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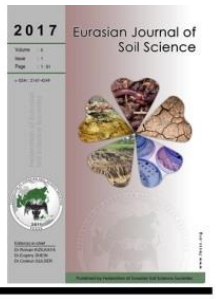

Glyphosate, 1,1'- dimethyl-4,4'-bipyridinium dichloride and Atrazine induces changes in Soil organic carbon, bacterial and fungal communities in a tropical alfisol

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Abstract

The increasing use of agrochemicals for weed control has raised concerns about their ecotoxicological effects on soil micro-biota communities and soil functions which serve as indicators of soil quality. Thus, this study was conducted to evaluate the effects of continuous field applied herbicides glyphosate, paraquat, atrazine and their combined forms over a period of five years on soil organic carbon, bacterial and fungal population in Akure, Ondo State Nigeria. Soil samples from farmer's field which have been exposed to continuous herbicide application were collected and analysed for physio-chemical properties, organic carbon, total bacterial and fungal population. Simultaneously, soil samples designated as control were collected from adjacent fields with no history of herbicide application and analysed. Results showed a significant (P=0.05) 86% and 128% increase in bacterial population from glyphosate and atrazine treated fields respectively and 42% decrease in paraquat and Glyphosate + paraquat fields when compared with the untreated field. A significant 35% decrease in fungal population was observed in fields applied with atrazine and a further 10% decrease in fungal populations in all herbicide treated fields irrespective of herbicide type and combinations when compared with the untreated field. These changes also correlates with the abundance of beneficial microbes such as *Pseudomonas aeruginosa, Pseudomonas fluorescens, Proteus mirabilis, Aspergillus flavius* with a probable influence on plant growth promotion and potentials for biodegradation of persistent herbicides. SOC, SOM and pH was significantly (P=0.05) increased in atrazine and atrazine + paraquat treated fields when compared with the untreated fields and other herbicide treatments.

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Introduction

Herbicides use are of primary concern in recent times due to their increasing application into the soil ecosystem. Intensively cultivated farmlands by farmers in south-western Nigeria are periodically treated with herbicides to combat weed infestation. In recent years, large quantity of pre and post-emergent herbicides have been consistently used to impede the activity of weeds which compete with grown crops for space, water, nutrients and ultimately affect crop yield (Varshney et al., 2012). A proportion of herbicides introduced as pre- or post-emergence weed killer have great residual activity in soil, which are ecologically destructive (Ayansina and Oso, 2006; Riaz et al., 2007; Pandey et al., 2007). Whereas, it is expected that herbicides applied should be of good efficacy and also pose minimum deleterious effects to crop and soil ecosystem (Hoerlein, 1994). Herbicides are extraneous to soil component pools, and these actions causes'

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changes in the catalytic efficiency and behaviour of soil enzymes (Sannino and Gianfreda, 2001), which induces microbial functions in the soil-plant environment. Various studies have stated that herbicides causes qualitative and quantitative change in enzyme activity (Sebiomo et al., 2011, Xia et al., 2012). The action of these herbicides in the soil is becoming increasingly important since they could be leached, in which case groundwater is contaminated and could persist on the top soil (Sebiomo et al., 2011 cited Ayansina et al., 2003). When applied, there are possibilities that these herbicides may exert certain effects on non-target organisms, including soil microorganisms (Simon-Sylvestre and Fournier, 1980). Many of the active ingredients in herbicides such as Glyphosate – Isopropylamine salt, Propanil – 3'3'-dichloropropionanile, 2, 4 – D acid – 2, 4-dichlorophenoxy acetic acid etc are persistent soil pollutants, whose impacts may persist for decades and adversely affect soil properties and soil biota (Shaner and Leonard, 2001). There is a growing concern that herbicides may not only affect target weeds but also the microbial communities present in soils and the performance of important soil functions (Hutsch, 2001). A study conducted by El-Ghamry et al., (2001), documented both positive and deleterious effect of herbicides on soil functions and activities of soil microbes.

Microorganisms degrade different kinds of carbonaceous substances including accumulated herbicides in soil to derive their energy and other nutrients for their cellular metabolism (Das et al., 2012). This reaction could favourably influence soil functions, the transformations of nutrients and soil biota activity. However, the interaction between the herbicides, soil functions and microorganisms could be dependent on the type of herbicides, soil type and microorganisms present (Nongthombam et al., 2008). Hence, the need to study the impact of herbicides on soil microbiota communities and address issues surrounding the environmental impacts of herbicide use. Glyphosate [N-(phosphonomethyl) glycine] is a broad-spectrum, non-selective, post-emergence herbicide that controls most of the annual and perennial weeds through inhibition of aromatic amino acids biosynthesis involved in protein synthesis (Battaglin et al., 2005). It also inhibits 5-enolpyruvylshikimic acid synthase *via* the shikimic acid pathway (Franz et al., 1997), which is ubiquitous in microorganisms (Bentley, 1990) that link primary and secondary metabolism. Glyphosate also acts as a competitive inhibitor of phosphoenolpyruvate, which is one of the precursors to aromatic amino acid synthesis. Soil and climate conditions affect glyphosate persistence in soil as it has been reported that the half-life of glyphosate in soil ranges between 2 and 197 days (NPIC, 2010). However, recent findings has disputed this with research now showing that a typical glyphosate field half-life of around 47 days in soil can increase up to 22 years becoming difficult to biodegrade due to strong complexes with metal ions (Jayasumana et al., 2014). 1,1'- dimethyl-4,4'-bipyridinium dichloride (Paraquat) is stable in acidic or neutral solutions, but is hydrolyzed at pH > 12 (Wauchope et al., 1992). It undergoes photolysis in aqueous solution to form N- methybetaine of isonicotinic acid, and subsequently methylamine hydrochloride (Slade, 1965). Paraquat is highly persistent in the soil environment, with reported field half-lives of greater than 1000 days (Wauchope et al., 1992). The reported half-life for paraquat in one study ranged from 16 months (aerobic laboratory conditions) to 13 years (field study) (Rao and Davidson, 1980), while Atrazine is a pervasive environmental contaminant (Cox, 2001). It is strongly persistent and is one of the most significant water pollutants in rain, surface, marine, and ground water (Wiegand et al., 2001). Its persistence (it has a half-life of 125 days in sandy soils (Wiegand et al., 2001)) and mobility in some types of soils because it is not easily absorbed by soil particles means it often causes contamination of surface and ground waters. The micro-biota community and enzymatic activities are sensitive to agrochemicals and have been regarded as potential indicators for measuring the degree of soil fertility, soil pollution and alterations in microbial communities (Kalia and Gosal, 2011; Bacmaga et al., 2015; Borowik et al., 2016). There is a growing need to preserve and monitor soil quality and the micro-biota community as they are considered as an indicator of soil health and pollution. However, recent studies on herbicides effect on soil microbial communities are short term, pot experiments and screen house based (Bacmaga et al., 2015; Borowik et al., 2016) and empirical findings from these studies may not provide a realistic evaluation of the effects of herbicides on soil microbes in field conditions.

This study was conducted to determine the long term effects of single and dual field applied herbicides (glyphosate, paraquat and atrazine) on soil organic carbon and selected soil microbial population (bacteria and fungi), of a 5 years intensively cultivated farm land. This study is expected to provide better understanding of the possible long term response of these selected microbes to different herbicides under field conditions.

Material and Methods

Study site

The present study was conducted in November, 2015 on a farmer's field located near the poultry section of the Teaching and Research Farm of the Federal University of Technology Akure, Ondo state, Nigeria. The location lies between Latitude 7°20'N and Longitude 5°30'E, a bimodal rainfall pattern, with a long rainy season, usually between March and July and a short rainy season, usually extending from September to early November, after a short dry spell in August and a longer dry period from December to February. The Soils at the experimental site are alfisol classified as clayey skeletal oxic-paleustaif. Alfisols are a soil order in USDA soil taxonomy formed in semiarid to humid areas, typically under a hardwood forest cover, moderately leached soils with a clay-enriched subsoil and relatively high native fertility (USDA, 2003). Annual daily average minimum and maximum temperature are 28.10˚c and 32.0˚c, while the relative humidity ranges between 75% - 85%.

Experimental layout

The experiment was laid out in plots set-up as completely randomized design, and each plot measured (3 x 5) m^2. The dominant weed species on these plots includes, *Chromolaena odorata, Euphorbia heterophylla, Tridax procumbens, Cyperus esculentus, Cyperus rotundus* etc. Seven different plots; Control (no herbicide applied plot), three singly and three dual applied (Pre and Post emergence) herbicide treatments were identified and mapped out based on the herbicides application information collected from the farmers through an informal unstructured interview method. The herbicides used by the farmers on the plots were Paraquat, Glyphosate and Atrazine. The fields were periodically sown to maize, cassava, yam, vegetables and were reportedly sprayed with these herbicides as pre and post emergent herbicides at manufacturer's recommended rates Paraquat, (200g/l), Glyphosate (360g/l) and Atrazine (500g/l) and half of recommended rates for combined dosage for over five years.

Soil sampling and bioassay

Soil sampling was in accordance with the general methods for soil microbiological study. Moist rhizosphere soil sample were collected from a depth of (0-5) cm from each plot. Each plot was divided into 5 sub-plot, and from each sub-plot, five sub samples were collected randomly. These samples were thoroughly mixed to form a composite sample. The composite samples were homogenized, sieved through 0.2 mm sieve to remove stone and plant debris, and were analyzed. The effect of the different herbicides applied on the soil were analyzed in response to changes in organic carbon, bacteria population, fungal population and identification and soil chemical properties with respect to control treatment in triplicates.

Soil physico-chemical determination

The soil particle size analysis was done using standard hydrometer method described by Gee and Bauder (1986), while the particle fraction was calculated using the formulae and the textural classes described by Okalebo et al. (2002). Soil pH was determined in 1:2.5 (Soil: water) and KCl solution (1:1) using glass electrode pH meter. Total nitrogen in the soil was analyzed using Kjeldahl method (Bremner, 1960). Available phosphorus was extracted using Olsen's extract while the P in the extract was determined by the use of spectrophotometer. Exchangeable cation (K, Ca, Mg) were extracted with 1 N Ammonium Acetate K in the extract was determined by flame photometry, Ca and Mg were determined by Atomic Absorption Spectrometer (AAS). Soil organic carbon (OC) in different herbicides treated and control soil samples were determined by partial oxidation method (Walkley and Black, 1934) through titration against 1N $(NH_4)_2Fe(SO_4)_2.6H_2O$ using diphenylamine indicator.

Microbial enumeration

Nutrient agar (NA) was used for the enumeration of total heterotrophic bacteria by the pour plate method. Incubation was done at 30°C for 24 - 48h. Potato dextrose agar (PDA) was used for enumeration, isolation of fungi and incubation was at 25°C for 48h. Bacterial isolates were characterized based on cultural characteristics, staining reactions and biochemical reactions. Identification was thereafter made with reference to Bergey's manual of systemic bacteriology and fungal isolates were characterized as described by Barnett and Hunter (1972).

Statistical analysis

The data were statistically analyzed using a one-way analysis of variance (ANOVA) and means separated using Tukey's HSD test by SPSS Statistical package20th edition.

Results and Discussion

Effects of herbicides on soil chemical properties

The effects of herbicide application on soil chemical properties are presented in Table 1. The soil textural analysis indicates that the soil was a sandy loam (Sand 70. 79%; Silt 16.22%, and clay 12.98%). Results from all treatments indicate that there was no significant (P=0.05) difference with respect to soil pH, however atrazine treated plots recorded the highest statistical soil pH (6.55) values while glyphosate + paraquat treated plots recorded the lowest values (4.96). The atrazine treated plot recorded a 14.3% increase in pH values when compared to the control which makes the soil on this plot to be slightly acidic and in a range where most plant nutrients are available for uptake. The increase in soil pH could become disadvantageous as this could increase the persistence of some herbicide due to restricted hydrolysis associated with high pH levels and increase in exposure period by soil microbes thereby causing mortality and a decrease in microbial biomass carbon (Abbas et al., 2014). Atrazine + paraquat treated plots and singly treated atrazine plots increased soil organic matter when compared with other treatments and the control (untreated plots). SOM values of (5.53 and 5.50%) respectively were recorded in these plots which was significantly (P = 0.05) higher than other treatments. Paraquat treated plots (1.05%) recorded the lowest SOM values while the control (untreated) plot had values of (3.98%). Soil available P was significantly (P=0.05) higher in Atrazine + Glyphosate treated plot (49.93mg/kg) while paraquat treated plots recorded the lowest value of available P (6.77mg/kg). There was no significant (P=0.05) difference observed across all treatments with respect to total soil nitrogen (N). However, Atrazine + paraquat treated plots recorded higher statistical values of total N (0.62%) while the untreated plot (control) recorded the lowest values (0.48%). Exchangeable cations was significantly (P=0.05) increased across all herbicide treatments, Untreated plot (control) recorded the highest values of calcium (2.07cmol/kg) and magnesium (1.07cmol/kg), while glyphosate and atrazine + paraquat both recorded the lowest calcium and magnesium values (0.53 and 0.27cmol/kg) respectively. Na was increased in glyphosate treated plot (1.78cmol/kg) which recorded the highest Na values but wasn't significantly (P = 0.05) different from other treatments. Extractable K was also increased in glyphosate treated plot (2.61cmol/kg), however this wasn't significantly (P=0.05) different from other treatments. Cation exchange capacity (CEC) was higher and significantly (P=0.05) different in untreated (control) plot (7.64) when compared with other treatments.

Table 1. Chemical properties of herbicides treated soils at (0 – 15 cm) depth

Treatments	pH (H_2O)	OM (%)	P (mg/kg)	N (%)	Ca	Mg	K	Na	CEC
					(cmol/kg)				
Atrazine	6.55a	5.50a	38.19a	0.55a	1.53a	0.77ab	2.40a	1.61a	6.91a
Glyphosate	5.26b	1.62c	12.83b	0.52a	0.53b	0.27b	2.61a	1.78a	5.35ab
Paraquat	5.40b	1.05c	6.77c	0.58a	0.60b	0.30b	2.55a	1.71a	5.52ab
Atrazine + Glyphosate	5.70b	4.82a	49.93a	0.55a	0.60b	0.30b	2.53a	1.76a	5.35ab
Atrazine + Paraquat	5.06a	5.53a	8.09c	0.62a	0.53b	0.27b	2.48a	1.25a	4.85ab
Glphosate + Paraquat	4.96c	3.81b	33.29a	0.55a	0.67b	0.33b	2.56a	1.45a	5.85ab
Control	5.61b	3.98b	14.39b	0.48a	2.07a	1.03[a]	2.48a	1.70a	7.64a

*Means followed by the same letter within each column are not significantly different (P=0.05) as indicated by Tukey's HSD Test

The long term changes in soil chemical properties of herbicide treated soils is a response to the disruption of biological and chemical equilibrium reaction in soil which in turn influence the activity of soil microorganisms. The fluctuating soil chemical properties level which was observed in this study could be ascribed to the adsorption of some herbicides on colloidal sites and organic matter which concealed the effects of these herbicides on soil microbial biomass, and subsequently led to increased hydrolysis of microbial cells (Jayamadhuri and Rangaswamy, 2005). It is obvious from this study that herbicides has various stimulatory effect on soil microbial activity which affects their biodegradation rates, and this mechanism is dependent on the type of herbicide, and application rates that can significantly alter the soil microbial pool quantitatively and qualitatively over an extended period (Anderson and Armstrong, 1981). Herbicides can also interfere with vital processes involving non-target microbial community; and these action includes respiratory activity, molecular composition, biosynthetic reactions, cell growth and division (De Lorenzo et al., 2001).

Effect of herbicides on soil organic carbon

Changes in soil organic carbon (SOC) values as observed in this study could have been induced by continuous herbicide application (Figure 1). Result indicate a significant (P=0.05) increase in SOC with atrazine + paraquat treated plots recording higher values of SOC (3.21%) while low values (0.61%) of SOC was observed in paraquat treated plots.

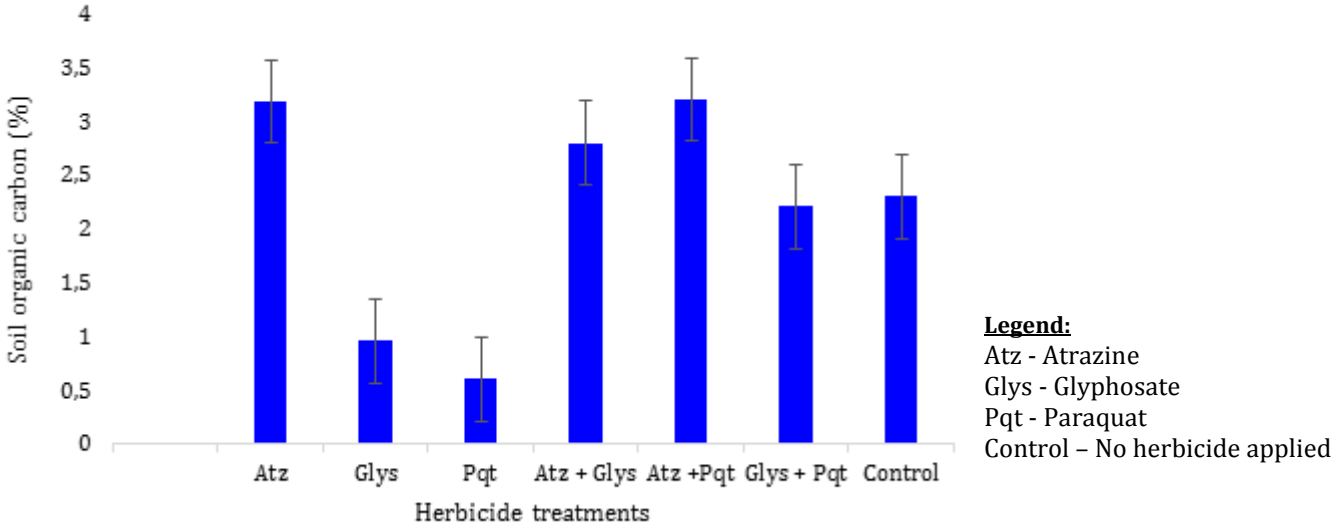

Figure 1. Effect of herbicide treatments on soil organic carbon

The increase of SOC in herbicide treated soil could be attributed to the faster putrefaction of herbicides by the dynamic microbial activity which further influence the movement and persistence of these herbicides due to increasing SOC levels which aids a faster biodegradation process (Ayansina and Oso, 2006). Result from this study was quite different from Sebiomo et al. (2011) who observed significant reduction in percentage organic matter after initial application of herbicides to soils in an incubatory study, although organic matter levels increased after continuous application from the second to the sixth week of treatment. Ayansina and Oso (2006) also reported that soil treatment with atrazine resulted in significant changes in soil organic matter levels. Considerable changes in soil organic matter were observed by Ayansina and Oso (2006) from herbicide treatment with atrazine, while in this study changes were only observed in glyphosate and paraquat treatments. Furthermore, Yaron et al. (1985) opined that soil containing high levels of organic matter would exhibit elevated microbial activity which would enable the soil to adsorb applied herbicide thereby decreasing its concentration in soil solution and reducing biodegradation and elongating its persistence in the soil. Suppression and poor root growth of weeds due to the actions of glyphosate, paraquat and their combined levels could also induce the decline in organic matter by interfering with the root-rhizosphere mechanism.

Effect of herbicides on microbial communities

Dominant fungal and bacteria communities isolated from the herbicide treated and untreated soil are presented in Tables 2, 3 and 4. Results indicate that beneficial bacteria's such as *Pseudomonas spp, Bacillus spp, Enterobacter spp* e.t.c were the dominant colonies across the herbicide treated fields. Under the atrazine treated plots, *Pseudomonas fluorescens* was the highest bacteria genera population (20 x 10^{-8} cfu/g) while the lowest population was *Chromobacterium lividium* and *Xanthomonas spp* (12 x 10^{-8} cfu/g) respectively. In the glyphosate treated plot, isolated bacteria genera; *Bacillus subtilis* and *Thiobacillus thiooxidans* were the dominant colonies at (20 x 10^{-8} cfu/g) respectively, while *Citrobacter freundii* was observed to have the lowest colony (8 x 10^{-8} cfu/g). Paraquat treated plots generally recorded low bacteria colonies, beneficial bacteria such as *Rhizobium, Bacillus subtilis,* were isolated under this plot, however, *Bacillus subtilis* recorded the highest colony count (5 x 10^{-8} cfu/g)

Table 2. Bacteria genera isolated from untreated and herbicides treated soil

Treatments	Isolated Bacteria	Colony count (10^{-8}cfu/g)
Atrazine	*Pseudomonas aeruginosa*	18
	Pseudomonas fluorescens	20
	Chromobacterium lividium	12
	Xanthomonas spp	12
	Enterobacter liquefacieus	15
	Flavobactererium spp	15
	Azotobacter spp	18
Total		110 x 10^{-8} (cfu/g)
Glyphosate	*Flavobacterium spp*	12
	Bacillus subtilis	20
	Proteus mirabilis	8
	Enterobacter spp	10
	Klebsiella aerogenes	10
	Yersinia spp	12
	Citrobacter freundii	8
	Thiobacillus thiooxidans	20
Total		100 x 10^{-8} (cfu/g)
Paraquat	*Proteus vulgaris*	4
	Bacillus subtilis	15
	Citrobacter freundii	3
	Enterococci spp	4
	Rhizobium spp	3
Total		29 x 10^{-8} (cfu/g)
Atrazine + Glyphosate	*Bacillus spp*	10
	Micrococus spp	6
	Pisteurella spp	5
	Leuconostoc spp	15
	Corynebacterium spp	10
	Kurthia spp	5
	Bacillus licheniformis	20
Total		71 x 10^{-8} (cfu/g)
Atrazine + Paraquat	*Chromobacterium lividium*	3
	Bacillus pumilus	6
	Klebsiella aerogenes	5
	E. coli	15
	Proteus mirabilis	20
Total		49 x 10^{-8} (cfu/g)
Glyphosate + Paraquat	*Citrobacter spp*	1
	Chromobacterium lividium	2
	Enterobacter spp	8
	Proteus mirabilis	7
	Acinetobacter anitratus	5
	Genella spp	1
	Branhamella catarrhalis	3
Total		27 x 10^{-8} (cfu/g)
Control (Untreated soil)	*Clostridium tetanomorphium*	20
	Brucella spp	3
	Bordetella spp	3
	Chromobacterium lividium	3
	Actinobacter antratus	5
	Acinetobacter spp	5
	Rhizobium spp	10
	Xanthomonas campestris	4
	Brevibacillus brevis	5
Total		58 x 10^{-8} (cfu/g)

Table 3. Fungi genera isolated from untreated and herbicides treated soil

Treatments	Fungi Isolated	Colony count (10^{-8} cfu/g)
Atrazine	*Aspergillus flavius*	15
	Doratomyces cordia	12
	Heterocerphalum spp	12
	Sclerothium spp	13
	Chaetomella spp	12
Total		64 x 10^{-8} (cfu/g)
Glyphosate	*Fusarium oxyporium*	8
	Geotrichum candidum	5
	Thallospora spp	6
Total		19 x 10^{-8} (cfu/g)
Paraquat	*Penicillium spp*	6
	Collectotrichum gleosporoides	3
	Geotricum candidum	2
	Fusarium spp	7
	Aspergillus nigericum	8
	Verticillium spp	5
Total		31 x 10^{-8} (cfu/g)
Atrazine + Glyphosate	*Fusarium spp*	1
	Trichoderma spp	2
Total		3 x 10^{-8} (cfu/g)
Atrazine + Paraquat	*Aspergillus niger*	3
	Coniothyrium spp	1
	Sphaeropsis spp	1
	Aspergillus fumigatus	3
	Trichothecium spp	2
	Botrytis cenerium	2
Total		12 x 10^{-8} (cfu/g)
Glyphosate + Paraquat	*Trichophyton spp*	3
Total		3 x 10^{-8} (cfu/g)
Control (Untreated soil)	*Aspergillus spp*	25
	Rhizoctonia solani	15
	Botryodiplodia spp	20
Total		60 x 10^{-8} (cfu/g)

Table 4. Bacterial and fungal population of herbicide treated and untreated soil

Treatments	Bacterial population (10^{-8} cfu/g)	Fungal population (10^{-8} cfu/g)
Atrazine	16^{a}	13^{b}
Glyphosate	13^{b}	6^{c}
Paraquat	6^{d}	5^{c}
Atrazine + glyphosate	10^{c}	2^{d}
Atrazine + paraquat	10^{c}	2^{d}
Glyphosate + paraquat	4^{d}	3^{d}
Control (Untreated)	7^{c}	20^{a}

Values assigned with different letters are statistically different at (P = 0.05)

Different bacteria genera's were isolated from the combined use of Atrazine and glyphosate treated fields. *Clostridium sporogenes,* and *Bacillus licheniformis* were the dominant isolated colonies (20 x 10^{-8} cfu/g) respectively. *Proteus mirabilis* (20 x 10^{-8} cfu/g) was observed to be the dominant bacteria colony in the atrazine and paraquat treated field. Different bacteria genera were isolated from the combined use of glyphosate and paraquat treated plot; however the colony counts were considerably lower when compared with herbicide treated and untreated (control) plots. Isolated bacteria genera *Enterobacter spp* was observed to have the highest colony count (8 x 10^{-8} cfu/g), while other less dominant bacteria colonies isolated include; *Chromobacterium lividium* (2 x 10^{-8} cfu/g), *Genella spp* (1 x 10^{-8} cfu/g) and *Citrobacter spp* (1 x 10^{-8} cfu/g).

Fungal colonies isolated from herbicide treated plots were generally low with respect to population count irrespective of the different herbicide types. However, *Aspergillus spp, Fusarium spp* and *Geotrichum spp* were the dominant colonies identified in all herbicide treated plots. *Aspergillus flavius* was the dominant colony in the atrazine treated plots (15 x 10^{-8} cfu/g). Glyphosate treated plots recorded the lowest fungal communities across all treatments, with population count as low as (5 x 10^{-8} cfu/g) observed for *Geotrichum candidum*. Paraquat treated plots also portrayed a linear decreasing trend in isolated fungal genera, *Aspergillus nigericum* (8 x 10^{-8} cfu/g) and *Fusarium spp* (7 x 10^{-8} cfu/g) were identified as the dominant fungal colonies under this plot. Combined atrazine and glyphosate treated plots remarkedly recorded the lowest fungal colonies amongst the herbicide treated plots with a total population count of (3 x 10^{-8} cfu/g). Despite the low population count, *Trichoderma spp* a beneficial fungi was observed to have the highest colony count (2 x 10^{-8} cfu/g). *Aspergillus spp* (6 x 10^{-8} cfu/g) were the dominant colonies isolated under the combined atrazine and paraquat herbicide treated plots. Although the fungal colonies were generally low, atrazine treated fields had the highest fungal colonies when compared with other herbicide treated plots (Table 3). Glyphosate and paraquat treated fields were also dominated by low fungal colonies. Fields under this herbicide treatment recorded a total fungal population count of (3 x 10^{-8} cfu/g) and was inhabited by the fungi genera *Trychophyton spp.* Diverse bacterial and fungal genera were identified and isolated from the untreated (control) fields. Bacteria colonies such as *Rhizobium spp* and *Clostridium spp* were the dominant colonies with respective population count of (10 x 10^{-8} cfu/g and 20 x 10^{-8} cfu/g). Fungal communities isolated from this field includes *Rhizoctonia spp*, *Aspergillus spp* and *Botryodiplodia spp* and their total population count (60 x 10^{-8} cfu/g) when compared with the herbicide treated fields was marginally higher except for Atrazine treated fields. Statistical analysis of total microbial population isolated and identified indicate a positive impact of herbicides on soil bacterial communities and a negative impact on fungal communities (Table 4). Fields treated with atrazine was significantly (P = 0.05) different from the untreated (control) soil and other herbicide treatments with respect to total bacterial population count as a high enumeration count was recorded (16 x 10^{-8} cfu/g). However, Atrazine + glyphosate and Atrazine + paraquat treated fields bacterial population count were not significantly (P=0.05) different from the untreated field except for glyphostate treated fields (Table 4). 128% increase in bacterial communities was observed in atrazine treated fields, 86% increase in bacterial communities isolated from glyphosate treated fields, 43% increase in Atrazine + glyphosate and Atrazine + paraquat treated fields respectively while over 42% decrease in bacteria population count was estimated from Glyphosate + paraquat treated fields relative to the untreated field.

Herbicide treatments exerted significant reduction in fungi colonies isolated and identified from treated fields. However, soil samples assayed from untreated fields was observed to have the highest fungi colonies amongst other treatments with a total fungal count of (20 x 10^{-8} cfu/g) (Table 4). Generally, fungal colonies enumeration count declined significantly across all herbicide treated plots when compared with the untreated field (control), with the lowest population count observed in atrazine + glyphosate, atrazine + paraquat and glyphosate + paraquat. A 35% reduction in fungal colonies was observed in atrazine treated plots, while over 10% decrease in fungal populations was observed in all herbicide treated fields irrespective of herbicide type and combinations when compared with the untreated field. Deleterious effect of herbicide application may lead to the reduction and negative stimulation of certain microbial communities with essential functions to perform in the soil environment and ecosystem. Herbicide treatments of glyphosate, paraquat, atrazine and their combined levels showed significant effects on isolated and identified fungal population within the soil environment. Fungal population increase and decrease observed due to the effects of herbicide treatments also varied among isolated fungal species and the types of herbicide treatments. The abundance of bacterial communities and fungal communities in atrazine treated fields suggest a stimulatory effect on select microbial growth and a high acclimatization to atrazine which could possibly create a colonies of microbes resistant to atrazine and can be used for degrading atrazine and bioaugmentation. This study also observed that the increase and decrease of some fungal species varied with differing herbicide types and this could be ascribed to the differing abilities of fungal mycelia to absorb herbicides for their utilization as some species are more active as herbicide degraders (Romero et al. 2009). Paraquat, glyphosate and their combined levels were found to be more inhibitory causing significant reductions in fungal populations. An earlier study had reported the Paraquat and glufosinate-ammonium were found to be inhibitory than glyphosate and metsulfuron-methyl, causing 80-100% growth inhibition to the fungal species. Paraquat was toxic *in vitro* to the radial growth of fungi as *Colletotrichum dermatium*, *Alternaria* sp., *Macrophomina phaseolina* and *Phomopsis* sp. isolated from soybean seeds at a concentration

as low as 600 µg a.i. mL-1 (Cerkauskas and Sinclair, 1982;Zain et al., 2013). A sharp decline in fungal specie population in response to glufosinate-ammonium was also observed for *Trichoderma harzianum* and *T. longipilus* (Ahmad and Malloch, 1995) and *Magnaporthe grisea* and *Cochliobolus miyabeanus* (Ahn, 2008). Glyphosate and its combined levels with other herbicide types also inhibited fungal population in this study and this could be due to the blocking of EPSPS enzyme in the shikimic acid pathway that ultimately affected the amino acid synthesis in fungal species. Previous *in vitro* studies also reported the growth-inhibitory effects of glyphosate on soil fungi communities such as *Fusarium solani*, *Pythium ultimum* and *Trichoderma viridae* at 100 and 140 ppm (Meriles et al., 2006), *Sclerotium rolfsi* at commercial recommended rate of 3.6 g L^{-1} (Westerhuis et al., 2007). Several studies have also reported the short-term effects on soil microbial communities upon single application of herbicide exposure (Hance, 1980) and at combined herbicides levels higher than the recommended field rate were observed to cause transitory effects on microbial biomass (Das et al., 2006; Weaver et al., 2007). Several studies have also reported significant decline of a number of cellulytic and pathogenic soil fungi by paraquat (Smith and Mayfield, 1977) and glyphosate (Anderson and Kolmer, 2005) in soil. The decline in the population of fungi and bacteria in this study was further corroborated by previous study conducted by Novak et al. (1999) who made use of post emergence herbicides and observed a decrease in the microbial biomass carbon (MBC). Decrease in bacteria, fungi and Actinomycetes population has also been reported by Sebiomo et al. (2011) from the use of atrazine, primeextra, paraquat and glyphosate herbicide treatments in an incubatory study. The reduction in MBC could be ascribed to the decrease in organic matter due to the mortality of soil microbes by herbicide residues (Abbas et al., 2014). The decline in bacteria population and some specific beneficial bacteria e.g phosphate solubilizing bacteria (*Enterobacter spp.* etc.) by glyphosate, paraquat and their combined herbicide treatment in this study could be attributed to the soil textural characteristics. High clayey contents could increase the persistence of herbicides in the soil and prolong interaction between herbicides and soil bacteria which could cause significant drop in their population. This result was in agreement with the findings of Cupples et al. (2005) who also observed prolong persistence of herbicides in the soil due to high clay content. The significant increase in both fungal and bacterial communities observed in atrazine treated soils could be ascribed to their intrinsic ability to tentatively mineralize and use the herbicides as energy source (Kunc et al., 1985). The decline in microbial counts exhibited in other herbicide treatments may be due to the fact that these microbial communities were less tolerant of the applied herbicides and were susceptible to the soil-herbicide interactions, which could have possibly been inhibitory (Taiwo and Oso, 1997). However, microbial population could be increased as these microbial communities adapt to the stress induced by the herbicides over a period of time. This could be ascribed to the recovery ability of different microbial species and enzymatic activities after the initial inhibition phase due to the microbial adaptation to these herbicides or biodegrading abilities.

Conclusion

Soil microbial communities are a very important component of the soil ecosystem. A functioning agro-ecosystem is dependent on these microbial communities as their abundance, activities and biodiversity are good indicators of a sound agro-ecosystem. This study indicated that glyphosate, paraquat, atrazine and their combinations induced both detrimental and beneficial changes in bacterial and fungal communities isolated and identified after a period of five year application and this could be attributed to organic matter levels, clay contents and the elongated persistence of these herbicides in the soil. Therefore, farmers are implored to observe a cautious approach by strictly following manufacturers recommended rates when applying these herbicides. The idea of combining herbicides for increased weed eradication efficacy so as to combat herbicide resistant weeds should be discouraged as this poses deleterious effect on soil microbes and ecosystem functioning. Farmers could also combat weed infestation by mulching their farmlands with mulch materials with allelopathic abilities for weed suppression which also exert beneficial effects on crop growth, soil health and are non-ecologically destructive. Soil microbial communities with increased population in herbicides treated fields as observed from this study could be isolated and cultured for use in bioaugmentation programs to biodegrade persistent herbicides in contaminated soils in the study area.

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