicator taxa identified to be strongly associated with week 2. Nevertheless, the relative abundance of *Paenochrobactrum* significantly increased at week 2 after application of IM and PMG in Alfisol compared to ORGH and control. It is possible that metabolites produced from Imazapyr and primextragold had components which served as good carbon source for the genus *Paenochrobactrum*, and that resulted to its increase in relative abundance compared to other genera also associated with week 2. However, herbicide biodegradation ability by *Paenochrobactrum* is yet to be known. The indicator taxa strongly associated with week 4 were *YC-ZSS-LKJ147* and *Methylophilus*, however, it was observed that application of ORGH resulted to significant increase in the relative abundance of *YC-ZSS-LKJ147* in Alfisol compared to other herbicide treatments as well as control but no significant

difference was observed in Inceptisol when ORGH herbicide was applied compared to control.

The degradation ability of YC-ZSS-LKJ147 is yet to be known, hence its increase in relative abundance after application of AA herbicide in Alfisol could be due to increase in concentration of organic matter and clay particles in this soil type. Thermaerobacter, are gram-positive, rod-shaped cells and they are strictly-aerobic. Their spores are usually round in shape, which terminates with bulged sporangium. Their growth occurs at range of pH 6.5–10.5 (while their optimum pH for growth is 8.0). NaCl concentrations higher than 1% inhibit their growth (Shuhei *et al.*, 2009) This could be the reason for their low relative abundance in this study since their optimum pH for growth is 8.0 and they do not grow in a stressful environment.

Application of IM and PMG herbicides significantly increased the relative abundance of *Morganella* at week 8 in Alfisol compared to control and Inceptisol. This could be as a result of significantly higher organic matter and clay particle content of Alfisol as we observed in this study which aided the persistent levels of IM and PMG herbicide and their metabolites in Alfisol. The ability of *Morganella* to metabolize IM and PMG herbicides as their source of carbon is also part of the factors to be considered since the capability of *Morganella* to biodegrade pesticide and polycyclic aromatic hydrocarbon (PAHs) had been previously reported (Tony *et al*., 2020; Haruna *et al.,* 2021; Zhao *et al* 2021). It is important to state however, that their report is in line with the present study.

Among the indicator taxa associated with week 0 and 2, relative abundance of *Delftia* was significantly increased at week 2 after application of IM, PMG, and ORGH compared to week 0 whereas application of IM resulted to significant reduction in the relative abundance of *Flavitalea* compared to PMG and ORGH herbicide treatments in Alfisol in both weeks 0 and 2 especially at week 2. *Delftia* have been reported to possess genetic element for biodegradation of soil pollutants like herbicides as well as being able to inhabit harsh environment like high saline soil (Shetty *et al.,* 2015; Wu *et al.,* 2016), hence, this may be the reasons for their increase in relative abundance in this present studies. Reduction in the relative abundance of *Flavitalea* could be as a result of reduction in nutrient due to

competition from biodegraders who utilize these indigenous soil nutrients before they can degrade the pollutants (Kadri *et al*., 2014).

Relative abundance of *Nitrosomonas*, an indicator taxon associated with weeks 0 and 4 was significantly reduced at week 4 after application of PMG and ORGH compared to week 0 in Alfisol where they were found to be highly dominant. However, ORGH herbicide was found to facilitate increase in the relative abundance of *Nitrosomonas* at week 4 in Inceptisol compared to week 0. *Nitrosomonas* is one of the genera which mediate for oxidation of ammonium (NH_4^+) ions in the presence of oxygen to form nitrite (NO_2^-) and nitrate (NO_3^-) during nitrification stage of organic matter mineralization (Maartje *et al.,* 2015). Therefore, reduction in relative abundance of *Nitrosomonas* may inhibit the completion of the mineralization process, hence formation of nitrate nitrogen will be inhibited.

Turneriella was the indicator taxon identified at week 2 and 4, however, it was observed that its relative abundance significantly reduced at week 2 after application of IM, PMG, and ORGH herbicides but increased at week 4 in Alfisol where they were mostly dominant. This could be as a result of toxic effect of some of the metabolites produced by the herbicides at week 2 on *Turneriella* as influenced by Alfisol characteristics like organic carbon, clay particle and soil pH (Tony *et al*., 2020) which could have hindered factors like leaching or movement of the herbicide in the soil profile at week 2. Leaching of herbicides after application at week 4 could be a factor to consider which has the capacity to facilitate slight reduction in persistence of herbicide in the soil. This could be influenced by the level of decomposition and availability of soil organic matter (Albarrán *et al*., 2004; Ahangar *et al*., 2008). It could unavoidably facilitate increase in relative abundance of microbes originally reduced due to toxic level of herbicide metabolites in the soil.

Cutibacterium was the indicator taxon associated with week 2 and 8. It was observed that the relative abundance of *Cutibacterium* increased significantly at week 2 after application of IM, PMG, and ORGH herbicides in Alfisol compared to Inceptisol. Conversely, application of PMG herbicide significantly increased relative abundance of *Cutibacterium* at week 8 in Inceptisol compared to Alfisol. However, the biodegradation ability and mechanism for metabolism of herbicides by *Cutibacterium* is yet to be reported. *SM1A02*, the indicator taxon associated with weeks 4 and 8 was observed to increase significantly in

its relative abundance at week 4 when IM herbicide was applied which later reduced at week 8 in both Alfisol and Inceptisol. *SM1A02* has been reported to exhibit varying metabolic capabilities with respect to nitrogen acquisition through nitrification process (Chen *et al.* 2019).

Among the indicator taxa which were strongly associated with weeks 6 and 8, it was observed that the relative abundance of *Enhydrobacter* and *Alkanindiges* were reduced significantly at week 6 after application of IM in Alfisol. This could be that the transformed products of IM were toxic to *Enhydrobacter* and *Alkanindiges* at week 6. However, relative abundance of *Enhydrobacter* were observed to increased significantly after application of PMG and ORGH compared to control in Afisol. This could be that the transformed products of PMG and ORGH herbicide at week 6 were easy for *Enhydrobacter* to metabolize as their source of carbon. Significant higher %OC and soil pH in Alfisol compared to Inceptisol could also be a facilitator to increase in the relative abundance of the genus due to the differential ways in which the soil microbes respond to the influence of these soil properties. Conversely, the three herbicide treatments increased the relative abundance of *Alkanindiges* compared to control in Inceptisol. The uniquely distributed soil properties and soil nutrients in Inceptisol as well as the chemostatic ability of certain microbes could have supported the increase in the relative abundance of the genus *Alkanindiges in* Inceptisol (Stocker 2012; Zhang *et al.,* 2014; Wenbing *et al.,* 2019; Fengshen *et al*., 2021; Nguyen *et al*., 2021).

At week 8, application of ORGH herbicides increased the relative abundance of *Enhydrobacter* in Alfisol. It is imperative to note additionally, that the general increase or decrease in relative abundance of the soil microbiome (genera) observed across the weeks could also be as a result of their response to seasonal variation and fluctuations with respect to soil sampling time (Rasche *et al*., 2010; Li *et al*., 2015; Wu *et al*. 2016; Li *et al.* 2018; Song-ping *et al.,* 2020). Previous studies had also reported that constant climatic changes can easily alter soil biodiversity as well as their distributions in the soil over time (Gottfried *et al*. 2012; van der Putten, 2012; Langley and Hungate 2014).

5.7 Relative Abundance

Majority of the agrochemical products used in agricultural production and weed management have been reported to have varying negative impacts on beneficial non-target

organisms involved in nutrient retention and recycling, it is also pertinent to say that, these chemicals can easily move within the environment by adhering to the organic matter in the soil or to the roots/aerial parts of the primary producers (Tejada and Benítez, 2017; Takeshita *et al*., 2019). Most of these agrochemicals have the ability to persist in the soil for prolonged periods, hence can negatively impact soil microbial flora (Pileggi *et al.*, 2020), either by reduction in population or by negatively modifying their mode of functions in the soil.

With this background in mind, it was observed from this study that IM and PMG herbicides reduced the relative abundance of the genera *Candidatus udaeobacter, Ellin516, Pedosphaera*, *AdurBin063-1 Candidatus xiphinematobacter* and *Luteolibacter* all belonging to the phylum *Verrucomicrobiot*a either at the early stage or towards the middle stage after their application compared to relative abundance of these genera in control Alfisol and Inceptisol. Previous studies on effect of herbicide application on soil microbiota reported the negative impact of herbicide application for weed control on non-target soil microbiota thereby affecting the dynamics of biogeochemical cycles and soil fertility (Elias and Bernot, 2014; Liu *et al.,* 2014; Qian *et al*., 2015).

It is imperative to mention that, the consistent decrease in the relative abundance observed in most of the genera such as *Candidatus xiphinematobacter, Luteolibacter*, *Ellin516, AdurBin063,Pedosphaera*, *Edaphobaculum*, *Chithoniobacte,* to mention but a few, after application of IM and PMG, in both Alfisol and Inceptisol either at the early stage, the middle stage or at later stage is in line with the previous findings who reported that application of herbicides for weed management decreased the bacteria abundance at the initial stage of application but may facilitated their increase at a later stage (Xu *et al*., 2017; Bezuglova *et al.,* 2019). It is also in line with the findings of Oladele and Ayodele, (2017) who reported a decrease in soil microbial abundance due to length time of soil exposure to certain herbicides.

The decrease in relative abundance of *Edaphobaculum, Puia* and *Chryseobacterium* all belonging to the phylum *Bacteroidota* observed in either week 4 or consistently from week 2 to 4 in both Alfisol and Inceptiol of this study could be due to the fact that the applied herbicide induced inhibition of nutrient cycling within the soil environment from the time of application to the middle stage of application hence causing reduction in abundance of

these microbes. This is in agreement with the findings of some studies who reported that application of herbicides can prevent the cycling of nutrients in soil ecosystem hence negatively impacting the growth and development of soil microbiomes with respect to reduction in their abundance as a result of reduction in nutrient availability (Suvi *et al.*, 2023). Application of some of these herbicides may also facilitate oxidative stress through production of reactive oxygen species which can generate lipid peroxidation while reacting with the microbial cellular membrane (da Silva Rovida *et al.*, 2021). Other studies reported a decrease in the abundance of soil microbiomes in the soil exposed to certain chemical herbicides (Oladele and Ayodele, 2017 ; Carpio *et al.*, 2020).

Increase in the relative abundance of the genera *Candidatus udaeobacter* observed at week 8 compared to week 2 after application of IM and PMG in Inceptisol, as well as increase in relative abundance of *Pedosphaer*a and *Chithoniobacter* observed when ORGH herbicides was applied, at week 4 and 8 in both Alfisol and Inceptisol, revealed that these bacteria genera belonging to the phylum *Verrucomicrobiota* have the ability to degrade and utilize IM, PMG and ORGH as their source of carbon over a period of time, irrespective of the soil type where the herbicide was applied. This confirms the report that when bacteria are exposed to some herbicides they have the ability to adapt to its hazardous effect, they could degrade those types of herbicides and utilize them as their carbon source and this can result to the increase in their relative abundance (Caracciolo *et al.*, 2010 ; Carles *et al*., 2018).

Consistent reduction in relative abundance of the genera *Candidatus udaeobacter* P*edosphaera, Candidatus xiphinematobacter,* Ellin 516 and *Luteolibacter* observed when IM and PMG herbicides were applied either in Alfisol or Inceptisol or both soils, either at week 4 to 6 or at week 2 to 8 as observed in this study could be as a result of inability of these genera to adapt to stress induced by the metabolites of IM and PMG while interacting with the soil. This result corresponds with previous findings who reported that application of certain herbicides whose components are made up of atrazine and metolachlor as well as those belonging to triazine groups are capable of inducing negative shift in the abundance of certain soil microbes which do not possess the mechanism for adaptation to stressful conditions generated when such herbicides are applied in soil for weed management (Ayansina and Oso, 2006; Kaiser *et al*., 2016; Chen *et al*., 2021).

Actinobacteriota which is a major phylum under Bacteria domain had been observed in a vast range of terrestrial and aquatic ecosystems. *Actinobacteriota* are recognized as filamentous bacteria due to their ability for substrate mycelium and aerial mycelium formation. *Streptomyces,* despite being the most dominant genus in *Actinobacteriota*, are definitely not the only one that are dominant in the phylum *Actinobacteriota* (Amin *et al*., 2020). *Streptomyces* is majorly the genus of *Actinobacteriota* which can be isolated through culture method to discover diverse species (Shepherd *et al*., 2010). However, most of other non-s*treptomyces* strains have low frequency of isolation under normal conditions and mostly require non-culture methods of isolation and cultivation (Baltz, 2006; Subramani and Aalbersberg, 2013).

Moreover, it is interesting to mention that previous studies had investigated the ability of *Actinobacteriota* to act as pollutant degrader and had reported the phylum as having ability to remove and metabolize pollutant as well as use them for their growth (Chaturvedi and Khurana, 2019). Barot and Chaudhari (2020) reported that *Actinobacteria* of the genus *Kocuria kristinae* degraded chlorpyrifos pesticide in Paddy field soil exposed to continuous chlorpyrifos applications. Sun *et al.* (2014) reported that the genus *Nocardioides sol* degraded carbendazim pesticide in soil under long-term applications of carbendazim while Fuentes *et al*. (2011) reported that *Streptomyces* sp. degraded Lindane an organochlorine pesticide which contaminated the soil where it was applied.

However, result observed from this present study differ a bit from the findings of these previous studies on some genera within the phylum *Actinobacteriota*, since *Streptomyces*, *Microlunatus*, *Ilumatobacte*r and *Acidothermus* decreased in their relative abundance after application of IM and PMG at either week 2 or 6 irrespective of the soil type. This means that these group of genera within the phylum *Actinobacteria* are not able to degrade and metabolize IM and PMG herbicides used for this study, however, the effect of these herbicides were observed to have caused a negative shift in their relative abundance. This finding is however, in line with Yang *et al.* (2022) who reported that microbial community and their relative abundance could differ in terms of level of stability in different soil layers and in terms of resistance to pesticides. ORGH herbicide used for this study however, was observed to increase relative abundance of the genera such as *Acidothermus* at week 2 and

Ilimatobacter at week 6 in Alfisol, this could be that these genera were able to easily metabolize ORGH herbicide at week 2 and 6 respectively as their source of carbon, however, there are dearth of information on the effect of organic herbicide on soil microbial abundance.

Negative shift in terms of reduction was observed in the relative abundance of *Acinetobacter*, *Ellin6067* and *Pseudomonas* either at week 2 to 8 or at week 8 after application of IM or PMG or IM and PMG in either Alfisol or Inceptisol or both soils. It is important to mention that, the relative abundance of *Acinetobacter* and *Ellin6067* were only reduced by either PMG or IM at week 8 in Alfisol but was reduced after application of IM and PMG in Inceptisol. This could be as a result of differential ability of microbes and their relative abundance to adapt to the toxicity of various pesticides as well as their varying level of stability in different soil types and this confirms previous reports (Yang *et al.*,2022).

Nevertheless, reduction in the relative abundance of *Pseudomonas* after application of the IM and PMG herbicides at week 8 in Alfisol and at weeks 2 and 8 in Inceptisol could be that the genus does not have the ability to tolerate as well as degrade any of these herbicides over the period of time it was exposed to each of the herbicides irrespective of the soil type, hence a reduction was observed in its relative abundance. This assertion is in line with previous studies, who reported that, the application of certain herbicides promoted the selection of bacteria which were able to tolerate such herbicides and as such same herbicides increased their abundance and diversity in the soil with increasing levels of exposure (Carles *et al*., 2018).

Summarily, the reduction in the relative abundance of these group of genera after their exposure to either IM or PMG herbicide could be as a result of the negative impact of the applied chemical herbicides on soil microbial diversity and abundance depending on the level of adsorption and desorption, degradation, bioavailability, persistence and toxicity as well as soil factors such as concentration of clay particle, vegetation, and soil organic matter level within the two soil types used for this study (Hussain *et al.,*2009 ; Yadav *et al*., 2017). Previous investigations also reported that herbicide compounds belonging to the triazines and imidazolines group are more toxic when applied over a long time due to their persistence level as well as residual effect in soil (Kaiser *et al.,* 2016). Similarly, it has been

reported previously about the ability of atrazine and metolachlor to cause negative shift in biodiversity of varying species of bacteria in the soil (Olofson *et al.,* 2005).

The genera *Acinetobacter, Sphingomonas, Bradyrhizobium* and *Pseudomonas* which belong to the phylum Proteobacteria had been reported to have the ability to fix atmospheric Nitrogen in the soil either through symbiotic association or non- symbiotic association methods (Geddes *et al.,* 2020; Chen *et al*., 2021; Mazoyon *et al*., 2023). Nevertheless, it was observed from this study that application of IM and PMG in Alfisol reduced the relative abundance of *Acinetobacter*, *Sphinomonas*, *Pseudomonas* as well as *Bradyrhizobium*. High concentration of soil organic matter and clay particles observed in Alfisol in this study could have increased the retention and persistent level of IM and PMG herbicides in Alfisol up to week 8 (Tony *et al*., 2020) which could have hindered factors like leaching or movement of the herbicide down the soil profile. This could have influenced reduction of the relative abundance of these genera present in Alfisol due to the extent of adsorption, bioavailability and toxicity of the herbicides on the Alfisol.

Reduction in the relative abundance of *Acinetobacter, Sphinomonas, Bradyrhizobium* and *Pseudomonas* genera in Alfisol could facilitate impediment in nitrogen fixation on this soil type in future with continuous chemical herbicide application. This finding is in line with previous studies which reported that application of most herbicides can cause disruption of certain vital processes of N-fixation during symbiotic association between leguminous plants and rhizobacteria and free-living nitrogen fixation processes (Singh, and Wright, 2002; Meena *et al.,* 2015). Application of herbicides in soybean in-vitro cultures study, had also been reported to inhibit of *Bradyrhizobium* spp as well as negatively impacted its nodulation under controlled experimental conditions (Powell *et al.,* 2007). Application of herbicides are capable of interacting with the bacterial cell to disrupt its metabolic process as well as influence the death of sensitive species of soil microflora (Cycon and Piotrowska-Seget, 2019).

The two phases which make up the life cycle of the phylum *Myxococcota* includes its social nature and its cooperative predation and multicellular development. However, the two processes comprise of multicellular phases which are controlled by modified movement of the cells using two motility systems. When there are available nutrients, cells move in

properly coordinated pattern, forming swarm like multicellular biofilms. These multicellular biofilms usually make contact with the source of nutrient, a process which will allow thousands of cells to penetrate the source (Berleman and Kirby, 2009; Pérez *et al.*, 2016). This strategy enables the multicellular biofilms to hydrolyze extracellular biopolymers making use of exoenzymes to make the most efficient possible use of the available sources of nutrient. Having this background about phylum *Myxococcota* in focus, the study indicated that only the genus *Pararoellobacteria* was able to utilize PMG herbicide as its source of carbon at week 8 especially in Aflisol compared to other genera within the group. Whereas in Inceptisol *Bacteriap25* was the genus which was able to utilize IM herbicide at week 8 at their source of carbon. This mean that irrespective of the soil type, application of the either PMG or IM was metabolizable by *Pararoellobacteria* and *Bacteriap25* which was observed to increase their relative abundance at week 8 in Alfisol and Inceptisol respectively.

TM7a and *TM7* genera which belong to phylum *Patescibacteria* were tolerant to applied IM herbicide at week 8 and this increased their relative abundance while the relative abundance of *LWQ8* and *TM7* reduced after application of PMG and ORGH herbicides at week 8. In Inceptisol, application of IM, PMG and ORGH reduced the relative abundance of *LWQ8* while application of PMG and ORGH reduced the relative abundance of *TM7a* and *TM7*. This means that length of exposure of herbicide to soil, could negatively or positively impact microbial composition depending on their level of tolerance to applied herbicide in the soil. Therefore, from observation made from this study, bacteria genera from same phylum tolerated and metabolized the different types of herbicides differentially, they also behaved differently in the different soil types (Ayansina and Oso, 2006; Yang *et al.,*2022).

Other studies reported that application of herbicides with imidazoline and triazine at recommended rate significantly reduced the bacterial population and diversity (Stanley *et al*., 2013). Observations from this study however, differ from the findings of Tyagi *et al.* (2018) who reported that bacterial population and their relative abundance were not significantly reduced from 40 days after herbicide application and beyond. Kaiser *et al.* (2016) reported that long-term use of the herbicides atrazine and metolachlor resulted in a reduction in the relative abundance of the methanotrophic bacteria. Nevertheless, reduction in the relative abundance of LWQ8 and TM7a in Alfisol as well as LWQ8, TM7a and TM7

in Inceptisol when IM, PMG and ORGH herbicide were applied could be that these herbicides and their metabolites were intolerable by these genera irrespective of the soil type where they were applied.

Relative abundance of the genera *Ammoniphilus, Lysinibacillus, Turicibacter, Pseudogracibacillus* and *Melghirimyces* belonging to the phylum Firmicutes were all reduced either at week 6 or 8 after application of either IM in Alfisol or Inceptisol. Studies have reported that herbicides with triazines and imidazoline components are more persistent in the soil with high concentration of organic matter and clay particle when applied over a long time due to their adsorption level as well as residual effect in soil (Tony *et al*., 2020) hence they can be toxic to soil microbiota. Other researchers have reported the ability of atrazine and metolachlor to negatively contribute to shift in bacteria community and their abundance in soil (Olofson *et al.,* 2005). Relative abundance of *Turicibacter and Pseudogracibacillus* increase after application of IM and PMG respectively at a later time point in Inceptisol. The application of certain herbicides had been reported to facilitate the selection of bacteria tolerant to such herbicide and can be used as biodegraders of such herbicides. This could be the reason for the increase in the abundance of these group of bacteria in the soil with long period of exposure to these herbicides (Carles *et al*., 2018).

CHAPTER 6

SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

This study was designed to investigate; the persistency level of organic herbicides and two commonly used chemical herbicides under the pre- and post-emergence types in Alfisol and Inceptisol, the level of biodegradation of pre- and post-emergence chemical and organic herbicides, the diverse population of bacteria community present in both Alfisol and Inceptisol soils and the impact of herbicides application on the bacteria community within the soils under study.

The metabolites of organic herbicide which is also a post-emergence herbicide used in this study was lower with respect to persistence level compared to the metabolites of Imazapyr and PrimextraGold herbicides which are pre-emergence and post-emergence chemical herbicide, respectively. Biodegradation level in metabolites of organic herbicide used in this study was higher compared with those of Imazapyr and PrimextraGold which are chemical herbicides and this implies that it is more environmentally friendly and easily metabolized and utilized by most of the bacterial genera as their source of carbon. High-throughput sequencing such as 16SrRNA metagenomics used in this study; revealed a significant variation in indicator taxa between Alfisol and Inceptisol. Application of bio-herbicides and chemical herbicides induced both modifications in microbial population and diversity and their potential functions in the both Alfisol and Inceptisol

Furthermore, the study revealed that most of the bacterial community in both soils where the herbicides were applied relied on these herbicides as their source of carbon due to the level of herbicide degradation as well as a corresponding increase in presence of soil pollutant-herbicides degrading bacterial taxa detected in this soil environments. However, further observations from this study revealed that bio-herbicide application served more as source of carbon to most bacterial genera which belong to the phyla *Verrucomicrobiota*,

Actinobacteriota, Myxococcota, Proteobacteria, Firmicute, Bacteriodota and *Patescibacteria* found in both Alfisol and Inceptisol and this resulted to increase in their relative abundance except for few of the bacterial genera. Conversely the chemical herbicides Imazapyr and PrimextraGold, although served as source of carbon to some of the bacterial genera, however, induced more reduction in the relative abundance of these genera within the different phyla as observed in this study irrespective of the soil type. Length of exposure time of these genera to herbicides as well as the properties of the two different soil types used for this study significantly influenced the shift in bacteria community population and diversity as observed from this study.

6.2 Conclusion

According to the results obtained, organic herbicide was the least persistent, most able to break down and least toxic; as a result, it was most suitable for the environment and microbes. It is therefore advocated for. In the study of bacterial communities, the cutting-edge molecular methodology revealed clearer information on Alfisol and Inceptisol bacterial population and diversity as well as herbicide effect on diverse bacterial population. In both soil types.

6.3 Recommendations

Therefore, the omics work flow and bioinformatics employed in this study opens avenue for further investigations such as

1. The investigations on the effect of chemical and bio-herbicides on fungi community and composition in Alfisol and Inceptisol using ITS biomarkers.
2. Use of omics application such as metatranscriptomics to study the effect of these bio-herbicides and chemical herbicides on the active genes of both bacterial and fungi genera within their community as found in both Alfisol and Inceptisol.

6.4 Contributions to knowledge

1. 16S rRNA metagenomic revealed distinct variations between Alfisol and Inceptisol bacterial populations and diversity as well as the effect of bio-herbicides and

chemical herbicides on the bacterial population and diversity in Alfisol and Inceptisol at genus level.

2. Relative abundance of bacteria taxa in the soil has no direct relationship with herbicide biodegradation.
3. There is a marked rapid increase in the rate of degradation of herbicides in Inceptisol compared to Alfisol
4. Organic herbicide was found to increase the relative abundance of most bacteria genera found in Alfisol and Inceptisol compared to chemical herbicides.

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Appendix 1A: Effect of Non-sterilized Alfisol and Inceptisol on Metabolite Persistence at 0 Week After Herbicide Application

Soil Type Non-sterilized Alfisol	Treatment	Peak Area (%)	Metabolites	Soil Type Non-sterilized Inceptisol	Treatment	Peak Area (%)	Metabolites
	IM	61.6	2-Amino-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one		IM	50.14	2-Amino-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one
	PMG	84.98	Metolachlor		PMG	52.55	Metolachlor
	ORGH	35.13	Acetamide		ORGH	28.0	Acetamide

Legend: CT= Control, IM =Imazapyr herbicide, PMG = PrimextraGold herbicide, ORGH= Organic (Vinegar) herbicide

Appendix 1B: Effect of Non-sterilized Alfisol and Inceptisol on Metabolite Persistency at 4 Weeks After Herbicide Application

Soil type non-sterilized Alfisol	Treatment	Peak Area (%)	Metabolites	Soil type non-sterilized Inceptisol	Treatment	Peak Area (%)	Metabolites
	IM	48.71	2-Amino-3-carboxymethy-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one		IM	35.31	2-Amino-3-carboxymethy-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one
	PMG	50.97	Metolachlor		PMG	37.05	Metolachlor
	ORGH	30.18	Acetamide		ORGH	25.0	Acetamide

Legend: CT= Control, IM =Imazapyr herbicide, PMG = PrimextraGold herbicide, ORGH= Organic (Vinegar) herbicide

Appendix 1C: Effect of Non- sterilized Alfisol and Inceptisol on Metabolite Persistence at 8 Weeks After Herbicide Application

Soil Type non-sterilized Alfisol	Treatment	Peak Area (%)	Metabolites	Soil Type non-Sterilized Inceptisol	Treatment	Peak Area (%)	Metabolites
	IM	42.55	2-Pyrrolidinone,1-methyl		IM	33.20	2-Pyrrolidinone,1-methyl
	PMG	43.85	Atrazine		PMG	35.09	Atrazine
	ORGH	22.08	Acetamide		ORGH	19.20	Acetamide

Legend: CT= Control, IM =Imazapyr herbicide, PMG = PrimextraGold herbicide, ORGH= Organic (Vinegar) herbicide

Appendix 1D: Effect of Non- sterilized Alfisol and Inceptisol on Metabolite Persistence at 12 Weeks After Herbicide Application

Soil Type non-sterilized Alfisol	Treatment	Peak Area (%)	Metabolites	Soil Type non-sterilized Inceptisol	Treatment	Peak Area (%)	Metabolites
	IM	38.35	2-Amino-3,4-dihydro-4-4-dimethyl-6-pyrimidinone		IM	30.75	2-Amino-3,4-dihydro-4-4-dimethyl-6-pyrimidinone -
	PMG	31.75	Atrazine		PMG	22.29	Atrazine
	ORGH	18.11	Acetamide		ORGH	15.20	Acetamide

Legend: CT= Control, IM =Imazapyr herbicide, PMG = PrimextraGold herbicide, ORGH= Organic (Vinegar) herbicide

Appendix 2A: Biodegradation Level of Metabolite in Alfisol at 4 Week After Herbicide Application

Herbicide in Sterilized Alfisol	Biodgradation level of metabolites (%)	Metabolites	Herbicides in non-sterilized Alfisol	Biodgradation level of metabolites	Metabolites
IM	16.17	2-Amino-3-carboxymethy-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one	IM	10.30	2-Amino-3-carboxymethy-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one
PMG	53.97	Metolachlor	PMG	43.46	Metolachlor
ORGH	17.08	Acetamide	ORGH	23.49	Acetamide

Legend: CT= Control, IM =Imazapyr herbicide, PMG = PrimextraGold herbicide, ORGH= Organic (Vinegar) herbicide

Appendix 2B: Biodegradation Level of Metabolites in Alfisol at 8 Weeks After Herbicide Application

Herbicide in Sterilized Alfisol	Biodegradation level of metabolites (%)	Metabolites	Herbicides in non-sterilized Alfisol	Biodegradation level of metabolites (%)	Metabolites
IM	54.40	2-Amino-3-carboxymethy-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one	IM	42.08	2-Amino-3-carboxymethy-4-5-dihydro-4-methyl-4-oxo-3H-pyrrol-3-one
PMG	59.68	Metolachlor	PMG	54.89	Metolachlor
ORGH	30.93	Acetamide	ORGH	41.58	Acetamide

Legend: CT= Control, IM =Imazapyr herbicide, PMG = PrimextraGold herbicide, ORGH= Organic (Vinegar) herbicide

Appendix 2C: Biodegradation Level of Metabolites in Alfisol at 12 Weeks After Herbicide Application

Herbicide in Sterilized Alfisol	Biodegradation level of metabolites (%)	Metabolites	Herbicides in non-sterilized Alfisol	Biodegradation level of metabolites (%)	Metabolites
IM	63.53	2-Amino-3,4-dihydro-4-4-dimethyl-6-pyrimidinone	IM	58.67	2-Amino-3,4-dihydro-4-4-dimethyl-6-pyrimidinone
PMG	71.56	Metolachlor	PMG	62.63	Metolachlor
ORGH	40.76	Acetamide	ORGH	45.89	Acetamide

Legend: CT= Control, IM =Imazapyr herbicide, PMG = PrimextraGold herbicide, ORGH= Organic (Vinegar) herbicide

Appendix 3A: Biodegradation Level of Metabolites in Inceptisol at 4 Weeks After Herbicide Application

Herbicide in sterilized Inceptisol	Biodegradation level of metabolites (%)	Metabolites	Herbicide in non-sterilized Inceptisol	Biodegradation level of metabolites (%)	Metabolites
IM	29.49	2-Amino-3,4-dihydro-4-4-dimethyl-6-pyrimidinone	IM	8.94	2-Amino-3,4-dihydro-4-4-dimethyl-6-pyrimidinone -
PMG	41.98	Atrazine	PMG	61.96	Atrazine
ORGH	12.47	Acetamide	ORGH	23.42	Acetamide

Legend: CT= Control, IM =Imazapyr herbicide, PMG = PrimextraGold herbicide, ORGH= Organic (Vinegar) herbicide

Appendix 3B: Biodegradation Level of Metabolites in Inceptisol at 8 Weeks After Herbicide Application

Herbicide in sterilized Inceptisol	Biodegradation level of metabolites (%)	Metabolites	Herbicide in non-sterilized Inceptisol	Biodegradation level of metabolites (%)	Metabolites
IM	31.62	2-Pyrrolidinone, 1-methyl	IM	9.46	2-Pyrrolidinone, 1-methyl
PMG	56.49	Atrazine	PMG	68.17	Atrazine
ORGH	23.63	Acetamide	ORGH	32.98	Acetamide

Legend: CT= Control, IM =Imazapyr herbicide, PMG = PrimextraGold herbicide, ORGH= Organic (Vinegar) herbicide

Appendix 3C: Biodegradation Level of Metabolites in Inceptisol at 12 Weeks After Herbicide Application

Herbicide in sterilized Inceptisol	Biodegradation level of metabolites (%)	Metabolites	Herbicide in non-sterilized Inceptisol	Biodegradation level of metabolites (%)	Metabolites
IM	34.75	2-Amino-3,4-dihydro-4-4-dimethyl-6-pyrimidinone	IM	31.72	2-Amino-3,4-dihydro-4-4-dimethyl-6-pyrimidinone
PMG	61.64	Atrazine	PMG	25.46	Atrazine
ORGH	30.7	Acetamide	ORGH	90.5	Acetamide

Legend: CT= Control, IM =Imazapyr herbicide, PMG = PrimextraGold herbicide, ORGH= Organic (Vinegar) herbicide