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Rhizosphere Microbial Population and Plant Species Diversity as Influenced by *Chromolaena odorata* Invasion

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Abstract

Invasive plant species have been commonly implicated to cause loss in plant species diversity. Attention had however not been paid to the effects of these species loss on the soil microbiome. A study was conducted in 18 farmers' fields within three states in southwestern Nigeria to examine the effect of Siam weed (*Chromolaena odorata*) invasion on native plant diversity as well as on the rhizosphere microbial population using randomized complete block design. Results indicated significant losses in plant species diversity and reduction in density per square meter compared with adjacent non infested fields. Results further showed *C. odorata* invasion exerted diverse influence on soil microbial population. Relationships were subsequently established among plant density, species diversity; and soil microbial population. Further studies were also recommended to accommodate more microbiological indices.

Keywords: *chromolaena odorata*, invasive species, soil microbiome, species diversity, weed density

1. Introduction

Soil microorganisms derive energy from organic or inorganic substrates, or from the products of biochemical processes; yet, many of these processes are mediated by the roots of plants

(Tabatabai, 1994). Therefore, the health of the soil partly depends on plants. Furthermore, the soil microbial population is altered by the significant alteration in soil physical and chemical properties caused by the constant interactions between plant roots, soil, and microbes (Nihorimbere *et al.,* 2011). In addition, exudates from the roots in the rhizosphere drive the interactions between plant roots and microbial community (Badri *et al*., 2009, 2013a; Chaparro *et al*., 2013). Up to 21% of carbon fixed through photosynthesis are released by the roots of plants as soluble sugars, amino acids, or secondary metabolites (Badri & Vivanco 2009; Badri *et al*., 2013*b*; Chaparro *et al*., 2013), and these form the substrates that support microbial activities at the root zone of plants in the soil. Since these rhizo-deposits are a major driving force in the regulation of microbial diversity and activity in the soil (Mendes *et al.,* 2013), it will be expedient to postulate that any process or activity capable of altering plant species diversity or density will have a direct bearing on soil microbial composition and population.

Invasive species have been commonly implicated to cause loss in plant species diversity. They are non-native, exotic plant species occurring outside their natural adapted ranges. They may become invasive when introduced into a new area, where they tend to establish because of the absence of their natural enemies (Sax & Brown, 2000). They also possess features that help them to out-compete native species (Raghubanshi *et al*., 2005). Changes in hydrology and ecosystem functioning as a result of loss of biodiversity and even species annihilation have been identified with invasive species (Raghubanshi *et al*., 2005).

Chromolaena odorata is among the world most important invaders and has been ranked as the second most noxious plant that requires urgent attention in some parts of Africa (Robertson *et al*., 2003). It negatively affects biodiversity by suppressing indigenous grassland and savanna vegetation through physical smothering and allelopathy (Zachariades & Goodall, 2002). Although they are known to have severe negative consequences for biodiversity, the effects of invasive alien plants species on soil microbial population are not well documented. The soil harbors the largest volume and mass of microorganisms, and it is the richest in microbial species diversity compared to any other habitat on earth owing to its heterogeneous and complex multi-substrate nature. The vast majority of these microscopic soil organisms are highly beneficial in terms of nutrient cycling, soil tilth, and soil health. These organisms are very critical to soil fertility and plant nutrition owing to their interaction with plants. The present study seeks to find out the response of soil microbial population to the vastly reported trim in plant species richness and density caused by *C. odorata* invasion (Goodall & Zacharias, 2002). The experiment takes cognizance of the fact that the stage of infestation with respect to time may play a key role on its influence on biodiversity, therefore the age of *C.odorata* at the sampling sites were taken into consideration.

2. Materials and Methods

2.1 The Study Area

The study was carried out between May – June 2012 in 18 farmers’ fields within three states in southwestern Nigeria. The states are Ondo, Ekiti and Osun. These states have high infestation of *C. odorata*, covering several hectares of land. The study area has a bimodal rainfall pattern with an annual rainfall between 1300mm - 1500mm and a mean annual temperature of 29 ^{0}C.

2.2 Experimental Design and Treatment Application

The experimental design used was randomized complete block design (RCBD), with each state representing a block and the age of infestation of *C. odorata* forming the treatments to be considered. These treatments include: 1-2 years old *Chromolaena odorata* invasion, 3-4 years old *Chromolaena odorata* invasion, invasion of five years and above, and a neighboring field free of *C. odorata* invasion as control. Each block contained all the treatments listed above. Samples of similar treatment in the two farmers' fields in a state were bulked to form a treatment.

2.3 Site Selection and Data Collection

Two communities showing high prominence of *C. odorata* were selected in each of the states, and weed sampling was done in farming settlements where farmers were on ground to provide information on the fields. The communities were: Oda (7^0 6'N, 5^0 17'E) and Iju (7^0 24'N, $5^0$15'E) in Ondo State; Ise (7.4563^0 N, 5.4332^{0}E) and Ikere (7.4991^0'N, 5.2319^{0}E) in Ekiti State; and Ikeji (7.4296^0 N, 4.9481^{0}E) and Ilesa (7.6395^{0}N, 4.7588^{0}E) in Osun State. The stages of *C. odorata* invasion were determined in each of the sampling sites with the help of local farmers.

2.4 Plant and Soil Sampling

Plant sampling was done using three fixed 50cm x 50cm quadrats within each of the selected sites. Samples from the various sites within a state were bucked to represent the treatment from that block. Soil samples were also collected along with the plant samples. To achieve this, the rhizosphere soils adhering to the roots of the collected plants were shaken off directly into labeled polythene bags, which were immediately sealed. Both the weed and soil samples were taken to the laboratory for analysis. The weed spectrum was determined through physical examination and identification aided by the practical manual of Akobundu and Agyakwa (1987), while density was determined by physical count.

2.5 Enumeration of Soil Microbial Population

Numbers of microflora were estimated by soil dilution technique on Nutrient and Potato Dextrose Agars as isolation media for bacteria and fungi, respectively. To achieve serial dilution, 5 grams of soil was suspended in 150 ml Erlenmeyer flask containing 95 ml of sterilized distilled water to obtain a 10^{-1} dilution and was kept under shaking conditions at 120 rpm for 15 minutes. From the flask 1 ml of suspension was transferred to 9 ml water blank to make 10^{-2} dilution. The water blank was vortexed and then again 1 ml of the suspension was transferred to a new water blank (9 ml) tube to obtain 10^{-3} dilution. In the similar manner dilutions were made up to 10^{-8}. The nutrient agar medium was composed of peptone 5 g, meat extract 3 g, agar 15 g and 1000 mL distilled water. For bacterial count 0.1 ml aliquot of the dilution to 10^{-8} was spread and plated on nutrient agar medium Petri plates in triplicates. Then the plates were incubated in an inverted position at 28˚C for 2 days. The constituents of the Potato Dextrose Agar (gL^{-1}) were Peptone 5.0, potato extract 5.0, dextrose 10.0, Agar 20.0, and distilled water 1000.0 ml at pH 6.5. A mixture of 1g soil and 10mL of saline solution was shaken on a mechanical shaker for 10 minutes to dislodge fungal propagules into the solution. This was followed by serial dilutions to the concentrations of 10^{-5}. 0.5 mL of the aliquot was spread on Potato dextrose extract agar to isolate fungal spores and this was incubated at 28^{0C} for 4 days. Dilution factors of 8 and 5 were used to determine the bacterial colony and fungal spore forming units, respectively.

2.6 Data Analysis

Data collected from the experiment were submitted to an analysis of variance using Minitab 17, while treatment means were compared using the Tukey Test. Plant count data were normalized using Square root transformation before being subjected to ANOVA. Simple linear correlation and regression analysis was performed between increasing time of *C. odorata* infestation (X) and plant density, plant species diversity, or soil microbial population (Y) with a scientific calculator (Casio *fx*-7400G PLUS POWER GRAPHIC Model). Multiple correlations was also done with Minitab 17 and results were indicated by a Matrixplot. Graphs were prepared using Microsoft Excel ® (2016 version) and error bars were determined using the standard error.

3. Results and Discussion

3.1 Plant Species Enumeration Under C. odorata Infestation

The species of plants collected under varying age of *C. odorata* infestation are presented in Tables 1, 2, 3 and 4. Plant species such as *Tridax procumbens*, *Biden pilosa*, *A. Coyzoides* and *Synedrella odiflora*, belonging to the family Asteraceae, which were observed in the control plots were completely absent in the *C. odorata* infested fields irrespective of the stage of infestation. Similarly, plants belonging to the families Boraginaceae, Nactaginaceae, Rubiaceae and Aizoaceae were all missing from fields infested with *C. odorata*, but they appeared in the control plots. Other classes of plants such as Solanaceae, Poaceae, loganinaceae and Portulaceae were present at the early stages of *C. odorata* infestation, but were not found as *C. odorata* infestation advanced. This confirms the ability of *C. odorata* to reduce plant diversity in infested areas (Zachariades & Goodall, 2002). The classes of plant species observed to be missing probably belong to those highly vulnerable to competition by *C. odorata*, which can either be allelospolic or ellelopathic competition, or both. *Choromolaena odorata* has been found to suppress indigenous grassland and savannah vegetation through physical smothering (Zachariades & Goodall, 2002). There have also been many findings showing that substances released by *C. odorata* and aqueous extracts of the plant remarkably influence the seed germination and growth of neighboring plants (Hu & Zhang, 2013; Suwal *et al*., 2010).

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Table 1. Plant species under *C. odorata* infestation (1--2 years)

Plant family	Weed taxa	Growth form	Sampling blocks		
			Ondo	Ekiti	Osun
Acanthaceae	*Asystasia gangatica*	ABL	X	X	✓
Asteraceae	*Chromolaena odorata*	PBL	✓	✓	✓
	Aspillia africana	PBL	✓	✓	✓
Caesalpinioideae	*Daniellia oliveri*	PBL	X	X	✓
Combretaceae	*Combretum hispidum*	PBL	X	✓	X
Convolvulaceae	*Merremia aegytia*	AS	X	✓	✓
	Ipomoea eriocarpa	AS	✓	X	X
	Ipomoea triloba	AS	X	✓	X
Loganiaceae	*Spidelia anthelmia*	ABL	✓	X	X
Poaceae	*Brachiaria deflexa*	AG	X	✓	X
	Andropogen tectorum	PG	X	X	✓
Solanaceae	*Physalis micranths*	ABL	✓	✓	X
Cyperaceae	*Cyperus esculentus*	AG	X	✓	X
Euphorbiaceae	*Acalypha fimbriata*	ABL	X	✓	X
	Monihot esculentus	PBL	✓	X	X
Lamiaceae	*Hyptis lanceolata*	ABL	X	X	✓
Malvaceae	*Sida acuta*	PBL	X	X	✓
	Sida rhombifolia	PBL	X	X	✓
Commelinaceae	*Aneilena beniniense*	ABL	X	✓	X

✓ = Present, X = absent, ABL = Annual broadleaf, PBL = Perennial broadleaf, AG = Annual grass, PG = Perennial grass, AS = Annual sedges and PS = Perennial sedges.

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Table 2. Plant species under *C. odorata* infestation (3-4 years)

Plant family	Weed taxa	Growth form	Sampling blocks		
			Ondo	Ekiti	Osun
Asteraceae	*Chromolaena odorata*	PBL	✓	✓	✓
	Aspillia africana	PBL	X	✓	✓
Euphorbiaceae	*Euphorbia heterophyla*	ABL	✓	X	X
	Phyllathus amarus	ABL	X	X	✓
	Alchornea laxiflora	ABL	X	✓	X
Poaceae	*Eragrostis tenella*	AG	✓	X	X
	Brachiaria deflexa	AG	X	✓	X
	Andropogon tectorum	PG	X	X	✓
Hippocrateaceae	*Reissantia indica*	PS	✓	X	✓
Convolvulaceae	*Ipomoea triloba*	AS	X	✓	✓
	Ipomoea eriocarpa	AS	✓	X	X
Caesalpinioideae	*Anthonotha macrophylla*	PBL	X	X	✓
Portulacaceae	*Talinum triangulare*	PBL	✓	X	X
Smilacaceae	*Smilax anceps*	PS	✓	X	X
Tiliaceae	*Triumfetta cordifolia*	PBL	✓	X	X
Lamiaceae	*Hyptis lanceolata*	ABL	X	✓	X
Malvaceae	*Abutilon mauritianum*	PBL	X	X	✓
	Sida acuta	PBL	X	✓	X
Fabaceae	*Mucuna pruriens*	AS	X	✓	X

✓ = Present, X = Absent, ABL = Annual broadleaf, PBL = Perennial broadleaf, AG = Annual grass, PG = Perennial grass, AS = Annual sedges and PS = Perennial sedges.

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Table 3. Plant species under *C. odorata* infestation (5 years and above)

Weed family	Weed taxa	Growth form	Sampling blocks Ondo	Ekiti	Osun
Asteraceae	*Chromolaena odorata*	PBL	✓	✓	✓
	Aspillia africana	PBL	✓	X	X
Convolvulaceae	*Ipomoea eriocarpa*	AS	✓	X	X
	Ipomoea triloba	AS	✓	X	X
	Hewittia sublobata	PS	X	X	✓
Urticaceae	*Pouzolzia guineensis*	ABL	✓	X	X
Euphorbiaceae	*Manniophyton fulvum*	PBL	✓	X	X
	Phyllanthus amarus	ABL	X	✓	X
	Acalypha fimbriata	ABL	X	X	✓
Caesalpinioideae	*Anthonotha macrophylla*	PBL	X	X	✓
Commelinaceae	*Commelina benghalensis*	PBL	X	✓	X
Icacinaceae	*Icacina trichanth*	PBL	X	✓	✓
Malvaceae	*Sida acuta*	PBL	X	✓	✓
Hippocrateaceae	*Reissantia indica*	PS	X	X	✓
Acanthaceae	*Asystasia gangetica*	ABL	X	✓	X
	Acanthus montanus	ABL	X	✓	X

✓ = Present, X = Absent, ABL = Annual broadleaf, PBL = Perennial broadleaf, AG = Annual grass, PG = Perennial grass, AS = Annual sedges and PS = Perennial sedges.

Table 4. Plant species in fields with no *C. odorata* infestation

Weed family	Weed taxa	Growth form	Sampling blocks Ondo	Ekiti	Osun
Asteraceae	*Tridax procumbumbens*	ABL	X	X	✓
	Bidens pilosa	ABL	✓	X	✓
	Ageratum conyzoides	ABL	X	X	✓
	Synedrella nodiflora	ABL	✓	✓	X
Amaranthaceae	*Amaranthus spinosis*	ABL	✓	✓	✓
	Gomphrena celosioides	ABL	X	X	✓
Malvaceae	*Sida acuta*	PBL	X	✓	X
	Sida rhombifolia	PBL	✓	✓	X
	Sida cordifolia	PBL	✓	X	X
	Malvastrum coromandelianum	ABL	X	✓	X
Solanaceae	*Physalis angulata*	ABL	✓	X	✓
Poaceae	*Eleusine indica*	AG	X	X	✓
	Panicum laxus	AG	✓	X	X
	Eragrosis tenella	AG	X	X	✓
Boraginaceae	*Heliotropium ovalifolium*	ABL	X	✓	X
Nyctaginaceae	*Boerhavia coccinea*	PBL	X	✓	✓
Euphorbiaceae	*Acalypha fimbriata*	ABL	X	✓	X
	Euphorbia hirta	ABL	X	X	✓
Loganinaceae	*Spigelia anthelmia*	ABL	X	X	✓
Portulacaceae	*Portulaca oleracea*	ABL	X	X	✓
Rubiaceae	*Mitracarpus villosus*	ABL	X	X	✓
Aizoaceae	*Trianthema portulacastrum*	ABL	X	X	✓

✓ = Present, X = Absent, ABL = Annual broadleaf, PBL = Perennial broadleaf, AG = Annual grass, PG = Perennial grass, AS = Annual sedges and PS = Perennial sedges.

3.2 Effects of Stage of C. odorata Infestation on Plant Species Richness and Density.

Effects of infestation stage of *C. odorata* on plant density and plant species diversity shown in figure 1 indicate that plant density appeared to decrease with age of *C. odorata*, and the decrease became significant ($P < 0.05$) after three years of invasion. Plant species was significantly ($P < 0.05$) more diverse in fields with no *C. odorata* infestation than in invaded fields regardless of the stage of infestation. Previous studies have shown that *C. odorata* produces a variety of allelochemicals, including flavonoids, terpenoids, and alkaloids (Ambika and Jayachandra, 1980). Production of these compounds is most likely to vary in concentration and proportion with age of *C odorata* as is the case with certain other plant species. Chaparro *et al*. (2013) have shown that Arabidopsis roots release more phenolic-related compounds at later stages of life. This phenomenon has been suggested to be correlated to defense strategies against pathogens as secondary metabolites are involved in plant immunity against bacterial and fungal pathogens (Rogers *et al*., 1996; Clay *et al*., 2009; Millet *et al*., 2010; An and Mou, 2011; Bednarek, 2012).

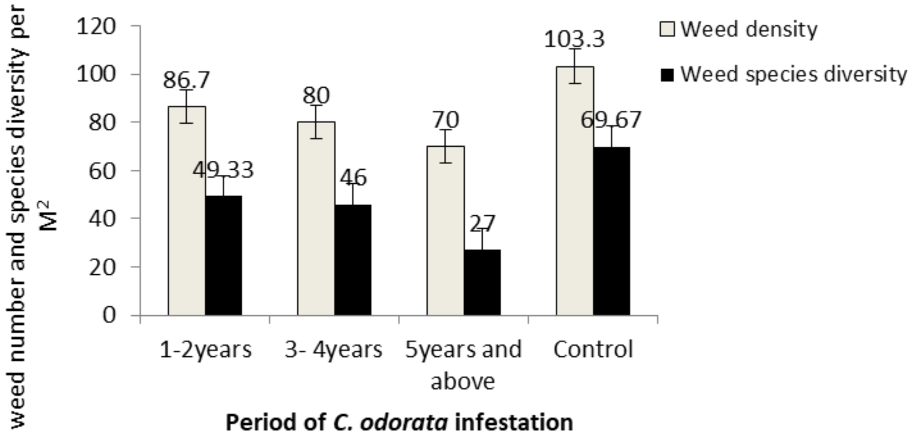

Figure 1. Effects of *C. odorata* invasion on weed density (m^{-2}) and species diversity

3.3 Responses of the Rhizosphere Microbial Population to C. odorata Infestation.

Chromolaena odorata infestation caused reduction in bacteria population in the rhizospere soils of the plants sampled, and this reduction (24.3%) became significant ($P < 0.05$) at about 5 years of continuous occupation by the invasive species (figure 2). Fungal population on the other hand was observed to be higher in the Siam weed infested plots than in the control, and this was also characterized by a drop in population density with time. The dynamics of yeast colonization of the rhizosphere in the sampled fields was similar to that of bacteria. Invasion of *C. odorata* at all times reduced yeast population relative to the control plot, and these reductions were by 39.6, 46.5 and 75.2%, respectively for invasion of 1-2 years, 3-4 years, and 5 years and above. Regressing stage of *C. odorata* infestation (x) against weed density, species diversity; or bacterial, fungal or yeast population (y) indicated negative relationships with prediction equation shown in Table 5.

The reduced bacteria population and increase in fungal count associated with *C. odorata* infestation suggested that *C. odorata* infestation exerted diverse influences on soil microbial activity in the soil. The rhizo-deposits (e.g. exudates, border cells, mucilage) have been identified as a major driving force in the regulation of microbial diversity and activity (Mendes *et al.*, 2013).

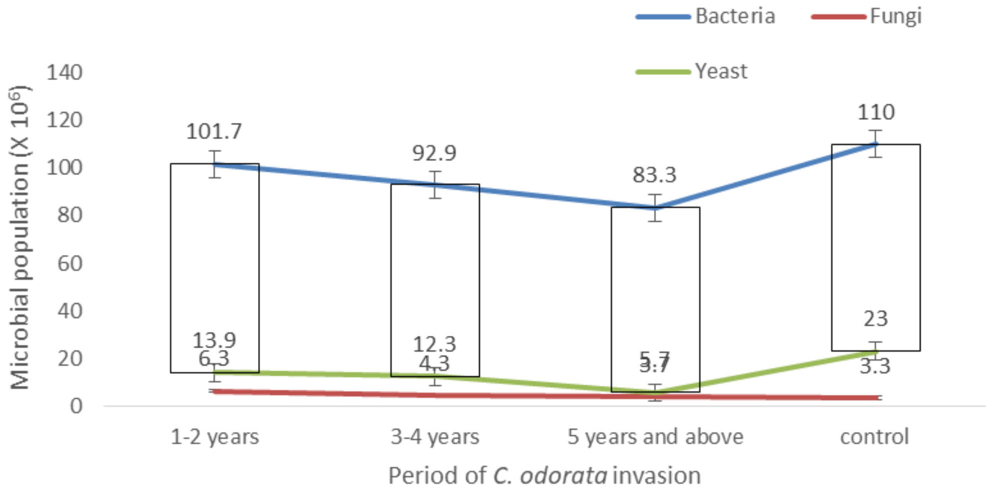

Figure 2. Effects of *C. odorata* infestation on soil microbial population (X 10^6)

Table 5. Linear correlation and regression analysis between increasing year of *C. odorata* infestation (x) and weed and soil microbial parameters (y)

Parameters	Correlation coefficient (r)	Regression equation
Weed density	- 0.99	Y = 9.14 – 0.42X
Weed diversity	- 0.93	Y = 57.52 – 5.58X
Bacteria	-0.99	Y = 111.03 - 9.2x
Fungi	-0.98	Y = 7.37 - 1.3x
Yeast	-0.91	Y = 18.83 - 4.1x

Since the composition of rhizo-deposits would vary with varying plant species composition, it is expected that microbial diversity and population will respond along plant population and diversity gradient. This presumably resulted from the ability of *C. odorata* to suppress the growth of certain classes of plants on whose root exudates certain groups of soil microorganisms depend for survival. Yeast population was highest in the control field, and this decreased from zero infestation as the years advanced. Juxtaposing this with the effects of stage of infestation on species diversity suggests that reduction in species diversity with time has a direct bearing on yeast population. One of the several classes of phytochemicals that constitute exudates from *C. odorata is* alkaloids (Hamidi *et al.*, 2014). Wink (1987) discovered in an in-vitro experiment with more than 70 alkaloids that most alkaloids are toxic or inhibitory to more than one group of organisms including plant seedlings, bacteria, insects and mammal. Einhellig (2002) also found that high amount of alkaloids can inhibit cell division and cell wall formation.

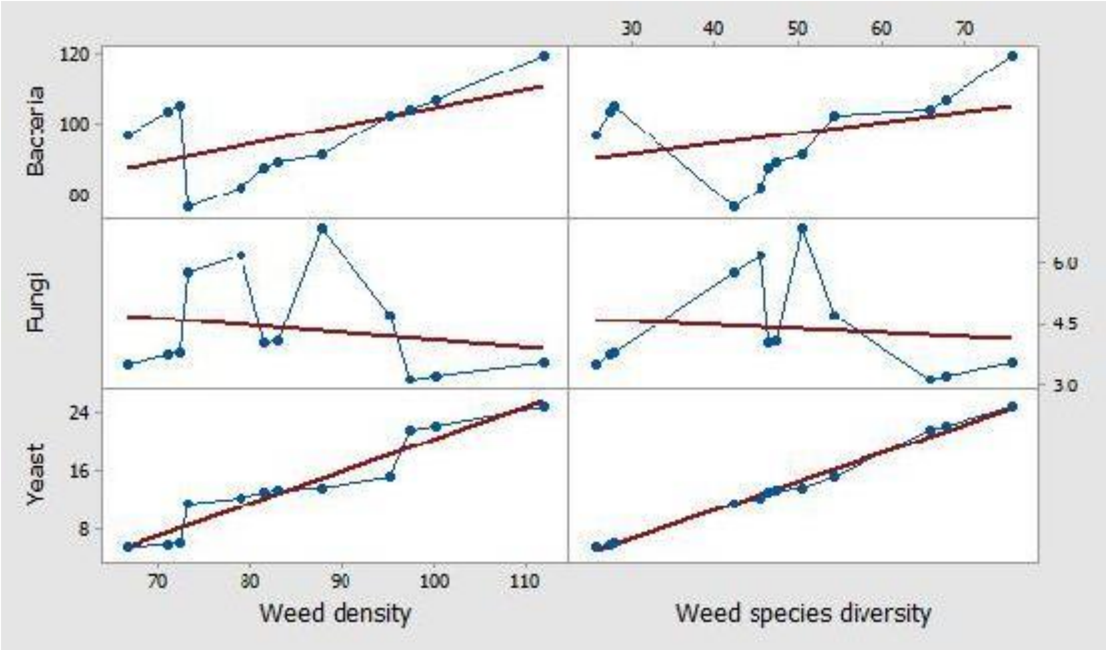

Figure 3. Matrix plot of bacteria, fungi, yeast Vs weed density, weed species

The relationships between weed density or species diversity and microbial population indicate positive correlation between weed density or species diversity and bacteria or yeast population (figure 3). Fungal population on the other hand was inversely related to the plant parameters of density and diversity.

4. Conclusions

There are empirical evidences to corroborate the widely reported suppressive influences of the Siam weed (*Chromolaena odorata*) on neighbouring plant species. Results showed that the suppression caused by this allelopathic plant species is species selective. The findings further revealed that the trim in species diversity and density also have a downward bearing on the populations of the rhizosphere colonizing microorganisms. *C. odorata* is therefore labeled as a threat to biodiversity whose management demands urgent attention.

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